UNSTEADY TRANSPORT AND HYDRATION DYNAMICS IN THE IN VIVO CORNEA

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ABSTRACT The unsteady response of the rabbit cornea to the normal periodic variations in tear tonicity which accompany the sleep-wake cycle is examined quantitatively in terms of a physical description of corneal mechanics and transport. Two different sets of experimental epithelial and endothelial flow conductivities and reflection coefficients are used, and the effect of variations in epithelial solute permeability and sodium pump rate is examined. The use of a set of experimental corneal parameters chosen earlier provides good agreement between calculated and observed in vivo corneal thickness dynamics when the tear tonicity is within the physiologic range. The factors affecting the time-course of corneal thickness dynamics are discussed, including the osmometric quality of the corneal stroma, the role of the epithelial sodium pump, the flow resistance of the limiting corneal layers, and cyclic changes in aqueous tonicity. The unsteady solutions presented here are related to the steady-state solutions given in earlier papers through the concept of the time-average steady state. Any realistic description of the normal in vivo cornea must recognize its unsteady character and the potential for transepithelial flow. On the average, the hypertonicity of the tears relative to the stromal fluid can be sufficient to account for rabbit corneal deturgescence. The absence of endothelial "pumping" from the in vivo rabbit cornea cannot be proven; neither is there any certain need to postulate such transport in the normal state.

INTRODUCTION

In an earlier paper (Friedman, 1972 *a*), it was shown that a set of mechanical equilibrium and flow equations could be written to describe the in vivo cornea, and that when normal corneal properties and boundary conditions, including a constant time-averaged tear tonicity, were substituted into the formulation, the normal corneal thickness was recovered. The steady corneal thickness computed in this fashion was found (Friedman, 1972 *b*) to exhibit a much stronger dependence on tear tonicity than had been observed experimentally, and it was hypothesized that this difference reflected the slowness with which the cornea responded to changes in the salt concentration on the tear side. This hypothesis was supported by estimates of the time constants for changing the tonicity of the stromal fluid.

The response of the cornea to tear tonicity is a critical aspect of the behavior of this tissue, for the living cornea normally experiences variations in this boundary condition. During sleep, the anterior surface of the cornea is covered by the palpebral conjunctiva which lines the inside of the eyelid, and the fluid ambient to the cornea may be expected to be isotonic to plasma. When the eye is open, water evaporates from the tear film, causing it to become more concentrated. The tear film is replenished with fresh, isotonic tears by blinking, but on the average, the open eye tonicity is hypertonic to the closed eye value. The degree of hypertonicity depends on the blink frequency; it is approximately 18 mosmol/liter in humans (Mastman et al., 1961), who blink about once every 5 s, and in excess of 30 mosmol/liter in rabbits, which blink only once every 10 min or so. The variations in open eye tonicity over the blink cycle will be neglected here and a constant open eye tonicity will be employed; if the corneal thickness responds slowly to diurnal variations in ambient salt concentration, it is surely unable to reflect the variations in this concentration between blinks.

A knowledge of the time-course of the corneal response to a changing environment is also important to the interpretation of in vivo and in vitro experiments directed towards an understanding of the factors which govern the thickness of the normal cornea. For instance, the conclusion that the cornea maintains a constant thickness under a given set of experimental conditions cannot be reached with certainty unless it can be shown that, if the cornea were in fact not at an equilibrium thickness, it would swell or thin by a measurable amount during the course of the experiment.

This paper extends the description of corneal mechanics and transport cited above to the unsteady state. The basic unsteady equations developed below can be used to examine unsteady corneal response to any change or changes in the corneal properties or boundary conditions, but the influence of tear tonicity will be emphasized here in recognition of its physiological relevance.

NOTATION

We summarize here the variables introduced in the steady-state corneal analysis (Friedman, 1972 *a*, *b*) and used in this paper without redefinition:

c_{Ik} Impermeant concentration at the kth station.

c_{sk} Salt concentration at the kth station.

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_s Stromal hydration, grams of H_2O per gram of dry tissue.

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, $P_3 \equiv 0$.

p_s Stromal swelling pressure.

R Gas constant.

T Absolute temperature.

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

$\bar{V}_s$ Molar volume of NaCl (solute).

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 to NaCl.

ψ_2 Thickness of dry stroma.

Subscripts

k (station index) 0, anterior chamber; 3, tear film.

k (membrane index) 1, endothelium; 3, epithelium.

DERIVATION OF THE UNSTEADY-STATE EQUATIONS

As in the time-average steady description of the cornea (Friedman, 1972 *a*), the tissue is represented as a series membrane system, consisting of (from anterior to posterior) epithelium, stroma, and endothelium. All fluxes are one dimensional, in the thickness direction, and the only species considered are sodium and chloride ions and water. The unsteady differential equations are ordinary if the stromal fluid is regarded as being uniform in salt concentration c_{ss} and hydrostatic pressure P_s; the neglect of gradients in these variables has been justified (Friedman, 1972 *b*) for the steady state in normal cornea, and the unsteady state is not expected to be dramatically different in this respect.

The flux equations and the endothelial mechanical equilibrium condition derived in Friedman (1972 *a*) apply here as well, except that the fluxes across the epithelium and endothelium need not be equal:

$$-\frac{4RT(c_{ss} - c_{s0})}{(c_{ss} + c_{s0})\Delta x_1} = (K_{s1}J_{s1} - J^a_{+1}) \cdot \frac{2f_{T1}}{c_{ss} + c_{s0}} - K_{s1}f_{+1}\bar{V}_0(J_{01} - J^a_{01}), \quad (1\,a)$$

$$-\frac{4RT(c_{s3} - c_{ss})}{(c_{s3} + c_{ss})\Delta x_3} = (K_{s3}J_{s3} - J^a_{+3}) \cdot \frac{2f_{T3}}{c_{s3} + c_{ss}} - K_{s3}f_{+3}\bar{V}_0(J_{03} - J^a_{03}), \quad (1\,b)$$

$$-\frac{P_s - P_0}{\Delta x_1} = \frac{RTc_{I0}}{\Delta x_1} + (f_{T1} - f_{+1})(K_{s1}J_{s1} - J^a_{+1}) + f_{01}(J_{01} - J^a_{01}), \quad (1\,c)$$

$$-\frac{P_3 - P_s}{\Delta x_3} = -\frac{RTc_{I3}}{\Delta x_3} + (f_{T3} - f_{+3})(K_{s3}J_{s3} - J^a_{+3}) + f_{03}(J_{03} - J^a_{03}), \quad (1\,d)$$

$$P_s + \gamma \exp(-\beta H_s) = P_0. \quad (1\,e)$$

Those variables introduced in the steady-state analysis are given in the preceding Notation section; a numerical subscript is appended to J_s and J_0, so J_{jk} is the anteriorly directed flux of j ($j = s$ or 0) through the kth membrane. To use the force balance (Eq. 1 e) in the unsteady state, the inertia of the endothelium is justifiably ignored.

