Last winter, the National Eye Institute invited a group of investigators interested in corneal problems to meet in Bethesda, Md. to define specific areas where increased research emphasis is needed.

Dr. Irving Leopold chaired the overall proceedings; Dr. James Elliott lead the discussion of clinical areas, and Dr. Claes Dohlman conducted the session on basic science. A complete list of Task Force participants appears at the end of this report.

After a preliminary discussion, each participant was assigned according to previous experience and interest to discuss a particular area of corneal investigation. Summaries of each discussion were prepared. These were circulated among all participants and, at the final meeting, were reviewed in detail. A priority was then assigned by closed ballot to each area on the basis of (1) importance of the particular research for enhancement of knowledge and the possibility of clinical usefulness and (2) the chance of, and the necessity for, success.

Although a multitude of approaches to the cornea problem were considered initially, each was subsequently included under one of the following main categories: (1) corneal opacification, (2) tear film, (3) interrelationships of inflammation, (4) edema, (5) epidemiology of morbidity and blindness from corneal disease, (6) herpes simplex, (7) drugs and delivery, (8) physical and toxic tolerance of cornea, (9) keratoplasties and keratoprosthesis, (10) tumor virology and immunology, (11) nonspecific inflammation, and (12) immunology.

1. Corneal opacification. Prospects of understanding and possibly controlling the physical, chemical, and biologic factors that underlie development of unwanted loss of transparency in the stroma and cornea were considered. Recent developments and technologic procedures have facilitated study of the uniform diameter of the collagen fibrils, regularity of intrafibrillar spacing, isolation and identification of many stromal macromolecules, and cell types from which each constituent originates. These advancements have also made possible a detailed analysis of how these constituents are synthesized, and their postsynthetic modification, excretion, and extracellular polymerization.

More knowledge is available concerning the collagens than there is for other glycoproteins or proteoglycans. Rapid advances have been made in understanding how stromal constituents are produced and assembled during embryonic development and how the opaque embryonic cornea achieves transparency.

However, specific gaps in present knowledge are preventing further progress in this area. How much of the physical arrangement of the fibril, its shape, position, and rotation is intrinsic to the collagen itself or is dictated by the noncollagenous matrix? What collagens are used in construction of the normal and scarred stromal collagenous matrix? Which are the cells that synthesize them, and what is the time and sequence of the production and rates of turnover?

It is obvious that we must learn as much about the chemistry of glycoproteins and proteoglycans as we now know about collagen. It is also evi-
dent that we need to know more about the cells that produce these substances and the time at which they are produced. A better understanding of the relationship between the proteoglycans, glycoproteins, and collagens is needed.

The same questions raised for normally developing stroma must be considered for scar tissue in the stroma. When we learn about the constituents of scars, the sequence in which the substances in scars are produced, and the rate at which they turn over, answers may follow.

Would it be possible to identify and control cells that normally produce constituents only during a brief period of development so they could be reactivated to produce that constituent later in life? Is there any possibility of reconstructing damaged corneas by grafting components of embryonic or fetal corneas? Could epidermal growth factors play a role in the healing of wounds in the cornea? Is it possible to change the refractive error of the ground substance to render it transparent?

2. Tear film. The precornea tear film coats the cornea, thus forming an optically smooth surface with a refractive index considerably different from that of air. This provides the eye with most of its refractive power in the form of a lens of high optical quality. The tear film also acts as a protective coating which guards the cornea against desiccation and perhaps infection.

Continuity of certain physical properties and chemical compositions are apparently necessary for the tear film to fulfill its role. But more needs to be known about its composition, ways of altering it, and the manner in which it is influenced by drugs.

Research is needed to correct the dry-eye syndrome, which can result from various causes, and to develop an adequate substitute for tears. Is it possible to increase the tear-film stability and its wetting properties? Is it necessary to obtain more facts on the chemistry of the secretion of the Meibomian glands and the conjunctival glands? Just how important is the mucin in the stability of the tear film?

More information is also needed about the stability of thin-liquid films. How does lid movement affect the mucin, lipid, and protein interaction in the tear film? Does the superficial epithelium play a role in tear film stability? What are the factors in various diseases which alter the tear film and how do they do this?

