A QUANTITATIVE DESCRIPTION OF EQUILIBRIUM AND HOMEOSTATIC THICKNESS REGULATION IN THE IN VIVO CORNEA

I. Normal Cornea

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ABSTRACT By combining a description of the coupled solute and water flows through the in vivo cornea with a set of appropriate mechanical equilibrium conditions, it is possible to calculate directly the corneal thickness, given the corneal temperature, the state of the aqueous and tears, the swelling pressure–hydration relation of the corneal stroma, and the transport properties of the corneal membranes. Active transport of ions or water by the corneal epithelium or endothelium, or both, are explicitly included. When published parameters are inserted into the formulation, the normal corneal thickness is recovered, and the corneal potential, anteriorly directed water flux, and stromal salt content are in reasonable to quantitative agreement with experiment. The analysis yields a simple physical explanation of the stromal imbibition pressure and the opposing forces which cause the cornea to assume its normal thickness.

INTRODUCTION

The mechanism by which the normal mammalian cornea maintains its thickness in spite of the swelling tendency of the stroma is of considerable clinical and research interest. The clinical importance of this mechanism derives from the fact that stromal edema is accompanied by a loss of corneal transparency and, in severe cases, by blindness. From a more fundamental point of view, the maintenance of corneal deturgescence represents a particularly interesting homeostatic problem, since the cornea is by virtue of its location directly affected by exogenous influences.

To date, no comprehensive quantitative description of the mechanism of corneal hydration control has been advanced; as a consequence, many aspects of the functioning of the intact cornea, such as the existence of the stromal swelling pressure in vivo and the site and role of the several active transport systems either demonstrated

or inferred to be present in this tissue, are still matters of debate. Indeed, virtually every possible mechanism of hydration control has been suggested in the literature at one time or another. Space does not permit a review of past and current theories of corneal hydration control; the reader is referred to Maurice's (1969) recent survey.

One might hope that the foregoing issues and others related to the means by which the cornea achieves its observed thickness could be clarified by examining the available in vivo data in terms of a sufficiently comprehensive, physically grounded description of the fluxes through and mechanical equilibria within the living cornea. This paper presumes to present a physical framework around which theories of hydration control may be constructed and in terms of which they may be examined. As might be expected, this framework is accompanied by a theory of its own, inasmuch as it is found that the normal corneal boundary conditions lead naturally to the normal corneal thickness.

The model, with which the next section of this paper will be concerned, is of the in vivo cornea in the time-average steady state. The mechanics of in vitro behavior are not necessarily the same as those in vivo, and, in any event, in vitro experiments are run because of their convenience, since it is of course the living eye in which the ultimate interest lies. In vitro data will be used only as a source of some of the property data required by the in vivo model. This last extrapolation, common to all discussions of corneal physiology, is sadly unavoidable, owing to the difficulties of in vivo measurement.

The description of the model is followed by the derivation of the flow and mechanical equilibrium equations implied by it. The predictions of these equations with respect to the normal cornea are then examined, to conclude part I of this paper. This comparison between experiment and theory is necessarily made for rabbit cornea, since not enough data are available for other species. The normal corneal thickness is correctly predicted by the theory, and reasonable values of stromal salt content, corneal potential, and transcorneal fluid flux are predicted.

In part II, the predictions of the theory developed in part I will be examined as they relate to the effects of variations in the corneal boundary conditions and corneal properties from their normal values. Part II will also include discussions of the possible role of metabolically coupled water and ion transport by the limiting membranes of the cornea.

MODEL

The anatomy and physiology of the cornea are well described by Maurice (1969) and Newell (1969) and in earlier ophthalmic reviews (Duke-Elder and Wybar, 1961; Adler, 1965), and will be described only briefly here. The tissue consists of three principal layers, each covering the entire corneal surface. Most anteriorly is the corneal epithelium, which is bathed by the tears and which possesses an electrogenic

sodium pump (Donn et al., 1959; Green, 1967; Ehlers and Ehlers, 1968), directed posteriorly. The corneal stroma lies behind the epithelium and by virtue of its structure exhibits a tendency to swell; this tendency is measured experimentally as the stromal swelling pressure (e.g., Hedbys and Dohlman, 1963). The swelling pressure falls from a normal value of approximately 60 Torr as the stroma thickens. Most posteriorly, separated from the stroma by Descemet's membrane and bathed by the aqueous in the anterior chamber of the eye, is a layer of endothelial cells. The hydrostatic (intraocular) pressure of the aqueous is normally 10–20 Torr above atmospheric.

It is generally accepted that the corneal epithelium and endothelium are permeable to both low molecular weight salts and water, but have nonzero reflection coefficients; that is, they may be classed as leaky semipermeable membranes. The stroma is an open structure through which salt and water pass with considerably more ease, and its reflection coefficient to small solutes is not measurably different from zero (Green and Green, 1969). Thus both water and salt can pass through the cornea under the influence of the driving forces which are present; these include the intraocular pressure (IOP), a time-average difference in tonicity between the tear film and aqueous, and at least one active transport system, the epithelial sodium pump cited above.

The tonicity of the tear film varies diurnally, being higher when the eye is open. The cornea responds with diurnal sleep-wake variations in thickness. In part II, evidence will be presented to suggest that the cornea does not equilibrate rapidly to these changes in ambient tonicity. The "steady-state" description of the cornea developed here is thus in fact a time-average state, a mean state about which the basically unsteady cornea oscillates diurnally. In subsequent discussion, the term "steady state" should be understood to signify this average condition.

The description of the steady state in vivo cornea divides naturally into two parts. First, the coupled flows of solute and solvent through a series membrane system must be treated; and second, a consistent description of the mechanical equilibrium of the tissue elements must be constructed. From the point of view of the model, the description of the coupled flows is straightforward. The corneal diameter/thickness ratio is large, so the flow problem is one-dimensional in a coordinate normal to the corneal surfaces. The only solutes included are sodium and chloride ions, and impermeants in either the tear film (epithelium-impermeable) or the aqueous (endothelium-impermeable). The omission of nonelectrolytes and minor ion fluxes is clearly an approximation, made here in the interest of simplicity. These flows may be included in a more complete description of the cornea, but they do not appear to be necessary to the reproduction of many aspects of corneal behavior.