Eqs. 1 are five equations in the seven time-dependent unknowns c_{ss}, J_{s1}, J_{01}, J_{s3}, J_{03}, P_s, and H_s. The two additional equations which are required describe the accumulation of salt and water in the stroma. These are of the form $dn_j/dt = J_{j1} - J_{j3}$, where n_j is the moles of j in the stroma per square centimeter of corneal area. The water content of the stroma is related to stromal hydration by $n_0 = H_s \psi_2/(\epsilon \bar{V}_0)$. Thus

$$dH_s/dt = (\epsilon \bar{V}_0/\psi_2)(J_{01} - J_{03}). \tag{2 a}$$

For the dilute solutions under consideration here, the volume of stromal fluid is $n_0 \bar{V}_0$ cubic centimeters per square centimeter of corneal area. Thus $c_{ss} = n_s/(n_0 \bar{V}_0) = n_s \epsilon/(H_s \psi_2)$. Differentiating with respect to time, and using Eq. 2 a

$$dc_{ss}/dt = [\epsilon/(H_s \psi_2)](J_{s1} - J_{s3}) - (c_{ss}/H_s)(\epsilon \bar{V}_0/\psi_2)(J_{01} - J_{03}). \tag{2 b}$$

The general procedure of solution is iterative. In general, the initial values of c_{ss} and H_s are specified. Eqs. 1 are solved for P_s and the fluxes, in which they are linear. Using these fluxes, the difference forms of Eqs. 2 are used to advance H_s and c_{ss} in time, after which a new P_s and a new set of fluxes are found. This procedure is used to calculate the response of the cornea to a change in either its properties or boundary conditions; the initial values $c_{ss}(0)$ and $H_s(0)$ are the stromal salt concentration and hydration at the time at which the cornea is perturbed.

In studies of the effect of periodic variations in tear tonicity, c_{s3} assumes the closed eye value $(c_{s3})_c$ for a time τ_c, followed by the open eye value $(c_{s3})_0$ for a time τ_0. Here, the initial conditions $c_{ss}(0)$ and $H_s(0)$ are arbitrary, since the integration is continued until a periodicity condition is satisfied to a desired accuracy: $c_{ss}(t) = c_{ss}(t - \tau_0 - \tau_c)$, $H_s(t) = H_s(t - \tau_0 - \tau_c)$.

PARAMETER SELECTION

The base case corneal parameters in Friedman (1972 a) were used here as well. Their values are summarized in Table I. It remains to discuss the additional parameters τ_c and τ_0 needed to define the unsteady cornea, and a major variation from the base case parameters whose implications were also explored.

Sleep-Wake Cycle Parameters (τ_c, τ_0)

It should be noted first that the term "sleep-wake" cycle is somewhat of a misnomer; what is really meant is "closed eye-open eye" cycle. The term "sleep" is used here

TABLE I

BASE CASE CORNEAL PARAMETERS

Environmental		
RT	$= 2.58 \times 10^3$ J/mol	
P_0	$= 2.67 \times 10^{-3}$ J/cm^3	
P_3	$= 0$	
c_{s0}	$= 1.49 \times 10^{-4}$ mol/cm^3	
$(c_{s3})_c$	$= 1.55 \times 10^{-4}$ mol/cm^3	
$(c_{s3})_0$	$= 1.83 \times 10^{-4}$ mol/cm^3	
c_{I0}	$= c_{I3} = 0$	
Dimensional		
$\bar{V}_0$	$= 18$ cm^3/mol	
ϵ	$= 0.72$	
ψ_2	$= 6.05 \times 10^{-3}$ cm	
γ	$= 0.1181$ J/cm^3	
β	$= 0.809$	
Δx_1	$= 5 \times 10^{-4}$ cm	
Δx_3	$= 4 \times 10^{-3}$ cm	
Transport	**Endothelium ($k = 1$)**	**Epithelium ($k = 3$)**
f_{0k}, J-s/mol-cm^2	2.17×10^9	4.92×10^8
f_{Tk}, J-s/mol-cm^2	4.06×10^{11}	7.94×10^{11}
f_{+k}, J-s/mol-cm^2	2.43×10^{11}	1.58×10^{11}
K_{sk}	1.66	1.84
J^a_{+k}, eq/cm^2-s	0	-1.2×10^{-10}
J^a_{0k}	0	0

colloquially; an animal who sleeps with his eyes open is regarded as "awake" in the present context.

Estimates of τ_c and τ_0 were obtained by observing over a 24 h period 14 caged healthy albino rabbits in a laboratory environment to which they had become accustomed. They were exposed to laboratory and ambient lighting during the day and a red safety light in an otherwise dark room between 6:00 p.m. and 7:00 a.m. The animals were observed every half-hour from 10:00 a.m. to 4:00 p.m. and from 8:00 a.m. to 9:30 a.m. the following day and hourly at night to note which were asleep and which were awake. Thus each observation of a sleeping animal during the day counted, on the average, as a half-hour nap time, and each such observation at night counted as an hour's nap. Of the 336 rabbit-h observed, 68, or 20%, were spent sleeping. For the 14 rabbits, the average sleep time was 4.9 $\pm$0.6 h.

The length of the sleep-wake cycle in rabbit was estimated by counting the number of naps taken by the animals during the experiment. An animal "napped" if it was found to have its eyes closed *and* it had been awake at the preceding observation time. The 14 animals took 54 such "naps" during the 24 h of observation. Thus the average number of naps is four, to the nearest integer, and the sleep-wake cycle time is 6 h. 20% of this, approximately 1¼ h, is spent with the eyes closed. Thus the following values of τ_c and τ_0 were used: $\tau_c = 4.5 \times 10^3$ s, $\tau_0 = 1.71 \times 10^4$ s.

Alternative Epithelial and Endothelial Transport Parameters

The values of f_{+k} in Table I are based on the reflection coefficients in Green and Green (1969), to which they are related by

$$\sigma_k = (1/f_{Tk})[f_{Tk} - f_{+k} - \bar{V}_s f_{ok}/(K_{sk}\bar{V}_0)]. \tag{3}$$

The last term is small, so

$$f_{+k} \simeq f_{Tk}(1 - \sigma_k). \tag{4}$$

Similarly, the values of f_{0k} in Table I are found from Green and Green's hydraulic conductivities $L_{p,k}$ using

$$f_{0k} = \frac{\bar{V}_0[f_{Tk} - L_{p,k}\Delta x_k K_{sk} c_R f_{+k}(f_{Tk} - f_{+k})]}{L_{p,k} f_{Tk} \Delta x_k} \tag{5}$$

where c_R is the concentration of salt solution (Ringer's, for Green and Green) used in the measurement of $L_{p,k}$. Eqs. 3 and 5, which relate the phenomenological and frictional coefficients for electrolyte transport, are analogous to the equations given by Ginzburg and Katchalsky (1963) for nonelectrolytes.

