The interrelationship of metabolism and deturgescence of the living cornea

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It is the purpose of this paper to review recent investigations, including some unpublished studies on the metabolic and physiologic dependence of small areas of stroma on the overlying cells, with the object of assessing the extent to which they have contributed to a fuller understanding of the mechanism by which aerobic metabolism maintains the deturgescence of the corneal stroma. The recent findings and conclusions of Herrmann on the metabolic interaction between the epithelium and underlying stroma are given. His contention that there is a structural linear metabolic interrelationship is discussed in the light of Langham and Pollack's observations that separation of stroma from either the overlying epithelium or its underlying endothelium by an impermeable thin polypropylene sheet neither decreases the ability of the isolated stroma to incorporate radioactive sulfate nor causes physiologic changes over periods of many weeks. In attempting to explain the specificity of the metabolic changes in the stroma after removal of epithelial or endothelial cells, attention is given particularly to the changes in swelling properties of corneal stroma that occur in the area lying immediately below or above the denuded layer. Thus, it is concluded that a decreased uptake of compounds by the stroma is associated with a change in swelling properties of the tissue and is not a direct effect of loss of epithelium.

During the last 3 or 4 years new experimental evidence concerning the vital role played by the corneal epithelium in maintaining the function and structure of the stroma has been reported. The biochemical and physiologic reactions of the cornea to partial removal of the epithelial layer have revealed a previously unsuspected spatial dependence of small areas of stroma on the overlying cells. Pari passu, removal of relatively large areas of the epithelium has been found to cause marked physiologic changes in the denuded area but little, if any, in the remaining area covered by epithelium. It is the purpose of this paper to review these findings, some of which have not yet been published, and to assess to what extent they have contributed to a fuller understanding of the mechanism by which aerobic metabolism maintains the deturgescence of the corneal stroma. The influence of metabolism on corneal hydration in the excised eye and in the living animal has been reviewed recently by Harris and Langham.

Experimental observations on the effect of partial removal of the epithelium on the uptake of glycine-1-C-14 by the corneal stroma of the chick embryo have been reported by Herrmann and Love. Their study arose from a previous observation of Herrmann that the uptake of glycine-1-C-14 into a collagenous fraction of the stroma decreased when the corneal epithelium was removed. Herrmann and Love incubated excised chick corneas in a medium containing glycine-1-C-14 after

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partial removal of the epithelium and then determined the distribution of the glycine uptake by a radioautographic technique. Their results showed that the uptake of the amino acid was uniformly distributed in the stromal areas bordered by intact epithelium and endothelium, and that a sharp transition took place at the junction of the normal and denuded areas with very little uptake in the latter.

Similar radioautographic studies were reported by Smelser\(^2\) in a study of the role of the epithelium in the uptake of inorganic S-35-O, into the mucopolysaccharide of the corneal stroma of rats. Animals were given an injection of S-35-labeled sulfate before or after portions of the corneal epithelium had been scraped off. Total removal of the epithelium led to a marked decrease in uptake of S-35-O, compared with that of the normal eye, and removal of either the central or peripheral areas of the epithelium resulted in a decrease in uptake in the abraded area but not in the remainder of the stroma. A similar dependence on an intact epithelium for uptake of inorganic S-35-O, by the rabbit stroma has been reported by Wortman.\(^2\) Evidence that this sulfate is incorporated into the mucopolysaccharide of the cornea may be found in several investigations.\(^1\)\(^-\)\(^10\)

In attempting to explain his findings with glycine, Herrmann\(^14\) drew attention to an earlier observation that the oxygen consumption of the excised bovine cornea was nearly completely restricted to the epithelium, and a new observation that the uptake of glycine by the cornea was dependent upon aerobic metabolism.\(^1\) From these observations he suggested that the uptake of glycine by the stroma was mediated by energy transference from the epithelium. Furthermore, in view of the spatial specificity of this phenomenon he suggested that the metabolic interaction took place across the epithelial-stromal barrier by means of structural components which accepted electrons from the stromal metabolites and transferred them to the active oxidative pattern of the corneal epithelium.

