Introduction: Factors influencing corneal hydration

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The swelling of the corneal buttons in aqueous solution can be prevented by adding high molecular weight solutes to the bathing medium. In appropriate concentrations of these colloidal substances the button can be dehydrated, a matter of some significance in clinical situations. Swelling of the cornea in the intact eye in vitro can be similarly prevented. The suggestion is made that swelling is due to Donnan osmotic pressure of the cornea. Current evidence favors the view that the swelling potential is present in vivo and that normal corneal hydration is maintained by secretion of fluid. The rate of secretion seems adequate to maintain normal corneal hydration in close proximity to a corneal puncture.

The members of the symposium committee have been asked by the Chairman of the Board of Trustees of the Association to assemble a panel which might review certain aspects of corneal structure, physiology, biochemistry, and disease. The task seems particularly appropriate because it has been 3 years since such a review was presented. It is planned, too, that the present discussion will provide material of clinical significance. The committee recognizes that much good work and many able investigators are not included on the program. Unfortunately, the time available was too short for a more inclusive program.

Many corneal diseases at some stage cause corneal hydration. This has, in some respects, an obscure but certainly a significant influence on the ultimate effect of the disease process. It is quite appropriate, therefore, to introduce this Symposium on the Cornea with a brief discussion of some factors known to alter corneal hydration. There is no particular unanimity of opinion concerning the interpretation of known facts. The present introduction is written with the knowledge that other opinions will be voiced by the panel of the Symposium and is intended as a point of departure for discussion purposes.

I would like to consider these factors from three different experimental situations: first, the hydration of excised pieces...
of cornea; second, the corneal hydration observed in the isolated but intact eye maintained in a moist chamber; and, third, corneal swelling in situ.

It has long been known that a corneal button immersed in water or normal saline takes on considerable fluid. The kinetics of this swelling at both 0° C. and 37° C. has been previously reported. As would be expected the rate of hydration of the corneal button at 37° C. is more rapid than at 0° C. The Q₁₀ is calculated to be in the neighborhood of 1.3. This is a reasonable figure for physical swelling. It should be noted, however, that the same degree of hydration is approached at both temperatures.

The swelling of the cornea is altered by many factors. First, the hydration varies with the pH of the solution. Loeven found the swelling potential of the cornea to be minimal at a pH of 4.5 to 4.7. The findings of others are in the same range. Second, the ionic milieu also is of significance. The swelling varies with the charge and valence of the ion. In addition, different ions with similar charge and valence have differing effects on corneal swelling. Third, the endothelial and epithelial barriers of the corneal button have a considerable influence on its swelling. This can be readily shown in vitro if corneal buttons are placed in an appropriate medium at 0° C. with or without the epithelium and endothelium. Those buttons stripped of endothelium and epithelium swell at a greater rate than do the intact pieces. Since metabolic influence is at a minimum at this temperature, the effect of the endothelium and epithelium can be considered simply as a barrier to the diffusion of fluid into the cornea.

It seems quite likely that the hydration of the cornea under these circumstances is due to its colloid constituents. The actual swelling pressure of the cornea has been measured by many different workers; perhaps the most accurate work is that of Dohlman and Anseth. This will be considered in greater detail by Dr. Dohlman.

Suffice it to say that isolated corneal stroma can be prevented from hydrating if the bathing solution has a sufficiently high colloidal osmotic pressure, somewhat greater than twice that of plasma.

One interesting feature of this phenomenon that has not been emphasized is that it is quite possible to dehydrate the cornea by increasing the colloidal osmotic pressure of the bathing medium. For example, if one bathes an isolated corneal button in a solution of 25 per cent serum albumin, its hydration is reduced to approximately two thirds of its original value (Fig. 1). The total osmotic pressure (in this case, one half that of the aqueous) is of no significance.

Parenthetically, it might be noted that there are some clinical situations in which it may be desired to dehydrate the cornea. We have tried 25 per cent serum albumin, and, although finding it somewhat effective have, nonetheless, thought that it is not the best agent for this purpose. First, it is quickly lost from the conjunctival sac, and, second, at least in the experimental animal, it has some toxic effect on the epithelium. Among the sub-
stances which appear useful are the carboxymethylcelluloses. The product marketed as low viscosity carboxymethyl-cellulose (CMC) has a molecular weight of about 40,000. A 5 per cent solution of this substance is quite viscous and remains in the conjunctival sac. It will dehydrate the corneal buttons or corneal stroma in vitro (Fig. 2). Addition of dextrans of low molecular weight further reduces hydration of excised pieces of cornea (Fig. 3). We have used 5 per cent CMC clinically. Its use in this manner seems to have certain merit in the treatment of corneal ulcers where the epithelium is absent and stromal hydration is a prominent feature and in the postoperative management of keratoplasty. The materials appear to be of no value in the management of bullous keratopathy or where epithelial edema is a prominent clinical feature.