Values of σ_k and $L_{p,k}$ quite different from those found by Green and Green (1969) have been reported by Mishima and Hedbys (1967). The latter authors did not use isolated membrane preparations, as did Green and Green; rather, their results are based on measurements of the thinning curves of entire cornea when either the epithelial or endothelial surfaces were exposed to solutions made hypertonic with either NaCl or solutes which apparently could not penetrate the limiting corneal layers. The endothelial properties were measured in a moist chamber, and the epithelial properties were determined in vivo, with the aqueous replaced by silicone oil to eliminate transendothelial fluxes. The values of $L_{p,k}$ found by Mishima and Hedbys are much larger than Green and Green's values, and their values of σ_k are somewhat greater. One might expect that the corneal layers were less traumatized in Mishima and Hedbys' experiments than in Green and Green's; this would explain the higher σ_k's found in the former study, but not the larger $L_{p,k}$'s. An explanation of the discrepancy in the $L_{p,k}$ values may be sought in the fact that the Mishima-Hedbys experiments were carried out at temperatures higher than those employed by Green and Green. In any event, since the Mishima and Hedbys results were so different from those reported in Green and Green, this study was extended to examine the implications of the former work as well as those of the latter.

Stanley et al. (1966) carried out a series of experiments similar to those performed by Mishima and Hedbys in their studies of the epithelium, except that Stanley et al. did not disturb the aqueous. The values of $\sigma_k L_{p,k}$ found by Stanley et al. were in fair (endothelium) to good (epithelium) agreement with those of Mishima and Hedbys. Inasmuch as Stanley et al. examined the cornea when it was in a more natural state, their results will be used to test the present theory rather than as a source of additional property data.

The results reported by Mishima and Hedbys (1967) were examined in two ways:

(*a*) Four artificial thinning curves were generated by substituting Mishima and Hedbys' values of $\sigma_k L_{p,k}$ into the equation (their Eq. 9) employed by them to interpret their own data. To simulate the endothelial properties experiment, a hypertonicity of 35 mosmol/liter salt or impermeant was used; to simulate experiments on the less permeable epithelium, the selected hypertonicity was 130 mosmol/liter, again of salt or impermeant. All the other quantities needed to construct the artificial curves are known or are in Mishima and Hedbys' paper. However, a difficulty was encountered with respect to the parameter n, which represents the reflection coefficient of the membrane of interest to the added solute divided by its reflection coefficient to salt. Clearly, $n = 1$ when the ambient hypertonicity is effected with salt, but $n > 1$ when an impermeant is used, particularly if the endothelium is under study. However, upon examining the calculated curves and asymptote in Mishima and Hedbys' Fig. 10 (endothelium experiment, 46 mosmol/liter raffinose added), it appears that $n = 1$ was used even when the added solute was an impermeant. Thus n was set equal to unity in their Eq. 9, irrespective of the identity of the added solute, to construct a set of thinning curves most representative of those found experimentally.

The Mishima-Hedbys experiments were then simulated using Eqs. 1 and 2 to find the values of f_{0k} and σ_k which best fit the thinning curves constructed in the fashion indicated above. The friction coefficients of the membrane *not* being studied in each "experiment" were raised by several orders of magnitude, thus making the membrane effectively impermeable, as assumed by Mishima and Hedbys in their analysis. For the "endothelial properties" experiment, the epithelial sodium pump rate was set equal to zero. The initial value of c_{s_3} was a reasonable 152 mM $= 1.52 \times 10^{-4}$ mol/cm^3, and $H_s(0) = 3.4453$, selected to give an initial corneal thickness of exactly 395 μm (the value in Mishima and Hedbys) with the Table I values of ϵ, ψ_2, Δx_1, and Δx_3.

The "epithelial properties" experiment was examined for two pairs of values of f_{T3} and J^a_{+3}; these parameters do not enter into the analysis of the endothelial properties experiment. As noted in Friedman (1972 *a*), Maurice (1967) has claimed that Green's (1967) measured epithelial sodium permeability, from which the value of f_{T3} in Table I is derived, is a factor of 10 too high, and these reservations are supported, at least qualitatively, by a comparison of calculated and experimental corneal potentials. The corneal potential is effectively proportional to $f_{T3}J^a_{+3}$ (Friedman, 1972 *b*), and when the parameter values in Table I are used, the calculated corneal potential is about half the experimental value of Maurice. Thus, if the value of J^a_{+3} in Table I is used with a 10-fold greater f_{T3} (equivalent to a 10-fold smaller epithelial sodium permeability), the experimental and calculated potentials would differ by a factor of five. This difference can be reduced by using a smaller epithelial pump rate; Donn et al.'s (1959) value of $J^a_{+3} = -5 \times 10^{-11}$ mol/cm^2-s was chosen. To

bring the corresponding calculated corneal potential closer to experiment, a fourfold greater value of f_{T3} was used; $f_{T3} = 3.12 \times 10^{12}$ J-s/mol-cm^2.

(*b*) The values of σ_k and $L_{p,k}$ given by Mishima and Hedbys were used directly to simulate the living cornea; Eqs. 4 and 5 were used to translate these values into the quantities f_{+k} and f_{0k}. It will be seen from Eq. 5 that f_{0k} is positive only if $f_{Tk} > L_{p,k}\Delta x_k K_{sk} c_R f_{+k}(f_{Tk} - f_{+k})$ or, using Eq. 4, if $L_{p,k}\Delta x_k K_{sk} c_R f_{Tk}\sigma_k(1 - \sigma_k) < 1$. This condition is not satisfied for either the epithelium or the endothelium when the values of $L_{p,k}$ and σ_k in Mishima and Hedbys ($\sigma_3 = 0.87$ from the $\sigma L_p RT$ values in their Table I) are used; that is, f_{0k} as given by Eq. 5 is negative. This is so for f_{03} even when the base case value of f_{T3} is used. This inconsistency of the Mishima-Hedbys parameters with the representation of corneal transport presented earlier (Friedman, 1972 *a*) is troubling; however, the phenomenological corneal behavior implied by the Mishima-Hedbys values of $L_{p,k}$ can still be examined in the present context by permitting f_{0k} to assume the negative values given by Eq. 5. The Mishima-Hedbys parameters translate to $f_{+1} = 1.58 \times 10^{11}$ J-s/mol-cm^2 ($\sigma_1 = 0.61$), $f_{01} = -2.09 \times 10^8$; $f_{+3} = 1.03 \times 10^{11}$ J-s/mol-cm^2, $f_{03} = -3.92 \times 10^8$, for $f_{T3} = 7.94 \times 10^{11}$ J-s/mol-cm^2; $f_{+3} = 4.12 \times 10^{11}$ J-s/mol-cm^2, $f_{03} = -1.77 \times 10^9$, for $f_{T3} = 3.12 \times 10^{12}$ J-s/mol-cm^2 (See Note Added in Proof).

The value of $L_{p,3}$ found by Green and Green (1969) yields a positive f_{03} when the Table I value of f_{T3} is used, but f_{03} is calculated to be negative when a fourfold larger value of f_{T3} is employed in its computation. For this reason, the alternative values of f_{T3} and J^a_{+3} discussed above were used to interpret only the Mishima and Hedbys (1967) data.

