The function of the corneal epithelium in health and disease
The Jonas S. Friedenwald Memorial Lecture

Claes H. Dohlman

The corneal epithelium fulfills important functions since its absence or disease can lead to serious consequences. In the presence of a long-standing epithelial defect, the stromal surface dries easily and becomes irregular, the stroma swells and clouds, ulceration and scarring may follow, and the entire eye becomes vulnerable to infection. In the healed stage, the epithelium may have developed surface irregularities and reduced transparency. Such residual damage of the epithelium, resulting from acute or chronic disease or injury, is responsible for reduction of vision in an enormous number of people the world over. Yet, our knowledge of how the epithelium functions in health and disease is quite modest, and past research in this field has raised a number of controversial questions. I will try to review some of the more important problems of the physiology and pathophysiology of the corneal epithelium and briefly discuss some recent studies in our laboratory.

Anatomy

The microscopic anatomy will not be discussed in any detail here. For that the reader is referred to recent articles and text. However, there are a few morphologic aspects that may be of particular importance to the subsequent discussion of epithelial functions.

The surface of the epithelium seems smooth enough in the light microscope, but with the electron microscope minute villi can be observed projecting from the surface cells. Scanning electron microscopy has revealed that the protrusions are more like minute plicae, rather than villi (Fig. 1). They range in height from 1 to 2 μ, and they may play a role in the anchoring of the tear film (see below under Tear film).

Another anatomical feature of relevance to function is the gradual flattening of the epithelial cells as they approach the surface (Fig. 2). The outermost cells become very thin and nearly 50 μ in width. The cell membranes of the epithelium are also remarkably close and interdigitated, particularly in the middle layers, and they are connected by numerous desmosomes. This means that substances that are not lipid soluble (and therefore do not pass...
through the cell membranes) will have a very long and tortuous path to travel along the narrow intercellular spaces in order to reach the other side of the epithelium. This is probably why the epithelium is so poorly permeable to electrolytes, glucose, and so forth.9 (See under Hydration.) This tightly packed anatomical arrangement with virtual absence of intercellular spaces partially explains the remarkable transparency of the epithelium (see under Optical considerations).

The presence of well-developed Golgi apparatus and endoplasmic reticulum in the epithelial cells suggests secretory activity (see under Hydration). Mitochondria are scarce, which would indicate low aerobic oxidation and more dependence on the pentose shunt.10

A third anatomical feature that deserves to be singled out here is the fine basement membrane which separates the epithelium from Bowman's membrane3 (Fig. 3). It is only about 0.05 μ thick11 and is stainable with periodic acid–Schiff (PAS) stain. The basement membrane of the corneal epithelium is not in any way unique—most epithelia of the body have a similar structure—but it may be of critical importance to the adhesion of the corneal epithelium in a way not quite understood. The basal cells are normally attached firmly to the basement membrane with hemidesmosomes. If the basement membrane is surgically removed, the development of epithelial adhesion is much slower.12 Long-standing epithelial defects and recurrent erosions in the eyes of patients may be explained by damage to the basement membrane.13 Eventually, however, the epithelium secretes new basement membrane and becomes well attached again to the rest of the cornea.
Optical properties

In order to allow the formation of a sharp image on the retina, the corneal surface must be extremely smooth, and the corneal epithelium (as well as the remaining media) must have a high degree of transparency (minimal scattering of the light). The smoothness of the surface is aided in all likelihood by the tear film.

As was pointed out in the preceding section, the normal anterior epithelial surface appears irregular in electron microscope pictures. It appears probable that the tear film is needed to cover these microscopic surface crevices. The transparency of the normal epithelium is explained on the basis of a virtual absence of fluctuations in refractive index throughout the cellular layer.\textsuperscript{14, 15}

Visible irregularity of the surface is extremely common after corneal disease and is, alone, responsible for reduction of vision on an enormous scale. Thus, after infectious keratitis, corneal edema, or dry eye, either the epithelium itself becomes of uneven thickness, or previous stromal ulceration causes the surface to lose its regular curvature. Not even an abundant tear film can restore sufficient smoothness to allow good vision. Such irregular astigmatism is clinically diagnosed with a Placido disc (Fig. 4) or keratometer and is significantly eliminated with a contact lens, or to some extent, with a pinhole.

