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

II. VARIATIONS FROM THE NORMAL STATE

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ABSTRACT The description of corneal mechanics and transport developed in part I and used there to describe normal corneal behavior is here applied to corneas whose properties or boundary conditions are abnormal. The predicted effects of changing intraocular pressure, aqueous concentration, and tear tonicity are examined, and these compare favorably with available experimental data. The periodic variation in tear tonicity which accompanies the sleep-wake cycle prevents the cornea from achieving a true steady state, but a time-average steady state, about which corneal behavior oscillates, can be defined. The in vivo effects of endothelial dystrophy and epithelial removal are explained, and it is suggested that the epithelial sodium pump may act homeostatically to maintain corneal thickness in the face of ambient temperature variations. Part II concludes with a discussion, from the standpoint of the present theory, of the role of metabolically coupled water transport in the maintenance of the normal corneal thickness.

INTRODUCTION

In part I of this paper, a description of corneal mechanics and transport was developed and shown to describe quite well the behavior of normal rabbit cornea. If, indeed, this theory is correct, it should predict correctly not only normal behavior but also the fashion in which the cornea responds to changes in the state of the solutions which bathe it and to changes in the transport properties of the corneal layers themselves. There is a fair amount of experimental data on the effects of such variations from the normal state, and most of this part is devoted to a comparison of these data with the theory. Since the theory was developed in part I to describe the in vivo cornea, only in vivo experiments are used here to test it. In this way, it is hoped, the comparison between theory and experiment which is sought will not be clouded by possible in vitro artifacts.

After a simplifying linearization of the governing equations derived in part I, the departures from normalcy are divided into two categories: *independent variable* effects (intraocular pressure, aqueous tonicity, tear tonicity), which represent changes in the corneal boundary conditions; and *exogenous/traumatic* effects (endothelial trauma, epithelial removal, temperature effects, poisons), which represent changes in the corneal properties.

There is some amount of in vitro evidence that either or both of the limiting corneal membranes may exhibit metabolically coupled water transport. Though such transport is not included among the normal corneal parameters in part I and indeed does not appear to be necessary to the maintenance of normal hydration, the analytic framework presented here can be used to assess the significance of such "water pumps" as have been proposed. This is done to conclude part II.

NOTATION

Those terms defined in part I and used in part II are listed below:

c_{Ik} Impermeant concentration at the kth station, $c_{I1} = c_{I2} = 0$.

c_{sk} Salt concentration at the kth station.

$\bar{c}_{sk}$ Mean salt concentration in the kth membrane $= (c_{sk} + c_{s,k-1})/2$.

$\Delta c_{s(k)}$ Salt concentration difference across the kth membrane $= c_{sk} - c_{s,k-1}$; $\Delta c_{I(k)}$ defined similarly.

f_{Tk} Frictional coefficient measuring solvent and membrane drag on sodium in the kth membrane.

f_{+k} Frictional coefficient measuring solvent drag on sodium in the kth membrane.

f_{0k} Frictional coefficient measuring membrane drag on solvent in the kth membrane.

H Stromal hydration.

J_0 Transcorneal fluid flux (anteriorly directed is positive).

J_s Transcorneal salt flux (anteriorly directed is positive).

J^a_{+k} Flux of actively transported sodium in the kth membrane (anteriorly directed is positive).

J^a_{0k} Flux of metabolically "pumped" solvent in the kth membrane (anteriorly directed is positive).

K_{sk} Parameter measuring the ratio of solvent and membrane drag on chloride (generic anion) to that on sodium in the kth membrane.

$L_{p,k}$ Hydraulic conductivity of the kth membrane.

P_k Free solution hydrostatic pressure at the kth station, $P_3 \equiv 0$.

ΔP_k Pressure drop across the kth membrane $= P_k - P_{k-1}$.

p Stromal swelling pressure; p_k = stromal swelling pressure at the kth station.

R Gas constant.

T Absolute temperature.

$\bar{V}_0$ Molar volume of water (solvent).

Δx_k Thickness of the kth membrane.

β, γ Constants in the relation between swelling pressure and hydration, $p = \gamma \exp(-\beta H)$.

ϵ Ratio of stromal fluid density to stromal dry tissue density.

σ_k Reflection coefficient of the kth membrane.

ψ_3 Thickness of dry stroma.

Subscripts

k (station index)	0, anterior chamber; 1, interface between endothelium and stroma; 2, interface between stroma and epithelium; 3, tear film.
k (membrane index)	1, endothelium; 2, stroma; 3, epithelium.

LINEARIZATION OF THE CORNEAL EQUATIONS

The set of eight equations derived in part I to describe the in vivo cornea are nonlinear, first, because of the use of a mean intramembrane salt concentration in the denominator of the concentration equations for the epithelium and endothelium (part I, equations 6), and second, because of the nonlinear dependence of stromal swelling pressure and flow conductivity on hydration, as manifested in the complexity of the stromal flow and concentration equations (part I, equations 13, 14). Although the full set of equations presented in part I will be used for all quantitative results to be reported here, it is nonetheless instructive to examine the possibilities and implications of linearizing the set. This linearization is accomplished by replacing the mean salt concentrations $\bar{c}_{s1}$ and $\bar{c}_{s3}$ by $(c_{s0} + c_{s3})/2$ and neglecting stromal gradients. These simplifications are clearly valid for the normal cornea. In terms of variations from the normal state, the range of validity of the substitution for $\bar{c}_{sk}$ is probably not much narrower than that of the use of $\bar{c}_{sk}$ itself. Stromal gradients will be small as long as the barrier functions of the limiting layers are retained.