RESULTS FOR NORMAL CORNEA

Base Case (Table I) Parameters

The calculated periodic behavior of the normal cornea, using the base case parameters, is shown in Fig. 1. The figure covers one 6-h cycle, from the beginning of one nap to the beginning of the next one. When the animal goes to sleep, the tear tonicity is regarded as undergoing a step change from $(c_{s3})_0$ to $(c_{s3})_c$. The fluxes across the epithelium change discontinuously in response to this discontinuous change in boundary conditions, but the endothelial fluxes, stromal salt content, and corneal thickness remain continuous, though their derivatives are not. Since c_{ss} changes slowly, the tears are initially hypotonic to the stromal fluid, and there is a brief time during which fluid flows posteriorly across the epithelium ($J_{03} < 0$). The epithelial salt flux contains a large constant component due to the sodium pump in this membrane, and the influence of c_{s3} on J_{s3} is relatively small. Water continues to enter the stroma across the endothelium, and since it is no longer extracted to any extent across the epithelium, the tissue swells. This swelling causes c_{ss} to fall towards the aqueous salt concentration, and the magnitude of the transendothelial salt flux diminishes as the driving force $c_{ss} - c_{s0}$ becomes less. At the same time, the driving

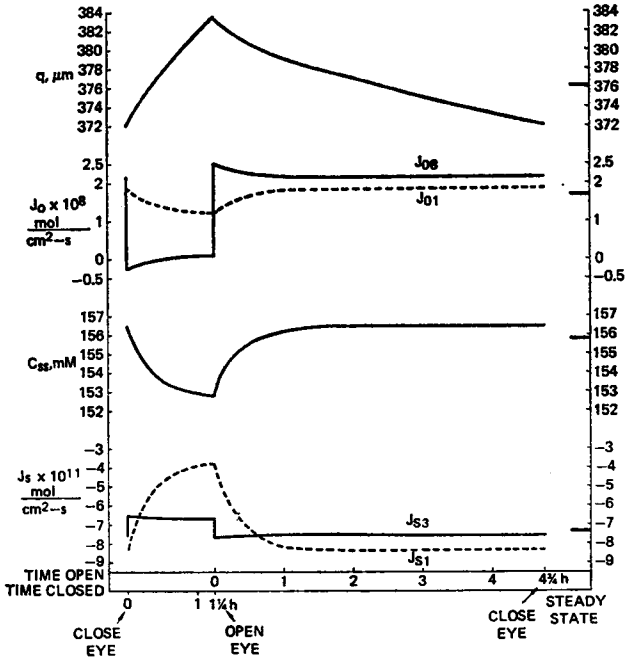

FIGURE 1 Calculated periodic behavior of normal rabbit cornea. q is the corneal thickness. The heavy lines on the right ordinate are the time-average steady values from Friedman (1972 *a*).

force $c_{s3} - c_{ss}$ for passive salt flow across the epithelium becomes larger, so $|J_{s3}|$ gradually increases. As c_{ss} falls, an osmotic driving force for anteriorly directed transepithelial flow develops and that for osmotic flow across the endothelium becomes less; accordingly, J_{03} eventually becomes positive and J_{01} slowly diminishes as the animal sleeps.

The response of the cornea when the animal awakes can be viewed similarly. The tear tonicity rises suddenly, and the epithelial water flow is suddenly greater than the inward flux across the endothelium. The tissue thins, causing c_{ss} to rise, this increase being augmented by the discontinuous rise in transepithelial salt flux following the jump in tear tonicity. The steady increase in c_{ss} causes $|J_{s1}|$ to increase with time and $|J_{s3}|$ to decrease slowly after its initial jump; similarly, the changing osmotic driving force causes J_{01} to rise gradually, and J_{03} becomes less positive during the open eye phase.

As the eye remains open, the fluxes assume nearly constant, *but unequal*, values. Thus more water leaves the stroma across the epithelium than enters across the endothelium ($J_{03} > J_{01}$) and more salt leaves the stroma across the endothelium than enters across the epithelium ($-J_{s1} > -J_{s3}$). The stroma thins slowly, because of the inequality of water fluxes; however, the rate of salt loss is such that c_{ss} changes only slightly with time. The difference between the nearly constant flux values after

the eye has been open for some time is such that, over a cycle, the algebraic area between the J_{01} and J_{03} curves, and the J_{s1} and J_{s3} curves, is zero.

The properties in Fig. 1 may be regarded as oscillating about steady-state values which correspond to some constant tear tonicity intermediate between $(c_{s3})_c$ and $(c_{s3})_0$. An estimate of this tonicity is $c_{s3} = [\tau_c(c_{s3})_c + \tau_0(c_{s3})_0]/(\tau_c + \tau_0) = 1.77 \times 10^{-4}$ mol/cm^3. It is this concentration which was used to define the time-average steady state in Friedman (1972 *a*). The corresponding steady values of q, J_0, c_{ss}, and J_s, taken from the previous work, are indicated on the right-hand ordinate in Fig. 1. They may be regarded as the limiting values of the curves in the figure as τ_0 and τ_c go to zero, with τ_0/τ_c fixed at the value used here.

Simulation of the Mishima-Hedbys Experiments

The results of the simulation of the epithelial properties and endothelial properties experiments will be discussed in turn.

Epithelial Properties Experiment. Initially, σ_3 was set equal to 0.87[1] (Mishima and Hedbys' Table I value) and the value of f_{03} which gave a calculated thinning curve in agreement with the artificial data when impermeant was added to the tears, was sought. Even when f_{03} was zero, the calculated cornea thinned too slowly, for either pair of f_{T3} and J^a_{+3}. No faster thinning rate could be obtained at fixed σ_3 without making f_{03} negative, an option excluded here. Hence, σ_3 was raised, with f_{03} held at zero. With the modified values of f_{T3} and J^a_{+3}, the calculated cornea still thinned too slowly, even when σ_3 was 0.99, so this option was dropped. For the base case values of f_{T3} and J^a_{+3}, the calculated cornea thinned too slowly when σ_3 was 0.96 and too rapidly when σ_3 was 0.99. Hence, σ_3 was tentatively set at 0.99 and f_{03} was raised from zero to reduce the thinning rate. A value of $f_{03} = 2.4 \times 10^7$ J-s/mol-cm^2 gave a thinning curve which agreed with the artificial data. When these parameters were used in a simulation of the experiments in which hypertonicity was effected by adding NaCl, agreement with these artificial data was also observed. Hence, the derived epithelial properties are $\sigma_3 = 0.99$, $f_{03} = 2.4 \times 10^7$ J-s/mol-cm^2.

