The movement of ions and water across the cornea

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The movement of solutes and water across the cornea and its boundaries has been reviewed, and new work on water diffusion and on endothelial pinocytosis has been summarized.

The cornea performs its main optical function not directly by cellular activity but by the physicochemical arrangement of its inert collagen and mucopolysaccharides. The swelling of the ground substance largely determines whether the collagen fibrils are in their normal lattice arrangement, transparent to light, or whether this delicate alignment is distorted. Ultimately, the movement of water and ions across the boundaries of the cornea influences this hydration and determines whether it will be clear or opaque.

The cornea is well suited to studies of diffusion because most of its area is remote from a blood supply and because the accessibility of two of its surfaces makes it readily available to physiologic investigation. The distribution of ions on either side of the semipermeable membranes of the cornea is influenced by the Gibbs-Donnan equilibrium, but this equilibrium is itself dependent upon the amount and charge of the colloids in the stroma and upon the permeability of the epithelium and endothelium. Cell metabolism undoubtedly influences the integrity of the layers and, in all likelihood, affects the nature of the stromal colloids. The movement of substances across the cornea is also of direct clinical concern. Corneal nutrition depends entirely upon diffusion from the limbus, tears, and aqueous humor. Disease of the limiting membranes, as in Fuchs' endothelial dystrophy or a herpetic infection of the epithelium, causes alterations in the fluid exchange of the cornea with well-known clinical sequelae. Surgical complications, such as adherent vitreous, may interfere with the normal permeability of the endothelium with disastrous results for the patient. When the workings of these membranes are understood, progress can be made in preventing or curing this sort of clinical problem.

Limbus

The cross-sectional area of the limbus through which diffusion occurs is roughly, 0.2 cm. Although this is only one seventh of the corneal surface, movement of ions across this area is not restrained by any selective membrane, and solutes and water can move freely in response to concentration and osmotic gradients. It is probable that the limbal capillaries are like...
those elsewhere in the body and that the fluid which is driven from them by hydrostatic pressure is essentially an ultrafiltrate of the blood, free of large molecules and similar to extracellular fluid throughout the body. The importance of the limbus, however, is limited by the relatively enormous distance that separates most of the stroma from the limbal capillaries; in the case of the central cornea, it would take many hours for even a small fraction of a substance liberated at the limbus to accumulate by diffusion. If solute and water flowed from the limbal capillaries into the stroma instead of moving only by diffusion, the rate of exchange between the limbus and much of the stroma might be appreciably increased. The most direct evidence, however, does not support the view that there is any flow at the limbus. Spots of fluorescein and of hemoglobin introduced into the stroma through a peripheral scratch in the epithelium show no over-all drift after several hours, and, if flow exists, it is less than 0.1 mm. per hour. Nevertheless, it has been known since publication of the work of Griiber that ions do effectively cross the limbus. In 1894 he showed that rust particles introduced into the corneal stroma turned blue from the periphery to the center when potassium ferrocyanide was injected into the blood stream. These qualitative observations were extended by Potts and Johnson, who injected various radioactive isotopes intravenously and found, after a given time, higher activity in the periphery of the cornea than in the center. When the limbus was cauterized before intravenous injection, the over-all radioactivity in the cornea was lower, presumably because the ions could not diffuse so readily across the cauterized tissue. Independently, Maurice found that intravenous sodium-24 resulted in higher activity in the periphery of the cornea; he also showed that when solutions containing sodium-24 were applied directly to the corneal epithelium of the rabbit, there was a lower activity in the periphery which reflected removal of this ion by exchange with the limbal capillaries. Maurice concluded that the exchange of sodium at the limbus was one fifth that across the endothelial surface of the cornea.

Stroma

Diffusion studies on the stroma provide information on the structure of the cornea as well as useful physiologic data. In general, it has been found that substances up to the size of hemoglobin diffuse readily through the stroma, although the movement of larger molecules is more hindered than the movement of smaller ones. Sodium ions, with a diameter of 6 Å, diffuse twice as slowly as they would in saline solution; fluorescein, somewhat larger is slowed by a factor of 5; and albumin and hemoglobin molecules, about 70 Å in diameter, diffuse 8 to 10 times more slowly in the stroma than they would in saline solution. Proteins much larger than this are prevented from diffusing; the limiting size in the normal cornea corresponds to a molecular weight of about 500,000. A swollen cornea allows larger proteins to diffuse, and an artificially dehydrated one inhibits the movement of smaller molecules.

