

Theoretical basis for an anomalous temperature coefficient in swelling pressure of rabbit corneal stroma

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ABSTRACT In the rabbit corneal stroma, the swelling pressure, P , has been reported to have an anomalous (negative) temperature coefficient, α_p , contradicting traditional Donnan swelling theory. A parallel-plate, diffuse double layer Gouy-Chapman model was used to resolve this discrepancy. The present model incorporates the possibility that surface charge, σ , is temperature dependent. It is shown that negative,

zero, or positive coefficients of swelling pressure change with temperature are not mutually exclusive conditions, but can be attributed to the *same* underlying mechanism. For likely values of α_p (range $-7 \times 10^{-3} \text{ K}^{-1}$ to $+3.2 \times 10^{-3} \text{ K}^{-1}$), the effective stromal charge has a negative temperature dependency, or $\text{dln}\sigma/\text{d}T < 0$. The present formalism is robust against variation in assumed α_p , and is able to simulta-

neously satisfy the known values of swelling pressure, its thermal dependency, and stromal charge. These results implicate significant coulombic forces behind P . Predicted stromal surface charge is $\sim 0.01 \text{ Cm}^{-2}$. The predictions were confirmed with macro-continuum Donnan swelling theory, suggesting that Donnan osmotic swelling is the principal macroscopic component of P .

INTRODUCTION

The mammalian corneal stroma exhibits an expansive pressure P of $\sim 8 \text{ kPa}$ (60 mmHg) at normal hydration ($3.1\text{--}3.5 \text{ kg H}_2\text{O/kg dry weight}$ [see Maurice, 1984]). This expansive or swelling pressure is exerted both in vivo (Hedbys et al., 1963) and in vitro (Kinsey and Cogan, 1942). Thus, the stroma demonstrates a propensity to imbibe water to many times the original weight, but corneal edema is normally prevented in vivo by the presence and activity of epithelia bounding the stroma.

The origin of the swelling pressure has remained largely controversial. The corneal stroma consists of several hundred lamellae with each lamella containing parallel collagen fibrils of 30 nm diam spaced $\sim 60 \text{ nm}$ apart in a quasiregular fashion. Fixed charges arise from dissociation of the sulphonic acid and carboxylic acid groups of the stromal proteoglycans which are in physicochemical association with the collagen fibrils. Hodson (1971) showed that on a macroscopic scale, P could be explained in terms of a Donnan effect, whereby fixed charges in the corneal stroma are able to provide a chemical potential difference favoring fluid influx from an external bath into the extracellular space of the stroma. Based on the apparent distribution of radiolabeled Na^+ , Hodson (1971) determined a stromal charge of $n_f = 0.048 \text{ M}$ in the rabbit cornea, which corresponds to the concentration he calculated that is required to

generate $P = 8 \text{ kPa}$. This matched earlier determinations of $0.055\text{--}0.072 \text{ M}$ (Catchpole et al., 1966; Joseph et al., 1969). In contrast, Friedman et al. (1972) and Friedman and Green (1971) inferred an effective fixed charge in the corneal stroma that was markedly lower than Hodson's (1971) figure, and concluded that Donnan swelling theory was unable to entirely account for P . However, the central assumption in Friedman and Green (1971) of no stromal Cl^- binding has been questioned (Elliott, 1980; Whitburn, 1981). Indeed, given a modicum of stromal Cl^- immobilization, the n_f predicted by Friedman et al. (1972) could have been significantly underestimated (Kwok, 1986). An n_f of 0.033 M was predicted in bovine corneal stroma by Elliott et al. (1980) using Donnan osmotic swelling theory. As intimated by Elliott et al. (1980), their model lacked accuracy in the physiological range of hydrations, a shortcoming subsequently confirmed in cat corneal stromas (Kwok, 1986). However, recent improvements to the approach (Kwok and Klyce, 1988) provide n_f values consistent with Hodson's (1971) proposals, suggesting that the Donnan effect is the principal component of the macroscopic stromal swelling pressure.