The mechanics of corneal equilibrium are best begun with the endothelium. When the in vivo cornea swells, irrespective of the cause, the epithelium itself does not generally move relative to the orb; rather the endothelium moves posteriorly (Ytteborg and Dohlman, 1965 *a*; Maurice, 1969). Similarly, deswelling is accompanied by anteriorly directed endothelial motion. It should be noted that this fact does not dem-

onstrate either that swelling (or deswelling) in vivo corneas (or isolated corneas where the anterior surface communicates with a reservoir of solvent) imbibe (or expel) solution through the endothelium or that the endothelium plays any active role in the maintenance of corneal deturgescence. It does show that the mechanical forces in the cornea are such that the epithelium is bowed anteriorly to the limit of its travel and that the rearward motion of the endothelium is not so limited. The limit of forward travel of the endothelium most likely arises from the effectively inextensible collagen fibrils of the posterior stroma, whose slack is taken up as the endothelium advances anteriorly; Descemet's membrane may also play a role.

Inasmuch as corneal thinning may be induced in vivo by bathing the eye with hypertonic saline (Stanley et al., 1966), it may be concluded that under normal conditions the endothelium is free to move in either direction, and since it does assume a steady position, corresponding to the normal corneal thickness, it must be in mechanical equilibrium. It thus becomes necessary to discuss the forces acting on this membrane.

First, we remark that the drag forces on the endothelium caused by the flow of solvent and salt through it are precisely equivalent to the hydrostatic forces acting at the entrances to the solute and solvent pathways through the membrane. The "osmotic driving force" is not a true mechanical force; the force which devolves from it under thermodynamic equilibrium conditions arises from the hydrostatic pressure required to prevent water flow into a region of relative hypertonicity. The hydrostatic pressure posterior to the endothelium is the IOP. There is ample evidence now that the stromal swelling pressure is present in vivo and has a value, at least under normal conditions, similar to that measured in vitro; the hydrogel studies of Klyce and Dohlman (1967) may be cited here. The swelling pressure is measured as the mechanical force (Hedbys and Dohlman, 1963) required to hold the stroma at a given hydration. The collagen fibers at the posterior stroma must be slack to permit endothelial motion in either direction, so this restraining force must be applied to the stroma by the endothelium. The endothelial mechanical equilibrium condition is thus $HP + SP_p = IOP$, where SP_p is the swelling pressure of the stroma at its posterior hydration and HP is a properly defined hydrostatic pressure at the anterior endothelial surface. The correct definition of HP is best elucidated by a "thought" experiment, Fig. 1. The mechanical equilibrium described above is shown in Fig. 1 *a*. In Fig. 1 *b*, a porous spacer has been placed between the stroma and endothelium. Stroma cannot enter the spacer and the endothelium is obviously still in equilibrium. The force exerted by the stromal swelling pressure is transmitted to the endothelium by the thin columns in the spacer. The proper hydrostatic pressure for the statement of endothelial mechanical equilibrium is seen to be that of the fluid within the spacer, that is, the hydrostatic pressure of a solution locally in thermodynamic equilibrium with the adjacent stroma. This is a pure solution hydrostatic pressure in the same sense as the IOP and will be termed *P*. It can be shown that this pressure is also the proper one to use in describing solvent flow through the

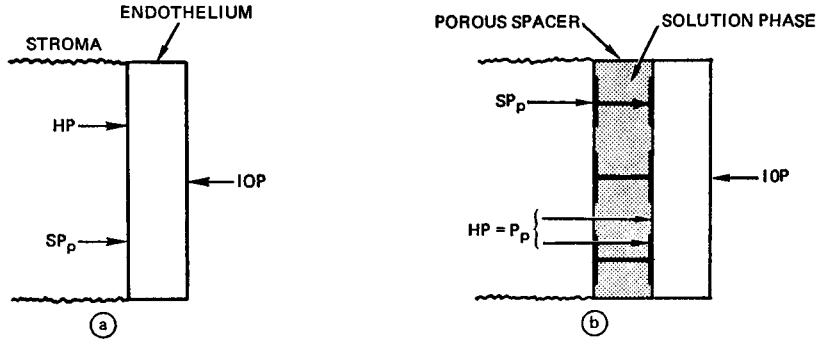

FIGURE 1 Endothelial equilibrium condition. IOP, intraocular pressure; SP_p, swelling pressure at posterior stroma; HP, consistent hydrostatic pressure at anterior endothelium.

corneal membranes. The endothelial mechanical equilibrium condition is, therefore,

$$P_p + SP_p = IOP. \tag{1}$$

This condition explains the "imbibition pressure" of Hedbys et al. (1963) and its dependence on the swelling and intraocular pressures as determined in vivo by these authors. When a fine needle is inserted into the stroma, the tissue cannot enter and the local P is measured. Thus the imbibition pressure is precisely the hydrostatic pressure of a pure solution phase in local thermodynamic equilibrium with the stroma at the tip of the cannula. We shall not at this point discuss the gradients of P within the stroma, but the value of P measured near the endothelium (P_p) is given by equation 1. Indeed, an equation almost identical with 1 was derived by Hedbys et al., but through less physically precise concepts, such as interstitial fluid pressure and structural framework tissue pressure. The agreement between equation 1 and the experiments of Hedbys et al. supports the thesis that the in vivo endothelium transmits no important stresses to the limbus, either directly or via the collagen of Descemet's membrane or the posterior stroma.

