

ANNUAL REPORT  
OF  
PROGRAM ACTIVITIES  
NATIONAL EYE INSTITUTE  
Fiscal Year 1981

U. S. DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service      National Institutes of Health











THE NATIONAL EYE INSTITUTE

60

ANNUAL REPORT

Fiscal Year 1981



TABLE OF CONTENTS

	<u>Page</u>
Statement of the Institute Director	1
Extramural and Collaborative Programs	3
Report of the Associate Director	5
Retinal and Choroidal Diseases	9
Corneal Diseases	31
Cataract	53
Glaucoma	89
Strabismus, Amblyopia, and Visual Processing	101
Office of Biometry and Epidemiology	123
Report of the Chief	125
Contract Narratives:	
Diabetic Retinopathy Study	133
Diabetic Retinopathy Vitrectomy Study	135
Early Treatment Diabetic Retinopathy Study	137
Visual Acuity Impairment Survey (VAIS) Pilot Study	139
Office of Program Planning, Analysis, and Evaluation	141
Report of the Chief	143
Office of Scientific Reporting	145
Report of the Chief	147
Intramural Research	151
Report of the Scientific Director	153
Clinical Branch	155
Report of the Clinical Director	157
Ballintine, Elmer J., M.D.	
Ocular Hypertension Study	165
Search for Diabetic Retinopathy in Acromegaly	167
Urokinase Central Retinal Vein Occlusion Trial	169

Kaiser-Kupfer, Muriel I., M.D.	
The Diagnosis, Pathogenesis and Treatment of Gyrate Atrophy of the Choroid and Retina	173
The HLA and ABO Antigens and Immunologic Studies in Cogan's Syndrome	177
Ophthalmologic Screening for Tamoxifen Toxicity to the Eye	179
Pigment Dispersion With and Without Glaucoma	181
Progressive Essential Iris Atrophy	185
Visual Function and Ocular Pigmentation in Albinism	187
Kupfer, Carl, M.D.	
Anterior Chamber Anomalies Associated with Glaucoma or Ocular Hypertension	189
Meyers, Sanford M., M.D.	
Laser Instrumentation for Vitreous Surgery	191
Septic Chorioretinitis in Animals	193
Section on Clinical Eye Pathology	
Rodrigues, Merlyn M., M.D.	
Clinicopathologic Studies of Human Ocular Diseases	195
Histopathologic Studies of Animal Models of Human Ocular Diseases	201
Histopathology and In Vitro Characteristics of Human Corneal Dystrophies and Degenerations	205
Section on Glaucoma	
Gaasterland, Douglas E., M.D.	
Aqueous Humor Flow Measurement by Fluorophotometry	209
Experimental Glaucoma in the Rhesus Monkey	211
Laboratory Studies of Aqueous Humor Dynamics	213
Laser Surgery for Glaucoma	217
Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma	221
Studies of Parameters of Intraocular Pressure	223
Treatment of Neovascular Glaucoma	227
Section on Neuro-Ophthalmology	
Cogan, David G., M.D.	
Contributions to Ophthalmic Pathology and Systemic Disease	229
The Eye and Metabolic Disease	231
Oculomotor Disorders in Human Subjects	235
Parametric Studies of the Pupillary Functions	239

Section on Ophthalmic Immunology

Nussenblatt, Robert, M.D.

Cataracts in Juvenile Guinea Pigs with Allergic Encephalomyelitis	241
Cyclosporin A Therapy in Uveitis	243
Double Masked Treatment of Ocular Toxoplasmosis	245
HLA, ABO, and B-cell Alloantigens and Ocular Inflammatory Disease	247
Immune Functions in Ocular Diseases of Obscure Etiology	249
Immune Mechanisms in Experimental Autoimmune Uveitis	253

Salinas-Carmona, Mario, M.D.

Suppression of Retinoblastoma Proliferation by Human Soluble Lymphocyte Factors	257
--	-----

Section on Retinal and Ocular Connective Tissue Diseases

Hess, Helen H., M.D.

Biochemistry of Retinal and Pigmented Epithelium in Health and Disease	261
---	-----

Newsome, David A., M.D.

Biochemistry and Biology of Normal and Pathologic Retinochoroidal Tissues	267
Clinical and Laboratory Studies in Macular and Retinal Degenerations	271
Effects of Vitamin A, Selected Hormones and Drugs on Corneal Epithelium and Collagenases	275
Ocular Connective Tissue Macromolecules and Their Function in Vision	279

Section on Visual Processing

de Monasterio, Francisco M., M.D., D.Sc.

Acquired Color Vision Deficiencies: Mechanisms and Diagnosis	283
Electrophysiological and Psychophysical Evaluation of Retinal Disorders	287
Physiological Studies of the Visual System of Primates	289
Retinal Function in Posterior Uveitis	293

Gunkel, Ralph D., O.D.

Research in Methods of Evaluating Visual Processes	297
--	-----

Higgins, Kent E., Ph.D.

Spatial Contrast Sensitivity Studies in Retinal Disease	301
---	-----

Jaffe, Myles, O.D.

Psychophysics in the Management of Chiasmatic Lesions	303
---	-----

	<u>Page</u>
Schein, Stanley J., M.D., Ph.D. Anatomical Studies of the Visual System of Primates	307
Laboratory of Sensorimotor Research	313
Report of the Chief	315
Neuro-ophthalmologic Mechanisms Section	
Goldberg, Michael E., M.D. Cerebral Cortical Mechanisms for Eye Movements and Visual Attention	319
Visual Processing in Brains following Cortical Ablation	323
Oculomotor Control Section	
Miles, Frederick A., D.Phil. Adaptive Regulation in the Vestibulo-ocular System The Neural Coupling between Vergence Eye Movements and Accommodation	327
	331
Optican, Lance M., Ph.D. Quantitative Modeling of Sensorimotor Systems	335
Real-time Behavioral Control and Data Acquisition	339
Visuomotor Integration Section	
Albano, Joanne E., Ph.D. Visual and Oculomotor Functions of the Primate Superior Colliculus	343
Richmond, Barry J., M.D. Visual Processing in Inferior Temporal Cortex	347
Robinson, David Lee, Ph.D. Visuomotor Properties of Neurons in the Thalamus and Extrastriate Cortex	351
Wurtz, Robert H., Ph.D. Role of Substantia Nigra in the Initiation of Eye Movements	355
Visual Motion Processing in the Primate Brain	359
Laboratory of Vision Research	363
Report of the Chief	365
Section on Biochemistry	
Kador, Peter, Ph.D. Cataracts	367

	<u>Page</u>
Russell, Paul, Ph.D. Chemistry and Metabolism of the Lens	371
Shichi, Hitoshi, Ph.D. The Biochemical Pharmacology of the Eye The Biochemistry of the Visual Process	375 377
Zigler, J. Samuel, Jr., Ph.D. Structure and Composition of Lens Crystallins with Respect to Cataract Development	381
Section on Experimental Biology	
Nelson, Ralph, Ph.D. Electrophysiology and Morphology of Mammalian and Avian Retinas	385
Puro, Donald G., M.D., Ph.D. Neuropharmacology of the Retina	391
Section on Experimental Embryology	
Zelenka, Peggy, Ph.D. Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia	395
Section on Experimental Immunology	
Gery, Igal, Ph.D. Immune Responses to Ocular Antigens Macrophage Interactions With Other Cells and Their Components	399 403
Section on Experimental Pathology	
Carter-Dawson, Louvenia, Ph.D. Role of Vitamin A in Maintenance and Development of Ocular Tissues	407
Robison, W. Gerald, Jr., Ph.D. Ultrastructure and Function of the Pigment Cells of the Eye	411
Section on Retinal and Corneal Metabolism	
Battelle, Barbara-Anne, Ph.D. Neurotransmitter Chemistry of Retinal Neurons	415
Chader, Gerald J., Ph.D. Metabolism of the Retina and Pigment Epithelium	419

	<u>Page</u>
Schein, Stanley J., M.D., Ph.D. Anatomical Studies of the Visual System of Primates	307
Laboratory of Sensorimotor Research	313
Report of the Chief	315
Neuro-ophthalmologic Mechanisms Section	
Goldberg, Michael E., M.D. Cerebral Cortical Mechanisms for Eye Movements and Visual Attention	319
Visual Processing in Brains following Cortical Ablation	323
Oculomotor Control Section	
Miles, Frederick A., D.Phil. Adaptive Regulation in the Vestibulo-ocular System The Neural Coupling between Vergence Eye Movements and Accommodation	327
	331
Optican, Lance M., Ph.D. Quantitative Modeling of Sensorimotor Systems Real-time Behavioral Control and Data Acquisition	335
	339
Visuomotor Integration Section	
Albano, Joanne E., Ph.D. Visual and Oculomotor Functions of the Primate Superior Colliculus	343
Richmond, Barry J., M.D. Visual Processing in Inferior Temporal Cortex	347
Robinson, David Lee, Ph.D. Visuomotor Properties of Neurons in the Thalamus and Extrastriate Cortex	351
Wurtz, Robert H., Ph.D. Role of Substantia Nigra in the Initiation of Eye Movements	355
Visual Motion Processing in the Primate Brain	359
Laboratory of Vision Research	363
Report of the Chief	365
Section on Biochemistry	
Kador, Peter, Ph.D. Cataracts	367

	<u>Page</u>
Russell, Paul, Ph.D. Chemistry and Metabolism of the Lens	371
Shichi, Hitoshi, Ph.D. The Biochemical Pharmacology of the Eye The Biochemistry of the Visual Process	375 377
Zigler, J. Samuel, Jr., Ph.D. Structure and Composition of Lens Crystallins with Respect to Cataract Development	381
Section on Experimental Biology	
Nelson, Ralph, Ph.D. Electrophysiology and Morphology of Mammalian and Avian Retinas	385
Puro, Donald G., M.D., Ph.D. Neuropharmacology of the Retina	391
Section on Experimental Embryology	
Zelenka, Peggy, Ph.D. Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia	395
Section on Experimental Immunology	
Gery, Igal, Ph.D. Immune Responses to Ocular Antigens Macrophage Interactions With Other Cells and Their Components	399 403
Section on Experimental Pathology	
Carter-Dawson, Louvenia, Ph.D. Role of Vitamin A in Maintenance and Development of Ocular Tissues	407
Robison, W. Gerald, Jr., Ph.D. Ultrastructure and Function of the Pigment Cells of the Eye	411
Section on Retinal and Corneal Metabolism	
Battelle, Barbara-Anne, Ph.D. Neurotransmitter Chemistry of Retinal Neurons	415
Chader, Gerald J., Ph.D. Metabolism of the Retina and Pigment Epithelium	419

	<u>Page</u>
Dudley, Peter, Ph.D. Retina Lipid Metabolism: Correlation with a Circadian Rhythm and Effect of Light	423
Liu, Y. P., Ph.D. Cyclic Nucleotides and Vision	425
O'Brien, Paul J., Ph.D. The Biochemistry of Normal and Dystrophic Retinas	429
The Cell Biology of the Vertebrate Retina	431
Wiggert, Barbara, Ph.D. Vitamin A and Ocular Tissues	435

STATEMENT OF THE INSTITUTE DIRECTOR

During the past year the National Eye Institute's budget increased to \$118 million, enabling the continuation and maintenance of both the extramural and intramural programs. In FY 1981 the NEI was able to fund over 1,000 extramural-initiated research project grants and 76 intramural projects. This was 100 less than NEI's high point of 1,100 grants in FY 1979.

Perhaps the most important management activity during the past year was the development of the National Advisory Eye Council's third comprehensive program evaluation and national plan which will cover the fiscal years 1983-1987. This effort involved staff from every branch of the Institute. We expect this document, which will be published early in 1982, to provide the Council and NEI staff with a structure and guidance for the development of the nation's vision research effort over the next few years. As has been the case with past planning reports, the new plan should be of great value to the NEI staff in formulating policy and in carrying out the day-to-day management of NEI programs. The plan will also provide a valuable resource for responding to requests for program information from higher governmental levels and from the public. And perhaps most important, we expect the new report to increase the Institute's capability for responding to emerging national policies concerning governmental support of biomedical science. It is clear that program planning will be even more crucial in the years to come than it has been in the past as the Administration and the Congress reexamine the Federal role in supporting a wide range of domestic programs.

In the meantime, the NEI must make every effort to maximize the return on its investment in vision research and to modify its policies and organization to meet changing needs. Because of the past growth and increased complexity in the programs and mechanisms supported within the Extramural and Collaborative Programs, this part of the Institute was officially reorganized during FY 1981 into the following four branches: the Retinal and Choroidal Diseases Branch; the Anterior Segment Diseases Branch; the Strabismus, Amblyopia, and Visual Processing Branch; and the Extramural Services Branch.

Also during the past year Dr. Jin H. Kinoshita was named Scientific Director of the NEI, and Dr. Gerald Chader took his place as Chief of the Laboratory of Vision Research. Dr. Joram Piatigorsky was named Chief of the newly-created Laboratory of Molecular and Developmental Biology. Under Dr. Piatigorsky's leadership, this laboratory will apply the most up-to-date advances in molecular biology to ophthalmic research.

The accomplishments of the NEI and those it supported over the past year, and examples of current and planned research and administrative endeavors, are presented in the following reports of NEI offices and branches.

  
Carl Kupfer, M.D.



EXTRAMURAL AND COLLABORATIVE PROGRAMS



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS  
Ronald G. Geller, Ph.D.

In keeping with the Institute's first priority, support for investigator-initiated individual research projects (R01), over 900 awards were made covering a wealth of scientifically exciting ideas relevant to the prevention, treatment, and cure of diseases and disabilities of the visual system. This represents about 90 percent of our extramural budget. The following sections highlight some of the issues and accomplishments in the NEI Extramural and Collaborative Programs during FY 1981.

For FY 1981, the National Eye Institute received an appropriation of \$117,983,000--an increase of \$4,983,000 over the previous year's appropriation. Of the \$117,983,000, a total of \$102,053,000 was allocated to Extramural and Collaborative Program activities in the following categories:

Research Grants	\$ 93,674,000
Research Training Awards	4,088,000
Research Contracts	<u>4,291,000</u>
Total	\$102,053,000

These funds were distributed among the Institute's five programs as follows:

	Research, Training, & Contract Dollars (in thousands)
Retinal and Choroidal Diseases	\$ 42,424
Corneal Diseases	16,811
Cataract	9,697
Glaucoma	9,949
Strabismus, Amblyopia, and Visual Processing	<u>23,172</u>
Total	\$102,053

The grant application receipt rate was 20 percent higher than in FY 1980. The National Advisory Eye Council approval rate was also higher. Ninety-five percent of grants submitted were approved for funding in FY 1981. The Institute was able to fund only 51 percent of all approved applications, essentially the same as in FY 1980. The data are given below:

Grant Application Rate<sup>1</sup>

	<u>Received &amp; Reviewed</u>	<u>Recommended for Approval</u>	<u>Approved &amp; Funded</u>	<u>% Funded of All Approved Applications</u>
FY 1978	681	562	343	61
FY 1979	579	495	308	62
FY 1980 <sub>2</sub>	516	432	225	52
FY 1981 <sup>2</sup>	636	606	309	51

<sup>1</sup>

<sup>2</sup> R01 and R23

FY 1981 figures are estimates based on current information.

The distribution of awards (for R01s and R23s) between competing and non-competing research grant applications was as follows:

	<u>FY 1979 Number of Grants</u>	<u>FY 1980 Number of Grants</u>	<u>FY 1981 Number of Grants</u>
Prior Year Commitments	577	679	601
New Research Awards	177	86	159
Renewal Awards	<u>115</u>	<u>115</u>	<u>150</u>
	869	880	911

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

FY 1981 Research Grants by Mechanism  
(Dollars in Thousands)

	<u>Number</u>	<u>Total Awarded</u>
Research Grants (R01, R10, R13, R23)	933	86,967
Core Grants (P30)	25	3,563
Specialized Clinical Research		
Center Grants (P50)	4	562
Research Career Development Awards (K04)	46	1,831
Academic Investigator Awards (K07)	<u>8</u>	<u>277</u>
	1,016	93,200

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).

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 \$4,078,000 was available for support of vision research training in FY 1981, most of it for the National Research Service Awards (NRSA). The individual NRSA fellowship awards accounted for \$1,433,000, or 35 percent of available training funds. The institutional NRSA training awards accounted for \$2,645,000, or 65 percent of the program. A summary of the training program for FY 1981 follows:

VISION RESEARCH TRAINING FY 1981  
(Amounts in Thousands)

	INSTITUTIONAL (NRSA T32)				INDIVIDUAL (NRSA F32)		Total (T & F)	Percent Training Budget
	No. of Inst. Awards	Pre-Doctoral	Post-Doctoral	Amount	No. of Ind. Awards	Amount		
Retinal and Choroidal Diseases	16	15	47	\$ 976*	30	\$ 582	\$1,558	39
Corneal Diseases	8	6	32	768	9	188	956	23
Cataract	2	0	6	133	4	72	205	5
Glaucoma	5	0	16	257	3	63	320	8
Strabismus, Amblyopia, and Visual Processing	7	17	22	511	28	528	1,039	25
<b>TOTALS</b>	<b>36</b>	<b>34</b>	<b>119</b>	<b>\$2,645</b>	<b>74</b>	<b>\$1,433</b>	<b>\$4,078</b>	<b>100</b>

\* \$13,000 of this total represents NEI's co-funding of two T35s (short-term training program) under the auspices of the NIGMS for four predoctoral positions.

The FY 1980 appropriation for the NEI included \$3,000,000 for research grants for construction of vision research facilities. The program was formally announced on September 19, 1980. Thirteen applications were received in response to this announcement.

The name of the Sensory and Motor Disorders of Vision and Rehabilitation program has been changed. The new name is the Strabismus, Amblyopia, and Visual Processing Branch.

## Small Grants Program

The National Eye Institute initiated a small grants program for pilot projects for all its program areas beginning with the October 1, 1981, application receipt date.

This is a one-year nonrenewable award intended to provide support for pilot projects, testing of new techniques, or feasibility studies of innovative and high-risk research, which would provide a basis for more extended research.

This program is designed to support:

Clinicians with limited research experience.

Recently trained, or less experienced, investigators.

Investigators whose research career was interrupted and is intended to be resumed.

Investigators changing field of research.

Investigators at minority institutions or located in a largely non-research environment.

Established investigators needing quick support for a pilot project.

The award may not be used to supplement support for an ongoing project.

The award will provide a maximum of \$15,000 (direct costs) for technical assistance, supplies, small equipment, and travel required by the project. The NEI expects to make approximately ten awards for each review cycle.

Applications will be evaluated with respect to the following criteria: The significance and scientific merit of the proposed project, and its characterization as an innovative and/or pilot project which provides a basis for more extended research; the methodology, including choice of experimental material; the investigator's background and training for carrying out the project; adequacy of the available and requested facilities; and the adequacy of justifications presented for budget requests.

## Staff Appointments in FY 1981

The following staff appointments occurred during FY 1981:

Constance Atwell, Ph.D., has been designated as Chief of the Strabismus, Amblyopia, and Visual Processing Branch

Ralph Helmsen, Ph.D., has been designated as Chief of the Anterior Segments Diseases Branch

Janet Cardenas, Ph.D., has been appointed as a Program Director in the Strabismus, Amblyopia, and Visual Processing Branch and is responsible for grants in the area of psychophysics, ocular motility, and strabismus

Jack McLaughlin, Ph.D., has been appointed Program Director for Retinal-Neural Diseases in the Retinal and Choroidal Diseases Branch

Anna Marie Perrell has been appointed as Chief of the Extramural Services Branch.

I. RETINAL AND CHOROIDAL DISEASES PROGRAM

FISCAL YEAR 1981

<u>Subprogram</u>	<u>Number of Grants</u>
A. Vascular, Inflammatory and Neoplastic Disorders of Retina and Choroid	[92]
1. Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities	57
2. Inflammatory Disorders	14
3. Tumors	19
4. Noninvasive Techniques in the Study of Retinal Disorders*	2
B. Degenerative Disorders of the Retina	[82]
1. Developmental and Hereditary Disorders	40
2. Macular Degeneration	22
3. Retinal Detachment and Vitreous Disorders	16
4. Rescue and Regeneration of Neurons in the Retina	2
5. Toxic, Nutritional, and Environmental Disorders	2
6. Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models*	0
C. Fundamental Processes and Retinal Disorders	[109]
1. Retinal Pigment Epithelium	25
2. Photoreceptors, Visual Pigments, and Phototransduction	114
3. Retinal Organization, Neurotransmission, and Adaptation	64
4. Glial Cells and the Retinal Micro-environment	6
TOTAL	383

\*Grants which are relevant to this subprogram but which are more specific to another subprogram are counted elsewhere.

## INTRODUCTION

During the past year, the staff of the Retinal and Choroidal Diseases Program and members of the Retinal and Choroidal Diseases Program Planning Panel have had many discussions related to the research needs of the program for the next five years. One of the major questions we discussed was how to logically categorize and integrate the basic and clinical research projects which relate to the many diseases associated with the retinal, choroidal, uveal, and vitreous tissues of the eye. The new program categories had to meet the following requirements:

- (1) the prevention of eye diseases should be emphasized,
- (2) the areas of need and research opportunity should be highlighted,
- (3) the need for more basic research related to medically relevant problems should be emphasized,
- (4) the desirability of more interaction between clinical investigators and basic researchers should be stressed, and
- (5) the new categorization should provide for effective management, both scientifically and administratively.

In the preceding table, the revised program categorization is presented together with the number of awards made during FY 1981.

This categorization contains all of the major elements of prior classifications used in the previous Program Plan. But, several significant changes should be noted. First, to highlight new research opportunities which relate to retinal and choroidal diseases, two new areas were added. These are Rescue and Regeneration of Neurons in the Retina and Noninvasive Techniques in the Study of Retinal Disorders. Each of these areas holds promise for some new discoveries in vision research. Second, projects in the area of low vision were consolidated under the Strabismus, Amblyopia and Visual Processing Program.

What follows in the rest of this chapter is a description of the research objectives for each subprogram area, a synopsis of significant research findings during the past year, and a discussion of the many challenges for vision scientists for the near future.

## A. Vascular, Inflammatory and Neoplastic Disorders of the Retina and Choroid

### Al. Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Objectives: The most important disease of the retinal blood vessels is diabetic retinopathy, which is a frequent complication in individuals who have diabetes mellitus. Diabetes mellitus is not the only such threat to vision; other diseases of the retinal blood vessels that can also produce loss of vision include hypertension and arteriosclerosis, vascular occlusions, sickle cell disease, retrolental fibroplasia and cystoid macular edema. These diseases can impair retinal function by causing blood vessels to grow abnormally, to close off, or to leak. The objectives of this subprogram which are directed toward helping us understand, correct and prevent these retinal blood vessel abnormalities are:

- o To develop better methods of prevention, diagnosis and treatment of diabetic retinopathy and other vascular diseases of the retina and choroid (such as sickle cell retinopathy, vascular occlusions, and retinopathy of prematurity).
- o To understand the anatomy, biochemistry, and physiology of the retinal and choroidal vasculature in normal and diseased states.
- o To understand how blood flow is controlled in the retina.

Recent Research Accomplishments: There are several advances which are noteworthy in attempting to understand retinal blood vessel abnormality. The isolation and maintenance of capillary endothelial cells <sup>1,2</sup> should enhance the possibility that new information about the biochemistry and physiology of retinal vascular cells will be forthcoming. In addition, progress in developing a retinal oximeter <sup>3</sup> should provide the opportunity for further knowledge of the retinal circulation and retinal metabolism.

Future Needs: With the gains that have been made in the treatment of diabetic retinopathy, the major research effort should now be in elucidating the mechanisms responsible for neovascularization, vessel closure, and vessel leakage. Given the frequency of retinal vascular occlusion, an innovative research project which should be attempted is the prolongation of retinal survival and the restoration of retinal function after such an occlusion (see program on Rescue and Regeneration of Neurons in the Retina). These areas of research will benefit greatly from interactions of research clinicians and basic vision scientists with other biomedical researchers.

## A2. Inflammatory Disorders

Objectives: Inflammatory diseases of the retina and the uvea can generally be divided into two major categories -- infectious and noninfectious. Infectious agents which destroy the retina include viruses, bacteria, fungi and protozoans. Noninfectious inflammatory diseases of the retina and choroid include a broad category of disorders related to the immunologic defense system of the body. Although inflammatory disorders of the retina can cause blindness, they can also cause other forms of morbidity such as floating spots, distortion of images, severe sensitivity to light and in some cases pain.

In an effort to increase our understanding of inflammatory diseases, the objectives of this subprogram are:

- o To establish the causes or etiologic factors responsible for uveitis.
- o To improve the accuracy with which uveitis can be diagnosed.
- o To establish the natural course and prognosis of various forms of uveitis.
- o To develop improved methods of therapy for uveitis.

Recent Research Accomplishments: The ability to diagnose several inflammatory diseases has been improved by applying immunological and biochemical microtechniques. Some examples of this are the ability to detect antibodies to toxoplasmosis using radio-immune and the enzyme-linked immunosorbent assays<sup>4</sup>, and to distinguish retinoblastoma from nematode endophthalmitis by the presence of abnormally high levels of lactate dehydrogenase in the aqueous humor of children with retinoblastoma<sup>5</sup>.

Future Needs: The application of techniques now being developed in the field of immunology to investigate the causes of uveitis is likely to provide unparalleled opportunities in elucidating the causes and pathogenesis of infectious and noninfectious uveitis. In addition, the development of animal models of uveitis which closely simulate the condition in humans will also greatly accelerate the accumulation of knowledge in this area.

## A3. Tumors

Objectives: Tumors of the eye originate primarily from the retina and the uvea, and less frequently from the lids, the conjunctiva and the orbit. Eye tumors such as uveal melanoma and retinoblastoma have an importance that far surpasses their frequency -- they can cause death as well as loss of vision.

Eye tumors can be diagnosed with some success. However, information about risk factors for developing tumors, about the most appropriate treatment for malignant tumors and the biology of the tumor is still lacking. To help address these problems, the objectives of the ocular tumor subprogram are:

Melanoma --

- o To determine the natural history and pathogenesis of ocular tumors with special emphasis on utilizing state-of-the-art biochemical, histochemical and immunologic techniques.
- o To define the epidemiology of ocular tumors with an emphasis on identifying possible risk factors for their development.
- o To determine the efficacy of treatments in randomized controlled clinical trials.
- o To understand the role of immunity in the disease process.
- o To develop and refine methods of diagnosing and characterizing ocular tumors.

Retinoblastoma --

- o To determine the etiology and pathogenesis of retinoblastoma.
- o To differentiate retinoblastomas which are genetic in origin from those which are "sporadic."
- o To improve the effectiveness of retinoblastoma treatment with emphasis on preserving vision.

Recent Research Accomplishments: A recent finding about risk factors for uveal melanoma was the demonstration of an increased incidence of choroidal melanoma in a cluster of chemical workers in the Eastern United States <sup>6</sup>. In retinoblastoma research, the use of established cell lines to study the biology of retinoblastoma cells has been <sup>7,8</sup> very successful. Investigators in two different research laboratories <sup>7,8</sup> have demonstrated the presence of cellular retinol and retinoic acid binding proteins on retinoblastoma cells grown in vitro.

Future Needs: These studies should prove useful in determining the cell of origin of retinoblastoma and in providing clues for the therapeutic management of the disease. Future opportunities in eye tumor research include epidemiologic studies and basic investigations about the biology of ocular tumors since such studies are essential to understanding the causes of and determining the appropriate management of these disorders.

#### A4. Noninvasive Techniques in the Study of Retinal Disorders

Objectives: Unlike other surgical specialities where biopsies are used to make a diagnosis, biopsies of retinal tissue are rarely performed because of the delicacy of the ocular tissue and the inherent risks of such an invasive procedure. Therefore, to obtain needed information about the disease process such as, which cells are mal- or non-functioning, what are the early signs of the anomaly, and does the capacity for recovery exist, the use of noninvasive tests for diagnosing retinal diseases is essential.

This is a research area which could be of great importance in the early detection and diagnosis of visual disorders, and in the evaluation of therapeutic modalities, but which has not received emphasis in accordance with its importance. The objectives of this subprogram encompass the following:

- o To encourage basic research in humans, animals, and other model systems so as to develop better noninvasive tests of retinal function.
- o To encourage research which enhances the application of these techniques in the diagnosis, treatment, prevention, and management of retinal disease in infants and adults.

The main thrust of this program is the application of noninvasive techniques (including the adaptation and modification of presently available techniques as well as the development of new techniques) to understanding ocular anomalies. Research is encouraged at the levels of both clinical and basic research.

#### B. Degenerative Disorders of the Retina

##### B1. Developmental and Hereditary Disorders

Objectives: Retinitis pigmentosa and related diseases are among the many important developmental and degenerative disorders of the retina, retinal pigment epithelium, choroid, and associated structures. These disorders generally afflict the newborn and the young and are tragically common. In the United States they are estimated to be responsible for one third of all blindness among children of school age. The economic burden imposed by these disorders is large, but the emotional impact to the victims and their families is incalculable.

Little can be done at present to prevent the onset or progression of these diseases, but the state of knowledge in this field has increased substantially in recent years, and there is reason to believe that progress can be made toward achieving the following objectives of this subprogram:

- o To discover specific causes in human retinal degenerative diseases and in appropriate animal models.
- o To develop the techniques needed to identify affected patients and seek techniques for prenatal diagnosis.

Recent Research Accomplishments: Much has been learned in recent years about the ultrastructure, function, and biochemistry of the normal retina and retinal pigment epithelium, and this knowledge has been applied in efforts to understand these diseases. Second, studies of patients using sophisticated measures of retinal function<sup>9</sup>, examination of postmortem donor eyes<sup>10,11</sup>, and biochemical studies of blood, urine, and cultured skin fibroblasts have led to new information on the causes of some of these conditions and in some cases to rational treatment trials. Third, delineation of specific biochemical defects in the photoreceptors or defects in the relationships of one retinal cell type to another in animals with hereditary retinal diseases has provided a scientific basis<sup>12</sup> for considering similar pathogenetic mechanisms in human diseases.

Future Needs: On the other hand, specific biochemical defects are not known in the overwhelming majority of patients, nor are the precise cellular and chromosomal loci of the abnormalities. To accomplish these objectives a major research effort remains, of necessity, directed at understanding the normal retina, at understanding how the retina develops, and how it is maintained in a healthy state. Greater effort is needed to provide baseline data on the structure, biochemistry, and metabolism of the normal human retina and choroid and to evaluate postmortem donor eyes from affected patients against this data base. Studies of animal models of these diseases, including those making full use of new methods of genetic analysis, continue to be stressed because they are an important source of the scientific rationale and strategies needed to discover the causes for the human diseases. Other areas of emphasis include the development of methods to detect carriers, the assessment of early stages of retinal malfunction with noninvasive techniques, the definition of the short-term natural histories of these diseases, and the search for aggravating or ameliorating factors through epidemiological studies. These considerations serve to emphasize that research on these disorders is closely related to research covered within other subprograms. Studies on the cell biology of the photoreceptors and retinal pigment epithelium, the interactions of cells within the retina, and mechanisms of visual adaptation are particularly relevant.

## B2. Macular Degeneration

Objectives: The macula is that area of the retina which is responsible for sharp, clear color vision. Diseases of the macula result in loss of this refined visual acuity and sometimes severe loss of vision. Macular diseases actually refer to a complex group of disorders which include senile macular degeneration, central

serous choroidopathy, hereditary macular degeneration, toxic macular disease, macular disease associated with other eye or systemic conditions, and myopia. Macular diseases affect primarily the elderly population and to a lesser extent juveniles.

The objectives of this subprogram are aimed at understanding the underlying or precipitating causes of macular diseases and include research:

- o To improve our understanding of macular diseases by interfacing research advances made in studies of the retinal pigment epithelium and photoreceptors, with the pathophysiology of generalized hereditary chorioretinal degenerations and other vascular abnormalities.
- o To obtain more information about the causes of macular degeneration by pursuing biochemical, histologic, and metabolic studies.
- o To document the natural courses of these diseases and to develop improved methods of treatment which will be tested in randomized controlled clinical trials.
- o To devise better methods of testing functional vision in infants, children and adults.

Future Needs: The major research thrust in this program remains a collaborative randomized controlled clinical trial to determine whether laser photocoagulation is effective in obliterating neovascularization in patients with senile macular degeneration. However, since the retina, the retinal pigment epithelium and the choroid all function as a unit, it is rather obvious that unless the research gains in any of these entities are applied to how the other work, our ability to prevent and to properly treat diseases of the macula will be severely hampered. (See subprograms on Photoreceptors, Visual Pigments, and Phototransduction and on Retinal Pigment Epithelium.)

### B3. Retinal Detachment and Vitreous Disorders

Objectives: The vitreous gel occupies approximately 80 percent of the volume of the eye and acts as an internal support to the eyeball. If for a variety of reasons the vitreous shrinks, it can pull on the retina and create a retinal break. Should the fluid component of the vitreous accumulate beneath the retina, a retinal detachment will result. Retinal detachment is not necessarily associated with an abnormality of the vitreous or of the retina; it can also develop from other eye disorders such as high myopia, diabetes mellitus, ocular trauma, and hereditary and congenital eye problems. To prevent retinal

detachment, more information on the relationship between the neural retina and the retinal pigment epithelium and how an abnormal vitreous gel affects this relationship is needed. The research needs of this subprogram are encompassed in the following objectives:

- o To investigate the mechanisms by which the retina remains in apposition to the pigment epithelium and the factors which lead to retinal detachment.
- o To improve our knowledge of the pathogenesis and treatment of abnormal membranes and blood vessels in the vitreous cavity.
- o To identify the causative factors and to develop treatment therapies for retinal detachment and for vitreoretinal membrane shrinkage.
- o To study the development, structure, metabolism, function and immunologic properties of the normal vitreous as related to aging and disease.
- o To improve diagnostic methods, instrumentation, and treatment modalities for vitreoretinal diseases.
- o To improve our knowledge of the effects of blunt and penetrating trauma on the retina and vitreous.

Future Needs: Significant research advances have been made in improving vitreous surgery through the development of miniature surgical instruments<sup>13,14</sup>. However, if retinal detachments are to be prevented, more basic knowledge is needed, such as, what causes the vitreous to become abnormal, what biochemical and physiological changes lead to retinal detachment, and what are the predisposing factors for retinal detachment?

#### B4. Rescue and Regeneration of Neurons in the Retina

Objectives: Repairing or replacing any part of the human visual system is not presently feasible despite the burgeoning interest of investigators in the development, plasticity, and regeneration of the nervous system. A more immediate and practical goal might be to apply the results of relevant experimental studies in an effort to rescue injured optic nerves and retinal neurons which might otherwise undergo degeneration. Such injuries are commonly traced to a single event such as physical injury, infection, or temporary deficiencies of blood supply and frequently result from adverse conditions present outside an otherwise intact optic globe. More chronic injuries may result from reversible processes such as glaucoma, toxic exposures, or treatable intracranial tumors. There is usually a lag between the time of injury and the dying

back of the affected nerve fibers or retinal neurons. This lag period could provide an opportunity for therapeutic interventions designed to redress the balance between degeneration and regeneration in the injured nerves and neurons. The present realities, however, and our as yet rudimentary understanding of the critical underlying processes indicate that the objectives of this subprogram constitute a long-term proposition:

- o To define at the genetic, molecular, cellular, and physiological levels the biology of retinal neurons, glial cells, and connective tissue cells (1) in normal development and maintenance, and (2) in response to injuries, including the physical and chemical conditions which influence degeneration, and these conditions which promote rescue and regeneration.
- o To facilitate and to promote awareness of clinicians and basic scientists concerning opportunities for the clinical application of research in the rescue and regeneration of neurons in the optic nerve and the retina in patients with nonprogressive disorders of these structures.

Future Needs: The detailed biochemical characterization of the optic nerve and retinal neurons and glia has really just begun. But, there are five areas of recent research which at the molecular level are helping to define factors which may be of significance in rescue and regeneration of retinal neurons:

1. Biochemical characterization of various components of nerve and glial cells under conditions of development, survival, degeneration, and regeneration;
2. The biochemistry of the directional interaction between nerve cells and the cells with which they synapse;
3. The role of specific genetically controlled molecules in organizing and guiding both the initial and regenerative development of the nervous system;
4. Biochemical tags for the identification of specific cell types and quantification of cell function in degenerating and regenerating neurons; and,
5. Pharmacologic influences on growth, degeneration, rescue, and regeneration.

Techniques now under development for the culturing of isolated retinal neurons and glial cells should make it possible to determine the conditions which optimize the growth and regeneration of these cells and to determine important features of neuron-glia interactions.

## B5. Toxic, Nutritional, and Environmental Disorders

Objectives: The retina differs from all other tissues of the body in its susceptibility to toxic agents; it has special nutritional requirements, an intricate and delicately balanced physiology, and it functions in a rigorously controlled environment. Although many other tissues have comparable individual limitations, no other tissue has precisely the same vulnerability. Thus, toxic or environmental agents, acting separately or at times together, can exert severely damaging effects on the retina although they are apparently harmless elsewhere. There is already abundant evidence that drugs that do little or no harm elsewhere in the body can produce a retinal degeneration. Consequently, the only tissue that can serve to predict the effect of a given drug or environmental hazard on the retina is the retina itself.

Taking these facts into consideration, the subprogram objectives are as follows:

- o To improve the scope and quality of clinical and basic research directed toward toxic and environmental disorders of the eye.
- o To gather laboratory and clinical information about such hazards in an efficient manner.
- o To disseminate information about potential hazards to the retina and the optic nerve at the earliest possible moment, and thus limit toxic and environmental exposure.

Future Needs: Powerful new techniques such as whole organ autoradiography, organ and tissue culture of the retina and lens, and other developments in the field make possible several important initiatives. These would include:

1. The description of new drugs in terms of their penetration of the blood-ocular barriers and its subsequent localization and metabolism within the eye;
2. The development of systems for the in vitro screening for potential ophthalmic toxicity;
3. An expansion of present studies of toxicity associated with the intravitreal administration of antibiotics; and,
4. The application of modern epidemiological techniques to selected populations to characterize environmental, genetic, and toxicological interaction.

## B6. Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models

Future Needs: Progress in understanding retinal and choroidal diseases depends on the acquisition and study of appropriate human and

animal tissues. Since biopsy material from the retina and choroid is presently not available to the research community, it is essential that human eyes enucleated for malignancy or for other reasons, or taken postmortem, and eyes from animals with pathology comparable to that occurring in humans become available for study. This is obviously an issue which is relevant to all Retinal and Choroidal Diseases subprograms and is highlighted in this program to provide the needed focus.

Current evidence suggests that specific biochemical defects may exist in different types of inherited human retinal degenerations. If this is the case, comparative biochemical studies of normal and diseased human donor retinas are crucial. So too are the corresponding studies in animals affected with hereditary retinal degenerations. The discovery of specific defects in animal eyes has now pinpointed areas for study in humans. Even in the absence of precise overlap in pathogenesis, the existence of animal models opens the door to practical trials of modes of therapy that could have important applications to humans. The animals too would surely benefit if a specific cause for these blinding diseases could be discovered.

Objectives: Very much the same arguments apply in the search for the causes and treatments of diabetic retinopathy, uveitis, retinal detachment, tumors and all other major types of retinal and choroidal disorders. As a result, the following subprogram objectives were instituted:

- o To make available postmortem human donor eyes affected by retinal and choroidal diseases for study by modern techniques.
- o To make available normal postmortem human donor eyes for comparative studies with diseased donor eyes.
- o To discover and develop animal models of retinal and choroidal diseases.
- o To provide a mechanism whereby promising animal models can be bred to provide adequate numbers for distribution to qualified investigators.

Recent Research Accomplishments: Research utilizing postmortem human donor eyes from normal individuals and from patients has increased substantially. Retinal pigment epithelial cell lines have been developed for subsequent biochemical and ultrastructural studies and studies have begun on cultured cells from postmortem donor eyes of patients with retinitis pigmentosa<sup>26</sup>. Photoreceptor cell-specific processes have been studied in short-term cultures including the capacity to synthesize rhodopsin, high-affinity uptake mechanisms for taurine, and

the ability to incorporate phosphate into rhodopsin with exposure to light<sup>27</sup>. Pericytes and endothelial cells from retinal blood vessels are increasingly being utilized for studies of possible pathogenic mechanisms involved in diabetic retinopathy. Postmortem human eyes have recently been used to locate the cellular sites of putative amino acid neurotransmitters in the retina (28). The successful NEI contract supporting breeding and distribution of the Irish setter model of hereditary retinal degeneration has continued. Finally, the promising miniature French poodle model has recently begun to be distributed to qualified investigators.

## C. Fundamental Processes and Retinal Disorders

### C1. Retinal Pigment Epithelium

Recent Research Accomplishments: The retinal pigment epithelium (RPE) is a remarkable tissue layer which we now know is a central element in the survival and function of the visual cells. The RPE is interposed between the choroidal capillaries and the photoreceptors and so positioned selectively regulates the exchange of materials between the photoreceptors and the bloodstream, and regulates the environment in which these visual cells live<sup>15,16</sup>. The RPE is thought to provide some of the forces needed to maintain the neurosensory retina in its proper anatomical position<sup>17</sup>. Moreover, the RPE absorbs the excess light energy not trapped by the photoreceptors, thereby reducing light scatter and enhancing the clarity of images. Finally, the RPE has been demonstrated to play a central role in keeping the photoreceptors youthful and vigorous. Each day it recognizes, ingests, and digests "older" portions of the continuously-growing photoreceptor outer segments that are discarded in a cyclical manner with the rising and setting of the sun. If any of these functions are disturbed, the photoreceptors and vision are adversely affected.

Based on what is now known, it is now reasonable to expect that the RPE is involved either primarily or secondarily in a variety of ocular disorders, including the hereditary and developmental disorders such as retinitis pigmentosa, senile macular degeneration, diabetic retinopathy, inflammatory disease, retinal detachment, and light- and drug-induced retinopathy. As the above examples demonstrate, this is an area of great opportunity. We are beginning to understand the cellular properties of the RPE which are crucial to the function and nurturing of the photoreceptors. As this understanding continues to deepen, increasing emphasis is being placed on the manner in which specific genetic or acquired dysfunctions of the RPE can affect retinal viability, and the manner in which noninvasive tests can monitor the function of the RPE<sup>18</sup>.

Objectives: Successful attainment of the following objectives should provide powerful tools for the prevention and treatment of retinal and choroidal diseases:

- o To gain fundamental knowledge about the RPE in structural, molecular, and physiological terms.
- o To understand the trophic influences of the RPE on other elements of the visual system.
- o To determine the role of the RPE in chorioretinal disease and retinal detachment, both as a primary and secondary factor.
- o To develop noninvasive methods for studying RPE function.

## C2. Photoreceptors, Visual Pigments, and Phototransduction

Recent Research Accomplishments: The highly specialized photoreceptor cells--the rods and cones--interact with light and initiate the process of vision. Light is absorbed by pigments within the photoreceptors, triggering a cascade of metabolic and ionic changes within the cell. The resultant "signal" is modified by other inputs and is transmitted to interacting neural cells of the retina for further processing of the visual information. Tremendous advances have been made in recent years and a broad outline of the primary photoreceptor physiological functions and mechanisms has emerged. Many investigators have redirected their research towards characterizing these mechanisms at the molecular level and at elucidating the metabolic processes by which the photoreceptors maintain, repair, and renew their structures. The latter focus comes from the realization that the unique specialization of the photoreceptors cells for the reception and transduction of light has important consequences for the health of these cells and, thus, for sight itself. The photoreceptors are the most metabolically active cells in the visual pathway and their needs may be compromised and blindness ensue from inherited metabolic defects, from overexposure to light, from dietary inadequacies (e.g., vitamin A deficiencies), and from aging. Strides have been taken toward defining the critical metabolic features of rod and cone photoreceptors the for establishing the limits beyond which metabolic insufficiencies become disease.

Objectives: The following are the subprogram objectives for present and future research in this area.

- o To determine the molecular structure and dynamics of the photoreceptor "machinery" (e.g., visual pigments, membranes, cilium, synapses, and the nucleus).
- o To elucidate the metabolic mechanisms by which photoreceptors maintain, repair, and renew their structures.
- o To characterize the major physiological functions of the photoreceptors (transduction, adaptation, and synaptic interactions) in terms of the underlying molecular mechanisms.

- o To discover the genetic factors and cell interactions which control photoreceptor growth, differentiation, and maintenance of photoreceptors in their fully differentiated state.
- o To discover the causes of photoreceptor degeneration and aging, and to develop methods for preventing or slowing these processes and for facilitating the recovery of photoreceptor function after injury.
- o To develop, and make full use of, noninvasive techniques for assessing photoreceptor function in humans.

### C3. Retinal Organization, Neurotransmission, and Adaptation

Objectives: Our perception of visual information depends on the orderly receipt of a visual image by the rods and cones, and the photoreceptor cells of the retina. But, the retina is far more than an array of photoreceptors and contains in addition at least five distinct types of neurons which serve to process visual information. In fact, the basic response properties of neurons throughout the visual system, of responding to illumination depending on the nature and position of the light stimuli, is established within the retina. Thus, if we are to understand how we see, we must first understand how the retina works. To accomplish this and to bring this understanding to bear on retinal and choroidal disease processes, the following subprogram objectives have been formulated:

- o To define the anatomical, biochemical, physiological, and pharmacological principles underlying retinal function.
- o To determine the metabolic processes and physiological conditions necessary for the maintenance and proper functioning of the retina.
- o To define the principles of retinal development, including the mechanisms of induction, and differentiation of various retinal cell types, synaptogenesis, the onset of retinal function, and retinal genetics.
- o To develop noninvasive methods for studying retinal function to understand retinal disease processes, and to develop rational therapies for retinal disorders.

Recent Research Accomplishments: Largely through the creative application of two techniques, electron microscopy and intracellular electrophysiological recording, spectacular progress has been made in our understanding of the functional organization of the retina. Thus, we now understand the basic response properties of all major classes of retinal neurons, and we are beginning to understand how the responses of the neurons proximal to the photoreceptors are formed by the synaptic interactions among the various retinal neurons. One of the most interesting and unexpected findings has been that in vertebrates the distal retina

behaves physiologically as though darkness maximally stimulates the system and illumination turns the system off. Why this is so remains a mystery (no other neurons in the brain are known to behave this way), but the answer may someday help to explain why cells of the distal retina, especially the photoreceptors, are so susceptible to damage from a number of factors including prolonged illumination, toxic substances, and inherited gene defects.

Future Needs: Recently, the attention of a number of workers in the field has turned toward attempting to understand the mechanisms underlying the interactions occurring within the plexiform layers of the retina. Substantial evidence for at least a dozen different substances serving as potential neurotransmitters in the retina has been presented, and we still do not have data on what the transmitters may be for about one-half of the retinal neurons<sup>19</sup>. In addition, recent studies have shown that there are a substantial number of neuropeptides present in many retinas<sup>20,21</sup>. The question of whether these neuropeptides serve as neurotransmitters or play some other role in the retina is under active investigation. This pharmacological work, although far from complete, has led to the important realization that each major type of retinal neuron may be subdivided on the basis of the neurotransmitter substance it contains and that there are distinct pharmacological subclasses of cells. It now seems highly likely that these subclasses make specific and different synaptic connections within the layers of the retina, and mediate different functions.

Retinal development is an area of retinal research that has not progressed as far as some others, but is of great potential importance. We have little information concerning the factors underlying neurogenesis, synaptogenesis, and the development of the neurotransmitter systems in the retina<sup>22</sup>. Since a number of the described inherited retinal lesions appear during development, such information is sorely needed.

We all experience the phenomenon known as visual adaptation when we enter a dimly-lit movie theater and find that it takes several minutes for our eyes to gain enough sensitivity for us to "see" again. While much of this visual adaptation has been shown to occur at the level of the photoreceptors, other retinal mechanisms are involved. A number of night-blinding conditions are known, and understanding the mechanisms underlying visual adaptation may provide clues concerning the defect in such diseases.

New approaches and techniques that appear to offer considerable promise include the use of monoclonal antibodies for the recognition and preparation of retinal neurons and membrane constituents, the use of isolated retinal neurons and glia and the maintenance of these cells in tissue culture, the use of ion-selective electrodes and new recording techniques for determining the retinal network contributions to retinal field potentials and visual adaptation, and the development of noninvasive methods for assessing visual function.

#### C4. Glial Cells and the Retinal Microenvironment

Recent Research Accomplishes: In recent years, important new concepts have been developed concerning the high degree of interdependence between retinal nerve cells and their surroundings. Because of the close packing of nerve cells in the nuclear and synaptic areas of the retina, the extracellular space and glial elements acquire the potential to serve as modulators of the chemical substances that influence neuronal behavior, as communication channels between neurons, and as the source of the transretinal potentials useful in the diagnostic evaluation of retinal disease. Although the electroretinogram (ERG) has long been one of the most valuable noninvasive techniques in the diagnosis of retinal diseases, its clinical usefulness has not yet been fully realized. Research has shown that these potentials arise partly from photoreceptors, partly from the Muller (glial) cells of the retina, and partly from the retinal pigment epithelium. The underlying mechanisms for these potentials appear to involve shifts of small ions across the membranes of the involved cells, but except for the photoreceptor currents, which give rise to the a-wave of the ERG, the details of how the other currents arise are still not fully understood. Clearly, if this were known, the site and nature of defective retinal function might be inferred from selective loss of one or another of the component potentials.

Objectives: From these considerations the following subprogram objectives were determined:

- o To identify the mechanisms which serve to control the dynamic extracellular milieu of the normal retina and which contribute to the prolonged upset due to pathological conditions.
- o To determine the specialized membrane properties which enable the unidirectional channeling of metabolites and other substances through the cell and surrounding media (e.g., the functional polarization which determines the nature and direction of transport).
- o To examine further the electrogenic properties of the retinal glia, including glial contributions to the electroretinogram, and the possibility that the electrical activity of these elements influences the information processing of neural circuits.
- o To determine the role of the glial elements in neuronal metabolism, e.g., in terminating the actions of neurotransmitters and in transporting the precursors required to replenish the supply of transmitter substances to the nerve endings.
- o To determine the role of neuroglia in retinal development and in the regenerative response of injured nerve cells.

Future Needs: Powerful new tools, ion-specific electrodes for example, are being used to study the structure and functional role of the retinal microenvironment, and to provide in quantitative terms a description of the homeostatic mechanisms that regulate its composition<sup>23</sup>. But, there are several other areas where greater effort is needed to take advantage of opportunities for progress. Regional heterogeneities may exist along the length of the Muller cells that may have relevance for our understanding of nerve-glia interactions, the inactivation of neurotransmitters, the interpretation of studies of neuronal degeneration, and the glial contribution to the ERG waveform.

## References

1. Buzney SM, Massicotte SJ: Retinal vessels: Proliferation of endothelium in vitro. Invest Ophthalmol Vis Sci 18:1191-1198, 1979.
2. Frank RN, Kinsey VE, Frank KW, et al: In vitro proliferation of endothelial cells from kitten retinal capillaries. Invest Ophthalmol Vis Sci 18:1195-1200, 1979..
3. Delori FC, Parker JS, Gragoudas ES: Oximetry of retinal vessels. Invest Ophthalmol Vis Sci 19(suppl):138, 1980.
4. Lin TM, Halbert SP, O'Connor GR: Standardized quantitative enzyme-linked immunoassay for antibodies to *Toxoplasma gondii*. J Clin Microbiol 11:675-681, 1980.
5. Shields JA, Lerner HA, Felberg NT: Aqueous cytology and enzymes in nematode endophthalmitis. Am J Ophthalmol 84:319-322, 1977.
6. Albert DM, Puliafito CA, Fulton AB, et al: Increased incidence of choroidal malignant melanoma occurring in a single population of chemical workers. Am J Ophthalmol 89:323, 1980.
7. Russell R, Wiggert B, Derr J, et al: Nuclear uptake of retinoids: Autoradiographic evidence in retinoblastoma cells in vitro. J Neurochem 34:1557-1560, 1980.
8. Saari JC, Futterman S, Stubbs GW, et al: Cellular retinol and retinoic acid-binding proteins in transformed mammalian cells. Invest Ophthalmol Vis Sci 17:988-992, 1978.
9. Geiser DK, Fishman GA, Cunha-Vaz J: X-linked recessive retinitis pigmentosa and vitreous fluorophotometry: A study of female heterozygotes. Arch Ophthalmol 98:307-310, 1980.
10. Ulshafer RJ, Garcia CA, Hollyfield JG: Sensitivity of photoreceptors to elevated levels of cGMP in the human retina. Invest Ophthalmol Vis Sci 19:1236-1241, 1980.
11. Flood MT, Gouras P, Kjeldbye H: Growth characteristics and ultrastructure of human retinal pigment epithelium in vitro. Invest Ophthalmol Vis Sci 19:1309-1320, 1980.
12. LaVail MM: Analysis of neurological mutants with inherited retinal degeneration: Friedenwald Lecture. Invest Ophthalmol Vis Sci, 1981, to be published.

References cont.

13. Machemer R, Parel J M, Hickingbotham D, Nose I: Membrane peeler cutter: Automated vitreous scissors and hooked needle. Arch Ophthalmol 99: 152-153, 1981.
14. Hickingbotham D, Parel JM, Machemer R: Diamond coated all purpose foreign body forceps. Am J Ophthalmol 92:267-268, 1981.
15. Steinberg R, Miller S: Transport and membrane properties of the retinal pigment epithelium, in Zinn KM, Marmor MF (eds): The Retinal Pigment Epithelium. Cambridge, Harvard University Press, 1979, pp. 205-225.
16. Bok D: Autoradiographic studies on the polarity of the plasma membrane receptors in retinal pigment epithelial cells, in Hollyfield JG (ed): IV International Symposium on the Structure of the Eye. New York, Elsevier-North Holland, 1981, to be published.
17. Marmor MF, Abdul-Rahim AS, Cohen DS: The effect of metabolic inhibitors on retinal adhesions and subretinal fluid resorption. Invest Ophthalmol Vis Sci 19:843-903, 1980.
18. Fitzgerald CR, Enoch JM, Birch DG, et al: Anomalous pigment epithelial photoreceptor relationships and receptor orientation. Invest Ophthalmol Vis Sci 19:956-966, 1980.
19. Lam DMK, Hollyfield JG: Localization of putative amino acid transmitters in the human retina. Exp Eye Res 31:792-832, 1980.
20. Karten HJ, Brecha N: Localization of substance P immunoreactivity in amacrine cells of the retina. Nature 283:87-88, 1980.
21. Yamada T, Marshak D, Basinger S, et al: Somatostatin-like immunoreactivity in the retina. Proc Natl Acad Sci USA 77:1691-1695, 1980.
22. Lam DMK, Fung SC, Kong YC: Postnatal development of GABA-ergic neurons in the a rabbit retina. J Comp Neurol 198:89-102, 1980.
23. Ripps H, Mehaffey L III, Siegel IM: "Rapid regeneration" in the cat retina. A case for spreading depression. J Gen Physiol 77:335-346, 1981.
24. Sarthy PV, Bunt AH: The ultrastructure of isolated glial (Muller) cells from the turtle retina. Anat Rec, 1981, to be published.
25. Szamier RB, Ripps H, Chappell RL: Changes in ERG b-wave and Muller cell structure induced by alpha-aminoadepic acid. Neurosci Lett 21:307-312, 1981.

References cont.

26. Edwards RB: Studies of cultured human retinal pigment epithelium from normal donors and a patient with retinitis pigmentosa. Proc Int Soc Eye Res 1, 1981.
27. Schmidt EY, Berson EL: Postmortem metabolic capacity of photoreceptor cells in human and rat retinas. Invest Ophthalmol Vis Sci 19:1274-1280, 1980.
28. Lam DM, Hollyfield JG: Localization of putative amino acid neurotransmitters in the human retina. Exp Eye Res 31:729-832, 1980.



II. CORNEAL DISEASES PROGRAM

FISCAL YEAR 1981

<u>Subprogram</u>	<u>Number of Grants</u>
A. External Ocular Infections and Inflammatory Disease	[36]
1. Herpes Simplex	17
2. Herpes Zoster	0
3. Adenovirus and Enterovirus	1
4. Bacterial and Fungal Keratitis	7
5. Chlamydial Keratoconjunctivitis	6
6. Chronic Blepharitis	2
7. Other	3
B. Ocular Surface Problems	[36]
1. Tear Film and its Abnormalities	6
2. Ocular Surface Disorders	26
3. Drug Delivery and Toxicity	0
4. Other	4
C. Refractive Problems and Contact Lenses	[14]
1. Modification of Refractive Error	14
2. Other	0
D. Corneal Edema, Endothelial Dysfunction, Dystrophies, and Inherited Disease	[31]
1. Endothelial Tissue Culture, Replacement, and Repair	5
2. In vivo Evaluation of Corneal Epithelial and Endothelial Membrane Function	2
3. In vivo Morphologic Evaluation--Specular Microscopy	2
4. Endothelial and Epithelial Transport Processes (Corneal Hydration and Edema)	11
5. Stromal Swelling Properties and Transparency	6
6. Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies	4
7. Normal Corneal Development	1
8. Other	0
E. Corneal Transplantation and Stromal Wound Healing	[26]
1. Inflammation and Repair	18
2. Corneal Transplantation	4
3. Other	4
Total Grants	143

## INTRODUCTION

The Corneal Diseases program of the National Eye Institute provides the focus within the federal government for the support of research investigations of disorders of the cornea, lids, conjunctiva, lacrimal gland, diseases of the orbit and external eye and refractive errors and injuries of the cornea. Although lesions of the cornea account for only approximately 6 percent of all cases of blindness in the United States, diseases and disorders which affect this tissue constitute 62 percent of the total incidence of acute and chronic disorders, diseases and injuries which affect the eyes. Ocular infections, conjunctival allergies and traumatic or foreign body injuries to the cornea constitute the bulk of these eye problems.

## A. External Ocular Infections and Inflammatory Disease

### Al. Herpes Simplex

Objectives: Herpes simplex virus, the most ubiquitous communicable infectious virus in man, is the etiologic agent of a wide variety of chronically recurring diseases. Ocular herpes simplex, due primarily to type 1 but occasionally to type 2 virus, is the leading cause of corneal blindness due to infection in the United States, with approximately 500,000 cases reported annually.<sup>1,2</sup> Recurrent herpes of the mouth and skin, also usually due to type 1 virus, afflicts approximately one-third of the world population, one-half of these people suffering more than one attack annually. Genital herpes, caused primarily by type 2 virus, is the second most common cause of venereal disease in the country, with approximately 100,000 cases currently reported annually, a number steadily rising as the disease, like ocular herpes, is recurrent and as yet incurable.<sup>2,3</sup> Medical therapy of ocular herpes requires frequent visits to the physician over periods ranging from several weeks to several months. Approximately 25 percent of those who develop herpetic ulcers, epithelial keratitis, will experience recurrence of these surface ulcers, a complication of deep corneal stromal keratitis, inflammation of the iris, or any combination of these three within two years.<sup>4,5</sup>

- o To elucidate further the respective and interacting roles of herpes simplex virus and host factors in primary and recurrent epithelial, stromal, endothelial and uveal disease of infectious and immunologic etiology.
- o To investigate mechanisms of herpetic neuronal latency, the relationship of latency to herpes viral strains and patterns of disease, and to develop further techniques for reactivation and suppression of neuronal reservoirs of chronically recurrent disease.
- o To develop more effective, specific and safe topical and systemic therapeutic drugs to prevent recurrent viral infections and enhance, ameliorate or block the resulting immunologic response.

Overview of Current Research Support: Of the 36 ocular infection and inflammatory disease research grants funded by the National Eye Institute funded in FY 1981 17 of these grants are exclusively concerned with herpes simplex. Four other studies deal with herpes as a smaller component of larger projects concerning viral or chlamydial infections. The distribution of NEI grants among areas of study are funded most heavily in determining the role of live herpes simplex virus, host factors in recurrent disease, and neuronal factors responsible for viral ganglionic (neuronal) latency and its role in recurrent disease. There is less funding using multiple routes of administration. No major NEI proposal has as yet been awarded for study of mechanisms triggering viral shedding or understanding neuronal and cell-mediated immunity

through modification of the host response. Thus, there is a definite need for development of a reproducible animal model of herpetic keratitis, e.g., the murine system, so that subsets of T-lymphocytes in such an animal can be genetically manipulated for the purpose of distinguishing protective from damaging constituents of such cells.

Recent Research Accomplishments: Ophthalmic investigators in herpes research have had a unique opportunity not only to prevent disabling eye disease but to develop findings which are of enormous importance to medicine as a whole. The drug, acyclovir, had its initial trials in ocular herpes but has proved so effective and nontoxic as to be currently under widespread investigation as both a topical and systemic agent for therapy of herpetic infection of the skin, genitalia, and brain.<sup>3,4</sup>

## A2. Herpes Zoster

Objectives: Herpes zoster (shingles), a virus identical to chicken pox, cause approximately 7 percent of all skin disease, a significant percentage of this involving the eye. Corneas scarred by zoster, unlike simplex, are not amenable to successful transplantation surgery because of poor healing abilities, lid abnormalities, and epithelial and tear dysfunction.

- o Develop a satisfactory animal model for evaluation of zoster infection and for testing new therapeutic modalities.
- o Define the roles of herpes zoster virus and zoster antigen in deep and superficial ocular and periocular disease.
- o Develop and evaluate new antiviral and anti-inflammatory therapy for use in acute ophthalmic zoster.

Overview of Current Research Support and Needs: These are at best only limited studies on ocular herpes zoster supported under NEI funding. Lack of an animal model of this disease has greatly interfered with progress in understanding the pathogenesis of all aspects of acute and chronic zoster regardless of its location in the body and the development of appropriate antiviral and anti-inflammatory therapeutic regimen.

## A3. Adenovirus and Enterovirus

Objectives: Adenovirus, the most frequent cause of epidemic ocular disease, predominantly attacks adults, thereby producing significant loss of production. Outbreaks of this disease disrupt places of work, educational institutions, and recreational facilities. The ocular route of infection may lead to diseases in other organs and disseminated infection in children can produce death.

- o To develop a reproducible animal model system to study the pathogenesis of acute adenoviral infections.
- o To determine the role of adenovirus and host response to it in epidemiologic and pathogenetic terms and develop new modalities for prevention and therapy of infection.

Overview of Current Research Support and Needs: There are currently three studies of adenoviral disease funded by the NEI; two of these are a small part of larger programs. A satisfactory animal model has not yet been developed for use in evaluating viral or host response factors in ocular disease or in testing therapeutic agents. It is essential that further work in this area be carried out before progress can be made with the studies essential to managing this widespread infection.

#### A4. Bacterial and Fungal Keratitis

Objectives: Bacterial and fungal infections of the cornea produce severe ocular pain, tearing, and reduction in vision which interfere with work and daily activity. Although the annual incidence cannot be determined from available data, it is likely that suppurative microbial keratitis accounts for a major portion of office visits and hospitalizations currently attributed to eye care in the United States. Approximately 5 percent of office visits for eye care in the United States in 1976 related to some form of keratitis excluding refractive errors and trauma.

- o To define the harmful and beneficial effects of the corneal inflammation in bacterial and fungal keratitis.
- o To develop methods of controlling the destructive components of corneal inflammation.
- o To define the mechanisms of corneal destruction by various microorganisms.
- o To develop new techniques of drug delivery which provide sustained, high concentrations of effective agents within the cornea.
- o To develop new, highly potent, and safe antimicrobial agents which can be administered by multiple routes.
- o To establish rapid, practical and reliable techniques for detection of microorganisms and microbial substances in corneal tissue.
- o To develop effective methods for prevention of microbial keratitis in relatively high risk conditions, e.g. corneal injury, contact lens wear, corticosteroid therapy of nonmicrobial keratitis, corneal exposure in hospitalized patients with sepsis.

Overview of Current Research Support and Needs: The NEI currently supports four projects directed exclusively at the pathogenesis and therapy of bacterial and fungal keratitis. Two other research grants are directed at the investigation of ocular pharmacokinetics of antibacterial and antifungal antibiotics following various routes of administration while a separate study is attempting to establish a reliable method for detection of certain microorganisms in corneal tissue.

Recent Research Accomplishments: New antibacterial antibiotics have been evaluated in animal models and, to a limited degree, in human keratitis. Based on animal and clinical trials of efficacy and safety, the first ophthalmic antifungal agent, natamycin, was approved and released by the FDA.

#### A5. Chlamydial Keratoconjunctivitis

Objectives: Chlamydial keratoconjunctivitis (trachoma) is an acute and chronic ocular infection caused by the agent chlamydia trachomatis and remains the leading cause of blindness throughout the world. The disease affects some 500 million people and causes blindness in approximately 2 million. As many as 2 to 6 percent of newborns in the United States may acquire neonatal chlamydial conjunctivitis as the result of the chlamydial cervicitis which affects 5 to 13 percent of pregnant women.

- o To define the role of local and systemic immunity in primary infection and reinfection by C. trachomatis.
- o To develop rapid, practical, and reliable methods of diagnosis of chlamydial keratoconjunctivitis.
- o To define the optimal method of preventing neonatal chlamydial conjunctivitis.
- o To develop newer, more effective antichlamydial antibiotics.
- o To define the role of concurrent bacterial infection in the pathogenesis of chlamydial keratoconjunctivitis.

Overview of Current Research Support and Needs: The current NEI support for chlamydial research consists of six grants devoted to the study of the pathogenesis and therapy of this ocular infection which is centered at four different academic institutions. Four additional projects in chlamydial basic and clinical research are supported by the National Institute of Allergy and Infectious Diseases, the Division of Research Resources, and the Indian Health Service. Nonhuman primate animal models of c. trachomatis keratoconjunctivitis should be established in order to elucidate the pathogenesis of disease, role of immunity, and optimal chemotherapy.

## A6. Chronic Blepharitis

Objectives: Chronic blepharitis is a common chronic ophthalmic inflammatory problem which involves the lids and also often the conjunctiva and the cornea. The entity, although common, is poorly understood by both ophthalmologists and patients, which results in the dissatisfaction of all involved.

- o To understand the pathogenesis of chronic blepharitis and its commonly associated keratoconjunctivitis and devise appropriate treatment.

Overview of Current Research Support and Needs: There is at present one NEI-supported grant which evaluates chronic blepharitis. There is an additional NEI grant which investigates the biochemical analysis of meibomian secretions in normals and patients with blepharitis. From a qualitative standpoint the current research endeavors would appear to be good; however, from a quantitative standpoint too little is presently being done to elucidate this complex and heterogeneous chronic disease process.

## B. Ocular Surface Problems

### B1. Tear Film and Its Abnormalities

Objectives: The tear film which covers the surface of the eye is directly in contact with the environment and the underlying surface cells. It is critically important in protecting the eye from adverse external factors, and for maintaining the health of the underlying cornea and conjunctiva. Numerous disease adversely affect the tear film, causing conditions that, in turn, affect the cornea and conjunctiva. Tear abnormalities may occur as primary and isolated manifestations of eye disease or may be found in conjunction with other ocular or systemic disease. Adverse effects of tear film abnormalities range from persistent eye irritation to severe discomfort and blindness. Although exact figures are not available, tear film disturbances probably account for eye symptoms in millions of Americans.

Several specific research objectives which are critical to understanding the tear film and its abnormalities can be identified:

- o To understand the normal composition, function, and control mechanisms of the precular tear film, and changes which occur in that film as a result of disease and aging.
- o To determine the interrelationship between the precular tear film and underlying ocular surface.
- o To better evaluate and diagnose patients with abnormalities of the precular tear film.

- o To develop new approaches to the therapy for dry eye and tear abnormalities.

Overview of Current Research Support: Currently, there are four principal investigators involved in the analysis of the precocular tear film. Among them, six major projects are underway; two of these involve fundamental studies to tear film physiology, and two others are involved in determining the effect of the tear film on the cornea. The mechanism of control of tear film formation is the subject of one other investigation.

Recent Accomplishments: Recent results suggest that tears contain a highly surface active, low molecular weight glycoprotein (8,000 daltons) component. Such a result, when confirmed, may drastically change our present view of tear film formation and rupture.

## B2. Ocular Surface Disorders

Objectives: The ocular surface has been defined as the epithelium and subjacent tissues which line the back of the lids, cover the globe, and extend over the cornea. With the exception of the cornea, the tissues underlying the epithelium are vascularized and contain large numbers of inflammatory and immunologic cells.<sup>9,10</sup> Consideration of the corneal and conjunctival surface as parts of single sheet of tissue has permitted a new approach to the study of surface disease, acknowledging the close relationship and interaction between these regions. While ocular surface abnormalities are not necessarily common, they frequently occur in young people (e.g., in the case of chemical and thermal injury) or are bilateral, leading to chronic pain, severe visual disability, and frequently, total blindness.

- o To understand healing and regeneration of ocular surface epithelium.
- o To understand the normal diseased ocular surface epithelium with specific reference to its biology biochemistry, metabolism, physiology and morphology.
- o To determine the influences responsible for specific differentiation of ocular surface epithelium into conjunctival or corneal types, and to explore the utility of ocular surface replacement.
- o To evaluate the normal immunologic mechanisms of the ocular surface and to elucidate the immunologic changes and roles in ocular inflammatory disease.
- o To investigate autoimmune mechanisms by isolating, identifying and characterizing ocular antigens relevant to the ocular surface.

Overview of Current Research Support: Twenty-six different projects are ongoing in this research area. Some of the major studies are; a) research into the factors responsible for maintenance and regeneration of the corneal epithelium and ocular surface epithelium; b) physiologic studies which includes research on the cornea and its epithelium in terms of permeability, function and transport; and c) immunologic aspects of the ocular surface.

Recent Research Accomplishments: There has been a demonstration that stabilization of the ocular surface can be accomplished by using transplanted tissue from the contralateral normal eye. Recently,<sup>11,a,12</sup> a new procedure, conjunctival transplantation, has been developed. This procedure, based on an understanding of basic biology of the ocular surface, relies on resurfacing of the cornea from peripherally placed healthy donor conjunctiva which replaces damaged host conjunctiva. Since the corneal epithelial surface may constantly be replaced by cells from conjunctiva, such peripheral graft placement insures a constant supply of new healthy cells for the corneal surface. Since this procedure can only be used in cases of unilateral injury or disease, other epithelial courses and alternative methods of applying such cells to diseased surfaces are being investigated.

### B3. Drug Delivery and Toxicity

Objectives: This is one of the most important areas in ocular care because drug delivery is a fundamental component of therapy. All new agents must be evaluated not only with regard to their penetration into the eye, but also with regard to their potential for being toxic and causing allergy. In general, there are three major methods by which drugs gain entrance to the eye: by topical applications through drops or ointment; by an injection around the globe; or through systemic administration. In general, disorders of the cornea, anterior chamber, and iris are most effectively treated by topical application.

- o To assess adverse drug influences on the ocular surface, including toxicity and immunological mechanisms.
- o To develop new and improved methods of drug delivery.

Overview of Current Research Support: At present there is no single NEI grant devoted solely to the study of drug effects on corneal tissue. Adverse effects of specific drugs have been studied on the corneal endothelium and epithelium.

Recent Research Accomplishments: It has also been determined that the corneal and conjunctival tissue contains cells (Langerhans cells) capable of interacting with sensitizing agents, and the presence of these cells suggests a mechanism for immunologic sensitization by these tissues.<sup>13</sup>

## C. Refractive Problems and Contact Lenses

### C1. Modification of Refractive Error

Objectives: It is estimated that nearly 100 million people in the United States have refractive errors that require correction. There are several methods of correcting refractive error. These include spectacles, contact lenses, orthokeratology, intraocular lenses, and refractive keratoplasty. Each of these treatments has certain advantages and disadvantages. Spectacles represent the easiest and safest method of correcting refractive error. For the vast majority of refractive errors, glasses represent an inexpensive and adequate method to correct myopia, hypermetropia, astigmatism, and presbyopia. The major disadvantage of spectacles is that with high refractive errors, such as those following cataract surgery, and irregular astigmatism, the vision cannot usually be corrected to levels required for normal visual function. Many patients, however, do not like glasses because of their cosmetic appearance and the reflections and aberrations that are especially bothersome in the higher lens powers.

Contact lenses can also correct a wide range of refractive errors. With the introduction of gas permeable soft and hard materials, some patients are able to wear lenses without developing any adverse corneal changes. In addition to the refractive problems that may require contact lenses, there are also many corneal diseases in which the use of a hydrogel contact lens has an important therapeutic effect. Diseases such as recurrent corneal ulcer, bullous keratopathy, keratoconus, and keratitis sicca frequently are helped when lenses are worn.

Orthokeratology is a method that uses specially designed contact lenses to reshape the cornea and thereby reduce myopic refractive error. Ideally, this represents a reasonably safe and inexpensive procedure. Unfortunately, the results of controlled clinical trials<sup>14,15</sup> indicate that only about 1 to 1.25D of change can be induced and that change is not permanent, thereby requiring the continued use of contact lenses.

Intraocular lenses (see Cataract report) are used in the visual correction of aphakia. Lens implants represent the optimal visual correction since the intraocular lens essentially replaces the cataractous lens and thus the optics are excellent. Unfortunately, in many aphakic patients lens implants are contraindicated because of certain ocular conditions such as glaucoma, corneal dystrophy, and chronic corneal edema.

Refractive keratoplasty consists of several different types of surgical procedures which are designed to correct various types of refractive errors. Theoretically, surgical correction provides a permanent cure and therefore is appealing to both patient and practitioners. Patients with high refractive errors who would otherwise require contact lenses are particularly interested in this type of correction.

Also, surgical correction of the high hyperopia following cataract surgery offers an additional treatment option to the aphakic patient. Because these procedures require surgery to the cornea they involve some degree of risk. Presently, there is insufficient scientific evidence to evaluate properly the safety and efficacy of refractive keratoplasty.

- o To develop and test new procedures and materials that improve visual acuity by lens application and/or modification of corneal refractive properties.
- o To study the biological effects of existing materials and/or proposed procedures for the correction of refractive error by contact lenses and/or surgical procedures.
- o To improve and develop methods for mensuration of optical and physiological properties of the cornea.

Overview of Current Research Support: The National Eye Institute currently sponsors five studies which are in part related to contact lenses. One study pertains to the oxygen need of patients and how this need relates to extended contact lens wear. Two of the projects are studying physiological effects on the cornea that relate to contact lens wear, such as hypoxia and changes in corneal sensitivity. Another study has measured tear film changes accompanying contact lens wear, and the fifth one deals with gas transmission of several contact lens materials. There has been limited research activity in this subprogram area until the last fiscal year in regard to the study of the safety and efficacy of refractive keratoplasty procedures. Since that time, three proposals which have been submitted have been awarded for such studies in various animal species. A multicenter randomized clinical trial composed of eight clinics and a coordinating center has also been funded for evaluation of the safety and efficacy of radial keratotomy as a procedure for the surgical correction of myopia.

Recent Accomplishments: One of the most important advances in contact lens research has been the introduction of an extended-wear lens for aphakia. These lenses have incorporated many design features which were derived from information gained under NEI research funding. For example, such lenses are made with high water content, which improves the transmissibility of the lens.<sup>14,15</sup>

#### D. Corneal Edema, Endothelial Dysfunction, Dystrophies, and Inherited Disease

##### D1. Endothelial Tissue Culture, Replacement, and Repair

Objectives: The corneal endothelium is a delicate layer of cells without natural regenerative and with limited reparative capabilities in man, which is the most vital layer in maintaining corneal clarity. If these cells are missing or malfunctioning, permanent corneal edema

with loss of corneal transparency occurs which requires corneal transplantation for return of normal vision. Stimulation or replacement of these cells without corneal transplantation has been a long-term goal in ophthalmology.

- o To evaluate epithelial, endothelial, and keratocyte growth and metabolism in normal, aging, degenerative, and dystrophic states in vivo and in vitro.
- o To evaluate the replacement, repair and stimulation of corneal endothelium.

Overview of Current Research Support: The evaluation of the transplantation of tissue cultured corneal endothelium is currently being funded under three NEI grants. There is one NEI-funded grant supporting the in vitro evaluation of tissue cultured corneal endothelium and one grant supporting the evaluation of the histocompatibility of tissue cultured endothelium.

Recent Research Accomplishments: A major accomplishment in this research area has been the successful transplantation of tissue cultured corneal endothelium<sup>16,17</sup> and vascular endothelium<sup>18</sup> in various animal species.

## D2. In Vivo Evaluation of Corneal Epithelial and Endothelial Membrane Function

Objectives: The preservation of corneal transparency depends upon the metabolic integrity of the epithelium and endothelium. Each of these layers prevents stromal edema by forming a barrier on the front and back of the stromal surface and by the process of active ion transport. These properties can be readily assessed in animal experiments. While most techniques developed to examine cell layer function have been designed for isolated tissues, some of these may be modified or extended for use in vivo. These procedures when done in vivo should fulfill the general criteria of being safe, noninvasive, and useful diagnostically. Currently, there is at present no quantitative procedure meeting these criteria that can be used to make an early diagnosis of endothelial dystrophies or degenerations.

- o To devise better methods for in vivo assessment of endothelial function and to make appropriate morphological correlation.

Overview of Current Research Support and Needs: There is no known support for the improvement of corneal pachometry. Fluorophotometry has been recognized as a potentially powerful clinical tool to measure the barrier functions of the corneal epithelial and endothelium. Currently, two grants from NEI are funded with this principal objective. There are major weaknesses at present in the in vivo evaluation of endothelial physiological function.

Recent Research Accomplishments: Continued research with animal models, particularly the rabbit, has provided the basis for much understanding of both epithelial and endothelial function and normal morphology. A variety of tools has been developed in the research laboratory which can be adapted to in vivo use. The thickness of the cornea can now be measured automatically with high precision in vitro using microprocessor technology.<sup>19,20</sup> This approach could be especially useful to measure thickness in situations where the cornea is opaque or the surface is irregular. New ophthalmic fluorophotometers have been developed for the research setting,<sup>21,22</sup> offering the promise that simple techniques and economical devices may be developed for the measurement of corneal barrier properties. Recently, a positive correlation between endothelial fluorescein permeability and corneal thickness was demonstrated in patients with corneal guttata.<sup>23</sup>

### D3. In Vivo Morphological Evaluation--Specular Microscopy

Objectives: The development of the specular microscope<sup>24</sup> and its extension to clinical use in man<sup>25,26</sup> has made possible the morphological evaluation of the endothelial layer in vivo. This valuable tool and its application have provided better understanding of the corneal endothelium and its participation in disease. Major advances in intraocular surgical technique and eye banking have occurred as the result of knowledge gained with specular microscopy.

- o To devise better methods for in vivo assessment of corneal endothelial morphology and make appropriate histological and functional correlations.

Overview of Current Research Support: There are six NEI-supported grants in which specular microscopy is central as well as one VA and one Fight For Sight grant. Also, there are multiple grants supported by the NEI which use specular microscopy as a tool. The strength of the current research activity includes the research that lead to the establishment that cell density alone does not reflect physiological function or reserve.

Recent Research Accomplishments: A number of significant accomplishments have occurred in the area of specular microscopy. It has been determined that endothelial cell density alone is inadequate to predict physiological function or reserve.<sup>27,28</sup> It has been determined that there is a tendency for decreasing cell density with age<sup>27,29</sup> but that variability within any age group is significant, i.e., one cannot predict the age of a cornea from the cell density observations or visa versa.

#### D4. Endothelial and Epithelial Transport Processes (Corneal Hydration and Edema)

Objectives: The control of corneal hydration depends upon a balance between dissipative forces (such as swelling pressure and passive inhibition) and active forces (such as intraocular pressure) and active flows (such as active ion transport and metabolic energy). The role of corneal metabolism in hydration control has been studied most extensively in the amphibian and in the rabbit using advanced biophysical, physiological, pharmacological and morphological procedures. Because transport processes underlie the principal function of the corneal membranes, a thorough examination of the details of these processes and mechanisms is necessary to explain the etiology of corneal disease involving stromal and epithelial edema which represent major causes of corneal blindness. It is hoped that ways to modulate corneal permeability and transport will result from these studies and lead to alternatives to keratoplasty.

- o To understand the normal function of the epithelium, endothelium, and connective tissues of the cornea and how they are affected by injury, disease, and aging.

Overview of Current Research Support: Support for the study of corneal transport processes has been good in the past. Currently, there are eleven NEI research grants in this area of research. Because the cornea has shown to be a good model tissue in which to study the physiology and biophysics of transport processes, it is anticipated that additional projects may be funded in the future by other Institutes within NIH.

Recent Research Accomplishments: Several aspects of ion transport processes across the epithelium have been revealed by NEI-supported programs. The epithelium transports Cl from the stroma to the periocular tear film by a process which is modulated by intracellular cyclic AMP which in turn is stimulated by beta-agonists. First messenger hormones having this action include epinephrine which has been shown to stimulate Cl secretion by selectively increasing membrane permeability.<sup>30</sup> The barrier properties of the epithelium have been studied in detail, and it has been reported<sup>31</sup> that most of these lie at the outermost cell layer of the epithelium. The corneal barrier is therefore accessible to chemical or pharmacological modulation, but also its location means that the epithelial barrier can be easily compromised by abrasions from foreign bodies and appliances.

#### D5. Stromal Swelling Properties and Transparency

Objectives: The stroma forms the bulk of the cornea and among the connective tissues in the body has unique structural and biochemical attributes that cause it to be normally transparent while retaining the mechanical properties necessary to ensure the integrity of the

eyeball. The collagenous fibrous elements in the corneal stroma run across the cornea without apparent interruption or splitting from limbus to limbus. They form distinct layers or lamellae within which all the fibers are equidistantly spaced and run parallel to one another. The areas around these fibers contains a ground substance which is rich in glycosaminoglycans and therefore very hydrophilic. While the small constant diameter and regular spacing of the collagen is necessary to sustain transparency, it also produces a meshwork within which there are minimal structural restrictions to prevent expansion or swelling between its anterior and posterior surfaces. Hence, corneal stroma, unprotected by its limiting cell layers will imbibe fluid until it is several times thicker than normal and becomes opaque. In order to devise better procedures and therapies to minimize stromal edema and associated opacity, we need to understand more fully how disease processes alter the structure and composition of tissue.

- o To understand the swelling properties of the corneal stroma and factors governing corneal transparency.

Overview of Research Support: The NEI and the Division of Research Resources are sponsoring one principal investigator in characterizing stromal glycosaminoglycans with special attention to their hydration properties. One extramural NEI investigator is using high energy particle scattering to determine structural relationships in hydrated stroma. Several NEI investigators are applying theoretical physical models and techniques to examine the basic principles of light scatter in stroma. Significant gaps in funding exist in the areas of changes in stromal properties that occur during development or with aging and disease, in understanding the physics of light scatter caused by scarring and disease, and in studying the mechanical properties of the stroma and how they altered by surgery and disease.