The stability, composition, delivery, and distribution of the tear film needs investigation. Attention must be paid to the formulation of tears in order to provide adequate substitutes, and particular attention must be directed toward the role of contact lenses and artificial tear pump mechanisms.

3. Interrelationships of inflammation. It has long been suspected that the collagengolytic enzymes are responsible for some aspects of pathologic connective tissue structure in various diseases in the body. Recent studies of the origin of perforations in alkali-burned corneas indicate that collagenase causes tissue destruction. It is obvious to those working in this area that isolation, purification, and identification of the characteristics of destructive proteases are necessary.

It is important to differentiate among collagenolytic enzymes because it has been shown that certain collagenases can be stimulated rather than inhibited by the usual collagenase inhibitors. Many proteases may be involved in corneal breakdown; these must be identified and their origin determined. Those factors which might stimulate the formation of these enzymes must also be identified.

What role do inflammatory cells have in the production and elaboration of proteolytic enzymes? How are these cells and enzymes involved in the destruction and repair of the cornea? What role does serum play in the inhibition or stimulation of many of these factors? What are the factors responsible for regulating epithelial cell mitosis, epidermal growth factors, and spreading of epithelial cells? Are protease inhibitors clinically effective?

4. Edema of the cornea. This condition, which usually causes cloudy vision, may be associated with swelling of the stroma and with accumulation of fluid in the epithelial layer of the cornea. Generally, corneal edema is believed to result not from a metabolic defect in the epithelium but from loss of function of the endothelium.

The endothelium normally pumps fluid out of the corneal stroma causing a negative fluid pressure within the tissue. This in turn draws the epithelium onto the corneal surface and maintains its integrity.

When the endothelium fails, the stroma swells and the epithelium itself becomes loose and edematous. Endothelial failure can result from gross defects due to trauma, inflammation, and dystrophies.

Little is known about the nature of the fluid pump in the endothelium. However, information about this mechanism is essential to understanding disturbances of the hydration control system and how these are compensated for. Measures which can be undertaken at the present time to correct defects in epithelium include transplantation, evaporation of the fluid from the anterior corneal surface, use of a soft contact lens, and application of various membranes to the posterior corneal surface.

Why do the endothelial cells seem to be limited in their capacity to divide and spread to cover defects in the cell layer? If the factors controlling such activity could be identified, it might be
possible to encourage the endogenous cell layer to repair its defects and thus in many cases obviate the need for full-thickness grafts.

What are the factors that promote adhesion of the epithelial cells? What are the optimal substrate requirements for endothelial cell growth? What is the role of aqueous ascorbate and lactate in endothelial cell metabolism?

The physiology of the epithelial and endothelial layers must be investigated. The system of pumps and barriers that contributes to corneal water balance and cohesion should be studied with a view to its possible stabilization under stress by means of drugs or physicochemical treatment.

Clarification of the embryonic origin of the corneal endothelial layer gives a rational basis for choosing a substitute layer more compatible with the endothelium, thereby enhancing the likelihood that a suspension of exogenous cells would adhere to the cornea and multiply. The nutrient requirements of the endothelium should be explored.

5. Epidemiology of morbidity and blindness from corneal disease. The incidence, prevalence, and morbidity of corneal disease should be investigated to aid in the identification of potentially fruitful areas for corneal research. The incidence of blindness due to corneal disease is only a small part (0.75 per cent) of the total number of newly registered blind. However, participants in the Task Force felt that diseases affecting the cornea constitute approximately 25 per cent of ophthalmic practice. Moreover, very little is known about the prevalence of various important corneal diseases such as herpes simplex and herpes zoster keratitis.

Specific information on the natural history of particular corneal diseases is scarce. Furthermore, little is known about how these diseases have been altered by newer therapeutic modalities such as the apparent prolongation of herpes simplex keratitis by topical steroids. There appears to be a certain geographic prevalence for certain diseases such as the large number of keratoconus cases in one segment of Pennsylvania. Are there geographic patterns of corneal disease throughout the United States.

There is no question that there are specific gaps in epidemiologic knowledge of corneal diseases. Such information may be obtained from indepth studies in small geographic areas such as surveying closely followed, prepaid medical plans used on the West Coast and in New York. In this way perhaps, information could be gathered concerning the influence of environment and socioeconomic factors in corneal blindness and morbidity.