As shown below, Donnan swelling theory predicts that the temperature coefficient of pressure change $\alpha'_p = (\Delta P/P)/\Delta T$ is positive. An apparent anomaly was found by Hara and Maurice (1972), who reported that $\alpha'_p \approx -7 \times 10^{-3} \text{ K}^{-1}$ for rabbit corneal stroma. Swelling pressure P was found to fall if the temperature was raised

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from 22 to 37°C, whereas P was observed to rise when the temperature was reduced from 22 to 3°C. In addition, Fatt (1971) applied a pachometric technique to monitor the thickness of bovine corneal stromas, and found a coefficient of thermal expansion of $-1.8 \times 10^{-3} \text{K}^{-1}$ (range of $-1.7 \times 10^{-3} \text{K}^{-1}$ to $-1.9 \times 10^{-3} \text{K}^{-1}$), in accord with the swelling pressure result of Hara and Maurice (1972). An apparent paradox developed: if Donnan swelling theory was claimed to work in corneal stroma, why was $\alpha_p < 0$?

An explanation for $\alpha_p < 0$ was advanced by Brenner and Parsegian (1976),¹ using a model of diffuse double layer electrostatic forces (Parsegian and Gingell, 1972). If Donnan swelling theory is valid for the corneal stroma, then significant electrostatic interactions should be predicted in a microcontinuum approach, because osmotic swelling forces have a formal equivalence to electrostatic repulsion forces associated with the net negative charge (Flory, 1953). Unfortunately, the n_i values predicted by Brenner and Parsegian (1976) were unphysiological and unlikely. For a swelling pressure $P = 8 \text{ kPa}$ to exhibit temperature coefficients between 0 and $-5 \times 10^{-3} \text{K}^{-1}$, Brenner and Parsegian (1976) predicted that effective stromal charge concentrations n_i ranging from 0.18 to 3.2 M were required, far exceeding known estimates of between 0.05 to 0.07 M (see above). The Brenner and Parsegian (1976) result holds for temperature coefficients $\text{dln } P/\text{d}T \leq 2.3 \times 10^{-3} \text{K}^{-1}$, and it is only in a restricted region near this upper bound that the n_i estimate approaches known values: for $\text{dln } P/\text{d}T = 2 \times 10^{-3} \text{K}^{-1}$, an effective stromal charge of 0.08 M is predicted. To date, no studies have reported that $\text{dln } P/\text{d}T > 0$. Therefore, the formalism introduced by Brenner and Parsegian (1976) was unable to give reasonable results for corneal stroma if various well-known colligative properties were included. Furthermore, the quantitative role of electrostatic repulsion forces remained unclear.

In view of recent results from this laboratory, this paper reconsiders the temperature dependency of corneal stromal swelling pressure using a microcontinuum approach. The present model incorporates the possibility of thermal dependence of the stromal charge, unlike the formalism of Brenner and Parsegian (1976) where surface charge is assumed to be temperature invariant. It is shown that negative, zero, or positive coefficients of pressure change with temperature are not mutually exclusive conditions, but can be attributed to the same underlying mechanism.

¹Brenner and Parsegian (1976) define the temperature dependency of swelling pressure P as $\text{dln } P/\text{d}T$, which is the limit as $\Delta \rightarrow 0$ of $\alpha'_p = (\Delta P/P)/\Delta T$, the conventional experimental definition. [Recall that $\text{dln } P/\text{d}T = P^{-1}(\text{d}P/\text{d}T)$.] For convenience, we will sometimes use the (nonsuperscripted) form α_p to denote $\text{dln } P/\text{d}T$.

METHODS

The swelling process in corneal stroma changes tissue volume mainly in the anteroposterior direction, with little lateral swelling (see Maurice, 1984). For known temperature coefficients, between 23 and 34°C corneal hydration H will change at most by $\pm 0.25 \text{ kg H}_2\text{O}/\text{kg dry wt}$ ($<6\%$ by weight of water). Within this range, the quasiregularity of interfibrillary spacing d of the stromal collagen fibrils is preserved, with both d^2 (Goodfellow et al., 1978) and total thickness (Hedbys and Mishima, 1966) being linearly related to H . Significant spatial variations in overall tissue stress are not evident in the corneal stroma at steady state (see Maurice, 1984). Thus, a one-dimensional, space-averaged description is adopted as a first approximation to model the temperature dependency of the swelling pressure.