In discussing the mechanics of the stroma, it is convenient to consider a differential thickness of this tissue, for which a force balance gives

$$dP + d(SP) + dT = 0, \tag{2}$$

where T refers to that portion of the pressure head across the tissue element which is transmitted to the limbus through collagen fibrils in tension. The condition 1 on the endothelium is based on the observation that the endothelium can move forward from its normal position, so the posteriormost collagen fibers are slack: $T_p = 0$. On the other hand, the fibrils immediately behind the epithelium are most likely in tension, as will be discussed later. Integrating equation 2 from the posterior of the stroma to some point within it, $P = P_p - SP + SP_p - T$. Since the hydration gradients in

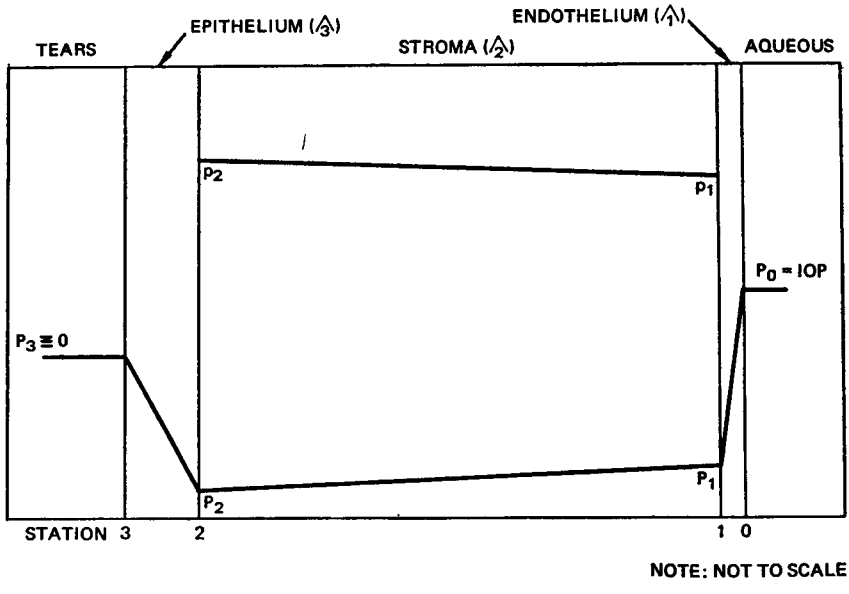

FIGURE 2 Pressures in the in vivo cornea. p, swelling pressure. Stromal pressure gradients are not strictly linear and are exaggerated for clarity.

the stroma are small, SP $\approx$ SP$_p$, and $P \approx P_p - T$.[1] Hedbys et al. (1963) found no significant variation of P with depth through the stroma, so it is likely that T is near zero except near the epithelium; that is, through most of the stroma, no load is transmitted to the limbus and the stromal elements "float" in the same sense as the endothelium: $dP + d(SP) = 0$.

Since neither the endothelium nor most of the stroma transmit any load to a structure external to the cornea, the force of the IOP must be taken up via the epithelium and the anteriormost stromal layers. The blebbing (Green, 1969) and edema (for instance, Ytteborg and Dohlman, 1965 *b*) of the epithelium when the IOP is elevated can be taken as indirect support of this conclusion. The load induced by the IOP is normally taken up by the anteroposterior component of the tension in the collagen fibers in the anterior segment of the stroma. The thickness of fibers required to support the load depends on the magnitude of the IOP, but need not be large under normal conditions. According to Maurice (1969), Descemet's membrane, less than 10 μ thick, "is probably not equal in tensile strength to a layer of stromal lamellae of the same thickness, [but] is able by itself to bear the intraocular pressure." In subse-

[1] It may be noted that there is some evidence (Kikkawa and Hirayama, 1970) that the swelling pressure–hydration relation of the stroma is not uniform throughout. The swelling pressure of the anterior segment would appear to be less than that posteriorly, in the normal cornea. When the swelling pressure–hydration curve of entire stroma is measured on excised tissue, average values are in fact obtained. The use of these values for SP is tantamount to assuming that most of the stroma is uniform in this regard. That segment of the stroma which appears to possess a below average swelling pressure may be the portion wherein the load imposed by the IOP is transmitted to the limbus.

quent discussion, when the epithelium-stroma interface is referred to, this interface more properly corresponds to that plane in the stroma which separates fibrils in tension from those which are not. This plane may be the rear of Bowman's layer, in those species possessing one.

The mechanical model of the cornea described above is shown in Fig. 2 with the notation that will be employed below. The pressure distribution is very similar to that given by Ytteborg and Dohlman (1965 *b*), if their fluid pressure, defined by them as that in the stromal interstices, is understood to mean the equilibrium hydrostatic pressure P. This would appear to be a proper substitution inasmuch as Ytteborg and Dohlman later identify the fluid pressure with the measurable imbibition pressure. The present model differs from that of the earlier workers with respect to the origin of the pressure drop across the endothelium and the character of the forces at the anterior region. In addition, the pressure gradients are here sited within the corneal membranes rather than in adjacent stoma.

GOVERNING EQUATIONS

In this section equations for the coupled flows of salt and water through the living cornea will be derived in terms of the properties of the corneal layers, and the mechanical equilibrium conditions discussed above will be presented in consistent terms. The flow relations are based on the frictional formulation of the equations of irreversible thermodynamics; this formulation is briefly reviewed below.

Underlying Thermodynamics

We start with Kedem and Katchalsky's (1961) force balance:

$$-\frac{d\tilde{\mu}_j}{dx} = \sum_{k \neq j} f_{jk}(v_j - v_k), \tag{3}$$

where $\tilde{\mu}_j$ is the electrochemical potential of the jth species, x is distance through the membrane, f_{jk} is a friction coefficient measuring the drag on a mole of j caused by a flow of the kth species relative to the jth, and v_j = species velocity = J_j/C_j, where J is transmembrane flux and C is concentration at x, per unit volume membrane. The index j includes Na^+ (subscript +), Cl^- (subscript −), and water (subscript 0); k includes these three species and the membrane (subscript m) whose velocity is set equal to zero. The Onsager reciprocal relation applied to the friction coefficients gives $f_{jk}/C_k = f_{kj}/C_j$.