6. Herpes simplex. More information is especially needed about recurrent and deep herpes simplex infections. Data are needed on new drugs used for active and prophylactic therapy. At the present time investigators are considering interferon, profavlin and light, and antiviral drugs. Whether human interferon will be useful in preventing recurrent herpes simplex infection must be determined. Studies of lysosomes, phagocytosis, and other body defense mechanisms would also be valuable.

Sufficient information is not available to determine when and why adrenal corticosteroids may be beneficial in some of these viral inflammatory states and detrimental in others. What is the mechanism for the adverse effect of steroids on certain cases of herpes simplex infections?

7. Drugs and delivery. More information is needed on antibacterial agents, antifungal agents, and antiviral medications. Each of these drugs needs to be evaluated for penetration and tolerance. Measures should be found for earlier diagnosis so that these drugs may be used most effectively.

Both nonsteroidal and steroidal anti-inflammatory agents should be developed and compared for their therapeutic and toxic penetration and anti-inflammatory potency. What are the potential and actual roles of sodium chromoglycolates and prostaglandin inhibitors in the management of ocular disorders?

The vehicles, mechanisms, and devices employed for delivering each of these agents must be considered in terms of reducing the number of applications needed to obtain the desired effect.

Some drugs are now kept off the market simply because there is not sufficient demand to make it worthwhile for a drug firm to seek the Food and Drug Administration's approval. Methods must be found to make such drugs available. For example, cooperative studies should be implemented for the investigation of uncommon corneal problems. Such studies may lead to improved therapy for these limited disorders, particularly when new drugs are ready for clinical trial.

8. Physical and toxic tolerance of cornea. Thousands of chemicals, drugs, plants, and venoms can injure the cornea and cause loss of vision and pain in varying degrees. Some of these toxic substances are present in the home, industry, agriculture, or medical practice. Remarkably little is known about how such substances causes corneal injury. This lack of knowledge hinders development of more specific and effective therapeutic measures. In the case of new drugs and chemicals, attempts should be made to relate the chemical properties to potential injury.

Differences in the reactions of various animals to toxic substances presents considerable difficulty for evaluating and investigating toxic actions in the cornea. This is particularly true in syste-
The importance of studies of immunologic rejection of tissues and organs can only be made by study of the cornea. This is due to the partial immunologic privilege of the corneal epithelium which allows grafting with little or no rejection. Although many organ transplantations have been performed, there is still a need for continued study of rejection processes and development of effective methods of treatment. The use of contact lenses for corneal grafts is an example of how such studies can make a valuable contribution to the field.

There are laws that require the testing of substances hazardous to the eyes. However, no effective and valid method of doing this has been established. The effects of noxious, as well as beneficial compounds, on the corneal epithelium obviously merit long-term study.

9. Keratoplasties and keratoprostheses. The problem of rejection from immunologic reaction and faulty technique in corneal transplantation must be considered. Can better use be made of tissue typing? Are there ways of blocking out the antigenic response of the donor cornea?

The importance of fully understanding the incidence and mechanisms of immunologic rejection of corneal grafts cannot be overemphasized. Criteria must also be developed for clinical diagnosis of specific rejection as a guide for therapy.

Perhaps the most important argument for the continuing study of corneal graft rejections is in the field of general transplantation biology. It became clear during a recent international symposium on corneal graft rejection that the ocular immunologist’s knowledge of experimental models can make a valuable contribution to the field. Simple descriptions of the mechanisms of rejection of tissues and organs can only be made by study of the cornea. This is due to the partial and controllable immunologic privilege of this graft site, the histologic simplicity of the structures involved, the nonobligatory nature of corneal vascularization, and the ability to induce at will slowly developing rejection processes which are readily visualized and easily interpreted. The importance of studies of immunologic rejection of the cornea for such programs as skin and kidney transplantation will become increasingly apparent during the coming years. These studies will probably provide a good demonstration that eye research can make very important contributions to other fields.