Following Brenner and Parsegian (1976), we applied Gouy-Chapman theory to two charged parallel plates to model the electrostatic repulsion forces arising from interaction of the two diffuse double layers.² At equilibrium, an elemental volume in the medium experiences two forces in balance: the gradient of hydrostatic (osmotic) pressure, and the force on the space charge (Verwey and Overbeek, 1948). For low potentials, the total pressure between two parallel plates of constant charge is (Ohshima, 1974, *a* and *b*; Ohshima et al., 1987):

$$P = (\epsilon_r \epsilon_0 / 2) \{ \kappa^2 [\psi(0)]^2 - (d\psi/dx|_0)^2 \},$$

where $P > 0$ implies repulsion, and $P < 0$ implies attraction. If the surface charge σe is smoothed out (negligible thickness), then³

$$P = (\sigma^2 e^2 / 2\epsilon_r \epsilon_0) \text{csc}^2(\kappa l / 2), \quad (1)$$

where ϵ_0 is the permittivity of free space ($8.854 \times 10^{-12} \text{ C}^2 \text{ N}^{-1} \text{ m}^{-2}$), ϵ_r is the dielectric constant of the medium, e is the electronic charge, l is the separation, and κ is the inverse Debye length.

$$\kappa^2 = (e^2 / \epsilon k T) 1000 N_A \sum c_i z_i^2, \quad (2)$$

where $\epsilon = \epsilon_r \epsilon_0$, k is the Boltzmann constant, T is the temperature, N_A is Avogadro's number, c_i (mole) is the concentration of ion i of valence z_i . For $T = 310 \text{ K}$ (37°C), and a 1:1 medium of 0.15 M, Eq. 2 yields an inverse Debye length $\kappa = 1.284 \times 10^9 \text{ m}^{-1}$.

Ohshima et al. (1987) derived an expression for the pressure in the case of two different surface charges of finite thickness d_1, d_2 . For equal surface charges, the result (Ohshima et al., 1987, Eq. 15) reduces to Eq. 1 in the limit $d \rightarrow 0$. For the case $\kappa l \gg 1$, the repulsive pressure is given by:

$$P = (\sigma^2 e^2 / 2\epsilon_r \epsilon_0) \exp(-\kappa l). \quad (3)$$

An analytical expression for the temperature dependency of swelling pressure P can be derived from Eq. 1 by allowing σ , ϵ , and l to vary with

²We are not suggesting that corneal stromal charge exists in parallel sheets. Rather, we utilize the one-dimensionality of anteroposterior corneal stromal expansion which is analogous to the volume expansion caused by repulsion forces in a classic two-plate electrostatic model. Total surface charge and plate separation are allowed to vary with temperature to determine the temperature dependency of the overall repulsion force.

³This follows from the well-known Poisson-Boltzmann result $P = 2n_0 k T [\cosh\{ze\psi(l/2)/kT\} - 1]$, for thick plates of equal charge (Ohshima, 1974a, Eq. 21). Putting $\sigma_1 = \sigma_2$ in Eq. 35 of Ohshima (1974b) yields the same result. Brenner and Parsegian (1976) took the large separation form (Eq. 3 below) to begin their analysis.

temperature. This leads to

$$d \ln P / dT = 2(d \ln \sigma / dT) - d \ln \epsilon / dT - \coth(\kappa l / 2) \kappa d l / dT, \quad (4)$$

in units of K^{-1} . Because κ and l are temperature dependent, $d \kappa / dT = l d \kappa / dT + \kappa d l / dT$. Putting $l^{-1} d l / dT \approx (\Delta l / l) / \Delta T = -1.8 \times 10^{-3} K^{-1}$ (Fatt, 1971) gives $\kappa d l / dT = -1.8 \times 10^{-3} \kappa l$. Eq. 2 helps to simplify $l d \kappa / dT$ and Eq. 4 becomes:

$$d \ln P / dT = 2(d \ln \sigma / dT) - (d \ln \epsilon / dT) + (T^{-1} + d \ln \epsilon / dT + 0.0036)(\kappa l / 2) \coth(\kappa l / 2). \quad (5)$$