The epithelium can interfere in ways other than surface irregularity. Following severe corneal disease, the epithelium may have been sufficiently damaged to scatter a significant amount of the light that passes
through it, which causes reduction in transparency. In the slitlamp, the epithelium now becomes visible because of the increased backscatter. This is due to the appearance of optical inhomogeneities in the tissue, and when these are spaced far enough from each other, light scattering becomes significant. In epithelial edema from Fuchs' dystrophy, for instance, the light scattering is explained by the accumulation of fluid between the cells. When the intercellular spacing exceeds approximately one half the wavelength of light, scattering takes place and becomes greater as the spacing gets wider. The scattered light can interfere with itself in the manner of a diffraction grating to produce haloes. Light scattering in the epithelium may not be severe enough to reduce visual acuity when tested in a sparsely lit ophthalmologist's office but could still cause considerable glare in bright sunlight. Glare sensitivity can be tested under office conditions with the aid of special equipment. As would be expected, the closer the corneal pathology that scatters the light is situated to the center of the cornea, the more pronounced is the glare (Fig. 5). In long-standing epithelial disease, connective tissue tends to form between the epithelium and Bowman's membrane. In chronic edema this degenerative pannus can become quite thick and it increases the corneal scatter significantly. It can be peeled off with the epithelium as a single leathery layer. New epithelium, thinner and less hazy, grows over the stroma, but edema reforms, and discomfort often returns.

Surface irregularity and light scattering by the epithelium is often much more destructive to visual acuity than stromal edema or scarring. Particularly, the influence of an irregular surface on visual acuity is often undetected or underestimated, whereas the role of stromal scarring is often overestimated.

The tear film

The ultrathin fluid layer on the front surface of the globe which makes up the tear film is virtually a part of the epithelium and, possibly, is held in place by the microplicae of the surface cells. The thickness of the normal precorneal tear film, as has been measured by two independent methods, is about 7 μm, approximately one tenth of the thickness of the epithelium. By instilling fluorescein into the cul-de-sac and following its dilution and rate of disappearance with a fluorimeter, the normal tear volume has been estimated to be about 6 μl and the average rate of turnover to be about 1.2 μl per minute.
Corneal epithelium in health and disease

and a mucous layer (Fig. 6).\textsuperscript{22} The lipid layer is derived from the meibomian glands. The thickness of the lipid layer varies with the lid movement. Even in the wide-open eye, it is probably thicker than a monolayer.\textsuperscript{23, 24} When the lids close, the film thickness may increase up to a few tenths of a micron, as indicated by interference colors.\textsuperscript{25} The aqueous fluid is secreted from the lacrimal glands, including the accessory glands. The mucus, which seems to be partly adsorbed to the epithelial surface and partly dissolved in the tear fluid (Fig. 6), is, in all likelihood, derived from the conjunctival goblet cells. The corneal epithelium lacks goblet cells and mucus cannot be seen histochemically in any of its surface cells. In contrast, the normal conjunctiva abounds with goblet cells filled with PAS-positive materials (Fig. 7), and, most likely, this mucous material is rubbed onto the corneal surface by the tarsal conjunctiva at every blink. The importance of the mucus component of tears and the necessity of periodic resurfacing of the corneal epithelium with this material has been demonstrated recently. (See below.)

One main function of the tear film obviously is to wet the surface and protect the epithelium from osmotic damage due to evaporation which could result in keratinization and ulceration. Whether the tears have any additional function as a vehicle for essential compounds is uncertain. Lysozyme, which normally exists in high concentration in the tear fluid, has a certain bacteriostatic effect, but its absence, as seen in cases of rheumatoid arthritis,\textsuperscript{26} does not seem to lead to an increased risk of infection. The tear film also contains immunoglobulins, but again their role is not clear.\textsuperscript{27} Though it would be logical to expect the tears to carry nutrients to the epithelium there is no evidence that this route of supply is important (see below under Nutrition).

When the corneal and conjunctival epithelium clinically suffer from “drying,” what is the sequence of events in this process? When the lids are opened, water evaporates from the tear film; or, in the absence of the tear film, from the epithelial surface.\textsuperscript{28} In an eye with scant tear fluid, such as in the case of Sjögren's syndrome, the fluid film could conceivably concentrate its content of solutes to a high concentra-
tion, thereby causing a diffuse osmotic damage to the underlying cells. This mode of action probably plays little role, however. Tears in keratoconjunctivitis sicca have not been registered to have a tonicity much higher than normal. What does seem to occur when the tear film is scant is loss of its stability. The tear film breaks up, baring the epithelium in areas of various sizes (Fig. 8). This formation of dry spots, or "holes," in the tear film, is not restricted to pathologic circumstances. If the lids are held apart long enough even in a normal person, dry spots usually form (Fig. 9). This phenomenon was described several decades ago, and its mechanism has more recently been elaborated upon further. It was found that the average time for the appearance of dry spots on the cornea upon opening the eye is about 30 seconds in normal adults. In patients with Sjögren's syndrome this breakup time is diminished.

In quite a few clinical situations the tear film shows an abnormal breakup despite adequate aqueous tears due to local non-wetting of the epithelium. Thus, in many cases of the Stevens-Johnson syndrome, early ocular pemphigoid, avitaminosis A, chronic bacterial or viral conjunctivitis, chemical burns, and sometimes in elderly people without obvious pathology, the Schirmer test can be normal, but the tear film still shows marked instability. This can lead not only to irritation and photophobia but also to frank damage to the epithelium with ulceration or keratinization as a consequence.