Several million people in this country wear contact lenses, yet the effect of contact lenses on the cornea needs clarification. The importance of the biophysical properties of surface coatings of the lenses and their interaction with the cornea is not clear. This applies to preservatives, cleaning solutions, and soaking solutions used with these lenses, and their possible effects on corneal metabolism and ocular microbiology. New plastic materials are being used for contact lenses, and a large number of people will likely be exposed to them.

The potential benefit and relative value of the various plastic substances used in contact lenses needs intensive study. The results of such investigations could greatly increase the therapeutic potential of contact lenses as protective devices, optical aids, and drug carriers.

Artificial corneal optical implants are indicated in a small number of people. However, those who are blind from severe acid or alkali burns or from other causes and who have had repeated graft failure, can certainly benefit from this approach. The major problem is the extrusion of the implant.

Is this due to the manufacture of enzymes by the epithelium bordering the prosthesis which destroy corneal stroma? Or is this simply due to the fact that stroma does not grow into the prosthetic device throughout its entire length and spontaneously retracts from it, thus causing extrusion? Some of the present devices cause the epithelium to grow around them into the anterior chamber which leads to fistula formation and infection. Various materials are being investigated for their ability to stimulate and encourage the growth of collagen stromal tissue into the implant.

The curvature of the cornea can be modified by thermokeratoplasty. It has been known for many years that heat will shrink corneal collagen. In fact, this was used by Ulschhang in the early 1900’s for the treatment of keratoconus, but no controlled method of applying heat was available. This is particularly crucial because excessive heat can cause corneal damage and perforation.

Thermokeratoplasty procedures have recently been used successfully in treating human keratoconus and may also be useful in correcting astigmatism and myopia, and perhaps even in the refractive correction required for aphakia. Certainly it warrants further exploration. Hypertonic salt concentrations have also been shown capable of modifying the corneal curvature.

10. Tumor virology and immunology. Blindness due to bilateral neoplastic disease involving the conjunctiva and cornea does not constitute a significant public health problem in this country. Nevertheless, basic information obtained from directed research may prove valuable in the treatment of systemic neoplasia, tumor immunology, and conjunctival melanoma.

Malignant melanomas of the conjunctiva may arise from pre-existing nevi or from areas of acquired melanosis. The incidence of conjunctival melanomas which arise from pre-existing nevi is quite small. On the other hand, acquired melanotic lesions of the conjunctiva frequently give rise to melanomas.

One of the features known histologically is the presence of chronic inflammatory cells associated with active areas of acquired melanosis, both in the premalignant and malignant phase. The significance of their presence has been overlooked. A similar inflammatory spot has been noted...
in areas of acquired melanomas of the skin where inflammatory cells are considered to represent an immune response.

Is the waxing and waning of areas of acquired melanosis related to an immune mechanism? Current modes of therapy, such as local excision, excision with grafting, exenteration, and radiation do not materially affect the course of the patient's disease once an established melanoma has developed in this area. There is evidence from collaborative studies throughout this country of possible beneficial effects of immunotherapy for patients with cutaneous melanomas.

Those with an adequate delayed hypersensitivity response seem to do better than those who lack such a hypersensitivity reaction. A number of survivors of malignant melanomas appear to have a certain transfer factor in their system which, when given to selective recipients, appears to have a deterring effect on the development of the recipient's melanoma. Augmentation or enhancement of delayed hypersensitivity response of patients with malignant melanoma is presumed to have a beneficial effect.

There is no information on this subject concerning conjunctival or, for that matter, intraocular melanomas. No coordinated attempts have been made to study the immunologic aspect of patients with conjunctival intraocular melanomas.

What are the critical factors which differentiate true neoplasia from pseudoneoplastic proliferative reactions? Lymphopseudo tumors have been shown to be related in some instances to systemic disease entities, such as Sjogren's syndrome, arthritis to a certain extent, and thyroid disease. No properly managed study has been made of the development of these lesions in the eye to investigate their association with other systemic manifestations of disease. The immune status should be assessed.

Does ultraviolet exposure trigger abnormal tumor virus replication? Exposure to the ultraviolet part of the sun spectrum has been associated with the majority of skin malignancies.