Measurements of the electric resistance of the stroma are the same in the plane of the cornea as across it, about 2½ times greater than the resistance of aqueous humor. It has also been observed that hemoglobin diffuses as readily across the stroma as along its plane. This suggests that movement of the ion in the stroma does not take place in any microscopic canals, in which case electric resistance and diffusion should be different in the two directions. Diffusion takes place through the substance of the stroma and is slowed by the viscosity of the ground substance and by the insoluble collagen fibrils. In view of the fact that proteins 70 Å in diameter can move through the stroma, the collagen fibrils must be at least this far apart. This observation supports the assumption that the normal collagen fibrils are not maximally hydrated, in which case they would be so swollen as to almost touch one another.
Fig. 1. Measurements of steady-state water flux across separate corneas.8

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<th>Average Values of 12 Experiments: Aqueous to Tears Direction</th>
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<td>Average R (ohms) During Flux Measurements</td>
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<td>Average Thickness (mm) of Cornea at end of Experiment</td>
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<tr>
<td>Water Flux (cc/cm²/hr)</td>
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The stroma is the main corneal barrier to water.8 In a series of unpublished experiments, Dr. Miller, Mrs. Mallett, and I measured the water flux across living corneas in vitro, using tritiated water as tracer. Briefly, we found that the amount of water moving across the cornea was the same, whether we measured the flux from aqueous humor to tears or from tears to aqueous humor (Fig. 1). Any difference in the movement of water which might be present because of the hydrostatic pressure on the endothelium was less than our experimental error. The amount of water which crosses the living unswollen cornea in each direction is 0.4 c.c. per hour per square centimeter, and this can all be accounted for by diffusion. We concluded that water does not flow through pores in the cornea. Although there is no evidence of a water pump, it is possible that one exists but that it is smaller than can be detected. When the epithelium or endothelium was scraped from the cornea during the experiments, the change in water flux was negligible.

Epithelium

The epithelium, as might be expected from its exposed position, is the principal barrier in the cornea; it is especially impermeable to ions and other lipoid insoluble substances. The permeability coefficient for sodium is 0.0004 cm. per hour; by way of comparison, this means that the epithelium is 40,000 times more resistant to the diffusion of sodium than a similar thickness of saline. In vitro measurements of the epithelial electrical conductance corroborate this resistance to the movement of the ion; and almost all lipoid-insoluble substances measured have the same low order of magnitude of diffusion. These findings could be explained if the predominant route across the epithelium were via a system of pores, possibly the intercellular spaces.

In 1957 it was shown that the epithelium generated an electrical potential up to 40 millivolts, when the stroma was positive as compared with the tear fluid. It has been demonstrated that the epithelium actively transports sodium ions from the sur-
face of the tear fluid into the stroma of the cornea.\textsuperscript{12} When the concentration of sodium is the same on each side of the living cornea in vitro and when the electric potential of the cornea is short-circuited, approximately twice as much sodium moves into the stroma across the epithelium as moves out. It is unlikely that other large ion pumps exist across the epithelium, for the net sodium flux is approximately equivalent to the neutralizing current. The significance of this inward-ion pump is not clear; however, its presence casts some doubt upon the possible existence of other outward ion pumps in the cornea.

The epithelial impermeability is not maintained for fat-soluble substances which pass this layer quite readily.\textsuperscript{13-15} The general explanation for this difference is that the cell membranes have a significant lipoid component, and fat-soluble material passes through the membranes while fat-insoluble substances must go through pores or around the cells. In the case of the cornea, substances which have a significant affinity for both aqueous solutions and lipoids pass the cornea most easily. Aqueous solubility is necessary so that substances can penetrate the tear film and gain access to the epithelial membrane; after substances have passed through the lipoid membrane, aqueous solubility again plays an important role in diffusing through the stroma.