Rearranging Eq. 5 gives

$$(\kappa l / 2) \coth(\kappa l / 2) = \frac{d \ln P / dT + d \ln \epsilon / dT - 2(d \ln \sigma / dT)}{T^{-1} + d \ln \epsilon / dT + 0.0036}. \quad (6)$$

The RHS of Eq. 6 is evaluated by recalling the temperature dependence of the permittivity of water (Vidulich et al., 1967), which leads to $d \ln \epsilon / dT = -4.5844 \times 10^{-3} K^{-1}$. Eq. 6 was solved for $\kappa l / 2$ using the Newton-Raphson numerical iteration method (Hildebrand, 1974). The termination criterion for the algorithm was a difference in successive estimates of $<10^{-5}$. The initial value of $\kappa l / 2$ was taken to be the RHS of Eq. 6, because $\coth(\kappa l / 2) \rightarrow 1$ for $\kappa l / 2 > 2$. Various values of the temperature coefficients $d \ln \sigma / dT$ and $d \ln P / dT$ were assumed, and Eq. 6 solved for κl thereby allowing separation l to be calculated, because κ is known (Eq. 2). The surface charge density σ (number of charges/m²) was deduced from Eq. 1 for different values of swelling pressure P . The charge density n_f (mole charges/liter stromal fluid) was calculated from $\sigma / (10^3 N_A l)$.

RESULTS

Solutions of Eq. 6 took 4–5 iterations. The comparison with the results of Brenner and Parsegian (1976) are shown in Fig. 1. For $-8 \times 10^{-3} < d \ln \sigma / dT < 0$, the present analysis is able to predict temperature coefficients of swelling pressure α_p that are negative, zero, or positive (Fig. 2). Taking the Hara and Maurice (1972) figure of $\alpha_p \approx -7 \times 10^{-3} K^{-1}$, and assuming $n_f = 0.048$ M, $P = 8$ kPa, $T = 310$ K, implies that $d \ln \sigma / dT = -8.5 \times 10^{-3} K^{-1}$. The average predicted surface charge σ_e is ~ 0.01 Cm⁻², with an interface potential $\psi(0)$ of 25 mV or less. Figs. 1 and 2 show only those calculations where $\kappa l > 4$.

DISCUSSION

The present analysis is able to account for a negative temperature coefficient in swelling pressure (Hara and Maurice, 1972), a zero coefficient (Hedbys and Dohman, 1963), or a positive coefficient of $3.2 \times 10^{-3} K^{-1}$ as predicted by conventional Donnan theory for $T = 310$ K. More interesting, however, is that over this entire range,

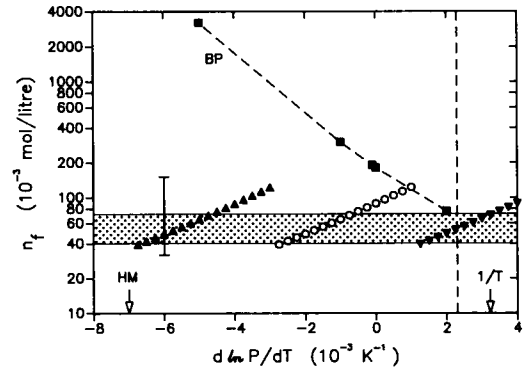


FIGURE 1. Stromal charge n_f predicted for the temperature coefficient of pressure change ($T = 310$ K). The n_f are calculated from Eq. 6, assuming values for $d \ln \sigma / dT$ of $-8 \times 10^{-3} K^{-1}$ (\blacktriangle), $-6 \times 10^{-3} K^{-1}$ (\circ) and $-4 \times 10^{-3} K^{-1}$ (\blacktriangledown). Vertical bars indicate the effect of varying the thermal expansion coefficient $\alpha_r = -1.8 \times 10^{-3} K^{-1}$ by $\pm 25\%$ (for $n_f = 0.048$ M, $d \ln \sigma / dT = -6 \times 10^{-3} K^{-1}$). As $\alpha_r \rightarrow 0^-$, n_f increases. The shaded region covers the known values of n_f , with lower bound from Hodson et al. (1988). The Brenner and Parsegian (1976) model, denoted by “BP”, is valid only to the left of the vertical broken line, and predicts excessively high stromal charges, failing to match either the Hara and Maurice (1972) value denoted by “HM” (lower left), or a T^{-1} characteristic from Donnan theory (lower right).