The mechanism of tear film stability in health and disease has been investigated recently with surface-chemical techniques in our laboratory. It was found that corneal epithelium, free of mucus, is quite hydrophobic, as would be expected from the lipid-protein composition of cell walls in general. A drop of fluid (saline, tears, etc.) placed on such a mucus-free corneal
surface spreads poorly. The drop remains convex with a large contact angle (Fig. 10). If, however, mucus from the conjunctival sac is smeared onto the surface and then a drop applied, it spreads completely (contact angle zero). These and other experiments have made it quite likely that conjunctival mucus is the natural wetting agent of the tear film, and that without mucus the fluid layer loses its stability.

In the clinical situations mentioned earlier wherein aqueous tear production is sufficient, but where the tear film still breaks up prematurely, could mucus be lacking? It seems so, because on conjunctival biopsy of such cases the goblet cell population is reduced drastically or totally absent. In the Stevens-Johnson syndrome (Fig. 11), pemphigoid, avitaminosis A, and so forth, the conjunctival goblet cells disappear early in the disease (Fig. 12). Most likely, mucus deficiency is the direct cause of the tear film instability and subsequent damage to the epithelium (Fig. 13).

The role of the oily layer in normal and pathologic eyes is not clear. In vivo experiments in rabbits have shown a retarding effect of the lipids on the evaporation of water from the aqueous layer. In vitro measurements, on the other hand, have indicated no such influence. By using more sensitive in vitro techniques, however, a retardation of water evaporation by purified meibomian gland secretion can be measured in vitro. This effect is greater if the water contains dissolved mucin. The lipids have not been measured quantitatively in humans, but in most clinical conditions Newtonian colors on the surface can be observed with the slit lamp, indicating the presence of a thin oily layer. Only when the lid margins and the meibomian glands have atrophied is the color pattern absent.

With the realization of the central role of mucus deficiency in dry eye syndromes, therapeutic efforts have taken new directions. A soft hydrogel lens constitutes a stable tear film of sorts, but oxygen deprivation can sometimes damage the pathologic epithelium. Probably more promising are new synthetic polymers with mucus-like action. They can be administered as drops or even continuously with the aid of a minipump.

**Barrier to microorganisms**

The epithelium is a remarkably efficient mechanical obstacle to the entry of bacteria and fungi into the stroma. In this function, it is undoubtedly aided by the flushing action of the tear film. Most infections of the corneal stroma are connected with trauma of the epithelium, usually minor, which opens the way for the invasion. Conococcus, Koch-Weeks bacillus, and diphtheria bacillus, however, are known to be...
Fig. 13. Tear film break-up time. (I) In normals; (II) in patients with decreased aqueous tear production (keratoconjunctivitis sicca); and (III) in patients with normal aqueous tears but decreased goblet cell population (Stevens-Johnson, ocular pemphigus, etc.). Mucus production is in all likelihood low in the last category which seems to lead to tear film instability and corneal damage.

able to penetrate into the stroma even without the aid of trauma. Debilitating disease is predisposing. Bacteria and fungi would not be expected to penetrate the dense basement membrane and force their way into the stroma unless the tissue is digested by enzymes released from either the microorganisms themselves or injured epithelial cells.

In the case of virus infection, the epithelium seems less of a barrier. Given the right immunologic and systemic circumstances, herpes virus is ingested by the cells by a pinocytosis-like mechanism. The virus then multiplies in the cells and spreads to the stroma in a large percentage of cases. The organisms would not be expected to penetrate the basement membrane without the aid of lytic enzymes from the epithelial cells, unless they can enter where nerve endings penetrate the membrane. The exact mode of propagation is unknown.

Nutrition

The epithelium has a relatively high rate of cell turnover. The average life span of mammalian epithelial cells is about one week, as has been demonstrated by measuring the mitotic rate in the basal cells and by following tritiated thymidine-labeled cells migrating to the surface. The presence of well-developed Golgi apparatus in the epithelial cells suggests secretory activity. These synthetic processes must require a high metabolic activity, and a steady supply of oxygen and nutrients is needed from the surroundings.

The epithelium could receive its nutrition theoretically from the tears, the aqueous humor, or the limbal capillaries. With regard to oxygen, epithelial consumption has been found to be between 3 and 7 µl per hour per milligram of dry weight, and it seems that most of it has to enter across the anterior surface. If this route is interfered with by a tight-fitting methylmethacrylate contact lens, intracellular edema occurs as well as a marked decrease in epithelial glycogen and an increase in lactic acid content. On the other hand, silicone contact lenses, which are permeable to oxygen, do not cause changes in glycogen or lactic acid amounts. When the lids are closed (e.g., during sleep), the epithelium is still exposed to 55 mm. Hg oxygen tension from the tarsal conjunctiva which seems sufficient for the need of the epithelium. Recent work has shown that an oxygen tension, even as low as 20 mm. Hg, is enough to keep the epithelium free from edema.