The system of interest contains three species and thus is defined by six independent phenomenological coefficients. These are reduced for simplicity to three by assuming $f_{+-} = 0, f_{-m} = Kf_{+m}$, and $f_{-0} = Kf_{+0}$, where K is a membrane property. The small effect of the direct interactions measured by f_{+-} on solute flow through the epithelium and endothelium has been noted elsewhere (Friedman, 1971 *a*). The assumption $f_{-m}/f_{+m} = f_{-0}/f_{+0}$ is supported by the similarity of the ratios of the membrane permea-

bilities to sodium and chloride (ω_{Na}/ω_{Cl}) to the ratio of their ion mobilities in NaCl solutions. Equation 3 becomes, for the ions

$$-\frac{d\tilde{\mu}_j}{dx} = \frac{J_j f_{jT}}{C_j} - \frac{J_0 f_{j0}}{C_0} \qquad (j = +, -), \tag{4 a}$$

where $f_{jT} = f_{j0} + f_{jm}$; for water

$$-\frac{d\mu_0}{dx} = \frac{1}{C_0}\sum_{j\neq 0} f_{j0}\left(\frac{J_0 C_j}{C_0} - J_j\right) + \frac{J_0 f_{0m}}{C_0}, \tag{4 b}$$

where reciprocity has been used.

In the steady state, the fluxes through the membrane are independent of x, and the friction coefficients are constant, averaged across the membrane. The concentration gradients across the cornea are generally small, so the right-hand sides of equations 4 are nearly constant. Since the electrochemical potentials of the mobile species are continuous at the membrane surfaces, the left-hand sides may be replaced by

$$-\frac{\Delta\tilde{\mu}_j}{\Delta x} = -\frac{1}{\Delta x}\left(\frac{RT\Delta c_j}{\bar{c}_j} + Z_j F\Delta\psi\right) \qquad (j = +, -), \tag{5 a}$$

$$-\frac{\Delta\mu_0}{\Delta x} = -\frac{\bar{V}_0}{\Delta x}(\Delta P - RT\sum_{j=+,-}\Delta c_j - RT\Delta c_I), \tag{5 b}$$

where Δx is membrane thickness, $\bar{V}$ is specific volume, Z_j is ionic charge; R, T, and F have their usual meanings; and the hydrostatic pressure P, the species concentration c_j (and an appropriately averaged concentration $\bar{c}_j$), the electrostatic potential ψ, and the concentration of impermeant c_I are measured in an equilibrium free solution adjacent to the membrane surfaces. In writing equation 5 *a*, the effect of pressure on the electrochemical potential of the ions is neglected, an assumption which has been justified a posteriori. Since c is a free solution variable, $c_+ = c_- \equiv c_s$, $\Delta c_+ = \Delta c_- = \Delta c_s$, and it is assumed that $\bar{c}_+ = \bar{c}_- = \bar{c}_s$.

Flow Equations: Limiting Membranes

Equation 5 *a* is used to construct the sum $-(\Delta\tilde{\mu}_+ + \Delta\tilde{\mu}_-)/\Delta x$, from which the electrostatic term is absent. This sum is equated to a similar sum of the right-hand side of equation 4 *a*, to give

$$-\frac{2RT\Delta c_s}{\bar{c}_s\Delta x} = \left(\frac{J_+^p}{C_+} + \frac{KJ_-^p}{C_-}\right)f_{+T} - (1 + K)\frac{f_{+0}J_0^p}{C_0},$$

where it is assumed that the frictional interactions defined by equation 3 take place in a common "passive" channel occupied by salt and water. The fluxes through this channel are denoted by the superscript p.

The concentration C_j is defined per unit volume membrane and it is necessary to relate it to c_j, the concentration in an equilibrium free solution. Passive flow of ions through the corneal endothelium is thought to be via intercellular pores (Maurice, 1961). A calculation of endothelial hydraulic conductivity based on fluid flow through the same network gives a value one-third of that deduced by Mishima and Hedbys (1967) from perfusion experiments on enucleated eyes; this has led Maurice (1969) to conclude that some water may flow through the endothelial cells. However, Green and Green (1969) also measured the endothelial flow conductivity, using isolated preparations, and found a value considerably less than that reported by Mishima and Hedbys, so the assumption that the passive flow of ions and water is exclusively through the same intercellular spaces is at least not unreasonable. Even less evidence is available regarding the pathways for flow through the epithelium and a similar assumption will be made for this tissue. Some support for this assumption is presented in Maurice (1951). In mathematical terms, the assumption made is that $C_j/c_j = \varphi_k (j = +, -, 0; \varphi_k$ constant) in the kth membrane.

The friction coefficients are next redefined in terms of φ_k : for the kth membrane, f_{+T}, f_{+0}, and f_{0m} are replaced by $\varphi_k f_{Tk}$, $\varphi_k f_{+k}$, and $\varphi_k f_{0k}$, respectively. Because the solutions bounding the cornea differ little in concentration, C_+ and C_- may be replaced by $\varphi_k \bar{c}_s$ in the right side of the preceding equation; because the solutions are dilute, C_0^{-1} may be replaced by $\bar{V}_0/\varphi_k$. Then,

$$-\frac{2RT\Delta c_{s(k)}}{\bar{c}_{sk}\Delta x_k} = (J^p_{+k} + K_k J^p_{-k})\frac{f_{Tk}}{\bar{c}_{sk}} - (1 + K_k) f_{+k} J^p_{0k} \bar{V}_0,$$

where $\Delta c_{s(k)}$ is the concentration difference across the kth membrane, $\bar{c}_{sk}$ is the average equilibrium free solution concentration within the kth membrane, and (for instance) J^p_{+k} is the passive sodium flux through the kth membrane.

The flow equations to be used here allow for the inclusion of active transport of cations (J^a_{+k}) or water (J^a_{0k})[2] through either the epithelium or endothelium; active chloride transport could be included as well at the price of somewhat greater complexity. The corneal current is zero (Maurice, 1969); thus $J^a_{+k} + J^p_{+k} = J^p_{-k}$. In the absence of active chloride transport, J^p_{-1} (chloride flux across endothelium) $= J^p_{-3}$ (chloride flux across epithelium) $= J_s$, the steady-state salt flux through the cornea. Similarly, $J^p_{0k} + J^a_{0k} = J_0$, the steady-state water flux through the cornea. The preceding equation may be written as

$$-\frac{2RT\Delta c_{s(k)}}{\bar{c}_{sk}\Delta x_k} = (K_{sk} J_s - J^a_{+k})\frac{f_{Tk}}{\bar{c}_{sk}} - K_{sk} f_{+k} \bar{V}_0 (J_0 - J^a_{0k}), \qquad (6)$$

where $K_{sk} = 1 + K_k$. The left-hand side of equation 6 represents the driving force

[2] J^a_{0k} is that portion of the water flux across the kth membrane which is not described by the passive flow equations. No implication that water itself is the substrate of the relevant active transport process is intended.

for salt diffusion, balanced by two terms on the right-hand side, which correspond to diffusional resistance and convective drag, respectively.