#### D6. Corneal Dystrophies, Inherited Disorders and Developmental Abnormalities

Objectives: The corneal dystrophies are a heterogenous group of relatively uncommon disorders which are usually inherited and can begin early in life or may become manifest during aging. Although the incidence of dystrophies is not high, these developmental disorders of the cornea result in significant morbidity in affected individuals. Corneal dystrophies and developmental anomalies can cause blindness but often only result in considerable discomfort and inconvenience to afflicted individuals.

- o To understand the pathogenesis and to find more effective methods for the treatment of corneal developmental abnormalities, corneal dystrophies and other inherited abnormalities of the cornea by determining the cellular and biochemical bases of the disorders.

Overview of Current Research Support: The NEI is currently supporting research on several aspects of corneal dystrophies and corneal fibroblast (keratocyte) metabolism. Only a few investigators are currently trying to delineate inherited disorders of the eye, and at present at least five funded grants are concerned with various aspects of the corneal dystrophies.

Recent Research Accomplishments: Several studies have shown that the biosynthetic activities of corneal organ cultures<sup>32,33</sup> more closely resemble the cornea in vivo than cell cultures. Several investigators have studied the macromolecular constituents of the normal cornea in cell and organ culture and have<sup>33</sup> begun in vivo studies of matrix constituents and their turnover. It is now established that the corneal proteoglycans produced by organ<sup>33</sup> cultures of the cornea closely resemble those synthesized in vivo. Understanding of these cellular and matrix interactions in the cornea may lead to determination of the location of the biochemical defect(s) in a variety of corneal dystrophies.

#### D7. Normal Corneal Development

Objectives: The mammalian (human) cornea is thought to result from complex cell-cell, cell-matrix and cell-humoral interactions. Much has been learned about such interactions by studies of model systems, chiefly the chick cornea. Increased knowledge of the mechanisms of corneal development would provide important contributions to diverse areas as corneal wound healing, the culture and transplantation of exogenous corneal cells and tissue, and tumor cell metastasis, invasion, and growth. Because of its relevance to these areas, the study of corneal development has an importance which extends widely across fundamental biomedical research.

- o To understand normal corneal development and apply such information to the understanding of related disease states.

Overview of Current Research Support: The NEI currently funds investigations on normal corneal development as components of grants listed under other subprograms, i.e., biosynthesis and control of corneal macromolecules like collagen, keratins and proteoglycans during development.

#### E. Corneal Transplantation and Stromal Wound Healing

##### E1. Inflammation and Repair

Objectives: The body responds to diverse insults by mechanisms which collectively result in inflammation. Such reactions in the cornea are associated with an infiltration of leukocytes (white blood

cells) into the corneal tissue. The actual severity of inflammation varies with the nature of the underlying insult and can be influenced by the simultaneous presence of microorganisms and their products. While the host responses to noxious insults can be beneficial, they can also be detrimental. The end result of inflammatory processes is all too frequently an opaque, scarred cornea. It has been estimated that 100,000 or more cases of corneal opacification occur annually due to infections and other disorders which produce decreased vision and severe pain. Such disorders result in numerous visits for eye care which can be associated with loss of productivity and jobs and millions of dollars of medical costs for rehabilitation.

- o To understand the biological and biochemical bases of tissue destruction and scarring in the cornea and sclera.
- o To understand the mechanism(s) of corneal nevascularization.
- o To prevent ulceration and to promote the synthesis and organization of corneal matrix components compatible with transparency.

Overview of Current Research Support: The NEI currently supports nineteen grants which wholly or in part investigate some aspect of corneal wound healing or vascularization.

## E2. Corneal Transplantation

Objectives: In cases of corneal opacification which occur following injury, infection, or hereditary diseases, the only current means of visual recovery is through excision and replacement of the scarred cornea, i.e., corneal transplantation.<sup>34</sup> In this form of ocular surgery, 70 to 90 percent of the cornea is replaced with tissue obtained from cadaver donors. The corneal transplant is the most successful and oldest organ transplantation. The procedure has been performed with regularity since the 1940s, and today over <sup>35</sup>10,000 transplants are performed annually in the United States. However, corneal transplants can become clouded due to immunologic rejection (allograft reaction), glaucoma, or failure of the transplanted endothelium. In such cases, it is necessary to understand the mechanism(s) of graft failure in order to ultimately restore vision.

- o To determine the process(s) of corneal transplant failure and how to prevent them.
- o To improve the recovery of vision after transplant.
- o To eliminate the cause(s) of astigmatism following corneal transplantation.
- o To improve the selection and handling of donor eyes to provide recipients with the best possible tissue.

Overview of Research Support: The NEI has funded four grants in fiscal years 1977-1980 whose emphasis is on the immunology of corneal transplantation. Presently funded projects include those which evaluate the cell antigens responsible for immunologic rejection, the cellular mechanisms of rejection, and means of suppressing the rejection process.

Recent Research Accomplishments: Definition of the process of graft rejection has been approached<sup>36</sup> by examining the cell types responsible for effecting the rejection. Although a specific type of lymphocyte, the T lymphocyte, seems a likely candidate for the rejection process, it is only recently that any solid evidence has suggested this to be the case.<sup>37</sup> Although certain antibodies have been demonstrated in vitro to be toxic to the corneal endothelium with or without the presence of complement, the role of antibodies in the immune process appears to be secondary to that of the lymphocyte.

## References

1. Dawson CR, Togni, B.: Herpes simplex eye infections: Clinical manifestation, pathogenesis and management. Surv Ophthalmol 21:121, 1976.
2. Workshop on the treatment and prevention of herpes simplex infection: News from the National Institutes of Health. J Infect Dis 127:117, 1973.
3. Kaufman H: Antimetabolite drug therapy in herpes simplex. Ophthalmology 87:135, 1980.
4. Selby P., Jameson B, Watson J, et al: Parenteral acyclovir therapy for herpes virus infections in man. Lancet:1267, 1979.
5. Jones DB, Forster RK, Rebell G: Fusarium solani keratitis treated with pimaricin (natamycin): Eighteen consecutive cases. Arch Ophthalmol 88:147, 1972.
6. Schachter J: Chlamydial infections. New Engl J Med 298:928, 1978.
7. Chandler JW, Alexander ER, Pfeiffer TA, et al: Ophthalmia neonatorum associated with maternal chlamydial infections. Trans Am Acad Ophthalmol Otolaryngol 83:302, 1977.
8. Holly FJ, Hong BS: Biochemical and surface chemical characterization of human tear proteins. Am J Optom Physiol Opt, to be published.
9. Allansmith MR, Greiner JV, Baird BS: Number of inflammatory cells in the normal conjunctiva. Am J Ophthalmol 86:250, 1978.
10. Franklin RM, Prendergast RA, Silverstein AM: Secretory immune system of rabbit ocular adnexa. Invest Ophthalmol Vis Sci 18:1093, 1979.
11. Thoft RA: Conjunctival transplantation. Arch Ophthalmol 95:1425, 1977.
12. Thoft RA: Conjunctival transplantation as an alternative to keratoplasty. Ophthalmology 86:1084, 1979.
13. Klareskog L, Forsum U, Tjernlund UM, et al: Expression of Ia antigen-like molecules on cells in the the corneal epithelium. Invest Ophthalmol Vis Sci: 18:310-313, 1979.
14. Fatt I, St. Helen R: Oxygen tension under an oxygen-permeable contact lens. Am J Optom 48:545-555, 1971.
15. Refojo MF: Mechanism of gas transport through contact lenses. J Am Optom Assoc 50:285-287, 1979.

References Cont.

16. Jumblatt M, Marurice M, M Culley J: Transplantation of tissue-cultured corneal endothelium. Invest Ophthalmol Vis Sci 17:1135-1141, 1978.
17. Gospodarowicz D, Greenburg G, Alvarado J: Transplantation of cultured bovine corneal endothelial cells to rabbit cornea: Clinical implications for human studies. Proc Natl Acad Sci USA 18(suppl):9, 1979.
18. Gospodarowicz DJ, Greenburg GB, Alvarado J: The transplantation in vivo of cultured bovine corneal and vascular endothelial cells in rabbit and cat corneas. Invest Ophthalmol Vis Sci 18(suppl):9, 1979.
19. Klyce SD, Maurice DM: Automatic recording of corneal thickness in vitro. Invest Ophthalmol Vis Sci. 15:550-553, 1976.
20. Klyce SD, Russell SR: System for monitoring the thickness of transparent layered structures. Rev Sci Instrum 49:1318-1321, 1978.
21. Brubaker RF, Coakes RL: Use of a xenon flash tube as the excitation source in a new slit-lamp fluorophotometer. Am J Ophthalmol 86:474-484, 1978.
22. Coakes RL, Brubaker RF: Method of measuring aqueous humor flow and corneal endothelial permeability using a fluorophotometry nomogram. Invest Ophthalmol Vis Sci 18:288-302, 1979.
23. Burns RR, Bourne WM, Brubaker RF: Endothelial function in patients with cornea guttata. Invest Ophthalmol Vis Sci 20:77-85, 1981.
24. Maurice DM: Cellular membrane activity in the corneal endothelium of the intact eye. Experientia 24:1094-1095, 1968.
25. Laing RA, Sandstrom MM, Leibowitz HM: In vivo photomicrography of the corneal endothelium. Arch Ophthalmol 93:143-145, 1975.
26. Bourne WM, Kaufman HE: Specular microscopy of human corneal endothelium in vivo. Am J Ophthalmol 81:319-323, 1976.
27. Laing RA, Sandstrom MM, Berospì AR, et al: Morphological changes in corneal endothelial cells after penetrating keratoplasty. Am J Ophthalmol 82:459-464, 1976.
28. Bourne WM, Kaufman HE: The endothelium of clear corneal transplants. Arch Ophthalmol 94:1730-1732, 1976.
29. Laing RA, Sandstrom MM, Leibowitz HM: Changes in the corneal endothelium as a function of age. Exp Eye Res 22:587-584, 1976.

References Cont.

30. Klyce SD, Wong RKS: Site and mode of adrenaline action on chloride transport across the rabbit corneal epithelium. J Physiol (Lond) 266:777-799, 1977.
31. Klyce SD: Electrical profiles in the corneal epithelium. J Physiol (Lond) 226:407-429, 1972.
32. Klintworth GK, Smith CF: A comparative study of extracellular sulfated glycosaminoglycans synthesized by rabbit corneal fibroblasts in organ and confluent cultures. Lab Invest 35:258, 1976.
33. Hassel J, Newsome DA, Hascall VC: Characterization and biosynthesis of proteoglycans of corneal stroma from rhesus monkey. J Biol Chem 254:12346, 1979.
34. Binder PS: Corneal transplantation today. J Cont Ed Ophthalmol 6:13-26, 1979.
35. Binder PS: Corneal preservation and eye banking, in Symposium on the Medical and Surgical Diseases of the Cornea. St. Louis, CV Mosby Co, 1980, pp320-354.
36. Ohashi K, Szymanska I, Basu PK, et al: Proportions of T- and B-lymphocytes in preauricular lymph nodes and aqueous humour of rabbits showing corneal graft reactions. Can J Ophthalmol 15:30-34, 1980.
37. Tagawa Y, Prendergast RA, Silverstein AM: Role of T-cells in rejection of corneal endothelium: A study of local graft verses host reactions. Presented at the Association for Research in Vision and Ophthalmology Annual Meeting, Orlando, Florida, April 1980.



### III. CATARACT PROGRAM

FISCAL YEAR 1981

<u>Subprogram</u>	<u>Number of Grants</u>
A. <u>The Normal Lens</u>	[37]
1. Molecular Biology	5
2. Physiology and Cell Communication	8
3. Lens Protein and Transparency	5
4. Lens and Aging	5
5. Lens Constituents	5
6. Lens Morphology	1
7. Cell Division and Protein Synthesis	8
B. <u>Epidemiology of Cataracts</u>	[0]
1. Cataract Risk Factors	0
2. Cataract Classification	0
3. Train Cataract Epidemiologists	0
4. Cataract Morbidity	0
5. Collaboration in Clinical Trials	0
C. <u>Senile Cataract</u>	[25]
1. Natural History	0
2. Human Lens Plasma Membrane	17
3. Light Scattering Elements	5
4. Antioxidant Defense Mechanisms	2
5. Environmental Factors	1
6. Culture System for Human Lens	0
7. Collection of Human Lens	0
D. <u>Diabetic and Metabolic Cataract</u>	[16]
1. Pathways in Diabetic and Metabolic Cataracts	9
2. Protein Metabolism in Cataracts	3
3. Drugs to Delay Diabetic Cataract	3
4. Tissue Culture of Cell Lines	0
5. Role of Nutrition in Cataract	1
E. <u>Nongenetic Congenital and Genetic Cataracts and Dislocated Lenses</u>	[8]
1. Genetic Cataracts in Families	0
2. Culture Lens Epithelial and Fiber Cells	2
3. Molecular Genetic Probes	5
4. Culture Ciliary Epithelial Cells	0
5. Role of Zonule in Lens-Globe Growth	0
6. Lens Dislocation - Animal Model	0
7. Zonular Fiber	1
8. Biochemical and Immunological Studies of Zonular Fiber	0
F. <u>Cataract Induced by Environmental and Toxic Effects</u>	[9]
1. The Cataractogenic Effects of Radiations	5
2. Systems for Study of Radiation and Toxicity	3
3. Mode of Action of Cataractogenic Drugs	1

cont.

G. <u>Treatment of Cataract and Correction of Aphakia</u>	[11]
1. Improve Cataract Extraction	0
2. Compare Methods of Cataract Surgery	1
3. Aphakic Correction	2
4. Cataract Management in Infants and Children	0
5. Cataract Prevention and Treatment	0
6. Lens Accommodation	5
7. Lens Induced Uveitis	3

TOTAL NUMBER OF GRANTS [106]

## INTRODUCTION

Cataract is a sizeable public health problem as attested to by data from several sources. Cataracts are the second leading cause of legal blindness in the United States.<sup>1</sup> The enormity of cataracts as a public health problem and the real limits on scientific and financial resources to deal with this problem demand ongoing appraisal of the impact of cataract-related research.

The Cataract program is divided into seven subprograms. Each of the subprograms will be briefly described. This will include: the types of research work being planned and those being conducted, a list of subprogram objectives, an overview of current research support, and the research accomplishments.

## A. The Normal Lens

The normal lens is a transparent, nonvascularized tissue encapsulated by a collagenous basement membrane and composed of anterior epithelial cells and posterior fiber cells.

The objective of cataract research is to cure or prevent human lens opacities. Detailed investigations of the human lens are thus important. It should be recognized, however, that age-dependent variables, the unavailability of normal control lenses, and multiple types of cataract are persistent problems with human lens research. Cataract research should, therefore, continue to use and develop a variety of animal models, both in vivo and in tissue and cell cultures.

Study of all aspects of the normal lens is of fundamental importance for understanding cataract since a multiplicity of factors has been linked to cataract. Such different phenomena as hydration, protein aggregation, phase separation of protein-water mixtures, epithelial cell hyperplasia, and defective fiber cell differentiation have all been implicated in cataract formation.

### A1. Molecular Biology

Objectives:<sup>3</sup> Apart from the isolation of alpha crystallin<sup>2</sup> and delta crystallin mRNAs, studies on molecular biology and gene expression in the lens have just begun.

- o To understand the mechanisms that give rise to growth, cell differentiation, and differential gene expression in the lens.

Overview of Current Research Support and Need: There are five grants being supported in molecular biology in FY 1981. The recommendation is to add two grants to this category for FY 1983. In future years this may be one of the most important area of lens research.

Recent Research Accomplishments:<sup>4</sup> Chicken delta crystallin cDNA has been cloned in a bacterial<sup>5</sup> plasmid<sup>4</sup> and used to isolate segments of the delta crystallin genes.<sup>5</sup> These experiments have demonstrated that there are at least two very similar genes for delta crystallin, each containing numerous intervening sequences. Gene analysis may also be used to understand protein polymorphism in the lens. An interesting recent example concerns the existence of a minor 24,000 dalton alpha crystallin polypeptide present in the rat lens. Amino acid sequencing studies have revealed that this polypeptide contains an insert of 22 residues. This suggests a special case of gene duplication or of unusual processing of alpha crystallin precursor mRNA. Direct examination of the alpha crystallin genes will unequivocally answer this and other similar questions. The ability to identify mRNAs for crystallin and noncrystallin proteins paves the way for further exploration of the genes important in the development and maintenance of the lens.

## A2. Physiology and Cell Communication

Objectives: Significant progress has been made in the area of membrane transport, electrical properties, and cell to cell coupling in variety of lenses.

- o To understand the mechanisms that give rise to growth, cell differentiation, and differential gene expression in the lens.
- o To understand the physiological, physicochemical, and biochemical mechanisms responsible for lens transparency.

Overview of Current Research Support: There are eight grants being supported in physiology and cell communication in FY 1981. The recommendation is to add two grants to this category for FY 1983.

Recent Research Accomplishments: 7-9 It is now clear that lens epithelium contains an electrogenic sodium pump. Such a pump has also been suggested for anterior fiber membranes. The existence of a monovalent cation pump in other membranes in the lens has not yet been established unequivocally.

Numerous studies have shown an effect of low  $Ca^{++}$  in the bathing medium on monovalent cation permeability. Recent studies utilizing electrical impedance techniques have shown that conductance properties of the lens are very complex with the inner fiber membranes having very different properties than the surface cells.

The normal lens of many species has been shown to abound in cell to cell gap junctions. Although the functions of these ubiquitous junctions are not totally understood, it is clear that they allow diffusion of low molecular weight substances from one fiber to another and provide pathways for electric current flow. A recent study has demonstrated that these junctions are lost during the formation of cataract in Nakano mouse and provides a link between cell communication and cataract formation.

## A3. Lens Protein and Transparency

Objectives: The factors which make the normal lens transparent are not yet known. Important advances have been made in the physicochemical basis of the loss of lens transparency.

- o To understand the physical and chemical properties of the constituents of the normal lens.
- o To understand the physiological, physicochemical, and biochemical mechanisms responsible for lens transparency.

Overview of Current Research Support: There are five grants being supported in lens protein and transparency in FY 1981. The plan is to add one more grant in this category in FY 1983.

Recent Research Accomplishments: The use of quasielastic light scattering theory and thermodynamic phase diagrams have provided new descriptions of nuclear <sup>10-12</sup> cold cataracts, and nuclear cataracts in galactosemic rat lenses. Substances which alter the phase curves<sup>10</sup> have been shown to have predictable effects in reducing the opacity. Other investigators using a somewhat more general theory of light scattering in inhomogeneous solids have been able to predict lens opacity on the basis of protein aggregation and syneresis.<sup>13,14</sup> A loss of the balance between form and intrinsic birefringence has also been implicated.

#### A4. Lens and Aging

Objectives: Studies on the posttranslational modifications of the lens crystallins are needed in order to understand the heterogeneity of the crystallin polypeptides and to explore the possible mechanism<sup>15</sup> of crystallin aggregation during aging and cataract formation.

- o To understand the anatomical and mechanical factors involved in lens deformation.
- o To understand the effects of cataractogenic agents (drugs and other external factors), ocular and systemic disorders, and aging on normal lens structure and function.

Overview of Current Research Support: In FY 1981 there are five grants in the area of lens and aging. In FY 1983 the plan is to add one more grant to this area.

Recent Research Accomplishments: Cleavage,<sup>16,17</sup> oxidation,<sup>18,19</sup> fluorogen production, and racemization of aspartic acid are among the list of naturally occurring modifications of crystallin polypeptides that may contribute to cataractogenesis. Oxidation in particular appears to be an important avenue of crystallin aggregation and association with lens membranes, and both have been implicated in cataract formation in man.

#### A5. Lens Constituents

Proteins: Much has been learned over the last few years about the synthesis and structure of the crystallins, which are the major protein constituents of the lens.

Lipids: During the past few years, lens lipids have begun to receive considerable attention.

Carbohydrates: Few investigators are presently studying carbohydrates metabolism in the lens.

- o To understand the physiological, physicochemical, and biochemical mechanisms responsible for lens transparency.
- o To understand the physical and chemical properties of the constituents of the normal lens.

Overview of Current Research Support: In FY 1981 there were five grants in this category. In FY 1983 the recommendation was to add no grant in this category.

Recent Research Accomplishments:

Proteins: Changes in the intralenticular concentrations of ions (especially  $\text{Na}^+$  and  $\text{K}^+$ ) appear able to differentially reduce crystallin synthesis in normal and cataractous lenses interfering with mRNA translation.<sup>20, 21, 22, 23</sup>

Lipids: The phospholipid content of the lens membranes of several species has been established.

Carbohydrates: The work of the Howe Laboratory of Ophthalmology in Boston continues on the control of pyruvates kinase and on human lens polyol dehydrogenase.

A6. Lens Morphology

Objectives: Appreciable progress in the understanding of lens anatomy has occurred over the past few years, in large measure due to use of the techniques of transmission and scanning electron microscopy.

- o To understand the anatomical and mechanical factors involved in lens deformation.

Overview of Current Research Support: In FY 1981 one grant was supported in this category. One more grant is recommended for this category in FY 1983.

Recent Research Accomplishments: Numerous studies describing the ultrastructure of lens epithelial cells have been published.<sup>24</sup> Few species have zonulae occludentes between epithelial cells, and thus it is anticipated that small molecules are free to move between these cells.<sup>25, 26</sup> Many lateral interdigitations between human lens epithelial cells have been visualized.<sup>27</sup>

Much work has been done on the structure of fiber cells both in the cortex and nucleus. The fibers are interconnected through an elaborate array of both tongue and groove structures and ball and socket structures.

## A7. Cell Division and Protein Synthesis

Objectives: The lens differentiates from the surface ectoderm by a series of inductive interactions culminating in a direct contact with the optic vesicle. This has been reviewed recently.<sup>28</sup>

- o To understand the mechanisms that give rise to growth, cell differentiation, and differential gene expression in the lens.
- o To understand the physiological, physicochemical, and biochemical mechanisms responsible for lens transparency.
- o To understand the physical and chemical properties of the constituents of the normal lens.

Overview of Current Research Support: In FY 1981 there were eight grants supported in this category. No additional grants are recommended in this category for FY 1983.

Recent Research Accomplishments: A culture method has been developed to study lens induction in the chicken embryo.<sup>29</sup> This advance has provided for the existence of an inducing substance with a maximum molecular weight of 20,000 daltons which is synthesized by the eye cup. These experiments raise the exciting possibility of isolating the naturally occurring lens inducer(s).

## B. Epidemiology of Cataracts

Epidemiologic methods have seldom been applied to noninfectious eye and vision problems, with cataract studies being especially infrequent. These studies, however, have made important contributions, such as the establishment of an association between cataracts and diabetes. The existence of such an association has been a longstanding clinical impression. However, it could not be established through clinical studies of extracted cataracts, because of the self-selection of patients undergoing cataract surgery. It was only through epidemiologic studies of populations that an association was shown to exist.

### B1. Cataract Risk Factors

Objectives: Progress has been made in identifying associations of senile cataracts with several factors that may play a role in pathogenesis.

Ultraviolet light: It is possible that sunlight and artificial light sources emitting light of 300-400 nm wavelengths increase the risk of senile and other types of cataracts.

Diabetes: The role of diabetes as a risk factor in senile cataracts has been a topic of some controversy.

Other Systemic Factors: As part of the Framingham Eye Study, the relationship between cataracts and the variables previously measured by the Framingham Heart Study was determined.<sup>30</sup>

- o To assist in the development of an objective, reproducible, and standardized classification of type and severity of cataracts.
- o To obtain valid and reliable data on the incidence and prevalence of cataracts.
- o To determine the role of various environmental and genetic factors in cataract etiology.
- o To evaluate diagnostic and therapeutic modalities in cataract management.

Overview of Current Research Support: In FY 1981 there was no grant supported in this category. In FY 1983 two grants are recommended for this category.

Recent Research Accomplishments:

Ultraviolet light: The hypothesis of an association between human cataracts and sunlight exposure has been supported by recent studies. In the MRA and NHANES data, a relationship was found between cataracts in different areas of the United States and the duration of sunlight<sup>31</sup> in these areas. This association was most evident among older persons.

Diabetes: Recent study at the NEI has shown the existence of an association<sup>32</sup> between diabetes and cataract in both FES and HANES population. This study found that the prevalence of cataracts was three to four times higher in diabetic than nondiabetics under 65 years of age. Among older persons, however, no marked differences could be shown. The results of this and other studies suggest that diabetics under the age of 65 are at greater risk of having cataracts than nondiabetics.

Other Systemic Factors: Casual blood sugar, systemic blood pressure, vital capacity, serum phospholipid, short stature, low educational status,<sup>30</sup> and hand strengths were associated with the presence of cataracts.

B2. Cataract Classification

Objectives: A major problem in cataract research has been the lack of a uniformly accepted definition of the disease and the lack of adequate measurement and classification schemes.

- o To assist in the development of an objective, reproducible, and standardized classification of type and severity of cataracts.

Overview of Current Research Support: In FY 1981 there was no grant supported in this category. In FY 1983 the recommendation is to add one grant to this category.

Recent Research Accomplishments: Progress has been made in the area of classification of cataracts through the work of the Cooperative Cataract Research Group (a consortium grantee of the NEI), but so far this has been confined to an in vitro classification rather than in vivo assessment. It has been recognized that the senile cataract category is too broad and that the various types of senile lens changes should be analyzed separately. An accomplishment in this area was the work of NEI investigators regarding the association of senile maculopathy with nuclear and cortical opacities. Using data from the FES, no relationship was found between maculopathy and all types of lens changes combined.<sup>33</sup> However, when these were evaluated separately, senile maculopathy had a negative association with nuclear sclerosis, but a positive association with cortical opacities.<sup>34</sup> This could possibly be attributed to a protective filtering effect of the nuclear pigment, which would prevent macular damage from light exposure.

### B3. Train Cataract Epidemiologists

Objectives: The epidemiology of cataracts is only beginning to develop. A major problem is the shortage of qualified investigators, since very few are working in this area.

- o To train investigators in the area of cataract epidemiology.

Overview of Current Research Support: In FY 1981 there were no grants in this area. In FY 1983 the recommendation is to add one grant to this area.

Recent Research Accomplishments: At the present time, few cataract researchers have received training in epidemiologic techniques, and few epidemiologists are knowledgeable in eye research. This situation reflects the generally limited interaction between eye care professionals and epidemiologists. Such an interaction can be increased through opportunities for formal training of qualified investigators in both fields. This training can take the form of course and field experience, following the format of the training programs sponsored by the NEI Office of Biometry and Epidemiology. The training of investigators would be facilitated by the establishment of collaborative ophthalmologic-epidemiologic centers.

In addition, a book on epidemiology and statistics for ophthalmologists has been recently published.<sup>35</sup> Although these efforts are not specifically directed to cataract investigators, they represent important steps in increasing knowledge of epidemiologic methods among eye researchers.

#### B4. Cataract Morbidity

Objectives: Descriptive studies are useful to measure the magnitude of cataracts as a cause of morbidity. Such studies define the extent of the public health problem caused by cataracts, by determining cataract incidence (number of new cases developing in a time period) and prevalence (number of existing case at a point in time) in the population. In addition to measuring the frequency of cataract occurrence, such studies can evaluate the extent of visual impairment associated with treated and untreated cataracts. Since surgical treatment is successful in most cases, it is important to determine the magnitude of treated and untreated disease and its associated visual deficit. All this information has important public policy implications.

- o To obtain valid and reliable data on the incidence and prevalence of cataracts.
- o To determine the role of various environmental and genetic factors in cataracts etiology.
- o To evaluate diagnostic and therapeutic modalities in cataract management.

Overview of Current Research Support: In FY 1981 there was no grant in this area. In FY 1983 the recommendation is to add one grant in this area.

Recent Research Accomplishments: The implementation of the Framingham Eye Study was an important accomplishment in cataract epidemiology. It permitted an assessment of the prevalence of carefully defined senile lens changes in a given population, as well as a study of the distribution of such changes in the population according to many variables.

#### B5. Collaboration in Clinical Trials

Objectives: Experimental studies provide a critical evaluation of the advantages and disadvantages of current treatment and diagnostic procedures. As such, they are essential to evaluate properly the different modalities of cataract management. With the advent of so many new diagnostic and surgical techniques, it is difficult for the clinician to select the most desirable approach to patient management. The relative value of different approaches can be rigorously evaluated through the careful design and implementation of controlled clinical trials.

- o To assist in the development of an objective, reproducible, and standardized classification of type and severity of cataracts.
- o To obtain valid and reliable data on the incidence and prevalence of cataracts.

- o To evaluate diagnostic and therapeutic modalities in cataract management.

Overview of Current Support and Needs: In FY 1981 there was no grant in this area. In FY 1983 the recommendation is to add one grant in this area.

Recent Research Accomplishments: New devices and procedures pertaining to cataracts are constantly being introduced into the practice of ophthalmology. Clinicians cannot possibly make informed decisions regarding optimum management without proper evaluation of such devices and procedures. Yet, many new techniques are being applied in the preoperative, operative, and postsurgical periods, often without comprehensive, well-designed studies of efficacy and long-term advantages.

Appropriately designed, controlled clinical trials are greatly needed to provide information on the value of the various options available for managing patients with cataract.

### C. Senile Cataract

Senile cataract is the opacification of the human lens that occurs with aging and consists of two main subgroups: cortical and nuclear opacities. Cortical cataracts consist of a heterogeneous group of opacities that involve the younger and more superficial fiber cells. The nuclear opacities are located in the central deep area of the lens and also consist of several types. Cortical and nuclear opacities often coexist in the same lens.

Cataract research is also relevant to human aging in general. Understanding of the events leading to protein aggregation, degradation, and insolubilization will be of considerable value to the study of age-related changes in other tissues of the human body. Although the specific mechanisms that cause senile cataracts remain to be elucidated, our knowledge of the changes in structure and chemistry that accompany the cataractous process has been greatly expanded.

#### C1. Natural History

Objectives: The study of the natural history of the cataractous process and of risk factors should be actively pursued using a multidisciplinary approach.

- o To determine the cause(s) and pathogenesis of senile cataract including its risk factors.

Overview of Current Support: In FY 1981 there was no grant in this area. In FY 1983 the recommendation is to have one grant in this area.

Research Accomplishments: The establishment of the Cooperative Cataract Research Group (a funded consortium of the NEI) has stimulated research in the area of human cataractogenesis. There is a great need to encourage epidemiologists and lens researchers to cooperate together to work on discerning the natural history of the cataractous process and of the risk factors.

## C2. Human Lens Plasma Membrane

Objectives: The role of the plasma membrane and cytoskeleton in the cataractous process is being extensively explored.

- o To determine the cause(s) and pathogenesis of senile cataract including its risk factors.
- o To seek means to prevent, delay the progress of, or reverse the cataractous process.

Overview of Current Support: In FY 1981 this area had 17 grants of a total of 25. The recommendation for FY 1983 is to add one grant. The panel needs to look over the future plans for this subprogram since it appears to be overly represented in this one area.

Recent Research Accomplishments: The isolation of plasma membrane of the fiber cells has been accomplished,<sup>36</sup> and a great deal is now known about its protein and lipid composition.

## C3. Light Scattering Elements

Objectives: The light scattering elements of cortical cataracts<sup>37</sup> are the large intercellular clefts and areas of fiber cell breakdown. High molecular weight aggregates present in the cortex may also play a role.<sup>38</sup>

- o To determine the cause(s) and pathogenesis of senile cataract including its risk factors.
- o To seek means to prevent, delay the progress of, or reverse the cataractous process.

Overview of Current Support: There are five grants in this area in FY 1981. In FY 1983 two additional grants are recommended in this area.

Recent Research Accomplishments: The greatly increased level of high molecular weight aggregates<sup>39,40</sup> probably accounts for the light scattering in nuclear cataracts. Syneresis, with or without an accompanying aggregation process,<sup>41</sup> could also account for a large part of the opacification in the nucleus. Changes in local hydration of protein enhances the refractive index fluctuation which contributes to the opacity. The phenomenon of phase separation of cell cytoplasm accompanied or

preceded by cell surface change may be intimately associated with the mechanism for the formation of scattering centers in the reversible stages of cold cataract opacification. Chemical reagents which improve phase transparency in the transition cataract also do so in human cataracts in vitro.

#### C4. Antioxidant Defense Mechanisms

Objectives: Direct evidence of the involvement of oxidation in cataractogenesis has been established experimentally. The antioxidants of the lens include glutathione and its regeneration systems, which are all markedly reduced in the human cataractous state.<sup>42</sup>

- o To determine the cause(s) and pathogenesis of senile cataract including its risk factors.
- o To seek means to prevent, delay the progress of, or reverse the cataractous process.

Overview of Current Support: In FY 1981 there are two grants supported in this area. For FY 1983 the recommendation is to maintain the number of grants in this area to two.

Recent Research Accomplishments: In addition to acting as an in situ reducing agent to maintain protein sulfhydryls, glutathione can also cleave the disulfide bonds in proteins from advanced nuclear cataractous lenses and in mixed disulfides of lens proteins and glutathione.<sup>43</sup>

#### C5. Environmental Factors

Objectives: The mechanism(s) that lead to oxidative damage remains to be elucidated. A prime factor appears to be sunlight acting via ambient ultraviolet light radiation (300-400nm).<sup>44</sup>

- o To determine the cause(s) and pathogenesis of senile cataract including its risk factors.
- o To seek means to prevent, delay the progress of, or reverse the cataractous process.

Overview of Current Research Support: In FY 1981 there is one grant supported in this area. For FY 1983 the recommendation is to add two more grants in this area.

Recent Research Accomplishments: Long-wave UV light readily penetrates the cornea and therefore can reach the lens which absorbs it more efficiently than any other ocular tissue. This radiation is capable of generating fluorescent pigments via a photochemically initiated free radical mechanism involving tryptophan and other aromatic amino acids as the absorbing chromophores.<sup>45</sup>

## C6. Culture System for the Human Lens

Objectives: The development of an in vitro culture system for the human lens is of great importance.

- o To seek means to prevent, delay the progress of, or reverse the cataractous process.

Overview of Current Research Support: In FY 1981 there was no grant being supported in this area. For FY 1983 the recommendation is to add one grant in this area.

Recent Research Accomplishments: Epithelial cell changes that occur with aging and cataract formation consist of a decrease in the number of cells, from 93 to 100  $\mu\text{m}^2$  in young adults to about 62  $\mu\text{m}^2$  in noncataractous lenses of 50- to 70-year-olds, to less than 50  $\mu\text{m}^2$  in cataractous lenses.<sup>46</sup>

Despite the loss of epithelial cells and the marked reduction in mitosis with aging, they have been successfully cultured for short periods of time.<sup>47</sup> There appears to be progressive age-related decline of growth capacity. Even epithelia from cataractous lenses contain a population of cells that can proliferate.<sup>48</sup> A permanent cell line of human lens epithelium has not been established.

## C7. Collection of Human Lens

Objectives: The advancement of research in Senile Cataract is directly related to the availability of the human lens for investigation.

- o To determine the cause(s) and pathogenesis of senile cataract including its risk factors.
- o To seek means to prevent, delay the progress of, or reverse the cataractous process.

Overview of Current Research Support: In FY 1981 there was no grant support for this area. For FY 1983 the recommendation is to add one grant to this area.

Recent Research Accomplishments: The establishment of the Cooperative Cataract Research Group has stimulated collaborative research<sup>49,50</sup> and greatly facilitated studies on the human lens. A contract was arranged by the NEI to obtain some special cataractous lenses from India. Through the auspices of the CCRG human lenses have been made more readily available to a wider variety of investigators than before but much greater improvement is required.

#### D. Diabetic and Metabolic Cataract

The research in this area is concerned with studies of cataracts produced as a result of diabetes, galactosemia (excess galactose in blood), or other types of sugars.

Metabolic cataracts are those which may be formed due to a specific metabolic block or abnormality; in the broadest sense they must include sugar, nutritional, and genetic cataracts.

##### D1. Pathways in Diabetic and Metabolic Cataracts

Objectives: An understanding of the nature of diabetic cataracts is evolving from detailed studies of sugar cataracts either produced experimentally or in some animal models with congenital diabetes. In this sequence of reactions, pyridine nucleotides and the mechanisms responsible for their generation in the lens play an important part,<sup>51,52</sup> thus necessitating an inquiry into the fundamental metabolic pathways in the lens.

- o To understand and identify the factors involved in the development of diabetic and metabolic cataracts.
- o To establish cell lines in tissue culture from cataracts of different etiology.
- o To elucidate further that diabetes can hasten the development of senile cataracts.

Overview of Current Research Support: In FY 1981 there were nine grants supported in this area. The recommendation for FY 1983 is to add no grant in this area.

Recent Research Accomplishments: A major accomplishment in cataract research is the elucidation of the initiating factors in diabetic or sugar cataracts. During the last five years, it has been well established that the formation of polyols is the major cause of cataractogenesis in diabetes and galactosemia and that the enzyme aldose reductase plays a key role in the formation of sugar cataracts. This enzyme, activated when sugar levels are elevated, converts sugars to polyols. The accumulation of polyols which do not freely diffuse out of cells or are actively metabolized accumulate to high levels, causing cells to swell and eventually rupture. The osmotic events caused by polyol retention initiate a series of events leading to opacification.

##### D2. Protein Metabolism in Cataracts

Objectives: An interesting and potentially significant line of research<sup>53</sup> is the effect of cation imbalance in the lens on protein synthesis.

- o To understand and identify the factors involved in the development of diabetic and metabolic cataracts.
- o To establish cell lines in tissue culture from cataracts of different etiology.
- o To elucidate further that diabetes can hasten the development of senile cataracts.
- o To develop additional animal models in the study of diabetic and metabolic cataracts.

Overview of Current Research Support: In FY 1981 there are three grants in this area. The recommendation for FY 1983 is to maintain the number of grants in this area to three.

Recent Research Accomplishments: Researchers at the National Eye Institute and the National Institute of Child Health and Human Development have shown that cation imbalance seen in sugar cataracts and in animal models with hereditary cataracts adversely affects protein synthesis.<sup>53</sup>

### D3. Drugs to Delay Diabetic Cataract

Objectives: The polyol hypothesis has led to the discovery and development of several inhibitors of aldose reductase which have been used in delaying or preventing diabetic cataracts in animals.

- o To develop effective drugs which will slow or block the development of diabetic cataract in man.
- o To evaluate the safety and efficacy of drugs used in delaying or preventing diabetic cataracts.

Overview of Current Research Support: In FY 1981 there were three grants in this area. For FY 1983 the recommendation is to add one more grant in this area.

Recent Research Accomplishments: The polyol pathway has also been observed in human lenses, and the production of sorbitol along with the resulting lens swelling can be delayed in in vitro cultured human lenses through the use of aldose reductase inhibitors.<sup>54</sup>

In the evaluation of aldose reductase inhibitors, differences in the susceptibility of human placental, human lens and rat lens aldose reductase to inhibitors have been observed. These studies are not only important but essential for designing specific aldose reductase inhibitors and for an eventual clinical trial of aldose reductase inhibitors in human diabetic cataracts.

#### D4. Tissue Culture of Cell Lines

Objectives: The use of lens tissue for establishment of well-characterized lens cells is one of great potential. The cultured lens cells from both normal animal and human sources with known medical history and pathology, age, and genetic abnormalities can provide tissue which could be subjected for detailed analysis.

- o To understand and identify the factors involved in the development of diabetic and metabolic cataracts.
- o To establish cell lines in tissue culture from cataracts of different etiology.

Overview of Current Research Support: There are no grants being supported in this area in FY 1981. The recommendation is to add one grant in this area in FY 1983.

Recent Research Accomplishments: In the Nakano mouse, the basic defect is ion transport as a result of an inhibitor of Na-K ATPase produced in the lens. The inhibitory factor, which is present only in the lens, has been isolated and characterized by growing cell lines of Nakano mouse lens epithelium in tissue culture.<sup>55,56</sup> This illustrates that such an approach is feasible in identifying specific factors involved in human genetic cataracts.

#### D5. Role of Nutrition in Cataract

Objectives: Turning to the role of nutrition in cataracts, it is important that systematic epidemiological studies should be undertaken, especially in areas where cataract development has been observed in younger age group.

- o To understand and identify the factors involved in the development of diabetic and metabolic cataracts.
- o To establish cell lines in tissue culture from cataracts of different etiology.
- o To establish the role of nutrition in human senile cataracts through epidemiological studies.

Overview of Current Research Support: There is one grant being supported in this area in FY 1981. The recommendation is to add one more grant in this area for FY 1983.

Recent Research Accomplishments: Malnutritional factors are important as suggested by the established production of cataracts in the young and experimental animals subjected<sup>57</sup> to a deficiency of proteins, amino acids, or certain vitamins.

## E. Nongenetic Congenital and Genetic Cataracts and Dislocated Lenses

Congenital cataracts fall into two broad categories, either acquired during uterine life and nongenetic, or genetic. Nongenetic cataracts are usually accompanied by other ocular features such as pepper and salt retinopathy or extraocular features such as deafness or cardiac disease, or even mental retardation as evidence of a widespread intrauterine viral disease. Genetic cataracts can occur either at birth or at a later stage as infantile or juvenile cataracts.

### E1. Genetic Cataracts in Families

Objectives: A strong genetic influence is often observed in cataracts. Senile cataracts often run in families as well. No studies have been done to date to elucidate whether these latter cataracts occur on a genetic basis or are due to environmental factors common to a family such as nutrition or sunlight exposure.

- o To analyze cataracts on a population genetic basis.
- o To determine genetic versus environmental influences on the production of cataracts.
- o To determine relationships to other ocular abnormalities and systemic disease.
- o To develop a reasonable classification of cataracts on the basis of genetic, biochemical, ultrastructural, and other parameters.

Overview of Current Research Support: In FY 1981 there was no grant in this area. For FY 1983 the recommendation is to add one grant in this area.

Recent Research Accomplishments: Isolated hereditary cataracts are in most instances inherited as an autosomal dominant trait, less likely as an autosomal recessive inheritance, and least likely an X-linked inheritance. Many clinical types have been described.

Congenital cataracts represent a major cause of childhood blindness; the statistics vary from 14 percent<sup>58</sup> in a large British survey to close to 40 percent in an Italian survey.

### E2. Culture Lens Epithelial and Fiber Cells

Objectives: Tissue culture of normal and cataractous lens cells from mouse, rat, chicken, frog, and rabbit have contributed to studies on the cataractous states.

- o To better understand the mechanism that lead to abnormal differentiation of the lens through biochemical and ultrastructural studies.

- o To develop techniques for in vitro culture of human lens epithelial and fiber cells.

Overview of Current Research Support: In FY 1981 there were two grants in this area. For FY 1983 the recommendation is to add one more grant in this area.

Recent Research Accomplishments: With the gradual improvement of techniques to culture animal cells, the ability for culturing human lens cells appears the only area which awaits a major effort. Once developed this system would be an important step in better understanding genetic cataracts in humans.

### E3. Molecular Genetic Probes

Objectives: Several genetic cataract animal models, primarily in mice, have recently been described.<sup>59</sup>

- o To better understand the mechanisms that lead to abnormal differentiation of the lens through biochemical and ultrastructural studies.
- o To develop techniques for in vitro cultures of human lens epithelial and fiber cells.
- o To further develop animal models of genetic cataracts.

Overview of Current Research Support: In FY 1981 there are five grants in this area. For FY 1983 the recommendation is to retain this number of grants in this area.

Recent Research Accomplishments: Animal models will allow detailed genetic and molecular biological studies to be carried out to determine the basis for the cataractous state. Molecular studies include molecular cloning of lens protein genes to allow investigation of transcription and translation of lens proteins. Also, such probes can determine whether observed chromosome abnormalities found with many cataractous conditions result in gene deletion of lens function or whether such genes are masked or mutated.

### E4. Culture Ciliary Epithelial Cells

Objectives: Most dislocation of the lens are traumatically induced. On a genetic basis, however, they occur either as isolated ocular disorders or in association with other ocular malformations as part of a systemic disease. To date, some 20 different genetic etiologies have been established. Although each entity is rare, their aggregate is significant.

For a definitive study of zonular secretion it will probably require tissue culture of ciliary nonpigmented epithelial cells so that the product can be analyzed.

- o To improve understanding of the mechanism that lead to abnormalities of the lens zonule present at birth or occurring later in life.

Overview of Current Research Support: In FY 1981 there is no grant being supported in this area. For FY 1983 the recommendation is to add one grant in this area.

Recent Research Accomplishments: From the studies with tissue culture of ciliary nonpigmented epithelial cells some antizonular antibodies may be raised and used for detecting intracellular molecules during the zonular synthetic period. Manipulation of the secretory product will be of great interest.

#### E5. Role of Zonule in Lens-Globe Growth

Objectives: Zonules have been found to be less aggregated ("dysplastic") and possibly more profuse in lid-suture myopia.

- o To understand better the biochemistry and ultrastructure of the lens zonule, which in turn may lead to understanding to the Marfan syndrome and various cardiovascular diseases.
- o To improve understanding of the mechanisms that lead to abnormalities of the lens zonule present at birth or occurring later in life.

Overview of Current Research Support: In FY 1981 there was no grant supported in this area. For FY 1983 the recommendation is to have one grant in this area.

Recent Research Accomplishments: It was found that zonules were less aggregated and possibly more profuse in lid-suture myopia. This latter finding opened the door to another facet of zonular-lens dislocation research: the frequent occurrence of small lenses and dislocation, suggesting the possible feedback relations of zonules, lens-globe proportions, and accommodation.

#### E6. Lens Dislocation - Animal Model

Objectives: There are many models of lens dislocation among the larger mammals which could be studied in the same way as the human.

- o To understand better the biochemistry and ultrastructure of the lens zonule, which in turn may lead to understanding of the Marfan syndrom and various cardiovascular diseases.
- o To develop animal models for dislocated lenses.
- o To develop treatment plans for the visual complication of dislocated lenses.

Overview of Current Research Support: In FY 1981 there is no grant in this area being supported. For FY 1983 the recommendation is to add one grant in this area.

Recent Research Accomplishments: Some of the animal models which are available are the glaucomatous beagle and the buphthalmic rabbit, and they form a reservoir for experimentation in the area of dislocated lenses. Other models should be sought. The possibility of inducing zonular dysgenesis by dietary changes related to sulfur metabolism should be attempted.

#### E7. Zonular Fiber

Objectives: Although some work has been done already in the ultrastructural morphology of human zonules and their lens-ciliary insertion,<sup>60</sup> more work needs to be done.

- o To improve understanding of the mechanisms that lead to abnormalities of the lens zonule present at birth or occurring later in life.

Overview of Current Research Support: In FY 1981 there is one grant being supported in this area. For FY 1983 the recommendation is to add another grant in this area.

Recent Research Accomplishments: Application of the knowledge arising from some of the morphological studies on the zonules and their lens-ciliary insertion have been made to pathologic dislocation of the lenses, encompassing earlier studies of zonular fragmentation in homocystinuria<sup>61</sup> and in the Marchesani syndrome.<sup>62</sup> One interesting report utilizing SEM describes that zonular fibrils are five to six times thicker than normal in the Marfan syndrome. It is possible that larger fibrils may indicate a thicker carbohydrate coating or that some of them are actually vitreal fibers.

#### E8. Biochemical and Immunological Studies of Zonular Fiber

Objectives: Considerable progress has been made in characterizing zonular protein.

- o To improve understanding of the mechanisms that lead to abnormalities of the lens zonule present at birth or occurring later in life.

- o To understand better the biochemistry and ultrastructure of the lens zonule, which in turn may lead to understanding of the Marfan syndrome and various cardiovascular diseases.

Overview of Current Research Support: In FY 1981 there is no grant being supported in this area. For FY 1983 the recommendation is to add one grant in this area.

Recent Research Accomplishments: In characterizing zonular proteins amino acid analysis of the bovine zonule has been completed.<sup>63</sup> Such analysis has been extended to man. The high cysteine content of the zonular protein correlated well with the fact that all enzyme defects known in ectopia lentis involve sulfur metabolism.

#### F. Cataract Induced by Environmental and Toxic Effects

The lens, which continues to grow throughout life, does not renew its cells, and mature fibers are expected to remain transparent through a person's lifetime. Continuous insult to lens from drugs or radiation may interfere with the normal repair and protective mechanism, thereby hastening the formation of senile cataract.

##### F1. The Cataractogenic Effects of Radiation

Objectives: It has long been recognized that ionizing radiation from gamma or x-ray sources will give rise to cataracts in animals and humans. A review of this subject has recently been published.<sup>64,65</sup>

- o To understand and identify the biochemical and biophysical changes which take place in the lens as a result of insults from environmental radiation, as well as insults from chemicals and drugs.
- o To protect the lens from such insults, thereby avoiding, or at least postponing significantly the onset and development of senile cataract.

Overview of Current Research Support: In FY 1981 there are five grants being supported in this area. For FY 1983 the recommendation is to keep the level of support at five grants in this area.

Recent Research Accomplishments: The cornea effectively absorbs all ultraviolet radiation shorter than 300 nm, thereby only "long" wavelength UV light will be considered (i.e., from 300 to 400 nm). In the 12 years, evidence has accumulated that this long wavelength UV light at radiances not greater than those in normal sunlight can lead to changes in lens proteins and thereby may contribute to lens opacity.<sup>65</sup> It is well known that infrared radiation at relatively high intensities can cause cataract.<sup>64,65</sup> Under certain conditions, radiofrequency radiation is known to be capable of inducing cataract.<sup>65</sup>

## F2. Systems for Study of Radiation and Toxicity

Objectives: A recent major accomplishment is the development of a reproducible method of objectively monitoring visible lens changes using the Scheimpflug Principle. <sup>66-68</sup>

- o To understand and identify the biochemical and biophysical changes which take place in the lens as a result of insults from environmental radiation, as well as insults from chemicals and drugs.
- o To protect the lens from such insults, thereby avoiding, or at least postponing significantly the onset and development of senile cataract.

Overview of Current Research Support: In FY 1981 there are three grants being supported in this area. For FY 1983 the recommendation is to maintain the level at three grants in this area.

Recent Research Accomplishments: The Scheimpflug Principle promises to be a powerful clinical tool for measuring the effects of drugs and radiations on the lens. Recently it was reported that such a camera is capable of monitoring lens fluorescence (at specific wavelengths) in the patient through the use of UV light. <sup>69</sup>

It is hoped that this system will be useful for monitoring drug and radiation effects on the lens even before they become clinically manifested by the usual slit-lamp examination.

## F3. Mode of Action of Cataractogenic Drugs

Objectives: It is well established that many drugs and chemicals, whether applied directly to the eye or taken, systemically for other disorders, can cause cataract in humans. <sup>70-72</sup>

- o To understand and identify the biochemical and biophysical changes which take place in the in the lens as a result of insults from environmental radiation, as well as insults from chemicals and drugs.
- o To protect the lens from such insults, thereby avoiding, or at least postponing significantly the onset and development of senile cataract.

Overview of Current Research Support: In FY 1981 there was one grant being supported in this area. For FY 1983 the recommendation is to add one more grant in this area.

Recent Research Accomplishments: The term "cataract" generally applies to opacification and changes of lens substance itself; however, there are many cases in which deposits of foreign material or drugs in the lens can result in local opacities. These deposits, which sometimes interfere with<sup>71</sup> visions, do not necessarily lead to further opacification of the lens. Many drugs and chemicals will induce cataracts in various animals. In many cases, the human lens and lenses from other animals show a dramatic difference in their susceptibility to certain drugs and chemicals. Considerable species differences have<sup>70-72</sup> been found in the response of the lens to various drugs and compounds.

#### G. Treatment of Cataract and Correction of Aphakia

At present, there is no proven medical treatment to prevent or cure cataract, the second leading cause of blindness in this country.<sup>1</sup> Therefore, surgery to remove the cloudy lens is the only available alternative.

##### G1. Improve Cataract Extraction

Objectives: Surgery of cataracts has become increasingly refined with the development of various techniques and instrumentation for performing intracapsular or extracapsular cataract extraction. About 85 percent<sup>73,74</sup> of such surgeries are successful with vision of better than 20/40.

- o To evaluate current techniques for cataract extraction and correction of aphakia and develop new approaches to these problems.

Recent Research Accomplishments: Significant complications may develop from cataract surgery including damage to the endothelium with resultant corneal decompensation and clouding, and cystoid macular edema accounting for decreasing central vision. New techniques for the removal of cataracts such as phacoemulsification, irrigation-aspiration, and other microsurgical instrumentation have resulted in further technical improvement in cataract extraction in infants, children, and young adults. Despite these technical advances, thousands of such young patients remain blind because no satisfactory method of correcting aphakia and preventing amblyopia is available. The role of glasses, contact lenses, intraocular lenses, and refractive keratoplasty warrants further study in these cases.

##### G2. Compare Methods of Cataract Surgery

Objectives: Although it is difficult to establish controlled trials of cataract surgery with or without intraocular lenses due to enthusiasm of ophthalmologic surgeons and their patients for the intraocular lens, better attempts should be made by the NIH to establish such trials in the United States.

- o To evaluate current techniques for cataract extraction and correction of aphakia and develop new approaches to these problems.

Recent Research Accomplishments: The removal of a cataract from the eye is made by an incision in the cornea or sclera. The lens is then either completely removed (intracapsular extraction) by grasping the capsule with forceps or a freezing probe, or the lens substance is extracted leaving the posterior capsule in the eye (extracapsular extraction).<sup>73</sup> Major accomplishments in the past five years include improved microsurgical techniques and instrumentation for both intracapsular and extracapsular cataract extraction, resulting in better wound closure and fewer complications.<sup>75</sup>

### G3. Aphakic Correction

Objectives: Aphakia still remains the major problem confronting cataract researchers today. The lack of correction of aphakia following cataract extraction in infants and young children causes amblyopia and, for all intents and purposes, blindness. Also, young adults who have undergone cataract extraction and are intolerant of contact lenses are essentially blind in the aphakic eye. Alternatives to correction of aphakia must be developed.

- o To evaluate current techniques for cataract extraction and correction of aphakia and develop new approaches to these problems.
- o To determine the influence of aphakia on visual development in infants and children and devise therapies to successfully overcome the defect.
- o To establish standards and guidelines for toxicological studies of intraocular lens materials.

Recent Research Accomplishments: Hard plastic contact lenses for aphakia in children and adults have been used for several years and are viable alternatives for correction of aphakia, at least in adults. Soft daily-wear contact lenses have been approved within the past five years. Extended-wear soft contact lenses are under investigation as alternatives to hard and daily-wear contact lenses, and certain types have been released by the FDA for use in aphakia.<sup>81</sup> This represents a major breakthrough in the management of aphakia in that it allows postoperative patients, who have problems inserting and removing contact lenses, the option of wearing lenses for extended periods of time--sometimes up to several months.

Major advances in the correction of aphakia by the use of intraocular lenses have been made in the past ten years. Plastic lenses placed within the eye at the time of cataract surgery (intraocular lenses) have been developed over the past two and one-half decades, and are being used increasingly in United States as an alternative to eyeglasses or contact lenses in the correction of aphakia.<sup>80</sup> Because they replace the extracted

natural lens at practically the same location, there appear to be optical advantages to the intraocular lenses.<sup>81</sup> However, certain complications, such as corneal edema and macular edema, may appear<sup>81</sup> more frequently in patients who have received implants.

#### G4. Cataract Management in Infants and Children

Objectives: The absence of the crystallin lens in infants and children not only results in the same optical problems as in adults but presents the special problem of the development of amblyopia ("lazy eye").

- o To evaluate current techniques for cataract extraction and correction of aphakia and develop new approaches to these problems.
- o To study the medical and preventive therapies of cataract.
- o To determine the influence of aphakia on visual development in infants and children and devise therapies to successfully overcome the defect.
- o To establish standards and guidelines for toxicological studies of intraocular lens materials.

Overview of Current Research Support: In FY 1981 there is no grant supported in this area. For FY 1983 the recommendation is to add 2 grants in this area.

Recent Research Accomplishments: Amblyopia is especially important in children who develop cataracts before the age of eight. Unless optical correction is available soon after surgery, permanent visual loss and sensory deprivation may occur. However, in some cases intraocular lenses and contact lenses may not be adequate to prevent amblyopia. Innovative approaches to this problem are required.

#### G5. Cataract Prevention and Treatment

Objectives: Although claims have been made that various drugs and chemicals have a therapeutic effect on human cataract, no well-controlled, prospective clinical trials have as yet been reported.

- o To study the medical and preventive therapies of cataract.

Overview of Current Research Support: In FY 1981 there is no grant being supported in this area. For FY 1983 the recommendation is to have one grant in this area.

Recent Research Accomplishments: There are no data to prove that any medical treatment for senile cataract is efficacious. Nevertheless, multiple medical remedies, poorly studied, have been marketed to satisfy public demand. Years of basic cataract research have now identified

aldose reductase inhibitors, as potential agents in prevention of certain sugar cataracts.<sup>82,83</sup> The only effective treatment available for advanced cataract is surgery.

#### G6. Lens Accommodation

Objectives: Accommodation is the neurophysiologic process by which the eye automatically refocuses when the visual need changes from distance to near objects.

- o To study the medical and preventive therapies of cataract.

Overview of Current Research Support: In FY 1981 there are five grants being supported in this area. The recommendation for FY 1983 is to reduce the number of grants in this area by three, leaving two grants.

Recent Research Accomplishments: Accommodation is gradually lost with age (presbyopia or "old sight") requiring reading glasses or bifocals, and immediately lost after any cataract surgery. Considerable research<sup>84,85</sup> has been performed on measuring the resting point of accommodation. More work is needed to clarify the nature of the accommodative response. The interaction between the lens and the ciliary body and changes<sup>86</sup> associated with aging in regards accommodation are still unclear.

#### G7. Lens Induced Uveitis

Objectives: Lens proteins have been regarded as classical examples of those proteins which, because they are sequestered from immunocompetent cells early during fetal life, have escaped the normal induction immunological tolerance to self-antigens.

- o To study the medical and preventive therapies of cataract.

Overview of Current Research Support: In FY 1981 there are three grants being supported in this area. The recommendation for FY 1983 is to decrease by two the number of grants in this area so that there will only be one grant.

Recent Research Accomplishments: In man phacoantigenic uveitis has been<sup>87</sup> associated with an immune reaction against autologous lens antigen. However, other evidence indicates that immunological tolerance to lens antigens is, indeed, normally present. By radio-immunoassay investigators have demonstrated the presence of alpha and gamma crystallins in aqueous humor from human eyes with clear lenses and increased amounts in aqueous humor from eyes with cataracts.<sup>88</sup> It is thought that the small amounts of lens crystallins which presumably leak through the lens capsule may be the source of the antigens that maintain low-zone tolerance.

Low concentrations of lens antibodies have been demonstrated by complement fixation test in sera from 50% of healthy blood donors. Increased anti-lens titers have been demonstrated by passive hemagglutination in patients with<sup>92</sup> uveitis<sup>90,91</sup> and as a consequence of extracapsular cataract extraction.

## References

1. Vision Problems in the U.S. New York, National Society to Prevent Blindness, 1980.
2. Bloemendal H: The vertebrate eye lens. Science 197:127-138, 1977.
3. Zelenka P, Piatigorsky J: Isolation and in vitro translation of delta-crystallin mRNA from embryonic chick lens fibers. Proc Natl Acad Sci USA 71:1896-1900, 1974.
4. Bhat SP, Piatigorsky J: Molecular cloning and partial characterization of delta-crystallin cDNA sequences in a bacterial plasmid. Proc Natl Acad Sci USA 76:3299-3303, 1979.
5. Bhat SP, Jones RE, Sullivan MA, et al: Chicken lens crystallin DNA sequences show at least two delta-crystallin genes. Nature 284:234-238, 1980.
6. Rae JL: The electrophysiology of the crystalline lens, in Zadunaisky JA, Davson H (eds): Current Topics in Eye Research. New York, Academic Press, 1979, vol 1, pp 37-90.
7. Candia OA: The influence of calcium-free media on the electrical properties of the isolated toad lens. Exp Eye Res 30:193-201, 1980.
8. Hightower KR, Kinsey VE: Studies on the crystalline lens: XXIII. Electrogenic potential and cation transport. Exp Eye Res 24:587-593, 1977.
9. Paterson CA, Neville MC, Jenkins RM, et al: An electrogenic component of the potential differences in the rabbit lens. Biochim Biophys Acta 375:309-316, 1975.
10. Benedek G, Clark JI, Serrelach E, et al: Light scattering and reversible cataracts in the calf and human lenses. Philos Trans R Soc Lond (Biol) A293:329-340, 1979.
11. Clark JI, Benedek GB: Phase diagram for cell cytoplasm from the calf lens. Biochem Biophys Res Commun 95:482-489, 1980.
12. Ishimoto D, Sun ST, Nishio I, et al: Cytoplasmic phase separation in galactosemic cataracts. Proc Natl Acad Sci USA 76:4414-4416, 1979.
13. Bettelheim FA, Siew EL: Light scattering and lens morphology, in Srivastava SK (ed): Red Blood Cell and Lens Metabolism. North-Holland, NY, 1980, vol 9, pp 433-446.
14. Bettelheim FA, Paunovic M: Light scattering of normal human lens: I. Application of random density and orientation fluctuation theory. Biophys J 26:85-100, 1979.

Referemces Cont.

15. Harding JJ, Dilley KH: Structural proteins of the mammalian lens: A review with emphasis on changes in development, aging and cataract. Exp Eye Res 22:1-73, 1976.
16. Stauffer J, Rothschild C, Wandel T, et al: Transformation of alpha-crystallin polypeptide chains with aging. Invest Ophthalmol Vis Sci 13:151-153, 1974.
17. Siezen RJ, Bindels JG, Hoenders HJ: The interrelationship between monomeric, oligomeric and polymeric alphacrystallin in the calf lens nucleus. Exp Eye Res 28:551-567, 1979.
18. Anderson EI, Wright DD, Spector A: The state of sulfhydryl groups in normal and cataractous human lens proteins. II. Cortical and nuclear regions. Exp Eye Res 29:233-243, 1979.
19. Goosey JD, Zigler JS, Kinoshita, JH: Cross-linking of lens crystallins in a photodynamic system: A process mediated by singlet oxygen. Science 208:1278-1280, 1980.
20. Shinohara T, Piatigorsky J: Regulation of protein synthesis, intracellular electrolytes and cataract formation in vitro. Nature 270:406-411, 1977.
21. Piatigorsky J: Intracellular ions, protein metabolism, and cataract formation, in Zadunaisky JA, Davson H (eds): Current Topics in Eye Research. New York, Academic Press, 1980, vol 3, pp 1-39.
22. Piatigorsky 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.
23. Shinohara T, Piatigorsky J: Persistence of crystallin mRNAs with reduced translation in hereditary cataracts in mice. Science 210:914-916, 1980.
24. Rae JL: The electrophysiology of the crystalline lens, in Zadunaisky JA, Davson H (eds): Current Topics in Eye Research. New York, Academic Press, 1979, vol 1, pp 37-90.
25. Goodenough DA, Dick JSB II, Lyons JE: Lens metabolic cooperation: A study of mouse lens transport and permeability visualized with freeze-substitution autoradiography and electron microscope. J Cell Biol 86:576-590, 1980.
26. Rae JL, Stacey T: Lanthanum and procion yellow as extracellular markers in the crystalline lens of the rat. Exp Eye Res 28:1-21, 1979.
27. Farnsworth PN, Burke-Gadomski P, Kulyk T, et al: Surface ultrastructure of the epithelial cells of the mature human lens. Exp Eye Res 22:615-624, 1976.

References Cont.

28. McAvoy JW: Induction of the eye lens. Differentiation 17:137-149, 1980.
29. van der Starre H: Biochemical investigation of lens induction in vitro: II. Demonstration of the induction substance. Acta Morphol Neerl Scand 16: 109-120, 1978.
30. Khan HA, Leibowitz HM, Ganley JP, et al: The Framingham Eye Study: II. Association of ophthalmic pathology with variables previously measured in the Framingham Heart Study. Am J Epidemiol 106:33-41, 1977.
31. Hiller R, Giacometti L, Yuen K: Sunlight and cataract: An epidemiologic investigation. Am J Epidemiol 105:450-459, 1977.
32. Ederer F, Hiller R, Taylor HR: Senile lens changes and diabetes: Two population studies. Am J Ophthalmol, to be published.
33. Sperduto RD, Seigel D: Senile lens and senile macular changes in a population-based sample. Am J Ophthalmol 90:86-91, 1980.
34. Sperduto RD, Hiller R, Seigel D: Lens opacities and senile maculopathy. Arch Ophthalmol, to be published.
35. Sommer A: Epidemiology and Statistics for the Ophthalmologist. New York, Oxford University Press, 1980.
36. Alcala J, Valentine J, Maisel H: Human lens fiber cell plasma membranes: I. Isolation, polypeptide composition and changes associated with aging. Exp Eye Res 30:659-677, 1980.
37. Philipson B: Changes in the lens related to the reduction of transparency Exp Eye Res 16:29-39, 1973.
38. Garner MH, Spector A: Sulfur oxidation in selected human cortical cataracts and nuclear cataracts. Exp Eye Res 31:361-369, 1980.
39. Benedek GB: Theory of transparency of the eye. Appl Optics 10:459-473, 1971.
40. Tanaka T, Benedek GB: Observation of protein diffusivity in intact human and bovine lenses with application to cataract. Invest Ophthalmol Vis Sci 14:449-456, 1975.
41. Bettelheim FA: Syneresis and its possible role in cataractogenesis. Exp Eye Res 28:189-197, 1979.
42. Reddy V: Dynamics of transport systems in the eye. Invest Ophthalmol Vis Sci 18:1000-1018, 1979.
43. Augusteyn RC: On the possible role of glutathione in maintaining human lens protein sulfhydryls. Exp Eye Res 28:665-672, 1979.

## References Cont.

44. Zigman S: Eye lens color: Formation and function. Science 171:807, 1971.
45. Lerman S: Human ultraviolet radiation cataracts. Ophthalmic Res 12:303-314, 1980.
46. Kuwabara T: Aging changes in the lens epithelium, in Srivastava SK (ed): Red Blood Cell and Lens Metabolism. New York, Elsevier North-Holland, 1980, pp 41-44.
47. Tassin J, Malaise E, Courtois Y: Human lens cells have an in vitro proliferative capacity inversely proportional to the donor age. Exp Cell Res 123:388-392, 1979.
48. Eguchi G, Kodama R: A study of human senile cataract: Growth and differentiation of lens epithelial cells in in vitro cell culture. Ophthalmic Res 11:308-315, 1979.
49. Anderson EI, Wright DD, Spector A: The state of sulfhydryl groups in normal and cataractous human lens proteins: II. Cortical and nuclear regions. Exp Eye Res 29:233-244, 1979.
50. Harding CV, Chylack LT Jr, Susan SR, et al: Morphological changes in cataract: The ultrastructure of human lens opacities localized by Cooperative Cataract Research Group procedures, in Srivastava SK (ed): Red Blood Cell and Lens Metabolism. New York, North-Holland, 1980, pp 27-40.
51. Varma SD, Kinoshita JH: Sorbitol pathway in diabetic and galactosemic rat lens. Biochim Biophys Acta 338:632-640, 1974.
52. Jedziniak J, Kinoshita JH: Activators and inhibitors of lens aldose reductase. Invest Ophthalmol 10:357-366, 1971.
53. Kador PF, Zigler JS, Kinoshita JH: Alterations of lens protein synthesis in galactosemic rats. Invest Ophthalmol Vis Sci 18:696-702, 1979.
54. Varma S, Schocket SS, Richards RD: Implications of aldose reductase in cataracts in human diabetics. Invest Ophthalmol Vis Sci 18:237-241, 1979.
55. Fukui HN, Merola LO, Kinoshita JH: A possible cataractogenic factor in the Nakano mouse lens. Exp Eye Res 26:477-485, 1978.
56. Russell P, Fukui HN, Kinoshita JH: A Na-K ATPase Inhibitor from cultured lens cells, in Srivastava SK (ed): Red Blood Cells and Lens Metabolism. New York, North-Holland, 1980, pp 411-413.
57. Bunce GE: Nutrition and cataract. Nutr Rev 37:337-343, 1979.

References Cont.

58. Fraser GR, Friedman AL: The Causes of Blindness in Childhood. Baltimore, The Johns Hopkins Press, 1967.
59. Zwann J: Genetically determined lens abnormalities, in Srivastava, SK (ed) Red Blood Cell and Lens Metabolism. North-Holland, NY, vol 9, pp 415-422, 1980.
60. Streeten BW: The zonular insertion: A scanning electromicroscopic study. Invest Ophthalmol Vis Sci 16:364, 1977.
61. Ramsey MS, Yanoff M, Fine BS: The ocular histopathology of homocystinuria. Am J Ophthalmol 74:377-385, 1972.
62. Yanoff M, Fine BS: Ocular Pathology. Hagerstown, Harper & Row, 1975.
63. Buddecke E, Wollensak J: Zur biochemie der zonulafaser des rinderauges. Z Naturforsch 216:337-341, 1966.
64. Bellows JG: Cataract and Abnormalities of the Lens. New York, Grune & Stratton, 1975.
65. Lerman S: Radiant Energy and the Eye. New York, Macmillan, 1980.
66. Brown N: Slit-image photography. Trans Ophthalmol Soc UK 89:397-404, 1969.
67. Brown N: An advanced slit-image camera. Br J Ophthalmol 56:624, 1972.
68. Dragomirescu V, Hockwin O, Koch HR, et al: Development of a new equipment for rotating slit-image photography according to Scheimpflug's Principle, in (ed): Interdisciplinary Topics in Gerontology, Basel, S Karger, 1978, pp 118-130.
69. Lerman S, Hockwin O, Dragomirescu V: A new in vivo method for monitoring aging changes in the human ocular lens. Science, to be published.
70. Bellows JG: Cataract and Abnormalities of the Lens. New York, Grune & Stratton, 1975.
71. Grant MW: Toxicology of the Eye, ed 2. Springfield, Charles C Thomas Publisher, 1974.
72. Kuck JFR Jr: in Dikstein S (ed): Drugs and Ocular Tissues. Basel, S Karger, 1977, pp 433-523.

References Cont.

73. Troutman RC, Clahane AC, Emery JM, et al: Cataract survey of the cataract-phacoemulsification committee. Trans Am Acad Ophthalmol Otolaryngol 79:178, 1975.
74. Worthen DM, Boucher JA, Buxton JN, et al: Interim FDA report on intraocular lenses. Ophthalmology 87:267-271, 1980.
75. Kwitko ML, Preaeger DL: Pseudophakia: Current Concepts and Trends. Baltimore, Williams and Wilkins, 1980.
76. New Orleans Academy of Ophthalmology: Symposium on Cataracts. St Louis, CV Mosby Co, 1979.
77. Balazs EA, Hultsch E: Replacement of the vitreous with hyaluronic acid, collagen and other polymers, in Irvine A, O'Malley C (ed): Advances in Vitreous Surgery. Springfield, IL, Charles C Thomas, 1976, p 601.
78. Woods AC: The adjustment to aphakia. Am J Ophthalmol 55:1268, 1963
79. Berens C, Bannon R: Aniseikonia: A present appraisal and some practical considerations. Arch Ophthalmol 70:181-188, 1963.
80. Nordlohne ME: Intraocular Implant Lens. Baltimore, Williams and Wilkins, 1975.
81. Stark WJ, Karacher GP, Cowan CL, et al: Extended-wear contact lenses and intraocular lenses for aphakic correction. Am J Ophthalmol 88:535-542, 1979.
82. Varma S, Schocket SS, Richards RD: Implications of aldose reductase in cataracts in human diabetics. Invest Ophthalmol Vis Sci 18:237, 1979.
83. Reddy VN, Schwass D, Chakrapani B, et al: Biochemical changes associated with the development and reversal and galactose cataracts. Exp Eye Res 23:483, 1976.
84. Provine RR, Enoch JM: On voluntary ocular accommodation. Perception and Psychophysics 17:209-212, 1975.
85. Blank K, Enoch JM: Monocular spatial distortions induced by marked accommodation. Science 182:393-395, 1973.
86. Saladin J, Stark L: Presbyopia: New evidence from impedance cyclography support the Hess-Gullstrand theory. Vision Res 15:537-541, 1975.
87. Sandberg HO, Closs O: Lens crystallins and low zone tolerance, in Silvertein AM and O'Connor GR (ed): Immunology and Immunopathology of the Eye. (ed). New York, Masson Publishing USA, INC, 1979, pp 325-330.
88. Sandberg HO, Closs O: Radioimmunoassay of sheep alpha and gamma crystallins. I. Exp Eye Res 27:61, 1978.

References Cont.

89. Hackett E, Thompson A: Anti-lens antibody in human sera. Lancet 2:663, 1964.
90. Perkins ES, Wood RM: Auto-immunity in uveitis. Br J Ophthalmol 48:61, 1964.
91. Luntz MH: Anit-uveal and anti-lens antibodies in uveitis and their significance. Exp Eye Res 7:561, 1968.
92. Wirostko E, Spalter HF: Lens-induced uveitis. Arch Ophthalmol 78:1, 1967.

IV. GLAUCOMA PROGRAM

FISCAL YEAR 1981

<u>Subprogram</u>	<u>Number of Grants</u>
A. Aqueous Humor Inflow	[28]
1. Define fluid exchange, flow and transport in the eye; physiologic controls of intraocular pressure.	13
2. Describe processes governing fluid entry into the eye in ocular hypertension and hypotension, the various types of glaucoma hypotony.	1
3. Develop improved therapeutic measures and test them clinically	14
B. Aqueous Humor Outflow	[23]
1. Describe biomechanics of normal and abnormal outflow	4
2. Define biologic bases of normal and abnormal outflow	12
3. Improve outflow: new drugs, surgery, clinical trials	7
C. The Optic Nerve	[17]
1. Understand mechanisms of nerve damage and risk factors	8
2. Improve methods for recognizing and measuring nerve damage and loss of vision	9
D. Primary Open-Angle Glaucoma	[ 7]
1. Identify outflow abnormalities	1
2. Study epidemiology and natural history; improve monitoring methods	2
3. Evaluate therapy	4
E. Angle-Closure Glaucoma	[ 3]
1. Understand and classify pathologic mechanisms	0
2. Develop predictive and diagnostic criteria	2
3. Evaluate and improve therapy	1
F. Developmental, Congenital, or Infantile Glaucoma	[ 1]
1. Understand normal and pathological development of anterior chamber angle	0
2. Improve methods of clinical evaluation	0
3. Develop and evaluate classification system	1
G. Secondary Glaucomas	[8]
1. Study natural histories	2
2. Investigate pathogenic mechanisms	6
3. Develop models	0
4. Evaluate therapies	0
Total Grants	[87]

## INTRODUCTION

Glaucoma is one of the major causes of blindness in the United States, accounting for about 12% of the defined cases of blindness. There are about 67,000 persons blinded by these diseases, another 207,000 who suffer severe visual impairment due to glaucoma, and about 1.5 million persons are defined as having glaucoma. Estimates of the number of people with elevated intraocular pressure run from 5-10 million; what number of these people will develop clinical glaucoma and suffer loss of visual field, and to what extent therapy should be applied to them remain to be determined. It is not known why some individuals suffer glaucomatous nerve loss at "normal" pressures while others with elevated pressures never develop glaucoma. Estimates suggest that perhaps 10-20% of ocular hypertensives develop glaucoma in the ten years following detection of their condition. Conversely, a significant number of persons, perhaps as many as one-third of patients initially seen with disc and field changes characteristic of glaucoma, have "normal" intraocular pressures, the so-called "low tension glaucoma" group; about half of these persons later develop higher intraocular pressure.

The Planning Panel, in preparing Vision Research: A National Plan -- 1983-1987, has reorganized the format for presenting the recommendations for glaucoma research. Formerly, the program was subdivided into four sections: Etiology of each of the major classes of glaucoma; Optic Nerve and Vision Changes; Hydrodynamics of the Eye; and Medical and Surgical Treatments. The present plan will stress the major disease categories in seven sections: primary open-angle glaucoma, angle-closure glaucoma, developmental, congenital and infantile glaucomas, secondary glaucomas, and subjects common to all of them, aqueous humor inflow, outflow, and damage to the optic nerve. This shift reflects both a philosophic change to a more direct commitment to immediate disease research and a practical consideration for providing an improved means of managing the program.

The direction and content of the Glaucoma program's research portfolio has evolved considerably since the current plan was published in 1978, and this review provides a 2 1/2 year overview of the response to the plan. Thirty-nine new grants were funded in 1981, while 24 others were dropped from the program, yielding 87 glaucoma grants funded in FY 1981, and representing a 50% turnover in the content of the program in 1981. The quality of incoming grants was markedly improved over that of recent years, judged by a pronounced improvement in priority scores. The changing nature of the grant portfolio was reflected in subject matter (e.g. a substantial shift to research on the biology of the trabecular meshwork, encouraged by new techniques to maintain the cells in culture) and in an increasing emphasis on human and primate studies (e.g. in 1980, 50% of the studies had as major experimental subjects man or primates, while by 1981 the percentage had increased to 70%); also 60% of the new grants were awarded to clinicians.

## A. Aqueous Humor Inflow

Objectives: Adequate production and flow of aqueous humor is required to maintain normal intraocular pressure and balance outflow, and to provide nutrients to and remove metabolites from tissues of anterior segment of the eye. While the processes involved in aqueous humor production are still poorly understood, most current antiglaucoma drug therapy acts to reduce the rate of aqueous humor formation. In the case of hypotony, it would be desirable to have a means of increasing the amount of aqueous humor produced. Thus, for developing improved modes of therapy, it is essential to understand the processes controlling aqueous humor formation.

- o To understand fluid exchange, flow, and transport mechanisms within the eye as they relate to the determination of pressure.
- o To determine how the processes responsible for the entrance of fluid into the eye differ from normal in ocular hypertension and hypotension, in the various types of glaucoma, and in hypotony.
- o To develop improved therapeutic measures to alter the rate of inflow of fluid into the eye, and to test them in controlled clinical studies.

Future Needs: Twenty-eight grants are presently divided about equally among projects examining aspects of fluid flow and others attempting to understand the mechanisms of drug action and evaluate drug therapy.

The Panel has strongly emphasized that new studies on production of aqueous humor be directed to clinically relevant models, looking toward studies with subhuman primates and man once the basic information has been obtained from investigations in other species. Most drugs now used to treat glaucoma diminish inflow of aqueous humor, but their mechanisms of action are poorly understood. It is important, therefore, both to define how such drugs act and to carefully evaluate new drugs in controlled clinical trials.

Nine new grants are recommended in this subprogram which would be directed to studies of applications of new quantitative methods to study the physiology and pharmacology of aqueous inflow in man and primates (with emphasis on noninvasive techniques), to understanding neural-humoral control mechanisms and for conducting clinical studies and clinical trials with new drugs.

Recent Research Accomplishments: A new drug with therapeutic potential, vanadate, has been studied in animals and is now undergoing preliminary testing in man.<sup>1</sup> Vanadate is an ATPase inhibitor which reduces aqueous humor production. A number of investigators are defining how timolol acts to reduce intraocular pressure at both the cellular and clinical levels, and there is considerable interest in determining how it acts in conjunction with epinephrine.<sup>2,3,4</sup>

Dipivalylepinephrine, a prodrug of epinephrine, has been found to be safe and effective and, because it is used in lower concentrations than epinephrine, it causes fewer clinical problems. The therapeutic potential for use of cannabinoids in treating glaucoma is under active study; it now appears that delta-9-tetrahydrocannabinol is not very effective in lowering intraocular pressure when administered topically or orally, although it is effective for many persons who smoke it. An exciting finding is that an aqueous noncannabinoid extract of marijuana leaf is a very potent agent in suppressing aqueous humor production; whether it will ever have clinical use is not known, but it may prove to be a powerful research tool in analyzing how aqueous humor is formed. Cholera toxin, which both increases cyclic AMP production in ciliary processes and lowers intraocular pressure over a long term, is another new tool of potential research importance.

## B. Aqueous Humor Outflow

Objectives: The predominant cause of elevated intraocular pressure in all forms of glaucoma is increased resistance of the anterior chamber filtration area tissues to outflow of aqueous humor. In some of the types of secondary glaucoma, the blocking mechanisms have been demonstrated experimentally. Major interest in this area is devoted to determining the mechanisms, from the basic cellular biology of involved tissues to clinical observations, of the reduced rate of aqueous outflow in primary open-angle glaucoma.

- o To define the biomechanics of normal and abnormal aqueous humor outflow by noninvasive methods, by posterior (uveal) outflow pathways, and by studying effects of composition of aqueous humor.
- o To define the biologic bases of the normal and abnormal outflow systems, especially by investigating cell biology in tissue samples and tissue culture and the morphologic basis of outflow system.
- o To improve outflow in eyes with glaucoma by drugs and surgery.

Future Needs: Twenty-three FY 81 grants are predominantly concerned with studies of aqueous humor outflow, about half of them dedicated to projects defining the biologic bases of the outflow pathways. The panel, in recognizing recent advances, has indicated that, as with aqueous inflow, outflow studies should increasingly be conducted in subhuman primates and man. Of considerable importance to our understanding of the pathology of glaucoma and improving treatment is the development of new noninvasive means for measuring aqueous humor hydrodynamics and obtaining continuous measurements of intraocular pressure and outflow facility.

New methods of organ culture and tissue culture should enable investigators to study the factors which cause resistance to outflow--anatomic, biochemical, or mechanical--and to determine how drugs interact with the tissues to relieve elevated intraocular pressure. The availability of cultured trabecular

meshwork cells is enabling a growing number of investigators to look directly to the cell biology and drug interactions of the outflow area to determine the basis for major resistance to outflow. Filtration surgery for glaucoma often fails, as the wound heals; why this occurs in some patients should be determined. Laser surgery has yet to be critically evaluated, although it is coming into increasing use as a substitute for conventional surgery. Also, many parameters of laser treatment still require study to determine optimum use of this tool.

Eleven new grants are recommended for work to develop new noninvasive methods of measuring parameters of outflow, for defining posterior outflow pathways, to determine how aqueous humor composition affects outflow, and to develop improved means of therapy (including studies on the mechanisms of drug action, the failures of filtration surgery, and evaluation of laser treatment in clinical trials).

Recent Research Accomplishments: With the demonstration over the last few years that trabecular meshwork endothelial cells may be maintained and propagated in culture (for several passages, at least) it has become feasible to study the cell biology of that part of the aqueous humor outflow system. A number of investigators are now engaged in studying the structure and ultrastructure, biology, pharmacology, and biochemistry of these cells, and their findings should shed light on the properties of the cells and how their intra- and extracellular products may obstruct outflow. This will continue to be a very lively area of research. Both in vivo and in vitro experiments now indicate that the trabecular meshwork is actively affected by drug therapy, and that, indeed, the meshwork cells respond to adrenergic drugs used to reduce intraocular pressure.<sup>10</sup> More attention will be devoted to studies of the posterior (uveal; unconventional) outflow pathways. The addition of aqueous flow fluorophotometry to the methods presently used to measure aqueous humor hydrodynamics in the living human eye will enhance these studies.