Endothelial Properties Experiment. Initially, σ_1 was set equal to 0.6 and the value of f_{01} which gave a calculated thinning curve in agreement with the artificial data when impermeant was added to the aqueous, was sought. Even when f_{01} was zero, the calculated cornea thinned too slowly, and this disagreement between the calculations and the artificial data remained even after σ_1 had been raised to 0.8. This discrepancy cannot result from the use of $n = 1$ in the construction of the artificial data; the artificial thinning curve from Mishima and Hedbys' Eq. 9 would have been even steeper had a value of $n > 1$ been employed. Furthermore, the calculated cornea, with $f_{01} = 0$ and $\sigma_1 = 0.8$, thins too slowly when salt is added to the aqueous,

[1] Note that σ_k always refers to the reflection coefficient to *salt*; that to impermeant is always unity.

and here there is no ambiguity regarding the proper value of n to use in constructing the appropriate artificial data. It seemed unrealistic to raise the endothelial reflection coefficient above *twice* the value found by Green and Green (1969). The only remaining alternative, apart from permitting f_{01} to be negative, was the inclusion of a "pump" in the endothelium to speed up the calculated thinning rate. Without considering the detailed mechanism of such a pump, it was assumed that its only effect was a vectorial transport of water from the stroma to the aqueous ($J^a_{01} < 0$). The inclusion of a pump whose rate was 1.3×10^{-7} mol/cm^2-s (84 μm/h) gave a thinning curve which fit the artificial data. This pump rate also gave a calculated thinning curve which agreed with the artificial data for the case in which hypertonicity was effected by adding NaCl. Hence the derived endothelial properties are $\sigma_1 = 0.80$, $f_{01} \approx 0$, $J^a_{01} = -1.3 \times 10^{-7}$ mol/cm^2-s.

The values of f_{+k}, f_{0k}, and J^a_{01} in Table I were replaced by the values derived from the Mishima-Hedbys experiments (using Eq. 4 to go from σ_k to f_{+k}), and an integration corresponding to that which led to Fig. 1 was carried out. The cornea swelled considerably as the integration proceeded and did not achieve a periodic state, even though a "water pump" of reasonable magnitude (Maurice, 1972) was sited in the endothelium.

Mishima and Hedbys Parameters

When the Mishima-Hedbys parameters were used at face value ($f_{0k} < 0$), in place of their Table I counterparts, the calculated cornea continued to swell as the integration proceeded. No periodic state was achieved as the tear tonicity alternated between 155 and 183 mM. A posteriorly directed endothelial water pump was arbitrarily sited in the cornea, to see if this would permit a reasonable periodic solution; instead, the calculated cornea swelled still more rapidly, again without achieving a periodic state.

It was possible to achieve a periodic state with the Mishima-Hedbys parameters by siting an isotonic, neutral posteriorly directed pump in the endothelium. The isotonic active transport rates which gave a normal time-average corneal thickness with the Mishima-Hedbys parameters were 43 μm/h for the base case values of f_{T3} and J^a_{+3} and 26 μm/h with the modified values. However, the thickness-time curves were strange. For the base case epithelial permeability and pump rate, the corneal thickness was 371 μm when the eyes were first closed. Exposure to 155 mM tears caused the cornea to swell to a thickness of 383 μm, but then the cornea began to thin, *with* c_{s3} *still at* $(c_{s3})_c$, its thickness reaching 374 μm by the time τ_o had elapsed. The tear tonicity then rose to $(c_{s3})_0$, simulating the opening of the eye, and the cornea continued to thin. However, after reaching a thickness of 361 μm, it began to *swell* in spite of the continuing tear hypertonicity and reached a thickness of 371

μm at the end of the cycle. Qualitatively identical behavior was observed with the alternative values of f_{T3} and J^a_{+3}.

DISCUSSION

Temporal Variations in Normal Corneal Thickness

It can be seen from Fig. 1 that, except for the epithelial water flux (J_{03}) and the endothelial salt flux (J_{s1}), the parameters in the figure oscillate with an amplitude which is small relative to their time-average values on the right-hand ordinate. These time-average values have already been compared with observed corneal properties (Friedman, 1972 *a*), and only the excursions of these properties about their time-average values need be discussed here.

The only corneal property whose time-dependence has been measured in vivo is thickness itself. The most thorough work is that of Mishima and Maurice (1961). They found that when the rabbit eye was opened, the cornea thinned by approximately 15 μm, in good agreement with the 12 μm excursion in Fig. 1. The shape of the experimental $q - t$ curves for the thinning which accompanied the opening of the eyes and the swelling which followed either eye closure or bathing in 0.9% NaCl, differs somewhat from the calculated shape, in that the $q - t$ curve in Fig. 1 is more gentle than those given by Mishima and Maurice. The experimental work shows a steady change in thickness during the first hour or so, but little change in thickness thereafter. The experimental data for transient corneal swelling under the above conditions is compared with the present theory in the left portion of Fig. 2. Considering the a priori nature of the calculation and the absence of any adjustable parameters from the analysis, the agreement with experiment is really quite good.

Mishima and Maurice (1961) also found that the swelling induced by eye closure

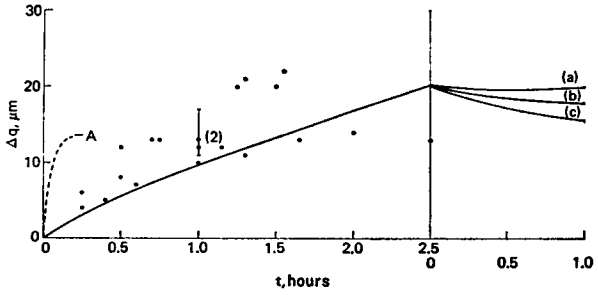

FIGURE 2 Variation with time of rabbit corneal thickness during eye closure or bathing with 155 mM (0.9%) NaCl. Δq equals the change in corneal thickness from initial value. ●, experimental data from Mishima and Maurice (1961); vertical bar summarizes Hara's (1970) experimental results; ——, theoretical curve, present work; ----, theoretical curve based on Stanley et al. (1966), including osmometer asymptote A. A new time scale begins after 2.5 h, showing calculated course of corneal thinning when eye bathed with (*a*) 172 mM (1%), (*b*) 177 mM, or (*c*) 183 mM NaCl.

or bathing with isotonic saline could be reversed by bathing the corneal surface with 1% NaCl. The calculated effect of raised tear tonicity on the swelling curve of a cornea previously exposed to 0.9% NaCl is illustrated in the right portion of Fig. 2, and it is evident that the experimental corneal response is not reproduced here. Even after the cornea has swelled in 0.9% NaCl for 2½ h, replacement of the tear solution by 1% NaCl does not cause much thinning (curve a); after a 30 min thinning transient, the cornea resumes its swelling, but at a much reduced rate. Both the origins and implications of this discrepancy will now be discussed.

As noted earlier, the time-average tear tonicity employed here is about 177 mM. The corneal thickness in Fig. 1 oscillates about that value which would obtain were the cornea exposed indefinitely to 177 mM NaCl. This base-line thickness was calculated in Friedman (1972 *a*) and found to be 376 μm. The *steady* corneal thickness is highly sensitive to c_{s3}; hence the range of thicknesses in Fig. 1 corresponds in this sense to a very small range in c_{s3}. That is, the tear tonicities which, if applied indefinitely, would give steady corneal thicknesses of 372 μm (open eye, just at closing) or 384 μm (closed eye, just at opening) are both very close to 177 mM. As a consequence, a cornea which is exposed to a tear solution less concentrated than 177 mM and is swelling is calculated to "reverse" *only* if the tear tonicity is raised to a concentration in the neighborhood of 177 mM or above (Fig. 2, curves b, c). This requirement is not met when a 1% NaCl bath is employed.