The actual colloidal constituent of the cornea responsible for its swelling is not known. By analysis, collagen is the colloid in highest concentration. Yet, it seems likely that the collagen fibrils per se do not swell. There is much evidence which contributes to this. First, the pH at which minimal corneal swelling is observed is not the isoelectric point of collagen. Second, Francois, Rabaey, and Vandermeerssche showed by electron microscopy that the diameter of the collagen fibril does not increase as the cornea swells. Third, as Maurice has pointed out, data on birefringence indicate that the hydration of the collagen fibril is not altered as the cornea swells. A similar conclusion is evident from the data of Kikkawa. Last, the fact that the cornea tends to swell only in its anteroposterior dimension rather than radially suggests that collagen does not become hydrated. This latter fact accounts for the feasibility of transplanting swollen corneas into tissue having normal hydration without fear that the donor tissue will retract in its radial dimension as it becomes dehydrated.

It seems quite likely then that the other colloidal constituents are responsible for corneal swelling. There is evidence that corneas devoid of mucopolysaccharides do not swell nearly as markedly as does the native tissue. Thus, the mucopolysaccharides are undoubtedly of considerable significance. In any event, swelling appears to take place between fibrils and tends to separate them further.

(Some speak of corneal hydration as a "binding" of water. Bound water is by definition insolvent water. Present estimates of the osmotic pressure of the cornea, assuming no bound water, suggest that...
the cornea may be normally somewhat hypertonic with respect to aqueous. If the cornea normally contains insolvent water, our estimates of the osmotic pressure of corneal fluid would have to be adjusted upward. Since it is unlikely that the osmotic pressure of the cornea would be more than the estimated values, there can be little or no bound water in the cornea. The best evidence indicates that this is true of biologic systems in general.

There is an additional feature of corneal swelling which I think is worthy of emphasis. As a corneal piece swells, the ratio of sodium between the stroma and the bathing medium (which is well in excess of 1.0) remains constant. This indicates that the number of anionic linkages of some colloidal component must increase. If this colloidal component is responsible for the swelling and if the swelling is on a Donnan basis, then the swelling pressure should remain constant as the cornea hydrates. The data of Dohlman and Anseth, however, demonstrated that the swelling pressure grew less as the cornea hydrated. The apparent discrepancy remains unresolved. If the former view is correct, it provides us with a clue as to why the cornea may swell to such a large degree rather than be restricted in some capacity by the mechanical restraints imposed by the tissue.

If the isolated but intact eye is immersed in an appropriate solution, the cornea swells to a certain degree, and, at 37°C, reaches a new steady state of increased hydration. On the other hand, in a moist chamber under sterile conditions it is quite possible to maintain the corneal hydration of the intact eye for a period of 12 hours at 37°C. Thereafter, probably because of a decrease in metabolic activity, the cornea does swell.

At 0°C a marked corneal swelling is observed when the isolated intact eye is maintained in a moist chamber. This cold-induced hydration can be prevented in the same manner that the swelling of corneal buttons at 37°C is prevented, i.e., by increasing the colloid osmotic pressure of the bathing medium (in this case, the aqueous). Thus, if the aqueous is removed and replaced with 25 per cent serum albumin, for example, the cornea of the isolated intact eye hydrates only slightly in the cold.

The swelling which occurs in the cold seems to result from a reduced metabolic rate. That this is true can be readily shown by reincubating the refrigerated eye at 37°C. At the higher temperature the fluid which accumulates during refrigeration is excreted. From this and a variety of other studies in which metabolism is reduced by use of metabolic poisons, deprivation of oxygen, etc., we have concluded that the maintenance of corneal hydration requires an actively metabolizing structure. The evidence further suggests that the cornea can excrete fluid and that the swelling of the cornea is normally prevented in this manner.

The epithelium and endothelium influence corneal hydration in the intact excised eye which is maintained in the moist chamber. When either is removed, the ability of the cornea which has been hydrated at 0°C, subsequently to excrete fluid at 37°C is considerably reduced. (Where the endothelium is damaged, much of the apparent reduction in ability of the cornea to excrete fluid can be attributed to the more rapid uptake of aqueous by a cornea stripped of its posterior barrier.) We have concluded, on the basis of present evidence, that an excretion of fluid occurs across some structure at or near the endothelium. In any event, however, both the epithelium and endothelium contribute to the dehydrating process.