The eight equations given in part I then collapse to four linear equations in J_s, J_0, and the mean stromal salt concentration (c_{ss}) and pressure (P_s), with the auxiliary endothelial equilibrium condition, $p_s = P_0 - P_s$, where p_s is mean stromal swelling pressure. In matrix form,

$$\mathbf{A} \cdot \mathbf{X} = \mathbf{C} \cdot \mathbf{V} \tag{1}$$

where

$$\mathbf{A} = \begin{pmatrix} \dfrac{2f_{T1}K_{s1}}{c_{s3} + c_{s0}} & -\bar{V}_0 f_{+1} K_{s1} & \dfrac{4RT}{\Delta x_1(c_{s3} + c_{s0})} & 0 \\ \dfrac{2f_{T3}K_{s3}}{c_{s3} + c_{s0}} & -\bar{V}_0 f_{+3} K_{s3} & -\dfrac{4RT}{\Delta x_3(c_{s3} + c_{s0})} & 0 \\ K_{s1}(f_{T1} - f_{+1}) & f_{01} & 0 & \dfrac{1}{\Delta x_1} \\ K_{s3}(f_{T3} - f_{+3}) & f_{03} & 0 & -\dfrac{1}{\Delta x_3} \end{pmatrix}$$

$$\mathbf{X} = \begin{pmatrix} J_s \\ J_0 \\ c_{ss} \\ P_s \end{pmatrix}$$

$$\mathbf{C} = \begin{pmatrix} \frac{2f_{T1}}{c_{s3}+c_{s0}} & -\bar{V}_0 f_{+1} K_{s1} & 0 & 0 & 0 & 0 & 0 & 0 & \frac{4RTc_{s0}}{\Delta x_1(c_{s3}+c_{s0})} \\ 0 & 0 & \frac{2f_{T3}}{c_{s3}+c_{s0}} & -\bar{V}_0 f_{+3} K_{s3} & 0 & 0 & 0 & 0 & -\frac{4RTc_{s3}}{\Delta x_3(c_{s3}+c_{s0})} \\ f_{T1}-f_{+1} & f_{01} & 0 & 0 & -\frac{RT}{\Delta x_1} & \frac{1}{\Delta x_1} & 0 & 0 & 0 \\ 0 & 0 & f_{T3}-f_{+3} & f_{03} & 0 & 0 & \frac{RT}{\Delta x_3} & -\frac{1}{\Delta x_3} & 0 \end{pmatrix}$$

$$\mathbf{V} = \begin{pmatrix} J^a_{+1} \\ J^a_{01} \\ J^a_{+3} \\ J^a_{03} \\ c_{I0} \\ P_0 \\ c_{I3} \\ P_3 \\ 1 \end{pmatrix}$$

When the linearized equations are applied to the normal cornea, the results which are obtained differ trivially from the solution of the complete nonlinear set.

It will be observed that none of the variable elements of **V** appear in **A** or **C**. It is easy to show that, for fixed **A** and **C**, the elements of **X** are linear in the elements of **V**. More specifically, the equilibrium stromal swelling pressure depends linearly on the active transport rates, the concentration of impermeants in the corneal environment, and the intraocular pressure. Furthermore, the degree to which p_s is affected by the variation of one element of **V** is independent of the values of the other vector elements.

With this as background, we now examine the effects of variations from the normal state of the cornea.

INDEPENDENT VARIABLE EFFECTS

Intraocular Pressure P_0

Ytteborg and Dohlman's (1965) clinical studies of the relation between P_0 and corneal thickness showed no significant dependence of the latter on the former. Subsequent work by Ehlers and Riise (1967) led them to the conclusion that reduced intraocular pressure led to an increase in corneal thickness. They further claimed that Ytteborg and Dohlman's data for eyes whose P_0 was subnormal were qualitatively consistent with theirs when correlations between the corneal thicknesses of contralateral eyes were taken into account.

The present analysis gives a linear dependence of stromal swelling pressure on intraocular pressure; for the base case cornea, $\partial p_s/\partial P_0 = 0.19$. Thus a decrease in P_0 is predicted here to lead to a small amount of corneal thickening, as found by Ehlers and Riise (1967). The weak dependence of p_s on P_0, coupled with the in-

trinsic variability of the population, may explain the difficulty in establishing the effect of P_0 on p_s with a high degree of statistical certainty.

The thicknesses of corneas in hypertensive eyes are dependent not only on P_0, but also on the extent to which the limiting membranes may have been damaged as a consequence of the elevated intraocular pressure. The present theory predicts that in the absence of such trauma, slightly thinner corneas should be evident. Ytteborg and Dohlman's (1965) data on this point again suggest no correlation, but Sbordone (1953) is cited by Ehlers and Riise (1967) as having found corneal thinning in chronically glaucomatous eyes. Ehlers (1970) has more recently reported clinical studies on 14 glaucomatous eyes, in which a mean reduction in P_0 of 24 Torr caused the corneas to swell by 20 ± 4 μ. For human corneas, the variation of corneal thickness with stromal hydration is $dq_c/dH_s = 142$ μ (Ytteborg and Dohlman, 1965); if this value is used with that of $\partial p_s/\partial P_0$ given above, a thickening of 12 μ is predicted, in reasonable agreement with Ehlers. Further support for the theoretical prediction may be drawn from Cogan's (1968) observation that stromal edema accompanies the reduction of the intraocular pressure in acutely glaucomatous eyes.