Since a 1% NaCl bath does in fact cause reversal experimentally, it may be inferred that the time-average tonicity employed here is too high, reflecting an overestimate of $(c_{s3})_0$. Mishima and Maurice (1961) stated that the responses of individual animals varied in their experiments, but that $(c_{s3})_0$ was certainly greater than 0.9% and less than 1.1% saline; the Table I value of $(c_{s3})_0$ lies within this range.

Comparison of Theory with the Experiments of Stanley et al. (1966)

Stanley et al. measured the thinning curves of in vivo rabbit corneas whose anterior surfaces were bathed by strongly hypertonic media (1.3–1.5% NaCl or glucose equivalent). Their experimental results for 1.5% NaCl perfusion (J. A. Stanley, personal communication) and the fit of their own thinning rate expression to these data are compared with the present theory in Fig. 3. It is evident that, as would be expected, when Stanley et al.'s values of $\sigma_k L_{p,k}$ are substituted into their own thinning equation, a calculated curve which agrees rather well with their experimental results is obtained. It is also evident that the present analysis, using the Table I corneal parameters, predicts a thinning rate which is much less than that found experimentally.

The inability of the present theory to reproduce the Stanley et al. (1966) data, as contrasted with its comparative success in describing the response of the cornea to small changes in tear tonicity (Fig. 2, left), reflects the inconsistency of the two sets

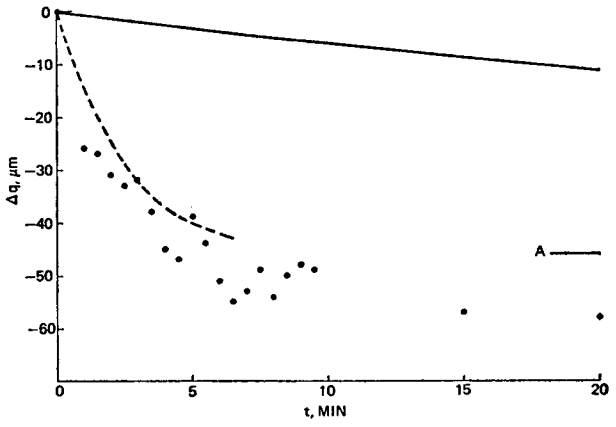

FIGURE 3 Variation with time of rabbit corneal thickness during anterior surface perfusion with 1.5% NaCl. Δq is the change in corneal thickness from initial value. ●, sample experimental data from Stanley et al. (1966); ----, thinning curve calculated from Stanley et al.'s $\sigma_k L_{p,k}$ values, using their thinning rate formula. The osmometer asymptote A from their formula is shown on the right ordinate. ——, theoretical curve, present work.

of experimental data rather than a fundamental defect in the theory. This is demonstrated in Fig. 2, where Stanley et al.'s permeabilities *and* correlating equation are used to predict the swelling rate of in vivo cornea when the tear salinity is lowered to 155 mM. As would be expected from Fig. 3, the swelling rate calculated on the basis of Stanley et al.'s numbers and equation is much more rapid than that given by the present work. It is also much more rapid than has been found experimentally, since according to the Stanley et al.-based calculation, the cornea should swell to within 1 μm of its osmometer asymptote in only 7–8 min. One can only speculate about the origins of this inconsistency; perhaps the osmotic shock imposed by Stanley et al. at the anterior corneal surface caused the epithelial properties to change from their normal values.

The values of $\sigma_k L_{p,k}$ found by Stanley et al. (1966) are similar to those of Mishima and Hedbys (1967) and were found using similar experimental and analytical procedures, the chief difference between the two being the more normal corneal state in the former experiments. The implications of the Stanley et al. numbers cannot be explored as fully as could those of Mishima and Hedbys, because σ_k cannot be found with confidence from the former work, but it is likely that the problems encountered with the latter data, both with respect to the sign of the derived f_{0k} and the inability to achieve a realistic periodic state even with a potent endothelial pump, are common to both sets.

Finally, it may be noted that Stanley et al.'s observation that corneas with damaged endothelia thin more slowly under topical hypertonicity has been confirmed qualitatively by numerical simulations using the techniques presented here.

The data reduction procedures used by Mishima and Hedbys (1967) and Stanley et al. (1966) were based on the approximation that the salt content (n_s, moles per square centimeter of corneal area) of the stroma had remained constant during their experiments; a justifying calculation was given by the former authors. That is, these investigators regarded the cornea as behaving osmometrically during their experiments. The cornea is bounded by solute-permeable membranes, so it is clearly not a *perfect* osmometer, and a more rigorous statement of the earlier workers' approximation is that the characteristic time constant for salt flux into or out of the stroma is much greater than the duration of their experiments: $\theta_s \gg \theta_{expt}$. The characteristic time for changing the water content of the stroma (θ_0) is clearly more comparable with θ_{expt}; were this not so, gross corneal swelling or thinning would not have been observed.

The shape of the corneal swelling and thinning curves can conveniently be discussed in terms of the osmometric quality of the stroma. This discussion begins with a qualitative description of how corneal thickness should vary with time if the salt content of the stroma responds very slowly to changes in ambient concentration. The unsteady calculations given earlier are employed to obtain quantitative measures of the constancy of n_s in the normal state; it is shown that when θ_{expt} is of the order of hours rather than minutes, variations in n_s become nonnegligible. The effect of the epithelial sodium pump on n_s is also mentioned briefly. Two additional influences on the shape of the $q(t)$ curves are discussed here as well, though they are more peripheral to the matter of stromal osmometry; these are the effects of the flow resistance of the limiting corneal layers and of possible cycles in aqueous tonicity.

When θ_0 is small and $\theta_s \gg \theta_{expt}$, the following variation of corneal thickness with time is to be expected: the tonicity of the tear film is (for example) raised, and the cornea begins to thin. After a time of the order of θ_0, the water fluxes through the epithelium and endothelium are nearly equal, but the salt content of the stroma has changed little from its initial value. Even though the corneal thickness is no longer changing measurably, since the water fluxes are now nearly equal, the cornea is *not* in a steady state because the stromal salt concentration (c_{ss}, moles per cubic centimeter of stromal fluid) is far removed from its steady value. The dependence of corneal thickness on tear tonicity in this *apparent* steady state has been derived (Friedman, 1972 *b*). The cornea then continues to thin at a much slower rate as the salt content of the stroma approaches its equilibrium value; however, since $\theta_s \gg \theta_{expt}$, the experiment is over before much additional thinning takes place. The thinning curve accordingly exhibits a pronounced change in slope, as is most evident in Stanley et al.'s (1966) experimental data (Fig. 3).

The change in salt concentration which accompanies an osmometric change in thickness is easily found. The salt concentration in the stroma is $n_s/(q_s - \psi_2)$, where

q_s is stromal thickness and $q_s - \psi_2$ is that portion of the stromal thickness which is occupied by fluid. If n_s is held constant at its time-average value $n_s^\circ = H_s^\circ \psi_2 c_{ss}^\circ / \epsilon$, then

$$(\Delta c_{ss})_{n_s^\circ \text{ const}} = \frac{H_s^\circ \psi_2 c_{ss}^\circ}{\epsilon} \cdot \Delta(q_s - \psi_2)^{-1}.$$

For the 12 μm excursion of corneal thickness in Fig. 1, $(\Delta c_{ss})_{n_s^\circ \text{ const}} = 6.8$ mM, where the time-average values H_s° and c_{ss}° are taken from Friedman (1972 *a*). The calculated excursion of c_{ss} is 3.7 mM, indicating that the inequality $\theta_s \gg \theta_{\text{expt}}$ does not hold too well when τ_c and τ_0, the normal in vivo counterparts of θ_{expt}, are used.