A similar treatment of equations 4 *b* and 5 *b*, using equation 6 for $\Delta c_{s(k)}$, gives

$$-\frac{\Delta P_{(k)}}{\Delta x_k} = -\frac{RT\Delta c_{I(k)}}{\Delta x_k} + (f_{Tk} - f_{+k})(K_{sk} J_s - J^a_{+k}) + f_{0k}(J_0 - J^a_{0k}), \quad (7)$$

where $\Delta P_{(k)}$ is the pressure drop across the *k*th membrane. The pressure gradient equals the sum of three terms: the first is a consequence of the requirement that the solvent chemical potential be continuous at the membrane surface; for a pore model membrane, this term corrects the pressure drop $\Delta P_{(k)}$ between free, impermeant-containing solutions outside the membrane to the pressure difference between free solutions in equilibrium with the pore liquid at the membrane boundaries (see, for instance, Dick, 1967). The remaining terms on the right-hand side of equation 7 correspond to membrane drag on the solute and the solvent, respectively.

Mechanical Equilibrium: Endothelium and Stroma

In terms of the notation used in the body of the paper, equation 1 is

$$P_1 + p_1 = P_0 . \quad (8)$$

For the stroma, equation 2 is integrated with $dT = 0$ to give

$$p_1 + P_1 = p_2 + P_2 . \quad (9)$$

Flow Equations: Stroma

The reflection coefficient to NaCl of the highly permeable stroma is near zero, so fluid flow through this structure is driven by the hydrostatic pressure gradient across it. By equation 9, this demands a trans-stromal swelling pressure gradient and hence a hydration gradient. Since the flow conductivity of the stroma and its salt diffusion constant are both hydration dependent, a differential analysis is indicated. Vectorial active transport systems are absent from the stroma, so equation 7 can be written in differential form as

$$-\frac{dP}{dx} = (f_{T2} - f_{+2})K_{s2} J_s + f_{02} J_0 . \quad (10)$$

Equations 4 may be used to describe a reflection coefficient experiment, in which the osmotic effect of NaCl at a concentration c^a_s is balanced across a membrane by that of an impermeant at a concentration c^b_I. The reflection coefficient of the membrane for the salt is found to be

$$\sigma = \frac{c^b_I}{2c^a_s} = \frac{1}{f_T}\left(f_T - f_+ - \frac{\bar{V}_s f_0}{K_s \bar{V}_0}\right). \quad (11)$$

When $\sigma = 0$, as for the stroma, $f_{T2} - f_{+2} = \bar{V}_s f_{02}/(K_{s2}\bar{V}_0)$. The value of f_{02} can be related to the stromal flow conductivity (k/η) by comparing equation 10 with Darcy's law for pure solvent flow: $J_0 = -f_{02}^{-1}(dP/dx) = -\bar{V}_0^{-1}(k/\eta)(dP/dx)$. Substituting for $(f_{T2} - f_{+2})$ and f_{02}, equation 10 becomes $-dP/dx = (k/\eta)^{-1}(J_s\bar{V}_s + J_0\bar{V}_0)$. Equation 2 describes the equilibrium condition for a differential stromal element; with $dT = 0$, $dP/dx + (dp/dH)(dH/dx) = 0$, where p is regarded as a function of only the hydration, H grams water per gram dry tissue. Thus

$$\frac{dH}{dx} = (dp/dH)^{-1}(k/\eta)^{-1}(J_s\bar{V}_s + J_0\bar{V}_0). \tag{12}$$

Since dp/dH and k/η are known functions of H, equation 12 can in principle be integrated from $H(x_1) = H_1$ to $H(x_2) = H_2$ (see Fig. 2 for station numbers). However, the stromal thickness $x_2 - x_1$ is itself a function of the hydration profile in the tissue, and it is convenient to replace x by a Lagrangian coordinate ψ (Fatt and Goldstick, 1965; Friedman, 1971 *b*) tied to the dry tissue. The two space coordinates are related by $dx = (\epsilon + H)\, d\psi/\epsilon$, where ϵ is the ratio of the density of the stromal fluid to that of the dry tissue. The integrated form of equation 12 is

$$\epsilon \int_{H_1}^{H_2} (\epsilon + H)^{-1} \frac{dp}{dH}\left(\frac{k}{\eta}\right) dH = (J_s\bar{V}_s + J_0\bar{V}_0)\psi_2,$$

where ψ_2 is the thickness of dry stroma.

Analytic expressions for $p(H)$ and $k(H)/\eta$ are available for substitution into the preceding equation. The swelling pressure relation can be described for steer (Fatt and Goldstick, 1965) and rabbit (Friedman and Green, 1971 *a*) as an exponential function: $p = \gamma \exp(-\beta H)$, where γ and β are species-dependent constants; thus $dp/dH = -\beta\gamma \exp(-\beta H)$. The stromal permeability can be correlated by (Friedman, 1971 *b*) $k/\eta = C_1H^3/(H + \epsilon)$, where C_1 is a species-dependent constant. Equation 12 then integrates to

$$\frac{(J_s\bar{V}_s + J_0\bar{V}_0)\psi_2}{C_1\epsilon} = \left\{\frac{\gamma}{\beta}e^{-\beta H}\left[2\beta\epsilon - \beta H - 1 + \frac{\beta^2\epsilon^3}{H + \epsilon}\right] + \gamma\beta\epsilon^2(3 + \beta\epsilon)e^{\epsilon\beta}Ei[-\beta(H + \epsilon)]\right\}_{H=H_2}^{H=H_1}. \tag{13}$$

The concentration equation 6 similarly becomes

$$-\frac{2RT}{c_s}\cdot\frac{dc_s}{dH}\cdot\frac{dH}{dx} = f_{+2}K_{s2}\left(\frac{J_s}{c_s} - J_0\bar{V}_0\right) + \frac{J_s\bar{V}_s}{c_s}\cdot\left(\frac{k}{\eta}\right)^{-1},$$

where the zero reflection coefficient of the stroma has been used to eliminate f_{T2}, and f_{02} has been expressed in terms of flow conductivity.