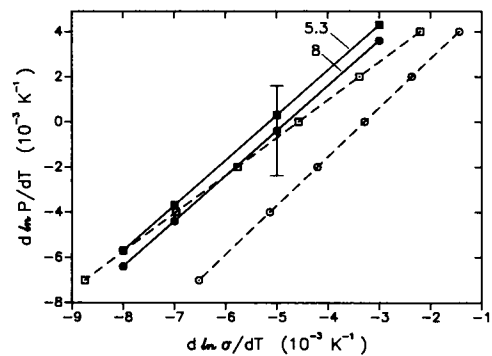


FIGURE 2. Predicted relationship between the temperature coefficients of P and σ . Solid lines represent the electrostatic model (Eq. 6) with $n_f = 0.043$ M, swelling pressures of (\bullet) 8 kPa (60 mmHg) and (\blacksquare) 5.3 kPa (40 mm Hg) calculated at $T = 310$ K. Recalculation for $T = 295$ K produces $<5\%$ change in the predicted α_p . Corneal stromal surface charge σ_e is ~ 0.006 Cm⁻² for $P = 8$ kPa, and ~ 0.01 Cm⁻² for $P = 5.3$ kPa. Vertical bars encompass a $\pm 25\%$ variation in the coefficient of thermal expansion, $\alpha_r = -1.8 \times 10^{-3} K^{-1}$, with α_p becoming more negative as $\alpha_r \rightarrow 0^-$. Broken lines are the Donnan model (Eq. 10) with $F = 0.043$ M, $P_D = 8$ kPa, $T = 310$ K, $\alpha_r = -1.8 \times 10^{-3} K^{-1}$, assuming $d \ln m_{Na} / dT = 0$ (\circ), and $d \ln m_{Na} / dT = 0.5 d \ln \sigma / dT$ (\square). The known values of α_p (units of K^{-1}) range over the interval $[-7 \times 10^{-3}, 0]$, where both models predict a negative thermal dependency of surface charge, or $d \ln \sigma / dT < 0$.

the temperature coefficient of surface charge density is predicted to remain negative, or $d\ln\sigma/dT < 0$ (Fig. 2).

It is possible to attempt more rigorous modeling of the double layer interaction by introducing additional factors such as finite ion size and ion-ion correlation effects (Blum and Jancovici, 1984; Kjellander and Marcelja, 1986). However, the formalism used here derives from a classic Gouy-Chapman approach (Gouy, 1910; Chapman, 1913) based on Poisson-Boltzmann theory, which is adequate for predicting long-range interactions between diffuse double layers in a 1:1 salt medium (see Lozada-Cassou et al., 1982). Thus, notwithstanding the approximations inherent in the present approach, the predicted charge densities n_f can be seen to retain plausibility over a wide range of variation in the parameters (Figs. 1 and 2). The predicted potential $\psi(0) < 25$ mV is comparable with Whitburn's (1981) microelectrode recordings in fresh bovine corneal stroma (29 ± 8 mV at pH 7.75). The calculated surface charge was checked against that predicted by a unit cell model of proteoglycan space charge (Eisenberg, 1983; Eisenberg and Grodzinsky, 1988). This suggested an effective surface charge in corneal stroma of ~ 0.01 Cm⁻² ($n_f = 0.05$ M), in agreement with present results. Thus, it appears that the major prediction of a negative temperature dependency of stromal charge is reasonably accurate. Indeed, preliminary data from this laboratory (Kwok and Klyce, 1989) support the notion that $d\ln\sigma/dT < 0$, at least in rabbit corneal stroma.