Tonicity of the Aqueous c_{s0} and Tears c_{s2}

As shown in part I, the normal corneal hydration is the result of a balance between principally osmotic water flow across the epithelium and largely hydraulic flow across the endothelium. Any decrease in the former flux, caused by decreasing the tonicity of the tears or increasing that of the aqueous, will by continuity decrease the latter flux. For the anteriorly directed hydraulic flow across the endothelium to decrease, the imbibition pressure must become less negative; hence, the stromal swelling pressure falls and the stroma thickens.

The predicted induction of corneal swelling by raising c_{s0} in vivo is a steady-state prediction, in the sense described in part I. The initial response of the cornea to aqueous hypertonicity will be thinning, as fluid is driven osmotically across the endothelium and into the anterior chamber. However, with time, this driving force is lessened as salt diffuses into the stroma, and the cornea should then swell past its initial thickness to a higher steady value. In the steady state, c_{ss} rises by 9.1 mM when c_{s0} is raised by 10.0 mM; thus the driving force for anteriorly directed flow across the epithelium is reduced by an effective 14.6 milliosmols ($\sigma_2 = 0.8$) while that across the endothelium is diminished by only 0.7 milliosmols ($\sigma_1 = 0.4$).

There seem to be few data available on the in vivo effects of aqueous hypertonicity. Cogan (1968) notes that epithelial edema, which can be associated with stromal swelling, may be induced experimentally by injecting hypertonic solutions into the anterior chamber.

The promotion of corneal swelling in vivo by topical hypotonicity and corneal thinning by the application of hypertonic solutions is well documented (Von Bahr,

1949, 1956; Mishima and Maurice, 1961 *b*; Stanley et al., 1966). However, the theoretical sensitivity of corneal thickness to tear tonicity is considerably greater than that observed experimentally. In the work of Mishima and Maurice and Stanley et al., solutions of various tonicities were applied to the cornea for a time which appeared to be sufficient for the establishment of a new steady state. The former authors observed (for example) that intact rabbit corneas swelled by approximately 10–15 μ after 1 hr contact with 0.9% NaCl; a like amount of thickening was exhibited after a similar period of eye closure. Stanley et al. found that the cornea achieved an apparently steady thickness after 20 min exposure to 0.9% NaCl and that it subsequently thinned by approximately 60 μ after 10 min perfusion with 1.5% saline. In contrast to these experimental results, the base case cornea of part I swells by 60 μ upon the reduction of c_{s3} by a mere 6 mM.

In trying to reconcile theory and experiment on this point, it will be assumed that the experimental corneas were undamaged and were indeed in contact with the indicated bathing solutions. A number of possible explanations may then be examined.

(*a*) *Corneal parameter or boundary condition changes.* Some corneal parameter or boundary condition may change in parallel to a change in c_{s3}, acting in a compensatory fashion. Such a function might for instance be performed by the epithelial sodium pump, were $| J^a_{+3} |$ highly sensitive to substrate concentration (which it is not [Friedman, 1971]).

Perhaps the most likely variation is in c_{s0}; the steady corneal thickness is directly dependent on $c_{s3} - c_{s0}$ and changes hardly at all if the concentrations of tears and aqueous are shifted by the same amount. Indeed, lowering both c_{s0} and c_{s3} by 13 mM results in a calculated corneal thinning of only 8 μ. There is reason to expect that changes in c_{s3} cause corresponding variations in c_{s0}, but is not clear that the latter are comparable in size to the former. Stanley et al. (1966) were unable to detect any significant change in the conductivity of the aqueous after bathing the anterior surface of the rabbit eye for 20 min with solutions as concentrated as 1.5% NaCl, though a rise of perhaps 10% from the normal aqueous conductivity value was observed when 1.7% NaCl or glucose equivalent solutions were used.

One may calculate the effect on aqueous concentration of changes induced in J_s and J_0 by variations in c_{s3}. For the base case cornea, with an area $A_c = 2$ cm², the outgoing water flow rate is 0.04 μl/min, compared with an aqueous generation rate of $V_a = 2$–5 μl/min. The volume flow of aqueous is thus insensitive to J_0. The effect of salt influx through the cornea on aqueous concentration is given by $-J_s A_c / V_a = 0.03$ mM for the base case. When c_{s3} is raised to 1.5% NaCl, the corresponding water flow is still less than 0.2 μl/min and $-J_s A_c / V_a = 0.04$ mM. Thus it does not appear that the concentration of the aqueous is sufficiently responsive to the tear film tonicity to explain the experimentally observed insensitivity of corneal thickness to variations in c_{s3}.

(*b*) *Error in the base case parameters.* There may be an error in the base case

parameters, leading to an overestimate of $\partial p_s/\partial c_{s3}$. To examine the possibilities of this explanation, it is convenient to evaluate $\partial P_s/\partial c_{s3}$ from the linearized equations. A further approximation is made in equation 1 with little loss in accuracy: the quantity $(c_{s3} + c_{s0})$ in the denominators of the elements of **A** and **C** is replaced by $2\bar{c}_a$ and this quantity is regarded as constant though c_{s3} varies. Let $\mathbf{B} = \mathbf{CV}$ and differentiate equation 1 with respect to c_{s3}: $\mathbf{AX}' = \mathbf{B}'$, where primes denote differentiation with respect to c_{s3}. Thus $\mathbf{X}' = (\partial J_s/\partial c_{s3}, \cdots, \partial P_s/\partial c_{s3})$ and $\mathbf{B}' = (0, -[2RT/(\bar{c}_a \Delta x_3)], 0, 0)$. Further examination of the matrix equation reveals that $\partial P_s/\partial c_{s3}$ is determined only by the friction coefficients and thicknesses of the epithelium and endothelium, and not by the magnitudes of any active transport rates or impermeant concentrations. The solution for $\partial P_s/\partial c_{s3}$ is