Perhaps a search for cell transformation in tissues excised from patients with interepithelial epitheliomas and frank squamous cell carcinomas of the conjunctiva should be undertaken. Why are true primary corneal neoplasms extremely rare? What are the underlying factors responsible for this phenomenon? What is the role of blood vessels in the development of neoplasia? How important is their presence?

11. Nonspecific inflammation. Over the past four decades extraordinary changes have taken place in the character of infectious corneal disease. In the 1920's, organisms such as diphtheria bacillus, gonococcus, pneumococcus, and vaccinia were the responsible etiologic factors. Today, opportunists such as pseudomonas, proteus, fungi, and the herpes simplex virus appear to be more important.

Socioeconomic factors are important in connection with the true corneal pathogens, helping them to get a foothold. Immunosuppressive drugs and antibiotics are apparently of some importance in dealing with the opportunists.

It is obvious that public health measures, improved medical care, and economic conditions have had a profound effect on reducing the prevalence of corneal damage formerly caused by such diseases as diphtheria, and smallpox. Smallpox has all but disappeared and, although there has been some recurrence of diphtheria, diphtheric ocular disease with its toxic corneal necrosis has, so far, not reappeared. Unfortunately, behavior of the opportunistic microbial forms has become worse. Formerly, herpes simplex, zoster, and vari-cellula virus produced serious corneal disease only in humans whose cellular immunity was compromised by infancy, old age, malignancy, or malnutrition. Today they also produce serious disease in otherwise immunologically competent people who have been compromised by various immunosuppressive and cytotoxic drugs, including the corticosteroids, and certain antibiotics that have immunosuppressive properties.

It is vitally important that all the factors which influence the prevalence and severity of infectious corneal disease be analyzed. It may be that suppression of inflammation can be responsible for the development of disease from opportunistic organisms. If so, the inflammatory response could be considered a desirable feature and not one to be suppressed.

Studies should therefore be undertaken to determine those situations in which inflammation is desirable and those in which it is not. Means of controlling the response should be identified in order to prevent undesirable invasion by opportunistic organisms. In addition, measures should be developed that will boost the defenses in the proper direction without heightening the destructive effect of excessive inflammation. The factors which control neovascularization of the cornea may have an adverse effect on resistance to infection. These must also be determined.

12. Immunology. The reaction of the host to infection can be divided into defense mechanisms which are beneficial and host reactivity which can cause additional harm.

Circulating antibodies can play a major role in preventing disease and controlling infection and inflammation in general. While a great deal is known about antibody response to infection, it is necessary to understand how serum antibodies can be employed diagnostically. The possible autoimmune and immunodeficiency problems must also be identified.

It is clear that the mucosal surfaces secrete...
antibodies, primarily IgA, in response to local infection or challenge. IgA secretion has been studied in the eye and differs from some other areas of mucosal exposure. Secretory antibodies to bacterial and viral organisms can be produced and antibodies to herpes can be made specifically. These may play some role in determining both initial infection and recurrence.

Cellular immunity has not been explored sufficiently. In part, this is because the tools for its study have been inadequate. More recently, however, measurements of cellular immunity by techniques such as lymphocyte transformation, macrophage inhibition, and lymphocyte toxicity have begun to yield important information which relates both to host defense and to inflammatory damage.

It is believed that cellular immunity probably plays a crucial role in the prevention of recurrent viral diseases and in a variety of other infectious processes. The defense aspect of cellular immunity is of vital importance. In addition, cellular defenses seem to be primarily involved in the production of many types of inflammation after infection that are associated with rejection of corneal transplants.

There has been little work on applying the measurement of cellular immunity factors to clinical problems of the eye. Such studies become important in view of the possibility that cellular immunity could be augmented and manipulated by administering transfer factors, BCG, vaccines, etc.

Autoimmunity must be investigated as a cause of corneal diseases such as corneal ulcer, conjunctival shrinking, and keratoconjunctivitis sicca. Additional studies of circulating antibodies and cellular immune factors in these conditions are also required.

What role does local injury play in initiating the autoimmune process? Autoimmunity may either continue the inflammation or heighten it and may affect the reaction of the eye to subsequent injury. Some of the secretory antibodies which are formed, such as IgE, also can be produced locally. Its presence has been correlated with vernal and atypical conjunctivitis in the eye, just as it has been correlated with asthma elsewhere in the body. Apparently it produces some of its damage through interaction with the mast cell where it stimulates the elaboration of serotonin, histamine, and perhaps other factors. Some of this effect of IgE can be blocked by chromoglycate.