**Endothelium**

In the opinion of many, the single-celled endothelial layer holds the key to the problem of corneal hydration in the mammal. Disease or trauma to this layer results in enormous corneal swelling, but the permeability of this layer does not suggest any unusual resistance to water or ions. The endothelium is approximately 100 times more permeable than the epithelium to sodium,\textsuperscript{9} and its electric conductance is also about 100 times greater.\textsuperscript{10} In rabbits, half of the sodium in the stroma exchanges with that in the aqueous humor every 14 minutes.\textsuperscript{9}

Recently Maurice\textsuperscript{7} compared the resistance of the endothelium to several small ions at low temperatures when cellular metabolism is inhibited. He found that, despite their different biologic and chemical properties, cesium, bromide, and sodium were all slowed similarly by the endothelial layer. In each case the endothelium was about 1,900 times less permeable than a layer of saline of the same thickness. These experiments were repeated at 37° C, when endothelial metabolism was active, and the three small ions were again obstructed by approximately the same amount. He interpreted these results as supporting the view that the greater part of the movement of these ions was by way of a system of relatively large uncharged pores. Maurice suggested that the ions move across the endothelium predominantly by diffusion through the intercellular spaces, and, from his data, he calculated that the intercellular distance was approximately 50 Å, this has been partly confirmed by electron micrographs.\textsuperscript{10, 17}

Recently, Dr. Kaye, Dr. Pappas, Mrs. Mallett, and I have found terminal bars between adjacent endothelial cells (Figs. 2 and 3). The fact that these structures are always present suggests that the bars exist throughout the breadth of the intercellular space. There is evidence that cells adhere to each other at the site of the terminal bars and that the space between adjacent terminal bars, approximately 100 Å, is filled with a condensation of the amorphous material which is normally present on the outside of cell membranes.\textsuperscript{19} The intercellular space may not be an uninterrupted column of aqueous humor; ions passing between cells may have to move through poorly defined organic material in which the diffusion characteristics are unknown.

In another series of experiments in which thorium dioxide was placed adjacent to the endothelial surface for several hours before fixation, the marker was found adsorbed to the apical surface of the endothelium and in cytoplasmic vesicles. In some cases vesicles were seen emptying into the intercellular space basal to the terminal bar,
Fig. 2. An electron micrograph of rabbit corneal endothelium which had been maintained in vitro and exposed to a suspension of thorium dioxide for 30 minutes before fixation. Particles are adsorbed to the surface, and numerous vesicles (V) containing marker are seen in the apical cytoplasm. Many thorium dioxide particles are found in the intercellular space (IC) below the terminal bar (T). N, Nucleus; D, Descemet’s membrane.¹⁸
Fig. 3. This electron micrograph shows a portion of the rabbit corneal endothelium which had been exposed in vitro for 3 hours to a suspension of thorium dioxide. There is a dense accumulation of particles at the surface. The intercellular space (IC) is filled with thorium dioxide, and numerous large and small vesicles (V) containing marker are seen primarily in the apical cytoplasm. Particles are seen accumulated at the junction of the endothelium and Descemet's membrane and diffusing into Descemet's membrane (D).\textsuperscript{18}
Fig. 4. An electron micrograph of the endothelium of a rabbit cornea which was cooled to 0° C. before thorium dioxide was added to the solution bathing the endothelial surface. There is some attachment of particles to the surface, and a few particles are found in the intercellular space (IC). Two vesicles (V) containing marker are seen in the apical cytoplasm. There is estimated to be a 70 per cent reduction of transport under these conditions. D, Descemet's membrane; T, terminal bar.
but the thorium dioxide particles were never seen in the space between the terminal bars. Even ferritin particles, which are as small as 30 to 40 Å, were found in cytoplasmic vesicles and on each side of the terminal bar but not in it. This activity was suppressed when the in vitro corneas were cooled to 0°C. (Fig. 4).

These electron micrographs demonstrate pinocytosis in the corneal endothelium of the rabbit. I do not imply that this process is responsible for the movement of ions across the endothelium, but it is probable that this metabolic transport system is important in the movement of some substances into the cornea.

REFERENCES