$$\frac{\partial P_s}{\partial c_{s3}} = \frac{2RT}{\bar{c}_a} \cdot \frac{(f_{T1} - f_{+1})K_{s1}f_{03} - (f_{T3} - f_{+3})K_{s3}f_{01}}{\Delta},$$

where $\Delta = [f_{T3}K_{s3}f_{03}/\bar{c}_a + \bar{V}_0 f_{+3}K_{s3}^2(f_{T3} - f_{+3})][\Delta x_3/\Delta x_1] + f_{T1}K_{s1}f_{03}/\bar{c}_a + \bar{V}_0 f_{+1}K_{s1}K_{s3}(f_{T3} - f_{+3}) + f_{T3}K_{s3}f_{01}/\bar{c}_a + \bar{V}_0 f_{+3}K_{s3}K_{s1}(f_{T1} - f_{+1}) + [f_{T1}K_{s1}f_{01}/\bar{c}_a + \bar{V}_0 f_{+1}K_{s1}^2(f_{T1} - f_{+1})][\Delta x_1/\Delta x_3]$.

The dependence of p_s on c_{s3} can be diminished by raising Δ or lowering the magnitude of the numerator of the preceding expression. Large changes in Δ cannot be effected without correspondingly large changes in the friction coefficients, because all the terms in Δ are positive and the individual friction coefficients enter each term linearly. Note that Δ does get very large as Δx_1 or Δx_3 go to zero. Thus the effect of tear tonicity decreases in the event of epithelial or endothelial trauma, as observed experimentally.

The numerator of $\partial P_s/\partial c_{s3}$ is negative for the base case; the second term is about 20 times the first. Again, large changes in the friction coefficients would be necessary to effect a major change in $\partial P_s/\partial c_{s3}$.

The linearized equations, it might be remarked, yield the equilibrium value of P_s, from which p_s is calculated by $p_s = P_0 - P_s$. It is then assumed that the in vitro relation between p_s and the mean stromal hydration H_s holds in vivo, and H_s is calculated accordingly. If γ and β are different in the living eye than in excised stroma, and particularly if swelling pressure depends more strongly on hydration in vivo, the large variations in P_s as c_{s3} is varied would induce relatively small changes in corneal thickness. The use of in vitro swelling pressure data to describe the behavior of the normal cornea is supported by the measurements by Hedbys et al. (1963) of the variation of imbibition pressure with in vivo corneal thickness.

In summary, it does not appear that the difference between experiment and theory under consideration here can be explained by any but the grossest error in the input parameters.

(*c*) *Cornea not in steady state.* The cornea may not in fact have reached the steady state at the time the experiments were concluded. Since an effect on hydration is

observed when c_{s3} is changed, the time constant for water flow into or out of the cornea cannot be very long, but it may be that the time constant for salt flux is considerably greater. This would explain the success of earlier treatments of the cornea as an osmometer for dynamic analysis purposes (Stanley et al., 1966; Mishima and Hedbys, 1967).

An estimate of the time constant for changing the tonicity of the stromal fluid as a result of passive nonconvective salt diffusion through a membrane can be obtained from equation 6 in part I, which relates the flux J_s through the membrane to the diffusional driving force $\Delta c_{s(k)}/\Delta x_k$: $J_s = -2RT\Delta c_{s(k)}/(K_{sk}f_{Tk}\Delta x_k)$. The rate of accumulation of salt in the stroma is given by $\mathrm{d}|\,c_{ss}\,|/\mathrm{d}t = \epsilon\,|\,J_s\,|/(H_s\psi_2)$, and the desired time constant is $\tau_k = H_s\psi_2 K_{sk}f_{Tk}\Delta x_k/(2RT\epsilon)$.

For the base case cornea, $\tau_1 \approx 30$ min and $\tau_3 \approx 9$ hr. Thus the epithelium does in fact behave almost as though it were impermeable to salt, for the experiments of interest here. Roughly speaking, in the time scale of the experiments, the cornea swells or thins only to the point that the water fluxes across the epithelium and endothelium are nearly the same; the total salt content of the stroma changes much more slowly. A rough estimate of the hydration in this *apparent* steady state can be obtained by assuming that the stromal salt content, proportional to $c_{ss}H_s$, is fixed at the base case value (denoted by the superscript o; $c_{ss}H_s = c^o_{ss}H^o_s$), neglecting the epithelial sodium pump, equating solvent flux through the epithelium to that through the endothelium, and requiring the endothelium to be in mechanical equilibrium. The resulting equation is transcendental, owing to the exponential dependence of p_s on H_s, but can be linearized about the base case to give the dependence of stromal thickness Δx_2 on c_{s3}: with $f^*_{ok} = \bar{V}_o/(L_{p,k}\Delta x_k)$,

$$\left(\frac{\partial[\Delta x_2]}{\partial c_{s3}}\right)^o = -\frac{\psi_2}{\epsilon}\cdot\frac{2RT\sigma_3 f^*_{01}\Delta x_1}{\beta p^o_s(f^*_{03}\Delta x_3 + f^*_{01}\Delta x_1) + \dfrac{2RTc^o_{ss}}{H^o_s}(\sigma_1 f^*_{03}\Delta x_3 + \sigma_3 f^*_{01}\Delta x_1)}.$$

When the corneal parameters and base case results given in part I are substituted into this equation, $(\partial[\Delta x_2]/\partial c_{s3})^o = -0.6\ \mu$/mM, describing closely the experimental results of Mishima and Maurice (1961 *b*) and Stanley et al. (1966) cited above.