Primarily, the problem involving graft rejection is its diagnosis. It is often difficult to determine if the clouding of a specific graft is immunologic rather than a function of tissue damage. Laboratory tests which detect early graft rejection would be valuable. Study of corneal antibodies before and after keratoplasty in rejecting and nonrejecting patients would also be worthwhile.

Graft rejections may be preventable by two major techniques: (1) it may be possible to modify the donor material's antigenicity by chemical means and (2) blocking antibody can be used on the donor to bind antigenic sites which may otherwise sensitize the recipient. Enzymatic treatment of antitissue antibodies with papain or pepsin might well remove a complement-fixing antigen and prevent cytotoxicity.

Tissue typing may also prevent graft rejection by particularly susceptible hosts. A specific study of corneal antigens is necessary to determine which tissue histocompatibility antigen or antigens are most important in the different layers of the cornea and whether these are the same, or as complex and varied, as those throughout the body. It is important that these studies be done in penetrating homografts and not with interlamellar heterografts. If tissue typing is to be effective, corneal preservation is almost essential to make this technique practical and to allow adequate time for collecting sufficient material so that it can be typed and matched with recipients.

The results of the Task Force's priority scores on each of these topics follow: (1) immunopathologic mechanisms, (2) herpes simplex, (3) scar of corneal opacification, (4) edema, (5) drugs and delivery, (6) epidemiology, (7) interrelationship of inflammation with tissue destruction, (8) tear film, (9) keratoplasty and keratoprosthesis, (10) physical factors in corneal disease, (11) nonspecific inflammation, and (12) tumor virology and immunology.

From the Information Office, National Eye Institute, National Institutes of Health, Bethesda, Md. 20014. Manuscript submitted April 2, 1973; manuscript accepted April 4, 1973.

Julian M. Morris

Group B participants: infectious disease and transplantation.

Dr. Chandler R. Dawson
Francis I. Proctor Foundation
University of California
Parnassus and Third Ave.
San Francisco, Calif. 94122

Dr. James H. Elliott
Division of Ophthalmology
School of Medicine
Vanderbilt University
Nashville, Tenn. 37203
Group A participants: wound healing and the prevention of scar formation.

Dr. George B. Benedek  
Department of Physics  
Room 13-2005  
Massachusetts Institute of Technology  
Cambridge, Mass. 02139

Dr. Michael Berman  
Department of Cornea Research  
Retina Foundation  
20 Stanford St.  
Boston, Mass. 02114

Dr. Stuart Brown  
Cornell University Medical College  
1300 York Ave.  
New York, N. Y. 10021

Dr. Charles Cintron  
Department of Cornea Research  
Retina Foundation  
20 Stanford St.  
Boston, Mass. 02114

Dr. Alfred Coulombre  
Section on Experimental Embryology  
Laboratory of Vision Research  
National Eye Institute  
Bethesda, Md. 20014

Dr. Claes Dohlman  
Department of Cornea Research  
Retina Foundation  
20 Stanford St.  
Boston, Mass. 02114

Dr. W. Morton Grant  
Howe Laboratory of Ophthalmology  
Harvard University Medical School  
243 Charles St.  
Boston, Mass. 02114

Dr. Frank Holly  
Department of Cornea Research  
Retina Foundation  
20 Staniford St.  
Boston, Mass. 02114

Dr. David Maurice  
Ophthalmology Division  
Department of Surgery  
Stanford University School of Medicine  
Stanford, Calif. 94305

Dr. Walter Scott  
Department of Ophthalmology  
Mount Sinai School of Medicine  
100 St. and Fifth Ave.  
New York, N. Y. 10029

Dr. George Smelser  
Department of Ophthalmology  
College of Physicians & Surgeons  
Columbia University  
630 W. 168 St.  
New York, N. Y. 10032

Dr. Robert Trelstad  
Developmental Biology Laboratory  
Harvard Medical School  
Massachusetts General Hospital  
Boston, Mass. 02114