Glucose is an essential metabolite which must reach the epithelium in sufficient quantity. Glucose consumption of the whole cornea has been estimated at approximately 100 µg per square centimeter per hour, and the epithelium probably accounts for at least one half of this on the basis of
cell volume. The source of the glucose—whether it comes from the tears, limbus, or aqueous humor—has been under investigation in our laboratory for some time. Our interest was stimulated by observing epithelial complications after fluid barrier procedures (artificial endothelium, etc.) in human beings. It was noted that when such fluid-impermeable barriers were inserted into or behind the stroma, epithelial edema could be prevented, but in many cases the epithelium appeared loose and could be torn away easily, resulting in long-standing defects and sometimes in stromal ulcerations. It was suspected on the basis of these observations and of earlier experimental work (see below) that the complications might be due to interference with nutritional pathways.

Although the tear film might be suspected to be a major source of glucose because of its proximity to the epithelium, the fact that tears have a very low glucose concentration of only about 3 mg. per 100 ml. and a small turnover rate rules them out. The amount of glucose available is far too low to meet the requirements of the epithelium. In addition, glucose as well as other water-soluble substances penetrate the epithelium very poorly, whereas entry across the endothelium seems to be facilitated. The limbal capillaries may serve as a source of glucose, but the distance from the limbus to the central cornea makes this unlikely. In addition, measurements of glucose flux across the stromal surface showed that when the aqueous was replaced with silicone oil, the flux dropped to a fraction of the control value. On this basis, it was calculated that the epithelium, even as peripheral as 1 to 2 mm. from the limbus, could receive glucose from the limbal vessels only at a rate of less than 20 per cent of the normal supply.

It seems very probable that the aqueous humor is the main source of glucose, not only for the endothelium and stroma but also for the epithelium. Early experiments with intrastromal membranes and silicone oil in the anterior chamber showed that corneal degeneration could result if the cornea was separated from the aqueous humor. It was calculated that the glucose concentration anterior to these barriers should be very low if the aqueous humor plays a major role. Actual determinations of glucose did indeed show a marked decrease of glucose both in stroma and epithelium in front of an intrastromal imper-
Fig. 16. Corneal changes in a patient with anterior segment necrosis following operation for retinal detachment.

meable membrane32, 33 (Fig. 14). Also, glycogen decreased drastically within hours after insertion of the membrane33 (Fig. 15). These experiments showed that normal limbal and tear glucose supplies could not maintain the epithelial glucose and glycogen in the absence of the aqueous supply. Measurements of glucose flux showed that 110 μg of glucose per square centimeter per hour can reach the epithelium from the aqueous humor—a value greater than that needed by the epithelium.74

These findings proving the necessity of aqueous glucose for the epithelium have clinical implications. For one thing, they dispel some previous assumptions on the pathogenesis of some corneal conditions. For example, it has been shown that marginal degenerations of the cornea are often accompanied by an obliterative vasculitis of the limbal vessels,75 and this observation might suggest that corneal nutrition has been secondarily impaired, especially since treatment with heparin has been reported successful.76 This interpretation does not seem likely in view of the strong evidence in favor of nutrition reaching the cornea from the aqueous humor.

Nutritional factors have also been invoked to explain graft failures in very leukomatosus corneas. It has been assumed that such scarring would prevent nutrients reaching the graft across the edges. Aside from being unlikely in view of the research cited above, it has proved untenable on clinical grounds. Totally leukomatosus corneas can now be grafted successfully and the transplants can remain clear for years.

More important from a practical point of view is the possibility that some forms of corneal disease are caused by a lack of nutrition. It seems very likely that the epithelial defects and stromal ulcerations occurring in surgery employing fluid barriers (artificial endothelium, intrastromal membranes, or buried mushroom prostheses) are due to blocking the pathway of nutrients from the aqueous to the epithelium.

In addition, it has been found that glucose in the anterior chamber can become very low after experimental alkali burns, presumably due to impairment of the ciliary body,77 indicating that there may be a nutritional element in the corneal pathology seen after chemical burns. Another example of a clinical situation which may be related to nutritional damage is anterior segment necrosis which can occur when the arterial supply to the iris and ciliary body is interrupted temporarily, as can happen after retinal detachment surgery.78

In these cases, the cornea becomes edematous and vascularized, often with an epithelial defect (Fig. 16). The same clinical picture can be caused experimentally in rabbits by cauterizing the long posterior ciliary arteries. Aqueous taps in such animals have shown a very low glucose content down to five per cent of normal79 (Fig. 17). Glycogen disappears early from the epithelium. These findings have opened up the possibility that the corneal damage in anterior segment necrosis is mediated via low aqueous concentration of essential nutrients and subsequent symptoms of starvation. Corneal nutrition, which has received scant attention in the past, may very well prove to be a factor in certain corneal diseases.