Thus it would appear that a considerable period of time must elapse before the living cornea can equilibrate to changes in tear film tonicity. The slow response of the stromal salt content to such variations causes the cornea to behave as an osmometer over reasonable experimental durations. Indeed, the cornea may so respond to diurnal variations in c_{s3}, so that the cornea is never truly in the steady state, but varies periodically about a base case hydration corresponding to an ambient tonicity between the open-eye value of approximately 183 mM (Iwata et al., 1969) and the closed-eye value, which is thought to approximate that of plasma. Thus the cornea rapidly thins some 15 μ upon awakening, remains relatively constant during the day because of the difficulty with which salt leaves the stroma, and thickens again

during sleep. The complete description of this behavior must await the extension of the present work to the unsteady state; such an analysis will be the subject of a subsequent communication. It is on the basis of these considerations that a value of c_{s3} less than that given by Iwata et al. was used in part I to define the base case.

EXOGENOUS/TRAUMATIC EFFECTS

Variations in the Friction Coefficients

All the effects to be discussed in this section derive to some extent from changes in the friction coefficients of the epithelium and/or the endothelium. It is thus appropriate to consider at the outset the predicted effect on corneal thickness of changing these coefficients, by whatever cause. Since they appear in the coefficient matrix **A** in equation 1, the equilibrium stromal swelling pressure does not depend linearly on their values. However, the changes in p_s and corneal potential ψ induced by small variations in the friction coefficients about their base case values can be computed, and these results are given in Table I. The reflection coefficient σ_k replaces the friction coefficient f_{+k} ; the two are nearly exactly related by $\sigma_k = (f_{Tk} - f_{+k})/f_{Tk}$.

It will be seen from Table I that the corneal potential, which originates essentially entirely across the epithelium, is sensitive to only the solute permeability of this membrane. Convective effects on the electrical behavior of the cornea are small, since ψ is insensitive to variations in f_{0k} or σ_k. The stromal swelling pressure, on the other hand, is quite insensitive to f_{T3} at fixed σ_3 , but it is very sensitive to σ_3 . The epithelial reflection coefficient has been thought by other experimenters (Mishima and Hedbys, 1967) to be larger than Green and Green's (1969) value used here. Maurice (1967) has claimed that Green's (1967) epithelial sodium permeability,

TABLE I

EFFECT OF VARIATION OF FRICTIONAL PROPERTIES OF LIMITING MEMBRANES OF THE CORNEA ON EQUILIBRIUM STROMAL SWELLING PRESSURE AND CORNEAL POTENTIAL

	Epithelium		Endothelium	
	Swelling pressure	|Potential|	Swelling pressure	|Potential|
	%	%	%	%
Raise f_{0k} 10%	−7	<1	+17	<1
Raise σ_k by 0.1 at fixed f_{Tk} (lower f_{+k})*	+73	<1	−21	<1
Raise f_{Tk} 10% at fixed σ_k (raise f_{+k} 10% too)	−8	+11	−14	<1

* $\sigma_1^\circ = 0.4$, $\sigma_2^\circ = 0.8$.

from which the base case value of f_{T3} is obtained, is too high. Since f_{T3} is inversely proportional to solute permeability, it is seen that both of these criticisms can be met by adjusting f_{T3} and σ_3 upward to raise the calculated corneal potential without causing the calculated corneal thickness to differ from the observed in vivo value.

Endothelial Trauma

It is well known (Leber, 1873; Maurice and Giardini, 1951; Larsen, 1966; Dohlman et al., 1968) that endothelial disease or damage lead to corneal swelling in vivo. This behavior is reproduced by the present corneal model; when all the friction coefficients of the endothelium are reduced to 75, 50, and 25% of their base case values, the equilibrium stromal hydration rises from its normal value of 3.2 to 3.4, 3.6, and 4.3, respectively.

In the limiting case of endothelial removal, $\Delta x_1 \rightarrow 0$. Since all the constituents of the aqueous are then free to enter the stroma, $\Delta c_{I(1)} = 0$, irrespective of the value earlier selected for c_{I0}. The transcorneal fluxes remain finite, so $\Delta P_{(1)} \rightarrow 0$ (see part I, equation 7); that is, $P_1 \rightarrow P_0$. In the absence of endothelium (and in the limit of the endothelial equilibrium condition, part I, equation 8), $p_1 \rightarrow 0$; that is, the posterior stroma is maximally swollen. From the mechanical equilibrium condition for the stroma (part I, equation 9), $p_2 \rightarrow P_1 - P_2$, so p_2 will have a small positive value equal to the hydrostatic pressure drop across the stroma. Since the principal barrier to fluid flow across the cornea is the still intact epithelium, destruction of the endothelium does not lead to much of an increase in J_0. The flow conductivity of the stroma is high, so the pressure drop across this structure remains small; when the endothelial friction coefficients are reduced by 75%, $P_1 - P_2$ is only 3.2 Torr. Thus endothelial removal is expected to cause considerable stromal swelling. The only effect of topical hypertonicity on a cornea lacking its endothelium would be to increase J_0, and hence p_2, but the stromal flow conductivity is such that only a minor effect on corneal thickness would be induced.