It is of importance to know the aerobic and anaerobic metabolic activity of the stromal cells, for, if these cells are devoid of oxygen uptake, there can be no doubt that the stromal metabolism and function are dependent upon the oxidative metabolism of either or both the epithelium and endothelium because the cornea swells in the absence of oxygen. However, in contrast to Herrmann’s findings on cattle corneas, there is much evidence that the stromal cells of the rabbit have an active aerobic metabolism.\(^17\)\(^-\)\(^19\) Studies of the oxygen uptake of the rabbit corneal stroma revealed that it accounted for approximately 25 per cent of the oxygen uptake of the total cornea and that the accumulation of lactic acid in isolated stroma increased by 35 per cent in the absence of oxygen. Moreover, if this metabolic activity is attributed to the stromal cells, calculation indicates that the oxidative activity of the stromal cells of the rabbit is similar to that of the cells of the epithelium. It has, therefore, not been possible to ascertain from this type of experiment on rabbits whether the swelling which follows inhibition of either oxygen uptake or oxidative phosphorylation is due to a primary effect on the metabolism of the limiting cellular layers.

An opportunity to examine whether the type of structural linear metabolic interrelationship between the epithelium and stroma, as suggested by Herrmann, does occur in rabbit and cat corneas was taken by Langham and Pollack\(^2\) during the course of studies on the properties of an area of stroma separated from the epithelium by a water-impermeable thin sheet of polypropylene. It was found that polypropylene discs 6 mm. in diameter could remain in the stroma of cat, monkey, and human eyes for periods of more than one year without causing pathologic changes. In similar experiments on rabbits, Knowles\(^2\) has shown that no pathologic change takes place in the area of stroma posterior to the
The stroma may function normally for prolonged periods when separated mechanically from its overlying epithelium.

The interesting question arises whether the incorporation of either glycine-1-C-14 or S-35-O, is affected by separation of the stroma from the overlying epithelium and this aspect was examined by Langham and Pollack using inorganic S-35-O, In these experiments pairs of eyes from individual rabbits were used. The eyes were excised and placed in a medium containing labeled sulfate, incubated for 1 hour at 37°C, and then prepared for radioautographic analysis. In initial control experiments partial removal of the epithelium was performed and the results confirmed the previous observation by Smelser26 that the uptake of S-35-O, was markedly decreased in the abraded area. Neither Herrmann nor Smelser studied the effect of partial removal of the endothelium, but it is of importance to know whether removal of endothelium would induce a similar effect. This was examined and a similar result was found in that incorporation of S-35-O, was markedly decreased in the abraded area. Thus, even with the epithelium in place, sulfate fixation was markedly decreased in the stroma directly above the denuded endothelium.

In similar studies on excised rabbit eyes the incorporation of S-35-O, was determined after the insertion of the polypropylene sheets into the stroma. The results were as follows: with the limiting layers intact the incorporation of sulfate in the stroma of the experimental and control corneas was uniformly distributed (Fig. 1); after removal of the epithelium anterior to the disc, incorporation of S-35-O, was significantly decreased in front of the disc, but was normal behind and in the remainder of the cornea; finally, after removal of the endothelium posterior to the disc, incorporation of S-35-O, was decreased in the area behind the disc, but normal in the remaining part of the cornea.

Fig. 1. A radioautograph showing that the incorporation of S-35-O, in areas of stroma on either side of a polypropylene disc are the same. The disc is indicated by the empty space. The disc was inserted into the middle of the normal stroma of a cat, S-35-O, was injected into the anterior chamber, and the cornea was removed for analysis 6 hours later.26
In further studies on the cornea of the living cat, two thin membranes were placed in the stroma to isolate a segment from the overlying and underlying limiting cellular layers, and under these conditions the uptake of S-35-O₄ was found to be normal throughout the cornea (Fig. 2). Thus, physical separation of the stroma from either or both the epithelium and endothelium did not modify its ability to esterify inorganic S-35-O₄. Consequently, if there was a metabolic dependency of the stroma on the epithelium and endothelium, exchange or transfer of substrates must have taken place by diffusion into the isolated stroma around the edges of the discs.