Various types of laser therapy are being applied to the trabecular meshwork in substitution for conventional surgery to improve aqueous humor outflow.<sup>11,12</sup> Several aspects of this presently empirical mode of treatment should shortly come under active investigation in controlled clinical trials.

### C. The Optic Nerve

Objectives: Irreversible damage to the optic nerve is the eventual outcome of the glaucoma when intraocular pressure cannot be controlled. However, the pathogenesis of nerve fiber loss and resultant loss of vision is still under investigation. Two major schools of thought hold that either ischemic or mechanical damage are the basis of the nerve damage, and each has presented experimental evidence to bolster its theory. As yet, no opportunities seem to be available to study either restoration of nerve function or protection of the nerve by means other than reduction of intraocular pressure.

- o To understand mechanisms of optic nerve damage by conducting experimental studies in primary open-angle and low tension glaucomas, and by studying risk factors.
- o To improve methods for recognizing and quantitating optic nerve damage and loss of visual function, by developing new noninvasive methods, by developing and evaluating new psychophysical tests, and by evaluating automatic perimetry.

Future Needs: Seventeen grants are presently classified under this subprogram, eight studying either experimental pathogenesis of nerve damage or risk factors, and nine devoted to improving methods of quantitating optic nerve function or devising new tests.

The major needs in this subprogram are for better ways to evaluate the status of the optic nerve and to predict which eyes at risk require aggressive treatment to lower intraocular pressure. Appropriate therapy and effective preventative measures ultimately will depend upon a thorough understanding of the pathogenesis of optic nerve damage. The Panel believes that the time and technology are now ripe for development of new types of noninvasive measurements.

Six new grants are recommended in this area to identify mechanisms of damage in open-angle and low tension glaucoma, to determine risk factors for nerve damage, to evaluate methods for automatic perimetry, and to develop new noninvasive psychophysical measurements for detecting and evaluating the extent of damage to the optic nerve.

Recent Research Accomplishments: A number of investigators have shown experimentally that elevated intraocular pressure can produce changes typical of glaucoma in the optic nerve, and they are now attempting to delineate the mechanisms by which damage to the optic nerve may occur in susceptible people.<sup>13,14</sup> Procedures for evaluating the status of the nerve in man, such as mapping the topology of the optic nerve head, evaluating blood vessel status by fluorescein angiography, and automatic perimetry are coming into use in clinical practice.<sup>15-17</sup> The great need is predict damage and detect the earliest stages of visual field loss by noninvasive methods to determine which persons with ocular hypertension should be treated. Methods for evaluating optic nerve head status presently under investigation include stereophotogrammetry and stereochronoscopy, and systems are being devised for obtaining and recording visual field data for longitudinal comparisons. Research is also under way on new psychophysical methods which may detect early glaucomatous field loss, such as determining changes from normal of color vision or contrast sensitivity.

#### D. Primary Open-Angle Glaucoma

Objectives: Primary open-angle glaucoma accounts for an estimated 50-80% of all glaucoma. This disease is characterized by elevated intraocular pressure, nerve loss detected as particular changes in the optic disc and the visual

field, and anatomically open angles. In spite of the open angle, the major cause for elevated pressure is obstruction of the outflow of aqueous humor by increased resistance in the filtration system.

- o To identify outflow abnormalities, with emphasis on establishing a human donor program to obtain glaucomatous eyes, and to identify naturally occurring animal models.
- o To study epidemiology and natural history of open angle glaucoma and the low tension glaucomas, and to improve methods for monitoring hydrodynamics of aqueous humor.
- o To evaluate methods of treatment, especially for low tension glaucoma, to develop permanent therapeutic interventions, to conduct drug studies in clinical trials, and to study compliance.
- o To conduct a case-control study of reasons for loss of vision in glaucoma patients.

Future Needs: Seven grants are presently concerned primarily with clinical studies and one is defining an animal model.

A critical need for furthering our understanding of glaucoma is to have available for study eyes from patients at various stages of the disease, since mainly end-stage eyes are now obtained. The Panel strongly recommends that a registry and donor program be established to obtain and distribute appropriately preserved tissues. Low tension glaucoma, which is difficult to detect before vision is affected, may represent a significant number of patients; an increased level of effort should be devoted both to determining its etiology and improving methods of treatment. To improve glaucoma therapy, we need to know why patients with glaucoma lose vision--what are the roles of time of entry into therapy, compliance with therapy, iatric factors, etc.? A good care-control study should answer these questions.

It is recommended that this subprogram be expanded by six high priority grants which would establish a glaucoma eye donor program, study the natural history and epidemiology of low tension glaucoma, evaluate treatment methods, and conduct a case-control study of loss of vision by glaucoma patients.

Recent Research Accomplishment: Efforts to delineate clinically the modes of action of timolol and epinephrine (discussed in the FY 80 Annual Report) are continuing, but definitive conclusions are elusive. A number of clinicians have presented favorable data on the use of laser treatment of the trabecular meshwork to treat open-angle glaucoma. However, these short-term results should be verified by long-term controlled clinical trials. Encouraging new drug therapy studies and possibilities for new tests of optic nerve function are noted in Sections 2 and 3.

## E. Angle-Closure Glaucoma

Objectives: Angle-closure glaucoma is estimated to account for 10-20% of adult glaucoma. This condition results from contact of the iris with the filtration area of the angle of the anterior chamber, the trabecular meshwork. It is caused mainly by pupillary block in which the iris comes into contact with the lens and prevents the flow of aqueous humor from the posterior to the anterior chamber. Once the condition is recognized, surgery can relieve it; however, it is difficult to predict before an attack of angle-closure which eyes that appear to be anatomically susceptible to this disease will actually develop it.

- o To understand pathogenetic mechanisms and classify subtypes of angle-closure glaucoma.
- o To develop predictive and diagnostic criteria for angle-closure.
- o To improve clinical management of angle-closure glaucoma.

Future Needs: Three grants are currently funded in this subprogram. One is an experimental study of laser iridotomy. The second is a collaborative study of natural history and evaluation of provocative tests, in which two principal investigators and three collaborating investigators are participating.

Definition of classes of angle closure is necessary in order to develop optimal clinical strategies. A critical need is to be able to predict closure in a given eye so that rational prophylactic measures may be taken; thus new reliable tests must be developed. Many surgeons now use laser burns to create an iridotomy. Iridotomy should be compared to the standard iridectomy procedure in a controlled clinical trial. The time is ripe for such a study, before laser therapy (at the present level of knowledge about its effects and side effects) supplants conventional surgery.

The Panel has recommended as program expansion priorities two new grants dedicated to defining pathogenetic mechanisms of angle-closure and one grant for conducting a controlled clinical trial of laser iridotomy.

Recent Research Accomplishments: An alpha-adrenergic blocker, thymoxamine, may have clinical application in treating acute angle-closure glaucoma as well as in preventing inadvertent precipitation of closure in susceptible individuals following pupil dilation.<sup>18</sup> Argon laser iridotomy is becoming a method of choice over conventional iridectomy for many surgeons in treating angle-closure,<sup>19</sup> and it has been reported to be very effective in a recent clinical study.

## F. Developmental, Congenital, or Infantile Glaucoma

Objectives: Developmental, congenital, and infantile glaucomas are severe diseases which must be detected early and treated aggressively to prevent life-long blindness. About 5% of legal blindness in children is attributed to these diseases. The disease entities are presently poorly defined which, when combined with their low incidence, makes difficult studies of their differentiation and natural history, as well as evaluation of various methods of therapy. Clearly, we must learn enough about these diseases to allow successful interventions at the earliest times feasible to prevent permanent visual handicaps in the affected children.

- o To increase our knowledge of the normal and pathologic development of the anterior chamber angle and aqueous humor drainage system.
- o To devise improved means of clinically evaluating glaucoma in children.
- o To classify glaucomas of infants and evaluate means of treating them.

Future Needs: One grant is presently funded in this subprogram, which is being used to develop a system for classifying these diseases.

Research on these serious types of glaucoma has been hampered both by their low incidence and the lack of common definitions which hinders analysis of data obtained by various investigators. Data are lacking for prevalence, natural history and therapy. Evaluation of aqueous humor dynamics in children often requires anesthesia, which itself can bias measurements; also noncontact methods of evaluation are needed. The Panel strongly recommends that a registry be organized and that it be part of one of three regional centers cooperating on research into understanding and treating glaucoma in infants and children.

## G. Secondary Glaucomas

Objectives: Secondary glaucomas, which result from a number of systemic and ocular diseases, all ultimately impede the outflow of aqueous humor. Certain of them are very difficult to manage and lead to a larger proportion of blindness than the far more prevalent primary open-angle glaucoma. The most severe secondary disease is neovascular glaucoma which is often a consequence of ischemic retinal vascular disease. Glaucoma following ocular inflammation is another important class of secondary glaucoma. Among other diseases in this category are glaucoma associated with pigment dispersion, exfoliation, trauma, lens disorders, hemorrhage, and certain degenerative disorders such as essential iris atrophy. While overall these diseases cause a disproportionate amount of blindness due to glaucoma, some secondary glaucomas are quite rare, which impedes improving our understanding of them.

- o To study the natural history of secondary glaucomas, especially neovascular glaucoma, inflammatory glaucoma, and the low frequency glaucomas.
- o To study the pathogenic mechanisms of secondary glaucomas.

- o To develop animal models of secondary glaucomas.
- o To test therapeutic modalities for treating secondary glaucomas.

Future Needs: Eight presently funded grants fit into this subprogram, two of which are natural history studies, and six of which are studying pathogenetic mechanisms (including four basic studies related to anterior chamber inflammation).

The major research need in this area is to gain better understanding of the pathogenesis and management of neovascular glaucoma, a devastating disease. Some of the low frequency types of glaucoma are poorly understood and therapeutic methods need to be compared and evaluated. To assure that sufficient numbers of patients with these disease may be seen and their diseases studied thoroughly, the Panel has recommended that a cooperative study among several centers be initiated.

Recommendations for program expansion are that six new grants be funded for studies on the pathogenesis and natural history of neovascular glaucoma, the natural histories of the rare glaucomas, and the treatment of inflammatory glaucoma and neovascular glaucoma.

Recent Research Accomplishments: Recent studies indicate that the incidence of neovascular glaucoma may be diminished as a consequence of panretinal photo-coagulation being administered to <sup>20</sup> eyes of patients with diabetic retinopathy or other ischemic retinal diseases. A small number of cases of neovascular glaucoma have had at least short-term benefit following <sup>21</sup> implantation of a valve permitting maintenance of lowered intraocular pressure. The previously designated "hemolytic" glaucoma, which follows intraocular hemorrhage, has been shown experimentally to be caused by rigid <sup>22</sup> erythrocytes (disintegrating ghost cells) clogging the trabecular meshwork. A recent finding, similar in nature, is that sickled red blood cells, also because of their rigidity, may fail to filter through the trabecular meshwork and thus obstruct aqueous outflow. <sup>23</sup> Since Blacks are especially prone to both sickle cell anemia and optic nerve damage, these observations should stimulate new research on therapeutic means of lysing the cells or ghosts trapped in the meshwork. Observations and experimental work have shown that pigmentary glaucoma is associated with pigment rubbed off the back of iris by the zonules as the pupil changes in diameter. <sup>24</sup> Finally, experiments have demonstrated that outflow <sup>25</sup> obstruction in phacolytic glaucoma can be caused by aggregated lens proteins.

## References

1. Krupin T, Becker B, Podos SM: Topical Vanadate lowers intraocular pressure in rabbits. Invest Ophthalmol Vis Sci 19:1360-1363, 1981.
2. Higgins RG, Brubaker RF: Acute effect of epinephrine on aqueous humor formation in the timolol-treated eye as measured by fluorophotometry. Invest Ophthalmol Vis Sci 19:420-423, 1980.
3. Yablonski M, Schenker H, Podos S: A fluorophotometric study of the effect on aqueous flow of topical epinephrine and timolol. Proc Int Soc Eye Res 1:77, 1980.
4. Thomas JV, Epstein DL: Timolol and epinephrine in primary open angle glaucoma. Arch Ophthalmol 99:91-95, 1981.
5. Kohn AN, Moss AN, Hargett NA, et al: Clinical comparison of dipivalyl epinephrine and epinephrine in the treatment of glaucoma. Am J Ophthalmol 87:196, 1979.
6. Green K, Zalkow LH, Deutch HM, et al: Ocular and systemic responses to water soluble material derived from cannabis sativa. Invest Ophthalmol Vis Sci 20(suppl):103, 1981.
7. Gregory DS, Mishima H, Stjernschantz J, et al: How does cholera toxin reduce intraocular pressure? Invest Ophthalmol Vis Sci 19(Suppl): 65, 1980.
8. Bartels SP, Neufeld AH: Intravitreal cholera toxin causes a decrease in intraocular pressure. Proc Int Soc Eye Res 1:78, 1980.
9. Polansky J, Weinreb R, Baxter J, et al: Human trabecular cells: I. Establishment in tissue culture and growth characteristics. Invest Ophthalmol Vis Sci 18:1043-1049, 1979.
10. Kaufman P, Barany EH: Adrenergic drug effects on outflow facility following ciliary muscle retrodisplacement in the cynomolgous monkey. Invest Ophthalmol Vis Sci, in press, 1981.
11. Wise JB: Long-term control of adult open angle glaucoma by argon laser treatment. Ophthalmology 88:197-202, 1981.
12. Wilensky JT, Jampel LM: Laser therapy for open-angle glaucoma. Ophthalmology 88:213-217, 1981.
13. Quigley HA, Addicks EM: Chronic experimental glaucoma in primates: II. Effect of extended intraocular pressure elevation on optic nerve head and axonal transport. Invest Ophthalmol Vis Sci 19:137, 1980.

References Cont.

14. Gaasterland D, Tanisihma T, Kuwabara T: Axoplasmic flow during chronic experimental glaucoma: Light and electron microscopic studies of the monkey optic nerve during development of glaucomatous cupping. Invest Ophthalmol Vis Sci 17:838, 1978.
15. Adam G, Schwartz B: Increased fluorescein filling defects in the wall of the optic disc cup in glaucoma. Arch Ophthalmol 98:1590, 1980.
16. Johnson CA, Keltner, JL: Comparative evaluation of the Autofield-I, CFA 120, and Fieldmaster model 101-PR automated perimeters. Ophthalmology 87:777, 1980.
17. Heijl A, Drance SM, Douglas GR: Automatic perimetry (Competer): Ability to detect early glaucomatous field defects. Arch Ophthalmol 98:1560, 1980.
18. Wand M, Grant WM: Thymoxamine hydrochloride: an alpha-adenergetic blocker. Surv Ophthalmol 25:75-84, 1980.
19. Pollack, IP: Use of argon laser surgery to produce iridotomies. Trans Am Ophthalmol Soc 77:674-706, 1979.
20. Wand M, Dueker DK, Aiello LM, Grant WM: Effects of panretinal photocoagulation on rubeosis iridis, angle neovascularization and neovascular glaucoma. Am J Ophthalmol 86:332-340, 1978.
21. Krupin T, Kaufman P, Mandell A, et al: Filtering valve implant surgery for eyes with neovascular glaucoma. Am J Ophthalmol 89:338-343, 1980.
22. Campbell DG, Essingmann EM: Hemolytic ghost cell glaucoma. Arch Ophthalmol 97:2141-2146, 1979.
23. Goldberg MF, Tso MOM: Sickled erythrocytes, hyphema, and secondary glaucoma. Ophthalmic Surg 10:89, 1979.
24. Campbell DG: Pigmentary dispersion and glaucoma: A new theory. Arch Ophthalmol 97:1667-1672, 1979.
25. Epstein DL, Jedziniak JA, Grant WM: Identification of heavy molecular weight soluble lens protein in aqueous humor in phakolytic glaucoma. Invest Ophthalmol Vis Sci 17:398-402, 1978.

V. STRABISMUS, AMBLYOPIA, AND VISUAL PROCESSING PROGRAM  
FISCAL YEAR 1981

<u>Subprogram</u>	<u>Number of Grants</u>
A. Visual Processing and Amblyopia	[185]
1. Normal and Abnormal Development	
a. Molecular Studies	7
b. Cell and Systems Research	42
c. Behavioral Research	8
2. Structure and Function	
a. Molecular Studies	8
b. Cell and Systems Research	61
c. Behavioral Studies	44
3. Disorders	
a. Amblyopia	13
b. Sensory Neuro-ophthalmic Disorders	2
B. Ocular Motility and Strabismus	[56]
1. Normal and Abnormal Development	3
2. Structure and Function	
a. Conjugate Eye Movements	38
b. Vergence and Accommodation	2
c. Muscle Physiology	1
3. Disorders	
a. Strabismus	7
b. Neuro-ophthalmology	5
C. Optics and Refractive Errors, Including Myopia	[3]
D. Rehabilitation	[2]
1. Low Vision	1
2. Blindness	1
	<hr/>
	Total
	246

## INTRODUCTION

Seeing involves a series of highly complex events that begins when images fall onto our retina and continues until we perceive objects in all their details, depth, and color. These events include: searching and scanning eye movements for orientation and general information gathering; convergence and focusing of the eyes to refine images of interest; and neurosensory processing of the visual stimulus. A disturbance of any part of this elaborate and precise system can lead to serious visual disturbances such as amblyopia, field defects, strabismus, nystagmus, myopia, or other high refractive errors. These disorders affect over 10 percent of the population and therefore constitute a serious problem from a public health point of view.

Research in the Strabismus, Amblyopia, and Visual Processing program has been subdivided into three major categories. First is the study of the processing of visual information by central pathways, including investigation of the structure, function, and development of these pathways in the normal individual and the disorders resulting from abnormal development, such as amblyopia. The second category is the study of ocular motility and disturbances of eye movement, such as strabismus. The third category deals with refractive errors, such as myopia, and includes the role of central and peripheral structures in their etiology.

## A. Visual Processing and Amblyopia

### A.1. Normal and Abnormal Development

Objectives: This subprogram includes research on the normal and abnormal growth and development of the visual nervous system. Research approaches include: Molecular Studies (subsection A.1.a.); Cell and Systems Research (subsection A.1.b.); and Behavioral Studies (subsection A.1.c.). The overall objectives, which include all three subsections, are to develop, evaluate, and apply new concepts and facts concerning the developing visual system. Specific subprogram objectives are:

- o To describe events in the normal development of visual system.
- o To elucidate the environmental and genetic controls of these events.
- o To understand the mechanisms underlying aberrations in development.
- o To develop the basic scientific groundwork for treatment of amblyopia.
- o To understand why mammalian optic nerves and tracts fail to regenerate effectively, preventing restoration of function.

Recent Research Accomplishments: Most of the Molecular Studies (subsection A.1.a.) deal with axonal transport, neuronal specificity, or synaptic transmission. Some workers are investigating the nature of molecules transported in pathways of normal infants of a variety of species and in infants deprived of normal visual stimulation in order to identify substances that may be involved in establishing and maintaining neuronal connections. Cell surface markers are being examined, particularly in the retinotectal system, to determine the proper connections in developing systems. Other studies utilize two-dimensional gel electrophoresis to identify changes in the population of protein molecules during normal development of reorganization of existing pathways. A very interesting result of one project is the finding that synaptic plasticity in the geniculostriate<sub>1</sub> pathway of cats is influenced by local levels of catecholamines. Other more recent studies show that the regeneration of optic nerve<sub>2</sub> axons in fish and amphibians can be accelerated by nerve growth factor.

These results, if applicable to humans, may mean that it will soon be possible pharmacologically to aid the regeneration of mammalian optic axons.

Cellular and Systems Research (subsection A.1.b.) concerns the differentiation of neurons and glia (including axonal growth and termination, synaptogenesis, axonal sprouting, and regeneration) and neuronal

connectivity (particularly the specificity of connections and their alterations under various conditions). The development of new techniques to manipulate embryonic animals has facilitated studies on the development of visual neural systems.

Recent studies show that the ultimate pattern of innervation of a structure is more restricted in mature animals than that seen early in development. This is demonstrated in the segregation of individual geniculate afferents<sub>3</sub> into a patchy terminal distribution from a more diffuse arbor. In a similar way, the postnatal development of interhemispheric connections through the corpus callosum proceeds from diffuse connectedness initially to sharper localization, to some extent dependent on experience.<sup>4,5</sup>

Studies with retinotectal pathway are modifying many of our concepts on the formation of neuronal connections. Previous suggestions of a rigid cell-to-cell specificity have been replaced by the notion of a modifiable set of connections which changes continually in the growing animal and may change<sub>3</sub> rapidly when either the retina or tectum is surgically altered.

Behavioral Research (subsection A.1.c.) focuses mainly on visual psychophysics with human infants. Reliable data on normal development of visual functions in the human infant have been obtainable only in the last few years. Studies on the development of visual and stereoscopic acuity in infants are now being extended to examine the development of contrast sensitivity and color vision. Studies with animals have given rise to the important concept of a "critical period," in which certain aspects of the ability of the infant visual system to analyze the objective world can be refined and modified by visual experience.

Future Needs: Overall, the research subprogram on Normal and Abnormal Development in Visual Processing and Amblyopia is very strong, as shown by the high general interest, the high quality of the research that includes both developmental biology and neuroscience, and the fairly direct relevance of the research to amblyopia. Priorities that have been identified for future research include: studies on the development of the visual systems of human infants by noninvasive methods; pre- and postnatal development of the visual system at the molecular, cellular, and behavioral level; studies of the effects of visual deprivation or abnormal stimulation at the molecular, cellular, and behavioral levels; and the search for factors important in the regeneration of the optic nerve and other visual pathways.

## A.2. Structure and Function

Objectives: Research in this subprogram, which includes Molecular Studies, Cell and Systems Research, and Behavioral Studies, is directed toward understanding the visual system and how it functions, both in normal individuals

and in the pathological conditions underlying amblyopia and other central visual disorders. Some specific objectives are:

- o To understand how visual information is encoded by single neurons or sets of neurons; and learning how neurons or neuronal sets are interconnected.
- o To elucidate the mechanisms for visual encoding via molecular, structural and electrophysiological studies.
- o To study the function of individual neurons or sets of neurons in the visual process.
- o To describe human visual processes in normal and dysfunctional states and improving specific diagnostic visual tests.

Recent Research Accomplishments: Identification and localization of neurotransmitters continues to be an area of very active research Molecular Studies (subsection A.2.a.). The transmitter that has perhaps been most clearly demonstrated in the visual cortex is gamma amino butyric acid (GABA). Studies show that GABA may be associated with smooth stellate cells, thought to be responsible for mediating inhibition in the cortex, and it may also function as an inhibitor in visual cortical cells.

The abundance of gut peptides and their receptors in mammalian brain suggests that these peptides could serve as long-term modulatory signals and may be involved in visual processing. Cholecystokinin and vasoactive intestinal polypeptide are both found in small neurons, possibly smooth stellate cells. Neurotensin and somatostatin are also found in the cortex, though in lesser quantities.

In Cell and Systems Research (subsection A.2.b.), new biochemical methods for identifying and characterizing cell types within the visual system are a powerful extension of standard morphological techniques. Monoclonal antibodies promise to lead us to new discoveries in the identification of cell subclasses. This technique has been used to study retinal development and may prove useful for the functional classification of cellular subpopulations in other parts of the visual system. Tritiated amino acids like <sup>3</sup>H proline and <sup>3</sup>H leucine, horseradish peroxidase, and acetylcholinesterase histochemistry can be used to identify neuronal pathways and synapses. Fluorescence histochemistry is an especially sensitive method for identifying the origin and distribution of neuronal terminals.

Deoxyglucose has been used as a metabolic marker to label cells in the lateral geniculate after visual stimulation. Alpha-bungarotoxin, a neurotoxin, can bind to tectal postsynaptic receptors which receive retinal input. By conjugating alpha-bungarotoxin or plant lectins

like concanavalin to fluorescent probes, it becomes possible to visualize receptors for neurotransmitters and to examine the distribution of surface carbohydrates on developing axons and dendrites.

Behavioral Studies (subsection A.2.c.) have historically provided one of the most important approaches to the study of vision; these studies provide data on the capabilities of the human visual system and thus furnish the benchmark against which other approaches must be measured. They also enable one to define normal vision and to describe and quantify the effect of various diseases on visual performance. Recent developments in psychophysics permit investigators to perform functional evaluations at the quantitative, as well as qualitative, level. We now know that visual information is transmitted separately by chromatic and nonchromatic channels that initiate in the retina but elaborate more centrally. Psychophysical studies in stereopsis and perception of movement continue to yield significant new information.

Future Needs: Research supported by the Visual Processing and Amblyopia - Structure and Function subprogram attracts some of the finest neurobiologists in the world and benefits from a remarkable array of powerful new methods. Results have included a great deal of new knowledge about classically recognized visual pathways, the definition of new pathways, and studies on many important parts of the visual system that remain to be explored in significant detail. Priorities for future research include: identification of neurotransmitters, peptides, and other chemicals important in signaling in visual pathways and in cell specificity and function; studies on the structural and functional specialization of cells in the visual pathways; elucidation of the roles of multiple afferent and of efferent pathways in visual processing; studies on the extrastriate cortical areas having visual representation; determination of the relationship of single cell activity to behavioral responses; evaluation of the "channel hypothesis" for human visual processing; the relationship of psychophysical data to behavioral and physiological findings; further delineation of normal and dysfunctional visual processes in humans and methods for studying these processes.

### A.3. Disorders

Objectives: Disorders of visual processing are among the most frequent causes of visual disability in our population. This category includes amblyopia, which may be considered a reduction in monocular vision, and disorders of binocular visual function, which include sensory adaptation to misalignment of the visual axes and impairment of stereoscopic depth perception.

Because disorders of visual processing appear to be at least in large part acquired and environmentally determined, they may be preventable or curable if detection and appropriate intervention occurs early enough.

Many neuro-ophthalmic sensory disorders are temporarily or permanently disabling, but the impact of these diseases on the health of the public is largely unknown since little descriptive epidemiology is available, and often this deals with only a few defined populations. The importance of neuro-ophthalmic disorders goes beyond their impact on patients or populations. Systems analysis applied to such "experiments of nature" continues to offer opportunities for investigators to learn about the structure and function of the human brain.

Specific objectives of the subprogram include:

- o To understand basic mechanisms underlying amblyopia and other neurogenic sensory disorders through (1) elucidation of physiological alterations, and (2) better characterization of the visual deficit in humans and appropriate animal models.
- o To determine the natural history of amblyopia and other sensory disorders and the prognosis for their successful treatment with varying degrees of impairment, age of the patient, and duration of the condition.
- o To develop and evaluate techniques for the diagnosis of sensory disorders and optic neuropathies at early ages.
- o To develop new treatment modalities for amblyopia and other sensory disorders that (1) are effective in older children and adults, and (2) do not risk creating a visual deficiency in the uninvolved eye or interfering with the development of normal binocularity.

Recent Research Accomplishments: The observation of physiologic abnormalities in retinal ganglion cells of strabismic cats supports the view that partial form vision deprivation as well as abnormal binocular interaction contributes to strabismic amblyopia. The likelihood of retinal abnormalities in human amblyopia is also supported by a number of psychophysical and electrophysiological studies.

Visual evoked potential and forced-choice preferential-looking techniques are promising means for diagnosing amblyopia in infants and for following the course of therapy; variations in contrast sensitivity function and in electrophysiological properties suggest a possible basis for predicting the outcome of treatment for amblyopia.

Recent research has assisted physicians in recognizing retinal changes associated with optic atrophy and has helped to clarify the anatomical basis for changes seen ophthalmoscopically. By use of red-free light and ophthalmoscopic and photographic techniques, it has been possible to detect and analyze changes in the thickness

of the retina due to drop-out of retinal nerve fibers resulting from lesions in the anterior visual pathway. This has proven to be a powerful tool for detecting and localizing lesions and for distinguishing between normal and atrophic optic nerves.

Future Needs: At present, thirteen research grants are being funded in amblyopic disorders and only two in neuro-ophthalmological disorders. Both of these very important areas have been targeted for major expansion by the Strabismus, Amblyopia, and Visual Processing Panel. Research is particularly needed for: developing methods for large-scale screening, evaluation, and monitoring of very young children for amblyopia and other sensory disorders; a better understanding of the nature and types of amblyopia and their associated histopathology; new treatment regimens for amblyopia, optic neuritis, ischemic optic neuropathy, and amaurosis fugax; developing animal models of optic neuropathies for electrophysiological and psychophysiological studies and for elucidation of the underlying factors in these disorders.

## B. Ocular Motility and Strabismus

### B.1. Normal and Abnormal Development

Objectives: Congenital disorders of the oculomotor system, which often handicap an individual for his entire life, are occurring with increased incidence due to the high percentage of premature and "small-for-dates" infants that now survive as the result of improved neonatal care. Disorders of the oculomotor system are especially common in this group of infants. Thus, understanding of the normal oculomotor system and of disorders that affect it is more important than ever before.

Specific subprogram objectives include:

- o To define the anatomic and physiologic maturation patterns of subsystems involved in the oculomotor system.
- o To assess the long-term oculomotor consequences of raising infants in various visual environments.
- o To delineate the various adaptive oculomotor strategies utilized by children to overcome congenital deficits such as congenital hemianopsia and other conditions associated with significant visual loss.

Recent Research Accomplishments: Preliminary studies of the maturation of the vestibulo-ocular system in human neonates indicates that this system matures at a much earlier period than other oculomotor systems<sup>8-10</sup> but that its maturation may be significantly

delayed in premature or "small-for-gestational dates" infants. It is not yet known whether these maturation delays result in long-term disorders of ocular vestibular function, but preliminary data suggest that specific patterns of maturation delay may be associated with some forms of horizontal comitant strabismus.<sup>11</sup>

Other research reveals that the extraocular muscles may originate at the corneal-scleral limbus as relatively undifferentiated mesodermal tissue. The orderly arrangement of these muscles is not complete at birth, and the location of the normal muscle insertion in relation to the limbus continues to change in the first several months of life.<sup>12</sup>

Future Needs: In FY 1981, only three research grants were supported by the Ocular Motility and Strabismus - Normal and Abnormal Development subprogram. The Strabismus, Amblyopia, and Visual Processing Panel has recommended that the number of research grants be increased and that the following priority areas receive particular emphasis: studies on the effects of early experience on development of strabismus, refractive error change, or loss of stereoacuity; development of quantitative recording techniques for studying eye movements in neonates and small children; additional studies on temporary neonatal strabismus and normal establishment of fusion; evaluation of adaptive oculomotor plasticity in human infants and animals with respect to disorders of the visual and oculomotor systems, including the cerebellum; molecular, cellular, and behavioral research on the development of oculomotor system, including that of extraocular muscles and associated cranial nerves, sensory inputs, and the cerebellum.

## B.2. Structure and Function

### B.2.a. Conjugate Eye Movements

Objectives: This subsection includes research on all aspects of the neural system that can produce and/or control conjugate eye movements. Specific subsection objectives are:

- o To classify and describe quantitatively all types of normal eye movements in humans and in animals.
- o To determine the role of neural circuits in conjugate eye movements and the mechanisms by which they generate normal or abnormal eye movements.
- o To identify and characterize all oculomotor subsystems and determining the interactions between them.

Nine of the current research projects are devoted to the more accurate description of eye movements, to determining the stimuli that produce such eye movements, or to examining the influence of eye movements on perception. Most of the remaining projects involve neuroanatomy or neurophysiology of systems related to oculomotor response.

Recent Research Accomplishments: Implantation of electrodes in various locations for antidromic and orthodromic stimulation in alert animals has permitted the elucidation of some of the anatomical connections of the neurons in oculomotor response. The use of complex training paradigms has enabled the exploration of cell behavior during a variety of visual and vestibular stimuli and alterations of mental set that expand the dimensionality of the behavior studies to more realistic levels. These procedures, in conjunction with greatly improved anatomic tracer techniques, have identified a number of structures as being important in oculomotor control. Some such structures are the vestibular nuclei, the nucleus prepositus hypoglossi, the flocculi, and many others.

The importance of studying neural transmitters in the oculomotor system has been dramatically emphasized by the recent discovery<sup>13</sup> that the drug baclofen, a synthetic analogue of GABA, stops periodic alternating nystagmus, a disorder in which jerk nystagmus to the left is cyclically replaced by nystagmus to the right about once every two minutes. This disorder produces illusory movement of the environment and degrades vision to the point that reading is impossible. Baclofen stops these oscillations and permits normal visual function.

Future Needs: Research in Conjugate Eye Movements should continue to emphasize the following areas: neuroanatomy, neurophysiology, and function of neural circuits involved in all types of eye movements; sensorimotor plasticity in the oculomotor system as well as the relevant neural mechanisms and cerebellar influences; identification of neurotransmitters in the oculomotor system; and pharmacological studies with drugs that affect the oculomotor subsystems.

#### B.2.b. Vergence and Accommodation

Objectives: Persons with normal binocular vision respond to near objects of interest by converging their eyes (to position images on the two foveas) and by increasing accommodation of the crystalline lens (to provide sharp retinal images). However, some persons with strabismus cannot achieve convergence and accommodation simultaneously, but can accomplish only one at the expense of the other, creating either singleness with blurred vision or clear but double vision.

Although there are known genetic precursors, the etiology of strabismus is unknown. As we learn how the neonate develops accommodation and how the child chooses clear or double vision, we will be better prepared to understand the genesis of strabismus and how to prevent and treat it.

The following objectives are designed to enhance our understanding of normal vergence and accommodation as well as abnormalities that may result in these functions:

- o To identify the stimuli (both visual and nonvisual) that produce specific responses of vergence and accommodation.
- o To extend what is known about the response characteristics of vergence and accommodation for the stationary head and body to situations where both are in motion.
- o To explore the interactions between vergence and accommodation, especially in terms of the effects of plasticity in normal development and aging.
- o To use animal research to elucidate the underlying neurophysiology of accommodation and vergence.

Recent Research Accomplishments: Objective methods such as preferential looking and visual evoked potentials have been used to assess development of acuity and contrast sensitivity of human infants during the first six months of life. Photorefraction, a relatively new technique that allows measurement of the accuracy of accommodation of human infants, shows that many newborn and one-month-old infants exhibit inconsistent fluctuations of accommodation over the full range and that accurate accommodation for most distances does not occur until about three months.<sup>14</sup>

Relaxation of accommodation has recently been reported<sup>15</sup> for a patient whose myopia was reduced while using feedback. These results, plus recent findings showing that the eccentric and unsteady fixation of amblyopic eyes<sup>16</sup> and the large and rapid ocular oscillations of persons with congenital nystagmus<sup>17</sup> can be improved by providing auditory feedback of eye position information, suggest that auditory or other feedback cues might be useful in extending the amplitude of accommodation and in delaying the onset of presbyopia.

Future Needs: Only two ongoing research grants deal with accommodation and convergence, along with development of instrumentation for binocular image stabilization. In view of the fact that deficits in the vergence system affect a significant portion of the general population, research in this subprogram needs to undergo considerable expansion. Specific research needs include: definition of accommodation and vergence stimuli for the neonate and characterization of the development of the associated motor responses; elucidation of accommodation/vergence relationships in neonates and determination of the critical period for influencing this synkinesis, both in normal development and in neonates with predilection for strabismus; definition of mechanisms responsible for plasticity (or rigidity) in

normal and strabismic individuals; and studies of the innervation of the ciliary muscle with respect to pathways, synaptic mechanisms, and possible transneuronal trophic influences.

### B.3. Disorders

#### B.3.a. Strabismus

Objectives: Strabismus, which is a misalignment of the two eyes, is a major problem. An estimated 38 million, or 19 percent of the civilian, noninstitutionalized population, ages 1-74, in the United States have a manifest or latent eye muscle imbalance condition.

Sixty-eight thousand strabismus operations are performed yearly, making surgery for this condition the second most common form of ophthalmic surgery. Prime objectives of the Strabismus Disorders subsection include:

- o To improve methods for detection, measurement, and evaluation of strabismus.
- o To improve methods, both surgical and nonsurgical, for the treatment of strabismus.
- o To investigate posttreatment strategies that create and promote the stability of the therapeutic result.

Of the projects currently supported by the Strabismus subsection one deals with normal cooperation between the fixing and the deviating eye and the phenomenon of prism compensation and addresses the issue of visual information processing in normal and strabismic subjects. Another investigates abnormal sensory aspects of binocular vision, including reduced stereopsis, in order to clarify factors contributing to reduced visual acuity, amblyopia and abnormal fusional vergence. Another seeks to develop and evaluate pharmacological means of correcting strabismus. Other investigations concern clinical implications of vergence anomaly, the interocular transfer of after-effects in strabismus, depolarizing nicotinic antagonists and eye positions, or clinical recording of human saccades to aid in diagnosing certain disease states.

Recent Research Accomplishments: A strain of monkey has been found with infantile strabismus which is somewhat comparable to that in humans. This provides us with a model upon which to study not only the etiology of strabismus but also nonsurgical and surgical treatment of the condition.<sup>18</sup>

In studies to develop nonsurgical alternatives for treating strabismus, botulinum toxin has been used to produce selective paralysis of an extraocular muscle. This toxin has been injected under electromyographic control into the antagonist of a paralyzed

extraocular muscle of human patients with severe strabismus to temporarily diminish or eliminate the deviation and to prevent contracture of this antagonist muscle.<sup>19,20</sup> This procedure appears to be a practical alternative to surgical correction.

Studies on the use of succinylcholine during surgery as an assist in recreating the pre-anesthetic strabismic deviation have provided some insights into the mechanism of muscle innervation and may eventually prove useful in determining the appropriate amount of strabismus surgery.<sup>21</sup>

Future Needs: A problem in human clinical strabismus management is that various authorities and medical centers tend to follow their own individual approaches, using past experience as a primary guide to the proper therapy. This leads to individual reports of the success or lack thereof for an individual treatment modality, based on experience with a limited number of patients treated and evaluated in a relatively noncontrolled manner. Multicenter studies and/or conferences on management techniques in strabismus could be most effective in setting up and evaluating treatment protocols.

Research in strabismic disorders needs to be increased greatly, as reflected by the recommendations of the Strabismus, Amblyopia, and Visual Processing Panel. Priority areas for additional research efforts include: surgical, pharmacological and other approaches for the treatment of strabismus; the role of sensory stimulations in strabismus; genetic and epidemiologic studies of the risk factors for strabismus; improved eye movement recording techniques for diagnosing strabismus; and studies on the chronology of treatment modalities for strabismus.

### B.3.b. Neuro-Ophthalmology

Objectives: Research in this subsection deals with all acquired or congenital eye movement disturbances exclusive of strabismus. This includes all forms of nystagmus and other types of ocular oscillations, gaze disturbances, oculomotor apraxia, ocular neuropathies, and transmission disturbances affecting the ocular muscles such as myasthenia gravis and Eaton-Lambert syndrome. Subsection objectives include:

- o To improve our ability to diagnose and treat neuro-ophthalmological disorders of ocular motility.
- o To develop a better understanding of the causes, mechanisms, and consequences of neuro-ophthalmological disorders.

Recent Research Accomplishments: Major clinical advancements have been through the utilization of quantitative eye movement recording (oculography) in patients with abnormal eye movements. This has enabled us to characterize and analyze wave forms in a precise manner. Previously, we had to rely on mere clinical description of eye movements which often precluded meaningful analysis and classification.

Considerable attention is being addressed to the concept of object and self motion preception correlating with vestibular, visual, somatosensory, and oculomotor control.<sup>22,23</sup> These studies are of potential clinical importance as further studies define multisystem interrelationships.

A number of specific disease states such as progressive supranuclear palsy,<sup>24</sup> cerebellar degeneration,<sup>25,26</sup> multiple sclerosis,<sup>27,28</sup> and myasthenia gravis<sup>29,30</sup> characteristically affect eye movements. These eye movement disorders are being studied carefully and are providing insights into disease mechanisms and, more importantly, facilitated diagnoses.

The finding of mitochondrial abnormalities in skeletal and extraocular muscles in patients with progressive external ophthalmoplegia and the recognition of a possible specific subtype of these disturbances holds future<sup>31</sup> promise for better understanding of this group of disorders.

Future Needs: Priorities for continuing and expanding research in neuro-ophthalmological disorders in NEI include: continued quantitative studies of eye movements in a variety of central and peripheral neurological diseases; determination of the cost effectiveness of computer analysis of oculomotor functions as compared to clinical analysis by a trained neuro-ophthalmologist; and the development of new technology for automated eye movement recording systems.

### C. Optics and Refractive Errors, Including Myopia

Objectives: Refractive error of the eye results when the eye fails to form a focused image on the retina because of an improper combination of power and spacing of the optical components of the eye. Research on refractive error including myopia is divided among NEI programs: The Cataract and Corneal Diseases programs support research in the surgical and nonsurgical correction of refractive error; the Retinal and Choroidal Diseases program supports research on refractive error as it relates to visual processing at the retina; the Strabismus, Amblyopia, and Visual Processing program is interested in basic mechanisms of refractive error and its development, in the overall shape of the eye in refractive error, and in psychophysical

testing in refractive error. The basic research goals are to correct refractive errors more efficiently and more effectively and to identify the mechanisms underlying the development and change of refractive errors. As such mechanisms are defined, preventive measures can be formulated and tested.

Specific objectives for research in Optics and Refractive Errors by the Strabismus, Amblyopia and Visual Processing Program are:

- o To identify mechanisms responsible for the control of eye growth and for the development of refractive errors, including myopia, hyperopia, astigmatism, and presbyopia.
- o To devise and evaluate methods for the prevention or control of refractive change.
- o To develop and test instrumentation for effective screening and for rapid and accurate measurement of refractive errors of the eye, especially in young children.

Recent Research Accomplishments: Until recently, little research progress has been made toward understanding the mechanisms underlying myopia and other refractive disorders, but an upsurge in interest for research in myopia is occurring. An international society has recently been formed, and major activity in both basic research and clinical investigation is beginning to appear.

Recent research findings may be setting the stage for additional productive research in optics and refractive errors. In 1977, Wiesel and Raviola<sup>32</sup> reported that lid closure in young monkeys led to the development of axial myopia. Subsequent papers by the same authors<sup>33,34</sup> indicate that this may indeed be a useful animal model for the development of axial myopia.<sup>35</sup> Similar observations have been made with lid closure in the tree shrew<sup>35</sup> and with field-restricting blinders in the chicken.<sup>36</sup> Furthermore, Hoyt and co-workers have reported eight cases of monocular axial myopia in human infants associated with neonatal lid closure.

Significant advances have been made in the automation of clinical refraction. Refractive measurements can now be performed in less than 1.5 seconds, using infrared light and computer-controlled electro-optical designs. Automated subjective refractors have now appeared which at least equal the accuracy of conventional techniques of clinical refraction.

Future Needs: Research in Optics and Refractive Errors needs to be expanded greatly in order to meet the objectives of this subprogram. Research priorities include: studies on the etiology and mechanisms of myopia using physiochemical approaches and animal models; epidemiological studies and limited clinical trials of treatment modalities for myopia; development of methods for mass screening for refractive errors; development and testing of special purpose refracting instruments of types that are not likely to be developed by industry.

#### D. Rehabilitation

Objectives: In 1980, the National Society to Prevent Blindness (NSPB) estimated that in the United States there were 11.4 million people with impaired vision, including 1.4 million with severe visual impairment and about 500,000 individuals who were legally blind.<sup>37</sup> Retinal and vitreous disorders are responsible for much of the partial and total loss of vision; other disorders that contribute to vision loss are: corneal and other forms of external eye disease; cataract; glaucoma; and diseases affecting the visual pathways and visual cortex.

Specific objectives of the Low Vision and Rehabilitation subprogram include:

- o To develop an infrastructure for research to meet the needs of the visually impaired.
- o To gather and analyze epidemiological data for blindness, partial loss of sight, and visual anomalies.
- o To improve the methods for specifying, measuring, and categorizing loss of visual function.
- o To advance research in the rehabilitation, training, and quality of life of the partially sighted.
- o To develop comprehensive research and training centers for training researchers and for broadening the research base in this field.

Recent Research Accomplishments: The Institute is making a concerted effort to identify needs and increase research toward the assistance and rehabilitation of blind persons and of those with low vision. Part of this effort has gone into the establishment of an interagency group to coordinate the Federal Government's support of research on low vision and to assist nonfederal efforts in this area.

One non-NEI project has dealt with the recognition that test chart design can have a substantial influence on visual acuity scores, especially for persons with low vision. A working group of the National Academy of Sciences/National Research Council Committee on Vision has recently recommended standard procedures for measuring and specifying visual acuity.<sup>38</sup> New charts have been designed with a view to improving the reliability of visual acuity measurements.<sup>39,40</sup>

Some important new aids to assist persons with visual loss have been developed recently. Important advances in telescopes and electronic voice synthesis have been accomplished independent of NEI support. For example, new spectacle-mounted bioptic telescopes of Keplerian design with considerable adjustability of focus have been made,<sup>41,42</sup> and several different hand-held monocular telescopes with wide ranges of focus have become available. Improved electronic voice synthesis has led to the development of a range of devices

such as talking calculators, clocks, scales, thermometers, multimeters, and calipers. Good progress has been made with reading machines<sup>43</sup> that convert typeface print into a synthesized voice output.

In an NEI-supported project, Rowell is developing auditory techniques for three-dimensional encoding of an image and is designing auditory cues for guiding a blind traveler along a path.<sup>44</sup> Levinson, whose research is supported by another NEI grant, is studying the usefulness of holographic images as a reading aid as well as the usefulness of temporal modulations or stimulus movement as a vision enhancer.<sup>45</sup>

Future Needs: Very little organized research is currently being conducted in Low Vision and Rehabilitation, and research training programs are nonexistent. Communication is poor among existing research groups in this area. Society should invest a reasonable national effort toward ameliorating the problems of those suffering visual loss. Specific research needs include: improving interaction, communication, and coordination among the available multidisciplinary research groups and service providers who render ocular and visual care rehabilitation and social services; developing an adequate epidemiological base, which in turn will require the development of standards for specification of visual impairment, for testing and recording visual functions, and for the specification of aids for the visually impaired.

## References

1. Kasamatsu T, Pettigrew JD: Depletion of brain catecholamines: Failure of ocular dominance shift after monocular occlusion in kittens. Science 194:206-209, 1976.
2. Turner FE, Delaney RK: Retinal ganglion cell response to axotomy and nerve growth factor in the regenerating visual system of the new (Notophthalmus viridescens): An ultrastructural morphometric analysis. Brain Res 171:197-212 1979.
3. Gaze RM, Keating MJ, Ostberg A, Chung SH: The relationship between retinal and tectal growth in larval Xenopus: Implications for the development of the retinotectal projection. J Embryol Exp Morphol 53:103-143, 1979.
4. Schmidt FT: Retinal fibers alter tectal positional markers during the expansion of the half retinal projection in goldfish. J Comp Neurol 177:279-300, 1978.
5. Finlay BL, Schneps ES, Schneider GE: Orderly compression of the retinotectal projection following partial tectal ablation in the newborn hamster. Nature 280:153-154, 1979.
6. Barnstable CJ: Monoclonal antibodies which recognize different cell types in the rat retina. Nature 286:231, 1980.
7. Oswald RE, Aswald RE, Schmidt JT, Norden JJ, Freeman JA: Localization of -bungarotoxin binding sites to the goldfish retinotectal projection. Brain Res 187:113, 1980
8. Eviatar L, Miranda S, Eviatar A, Freeman K, Borkowski M: Development of nystagmus in response to vestibular stimulation in infants. Ann Neurol 5:508-514, 1979.
9. Donat JF, Donat DR, Lay KS: Changing response to caloric stimulation with gestational age in infants. Neurology (NY) 30:776-778, 1980.
10. Kremenitzer JP, Vaughan HG, Kurtzberg D: Smooth pursuit eye movements in newborn infants. Child Dev 59:442-448, 1979.
11. Hoyt CS, Mousel DK, Weber AA: Transient supranuclear disturbances of gaze in healthy neonates. Am J Ophthalmol 89:708-713, 1980.
12. Sevel DR: Change of extraocular muscle insertions with normal maturation of the human infant. Br J Ophthalmol, to be published.
13. Halmagyi GM, Rudge P, Gresty MA, et al: Treatment of periodic alternating nystagmus. Ann Neurol 8:609-611, 1980.
14. Braddick O, Atkinson J: Accommodation and acuity in the human infant, in Freeman RD (ed): Developmental Neurobiology of Vision. New York, Plenum Press, 1979.

References Cont.

15. Trachtman JN: Biofeedback of accommodation to reduce functional myopia: A case report. Am J Optom Physiol Opt 55:400-406, 1978.
16. Flom MC, Kirschen DG, Bedell HE: Control of unsteady fixation in amblyopic eyes by auditory feedback of eye position. Invest Ophthalmol Vis Sci 19:1371-1381, 1980.
17. Kirschen DG, Flom MC, Bedell HE: Auditory feedback in the control of congenital nystagmus, in Fender D (ed): Oculomotor Symposium, to be published.
18. Kiorpes L, Boothe RG: Naturally occurring strabismus in monkeys. Invest Ophthalmol Vis Sci 20:257-263, 1981.
19. Scott AB: Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery. Ophthalmology 87:1044-1049, 1980.
20. Scott AB: Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery. J Pediatr Ophthalmol Strabismus 17:21-25, 1980.
21. Mindel JS, Raab EL, Eisenkraft JB, et al: Succinyl dicholine-induced return of the eyes to the basic deviation. Ophthalmology 87:1288-1295, 1980.
22. Dichgans J, Brandt T: Visual-vestibular interaction: Effects on self-motion perception and postural control, in Teuber H-L, Held R, Leibowitz H (eds): Handbook of Sensory Physiology. New York, Springer, 1978, pp 753-804.
23. Brandt T, Daroff RB: The multisensory physiological and pathological vertigo syndromes. Ann Neurol 7:195-203, 1980.
24. Troost BT, Daroff RB: The ocular motor defects in progressive supranuclear palsy (PSP). Ann Neurol 2:397-403, 1977.
25. Zee DS, Yee RD, Cogan DG, et al: Ocular motor abnormalities in hereditary cerebellar ataxia. Brain 99:207-234, 1976.
26. Baloh RW, Jenkins HA, Honrubia V, et al: Visual-vestibular interaction and cerebellar atrophy. Neurology 29:116-119, 1979.
27. Solingen LD, Baloh RW, Myers L, Ellison G: Subclinical eye movement disorders in patients with multiple sclerosis. Neurology 27:614-619, 1977.

References Cont.

28. Masteglia FL, Black JL, Collins DWK: Quantitative studies of saccadic and pursuit eye movements in multiple sclerosis. Brain 102:817-839, 1979.
29. Yee RO, Cogan DG, Zee DS, et al: Rapid eye movements in myasthenia gravis: II. Electrooculographic analysis. Arch Ophthalmol 94:1465-1472, 1976.
30. Schmidt D, Dell'Osso LF, Abel LA, et al: Myasthenia gravis: Dynamic changes in saccadic waveform, gain, and velocity. Exp Neurol 68:365-377, 1980.
31. Berenberg RA, Pellock JM, DiMauro S, et al: Lumping or splitting? "Ophthalmoplegia-plus" or Kearns-Sayre syndrome? Ann Neurol 1:37-54, 1977.
32. Wiesel TN, Raviola E: Myopia and eye enlargement after neonatal lid fusion in monkeys. Nature 266:66-68, 1977.
33. Raviola E, Wiesel TN: Effect of dark-rearing on experimental myopia in monkeys. Invest Ophthalmol Vis Sci 17:485-488, 1978.
34. Wiesel TN, Raviola E: Increase in axial length of the macaque monkey eye after corneal opacification. Invest Ophthalmol Vis Sci 18:1232, 1979.
35. Sherman SM, Norton TT, Casagrande VA: Myopia in lid-sutured tree shrew (*Tupaia glis*). Brain Res 124:154-157, 1977.
36. Wallman J, Turke J: Extreme myopia produced by modest change in early visual experience. Science 201:1249-1251, 1978.
37. Vision Problems in the U.S. National Society to Prevent Blindness, 1980.
38. Committee on Vision, Working Group 39: Recommended standard procedures for the clinical measurement and specification of visual acuity. Adv Ophthalmol 41:103-148, 1980.
39. Bailey IL, Lovie JE: New design principles for visual acuity letter charts. Am J Optom Physiol Opt 53:740-745, 1976.
40. Sloan LL: Needs for precise measures of acuity. Arch Ophthalmol 98:286-290, 1980.
41. Bailey IL: New "expanded field" bioptic systems. Optom Monthly 69:981-984, 1979.
42. Fonda G: A bioptic telescopic spectacle: Advantages and limitations Sight Sav Rev 48:125-128, 1979.

References Cont.

43. Scadden LA: Kurzweil reading machine evaluation of Model One.  
J Vis Impair Blindness 72:389-399, 1979.
44. Rowell D: Annual Progress Report, EY02463-03, May, 1981.
45. Levinson EM: Annual Progress Report, EY 03077-02, May, 1981.



OFFICE OF BIOMETRY AND EPIDEMIOLOGY



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

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 effectiveness of photocoagulation in reducing the incidence of diabetic blindness. Three more papers have been published by the Diabetic Retinopathy Study Research Group and numerous presentations have occurred. The publications include a supplement to Investigative Ophthalmology and Visual Science, discussing the design, methods and baseline results and a separate paper within the supplement on the photographic classification of diabetic retinopathy. The third paper, which was presented at the American Academy of Ophthalmology and has been published in Ophthalmology, discusses the clinical implications of the DRS results. Data tapes from which further analyses can be done have been delivered to the National Eye Institute. These archival tapes can be made available to investigators who wish to use this research resource.

The ETDRS was designed to provide a better understanding of the optimum time to use photocoagulation in the course of diabetic retinopathy. Patients with macular edema, preproliferative, or proliferative retinopathy are included. Three forms of photocoagulation treatment, ranging from a restricted focal treatment to a full scatter, are being assessed. In addition, half the patients are 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 patients. The study also provides the opportunity to investigate factors that are associated with the progression of disease. As of June 1981, nearly 900 treatment allocations had been issued and over 700 patients had been treated with recruiting likely to last until the end of 1983.

The DRVS has succeeded in recruiting over 500 patients whose vision has been reduced by hemorrhage into the vitreous (group H) and over 200 patients who still have useful vision but with serious risk of complications leading to retinal detachment (group NR). Half of the eligible eyes have been randomized to prompt vitrectomy, and half to vitrectomy one year later, if still indicated (in group H), or to "traditional" care in group NR.

The Institute has funded a randomized clinical trial to evaluate radial keratotomy, a relatively new surgical procedure to correct myopia. Dr. Robert Sperduto has been designated as the Institute's scientific project officer. The Prospective Evaluation of Radial Keratotomy Study (PERK) includes eight clinics with one or two shortly to be added. The primary study goals are to evaluate the effectiveness and complication rates associated with radial keratotomy. Patients will be followed for at least five years. Recruiting of patients began in August. Support of the study has been criticized by a small number of ophthalmologists who are performing a large number of these procedures. They have contended that the data which PERK is designed to collect are already available. Several members of OBE staff, particularly Drs. Sperduto and Seigel, have spent a large amount of time evaluating these contentions and preparing materials for the NEI Director.

Dr. Seigel and Mr. Podgor are collaborating with Dr. Robert Nussenblatt, Clinical Branch, NEI, in design and analysis of a clinical trial for treatment of ocular toxoplasmosis.

Epidemiology. 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. 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 prepared and implemented a plan for a pilot study for the Visual Acuity Impairment Survey (VAIS), a two-stage national survey of visual impairment in the United States. The primary objective of the full study will be to determine the prevalence of visual impairment, by

cause, in large metropolitan areas of the United States. A secondary objective will be 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. In the first stage, visual acuity examinations will 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 given a detailed ophthalmological examination. The one-year pilot study was started in August 1981 in three metropolitan areas. The manual of operations was prepared by OBE staff and consultants. If the pilot study is successful, the full study will be carried out in about 15 metropolitan areas.

OBE staff continued to exploit data from the Framingham Eye Study (FES) and the Health and Nutrition Examination Survey (HANES). A recent publication had questioned the existence of an association between senile cataract and diabetes. In response, a paper published by Mr. Ederer, Mrs. Hiller, and Dr. Taylor found diabetics under the age of 70 to be at increased risk of having senile cataract both in the Framingham and HANES investigations.

Dr. Sperduto, Mrs. Hiller, and Dr. Seigel published a paper demonstrating a negative association between nuclear lens changes and senile macular changes in the Framingham Eye Study data. They suggested that the yellow brown pigmentation of nuclear sclerosis might protect the macula from radiation induced damage.

Drs. Kahn and Milton published a study of the effect of alternative definitions of glaucoma in the Framingham Eye Study on the prevalence of glaucoma and its associations with Framingham Heart Study variables. They found an association, not previously reported, between glaucoma and alcohol use.

Mr. Podgor and Mr. Ederer collaborated with Dr. M. Cristina Leske, State University of New York at Stony Brook, in a study of screening methods for glaucoma using the Framingham Eye Study data. They have also begun to use Framingham data to estimate the incidence of late lens changes and of senile macular degeneration according to a method they had developed for glaucoma. The paper describing this method has been published. Mr. Podgor has continued to work with Dr. Robert Frank, Kresge Eye Institute, Wayne State University, in an extension of a study of retinopathy in juvenile-onset diabetes of short duration.

The National Oceanic and Atmospheric Administration has constructed new climatological maps of UV-B radiation for the United States. These maps in conjunction with the HANES data will be used for studying the association between UV light and senile lens changes.

Dr. Colton and Mr. Ederer published a literature review of the distribution of intraocular pressures in various populations.

Little is known about the etiology of senile macular degeneration, a major cause of blindness in the United States. In an attempt to test various etiologic hypotheses about the disease, a case-control study has been conducted by OBE staff collaborating with epidemiology and ophthalmology staff at Johns Hopkins University. The data have been collected and partially analyzed. Risk factors, including family history of senile macular disease, chemical work exposure, cigarette smoking, sunlight exposure, iris color, refractive error, hand grip strength, and cardiovascular disease, have been identified. This material was presented at the ARVO meeting in May of 1981 and is being prepared for publication.

Mr. Podgor collaborated with Dr. Muriel Kaiser-Kupfer, Clinical Branch, NEI, in a study showing decreased ocular rigidity in patients with osteogenesis imperfecta. A paper has been published. Mr. Podgor has also worked with Clinical Branch investigators on electroretinogram data from normal volunteers.

Dr. Milton collaborated with Dr. Kirsti Takki, Kivela City Hospital, Helsinki, in describing the natural history of gyrate atrophy, a rare chorioretinal dystrophy characterized by hyperornithinemia. A paper was presented at the 1980 annual meeting of the American Academy of Ophthalmology and subsequently published.

The role of afferent pupillary defect in disciform macular degeneration was described and quantified in a collaborative effort of Dr. Milton with Dr. David Newsome, Clinical Branch, NEI, and Dr. J. Donald M. Gass, Bascom Palmer Eye Institute, University of Miami. Dr. Milton also worked with Dr. Newsome in the analysis of a survey of eye disease in an area in Haiti.

In collaboration with Dr. Arin Chatterjee, Christian Medical College, Ludhiana, India, Dr. Milton completed a study of cataract etiology and prevalence in Punjab which confirms the high prevalence of senile cataract previously reported in that area and which suggests increased risk of cataract with low protein intake.

Dr. Milton continued his work in nutritional eye disease with the completion of a paper for the American Journal of Clinical Nutrition on evaluating the efficacy of programs for the control of xerophthalmia. He also participated with Indian and U.S. investigators, through workshops in Hyderabad, India, and at the NEI, in the preparation of a proposal for a clinical research center for the prevention of nutritional blindness in Hyderabad. This proposal, currently being reviewed by the NIH for approval as a special foreign currency project, includes a case-control study of risk factors in severe xerophthalmia in preschool children. Dr. Milton also met in Bethesda with the management team of the Royal Commonwealth Society for the Blind to discuss their intervention program for preventing xerophthalmia blindness in India.

#### Education and Consultation

Dr. Kupfer, Dr. Ferris, and Mr. Ederer participated as faculty in the second of a series of annual courses on epidemiologic and biostatistical approaches to clinical vision research. Along with university colleagues,

they presented a three-day course at Sarasota to clinical investigators. Attendance was excellent, and written evaluation indicated that the course material was quite appropriate. A third course is planned for 1982.

Drs. Kupfer, Ferris, Seigel, and Davis also presented a three-hour course on methods of clinical research at the American Academy of Ophthalmology.

Mr. Ederer serves as Epidemiology Editor for the Survey of Ophthalmology and is on the Editorial Board of the American Journal of Ophthalmology. Dr. Seigel is an Associate Editor for the American Journal of Epidemiology and is a member of the Editorial Board of the Archives of Ophthalmology. Mr. Ederer is a member of the Board of Directors of the Society for Clinical Trials and of the American College of Epidemiology.

Mr. Ederer was a consultant to the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases on the conduct of clinical trials.

In March 1981, NIH held the international symposium "Current Concepts in Biometry and Epidemiology" in honor of Jerome Cornfield, a renowned biostatistician and former consultant to NEI who had died in 1979. The proceedings, including a paper by Mr. Ederer, will be published as a supplement to Biometrics. Dr. Seigel chaired and Mr. Ederer served on both the planning committee for the symposium and the editorial board for the publication.

The Office is planning a national symposium on the epidemiology of eye diseases and conditions for June 9-10, 1982 at the National Institutes of Health. Papers reviewing the literature on the epidemiology of five eye major diseases and conditions and abstracts of results of current work and of work in progress are being invited.

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

Dr. Milton continued his consultation with Dr. Douglas Gaasterland, Clinical Branch, NEI, in analysis and computer applications in aqueous humor dynamics.

Dr. Seigel served as a consultant to the National Institute of Arthritis, Metabolism, and Digestive Diseases in the selection of a coordinating center for a clinical trial of control in the diabetic patient. He served as a member of a panel reviewing drug epidemiology studies for the Bureau of Drugs, FDA, a committee to evaluate intraocular lens studies for the Bureau of Medical Devices, and has been appointed a member of the Ophthalmic Drug Advisory Committee. He continues to serve as an advisor to the Boston University Drug Epidemiology Unit.

Drs. Milton and Seigel were active in assisting the NEI Director in consulting on protocols for studies in other countries. Examples were the ICMR study on the prevalence of cataract, an intervention program in nutritional blindness in India sponsored by the Royal Commonwealth Society for the Blind, and a proposed study of metal sutures in eye surgery.

Dr. Sperduto served as a consultant to the cataract panel of the National Advisory Eye Council Program Planning Subcommittee.

## Publications

### Office of Biometry and Epidemiology

1. Ederer F: Comment on Leske MC and Rosenthal J: Epidemiologic aspects of open-angle glaucoma. Surv Ophthalmol 24:258-259, 1980.
2. Kahn HA, Milton RC: Revised Framingham Eye Study prevalence of glaucoma and diabetic retinopathy. Am J Epidemiol 111:769-776, 1980.
3. Colton T, Ederer F: The distribution of intraocular pressures in the general population. Surv Ophthalmol 25:123-129, 1980.
4. Kahn HA, Milton RC: Alternative definition of open-angle glaucoma: Effect on prevalence and associations in the Framingham Eye Study. Arch Ophthalmol 98:2172-2177, 1980.
5. Ederer F, Hiller R, Taylor HR: Senile lens changes and diabetes in two population studies. Am J Ophthalmol 91:381-395, 1981.
6. Seigel D: Clinical trials, in Pharmacology of the Eye. Heidelberg, Springer-Verlag (in press).
7. The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: A short report of long range results. Diabetic Retinopathy Study (DRS) Report Number 4. Excerpta Medica (in press).
8. The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: Relationship of adverse treatment effects to retinopathy severity. Diabetic Retinopathy Study (DRS) Report Number 5. Chapter in Modern Problems in Ophthalmology (Proceedings of the 1979 Meeting of Club Jules Gonin) (in press).
9. Ederer F: Comment on Angle J and Wissmann DA: The epidemiology of myopia. Surv Ophthalmol 25:282, 1981.
10. Takki KK, Milton, RC: The natural history of gyrate atrophy of the choroid and retina. Ophthalmology 88:292-301, 1981.
11. Seigel D: Some basic principles in clinical trials. Am J Optom 58:277-280, 1981.
12. Kupfer MIK, McCain L, Shapiro JR, Podgor MJ, Kupfer C, Rowe D: Low ocular rigidity in patients with osteogenesis imperfecta. Invest Ophthalmol Vis Sci 20:807-809, 1981.
13. Milton R: Evaluation of the efficacy of program for the control of severe xerophthalmia. Am J Clin Nutr (in press).
14. Chatterjee A, Milton RC, Thyle S: Cataract prevalence and etiology in Punjab. Br J Ophthalmol (in press).

15. Sperduto RD, Seigel D: Senile lens and senile macular changes. Letter to Editor. Am J Ophthalmol 91:418, 1981.
16. Sperduto RD, Hiller R, Seigel D: Lens opacities and senile maculopathy. Arch Ophthalmol 99:1004-1008, 1981.
17. Ederer F, Hiller R, Taylor H: Diabetes and senile cataract. Reply to Letter to the Editor. Am J Ophthalmol 92:135-136, 1981.
18. The Diabetic Retinopathy Study Research Group: Diabetic Retinopathy Study: Design, methods, and baseline results. Report 6. Invest Ophthalmol Vis Sci 21(1, pt2):149-209, 1981.
19. The Diabetic Retinopathy Study Research Group: Diabetic Retinopathy Study: A modification of the Airlie House classification of diabetic retinopathy. Report 7. Invest Ophthalmol Vis Sci 21(1, pt2):210-225, 1981.
20. Ederer F: Jerome Cornfield's contributions to the conduct of clinical trials. In Proceedings of Current Topics in Biostatistics and Epidemiology, A Memorial Symposium in Honor of Jerome Cornfield. Biometrics, March 1982, supplement (in press).
21. The Diabetic Retinopathy Study Research Group: Photocoagulation treatment proliferative diabetic retinopathy: Clinical application of Diabetic Retinopathy Study (DRS) findings. Report 8. Ophthalmology 88:583-600, 1981.
22. Kupfer C: A new patient group in the Diabetic Retinopathy Vitrectomy Study (DRVS). Editorial. Arch Ophthalmol 99:65, 1981.
23. Newsome DA, Milton RC, Gass JDM: Afferent pupillary defect in disciform macular degeneration. Am J Ophthalmol (in press).
24. Leske MC, Ederer F, Podgor M: Estimating incidence from age-specific prevalence in glaucoma. Am J Epidemiol 113:606-613, 1981.

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: (No new funds allocated in this fiscal year.)

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 1,758 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 studies: 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 on May 31, 1979. The data have been edited and data tapes have been furnished to the National Eye Institute. Further analysis of these data is underway. The baseline monograph has been published as has a paper on the modification of the Airlie House Classification of diabetic retinopathy. A final report on the long-term follow-up of Diabetic Retinopathy Study patients is being prepared. Papers on the following topics: assessment of risk factors for severe visual loss, mortality of individuals with proliferative diabetic retinopathy, effects of treatment on diabetic macular edema, association of renal disease and diabetic retinopathy.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications:

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: A short report of

long range results. Diabetic Retinopathy Study (DRS) Report Number Four. Excerpta Medica (in press).

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment of proliferative diabetic retinopathy: Relationship of adverse treatment effects to retinopathy severity. Diabetic Retinopathy Study (DRS) Report Number Five. Chapter in Modern Problems in Ophthalmology (Proceedings of the 1979 meeting of Club Jules Gonin). S. Karger AG, Basel (in press).

The Diabetic Retinopathy Study Research Group: Diabetic Retinopathy Study: Design, Methods, and baseline results. Report six. Invest Ophthalmol Vis Sci 21(1, pt2):149-209, 1981.

The Diabetic Retinopathy Study Research Group: Diabetic Retinopathy Study: A modification of the Airlie House classification of diabetic retinopathy. Report seven. Invest Ophthalmol Vis Sci 21(1, pt2):210-225, 1981.

The Diabetic Retinopathy Study Research Group: Photocoagulation treatment proliferative diabetic retinopathy: Clinical application of Diabetic Retinopathy Study (DRS) findings. Report eight. Ophthalmology 88:583-600, 1981.

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: \$1.6 million for fiscal year 1981.

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 August 1981, a total of 495 eyes with severe hemorrhage had been randomized to early or deferred vitrectomy. Follow-up continued on the 742 eyes recruited in the natural history study, where recruiting has stopped. A total of 200 eyes have been randomized in groups NR.

Meeting recruiting goals remains the most difficult aspect of the study. Two clinics were added this year, one at Duke University and the other at Emory University. These replace four clinics in which recruiting was discontinued last year because of low recruiting levels.

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: Consideration is being given to setting a date for termination of recruiting. The date likely to be selected is end of 1982. The number of patients accrued as of that date may not meet the goals originally established. The slight loss of precision that will result may be preferred to undue delay of publication of study findings.

Preparation of a manuscript on the first two years of follow-up in the natural history study.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publication:

Kupfer C: A new patient group in the Diabetic Retinopathy Vitrectomy Study. (Editorial) Arch Ophthalmol 99, 1981.

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; a Central Laboratory at the Centers for Disease Control, Atlanta, Georgia; and an Electrocardiogram Reading Center at the University of Minnesota, Minneapolis, Minnesota

Title: Early Treatment Diabetic Retinopathy Study (ETDRS)

Principal Investigator: Dr. Lloyd Aiello (Chairman)

Current Fund Allocation: \$1.9 million this fiscal year.

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: As of June 19, 1981, 1,268 patients had started qualifying visits for this study with 1,043 completing this visit. A total of 886 treatment allocations have been issued and 718 patients have been treated. Recruitment is continuing to increase and it is expected that each clinic will recruit five patients per month. Recruitment is projected to end in October of 1983.

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 improving eye care for patients with diabetes.

Proposed Course: Follow-up of all ETDRS patients is planned for five years. Monitoring of accumulated data is performed at quarterly intervals.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications: None



CONTRACT NARRATIVE

Three Clinical Centers, plus a Coordinating Center at the University of Minnesota, Minneapolis, Minnesota; and a Fundus Photograph Reading Center at the Office of Biometry and Epidemiology, National Eye Institute, Bethesda, Maryland

Title: Visual Acuity Impairment Survey (VAIS) Pilot Study

Principal Investigator: Fred Ederer (Project Officer)  
Richard Mowery (Deputy Project Officer)

Current Fund Allocation: \$480,070 for the period June 1, 1981, through November 30, 1982.

Objectives: The Visual Acuity Impairment Survey is a planned multicentered, epidemiological study of the prevalence of central distance visual acuity impairment in the United States and of the eye diseases responsible for the impairment. The main goals of the 1-year study are:

- a. To determine the feasibility of the VAIS.
- b. To pretest home interview screening procedures and clinic examination procedures.
- c. To gather information that will help to plan the full study.

Major Findings: All clinic staff were trained and certified to begin subject examinations in August 1981. The Project Team and Data Center completed the preliminary versions of the Clinic Examination Form and the Manual of Procedures in July 1981. All equipment to be used by the regional Census Bureau offices and the three clinical centers was received in July 1981.

Significance to Biomedical Research and the Program of the Institute: The Visual Acuity Impairment Survey originated from the Institute's past involvement with the Model Reporting Area of Blindness, the Health and Nutrition Examination Survey, and the Framingham Eye Study. These studies attempted to measure the frequency of eye diseases or of visual impairment but were limited in scope or assurance of quality, or were hampered by logistical problems. The VAIS represents an extension of the Institute's interest in epidemiologic research and in gathering high quality population-based data to be used in program planning and for public information. The Study further offers the opportunity to introduce vision researchers to epidemiologic concepts and methods.

Proposed Course: Home screening by Census Bureau interviewers in mid-August 1981 and clinic examinations in early September. Approximately 200 subjects having visual acuity of 20/40 or worse and a control group of 200 subjects without visual impairment are expected to be examined by the three clinics between September 1981 and August 1982. Data collected at home and in the clinic will be sent to the Data Center in Minnesota for processing and analysis. The data will be reviewed on a regular basis by the Institute

staff, the Data Center, and the clinic Principal Investigators to evaluate study progress and to make necessary changes in examination procedures.

NEI Research Program: Retinal and Choroidal Diseases

Publications: None

OFFICE OF PROGRAM PLANNING, ANALYSIS, AND EVALUATION



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

REPORT OF THE CHIEF, OFFICE OF PROGRAM PLANNING, ANALYSIS, AND EVALUATION  
Julian M. Morris

During the past year the Office continued work on the development of the National Advisory Eye Council's comprehensive program evaluation and national plan for the years 1983-1987. The Office helped organize and conduct a score of Program Planning Subcommittee and program-specific panel meetings during 1981.

The Office coordinated the development of NEI program evaluations, and prepared the annual NEI Evaluation Plan, Research Plan (FY 1983-85) and a program performance summary for Cataract. In support of these plans and evaluations the Office prepared data and program analyses for the five major programs of the Institute. The Office also prepared the copy for the NEI exhibit on program planning at the Association for Research in Vision and Ophthalmology annual meeting. A separate program evaluation project, "Anatomy and Assessment of Program Planning at the National Eye Institute," was completed during the fiscal year. This project, which was supported by set-aside evaluation funds, assembled for the first time in one document information on the rationale and methodology for NEI program planning and evaluation, and aspects of its execution over the past several years. The report concluded with an assessment of these activities.

In fiscal year 1981 the responsibilities and resources of the Program Analysis Section, formerly called the Program Information Section, were transferred from the Extramural and Collaborative Programs to the newly-named Office of Program Planning, Analysis, and Evaluation. A new section head, David E. Scheim, Ph.D., was recruited. A fiscal analyst and a senior programmer were also hired. Recruitment to fill the remaining vacancy in the section, a secretary/computer clerk, is underway.

Under Dr. Scheim's direction NEI's centralized computer-based program data system will be refined, modernized, and upgraded to make it more efficient and comprehensive. It should soon be possible to provide further responses to staff data requests and to offer a greater range of analytic services.

In such cross-cutting areas as diabetes, toxicology, and environmental health, the Office collaborated with other NIH Institutes and outside organizations in analyses and evaluations of NEI research. The Office has also advised the Director, NEI, on program evaluation and planning policy and wrote, contributed to, commented upon, or coordinated NEI's contributions to the following reports:

- o Prevention Program Descriptions and Budget Figures
- o ASPE Request for Information on the Utilization of Completed Evaluations
- o Five-Year Outlook for Science and Technology and the Annual Technology Report

- o Research on Relationship Between Water Quality and Human Health
- o Support of Interferon Research
- o Federal Inventory of Population Research
- o Indian Health Service Annual Survey
- o Background Materials for NEI Planning/Appropriations Briefing Session with the Director, NIH
- o Quantitative Illustrations of the Impact of Biomedical Research on the Nation's Health
- o Highlights of Research in Prevention
- o ADP Financial Data Report
- o Research on Skin and Skin Diseases
- o Background Material for Secretary Schweiker's Visit
- o The Fourth Annual Science and Technology Report
- o The NIH Contribution to PHS Health Services Research Plan
- o NEI Risk Assessment Activities
- o Update of NEI Orientation Manual
- o Implementation Plans for Accomplishing 1990 Prevention Objectives
- o Preliminary Design of a Study of the Planning Processes at NIH
- o Fourth National Toxicology Program Annual Plan

Again this year, the Office coordinated the preparation and supervised the publication of the NEI Annual Report. Mr. Morris served as a reviewer on conference support contract proposals for the Fogarty International Center. He also served on a editorial advisory committee for the forthcoming first national plan of the National Institute of Child Health and Human Development.

OFFICE OF SCIENTIFIC REPORTING



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

REPORT OF THE CHIEF, OFFICE OF SCIENTIFIC REPORTING  
Marsha S. Corbett

During the past fiscal year, the Office of Scientific Reporting (OSR) has been very successful in increasing awareness of the National Eye Institute (NEI) and its programs among health care providers and the general public. The Office has stimulated nationwide publicity on vision research and prevention of blindness issues, and also worked to establish the Institute as a reliable source of information on such subjects. A short-term objective of these efforts is to keep the general public informed of how its tax dollars are spent. The long-range goal is to improve the prevention, diagnosis, and treatment of eye disease and blindness by disseminating information to health care professionals and the general public on research results that are or will be clinically applicable to the practice of medicine. A description of these information dissemination and knowledge transfer activities, beginning with some projects which have required a major expenditure of staff time and effort, appears below.

Fiscal Year 1981 also marked the official division of information and program planning functions within the Institute, with two separate offices resulting: the Office of Scientific Reporting and the Office of Program Planning, Analysis and Evaluation. Two experienced writers, Ms. June Wyman and Ms. Robin de Silva, joined the staff of the OSR and will be handling a full range of information office activities. Also joining the staff is Ms. Claudia Feldman, formerly a secretary in the Clinical Branch. Ms. Maureen Mylander, who served as a writer-editor, took a new position in the Office of Communications, NIH.

Prospective Evaluation of Radial Keratotomy (PERK)

Radial keratotomy is a controversial new eye operation to correct myopia (near-sightedness). Inquiries on this subject have flooded the OSR whenever proponents of the procedure sought publicity. In response to the inquiries, staff members provided information on radial keratotomy and referred people to medical societies representing ophthalmologists and radial keratotomists. In addition, a fact sheet on refractive keratoplasty was prepared to facilitate handling of requests for information.

A grant proposal for a clinical trial of radial keratotomy was subsequently submitted to NIH. After it was reviewed, approved, and funded, a news release announcing the NEI-supported Prospective Evaluation of Radial Keratotomy (PERK) was prepared for distribution. Ophthalmologists in states where participating centers are located were also informed about the study in a mass mailing. In addition, OSR staff prepared a brochure which discusses radial keratotomy and describes the PERK study.

The Office helped to organize an NEI workshop on radial keratotomy held in June. When surgeons coming to the meeting invited reporters to attend, the OSR staff responded quickly to provide the requested background materials and access to NEI officials. The staff also drafted the report prepared for distribution to workshop participants after the meeting and answered questions about the PERK study asked by members of Congress and administrative officials of the Federal Government.

In summary, OSR efforts to provide complete, accurate, up-to-date information about radial keratotomy and the PERK study to reporters, prospective patients, health care providers, and interested members of Congress required a major commitment of staff time and energy during the past year.

### Early Treatment Diabetic Retinopathy Study (ETDRS)

Another major undertaking was an information program to acquaint health care professionals, diabetics, and the press with laser treatment for diabetic retinopathy and with the ETDRS, which had just begun full scale patient recruitment. OSR staff collaborated with the Office of Biometry and Epidemiology and the ETDRS Research Group in carrying out the first steps of this information campaign during Fiscal Year 1981.

An exhibit on the ETDRS was displayed at the annual meeting of the American Academy of Ophthalmology. Included in this exhibit was an audiovisual slide program summarizing the aims of the ETDRS and the evidence in favor of photocoagulation as an effective treatment for advanced diabetic retinopathy. An exhibit hand-out was prepared to illustrate patient eligibility criteria. As an ongoing aid to recruitment, copies of the hand-out and slide show were also made available for distribution after the meeting. In addition, the panels from the exhibit have been lent to clinics so that they can display them at local meetings where information about diabetic retinopathy, photocoagulation, and patient recruitment for the ETDRS can be disseminated to health care providers and to the general public through the local media.

Two brochures have been prepared to assist the clinics in alerting physicians to the study and recruiting patients. One of these is a flyer which describes the study and its goals. The other is a more detailed explanation of the aims, risks, and benefits of the study for all patients considering enrollment in the ETDRS, and for their physicians as well.

New fact sheets on the ETDRS and diabetic retinopathy have been produced as a convenient, inexpensive means of conveying information to persons inquiring casually about diabetic retinopathy. And a new public education brochure, Diabetes and Your Eyes, has been prepared to alert diabetics to the ocular complications of their disease and the existence of an effective treatment. These new publications will greatly enhance the Office's ability to handle inquiries about diabetic retinopathy efficiently and to educate reporters, the public, and health care providers about diabetic retinopathy and NEI-supported research in this field.

Physicians across the country were alerted to the onset of full-scale recruitment for the ETDRS by an OSR-produced article which appeared in the Journal of the American Medical Association's "From the NIH" column. And many diabetics learned about the study from stories appearing in Parade, Better Homes and Gardens, Family Health, and other nationally distributed publications. Photographs and background information for these stories were supplied by OSR. The Office also helped to arrange nationwide television news and feature coverage of the ETDRS. For example, one documentary sequence on the study was broadcast nationwide on PM Magazine. Also, an interview with Dr. Fredrick Ferris, ETDRS Project Officer, was aired on a CBS affiliate and transmitted to stations from coast to coast. OSR notified ETDRS clinics around the country that their own CBS stations would be receiving network footage on the study and suggested that they might wish to offer a local tie-in and encourage a broadcast of the interview in their area.

## Scientific Reporting and Knowledge Transfer

OSR involvement with the two clinical trials described above has meant a major commitment of staff time and resources to scientific reporting and knowledge transfer activities during the past year. In addition to the printed and audiovisual materials mentioned, the Office distributed information about other NEI-supported clinical trials and programs to ophthalmologists, optometrists, epidemiologists, and neuroscientists. The Office responded to inquiries from the medical and scientific press and stimulated press coverage of NEI programs, policies, and research results in appropriate journals and scientific publications. Assistance was provided to various components of the Institute seeking to make their programs and projects known to the scientific community and to enlist the cooperation of scientists. The Office also updated a brochure on NEI-supported clinical trials in vision research to provide current information about these studies.

### Consumer Education

The Office of Scientific Reporting expanded its public information and education program during FY 1981. The first steps in a major information campaign on diabetic retinopathy and its treatment were carried out (see Early Treatment Diabetic Retinopathy Study above). These preliminary efforts to obtain both broadcast and print media coverage were extremely successful, and the experience has been helpful in the planning of further efforts in this regard. Progress has been made in enlisting the cooperation and assistance of voluntary organizations and professional societies, which have expressed an interest in helping to promote and distribute a key document in the campaign. The document, Diabetes and Your Eyes, was printed late in the Fiscal Year, and comprehensive plans for its distribution will be implemented next year. The Office also will collaborate with the Audiovisual Branch of NIH on production of several public service announcements featuring diabetic retinopathy and its treatment. New fact sheets on diabetic retinopathy and the ETDRS were prepared and are now being distributed.

In addition to providing information on diabetic retinopathy, radial keratotomy (see Radial Keratotomy above), and a host of other eye disorders, the OSR staff drafted an information brochure on cataract, its treatment, and research advances in the field. Another booklet on macular degeneration is also in draft.

### Press Relations

Efforts by the Office to establish good working relations with reporters have been so successful in recent years that the NEI has become a touchstone for reporters seeking objective information about the eye, treatment of eye disease, and vision research. In many cases, the NEI has no position on the issue raised, and serves merely as a clearinghouse for such inquiries, referring reporters to the appropriate professional societies, Federal agency, or university research team. Because this is perceived to be a valuable service, the groundwork is laid for future contacts with these same reporters.