Epithelial Removal

The promotion of swelling by epithelial removal is also well documented (Maurice and Giardini, 1951; Dohlman et al., 1968). The extent of swelling is not as great as when the endothelium is removed, and this observation is predicted by the present model. When $\Delta x_3 \rightarrow 0$, $\Delta c_{I(3)} = 0$, and $P_2 \rightarrow P_3 = 0$ (see part I, equation 7). Since there is still a driving force for water flow anteriorly through the stroma, $P_1 > 0$ and, by the endothelial equilibrium condition, $p_1 < P_0$. Thus the posterior stroma swells to the point that the local swelling pressure (p_1) falls below the intraocular pressure. The difference $P_0 - p_1 = P_1$ can in this case be rather large because the value of J_0 rises considerably upon removal of the epithelial flow barrier. The endothelial and stromal equilibrium conditions, with $P_2 = 0$, give $p_2 = P_0$, so the anterior stroma swells until its swelling pressure reaches the intraocular pressure.

Since p_1 does not fall below zero, the average stromal swelling pressure will lie between P_0 and $P_0/2$. When Δx_3 and J^a_{+3} are both set equal to zero, solution of the eight equations given in part I yields $p_s = (p_1 + p_2)/2 = 17$ Torr, $H_s = 4.9$; the stromal hydration thus rises by 1.7 g water/g dry tissue, and the cornea swells to 126% of its thickness before epithelial denudation. This result is in excellent agreement with Dohlman et al. (1968) and in satisfactory agreement with Maurice and Giardini (1951), who found somewhat larger increases in corneal thickness than did the later authors.

The mechanical opposition of the intraocular pressure to swelling in the case of epithelial removal has been noted earlier (Anseth and Dohlman, 1957). Their interpretation of the consequences of the destruction of either of the limiting layers is the same as that given here if the pressure drop through the stroma is neglected. The transcorneal pressure drop $P_0 - P_3$ is then entirely across the surviving layer. If this layer is the epithelium, $P_s = P_0$, $p_s = 0$, and the stroma swells considerably. If the endothelium is the surviving membrane, $P_s = P_3 = 0$, $p_s = P_0$, and the stroma swells until the swelling pressure falls to the intraocular pressure.

Finally, it should be noted that, similarly to the case of endothelial removal, no degree of hypertonicity in the tears can be expected to thin the cornea to the point that p_s exceeds P_0, once the epithelium has been removed.

Temperature Effects, Particularly Cooling: a Role for the Epithelial Sodium Pump?

Compared with excised tissue, the in vivo cornea exhibits a relatively weak dependence of thickness on external temperature. Normally, the temperature excursions experienced by the cornea are small compared with ambient variations. The surface temperature of the central cornea of the rabbit varies from 27.5°C in a 4°C environment (Schwartz, 1965) to near body temperature during sleep. Much larger changes in in vivo corneal temperature can be effected experimentally, but these too are accompanied by only minimal variations in thickness (Langham and Taylor, 1956; Langham, 1960; Mishima and Maurice, 1961 *b*). The maximum observed swelling of in vivo cornea exposed to cold is that recently reported by Kikkawa and Hirayama (1970). These authors were able to achieve corneal thickenings of up to 40% by irrigation with 4°C Ringer's solution for up to 18 hr.

Lowering the corneal temperature will affect principally the friction coefficients of the membranes and the rates of any active transport system. An effect on the swelling pressure–hydration curve is also expected, but this effect is small (Hedbys and Dohlman, 1963), at least in the neighborhood of normal hydration (Katano, 1966). If permeation through the corneal membranes is by way of intercellular pores, the friction coefficients should be more or less proportional to the solvent viscosity. This variation is evident for the solute permeability of the endothelium (Maurice, 1961) and has been assumed (Mishima, 1968) to hold as well for the

hydraulic conductivity of this membrane. Since there is some evidence (Maurice, 1951) for pore flow through the epithelium, we will assume that the temperature dependence of all the friction coefficients is the same as that of solvent viscosity. The activation energy of this variation is approximately 3.9 kcal/mole between 20 and 37°C.

The activation energy of the epithelial sodium pump is uncertain. An estimate of the relative variations of the epithelial friction coefficients and its pump rate can be inferred from the temperature dependence of the corneal potential. It was shown in Table I that ψ is essentially proportional to f_{T3}, at fixed σ_s, and it is effectively independent of f_{03} and the endothelial friction coefficients. It is also found that, as might be expected, ψ is nearly proportional to J^a_{+s} if all other corneal parameters are held constant (when $J^a_{+s} = (J^a_{+s})^o/2$, $\psi = 5.0$ mv). Thus, for temperature variations, $\psi \propto f_{T3} J^a_{+s}$. Experimentally, ψ does not vary too strongly with temperature in the range which characterizes the in vivo cornea (in vitro observation, Ehlers and Ehlers, 1968); the implication is that the epithelial solute permeability ($\propto f_{T3}^{-1}$) and pump rate have similar activation energies at these temperatures.

The interaction among pump rate, corneal temperature (assumed to be uniform), and equilibrium swelling pressure, as given by the basic equations derived in part I, is presented in Fig. 1. At any temperature, the epithelial sodium pump acts to cause swelling (lower p_s) by increasing the tonicity of the stromal fluid over the value