Metabolic interaction with the stroma

Whether or not the epithelium and the stroma supply each other with essential
metabolites has been debated back and forth in several publications. In an early study on the carbohydrate metabolism of the cornea, a definite pattern of exchange between epithelium and stroma was found.\(^{53}\) Also, by removing the epithelium in chicks, rats, or rabbits in vitro or in vivo, the uptake of glycine-\(^{14}\)C\(^{6}\) or of sulfate-\(^{35}\)S\(^{2}\) into the stroma was reduced markedly. It was realized later, however, that by vigorous scraping of the epithelium, keratocytes could become damaged or killed, which could explain the lack of uptake.\(^{83}\)

In vitro incubation of the cornea with sulfate-\(^{35}\)S gave variable results. In some studies, there was lower incorporation of the isotope when the epithelium was absent,\(^{84}\) but in others there was little difference.\(^{85-88}\) Our results gave little indication that the stromal keratocytes were metabolically dependent on the epithelium under normal circumstances. We could also confirm the vulnerability of the keratocytes to manipulation and drying which is likely to influence the results in experiments of this kind.

The problem of interaction has practical importance for further work on corneal prostheses of various kinds and on surgical adhesives. In both situations, the removal of large areas of the epithelium is sometimes required. Replacing the epithelium with a glued-on membrane or lens is of some practical importance in patients (and, in addition, it is of interest in corneal physiology).\(^{89-92}\) The fact that one can glue a...
Fig. 19. The effect of removal of epithelium on the buildup of tensile strength in a stromal wound. 

Tensile strength, in grams, is indicated below each figure. Wound healing is retarded in the absence of the epithelium but can be restored with a conjunctival flap. If an impermeable membrane is inserted between the flap and the stroma, wound healing is again delayed.

Large contact lens to Bowman's membrane in animal corneas virtually without affecting the normal histologic appearance of the stroma is an additional indication that the stromal cell population can survive without the proximity of the epithelium.

In human beings, the stroma beneath a glued-on lens can retain normal transparency and thickness for years (Fig. 18).

What was concluded above referred to the noninjured stroma. In stromal wound healing, on the other hand, the epithelium may well play a vital role. In short-term experiments with corneal incisions in rats, it has been shown that removal of the epithelium delays the transformation of keratocytes into fibroblasts. In another study, the build-up of tensile strength of stromal wounds in rabbit corneas with and without epithelium was followed. We found that in the absence of epithelium, wound healing was extremely retarded (Fig. 19). If, following removal of the epithelium, a full conjunctival flap was pulled over the stroma, the normal rate of wound strength build-up was restored. On the other hand, if a thin silicone rubber membrane was inserted between the conjunctival flap and the cornea, stromal wound healing was again delayed very markedly.

The evidence from the wound healing studies cited above suggests that the epithelium (or conjunctival blood vessels) provides substances to the stroma cells that are vital to the laying down of new collagen fibrils. The nature of these substances is unknown. It might be argued that, in the absence of epithelium, important metabolites leak out of the stroma. However, when the epithelium was replaced with an impermeable membrane, healing was still retarded.

These findings have clinical significance in lacerations and keratoplasty as well as in long-standing epithelial defects in herpetic keratitis, in perforations covered with adhesives, and other conditions. Also, these results give a plausible rationale for the use of a conjunctival flap in slow-healing corneal ulcers where the clinical benefit is undisputable.

Role in stromal hydration

The exact role of the epithelium in the normal regulation of stromal hydration and in corneal edema is somewhat controversial. Considerable information has been obtained, but how the isolated facts fit into the over-all picture of corneal hydration is uncertain in many cases.

The stroma has a well-known tendency to swell in vitro and in vivo when the limiting layers are damaged. The swelling pressure of the stroma has been measured in vitro and found to be in the range of 40 to 60 mm Hg at normal hydration in several species. The possibility has been raised that little or no stromal swelling pressure exists in the undisturbed cornea in vivo due either to structural forces or to metabolic influence by the epithelium.