The diffusional resistance of the stroma is measured by f_{+2} and depends on H.

Since the stroma is an open structure, it seems reasonable that f_{+2} can be approximated by f_+^0/φ_2, where f_+^0 measures the resistance to sodium diffusion in pure saline and φ_2 is the porosity of the stroma. In terms of the variables used here, $\varphi_2 = H/(H + \epsilon)$. This formulation gives a salt diffusivity in the stroma somewhat larger than that found by Maurice (1961). Using equation 12 for dH/dx, the concentration equation for the stroma becomes, upon rearrangement,

$$-2RT(J_s \bar{V}_s + J_0 \bar{V}_0)\, dc_s = \left(\frac{dp}{dH}\right)\left[\frac{(H + \epsilon)(k/\eta)f_+^0 K_{s2}}{H}(J_s - J_0 \bar{V}_0 c_s) + J_s \bar{V}_s\right] dH.$$

This equation can be solved exactly, but the solution is simpler if the smallness of the transcorneal concentration difference is again invoked to replace c_s on the right-hand side by an average value $\bar{c}_{s2}$; integration, using the above forms of $p(H)$ and $k(H)/\eta$, yields

$$\frac{2RT(J_s \bar{V}_s + J_0 \bar{V}_0)(c_{s2} - c_{s1})}{C_1 \gamma} = \left\{e^{-\beta H}\left[\frac{f_+^0 K_{s2}}{\beta^2}(J_s - J_0 \bar{V}_0 \bar{c}_{s2})(\beta^2 H^2 + 2\beta H + 2) + \frac{J_s \bar{V}_s}{C_1}\right]\right\}_{H=H_2}^{H=H_1}. \quad (14)$$

Summary

In summary, eight equations have been derived to describe the living cornea in the steady state:

(*a*), (*b*) Concentration equations 6 across the endothelium and epithelium. Here and below, $\bar{c}_{sk} \equiv (c_{sk} + c_{s,k-1})/2$, (note that k on the left-hand side of the definition of $\bar{c}_{sk}$ is a membrane index; k on the right-hand side is a station index). $\Delta c_{s(k)} = c_{sk} - c_{s,k-1}$.

(*c*), (*d*) Pressure equations 7 across the endothelium and epithelium. $\Delta P_{(k)} = P_k - P_{k-1}$. $\Delta c_{I(k)} = c_{Ik} - c_{I,k-1}$, with $c_{I1} = c_{I2} = 0$.

(*e*) Endothelial mechanical equilibrium condition 8, with $p_1 = \gamma \exp(-\beta H_1)$.

(*f*) Stromal mechanical equilibrium condition 9, with $p_k = \gamma \exp(-\beta H_k)$.

(*g*) Stromal flow equation 13.

(*h*) Stromal concentration equation 14.

These eight equations can then be solved for the eight unknowns J_s, J_0, c_{s1}, c_{s2}, H_1, H_2, P_1, and P_2.

BASE CASE: NORMAL CORNEA

Base Case Parameters

The parameters required to define the properties of the normal rabbit cornea are given, referenced, and occasionally discussed below. They can be divided into three

broad categories: environmental, dimensional, and transport. The number of significant figures used below do not necessarily reflect the accuracy to which the parameters are known; the "insignificant" digits are introduced upon conversion of the values of these parameters to consistent units.

Environmental

RT = 2.58×10^3 J/mole, corresponding to a (slightly high) corneal temperature of 37°C.

P_0 = 2.67×10^{-3} J/cc = 20 Torr, a nominal IOP.

P_3 = 0, gauge atmospheric.

c_{s0} = 1.49×10^{-4} moles/cc. The average cation content of rabbit aqueous, as summarized in Otori (1968), is 149 meq/liter, of which 144 meq/liter is Na^+. Of the 149 meq/liter of anions in the anterior chamber, only 105 meq/liter is Cl^- (Kinsey, 1953). Since the only permeating solutes in the model are Na^+ and Cl^-, the indicated value of c_{s0} was used to represent more fairly the osmolarity of the anterior aqueous (301 milliosmols/liter; Levene, 1959), at the price of underestimating the passive chloride flux through the cornea.

c_{s3} = 1.77×10^{-4} moles/cc. A weighted average between the open-eye value of 183 mM (Iwata et al., 1969) and the closed-eye value, presumably that of plasma.

$c_{I0} = c_{I3}$ = 0. These terms are not needed for the base case but may be used to study the effect of adulteration of tears or aqueous for experimental or therapeutic purposes.

Dimensional

$\bar{V}_0$ = 18 cc/mole.

$\bar{V}_s$ = 17 cc/mole (cited in Barry and Hope, 1969).

ϵ = 0.72 (Hedbys and Mishima, 1966). Hedbys and Mishima's value of ϵ is for entire cornea; the use of this value to describe stroma is justified in Friedman and Green (1971 *a*).

ψ_2 = 6.05×10^{-3} cm. This value is obtained by adjusting Hedbys and Mishima's (1966) experimental thickness-hydration line for rabbit cornea to give a hydration intercept of $-\epsilon$ and subtracting the dry thickness (Adler, 1965; Trenberth and Mishima, 1968; Maurice, 1969) of the cellular layers of the cornea.

γ = 0.1181 J/cc = 886 Torr; β = 0.809. Fit to Hedbys and Dohlman's (1963) data.

Δx_1 = 5×10^{-4} cm (Maurice, 1969).