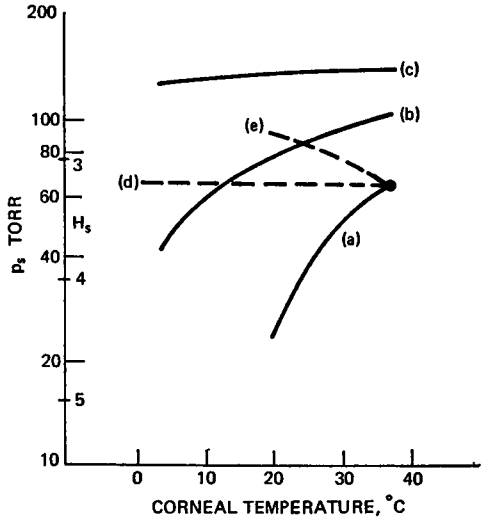

FIGURE 1 Effect of epithelial pump rate and corneal temperature on equilibrium stromal swelling pressure and hydration. Base case friction coefficients are taken to be those at 37°C, varying with temperature like viscosity. Solid lines show predicted variations with temperature at constant pump rate. (*a*) $J^a_{+s} = (J^a_{+s})^o$, base case value (●, base case); (*b*) $J^a_{+s} = (J^a_{+s})^o/2$; (*c*) $J^a_{+s} = 0$. Dashed lines show variations of swelling pressure and hydration with temperature if pump rate varies with temperature. (*d*) Activation energy = 5 kcal/mole; (*e*) activation energy = 10 kcal/mole.

which would obtain in the absence of a pump. As the stroma becomes more hypertonic, the osmotic driving force for fluid flow across the epithelium becomes less and that for flow across the endothelium increases. Thus a smaller hydrostatic pressure drop across the endothelium is necessary for the water fluxes across the limiting corneal membranes to be identical, the imbibition pressure P_s becomes less negative and the stroma swells until the swelling pressure falls to $P_0 - P_s$. An examination of the partial effects of varying the individual membrane friction coefficients (Table I) shows that if all of them are raised proportionately, the equilibrium swelling pressure will fall; this behavior is illustrated in Fig. 1 by the variation of p_s with temperature, at constant pump rate.

The normal cornea must maintain its transparency and hence near normal thickness over a temperature range which is wide relative to the environment of most body tissue. The epithelial sodium pump may therefore function as a homeostatic temperature compensator. Cooling tends to promote swelling by lowering membrane permeability, but at the same time it reduces the turgescent contribution of the epithelial active transport system. This possibility is illustrated by the dashed lines in Fig. 1 which show how the stromal swelling pressure and hydration would vary with temperature for two possible pump activation energies. For instance, if the activation energy of the pump were 5 kcal/mole, J^a_{+s} would fall to half its normal value at 12.6°C. Thus the swelling pressure locus for this activation energy (curve *d*) crosses the $J^a_{+s} = (J^a_{+s})^\circ/2$ curve *b* at 12.6°C. The corneal potential data discussed above suggest that the activation energies for corneal permeability and epithelial pump rate are similar at normal corneal temperatures, so curve *d* may be quite realistic in this range.

The conclusion that corneal thickness remains constant if the pump rate and permeability activation energies are comparable appears to hold irrespective of their value. If the activation energy of the pump were 10 kcal/mole, the pump rate would be halved at 24.3°C. If the corneal resistance were to rise twice as fast as viscosity as the tissue was cooled, the solid curves in Fig. 1 would then be steeper. For $J^a_{+s} = (J^a_{+s})^\circ/2$, the swelling pressure and hydration at 24.3°C would then be the same as those given in Fig. 1, curve *b*, at $37 - 2 \times (37 - 24.3) = 11.6$°C, that is, 63 Torr and 3.3 g water/g dry tissue.

For completeness, two compensating deviations from this simple homeostatic picture should be noted. First, it is likely that the pump rate falls more rapidly with decreasing temperature than does corneal permeability, particularly at very low temperatures, where in vitro experiments show the pump to have stopped (Donn et al., 1959). This deviation would, by itself, tend to cause corneal thinning (Fig. 1, curve *e*). Second, when the cornea is exposed to a cool environment, the epithelium is cooler than the endothelium. The cornea behaves as though it were uniformly at the epithelial temperature but had a leaky endothelium. This deviation causes the cornea to be more swollen than it would be were it cooled uniformly to the epi-

thelial temperature. A similar effect will be observed in the absence of temperature gradients through the cornea if the epithelial permeabilities to salt and water fall more rapidly with decreasing temperature than do those of the endothelium.

It was observed in the discussion of the effect of tear tonicity on corneal thickness that a long time might be required for the stromal sodium content to reach its new steady value subsequent to changes in the corneal parameters or environmental variables. This does not appear to be a problem insofar as corneal adjustment to temperature changes is concerned, since c_{ss} varies by only 0.4 mM along curve *d* in Fig. 1 between 37 and 4°C.

The Effect of Metabolic and Transport Inhibitors

The in vivo cornea responds only slightly to the topical application of metabolic inhibitors, at concentrations sufficient to cause isolated tissue to swell considerably (Langham and Taylor, 1956; Langham and Kostelnik, 1965). Brown and Hedbys (1965) found that the topical application of 10^{-3} M ouabain causes the rabbit cornea to swell by approximately 25%; this swelling is still modest compared with Langham and Kostelnik's measurements of the effect of ouabain on enucleated eyes.

The small effect of inhibitors on in vivo corneal thickness does not appear to be the result of any compensatory behavior by the tissue similar to that adduced to explain the insensitivity of thickness to ambient temperature variations. Rather it seems that, at the concentrations used in the bulk of the above experiments, the inhibitor had only a small effect on the corneal parameters themselves, and hence no large effect on corneal thickness either. For instance, Mishima (1968) and Trenberth and Mishima (1968) report no effect of ouabain on the friction coefficients of the corneal endothelium, in isolated preparations.

The application of inhibitors to the corneal surface might be expected to decrease the rate of any resident pump. It has already been shown that any agent which lowers the epithelial pump rate and affects no other corneal property would be expected to induce corneal thinning. The limited available evidence indicates that the epithelial sodium pump is not excessively sensitive to topically applied inhibitors. Friedman and Kupfer (1960) found the in vivo corneal potential to be unaffected by 5×10^{-4} M iodoacetic acid, 10^{-3} M 2,4-dinitrophenol, or 10^{-3} M NaCN; since the solute permeability of the epithelium is unlikely to have been decreased by these treatments, the constancy of ψ is good evidence that the pump was substantially unaffected.