On the other hand, we presented evidence recently in favor of an in vivo swelling pressure of the same magnitude as measured in vitro by inserting a transparent hydrogel membrane into the corneal stroma of rabbits. The gel then behaves as a pressure transducer by changing its...
Corneal epithelium in health and disease

thickness in proportion to the stromal swelling pressure. Also, the imbibition pressure of the corneal stroma has been measured to be negative, which would be a consequence of the swelling pressure of the stromal tissue.\textsuperscript{102}

If it is accepted that the stroma attempts continuously to imbibe fluid from its surroundings, what prevents it from doing so? One factor is the barrier function of the limiting membranes. In particular the epithelium has a very high resistance to the diffusion of electrolytes\textsuperscript{103,104} as well as a high resistance to the flow of water\textsuperscript{105} (Fig. 20).

The close packing and marked interdigitation of the epithelial cells might explain these properties (see under Anatomy). In the long run, however, fluid would be expected to leak in across the limbus and across the endothelium which would result in edema unless some mechanism continuously removed the imbibed fluid. Temperature reversal experiments (Fig. 21) have provided strong evidence in favor of such an active process working across one or both of the limiting cellular layers.\textsuperscript{106-110,116}

Which one—the epithelium or the endothelium? The epithelium has been shown to transport sodium actively, but inwardly from the tears to the stroma, which does not explain transport of fluid out of the stroma.\textsuperscript{111} Chloride has also been implicated as being actively transported across the epithelium into the eye.\textsuperscript{112} In the bullfrog there is a well-established active chloride transport in the opposite direction out of the eye.\textsuperscript{113,114} A search for an electrical potential across the epithelium has given values from a few millivolts up to 60 mV.\textsuperscript{9,113,115-118,149} in several species, with the outside negative.

The significance of these signs of active secretion by the epithelium is hard to interpret. It has been pointed out that since this secretion would create a movement of salt into the stroma, it is unlikely that it has anything to do with the active control of its thickness.\textsuperscript{9} It has been argued, however, that there is a direct relationship between corneal thickness and the rate of sodium transport across the epithelium, and that the maintenance of a high sodium level in the stroma reduces the swelling pressure of this tissue.\textsuperscript{112}

In contrast, the evidence in favor of the active dehydrating mechanism being situated in the endothelium is very strong. Thus, a complete temperature reversal effect can be demonstrated with the epithelium removed but not with the endothelium absent.\textsuperscript{100,110} We have added recently further evidence indicating that there is no fluid transport occurring across the epithelium that would affect stromal hydration.\textsuperscript{93} In cats, most of the corneal epithelium was removed and replaced with a large contact lens glued peripherally to Bowman's membrane. In all the uncom-
complicated cases the cat corneal stroma maintained its normal thickness (Fig. 22). Also, in human patients, normal stromal thickness could be maintained. Therefore, it seems that the epithelium is essential as a barrier to fluid, but it does not participate in a direct manner to active transport of fluid out of the stroma.

Epithelial edema

There are several forms of epithelial edema with different appearances and etiologies. In corneal infections (viral, bacterial, or fungal), the epithelium is often swollen, loose, and easily abraded in the area of maximal involvement, presumably due to the toxic influence of the microbes, tissue breakdown products, and so forth. The situation is usually reversible as far as the epithelium is concerned, and when the infection has cleared up, the epithelium regains its original transparency (in contrast to the frequent stromal scarring).

Epithelial edema in connection with contact lens wear has a different appearance and pathophysiology. Known as Sattler's veil, it is best visualized by utilizing the slit lamp technique of sclerotic scatter. There is no question that it is due to oxygen starvation caused by a tight-fitting lens which, in turn, leads to glycogen depletion and accumulation of fluid within epithelial
Fig. 25. Another type of edematous epithelium, typical in endothelial malfunction and in acute glaucoma. Intercellular spaces are enlarged markedly and filled with fluid. Desmosomes between cells are well maintained. (Original magnification ×25,000, Courtesy of Dr. T. Kuwabara.)

cells (Fig. 23)." (See discussion under Nutrition.) The symptoms of overwear include decreased vision, photophobia and glare, and discomfort, all of which are reversible.

Epithelial edema in connection with malfunction of the endothelium is clinically the most severe form of fluid accumulation, because it is usually irreversible. It occurs in Fuchs' dystrophy (Fig. 24), in connection with cataract extraction, in graft failure, and so on. Its influence on visual acuity is substantial from the start as even minor bulla formation creates irregularity of the surface as well as haziness throughout the epithelium. The latter is caused by the light scattering effect of fluid that lodges not only within the cells, but also between them, distending their interface space except at the site of the desmosomes (Fig. 25).

Several pathophysiologic mechanisms for the development of epithelial edema have been put forth. According to one theory, fluid would accumulate in the epithelium because of absorption of tear fluid. A few years ago an alternate possibility was suggested: that the fluid of the aqueous and stroma would be driven forward and pushed into the epithelium with intraocular pressure (IOP) as the driving force. This situation would occur when the endothelium is malfunctioning severely (and the IOP normal, as in Fuchs' dystrophy), when the IOP is very high (and the endothelium is normal, as in acute glaucoma), or in a combination of endothelial damage and ocular hyper-
Fig. 26. Epithelial edema in endothelial dysfunction (and in acute glaucoma) seems to be due to a slow flow of fluid that is pushed into the epithelium by the intraocular pressure (see text).

tension of sufficient severity (Fig. 26).