Measurement of corneal thickness in these experiments revealed that the decreased uptake of inorganic S-35-O₄ was always associated with swelling of the affected compartment. The question arises whether the strict demarcation of uptake between denuded and normal cornea is associated with a similar marked change in the swelling properties of the stroma. This aspect of the problem will now be considered.

The influence of partial removal of the epithelium on the swelling properties of the corneal stroma has been studied recently by Langham and Langham and Pollack. In the former study the epithelium was scraped off corneas of rabbits and the swelling measured during the recovery as new cells moved in from the periphery. Within 24 hours approximately 50 per cent of the stroma was covered by new epithelium and the corneal thickness under this new layer had returned to normal. After 48 hours about 75 per cent of the cornea was covered by epithelium and was of normal thickness, whereas the denuded area remained grossly swollen. Thus, the ability of the epithelium and the endothelium to induce recovery was confined to areas of stroma bordered posteriorly and anteriorly by endothelial and epithelial cells.

The marked change in swelling between the denuded and normal stroma was further investigated by measurement of the imbibition pressure after partial removal of the epithelium. The imbibition pressure of the stroma is defined as the hydrostatic pressure developed within a manometric system filled with physiologic saline when connected by a needle to a point source in the stroma; it results from the hydrophilic properties of the stromal constituents. It is distinguished from the swelling pressure of the cornea which is the hydrostatic pressure that must be applied to prevent swelling. The two pressures will be equal if there is no restraining or cohesive force. With the use of a technique similar to that described by Dohlman, Hedbys, and Maurice, two needles were
inserted into the stroma of a living rabbit (Fig. 3). The needles were connected to a differential transducer and the whole system was designed to have a low volume capacity (approximately 0.5 μL 100 mm. Hg⁻¹) and minimal leak (approximately 10⁻⁵ μL min⁻¹ for a pressure gradient of 100 mm. Hg). At the start of the experiment the pressures in both arms of the system were brought to atmospheric pressure, and then the taps to the pressure reservoir were turned off. Under these conditions it could be shown that the absolute pressure in both arms of the system became negative at a similar rate, and the pressure differential across the transducer remained close to zero. When the epithelium lying immediately above one needle was scraped off, a pressure gradient began to develop between the normal and abraded stromal areas, and, in a typical experiment, reached a steady-state value of approximately 60 mm. Hg, with the normal stroma showing an absolute imbibition pressure of approximately -65 mm. Hg (Fig. 4). Thus, marked differences in imbibition pressures can exist between neighboring regions of the stroma when there is localized epithelial trauma.

These biochemical and physiologic observations show that a decreased uptake of compounds is associated with a change in the swelling properties of the tissue and is not due to loss of epithelium. They fail to support the concept that the uptake of compounds by the stroma is dependent upon metabolic energy transmitted from the epithelial cells along linear structural components bridging the epithelial-stromal barrier. On the contrary, the function of the stroma appears normal in the absence of either or both the adjacent epithelial and endothelial cells provided no stromal swelling occurs. Therefore, if epithelial metabolism is needed to sustain stromal metabolism and function, it must occur by transference in all planes of the cornea.

These conclusions lead to a further consideration of the rapid gradation that occurs in the metabolic and swelling properties of the stroma between denuded and adjacent normal areas of the cornea. As the S-35-O⁻ uptake and thickness of the denuded stroma remain normal, provided there is an artificial barrier taking the place of the abraded epithelium, it is logical to conclude that the rapid gradation is due to a change in the biochemical or physical
The relationship between aerobic metabolism and corneal swelling. The experimental results reviewed in this paper emphasize the rapidity of changes that can occur in both the biochemical and swelling properties of the cornea when the epithelium or the endothelium is damaged, and one is faced with the fundamental question of whether biochemical changes induce alterations in the swelling properties or vice versa. At the present time the mechanism by which metabolism effects fluid movement from the cornea is unknown. The results of metabolic studies are consistent with the view that secretion of fluid takes place across the epithelial and endothelial cells, but it is still not clear to what extent, if any, it is necessary to invoke such a mechanism. In the normal cornea the function of the epithelium and endothelium is either to maintain an osmotic force of active transport in the outward direction sufficient to balance a finite swelling pressure of the stroma, or, alternatively, to sustain the cohesive forces that restrict the swelling of the stromal mucoid.