Δx_3 = 4×10^{-3} cm (Trenberth and Mishima, 1968; Maurice, 1969).

Transport

C_1 = 2.53×10^{-6} cm⁵/sec-J. This value of C_1 gives a flow conductivity–hydration relation falling between the data of Fatt (1968) and Friedman and Green (1971 *a*).

f_+^0 = 1.51×10^8 J-sec/mole-cm². This property is related to the diffusion constant D^o of NaCl in water by $f_+^0 = 2RT/(K_s^o D^o)$; D^o is from Perry (1950) and K_s^o from limiting mobilities at infinite dilution (Edsall and Wyman, 1958).

f_{01} = 2.17×10^9 J-sec/mole-cm², f_{03} = 4.92×10^8 J-sec/mole-cm². From Green and Green's (1969) values of hydraulic conductivity $L_{p,k}$, and f_{Tk} and f_{+k} below, using $f_{0k} = \bar{V}_0[f_{Tk} - L_{p,k}\Delta x_k K_{sk} c_R f_{+k}(f_{Tk} - f_{+k})]/(L_{p,k} f_{Tk} \Delta x_k)$, where c_R is the concentration of Ringer's solution used in the measurement of $L_{p,k}$. These values are larger than

those deduced by Mishima and Hedbys (1967) from unsteady corneal thinning experiments and are preferred for the time being because they were obtained more directly.

f_{T1} = 4.06×10^{11} J-sec/mole-cm², from Maurice's (1961) value of the obstruction of the endothelium to diffusion.

f_{T3} = 7.94×10^{11} J-sec/mole-cm² (Green, 1967). This value has been claimed by Maurice (1967) to be an order of magnitude too low, but Maurice's measurements (1955) and their interpretation were made before the epithelial sodium pump had been discovered.

f_{+1} = 2.43×10^{11} J-sec/mole-cm², $f_{+3} = 1.58 \times 10^{11}$ J-sec/mole-cm². From Green and Green's (1969) values of reflection coefficient, using equation 11. Green and Green's reflection coefficients are somewhat lower than Mishima and Hedbys' (1967), but are preferred for the time being for consistency with f_{0k}.

K_{s1} = 1.66. The obstruction of the endothelium to small cations is the same as that to small anions (Maurice, 1961), so K_{s1} was found from limiting ion mobilities at infinite dilution (Edsall and Wyman, 1958).

K_{s2} = 1.81. Similar to K_{s1}, but corrected for the somewhat larger obstruction of the stroma to small anions than to small cations (Maurice, 1961). This apparent dependence of stromal obstruction on ionic charge may result from a mild Donnan exclusion effect of the free acid sites (Catchpole et al., 1966; Otori, 1967; Friedman and Green, 1971 *b*) in the stroma. The influence of stromal charge is otherwise neglected, except insofar as the Donnan osmotic pressure contributes to the stromal swelling pressure, an effect "buried" in Hedbys and Dohlman's (1963) measurements.

K_{s3} = 1.84, from f_{T3} and Green's (1966) measurements of unidirectional chloride flux.

J^c_{+1} = 0, in the absence of any explicit evidence to the contrary.

J^c_{+3} = -1.2×10^{-10} eq/cm²-sec, from the short-circuit current of rabbit cornea in normal Ringer (Green, 1968), taking the series membrane character of the cornea into account (Friedman, 1971 *a*). Fluxes directed anteriorly are positive.

J^c_{01} = 0, for the base case. The implications of an endothelial "water pump" will be discussed in part II.

J^c_{03} = 0, for the base case. The implications of the epithelial water pump reported by Green and Green (1969) will be discussed in part II.

Base Case Results and Comparison with Living Tissue

The solution of equations 6–9, 13, and 14 using the parameters given above is presented graphically in Fig. 3. The cornea so calculated possesses many features in common with living cornea. Several of these will now be discussed.

Most important, the calculated corneal thickness is 376 μ and the average stromal hydration is 3.22 (76.3% water), in the experimental range for rabbit. This value is obtained without including any active transport system except the epithelial sodium pump. For this base case, the maintenance of normal hydration against some 60 Torr of swelling pressure can be viewed most clearly in terms of the continuity of solvent flow. The reflection coefficient and resistance to salt diffusion of the epithelium are substantially greater than those of the endothelium. As a consequence, the salt concentration difference across the endothelium is smaller than that across the epithelium, and the osmotic effect of this difference, "drawing" water anteriorly, is smaller still; but the water flow across each membrane must be the same in the

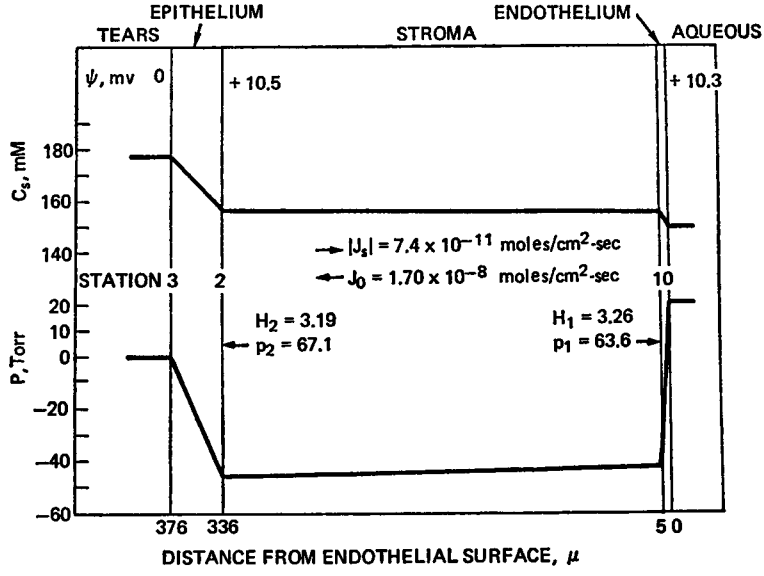

FIGURE 3 Calculated behavior of normal cornea. ψ, potential relative to tear side. Concentration and pressure profiles are drawn as linear between known values of c_s and P at stations 0–3.