This theory is based on the following facts: It has been established earlier that in the isolated stroma the imbibition pressure (IP) has the same numeric value (but opposite sign) as the swelling pressure (SP). In vivo, however, the imbibition pressure is modified by the intraocular pressure; thus $SP = IOP - IP$. The swelling pressure of the stroma has been correlated with hydration, and hydration correlated with thickness. Therefore, by measuring the corneal thickness of a patient’s eye, the swelling pressure can be calculated. By also determining the IOP by regular tonometry, stromal imbibition pressure can be calculated. In 90 patients with or without epithelial edema, it was found that virtually all of those with epithelial edema were calculated to have a positive imbibition pressure (Fig. 27). This means that the pressure of the stromal fluid, now being positive instead of negative, is bearing on the epithelium, and the stage is set for a slow flow of fluid across the stroma and into the epithelium. Thus, the mechanism would be the same whether epithelial edema resulted from acute glaucoma or from endothelial dysfunction. These relationships are depicted in Fig. 28.

There is additional evidence in favor of epithelial edema being caused by fluid pushed in from behind by the IOP. In phthisis there is never any epithelial edema, regardless of how thick the stroma is from severely malfunctioning endothelium. The driving force is lacking. Also, if a water-impermeable membrane is inserted into the stroma of a patient with advanced endothelial dystrophy, the stromal portion behind the membrane swells markedly and the anterior portion thins. This could only occur if a fluid flow was interrupted.

Thus, it seems that the epithelium in edema is the passive victim of the level of the IOP and the physiologic status of the endothelium, as well as its own lack of permeability to fluid and electrolytes.

Role in stromal ulceration

With a corneal ulceration we generally mean an epithelial defect which has become complicated by the disappearance of some underlying stromal substance, usually following infection, chemical burns, trauma, or desiccation (Fig. 29). Eventually the epithelium heals, leaving an area of stroma thinner than normal and often scarred. Very rarely do we see stromal thinning without epithelial defect (e.g., keratoconus), and in such instances, the process of thinning is extremely slow.

There are now good reasons to believe that stromal ulcerations are caused by enzymes that are released from cells rendered abnormal by the disease process (Fig. 30). Thus, the initial injury (viral, bacterial, or chemical) causes changes in corneal cells, particularly in the epithelium, which in turn respond by releasing proteolytic enzymes that flow out into the tear film and, given the right circumstances, digest some of the stroma.

The reasons for this enzymatic concept are, in short, the following (for details, see recent reviews):

1. Proteolytic enzymes seem to be re-
Fig. 27. Patients with epithelial edema (circles) and without (crosses) are plotted according to corneal thickness and intraocular pressure. In the area to the right of the curved line, the imbibition pressure of the stroma is calculated to be positive, and in the area to the left it should be negative. It is concluded that epithelial edema occurs when the stromal imbibition pressure is positive, most likely resulting in a flow through the stroma in the anterior direction.124

Fig. 28. Examples of imbibition pressure gradients in the normal cornea,102 in acute high-tension glaucoma, and in endothelial dysfunction, the latter two situations resulting in epithelial edema.124 (SP = swelling pressure of the stroma. FP = fluid pressure, or imbibition pressure). See text.
Enzymes have been implicated in the pathogenesis of corneal ulcers. Significant loss of stromal substance occurs only in connection with epithelial defects released from damaged epithelial cells. Collagenase has been the most extensively studied, but doubtless there are others. Thus, epithelial extracts can split a synthetic collagen-like substrate. Alkali-burned epithelium from rabbits, when cultured in a Rose (or similar) chamber can lyse a collagen gel and, even more important, it can lyse corneal stroma. Harvested enzymes from cultured full-thickness explants, when injected into alkali-burned stroma, create an ulceration. It was shown recently that epithelium from dendritic figures (herpetic) in rabbits also releases large amounts of collagenase in culture. The epithelium seems to be an essential source of collagenase; whether keratocytes and white blood cells contribute significantly is more uncertain. A stromal enzyme has been characterized recently as a collagenase. Polymorphonuclear cells can release a collagenase which, however, does not attack undenatured collagen fibrils.

The collagenase released from the alkali-burned cornea has been characterized and has been found to meet the criteria of a true collagenase. Thus, it reduces viscosity of a solution of collagen to about 50 per cent and it can split the tropocollagen molecule into the typical 3/4 and 3/4 fragments as seen with the electron microscope.