Last, I would like to discuss swelling in vivo. It is a common experience that injury to the epithelium or the endothelium, particularly the latter, is likely to lead to a hydration of the cornea. Anseth and Dohlman have demonstrated that the greater hydration resulting from endothelial damage is due to the intraocular pressure.
In vivo swelling of the cornea can be quite local. This is evident particularly in a corneal perforation where the cornea swells in the immediate region of the perforation because of the movement of fluid into the cornea whereas the surrounding structure maintains a relatively normal thickness. Since the lateral diffusion of fluid is quite rapid, one would expect that a break in the endothelial surface of the cornea would cause the entire cornea to hydrate. It seems likely that the intact portions of the cornea can excrete fluid with sufficient rapidity to remove completely that which enters through the corneal break. This can, as a matter of fact, be demonstrated in vitro to a certain degree. If two buttons of cornea are placed, one at 37° C. and one at 0° C., that at the lower temperature becomes uniformly thick throughout its radial dimension. On the other hand, at 37° C. the button assumes a biconcave shape, the thickness at any particular site presumably representing a balance between the amount of fluid which enters the cut end of the cornea and the amount excreted across its endothelial surface. Since at 0° C. no excretion occurs, the cornea swells uniformly. This ability of the cornea to dehydrate locally is of particular importance in corneal transplants.

The question of whether an active excretion of fluids is essential in vivo has never been fully answered. It has been suggested that the structural rigidity in situ or some force not requiring metabolic activity may be sufficient to balance the swelling pressure. Langham\textsuperscript{15} demonstrated that exposure of the eye in vivo to cold solution or to fairly critical concentrations of dinitrophenol did not cause corneal hydration. We have confirmed his results, but, for reasons which need not be elaborated here, do not agree with his conclusion that the metabolism of the cornea in vivo is not essential for maintenance of normal hydration.

Iodoacetate appears to be a better test material. When a small amount of iodoacetate is injected into the anterior chamber in vivo, appreciable swelling of the cornea can be measured 6 hours later (Fig. 4). Similarly, when iodoacetate is applied to the epithelium for 5 minutes, an increased hydration can be observed after 6 hours (Fig. 5). Such hydration could presumably be due to an increase in permeability of the limiting barriers. However, our previous studies with iodoacetate did not demonstrate this to be true. We

\begin{figure}[h]
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\caption{Water content of corneas from rabbit eyes which have received in vivo injections of 0.05 c.c. of iodoacetate into the anterior chamber. Determinations were made 6 hours after injection.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=0.5\textwidth]{Fig5.png}
\caption{Water content of corneas from rabbit eyes which were flooded in vivo for 5 minutes with iodoacetate. Determinations were made 6 hours later.}
\end{figure}
must accordingly conclude that in vivo iodoacetate alters the metabolism in such a manner as to reduce the rate of fluid excretion and permit the cornea to swell in situ.

Anseth and Dohlman suggested that the intraocular pressure acting against rigid and intact structures would balance to a certain extent the swelling pressure of the cornea. If this were true, then the cornea should hydrate at lower pressures. This is not the typical clinical experience. Nor does this conform with certain other data. We have previously demonstrated that the recovery from cold-induced hydration is greater at the lower pressures in the isolated but intact eye. Obviously, the intraocular pressure might induce two forces which tend to move fluid in opposite directions: first, a filtration pressure acting across the endothelium forcing fluid into the cornea; and, second, a mechanical pressure acting between the epithelial and endothelial surfaces expressing fluid from the cornea. The latter force has not been proved. Suffice it to say that the measurable dehydrating mechanism of the cornea operates more efficiently at lower pressures.

We have not considered the effect of the total osmotic pressure across the various surfaces involved. However, since the rate of movement of water across any particular surface is greater than that of any of the solutes such as sodium chloride or sodium bicarbonate, a movement of water in the direction of the greater osmotic pressure would be anticipated. This was suggested by Cogan and Kinsey several years ago and has been confirmed by others. In a steady-state condition this cannot be a means by which the cornea maintains its normal hydration since the endothelial and epithelial barriers are permeable to the solutes. However, in a given acute situation it is quite possible to alter the hydration of the cornea by applying solutions of differing tonicity.

In conclusion, I would like to summarize our current concept of fluid movement in the cornea. At the limbus an exchange of water solutes would tend to hydrate the cornea since the colloidal osmotic pressure of the cornea is greater than that of the plasma. Across the epithelial and endothelial surfaces there is an exchange of the majority of constituents to which these two surfaces are exposed. Across the epithelial barrier there may be a certain osmotic pressure gradient, which, in an acute but not in a chronic situation, may alter corneal hydration. Generally, however, there will be a movement of fluid into the cornea from the tears. Across the endothelial surface and at a considerably higher rate there is a similar movement. The net effect of exposure of the cornea at the limbus, epithelium, and endothelium under steady-state conditions is a movement of fluid into the cornea. Corneal hydration is prevented by the excretion of fluid, probably across the endothelial surface. We have not considered which constituent of the corneal fluid is actively transported. Present evidence is not sufficiently conclusive to identify it.

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