The variation of n_s with time in the in vivo cornea follows from the work presented above, since $dn_s/dt = J_{s1} - J_{s3}$. From the graphs of salt flux vs. time in Fig. 1, it can be seen that n_s falls ($J_{s3} > J_{s1}$) after the eye has been opened for 40 min and continues to fall until the eye has been closed for 10 min. During the remainder of the cycle, $J_{s1} > J_{s3}$ and n_s rises. The physical origins of this variation follow directly from the discussion of Fig. 1.

The magnitude of the excursions of n_s is shown in Fig. 4. The ordinate is $c_{ss}H_s$, millimoles per kilogram of dry tissue for unit density stromal fluid, to which n_s is directly proportional. The range of the excursion is about 3% of the mean value of n_s; since the corresponding percentage for the tear salt concentration is 17%, the stroma does act as a rather good osmometer.

The cornea acts as an imperfect osmometer not only because salt can diffuse across all of the corneal layers, but also because of the epithelial sodium pump, which is responsible for most of the inward salt flux across the epithelium. Indeed, were the corneal layers impermeable to salt, apart from the pump-related influx, the pump would cause the stromal salt content to rise at a rate of 28 mmol/kg dry tissue-h; at normal hydration, c_{ss} would rise by about 9 mM/h.

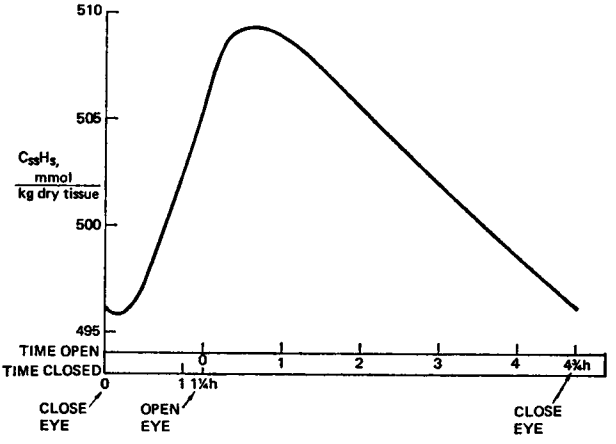

FIGURE 4 Calculated periodic variation of the salt content of normal rabbit corneal stroma.

As noted earlier, the corneal thickness curves would be expected to exhibit a "knee" when $\theta_s \gg \theta_{expt}$. One might ask why, if the stroma is a rather good osmometer, the calculated $q(t)$ curves in Figs. 1–3 are so gentle. The reason is that the sharpness of the break in the curve depends not only on the osmometric quality of the cornea, but also on the rate at which the apparent steady state is approached. The faster water enters or leaves the stroma during the initial transient, the sharper the break will appear to be. This rate is, of course, determined by the flow conductivities and reflection coefficients of the limiting corneal layers.

It can readily be shown that the flow resistance of these layers is sufficiently large to account for the apparent gentleness of the calculated swelling/thinning curves for the base case cornea; simply stated, water does not flow into or out of the stroma very fast. If the equations of Stanley et al. (1966), in which the cornea is regarded as a *perfect* osmometer, are used with the Table I parameters to describe the transient which follows eye closure, the cornea is predicted to swell from its starting thickness of 372 μm to a thickness of 380 μm only after 38 min have elapsed. The detailed numerical solution in Fig. 1 gives a corneal thickness of 380 μm after 47 min, indicating that the resistance of the corneal cell layers to water flow acts importantly to cause the absence of a knee from the calculated thickness-time curves. No better agreement between the Stanley et al. equations and the present work can be expected, since the former formulation neglects not only passive ion transport across the epithelium and endothelium, but also active epithelial transport and all hydrostatic driving forces.

Finally, mention should be made of the changes in aqueous tonicity (measured by c_{s0}) which accompany the sleep-wake cycle. Because of the capacity of the anterior chamber and the high aqueous flow rate (cubic centimeters per minute) compared with the flows across the cornea, c_{s0} is not particularly sensitive to c_{s3}. Indeed, Mishima and Maurice (1961) have found the aqueous of the open eye to be only about 2 mM more concentrated than that of the closed eye. Numerical experiments have shown that in the normal regime, the steady corneal thickness is determined by $c_{s3} - c_{s0}$ rather than by c_{s3} alone; this distinction has hitherto been moot since c_{s0} has been regarded as constant. In view of the important role of the transcorneal tonicity difference, the concomitant variation of c_{s0} with c_{s3} mitigates to a small extent the sensitivity of corneal thickness to tear tonicity. The unsteady-state calculations in Fig. 1 support Mishima and Maurice's result; for instance, when the eye is closed, less salt enters the anterior chamber across the endothelium (J_{s1} becomes less negative) and less water leaves the anterior chamber across the endothelium (J_{01} falls). These variations both cause the aqueous to become more dilute.

Interestingly, the variation in c_{s0} enhances the osmometric character of the cornea by promoting salt loss from the cornea when the eye is closed ($c_{ss} - c_{s0}$ is raised when c_{s0} falls) and inhibiting it when the eye is open. Since the salt content of the stroma rises during eye closure and falls while the eye is open, it is evident that by

its effect on J_{s1} the sleep-wake variation in c_{s0} acts to make the cornea a better osmometer than Fig. 4 would indicate.

The Time-Average Steady State (TASS) of the Cornea

The cornea is now seen as a structure which responds slowly to the periodic variations in tear tonicity which accompany the normal sleep-wake cycle. Accordingly, its thickness oscillates with an amplitude which is much less than the difference between the steady-state thicknesses that would result from continued exposure to $(c_{ss})_e$ and $(c_{ss})_0$. Intuition suggests that the mean state about which these oscillations take place is the TASS; that is, the steady state which would result were the cornea exposed continuously to a tear tonicity $(c_{ss})_{\text{time av}} = \int_0^\tau c_{ss} dt/\tau$. This intuitive conclusion is supported by the steady-state calculations (Friedman, 1971 *a*) whose results are presented on the right-hand ordinate of Fig. 1. Furthermore, for small excursions from the mean values of c_{ss} and H_s, Eqs. 1 and 2 can be linearized. The corneal oscillations are driven, in the present simple model, by variations in only tear tonicity, and it can be shown analytically that the mean state of the dynamic cornea does approach the TASS as the validity of the linearization improves.