In addition to responding to requests for information, OSR staff members arrange for reporters to have access to NEI officials, upon request, and stimulate press coverage of NEI clinical trials and research results. The OSR also contributes articles about Institute programs and policies to the NIH Record and to the NIH's Search for Health newspaper series.

## Public Inquiries

As expected, the volume of public inquiries usually increases when the Office is successful in stimulating press coverage of NEI programs and advances in vision research. However, increasing reliance on use of the telephone to respond to inquiries and the availability of new brochures and fact sheets continue to reduce the relative amount of paperwork and OSR's response time for routine inquiries. This, in turn, allows more staff hours to be devoted to other essential information, press relations, and knowledge transfer activities.

## Special Requests

Serving in an advisory capacity to the senior staff of the NEI, the Office of Scientific Reporting is frequently consulted about the impact of NEI policies on the general public, scientific community, and Congress. The Office is also responsible for preparing the NEI Director's Opening Statement before the House and Senate Appropriations committees, editing the transcripts from these hearings, and researching and writing answers to questions asked by committee members. OSR staff prepares the Special Reports to Congress as well and assists in the review and editing of other politically sensitive documents, including the Institute's annual budget statement and the NEI's annual submission to the NIH Diabetes Mellitus Coordinating Committee. The OSR staff members also advise on preparation of scientific manuscripts and audiovisual materials, distribution of information to the scientific community, and responses to Freedom of Information Act requests. The annual Presidential proclamation for Save Your Vision Week is prepared by OSR, and the staff has also collaborated on the development of questionnaires and information materials to be used in conjunction with the national Visual Acuity Impairment Survey.

INTRAMURAL RESEARCH



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

REPORT OF THE SCIENTIFIC DIRECTOR  
Jin H. Kinoshita, Ph.D.

Major changes in the administration of the NEI intramural program have taken place during the past year. Dr. Jin H. Kinoshita has been named the Scientific Director and Dr. Gerald Chader is the new Chief of the Laboratory of Vision Research. In addition, the Laboratory of Molecular and Developmental Biology with Dr. Joram Piatigorsky as its head has been newly created.

The Laboratory of Molecular and Developmental Biology fills a void in the vision research field because there existed no single center for molecular biology of the eye and visual system in the world. The goals of the Laboratory are to understand the genetic and molecular foundations of the eye and examine eye diseases in molecular terms. These goals will be accomplished by applying the rapidly expanding new technologies in the understanding of gene expression and molecular interactions in problems related to the eye. Dr. Joram Piatigorsky is uniquely qualified to lead this group having come from Dr. Philip Leder's National Institute of Child Health and Human Development laboratory, world famous for their pioneering work in molecular biology. Because of Dr. Piatigorsky's extensive contributions to modern molecular biology as applied to eye problems, he is already a recognized leader in this field. The Laboratory adds a new dimension to attack problems in ophthalmic research.

The intramural program which began with two branches at its inception now enters the second decade of existence with four strong components. The two oldest branches are the Clinical Branch headed by Dr. Elmer Ballintine and the Laboratory of Vision Research led by Dr. Chader. The newer branches are the Laboratory of Sensorimotor Research with its Chief, Dr. Robert Wurtz and the Laboratory of Molecular and Developmental Biology. During the next decade, I am certain the members of the intramural program will continue to provide leadership, maintain their preeminent status in the community of vision researchers, and bring much distinction to the NEI.



Clinical Branch



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

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

The program of clinical research has continued to expand with twenty-four approved clinical research protocols now active.

In order to execute this program effectively the Clinical Branch has, during the past year, been reorganized into six sections each with a section head: Ophthalmic Immunology, Dr. Robert Nussenblatt; Retinal and Ocular Connective Tissue Diseases, Dr. David Newsome; Glaucoma, Dr. Douglas Gaasterland; Neuro-ophthalmology, Dr. David Cogan; Clinical Eye Pathology, Dr. Marilyn Rodrigues and Clinical Director's Office.

One of these protocols, "Cyclosporin A Therapy in Uveitis", is the direct culmination of a series of laboratory studies conducted by the Ocular Immunology Section. In these experiments, uveitis developed when Lewis rats were immunized with retinal S-antigen. The severity of the uveitis was determined by the amount of S-antigen injected into the hind foot. It was shown that by varying the dose of cyclosporin A, the uveitis could be ameliorated or abolished even when the treatment with cyclosporin "A" was delayed until the inflammatory reaction had begun. Cyclosporin A is an endecapeptide that has been shown to suppress T-cell activity. Some patients with chronic uveitis had evidence of cellular immunity to S-antigen. Correlation of S-antigen cellular immunity with the chronicity and recurrence of some cases of uveitis support the idea that these patient's disease is the result of autoimmune processes and that T-cell suppression might be beneficial.

The idea that autoimmunity is involved is further supported by the demonstration that HLA B8, an antigen associated with several autoimmune diseases is associated with iridocyclitis in Black Americans.

The clinical trial will accept patients that have bilateral sight-threatening uveitis that is chronic, noninfectious and not controlled with conventional treatment. The patients lymphocytes will be tested against a variety of ocular antigens including purified S-antigen to demonstrate cellular immunity memory. These patients will be treated for six months with cyclosporin A while their clinical, immunologic, and ocular electrophysiologic course will be recorded.

Since some of the patients with chronic uveitis do not show evidence of cellular immunity to S-antigen, and do have a cellular response to crude retinal and uveal extracts, the possibility remains that other components of the retina and uvea may be antigens responsible for an autoimmune response. The search for evidence of a cellular immune sensitivity to other ocular antigens in uveitis patients is being continued.

A clue to the mechanism by which mononuclear cells when stimulated with concanavalin A exert suppressor activity on the proliferation on lymphocytes.

It was shown that supernatants from ConA stimulated peripheral human monocytes suppress the proliferation of retinoblastoma cells in tissue culture. The effect was greater when T-cell enriched fractions were stimulated. The suppressor activity is nondialyzable, heat stable, species specific, and is not interferon.

The Glaucoma Section has continued the development of laser instrumentation and techniques for antiglaucoma surgery. A delivery system for Q-switched ruby laser has been developed that delivers the high power pulses through an articulated arm to a Zeiss slit lamp. The system incorporates appropriate safety features and monitors the energy of each laser pulse. A study of the changes induced in monkey eyes two months after Q-switched ruby laser treatment of the trabecular meshwork was completed. The study demonstrated that the instrumentation was adequate for reliable and reproducible trabecular treatment, the Q-switched ruby laser explodes target tissue, and after healing, the site appears similar to healed argon laser treatment sites. There is little residual physiologic effect after treatment in normal eyes.

The study of the effects on the monkey eye of argon laser glaucoma treatments has been completed. The study demonstrated that there was no damage to the cornea, lens, or retina, and that the post-operative inflammation was transient. There was a small persistent dilation of the pupil after treatment and the intraocular pressure was transiently elevated for a few days in a few of the eyes. There was pitting and scarring of the trabecular site with proliferation of nearby endothelial cells. The outflow facility was not increased above normal in any of the treated animals.

A controlled random assignment trial of trabeculectomy compared to argon laser trabecular treatment was begun. The patients must have evidence of progressive glaucoma despite maximally tolerated medical treatment and inadequate control of the intraocular pressure. The worse eye is assigned to either laser treatment or trabeculectomy by randomization. If the second eye becomes eligible for treatment, it receives the alternate treatment to the one assigned to the first eye. In this study, six trabeculectomies and four laser treatments have been performed. Although the small numbers permit no definite conclusions, it already appears that the pressure lowering effects of the two procedures are similar but the postoperative complications are more frequent in the group receiving trabeculectomy. The long-term complications of laser treatment are not known.

The mechanism by which the beta-adrenergic blocker timolol lowers the intraocular pressure in normal and glaucomatous eyes continues to elude description. A series of observations in normal human volunteers showed that the impressive reduction of intraocular pressure produced by timolol was the result of marked reduction in the rate of aqueous flow. It had been shown previously that topical isoproterenol lowers the intraocular pressure by a similar effect on aqueous flow. When the two drugs were administered simultaneously there was no significant change either in intraocular pressure or in the rate of aqueous flow. A similar result was observed in two patients with ocular hypertension. In summary, both a beta sympathetic agonist and a beta antagonist each produced a reduction of aqueous flow but the two together have no effect. It is difficult to imagine how these effects could be the result of action on a single

group of receptors at a single site. In three pigtail monkeys after unilateral superior cervical ganglionectomy, the effects of timolol were the same in both eyes. Thus the effect of timolol is not dependent upon an intact sympathetic innervation.

The Section on Retinal and Ocular Connective Tissue Diseases has continued to study the defects of collagen, basement membrane, and proteoglycan metabolism in ocular diseases. The methods developed by this laboratory include the incubation of surviving tissue specimens in radiolabeled precursors of the structural elements, deaggregation and extraction of the intact macromolecules, fractionation by molecular sieve chromatography and characterising the isolated glycoconjugates by enzymatic degradation and isolation of radiolabeled fragments. Specific fluorescent antibody to components of extracellular matrix are used to study the localization of components in tissues and tissue cultures. Using these methods, it has been shown that the normal mammalian corneal stroma consists mainly of collagen, a chondroitin sulfate proteoglycan, and a keratan sulfate proteoglycan.

Corneal buttons obtained at surgery during corneal transplantation from patients with macular corneal dystrophy were found to be deficient in the keratan sulfate proteoglycan. The immunologic methods showed that these dystrophic corneas did contain a glycoprotein with a protein core immunologically similar to that of normal keratan sulfate proteoglycan. These observations suggests that the glycoprotein in macular dystrophy corneas may be a precursor to the keratan sulfate proteoglycan.

After corneal grafting, the macular dystrophy may recur in the clear corneal graft. A specimen consisting of both the successful graft which had become dystrophic and a rim of host cornea was obtained at a regrafting operation and subjected to histologic and biochemical study. Stromocytes from host and graft were propagated in tissue culture and their HLA types determined. The results indicated that host stromocytes had not invaded the graft and that the graft stromocytes synthesized normal proteoglycans. There was excessive synthesis of abnormal proteoglycan by host stromocytes and accumulation of this material in the host cornea. Smaller amounts of this material accumulated in the cloudy corneal graft. The practical implication of these observations is that the abnormal proteoglycan that clouds the corneal graft is produced by the remaining rim of host cornea and that a large transplant which would leave a minimum of host corneal tissues may be conducive to a longer period of graft transparency.

In the United States senile macular degeneration is the leading cause of visual disability and impairment less severe than legal blindness. There is no effective treatment for this disease and its continued investigation is a major long-term commitment of the Clinical Branch. As part of this effort, methods are being developed to grow retinal pigment epithelial cells and choroidal cells in tissue culture using media that are free of serum. Although culture of RPE still requires supplementing with an extract of mammalian retina, choroidal cells are being cultured and maintained in serum free medium. Studies with fluorescent antibodies to a variety of extracellular matrix components showed that cultured choroidal cells synthesize and deposit fibronectin whereas pigment

epithelial cells do not. Choroidal cells also deposit significantly more type I collagen in vitro than do pigment epithelial cells. A technique to harvest interphotoreceptor matrix from freshly enucleated primate and human eyes is being developed. The harvested matrix macromolecules are being isolated, identified and compared with those synthesized by pigment epithelial cells in culture. These studies establish methods by which the drusenoid changes in Bruch's membrane that accompany or predispose to macular degeneration can be studied.

A large panel of patients that includes several families having senile macular degeneration in various stages of development is being studied. The extensive programs of psychophysical testing and a variety of measurements of blood and urine chemistry are performed periodically in an effort to find correlations which might give some hint of possibilities for prevention or treatment. Among these data, there are indications that the thyroid function may be abnormal and TSH secretion may be abnormal.

A continuing study of copper metabolism in patients with retinitis pigmentosa has shown that the rate of copper excretion in the urine is normal in 200 patients. These data do not confirm previous reports of elevated copper excretion in retinitis pigmentosa patients in India. It is possible that there is a genetic isolate of RP patients in India that have abnormal copper metabolism or it may be that some aspect of diet may be involved in the high copper excretors.

A review of 62 patients with ocular pigment dispersion syndrome showed that some patients may escape glaucoma indefinitely. In one case glaucoma has not appeared during a twenty year period of observation. When glaucoma does occur, it is not related to the severity of the pigment dispersion. There is an hereditary predisposition in some families: for example, a mother and a daughter two brothers and one son and a brother and sister have the syndrome. The inability to taste phenylthiourea and the elevation of intraocular pressure after topical antiinflammatory steroids do not correlate with the presence or eventual development of glaucoma in these patients, in contrast to what has been reported in patients with ordinary open angle glaucoma. The HLA antigens are normally distributed in these patients. Repeated gonioscopy has shown that pigment dispersed on the surface of the trabecular meshwork may disappear over a period of years. Thus, the glaucoma associated with the pigment dispersion syndrome seems to have a different pathogenesis than does simple glaucoma

The clinical trial of a low arginine, low protein diet to prevent the visual loss in patients having hypo-ornithinemia and gyrate atrophy of the retina and choroid was continued. One patient has been maintained on the diet for 39 months and was found to show an improvement in dark-adaptation, averaged ERG, and color vision testing. In nine additional patients, all had a reduction of plasma ornithine while they were maintained on the diet in the hospital. Upon discharge from the hospital only two continued to maintain consistent reduction in plasma ornithine. Compliance with the inconveniences imposed by the diet are a major difficulty. Detailed study of the characteristics of this condition have revealed hair abnormalities, EEG abnormalities, and tubular aggregates in skeletal muscle.

Tamoxifen is an antiestrogenic substance which has been shown to be useful in the control of some metastatic breast cancers. When given in large doses in

some patients it has produced reduced visual acuity, subepithelial corneal opacities, macular edema, and refractor white deposits in the retina. Review of the affected patients has shown that doses of 10 to 20 milligrams per day do not result in retinal toxicity. Ocular toxicity occurs only at higher doses and after at least 100 grams of the drug have been given. Two eyes having the retinal toxicity were examined histopathologically. The retinal lesions were corpora amylacea like deposits within the axons of ganglion cells.

Vitreotomy, especially for removal of unresolved vitreous hemorrhage in diabetic retinopathy and in the management of infections in the vitreous cavity, is often accompanied by intraoperative complications. One important source of these is organized tissue bands in the vitreous which produce tension on the retina or retinal detachment. When these bands are removed surgically, large retinal tears or detachments may occur because of increased tension in the bands produced by the cutting process. The carbon dioxide laser offers the possibility of cutting these bands without producing tension and without risks of retinal burns. The infrared radiation of the laser (wavelength: 10.6 micrometers) does not penetrate water. Therefore, instruments are under development which will permit the surgeon to bring the infrared beam through an articulated arm and probe directly to the band to be cut. The pulse of laser energy then vaporizes the tissue but does not travel beyond the immediate site of its absorption by the tissue to be cut.

A model for vitreous band has been developed in rabbits and the feasibility of removing them by cutting the CO<sub>2</sub> laser has been demonstrated. Refinement of the delivery system for use in man is in progress.

In cooperation with the Developmental and Metabolic Neurology Branch of NINCDS, groups of patients with inborn errors of metabolism affecting the central nervous system and the visual motor functions of the eye are being studied. Some of these patients seem to have a new syndrome designated DAF, in which there is Down-gaze, paralysis, Ataxia or athetosis, and Foam cells containing large lipid vacuoles distributed in various organs. An additional 30 such cases, some of which were previously labeled with such names as ophthalmoplegic lipidosis, Niemann Pick variant, etc. have been gleaned from the literature. Frequently, the sphingo-myelinase activity in cultured white cells and fibrocytes from these patients has been reduced. The onset of the disease typically is in the first or second decade of life. The course is variable but the earlier the onset, the more severe the ophthalmoplegia and neurologic complications.

Another association has been described in which diseases that are accompanied by a deficiency of high density lipoprotein are predisposed to coronary artery disease and have peculiar lipid infiltrations of the corneal epithelium.

Evidence continues to accumulate that very early in the course of uveitis which is in the process of spreading to and involving the posterior segment of the eye, electroretinographic waveform changes in cone responses and color vision deficiency occur which are reliable indicators that the macular area of the retina is becoming involved. The electroretinographic changes are the result of changes in the responses from red- and green-sensitive cones and they are accompanied by reduction or extinction of responses mediated by blue-sensitive cones. These alterations appear to be specific for inflammatory

disease of immune origin involving the macular region. These results have been confirmed in electrophysiologic study of S-antigen induced posterior uveitis in Old World monkeys.

In a continuing anatomical study of the visual system of primates it has been found that intravitreal injection of the dye procion yellow results in the complete and systematic staining of a cone population in the monkey retina. These cones form a regular mosaic with the separation of the elements increasing with increasing distance from the fovea. A variety of comparisons to other investigations in the literature strongly indicate that these preferentially staining cones are the blue-sensitive ones. Apparently, the dye preferentially injures and eventually destroys these blue cones. The staining is the result of the leakiness of the cone which results from the metabolic injury produced by the dye. Two additional dyes have been found which are electron dense and stain the blue cone population as does procion yellow. The localization of the dye within the cone can then be studied by electronmicroscopy.

It is expected that eventually this method will be developed so that it can produce, in surviving monkeys, retinas which are completely lacking in blue cones. These will serve as a model for some of the situations in man in which retinal disease is accompanied by defects in blue cone function.

Other studies in monkey visual cortex have developed methods for staining that permit precise determination of the borders of the various areas of the cerebral cortex in and surrounding the visual cortex that are characterized by identifiable patterns of neural elements. These include improved methods for myelin and for silver staining of neural cells. With these methods, the extrastriate region V4 has been sharply delimited and a second new visual area within this area demarcated.

The activity labeling of central nervous elements using autoradiographic mapping of the uptake of deoxyglucose has been applied to the study of ocular dominance columns in the monkey striate visual cortex. Visualization of ocular dominant zones in layer 4CB have been visualized by staining for cytochrome oxidase activity using nitro blue tetrazolium.

These architectonic studies have been combined with intracellular and extracellular recordings from single neurons, intracellular staining with fluorescent dyes and extracellular recordings of mass responses to study the functional neural organization of the visual processing areas in the cerebral cortex, particularly with respect to color vision. Recordings from neurons of the V4 areas of the extrastriate visual cortex in monkeys permitted their classification with respect to responses to color and white light stimulation of the eye. Recent reports have claimed that area V4 has a high concentration of color selective cells and that it is specialized for the detailed analysis of color information. This study of a large number of cells showed that the most common ones were those that were not color coded, that about 25 percent of the cells could not be driven with any of the test stimuli used, indicating that they are driven by extraretinal signals, and that the number of color selective cells with either color biased or color opponent properties was not different from that of the foveal parts of the V2 area of the visual cortex. Studies of

the spectral response bandwidth of color opponent retinal ganglion cells showed that the average spectral band widths were not different than published values for higher levels of geniculate cortical pathways including the extrastriate area, V4. These studies indicate that area V4 is not particularly involved with color information processing.

The results of a study of septic chorioretinitis in dogs and pigtail monkeys were accepted for publication. The work was undertaken to investigate the nature of the fundus lesions observed in human patients having bacteremia. The results of this study strongly support the idea that the human lesions are the result of embolization of choroidal and retinal vessels by live bacteria. It is interesting that in the animal model, the severity and number of retinal lesions that resulted from intracarotid injection of *Streptococcus mutans* were not changed by pre or subsequent treatment with antibiotics.

Four corneal buttons obtained at corneal transplantation from patients with hereditary posterior polymorphous dystrophy were examined. The posterior surface of Descemet's membrane has a mixture of epithelial-like and endothelial cells. Cells cultured from the posterior corneal surfaces also were of two distinct types; one epithelial and one endothelial. The epithelial-like cells were positively stained with fluorescent antibody to human epidermal keratin while the endothelial cells were unstained. Other differences were demonstrated by electronmicroscopy.

During the year the Section on Clinical Eye Pathology processed fifty autopsy eyes. One-hundred and seventy eyes were obtained from the Eye Bank and the tissues distributed to investigators in the Clinical Branch and the Laboratory for Vision Research. Fifty-five biopsies and surgical specimens were processed.

Three-hundred and twenty animal eyes and other tissues were processed for investigations related to diabetes, uveitis, and retinal degeneration. Two-hundred and fifty tissue specimens were processed for transmission electron-microscopy. Two-hundred and forty specimens were processed for scanning electronmicroscopy.

There were 1040 outpatient and inpatient visits referred from other Institutes to the NEI clinical facilities. Fifty-nine inpatients were admitted and 15 surgical operations were performed. The Clinical Branch continued to cooperate with other NIH Institutes in pursuit of timely research opportunities. A cooperative study of breast cancer patients continues in cooperation with the National Cancer Institute, the study of diabetic retinopathy in the Pima Indian was continued with the Southwestern Fields Studies Section of NIAMD, a study of the effect of plasmaphoresis and lymphophoresis on the ocular lesions of Graves disease is underway with the investigators in NIAMD, and the Neuroophthalmology Section continues to cooperate in the study of the ocular lesions of patients in the NINCDS Developmental and Metabolic Neurology Branch. The Clinical Branch provides a pediatric ophthalmology representative to the Inter Institute Genetics group. Clinical Branch scientists continue to serve as consultants to the National Institutes on Drug Abuse, Interagency Committee on New Therapies for Pain and Discomfort, and the International Vitamin A Consultative Group.



## PERIOD COVERED

October 1, 1980, to September 30, 1981

## 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. Chief, Section on Glaucoma	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:

1.0

## PROFESSIONAL:

0.6

## OTHER:

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.

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.

Although visual field losses are said to be preceded by glaucomatous changes in the optic discs, in this study we have documented the development of an undoubted glaucomatous visual field loss in the absence of any change in optic disc appearance. A report of one of the cases has been submitted for publication.

Over 120 patients have been examined to determine eligibility, and 35 are under continuing observation following randomization to treatment groups.

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: We expect that the project will continue for at least five years, and that 100 subjects will be randomized to the treatment groups.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00099-03 CB

PERIOD COVERED

October 1, 1980, to September 30, 1981

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

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:

0.3

PROFESSIONAL:

0.3

OTHER:

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. 76 A 6 were examined ophthalmologically with special attention to visual field measurement to reveal chiasmatic 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.

Project Description:

Protocol Number: 76 A 6 (NIAMDD)

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 have 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. 76 A 6 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 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 longstanding diabetes. No elements of diabetic retinopathy were found among 29 patients with serum growth hormone elevated to 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. A comparison of fluorescein angiography with stereo fundus photographs showed that no angiopathy was seen in the angiograms that was not seen on the fundus photographs.

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 interfere with its action on target organs. This study suggests that such attacks on diabetic retinopathy are not likely to be successful. The results also indicate that in epidemiologic studies of early diabetic retinopathy, fluorescein angiography is not likely to increase the sensitivity of detection over that of stereo fundus photographs.

Proposed Course: Patient recruitment and follow up has been completed.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications:

Ballintine EJ, Foxman S, Gorden P, and Roth J: Rarity of diabetic retinopathy in acromegalics. Arch Intern Med (in press).

PERIOD COVERED

October 1, 1980, to September 30, 1981

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
	Daniel G. Seigel	Ph.D.	Deputy Chief, Office of Biometry	OBE	NEI

COOPERATING UNITS (if any)

Office of Biometry and Epidemiology, NFI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

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.

Project Description:

Protocol Number: 75 EI 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 retinal 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. Recruitment has been slow, mainly because the present protocol requires two weeks hospitalization for each patient. The protocol is now being revised to shorten the period of hospitalization. We will continue to recruit until 75 patients have been treated.

Project No. Z01 EY 00022-07 CB

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00083-04 CB
PERIOD COVERED October 1, 1980 to September 30, 1981		
TITLE OF PROJECT (80 characters or less) The Diagnosis, Pathogenesis and Treatment of Gyrate Atrophy of the Choroid and Retina		
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 Francisco de Monasterio M.D. Chief, Section Vial Processing CB NEI		
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: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Patients with <u>gyrate atrophy</u> of the <u>choroid and retina</u> are examined systematically to confirm the diagnosis. Skin fibroblasts of affected patients and family members grown in tissue culture are assayed for ornithine <u>amin- - transferase</u> activity. The results will be examined for correlation with the presence of homo- or heterozygosity for the disease trait. Patients will be given a trial of pyridoxine to see if 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.		

Project Description:

Protocol Number: 78 EI 01

Objectives: To determine the biochemical processes responsible for the elevated serum ornithine and the chorioretinal 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/or 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. Plasma 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 in this study has been followed for 39 months on a low protein, low arginine diet and was found to show an improvement in dark adaptation averaged ERG and color vision testing after 13.5 months on this regime with lowered plasma ornithine levels. Nine additional patients have been placed on the diet. All sustained a significant reduction of plasma ornithine while in the hospital. However, following discharge one patient discontinued the diet, five showed poor control, one showed fair control and two showed good control.

Systemic findings in this condition have been documented and confirmed which include abnormalities of hair, EEG abnormalities and tubular aggregates in muscle.

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 marker and concomitant enzyme 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 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, Walser M, Brusilow SW, Kaiser-Kupfer MI: Gyrate atrophy of the choroid and retina: Amino acid metabolism and correction of hyperornithinemia with an arginine deficient diet. J Clin Invest 65:371-378, 1980.

Kaiser-Kupfer MI, de Monasterio FM, Valle D, Walser M, Brusilow S:  
Gyrate atrophy of the choroid and retina: Improved visual function  
following reduction of plasma ornithine by diet. Science 210:1128,  
1980.

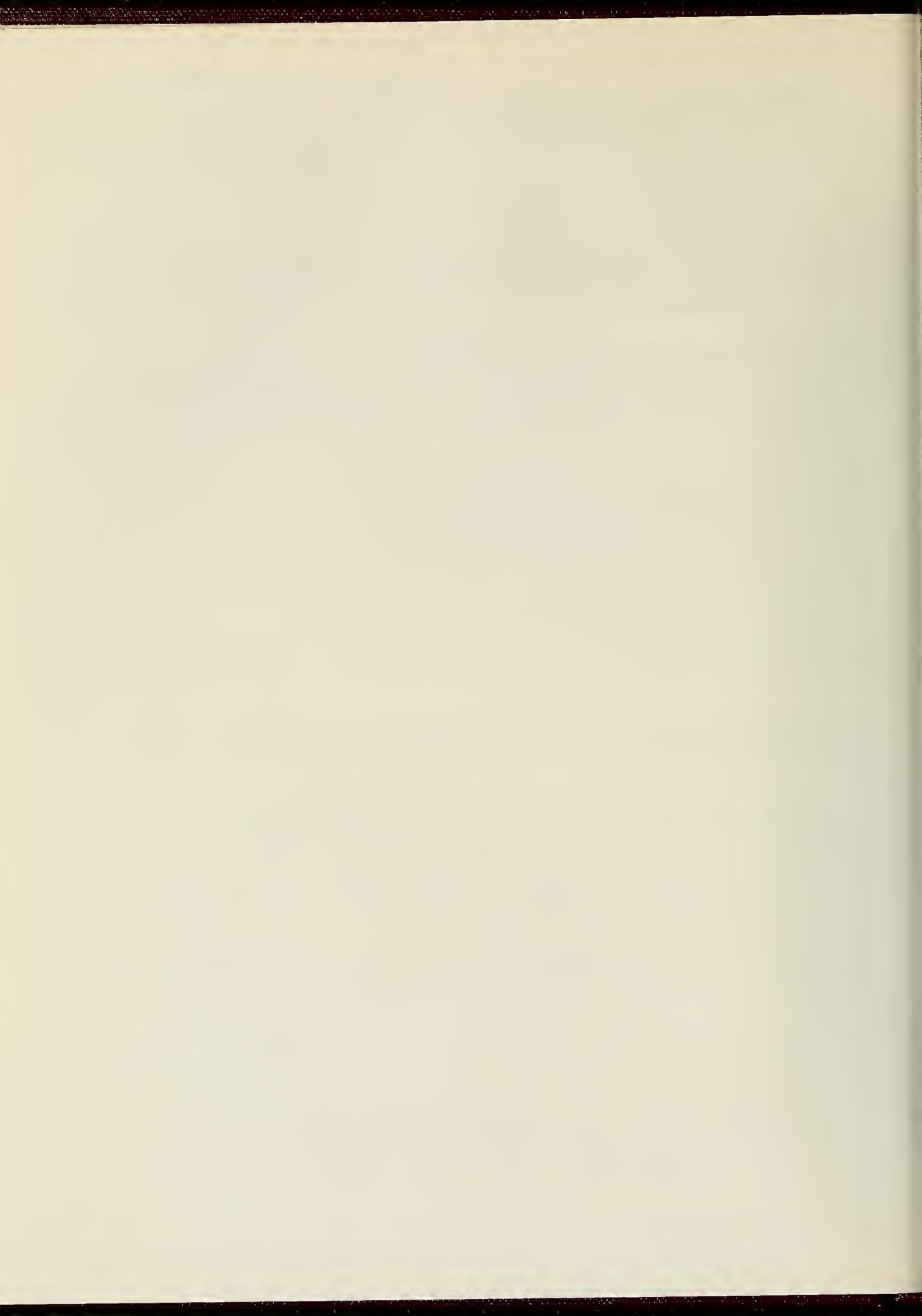
Kaiser-Kupfer MI: Gyrate Atrophy Symposium, Introduction.  
Ophthalmology 88:291, 1981.

Kaiser-Kupfer MI, Kuwabara T, Askanas V, Brody L, Takki K, Dvoretzky I,  
Engel WK: Systemic manifestations of gyrate atrophy of the choroid and  
retina. Ophthalmology 88:302, 1981.

Valle D, Walser M, Brusilow S, Kaiser-Kupfer MI, Takki K: Gyrate  
atrophy of the choroid and retina: Biochemical considerations and  
experience with an arginine restricted diet. Ophthalmology 88:325, 1981.

Kaiser-Kupfer MI, de Monasterio FM, Valle D, Walser M, Brusilow S:  
Visual results of a long-term trial of a low-arginine diet in gyrate atrophy  
of choroid and retina. Ophthalmology 88:307, 1981.

Kuwabara T, Ishikawa Y, Kaiser-Kupfer MI: Experimental model of gyrate  
atrophy in animals. Ophthalmology 88:331, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00085-04 CB																														
PERIOD COVERED October 1, 1980, to September 30, 1981																																
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  <table border="0"> <tr> <td>PI:</td> <td>Muriel I. Kaiser-Kupfer</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>David G. Cogan</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Kamal K. Mittal</td> <td>Ph.D.</td> <td>Research Microbiologist</td> <td>BB</td> <td>DBBP</td> </tr> <tr> <td></td> <td>Barton Haynes</td> <td>M.D.</td> <td>Staff Fellow</td> <td></td> <td>LCI NIAID</td> </tr> <tr> <td></td> <td>Anthony Fauci</td> <td>M.D.</td> <td>Senior Physician</td> <td></td> <td>LCI NIAID</td> </tr> </table>			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
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) Laboratory of Clinical Investigation, NIAID Bureau of Biologics, Food and Drug Administration																																
LAB/BRANCH Clinical Branch																																
SECTION																																
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.7	OTHER: 0.3																														
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this protocol was to determine the phenotype frequency of the <u>HLA and ABO antigens</u> as well as to explore the possibility of <u>altered immune response</u> in patients with <u>Cogan's syndrome</u> .																																

Project Description:

Protocol Number: 77 EI 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 were examined according to a standard set of procedures to confirm the diagnosis. Blood specimens were analyzed for HLA and ABO antigens and a prescribed battery of in vitro immunologic studies.

Major Findings: Patients with Cogan's syndrome did not have a specific HLA type. As a result of this study, a classification of Cogan's syndrome has been possible. Typical Cogan's syndrome is a disease of young adults characterized by flare-ups of interstitial keratitis, sudden onset of Meniere's-like attacks and deafness. The prognosis of typical Cogan's syndrome is excellent with only 10% of the patients developing life-threatening aortic insufficiency. Atypical Cogan's syndrome (vestibuloauditory dysfunction with ocular inflammation other than interstitial keratitis) overlaps with other rheumatologic syndromes and carries a less favorable prognosis being associated with vasculitis in 21% of patients. Treatment of interstitial keratitis consists of topical corticosteroids, and a short trial of systemic steroids is warranted as soon as possible after the onset of hearing loss.

Significance to Biomedical Research and the Program of the Institute: To determine the immunologic basis of an eye disease, clarify prognosis and recommended treatment.

Proposed Course: Project was terminated

NEI Research Program: Corneal Diseases--External Ocular Infections and Inflammatory Diseases

Publications:

Haynes B, Kaiser-Kupfer MI, Mason P: Cogan's Syndrome: Studies in thirteen patients, long term follow up, and a review of the literature. Medicine 59:426, 1980.

Haynes B, Pikus A, Kaiser-Kupfer MI, Fauci A: Successful treatment of sudden hearing loss in Cogan's syndrome with corticosteroids. Arthritis and Rheum 24,3:501-503, 1981.



Project Description:

Objectives: To determine in patients placed on tamoxifen the minimum level at which ocular changes are noted. To determine whether an animal model could be produced in oophorectomized female monkeys.

Methods Employed: All NCI metastatic breast carcinoma patients placed on tamoxifen are examined ophthalmoscopically. 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 prolonged periods. A clinicopathologic correlation became possible and corpora amylacea-like lesions were seen within the axons of the ganglion cells.

Significance to Biomedical Research and the Program of the Institute: It appears that tamoxifen is effective as a chemotherapeutic agent at doses of 10-20 mg and toxicity does not occur until approximately 100 gram has been taken. Therefore, there is a wide margin of safety before one needs to monitor for toxicity.

Proposed Course: The project will continue for one additional year.

NEI Research Program: Retinal and Choroidal Diseases--Toxic, Nutritional and Environmental Disorders

Publications:

Kaiser-Kupfer MI, Kupfer C, Rodrigues M: Tamoxifen retinopathy: A clinicopathologic report. Ophthalmology 88:89, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00011-07 CB

PERIOD COVERED

October 1, 1980, to September 30, 1981

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
	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:

0.8

PROFESSIONAL:

0.4

OTHER:

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)

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.

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<sub>2e</sub> and I<sub>4e</sub>)  
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 (2 1/2% 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 one son, 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.

More than 62 patients are currently enrolled in the study. Four patients have been demonstrated to have unilateral involvement. Two

patients have very marked evidence of PDS with deep anterior chambers, marked transillumination and heavy pigment in the angle structures. However, neither have developed elevated intraocular pressure.

Significance to Biomedical Research and the Program of the Institute:

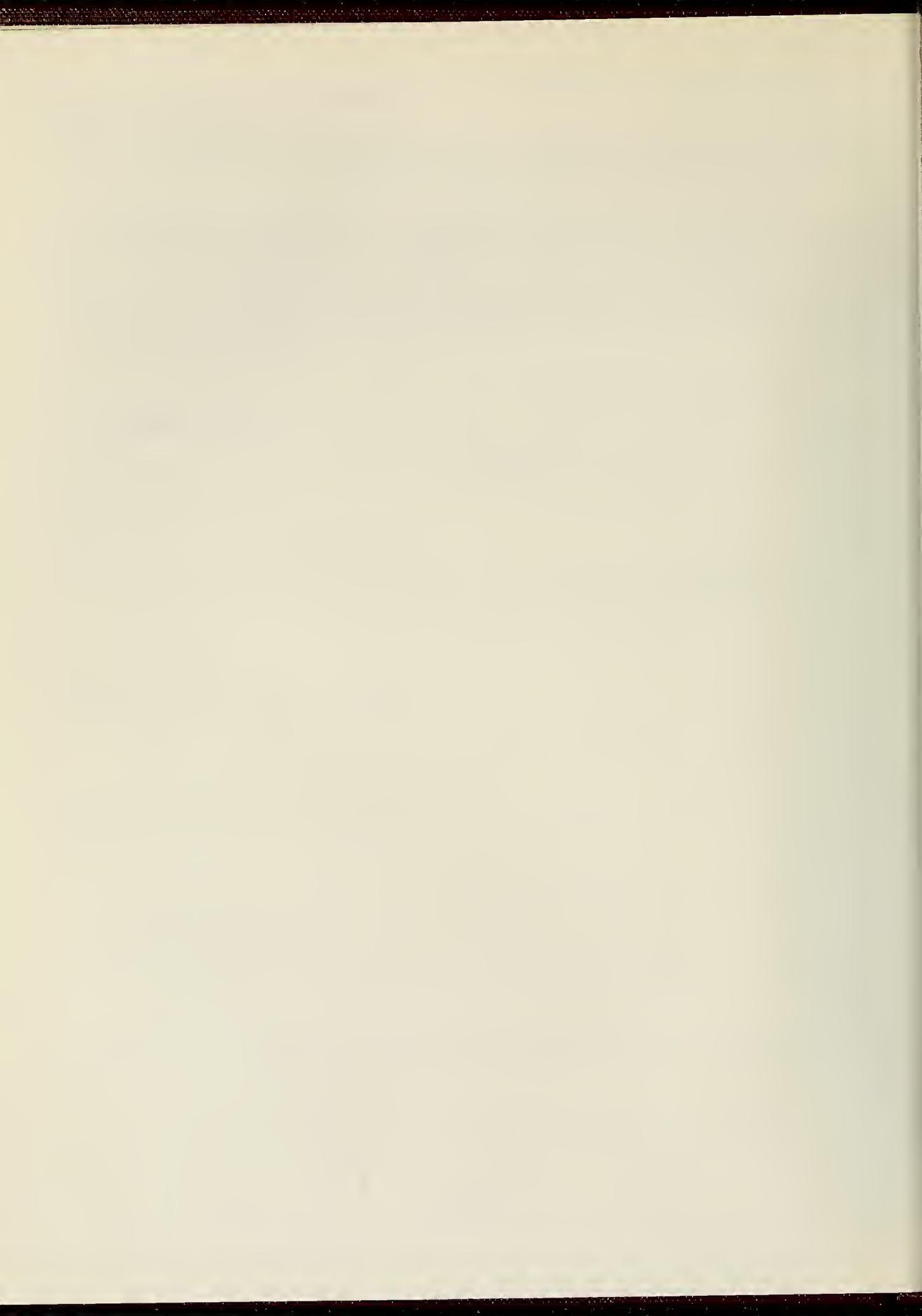
These data may enable a determination to be made of the risk of patients having pigment dispersion to develop glaucoma. Specifically, it may be possible to identify which features of these determinations have predictive value in forecasting which of those patients having pigment dispersion will develop 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--Developmental, Congenital, or Infantile Glaucoma

Publications:

Kaiser-Kupfer MI: Clinical research methodology in ophthalmology.  
Trans Am Ophthalmol Soc 128:896, 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00062-05 CB															
PERIOD COVERED October 1, 1980, to September 30, 1981																	
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  <table style="width:100%; border: none;"> <tr> <td style="width:30%;">PI:</td> <td style="width:40%;">Muriel I. Kaiser-Kupfer</td> <td style="width:20%;">M.D. Senior Staff Ophthalmologist</td> <td style="width:10%;">CB</td> <td style="width:10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Carl Kupfer</td> <td>M.D. Director</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Lessie McCain</td> <td>R.N. Clinical Technical</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Muriel I. Kaiser-Kupfer	M.D. Senior Staff Ophthalmologist	CB	NEI	Other:	Carl Kupfer	M.D. Director	CB	NEI		Lessie McCain	R.N. Clinical Technical	CB	NEI
PI:	Muriel I. Kaiser-Kupfer	M.D. Senior Staff Ophthalmologist	CB	NEI													
Other:	Carl Kupfer	M.D. Director	CB	NEI													
	Lessie McCain	R.N. Clinical Technical	CB	NEI													
COOPERATING UNITS (if any) None																	
LAB/BRANCH Clinical Branch																	
SECTION																	
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																	
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.2	OTHER: 0.3															
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (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 <u>aqueous humor dynamics</u> in both affected and unaffected eyes, and to attempt to find <u>genetic markers</u> such as <u>HLA and ABO antigens</u> or physical correlates with the disease process.																	

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<sub>2e</sub> and I<sub>4e</sub>)  
Photography of iris and iris transillumination  
Gonioscopy and gonioscopy photography  
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--Developmental, Congenital, or Infantile Glaucoma

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 BY 00060-05 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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: 1.2	PROFESSIONAL: .04	OTHER: .08
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  Patients with <u>hypomelanotic disorders</u> such as <u>ocular albinism</u> , <u>oculocutaneous albinism</u> , <u>Chediak-Higashi Disease</u> , <u>Hermansky-Pudlak Syndrome</u> and <u>iris transillumination defects</u> 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.		

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 color, 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 AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00084-03 CB
--	--	--

PERIOD COVERED  
October 1, 1980, to September 30, 1981

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
	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: 0.3	PROFESSIONAL: 0.1	OTHER: 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)

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.

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, lens 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--Developmental, Congenital, or Infantile Glaucoma

Publications:

Russell P, Uga S, Zigler JS, Kaiser-Kupfer MI, Kuwabara T: Studies using human lenses from a family displaying hereditary congenital cataracts. Vision Res 21:169, 1981.

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 FY 00120-01 CB																		
PERIOD COVERED October 1, 1980, to September 30, 1981																				
TITLE OF PROJECT (80 characters or less)  Laser Instrumentation for Vitreous Surgery																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0"> <tr> <td>PI:</td> <td>Sanford M. Meyers</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Robert F. Bonner</td> <td>Ph.D.</td> <td>Physicist</td> <td>DRS</td> <td>BEIB</td> </tr> <tr> <td></td> <td>Stephen B. Leighton</td> <td>Ph.D.</td> <td>Engineer</td> <td>DRS</td> <td>BEIB</td> </tr> </table>			PI:	Sanford M. Meyers	M.D.	Senior Staff Ophthalmologist	CB	NEI	Other:	Robert F. Bonner	Ph.D.	Physicist	DRS	BEIB		Stephen B. Leighton	Ph.D.	Engineer	DRS	BEIB
PI:	Sanford M. Meyers	M.D.	Senior Staff Ophthalmologist	CB	NEI															
Other:	Robert F. Bonner	Ph.D.	Physicist	DRS	BEIB															
	Stephen B. Leighton	Ph.D.	Engineer	DRS	BEIB															
COOPERATING UNITS (if any) Division of Research Services Biomedical Engineering Instrumentation Branch																				
LAB/BRANCH Clinical Branch																				
SECTION																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																				
TOTAL MANYEARS: 0.60	PROFESSIONAL: 0.35	OTHER: 0.25																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords)  A <u>carbon dioxide laser</u> with a special delivery system for use in <u>vitreous surgery</u> has been developed and is undergoing testing in animals. Preliminary data reveal that the carbon dioxide laser instrument appears beneficial in certain aspects of vitreous surgery.																				

Project Description:

Objectives: This study is designed to develop and test the efficacy of a carbon dioxide laser instrument for use in vitreous surgery.

Methods Employed: A carbon dioxide laser with a special delivery system adapted for use in vitreous surgery is being developed. The safety and efficacy of the prototype unit will be determined in rabbits and monkeys with vitreal membranes. In creating the vitreal membranes in these animals, standardly accepted methods will be used. The findings will be documented with photography during surgery. The animal eyes will be examined clinically and pathologically at selected times after surgery.

Major Findings: Preliminary data reveals that the carbon dioxide laser can cut experimentally created membranes in rabbits.

Significance to Biomedical Research and the Program of the Institute: Although the present mechanical vitrectomy instruments perform well in most cases, there is a risk of intraoperative complications (retinal tears and hemorrhage) when vitreal membranes are cut, especially if the membranes are taut and have a strong adhesion to the retina. "Tension" on the membranes is increased as the "cutter" of the vitrectomy instrument or the vitreous scissors cuts the tissue. This tension is transmitted to the vitreoretinal adhesion and surrounding retina predisposing this area to retinal tears and hemorrhage. The carbon dioxide laser vitrectomy may decrease the incidence of intraoperative complications and increase the facility in cutting vitreal membranes.

Proposed Course: If further investigation of the prototype laser in animals documents the efficacy and safety of the instrument in vitreous surgery, a clinical trial in selected patients in need of vitreous surgery will be initiated over the next three years.

NEI Research Program: Retinal and Choroidal Diseases--Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities/  
Retinal Detachment and Vitreous Disorders

Publications: None.



Project Description:

Objectives: This study is designed to correlate the clinical and pathologic features in the dog model of septic chorioretinitis, to investigate the pathophysiologic mechanisms involved in this model, and to determine if similar fundus lesions occur in pigtail monkeys.

Methods Employed: Mongrel dogs and pigtail monkeys (Macaque Nemestrina) will undergo carotid injection of certain bacteria. After preliminary results it was decided to use a dextran producing strain of Streptococcus mutans in most of the experiments because it consistently caused fundus lesions with only minimal systemic effects. Fundus lesions will be documented with fundus photography and correlated with the histopathologic findings. To investigate the pathophysiologic mechanisms involved in this animal model, various bacteria will be used and the effects of antibiotics and altering dextran production of a dextran producing strain of Streptococcus mutans will be studied.

Major Findings: Carotid injection of certain bacteria consistently cause fundus lesions in dogs and pigtail monkeys. The major pathophysiologic mechanism appears to be embolization of choroidal and retinal vessels by "live" bacteria, which clump and adhere well to tissues. In the dosages used antibiotics did not prevent or alter the severity of the fundus lesions.

Significance to Biomedical Research and the Program of the Institute: The chorioretinal lesions observed in this animal model resemble the fundus lesions which have been described in human cases of bacteremia unassociated with diabetes mellitus, blood dyscrasias, hypertension, or collagen vascular diseases. The preliminary data in this study support the hypothesis that the fundus lesions observed in human cases of bacteremia result from embolization by "live" bacteria. Thus, it appears that a detailed fundus examination can be helpful in assessing the extent of a systemic infection and new fundus lesions may signify recurrent infection or incomplete treatment. Additionally, fundus lesions, at times, may be the initial sign of a systemic infection.

Proposed Course: This project will continue for the next year to conclude the ongoing studies in the pigtail monkeys.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Meyers SM, Vasil ML: Septic choroiditis with serous retinal detachment in Streptococcus mutans-injected dogs. Infect Immun 29:714-718, 1980.

Meyers SM, Vasil ML, Yamamoto L: Pathophysiology of multifocal choroiditis with retinal detachment after carotid injection of Streptococcus mutans and other bacteria in dogs. Invest Ophthalmol Vis Sci (in press).

CYTHOSOLIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00096-03 CB
--	--	--

PERIOD COVERED  
 October 1, 1980 to September 30, 1981

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.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	Patricia Donohoo	M.S.	Biologist	CB	NEI
	Joseph Hackett	B.S.	Biologist	CB	NEI
	Reginald Gaskins		Histologist	CB	NEI
	Nicole Newman		Histologist	CB	NEI

COOPERATING UNITS (if any)  
 Wills Eye Hospital, Philadelphia  
 Department of Ophthalmology, University of Louisville, Louisville, Kentucky

LAB/BRANCH  
 Clinical Branch

SECTION  
 Section on Clinical Eye Pathology

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

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 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)

Patients with localized ocular diseases or with ocular manifestations of systemic disease are examined clinically, and photographic documentation is made of significant findings. 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 glaucoma, ocular and adnexal tumors, vitroretinal membranes, ocular manifestations of systemic diseases, and laser-induced ocular lesions. Histological studies are also performed on normal human and rhesus monkey cornea, iris, and trabecular mesh-work and include scanning and transmission microscopy of tissue specimens as well as of cell cultures.

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 is also helpful in the diagnosis of certain conditions.

Methods Employed: Specimens are obtained from patients at the National Eye Institute as well as 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 immunological studies. In other cases, routine histopathology is performed.

Major Findings:

I. Histologic studies of selected normal human ocular tissues

Scanning and transmission electron microscopy was 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

A. Corneal involvement in 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.

B. Corneal manifestations in monoclonal gammopathy

One patient had bilateral superficial corneal stromal opacities that resembled Reis-Buckler dystrophy clinically. The other had deep stromal lesions. Electron microscopy revealed extracellular parallel linear deposits. Immunoelectrophoresis of serum and urine in both cases disclosed elevated Kappa light chains. X-ray and bone marrow examinations were normal. In other patients, the corneal lesions were the first clue to the systemic diseases.

III. Glaucoma

A. Primary open-angle glaucoma

i. Twenty-five trabeculectomy specimens from patients with

primary open-angle glaucoma or chronic angle closure glaucoma, and eleven age-matched controls were examined by immunofluorescence and immunoperoxidase techniques to determine the types of collagen, immunoglobulins, and the presence of factor VIII-related antigen in the human aqueous drainage channels. In the glaucoma cases and in controls we demonstrated that the electron dense basement membrane-like material in the peripheral portion of the trabecular beams and in the juxta-canalicular meshwork, consists at least in part, of type IV collagen, a noncollagenous protein ("laminin") and fibronectin. Factor VIII-related antigen was demonstrated in conjunctival vessels of the control eyes. Schlemm's canal and the trabecular endothelial cells did not stain for factor VII-related antigen in any of the specimens examined. No deposits of IgA, IgM, IgG, and the C3 component of complement were detected in the aqueous drainage channels.

ii. Electron microscopy of argon laser therapy in open-angle glaucoma

Scanning and transmission electron microscopy were performed on trabeculectomy specimens from patients with medically uncontrolled progressive primary open angle glaucoma. Trabeculectomy specimens were obtained three hours to one year after continuous argon laser trabeculopexy. The laser-treated sites showed irregular areas of disrupted or obliterated trabecular beams. In more recent burns, fibrinous material and occasional macrophages were present. Specimens examined after a longer interval following laser showed considerable fibrosis at the treated sites. The laser burns primarily involved superficial and midtrabecular meshwork and did not extend to Schlemm's canal.

B. 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 Descemet's 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.

C. Glaucoma associated with endothelialization of the trabecular meshwork in two cases of posterior polymorphous dystrophy

The cells lining the trabecular meshwork disclosed features of epithelial-like cells with desmosomal junctions, scant mitochondria and numerous microvillus projections. These cells were a direct extension from the corneal endothelium which also exhibited similar features.

IV. Studies on ocular lesions associated with systemic diseases

Conjunctival biopsies from patients with Gaucher's disease showed elastoid degeneration without evidence of Gaucher cells.

Light and electron microscopy were performed on lesions from patients with midline and granuloma, Behcet's disease, allergic conjunctivitis, cutaneous leishmaniasis, ocular anomalies including 13 trisomy and optic nerve pit.

V. Vitreoretinal disorders

A. Vitreoretinal membranes

Vitreoretinal membranes were examined in culture from cases 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.

B. Corpora amylacea of the optic nerve and retina

These deposits were studied with histochemical stains and electron microscopy, and were shown to represent products of axonal degeneration.

Significance to Biomedical Research and the Program of the Institute:

These studies are directly concerned with mechanisms involved in primary and secondary glaucoma, corneal, conjunctival and retinal as well as ocular manifestations of systemic diseases.

Proposed Course: These projects will continue in the next fiscal year.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma; Corneal Diseases--External Ocular Infections and Inflammation Diseases; Retinal and Choroidal Diseases--Diabetic Retinopathy and Other Vascular and Circulatory Disorders.

Publications:

Rodrigues M, Katz S, Foidart J, Spaeth G: Collagen, Factor VIII antigen and immunoglobulins in the human aqueous drainage channels. Ophthalmology 87:337, 1980.

Rodrigues M, 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 98:688, 1980.

Eiferman R, Rodrigues M: Unusual superficial stromal corneal deposits in IgG monoclonal gammopathy. Arch Ophthalmol 98:78, 1980.

Rodrigues M, Waring G, Hackett J, Donohoo P: Histology of the normal human cornea, in Duane D and Jakobiec F (eds): Histology of the Eye. Hagerstown, Harper and Row (in press).

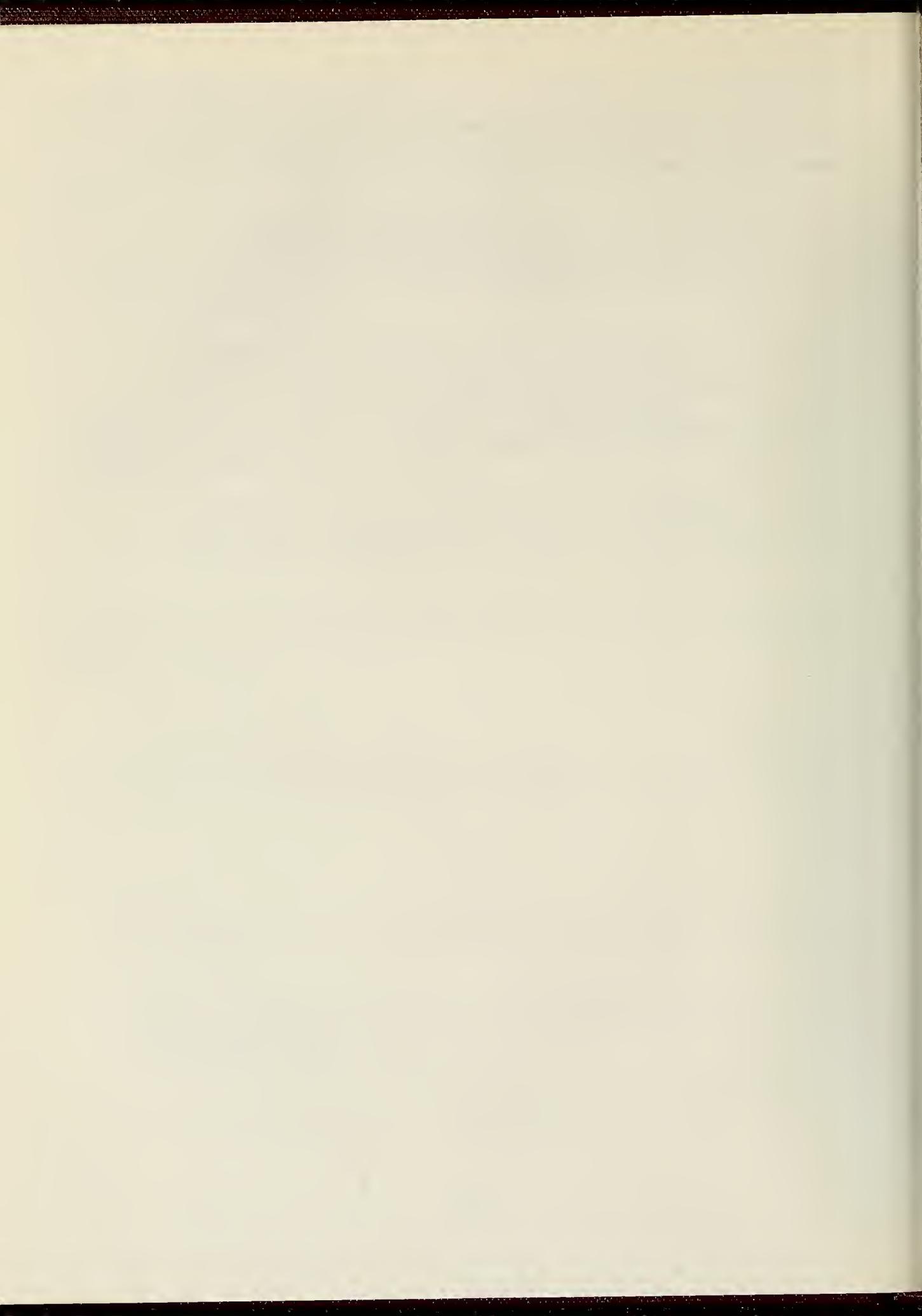
Rodrigues M, Spaeth G, Donohoo P: Electron microscopy of argon laser therapy in open-angle glaucoma. Ophthalmology. (in press).

Newsome D, Rodrigues M, Macheimer R: Human massive periretinal proliferation: In vitro characteristics of cellular components. Arch Ophthalmol 99:873, 1981.

Rodrigues M, Gaasterland D: Current concepts in the pathology of the glaucomas, in Nicholson D (ed). Ocular Pathology Update. New York, Masson Publishing Company 1980, pp 55-62.

Avendano J, Rodrigues M, Hackett J, Gaskins R: Corpora amylacea of the optic nerve: A form of neuronal degeneration. Invest Ophthalmol Vis Sci 19:50, 1980.

Waring G, Rodrigues M: Ultrastructure and successful keratoplasty of sclerocornea in Mieten's syndrome. Am J Ophthalmol 90:467, 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00114-01 CB
--	--	--

PERIOD COVERED  
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)  
Histopathologic Studies of Animal Models 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.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	Robert Nussenblatt	M.D.	Chief, Section on Clinical Ophthalmic Immunology	CB	NEI
	Carol Carrier	M.D.	Senior Staff Fellow	CB	NEI

COOPERATING UNITS (if any)

LAB/BRANCH  
Clinical Branch

SECTION  
Section on Clinical Eye Pathology

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

TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 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 Lewis rat was used to study the induction of autoimmune uveitis, particularly induced by retinal S-antigen. Modulation of the immune response occurred with cyclosporin A. Another model of virus-induced diabetes mellitus showed oculo-renal changes that were similar to human diabetes.

Project Description:

Objectives: Light and electron microscopic examinations of eye and other tissues in these models provide essential information related to the course of the disease and the type of cellular inflammation response.

Methods Employed:

1. Modulation of experimental autoimmune uveitis with cyclosporin A: Lewis rats were immunized with a total of 30 $\mu$ g of bovine S-Ag injected into the hind footpads. Animals were treated with varying doses of cyclosporin A (CsA) administered for different intervals. The eyes were processed for light and electron microscopy.

2. Virus-induced diabetes in mice: (DBA, NZB and SJL strains) Mice infected with the D (diabetogenic) variant of the M strain of the encephalomyocarditis (EMC) virus resulted in diabetes monitored by hyperglycemia and glycosuria within one month. Light microscopic examination was performed on the eyes and kidneys.

Major Findings:

1. Modulation of experimental autoimmune uveitis with CsA: CsA was capable of totally preventing the appearance of experimental autoimmune uveitis even when administered on alternating days in lower doses (10mg/k) or when begun 7 days after immunization (40 mg/kg). Lower doses of CsA resulted in a modulation of the disease from acute inflammation to a more chronic, granulomatous reaction. Electron microscopy clearly documented the changes in cellular responses.

2. Oculo-renal changes in EMC virus-induced diabetes mellitus in mice: Diabetic mice with the longest duration (six months) of diabetes showed the most marked alterations. Fasting blood sugar levels were 320-395 mg/dl and glucosuria was present. Clinically, based on ophthalmoscopy and fluorangiograms retinal vessels were normal; the only abnormality was decreased numbers of pericytes by trypsin digestion. Corneal epithelial edema was present and surface microvillous projections were decreased compared to controls. The kidneys of the same diabetic animals showed nodular and diffuse glomerulosclerosis and mesangial thickening similar to human Kimmelstiel Wilson disease. Histologically, moderate to advanced kidney disease was associated with relatively early retinopathy.

Significance to Biomedical Research and the Program of the Institute: These studies have direct clinical applications. In the first model (CsA in experimental autoimmune uveitis), CsA could be an effective therapeutic agent for T-cell mediated intraocular inflammation. In the second model (virus-induced diabetes mellitus) the renal and ocular changes appear similar to those seen in the human disease and will be investigated further.

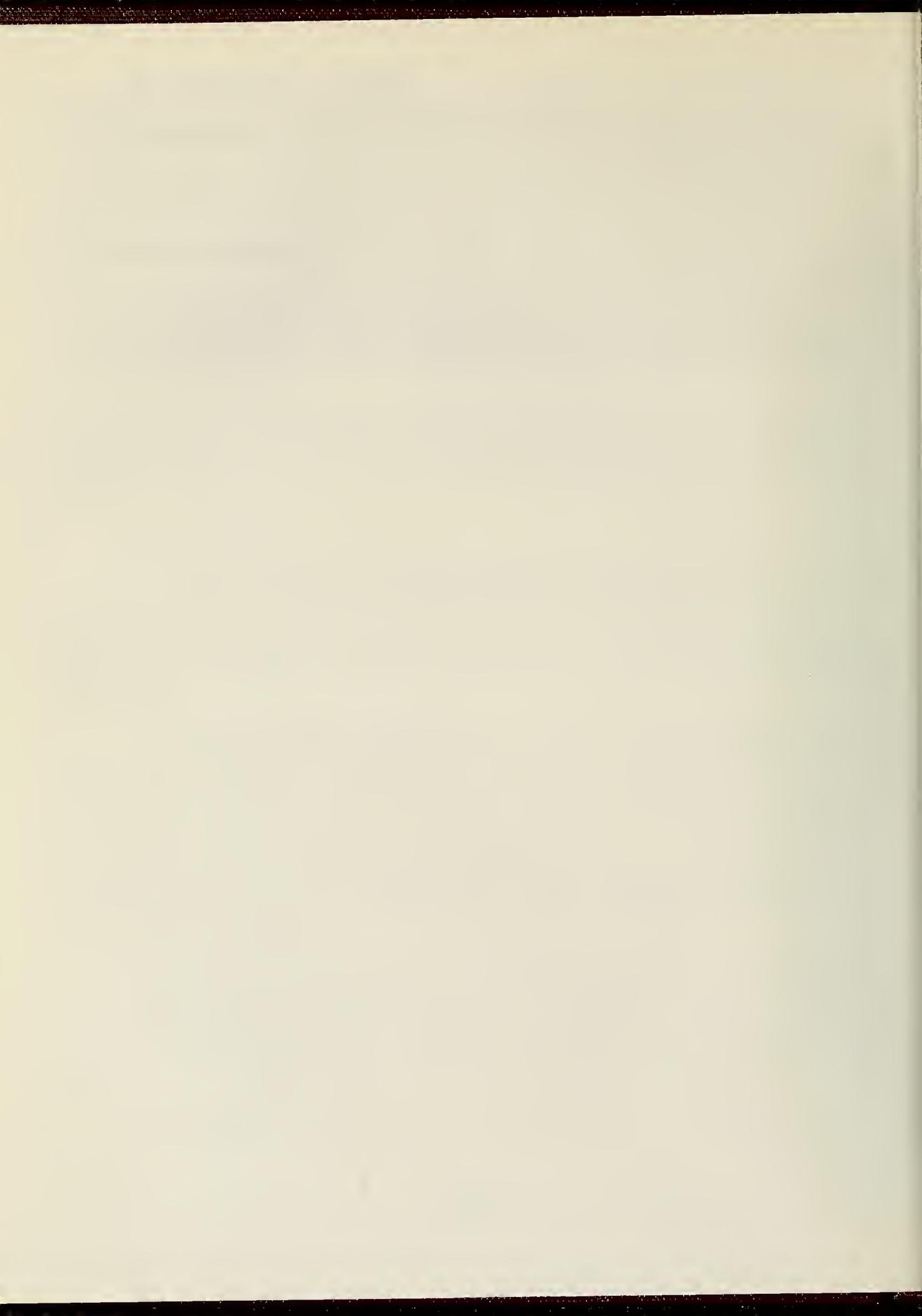
Proposed Course: These projects will continue in the next fiscal year.

NEI Research Program: Retinal and Choroidal Diseases; Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models; Diabetic Retinopathy; Inflammatory Disorders.

Publications:

Rodrigues MM, Currier C, Yoon J: Renal and ocular involvement in encephalomyocarditis (EMC) virus-induced diabetic mice. Fed Proc (Abstract) 40:741, 1981.

Nussenblatt RB, Rodrigues MM, Wacker WB, Cevalario SJ, Salinas-Carmona M, Gery I: Cyclosporin A. Inhibition of experimental autoimmune uveitis in Lewis rats. J Clin Invest 67:1228, 1981



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER  
Z01 EY 00078-04 CB

PERIOD COVERED  
October 1, 1980 to September 30, 1981

TITLE OF PROJECT (80 characters or less)  
Histopathology and In Vitro Characteristics of Human Corneal Dystrophies and Degenerations

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.	Chief, Section on Clinical Eye Pathology	CB	NEI
Other:	David Newsome	M.D.	Chief, Section on Retinal and Ocular Connective Tissue Diseases	CB	NEI
	Joseph Hackett	B.S.	Biologist	CB	NEI
	Patricia Donohoo	M.S.	Biologist	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 Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 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)

Human corneal dystrophies and degenerations, which have been clinically documented are studied as keratoplasty specimens with histochemical stains, scanning 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. In patients with macular corneal dystrophy intracellular and extracellular accumulation of fibrillogranular material was observed in the corneal stroma, Descemet's membrane and corneal endothelium. The presence and production of collagen, glycoconjugates, and collagenase have been investigated with immunofluorescent, electrophoretic, and chromatographic methods. Electron microscopic studies were performed on keratoconus and pellucid degeneration.

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 are divided into portions and used separately for light, scanning, and transmission electron microscopy. These data provide insight into the morphological appearance of the cells and the extracellular materials of the corneal layers. Other portions of the surgical specimens are 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 provide information about the collagen and glycoconjugate biosynthetic patterns of abnormal tissues.

Major Findings:

A. Corneal Dystrophies: 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 microvillus 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. The epithelial-like cells stained positive with antibody to human epidermal keratin, while the endothelial cells were unstained. 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 patients 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.

B. Corneal Degenerations: Keratoconus specimens had the same range of collagen types as normal cornea, with predominantly 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. Two patients with pellucid corneal degeneration showed thinned corneas inferiorly with no evidence of vascularization. Light and electron microscopy of a corneal button from each patient revealed irregularity of the epithelium in the peripheral thinned areas with a normal Bowman's layer in one case and focal dehiscences in the other. Marked thinning of the corneal stroma accompanied by the presence of a small number

of histiocytes was present peripherally in both cases. Descemet's membrane and endothelium were normal. Stromal collagen was normal in diameter and periodicity. In one case, CM-cellulose and <sub>3</sub>SDS gel profile of the collagens synthesized by these stromocytes in vitro (<sup>3</sup>H proline label) was similar to those of control corneas and keratoconus specimens. Collagenase activity levels in the culture medium from explanted pellucid tissue were comparable to or slightly higher than those observed in keratoconus. Pellucid degeneration may represent a peripheral form of keratoconus.

In vitro studies on normal human corneal and scleral collagens showed that human scleral collagen influences corneal collagen fibril formation. The collagenous component of connective tissues from many parts of the body including the eye is heterogeneous with respect to collagen types. Corneal collagen is largely type I with small amounts of types III and V. Scleral collagen, in contrast, has a very large proportion of type III with much larger diameter fibrils than those of cornea. We examined fibril formation in vitro of pepsin-acetic acid extracted and purified preparations of human ocular (types I and III) and placental (type V, gift from Dr. J-M Foidart) collagens alone and in various combinations. Solutions of collagens were prepared in 0.15 M phosphate 0.01 M tris, gelled at 37°C, centrifuged into pellets, fixed in glutaraldehyde and examined by scanning and transmission electron microscopy. Fibril diameters varied with type III and the largest (175 to 190 nm) and type I and V smaller (70 to 90 nm). Mixtures (1:1 v/v) of types I and III and I and V contained an intermediate sized fibril of about 140 nm plus some small and some large fibrils. Mixtures produced a marked heterogeneity of fibril diameters as compared with pure gels. These results reflect the collagen fibril diameter patterns seen in vivo in cornea and sclera, and demonstrate the important effect of collagen type mixture on uniformity and size of collagen fibrils.

#### 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 pathogenic 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 Dystrophies, Inherited Disorders and Developmental Anomalies.

#### Publications

Rodrigues M, Waring G: Anterior and posterior corneal dystrophies, in Klintworth G, Garner A (eds.): Pathobiology of Ocular Diseases. New York, Merce! Dekker Co. (in press).

Newsome D, Foidart J-M, Hassell J, Krachmer J, Rodrigues M, Katz S:  
Detection of specific collagen types in normal and keratoconus corneas.  
Invest Ophthalmol Vis Sci 20:738, 1981.

Rodrigues M, Newsome D, Krachmer J, Sun T-T: Posterior polymorphous  
dystrophy: Cell culture studies. Exp Eye Res (in press).

Hassell J, Newsome D, Krachmer J, Rodrigues M: Macular dystrophy: Failure  
to synthesize a mature keratan sulphate proteoglycan. Proc Natl Acad Sci  
USA 77:3750, 1980.

Mannis M, Fiori C, Krachmer J, Rodrigues M, Pardos G: Keratopathy  
associated with intracorneal glass. Arch Ophthalmol 99:850, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00050-05 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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. Chief, Section on Glaucoma CB NFI Other: Lessie McCain R.N. Clinical Technician CB NFI		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Clinical Branch		
SECTION Section on Glaucoma		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.02	PROFESSIONAL: 0.02	OTHER: 0.00
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The <u>aqueous humor flow</u> in humans is measured by determining the rate of loss of <u>fluorescein</u> from the eye after <u>iontophoresis</u> into the cornea in <u>normal volunteers</u> and in <u>patients</u> with <u>ocular hypertension</u> or <u>glaucoma</u> .		

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 polyacrylamide 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 slitlamp 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 1981 was the fourth year of this project.

Calculation of aqueous humor flow by fluorometric decay after iontophoresis can be done using the Mayo Clinic/Brubaker nomographic technique only if the original iontophoretic spot on the cornea is sufficiently large to avoid an excessively high concentration of fluorescein between the epithelial cells. We have been using an iontophoretic spot of 2.5 millimeters diameter with approximately .1 to .2 micrograms of fluorescein applied. The Mayo Clinic group has been using a 4 millimeter diameter iontophoretic spot with the same amount of fluorescein. Attempts to analyze our data using their technique proved to be impossible. The original total mass of fluorescein in the cornea in our studies cannot be determined from the Mayo Clinic equations. Without this information the subsequent steps in the calculation could not be employed. Thus, with our iontophoresis technique we have learned we must confine our analysis to using the Jones and Maurice methods as originally described in 1965.

Significance to Biomedical Research and the Program of the Institute: The aqueous humor flow rate is a primary determinant of the intraocular pressure. This accurate, safe, reproducible, noninvasive, direct determination of the flow in humans under normal and pathological conditions is leading to increased understanding of glaucoma and hypotony.

Proposed Course: The studies will continue.

NEI Research Program: Glaucoma--Aqueous Humor Dynamics: Inflow/Aqueous Humor Dynamics: Outflow

Publications:

Gaasterland DF: Discussion of papers by RF Brubaker and S Nagataki. Ophthalmology 88:287, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00154-08 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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 F. Gaasterland M.D. Chief, Section on Glaucoma CB NEI Other: None		
COOPERATING UNITS (if any)  None		
LAB/BRANCH Clinical Branch		
SECTION Section on Glaucoma		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.02	PROFESSIONAL: 0.02	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)  <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this investigation is to study the <u>morphology</u> , <u>physiologic function</u> , and <u>pharmacologic responses</u> in the eye of the rhesus monkey in its <u>normal state</u> compared to its state when <u>experimental glaucoma</u> has been induced by argon laser photocoagulation of the <u>trabecular meshwork</u> .		

Project Description:

Objectives: To study physiologic function, pharmacologic responses, 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 radiiodinated serum albumin injected into the anterior chamber. Retinal and optic nerve function are studied clinically and by autoradiography and morphologically to evaluate evidence of altered axoplasmic flow. The retina is also 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 1981 one eye of one additional monkey was treated. The eye received a single set of confluent applications of photocoagulation to eleven hours of the circumference of the trabecular meshwork. During the first month thereafter no elevation of intraocular pressure occurred. The inflammatory response cleared within two weeks. Modest anisocoria was noted, with the pupil in the treated eye being larger than the normal eye.

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 is being completed.

NEI Research Program: 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 00046-05 CB																																				
PERIOD COVERED October 1, 1980, to September 30, 1981																																						
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  <table border="0"> <tr> <td>PI:</td> <td>Douglas E. Gaasterland</td> <td>M.D.</td> <td>Chief, Section on Glaucoma</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>John A. Barranger</td> <td>M.D.</td> <td>Chief, Clinical Section</td> <td>DMNB</td> <td>NINCDS</td> </tr> <tr> <td></td> <td>Toichiro Kuwabara</td> <td>M.D.</td> <td>Chief, Section on Experimental Pathology</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Erik Linner</td> <td>M.D.</td> <td>Visiting Scientist</td> <td>CR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Roy Milton</td> <td>Ph.D.</td> <td>Chief, Section on Biometry</td> <td>OBE</td> <td>NEI</td> </tr> <tr> <td></td> <td>Elmer J. Ballintine</td> <td>M.D.</td> <td>Clinical Director</td> <td>CR</td> <td>NEI</td> </tr> </table>			PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI	Other:	John A. Barranger	M.D.	Chief, Clinical Section	DMNB	NINCDS		Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR	NEI		Erik Linner	M.D.	Visiting Scientist	CR	NEI		Roy Milton	Ph.D.	Chief, Section on Biometry	OBE	NEI		Elmer J. Ballintine	M.D.	Clinical Director	CR	NEI
PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI																																	
Other:	John A. Barranger	M.D.	Chief, Clinical Section	DMNB	NINCDS																																	
	Toichiro Kuwabara	M.D.	Chief, Section on Experimental Pathology	LVR	NEI																																	
	Erik Linner	M.D.	Visiting Scientist	CR	NEI																																	
	Roy Milton	Ph.D.	Chief, Section on Biometry	OBE	NEI																																	
	Elmer J. Ballintine	M.D.	Clinical Director	CR	NEI																																	
COOPERATING UNITS (if any) Developmental and Metabolic Neurology Branch, NINCDS, NIH																																						
LAB/BRANCH Clinical Branch																																						
SECTION Section on Glaucoma																																						
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																						
TOTAL MANYEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0																																				
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																						
SUMMARY OF WORK (200 words or less - underline keywords)  <p>Various projects are being carried out to clarify <u>intraocular fluid movement in rhesus monkeys and humans</u>. A method has been perfected for spectrophotometric determinations of <u>ascorbic acid concentration</u> in ocular and systemic fluids. <u>Clinical, physiologic, and morphologic studies</u> of the ocular alterations following an insult from <u>intracarotid hyperosmotic mannitol</u> show transient alteration of clinical and physiological status, despite permanent disruption of the pigmented epithelial layer of the <u>pars plana</u> and <u>pars plicata</u> of the ciliary body.</p>																																						

Project Description:

Objectives: This project is designed to examine the physiology of intra-ocular fluid movement under varied experimental conditions.

Methods Employed: Standard methods of cannulation and perfusion with non-invasive and invasive pressure measurements and with subsequent determination of volumes and flow by weight changes, dilution or turnover techniques have been used. Aqueous obtained by ocular cannulation is analyzed spectrophotometrically for ascorbate concentration.

Major Findings: During FY 1981, the spectrophotometric method for ascorbate determination has been applied to human samples obtained at the time of ocular surgery in the Clinical Center. Reliable tests have been done upon aqueous humor from five eyes. In all, the ascorbate concentration was observed to be within the range from 23 to 40 milligrams percent. Two of the eyes were receiving surgery for cataract, two for chronic simple glaucoma, and one for pigmentary glaucoma. The patients ranged in age from the late twenties to the seventies.

Transmission electron microscopic evaluation of the ciliary epithelium in monkeys following intracarotid mannitol has documented a persistent defect of some of the pigmented epithelial cells on the pars plicata.

A calibration for applanation of the monkey cornea has been performed in vivo and in vitro to relate applanated area, applanating force, pressure within the anterior chamber, and volume of fluid displaced from the anterior chamber. This shows that the in vivo calibration differs from in vitro. The apparent applanated area is over-estimated in the absence of an opacifier added to the tear film during measurements. The slope of the line relating displaced volume to the ratio of applanating force over pressure in the eye during applanation is nearly the same during in vivo and in vitro calibrations. An extrapolation is that in vitro calibrations of human eyes can be used to approximate the volume change in vivo during human applanation tonographic procedures wherein applanating force and pressure in the eye are recorded. During this study it was noted that the change in the calculated volume displaced, obtained from the radius of curvature of the cornea and the applanated area, approximates closely the actual change in volume measured either in vivo or in vitro.

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. These studies are yielding information applicable to understanding and treating glaucoma and hypotony.

Proposed Course: These studies will continue, emphasizing aqueous humor inflow, outflow, and composition.

NEI Research Program: Glaucoma--Aqueous Humor Dynamics: Inflow

Publications:

Rodrigues MM, Gaasterland DE: Current concepts in the pathology of the glaucomas (anterior segment), in Nicholson DH (ed): Ocular Pathology Update. New York, Masson Publishing Co, 1980, p. 55.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00168-06 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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 F. Gaasterland M.D.  Other: Charles Bonney Elmer J. Ballintine Robert Bonner Claude Cummins John Raymond Toichiro Kuwabara  Alan H. Rich	M.D.  D.V.M., Ph.D. M.D. Ph.D. B.S. B.S. M.D.  B.S.	Chief, Section on Glaucoma  Visiting Scientist Clinical Director Physicist Biologist Medical Photographer Chief, Section on Experimental Pathology Engineer
		CB NEI  CB NEI CB NEI BEIB DRS CB NEI ATGP AFFRI LVR NEI  BEIB DRS
COOPERATING UNITS (if any) Biomedical Engineering and Instrumentation Branch, DRS; Armed Forces Radiobiology Research Institute		
LAB/BRANCH Clinical Branch		
SECTION Section on Glaucoma		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.7	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The high energy and power of <u>lasers</u> offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, <u>iridotomy</u> and <u>trabeculotomy</u> are possible. This has importance for <u>glaucoma patients</u> 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 <u>simian</u> (rhesus) <u>eyes</u> and the application of promising systems and procedures to human glaucoma eyes under controlled conditions.		

Project Description:

Protocol Number: 80 FI 91

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 humans.

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. The NEI laser laboratory is equipped with a modified model 800 Coherent Radiation argon laser, which has been used for this and other projects, a Q-switched ruby laser, a Q-switched neodymium-YAG laser, and delivery systems.

Major Findings: Instrument development has continued to be an important activity during FY 1981. The Q-switched ruby laser system has been redesigned to allow delivery of the high power pulses through an articulated arm system to a clinical Zeiss slitlamp. The eyes of the operator are protected by a shutter system, which contains a feedback loop that prevents laser firing should it fail to close. An appropriate enclosure for the laser system and its mounting upon an optical bench has been designed and fabricated. This new delivery system is being tested and additional refinements will be introduced.

Before the Q-switched ruby laser system was dismantled to allow the above alterations, a study of the changes induced in the monkey eye at two months after Q-switched ruby laser treatment of the trabecular meshwork was completed. In six monkeys nine eyes were treated. Four eyes received either three or six applications in the nasal quadrants. Five eyes received between 10 and 13 applications in the nasal quadrants. The energy in air varied between 8 and 21 millijoules. The spot size in air measured between 75 and 100 micrometers. The total facility at two months after trabecular treatment with a Q-switched ruby laser was 0.51 in the eyes treated with three to six applications and 0.55 in the eyes treated with 10 to 13 applications. This compares with a mean value of 0.62 in the three control eyes. The standard error for each of these numbers is approximately 0.08. Several conclusions have been drawn. The instrumentation was sufficient for reliable trabecular treatment. The Q-switched ruby laser explodes target tissue, and after healing the site appears quite similar to healed argon laser treatment sites and there is little residual physiologic effect on normal tissue.

The study of the effects on the monkey eye of argon laser glaucoma treatment has been completed. In seven monkeys there were nine treated eyes and five control, untreated eyes. The intraocular pressure at the end of follow-up had a mean value of 15.3 in the treated eyes and 16.1 in the control eyes. The facility of outflow at the end of follow-up had a mean value of 0.43 in the treated eye and 0.46 in the control eyes. The clinical, physiologic and histologic examination allows several conclusions: no damage occurred to the cornea, lens, or retina; inflammation was transient; iris effects were indicated by a small, persistent dialation of the pupil; and the intraocular pressure was elevated rather high between 8 and 11 days in one of the nine eyes and in the

same eye was in the twenties for fourteen weeks. Two of the five eyes followed for six months developed numerous peripheral anterior synechia with a hillock configuration. The trabecular damage is pitting and scarring with proliferation of nearby endothelial cells. The outflow facility was decreased in one of nine treated eyes. The others showed no increase above normal.

High speed motion picture photography (Hycam unit) was done of cat eyes during Q-switched ruby laser iridotomy. Intraocular cavitation was observed in several eyes. This has not previously been recorded or reported. In one eye the laser light reflected from the retina, presumably from the tapetum, and exited through the iris creating a second iris hole. Several eyes, including this one, showed chorioretinal lesions which were not present before the iris treatment.

Seven patients have been enrolled in the prospective controlled random assignment trial of trabeculectomy compared to argon laser trabecular treatment. Each patient had progressive glaucoma changes and maximally tolerated medical therapy with inadequate pressure control, and each required intervention. In four patients one eye was treated; three of these eyes received trabeculectomy and one was assigned to laser treatment. In three patients binocular intervention was required. The worst eye was randomly assigned and the other eye received the other treatment. Thus in the trial at this time seven patients have undergone six trabeculectomies and four laser treatments. Follow-up varies from one month to one and one-half years. The numbers are inadequate to allow definitive comparison of effects, but there appear to be more complications in the group receiving surgical intervention. The pressure lowering effects appear similar in a preliminary analysis.

A study has been initiated to determine the power and energy density thresholds for damage of polymethylmethacrylate (PMMA) intraocular lenses as used in human cataract surgery. The lenses are being supported in saline in an artificial model eye. Results are not yet available.

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 and other anterior segment anomalies. This possibility is being investigated.

Proposed Course: The project will continue. Instrument development will include adaptation of pulsed lasers to the operating microscope and the slitlamp using the articulated arm if possible. A frequency doubled ND/YAG Q-switched laser has been ordered. This will update the laser equipment and allow expansion of the studies to the green wavelengths. The clinical trial will be expanded to include Q-switched ruby laser and/or ND/YAG laser as adequate equipment becomes available.

NEI Research Program: Glaucoma--Primary Open-Angle Glaucoma/Angle-Closure Glaucoma/Developmental, Congenital, or Infantile Glaucoma/Secondary Glaucomas/Aqueous Humor Dynamics: Outflow

Publications:

Bonney CH, Gaasterland DE, Rodrigues MM, Raymond JJ, and Donohoo P: Acute effects of Q-switched ruby laser on monkey anterior chamber angle. Invest Ophthalmol Vis Sci (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00143-08 CB															
PERIOD COVERED October 1, 1980, to September 30, 1981																	
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  <table style="width:100%; border: none;"> <tr> <td style="width:15%;">PI:</td> <td style="width:45%;">Douglas E. Gaasterland</td> <td style="width:20%;">M.D. Chief, Section on Glaucoma</td> <td style="width:10%;">CB</td> <td style="width:10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Elmer J. Ballintine</td> <td>M.D. Clinical Director</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Carl Kupfer</td> <td>M.D. Director</td> <td></td> <td>NEI</td> </tr> </table>			PI:	Douglas E. Gaasterland	M.D. Chief, Section on Glaucoma	CB	NEI	Other:	Elmer J. Ballintine	M.D. Clinical Director	CB	NEI		Carl Kupfer	M.D. Director		NEI
PI:	Douglas E. Gaasterland	M.D. Chief, Section on Glaucoma	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 Section on Glaucoma																	
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																	
TOTAL MANYEARS: 0.02	PROFESSIONAL: 0.02	OTHER: 0.0															
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords)  Thirty-six patients with <u>pigmented ocular lesions</u> originally participated in this study. The early results of the study show that the <u>diagnostic technique</u> used had <u>inadequate specificity</u> . For most patients a clear diagnosis has been made, and their ocular problem resolved. Except for occasional <u>follow-up examinations</u> of some of the patients, work on this project has ended.																	