1) Epithelium cultured from human corneal ulcers releases collagenase. It does not seem to matter what the original etiology of the ulcer is, rather, it is the severity of the corneal insult that grossly determines the amount of lysis. A single traumatic abrasion of the epithelium causes little lysis, and normal epithelium causes none. These circumstances are quite suggestive that collagenase plays a role in stromal ulceration. They still do not constitute absolute proof, however, as it might be argued that enzymatic release in tissue culture might not necessarily reflect the in vivo situation.

2) Stromal ulceration seems to be mediated by enzymes that are released into the tear film mainly by altered epithelial cells. Enzymes released from damaged epithelial cells, whether keratocytes and white blood cells contribute significantly is more uncertain. A stromal enzyme has been characterized recently as a collagenase. Polymorphonuclear cells can release a collagenase which, however, does not attack undenatured collagen fibrils.

3) By covering the corneal stroma with an impermeable membrane, stromal ulcerations can be prevented. If a large contact lens is glued onto the stroma after a severe chemical burn, for instance, no ulceration occurs beneath the lens as long as the latter adheres to the tissue properly. This finding greatly strengthens the assumption that, in disease, enzymes are released into the tear film and subsequently attack the stromal surface. It does not tell us about the source of the enzymes, which can be either epithelium or polymorphonuclear cells. This "artificial epithelium" technique is being evaluated presently as a means of protecting the stroma in severe chemical burns in human beings. Within a few days after the accident, a lens covering the entire cornea is attached with cyanoacrylate adhesive. As long as the lens stays in place and epithelium is prevented from growing in, no ulceration occurs beneath it.
Figs. 31 and 32. (Fig. 31) Diseased corneal epithelium, biopsied and cultured, causes lysis of a collagen gel. (Fig. 32) Also, corneal stroma (left) can be lysed in culture by an explant of corneal epithelium (right).

Figs. 33 and 34. (Fig. 33) Reduction of viscosity of soluble collagen incubated with corneal enzymes. The restricted viscosity drop is typical of animal collagenase. (Fig. 34) Cleavage of tropocollagen molecule in typical 1/4-1/2 fragments by corneal collagenase.

(4) Enzyme inhibitors can prevent stromal ulceration. Thus, it has been shown in rabbits with alkali-burned corneas that topically applied ethylenediaminetetraacetic acid (EDTA), EDTA-Ca, cysteine, and acetylcysteine can retard or prevent ulceration in a considerable percentage of the animals (Fig. 36). Also in human beings, in a series of ulcers of various etiologies treated double-blind with EDTA-Ca drops, this treatment was clearly efficacious (p < 0.001) (Table I). On the other hand, the action of the enzyme inhibitors can prevent stromal ulceration.

Table 1. Reduction of stromal ulceration when 0.2M EDTA-Ca is given as eyedrops in a variety of corneal diseases on a double-blind basis

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of patients</th>
<th>Patients continuing to ulcerate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2M EDTA-Ca (in polyvinyl alcohol vehicle)</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td>Control (polyvinyl alcohol vehicle)</td>
<td>26</td>
<td>54</td>
</tr>
</tbody>
</table>

*P < 0.001.
Fig. 35. By gluing a contact lens to Bowman's membrane after a chemical burn, stromal ulceration can be prevented.139

Fig. 36. Enzyme inhibitors, such as cysteine, applied as drops after alkali burns in rabbits, can prevent or retard stromal ulceration.142

Enzyme inhibitors tested so far is not overly strong. They cannot prevent ulceration in all cases, but their apparent lack of toxicity in the concentrations used suggests that they should be used clinically in impending ulceration. Serum contains collagenase inhibitors,144 which possibly explains why a vascularized cornea has less tendency to ulcerate than a nonvascularized one.

The reasons cited in favor of enzymes as mediators in stromal ulcerations do not provide complete proof of this concept, but the evidence is certainly strong. Another variable in this connection is the susceptibility of the stroma to enzymatic action. After an alkali burn, the tissue is considerably more readily digested than a normal stroma.145 It is possible that in other corneal diseases the stroma similarly offers less resistance to epithelial enzymes.

A final factor is the release of enzymes from invading bacteria. Particularly pyocyaneus is notoriously invasive due to the release of a collagenase. EDTA as eye drops has been tried experimentally with some effect in order to decrease the severity of pyocyaneus infections.146

Thus, in summary, it is very likely that the epithelium, once damaged, contributes to the destruction of the underlying stroma by the release of enzymes. This concept has opened up exciting possibilities for
clinical prevention of corneal ulcers, either mechanically with a glued-on lens or the like, or with enzyme inhibitors.

REFERENCES

101. Klyce, S. D., Jr., Dohlman, C. H., and Tol-


121. Cogan, D. G.: Experimental production of so-called bullous keratitis, 23: 918, 1940.