In short, the observed effect of topically applied inhibitors is consistent with the present corneal model, but lends no new insights into in vivo corneal behavior. At the concentrations used by most experimenters, no effect on corneal thickness was observed, apparently because neither the friction coefficients of the corneal membranes nor the epithelial pump rate were altered significantly.

METABOLICALLY COUPLED WATER TRANSPORT IN THE CORNEA

It is surely apparent that the combination of corneal and environmental parameter values which have been used here to define the normal cornea are not the only set capable of providing good agreement between theory and experiment. What is significant is that all the members of the set were found experimentally; no arbitrary parameters have been used. As has already been noted, not all of these experimental parameter values are universally accepted. In this section, we will examine the implications of the present theory with respect to the two parameters whose role in corneal hydration control is of uncertain but possibly major significance. These parameters are J_{01}^{c} and J_{03}^{c}, the fluid fluxes across the limiting layers of the cornea which are not directly coupled to the electrochemical potential gradients acting across these membranes.

The possibility that the cornea maintains its normal thickness in the face of the stromal swelling pressure by means of active extrusion of water, likely solute coupled, has long been entertained by corneal physiologists. The "pump" is thought by the majority of those who believe in it to reside in the corneal endothelium. Maurice (1972; and private communication) has observed a water flux across isolated endothelium against or in the absence of a solvent chemical potential gradient; the transport rate is 6–9 $\times$ 10^{-8} moles/cm^2-sec, directed posteriorly. A concise review of the arguments relating to active hydration control is given in Maurice (1969). At this writing, the only other published measurement of a water flux across a corneal membrane in the absence of a driving force is that reported by Green and Green (1969), who found a rate of 9 $\times$ 10^{-9} moles/cm^2-sec, directed posteriorly across isolated *epi*thelium. The flux observed by Green and Green would thus be expected to favor rather than oppose corneal swelling.

It is not within the scope of this paper to establish or deny the existence of these water pumps (though they are found here to be unnecessary to the maintenance of normal hydration in vivo) nor to speculate as to their mechanism; it might yet be useful to determine on the basis of the present analysis what order of magnitude of pump rate is "significant" in the present context. It was shown above that the equilibrium swelling pressure is practically linear in these rates; the coefficients for the base case are -6×10^{9} Torr/(mole/cm^2-sec) for $k = 1$ and 6×10^{9} Torr/(mole/cm^2-sec) for $k = 3$. A water pump rate of 1.1×10^{-8} moles/cm^2-sec would change p_s by an amount equal to the normal in vivo swelling pressure. Thus the pump rates reported by Maurice and the Greens are surely significant and merit confirmatory studies.

The smallest pump rate likely to be relevant to the mechanism of corneal deturgescence would be of the order 1×10^{-9} moles/cm^2-sec $\approx 6 \times 10^{-5}$ cm/hr. This rate is 6% of the base case value of J_0 and is an even smaller fraction of the value inferred by Mishima and Maurice (1961 *a*), so it may be exceedingly difficult to

demonstrate in vivo the presence of metabolically coupled water transport, even when such transport does in fact exist.

CONCLUSIONS

The salient conclusions and observations regarding in vivo corneal behavior which have been made here are summarized below.

(*a*) The equilibrium stromal swelling pressure depends linearly on the corneal active transport rates, the salt and impermeant concentrations of the aqueous and tears, and the intraocular pressure. The slopes of these linear relations are independent of the values of all of the above quantities.

(*b*) The theory based on the physical description of the in vivo cornea given in part I predicts that, in the absence of damage to the corneal membranes, increases in intraocular pressure should cause very slight corneal thinning.

(*c*) The theory predicts that swelling should be induced by raising the tonicity of the aqueous or lowering that of the tears. The theoretical dependence of steady corneal thickness on tear tonicity is greater than is found experimentally, probably because a true steady state had not been achieved during the time course of the experiments. The time constant for salt flux across the epithelium is of the order of 10 hr or more. For experiments of relatively short duration, an apparent steady state is achieved, in which the cornea exhibits the osmometer-like behavior observed by earlier workers (Stanley et al., 1966; Mishima and Hedbys, 1967). The theory predicts closely the corneal thickness in this apparent steady state.

(*d*) The corneal potential is determined almost entirely by the epithelial salt permeability and sodium pump rate.

(*e*) The theory predicts considerable stromal swelling to follow endothelial damage, and less to follow epithelial removal. These conclusions are derived in the framework of the present theory but are physically equivalent to Anseth and Dohlman's (1957) concepts, with the inclusion of stromal pressure gradients. The theory predicts that topical hypertonicity will not cause corneas swollen in either fashion to deswell a significant amount.

(*f*) The epithelial sodium pump appears to act homeostatically to maintain a normal corneal thickness in the face of ambient temperature variations.

(*g*) Since the normal corneal thickness is predicted by the theory with $J_{01}^{c} = J_{03}^{c} = 0$, the maintenance of the normal corneal hydration is by itself no basis for inferring the presence of a water pump in the cornea; as noted in (*e*), the observation of swelling consequent to endothelial dysfunction is no basis for siting this inferred pump in the endothelium. Pump rates as low as 1% of that claimed by Maurice (1972) and 10% of that published by Green and Green (1969) can affect stromal thickness. It will therefore be no easier to prove that such a pump does not exist in vivo than it has been to confirm that it does.

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