Project Description:

Protocol Number: 76 RI 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.

Major Findings: Several patients with lesions originally thought to be benign have been reexamined: none has had a change of diagnosis. One patient with a mass lesion that has been regarded as "suspicious" continues to have no increase of size of the lesion after seven years of followup. Two patients who have repeatedly refused to have enucleation continue to show slow growth of the choroidal melanomas without evidence of metastatic disease seven and eight years after diagnosis. In both, visual function remains good. One of these patients is now 86 years old: the other is in his 50's.

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 examination 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 AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00030-10 CB																														
PERIOD COVERED October 1, 1980, to September 30, 1981																																
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  <table style="width:100%; border: none;"> <tr> <td style="width:10%;">PI:</td> <td style="width:30%;">Douglas E. Gaasterland</td> <td style="width:10%;">M.D.</td> <td style="width:30%;">Chief, Section on Glaucoma</td> <td style="width:10%;">CB</td> <td style="width:10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Carl Kupfer</td> <td>M.D.</td> <td>Director</td> <td></td> <td>NEI</td> </tr> <tr> <td></td> <td>Lessie McCain</td> <td>R.N.</td> <td>Clinical Technician</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Roy Milton</td> <td>Ph.D.</td> <td>Chief, Section on Biometry</td> <td>OBE</td> <td>NEI</td> </tr> <tr> <td></td> <td>Elmer J. Ballintine</td> <td>M.D.</td> <td>Clinical Director</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI	Other:	Carl Kupfer	M.D.	Director		NEI		Lessie McCain	R.N.	Clinical Technician	CB	NEI		Roy Milton	Ph.D.	Chief, Section on Biometry	OBE	NEI		Elmer J. Ballintine	M.D.	Clinical Director	CB	NEI
PI:	Douglas E. Gaasterland	M.D.	Chief, Section on Glaucoma	CB	NEI																											
Other:	Carl Kupfer	M.D.	Director		NEI																											
	Lessie McCain	R.N.	Clinical Technician	CB	NEI																											
	Roy Milton	Ph.D.	Chief, Section on Biometry	OBE	NEI																											
	Elmer J. Ballintine	M.D.	Clinical Director	CB	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 Section on Glaucoma																																
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																
TOTAL MANYEARS: 0.55	PROFESSIONAL: 0.05	OTHER: 0.50																														
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																
SUMMARY OF WORK (200 words or less - underline keywords)  In this continuing study of the <u>parameters of intraocular pressure</u> , young and old <u>normal volunteers</u> and patients with <u>glaucoma</u> and <u>ocular hypertension</u> 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 <u>effects of antiglaucoma medications</u> alone and in combination upon the parameters in normal and in diseased eyes.																																

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 antiglaucoma 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. Z01 EY 00150-08).

Major Findings: Preliminary analyses of the data from the masked trial to test the effects of dilute atropine eye drops showed equivocal results. Pursuit of this project has been discontinued.

The computer programs for data analyses in this project have been re-written. The programs are now simplified and directly reflect the Friedenwald equations for tonographic results. The programs allow data analysis from the terminal in the examination area. A number of completed studies which are still being pursued are being reanalyzed with the new programs and the data thus made available for future analysis.

Two young and one older normal volunteers had replicate sessions of measurements before and after timolol 0.25% applied topically, and before and after the combination to timolol 0.25% and isoproterenol 1% applied topically. These three volunteers showed a response after topical timolol alone identical to that observed in a larger group. The impressive reduction of intraocular pressure was due to a marked reduction in calculated aqueous flow. One volunteer showed a modest increase in outflow facility. The others did not. After the combination of topical medications the parameters of intraocular pressure did not change. The intraocular pressure was minimally lower. The calculated aqueous flow was slightly higher. None of the changes was statistically or clinically significant. Two patients with ocular hypertension were also tested with either timolol or timolol plus isoproterenol during replicate determinations. After the combination, the mean pressure rose slightly in these two patients. This was due to a combination of slight reduction of calculated aqueous flow and modest, statistically significant reduction of true facility and total facility. The mechanism of the timolol effect was studied in three pigtail monkeys two to three weeks after unilateral superior cervical ganglionectomy. Both eyes received topical 0.25% timolol after the intraocular pressure was measured. Phencyclidine sedation was employed. One hour after topical medication the intraocular pressure had fallen in the eyes on the intact side and the eyes on the side with the histologically and pharmacologically proven superior cervical ganglionectomy and third neuron Horner's Syndrome. We must conclude that the effect of timolol is not dependent upon an intact sympathetic innervation. The antagonistic interaction of timolol

and isoproterenol could imply either an effect at a single set of receptors or an effect due to activation and deactivation of two sets of receptors.

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--Aqueous humor dynamics: Inflow/Aqueous Humor Dynamics: Outflow/Primary Open-Angle Glaucoma

Publications:

Gaasterland DE: Control of intraocular pressure, in Anderson RE (ed): Manual on Biochemistry of the Eye. Am Academy of Ophthalmol (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00077-04 CB
PERIOD COVERED <u>October 1, 1980, to September 30, 1981</u>		
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. Chief, Section on Glaucoma CB NEI Other: Elmer J. Ballintine M.D. Clinical Director CB NEI		
COOPERATING UNITS (if any)  None		
LAB/BRANCH <u>Clinical Branch</u>		
SECTION <u>Section on Glaucoma</u>		
INSTITUTE AND LOCATION <u>National Eye Institute, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>0.02</u>	PROFESSIONAL: <u>0.02</u>	OTHER: <u>0.0</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  Patients with <u>rubeosis iridis</u> and <u>neovascular glaucoma</u> 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 <u>visual function</u> ; adequate control of <u>intraocular pressure</u> , with or without medications; and control of <u>discomfort</u> . It is estimated that approximately 40 nondiabetic and 40 diabetic patients will be needed for this project.		

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 consent to participate, are randomly assigned to receive one of the two methods of treatment. Follow-up is aimed at identifying adequacy of treatment and identifying complications.

Major Findings: No new patients have entered the study during FY 1981. The protocol was revised to reduce the amount of cyclocryotherapy to be given during the first session and to simplify the progressive steps of cyclocryotherapy. Continued follow-up of the patients previously treated has revealed that the two cryotherapy treated eyes remain atrophic and one appears to be becoming phthisical.

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--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 00086-03 CB		
PERIOD COVERED October 1, 1980 to September 30, 1981				
TITLE OF PROJECT (80 characters or less)  Contributions to Ophthalmic Pathology and Systemic Disease				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT				
PI: Other:	David G. Cogan Toichiro Kuwabara Merlyn Rodrigues Fred C. Chu David M. Bachman W. Gerald Robison	M.D. Chief, Neuro-Ophthalmology Section M.D. Chief, Pathology Section M.D. Chief, Glaucoma Section M.D. Senior Staff Fellow M.D. Staff Fellow M.D. Geneticist, Cell Biologist	CB LVR CB CB CB LVR	NEI NEI NEI NEI NEI NEI
COOPERATING UNITS (if any) None				
LAB/BRANCH Clinical Branch				
SECTION Section on Neuro-Ophthalmology				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205				
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1		
CHECK APPROPRIATE BOX(ES)				
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER				
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords)				
<p>Tissue obtained either by biopsy or necropsy is studied with the aim of elucidating clinical signs and symptoms. Specific studies of the past year have included: lesions of the brain stem in patients with "<u>ocular bobbing</u>"; palpebral changes in <u>Leishmania</u>; corneal changes in disturbances of <u>lipid metabolism</u>; and ocular manifestations of <u>systemic vascular disease</u>.</p>				

Project Description:

Objectives: To interpret ophthalmic manifestations of disease through the study of pathologic processes in tissues and associated clinical signs during life.

Methods Employed: Tissue obtained by biopsy or necropsy are subjected to microscopy and, where indicated, to electron microscopy.

Major Findings: Opportunities of the past year enabled: correlative studies of "ocular bobbing" and brain stem pathology; palpebral manifestations of Leishmania; and unusual corneal signs in hypertension and stroke.

Significance to Biomedical Research and the Program of the Institute: Tissue changes provide the traditional means of understanding disease. Clinicians must have an awareness of, and access to, pathologic material in order to interpret clinical signs.

Proposed Course: To take full advantage of opportunities to study patients and tissue that become available and to report the results to colleagues who are involved in patient care.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing-- Disorders--Ocular Motility and Strabismus - Neuro-ophthalmology. Corneal Disease--Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies.

Publications:

Cogan DG: Stroke, in Van Dalen JTW, Lessell S (eds): Neuro-ophthalmology. Elsevier, North Holland Publishing Company, 1980.

Cogan DG: Stroke, in Van Dalen JTW, Lessell S (eds): Neuro-ophthalmology II (in press).

Cogan DG: The ocular fundus and hypertension, in Amery A (ed): Hypertensive Cardiovascular Disease: Pathophysiology and Treatment. Martinees Nyhoff (in press).

Cogan DG: Radiation effects on the eye and adnexa, in Chang CH, Ellsworth R, Tretter P (eds): Tumors of the Eye and Orbit. New York, Masson Publishing Inc., 1981.

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 00089-03 CB																																				
PERIOD COVERED October 1, 1980 to September 30, 1981																																						
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																																						
<table style="width:100%; border: none;"> <tr> <td style="width:15%;">PI:</td> <td style="width:35%;">David G. Cogan</td> <td style="width:10%;">M.D.</td> <td style="width:30%;">Chief, Neuro-Ophthalmology Section</td> <td style="width:5%;">CB</td> <td style="width:5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Fred C. Chu</td> <td>M.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>David M. Bachman</td> <td>M.D.</td> <td>Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Toichiro Kuwabara</td> <td>M.D.</td> <td>Chief, Pathology Section</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>W. Gerald Robison</td> <td>Ph.D.</td> <td>Geneticist, Cell Biologist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>John Barranger</td> <td>M.D.</td> <td>Chief, Clinical Investigation Service</td> <td>DMN</td> <td>NINCDS</td> </tr> </table>			PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI	Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI		David M. Bachman	M.D.	Staff Fellow	CB	NEI		Toichiro Kuwabara	M.D.	Chief, Pathology Section	LVR	NEI		W. Gerald Robison	Ph.D.	Geneticist, Cell Biologist	LVR	NEI		John Barranger	M.D.	Chief, Clinical Investigation Service	DMN	NINCDS
PI:	David G. Cogan	M.D.	Chief, Neuro-Ophthalmology Section	CB	NEI																																	
Other:	Fred C. Chu	M.D.	Senior Staff Fellow	CB	NEI																																	
	David M. Bachman	M.D.	Staff Fellow	CB	NEI																																	
	Toichiro Kuwabara	M.D.	Chief, Pathology Section	LVR	NEI																																	
	W. Gerald Robison	Ph.D.	Geneticist, Cell Biologist	LVR	NEI																																	
	John Barranger	M.D.	Chief, Clinical Investigation Service	DMN	NINCDS																																	
COOPERATING UNITS (if any)  Development and Metabolic Neurology Branch, NINCDS																																						
LAB/BRANCH Clinical Branch																																						
SECTION Section on Neuro-Ophthalmology																																						
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																						
<table style="width:100%; border: none;"> <tr> <td style="width:30%;">TOTAL MANYEARS:</td> <td style="width:30%;">PROFESSIONAL:</td> <td style="width:40%;">OTHER:</td> </tr> <tr> <td>0.6</td> <td>0.4</td> <td>0.2</td> </tr> </table>			TOTAL MANYEARS:	PROFESSIONAL:	OTHER:	0.6	0.4	0.2																														
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																																				
0.6	0.4	0.2																																				
CHECK APPROPRIATE BOX(ES)																																						
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER																																						
<input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																						
SUMMARY OF WORK (200 words or less - underline keywords)																																						
<p>Characteristic dysfunctions of the <u>visual</u> and <u>eye motor systems</u> occur in certain <u>inborn errors</u> of <u>metabolism</u>. The abnormalities presumably stem from the intracellular accumulation of abnormal storage materials, which are <u>cytotoxic</u>.</p>																																						

Project Description:

Objectives: To identify and characterize the ophthalmic abnormalities in metabolic disease with especial emphasis on those affecting the nervous system.

Methods Employed: Appropriate patients referred by ophthalmic and neurologic colleagues are screened for visual and ocular motor abnormalities. Those patients who are found suitable for further studies are, subject to their consent, enrolled in a battery of tests including electroretinography, measurement of visual evoked potentials, electro-oculography, and neurologic examination. Abnormalities are regularly documented by photography and/or video taping. Ancillary tests are enzyme assays and conjunctival biopsies when indicated.

Major Findings: Nine patients have been identified as having an abnormality that we have designated the DAF Syndrome, an acronym for down-gaze paralysis, ataxia and/or athetosis, and foam cells. We have found reports of 30 relevant cases in the literature under a variety of names such as ophthalmoplegic lipidosis, Niemann-Pick variant, etc. The sphingomyelinase level in cultured white cells and fibrocytes has been inconstantly reduced. The onset of the disease typically dates to the first or second decade of life. The course is variable, but our series suggests that the earlier the onset the more severe the ophthalmoplegia and neurologic complications.

Another metabolic abnormality characterized by a granular opacity about the macula and by visceromegaly has been observed in one patient who is being currently studied. This rare disease has been reported in only two patients previously. It runs a benign course without inducing significant ophthalmic or systemic incapacity.

Corneal changes with several types of systemic lipid disease have been documented by us in the past year and previous years (mostly through collaboration with Dr. Ernest Schaefer). Some of the patients have been diagnosed as having Tangier's disease or a Tangier-like entity. It would appear that those patients with a deficiency of high density lipoprotein have corneal infiltration and are prone to coronary disease. A few patients, especially those with abetalipoproteinemia have ocular motor disturbances.

Significance to Biomedical Research and the Program of the Institute: The accessibility of the eye and the transparency of its media provide a unique opportunity to recognize abnormal deposits resulting from metabolic disease. Ophthalmologists are thus in a privileged position to contribute importantly to the identification of pathologic states, to the monitoring of treatment and to the elucidation of pathogenetic processes. But the primary essence is to establish the validity of the observed associations and to correlate the ophthalmic abnormalities with those of other disciplines.

Proposed Course: So long as patients with metabolic faults come to our attention and so long as we have the means to make adequate studies we will continue as we have in the past few years.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--  
Visual Processing and Amblyopia - Disorders. Retinal and Choroidal Diseases--  
Developmental and Hereditary Disorders. Corneal Diseases--Corneal Dystrophies,  
Inherited Disorders, and Developmental Anomalies.

Publications:

Cogan DG: Optic atrophy, cataract and neurologic disorders (Garcin),  
in Myriantopoulos NC (ed): Handbook of Clinical Neurology, volume 42,  
"Neurogenetic Directory, Part 1". Amsterdam, North-Holland Publishing  
Company, 1981.

Cogan DG: Optic atrophy, congenital, in Myriantopoulos NC (ed):  
Handbook of Clinical Neurology, volume 42, "Neurogenetic Directory,  
Part 1". Amsterdam, North-Holland Publishing Company, 1981.

Cogan DG: Optic atrophy, infantile (Kjer), in Myriantopoulos NC (ed):  
Handbook of Clinical Neurology, volume 42, "Neurogenetic Directory,  
Part 1". Amsterdam, North-Holland Publishing Company, 1981.

Cogan DG: Radiation effects on the eye and adnexa, in Chang CH,  
Ellsworth R, Tretter P (eds): Tumors of the Eye and Orbit. New York,  
Masson Publishing Company, 1981.

Cogan DG: Stroke, in Lessell S, Van Dalen JTW (eds): Neuro-ophthalmology:  
A Series of Critical Surveys of the International Literature, vol 1.  
Amsterdam Elsevier-North Holland, 1980.

Cogan DG: Stroke, in Lessell S, Van Dalen JTW (eds): Neuro-ophthalmology:  
A Series of Critical Surveys of the International Literature, vol 2.  
Amsterdam Elsevier-North Holland (in press).

Cogan DG: The ocular fundus and hypertension, in Amery A (ed):  
Hypertensive Cardiovascular Disease, Pathophysiology and Treatment.  
Martinees Nyhoff (in press).

Cogan DG, Chu FC, Bachman D, Barranger J: The DAF syndrome, in Lessell S,  
Van Dalen JTW (eds): Neuro-ophthalmology: A Series of Critical Surveys  
of the International Literature, vol 2. Amsterdam, Elsevier-North  
Holland (in press).

Cogan DG, Chu FC, Gittinger J, Tychsens L: Fundus abnormalities of  
Gaucher's disease. Arch Ophthalmol 98:2202-2203, 1980.

Cogan DG, Chu FC, Reingold DB, Barranger J: Ocular motor signs in some  
metabolic disease. Arch Ophthalmol (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 00117-01 CB																									
PERIOD COVERED October 1, 1980 to September 30, 1981																											
TITLE OF PROJECT (80 characters or less) Oculomotor Disorders in Human Subjects																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																											
<table style="width:100%; border: none;"> <tr> <td style="width:10%;">PI:</td> <td style="width:30%;">David G. Cogan</td> <td style="width:30%;">M.D. Chief, Neuro-Ophthalmology Section</td> <td style="width:10%;">CB</td> <td style="width:10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Fred C. Chu</td> <td>M.D. Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>David M. Bachman</td> <td>M.D. Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Douglas B. Reingold</td> <td>M.A. Computer Specialist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Nina C. Walther</td> <td>B.S. Biologist</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	David G. Cogan	M.D. Chief, Neuro-Ophthalmology Section	CB	NEI	Other:	Fred C. Chu	M.D. Senior Staff Fellow	CB	NEI		David M. Bachman	M.D. Staff Fellow	CB	NEI		Douglas B. Reingold	M.A. Computer Specialist	CB	NEI		Nina C. Walther	B.S. Biologist	CB	NEI
PI:	David G. Cogan	M.D. Chief, Neuro-Ophthalmology Section	CB	NEI																							
Other:	Fred C. Chu	M.D. Senior Staff Fellow	CB	NEI																							
	David M. Bachman	M.D. Staff Fellow	CB	NEI																							
	Douglas B. Reingold	M.A. Computer Specialist	CB	NEI																							
	Nina C. Walther	B.S. Biologist	CB	NEI																							
COOPERATING UNITS (if any)																											
None																											
LAB/BRANCH																											
Clinical Branch																											
SECTION																											
Section on Neuro-Ophthalmology																											
INSTITUTE AND LOCATION																											
National Eye Institute, NIH, Bethesda, Maryland 20205																											
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:																									
3.0	1.0	2.0																									
CHECK APPROPRIATE BOX(ES)																											
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER																											
<input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline keywords)																											
<p>Unwanted or inadequate <u>eye movements</u> impair vision. By <u>neurological evaluation</u> we can sometimes localize the damage causing eye movement disorders. Our method is to combine in selected cases the best neurological evaluation possible with quantitative recording of eye movement responses to calibrated <u>vestibular</u>, <u>optokinetic</u>, and discrete <u>visual stimuli</u>. Eye movements are recorded by <u>electro-oculography</u> or <u>infrared oculography</u>, and response parameters are analyzed by <u>computer</u>. We have studied patients with <u>cerebellar disease</u>, <u>myasthenia gravis</u>, <u>internuclear ophthalmoplegia</u>, and <u>epileptiform eye movements</u>. Cerebellar eye movement disorders can be classified into proprioceptive and visual disturbances, and appear to correlate with mid-vermal and posterior vermal damage. We have outlined the features which distinguish Myasthenia Gravis from other conditions with similar presentations. We have modelled internuclear ophthalmoplegia as impaired excitation of ipsilateral medial rectus with partial impairment of contralateral lateral rectus.</p>																											

Project Description:

Protocol Number: 77-EI-140

Objectives: Abnormal eye movements occur as prominent features of various neurological diseases affecting the central nervous system. Our objective in this project is to provide evidence on the localizing value of eye movement disorders in neuro-ophthalmic diagnosis.

Methods Employed: In selected patients with metabolic or neurological disorders, we have attempted to quantitate the type and degree of ocular motor abnormality.

We record eye movements with electro-oculography or infrared oculography. Eye movements are evoked by rotating a subject in a Bárány chair or by projecting moving visual targets. Chair and visual targets are computer controlled, and eye movement responses are recorded online. Data are analyzed to evaluate eye movement accuracies, velocities, and latencies, and results are correlated with other probes of central nervous system structure and function.

Major Findings: We have monitored the eye movements of a series of patients with documented lesions of the cerebellum and have suggested a classification of these disorders into visual and proprioceptive abnormalities. The former manifest as non-smooth pursuit, decreased optokinetic nystagmus. We have shown dysmetric eye movements, previously thought to involve erroneous visually guided attempts to correct eye position, to occur in the dark. These defects appear to correlate with posterior vermal lesions, and the area containing the flocculi, still known as the vestibulocerebellum, would be better described as the visual cerebellum. Downgaze nystagmus, for as yet unknown reasons, appears to result from lesions to this area. Proprioceptive eye movement disturbances which manifest as dysmetria, flutter, and instability of gaze appear to correlate with mid-vermal lesions. We have contrasted the ocular involvement of myasthenia gravis with that of other conditions that sometimes have similar presentations, and have catalogued the abnormalities we have found to be associated.

Analysis of cases of internuclear ophthalmoplegia has allowed us to confirm and extend the understanding of this disturbance associated with lesions of the medial longitudinal fasciculus. We have offered a new suggestion for the basis of the abducting nystagmus seen in this disease, as impaired excitation of contralateral lateral rectus associated with compromise of the MLF.

Epileptiform eye movements in a case of methylmalonic aciduria were documented, forming the fourth published case of this rare metabolic disorder.

A study of the transitions between saccades and smooth eye movements in normal subjects showed that smooth (vestibular or pursuit) eye movements may persist to within a few tens of milliseconds of saccades. However,

stepped visual targets produce a consistent tendency for smooth eye movements to be attenuated over 100 milliseconds before saccades.

Significance to Biomedical Research and the Program of the Institute: Quantitation and modelling of ocular motor disturbances aids in diagnosing lesions within the central nervous system and contributes to our knowledge of how the brain programs eye movements.

Proposed Course: The project will be continued.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing-- Ocular Motility Disorders - Neuro-ophthalmology.

Publications:

Chu FC, Reingold DB, Cogan DG: Lid-triggered synkineses. Ophthalmology 88:1019-1123, 1981.

Cogan DG: Ocular motor apraxia, congenital, in Myriantopoulos NC (ed): Handbook of Clinical Neurology, Volume 43, "Neurogenetic Disorders Part 1", 1980.

Cogan DG: Oculomotor paralysis, cyclic, in Myriantopoulos NC (ed): Handbook of Clinical Neurology, Volume 24, "Neurogenetic Disorders, Part 1", 1981.

Cogan DG: Ophthalmoplegia, retinitis pigmentosa, deafness, mental retardation and cerebellar symptoms (Kearn-Sayre), in Myriantopoulos NC (ed): Handbook of Clinical Neurology, Volume 43, "Neurogenetic Disorders, Part 2" (in press).

Cogan DG: Internuclear ophthalmoplegia, in Neetens A (ed): Disorders of Myelin (in press).

Cogan DG, Chu FC, Reingold DB: Illusory movement of the environment in the presence of normal eye movements, in Honrubia V et al (eds): Nystagmus and Vertigo: Clinical Approaches to the Patient With Dizziness (in press).

Cogan DG, Chu FC, Reingold DB: Notes on congenital ocular motor apraxia: associated abnormalities, in Glaser J (ed): Neuro-ophthalmology Vol 10. St. Louis, CV Mosby Publishing Co, 1980, pp 171-179.

Cogan DG, Chu FC, Reingold DB, Tychsen RL: A long term follow-up of Congenital ocular motor apraxia: Case report. Neuro-ophthalmology Vol 1. Amsterdam, Aeolus Press, 1980, pp 145-147.

Cogan DG, Schulman J, Porter RJ, Mudd SH: Epileptiform ocular movements with methylmalonic aciduria and homocystinuria. Am J Ophthalmol 90(2): 251-253, 1980.

Reingold DB, Chu FC, Cogan DG, Leighton SB, McMinn WO: A computerized testing facility for clinical study of versional eye movement control, in Greenfield RH (ed): Computers in Ophthalmology. Silver Spring, MD, IEEE Computer Society, 1979, pp 220-222.

Reingold DB: The transitions between saccades and smooth eye movements, in Honrubia V et al (eds): Nystagmus and Vertigo: Clinical Approaches to the Patient with Dizziness (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 00087-03 CB
PERIOD COVERED October 1, 1980 to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  Parametric Studies of the Pupillary Functions		
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. Computer Specialist CB NEI
	J. Christian Gillin	M.D. Chief, Unit on Sleep Studies BPH MH
	Richard Lowenstein	M.D. Staff Psychiatrist BPH MH
	Natraj Sitaram	M.D. Staff Psychiatrist BPH MH
	John Nurnberger	M.D. Senior Staff Fellow BPH MH
	Elliot Gershon	M.D. Chief of Psychogenetics Section BPH MH
COOPERATING UNITS (if any)  Section on Psychogenetics, Biological Psychiatry Branch, National Institute of Mental Health		
LAB/BRANCH Clinical Branch		
SECTION Section on Neuro-Ophthalmology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 0.7	OTHER: 0.4
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input checked="" type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p><u>Pupillary dysfunction</u> 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: hypersensitive miotic response to <u>cholinergics</u> (1) in patients with affective <u>disorders</u>; (2) in two patients with <u>progressive external ophthalmoplegia</u>; and (3) in a patient with spasm of <u>accommodation</u>.</p>		

Project Description:

Protocol Number: 80-EI-59

Objectives: To measure pupillary reactions in patients with selected diseases and to create a new method for documenting pupillary abnormalities.

Methods Employed: Pupillary reactions in patients and normal subjects are evoked using visual stimuli and pharmacological agents. Changes in pupillary diameters are recorded on videotape using an infrared camera. Rate and extent of constriction are monitored with a dynamic analog readout of pupillary area. Differential rates of constriction and dilation of a pupil along different axes are monitored with a digital reconstruction of dynamic pupil shape.

Major Findings: We have documented that the presumed normal pupils in patients with primary affective illness display abnormal reactions to pilocarpine. In progressive external ophthalmoplegia, presumed by some to be a myopathic condition, findings of abnormal pupils suggest a neurodegenerative basis to the condition. We are documenting the tonic and segmental constriction of pupils in patients with Adie's syndrome.

Significance to Biomedical Research and the Program of the Institute: There is potential utility for using the pupils as an index of central neurochemical function in affective disorders. We have shown abnormal pupillary responses to either sympathomimetic and parasympathomimetic agents in affective disorders for the first time. Segmental palsy of the pupil frequently occurs as a manifestation of Adie's syndrome. This sign is poorly documented and understood. The clarification of the parameters to describe normal and abnormal pupillary movement will facilitate the classification of related tonic pupil syndromes.

Proposed Course: Further studies of the pupil will be done in patients with affective disorders and where indicated as part of a neuro-ophthalmological examination.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing -- Ocular Motility and Strabismus - Neuro-Ophthalmology

Publications:

Sitaram N, Nurnberger JI, Gershon ES, Vanskiver C, Chu FC, Reingold DB, and Gillin JC: Cholinergic involvement in primary affective illness: Greater miotic response to pilocarpine. Life Sciences (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00093-03 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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. Chief, Ophthalmic Immunology CB NEI Section Other: Sanford Stone M.D. Head, Immunology Unit OSD NIAID		
COOPERATING UNITS (if any) Department of Pathology, Albert Einstein College of Medicine Office of the Scientific Director, NIAID		
LAB/BRANCH Clinical Branch		
SECTION Section on Ophthalmic Immunology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.3	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  <u>Allergic encephalomyelitis</u> is a <u>central nervous system</u> disease of immunologic origin. In <u>juvenile strain 13 guinea pigs</u> , <u>cataracts</u> developed during severe allergic (autoimmune) encephalomyelitis syndromes produced <u>actively</u> or by <u>transfer of living lymph node cells</u> from sensitized strain 13 donors. These lens changes were manifested bilaterally <u>within a two-week period</u> of active sensitization or transfer of sensitized cells. The morphologic in vivo appearance of these cataracts is similar to both the <u>galactosemic induced</u> and <u>tryptophan deficiency cataract</u> 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.		

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.

Each animal is observed carefully 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 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. Initial biochemical analysis indicates the presence of unusual soluble lens proteins.

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 of how systemic diseases may alter the ocular environment so as to induce lenticular opacities.

Proposed Course: We will study the biochemical basis for the lens changes and attempt to prevent the induction of these cataracts during the disease.

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

Publications:

Raine CS, Tranpott U, Nussenblatt RB, and Stone SH: Association of optic uveitis with chronic relapsing experimental allergic encephalomyelitis: Relevance to multiple sclerosis. Lab Invest 42:327, 1980.

Stone SH, Nussenblatt RB, Cross FL, and Raine CS: Cataracts and allergic encephalomyelitis: Acute opacification of the lens in paralyzed juvenile guinea pigs. Ophthalmic Res 13:129-138, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00115-01 CB
PERIOD COVERED October 1, 1980 to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  Cyclosporin A Therapy in Uveitis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI:	Robert B. Nussenblatt	M.D. Chief, Ophthalmic Immunology Section CB NEI
Other:	Francisco de Monasterio	M.D., D.Sc. Chief, Section on Visual Processing CB NEI
	Kent F. Higgins	Ph.D. Senior Staff Fellow CB NEI
	Mario Salinas-Carmona	M.D. Visiting Fellow CB NEI
	Igal Gery	Ph.D. Visiting Scientist LVR NEI
COOPERATING UNITS (if any) Department of Immunology, National Naval Medical Center, Bethesda, Maryland		
LAB/BRANCH Clinical Branch		
SECTION Section on Ophthalmic Immunology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
Cyclosporin A, a endecapeptide product with specific <u>anti-T-cell characteristics</u> , will be administered to <u>patients with sight threatening ocular inflammatory disease of non-infectious origin</u> . This will be done in order to test Cyclosporin's efficacy in the <u>treatment of uveitis</u> .		

Project Description:

Protocol Number: 81-EI-33

Objectives: Cyclosporin A (CsA), an endecapeptide obtained from fungi, has been shown to have specific anti-T-cell activity (Transplantation Proc. 12:234, 1980). We have recently reported CsA's exceptional effectiveness in preventing the induction of S-antigen autoimmune uveitis in rats, as well as the inhibition of the disease once immunization has occurred (J Clin Invest 67:1228, 1981). The goal of this study will be to test CsA's efficacy in treating patients with bilateral sight threatening posterior uveitis of an autoimmune nature.

Methods Employed: Ten patients will be initially selected in this pilot project. Patients twenty-one years of age or older, of either sex (females not pregnant), will be admitted to this study. All patients should have a bilateral sight threatening uveitis of non-infectious etiology. Lymphocyte cultures are prepared where the immune cells are tested against various crude ocular extracts, as well as purified human 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. Patients chosen will be treated with CsA for six months. During this period, the patients' clinical, immunologic, and ocular electrophysiologic course will be closely monitored.

Major Findings: This study has just begun and no results can be given at this time.

Significance to Biomedical Research and the Program of the Institute: Uveitis is one of the most frustrating problems in all of ophthalmology. Present modes of therapy for patients with severe ocular inflammatory disease are inadequate, non-specific, and have a myriad of side effects. CsA, if effective, will be an important adjunct to therapy, and will, by virtue of its known immune properties, add to our knowledge of mechanisms in this disease.

Proposed Course: Studies of Cyclosporin A Therapy in 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 AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00116-01 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  Double Masked Treatment of Ocular Toxoplasmosis		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Robert B. Nussenblatt  Other: Elmer Ballintine Francisco de Monasterio  Daniel Seigel Igal Gery Marvin Podgor	M.D.  M.D. M.D., D.Sc.  D.Sc. Ph.D. M.S.	Chief, Ophthalmic Immunology Section Clinical Director Chief, Section on Visual Processing Deputy Chief Visiting Scientist Statistician
CB NEI  CB NEI CR NEI  OBE NEI LVR NEI OBE NEI		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Branch		
SECTION Section on Ophthalmic Immunology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>The purpose of the project is to evaluate whether <u>clindamycin</u> combined with <u>sulfadiazine</u> will prove as or more <u>effective therapy</u> for <u>ocular toxoplasmosis</u> than the combination of <u>sulfadiazine</u> and <u>daraprim</u>. Patients with <u>active toxoplasmosis</u> will be <u>randomized</u> within strata (determined by size of lesion and proximity to the macula) to one of the two treatments, in this <u>double masked</u> study.</p>		

Project Description:

Protocol Number: 81-EI-92

Objectives: Ocular toxoplasmosis represents a sizable number of cases seen in an uveitis clinic. Sulfadiazine and daraprim have been considered the combination of choice in the therapy of sight-threatening toxoplasma lesions. Reports have now suggested that clindamycin may be effective therapy for toxoplasmosis. The objective of this study is to randomize patients in a double masked study in order to compare the efficacy of sulfadiazine/clindamycin and sulfadiazine/daraprim therapy.

Methods Employed: Patients 18 years or older, of either sex (females not pregnant), will be admitted to this study. They should manifest an active retinal lesion due to toxoplasmosis. Patients will receive a standard ophthalmic examination and will be randomized into a therapy group on the basis of the size and position of the active lesion. The cause of the disease will be followed clinically, as well as with electrophysiologic testing. The data is to be collected by the OBE-NEI and evaluated.

Major Findings: This study has just begun, and no significant findings can be reported.

Significance to Biomedical Research and the Program of the Institute: Toxoplasmosis is the cause of a large number of uveitis cases in the United States. Daraprim has potentially serious side effects. If another form of therapy can be demonstrated equally or more effective than sulfadiazine and daraprim, then clinicians will be given an expanded choice in dealing with this potentially sight threatening problem.

Proposed Course: Studies on Double Masked Treatment of Ocular Toxoplasmosis will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders.

Publications: None



Project Description:

Protocol Number: 79 BI 48

Objectives: To determine whether patients with 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: HLA-B8 has been found to be associated with iridocyclitis in black Americans. This antigen has been associated with a wide range of autoimmune diseases, and its presence in patients with this disorder strongly suggests a similar mechanism for this disease.

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 whether 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:

Nussenblatt RB: HLA and ocular diseases: A review of four entities with close HLA associations. Immunology of the Eye, Workshop I, Immunogenetics and Transplantation Immunity. Immunology Abstracts, Information Retrieval, Inc, Washington, DC, 1980.

Nussenblatt RB, Mittal KK: Association of anterior uveitis in American blacks with HLA-B8, and not B27, in Terasaki PI (ed): Histocompatibility Testing. University of Southern California Press, 1980, p. 942.

Nussenblatt RB, Mittel KK: Iridocyclitis in Blacks: Association with HLA-B8 suggests an autoimmune etiology. Br J Ophthalmol 65:329-332, 1981.

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 00075-03 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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  Other: Igal Gery Stanley Cevario	M.D.  Ph.D. B.S.	Head, Ophthalmic Immunology Section Visiting Scientist Biologist
		CB NEI  LVR NEI CB NEI
COOPERATING UNITS (if any)  Department of Ophthalmology, University of Louisville, Louisville, Kentucky Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, Maryland		
LAB/BRANCH Clinical Branch		
SECTION Section on Ophthalmic Immunology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 0.5	OTHER: 0.8
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p> <u>In vitro cellular immune functions</u> are being studied in a masked method in patients with <u>ocular toxoplasmosis</u>, <u>presumed ocular histoplasmosis</u>, <u>pars planitis</u>, <u>Behcet's disease</u>, <u>ocular sarcoid</u>, <u>birdshot choroidopathy</u>, and <u>chorio-retinitis of unknown origin</u>. <u>Crude ocular antigens</u> as well as the purified <u>uveitogenic soluble antigen (S-antigen)</u> of the retina are being used in a <u>lymphocyte microculture technique</u> in order to evaluate the presence of cellular immune memory to ocular tissues. Immune memory is also evaluated by the production of <u>lymphokine</u> in a <u>capillary migration system</u>. A <u>subgroup of patients</u> with <u>posterior uveitis</u> has been identified as having this immunologic memory. Other studies concentrate on the presence of <u>suppressor cell activity</u> functioning of <u>macrophages</u> and lymphocyte subsets as defined by monoclonal antibodies in these patients. These results shed light on the basic mechanisms of uveitis and may be used as a guide for specific immunologic therapy.         </p>		

Project Description:

Protocol Number: 79 EI 49

Objectives: The objective of this study is to investigate several immunological 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 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 concanavalin 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 concanavalin 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. Monoclonal antibodies to T cell subsets, in conjunction with the fluorescein activated cell sorter, are, in addition, being used in an attempt to identify alterations in lymphocyte subgroups.

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.

Posterior uveitis patients manifested increased Con A induced suppression when compared to controls. But these same patients had decreased suppression when measured by the method described by Bresnihan and Jasin.

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, and that alterations in suppressor cells are also present.

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:

Nussenblatt RB, Gery I, Ballintine EJ, and Wacker WB: Cellular immune responsiveness of uveitis patients to retinal S-antigen. Am J Ophthalmol 89:173, 1980.

Nussenblatt RB: Uveitis: The role of immunity. Palestra Oftalmologia Panamericana 4:10, 1980.

Nussenblatt RB, Cevalario SJ, Gery I: Alterations in suppressor cell activities in intraocular inflammatory disease. Lancet ii:722-724, 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00094-03 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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  Other: Igal Gery Mario Salinas Toichiro Kuwabara  Merlyn Rodrigues  Stanley Cevario Francisco de Monasterio	M.D.  Ph.D. M.D. M.D.  M.D.  B.S. M.D., D.Sc.	Chief, Ophthalmic Immunology Section Visiting Scientist Visiting Fellow Head, Section on Experimental Pathology Head, Section on Clinical Eye Pathology Biologist Chief, Section on Visual Processing
CB NEI  LVR NEI CB NEI LVR NEI  CB NEI  CB NEI CB NEI		
COOPERATING UNITS (if any)  Department of Ophthalmology, University of Louisville, Kentucky		
LAB/BRANCH Clinical Branch		
SECTION Section on Ophthalmic Immunology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  <u>Guinea pig strain 13 animals, Lewis rats, and non-human primates immunized at a site distant to the eye with the Soluble antigen (S-antigen) of the retina in complete Freund's adjuvant develop experimental allergic uveitis (EAU).</u> Depending on the antigen immunizing dose and the animal, the ocular lesions can vary from an iridocyclitis to a panuveitis. <u>Lymph node cells, nonadherent T-cells obtained from peritoneal exudate cells, and peripheral lymphocytes 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. Ocular electrophysiologic (ERG) alterations seen in non-human primates with S-antigen uveitis are similar to those seen in patients with posterior uveitis.</u> Cyclosporin A, a drug with specific <u>anti-T-cell activity</u> , has been found to be exceptionally effective in <u>preventing the induction of EAU</u> in rats, and in suppressing the inflammation once immunization has occurred.		

Project Description:

Objectives: We have previously reported that experimental uveitis may be induced in animals by immunization with a purified component of the retina (S-antigen). 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: Strain 13 guinea pigs, Lewis rats, and non-human primates are immunized with purified S-antigen in complete Freund's adjuvant in one hind footpad or the nuchal region. Evidence of ocular inflammatory disease is monitored via slit lamp and ophthalmoscopic examinations. After two to four weeks, lymph node, peritoneal exudates, or peripheral blood cells are collected and used for several cellular immune studies. Lymphocyte cultures are prepared in microtiter plates and are stimulated with S-antigen as well as other antigens. Other immune cells from immunized animals are mixed with isogenic macrophages in order to demonstrate the release of migration inhibition factor in the presence of S-antigen. Lewis rats immunized with the S-antigen are "protected" by daily injections of Cyclosporin A. Antibodies are evaluated by gel diffusion, ELISA, and indirect hemagglutination techniques, and eyes are taken for histology.

Major Findings: Animals immunized with S-antigen 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.

The EAU model in non-human primates parallels closely the disease seen in some posterior uveitis patients. The finding that CsA therapy inhibited the development of the S-Ag induced uveitis. In addition, anti-S-Ag antibody titers were observed to be similar in rats protected and not protected with CsA. Knowing CsA's anti-T-cell effects, this study would support the need for T-cell participation in EAU.

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 will be utilized in future human clinical trials, including Cyclosporin A.

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:

Nussenblatt RB, Gery I, Salinas-Carmona M, Kuwabara T, and Wacker, WB: S-antigen induced uveitis in primates and guinea pigs. Fed Proc 39:470, 1980.

Nussenblatt RB, Kuwabara T, de Monasterio FM, and Wacker WB: S-Antigen uveitis in primates: A new model for human disease. Arch Ophthalmol 99: 1090-1092, 1981.

Nussenblatt RB, Rodrigues MM, Wacker WB, Cevario SJ, Salinas-Carmona MC, and Gery I: Cyclosporin A: Inhibition of experimental autoimmune uveitis in Lewis rats. J Clin Invest 67:1228-31, 1981.

Nussenblatt RB, Gery I, and Wacker WB: Experimental autoimmune uveitis: Cellular immune responsiveness. Invest Ophthalmol Vis Sci 19:686, 1980.

Nussenblatt RB, Gery I, Kuwabara T, de Monasterio FM, and Wacker WB: The role of the retinal S-antigen in primate uveitis. Immunology of the Eye, Workshop II, Autoimmune Phenomena. Immunology Abstracts (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 FY 00107-02 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  Suppression of Retinoblastoma Proliferation by Human Soluble Lymphocyte Factors		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Mario Salinas-Carmona Other: Robert Nussenblatt  Paul Russell John Hooks	M.D. Visiting Fellow M.D. Chief, Ophthalmic Immunology Section Ph.D. Research Chemist Ph.D. Research Microbiologist	CB NEI CB NEI  LVR NEI LOW NIDR
COOPERATING UNITS (if any)  Laboratory of Oral Medicine, NIDR		
LAB/BRANCH Clinical Branch		
SECTION Section on Ophthalmic Immunology		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.25	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  <u>Mononuclear cells</u> when stimulated with <u>concanavalin A</u> develop different biological activities, including <u>suppressor activity</u> . The objective of this work is to find out whether the inhibitory activity is mediated through soluble factors, and to characterize these factors' <u>biological</u> and <u>physiochemical</u> properties. We have found that the factors responsible for suppression are <u>non-dialyzable</u> , <u>heat stable</u> , <u>resistant to pH2 treatment</u> and inhibits proliferation of a variety of cells including <u>human lymphocytes</u> , <u>retinoblastoma cells</u> and <u>stromal keratocytes</u> .		

Project Description:

Objectives: Human mononuclear cells when stimulated with concanavalin A (Con A) develop different biological activities. Under specific culture conditions, and in the presence of that mitogen, some lymphocytes inhibit proliferation of fresh autologous or allogeneic lymphocytes. The mechanism by which those cells exert their suppressor activity is not known. The objectives of the present work is to investigate whether the inhibitory effect of Con A activated human lymphocytes is mediated through soluble factors, and if so to characterize their biological and physiochemical properties.

Methods Employed: Purified lymphocyte populations are stimulated with Con A for different periods of time. The resultant cell supernatants are sterilized by membrane filtration and tested against fresh allogeneic lymphocytes, retinoblastoma cells, and stromal keratocytes. Tritiated methyl thymidine uptake is used to assess cell proliferation. Biochemical methods such as membrane ultrafiltration, sieve chromatography, pH2 and enzyme treatment of crude supernatants as well as the semi-purified fractions are performed to determine some properties of the suppressor factors; bioassays such as interferon determinations are also done.

Major Findings: Supernatants from Con A stimulated peripheral human mononuclear cells produce 40-60% suppression of retinoblastoma cell proliferation in culture; T-cell enriched fractions are active in producing the inhibitory effect as compared to the non-T cell fractions. The suppressor activity is non-dialyzable, heat stable (56 C x 45 °), and pH2 resistant. Type II interferon has also been found in the suppressor supernatants, but methods by which interferon activity can be abrogated have little or no effect on the inhibitory action of the supernatant. The mechanism of suppression does not include cytotoxicity of the target cell. We found that lymphocytes from a normal donor do not produce IFN- $\gamma$  and still suppress when stimulated with concanavalin A, suggesting that factors other than IFN- $\gamma$  are responsible for suppression. The inhibitory activity of the Con A induced suppressor supernatants are species specific since they only affect human cells.

Significance to Biomedical Research and the Program of the Institute: A purified suppressor factor from normal human mononuclear cells has been sought for some time. Its identification would be of great benefit in understanding basic mechanisms of immuno-regulation.

Proposed Course: Sieve chromatography and other biochemical techniques are being used in order to isolate the suppressor substance. The mechanism of the suppressor factors against target cells will also be investigated. I will try to define the cell responsible for the production of the suppressor factors.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders

Publications:

Salinas-Carmona MC, Russell P, Hooks J, Gery I, Green I, Nussenblatt RB:  
Inhibition of retinoblastoma cell proliferation by mitogen induced human  
suppressor lymphocytes and their soluble factors. Fed Proc 40:3, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00135-09 CB		
PERIOD COVERED October 1, 1980, to September 30, 1981				
TITLE OF PROJECT (80 characters or less) Biochemistry of Retinal and Pigmented Epithelium in Health and Disease				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT				
Pl: Helen H. Hess Other: David A. Newsome  Gloria E. Westney Roy C. Milton Carol A. Currier Merlyn M. Rodrigues  Joseph J. Knapka  John G. Bieri	M.D. M.D.  B.S. Ph.D. M.D. M.D.  Ph.D.  Ph.D.	Medical Officer (Research) Chief, Section on Retinal and Ocular Connective Tissue Diseases Biological Aide Chief, Biometry Section Staff Fellow Chief, Section on Clinical Eye Pathology Nutritionist, Small Animal Section Chief, Nutritional Biochemistry Section	CB CB  CB CB OBE CB  VRB  LNE	NEI NEI  NEI NEI NEI NEI  DRS  NIAMDD
COOPERATING UNITS (if any) Section on Clinical Eye Pathology, Clinical Branch, NEI Veterinary Resources Branch, DRS, NIH Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA				
LAB/BRANCH Clinical Branch				
SECTION Section on Retinal and Ocular Connective Tissue Diseases				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205				
TOTAL MANYEARS: 2.2	PROFESSIONAL: 2.0	OTHER: 1.2		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords) Investigations are being conducted into the <u>biochemical composition</u> of the <u>sensory retina</u> , <u>pigmented epithelium</u> and <u>choroid</u> in normal and disease states, particularly in <u>animal models</u> of <u>human retinal degenerations</u> and diseased human ocular tissues. The tissue specific distributions of <u>inorganic constituents</u> are studied by flameless atomic absorption, with concentrations of <u>Ca</u> , <u>Cu</u> and <u>Zn</u> of particular interest. The effects of <u>nutrition</u> and <u>genetic background</u> on the progress of <u>chorioretinal degeneration</u> in the <u>retinal dystrophic pigmented RCS rat</u> and of <u>cataract formation</u> and prevention in the <u>tan-hooded pink-eyed retinal model</u> for human <u>retinitis pigmentosa</u> is being investigated, including use of <u>genetic linkage studies</u> to attempt to identify a RCS-like human disease. <u>Diabetic rodents</u> are being followed for the development of retinal disease that may model human <u>diabetic retinopathy</u> .				

Project Description:

Objectives: To study the biochemical composition of retinal photoreceptor, neuronal, glial, and pigmented 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. This exploration would extend to possibilities of prevention or therapy of cataracts which often accompany retinal and/or choroidal diseases. Diseases in which pigmented epithelium (PE) is involved are of particular interest.

Methods Employed: Twenty-four hour urine samples from humans with retinal disease are examined for trace metal content. Defined diets are prepared and fed to affected and congenic unaffected retinal dystrophic animals in controlled experiments. Clinical findings are recorded after indirect ophthalmoscopic and biomicroscopic examination and fundus photography, slit lamp examination and slit lamp photography. Analytical methods include flameless atomic absorption spectroscopy, light and electron microscopy, enzymatic assays and standard quantitative biochemical determinations as appropriate.

Major Findings:I. Mineral Metabolism in Human Retinal Disease:

The trace elements copper and zinc are being studied by flameless atomic absorption spectrophotometric assay of 24-hour urine specimens from patients with pigmentary retinal degenerations as well as angioid streaks.

Copper: It has been suggested that RP might be caused by an inborn error of copper metabolism. Using flameless atomic absorption, we did not confirm the finding in our initial studies, as all patient values were within 0-30  $\mu\text{g Cu}/24$  hr, in the same range as normals. Subsequently, we extended the study to other types of pigmentary retinal degeneration, including macular degeneration and angioid streaks. In a series of more than 200 patients, only one urine (from a patient with RP) had a value outside the normal range (60  $\mu\text{g}/24$  hr). These data indicate that previous reports of elevated Cu from India do not apply to all RP patients. It is possible that a genetic isolate of RP patients may exist in India characterized by an altered Cu metabolism, or that some aspect diet may be involved in the high Cu excretion. U.S. studies have suggested that low dietary Cu may be more common than realized and a comparison with the Indian diet consumed by the RP patients in the high copper excretion group could be useful.

Zinc: We have been determining Zn in the same urines in which Cu has been done. We are not aware of any other studies on Zn excretion in retinal diseases. A total of more than 200 urines (more than 300 specimens will be included eventually) have been analyzed. The normal values for both males and females agree well with those in the recent literature. Values below 100  $\mu\text{g}/24$  hr have been regarded as beyond the normal range. Seven females and 23 males have shown above normal excretion of Zn, and some clustering within families was evident. Fourteen females excreted less zinc than normals. Some of the patients in the study are children, and controls of the same age and sex will be used in their evaluation; these data are not all available as yet. Because of the masked design of the experiments and the large number in the series, analytical results and clinical

correlations remain to be completed on many cases. Replicate samples of urine at different times have been obtained on some patients, with similar results. We intend to go beyond the current type of experimental design to try to investigate what appear to be abnormal Zn excretion tendencies in certain families. In the RP group, all the patients with high values were in the younger age range below 35 years. In the group with drusenoid macular degeneration, all but one of the patients had a normal value for Zn excretion; that one patient admitted having taken oral Zn over a considerable period of time.

In addition to drusenoid macular degeneration (MD) and juvenile hereditary MD cases, we also studied three patients with a special type of MD secondary to idiopathic angioid streaks. In three siblings (2 males, 1 female, in the age range 45-50 yrs), the Zn excretion was elevated (1015-1500  $\mu\text{g Zn}/24 \text{ hr}$ ), and the value for the female (1322  $\mu\text{g Zn}/24 \text{ hr}$ ) was twice the highest value for normal females. Each of the males had a daughter and a son (age range 10-20 yrs) and the values for the two daughters were at the highest range of normal. In summary, idiopathic angioid streak was a special type of MD in which Zn excretion was markedly increased, although in the major group of MD it was not. Additional familial cases of angioid streak are being recruited for study. This study is a good example of the fact that as compared with sporadic cases, family groups have greater potential for revealing possibly significant findings with an economy of laboratory and clinical effort.

## II. Effects of Sunflower Seed Supplements on Reproduction and Growth of RCS Rats with Hereditary Retinal Dystrophy, and Congenic Controls:

The Royal College of Surgeons (RCS) rat has been proposed as an animal model of human retinitis pigmentosa because it has a hereditary retinal dystrophy characterized by almost total loss of rod photoreceptors. The model now consists of dystrophic strains with either pink or pigmented eyes and matching control strains having the same genome except for the retinal dystrophy (*rdy*) locus and loci closely linked with it. We found that both control and dystrophic pink-eyed strains reproduced poorly when they were fed a standard laboratory rodent diet and were housed in conventional animal rooms unshielded from pathogenic influences. More prolific reproduction and improved growth of young were obtained with a commercial unsterilized closed formula pelleted rodent ration, supplemented with 25% sunflower seed kernels with 95% survival of the control strain and 75% survival of the dystrophic strain. Sunflower kernels contain a high concentration of vitamin E and selenium with 47% fat, mostly linoleic acid. Effective absorption of the high vitamin E of the diet was shown by analyses of blood plasma of 50 day old dystrophic and control rats, in which the  $\alpha$ -tocopherol level was three-fold that in animals fed standard laboratory rodent diet. Progeny fed the diet for 8-10 months after weaning did not manifest cataracts, which occurred in 23% of the pink-eyed dystrophic animals fed standard rodent diets.

Fundus observations have been carried out on pink-eyed and black-eyed dystrophic and control rats aged 24 days to 17 months. Fundus photographs compared with those published previously by Herron et al (1974), appear to indicate a slower progress of the disease in our animals on the supplemented diet. These clinical observations are being expanded into controlled experiments with larger numbers of animals at successive ages and correlated with histopathology.

### III. Sunflower Kernel Supplemented Diet and Prevention of Cataracts in Pink-eyed Tan-hooded Retinal Dystrophic Rats:

After noting that retinal dystrophic rats on the sunflower kernel supplemented diet were not developing cataracts, we looked at the incidence of cataracts in our tan-hooded pink-eyed dystrophic rats fed standard laboratory rodent diet. At nine months of age, 22.5% of 40 rats had cataracts visible to the unaided eye, in agreement with a report of 24% by LaVail et al (1975). However, a 60% incidence of cataracts had been reported in 1939 by Bourne and Gruneberg in the original colonies of the retinal dystrophic strain, and these observations were made by direct ophthalmoscopy. To determine whether the incidence of cataracts was decreasing after many years of inbreeding the strain, we re-examined our rats by indirect ophthalmoscopy and photography and found cataractous change in 74%. Furthermore, we found that the remaining 26% all had posterior subcapsular cataractous changes demonstrable by slit lamp examination and photography. For the first time, therefore, this shows that the cataractous pathology which accompanies the retinal degeneration in the RCS rat is inherited in a simple mendelian fashion. The irregularity of manifestation of the mature cataract points to a factor or factors in the environment affecting full expression of the pathology.

The posterior subcapsular cataract of the RCS dystrophic rat is a type similar to that occurring in some persons after steroid administration, and in 20-25% of persons with retinitis pigmentosa. Our observation that the sunflower kernel supplemented diet has a beneficial effect upon the incidence of mature cataracts in retinal dystrophic rats may have theoretical and possible clinical importance. Consequently, we have undertaken controlled experiments including the use of defined-composition diets to attempt to elucidate the relative importance of sunflower kernel associated nutritional factors in the prevention of cataracts in the RCS rat.

### IV. Linkage of Seminal Vesicle Protein and Retinal Dystrophy in the Rat Genome: Significance for the RCS Rat Model of Retinitis Pigmentosa:

In collaboration with Dr. David Gasser in the Department of Human Genetics, University of Pennsylvania School of Medicine, we carried out linkage studies that represent a beginning in the attempt to identify on a particular chromosome in humans a segment of the genome that may be homologous to the region around the rat rdy locus. A human retinal disease found to map to the same chromosome or chromosomal locus might prove to be the disease fitting the RCS model.

These studies show that rdy and SVP-1 are on opposite sides of the locus for agouti. The SVP-1 and rdy are  $28 \pm 7$  centimorgans (cM) (crossover units) from each other. The SVP-1, agouti and rdy are the only genes known in the fourth linkage group of rats. However, Gasser (1972) showed that the SVP-1 to agouti region of rat LG IV is homologous to mouse LG V, where those genes occur on mouse chromosome 2. This homology was demonstrable because of gene polymorphism detected by coat color for agouti and electrophoresis for SVP-1. It is not known how far the homology of rat LG IV extends beyond agouti towards the centromere of chromosome 2, but now that the location of rdy has been clarified the question is whether any genes within 20-35 cM of SVP-1 on chromosome 2 also occur in LG IV of rat, especially ones that may have polymorphism due to different alleles. The mouse represents a convenient interspecies genetic scaffold because its genome has been worked out relatively well. However, so far as is known, the mouse has no mutant rdy gene.

If rat/mouse homology extends from SVP-1 to rdy, then rdy would map near three immunological loci (EA-6, Ir-2, and H-3) which have yet to be found in rats. However, a gene closely linked to mouse H-3 has been found that controls electrophoretic variants of B<sub>2</sub>-microglobulin (B<sub>2</sub>-M) which could be tested in rats.

In summary, by clarifying the location of rdy in the genetic map of rodents these studies point the way to possible discovery of a homologous chromosomal region in humans and identification of the specific type of human retinal degeneration for which the RCS rat may be a model.

#### V. Models of Diabetic Retinopathy:

The kidneys, eyes and pancreata of diabetic and control DBA/2J, SJL/J and NZB mice were examined by light and electron microscopy. Immunofluorescent reactions of the kidneys and eyes was performed with antibodies to mouse procollagen type IV, collagen type V, laminin, proteoglycan basement membrane (PBM) and fibronectin. The pancreas showed degranulation and necrosis of the beta cells of the islets of Langerhans 24-hours after infection. The most marked renal and ocular changes occurred in animals with the longest duration of diabetes (6 months) with fasting blood sugar levels of 350-390 mg/dl and glycosuria. The kidneys showed marked nodular and diffuse glomerulosclerosis and mesangial thickening. Scanning electron microscopy of the diabetic glomeruli revealed moderate to marked alteration of the normal surface contour of the podocytes and pedicels. Transmission electron microscopy disclosed marked accumulation of basement membrane-like material in the mesangium and subendothelial space that reacted with antibodies to procollagen IV, type V collagen, laminin, fibronectin and PBM. Ocular changes included decreased pericytes of the retinal vessels and corneal epithelial edema.

#### Significance to Biomedical Research and the Program of the Institute:

Retinal deteriorations are the major cause of untreatable legal or worse blindness in the United States and probably, taken as an aggregate, in the world. Nutritional and genetic factors are thought to play key roles in human diseases, and can often be studied in detail in animal models. The retinal pigmented epithelium is becoming increasingly appreciated as the primary site of many of these disease processes. Information gained from these studies should contribute to our understanding of human diseases and to initiating and conducting trials of possible therapeutic measures.

Proposed Course: The project will be continued with emphasis on controlled trials of nutritional regimens and rigorous elucidation of nutritional variables in retinal dystrophic animals. Human specimens will be analyzed as they become available.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

#### Publications:

Hess HH, Newsome DA, Knapka JJ, Bieri JG: Effects of sunflower seed supplements on reproduction and growth of RCS rats with hereditary retinal dystrophy. Lab Anim Sci (in press).

Hess HH, Newsome DA: Diet, reproduction and growth of dystrophic and control RCS rats. Invest Ophthalmol Vis Sci 19(3, Suppl.):91, 1980.

Hess HH, Gasser DL, Rodrigues MM, and Newsome DA: Quantitation of linkage between genes for seminal vesicle protein and retinal degeneration in RCS rats. Invest Ophthalmol Vis Sci 20(3, Suppl):79, 1981.

Rodrigues MM, Currier CA, Yoon J-W: Renal and ocular involvement in encephalomyocarditis (EMC) virus-induced diabetic mice. Fed Proc 40: 791, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00097-03 CB																								
PERIOD COVERED October 1, 1980, to September 30, 1981																										
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  <table border="0"> <tr> <td>PI:</td> <td>David A. Newsome</td> <td>M.D.</td> <td>Chief, Section on Retinal &amp; Ocular Connective Tissue Diseases</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Bruce A. Pfeffer</td> <td>Ph.D.</td> <td>Guest Worker</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Elsira Pina</td> <td>B.S.</td> <td>Biologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Louanne Krawczewicz</td> <td>B.S.</td> <td>Biologist</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	David A. Newsome	M.D.	Chief, Section on Retinal & Ocular Connective Tissue Diseases	CB	NEI	Other:	Bruce A. Pfeffer	Ph.D.	Guest Worker	CB	NEI		Elsira Pina	B.S.	Biologist	CB	NEI		Louanne Krawczewicz	B.S.	Biologist	CB	NEI
PI:	David A. Newsome	M.D.	Chief, Section on Retinal & Ocular Connective Tissue Diseases	CB	NEI																					
Other:	Bruce A. Pfeffer	Ph.D.	Guest Worker	CB	NEI																					
	Elsira Pina	B.S.	Biologist	CB	NEI																					
	Louanne Krawczewicz	B.S.	Biologist	CB	NEI																					
COOPERATING UNITS (if any) Duke University Department of Ophthalmology Hazleton Laboratories, Vienna, Virginia Section on Clinical Eye Pathology, Clinical Branch, NEI																										
LAB/BRANCH Clinical Branch																										
SECTION Section on Retinal and Ocular Connective Tissue Diseases																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.8	OTHER: 0.7																								
CHECK APPROPRIATE BOX(ES)  <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) Investigations are being conducted into aspects of specialization of the <u>primate</u> and <u>human retinochoroidal complex</u> with emphasis on the <u>pigmented epithelium</u> . Mammalian (including human) pigmented epithelial <u>choroidal cells</u> are being cultured on various substrata with media containing various <u>growth factors</u> and <u>hormones</u> so that these cells may exhibit maximal proliferative capacity. Cell- and tissue-specific marker enzymes such as <u>tyrosinase</u> are being assayed to determine the extent to which the cells are as differentiated as their in vivo counterparts. The <u>extracellular matrix</u> material from cultured RPE and choroid is being analyzed biochemically and by means of light and electron microscopic <u>immunocytochemistry</u> and the results compared with those from normal and pathologic tissues.																										

Project Description:

Objectives: Although the functional and anatomical specialization of the macula versus peripheral retinochoroidal tissues 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 describe the special biologic capabilities of the retinal pigmented epithelial cell which contribute to cell-tissue function and to responses to disease states including the formation of abnormal tissues in the vitreous cavity and on the retinal surface. We also wish to investigate, using a variety of techniques, possible alterations in normal enzyme systems such as tyrosinase and in extracellular matrix-basement membrane synthesis which may explain disease processes. A necessary adjunct goal is to develop a defined culture system for retino-choroidal cells, especially the pigmented epithelium, which will make possible experiments which cannot be accurately conducted in serum-containing media.

Methods Employed: Fresh and cultured pigmented epithelial, choroidal and, in some cases, retinal cells are harvested enzymatically and maintained in culture media with varying amounts and types of hormones and growth factors. Proliferative rate, morphology and enzymatic activity are studied in an attempt to determine the defined medium which supports the most differentiated growth. Synthesis of particular extracellular matrix proteins is determined by both direct immunofluorescent reactions with monospecific antibodies as well as fractionation and analysis of macromolecules radiolabeled in culture. Other techniques include a radioactive water release assay for tyrosinase, microdissection, photomicrography with phase and epifluorescent illumination, scanning and transmission electron microscopy, culture on various substrates including collagen and cell attachment protein films.

Major Findings: We have been able to reduce the serum component of growth medium for RPE cells ten-fold by supplementing the medium with an extract of mammalian retina (monkey or bovine). By modifying the  $[Ca^{++}]$  in the medium we have been able to passage RPE cells non-enzymatically. These cells then proliferate and maintain an epithelial morphology.

Choroidal cells have proved more adaptable to hormone supplemented defined media than pigmented epithelial cells and now can be cultured and maintained in serum-free conditions. By weaning pigmented epithelial cells slowly from serum-containing to defined media limited survival can be achieved at present. Fluorescent antibody reactions with antibodies to a variety of extracellular matrix (collagenous and non-collagenous) portions revealed that cultured choroidal cells synthesize and deposit fibronectin whereas pigmented epithelial cells do not. Choroidal cells also deposit significantly more type I collagen in vitro than do pigmented epithelial cells. These differences can help distinguish these cells in vitro.

Frozen sections of monkey posterior eye wall were reacted for the presence of serum proteins using the immunofluorescence method. A positive reaction occurred not only in the choroid but in extracellular spaces of the neural retina. These serum proteins have been shown biochemically to be present also in vitreous.

A technique to harvest interphotoreceptor matrix from freshly enucleated primate and human eyes has been developed. The harvested matrix macromolecules are

being isolated, identified and compared with those synthesized by pigmented epithelial cells in culture.

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. The techniques described aid in creating an in vitro system in which metabolic functions of normal and pathologic pigmented epithelial and choroidal cells can be accurately studied and manipulated. By coupling morphology with immunologic techniques, it is increasingly possible to identify pathologic cells of vitreous bands, epiretinal and macular fibrosis, all important causes of severe visual impairment or blindness. Increased understanding of the pathologic mechanisms and cell sources should aid in devising improved methods of treating several serious disorders. The creation of defined media for culturing retinochoroidal cells will allow screening of retinal degeneration patient sera for detrimental factors and perhaps the creation of an in vitro model of characteristic pathologic changes.

The pigmented epithelium is an archetypal epithelium with basement membrane and attachment proteins. Knowledge of normal and pathologic changes in these macromolecules could improve our understanding of macular degeneration as well as basic biologic phenomena of wide interest. One function of the pigmented epithelium is stabilization, alignment and attachment of the photoreceptors. Our investigations into the nature and source of the interphotoreceptor matrix could provide new insights into photoreceptor renewal, function, and retinal attachment.

Proposed Course: Studies to investigate the sources and methods of turnover and renewal of Bruch's membrane will be continued and expanded. Additional emphasis will be placed on adding quantitative determinations to the qualitative immunofluorescent studies and on alterations in the retinochoroidal complex with aging. Investigations of the interphotoreceptor matrix will be continued. It is expected that these investigations will be crucial to our understanding of the functioning of the pigmented epithelium as a metabolically active support layer for photoreceptors, of the pigmented epithelial-Bruch's membrane selective barrier for nutrients and other vital factors, and of the responses of these cells to various disease processes.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Newsome DA, Fletcher RT, Chader GJ: Human retinal cyclic nucleotides vary by area. Invest Ophthalmol Vis Sci 19:864-869, 1980.

Newsome DA, Rodrigues MM, Machemer RM: Human massive periretinal proliferation: In vitro characteristics of cellular components. Arch Ophthalmol 99:873-880, 1981.

Pfeffer BA, Fisher SK: Development of retinal pigment epithelial surface structures ensheathing cone outer segments in the cat. J Ultrastruct Res (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00098-03 CB		
PERIOD COVERED October 1, 1980, to September 30, 1981				
TITLE OF PROJECT (80 characters or less)  Clinical and Laboratory Studies in Macular and Retinal 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. Chief, Section on Retinal & Ocular Connective Tissue Diseases	CB	NEI
Other:	Helen H. Hess	M.D. Medical Officer (Research)	CB	NEI
	Alfred J. Lewy	M.D. Guest Worker	CB	NEI
	Kristi Silver	B.S. Microbiologist	CB	NEI
	Consuelo G. Muellenberg	B.S. Chemist	CB	NEI
	Ralph Gunkel	O.D. Ophthalmic Physicist	CB	NEI
	Patrick Stilwell	B.S. Guest Worker	CB	NEI
	Bruce Weintraub	M.D. Medical Director	CE	NIADDK
	Steven M. Sykes	B.S. Biologist	DBE	FDA
COOPERATING UNITS (if any) Clinical Endocrinology Branch, NIADDK Laboratory of Clinical Science, NIMH				
LAB/BRANCH Clinical Branch				
SECTION Section on Retinal and Ocular Connective Tissue Diseases				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205				
TOTAL MANYEARS: 3.1	PROFESSIONAL: 2.1	OTHER: 1.0		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords) Clinical investigations are underway to determine the early <u>natural history</u> and possible causes of <u>drusenoid macular degeneration</u> . <u>Drusen, serous and hemorrhagic detachments</u> of the retina and retinal pigmented epithelium, and <u>choroidal neovascularization</u> are manifestations of macular degeneration and will be studied by serial recordings of the anatomical appearance and visual function of eyes at high risk of developing disease. Results will be compared with those obtained from fellow eyes with more advanced disease, age matched normals, and those with <u>other maculopathies</u> including that of <u>retinitis pigmentosa</u> . Serum, and in some cases, 24-hour urine, levels of hormones including <u>melatonin, cortisol, thyroid</u> and zinc are being determined. By emphasizing studies of affected and unaffected family members and using various <u>genetic markers</u> such as <u>HLA antigens</u> , it is hoped that those factors materially associated with the appearance and/or progression of various <u>retinal degenerative conditions</u> will be elucidated. <u>Melatonin rhythms</u> are being studied in individuals blinded from various causes. <u>Blind subjects</u> can be either <u>free-running</u> or <u>entrained</u> to time cues other than the dawn-dusk cycle.				

Project Description:

Protocol Number: 79 EI 16, 81 EI 53

Objectives: Drusenoid (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 significant 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 retino-choroidal complex as the disease advances, and learning more about the possible genetic linkage of senile macular disease and other retinal deteriorations.

Environmental light is an important synchronizer of circadian rhythms in experimental animals and in humans. Light is known to suppress the nocturnal secretion of melatonin by the pineal gland in experimental animals; however, it was not thought to suppress in man. Concentrations of melatonin may also influence retinal metabolism in health and disease. We wish to learn more about the regulation of melatonin in patients with retinal disease or blindness.

Methods Employed: Patients are recruited into the study upon referral from an ophthalmologist, and are admitted into the study according to the NEI protocol (79 EI 16) or for melatonin studies under the NEI protocol (81 EI 53). Volunteers are obtained for use as age-matched controls through the Clinical Center volunteer office. Patients, after giving informed consent to participate in the study, provide a complete history and receive a routine ophthalmic examination and fundus photography complemented with blood and urine studies, genetic typing, psychophysical and electrophysiological studies, and fluorescein angiography when indicated. Patients are followed at regular intervals to evaluate changes, if any, with time. Twenty-four hour melatonin studies are done on overnight admission. Hormone levels are analyzed by mass spectrometry or radioimmunoassay as appropriate.

Major Findings: We discovered, using adequate ambient light levels, that light does suppress the nocturnal secretion of melatonin in man (Science 210:1267-1269, 1980). This effect is probably mediated by the same neural circuits which synchronize circadian rhythms with dawn and dusk light-dark transitions. We found that a white light of 1500 lux was adequate to produce the effect, while one of 500 lux was not. In order to determine the action spectrum, we used a green light, peak 524 nm and achieved suppression in human subjects. A light of equivalent lux with

the 480 to 550 nm light subtracted is currently being investigated. Since melatonin has been shown to be, in some species, extremely active in the control of pigment migration within pigmented epithelial cells of the retina, the intriguing possibility exists that melatonin may be involved in chorioretinal mechanisms in man. Our pilot studies have provided evidence that melatonin cycles are definitely abnormal and even free-running in subjects with congenital and traumatic blindness, blindness due to retinitis pigmentosa, and even severe visual loss due to retinal deterioration. Sleep and activity and temperature circadian rhythms may also be abnormal in blind subjects.

Drusenoid senile macular degeneration is the most prevalent cause of untreatable visual loss in the United States population over 60. We have undertaken serial clinical and laboratory investigations of a selected panel of patients with documentable macular degeneration in order to find parameters which accurately indicate the biological activity of the disease. Changes in cone threshold determinations, photostress recovery times, and color vision test results appear to predict or indicate progression or stabilization of the disease process as judged by visual function. These tests are easily performed, and may provide valuable tools for assessing the functional status of the macular chorioretinal complex. Alterations in thyroid function indicators indicate that some macular degeneration patients may also have inappropriate TSH secretion. The state of thyroid function in patients and the effects of thyroid substances of the RPE are under intensive investigation.

In the progressive forms of retinitis pigmentosa (RP), macular vision is usually spared to some degree until late in the course of the disease. The variability in the biological activity of the disease makes the prognostication of an affected individual's course highly uncertain, leading to a significant amount of additional suffering and anguish. Detailed serial examinations of selected well-documented RP patients using clinical observation and functional testing have indicated that the presence and degree of macular changes, particularly epiretinal membrane formation and cone functions (thresholds, color vision), coupled with blood vessel caliber may be sensitive indicators of the general rate of progress of the disease.

Shortly after the introduction of antimalarials (chloroquine and hydroxychloroquine) for the treatment of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), concern for retinal toxicity (RT) limited its widespread use. Since the incidence of RT seemed to be declining recently, we undertook a retrospective study to assess the frequency of RT with antimalarial use and whether there were any factors that might predict patient susceptibility to toxicity. A diagnosis of RT, defined as anatomical macular pigment changes, abnormal cone threshold testing, and decreased color vision was made. With increasing total dose, there was a striking increase in the frequency of RT and RT was associated most often with chloroquine use alone ( $p < 0.001$ ) in contrast to either hydroxychloroquine alone or the combination of both, and older age ( $p < .05$ ). No differences in incidence or RT were observed between patients with SLE and RA. Of the patients developing retinopathy, only one had evidence of worsening eye function after the drug was discontinued. In 8 patients, pre-drug cone threshold or color vision results were abnormal; none subsequently developed evidence of RT. Our data indicate hydroxychloroquine can be used safely and with minimal retinal toxicity even in total doses greater than 400 gm and in patients with SLE. Older patients ( $>50$ ) and patients receiving chloroquine alone may be more susceptible to retinal damage. Initial

cone threshold and color vision abnormalities are not absolute contraindications to starting antimalarials.

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. Melatonin may be the "master" hormonal regulator of human biological clocks, and may be involved in renewal systems in the retina.

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. Clinical melatonin studies will concentrate on RP patients, and the possible role of melatonin in retinal renewal and recovery from light toxicity is under active investigation in the laboratory.

NEI Research Program: Retinal and Choroidal Diseases--Macular Degeneration; Retinal Organization, Neurotransmission, and Adaptation

Publications:

Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP: Light suppresses melatonin secretion in man. Science 210:1267-1269, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 0079-04 CB	
PERIOD COVERED October 1, 1980, to September 30, 1981			
TITLE OF PROJECT (80 characters or less) Effects of Vitamin A, Selected Hormones and Drugs on Corneal Epithelium and Collagenases			
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. Chief, Section on Retinal & Ocular Connective Tissue Diseases	CB NEI
Other:	Carol A. Currier Louvenia Carter-Dawson John R. Hassell Leslie Harne  Kristi Silver Lance Liotta	M.D. Staff Fellow Ph.D. Staff Fellow Ph.D. Research Biologist Biological Laboratory Technician B.S. Microbiologist M.D. Senior Investigator Ph.D.	OBE NEI LVR NEI LDBA NIDR LDBA NIDR  CB NEI LPP NCI
COOPERATING UNITS (if any) Laboratory of Pathophysiology, NCI Laboratory of Biology and Developmental Anomalies, NIDR			
LAB/BRANCH Clinical Branch			
SECTION Section on Retinal & Ocular Connective Tissue Diseases			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Controlled experiments are being conducted to study the efficacy of topical and systemic <u>vitamin A therapy</u> in ameliorating a spontaneous <u>inherited corneal dystrophy</u> which resembles <u>human keratomalacia</u> in an <u>inbred mouse strain</u>. <u>Corneas</u> from these animals, <u>normal animals</u>, <u>vitamin A deficient animals</u> and <u>human donors</u> are being radioactively labeled in organ culture to determine the influence of vitamin A therapy on <u>glycoconjugate synthesis</u> and on <u>collagenase</u> activity. Mouse corneas have also been characterized by <u>scanning</u> and <u>transmission electron microscopy</u>. Results indicate that the <u>epithelium</u> of the dystrophic mouse cornea elaborates an unusual <u>basement membrane</u>. Vitamin A stimulates the synthesis of a major high molecular weight epithelial <u>glycoprotein</u> but does not appear to affect the course of the corneal destructive process in controlled therapeutic trials. Normal human and <u>keratoconus</u> corneas are being maintained in organ culture to determine the types of collagen-specific <u>metalloenzymes</u> elaborated and to search for <u>inhibitors of collagenase</u>.</p>			

Project Description:

Objectives: Keratoconus is a potentially serious corneal disorder. We have detected an uncommon basement membrane collagen destructive enzyme and now are attempting to define the cellular source of the enzyme and identify specific inhibitors.

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. An additional purpose is to assess the applicability of the inbred corneal dystrophic mouse as a model of human corneal disease.

Methods Employed: Corneas and conjunctival tissue were excised separately 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. Vitamin A therapeutic trials were conducted in a double masked controlled fashion utilizing topical and systemic routes of retinoid administration. Clinical observations were recorded, documented photographically and further confirmed by histologic examination.

Keratoconus surgical specimens and human normal autopsy corneas were cultured as explants and the medium assayed for collagenolytic activity. Specifically purified individual collagen types were radiolabeled in vitro and used as substrates in the assays. By utilizing different radiolabels, activity against types I and IV collagens could be determined simultaneously.

Major Findings: Keratoconus explants secrete a metalloenzyme which preferentially degrades type IV (basement membrane) collagen. The presence of type IV collagenase in the actual tissue was confirmed by immunofluorescent antibody reactions. Keratoconus conjunctiva, normal cornea and normal conjunctiva secrete little or none of this type IV degrading enzyme. Specific inhibitors of collagenase are being tested.

Ultrastructural examination of the inbred dystrophic mouse corneas revealed a thickening and irregularity of the corneal epithelial basement membrane, and a hypercellularity of the stroma. Vitamin A administration had no consistent effect in reducing corneal keratinization, ulceration, or in vitro elaboration of collagenase. Biochemically, a stimulation of synthesis of certain glycoproteins was detectable.

Significance to Biomedical Research and the Program of the Institute: The importance of metalloenzymes against basement membranes in metastasis is just becoming widely appreciated. Our work provides the first demonstration of such an enzyme in a non-cancerous human disease. Understanding the role of this enzyme may lead to knowledge of the pathogenesis of keratoconus, and discovery of appropriate inhibitors to therapeutic trials.

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 continue to characterize further the functional role of collagen destructive enzymes in keratoconus, their sources and effective inhibitors. Results of studies of the biochemically detectable effects of vitamin A on the dystrophic mouse cornea will be prepared for publication. This project will then be terminated.

NEI Research Program: Corneal Diseases--Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies; Inflammation and Wound Healing

Publications:

Hassell JR, Newsome DA: Vitamin A induced alterations in corneal and conjunctival epithelial glycoprotein biosynthesis. Ann NY Acad Sci USA 359:358-365, 1981.





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 the cornea, the outflow rate of the trabecular meshwork and filtration through Bruch's membrane. The purposes of this study include characterization of the collagens, proteoglycans, and glycoproteins normally present in the cornea, sclera, trabecular meshwork, vitreous, choroid and Bruch's membrane and the determination of the alterations that occur in these macromolecules in certain ocular diseases.

Methods Employed: Ocular connective tissue samples will either be radiolabeled in organ culture or cells derived from these tissues are grown and labeled in cell culture. The naturally occurring macromolecules are also extracted and characterized. Biosynthetically labeled as well as unlabeled matrix components are characterized using molecular sieve chromatography, DEAE-cellulose chromatography, CMC-cellulose chromatography, immunoprecipitation, gel electrophoresis, cesium chloride density gradient centrifugation, as well as with specific enzymes, such as collagenase, chondroitinase, keratanase, glycosidases, papain, and pepsin. Chemical characterizations, in terms of amino acid and carbohydrate analysis, are also conducted. Assays for collagenolytic activity are being carried out using radioactively labeled purified native collagen substrates with appropriate controls included.

Major Findings: Normal human corneas contain chondroitin and keratan sulfate proteoglycans. However, corneas from patients with corneal macular dystrophy contain only the chondroitin sulfate proteoglycan and not the keratan sulfate proteoglycan. Macular dystrophy corneas do contain an unusual glycoprotein not detected in normal corneas. The unusual glycoprotein in macular corneas may represent the accumulated material which produces the corneal clouding. This material cross-reacts with antibodies to purified normal keratan sulfate proteoglycan indicating that the abnormality lies outside the protein core. The structure of the linkage region has been partially determined by ultra HPLC and methylation procedures, and will be compared with the linkage region of the unusual glycoprotein from macular corneal dystrophy specimens.

Keratoconus corneas in vitro elaborate a metalloenzyme which is preferentially active against basement membrane (type IV) collagen. This is the second known enzyme with this activity (the other is from a mammary carcinoma). The presence of this enzyme in actual fresh keratoconus tissues was documented by a positive immunofluorescent reaction with specific antibodies. Inhibition of this enzyme by retinoids, phenytoin, progestational and steroidal hormones is under study.

Trabecular meshwork from rhesus monkeys in organ culture synthesized an unusually large amount of hyaluronic acid. Further studies on the types of collagen synthesized are also underway.

A heparan sulfate proteoglycan was isolated from a murine tumor which elaborates large amounts of basement membrane material. Antibodies against this proteoglycan react with basement membrane structures in the eye of lower species but not well with human or monkey. The heparan sulfate proteoglycan has been isolated from

human placenta and antibodies are being raised for use in human and monkey eye studies.

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 collagen and glycoconjugates will be prepared for use in clinical and biomedical research.

NEI Research Program: Corneal Diseases--Corneal Dystrophies, Inherited Disorders, and Developmental Anomalies; Corneal Transplantation and Stromal Wound Healing

Publications:

Hassell JR, Robey PG, Barrach H, Wilczek J, Rennard SI, Martin GR: Isolation of a heparan sulfate-containing proteoglycan from basement membrane. Proc Natl Acad Sci USA 77:4494-4498, 1980.

Hassell JR, Newsome DA, Krachmer JH, Rodrigues MM: Macular corneal dystrophy: Failure to synthesize a mature keratan sulfate proteoglycan. Proc Natl Acad Sci USA 77:3705-3709, 1980.

Hassell JR, Newsome DA, Martin GR: Isolation and characterization of the proteoglycans and collagens synthesized by cells in culture. Vision Res 21:49-53, 1981.

Rodrigues MM, Krachmer JH, Miller SD, Newsome DA: Posterior corneal crystalline deposits in benign monoclonal gammopathy. Arch Ophthalmol (in press).

Rodrigues MM, Sun T-T, Krachmer JH, Newsome DA: Epithelialization of the corneal endothelium in posterior polymorphous dystrophy. Invest Ophthalmol Vis Sci 19:832-835, 1980.

Newsome DA, Foidart J-M, Hassell JR, Krachmer JH, Rodrigues MM, Katz SI: Detection of specific collagen types in normal and keratoconus corneas. Invest Ophthalmol Vis Sci 20:738-750, 1981.

Newsome DA, Gross J, Hassell JR: Human corneal stroma contains three distinct collagens. Invest Ophthalmol Vis Sci (in press).

Newsome DA, Hassell JR, Rodrigues MM, Rahe AE, Krachmer JH: Biochemical and histological analysis of recurrent macular corneal dystrophy. Arch Ophthalmol (in press).

Hassell JR, Newsome DA: Vitamin A induced alterations in corneal and conjunctival epithelial glycoprotein biosynthesis. Ann NY Acad Sci 359: 358-365, 1981.

Rodrigues MM, Newsome DA, Krachmer JH, Sun T-T. Posterior polymorphous dystrophy of the cornea: Cell culture studies. Exp Eye Res (in press).