steady state. The difference between the osmotically induced flows across the epithelium and endothelium must be compensated for hydraulically. The IOP is insufficient for this purpose. Since P_0 and P_3 are fixed, only the stromal hydrostatic pressure can change to accomplish this compensation. Naturally, it falls, thereby enhancing endothelial water flow and opposing the outward osmotic flux across the epithelium. For the base case, the stromal hydrostatic pressure falls to an average gauge value of approximately −45 Torr (the imbibition pressure). Consequently, there is a 65 Torr, anteriorly directed hydrostatic pressure difference across the endothelium. For the endothelium to be stationary, this pressure drop must be balanced by an equal and opposite mechanical pressure which is delivered by the stroma. The stroma therefore assumes a hydration such that its swelling pressure is approximately 65 Torr. Or viewed in another way, the hydrostatic pressure difference across the endothelium pushes it anteriorly, compressing the stroma (whose anterior elements are already at their furthest forward travel). As the stroma is compressed, its swelling pressure rises to oppose the force imposed on it by the endothelium, and the endothelium ceases to move forward when the stroma pushes back with equal force.

Because of the low resistance of the stroma to flow and diffusion, the stromal gradients of c_s, H, p, and P are small.

The transmembrane potentials are calculated from equations 4 *a* and 5 *a*. In the preceding derivation, these equations were evaluated for Na^+ and Cl^- and the results were summed to eliminate the electrostatic term. Here, $\Delta\psi$ is sought, so the equations are evaluated for only one ion. Chloride is selected because there is no active trans-

this ion in the present formulation: $J_s^p = J_s$. Solving for $\Delta\psi$,

$$= \frac{1}{F}\left\{[K_{sk} - 1]\Delta x_k \left[\frac{J_s f_{Tk}}{\bar{c}_{sk}} - (J_0 - J_{0k}^a)\bar{V}_0 f_{+k}\right] + \frac{RT\Delta c_{s(k)}}{\bar{c}_{sk}}\right\} = \psi_k - \psi_{k-1}.$$

this equation is applied to the base case results, $\Delta\psi_{(1)} = +0.2$ mv and $\Delta\psi_{(3)} =$ mv. The stromal diffusion potential is only a few microvolts. The endothelial of the base case cornea is thus 10.3 mv positive, relative to the tears.

in vivo transcorneal potential measured by Maurice (1967) is about twice lculated here, and the difference is no doubt related to the epithelial properties r the base case. It will be shown in part II that, among the three epithelial fric- efficients, the epithelial potential depends strongly on only f_{Ts}. Thus it seems aurice's (1967) reservations regarding Green's (1967) epithelial sodium per- ity measurements are probably justified to some extent.

anteriorly directed solvent flow rate for the base case is 1.7×10^{-8} moles/ . Mishima and Maurice (1961) replaced the aqueous in proptosed rabbit eyes raffin and from the rate of the subsequent corneal thinning deduced a value of 10^{-8} moles/cm²-sec. In view of the difference between the anterior and pos- oundary conditions in the present work and that of Mishima and Maurice, eement between the value of J_0 given here and that obtained by them is prob- worse than might be expected.

average stromal salt concentration $(c_{s1} + c_{s2})/2$ is 156 mM. According to the on of c_s, this is the concentration of a free saline solution in equilibrium with e stromal fluid. The stroma is negatively charged and the composition of the l fluid must be found from a Donnan calculation. Friedman and Green) showed that the stroma binds sodium but does not bind chloride from NaCl ns and that at normal thickness the stromal free charge density is 14 meq/liter l fluid. If the stroma binds no anions (recall that Cl^- represents all the anions cornea and its environment), then the true anion content of the stroma should $- 14/2 = 149$ meq/liter stromal fluid, the same as that of the aqueous. This ation agrees with Otori's (1967, 1968) experimental results which showed the e contents of the stroma and aqueous to be essentially identical. The small n exclusion of chloride from the stromal interstitium is balanced by the excess rolyte needed to drive salt across the endothelium.

CONCLUSIONS

lient conclusions and observations regarding in vivo corneal behavior which een made here are summarized below.

Corneal swelling or thinning is effected by motion of the endothelium relative

to the orb; the epithelium does not generally move (Ytteborg and Dohlman, 1965 *a*). This is shown here to be a consequence of the mechanics of the cornea and has no implications with respect to the role of the endothelium in corneal hydration control. Since the epithelium is permeable to water, fluid fluxes through the endothelium are not simply related to changes in stromal hydration. When the cornea (say) thins, the endothelium moves forward, but it is incorrect to say that the "lost" stromal fluid has crossed the endothelium. Indeed, the fluid flow through the endothelium may be anteriorly directed throughout the thinning process.

(*b*) The corneal endothelium and most of the stroma (excluding the anteriormost lamellae) "float" in mechanical equilibrium and transmit no load to the limbus.

(*c*) The load imposed on the cornea by the IOP is normally taken up by the collagen fibrils of the anteriormost layers of the stroma; the load can be taken up differently when the epithelium is edematous.

(*d*) The imbibition pressure at any point in the stroma is the hydrostatic pressure of a pure solution phase in equilibrium with the stroma at that point. The equation of Hedbys et al. (1963) for imbibition pressure, applied at the posterior stroma, is equivalent to the mechanical equilibrium condition of the endothelium. The swelling pressure–hydration relation of the in vivo cornea is the same as that in vitro, as indicated by Hedbys et al.

(*e*) A physical description of the in vivo cornea, regarding the epithelium and endothelium as leaky semipermeable membranes, provides good agreement with experiment without requiring any active transport system to be sited in the endothelium. Normal hydration is achieved because in the time-average steady state the principally hydraulic water flow across the endothelium, driven by a negative imbibition pressure, must equal the osmotic flow across the epithelium, driven by hypertonic tears.

(*f*) The corneal properties and boundary conditions define an equilibrium stromal swelling pressure, and the stroma assumes a hydration such that this pressure obtains. The swelling pressure rather than the hydration is the paramount dependent variable of the analysis because the stromal resistance to water and salt flow is trivial at any realistic hydration.

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