The TASS is a most important corneal state, since the properties of this state are the ones most likely to be employed to describe the cornea. It can be found for the base case corneal parameters in Table I, with $(c_{ss})_{\text{time av}} = 177$ mM, and the TASS in the normal state and under abnormal conditions has been the subject of earlier work (Friedman, 1972 *a*, *b*). Use of the Mishima-Hedbys parameters, either those found from computer simulations of their experiments or those taken from their paper, leads to corneal simulations which do not suggest the existence of a TASS; in fact, a TASS does exist, but with a *negative* swelling pressure which cannot be reached numerically because of the assumed form of the swelling pressure-hydration relationship. The combination of active endothelial isotonic transport with the parameters reported by Mishima and Hedbys does yield a TASS, but the oscillations about it are unusual, to say the least. The reason for this behavior is that the steady swelling pressure of a cornea possessing the Mishima-Hedbys parameters *increases* as the tear tonicity is lowered; thus, when the eye is closed, there is an osmotic transient which causes swelling, but, with time, a maximum thickness is reached as the cornea "reverses" and tends towards the smaller steady thickness which corresponds to the lower tear tonicity.

These unusual features of "Mishima-Hedbys corneas" follow from the high epithelial and endothelial hydraulic conductivities found by these authors. The state of the aqueous and tears is the same for all model corneas, and the TASS transcorneal water flux is 11 times the base case TASS value when the Mishima-Hedbys conductivities are used. The higher reflection coefficients found by Mishima and Hedbys also play a role here. In the absence of any endothelial active transport, salt is convected into the stroma at a rate which is seven times the base case rate, and c_{ss} is

accordingly raised. Thus the osmotic driving force for flow into the tears becomes less, and the stromal fluid pressure must be greater to maintain identical flows across the endothelium and epithelium. This raised P_s is positive and greater than P_0 ; by Eq. 1 *e*, the TASS p_s is negative.[2]

When water is actively transported across the endothelium ($J^a_{01} < 0$) in Mishima-Hedbys corneas, flow continuity is maintained in the TASS by an increase in J^p_{01} and a decrease in J^p_{03} from their values when there is no active transport. Here the superscript p denotes passive flux: $J_{0k} = J^a_{0k} + J^p_{0k}$. Thus more salt is convected into the stroma since the rate of convection is determined by the passive flux of solvent, and less is convected out, leading to a further increase in c_{ss} ; this in turn causes the osmotic flow out across the epithelium to diminish. The decrease in osmotic flow is greater than the drop in J^p_{03} which is required to maintain identical flows across all corneal layers, so the driving force for hydraulic flow across the epithelium rises; that is, P_s increases. Hence, insertion of an endothelial water pump into a cornea possessing the Mishima-Hedbys parameters causes the equilibrium stromal swelling pressure to become still more negative.

A normal stromal swelling pressure can be reached by inserting a posteriorly directed isotonic pump into a "Mishima-Hedbys cornea," since the active solute transport cancels most of the convective effect of J^p_{01}. When the tear tonicity is reduced, the passive fluid flow across the cornea is diminished because the osmotic driving force for this flux falls. The stromal salt concentration decreases three times as much as in the base case cornea under similar circumstances, because not only is the driving force for diffusional entry of salt across the epithelium reduced by lowering c_{s3}, but, in addition, substantially less salt is convected in across the endothelium. The greater decrease in c_{ss} causes the osmotic driving force for transepithelial water flow ($c_{s3} - c_{ss}$) to fall by less in the Mishima-Hedbys cornea than in the base case cornea, when the tear tonicity is lowered. If no change in P_s accompanied a reduced tear tonicity in the base case, the remaining osmotic pressure difference across the epithelium would be insufficient to balance the passive fluid inflow across the endothelium, and P_s accordingly rises (p_s falls) when c_{s3} is reduced, in the base case. However, in the Mishima-Hedbys cornea, the remaining osmotic pressure difference would drive *more* water across the epithelium than enters across the endothelium, in the absence of a change in P_s. Hence, when the tear tonicity bounding a Mishima-Hedbys cornea is reduced, P_s falls (and p_s rises) to inhibit flow across the epithelium and to enhance flow across the endothelium. As a result, the TASS thickness of the Mishima-Hedbys cornea is reduced when the tears are made more hypotonic.

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[2] To review the relation between steady stromal fluid pressure (P_s) and corneal thickness, the relation $P_s + p_s = P_0$ (Eq. 1 *e*) shows that, at constant intraocular pressure, an increase in P_s is accompanied by a decrease in p_s. The stroma assumes a hydration such that its swelling pressure is $P_0 - P_s$; hence, if TASS flow continuity ($J_{01} = J_{03}$) demands a rise in P_s, then the cornea swells.

CONCLUDING REMARKS

The picture of the cornea developed and discussed above represents a considerable departure from earlier views of corneal behavior. The cornea is seen here to respond to variations in tear tonicity with time constants of such magnitude that it is normally never in the steady state. The time constants for salt entry into and exit from the stroma are such that the cornea responds as an imperfect osmometer to sleep-wake variations in tear tonicity. Because the corneal time constants are long compared with the normal sleep-wake cycle of the animal, the corneal thickness oscillates with a relatively small amplitude about a value which is the steady thickness which would result from continued exposure to a time-average tear tonicity. The time-average tonicity is hypertonic to the stromal fluid and the calculations presented here indicate that it can be sufficiently hypertonic to maintain corneal deturgescence in the absence of any active dehydration mechanism. The importance of transepithelial osmosis is such that the inclusion of the fluxes across this cell layer is a requirement of any realistic description of in vivo corneal hydration dynamics.

Although the analysis does show that for a reasonable set of corneal properties and boundary conditions, the normal corneal thickness can be achieved solely by the deturgescent effect of transepithelial osmosis, the uncertainties in the input parameters are such that it cannot be inferred that other dehydrating mechanisms must be absent, even in the normal state. Even so, the basically unsteady nature of in vivo corneal dynamics and the novelty of this theory based upon it would not be altered were any additional active transport system proven to be in the cornea. For instance, one would get a similar picture if there were significant imbibition from the limbal capillaries and this additional fluid were actively excreted by the endothelium, or if the open-eye tear tonicity were less than the value (Iwata et al., 1969) employed here and a deturgescent pump acted in a compensatory fashion. It might be noted that the key in vivo observation, in particular, the small amount of corneal swelling when the eye is bathed by isotonic or mildly hypotonic media (Von Bahr, 1949; Mishima and Maurice, 1961), which argues against hydration control solely by osmosis across the epithelium, is suggested here to reflect the slowness with which the cornea responds to changes in its environment. Since the a priori calculations presented here yield a normal corneal thickness without including any active vectorial transport system save for the epithelial sodium pump, the fact that such a thickness is normally achieved does not imply that additional transport systems *must* act in the normal state.

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Note Added in Proof. The transport equations used here and in earlier (Friedman, 1972 *a*) work are based on the assumption that salt and water share a single common pathway as they traverse the limiting corneal layers. Recent calculations have shown that if multiple parallel pathways are allowed, the negative values of f_{0k} found from the Mishima-Hedbys parameters can be explained, and the passive salt fluxes which are obtained when these parameters are used in corneal simulations are reduced from the values found in the present work. The implications of these observations, which increase the credibility of the Mishima-Hedbys parameters, will be the subject of a subsequent communication.