Project Description:

Protocol Number: 79 EI 92

Objectives: To characterize and document color vision abnormalities mediated by dysfunction of blue-sensitive, red-sensitive, and green-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 saturation discrimination tests) and electrophysiological studies of cone responses.

Major Findings: Early observations by H. Köllner indicated that whereas most types of retinal disease result in an acquired "blue-yellow" defect in color vision, disease of the optic nerve commonly leads to a "red-green" defect. Köllner's rule is interpreted today as indicating an undue vulnerability of blue-cone function to retinal insult. We have obtained evidence that the introduction of fluorescent dyes to the eye of Old-World macaque monkeys results in the specific staining of blue-sensitive cones. The complete staining of the cell body of these cones, accompanied by the lack of staining of other cones and rods, provides experimental support for the clinical observations of Köllner. The staining of blue cones of monkey retina can be explained by their lower threshold to metabolic alterations. In the case of the dyes we have used, it appears that they alter membrane function of blue cones, but not of other photoreceptors, and that this dysfunction results in cell death and the consequent penetration of the dye into the blue cones. No specific blue-cone staining is obtained when dyes that do not affect membrane function to a significant degree are injected intravitreally.

S-antigen induced uveitis in monkeys produces a "blue-yellow" defect similar to the one observed in clinical cases of inflammation of the posterior segment. We are at present attempting to combine both techniques, S-antigen induction of uveitis and blue-cone staining by intravitreal injection of dyes, in order to detect the retinal level at which immunological activity disrupts the processing of signals by blue-sensitive cones and their pathway.

Based on these techniques we are also attempting to develop a sub-human private model for acquired "blue-yellow" defects due to retinal insult. This model should provide an experimental tool to help improving clinical electrodiagnostic techniques.

Significance to Biomedical Research and the Program of the Institute:

The results help the understanding 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 color vision defects will be continued.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

de Monasterio FM, Schein SJ, McCrane EP: Staining of blue-sensitive cones of the monkey retina by a fluorescent dye. Science (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 FY 00059-03 CB																																																												
PERIOD COVERED October 1, 1980, to September 30, 1981																																																														
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  <table style="width:100%; border: none;"> <tr> <td style="width:10%;">PI:</td> <td style="width:40%;">Francisco M. de Monasterio</td> <td style="width:15%;">M.D., D.Sc.</td> <td style="width:25%;">Chief, Section on</td> <td style="width:5%;">CB</td> <td style="width:5%;">NEI</td> </tr> <tr> <td></td> <td></td> <td></td> <td style="text-align: center;">Visual Processing</td> <td></td> <td></td> </tr> <tr> <td>Other:</td> <td>Kent E. Higgins</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Rafael Caruso</td> <td>M.D.</td> <td>Expert</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Ralph D. Gunkel</td> <td>O.D.</td> <td>Ophthalmic Physicist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Myles Jaffe</td> <td>O.D.</td> <td>Guest Worker</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Stanley J. Schein</td> <td>M.D., Ph.D.</td> <td>Expert</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Doris Collie</td> <td>A.A.</td> <td>Health Technician</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Mary Fuhrman</td> <td></td> <td>Health Technician</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Patricia Christian</td> <td>B.S.</td> <td>Health Technician</td> <td>CB</td> <td>NEI</td> </tr> </table>			PI:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on	CB	NEI				Visual Processing			Other:	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI		Rafael Caruso	M.D.	Expert	CB	NEI		Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI		Myles Jaffe	O.D.	Guest Worker	CB	NEI		Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI		Doris Collie	A.A.	Health Technician	CB	NEI		Mary Fuhrman		Health Technician	CB	NEI		Patricia Christian	B.S.	Health Technician	CB	NEI
PI:	Francisco M. de Monasterio	M.D., D.Sc.	Chief, Section on	CB	NEI																																																									
			Visual Processing																																																											
Other:	Kent E. Higgins	Ph.D.	Senior Staff Fellow	CB	NEI																																																									
	Rafael Caruso	M.D.	Expert	CB	NEI																																																									
	Ralph D. Gunkel	O.D.	Ophthalmic Physicist	CB	NEI																																																									
	Myles Jaffe	O.D.	Guest Worker	CB	NEI																																																									
	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI																																																									
	Doris Collie	A.A.	Health Technician	CB	NEI																																																									
	Mary Fuhrman		Health Technician	CB	NEI																																																									
	Patricia Christian	B.S.	Health Technician	CB	NEI																																																									
COOPERATING UNITS (if any) None																																																														
LAB/BRANCH Clinical Branch																																																														
SECTION Section on Visual Processing																																																														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																																														
TOTAL MANYEARS: 6.0	PROFESSIONAL: 6.0	OTHER: 0.0																																																												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																																														
SUMMARY OF WORK (200 words or less - underline keywords)  The purpose of this project is to provide <u>diagnosis or evaluation of toxic, inflammatory, degenerative, or congenital retinal disorders</u> , and to conduct tests and experiments directed towards the clinical application and development of <u>electrophysiological and psychophysical procedures</u> for measuring <u>visual function in patients</u> of NEI's Eye Clinic and of other services in the NIH Clinical Center.																																																														

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, electroculography, spatial contrast sensitivity, sensory rod and cone thresholds, color-vision testing, retinal image stabilization, visual perimetry and other psychophysical functions.

Major Findings: Psychophysical and electrophysiological evaluations were performed on patients for diagnostic purposes in collaboration with clinical associates and staff members of the NEI.

Present efforts are directed towards the development of new color vision tests and 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 NIH Eye Clinic. 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: Retinal and Choroidal Diseases--Non-invasive Techniques in the Study of Retinal Disorders.

Publications:

Higgins KE, Daugman JG, Mansfield RJW: Amblyopic contrast sensitivity: insensitivity to unsteady fixation. Invest Ophthalmol and Vis Sci (in press).

Kaiser-Kupfer MI, de Monasterio FM, Valle D, Walser M, and Brusilow S: Gyrate atrophy of the choroid and retina: Improved visual function following reduction of plasma ornithine by diet. Science 210:1128-1131, 1980.

Kaiser-Kupfer MI, de Monasterio FM, Valle D, Walser M, and Brusilow S: Visual results of a long-term trial of a low-arginine diet in gyrate atrophy of choroid and retina. Ophthalmology 88:307-310, 1981.



Project Description:

Objectives: To study the neural organization underlying the processing of visual data in retina to cortex, with particular emphasis on color vision.

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 recordings from neurons of the extrastriate cortex of Old World macaque monkeys. (FM de Monasterio, SJ Schein, EP McCrane).

Recordings were made from neurons located within the foveal representation of the V4 area of extrastriate visual cortex using a semi-chronic, nitrous oxide preparation: the properties of the cells were examined in enough detail to permit their classification in terms of spatial and color properties. Cyto-architectural and myeloarchitectural studies of the cortical region confirmed the identification of the area where the recordings were made.

Color selective cells with either color-biased or color-opponent properties represented about 20% of the examined population. Their incidence was not significantly different from that of similar cells encountered in penetrations in the foveal part of the V2 area (area 18) of the visual cortex. Most color-selective cells had color-biased properties, best responding to wavelengths shorter than 460 nm or longer than 580 nm, or both. Some color-biased cells responded to photopically-matched white light, while others did not. No examples of "green-biased" cells were found. Very few cells showed overt color-opponent responses. The spectral sensitivity of V4 color selective cells was not unusually narrow. Cells lacking color-selective properties, and responding equally well to chromatic and achromatic lights of equal luminosity, were the most commonly encountered cell type in penetrations of different parts of the V4 area (56%). Other than color, these cells showed stimulus preferences similar to those of color-selective cells. Throughout V4, about 25% of the cells could not be unequivocally driven with the various test stimuli used. This finding is consistent with recent results from cell recordings in the prelunate gyrus of the awake, behaving animal indicating that some V4 cells receive extraretinal signals.

The results do not support recent claims that V4 has a high concentration of color-selective cells and that it is specialized in the detailed analysis of color information.

II. Extracellular recordings from retinal ganglion cells of Old-World macaque monkeys.

A. Studies of spectral response bandwidths. (FM de Monasterio, SJ Schein)

The spectral response bandwidth of color-opponent retinal ganglion cells were examined in conditions of neutral adaptation. Color-opponent cells show specific "signatures" in plots of response bandwidth vs. the wavelength of the peak sensitivity that allow for an acceptable estimate of the type(s) of cone input mediating cell responses.

Averaged spectral bandwidths of color-opponent ganglion cells were compared with published data from neurons of subsequent levels of the geniculocortical pathway, including the extrastriate area termed V4. No significant differences were found between color selective cells of the retina, dorsal lateral geniculate body, striate cortex and V4, which on the average have a half-bandwidth of 25 nm at half maximum sensitivity.

The spectral location of the peak sensitivity of responses of the various types of color-opponent ganglion cells shows a comparatively broad distribution, which loosely clusters at some spectral loci. Comparison of this distribution with that reported in several studies of V4 cell responses indicates a nearly complete absence of "blue-yellow" opponent responses in those cortical studies. In association with more recent electrophysiological studies of V4 cells, carried out by ourselves and other workers, the results of the present study do not support current claims of a color-specialization of this extrastriate cortical area.

B. Studies of "blue-yellow" and "red-green" opponent channels.  
(FM de Monasterio, KE Higgins)

We are currently examining the properties of two major types of color-opponent ganglion cells of the Old-World monkey retina, viz. "blue-yellow" and "red-green" opponent cells. In addition to their different spectral sensitivities, these two cell types differ in a number of other properties, such as (i) receptive-field size, (ii) conduction times, (iii) incidence in central retina, (iv) incidence of "On-center" and "Off-center" varieties, (v) incidence of concealment of surround opponency. The results indicate that these cell types represent two anatomically and physiologically different channels of the retinal output directed to the parvocellular layers of the lateral geniculate body.

III. Intracellular recordings and staining of single cells in the isolated monkey retina. (FM de Monasterio)

We are attempting to develop an isolated, superfused monkey retina preparation to study the anatomical organization responsible for the physiological functioning of the primate retina, using conventional intracellular recording and staining techniques to identify functional cell types. Development of a successful preparation would open the possibility of studying human retinal material with this technique.

Significance to Biomedical Research and the Program of the Institute:

Understanding the organization of the visual system of non-human primates is most valuable for understanding the mechanisms of visual processing of the human visual system, which at present can only be studied by indirect methods.

Proposed Course: Both extracellular and intracellular recordings from single cells of the monkey visual system will be continued.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing-- (Structure and Function).

Publications:

Schein SJ, Marrocco, RT, de Monasterio FM: Spectral properties of cells in the prestriate cortex of the monkey. Neurosci Abstr 580, 1980.

Nakamura RN, Desimone R, Schein SJ: Visually responsive units in striate cortex of blind monkeys. Neurosci Abstr 578, 1980.

de Monasterio FM: Functional properties and presumed roles of retinal ganglion cells of the monkey, in Szentágothai J, Hátori J, Palkovits M, (eds): Regulatory Functions of the CNS Subsystems: Advances in Physiological Sciences. New York, Pergamon Press, 1981, pp 261-270.

Schein SJ, Marrocco RT, de Monasterio FM: Is there a high concentration of color-selective cells in the V4 area of monkey visual cortex? J Neurophysiol (in press).

de Monasterio FM, Schein SJ: Spectral bandwidths of color-opponent cells of the geniculo-cortical pathway of macaque monkeys. J Neurophysiol (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 BY 00061-03 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
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  Other: Robert Nussenblatt  Kent F Higgins Stanley J. Schein Myles J. Jaffe	M.D., D.Sc.  M.D.  Ph.D. M.D., Ph.D. O.D.	Chief, Section on Visual Processing  Chief, Ophthalmic Immunology Section Senior Staff Fellow Expert Guest Worker  CB NEI CB NEI CB NEI CB NEI CB NEI CB NEI
COOPERATING UNITS (if any)  None		
LAB/BRANCH Clinical Branch		
SECTION Section on Visual Processing		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
Abnormalities of retinal function at the level of <u>rods</u> and <u>cones</u> or their <u>pathways</u> are being documented by <u>electrophysiological</u> and <u>psychophysical</u> studies of <u>patients</u> with <u>posterior uveitis</u> of suspected <u>immunological</u> origin, in addition to experimental studies of posterior uveitis in animal models.		

Project Description:

Protocol Number: 79 FI 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), electrocuculography, sensory dark-adaptation thresholds, visual perimetry, color vision tests and contrast sensitivity functions in cases of ocular toxoplasmosis, pars 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 patients with inflammation of the posterior segment indicate that diffuse and central involvement of the retina produces early electroretinographic waveform changes of cone responses and color vision deficiencies. The electroretinogram changes primarily involving responses mediated by signals from red- and green-sensitive cones, are accompanied by reduction or extinction of responses 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, titan or tetartan-like defects of central vision. We are at present examining specific losses in spatial contrast sensitivity.

The observed changes in electroretinography, coupled with color vision alterations and other psychophysical findings represent a nearly pathognomonic sign of central retinal involvement which seems to be associated with cell-mediated responses to the retinal S-antigen. These results have been confirmed in electrophysiological studies of S-antigen induced posterior uveitis in Old-World monkeys. We are at present attempting to determine by the use of fluorescent dyes alterations in the density and morphology of blue-sensitive cones in experimental uveitis.

Significance to Biomedical Research and the Program of the Institute: The detected electrophysiological signs 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:

Nussenblatt RB, Kuwabara T, de Monasterio FM, and Wacker WB: S-Antigen uveitis in primates: A new model for human disease. Arch Ophthalmol 99:1090-1092, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00006-10 CB	
PERIOD COVERED October 1, 1980, to September 30, 1981			
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 D. 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
COOPERATING UNITS (if any) None			
LAB/BRANCH Clinical Branch			
SECTION Section on Visual Processing			
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205			
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0	
CHECK APPROPRIATE BOX(ES)			
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
<p>Appropriate <u>psychophysical</u> procedures are used to measure the various visual functions of patients in the Eye Clinic, particularly <u>thresholds of visibility</u> for the retinal <u>rods</u> and <u>cones</u> and for <u>discrimination of colors</u>, all under standard conditions. Efforts continue in attempts to find or devise test methods which are more effective, more objective, and less demanding on the patients.</p> <p>Tests were conducted on 435 patients during the past year.</p>			

Project Description:

Protocol Number: 80 EI 08

Objectives: Emphasis has shifted somewhat from the former general aim of improvement in psychophysical test methods to more specifically "The Measurement of Color Vision", which is the title of the included protocol.

Since the conventional tests for color vision defects do not result in measurements, an instrument called the "chromagraph" was developed to fill this need. Findings are automatically plotted in the form of thresholds on a polar graph, which is easily understood at a glance, but is not convenient for statistical analysis. Progress is being made toward attaining a means for rapid digitizing of chromagraph results, which will facilitate statistical studies and correlations.

Methods Employed: All patients referred for psychophysical testing are given the Hardy-Rand Rittler Pseudoisochromatic test for color blindness, the Farnsworth Dichotomous Test Panel D-15, the Nagel anomaloscope for the Rayleigh equation, and the Gunkel Chromagraph test for color discrimination. Due to its tedious nature, the Farnsworth-Munsell 100-Hue test is used only in selected cases.

Computer programs have been devised for scoring the 100-Hue tests and for analysis of the chromagraph findings, but they still require more time than is justified because of the need for manual transcription of data.

Major Findings: The conventional tests for color vision do not appear to be useful in quantitating the severity of color defects or defining their spectral limits. Furthermore, any one of the conventional tests is frequently inconsistent with others in indicating the type of a defect as designated by traditional terminology. Even if a classification is agreed upon, very little is indicated as to what precise colors or saturations the subject sees or fails to see.

The striking difference between the colors a defective subject perceives in the Nagel anomaloscope and those he describes correctly when observing papers and fabrics suggests that confidence in color tests relying on paints, dyes, or pigments may not be justified. Since dichromats never see the true colors (red and green) in the Nagel anomaloscope, they are matching luminosities, which is in no way comparable to a color measurement. Subjects with less severe defects may perceive the red and/or the green more or less properly, but the degree remains unknown, as well as any weakness to other colors.

With the Gunkel Chromagraph thresholds of discrimination for as many colors as desired are plotted on a simple chromaticity circle in a matter of minutes. Size, shape, and location of the neutral area where no color was seen constitute an adequate and logical description of any type of color defect. Color and percentage loss can be approximated at a glance, but for rapid data-handling, this and other patient information should be digitized on tape. This suggests electronic recording directly from the chromagraph, which will require further development.

Significance to Biomedical Research and the Program of the Institute: A poster presentation was made at the April meeting of the Association for Research in Vision, and Ophthalmology demonstrating the utility of the color circle in plotting and understanding color vision and its defects. It is believed that this concept will greatly diminish the aura of mystery and confusion which has long surrounded these subjects.

Data presently on hand confirms the consistency and repeatability of chromagraph measurements. It will also show definite correlations between certain types of color defects and some specific disorders of the eye. It is observed that many subtle defects which are consistently plotted with the chromagraph are not even suggested by the conventional tests.

It seems certain that some form of the chromagraph will eventually replace the other color tests, since it provides better information and in much less time.

Proposed Course: It is desirable that the present studies continue in order to further confirm the validity of chromagraph data and its correlation with specific ocular and systemic disorders. Several months will be required to complete the electronic storage-retrieval system.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

Gunkel RD: Congenital color blindness. Arch Ophthalmol 99:1023-1027, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00121-01 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  Spatial Contrast Sensitivity Studies in Retinal Disease		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Kent E. Higgins Other: Francisco M. de Monasterio  Myles J. Jaffe Rafael C. Caruso	Ph.D. M.D., D.Sc.  O.D. M.D.	Senior Staff Fellow Chief, Section on Visual Processing Guest Worker Expert  CB NEI CB NEI  CB NEI CB NEI
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Branch		
SECTION Section on Visual Processing		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
<p>The purpose of this project is to provide <u>diagnosis</u> of specific alterations of <u>visual acuity</u> at different <u>spatial frequencies</u> in <u>toxic, inflammatory, degenerative, or congenital retinal disorders</u> using measurements of spatial contrast sensitivity.</p>		

Project Description:

Objectives: Diagnosis or evaluation of alterations of contrast sensitivity function in toxic, inflammatory, degenerative and congenital visual disorders affecting the retina and visual pathways. Development of clinical procedures for the study of spatial contrast losses at different spatial frequencies.

Methods Employed: Two different psychophysical techniques are being used to measure spatial contrast sensitivity in normal volunteers and clinical patients. The first technique (method of adjustment) is in common use since it does not require a computer and, presumably, takes less time. Unfortunately, it is not possible to evaluate the exact frequency-of-seeing criterion (or changes therein) that a given patient may be using as a basis for the judgment of threshold visibility. Consequently, changes in sensitivity across spatial frequencies and across time may reflect criterion fluctuations and not a change in the status of the visual pathways. The second technique (forced-choice), in contrast, is essentially free from patient criterion fluctuations. This technique is less commonly used in a clinical setting, primarily because it is extremely time-consuming unless stimulus presentation and data storage are under computer control.

Major Findings: For normal subjects our results indicate that the computer-controlled forced-choice technique requires generally less time than the method of adjustment. More importantly, the within and between subject variability is smaller using the forced-choice technique. Data from these volunteers is being analyzed to obtain normative limits for age and sex.

Preliminary measurements in diverse clinical patients indicate that the forced-choice technique with equal efficiency and reliability in a naive patient population. These measurements also indicate the types and amounts of contrast sensitivity loss that may be produced by different ocular disorders.

Significance to Biomedical Research and the Program of the Institute: This project will facilitate diagnosis and evaluation of retinal disorders in inpatients, outpatients and referred patients of the NIH Eye Clinic. Of particular significance is the preliminary finding that the forced-choice technique is likely to represent the most reliable and accurate technique for the early-detection and follow-up of contrast sensitivity changes produced by diverse ocular disorders.

Proposed Course: Studies of contrast sensitivity function will be continued.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--  
Visual Processing and Amblyopia/Disorders.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00123-01 CB
PERIOD COVERED October 1, 1980, to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  Psychophysics in the Management of Chiasmatic Lesions		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT		
PI: Myles Jaffe Other: Rafael Caruso Kent Higgins Francisco de Monasterio  Elmer Ballintine Pat Christian Doris Collie Mary Fuhrman	O.D. M.D. Ph.D. M.D., D. Sc.  M.D. B.S. A.A.	Guest Worker Expert Senior Staff Fellow Chief, Section on Visual Processing Clinical Director Health Technician Health Technician Health Technician
CB NEI CB NEI CB NEI CB NEI  CB NEI CB NEI CB NEI CB NEI		
COOPERATING UNITS (if any) Department of Radiology, CC Branch of Developmental Endocrinology, NICHD		
LAB/BRANCH Clinical Branch		
SECTION Section on Visual Processing		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  <u>Psychophysical studies</u> will be performed to evaluate the earliest effects of <u>chiasmal compromise</u> on visual function in selected patients with signs and symptoms of pituitary disease. These patients will be evaluated with computerized tomography to define the size of the lesion and its relation to the optic chiasm. The psychophysical tests include <u>color discrimination</u> , <u>Goldmann perimetry</u> , and <u>contrast sensitivity measurements</u> . The results of these tests will assist in the <u>early recognition</u> of an expanding mass. These results will also permit <u>quantitative longitudinal follow-up</u> of the therapy selected.		

Project Description:

Objectives: To find the earliest clinical expression of visual impairment secondary to chiasmal involvement by sellar or parasellar lesions.

Methods Employed:

Conventional methods  
Goldmann kinetic fields  
Nagel anomaloscopy  
Farnsworth Munsell 100 hue  
Farnsworth Munsell Panel D-15  
HRR Pseudoisochromatic plates

Experimental Methods

Goldmann static perimetry: The Goldmann 940 perimeter is used with a modified kinetic perimetry chart adapted for vertical meridian static perimetry. Using the traditional static quantitative perimetric technique, the differential threshold for light perception is determined in a selected set of points in the visual field. One eye is examined at a time using the precise near correction. Only patients with good central fixation are selected for this test.

Two sets of seven points have been selected. These are located along two vertical lines, 5° temporal and 5° nasal to either side of the central vertical meridian and parallel to it. Along each of these two individual vertical lines, one point is placed on the horizontal meridian. The remaining points are located at 10°, 20°, and 30°. The background luminance of the sphere is set at 31.5 asb and the initial luminance of the target is set at 1000 asb. A 1 mm<sup>2</sup> target (Goldmann II) is selected.

The patient should fixate the small central fixation point in the perimeter following adaptation to the luminance level of the sphere for 2 to 3 minutes. The horizontal meridian points are explored first.

Initially, a suprathreshold stimulus (e.g. II 4e, 1000 asb) is presented so that the patient is able to see where the target will appear. If the patient is unable to see the most intense (1000 asb) target, another point is selected. An infrathreshold stimulus is now selected (e.g. a target with a luminance of 4 asb [4a], which will not be perceived by a normal observer at 5° eccentricity). The target is presented for one second, allowing an interval of three seconds between presentations to avoid local adaptation. The luminance difference between two consecutive stimuli is 0.1 log unit. Successive targets of increasing luminance are presented until the patient indicates that the target is perceptible. An identical stimulus is presented for a second time: if the patient perceives it again, the next lower intensity is presented. The stimulus intensity is decreased each time the patient perceives the target on two consecutive presentations, and increased each time the patient is unable to identify it. The lowest stimulus intensity that can be perceived in two successive presentations will be regarded as the threshold, and its luminance recorded. The threshold of the points above and below the horizontal meridian is

determined using the same method, preceeding towards the periphery of the vertical meridian. The luminance of the stimulus that has been identified as the threshold intensity for the neighboring point on the same side of the vertical meridian is usually selected as the initial infrathreshold stimulus for each successive new point.

At the end of the examination, the two sensitivity profiles from opposite sides of the vertical meridian may be compared quantitatively with each other and with corresponding sensitivity profiles of normal subjects.

Forced - Choice Contrast Sensitivity: Contrast sensitivity will be measured using oscilloscope generated, sinusoidal luminance gratings at nine spatial frequencies over the range of 0.9 to 18.0 cycles/degree. The technique used will be a two-alternative temporal forced-choice procedure which is a modification of the double staircase technique. A computer randomly assigns the grating to the first or second time interval and randomly determines the order of spatial frequency presentation in each block of nine trials. The patient indicates by pressing one of two buttons in which of two time intervals a grating appears on an oscilloscope.

The oscilloscope screen subtends 2 degrees of visual angle at 2 meters and is masked by a white card board plaque with a centered square aperature. The illumination falling on to the cardboard is both hue and luminancematched to the luminous flux of the oscilloscope screen.

The cyclopleged patient views this stimulus display through a Badal optometer. This is modified to provide a constant pupil size as well as precise correction for the patient's astigmatism and spherical correction for the viewing distance of the stimulus display.

Major Findings: In patients with no field loss to the (kinetic) Goldmann I1e isopter, (central isopter) the other psychophysical parameters have not showed a consistent significant change. When the I1e isopter has evidenced a vertical meridian defect other psychophysical parameters now being explored have shown a quantifiable alteration.

In conditions where the I1e exclusively has demonstrated field loss, the 100 hue test has generated deutan and tetartan axes together with an overall poor discrimination pattern and an elevated error score. The HRR has demonstrated corroborating findings, specifically, errors indicating red-green deficiencies. The Nagel anomaloscope has not shown an alteration in the Raleigh equation but has demonstrated an extended range in both the light and dark adapted range test. The bias, putatively, is towards the protan direction.

Under the same conditions of hemicentral field loss, patients who presumably have "macular sparing" do show an almost uniform decrease in sensitivity to gratings in all spatial frequencies: the high spatial frequencies tend to be slightly more depressed than the middle and lower spatial frequencies. These

findings in conjunction with the color deficiencies tentatively suggest that in conditions of chiasmal compromise, macular fibers from both halves of the vertical midline are frequently involved.

Significance to Biomedical Research and the Program of the Institute:

The application of the studies to patient care is that psychophysical evaluation of central vision may be a more accurate and quantifiable method both in an initial assessment and in follow-up care to check for expansion or regression of the condition giving rise to chiasmal compromise. It should be noted that the patients in which these studies yield significant results are those with radiologically-documented suprasellar extension.

Proposed Course: We plan to continue these investigations until the number of patients stipulated in the protocol have been seen.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--  
Visual Processing and Amblyopia/Disorders

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00122-01 CB																																										
PERIOD COVERED October 1, 1980, to September 30, 1981																																												
TITLE OF PROJECT (80 characters or less)  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																																												
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Stanley J. Schein</td> <td style="width: 15%;">M.D., Ph.D.</td> <td style="width: 25%;">Expert</td> <td style="width: 10%;">CB</td> <td style="width: 10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Francisco de Monasterio</td> <td>M.D., D.Sc.</td> <td>Chief, Section on Visual Processing</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Rafael C. Caruso</td> <td>M.D.</td> <td>Expert</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Edna P. McCrane</td> <td>B.S.</td> <td>Biologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Jorge A. Martinez</td> <td>B.S.</td> <td>Technician</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Marvin B. Shapiro</td> <td>M.A.</td> <td>Research Mathematician</td> <td>LSMM</td> <td>DCRT</td> </tr> <tr> <td></td> <td>Joanne E. Albano</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> </table>			PI:	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI	Other:	Francisco de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI		Rafael C. Caruso	M.D.	Expert	CB	NEI		Edna P. McCrane	B.S.	Biologist	CB	NEI		Jorge A. Martinez	B.S.	Technician	CB	NEI		Marvin B. Shapiro	M.A.	Research Mathematician	LSMM	DCRT		Joanne E. Albano	Ph.D.	Staff Fellow	LSR	NEI
PI:	Stanley J. Schein	M.D., Ph.D.	Expert	CB	NEI																																							
Other:	Francisco de Monasterio	M.D., D.Sc.	Chief, Section on Visual Processing	CB	NEI																																							
	Rafael C. Caruso	M.D.	Expert	CB	NEI																																							
	Edna P. McCrane	B.S.	Biologist	CB	NEI																																							
	Jorge A. Martinez	B.S.	Technician	CB	NEI																																							
	Marvin B. Shapiro	M.A.	Research Mathematician	LSMM	DCRT																																							
	Joanne E. Albano	Ph.D.	Staff Fellow	LSR	NEI																																							
COOPERATING UNITS (if any) Laboratory of Statistical and Mathematical Methodology, DCRT Laboratory of Sensorimotor Research, NEI																																												
LAB/BRANCH Clinical Branch																																												
SECTION Section on Visual Processing																																												
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																																												
TOTAL MANYEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0.0																																										
CHECK APPROPRIATE BOX(ES)																																												
<input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER																																												
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																												
SUMMARY OF WORK (200 words or less - underline keywords)																																												
<p>The aim of this project is to study the <u>functional anatomical</u> organization of neurons of the visual system of non-human primates that can serve as a model for the human visual system. <u>Parcellation of the monkey visual cortex</u> is based on silver cell, silver myelin and cytochrome oxidase staining and on connectional studies. <u>Cytochrome oxidase staining</u> is also being used to <u>activity label chronic stimulation states</u> in the brain, and improvements in the <u>2-deoxy-glucose activity labelling method</u> for <u>acute stimulation states</u> are being developed. Retinal studies have focused on <u>blue-sensitive cones</u>, their <u>pattern</u> and <u>synaptology</u> and the <u>mechanism</u> by which <u>blue-sensitive cones</u> may be specifically stained.</p>																																												

Project Description:

Objectives: To study the neural organization underlying visual processing in retina and cortex.

Methods Employed: Silver cell, silver-myelin and cytochrome oxidase staining of monkey visual cortex; retrograde transport studies to mark cortical projection zones; activity labelling of chronic states with cytochrome oxidase; activity labelling of acute states with 2-deoxyglucose; intravitreal injection of dyes; histological processing of retina, including whole mounts, plastic and celloidin; modelling and statistical analyses of patterns; electron microscopy.

Major Findings:

I. Parcellation of visual cortex:

Cell and myelin stains have been used traditionally to subdivide the cerebral cortex into areas. This approach assumes that the pattern of elements reflects differences in function and connectivity among individual visual areas. Dissatisfaction with the so-called architectonic approach was derived from two sources. First, the staining patterns were not sharp, and borders (subtle in fact) were difficult to mark with certainty. Second, confirmatory evidence for the assignment of borders on the basis of functional and connectational differences was unavailable.

A. Silver staining for parcellation of visual cortex (Schein, de Monasterio, McCrane, and Martinez): Recent discoveries of silver methods for cell staining (Merker's method) and myelin staining (Gallyas' method) provide powerful tools for study of the cyto- and myelo-architecture of visual cortex. Further, we have markedly improved the new silver cell stain to the point where the neuronal soma are stained in their entirety, along with the initial portions of primary processes. Striate visual cortex was used as a testing ground for the improved silver cell stain: We identified and photographed representative examples of every cell type in striate cortex. We have begun applying these two powerful staining methods to extrastriate visual cortex.

In conjunction with physiological studies of V4, an extrastriate region once thought to be specialized for color processing, we applied these silver staining methods. The borders of the V4 region were readily marked, and a second, new visual area within this region could be demarcated.

B. Cytochrome oxidase staining for parcellation of visual cortex (Schein, de Monasterio, McCrane): Cytochrome oxidase, a mitochondrial enzyme, provides staining patterns based on mitochondrial labelling in cells of cerebral cortex. The intensity of staining reflects chronic (aerobic) activity. Areas of visual cortex differing in function and positioning of mitochondrial reservoirs (cell bodies and nerve terminals) would be expected to differ in their cytochrome oxidase staining patterns. The patterns are not crisp, since the "background" of mitochondria is high, but borders of extrastriate visual areas can be marked.

Borders based on cytochrome oxidase staining reflect both structural as well as long-term functional differences between visual areas. The 2-deoxyglucose method, described below, should mark borders based on short-term functional differences.

C. Connectional studies (Schein, Albano, de Monasterio): In addition to receiving direct retinal input, superficial layers of the superior colliculus receive topographic input from extrastriate visual areas. Retrograde transport of fluorescent dyes from superior colliculus should mark extrastriate visual areas and permit correlation of connectional organization with other parcellation methods.

We have used rat visual cortex to test fluorescent dyes for use in retrograde transport. We have been able to establish a group of four retrogradely-transported dyes, all four separable on the basis of fluorescence properties. We are now studying the monkey visual system with these dyes.

## II. Activity-labelling of central nervous system

A. Acute studies with 2-deoxyglucose (Schein, de Monasterio): We have been developing and studying the principles of techniques aimed at improving the resolution of the quantitative deoxyglucose method. The method, developed by Sokoloff, Kennedy and their associates at the NIH, results in autoradiographic mapping of glucose utilization. Such a "glucogram" reflects metabolism modulated by short-term nervous activity. The coarse autoradiographic resolution presently available smears local peaks and valleys of activity. We expect that with fine resolution glucograms (giving higher peaks and lower valleys as well as crisper patterns) it will be possible to assign functional roles to the different areas of the visual cortex.

Each of the steps proposed in the development of fine-resolution glucography must operate within severe constraints: The large size of the tissue involved, the necessity of quantitative retention and of strict localization of the water-soluble label. We have observed ocular dominance columns in the monkey striate visual cortex, one eye being closed during exposure to 2-deoxyglucose. Further studies are in progress. The methods developed for deoxyglucose may be applicable to other water-soluble molecules, and we have initiated studies along these lines.

B. Chronic studies with cytochrome oxidase (Schein, McCrane, de Monasterio): The richness of mitochondrial enzymes, among them cytochrome oxidase, in brain is a function of chronic aerobic demand. We enucleated a monkey and waited two months before sacrifice. Portions of the striate visual cortices were sectioned perpendicular and parallel to the surface. The normal pattern of staining was radically altered. Among the many changes, most noteworthy is the staining of layer 4CB, the later of striate cortex which receives a heavy parvocellular lateral geniculate projection. When sectioned parallel to the surface, through layer 4CB, the slabs of ocular dominance

columns were readily visualized. This example indicates that obvious changes can be observed after long-term changes of visual function.

### III. Studies of blue-sensitive cones.

A. Identification of procion-stained photoreceptors as blue-sensitive cones (de Monasterio, Schein, McCrane): Intravitreal injections of a fluorescent dye, Procion Yellow, results in the complete and systematic staining of a cone population in monkey retina. These cones form an approximately regular mosaic whose separation varies with retinal eccentricity. They are absent in the very center of the fovea and their peak density occurs at  $1^\circ$  eccentricity. The retinal distribution of these stained cones resembles that reported for blue-sensitive cones of other primates. Consistent with the idea that cones completely stained by the dye are blue-sensitive cones, they are found with much less incidence in cat and rabbit retina.

B. Mechanism of blue-cone selectivity of procion staining (Schein de Monasterio, Caruso): Köllner's Rule notes that disease of the retina causes a "blue-yellow" defect indicating that blue-sensitive cones have an excessive vulnerability to retinal insult. We hypothesize that the blue cones are most sensitive to the chemical toxicity of tissue-reactive dyes like Procion Yellow. When the blue cones die, the dye penetrates to give a complete staining, producing Golgi-like silhouettes of the blue cones. To test this hypothesis, we have sought a dye which could be used as a "leakiness detector", i.e. which would not stain photoreceptors in a normal retina but would concurrently stain procion affected cells. If this hypothesis could be confirmed, then it should be possible to titrate other 'poisons' to generate retinas free of blue-cones. Such animals would be of use in basic psychophysical and physiological studies as well as in the development and understanding of clinically-related electro-diagnostic methods.

C. Analysis of blue-sensitive cone pattern (Schein, Shapiro, de Monasterio): A pattern of points, such as that described by the blue cones, may be described as irregularly or regularly spaced. Furthermore, the pattern may be described according to a model which generates a similar pattern. The simplest models of a regular pattern are triangular, square, and hexagonal packing. More complex models discuss the packing of "hard spheres", "soft-spheres", etc. How best to describe a spatial pattern is a problem of wide-spread theoretical interest, particularly to biologists. We are able to whole-mount a horizontal strip of blue-cone-stained monkey retina, without distortion or loss of labelled cones. Computerized digitization of blue-cone positions is followed by pattern analysis: (1) Since the blue cone pattern is regular and does not match any of the simple lattice structures, the analysis requires the more complex models. (2) In order to test a model against the actual pattern, appropriate statistics must be chosen. (3) In order to appreciate the quality of the match between the model and the actual patterns, a method of display is required. We have all of the analytical steps operating on computer programs and have begun trials of models.

D. Electronmicroscopic studies of monkey blue cones (Caruso, de Monasterio, and Schein): We have found two electron-dense dyes which stain blue cones as does Procion Yellow. Stained retinæ are in process now. The

blue-cone system differs from the red and green-cone system in a number of psychophysical and physiological properties. Investigation of the synaptology of the blue cones may provide insight into some of these properties.

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. Functional description of cortical organization in monkey should, in itself, provide insight in human visual deficiencies. More specifically, a positron-emitter labelled 2-deoxyglucose is already in use for non-invasive activity labelling in humans. The resolution of the PET scans is poor at present, nearly one centimeter, and the theoretical limit is about five millimeters. Understanding the function of separate visual areas in monkey is therefore of special importance to maximally and differentially stimulate entire areas, encompassing large volumes of tissue.

Our investigations of the blue-cone mosaic has potentially direct clinical application in understanding "blue/yellow" defects in humans with retinal disease. In particular, we expect these studies to extend our understanding of electrodiagnostic methods. In the longer run, the biological specialization of blue cones for color-processing makes our interest on blue cones a strategic approach to color processing problems in general.

Proposed Course: All of the neuroanatomical studies described will be continued. Retinal studies, already productive, should continue to be so. The cortical studies are just now at a stage where their promise may be fulfilled.

NRI Research Program: Strabismus, Amblyopia, and Visual Processing-- Structure and Function/ Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

de Monasterio FM, Schein SJ, McCrane EP: Staining of blue-sensitive cones of the monkey retina by a fluorescent dye. Science (in press).

de Monasterio FM, Schein SJ, McCrane EP: Staining of blue-sensitive cones of macaque retina by a fluorescent dye. Invest Ophthalmol Vis Sci 20 (suppl):151, 1981.

Caruso RC, de Monasterio FM, Schein SJ, and McCrane EP: Differential staining of monkey retinal cells by procion and lucifer yellow. Invest Ophthalmol Vis Sci 20 (suppl):202, 1981.

Schein SJ, de Monasterio FM: Improved localization of 2-deoxyglucose in brain, by freeze-substitution. Trans Am Soc Neurochem 12:85, 1981.



Laboratory of Sensorimotor Research



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

REPORT OF THE CHIEF, LABORATORY OF SENSORIMOTOR RESEARCH  
Robert H. Wurtz, Ph.D.

During the year covered by this third annual report of the Laboratory of Sensorimotor Research, the laboratory was organized into three sections: a section on visuomotor integration; a section on neuro-ophthalmologic mechanisms; and a section on oculomotor control. Pending availability of space in the ACRF and Building 10, the laboratory remained scattered in temporary space in Buildings 9 and 36 and, through collaborative arrangements, at Johns Hopkins University. Preparation for the new laboratory this year included the development of a computer system for real-time experimental control (the REX system), a prototype of which is operating within the laboratory.

Research in the laboratory this year concentrated largely on the visuomotor control of eye movements. Nearly all types of eye movements were studied: rapid or saccadic eye movements that move the eye rapidly from one part of the visual field to another; pursuit eye movements that allow the eye to follow a moving target; vergence eye movements that allow the eyes to fixate on objects at different distances with concomitant adjustment of accommodation to maintain a clear image; and the vestibulo-ocular reflex that steadies the eye in spite of head and body movements.

Saccadic eye movements were investigated in two areas of the brain: the frontal eye fields of the cerebral cortex and the substantia nigra of the basal ganglia. The frontal eye fields have been known for over a century to be related in some way to the initiation of eye movements, and previous work in this laboratory has shown that the area is specifically involved in visually-initiated saccades. Work this year has elucidated the neural mechanisms that might underlie the generation of these visually-guided saccades. Neurons in this area of cortex include those that are active in anticipation of the onset of the visual signal to make an eye movement, neurons that respond to that visual stimulus, neurons that discharge before eye movements, and neurons that discharge after eye movements. The critical information for frontal eye field neurons has been shown to be not the retinal location of the eye movement target but rather the required direction of the eye movement. These experiments indicate that the frontal eye fields integrate visual information with recent oculomotor and motivational information to determine the appropriateness, direction, and amplitude of an eye movement.

The basal ganglia are known to be related to the initiation of movement, but it is only recently that experiments in this laboratory have shown that cells in one of the output structures from the basal ganglia, the lateral part of the pars reticulata of the substantia nigra, discharge in relation to saccadic eye movements. Cells in the substantia nigra show a decrease in their tonic rate of discharge before onset of saccades and, like the cells in the frontal eye fields, this response is related primarily to saccades to visually-guided targets. Cells have now been identified that discharge when a saccade is made, not to a spot of light currently present, but to a spot of light previously present--to a remembered target. The response is related to

the visual stimulus that must be remembered or to the saccade to that remembered stimulus or to both of these events. Anatomical experiments done this year show that the substantia nigra is one of the major subcortical sources of projections to the superior colliculus which, in turn, previously has been shown to contain cells that increase their discharge rate before saccadic eye movements. Electrical stimulation experiments have now shown that many cells in the substantia nigra send fibers to the superior colliculus. This connection between substantia nigra and superior colliculus suggests that the decrease in discharge rate of tonically firing neurons in the substantia nigra might act on the superior colliculus cells by releasing them from inhibition. These stimulation and recording experiments in awake behaving monkeys also demonstrate the possibility of determining the inter-relationships between areas related to the initiation of saccades such as the frontal eye fields, the substantia nigra, and the superior colliculus.

While considerable effort has been devoted in the last ten years to an analysis of neural mechanisms and the areas of the brain involved in the initiation of saccadic eye movements, much less effort has been devoted to smooth pursuit eye movements. Experiments this year in monkeys with unilateral ablation of the primary visual (striate) cortex show that while the monkeys can make saccades into their "blind" visual field, they cannot pursue a moving target in that field. This implies that striate cortex is necessary for processing stimulus velocity or position information required in the generation of specific eye movements. In addition, analysis of cellular activity in areas anterior to the striate cortex has shown that some cells discharge during smooth pursuit eye movements; in areas medial and anterior to the middle temporal visual area in the superior temporal sulcus, a prestriate visual area, cells have been found that respond during smooth pursuit eye movements in ways which cannot be fully explained by their passive visual properties observed during fixations. Cells also have been found in other regions of the superior temporal sulcus which become active when the monkey makes smooth pursuit eye movements in a particular direction but are inhibited by eye movements in the opposite direction. In net, these experiments begin to indicate the visual areas of the cerebral cortex that are involved in pursuit eye movements.

Transfer of fixation between targets at different distances involves a change in the vergence of the eyes which operates to eliminate disparity so that the image of the object being looked at falls on equivalent parts of the retina. At the same time there is a change in the curvature of the lens, accommodation, which operates to eliminate blur. These two processes are linked. During viewing with one eye, accommodation changes are accompanied by vergence changes even though the cue of double vision is absent. This implies that vergence is reflexly linked to accommodation. Experiments done this year have shown that this link between vergence and accommodation is subject to adaptive control by the brain. Human subjects were fitted with specially constructed spectacles to increase the apparent separation of the two eyes. This increased the amount of vergence per unit change in accommodation necessary to maintain single, clear vision. After a short period of exposure, this modified vision was sufficient to cause large increases in the vergence change associated with the unit change in accommodation. This finding supports the view that the close coupling between vergence and accommodation is subject to adaptive regulation as a result of visual experience. However, decreasing the apparent separation of the eyes with different spectacles failed to affect the

magnitude of the accommodation-vergence response implying considerable asymmetry in the adaptive control exerted by the brain.

Another reflex that has been shown to be under adaptive control is the vestibulo-ocular reflex. Previous work has shown that modifying the visual input to the system results in a modification of the reflex. Work this year has shown that adaptation of this reflex is specific to head movements of a certain frequency. Exposing the system to passive oscillations at one frequency resulted in changes of the reflex that were most marked at that frequency and that did not affect the reflex as much at other frequencies. These results suggest that the vestibulo-ocular reflex has frequency selective channels.

The goal of these experiments on visuomotor control is an understanding of the brain circuits which underlie a particular visuomotor mechanism. One key step in such understanding is the formulation of a quantitative model which summarizes the known experimental results and allows new relationships to be seen. Such a model was successfully developed in relation to experiments on the interaction between the vestibulo-ocular reflex and the optokinetic response. Previous experimental observations showed that the optokinetic response could be divided into an initial rapid and a later gradual response and that the gradual component changed up and down as the gain of the vestibulo-ocular reflex was changed up and down. The model, which includes a vestibular system and a gradual optokinetic subsystem that converge upon a single gain element, was tested using a digital computer. Modifications of only the single gain element in the model produced changes similar to the results of the experiments. Models such as this, developed in close association with data generated in the laboratory, will find increasing use in future work in the laboratory. This is particularly true in our attempts to link insights gained from basic research on monkeys and man with clinical problems of dysfunction in man.

In addition to investigating the neuromechanisms of visuomotor control, several experiments were done on cortical visual areas related to the neural mechanisms of visual perception. One of these investigations concentrated on an area in the middle temporal area of cerebral cortex that has been implicated in the perception of movement since neurons in this area respond selectively to the direction of motion of visual stimuli. Initial experiments have replicated these observations in awake monkeys. In addition, when the stimulus was moved across the receptive field as the monkey fixated, the response was very similar to that when the monkey tracked a moving target and thereby moved the receptive field as a stationary stimulus. These experiments show that extraretinal influences have little effect on the response of these cells. Another investigation concentrated on the inferior temporal area of cerebral cortex of the monkey that has been implicated as the critical structure related to form and pattern vision. As in other experiments in awake monkeys done in the laboratory, the responses of single neurons to a visual stimulus were investigated while the monkey fixated on a spot of light. The effect of the fixation spot was dramatic: removal of the fixation point increased the visual response and expanded the size of the visual receptive fields. Subsequent control experiments suggest that this increased response is related to visual interaction within the visual field rather than a shift in visual attention. These experiments show clearly that the receptive field properties of inferior temporal cells are altered during fixation on a visual target, and this fixation is an integral part of our normal vision.

An understanding of the organization of the primate brain underlying visuomotor control and perception clearly requires at least the range of approaches (anatomical, physiological, behavioral, and theoretical) that are summarized here and described in the following project reports. Our hope is that an understanding of these mechanisms derived from this research will aid in the diagnosis and treatment of human disease.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00049-03 LSR

PERIOD COVERED

October 1, 1980, to September 30, 1981

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. Chief, Neuro- LSR NEI  
ophthalmologic  
Mechanisms Section  
Other: Charles J. Bruce Ph.D. Senior Staff Fellow LSR NEI

COOPERATING UNITS (if any)

Department of Neurology, Georgetown University School of Medicine

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Neuro-ophthalmologic Mechanisms Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

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)

Studies are being conducted to determine the mechanisms through which the frontal eye fields of the cerebral cortex exert control over eye movements and visual attention in the monkey. Single cell recordings are made while the monkeys perform a series of visual tasks involving eye movements or visual fixation. We have found that the frontal eye fields contain a neural mechanism for the generation of visually-guided eye movements. Component neurons include neurons anticipating the onset of a signal to make an eye movement, neurons responding to the stimulus used to evoke an eye movement, neurons discharging specifically before eye movements (both to visual targets and to remembered visual targets), and neurons discharging after eye movements. Experiments creating a dissonance between retinal stimulation and required eye movement direction have shown that the critical information for frontal eye field neurons is not the retinal location of an eye movement target but rather the required direction of the eye movement. These data imply that the frontal eye fields contain a neural mechanism to integrate visual information, recent oculomotor information, and motivational information to determine the appropriateness, direction, and amplitude of an eye movement.

PHS-6040

(Rev. 2-81)

319

Project Description:

Objectives: Previous work in this laboratory established that of the two visual association areas most likely to be involved in the visual initiation of eye movements, the posterior parietal cortex and the frontal eye fields, the latter was by far the better candidate. In this area, neurons with visual receptive fields were shown to give enhanced discharges before eye movements to the stimulus in their receptive field but not when the same stimulus was used in other kinds of behavior. Conversely, in the parietal cortex, neurons which gave enhanced responses were shown to yield these responses whenever the animal attended to the target, not only when it made a saccadic eye movement to it. In addition, low threshold microstimulation through the recording microelectrode revealed that the eye movements evoked by this stimulation were predicted by the visual receptive field of the neurons at the stimulated site. Given these data, it was decided to investigate in greater detail the visual and oculomotor properties of the frontal eye fields in order to gain a greater understanding of the cortical processing involved in the generation of eye movements. It was also decided to use axoplasmic transport methods to delineate the pathways by which the cerebral cortex communicated this information to the brainstem oculomotor areas.

Methods Employed: A digital computer was used for behavioral control, data acquisition, and on- and off-line analysis of monkey behavior, eye movement, and neuronal discharge time patterns. Monkeys were trained on a series of visuomotor tasks, including visual fixation, single saccadic eye movements, saccadic eye movements to flashed targets, saccadic eye movements to remembered targets, and successive saccadic eye movements to briefly flashed targets. Activity of single units was measured during these tasks. Eye movements were measured using the magnetic search coil technique so that accurate quantitative measures of eye position and velocity could be obtained. A computerized system was developed so that eye movements of different targets could be presented in random and pseudo-random fashion. Several untrained monkeys were prepared for semichronic experiments in which large areas of the frontal cortex could be explored under direct vision in order to find low threshold visual areas and make a map of eye movements evoked by stimulation and also to delineate areas into which radioactively labeled amino acids could be placed in order to determine the neuroanatomic targets of areas from which eye movements could be evoked by low threshold electrical stimulation.

Major Findings: There is a continuum of electrical discharge in the frontal eye fields from the anticipation of a saccade to the registration of its accomplishment. A class of cells was described that began to discharge before the stimulus to make an eye movement occurred. These anticipatory neurons were directionally selective in that they began to discharge before the presentation of a stimulus when that stimulus occurred repeatedly in the required eye movement direction for the animal. However, the anticipatory discharge did not occur when the animal either was not required to make an eye movement or required to make an eye movement in an opposite direction. The anticipatory discharge did not occur on the first trial in any given direction, but required two to four trials to build up, and similarly, decayed

after two to four trials. A class of visually responsive neurons was described whose visual responsiveness was independent of eye movements. A second class of visually responsive neurons gives an enhanced discharge before eye movements into the receptive field of the neuron. A series of visuomotor neurons is present in the frontal eye field which give enhanced discharges to stimuli which are the targets of eye movements but also respond to some extent when the animal makes an eye movement to a remembered spot of light in total darkness. They do not discharge briskly before spontaneous eye movements made in total darkness. The bursts of these neurons tend to begin with the onset of the visual stimulus and continue to the eye movement.

Postmotor neurons discharge during and after eye movements. These neurons tend to discharge regardless of the circumstances under which the eye movement is made. A class of neurons has both visual and premotor discharge for eye movements in one direction, and postmotor discharge for eye movements in an opposite direction.

Because of the dominant visual input to neurons in the frontal eye fields, it was relevant to know if the important factor in determining the function of these neurons was the retinal location of a visual stimulus or the eye movement necessary to bring that stimulus to the fovea. These two factors were dissociated by requesting the animal to make two eye movements in total darkness to successively flashed spots of light. Since both spots of light were flashed and disappeared before the onset of the first movement, the eye movement to the second spot of light was made in the direction not predictable alone from the retinal location of the light but predictable from the vector subtraction of the preceding eye movement from the retinal location. Thus, the monkey could be asked to make a downward eye movement to a target that appeared only in its upper visual field. Most visuomotor cells gave a brisk discharge when the eye movement direction and amplitude was appropriate, regardless of the retinal location of the stimulus. Although some of this discharge could be attributed to the motor or postmotor components of the neuronal discharge, some component of discharge could only be explained as due to the visual stimulation arriving from a retinal area not usually in the receptive field of the neuron. These data imply that the frontal eye fields integrate both retinal stimulation and recent eye movements in order to issue a signal to initiate an eye movement of a certain direction and amplitude.

Electrical stimulation evokes eye movements at low current threshold in the frontal eye fields. In a series of experiments using untrained monkeys, penetrations were made normal to the cortical surface and eye movements measured. The direction of eye movements made from each site is reproducible and, as the electrode progresses through the frontal eye fields, there is a progression of change of eye movement direction which can undergo several absolute reversals. This indicates that, although there is a definite organization of frontal eye field topography, the organization is not that of simple retinotopic map.

The frontal eye fields, as defined by the area which produces eye movements at low threshold to electrical stimulation, project to cortical and brainstem areas involved in visual behavior. Ipsilateral cortical projection

areas include the immediate post arcuate area, the cortex surrounding the principal sulcus, and the posterior parietal cortical cortex. There is also a discrete projection to the contralateral frontal eye field. Deeper projection targets include the claustrum, caudate, several thalamic nuclei including the lateral rim of the medial dorsal nucleus and the medial pulvinar, the intermediate layers of the superior colliculus, and the parabigeminal nucleus. This pattern indicates that the oculomotor signal from the frontal eye fields interacts with visual and oculomotor information from the superior colliculus, both directly and through intermediate nuclei. It also interacts with the attentional and polymodal activity in the posterior parietal cortex, through the pulvinar and directly. The animal's oculomotor behavior must be constructed to some extent by these overlapping neural loops.

Significance to Biomedical Research and the Program of the Institute:

The frontal eye fields are important in their own right as the cerebral cortical region concerned with the guidance of eye movements and also as a cerebral cortical model for a premotor cortex. By understanding the mechanisms by which the frontal eye fields convert visual information into a signal for an eye movement, we can begin to understand the basic mechanisms by which the brain integrates sensory information into motor programs. We can also begin to understand the mechanisms of the deficits in patients with lesions in visual-motor control areas and use this understanding for improved diagnosis and the development of rehabilitative strategies.

Proposed Course: Stimulating electrodes will be placed in several of the areas to which the frontal eye fields project, and the projection properties of physiologically identified frontal eye field neurons will be analyzed. The functional anatomy of the region will be studied from two different points of view: the preliminary mapping data will be expanded to provide a functional topography, and a correlation between cell type and cortical layer will be undertaken.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Goldberg ME, Bushnell MC: The role of the frontal eye fields in visually guided saccades, in Fuchs AF, Becker W (eds): Progress in Oculomotor Research. New York, Elsevier, 1981, pp 185-192.

Bushnell MC, Goldberg ME, Robinson DL: Behavioral enhancement of visual responses in monkey cerebral cortex: I. Modulation in posterior parietal cortex related to selective visual attention. J Neurophysiol (in press).

Goldberg ME, Bushnell MC: Behavioral enhancement of visual responses in monkey cerebral cortex: II. Modulation in frontal eye fields specifically related to saccades. J Neurophysiol (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00047-03 LSR
PERIOD COVERED October 1, 1980, to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  <u>Visual Processing in Brains following Cortical Ablation</u>		
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. Chief, Neuro-ophthalmologic Mechanisms Section LSR NEI  Other: Charles J. Bruce Ph.D. Senior Staff Fellow LSR NEI Leslie G. Ungerleider Ph.D. Senior Staff Fellow LN NIMH Mortimer Mishkin Ph.D. Research Psychologist LN NIMH		
COOPERATING UNITS (if any)		
Department of Neurology, Georgetown University School of Medicine <u>Laboratory of Neuropsychology, NIMH</u> LAB/BRANCH		
<u>Laboratory of Sensorimotor Research</u> SECTION		
<u>Neuro-ophthalmologic Mechanisms Section</u> INSTITUTE AND LOCATION		
<u>National Eye Institute, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER		
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)		
The <u>striate cortex</u> of one <u>hemisphere</u> of a <u>rhesus monkey</u> is removed surgically under direct vision. The monkeys are allowed to recover from the effects of surgery in a normally lit environment. The monkeys are then trained on a series of tasks requiring visual perception and visually-guided eye movements. They are then prepared for chronic neurophysiological recording and for eye position recording. The activity of <u>single neurons</u> in the <u>posterior parietal cortex</u> both ipsilateral and contralateral to the lesion, is studied. The monkey's oculomotor capacity is studied quantitatively. Preliminary results indicate that the great bulk of the parietal cortex has its visual responsiveness to stimuli contralateral to the damaged hemisphere eliminated. The animals can make normal saccadic eye movements into the contralateral field and normal pursuit movements. However, they cannot make normal pursuit movements when the stimulus is artificially maintained in the visual field contralateral to the lesions. This indicates that visual activity in the posterior parietal cortex is partially dependent upon an intact visual cortex as is normal pursuit movement.		

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 and prestriate cortex; second, via the superior colliculus, then through posterior thalamic nuclei. These pathways interact: the striate cortex projects to the superior colliculus and the pulvinar of the thalamus, and the pulvinar projects to the prestriate cortex. Nonetheless, these areas are each capable of contributing to visual behavior in the absence of the other. Even in humans, where it was traditionally thought that striate damage led to total visual impairment, it has now become clear that there is some residual visual function in the absence of striate cortex which can be accessed using forced choice or nonverbal methods of evaluation. Since parietal cortex should receive the subcortical visual pathway in the absence of striate cortex, it was of interest to see if this area had enough visual processing to support the behavior found in the presence of striate lesions. Initial work in this laboratory showed that this residual visual processing could be performed in the cerebral cortex by parietal neurons that are visually responsive even in the absence of striate cortex. This visual activity was limited to a small area of the parietal cortex, but within this area the area seemed quite normal. Histological analysis found this striate independent function to be present in at least two discrete areas of parietal cortex: one on the dorsum of the inferior parietal lobule at the interparietal sulcus and one in the posterior part of the inferior parietal lobule in the superior temporal sulcus. Recent work on this project has concentrated on preparing animals for a further evaluation of these striate independent parietal areas and a detailed examination of the oculomotor strategies employed by animals with striate cortex lesions.

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 smooth pursuit, and saccadic eye movements. The monkeys were implanted with the magnetic search coils in order to measure accurately the eye position in space. Computer programs were written in order that smooth pursuit movements could be evaluated.

Major Findings: Animals with striate lesions are capable of saccadic eye movements of normal amplitude and velocity into their striate independent visual field. This implies that striate cortex and the bulk of parietal cortex are not critical for the localization of visual targets in space. Although hemidestriate animals can perform smooth pursuit eye movements under certain circumstances, there are two conditions under which these animals cannot: the first is when the computer constantly keeps the stimulus in the hemianopic field. The second is when the stimulus jumps into the hemianopic field. This implies that striate cortex is necessary for processing the stimulus velocity or position information required in the generation of smooth pursuit movements.

Significance to Biomedical Research and the Program of the Institute:

These results show that striate cortex and the bulk of parietal cortex are not necessary for the determination of a target's position in space but are necessary for determining its velocity of movement. Understanding how the brain parcels out different aspects of the process of visual analysis to different areas will not only further our knowledge of the functions of the normal brain but may also contribute to the development of strategies for the rehabilitation of patients with damage to those brain areas involved in some aspects of vision.

Proposed Course: A further survey of visual responsiveness of striate neurons will be undertaken to verify the existence and location of the previously described areas of spared function. The oculomotor capability, including optokinetic nystagmus, of animals with striate lesions will be further analyzed. A clinical protocol to study visual and oculomotor function in hemianopic patients will be initiated.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--  
Visual Processing and Amblyopia (Disorders)

Publications: None



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00112-01 LSR

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Adaptive Regulation in the Vestibulo-ocular System

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

PI:	Frederick A. Miles	D.Phil.	Research Physiologist	LSR NEI
Other:	Lance M. Optican	Ph.D.	Staff Fellow	LSR NEI
	Stephen G. Lisberger	Ph.D.	Staff Fellow	LNP NIMH

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Oculomotor Control Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

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 aim of this project is to increase our understanding of the adaptive mechanisms responsible for maintaining appropriate performance of the vestibulo-ocular reflex. Our previous work had used various optical devices that disturb the visual inputs associated with head movements to show that the gain and the phase of this reflex are both subject to visually-mediated regulation. The present project used 2x telescopic spectacles and fixed-field goggles in conjunction with passive sinusoidal oscillations to adapt the vestibulo-ocular reflex in four rhesus monkeys. Large changes in gain were seen at the adapting frequency, while at adjacent frequencies gain changes were less pronounced and associated with small but highly consistent amounts of phase lag or phase lead. Such frequency selective effects suggest the existence of temporal frequency channels in vestibulo-ocular pathways that are differentiated on the basis of phase shift.

Project Description:

Objectives: Our previous studies had used various optical devices to establish the existence of long-term adaptive mechanisms that are important for the maintenance of appropriate performance levels in the vestibulo-ocular reflex. These experiments suggested that the adaptation resulting from passive, sinusoidal oscillations might show frequency selective effects and the present experiments were undertaken to test this possibility systematically.

Methods Employed: The gain of the vestibulo-ocular reflex in four rhesus monkeys was increased by prolonged exposure to 2x telescopic spectacles or decreased by exposure to goggles providing a visual field that was fixed with respect to the head. While previous studies allowed the monkeys to undergo adaptation with free head, for the present experiments the monkeys were placed in a special primate restraining chair which could be oscillated about the vertical axis. After the monkey's head was secured to the chair, it was subjected to continuous sinusoidal oscillations at one selected frequency. Eye movements were measured using the electromagnetic search coil technique and monitored continuously. At regular intervals, the spectacles were removed, all lights extinguished and the horizontal vestibulo-ocular reflex tested over a wide range of sinusoidal frequencies in complete darkness.

Major Findings: The passive oscillation paradigm produced larger changes in vestibulo-ocular reflex gain at the adapting frequency than at adjacent frequencies. For example, adaptation at 0.2 hz produced larger changes in vestibulo-ocular reflex gain when tested at 0.2 hz (average for the four monkeys reaching high gains of 1.66 and low gains of 0.42) than when tested at 2.0 hz (up to 1.35 and down to 0.63). Similarly, adaptation at 2.0 hz produced larger changes in vestibulo-ocular reflex gain at 2.0 hz (up to 1.62 and down to 0.39) than at 0.2 hz (up to 1.10 and down to 0.62). While eye velocity remained 180° out of phase with head velocity at the frequency of adaptation, consistent small changes in phase shift were seen at adjacent frequencies. At frequencies above that used for adaptation, increases in vestibulo-ocular reflex gain caused eye velocity to lag the normal reflex, while decreases caused eye velocity to show phase lead. Conversely, at frequencies below that used for adaptation, increases in vestibulo-ocular reflex gain caused eye velocity to lead the normal reflex, while decreases caused phase lag. Such frequency-selective adaptation suggests the existence of temporal frequency channels in vestibulo-ocular pathways. The complex dependence of vestibulo-ocular reflex phase on gain and frequency can be explained if individual channels are also differentiated on the basis of phase shift.

Significance to Biomedical Research and the Program of the Institute:

A great deal is known about the anatomy and physiology of eye movements. The discrete, rather machine-like quality of the oculomotor system has proved particularly amenable to the analysis of the information processing on the level of the system and the single neuron. The fact that the system also displays plasticity opens up new approaches to the subject of motor

learning at the cellular level in the primate central nervous system. Advances toward understanding the mechanism of motor learning in the oculomotor system represent an important step toward understanding the maturation of the normal nervous system and the self-repairing abilities of the diseased nervous system. An adaptive mechanism that can operate selectively over narrow band widths constitutes a highly flexible and tunable interface between afferents and motor neurons. By compensating in narrow band widths for frequency-dependent variations in primary afferent responses, this interface could provide the means by which the frequency-independent performance of the mature vestibulo-ocular reflex is established during development and subsequently maintained through adulthood.

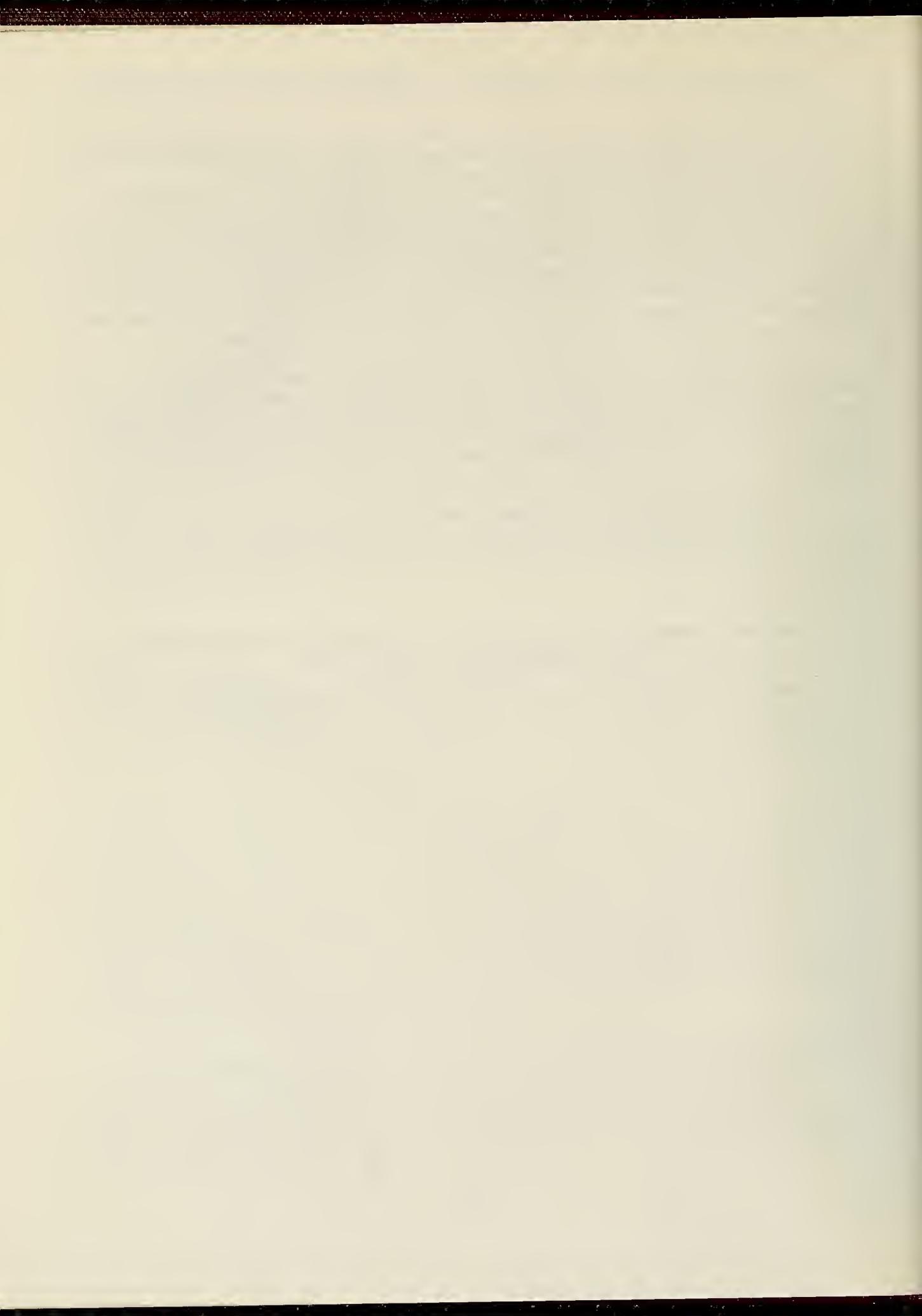
Proposed Course: The adaptive capability of the vestibulo-ocular reflex will continue to be the main concern in these studies. While previous studies have concentrated largely on the regulation of gain, future studies will emphasize the regulation of phase, using adaptation paradigms that introduce a modest phase shift between visual inputs and head movements.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Miles FA, Lisberger SG: Plasticity in the vestibulo-ocular reflex: A new hypothesis. Ann Rev Neurosci 4:273-299, 1981.

Miles FA, Lisberger SG: The "error" signals subserving adaptive gain control in the primate vestibulo-ocular reflex. Ann NY Acad (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00113-01 LSR
PERIOD COVERED October 1, 1980, to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  The Neural Coupling between Vergence Eye Movements and Accommodation		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  PI: Frederick A. Miles D. Phil. Research Physiologist LSR NEI		
COOPERATING UNITS (if any)  Laboratory of Physiology, Oxford University, England		
LAB/BRANCH Laboratory of Sensorimotor Research		
SECTION Oculomotor Control Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Transfer of fixation between targets at different viewing distances involves changes in <u>vergence eye movements</u> and <u>accommodation</u> that operate to eliminate disparity and blur, respectively. During monocular viewing, when disparity cues are absent, changes in accommodation due to blur also result in linear changes in vergence: the <u>accommodation-vergence response</u> . The present experiments were undertaken to determine whether these open-loop responses are subject to visually-mediated <u>adaptive regulation</u> . <u>Human subjects</u> were fitted with specially made laterally-displacing periscopic spectacles to increase the apparent separation of the two eyes and thereby increase the required change in convergence per unit change in accommodation necessary to maintain single, clear vision. Thirty minutes of exposure to these spectacles was enough to cause large increases in the vergence change associated with a unit change in accommodation during monocular testing. This finding supports the view that the close coupling between vergence and accommodation is subject to adaptive regulation. Decreasing the apparent separation of the eyes with medially-displacing (cyclopean) spectacles failed to affect the magnitude of the accommodation-vergence response. Thus, the adaptive mechanism shows considerable asymmetry.		

Project Description:

Objectives: Transfer of fixation between targets at different distances during monocular viewing results in changes in both accommodation and vergence. While the former operates to reduce blur and can be regarded as the response of a negative feedback control system to errors, the vergence changes--that normally would operate to reduce disparity--are operating open-loop in the monocular viewing situation; being regarded as a corollary of the change in accommodation, these vergence changes are referred to as the accommodation-vergence response and tend to maintain the alignment of the covered eye with respect to the object of regard. As with all open-loop responses, there is a gain problem--how does the system know by how much to alter vergence for a given alteration in accommodation in the monocular situation? Experiments were undertaken to determine whether the accommodation-vergence response was subject to adaptive control and could be modified by visual devices that alter the strength of the required coupling between accommodation and vergence responses.

Methods Employed: The normal coupling between vergence and accommodation was disturbed with specially constructed spectacles that alter the apparent separation of the two lines of sight. The apparent separation was increased by a factor of more than two by means of laterally-displacing, binocular periscopes (magnifying spectacles) or decreased to zero with medially-displacing binocular periscopes (cyclopean spectacles). Some control observations were made using base-in and base-out prism spectacles that induce a step alteration in the relationship between vergence and accommodation but, unlike the periscopic spectacles, do not alter the slope of the relationship. Thus, while the periscopic spectacles called for a change in the "gain" of the coupling between vergence and accommodation, the prismatic spectacles only called for a "DC shift". Accommodation-vergence responses were measured in human subjects using a haploscope incorporating a laser speckle optometer with Badal lens viewing to determine the accommodative state of the right eye. The latter fixated cross hairs through a mixing cube and Badal lens, providing accommodative stimuli of 0-4 diopters with fixed size and brightness cues. The left eye viewed a blank screen with alignment markings (suitably offset to avoid fusion with images in the right eye) through a mirror galvanometer that, when aligned by the subject, indicated the horizontal position of the left eye.

Major Findings: Accommodation-vergence was expressed as a dimensionless gain parameter equal to the measured change in vergence per unit change in accommodation divided by the required change in vergence to maintain correct alignment of the eyes during that same change in accommodation. Initial accommodation-vergence gains for the two and after wearing the magnifying spectacles for 30 minutes, these increased to 1.43 and 1.89, respectively. Neither the cyclopean spectacles nor the prismatic spectacles had any significant effect on the gain of the accommodation-vergence response, though the base-out prism arrangement caused a step increase in the vergence coupled to any given accommodative state. Thus, challenged with optical devices that increased the apparent separation of the two lines of sight, the gain of the accommodation-vergence response

shows an increase that is appropriate for improving the rapid alignment of the two eyes during accommodation. This strongly suggests that the normal coupling between vergence and accommodation as represented by the accommodation-vergence response is subject to adaptive regulation.

Significance to Biomedical Research and the Program of the Institute:

One of the major new developments in the field of oculomotor physiology in the last decade concerns the brain's ability to use visual inputs to regulate its performance. This adaptive capability is the key to achieving the extraordinary precision that characterizes the neural control of eye movements and is so imperative for clear vision. That the alignment of the two eyes, under the influence of accommodation-vergence, is subject to adaptive regulation is especially interesting since by far the most common clinical disorders of eye movements involve misalignment of the two eyes with respect to one another. The new optical techniques used in the present study to demonstrate this were based on a systems theory approach to the regulation of eye movements, rather than the "exercise approach" usual in conventional orthoptics, and they may prove useful in the diagnosis and treatment of binocular misalignments in human patients.

Proposed Course: The role of adaptive mechanisms in establishing and maintaining appropriate coupling between the vergence eye movements and accommodation will continue to be the main concern of these studies. During pinhole viewing, when blur cues are minimal, changes in vergence due to disparity also result in linear changes in accommodation: the vergence-accommodation response. Future studies will examine the effect of perisopic spectacles on the gain of this important open-loop, vergence-accommodation response.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Vergence and Accommodation)

Publications:

Judge SJ, Miles FA: Gain changes in accommodative-vergence induced by alteration of the effective interocular separation, in Fuchs AF, Becker W (eds): Progress in Oculomotor Research. New York, Elsevier (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00104-02 LSR

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Quantitative Modeling of Sensorimotor Systems

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

PI:	Lance M. Optican	Ph.D.	Staff Fellow	LSR	NEI
Other:	Frederick A. Miles	D.Phil.	Chief, Oculomotor Control Section	LSR	NEI
	Stephen G. Lisberger	Ph.D.	Staff Fellow	LNP	NIMH

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Laboratory of Sensorimotor Research

SECTION

Oculomotor Control Section

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

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)

Quantitative models of sensorimotor systems allow one to combine data from different types of experiments. The advantages of a model include its ability to test the internal consistency of the data and to make predictions which can form the bases for new experiments. A study of the interaction between the visual system and the vestibular system revealed a close coupling between the gradual component of the optokinetic response (OKR) and the gain control of the vestibulo-ocular reflex (VOR).

The coupling manifested itself in both linear and nonlinear ways, depending on the measure chosen. This made it impossible to decide whether a single variable gain element, shared by the VOR and the OKR, could explain the results or whether a more complicated arrangement would be required. Existing models of the VOR and OKR were modified to include a single variable gain element, common to both the VOR and the gradual component of the OKR. Simulation of the model on a digital computer reproduced both the linear and nonlinear interactions of the VOR gain change with the OKR.

Project Description:

Objectives: Understanding how the brain integrates sensory information to form a motor response requires an interdisciplinary approach. Data on the behavior of sensory and motor systems must be synthesized to form a unified sensorimotor model. The goal of the model is to summarize what is known about the system and to organize that information in such a way that new relationships can be seen. Such models make clear what is missing from the existing data base and thus often suggest new experiments. Because of their conciseness, these models also find clinical use in describing abnormal function and in designing new clinical tests.

Developing a quantitative model requires theoretical considerations of data from behavioral and neurophysiological experiments. This project studied the interaction between the vestibulo-ocular reflex (VOR) and the optokinetic response (OKR). A model was made to quantify the nature of this interaction.

Methods Employed: A behavioral approach was used to study the interaction of the VOR and the OKR in monkeys. Animals were seated inside a full-field visual stimulator for testing and had eye position accurately monitored by the Robinson magnetic field/search coil technique. The VOR was measured by rotating the animal in the dark, while the OKR was measured by turning a full-field drum around the animal in the light. The animal's VOR gain was changed by having the animal wear fixed-field or magnifying spectacles. The OKR was quantified by measuring the amplitude and time course of the slow-phase components of the optokinetic nystagmus (OKN) and the optokinetic after nystagmus (OKAN).

The model of the OKR and VOR systems was described by Laplace transforms and nonlinear operators. This model was then simulated on a digital computer using our own program for discrete time approximations to systems of continuous differential equations.

Major Findings: It was found that the OKR could be divided into two parts, an initial, or rapid component and a later, more gradual one. As the gain of the VOR was raised and lowered, the fast component of the OKR remained the same, while the gradual component changed. The maximum eye velocity obtained by the gradual component appeared to be proportional to the VOR gain. These results suggested that the OKR was generated by two subsystems and that the gradual subsystem shared a common gain element with the VOR. However, certain measures of the OKR, such as the final OKN eye velocity, varied nonlinearly with VOR gain. From this result, one might infer that the OKR and VOR systems were related by a more complicated interaction. There appeared to be no way to collect more data to decide on the detailed nature of the VOR and OKR interaction. Instead, a model was formed and simulated in the hopes that the simpler hypothesis could, by itself, account for the complex, nonlinear interactions.

Two models of the OKR and VOR interaction, based on studies in normal animals, have been presented by T. Raphan and by D. A. Robinson. The new model incorporated our new observations concerning the effects of VOR gain change data into these models. This model included a vestibular system and a gradual OKR subsystem which converged before a single variable gain element, and a rapid OKR

subsystem that converged after the variable gain element. The model was analyzed in detail and converted to equations for solution by a digital computer. The results of the simulation showed that, when the model was adjusted to reproduce the responses of normal monkeys, only the single variable gain element had to be changed to simulate all the effects of the VOR gain change. Both the qualitative and quantitative nature of the interaction were simulated successfully. Hence it has been shown that it is possible to explain all of the experimental results without requiring a more complicated model of the interaction between the VOR and the OKR.

Significance to Biomedical Research and the Program of the Institute:

Quantitative modeling allows data from widely disparate areas to be unified. The resulting model forms a convenient summary of that part of the data which is understood and a clear definition of those areas which remain mysterious. Such models require internal consistency and can thus be used to test old hypotheses and design new ones. Since the model describes how different results can be combined, data from animal research and clinical results from human patients can be joined in the same model. Since the range of these models can extend far beyond the scope of the original data used in their construction, they are useful in designing new clinical tests.

Proposed Course: The LSR provides an excellent opportunity for collaborative work in modeling the interface between sensory input and motor output in the oculomotor system. In the next year, efforts will be directed toward improving models of the saccadic eye movement system. To this aim, data from experiments on the cerebellum, the frontal eye fields, and the superior colliculus will be studied in order to understand how the visual system controls the saccadic motor system.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Lisberger SG, Miles FA, Optican LM, Eighmy, BB: Optokinetic response in monkey: Underlying mechanisms and their sensitivity to long-term adaptive changes in vestibulo-ocular reflex. J Neurophysiol 45:869-890, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 EY 00110-01 LSR																		
PERIOD COVERED October 1, 1980, to September 30, 1981																				
TITLE OF PROJECT (80 characters or less)  Real-time Behavioral Control and Data Acquisition																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width:100%; border: none;"> <tr> <td style="width:30%;">PI:</td> <td style="width:30%;">Lance M. Optican</td> <td style="width:10%;">Ph.D.</td> <td style="width:20%;">Staff Fellow</td> <td style="width:10%;">LSR</td> <td style="width:10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Barry J. Richmond</td> <td>M.D.</td> <td>Medical Officer</td> <td>LN</td> <td>NIMH</td> </tr> <tr> <td></td> <td>Arthur V. Hays, Jr.</td> <td>B.E.S.</td> <td>Electronics Engineer</td> <td>LSR</td> <td>NEI</td> </tr> </table>			PI:	Lance M. Optican	Ph.D.	Staff Fellow	LSR	NEI	Other:	Barry J. Richmond	M.D.	Medical Officer	LN	NIMH		Arthur V. Hays, Jr.	B.E.S.	Electronics Engineer	LSR	NEI
PI:	Lance M. Optican	Ph.D.	Staff Fellow	LSR	NEI															
Other:	Barry J. Richmond	M.D.	Medical Officer	LN	NIMH															
	Arthur V. Hays, Jr.	B.E.S.	Electronics Engineer	LSR	NEI															
COOPERATING UNITS (if any)  Laboratory of Neuropsychology, NIMH																				
LAB/BRANCH Laboratory of Sensorimotor Research																				
SECTION Oculomotor Control Section																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																				
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords)  Research in psychology and neurophysiology requires the correlation of stimuli with the responses they elicit. In studies of the sensory and motor systems, this requires that the behavior of the subject be controlled and that the data be collected by a <u>high-speed digital computer</u> . The current state of software development for such laboratory computer systems is limited to three types of programs. The first type includes special purpose programs which are designed to elicit a certain class of behavior from the subject and collect specific quantities as data. The second type includes general purpose behavioral controllers with only nominal data collection provisions. The third type includes general purpose data collection programs with little or no provision for behavioral control. This project has been devoted to creating a fourth class of <u>experimental research programs</u> . Known as the REX (Real-time EXperimentation) system, it is actually a collection of <u>program modules</u> which can be combined at the time of the experiment to perform both <u>behavioral control</u> and <u>data collection</u> in either laboratory or clinical applications.																				

Project Description:

Objectives: Because of the high temporal resolution required when studying sensory and motor systems in human and subhuman primates, the digital computer is becoming an ubiquitous tool in the neurophysiological and psychophysical laboratory. The design of experiments often requires that the behavior of the subject be controlled and the data collected with resolutions of one thousandth of a second. Active scientific and clinical laboratories also require great flexibility so that new experiments, or modifications to existing ones, can be made quickly and easily. The only way to accomplish this is with high-speed, general purpose digital computers. This shifts the burden of designing new experiments to one of developing computer programs, rather than designing new control electronics. While programming general purpose computers is very fast when compared with designing logic circuits, it can be quite difficult when the task is complex.

The evolutionary approach to the problem of providing laboratory programs has led to three classes of programs. One class is an operating system with simple (and thus limited) commands for controlling behavior. These are usually easy to learn and use but provide only primitive data collection capabilities. The second class is another type of operating system but one which uses simple commands to collect and transform data. Again, the ease of use of such systems is offset by their inability to account for the behavioral control needed in sensory-motor experiments. The third class consists of large, special purpose programs which are designed to handle a limited range of experimental protocols. These programs are usually run with the assistance of a simple operating system, such as RT-11 (from Digital Equipment Corporation). The operating system usually handles only transfers of data to and from the disk and perhaps the overlaying of program segments to allow an increase in the virtual size of the computer. All the rest of the work, such as interrupt processing and scheduling, is handled by the program. These programs are very effective in performing their tasks but are usually so complex that learning to use them, or trying to modify them, is a very demanding task.

Methods Employed: The goals of this project are to create a fourth class of programs which will allow experimental control and data collection to be specified quickly and easily. The revolutionary approach to the problem is to use a more sophisticated operating system, UNIX (from the Bell Telephone Laboratories), and a collection of programs for real-time experimentation (known as REX). The REX system breaks the tasks of a laboratory program into functional units and assigns one module for each unit. The UNIX operating system, which we have modified to provide real-time support features, then arbitrates among the different modules. The experimenter can select which modules he needs at the time he actually runs the experiment. One basic module is a program which interacts with the other modules through commands typed on a terminal by the experimenter. Another module maintains the data buffers in memory, while yet another transfers the data to the disk storage as necessary. Other modules, such as interrupt handlers and display processors, are added as needed to meet the special needs of a given experiment. Since the REX system is truly modular, only the new piece written for a novel experiment need be compiled. This prevents unknown interactions from creeping into the computer

program and causing (often very subtle) mistakes in the behavioral control or data collection.