The fact that stripping of the epithelium or endothelium leads to stromal swelling and the assumption that the swelling pressure of the excised cornea reflects the properties of the normal cornea in the living animal has led investigators to consider active transport as the most probable mechanism whereby the hydration of the tissue is maintained. Thus, it is widely believed that either water or a solute is transported out of the cornea at a rate equal to the passive net inward movement of water and salt.

Unfortunately, all attempts to identify and relate active transport mechanisms in the epithelium to the outward movement of fluid have so far been unsuccessful, and it is evident that if such a mechanism exists it must differ qualitatively from the well-known cationic active transport mechanism found in frog skin and mammalian tissues. Thus, while sodium is for thermodynamic and physiologic reasons the likely ion to be transported outward across the corneal epithelium, Donn, Maurice, and Mills have shown that sodium is actively transported into the stroma across the epithelium. In other studies, dinitrophenol (DNP) has been found to inhibit deturgescence of the cornea only in concentrations which induced a simultaneous depression of oxygen uptake, whereas inhibition of active transport of ions in other tissues by DNP has been found to be independent of a depressant effect on oxygen uptake. Finally, it has been observed that replacement of over 50 per cent of the corneal sodium by lithium did not inhibit the ability of aerobic metabolism to effect deturgescence of the swollen cornea, whereas lithium is known to inhibit active transport mechanisms in other tissues.

In this circumstance it would appear logical to give serious consideration to an alternative hypothesis that the swelling pressure is normally zero due to a balance between the imbibition pressure of the corneal mucopolysaccharide and the restraining or cohesive forces between the structural components of the stroma. Swelling would then result from a decrease in the cohesive forces, and, in turn, the uptake of fluid by the mucopolysaccharide would cause a concurrent decrease in the imbibition pressure. In this way a new equilibrium could develop in which the absolute values of the opposing forces were significantly decreased. In such a scheme it would have to be proposed that aerobic metabolism was essential to the maintenance of the cohesive forces in the normal cornea and to their restoration in the swollen cornea undergoing recovery.

Recent studies of the structure of the normal and swollen cornea indicate that a system of this nature could exist in the stroma. Collagen, which forms the prin-
Principal structural protein of the stroma, exhibits both close- and long-range intermolecular forces of attraction during development of the collagen fibril. The corneal fibril of diameter 250 Å comprises an association of many collagen molecules, each having a diameter of approximately 15 Å. In the cornea these fibrils form part of a regularly spaced two-dimensional lattice, and corneal swelling is characterized by an increase in the distance between the fibrils.2 The distance between the fibrils is approximately 250 Å which is well within the distance in which long-range intermolecular forces of attraction have been found to operate in protein gels.7 The significance of a lattice network of great uniformity is seen in that the collagen fibrils of most body tissues continue to grow throughout life. However, in the stroma, the aorta, and the lung—all of which are tissues of high tensile strength—the collagen fibril remains of constant diameter.8 The mechanism controlling the growth of these fibrils is not known, but it is widely believed that it involves the interaction between mucopolysaccharide and collagen. In this regard a chemical interaction between mucopolysaccharide and collagen of the cornea has been demonstrated by Woodin.27 Thus, there exists within the stroma a lattice gel of macromolecules possessing great swelling properties interspersed with molecules showing little or no swelling tendency at physiologic pH. The highly specific properties of this network may be vitally dependent for its normal biologic function on the environmental conditions which, in turn, would be dependent on the integrity of the epithelial and endothelial cellular layers.

REFERENCES