Another source of flexibility in the REX system is the nature of the behavioral controller. The steps in a control paradigm are broken into discrete states. These states are divided into sets of states, known as chains, that are processed independently of each other. Within each state chain, the states are linked by the conditions which cause a transition from one state to another. There is a special program to help set up these state chains, allowing very complex behavioral paradigms to be entered quickly.

The foundation of the REX system is a dual file scheme. The data are divided into two types: events which can be regarded as temporal epochs and analog signals. In a neurophysiological experiment, for example, the occurrence of an action potential in a neuron would be an epochal event, while an associated eye movement would have to be saved as a continuous position signal. (The state transitions of the behavioral lists are also regarded as events, as are the changing of parametric values and the altering of the configuration of run-time modules.) The data are stored in either the E-file or the A-file (for event and analog files). The purpose of this is first to provide a complete record of what went on during the experiment, and second to keep it in such a form that rapid access can be obtained to any part of the data. Hence the E-file serves as an index into the A-file, allowing one to quickly correlate events with analog signals. The formats of the E-file and the A-file were chosen to minimize the amount of damage that could be caused by errors in reading or writing the data. Since the data are often stored on some archival medium (such as magnetic tape), it is important that some way be available to check the consistency of the data files when they are used for analysis. The REX system includes several data file consistency checkers which can be used to verify that files are intact.

The run-time configuration of REX modules and the behavioral chains can be specified by tables or interactive design. However, it must happen that sometime the experimenter will require a module or a subroutine that does not exist. To make the addition of these modules or subroutines as easy as possible, all of REX is written in a higher level, structured programming language. The language chosen for this project was 'C', developed by Bell Laboratories. There are many advantages of 'C' for this project. First, since it is a higher level language, it is much easier for the novice to learn than assembler language. Also, since it is machine independent, code written for, say, a PDP-11 minicomputer can also be run on an Intel-8080 or a Motorola M-68000, microcomputer. Since all the REX code is written in 'C', it is accessible to anyone after a moderate investment in learning that language, unlike assembler, FORTRAN or BASIC language programs, which are virtually unreadable by anyone (even their authors).

We hope that the combination of the above features will make the REX system quick and easy to use by anyone, even scientists and physicians with little knowledge of computers. The design goal for REX was to achieve behavioral control with eye movement and single-unit recording at a sampling rate of one kilohertz. In terms of programming, the time to change from one paradigm to another should be less than 20% of the time required to set up the new

experiment. Also, the computerized record of the experiment should be complete without extra notes and be easily accessible to analysis programs. So far, all of these goals have been met in the first version of the REX implementation.

Significance to Biomedical Research and the Program of the Institute:

The importance of the REX system lies partly in its ability to reduce redundant or wasteful programming efforts and partly in its ability to allow the design of new and different experiments. The concept of independent program modules and a common data file format makes it possible, and desirable, to share these modules, thus saving considerable time and effort. Since the REX system is not constrained by any presuppositions about the type of experiment to be performed, it remains highly flexible. As the ease of programming the laboratory computer increases, so does the scientific productivity of the laboratory in both experimental and clinical programs.

Proposed Course: The REX system is currently running in several of our laboratories. We hope to gain valuable experience in the next few months on the ease with which scientists who are not computer experts interact with the system. This will enable us to redesign some of the basic REX modules to make them easier to use, and to provide special programs (like the behavioral setup dialogue program) to make the system more accessible to people who cannot program in 'C'. More REX modules will, of course, become available as they are needed by different researchers. As a wider range of experimental needs are answered with REX modules, we will also be able to improve the optimization of the REX system to provide smaller, faster, modules and more efficient data files.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Structure and Function)

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  701 EY 00055-03 LSR
--	--	---

PERIOD COVERED  
October 1, 1980, to September 30, 1981

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.	Staff Fellow	LSR	NEI
Other:	George F. Creswell	B.S.	Histologist	LSR	NEI

COOPERATING UNITS (if any)

LAB/BRANCH  
Laboratory of Sensorimotor Research

SECTION  
Visuomotor Integration Section

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

TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 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 afferent connections to the primate superior colliculus have been investigated using horseradish-peroxidase histochemistry. Rhesus monkeys were prepared for chronic neurophysiological recording in order to localize the deep eye movement-related activity within the colliculus. Injections of horseradish peroxidase were made into these deep layers. After a survival period that permitted the enzyme to be transported from the terminals to the cell bodies of origin, the monkeys were sacrificed, perfused, and the tissue sectioned and reacted for peroxidase. Individual sections of the brainstem were examined for labeled cells. Preliminary experiments indicate that the major sources of afferent input to the primate colliculus arise from the substantia nigra (pars reticulata) and the parabigeminal nucleus. Other structures that project to the colliculus include the contralateral superior colliculus and the mesencephalic reticular formation. The presence of labeled cells in several other structures was variable, suggesting that labeling of these neurons may be due to spread of the injection site into extracollicular structures. Additional experiments will be needed to determine an inventory of brainstem structures that project to the colliculus and which may be providing saccade-related input to the deep layers.

Project Description:

Objectives: The superior colliculus is an important center in visual and oculomotor processing. Neurons in the superficial layers receive direct input from the retina and discharge in relation to visual stimulation. Neurons in the deep layers fire in relation to saccadic eye movement, but the structures that contribute to this saccade-related discharge are unknown. It has been shown that in the cat more than 40 brainstem structures project to the deep layers, but no such extensive survey has yet been made in a primate. The purpose of these experiments was to determine which brainstem structures project to the colliculus in the monkey and to determine which of these structures may be providing the major input to the eye movement-related neurons in the deep layers.

Methods Employed: Two rhesus monkeys were anesthetized and surgically prepared for chronic neurophysiological recording. After recovery, microrecording and stimulation were used to identify the deep eye movement activity within the colliculus. Large injections of horseradish peroxidase (HRP) were made in order to label cell bodies projecting to the colliculus. After a survival time sufficient to allow retrograde transport of the enzyme, the animals were sacrificed, perfused, and their brains sectioned and reacted for peroxidase using the Mesulam TMB method to produce an optically-dense reaction product within the cell bodies. The tissue was examined for cells containing the reaction product, and the locations of labeled neurons were plotted on projected drawings.

Major Findings: The large injections in two monkeys were centered in the intermediate layers but also involved significant spread into the superficial and deep layers. In both cases, numerous HRP-filled neurons were located ipsilaterally in the anterolateral portion of the substantia nigra (pars reticulata), the parabigeminal nucleus, and in the contralateral intermediate and deep colliculus layers. In addition, both cases also contained a few labeled cells bilaterally, in the mesencephalic reticular formation, and ipsilaterally, in the nucleus reticularis pontis oralis.

In addition to these structures that contained labeled cells, other structures contained cells in only one of the two cases. In one case, the injection site involved the anteromedial portion of the superior colliculus and spread into the nucleus of the posterior commissure. In this case, numerous HRP-filled cells were seen in the contralateral nucleus of the posterior commissure and in a region corresponding to the rostral interstitial nucleus of Cajal, below the fasciculus retroflexus and nucleus parafascicularis adjacent to the central gray. In the other case, with a lateral and posterior injection that spread into regions near the parabigeminal nucleus and inferior colliculus, HRP-filled cells were seen in the ventral lateral geniculate nucleus, zona incerta, and nucleus of the brachium of the inferior colliculus.

In a third monkey, the injection was restricted to the superficial layers. In this case, HRP-labeled cells were not seen in either the substantia nigra or the parabigeminal nucleus.

Previous anatomical studies, in the cat, have found that numerous subcortical structures project to the superior colliculus. However, these

preliminary results suggest that, in the monkey, the most prominent subcortical inputs to the colliculus arise from two midbrain structures: the substantia nigra (pars reticulata) and the parabigeminal nucleus.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the organization of pathways that give rise to preoculomotor signals in the brainstem is a prerequisite toward understanding oculomotor disorders that result from trauma or disease.

Proposed Course: Experiments in the next year will attempt to determine whether these differences in afferent connections are a result of spread to adjacent structures and to determine whether different structures project to specific portions of the visual and oculomotor map of the colliculus.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function)

Publications:

Albano JE, Wurtz RH: The role of the primate superior colliculus, pretectum, and posterior-medial thalamus in visually-guided eye movements, in Fuchs AF, Becker W (eds): Progress in Oculomotor Research. New York, Elsevier, 1981, pp 153-160.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00103-02 LSR												
PERIOD COVERED October 1, 1980, to September 30, 1981														
TITLE OF PROJECT (80 characters or less)  Visual Processing in Inferior Temporal Cortex														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width:100%; border: none;"> <tr> <td style="width:33%;">PI:</td> <td style="width:33%;">Barry J. Richmond</td> <td style="width:10%;">M.D.</td> <td style="width:15%;">Medical Officer</td> <td style="width:10%;">LN</td> <td style="width:10%;">NIMH</td> </tr> <tr> <td>Other:</td> <td>Robert H. Wurtz</td> <td>Ph.D.</td> <td>Chief</td> <td>LSR</td> <td>NEI</td> </tr> </table>			PI:	Barry J. Richmond	M.D.	Medical Officer	LN	NIMH	Other:	Robert H. Wurtz	Ph.D.	Chief	LSR	NEI
PI:	Barry J. Richmond	M.D.	Medical Officer	LN	NIMH									
Other:	Robert H. Wurtz	Ph.D.	Chief	LSR	NEI									
COOPERATING UNITS (if any)  Laboratory of Neuropsychology, NIMH														
LAB/BRANCH Laboratory of Sensorimotor Research														
SECTION Visuomotor Integration Section														
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords)  <p>An area of the temporal lobe of the monkey has been implicated as the critical structure in the brain related to <u>form vision</u>. In order to examine the physiological basis of <u>visual processing</u> related to form vision, the activity of <u>single neurons in the inferior temporal cortex</u> was studied in awake behaving <u>monkeys</u>. We found that, when the monkeys were fixating on a spot of light, a stimulus falling in the receptive field of an inferior temporal cortex cell produced only a marginal response. Removal of the fixation point improved the visual response and expanded the size of the receptive field. Control experiments suggest that this effect of the fixation point is a result of visual interaction within the visual field rather than a shift in <u>visual attention</u>. These experiments indicate the receptive field properties of inferior temporal cells are altered by visual fixation.</p>														

Project Description:

Objectives: An area of the temporal lobe of the monkey, the inferior temporal cortex, has been implicated in form vision because ablation of this area produces severe deficits in visual discrimination. The physiological basis of this function has remained obscure in part because the stimuli necessary to consistently activate cells in this area have not been determined. The pioneering work of Charles Gross and his collaborators has indicated three points about these cells: they sometimes require complex stimuli; they have large visual receptive fields frequently extending 40 degrees from the fovea; and these fields almost always include the fovea and usually cross the vertical meridian. The goal of the present experiments was to investigate cells in the inferior temporal cortex of the awake behaving monkey in order to determine what stimuli are effective in activating them.

Methods Employed: Monkeys were recorded for looking at a small spot of light on a tangent screen. During this period of fixation, a second visual stimulus was projected onto the screen and was used to activate the cells under study. In addition to this conventional fixation paradigm, another paradigm was also used in which the fixation point was blinked off for one second during the fixation period and the receptive field stimulus came on while the fixation point was turned off. The monkey was required to continue to fixate because any eye movement beyond about .5 to 1 degree would terminate the trial. Eye movements were measured using the magnetic search coil technique and the experiments were controlled by an on-line digital computer.

Major Findings: Attempts to activate cells in inferior temporal cortex when the monkey was performing visual fixation were only marginally successful. Visual stimuli, such as spots or slits of light, complex stimuli including brushes, and sine or square wave gratings, produced responses that were weak and irregular. In addition, receptive fields were comparatively small, frequently only about 5 degrees in diameter. We noticed, however, that the fixation point itself and these stimuli seemed to be more effective when they incidentally fell on the receptive field between periods of active fixation. We therefore investigated whether the fixation point had some influence on the response of cells to the extrafoveal visual stimuli. To do this we first determined the response of a cell to the visual stimuli while the monkey was fixating and then found the response when the fixation point was blinked off. Response of the cell to the stimulus presented during the blink was greater than the response to the same stimulus when the fixation point was present. Furthermore, the response obtained during the blink of the fixation point was obtained over a larger area of the visual field than was the case during fixation; the effective size of the receptive field increased.

There are at least two hypotheses that might explain the effect of the fixation point on the receptive fields of the inferior temporal cells. The first hypothesis is based on a visual interaction: the fixation point falls on an antagonistic area of the receptive field and its presence suppresses the response of the stimulus in another part of the receptive field. The second hypothesis relies on some extravisual form of modulation; for example, a shift of the monkey's attention to the stimulus when the fixation point blinks off. We tested this latter hypothesis by using a task used previously on cells in the

superior colliculus, parietal cortex, and frontal eye fields: the monkey was required to respond to the visual stimulus falling in the receptive field of the cell but not make an eye movement to it. In this experiment we know that the monkey attended to the stimulus because his response to the stimulus indicates this attention. We found that the response to a stimulus when the monkey was known to attend to it was no greater than the response when he just fixated or when the stimulus occurred during the blink of the fixation point. While these experiments are not yet conclusive, they suggest that the fixation point modifies the visual response of the cell through a visual interaction and not by any extravisual modulation such as attention.

The difference in properties of inferior temporal cells in the awake and paralyzed monkey made us wonder whether we were in fact recording from a different population of cells in those experiments or whether the differences were related to the lack of anesthesia or the procedures used in the awake monkey. We therefore decided to perform several experiments with anesthesia and paralysis using conditions which were very close to those used by Gross and his collaborators. We recorded from the inferior temporal cortex in the same monkeys we had used in the awake state using the same cylinder, same microelectrodes, and the same depth of recording as we had used when the monkey was awake. We had no intention of doing an intensive survey of these cells, but what we found was in most respects similar to what Gross and his collaborators had reported. All cells responded more vigorously to brushes than to spots of light; the size of the receptive field was also larger with use of a brush than with use of the spot of light; fields were frequently large extending 20-30 degrees from the fixation point into the contralateral visual field. We did not, however, usually see extension of the receptive field into the ipsilateral visual field by more than a few degrees. In most respects, however, we think these experiments were consistent with the observations that Gross and his collaborators have made, and we conclude that we are probably sampling from the same population of cells in the awake animal that previous experiments had sampled from in the anesthetized, paralyzed monkey.

While the visual fixation task we have used is a laboratory oddity, visual fixation itself is no such oddity. The unusual case would be represented by lack of visual fixation such as in the blink experiments or the experiments on anesthetized and paralyzed monkeys. Our experiments indicate that the functional receptive fields in awake monkeys may be quite different from the receptive fields analyzed in the absence of visual fixation.

Significance to Biomedical Research and the Program of the Institute:

Different types of visual processing in the primate brain may occur in different areas of the brain. One of these areas is the inferior temporal cortex and a hypothesis about its function is that this area is important for that aspect of vision related to form discrimination. A better understanding of this area will aid not only in the understanding of the nature of visual processing in man but possibly reveal ways for diagnosing and treating patients with damage to this area of the brain.

Proposed Course: These experiments will continue as a major project of Barry J. Richmond in the Laboratory of Neuropsychology in the NIMH. In light of

this, no further experiments are planned in the immediate future in the Laboratory of Sensorimotor Research.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Structure and Function)

Publications:

Richmond BJ, Wurtz RH: Inferotemporal cortex in awake monkeys, in Morrison AR, Strick PL (eds): Changing Concepts of the Nervous System. New York, Academic Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00045-03 LSR
--	--	---

PERIOD COVERED  
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)  
  
Visuomotor Properties of Neurons in the Thalamus and Extrastriate Cortex

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 Physiologist LSR NEI  
Other: William Keys Ph.D. Staff Fellow LSR NEI

COOPERATING UNITS (if any)

LAB/BRANCH  
Laboratory of Sensorimotor Research

SECTION  
Visuomotor Integration Section

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

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	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)

Two cortical areas outside of the geniculoc-striate system have been studied in awake, behaving monkeys to understand their contribution to vision and eye movements. Neurons in a cortical region in the superior temporal sulcus respond to visual stimuli over large areas of the visual field. The majority are directionally selective, responding to stimuli moving in many, but not all, directions. Other cells become active when the monkey makes smooth pursuit eye movements in several but not all, directions; such cells appear to be unresponsive to visual stimuli while the eye is stationary. Other cells become active after saccadic eye movements whether the eye movements are visually guided or made spontaneously in total darkness. In cortical area 7 there are neurons which discharge when the eyes are located at specific positions in the orbit and visual stimulation is presented at a particular point in visual space. Such cells do not respond when the eyes are positioned elsewhere and comparable visual stimulation is presented. Thus, there are cortical cells which have passive visual responses, eye movement responses, or responses which are dependent upon appropriate visual and eye position information.

Project Description:

Objectives: Visual information reaches the cerebral cortex by two different pathways. The first route courses from the retina to the lateral geniculate nucleus of the thalamus and then to striate cortex. A second, less direct pathway, travels from the retina to the superior colliculus; information then ascends to the pulvinar nucleus of the thalamus and then to several different cortical targets. Recent studies have shown that neurons in these various cortical areas are involved in different types of visual and eye movement relations. This project is designed to study the visual properties of neurons in two different cortical areas and to examine various types of eye movements which may influence the visual responses of these neurons. Finally, the work attempts to determine if there are neurons in these areas which could function in the generation of eye movements and mediation of visual attentional processes.

Methods Employed: The activity of individual neurons in two regions of the cerebral cortex was studied while awake monkeys performed several different visuomotor tasks. The animals learned to fixate a spot of light (fixation point) projected on a tangent screen and detect its dimming in order to obtain water reinforcement. The location of the fixation point could be placed anywhere in the visual field. While the animal gazed at the fixation point, lights of various sizes, shapes, and intensities were either flashed stationary or moved across the tangent screen to determine the types of visual stimuli which caused cells to discharge. In other tests, smooth pursuit eye movements were elicited by slowly moving the fixation light across the tangent screen; saccadic eye movements were evoked by turning off the fixation point and turning on another light. Since the animal was required to detect the dimming of this second light to obtain reinforcement, the animal consistently made saccades to this light. A digital computer was utilized on-line to evaluate the discharge patterns of individual neurons as well as operate the various optical and behavioral devices. Recordings were made from two cortical sites; the first was deep within the superior temporal sulcus on the dorsal surface. This region lies anterior to the junction of the sylvian fissure and the superior temporal sulcus but posterior to the interaural plane. The second recording site was posterior to the interparietal sulcus in cytoarchitectural area 7. Small marking lesions were made at the sites of interesting cells; these marks were located after perfusion and histological section of the brain and were used to correlate the anatomical locations and functional properties of single neurons.

Major Findings: Nearly two thirds of the neurons on the dorsal bank of the superior temporal sulcus respond to visual stimuli. Most have visual receptive fields which include at least one quadrant of the visual field. Most receptive fields are located in the contralateral visual field, but many include large parts of the ipsilateral field. Many of these cells have been classified as directionally selective because they respond to the presence of stimuli moving through their receptive field in some, but not all, directions. Directionally-selective cells in other regions of visual cortex respond to a much smaller range of directions of stimulus movement

than do the cells on the anterior bank of the superior temporal sulcus. Almost any stimulus configuration which is moving in the appropriate direction through the receptive field will excite these cells.

Another set of neurons in this region discharges when the animal makes smooth pursuit eye movements to maintain fixation on a slowly moving spot of light. These cells are active for pursuit movements in a particular direction, independent of eye position, but are inhibited by eye movements in the opposite direction. Such cells do not appear to respond to stimuli which are moved while the eye is stationary. These cells are also active during smooth pursuit eye movements when background illumination is significantly reduced. These data suggest that the activity of such cells is related to the maintenance of eye motion, rather than the visual stimulation initiating or generated by the eye motion.

A third group of cells discharges after saccadic eye movements. This activity is present whether the eye movements are made in the light to visual targets, in the dark to briefly flashed targets, or spontaneously in total darkness. Such neurons become active after eye movements directed into the contralateral visual field and discharge independent of eye movement direction or amplitude.

Cells in a final group discharge tonically when the animal fixates a spot of light in certain locations but not others. These cells are not activated when the animal spontaneously fixates this region of visual space. Some of these cells are not responsive to visual stimuli when the animal is looking straight ahead but are responsive when the animal fixates eccentrically and stimuli are presented in certain portions of the visual field. Such cells may be said to have visual receptive fields, whose excitation is contingent upon the eye being located in a specific position in the orbit. Some of these cells have foveal receptive fields, while others have extrafoveal receptive fields, which become apparent only when the animal's eyes are in certain positions in their orbits.

In area 7 of posterior parietal cortex there is also a set of neurons which appear to be similar to those just described in the superior temporal sulcus; they possess visual receptive fields which are responsive to visual stimuli only when the eye is in certain positions.

Significance to Biomedical Research and the Program of the Institute:

Visual information is transmitted to many regions of the central nervous system. Different parameters of visual information appear to be channeled to different brain areas, suggesting that aspects of visual behavior such as motion perception, eye movements, and visual attention are mediated in these different areas. The studies described here attempt to examine how visual and eye movement functions are carried out by cortices located in area 7 and in the superior temporal sulcus. Since area 7 has cells which appear to integrate vision and eye position information, this part of the brain may contribute to the perception of visual space. The neurons in the superior temporal sulcus may serve to initiate smooth pursuit eye movement because some cells here are selectively active in relation to this type of behavior.

Knowing the brain regions which subserve various aspects of visual behavior and knowing the mechanism by which the brain mediates these functions will aid in diagnosing deficits in visual perception and oculomotor control seen in humans with damage to the parietal lobe and/or brainstem.

Proposed Course: Future experiments will attempt to determine what visual and eye movement conditions are necessary to activate those cells in the superior temporal sulcus which discharge in relation to tracking eye movements. Other studies are planned to determine if area 7 receives proprioceptive signals from the extraocular muscles. Such signals may provide the eye position information which influences the visual responsiveness of some cells in area 7. The pulvinar nucleus, which sends and receives fibers from the superior temporal sulcus and area 7 will be studied to understand the subcortical influences on these cortical regions.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Robinson DL, Baizer JS, Dow BM: Behavioral enhancement of visual responses of prestriate neurons in the rhesus monkey. Invest Ophthalmol Vis Sci 9:1120-1123, 1980.

Robinson DL, Bushnell MC, Goldberg ME: The role of posterior parietal cortex in selective visual attention, in Fuchs AF, Becker W (eds): Progress in Oculomotor Research. New York, Elsevier, 1981, pp 203-210.

Goldberg ME, Robinson DL: The significance of enhanced visual responses in posterior parietal cortex. Behavior Brain Sci 3:503-505, 1980.

Robinson DL, Keys W: Visuo-motor properties of neurons in superior colliculus and pulvinar nucleus of the monkey. Adv Physiol Sci 2:279-285, 1981.

Keys W, Goldberg ME: Single neuron studies of attention, in Sheer D (ed): Attention: Theory, Brain Function, and Clinical Application. New York, Academic Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 EY 00102-02 LSR
PERIOD COVERED October 1, 1980, to September 30, 1981		
TITLE OF PROJECT (80 characters or less)  Role of Substantia Nigra in the Initiation of 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:        Okihide Hikosaka        M.D., Ph.D.    Visiting Scientist    LSR    NEI		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Sensorimotor Research		
SECTION Visuomotor Integration Section		
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords)  The initiation of rapid or <u>saccadic eye movements</u> depends in part on the <u>basal ganglia</u> . An analysis of the discharge of <u>single neurons in the substantia nigra, pars reticulata</u> , which can be regarded as an important output from the basal ganglia, has shown that these cells discharge in relation to visually-guided saccades. Cells have now been identified which discharge when a saccade is made to a previously present spot of light--to a <u>remembered target</u> . The decrease in discharge rate is related to the visual stimulus that must be remembered, to the saccade to that remembered stimulus, or to both of these events. Many of these cells in the substantia nigra have been shown to project to the <u>superior colliculus</u> by showing that they can be antidromically activated from the superior colliculus. These experiments show that the substantia nigra is involved in the initiation of saccades, particularly those saccades to visual or remembered visual targets.		

Project Description:

Objectives: The initiation of movement, including rapid or saccadic eye movements, depends in part on the integrity of an area of the diencephalon, the basal ganglia. An output of this structure is to the substantia nigra, pars reticulata, in the midbrain. The substantia nigra is likely to be relevant to oculomotor control since it projects to cells in the superior colliculus which previously have been identified in this laboratory as being related to the initiation of eye movements. Last year's annual report described our initial findings showing that cells in the substantia nigra are related to the initiation of saccades, primarily those to visual targets. This report concentrates on additional cell types which have been studied in detail this year and upon the relationship of substantia nigra cells to the superior colliculus.

Methods Employed: Awake monkeys trained to fixate on a spot of light were used in these experiments. In addition to requiring the monkey to make a saccade from one spot of light to another, the monkeys were also required to make a saccade to the point where a spot of light had been and, if the eye movement was correct, the monkey was rewarded with a drop of water. Eye movements were recorded using the magnetic search coil technique, and recognition of correct eye movements was done by an on-line computer.

The single cell recording was facilitated by the introduction of a new recording technique in the laboratory which involved implantation of a 20 gauge guide tube over the substantia nigra. Through this guide tube which stayed in place for a period of days to weeks, a tungsten wire electrode was inserted into the substantia nigra. This technique has increased the stability of recording which is essential for the long-term analysis of cells with complicated relationships to behavior and has enabled longer recording periods from the monkeys with less damage to the brain.

Major Findings: We previously reported that cells in pars reticulata of the substantia nigra have a high frequency discharge, frequently about 100 spikes per second. The response of the cells is always a decrease in discharge rate. This response occurs during a visually-guided saccade in which the monkey makes a saccade to a spot of light and the response is coupled either with the stimulus onset or with the saccade onset. We have found several other types of cells but one which is particularly striking is characterized by little response during a visually-guided saccade and a stronger response when the saccade is made to a previously present spot of light. To demonstrate these relationships between cell activity and behavior, a spot of light is flashed in one part of the visual field for 50 msec while the monkey is looking at the fixation light. The fixation light then goes out and, if the monkey makes a saccade to the location of the previously flashed spot of light, it is rewarded. We find that while many cells will not respond before the saccade if the visual stimulus is present, they will respond when the monkey makes the saccade to the remembered target. However, this response is not related just to any spontaneous eye movement because, if we correlate the cell discharge to appropriate saccades made spontaneously by the monkey in the dark, no such decrease in discharge is observed. Just as other cells are related to a visual stimulus or a saccade to one part of the visual field, these saccade memory

cells are also related to saccades to one part of the visual field, usually the contralateral visual field.

Other cells discharge not in relation to a saccade to a remembered target but to a stimulus that must be remembered. Experiments that demonstrate this phenomena also use a brief flash of light 50 msec duration, which at a later time is a target for a saccadic eye movement. These cells, however, show a response at the time of the flashed light, not at the time of a subsequent saccade. Another type of cell seems to incorporate both characteristics of visual memory and saccade memory cells. In these cells, a flash of light, indicating the target for a future saccade, produces a decrease in discharge at the onset of the flash which continues until the saccade occurs at a later time to the location of this flash of light. These cells act like a register that holds the location of the visual target until the saccade to that visual target occurs.

The cells related to eye movements or to visual targets or to remembered targets are concentrated in the lateral region of the pars reticulata as demonstrated by histological localization of marks made at the time of recording. This area is also the area that has been demonstrated to be the major source of projecting fibers to the superior colliculus and particularly to the layer related to the initiation of eye movements. It does not follow, however, that these cells are the same cells that project to the superior colliculus. In order to see the relation of the substantia nigra cells to the superior colliculus, we introduced a stimulating electrode into the superior colliculus which allowed antidromic stimulation from the superior colliculus to the substantia nigra cells. Antidromic responses were identified by both fixed latencies to single and double stimuli with a short interval and by collision with spontaneous spikes. The threshold and latency for antidromic activation was measured at 100 micron steps through the superior colliculus and this yielded a depth threshold and depth latency plot. The latency of antidromic response usually ranged between 0.7 and 1.3 msec. The depth threshold plot typically showed low threshold peaks of less than 25 microA. These peaks usually corresponded to the depths for visual-motor or motor-related cells recorded in the superior colliculus. These multiple peaks indicate that the axons of pars reticulata cells arborize to several branches in the intermediate and deep layers and possibly terminate on some cells in these layers.

We suggest that these pars reticulata cells have monosynaptic connections with superior colliculus cells which increase discharge rate in relation to visually or nonvisually guided saccades. One hypothesis explaining this inter-relationship is that the pars reticulata provides a tonic inhibitory drive to the superior colliculus; release of this drive during the decrease in discharge preceding saccades releases the superior colliculus cells and allows the burst of activity in these cells to occur before a saccadic eye movement.

Significance to Biomedical Research and the Program of the Institute:  
One of the goals of our research is to understand the circuits within the brain that produce saccadic eye movements. These experiments have indicated that the substantia nigra, an output pathway from the basal ganglia, is part of that circuitry. The basal ganglia are suspected to be involved in several disease processes, including Parkinson's Disease and Huntington's Disease, both of which

involve abnormalities of oculomotor control. The present experiments demonstrate the relationship of these cells not only to eye movement but to an established oculomotor area, the superior colliculus. An understanding of this part of the circuitry will not only expand our understanding of the organization of the brain related to oculomotor control but hopefully will lead also to an evaluation of the deficit observed in patients with diseases of the basal ganglia.

Proposed Course: Experiments aimed at characterizing the types of cells in the pars reticulata will continue as will the analysis of which of these cell types project to the superior colliculus. Efforts will also be made to analyze the effect of damage to this area on the initiation of saccades.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Ocular Motility and Strabismus (Structure and Function--Conjugate Eye Movements)

Publications:

Hikosaka O, Wurtz RH: The role of substantia nigra in the initiation of saccadic eye movements, in Fuchs AF, Becker W (eds): Progress in Oculomotor Research. New York, Elsevier, 1981, pp 145-152.

Wurtz RH, Albano JE, Hikosaka O: Relation of the superior colliculus to the initiation of eye movements. Adv Physiol Sci 1:251-257, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 EY 00109-01 LSR										
PERIOD COVERED October 1, 1980, to September 30, 1981												
TITLE OF PROJECT (80 characters or less)  Visual Motion Processing in the Primate Brain												
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width:100%; border: none;"> <tr> <td style="width:33%;">PI: Robert H. Wurtz</td> <td style="width:15%;">Ph.D.</td> <td style="width:20%;">Chief</td> <td style="width:15%;">LSR</td> <td style="width:17%;">NEI</td> </tr> <tr> <td>Other: William T. Newsome</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LSR</td> <td>NEI</td> </tr> </table>			PI: Robert H. Wurtz	Ph.D.	Chief	LSR	NEI	Other: William T. Newsome	Ph.D.	Staff Fellow	LSR	NEI
PI: Robert H. Wurtz	Ph.D.	Chief	LSR	NEI								
Other: William T. Newsome	Ph.D.	Staff Fellow	LSR	NEI								
COOPERATING UNITS (if any)												
LAB/BRANCH Laboratory of Sensorimotor Research												
SECTION Visuomotor Integration Section												
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205												
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.5	OTHER: 0.5										
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS												
SUMMARY OF WORK (200 words or less - underline keywords) The <u>processing of visual information</u> in the <u>primate cerebral cortex</u> is initiated in the primary visual receiving area, striate cortex, and further elaborated in surrounding <u>extrastriate visual areas</u> . One of these extrastriate areas, <u>MT</u> , is rich in neurons which respond selectively to the <u>direction of motion</u> of visual stimuli. It therefore seems likely that MT is involved in the <u>animal's perception of motion</u> and may also direct such information to other neural systems which initiate appropriate <u>behavioral responses</u> . We have begun to test these hypotheses by combining behavioral and single-neuron recording techniques to investigate MT in <u>awake rhesus monkeys</u> . We have found cells that show directional responses similar to those observed previously in anesthetized monkeys. Neuronal responses were similar when the stimulus was moved across the receptive field as the monkey fixated and when the monkey tracked a moving target and thereby moved the receptive field across a stationary stimulus. While this similarity of response indicates a lack of extraretinal influences on these cells, preliminary evidence suggests that many direction-sensitive neurons medial and anterior to MT do respond during smooth pursuit eye movements in ways which do require an extraretinal input.												

Project Description:

Objectives: Extrastriate visual cortex has been thought for many decades to mediate higher visual functions involved in the perception of, and response to, visual stimuli. In the past ten years, experiments in anesthetized animals have demonstrated the existence of several distinct extrastriate visual areas which can be distinguished on the basis of cell structure, anatomical connections, visual topography and neuronal response properties. We have begun an investigation of one of these areas, the middle temporal visual area (MT), employing single-cell recording techniques in awake, behaving monkeys. We hope to exploit the unique advantages of the awake monkey to gain insight into the functional roles played by MT in the perception of, and response to, visual stimuli. MT is a particularly advantageous subject for this study because of its small size (10 mm x 5 mm), myeloarchitectonically identifiable boundaries, robust visual responses, and the relatively homogeneous response properties of its neurons.

Methods Employed: A digital computer was used for behavioral control, stimulus presentation, data acquisition, and on-line analysis of monkey behavior and neuronal response patterns. Eye movements were monitored by the magnetic search coil technique. Rhesus monkeys were trained on four tasks: fixate a spot of light and ignore other stimuli; make a saccade from the fixation point to a peripheral stimulus; track a moving stimulus with smooth pursuit eye movements; make a saccade from the fixation point to a moving peripheral stimulus and pursue the stimulus with smooth pursuit eye movements. Small electrolytic marking lesions were made at selected recording sites for subsequent histological identification of recording locations.

Major Findings: Initially, the passive response properties of MT neurons were studied while the monkeys fixated a small spot of light. Of 151 cells examined, 67% were selective for the direction of motion of visual stimuli within the receptive field, 17% were biased for direction of motion, and 15% were not directional. For 72 cells for which quantitative data were obtained, indices of directionality were similar to those observed previously in anesthetized monkeys. Responses of MT cells were not dependent on the form of the stimulus; robust responses were generally elicited using small spots of light as well as larger slits and bars. Neuronal responses were frequently biased for a range of velocities. In 13 cells for which quantitative data on velocity were obtained, the preferred velocities ranged between 10°/sec and 150°/sec with the majority lying between 10°/sec and 40°/sec. In all these aspects, passive visual properties of MT neurons in the alert monkey are consistent with the results of previous studies in anesthetized animals.

In order to determine whether the response of MT cells was modulated by the monkey's use of the stimulus, we studied single cell responses (n = 26) as monkeys used a spot of light within a neuron's receptive field as the target for a saccadic eye movement. We have not observed any enhancement of the visual response of the cells or any discharge related to the eye movement itself when the visual stimulus was the target for a saccadic eye movement.

In order to test the possible effects of smooth pursuit eye movements on MT cells, neuronal responses were compared under two conditions of retinal

stimulation: 1) when the stimulus was moved across the receptive field as the monkey fixated; and 2) when the monkey tracked a moving target and thereby moved the receptive field across a stationary stimulus. The large majority of MT cells responded similarly to equivalent retinal stimulation whether that stimulation was caused by moving stimuli during fixation or by stationary stimuli during smooth pursuit eye movements. However, preliminary evidence suggests that many direction-sensitive neurons medial and anterior to MT respond during smooth pursuit eye movements in ways which cannot be fully explained by their passive visual properties observed during fixation. Such neurons are indicative of the integration of extraretinal signals with passive visual signals at these cortical loci.

Significance to Biomedical Research and the Program of the Institute:

Knowledge of the basic mechanisms of visual function is a prerequisite for the diagnosis and treatment of pathologies of central visual structures. While anatomically oriented studies tell us much about the organization of visual structures and pathways, behavioral and physiological experiments with perceiving, functioning animals are required before the very difficult and subtle issues of visual information processing and visuomotor integration can be addressed incisively. We are engaged in an effort to apply these approaches to visual function in a higher-order extrastriate visual area. It seems probable that these studies will constitute a significant step in the attempt to understand the intricate stages of normal visual processing in monkey and, by inference, in man.

Proposed Course: The middle temporal visual area (MT) of alert monkeys contains a large majority of cells which respond selectively to the direction of motion of visual stimuli. Such results suggest that MT is involved in the perception of direction of motion and in the distribution of such information to neural structures which mediate appropriate behavioral responses to such information (for example, smooth pursuit eye movements). Future experiments will be designed to test these possibilities. Physiological and behavioral studies will be performed in monkeys trained to report directional discriminations and in monkeys trained to respond to moving stimuli with smooth pursuit eye movements. Studies of normal monkeys will be complemented with studies of animals with MT lesions. These approaches should allow us to address directly the relationship of neural signals in MT to perception and behavior.

NEI Research Program: Strabismus, Amblyopia, and Visual Processing--Visual Processing and Amblyopia (Structure and Function)

Publications: None



Laboratory of Vision Research



ANNUAL REPORT  
NATIONAL EYE INSTITUTE  
October 1, 1980 - September 30, 1981

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH  
Jin H. Kinoshita, Ph.D.

It is difficult to review the hundred publications emerging from the LVR during the past year and highlight one that is most significant. There is one exciting study, however, that captures the essence of biomedical research in that it required ingenuity along with a measure of serendipity extending laboratory research to relate directly to a clinical problem and resulting in a major advance.

One often wonders when an area of research is extensively studied whether the answer to a particular question is already available but hidden in the mass of accumulated data and the solution sometimes merely requires the fitting together of the isolated pieces of information. An example of these circumstances and the unraveling of an interesting riddle involves the retinal "S" antigen. For a number of years the retinal soluble antigen was the focus of attention of many ophthalmic immunologists. This substance found in the retinal outer segments, when injected into animals produced an inflammatory reaction which had features similar to those found in human uveitis cases. Since uveitis is a puzzling disease difficult to treat the development of an animal model raised the hopes that an understanding of the nature and possible treatment of this disease process may be forthcoming. This possibility generated increased activity in this country and abroad to learn more of the nature and properties of the S antigen. The antigen was purified, and as its properties were revealed many began to wonder about its identity. Scientists probed into the possibility that it was similar to a retinol receptor protein; others thought it was similar to this or that enzyme. But its identity remained obscure.

While the immunologists were involved with the S antigen the retinal biochemists were busy with challenges of their own. The fundamental question that has eluded the visual pigment scientists was how the bleaching process of rhodopsin triggers the neural response. The link between the photochemical events and the initiation of neural response has been extensively researched but remain unidentified. Search for support that cyclic nucleotides or calcium may be involved is still ongoing. The demonstration that during rhodopsin bleaching the visual pigment becomes phosphorylated suggested the possibility that this process may be involved in the transformation of the photochemical events to the neural response. Dr. Shichi of the LVR has been examining the rhodopsin phosphorylation process and found a specific kinase for rhodopsin. Interestingly this enzyme was different from other protein kinases and was located only in the outer segments. As the properties of rhodopsin kinase were unraveled Dr. Shichi was struck with the fact that many of the properties of the enzyme were similar to S antigen. He brought this to the attention of the NEI immunologists, Drs. Nussenblatt and Gery, and together they set forth to test the thesis that rhodopsin kinase is S antigen. The animal test was the crucial experiment and it was positive. Injection of rhodopsin kinase into rats

produced a severe anterior and posterior uveitis indistinguishable from that elicited by S antigen injection. Moreover, S antigen was shown to act as an enzyme capable of phosphorylating rhodopsin. Other chemical and immunological properties support the thesis. The weight of evidence appears heavily in favor of the fact that S antigen is indeed rhodopsin kinase.

The study represents a major advance in ophthalmic immunology. The story does not end here as Drs. Nussenblatt and Gery are in the process of developing a chemical modality to block the inflammatory process evoked by S antigen. This information can be found in this annual report.

I am pleased to record the honors and distinctions bestowed on several members of the Laboratory of Vision Research during the past year. Dr. Toichiro Kuwabara was honored at the 83rd Congress of the Japanese Ophthalmological Society for his outstanding contributions to ophthalmic pathology. The award was presented at Kyushu, University Medical School in Japan. Dr. Paul O'Brien, well known for his contribution to the retinal biochemistry field, is serving as the Chairman of the Board of Trustees of the Association for Research in Vision on Ophthalmology. This organization is the leading vision and ophthalmic research group in this country. Dr. Hitoshi Shichi, after 15 years at the National Institutes of Health, has resigned to assume the post as Assistant Director and Professor at Institute of Biological Sciences at Oakland University, Rochester Michigan. Dr. Shichi has been outstanding in his field and it was a matter of time before he received a position where he would have greater influence in the further development of vision research field. Dr. Peter Kador was the recipient of the first International Rhoto Award to a young cataract researcher. The work cited for this award was his pioneering work in the development of new classes of aldose reductase inhibitors. Ms. Deborah Carper was selected as the top winner of the American Chemical Society Award to technicians. It is very reassuring and encouraging that LVR members at various stages in their career have been duly recognized for their outstanding contributions.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF</b> INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00003-09 LVR		
PERIOD COVERED October 1, 1980, to September 30, 1981				
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: Other:	Peter Kador Jin H. Kinoshita Manuel Datiles Henry N. Fukui Lorenzo Merola Deborah A. Carper	Ph.D. Ph.D. M.D. Ph.D. M.S. B.A.	Research Chemist Scientific Director Visiting Scientist Research Chemist Chemist Biologist	LVR NEI  LVR NEI LVR NEI LVR NEI 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: 5.3	PROFESSIONAL: 4.3	OTHER: 1.0		
CHECK APPROPRIATE BOX(ES)				
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER				
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords) Current investigations are being conducted on the events leading to the formation of several types of cataracts. <u>Hereditary cataract</u> formation is being studied in two strains of mice developed in our laboratory. Both develop <u>osmotic cataracts</u> ; however, subtle significant differences suggest different modes of cataract formation in these strains.  <u>Diabetic or sugar cataract</u> formation is also being studied. Initiated by the enzyme <u>aldose reductase</u> , methods for controlling the onset of these cataracts through the regulation of this enzyme are being developed.				

## Project Description:

Objectives: To study the mechanism of diabetic cataract formation and to develop methods for its regulation.

Methods Employed: Sugar cataracts can be induced in animals by either making them diabetic through the use of appropriate chemical agents or by making them galactosemic through the use of a galactose enriched diet. Biochemical methods used for the purification of the enzyme include column chromatography, affinity chromatography, polyacrylamide gel electrophoresis (PAGE) and high pressure liquid chromatography (HPLC). Computational methods for enzyme analysis, inhibitor structure activity studies and corneal reepithelialization studies required the use of the PROPHET and DCRT computer systems.

Major Findings: A method for the rapid purification of human placental aldose reductase has been developed. Using affinity chromatography, the enzyme can be obtained with apparent homogeneity in three steps. An initial 30-70% ammonium sulfate fraction is placed on a 4-carboxybenzaldehyde coupled AH-Sepharose 4B column where upon elution with 0.1 M Na,K-phosphate buffer, pH 6.2 an enzyme fraction separated from the major protein peak. Chromatography of this enzyme fraction on an Amicon Matrix Orange A dye liquid column and elution with phosphate buffer containing 0.1 mM NADPH yielded aldose reductase of high purity. The enzyme, MW 37K, displayed an apparent  $K_m$  of 0.36 mM for DL-glyceraldehyde. Only trace activity was observed when L-gulonate was used as substrate indicating the absence of hexonate dehydrogenase activity.

Antibodies produced in rabbits against the purified enzyme gave a single line of identity with either human placental or lens aldose reductase but no line of identity with rat lens aldose reductase suggesting immunological differences. Differences in the susceptibility of rat lens, human lens and human placental aldose reductase to be inhibited have also been observed suggesting that inhibitors designed for clinical use must eventually be evaluated with human enzyme. Currently 'no universal' inhibitor exists.

A method for measuring the effect of aldose reductase and its inhibition on the delayed rate of corneal reepithelialization has also been developed. After complete denudation, corneas of galactosemic rats were photographed at various time intervals. The outlined photographs were then digitally entered into a computer and the rate of reepithelialization calculated. Statistical analysis of the data showed a marked decrease in the healing rate of galactosemic rats when compared to normal rats. Galactosemic rats treated with the aldose reductase inhibitor Sorbinil (6-fluoro-spiro (chroman-4,4'-imidazolidine)-2'-5'-dione) had healing rats no different from that of normal rats.

In the early phase of healing, SEM of the leading edge of the regenerating epithelium of normal rats showed a predominance of ruffled basal cells with abundant filopodia. In the galactosemic rats cells of the basal layer appeared flat and had few filopodia. Galactosemic rats treated with Sorbinil showed the same ruffled filopodia abundant cells found in normal rats. These studies suggest that the defect in the process of reepithelialization of the cornea in galactosemic and diabetic rats involves aldose reductase quite possibly in the

basal layer of the cells.

Significance to Biomedical Research and the Program of the Institute:

Worldwide, cataract is one of the major causes of blindness while vision loss prior to surgery due to cataract formation in the United States presents a major public health problem. The diabetic population is especially prone to cataract formation and other ocular complications including retinopathy and corneal reepithelialization. Through the study of sugar cataracts and aldose reductase regulation methods for the control of diabetic cataract formation and possibly the control of other diabetic complications can be developed.

Proposed Course: These studies will be continued. Aldose reductase from human and rat lens will be purified and compared. The mechanism of action of aldose reductase inhibitors will be studied in detail along with the structure activity relationships of the inhibitors. Through such studies the minimum requirements of the inhibitory site may be determined so that more active inhibitors may be designed.

NEI Research Program: Cataract--Diabetic and Metabolic Cataract

Publications:

Kador PF, Carper D, Kinoshita JH: Rapid purification of human placental aldose reductase. Anal Biochem (in press).

Kador PF, Goosey JD, Sharpless NE, Kolish J, Miller DD: Stereospecific inhibition of aldose reductase. European J Med Chem (in press).

Jernigan HM Jr, Kador PF, Kinoshita JH: Carrier mediated transport of choline. Exp Eye Res (in press).

Kinoshita JH, Kador P, Datiles M: Aldose reductase in diabetic cataracts. JAMA 246,257, 1981.

Datiles M, Fukui H, Kuwabara T, Kinoshita JH: Galactose cataract prevention with Sorbinil, an aldose reductase inhibitor: A light microscopic study. Invest Ophthalmol Vis Sci (in press).

Kador PF, Kinoshita JH: Immunological recognition of lens aldose reductase with antibodies prepared from human placental aldose reductase. Invest Ophthalmol Vis Sci 20(suppl):35, 1981.

Datiles M, Kador PF, Fukui H, Kuwabara T, Kinoshita JH: Corneal reepithelialization in the galactosemic rats. Invest Ophthalmol Vis Sci 20(suppl): 39, 1981.

Fukui H, Datiles M, Kuwabara T, Kinoshita JH: Histological studies of the inhibitor effects on sugar cataract formation. Invest Ophthalmol Vis Sci 20:128, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER Z01 EY 00136-09 LVR		
PERIOD COVERED October 1, 1980, to September 30, 1981				
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: Other:	Paul Russell Jin H. Kinoshita Tien-sheng Hu Geeta Pararajasegaram	Ph.D. Ph.D. M.D. B.S.	Research Chemist Scientific Director Guest Worker Guest Worker	LVR NEI LVR LVR LVR NEI NEI NEI 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: 3.2	PROFESSIONAL: 2.2	OTHER: 1.0		
CHECK APPROPRIATE BOX(ES)				
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER				
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS				
SUMMARY OF WORK (200 words or less - underline keywords)				
<p>Work on membrane <u>proteins</u> from <u>human</u> and <u>animal lenses</u> has continued. Changes of the lens membrane constituents have been studied during <u>cataract development</u>. An <u>in vitro model system</u> using glucose deprived incubated lenses mimics many of the changes observed with cataract formation in vivo. Changes in membrane proteins as well as <u>enzyme inactivation</u> can be followed with the system.</p> <p><u>Tissue culture</u> of lens <u>epithelial cells</u> is being refined. Characterization of a defined culture medium for optimal growth of human and animal lens cells has been undertaken. The isolation of the <u>Na-K ATPase inhibitor</u> present in cultured lens cells from the <u>Nakano</u> mouse has also been carried out.</p>				

Project Description:

Objectives: To investigate the processes leading to cataract, it is essential to understand the changes in lens components. Our objective is to study those changes associated with the membranes and the membrane proteins of the lens that occur during cataract formation. Cell culture of lens epithelium is also undertaken to define a system where human lens specific components will be available for further investigation.

Methods Employed: Proteins from the lens as well as enzymatic activities from cells in the lens have been studied. Spectroscopic, gel electrophoretic and immunochemical methods have been employed. Translation of lens mRNA's as well as rocket immunoelectrophoresis of these translation products has been performed. Electron microscopic methods in collaboration with the LVR Experimental Pathology Section have also been used.

Major Findings: One of the more intriguing aspects of lens morphology is the large number of gap junctions on the mature lens fiber cell. These gap junctions have been isolated and the main intrinsic membrane polypeptide, associated with the gap junction fraction from lenses of young animals, has a molecular weight of 26,000. In older lenses and in cataractous animal lenses, a 23,000 MW component can also be observed. The new method for isolation of the membrane proteins using alkali extraction has facilitated the preparation of these proteins. Changes in the amounts of 26,000 and 23,000 MW polypeptides can be followed during cataract development.

Previous work has shown the correlation between the decrease in 26,000 MW polypeptide in cataract formation and the decrease in gap junctions in the fiber cells. The concomitant increase in 23,000 MW component suggests a post translation modification of the 26,000 MW polypeptide to the 23,000 MW one. The mechanism of this modification remains unclear. Some published reports suggest enzymatic degradation of the 26,000 MW polypeptide while other observations point toward some other mechanism. Radioactive labeling of this polypeptide in vivo or with the in vitro incubated lens has not resolved this controversy. The amount of label incorporated into this polypeptide has not been substantially above background levels to facilitate further analysis.

Labeling of the 26,000 MW component is possible if mRNA is extracted from the insoluble fraction of bovine or mouse lenses. This mRNA can be translated using the rabbit reticulolysate system. The translation product has a molecular weight equivalent to the 26,000 MW component. This result suggests no addition post translational modification is necessary for this polypeptide to be associated with the gap junction fraction initially. The translated 26,000 MW polypeptide has similar immunological properties to the lens polypeptide as shown by rocket immunoelectrophoresis. Thus it is now possible to get radioactively labeled 26,000 MW polypeptide to study the mechanism by which it changes to 23,000.

In the Nakano and Philly mice, two cataractous mouse strains, the transition from 26,000 MW to 23,000 MW polypeptide occurs at the time of cataract formation. Use of lenses from animals of these two strains to study the possible modifica-

tion of radiolabeled 26,000 MW polypeptide is hindered by the fact that individual differences in the animals of these strains might suggest an artifactual time course for this alteration. To obtain a more reproducible and consistent model system, lenses from normal rats and mice have been incubated in culture medium without glucose for two days. After deprivation of glucose, modification of the 26,000 MW polypeptide can be observed. Use of these in vitro incubated lenses with radiolabeled 26,000 MW polypeptide should make analysis of the type of post translation change possible.

Data from the glucose deprived lenses also show changes which are similar to those observed in cataractous lenses. There is an increase in the heavy molecular weight aggregates during incubation of the lenses which is similar to the increase observed in cataractous animal lenses. Loss of a specific, water-soluble, beta crystallin in vitro parallels the loss observed with cataract formation. In addition there is an increase in the water insoluble protein which mimics the change seen with the animal lenses. These alterations seen with the incubated lens allow study of these changes in a well defined system which is easily manipulated.

Work has also progressed with the culture of the epithelial cells of the lens. Investigations with lens cells from Nakano mice as well as normal mice have shown the presence of large aggregates of cells. These lentoid bodies also occur in the culture of human lens cells. Work has been directed to the growth of cells in serum-free defined medium. Use of epidermal growth factor as well as transferrin, insulin and selenium in cell culture has reduced the serum requirement of cell lines to one tenth the original. Collaborative work has shown that epithelial cells from the lens can be maintained in serum-free medium if factors released from retinal cells are added to the culture medium.

Significance to Biomedical Research and the Program of the Institute:

Alterations in the gap junctions of the lens fiber cell are related to cataract development in vivo. To understand the cataract process, the concomitant changes in membrane polypeptides and gap junctions must be resolved. Understanding the mechanism of gap junction changes may prove significant not only to cataractogenesis but also to other biological processes.

Current growth conditions for human lens epithelium are inadequate. Some growth does occur but isolation of lens specific polypeptides is not possible. Improvement and definition of culture condition should aid in the problem as well as afford an opportunity to observe possible developmental changes in vitro.

Proposed Course: Investigation into the mechanism by which the 26,000 MW polypeptide is modified will be studied with the in vitro system. In addition studies of the inactivation of specific enzymes during cataract development will be attempted. Culture of lens epithelial cells in defined media will be continued as an approach to determine possible cataractogenic factors.

NEI Research Program: Cataract--The Normal Lens

Publications:

Russell P, Fukui HF, Kinoshita JH: A Na-K ATPase inhibitor from cultured

lens cells, in Srivastava S (ed): Proceeding of the Second International Symposium of the Red Blood Cell and the Lens, 1980, pp 411-413.

Russell P, Fukui HF, Kinoshita JH: Properties of a Na-K ATPase inhibitor in cultured lens epithelial cells. Proceeding of the ocular tissue culture symposium. Vision Res 21:37-39, 1980.

Russell P, Uga S, Zigler JS Jr, Kaiser-Kupfer M, Kuwabara T: Studies using human lens from a family displaying hereditary congenital cataracts. Proceedings of the ocular tissue culture symposium. Vision Res 21:169-172, 1980.

Zigler JS Jr, Carper DA, Russell P, Kinoshita JH: Analysis of immunochemical properties of human  $\beta$ -crystallin by radioimmunoassay. Exp Eye Res 31:389-397, 1980.

Russell P, Robison WG Jr, Kinoshita JH: A new method for rapid isolation of the intrinsic membrane proteins from lens. Exp Eye Res 32:511-516, 1981.

Russell P, Carter-Dawson L, Kinoshita JH: Changes in incubated rat lenses mimicking cataract development. Invest Ophthalmol Vis Sci 20 (suppl):131, 1981.

Huang K, Roy D, Garner MH, Spector A, Carper D, Russell P: Investigation of normal and Nakano lens polypeptides. Invest Ophthalmol Vis Sci 20(suppl): 168, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00007-06 LVR
PERIOD COVERED October 1, 1980, to September 30, 1981		
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:       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: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) <u>Drug metabolizing and detoxifying enzymes of the eye</u> are localized mainly in the <u>ciliary body</u> and the <u>pigmented epithelium</u> .		

Project Description:

Objectives: Distribution of drug metabolizing enzyme activities in microsomal subfractions was studied.

Methods Employed: Fresh bovine eyes were dissected to collect various ocular tissues. Microsomal fractions were prepared and further fractionated into subfractions in a sucrose gradient. Drug metabolizing enzyme activities were then determined.

Major Findings: Activities of drug metabolizing enzymes (aryl hydrocarbon hydroxylase) and drug detoxifying enzymes (UDP-glucuronyl transferase, glutathione S-transferase, gamma-glutamyl transpeptidase, cystine aminopeptidase, N-acetyl transferase) are high in the ciliary body and pigmented epithelium.

Significance to Biomedical Research and the Program of the Institute: Little is known about the fate of drugs and environmental chemicals entering the eye through blood circulation or by topical administration. Elucidation of the mechanism of drug metabolism and drug detoxification in the eye provides important knowledge in designing drugs for ocular diseases and for prevention of drug toxicity in the eye.

Proposed Course: This project is terminated.

NEI Research Program: Retinal and Choroidal Diseases--Toxic, Nutritional and Environmental Disorders.

Publications:

Das ND, Shichi H: Enzymes of mercapturate synthesis and other drug-metabolizing reactions - specific localization in the eye. Exp Eye Res (in press).

Shichi H, Nebert DN: Genetic differences in drug metabolism associated with ocular toxicity. Env Health Perspect (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF          INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 EY 00004-07 LVR			
PERIOD COVERED October 1, 1980, to September 30, 1981					
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: Other:	Hitoshi Shichi Charles N. Rafferty Hiroyuki Fukui Robert L. Somers	Ph.D. Ph.D. M.D., Ph.D. B.S.	Research Chemist Research Chemist Postdoctoral Fellow Chemist	LVR LVR LVR LVR	NEI NEI NEI 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: 3.5	PROFESSIONAL: 1.5	OTHER: 2.0			
CHECK APPROPRIATE BOX(ES)					
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER					
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS					
SUMMARY OF WORK (200 words or less - underline keywords) A protein that <u>couples the photic bleaching of rhodopsin</u> to the <u>activation of cyclic nucleotide phosphodiesterase</u> has been purified to homogeneity and characterized. The protein binds either GDP or GTP and an <u>exchange of GDP with GTP</u> is catalyzed by opsin. The protein complexed with GTP but not with GDP activates phosphodiesterase.					

Project Description:

Objectives: The overall 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, the investigations presented in this report deal with (1) a protein that couples the photosignal reception by rhodopsin to cyclic nucleotide phosphodiesterase in rod outer segments, (2) 5'-nucleotidase that regulates 5'-GMP level of rod outer segments, and (3) the nature of protonation of rhodopsin chromophore.

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

Major Findings: I. A protein that couples the photic bleaching of rhodopsin to the activation of cyclic nucleotide phosphodiesterase was purified to homogeneity. The protein exchanges bound GDP for GTP very rapidly in the presence of opsin and hydrolyzes GTP to GDP slowly. The protein complexed with GTP (or its non-hydrolyzable analog GPPNP) activates cyclic nucleotide phosphodiesterase. II. The 11-cis retinylidene chromophore of rhodopsin is protonated by hydronium ion. Upon photon absorption at  $-190^{\circ}\text{C}$  the chromophore is isomerized to a transoid form (bathorhodopsin). The chromophore is still protonated by hydronium ion. The hydronium ion is replaced by proton as bathorhodopsin decays thermally to metarhodopsin I. III. There are two types of 5'-nucleotidase in rod outer segments: membrane bound (integral) enzyme and extractable (peripheral) enzyme. Both enzymes have been purified to homogeneity. The integral enzyme does not seem to have the catalytic center on the cytosolic side of rod membranes. Buffer extracts the peripheral enzyme more efficiently in the dark than in the light. The extracted enzyme binds to rod membranes in the presence of  $10^{-4}$ - $10^{-3}$  M  $\text{Ca}^{++}$  and light.

Significance to Biomedical Research and the Program of the Institute: The findings on the coupling protein and 5'-nucleotidase contribute to our understanding of the biochemical aspects of visual photoreceptor function. The findings on the retinylidene chromophore of rhodopsin provide important clues to elucidating the early photochemical events of vision.

Proposed Course: This project is terminated.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Transduction.

Publications:

Shichi H, Williams TC: Rhodopsin phosphorylation suggests biochemical heterogeneities of retinal rod disks. J Supramol Struct 12:419-424, 1979.

Tsunasawa S, Narita K, Shichi H: The blocked N-terminal residue of rhodopsin is acetyl methionine. Biochim Biophys Acta 624:218-225, 1980.

Shichi H: Molecular biology of the visual process, in Siegel GJ, Albers RW, Katzman R, Aganoff BW (eds): Basic Neurochemistry ed 3. Boston, Little, Brown and Co. (in press).

Shichi H, Somers RL: Distribution of enzymes involved in nucleotide metabolism in the disk and other rod membranes. Photochem Photobiol 32:491-495, 1980.

Adams AJ, Tanaka M, Shichi H: Isolation of intact disks by concanavalin A columns, in Packer L (ed): Methods Enzymol (in press).

Shichi H, Somers RL: Photoregeneration, in Packer L (ed): Methods Enzymol (in press).

Shichi H: Possible identity of experimental uveitogenic antigen (S-antigen) with rhodopsin kinase. Japan J Ophthalmol (in press).

Nussenblatt RB, Shichi H, Kuwabara T, Cevario S, Gery I: Resemblance between rhodopsin kinase and S-antigen induced uveitis. Br J Ophthalmol (in press).

Shichi H: Guanosine nucleotide metabolism in the bovine rod outer segment: Distribution of enzymes and a role of GTP. Current Topics in Membranes and Transport, Academic Press, Vol. 13 (in press).

Rafferty CN, Shichi H: The involvement of water at the retinal binding site in rhodopsin and early light-induced intramolecular proton transfer Photochem Photobiol 33:229-234, 1981.

Shichi H: Rhodopsin kinase - with emphasis on function and immunopathogenicity. J Japan Biochem Soc (Seikagaku) 53:20-24, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00105-02 LVR												
PERIOD COVERED October 1, 1980, to September 30, 1981														
TITLE OF PROJECT (80 characters or less)  Structure and Composition of Lens Crystallins with Respect to Cataract Development														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width:100%; border: none;"> <tr> <td style="width:15%;">PI:</td> <td style="width:35%;">J. Samuel Zigler, Jr.</td> <td style="width:15%;">Ph.D</td> <td style="width:25%;">Senior Staff Fellow</td> <td style="width:10%;">LVR</td> <td style="width:10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Igal Gery</td> <td>Ph.D.</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	J. Samuel Zigler, Jr.	Ph.D	Senior Staff Fellow	LVR	NEI	Other:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI
PI:	J. Samuel Zigler, Jr.	Ph.D	Senior Staff Fellow	LVR	NEI									
Other:	Igal Gery	Ph.D.	Visiting 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, MD 20205														
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:												
1.1	1.1	0.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) In human senile nuclear cataracts the lens crystallins undergo <u>oxidative damage</u> which leads to the formation of proteins that are <u>pigmented</u> , develop a characteristic <u>non-tryptophan fluorescence</u> and are <u>cross-linked</u> by non-disulfide covalent bonds. It has been believed that <u>photo-oxidation</u> of aromatic amino acid residues may be responsible for these changes. We have now demonstrated what we believe to be a more attractive mechanism for formation of these altered lens crystallins, i.e. <u>photosensitized oxidation</u> mediated by <u>singlet oxygen</u> . We have shown that such a system which involves light, a photosensitizing molecule and <u>oxygen</u> can mimic these changes <u>in vitro</u> since solutions of lens crystallins exposed to such a system undergo each of the modifications outlined above. In addition crystallins in <u>intact lenses</u> irradiated in the presence of <u>photosensitizers</u> are also susceptible to these changes. In contrast to the <u>direct photo-oxidation</u> of aromatic residues this system is dependent upon <u>near UV light</u> of wavelengths which readily penetrate to the lens. Since we have been able to demonstrate the presence of effective near UV absorbing photosensitizers in human lens we believe that this mechanism may be a major factor in the etiology of human senile cataract.														

Project Description:

Objectives: A number of structural modifications are well documented in human lens crystallins during normal aging and senile cataract development. It is believed that most of these changes are the result of oxidative insult to the lens although the particular mechanism(s) is not clear. The aim of this study is to demonstrate that the oxidative changes to lens crystallins may result from photosensitized oxidation involving singlet oxygen as the primary injurious species. In addition studies have been conducted to determine whether human cataracts associated with chronic uveitis may result from oxidative damage as well.

Methods Employed: Crystallins were isolated from human lenses by column chromatography and evaluated for purity by standard techniques including electrophoresis, isoelectric focusing and immunochemical techniques. Protein solutions or intact lenses were irradiated in the presence of photosensitizers with either visible or near ultraviolet radiation. Photo-oxidative changes were evaluated by spectrofluorometry, absorption spectroscopy and gel electrophoresis. Rat lenses were organ cultured in modified TC-199 medium under standard tissue culture conditions with or without activated murine peritoneal macrophages present in the culture dishes.

Major Findings: We have demonstrated that solutions of lens crystallins exposed to light in the presence of photosensitizers such as rose bengal undergo a number of structural modifications which closely parallel those known to occur in the human lens in vivo. These modifications include the development of non-disulfide covalent cross-links, pigmentation, increased absorbance in the 300-400 nm range and a characteristic non-tryptophan fluorescence. Studies using specific scavengers have suggested that singlet oxygen, a highly reactive excited state of oxygen is responsible for producing these changes. Confirmation was obtained by demonstrating that such modifications are produced in crystallins exposed to photophysically generated singlet oxygen, i.e. singlet oxygen produced by laser radiation in the absence of photosensitizer. We have also demonstrated that endogenous photosensitizers exist in human lens which are capable of producing singlet oxygen when exposed to near UV light at wavelengths which freely penetrate into the lens.

Extension of these studies to intact rat lenses irradiated in culture in the presence of rose bengal or the photosensitizing compound kynurenine, which is known to exist in human lens, has shown that production of non-disulfide covalent cross-link also occurs in the crystallins of such lenses. This finding indicates that crystallins in their normal environment within the intact lens are also susceptible to singlet oxygen oxidation. Thus we believe that photosensitized oxidation mediated by singlet oxygen may play a significant role in the changes involved in formation of human senile cataracts, particularly those of the nuclear type. The oxidative alterations are most prominent in the lens nucleus which is consistent with the fact that the nuclear crystallins are the oldest proteins in the lens and do not turnover. The greatly reduced concentration of endogenous antioxidants in the lens nucleus may also play a role in the localization of damage in the nucleus.

We have also considered the possibility that cataracts secondary to chronic ocular inflammation (uveitis) may result from oxidation produced by oxidants (e.g.  $H_2O_2$ ,  $O_2^-$ ) released by inflammatory cells. Damage to cultured rat lenses in terms of their ability to actively accumulate labeled compounds has been demonstrated following exposure of the lenses to activated murine macrophages. Attempts to block the damaging effects by adding specific scavengers of the various activated forms of oxygen have not been successful, although nonspecific antioxidants such as dithiothreitol and glutathione do partially block the lens damage. This suggests that oxidation is responsible in part for the observed effects, but it also indicates that other mediators released by the macrophages must play a role in the process as well. Studies are in progress aimed at determining which of the many other potential mediators released by activated macrophages may be involved.

Significance to Biomedical Research and the Program of the Institute:

At present the etiology of senile cataract is unknown. Elucidation of the mechanism underlying formation of even one class of senile cataracts (e.g. those involving primarily changes in the lens nucleus) would be a great step towards understanding and possibly preventing the development of such cataracts. If near UV light is the initiating factor in the formation of nuclear cataracts, prevention of the disease is a realistic goal.

Proposed Course: Since we now have the means of producing in vitro modifications to crystallins which appear to be identical to those found in vivo, we have a powerful tool for studying in detail the structure of these altered proteins in terms of amino acid residues involved and the precise nature of the fluorescent and cross-linking products formed. Attempts at isolating and identifying these products from human lens have failed because of their low concentration and the difficulty in working with the very heterogeneous and highly insoluble proteins with which they are associated. The ability to produce high concentrations of the oxidative products in purified water-soluble crystallin preparations will make analysis much easier. Once structures are identified from these in vitro studies it will be much easier to identify their presence in material from aging or cataractous human lenses.

NEI Research Program: Cataract--Senile Cataract

Publications:

Zigler JS Jr, Goosey JD: Photosensitized oxidation in the ocular lens: Evidence for photosensitizers endogenous to the human lens. Photochem Photobiol 33:869-874, 1981.

Goosey JD, Zigler JS Jr, Matheson, IBC, Kinoshita JH: Effects of singlet oxygen on human lens crystallins in vitro. Invest Ophthalmol Vis Sci 20: 679-683, 1981.

Zigler JS Jr, Goosey JD: Aging of protein molecules: Lens crystallins as a model system. Trends in Biochem Sci 6:133-136, 1981.

Zigler JS Jr, Carper DA, Kinoshita JH: Changes in lens crystallins during

cataract development in the Philly mouse. Ophthalmic Res (in press).

Gery I, Zigler JS Jr, Brady RO, Barranger JA: Selective effects of glucocerebroside (Gaucher's storage material) on macrophage cultures. J Clin Invest (in press).

Zigler JS Jr, Gery I, Kessler D: Effects of activated macrophages on cultured lenses. Invest Ophthalmol Vis Sci 20(suppl):134, 1981.

Jernigan HM Jr, Zigler JS Jr: Photodynamic crosslinking of proteins in cultured lenses. Invest Ophthalmol Vis Sci 20(suppl):129, 1981.

Gery I, Zigler JS Jr, Nussenblatt, RB: Dissociation between the humoral and cellular autoimmune responses to lens crystallins. Fed Proc 40:1137, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00005-09 LVR
--	--	---

PERIOD COVERED  
October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)  
Electrophysiology and Morphology 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.	Staff Fellow	LVR	NEI
Other:	Thomas Lynn		Biological Aid	LVR	NEI

COOPERATING UNITS (if any)  
Department of Physiology, University of Utah, Salt Lake City

LAB/BRANCH  
Laboratory of Vision Research

SECTION  
Section on Experimental Biology

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

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.75	OTHER: .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 the electrophysiology of single retinal neurons, intracellularly, and stain the cells through the electrode with horseradish peroxidase (HRP) so that the synaptic connections of physiologically identified neurons can be identified with the electron microscope. All amacrine cells, which are prominent among several types of amacrine cells intervening between rod bipolar cells and ganglion cells in the cat retina, quicken the initial sluggish rod waveform providing a faster rod response. Using Golgi impregnation, a new class of retinal neuron has been identified in rhesus monkey retina. These cells are termed biplexiform cells since they arborize in both plexiform layers. Golgi-EM shows that in the outer plexiform layer (OPL) they form central elements at rod spherules, while in the inner plexiform layer (IPL) they are postsynaptic to rod bipolar cell axon terminals. Electron microscopy of excitatory, cholinergic synapses demonstrates interactions between synaptic vesicles and the presynaptic membrane. Release of transmitter is accompanied by selective depletion of synaptic vesicles aligned at the active zone, loss of half of the synaptic vesicles in the terminal, and synaptic vesicle openings at active zone.

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 or by disease states of the retina.

Methods Employed: Golgi impregnation which stains widely-separated individual cells in their entirety is used to study the form, shape, size and spatial relationships of retinal neurons. Their synaptic connections with other neurons are then examined by Golgi-EM, thus providing insight into their function. Electron microscopy of synaptic terminals in either resting or stimulated states reveals the function of interneuronal contacts.

We characterized the response properties of neurons in retinas by intracellular recording of their transmembrane potentials and extracellular electroretinographic (ERG) recording during photic stimulation. Viable retina-eyecup preparations are maintained in vitro by perfusion of the ophthalmic arteries and retinal surface with synthetic media. HRP, injected into neurons through the electrodes, fills their axons and dendrites, and 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.

Major Findings: I. Biplexiform cells of primate retina: a new class of retinal neuron. Neurons of the vertebrate retina are considered, generally, to consist of five classes of cells, each of which may contain many different morphological and functional types. Basically, ganglion cells convey information to other parts of the central nervous system via their axons, but light transduced by photoreceptors is transmitted in the form of electrical and chemical signals to the ganglion cells by interneurons, the bipolar cells. Horizontal and amacrine cells interact in this chain of transmission at the levels of the photoreceptor-bipolar cell synapse (outer plexiform layer) and the bipolar-ganglion cell synapse (inner plexiform layer) respectively. Ganglion cells are not thought to contact photoreceptors directly. Now, a new class of retinal neuron has been found in Golgi preparations of the rhesus monkey retina which has the characteristic features of a ganglion cell, but which directly contacts photoreceptors. These cells are termed biplexiform cells since they arborize in both plexiform layers. Biplexiform cells have small cell bodies in the ganglion cell layer, fine wavy, radiate dendrites which branch mainly in the inner one-half of the inner plexiform layer and have a relatively moderate span. A single axon arises from either the soma or one of the dendrites. Three to five very fine telodendritic processes arise from the main dendritic arborization and ascend to the outer plexiform layer where they follow a long, tortuous, horizontal course and sometimes terminate as small individual knobs at the level of the receptor bases. Golgi-RM shows that in the outer plexiform layer, biplexiform cell processes ter-

minate as central elements at the ribbon synaptic complex of rod spherules, while in the inner plexiform layer its dendrites are post-synaptic to rod bipolar cell axon terminals at conventional (non-ribbon) synapses. Due to their unique morphology and synaptic connections biplexiform cells represent a totally new class of retinal neuron.

II. Structural changes during transmitter release at synapses: Interactions between synaptic vesicles and the presynaptic membrane which accompany transmitter release were examined at excitatory, cholinergic synapses in bullfrog sympathetic ganglia. Ganglia were fixed at rest or during electrical stimulation of the preganglionic axons and then either thin-sectioned or freeze-fractured. Release of transmitter for brief periods is accompanied by selective depletion of four fifths of the synaptic vesicles aligned at active zones, overall loss of half the synaptic vesicles in the terminals and synaptic vesicle openings at active zones. These findings are consistent with the hypotheses that synaptic vesicles which are ready to be released are aligned at active zones and that these vesicles fuse with, and add their membrane to, the presynaptic membrane as they release transmitter. Larger vesicles with dense cores also contact and open onto the presynaptic membrane at the active zone, appearing to release their contents by exocytosis. The arrangement of intramembrane particles at fractured postsynaptic specializations resembles that at other excitatory, cholinergic synapses. These findings are relevant to studies of retinal circuitry and synaptic function since similar synapses are found in the retina.

III. Amacrine cells of the cat retina: intracellular responses, HRP stains and synaptic connections. AII amacrine cells are prominent among the several classes of amacrine cell intervening between rod bipolar cells and ganglion cells in the cat retina. Because of the narrow dendritic spread of AII cells it has been suggested that they act to transmit 'simple,' 'sustained' rod signals to the center mechanisms of ganglion cells. But rod signals enter cones through interreceptor contacts and are passed directly to ganglion cells through the cone bipolar cells. Rod signals in this pathway more surely fulfill the criteria of being 'simple' and 'sustained'. Investigation of the dynamics of the responses of AII cells and such rod-dominated S-potentials as recorded in rod bipolar cells, horizontal cell axon terminals, and a center-hyperpolarizing rod-dominated amacrine response, suggests that the AII cell acts to quicken the initial phase of the sluggish rod waveform. As measured by the latency to half-maximum amplitude for the response, AII amacrine cells are faster than rod-dominated S-potentials at all stimulus energies, and in the lower half of the rod dynamic range, are some 35 to 40 msec or 1/3 again faster. Here they achieve their peak when the rod bipolar response has achieved only 1/3 of its maximum value. The rise-time of the AII response is correspondingly quick and requires only 5 msec for saturating stimuli (25% to 75% transit time). The evolution of a system providing a quick rod response would seem advantageous to a twilight hunter such as the cat.

Three morphologically different types of amacrine cell have been studied by intracellular recording and light and electron microscopy.  $A_4$ , with a narrow-field dendritic tree restricted to S2 of sublamina a, is pre- and post-synaptic to flat cone bipolars, post-synaptic to amacrines, and pre-synaptic to ganglion cells of the off-center system. Electrophysiology shows this amacrine cell to have a sustained hyperpolarizing response driven mainly by the cone system. Its

receptive cell field is small with no surround evident.  $A_8$  is another narrow-field amacrine that resembles morphologically an inverted AII amacrine cell. An HRP injected example shows that its processes in sublamina a are post-synaptic to flat cone bipolars and its beaded dendrites in sublamina b are post-synaptic to rod bipolars, amacrines and cone bipolars. Intracellular recordings indicate that  $A_8$  has impulse activity and receptive field properties similar to an off center ganglion cell.  $A_{17}$ , on the other hand, is a wide-field amacrine cell with a radiating dendritic tree ramifying primarily in sublamina b. Its fine caliber dendrites bear distinctive swellings or beads every 10 $\mu$ m or so. EM study of an HRP-injected cell indicates that the beads are sites of reciprocal synaptic interactions with rod bipolars.  $A_{17}$  has a large (900 $\mu$ m) receptive field and sustained, depolarizing, rod-driven responses.

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. 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 neurons the retina contains, and what their physiological properties and roles in vision may be, provides a necessary substrate for interpreting and testing retinal dysfunction.

Proposed Course: This project will be continued along lines indicated in the project description. Emphasis will be given to neurons participating in the inner and outer plexiform layers of primates and cats. Comparisons will continue to be made between homologous cells types in the retinas of different species using the Golgi staining technique. Particular emphasis will be given to inter-receptor contacts in primates. Existing data supplemented by additional data currently being obtained on amacrine cells in the cat retina will be organized and made ready for publication. The interrelationships of amacrine, bipolar and ganglion cells in the cat IPL will be studied further.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation.

Publications:

Nelson R: All amacrine cells quicken the time course of rod signals in the cat retina. J Neurophysiol 1982 (in press).

Nelson R, Kolb H, Robinson M, Mariani A: Neural circuitry of the cat retina: Cone pathways to ganglion cells. Vision Res 1981 (in press).

Kolb H, Nelson R: Morphology and circuitry of some amacrine cells in the cat retina. Vision Res 1981 (in press).

Mariani AP: A diffuse, invaginating cone bipolar cell in primate retina. J Comp Neurol 197:661-671, 1981.

Kolb H, Nelson R, Mariani AP: Amacrine, bipolar and ganglion cells of the cat retina. Vision Res 21:1081-1114, 1981.

Nelson R: All amacrine cells quicken the time course of rod signals in the cat retina. Invest Ophthalmol Vis Sci 20(suppl):184, 1981.

Mariani AP: Biplexiform cells of primate retina. Invest Ophthalmol Vis Sci 20(suppl):203, 1981.

Kolb H, Nelson R: Three amacrine cells of the cat retina: Morphology and intracellular responses. Invest Ophthalmol Vis Sci 20(suppl):184, 1981.

Dickinson-Nelson A, Reese TS: Structural changes during transmitter release at synapses in the frog sympathetic ganglion. J Neurosci 1982 (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00125-01 LVR															
PERIOD COVERED October 1, 1980, to September 30, 1981																	
TITLE OF PROJECT (80 characters or less)  Neuropharmacology of the Retina																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" data-bbox="65 430 1332 534"> <tr> <td>PI:</td> <td>Donald G. Puro</td> <td>M.D., Ph.D</td> <td>Medical Officer</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td></td> <td></td> <td>Ophthalmologist</td> <td></td> </tr> <tr> <td>Other:</td> <td>Barbara-Anne Battelle</td> <td>Ph.D.</td> <td>Senior Staff Fellow</td> <td>LVR NEI</td> </tr> </table>			PI:	Donald G. Puro	M.D., Ph.D	Medical Officer	LVR NEI				Ophthalmologist		Other:	Barbara-Anne Battelle	Ph.D.	Senior Staff Fellow	LVR NEI
PI:	Donald G. Puro	M.D., Ph.D	Medical Officer	LVR NEI													
			Ophthalmologist														
Other:	Barbara-Anne Battelle	Ph.D.	Senior Staff Fellow	LVR NEI													
COOPERATING UNITS (if any)  None																	
LAB/BRANCH Laboratory of Vision Research																	
SECTION Section on Experimental Biology																	
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205																	
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0															
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords)  Information basic to understanding <u>retinal disorders</u> at a cellular level is acquired from experiments which combine the technologies of <u>neuropharmacology</u> , <u>intracellular electrophysiology</u> , and <u>cell biology</u> , including <u>cell culture</u> . The actions and interactions of <u>neurotransmitters</u> , <u>neuromodulators</u> , <u>hormones</u> and selected <u>drugs</u> on <u>retinal neurons</u> are examined. The specificity and stability of <u>synapses</u> made by embryonic retinal neurons under various controlled conditions of culture are studied quantitatively.																	

Project Description:

Objectives: The general goal of this project is to establish a framework of knowledge so that the mechanisms by which diseases, developmental abnormalities, drugs and injuries alter normal retinal function can be elucidated at the cellular level. Greater understanding of pathophysiology is fundamental to the prevention, diagnosis and treatment of retinal problems. Specific objectives are to apply technical advances in electrophysiology, neuropharmacology and cell biology, including cell culture, in order to (1) study the effects of neurotransmitters, neuromodulators, hormones and selected drugs on the function of retinal neurons, (2) characterize steps in the maturation of neuronal function, including neurotransmitter release and synapse formation and (3) establish parameters for assaying the effects of therapeutic and toxic agents on developing retinal neurons.

Methods Employed: Using techniques of intracellular electrophysiology and cell culture, methods have been developed which allow the continuous monitoring of neurotransmitter output from a single cholinergic retinal neuron. Putative neurotransmitters, neuromodulators and drugs are applied near a cholinergic neuron by either microiontophoresis or pressure ejection from a single or multi-barrel micropipette.

Major Findings: The following findings were derived from studies of cultured retinal neurons.

(1) The putative neurotransmitters glutamate, gamma-amino butyric acid, glycine and dopamine have direct effects on some cholinergic neurons of the vertebrate retina. Glutamate is excitatory, the others inhibitory. The use of a cell culture system has permitted for the first time the demonstration that these neurotransmitters can act at cholinergic retinal neurons.

(2) At least six classes of cholinergic neurons have been isolated from the chick retina. This classification is based on a cell's responses to a spectrum of putative neurotransmitters. Identifying types of cells in the retina is important in attempting to study retinal disorders at a cellular level.

(3) Functional synapses can be made by at least some neurons of the rat retina very early in development - many days before synapse formation has been detected by morphological criteria. Initially, the specificity of synapse formation by these neurons is low since they can transiently synapse with cultured muscle cells. These physiological findings were correlated with a biochemical analysis (Z01 EY 00066-03 LVR) in the first study of acetylcholine synthesis and release by developing neurons of a mammalian retina. Parameters quantitated in this study may be useful in the detection of agents toxic to developing retinal neurons.

(4) Hormones may play a vital role in the development of retinal neurons. With rat retina, glucocorticoid hormones were found to accelerate the maturation of a cholinergic neuron's ability to release acetylcholine in response to excitation by a neurotransmitter. Work currently in progress indicates that these steroids induce the formation of functional voltage dependent calcium channels.

The finding of hormonal modulation of a crucial step in the development of retinal neurons opens a new area of investigation.

Significance to Biomedical Research and the Program of the Institute:

Retinal disorders are a major cause of irreversible visual loss. To more effectively combat retinal problems, greater understanding of pathophysiology at the cellular level is required. This project has focused on the development of techniques which allow the detailed study of single retinal neurons. New information about parameters vital to the maturation and function of retinal neurons has been obtained.

Proposed Course: Emphasis will be placed on the study of interactions of neurotransmitters and neuromodulators, including certain peptides, at the neuronal level. Further analysis of the glucocorticoid hormone effect on the development of retinal neurons will be pursued. In addition, other hormones, such as insulin, will be evaluated in the cell culture system for possible developmental of functional roles.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation

Publications:

Puro DG, Battelle B-A: Development of cholinergic function in rat retina. Invest Ophthalmol Vis Sci 20(suppl):188, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00177-06 LVR																									
PERIOD COVERED October 1, 1980, to September 30, 1981																											
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																											
<table style="width:100%; border: none;"> <tr> <td style="width:10%;">PI:</td> <td style="width:30%;">Peggy Zelenka</td> <td style="width:15%;">Ph.D.</td> <td style="width:25%;">Geneticist</td> <td style="width:30%;">LVR NEI</td> </tr> <tr> <td>Other:</td> <td>Gloria Chepko</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>Ngoc-Diep Thi Vu</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>Howard Jernigan</td> <td>Ph.D.</td> <td>Visiting Scientist</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>David Beebe</td> <td>Ph.D.</td> <td>Ass't. Professor</td> <td>USUHS</td> </tr> </table>			PI:	Peggy Zelenka	Ph.D.	Geneticist	LVR NEI	Other:	Gloria Chepko	Ph.D.	Staff Fellow	LVR NEI		Ngoc-Diep Thi Vu	Ph.D.	Staff Fellow	LVR NEI		Howard Jernigan	Ph.D.	Visiting Scientist	LVR NEI		David Beebe	Ph.D.	Ass't. Professor	USUHS
PI:	Peggy Zelenka	Ph.D.	Geneticist	LVR NEI																							
Other:	Gloria Chepko	Ph.D.	Staff Fellow	LVR NEI																							
	Ngoc-Diep Thi Vu	Ph.D.	Staff Fellow	LVR NEI																							
	Howard Jernigan	Ph.D.	Visiting Scientist	LVR NEI																							
	David Beebe	Ph.D.	Ass't. Professor	USUHS																							
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: 2.5	PROFESSIONAL: 2.5	OTHER: 0.0																									
CHECK APPROPRIATE BOX(ES)																											
<input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER																											
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline keywords) This project seeks to determine whether the regulation of <u>lens fiber differen-</u> <u>tiation</u> and maturation is associated with alterations in the <u>plasma membrane</u> . To this end, the principal <u>lipid</u> and <u>protein</u> components of embryonic and adult chicken lens membranes are being identified, and their metabolism is being investigated. Because of the known involvement of <u>phosphatidylinositol (PI)</u> <u>turnover</u> and <u>phosphatidylethanolamine (PE) transmethylation</u> in regulatory mechanisms in other cell types, this study has focused on lens phospholipid metabolism using <u>isotopic labeling techniques</u> and <u>computer modeling</u> to analyse the kinetics of radio-isotope incorporation. The relationships between phos- pholipid metabolism and differentiation have been studied in vitro, under defined conditions, using explants of <u>embryonic chick lens epithelia</u> . These tissues differentiate to form lens fibers in the presence of <u>fetal calf serum</u> , <u>insulin</u> , or <u>vitreous humor</u> . Results obtained in vitro have been compared with results obtained in vivo in lenses of embryonic chicks and other species.																											

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: In vitro studies of lens phospholipid metabolism employ cultured explants of lens epithelia from 6-day-old or 19-day-old embryonic chicks. Various cultured conditions are used to maintain the cells in an epithelial state or to permit their differentiation into lens fibers. Phospholipids are labeled with  $^{32}\text{P}$ -orthophosphate,  $^3\text{H}$ -glycerol,  $^3\text{H}$ -methionine, or  $^3\text{H}$ -labeled fatty acids. Drugs which interfere with phospholipid metabolism are added to the culture medium to test their effect of differentiation. Labeled phospholipids are analysed by thin layer chromatography and scintillation counting.

Polyphosphoinositides from lenses of mouse, rat, and chicken are isolated by affinity chromatography on glass bead columns containing covalently bound neomycin. Phosphate analysis is then used to determine the amount of polyphosphoinositide present.

Precursors of phospholipid synthesis are isolated by thin layer chromatography or high performance liquid chromatography, and their concentrations are determined by means of a colorimetric assay for phosphate. Knowledge of precursor concentrations allows calculation of precursor specific radioactivities, making possible determinations of rates of synthesis and degradation of individual phospholipids by computer modeling.

Major Findings: The intralenticular concentrations of two precursors of phospholipid metabolism, phosphorylcholine and phosphorylethanolamine, have been determined for a variety of species at different stages of development and maturation. The results showed large differences among the species tested. Within each species the concentrations of both substances decreased with age. Furthermore, cataractous human lenses showed decreased concentrations of both substances. These results will facilitate further studies of lens phospholipid metabolism.

Studies of phospholipid metabolism in cultured explants of 6-day-old embryonic chick lens epithelia in Hams F-10 minimal medium have shown that an abrupt increase in the rate of degradation of all major phospholipids occurs after about 9 hrs in culture. This result can not be explained by loss of cells, cell death, or changes in precursor specific activity. Previous studies of embryonic chick lens epithelia cultured under these conditions have shown that between 6 and 12 hrs in culture the cells lose their ability to differentiate into lens fibers in response to fetal calf serum. Thus, the observed increase in phospholipid degradation is temporally correlated with the loss of the cells' ability to differentiate. This result suggests that phospholipid degradation may be coupled to degradation of specific receptors in the plasma membrane.

A transient increase in the transmethylation of PE to phosphatidylcholine (PC) has been observed in explants of embryonic chick lens epithelia stimulated to differentiate into lens fibers by the addition of fetal calf serum, vitreous humor, or insulin to the culture medium. The response reaches a maximum 6 sec after the addition of fetal calf serum or vitreous humor, 30 sec after the addition of insulin. Deaza-adenosine, an inhibitor of methylation, produces a concentration dependent inhibition of the cellular elongation that is characteristic of lens fiber formation. Following the increase in phospholipid methylation there is an increase in the release of arachidonic acid, or its metabolites, to the medium. This release is also inhibited by deaza-adenosine. This study provides the first evidence that alterations in phospholipid metabolism play a regulatory role in the differentiation of lens epithelial cells into lens fibers.

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. Additional experiments will be undertaken to characterize the changes in phospholipid metabolism that accompany lens fiber formation and to ascertain their biological significance.

a) Studies of PI metabolism during lens fiber formation will be continued. Phosphorylation of PI to polyphosphoinositides will be studied during the initiation of differentiation in cultured explants. Specific inhibitors of phospholipid metabolism will be used to determine whether phospholipid methylation and phosphatidylinositol turnover are coupled.

b) The observed release of arachidonic acid, or its metabolites, during in vitro differentiation of lens epithelial cells into lens fibers raises the possibility that prostaglandins may be involved in the process. To investigate this possibility, the identity of the material released to the medium must first be determined. Methods for analysing prostaglandins and other metabolites of arachidonic acid will be introduced. In addition, the effect of inhibitors of arachidonic acid metabolism will be tested.

c) Comparative studies will be undertaken to determine the ability of lenses of various species to metabolized arachidonic acid.

NEI Research Program: Cataract--The Normal Lens

Publications:

Zelenka P, Jernigan HM Jr: Phosphorylcholine and phosphorylethanolamine concentrations in the lens. Exp Eye Res (in press).

Zelenka PS, Beebe DC: Phospholipid methylation during in vitro differentiation of lens fiber cells. Fed Proc 40:1804, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00069-04 LVR																																				
PERIOD COVERED October 1, 1980, to September 30, 1981																																						
TITLE OF PROJECT (80 characters or less)  Immune Responses to Ocular Antigens																																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0"> <tr> <td>PI:</td> <td>Igal Gery</td> <td>Ph.D.</td> <td>Visiting Scientist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Robert B. Nussenblatt</td> <td>M.D.</td> <td>Senior Staff Ophthalmologist</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Magda El Saied</td> <td>M.D.</td> <td>Visiting Fellow</td> <td>CB</td> <td>NEI</td> </tr> <tr> <td></td> <td>Hitoshi Shichi</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>W. Gerald Robison, Jr.</td> <td>Ph.D.</td> <td>Geneticist/Cell Biologist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Julia Derr</td> <td>B.S.</td> <td>Biologist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI	Other:	Robert B. Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI		Magda El Saied	M.D.	Visiting Fellow	CB	NEI		Hitoshi Shichi	Ph.D.	Research Chemist	LVR	NEI		W. Gerald Robison, Jr.	Ph.D.	Geneticist/Cell Biologist	LVR	NEI		Julia Derr	B.S.	Biologist	LVR	NEI
PI:	Igal Gery	Ph.D.	Visiting Scientist	LVR	NEI																																	
Other:	Robert B. Nussenblatt	M.D.	Senior Staff Ophthalmologist	CB	NEI																																	
	Magda El Saied	M.D.	Visiting Fellow	CB	NEI																																	
	Hitoshi Shichi	Ph.D.	Research Chemist	LVR	NEI																																	
	W. Gerald Robison, Jr.	Ph.D.	Geneticist/Cell Biologist	LVR	NEI																																	
	Julia Derr	B.S.	Biologist	LVR	NEI																																	
COOPERATING UNITS (if any)  None																																						
LAB/BRANCH Laboratory of Vision Research																																						
SECTION Section on Experimental Immunology																																						
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205																																						
TOTAL MANYEARS: 1.7	PROFESSIONAL: 1.6	OTHER: 0.1																																				
CHECK APPROPRIATE BOX(ES)  <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																																						
SUMMARY OF WORK (200 words or less - underline keywords)  <u>Experimental autoimmune uveitis (EAU)</u> in rats is being used as a possible model for certain human ocular diseases. Two aspects of EAU induction have been studied: (1) The <u>role of lymphocytes</u> as the major component of the pathogenic mechanism was confirmed in experiments in which <u>transfer of EAU</u> was achieved: EAU was transferred to normal, naive rats by injection of lymphocytes from donors with active disease. (2) The <u>effect of the genetic makeup on susceptibility to EAU</u> induction was examined. Various <u>inbred rat strains</u> were compared and showed great variations in their development of EAU.																																						

Project Description:

Objectives: Experimental autoimmune uveitis (EAU) has been found in recent years to be a useful tool for investigating the mechanisms involved in autoimmune processes of the eye. The present studies addressed specifically two aspects related to induction of EAU: (1) the pathogenic role of lymphocytes, tested by their capacity to transfer the disease and (2) the influence of the genetic makeup on the susceptibility to the disease, examined by comparing the development of EAU in rats of different genetic backgrounds.

Methods Employed: Rats of inbred strains were supplied by commercial sources or the NIH breeding facilities. EAU was induced by immunization with the retinal S-antigen emulsified in complete Freund's adjuvant (CFA). The S-antigen was prepared from bovine retinae, according to the method of Wacker et al (1977). The severity of disease was measured by both clinical and histological changes. Experimental autoimmune encephalomyelitis (EAE) was induced by immunizing rats with guinea pig myelin basic protein, emulsified in CFA. Spleen cells used for disease transfer were collected from rats with active EAU and cultured for 3 days with concanavalin A. Different numbers of the cultured cells were injected to normal recipients.

Major Findings: EAU may be transferred into naive normal recipients by injection of spleen cells from actively immunized donors. In some cases, as few as  $2 \times 10^7$  lymphocytes could transfer the disease. However, the transfer was entirely reproducible when using  $10^8$  cells. A prerequisite for successful transfer of disease was the preculturing of the spleen cells in the presence of a mitogenic lectin, concanavalin A.

Rats of different inbred strains showed a remarkable degree of variation in their susceptibility to EAU induction. Thus, all tested rats of certain strains (Lewis, CAR, LBNF) developed EAU, while only about 10-20% of the BN or MAXX strains developed the disease. A partial correlation was found between the susceptibility to EAU and to another autoimmune disease, EAE: all rats of the strains with complete susceptibility to EAU were also susceptible to EAE, while the strains with partial resistance to EAU were completely resistant to EAE.

Significance to Biomedical Research and the Program of the Institute: The transfer of EAU by lymphocytes from donors with active disease provides direct evidence supporting the assumption that this disease is caused by a cell-mediated immune response. Moreover, incubation with concanavalin A, which was essential for disease transfer, selectively stimulates the T-lymphocyte population. These findings thus indicate that T-cells are a major component of this process. This conclusion is also in accord with data accumulated by Drs. Nussenblatt and Salinas-Carmona, at the Clinical Branch, NEI (see corresponding reports in this volume). The identification of the cell type involved in the etiology of autoimmune ocular diseases is essential for the course of treatment experimentation for these entities.

The finding that rats of various inbred strains differ in their susceptibility to EAU induction indicates that genetic factors play a pivotal role in determining the development of this autoimmune disease. Despite the close similarity between the patterns of susceptibility to EAU and EAE, the results indicate that different immune genes may control the development of these two experimental diseases, since rats of strains which are completely resistant to EAE may develop EAU. These data may suggest, therefore, that the development of both EAU and EAE is controlled by both the well defined immune response gene system and another set of genes, which are yet to be characterized and may control the development of the pathogenic processes of both diseases. Ocular diseases of autoimmune origin in man have been long suspected to be regulated by genetic factors. The preliminary data accumulated in this study are in line with this notion and suggest that the animal model may be useful for investigating these factors.

Proposed Course: Future studies will focus on further investigations of the processes and cells involved in the induction and regulation of EAU. In particular, attempts will be made to characterize the population of lymphocytes which transfer the disease and to further analyze their mode of action. In addition, the possible suppression of EAU by immune mechanisms (e.g. suppressor lymphocytes or their products) will be tested.

The relationship between the genetic makeup and susceptibility to EAU will be further examined by including in the study more inbred strains with partial genetic homology with the high or low responding strains.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Diseases

Publications:

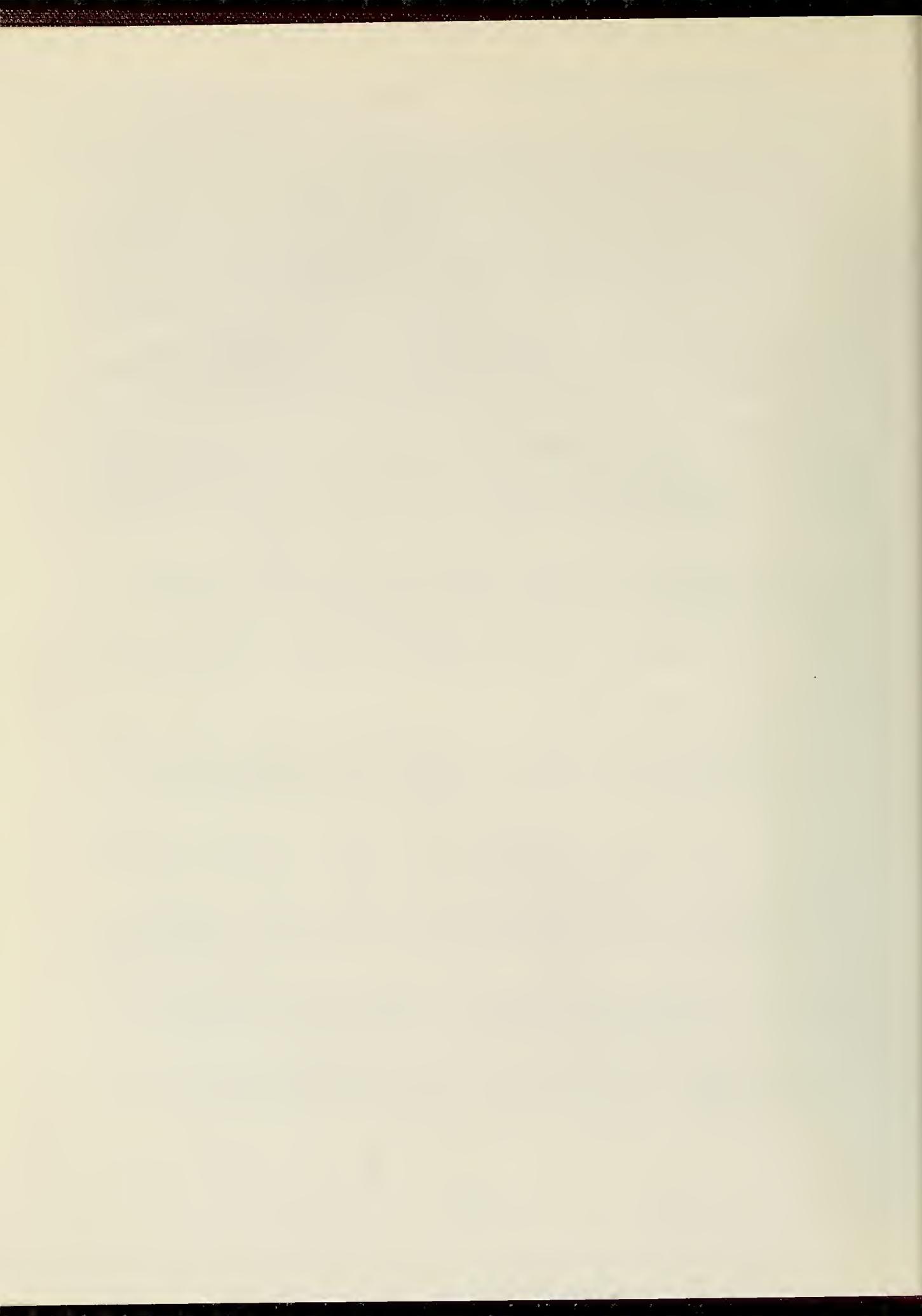
Gery I, Nussenblatt R, BenEzra D: Dissociation between humoral and cellular immune responses to lens antigens. Invest Ophthalmol Vis Sci 20:32-39, 1981.

Nussenblatt RB, Rodrigues MM, Wacker WB, Cevalario SJ, Salinas-Carmona MC, Gery I: Cyclosporin A: Inhibition of experimental autoimmune uveitis in Lewis rats. J Clin Invest 67:1228-1231, 1981.

Nussenblatt RB, Shichi H, Kuwabara T, Cevalario SJ, Gery I: Resemblance between rhodopsin kinase and S-antigen induced uveitis. Br J Ophthalmol (in press).

Nussenblatt RB, Rodrigues MM, Salinas-Carmona MC, Gery I, Cevalario S, Wacker W: Modulation of experimental autoimmune uveitis with cyclosporin A. Arch Ophthalmol (in press).

Gery I, Shichi H, Kuwabara T, Cevalario S, Nussenblatt RB: Rhodopsin kinase resembles S antigen in uveitogenicity and immunogenicity. Invest Ophthalmol Vis Sci 20(suppl):99, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE  
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF  
HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
NOTICE OF  
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 EY 00023-03 LVR

PERIOD COVERED

October 1, 1980, to September 30, 1981

TITLE OF PROJECT (80 characters or less)

Macrophage Interactions With Other Cells and Their Components

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:	John A. Barranger	M.D.	Chief, Clinical Section	DMNB	NINCDS
	J. Samuel Zigler, Jr.	Ph.D.	Senior Staff Fellow	LVR	NEI
	John A. Schmidt	M.D.	Research Associate	LI	NIAID
	Julia Derr	B.S.	Biologist	LVR	NEI

COOPERATING UNITS (if any)

Developmental and Metabolic Neurology Branch, NINCDS  
Laboratory of Immunology, NIAID

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Immunology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

0.6

OTHER:

0.9

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 are involved in a variety of processes, many of which affect the eye. The present studies have dealt with three macrophage interactions: (1) Macrophages produce and release a mediator, lymphocyte activating factor (or Interleukin 1), which is essential for stimulation of the immune system. The relationship between production of Interleukin 1 and the degree of differentiation of human monocytes to fully mature macrophages was examined. (2) Macrophages release also a mediator which stimulates fibroblasts and is believed to participate in processes of fibrosis and wound healing. The possible identity between this mediator and Interleukin 1 is suggested by data accumulated in this study, mainly from experiments using silica particles for macrophage stimulation. (3) Macrophages are a major component of the inflammatory infiltration and are known to adversely affect adjacent tissues. The possibility that macrophages may participate in cataract formation, a common feature of chronic uveitis, was examined by determining the cytotoxic effects of inflammatory macrophages on the metabolism of lens epithelial cell cultures.

403

Project Description:

Objectives: These studies focus on three types of macrophage (M $\phi$ ) interactions with other cells: (1) The production and release of a mediator, lymphocyte activating factor (or Interleukin 1), which stimulates certain lymphocyte responses (see Reports of FY 1979, 1980). The present study was aimed at monitoring the changes in Interleukin 1 production and release which follow the differentiation of human monocytes to fully mature M $\phi$ . (2) Another M $\phi$  product increases the metabolism and proliferation of fibroblasts and was suggested to be involved in certain fibrotic and wound healing processes. Studies concerning this mediator examined its possible common identity with Interleukin 1 and the release of both mediators by M $\phi$  incubated with a fibrosis-inducing agent, silica particles. (3) Cataract formation is a common feature of chronic uveitis, but its pathogenic mechanism is unknown. The present study was aimed at testing the hypothesis that inflammatory M $\phi$  may participate in cataract formation by damaging the lens; inflammatory M $\phi$  are known to carry cytotoxic capacity.

Methods Employed: Differentiation of monocytes to "mature M $\phi$ " was induced by culturing human peripheral blood monocytes for various periods in medium with autologous serum. Mediator activities were determined in supernatants or lysates of the monocyte monolayer cultures, or in their chromatographic (Sephacryl S-200) fractions. Interleukin 1 was assayed by its capacity to stimulate DNA synthesis (increased thymidine incorporation) in cultures of murine thymocytes, while the fibroblast activating factor was measured by its effect on the DNA synthesis of human skin fibroblasts (the latter assay was carried out by Dr. J.A. Schmidt, LI, NIAID). Inflammatory ("activated") M $\phi$  were collected from the peritoneal cavity of mice injected with Corynebacterium parvum or Bacillus Calmette Guerin (BCG). The damaging effects of these cells was determined according to their capacity to reduce the DNA synthesis of murine lens epithelial cells (a cell line kindly provided by Dr. P. Russell, LVR, NEI).

Major Findings: (1) Human monocytes that transform in culture to fully differentiated macrophages were clearly inferior to freshly cultured monocytes in their capacity to produce and release Interleukin 1. The transformed cells did not react at all to glucocerebroside (the storage lipid of Gaucher's disease) or silica particles and produced only low levels of intracellular Interleukin 1 when stimulated with lipopolysaccharide. (2) Human monocytes stimulated with silica particles produce and release high levels of both Interleukin 1 and the factor which stimulates DNA synthesis in fibroblasts. Furthermore, a complete correlation was found between the levels of these two biologic activities in different supernatants and lysates of the monocyte cultures, or their chromatographic fractions. (3) Activated M $\phi$  inhibited markedly the metabolic activity of lens epithelial cells. The inhibitory effect was counteracted in part by catalase and by indomethacin; superoxide dismutase had no protective effect.

Significance to Biomedical Research and the Program of the Institute:

The knowledge about M $\phi$  and their products is essential for a better understanding of processes in which M $\phi$  are actively involved and which affect ocular and other tissues. These processes include in particular the immune response, inflammation, fibrosis and wound healing. The data collected in this study shed new light on certain of the M $\phi$  activities.

(1) The finding of a partial loss of Interleukin 1 production during the transformation of monocytes to "mature" M $\phi$  presents a new feature of the differentiation process and may suggest that differentiated M $\phi$  have a smaller role than the monocytes in affecting the immune system by mediators. This notion is in line with previously reported results (FY 1980), that murine M $\phi$  activated in vivo (by bacteria) are inferior to "resident" M $\phi$  in their Interleukin 1 production.

(2) The results indicating that the fibroblast activating factor may be identical to Interleukin 1 suggest that the processes of fibrosis and wound healing are regulated by mechanisms similar to those which affect the immune response. The finding that silica particles stimulate the release of the fibroblast activating factor provides direct evidence for the hypothesis that M $\phi$ -made products stimulate the cellular processes of silicosis. It is noteworthy that silica particles induce granulomatous and fibrotic reactions in the eye, similar to the reactions in other tissues.

(3) The data showing the cytotoxic effect of activated M $\phi$  on lens epithelial cells support our working hypothesis, that these inflammatory cells may contribute to cataract formation in uveitic eyes. These results are also in accord with data reported by Dr. J.S. Zigler (see Report Z01 EY 00105-02 LVR) in which activated M $\phi$  damaged whole lenses in culture. The partial protective effects of indomethacin and catalase may suggest that metabolites of arachidonic acid and oxygen radicals may participate in the damaging processes.

Proposed Course: The role and mode of action of M $\phi$  products in mediating the immune response, granulomatous reaction, fibrosis and tissue damage will be further investigated. The use of specific drugs to block these processes will be further introduced in order to learn more about the processes and to form a possible basis for future treatment of certain pathologic conditions.

NEI Research Program: Retinal and Choroidal Diseases--Inflammatory Disorders; Corneal Diseases--Inflammation and Wound Healing; Cataract--Cataracts Induced by Environmental and Toxic Effects

Publications:

Gery I, O'Brien PJ: RCS rat macrophages exhibit normal ROS phagocytosis. Invest Ophthalmol Vis Sci 20:675-679, 1981.

Bonney RJ, Davies P, Staruch MJ, Gery I, Kuehl FA, Humes JL: Possible roles of prostaglandins synthesized and secreted by macrophages in regulating immune responses. Agents and Actions Suppl 7:3-9, 1980.

Ben-Zvi A, Rodrigues MM, Gery I, Schiffmann E: Induction of ocular inflammation by synthetic mediators. Arch Ophthalmol 99:1436-1444, 1981.

Gery I, Zigler JS Jr, Brady RO, Barranger JA: Selective effects of glucocerebroside (Gaucher's storage material) on macrophage cultures. J Clin Invest (in press).

Gery I, Seminara D, Derr J, Barranger JA: Production and release of lymphocyte activating factor (Interleukin 1) by human monocytes and their derived macrophages, in Resch K, Kirchner H (eds): Mechanisms of Lymphocyte Activation (Proceedings of the 14th Leucocyte Culture Conference). Amsterdam, Elsevier/North Holland (in press).

Gery I, Davies P, Derr J, Krett N, Barranger JA: Relationship between production and release of lymphocyte activating factor (Interleukin 1) by murine macrophages: I. Effects of various agents. Cell Immunol (in press)

Barranger JA, Gery I: Differences between resident and activated macrophages (M $\phi$ ) in production and release of lymphocyte activating factor (LAF, or Interleukin 1). Fed Proc 40:1057, 1981.

Zigler JS Jr, Gery I, Kessler D: Effects of activated macrophages on cultured lenses. Invest Ophthalmol Vis Sci 20(suppl):134, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00032-05 LVR																								
PERIOD COVERED October 1, 1980, to September 30, 1981																										
TITLE OF PROJECT (80 characters or less)  Role of Vitamin A in Maintenance and Development of Ocular Tissues																										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table border="0" data-bbox="78 472 1326 654"> <tr> <td>PI:</td> <td>Louvenia Carter-Dawson</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Toichiro Kuwabara</td> <td>M.D.</td> <td>Head, Section on Experimental Pathology</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>W.G. Robison</td> <td>Ph.D.</td> <td>Geneticist/Cell Biologist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>John G. Bieri</td> <td>Ph.D.</td> <td>Chief, Section on Nutritional Biochemistry</td> <td>LNE</td> <td>NIAMDD</td> </tr> </table>			PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI	Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI		W.G. Robison	Ph.D.	Geneticist/Cell Biologist	LVR	NEI		John G. Bieri	Ph.D.	Chief, Section on Nutritional Biochemistry	LNE	NIAMDD
PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR	NEI																					
Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR	NEI																					
	W.G. Robison	Ph.D.	Geneticist/Cell Biologist	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: 1.2	PROFESSIONAL: 1.2	OTHER: 0.0																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords)  A deficiency in <u>vitamin A</u> during <u>retinal development</u> results in failure of <u>hyaloid vessel regression</u> , abnormal arrangement of photoreceptor cells in <u>clusters (rosettes)</u> with other segments facing the center, focal <u>degeneration of photoreceptor cells</u> and incomplete organization of the retinal neurons into layers. This vitamin is not only essential for maintenance of mature retinal structure and function but also for normal retinal development.																										

Project Description:

Objectives: Vitamin A is essential for the maintenance of normal visual function and structure of mature ocular tissues. A deficiency in this vitamin results in poor or complete loss of vision, xerophthalmia and keratomalacia. Little is known about the role of vitamin A in developing ocular tissues and abnormalities which may result from vitamin A deficiency. This project was designed to investigate the effects of vitamin A deficiency on the development of ocular tissues.

Methods Employed: Pregnant mice were fed a vitamin A deficient diet beginning 7 to 8 days before delivery. The first and second generations were maintained on the deficient diet through 15 months. The degree of deficiency was determined by examining the serum and liver levels of vitamin A using high pressure liquid chromatography. Retinas were examined by light microscopy from the day of birth to 15 months.

Major Findings: Although the mice were fed a vitamin A deficient diet, trace amounts of the vitamin were detectable in the serum and liver of most animals. However, normal development of the retina was not supported in many mice. Abnormalities included failure of hyaloid vessel regression, rosette formation in the photoreceptor layer, focal degeneration of photoreceptor cells, migration of pigment epithelial cells into the neural retina, and incomplete separation of retinal layers.

Significance to Biomedical Research and the Program of the Institute: Many people, especially children, suffer from vitamin A deficiency in several less developed areas of the world. These children are often the offspring of mothers who have not received adequate levels of vitamin A during pregnancy. Precisely what abnormalities result from vitamin A deficiency during development are not clearly defined. However, studies which we are conducting on ocular tissues exposed to very low levels of vitamin A during development can provide useful information on the role of this vitamin in development. These studies can provide information instrumental in identifying human disorders which may involve defects associated with storage, utilization or uptake of this vitamin during the development of ocular tissues.

Proposed Course: Other ocular tissues will be examined and mice from a different genetic background, that appear to be more easily depleted of vitamin A will be deprived of the vitamin and the ocular tissues from the second generation will be examined for developmental abnormalities. Biochemical analyses of vitamin A receptors and glycoprotein synthesis in the ocular tissues of experimental and control animals will be made.

NEI Research Program: Retinal and Choroidal Diseases--Toxic, Nutritional, and Environmental Disorders

Publications:

Carter-Dawson L, Kuwabara T, Bieri JG: Effects of moderate-intensity light on vitamin-A deficient rat retinas. Invest Ophthalmol Vis Sci 20:569, 1981.

Project No. Z01 EY 00032-05 LVR

Carter-Dawson L, Robison WG, Kuwabara T: Differential sensitivity of Mouse "tapetal" retina to light. Invest Ophthalmol Vis Sci 20(suppl):80, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00149-08 LVR																								
PERIOD COVERED October 1, 1980, to September 30, 1981																										
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  <table border="0"> <tr> <td>PI:</td> <td>W. Gerald Robison, Jr.</td> <td>Ph.D.</td> <td>Geneticist/Cell Biologist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>Toichiro Kuwabara</td> <td>M.D.</td> <td>Head, Section on Experimental Pathology</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>John G. Bieri</td> <td>Ph.D.</td> <td>Chief, Section on Nutritional Biochemistry</td> <td>LNE</td> <td>NIAMDD</td> </tr> <tr> <td></td> <td>Stephen M. Sykes</td> <td>M.S.</td> <td>Biologist, Experimental Studies Branch, Division of Biological Effects</td> <td></td> <td></td> </tr> </table>			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		Stephen M. Sykes	M.S.	Biologist, Experimental Studies Branch, Division of Biological Effects		
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																					
	Stephen M. Sykes	M.S.	Biologist, Experimental Studies Branch, Division of Biological Effects																							
COOPERATING UNITS (if any) Laboratory of Nutrition and Endocrinology, NIAMDD Bureau of Radiological Health, FDA																										
LAB/BRANCH Laboratory of Vision Research																										
SECTION Section on Experimental Pathology																										
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																										
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.3	OTHER: 1.0																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords)  We found large (>10µm) <u>lipid droplets</u> with bright, <u>vitamin A-specific auto-fluorescence</u> in the <u>pigment epithelium</u> and other cells of the transition zone (" <u>ora serrata</u> ") between the peripheral retina and ciliary epithelium of mice and rats. In order to elucidate the structure and function of these droplets we utilized <u>fluorescence microscopy</u> , <u>histology</u> , and <u>electron microscopy</u> on eyes of animals under various experimental conditions. <u>C57BL/6J</u> mice injected intramuscularly with <u>retinyl acetate</u> in cumulative doses up to 94,000 IU showed increases in the number, size, and fluorescence of lipid droplets in the transition cells of the "ora serrata" region when compared to controls injected with <u>retinoic acid</u> or with the peanut oil solvent alone. Sprague-Dawley rats fed purified diets lacking <u>vitamin A</u> or supplemented with 0.23 to 23.0 mg/kg of <u>retinol</u> showed dose-related changes in the "ora serrata" lipid droplets. These droplets could represent significant storage sites for retinal vitamin A.																										

Project Description:

Objectives: To study the role of dietary vitamin A in visual function and the maintenance of the retina. This is part of our continued effort to examine what specific functions of the pigment epithelial cells are altered or lacking under various experimental and pathological conditions that might influence their ability to provide proper maintenance of the visual apparatus.

Methods Employed: We designed experiments to produce mice and rats with different levels of tissue vitamin A by injection and diet in order to determine the dynamics of vitamin A storage in the most peripheral part of the retina. Retinas were analyzed by fluorescence microscopy, histology and electron microscopy after a few weeks of vitamin A injection or after 6 and 8 months of vitamin A diet. Both color and black and white photographs were used in the analysis.

Major Findings: Large lipid droplets which show an autofluorescence specific for vitamin A change in number, size and amount of fluorescence according to the vitamin A which is available, suggesting that they may serve as storage sites for the vitamin A utilized by the retina.

Significance to Biomedical Research and the Program of the Institute: Vitamin A (retinol) has a central role in the visual process and undergoes dynamic exchange between the photoreceptor cells and the retinal pigment epithelium upon light adaptation and during the daily cycle. The pigment epithelium contains more than 90% of the vitamin A stores of the retina and these occur in the form of lipid droplets, as we showed previously. The present findings reveal an exceptionally large store of vitamin A in large lipid droplets which form a brightly fluorescent ring in the ora serrata region at the extreme periphery of the retina. Because the published biochemical studies on vitamin A determinations of the retina and pigment epithelium have not included this peripheral region, the reported amounts of total vitamin A in the retina and pigment epithelium must be significantly lower than the actual amounts. New biochemical determinations are being made by collaborators who are now trained in obtaining the entire retina for analysis.

Proposed Course: We plan to enter a new phase of studying the inter-relationships between photoreceptor cells and the pigment epithelium of the retina by attempts to reverse lipofuscin (aging pigment) formation in the retinal pigment epithelium by using the drug centrophenoxine which has been shown to decrease the amount of lipofuscin accumulated in the central nervous system and to restore functions lost by such accumulation.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Carter-Dawson L, Robison WG Jr, Kuwabara T: Differential sensitivity of the mouse "tapetal" retina to light. Invest Ophthalmol Vis Sci 20(suppl):80, 1981.

Robison WG Jr, Kuwabara T, Bieri JG: Large vitamin A droplets in "ora serrata" of rodent retinas. Invest Ophthalmol Vis Sci 20(suppl):76, 1981.

Robison WG Jr, Kuwabara T, Zwaan J: The mouse in biomedical research, in Foster HL, Small JP, Fox J (eds): Eye Research, Chapter 62. New York, Academic Press, Inc. (in press).

Russell P, Robison WG Jr, Kinoshita JH: A new method for rapid isolation of the intrinsic membrane proteins from lens. Exp Eye Res 32: 511-516, 1981.

Sykes SM, Robison WG Jr, Bieri JG: Retinal damage by cyclic light and the effect of vitamin E. Symposium on Biological Effects and Measurement of Light Sources, in Hazzard D (ed): DHHS (FDA) Publication 81-8156:86-99, Washington DC.

Sykes SM, Robison WG Jr, Waxler M, Kuwabara T: Damage to the monkey retina by broad spectrum fluorescent light. Invest Ophthalmol Vis Sci 20:425-434, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00066-04 LVR
--	--	---

PERIOD COVERED  
October 1, 1980, to September 30, 1981

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: Judith A. Evans Ph.D. Extramural Postdoctoral Fellow LVR NEI

COOPERATING UNITS (if any)  
  
Institute for Sensory Research, Syracuse University, Syracuse, N.Y.

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: 1.2	PROFESSIONAL: 1.2	OTHER: 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)

Studies are underway to identify chemical neurotransmitters in retinal neurons and other neurons in visual pathways, to examine the development of neurotransmitter systems in retina, and to determine the role of chemical neurotransmitters in processing visual information. Biochemical and anatomical techniques are used in these studies. Two systems are currently being investigated: (1) the relatively simple visual system of Limulus polyphemus and (2) developing mammalian retinal neurons in intact retinas and in monolayer cell culture. Work with the simple visual system has led to the first identification of an efferent chemical neurotransmitter probably involved in control of circadian changes in photoreceptor cell sensitivity and of circadian photoreceptor or cell membrane turnover. Studies of the intact developing mammalian retina have shown that some neurotransmitter development occurs very early in retinal development. Information on the development of the intact mammalian retina will form the basis for future studies of mechanisms controlling normal and abnormal development of retinas using the cell culture system.

Project Description:

Objectives: Superimposed upon the anatomical wiring diagram of visual systems is a complex neurochemical circuitry. Critical to an understanding of how the visual systems function is knowledge of (1) the identity of molecules involved in neurotransmission in the visual system (2) how the neurochemical circuitry is established during development and (3) how individual neurotransmitters function in the processing of visual information.

Methods Employed: High voltage electrophoresis and other chromatographic techniques are employed to study synthesis, accumulation and metabolism of putative neurotransmitters from radioactively labeled precursors. Sensitive enzymatic assays and high performance liquid chromatography coupled with electrochemical detection are used to measure endogenous levels of amines. Sites of synthesis and uptake of putative neurotransmitters are localized using light autoradiography and high resolution electron microscope autoradiography.

Major Findings: (1) Neurotransmitters in the visual system of Limulus polyphemus. Results of this study have led to identification of the biogenic amine octopamine as a likely efferent neurotransmitter to retinas. Although efferent innervation to retinas is common among both vertebrate and invertebrate species, our study represents the first identification of an efferent neurotransmitter candidate. Previous biochemical studies showed that octopamine was synthesized and present within each of the eyes of Limulus, and light autoradiographic studies revealed that octopamine was synthesized within a population of small efferent fibers that project from the CNS to ventral photoreceptor cells. More recent studies using high resolution electron microscope autoradiography confirmed the localization of octopamine to efferent fibers of both the ventral and lateral eyes of Limulus. Endings of octopamine-containing efferent fibers within ventral photoreceptor cells are closely associated with membranes that contain photosensitive pigment, thus release of octopamine could directly effect the response of these cells to light. In lateral eye, octopamine-containing efferent fibers contact pigment cells as well as photoreceptor cells. We hypothesize that octopamine released from efferents in lateral eye influences known circadian changes in photoreceptor cell sensitivity indirectly by modulating pigment cell migration and directly by affecting the photoreceptor cell itself. (2) Development of neurotransmitter function in mammalian retinal neurons. We found that rat retinal neurons grown in monolayer cell culture express the neuronal-specific function of neurotransmitter synthesis. In order to evaluate differentiation of rat retinal cells in culture, more information is needed on the normal development of transmitter function in intact retinas. Therefore, we have undertaken a series of studies of the normal development of neurotransmitter function in intact retinas. We began by investigating the development of cholinergic function. Acetylcholine is certainly a neurotransmitter in rat retina as well as in other mammalian retinas. Major increases in acetylcholine synthesis, choline acetyltransferase activity (choline acetyltransferase is the enzyme responsible for the synthesis of acetylcholine from choline) and high affinity choline uptake occur postnatally during a period of rapid synaptogenesis. However,

acetylcholine synthesis and choline acetyltransferase activity are also detected very early in retinal development (embryonic day 16). Electrophysiological studies (Puro, Z01 EY 00125-01 LVR) done in conjunction with these analyses revealed that acetylcholine is released from cholinergic neurons soon after synthesis was detected (embryonic day 18). Significance of the early development of cholinergic function in mammalian retinas is unclear, but it may be important in establishing proper connections among retinal neurons.

Significance to Biomedical Research and the Program of the Institute:

(1) Efferent innervation to retinas is common among both vertebrate and invertebrate species. In most animals the function of efferent fibers is unknown. In Limulus, however, there is good evidence that efferent innervation controls circadian changes in photoreceptor cell sensitivity, and in both Limulus and rat, photoreceptor cell membrane turnover is in part controlled by efferents. It seems clear from these examples that efferent innervation to retinas can influence photoreceptor cell metabolism, therefore the possibility should be explored that some retinal diseases involving photoreceptor cells may be caused by improper efferent innervation. Our identification of an efferent neurotransmitter candidate will allow detailed biochemical and electrophysiological studies of efferent control of photoreceptor cells. These studies should contribute significantly to an understanding of the requirements for normal photoreceptor cell function and possible causes of photoreceptor cell malfunction. (2) Proper processing of visual information by the adult retina requires establishment of appropriate anatomical and neurochemical connections during development. Studies of neurotransmitter systems in the normal developing mammalian retina, in addition to revealing fundamental features of neuronal differentiation in a healthy tissue, lay the necessary ground work for future studies of possible environmental and genetic influences on retinal development.

Proposed Course: (1) Studies of octopamine-containing efferent fibers in Limulus will continue. Special emphasis will be given to elucidating mechanisms of octopamine release and the pharmacology of octopamine effects on photoreceptor cells. An effort will be made to localize the cells of origin of efferent fibers and determine their connectivity with the rest of the Limulus visual system. We also anticipate beginning studies of the neurochemistry of efferent fibers in rat retinas. (2) With the analysis of cholinergic development in intact rat retinas completed, we are turning attention to the development of the GABAergic and dopaminergic systems. GABA and dopamine synthesis in developing rat retinas will be measured along with changes in dopamine content and tyrosine hydroxylase activity. The effect of light deprivation on development of neurotransmitter systems will be examined along with possible circadian changes in neurotransmitter synthetic activity.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Organization, Neurotransmission, and Adaptation.

Publications:

Battelle BA, LaVail MM: Protein synthesis in retinas of rats with inherited retinal dystrophy. Exp Eye Res 31:251-269, 1980.

Battelle BA: Neurotransmitter candidates in the visual system of Limulus polyphemus: Synthesis and distribution of octopamine. Vision Res 20: 911-922, 1980.

Battelle BA, Chamberlain SC: Autoradiographic localization of sites of octopamine synthesis in Limulus ventral eye. Society for Neuroscience Abstracts 6:702, 1980.

Puro DG, Battelle BA: Development of cholinergic function in rat retina. Invest Ophthalmol and Vis Sci 20(suppl):188, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00124-01 LVR																									
PERIOD COVERED October 1, 1980, to September 30, 1981																											
TITLE OF PROJECT (80 characters or less)  Metabolism of the Retina and Pigment Epithelium																											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width:100%; border: none;"> <tr> <td style="width:10%;">PI:</td> <td style="width:35%;">Gerald J. Chader</td> <td style="width:15%;">Ph.D.</td> <td style="width:20%;">Research Chemist</td> <td style="width:20%;">LVR NEI</td> </tr> <tr> <td></td> <td>Eileen Masterson</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>Elizabeth Barbehenn</td> <td>Ph.D.</td> <td>Expert</td> <td>LVR NEI</td> </tr> <tr> <td></td> <td>Shay-Whey Margaret Koh</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR NEI</td> </tr> <tr> <td>Other:</td> <td colspan="4">None</td> </tr> </table>			PI:	Gerald J. Chader	Ph.D.	Research Chemist	LVR NEI		Eileen Masterson	Ph.D.	Staff Fellow	LVR NEI		Elizabeth Barbehenn	Ph.D.	Expert	LVR NEI		Shay-Whey Margaret Koh	Ph.D.	Staff Fellow	LVR NEI	Other:	None			
PI:	Gerald J. Chader	Ph.D.	Research Chemist	LVR NEI																							
	Eileen Masterson	Ph.D.	Staff Fellow	LVR NEI																							
	Elizabeth Barbehenn	Ph.D.	Expert	LVR NEI																							
	Shay-Whey Margaret Koh	Ph.D.	Staff Fellow	LVR NEI																							
Other:	None																										
COOPERATING UNITS (if any)  Laboratory of Neurochemistry, NINCDS																											
LAB/BRANCH Laboratory of Vision Research																											
SECTION Section on Retinal and Corneal Metabolism																											
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, MD 20205																											
TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0.0																									
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline keywords) General <u>metabolism</u> of the <u>retina</u> and <u>pigment epithelium</u> was studied in cultured cells and in freshly dissected tissues. <u>Glucose uptake</u> was found to be by a facilitated diffusion mechanism. <u>Phagocytosis</u> was dependent on the continuous production of ATP via the TCA cycle. Glucose is the predominant energy source for cultured PE cells although <u>glutamine</u> can be used to maintain cells in a stable, non-dividing state and thus can probably function as an important alternative energy source in vivo.																											

Project Description:

Objectives: To better understand the general metabolism of the retinal pigment epithelial unit and to apply this information to diseases which affect these tissues.

Methods Employed: PE and retinal cell cultures were maintained from tissues of the chick embryo. When appropriate, fresh tissues were dissected from the eye using a stereomicroscope. Biochemical analyses were performed using standard assay procedures from the literature as adapted in our laboratory for the particular ocular tissue.

Major Findings: 1) The growth characteristics and biochemical differentiation of PE cells were found to be different in different growth media. In standard Eagle's MEM, cells grew slowly, differentiated well morphologically and demonstrated a specific intracellular receptor for vitamin A (retinol). In Ham's F-12 medium, cell proliferation was greatly enhanced but the cells appeared to lose some differentiated characteristics including the vitamin A receptor. 2) Chick PE cells in culture were found to require glucose as their major energy source for long term growth; pigment formation and colony organization. The cells, however, can adequately be maintained on glutamine under conditions of low glucose or starvation. During periods of energy starvation, phosphocreatine levels fell dramatically but the ATP level is maintained at a relatively constant level. The combination of glucose plus glutamine is required to reverse this drop as indicated by the phosphocreatine levels measured five minutes after refeeding. The ATP/P-creatine ratio therefore becomes a useful measure of the energy state of the cell.

Significance to Biomedical Research and the Program of the Institute: The pigment epithelium is an important cell layer which acts as a partner with the retinal outer segments in the visual process. Understanding the basic factors which promote differentiation and the basic factors which control PE cell energy metabolism should aid us in better understanding dysfunction of the PE-retina unit.

Proposed Course: The metabolic capabilities of the PE cell will be further investigated. Correlations with specific diseases of the PE-retina unit will be made.

NEI Research Program: Retinal and Choroidal Diseases--Retinal Pigment Epithelium

Publications:

Israel P, Masterson E, Goldman AI, Wiggert B, Chader GJ: Retinal epithelial cell differentiation: Influence of culture medium in vitro. Invest Ophthalmol Vis Sci 19:720-727, 1980.

Masterson E, Goldman AI, Chader GJ: Phagocytosis of rod outer segments by cultured epithelial cells. Vision Res 21:143-145, 1981.

Masterson E, Chader GJ: Pigment epithelial cells in culture: Metabolic pathways required for phagocytosis. Invest Ophthalmol Vis Sci 20:1-7, 1981.

Masterson E, Chader GJ: Characterization of glucose transport by cultured chick pigmented epithelium. Exp Eye Res 32:279-289, 1981.

Tamai M, Mizuno K, Chader, G: In vitro studies on shedding and phagocytosis of rod outer segments in the rat retina: Effects of oxygen concentration. Invest. Ophthalmol Vis Sci (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 EY 00041-03 LVR												
PERIOD COVERED October 1, 1980, to September 30, 1981														
TITLE OF PROJECT (80 characters or less)  Retina Lipid Metabolism: Correlation with a Circadian Rhythm and Effect of Light														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT  <table style="width:100%; border: none;"> <tr> <td style="width:15%;">PI:</td> <td style="width:30%;">Peter Dudley</td> <td style="width:15%;">Ph.D.</td> <td style="width:20%;">Staff Fellow</td> <td style="width:10%;">LVR</td> <td style="width:10%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Paul J. O'Brien</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Peter Dudley	Ph.D.	Staff Fellow	LVR	NEI	Other:	Paul J. O'Brien	Ph.D.	Research Chemist	LVR	NEI
PI:	Peter Dudley	Ph.D.	Staff Fellow	LVR	NEI									
Other:	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: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) The shedding of <u>rat rod outer segment</u> (ROS) discs, and the process of phagocytosis, is a <u>circadian</u> phenomenon characterized morphologically by the engulfment of ROS discs by the <u>pigment epithelium</u> . This process occurs over a period of a few hours beginning two hours after the start of the light period. The synthesis of ROS disc <u>membrane phospholipids</u> was also found to follow a circadian rhythm, being elevated during the daylight hours and depressed during the night. This rhythm persisted after three days of constant darkness as well as under constant light conditions. <u>Phosphatidyl inositol</u> exhibited an additional increase in synthesis in response to light at any time of the day or night.														

Project Description:

Objectives: Biochemical events associated with circadian shedding of vertebrate photoreceptor disc membranes have not been documented. The objectives of this project are (1) to determine if labeling of retina membrane phospholipids in the rat occurs at a specific time on a daily basis, (2) to ascertain whether this is a circadian phenomenon, and (3) to determine the relationship of membrane synthesis to disc shedding.

Methods Employed: Ordinary biochemical techniques were employed such as incubation of retinas, extraction of lipids and separation of neutral and polar lipids by thin layer chromatography.

Major Findings: Rat retinas incubated in vitro with (<sup>3</sup>H)-glycerol exhibited elevated rod outer segment (ROS) phospholipid synthesis during the daylight hours when the animals had been maintained on a 7 a.m. to 7 p.m. light schedule. Rates of synthesis were three fold lower at night. This diurnal rhythm persisted after three days in constant darkness or under constant light conditions and is thus a circadian rhythm. In addition, phosphatidyl inositol synthesis was stimulated by light during the day or night.

Significance to Biomedical Research and the Program of the Institute: Specific biochemical events associated with the circadian shedding of photoreceptor membranes have not been demonstrated. It is known that the circadian oscillator that controls shedding is insensitive to constant light. Thus the ROS phospholipid synthetic rhythm could qualify as the heretofore unknown oscillator. Furthermore, light-stimulated phosphatidyl inositol synthesis implicates this lipid in the process of transduction.

Proposed Course: This project will be terminated with some aspects carried over into the Cell Biology of the Vertebrate Retina.

NEI Research Program: Retina and Choroidal Diseases--Photoreceptors, Visual Pigments, and Transduction.

Publications:

Dudley PA, O'Brien PJ: Circadian synthesis of rat retina membrane phospholipids. Invest Ophthalmol Vis Sci 20(suppl):4, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00148-08 LVR																		
PERIOD COVERED October 1, 1980, to September 30, 1981																				
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  <table border="0"> <tr> <td>PI:</td> <td>Y.P. Liu</td> <td>Ph.D.</td> <td>Staff Fellow</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>G.J. Chader</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td>Other:</td> <td>R.T. Fletcher</td> <td>M.S.</td> <td>Chemist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Y.P. Liu	Ph.D.	Staff Fellow	LVR	NEI		G.J. Chader	Ph.D.	Research Chemist	LVR	NEI	Other:	R.T. Fletcher	M.S.	Chemist	LVR	NEI
PI:	Y.P. Liu	Ph.D.	Staff Fellow	LVR	NEI															
	G.J. Chader	Ph.D.	Research Chemist	LVR	NEI															
Other:	R.T. Fletcher	M.S.	Chemist	LVR	NEI															
COOPERATING UNITS (if any) <table border="0"> <tr> <td>1) Lab. Chem. Pharmacology NHLBI</td> <td>3) Dept. of Pathology</td> </tr> <tr> <td>2) Section Ophthalmology School Vet. Medicine Univ. Penn., Philadelphia PA</td> <td>Yale Medical School New Haven, CT</td> </tr> </table>			1) Lab. Chem. Pharmacology NHLBI	3) Dept. of Pathology	2) Section Ophthalmology School Vet. Medicine Univ. Penn., Philadelphia PA	Yale Medical School New Haven, CT														
1) Lab. Chem. Pharmacology NHLBI	3) Dept. of Pathology																			
2) Section Ophthalmology School Vet. Medicine Univ. Penn., Philadelphia PA	Yale Medical School New Haven, CT																			
LAB/BRANCH Laboratory of Vision Research																				
SECTION Section on Retinal & Corneal Metabolism																				
INSTITUTE AND LOCATION National Eye Institute, NIH, Bethesda, Maryland 20205																				
TOTAL MANYEARS: 1.75	PROFESSIONAL: 0.75	OTHER: 1.0																		
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input checked="" type="checkbox"/> (b) HUMAN TISSUES <input type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords) 1) Studies on <u>cyclic GMP phosphodiesterase (PDE)</u> in human, dog, cow and rat show that the enzyme is normally independent of <u>calcium</u> and <u>calmodulin</u> . In the <u>retinas</u> of <u>Irish Setter dogs</u> affected with rapid onset <u>retinal degeneration</u> , however, the PDE enzyme is calcium-dependent; also the PDE activity and the calmodulin concentration are abnormally low. Results obtained from intraocular injection of purified bovine brain calmodulin in affected Irish Setter dogs showed higher levels of PDE and calmodulin in some retinas injected with calmodulin than in the control retinas. 2) Biochemical studies of the inter-cellular fluids of the subretinal and subchoroidal spaces of the rabbit eye were also performed to begin to investigate possible enzyme activities in these important fluids. The subretinal fluid of the retinal fraction demonstrated high cyclic GMP PDE and calmodulin activities that did not appear to result from serum or tissue contamination.																				

Project Description:

Objectives: 1) To study the role of PDE and calmodulin in normal and diseased eye tissues and to explore possible vehicles for the delivery of calmodulin into the retina of the dog. 2) To elucidate the biochemical activities and metabolic events associated with the subretinal and subchoroidal fluids.

Methods Employed: Cyclic nucleotide PDE and calmodulin are assayed with radiolabeled substrates and ion-exchange resins. Retinas from humans, dogs, cows and rats are dissected by conventional techniques. Fluids from the subretinal and subchoroidal spaces are collected in the laboratory of Dr. Y. Lai by gentle irrigation and aspiration of the appropriate tissue surfaces. Sucrose density gradient ultracentrifugation was performed to isolate photoreceptor units.

Major Findings: 1) With the availability of Irish Setter dogs with retinal degeneration, intraocular injections were performed in hopes of delivering calmodulin into the retina. Results obtained from our preliminary experiments showed higher levels of calmodulin in the retinas of dogs injected with calmodulin, indicating that calmodulin may actually be transported from the vitreous humor into the retina. Some retinas of injected eyes showed lowered cyclic GMP concentration indicating that the calmodulin injection in some cases was efficacious. Other than the retinas of Irish Setter dogs with early onset retinal dysplasia, retinas of other dog types and of all species tested including man demonstrate a calcium-independent type of PDE. The affected dog retinas therefore appear to be unique in that they demonstrate both low PDE activity and calmodulin concentration as well as an altered PDE enzymatic type.

2) Subretinal fluids and washings of the subretinal and subchoroidal spaces were examined for PDE activity and for the presence of calmodulin. The fluid of the subretinal space demonstrated the highest cyclic GMP PDE activity and calmodulin concentration. The subchoroidal fluid also exhibited substantial activity. Lower PDE activity and calmodulin was observed in washings of the PE cell surface and the sub-pigment epithelial space. Control experiments with serum and homogenized tissue preparations indicate that these activities are not artifacts of tissue breakdown and disruption.

Significance to Biomedical Research and the Program of the Institute:

1) With the possibility of delivering calmodulin into the retina by intraocular injections, the exogenous calmodulin may be able to activate the retina PDE, to hydrolyze cyclic GMP and to slow down or even stop the degenerative progress of the disease in affected retinas.

2) The development of new biochemical research approaches and techniques in the area of the subretinal and subchoroidal space has resulted in the identification of cyclic nucleotide phosphodiesterase and calmodulin in these areas. The development of this technique and the resulting findings will further our knowledge of the interaction of retina and PE and also possibly of the alterations which occur in the permeability of the retina-blood barrier in retinal disorders.

Proposed Course: (1) Studies on intraocular injections of calmodulin into retinas of Irish Setter dogs with retinal degeneration will continue. (2) The characterization of cyclic nucleotide phosphodiesterases and calmodulin associated with the subretinal and subchoroidal fluids will be continued with light-adapted and dark-adapted eyes to assess possible differences under differing physiological conditions.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Newsome D, Fletcher RT, Chader GJ: Cyclic nucleotides vary by area in the retina and pigmented epithelium of the human and monkey. Invest Ophthalmol Vis Sci 19:864-869, 1980.

Chader GJ, Fletcher RT, Russell P, Krishna G: Differential control of protein kinase activities of the retinal photoreceptor: cation effects on phosphorylation by ATP and GTP. Biochemistry 19:2634-2638, 1980.

Chader GJ, Fletcher RT, Krishna G: Guanine nucleotides: Importance in visual process of the rod outer segment, in Sears, M (ed): New Directions in Ophthalmic Research. New Haven, Yale Univ Press (in press).

Chader GJ, Liu YP, Fletcher RT, Aguirre GA, Santos-Anderson R, T'so MOM: Cyclic GMP phosphodiesterase and calmodulin in early onset inherited retinal degenerations, in Miller W (ed) Current Topics in Membranes and Transport. New York, Academic Press (in press).

Liu YP, Cheung WY: Cyclic nucleotide phosphodiesterases. Molecular and Cellular Biochemistry (in press).

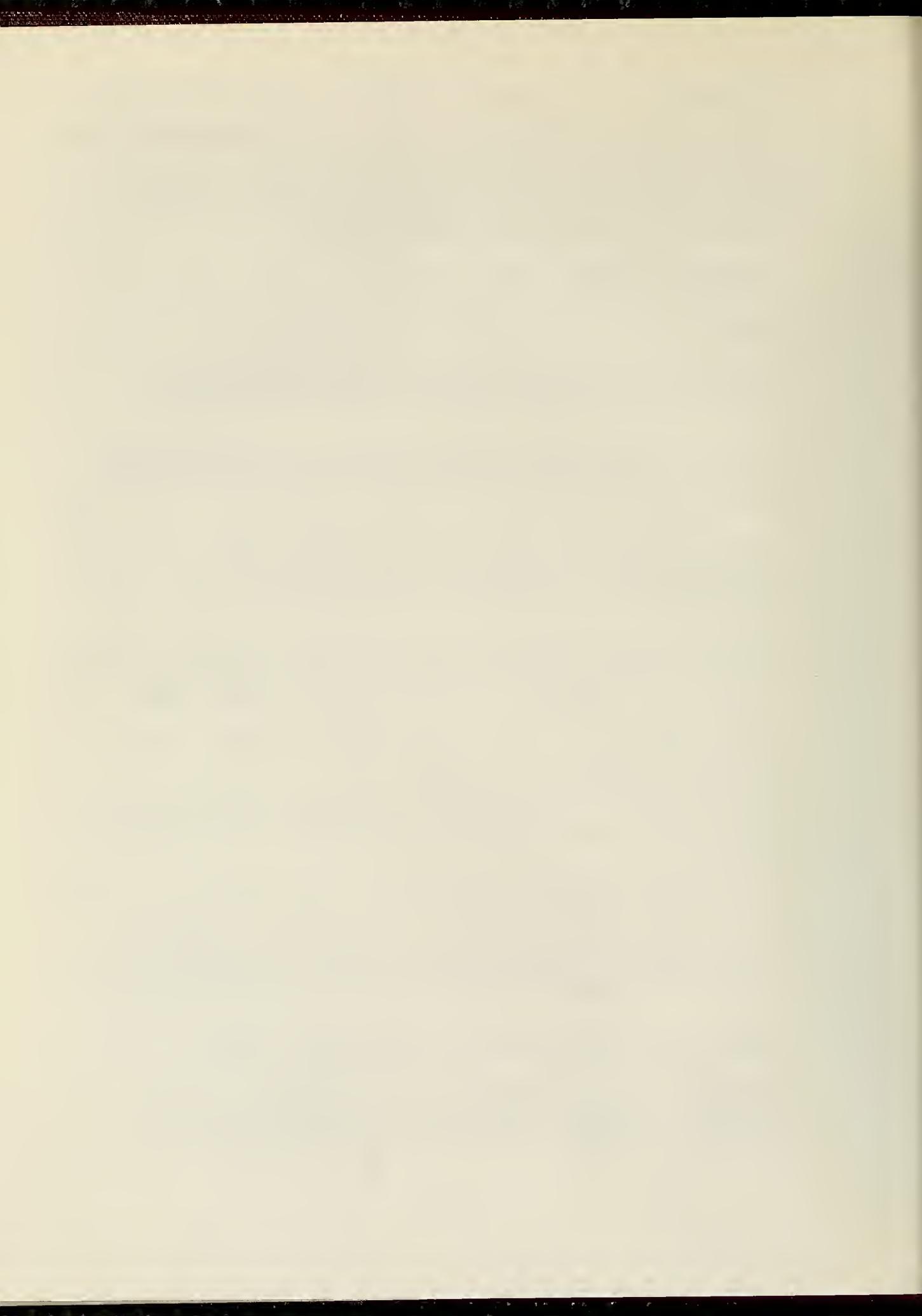
Liu YP, Chabner BA: L-Asparaginase, in Chabner BA (ed): The Clinical Pharmacology of Anti-tumor Drugs. Philadelphia WB Saunders Co (in press).

Lai YL, Masuda K, Hayasaka S, Suzuyama Y, Lin TC, Lug R, Liu YP: Study of subretinal intercellular space. Exp Eye Res (in press).

Lai YL, Lug R, Masuda K, Liu YP: Mechanism and significance of photoreceptor cell loss in the Fischer rat retina, in Hollyfield J (ed): Structure of the Eye. New York, Elsevier North Holland (in press).

Lai, YL, Lug R, Masuda K, Liu YP: Subretinal displacement of photoreceptor nuclei in human retina. Exp Eye Res (in press).

Woodford BJ, Liu YP, Fletcher RT, Chader GJ, Farber D, Santos-Anderson R, T'so MOM: Cyclic nucleotide metabolism in inherited retinopathy in collies: A biochemical and histochemical study. Exp Eye Res (in press).



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00016-14 LVR
--	--	---

PERIOD COVERED  
October 1, 1980, to September 30, 1981

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
	Igal Gery	Ph.D.	Visiting Scientist	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: 0.6	PROFESSIONAL: 0.3	OTHER: 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)

Opsin synthesis was measured in miniature poodles affected with progressive rod-cone degeneration. Photoreceptors develop normally but begin to degenerate after the dog is fully grown. At all ages studied the rate of rod outer segment renewal was about half the normal value. Opsin synthesis, however, occurred at the normal rate until advanced stages of the disease when photoreceptor cell death was apparent. Thus the defect may involve photoreceptor membrane assembly rather than synthesis.

Peritoneal macrophages from RCS rats exhibited normal phagocytic capabilities toward bovine or rat rod outer segments as well as toward RCS retinal debris. Thus the genetic defect is expressed in the RCS pigment epithelium but not in all phagocytic cells.

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 examine biochemical events unique to the retina, especially the synthesis of photoreceptor membrane components, in the retinas of vertebrates which can be affected by inherited retinal degenerations.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of peritoneal macrophages, cell fractionation, isolation of rod outer segments by density gradient centrifugation, and SDS gel electrophoresis.

Major Findings: The synthesis of membrane proteins, particularly opsin, was found to occur at an identical rate in 14 month old normal miniature poodles and in littermates affected with progressive rod-cone degeneration, an inherited disease. The rate of rod outer segment renewal, revealed by autoradiography following intravitreal injection of labeled leucine, was half the normal value. Only when photoreceptor cell death was apparent did the synthesis of opsin begin to decrease.

Peritoneal macrophages from RCS or normal rats phagocytized bovine or rat rod outer segments as well as RCS retinal debris at identical rates.

Significance to Biomedical Research and the Program of the Institute:

The reduced rate of rod outer segment renewal in the affected miniature poodle is not the result of reduced membrane synthesis since opsin synthesis occurs at a normal rate. Thus it is possible that the assembly of membranes may be defective. This represents a new class of degenerative disorders which closely mimics retinitis pigmentosa in humans.

In the RCS rat, the genetic defect is known to reside in the pigment epithelium. Since other phagocytic cells are not affected, the lesion very likely involves a unique protein in the retina or pigment epithelium. Identification of this protein will provide a probe to study human retinal degenerations.

Proposed Course: Both Irish setters and miniature poodles with inherited retinal degenerations will be studied further to search for specific biochemical defects in the assembly of photoreceptor membrane components. Plasma membrane proteins will be studied in pigment epithelium from normal and RCS rats in search of a missing or defective receptor protein involved in recognition and phagocytosis of rod outer segments.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Gery I, O'Brien PJ: RCS rat macrophages exhibit normal ROS phagocytosis. Invest Ophthalmol Vis Sci 20:675-679, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE <b>NOTICE OF INTRAMURAL RESEARCH PROJECT</b>	PROJECT NUMBER  Z01 EY 00015-16 LVR	
PERIOD COVERED October 1, 1980, to September 30, 1981			
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
	Nancy Philp	Ph.D.      Post-Doctoral Fellow	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:	
2.0	1.3	0.7	
CHECK APPROPRIATE BOX(ES)			
<input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER			
<input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS			
SUMMARY OF WORK (200 words or less - underline keywords)			
Biochemical correlates to <u>circadian photoreceptor outer segment shedding</u> in rats were sought. Opsin synthesis as a function of the ambient light cycle was followed by studying radioactive <u>leucine</u> and <u>glucosamine</u> incorporation in vitro. Neither precursor exhibited any diurnal rhythm of incorporation into opsin, eliminating opsin synthesis and glycosylation as elements contributing to circadian rhythms in photoreceptors.			
<u>Iodination</u> of bovine <u>pigment epithelium</u> followed by <u>SDS gel electrophoresis</u> revealed a reproducible pattern of plasma membrane proteins, some of which bound to mannose affinity columns. Thus tissue lectins could mediate outer segment recognition and binding in preparation for phagocytosis by the pigment epithelium.			

Project Description:

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. This project was designed to determine where and when sugars are added to rhodopsin 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. In addition, biochemical correlates to circadian photoreceptor shedding will be sought, particularly in relation to glycoprotein synthesis and function. Finally, specific carbohydrate receptors will be sought on pigment epithelial microvilli which could mediate interactions with photoreceptors.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas and pigment epithelium with radioactive precursors, cell fractionation, SDS gel electrophoresis, scintillation and gamma counting, and autoradiography.

Major Findings: Radioactive leucine and glucosamine were incorporated into opsin in rat retinas incubated in vitro at various times throughout a normal 12 hour light and 12 hour dark cycle. The rate of opsin labeling with either precursor was constant throughout the 24 hour period. Thus, opsin synthesis and glycosylation do not appear to undergo any circadian rhythms such as photoreceptor shedding does. Iodination of bovine pigment epithelium revealed a reproducible pattern of plasma membrane proteins, some of which could be retained on mannose affinity columns.

Significance to Biomedical Research and the Program of the Institute: There are two known prerequisites to circadian photoreceptor shedding in the rat retina: (1) at least two hours of darkness and (2) approximately a 24 hour interval between shedding events. It is presumed that this reflects two hours of light-sensitive reactions and 24 hours of preparatory, possible biosynthetic, reactions. These results suggest that opsin synthesis could represent the 24 hour interval requirement for shedding but is probably not involved in the two hours of light-sensitive reactions.

The presence of mannose-specific binding proteins on the pigment epithelial plasma membrane suggests a mechanism for the recognition and binding of rod outer segment membranes prior to phagocytosis. Rhodopsin in these membranes contains mannose along with other sugars. Identification of mechanisms such as this provides an opportunity to examine potential sites of specific lesions in retinas with inherited degenerative disorders.

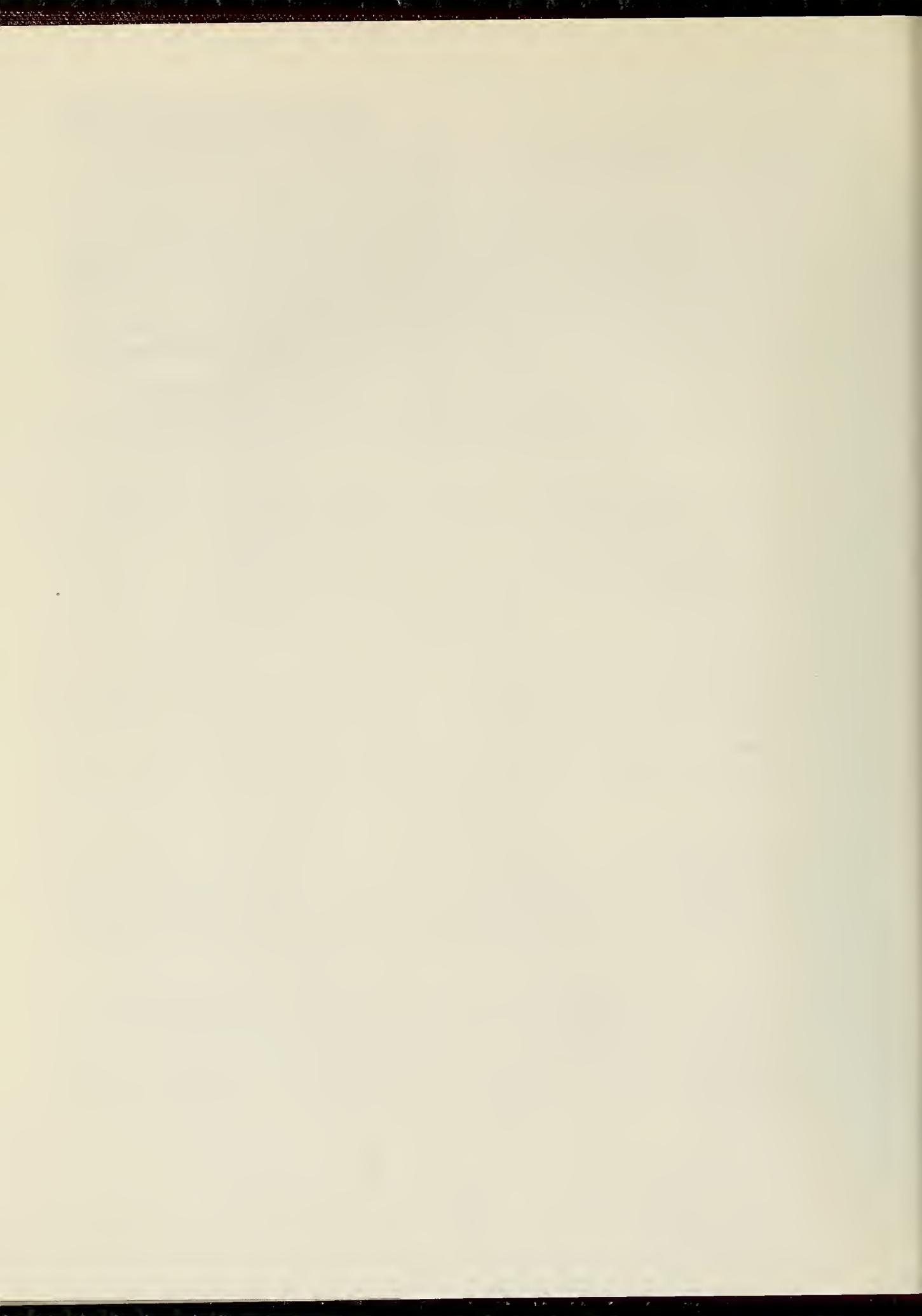
Proposed Course: Modifications of newly synthesized opsin such as the addition of galactose or fucose residues will be studied in a search for membrane assembly markers or evidence of circadian rhythms. The appearance of specific receptor proteins in plasma membranes of the pigment epithelium will be followed as a function of developmental age. This will be correlated with known changes in the ability of the pigment epithelium to phagocytize rod outer segments.

NEI Research Program: Retinal and Choroidal Diseases--Photoreceptors, Visual Pigments, and Transduction.

Publications:

Goldman AI, Teirstein PS, O'Brien PJ: The role of ambient lighting in circadian disc shedding in the rod outer segment of the rat retina. Invest Ophthalmol Vis Sci 19:1257-1267, 1980.

Teirstein PS, Goldman AI, O'Brien PJ: Evidence for both local and central regulation of rat rod outer segment disc shedding. Invest Ophthalmol Vis Sci 19:1268-1273, 1980.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER  Z01 EY 00070-04 LVR																								
PERIOD COVERED October 1, 1980, to September 30, 1981																										
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  <table style="width:100%; border: none;"> <tr> <td style="width:15%;">PI:</td> <td style="width:35%;">Barbara Wiggert</td> <td style="width:15%;">Ph.D.</td> <td style="width:20%;">Research Chemist</td> <td style="width:10%;">LVR</td> <td style="width:5%;">NEI</td> </tr> <tr> <td>Other:</td> <td>Ling Lee</td> <td>M.S.</td> <td>Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Paul Russell</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> <tr> <td></td> <td>Gerald J. Chader</td> <td>Ph.D.</td> <td>Research Chemist</td> <td>LVR</td> <td>NEI</td> </tr> </table>			PI:	Barbara Wiggert	Ph.D.	Research Chemist	LVR	NEI	Other:	Ling Lee	M.S.	Chemist	LVR	NEI		Paul Russell	Ph.D.	Research Chemist	LVR	NEI		Gerald J. Chader	Ph.D.	Research Chemist	LVR	NEI
PI:	Barbara Wiggert	Ph.D.	Research Chemist	LVR	NEI																					
Other:	Ling Lee	M.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) Yale University Medical School Department of Ophthalmology, the Medical College of Wisconsin																										
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: 2.4	PROFESSIONAL: 1.4	OTHER: 1.0																								
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) HUMAN SUBJECTS <input type="checkbox"/> (b) HUMAN TISSUES <input checked="" type="checkbox"/> (c) NEITHER  <input type="checkbox"/> (a1) MINORS <input type="checkbox"/> (a2) INTERVIEWS																										
SUMMARY OF WORK (200 words or less - underline keywords) 1) The <u>7S retinol-binding protein</u> previously detected in <u>outer segments</u> was present in saline washes of <u>interphotoreceptor (IP)</u> and <u>RPE surfaces</u> . <u>CRBP</u> was also present in these washes as well as in washes of the <u>suprachoroidal space</u> .  2) ( <sup>3</sup> H)- <u>Retinol binding to CRBP</u> was markedly reduced in <u>corneal and conjunctival epithelia</u> of <u>vitamin A-deficient</u> as compared with normal or control rabbits. ( <sup>3</sup> H)- <u>retinoic acid binding to CRABP</u> was greater in <u>corneal and conjunctival epithelia</u> from <u>vitamin A-deficient rabbits</u> .  3) A low molecular weight (14,800) <u>protein</u> which specifically binds ( <sup>3</sup> H)- <u>arachidonic acid</u> was found in chick embryo <u>retina cytosol</u> .																										

Project Description:

Objectives: To elucidate the mechanism of action of retinoids in ocular tissues.

Methods Employed: Sucrose density gradient centrifugation, gel filtration, thin layer chromatography, SDS-polyacrylamide gel electrophoresis, preparative isoelectric focusing, and isoelectric focusing on thin layer polyacrylamide gels were employed in studies of cellular retinoid-binding proteins, fatty acid-binding proteins, and vitamin A metabolism.

Major Findings:

1) Distribution of retinoid-binding proteins in rabbit retina

A study was made of the distribution of retinoid-binding proteins in 5 compartments of the rabbit retina: 1) cytosol of homogenized neural retinal tissue 2) saline washes of the interphotoreceptor (IP) space 3) washes of the apical RPE surface 4) cytosol of homogenized RPE-choroidal tissue 5) washes of the suprachoroidal space. Cellular Retinol-Binding Protein (CRBP) was present in all five compartments. The 7S retinol-binding protein previously detected in outer segments was present in washes of IP and RPE surfaces. Very little cellular Retinoic Acid-Binding Protein (CRABP) was detected in the washes.

2) Vitamin A-Deficient Rabbit Tissues

(<sup>3</sup>H)-Retinol binding to CRABP was reduced by 55-60 percent in corneal epithelium and by 80-85 percent in conjunctival epithelium of vitamin A-deficient as compared with normal or control rabbits. There was no reduction of (<sup>3</sup>H)-retinol binding to CRBP in retina, brain or liver of vitamin A-deficient animals. There was an apparent increase in (<sup>3</sup>H)-retinoic acid binding to CRABP in corneal and conjunctival epithelia from vitamin A-deficient rabbits.

3) Fatty Acid Binding

A protein which specifically binds (<sup>3</sup>H)-arachidonic acid was found to be present in chick embryo retina and adult bovine retina cytosol. This protein sediments at 2S on 5-20 percent sucrose gradients but is separable from CRBP and CRABP by means of isoelectric focusing. SDS gel electrophoresis following preparative isoelectric focusing and gel filtration revealed a major protein band of approximately 14,800 molecular weight. Specific (<sup>3</sup>H)-arachidonic acid binding was found to be present in developing chick embryo retina cytosol at all stages examined from 8 through 16 days of incubation, with the highest levels being observed at the earlier stages. Specific (<sup>3</sup>H)-arachidonic acid binding was also present in the cytosol of chick pigment epithelium-choroid, brain and heart.

Significance to Biomedical Research and the Program of the Institute:

Vitamin A is necessary for the normal growth and differentiation of most tissues. In addition, it also plays a specialized role in the visual process. Thus, a better understanding of its mechanism of action in ocular tissues is of importance not only in determining how ocular tissues such as the retina function normally but also in the effort to prevent or treat ocular diseases involving vitamin A metabolism.

Proposed Course: Further studies are planned on the role of cellular retinoid-binding proteins and on the metabolism of retinoids in both fresh ocular tissues and in cells grown in tissue culture.

NEI Research Program: Retinal and Choroidal Diseases--Developmental and Hereditary Disorders

Publications:

Wiggert B, Chader GJ: Cytosol binding of retinyl palmitate and palmitic acid in pigment epithelium and retina. Proc NY Acad Sci 359:427-428, 1981.

Chader GJ, Wiggert B, Russell P, Tanaka M: Retinoid binding proteins of retina and retinoblastoma cells in culture. Proc NY Acad Sci 359:115-133, 1981.

Wiggert B, Derr JE, Israel P, Chader GJ: Cytosol binding of retinyl palmitate and palmitic acid in pigment epithelium and retina. Exp Eye Res 32:187-196, 1981.

Lai YL, Wiggert B, Liu YP, Chader GJ: Retinoid-binding proteins: Possible transport vehicles between retinal compartments. Invest Ophthalmol Vis Sci 20(suppl):210, 1981.

Wiggert B, VanHorn DL, Fish BL: Vitamin A deficiency and (<sup>3</sup>H)-retinoid binding in cornea and conjunctiva. Invest Ophthalmol Vis Sci 20(suppl): 155, 1981.

Lee L, Wiggert B: (<sup>3</sup>H)-Arachidonic acid binding in developing chick retina cytosol. Invest Ophthalmol Vis Sci 20(suppl):215, 1981.

Chader G: Retinoids: binding proteins, transport and mechanism of action, in McDevitt D (ed): Cellular Aspects of the Eye. New York, Academic Press, 1981 (in press).

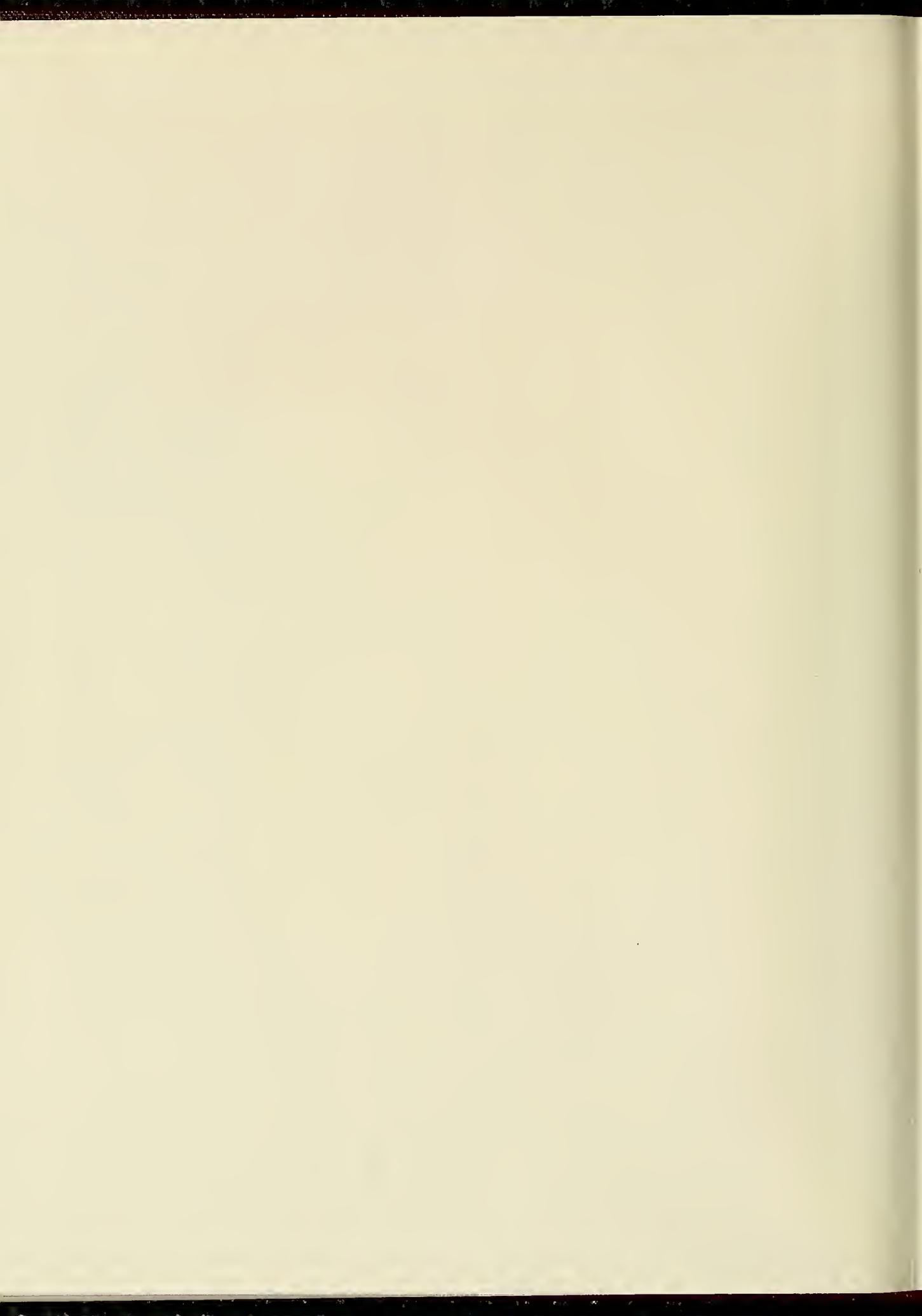
Chader G: Vitamin A, in Sears M (ed): Handbook of Experimental Pharmacology. Berlin, Springer-Verlag (in press).













NIH LIBRARY



3 1496 00195 1857