The previous approach by Brenner and Parsegian (1976) led to unexpectedly high predictions for n_f (see Fig. 1), on the assumption that surface charge was temperature invariant, or $d\ln\sigma/dT = 0$. For P in the range 4–8 kPa, the present model predicts that the condition $d\ln\sigma/dT = 0$ leads to $d\ln P/dT \approx 10 \times 10^{-3}$ K⁻¹, a temperature coefficient that has never been reported, and which exceeds the classical Donnan prediction, as now shown.

The temperature dependency of stromal charge can be calculated for the macroscopic level by applying an idealized Donnan theory of corneal stromal swelling. Following the arguments presented by Hodson (1971), the Donnan osmotic swelling pressure of corneal stroma immersed in saline is given by:

$$P_D = RT[\sqrt{(F^2 + 4C_0^2)} - 2C_0], \quad (7)$$

where fixed charge concentration $F = n_f$ (in mol/liter), and C_0 is the molar concentration of sodium in the external bath, assuming an ideal solution. Eq. 7 leads to (Friedman and Green, 1971; Elliott et al., 1980):

$$P_D = RTF^2/4C_0. \quad (8)$$

For $P_D = 8$ kPa, Eq. 8 implies that $F = 0.043$ M ($T = 310$ K, $C_0 = 0.15$ M). Conventional Donnan osmotic swelling

theory (constant F in Eq. 8) predicts a positive temperature dependency of $d\ln P_D/dT = T^{-1}$, or 3.2×10^{-3} K⁻¹. More generally, if $F = f(T)$, and $-\infty < d\ln F/dT < +\infty$, the temperature dependency of the fixed stromal charge is obtained by differentiating Eq. 8 with respect to T , yielding

$$d\ln F/dT = \eta(\alpha_D - T^{-1} + \xi' d\ln m_{Na}/dT), \quad (9)$$

where $\xi = \sqrt{(F^2 + 4C_0^2)}$, $\xi' = \xi(F + \xi)/4C_0^2$, $\eta = 4C_0^2/[8C_0^2 + F(F + \xi)] \geq 0$, and $\alpha_D \equiv d\ln P_D/dT$. The temperature dependence of free stromal sodium m_{Na} (molar) appears in Eq. 9 from the relation $m_{Na} = 0.5(F + \xi)$, which is a consequence of bulk electroneutrality in the corneal stroma (see Hodson, 1971).

The macrocontinuum Donnan model predicts a negative temperature dependence of stromal charge. In Eq. 9, putting $\alpha_D = 0$ and $d\ln m_{Na}/dT = 0$ leads to $d\ln F/dT = -\eta/T = -1.5 \times 10^{-3}$ K⁻¹ ($F = 0.043$ M, $C_0 = 0.15$ M, $T = 310$ K). It can be shown that $d\ln m_{Na}/dT$ is directly proportional to $d\ln F/dT$, and of the same sign, if C_0 is assumed to be temperature invariant. Thus, if $\alpha_D \leq 0$ in Eq. 9, then $d\ln F/dT < 0$ and generally, $d\ln F/dT < 0$ when $\alpha_D < T^{-1} = 3.2 \times 10^{-3}$ K⁻¹ ($T = 310$ K). The same trend applies to the implied surface charge σ . This can be seen by recalling $F = n_f = \sigma/l$, so $d\ln F/dT = d\ln\sigma/dT - d\ln l/dT = d\ln\sigma/dT - l^{-1}dl/dT$; the second ansatz is (minus) the coefficient of thermal expansion α_l , $\sim -1.8 \times 10^{-3}$ K⁻¹ (Fatt, 1971) which leads to $d\ln F/dT = d\ln\sigma/dT + 1.8 \times 10^{-3}$, or $d\ln\sigma/dT = d\ln F/dT - 1.8 \times 10^{-3}$ (note that $d\ln F/dT \leq 0$ implies that $d\ln\sigma/dT < 0$). Combining this result with Eq. 9 gives

$$d\ln\sigma/dT = \eta(\alpha_D - T^{-1} + \xi' d\ln m_{Na}/dT) - 1.8 \times 10^{-3}. \quad (10)$$

Eqs. 9 and 10 indicate that the prediction $d\ln\sigma/dT < 0$ still holds for the classical condition $d\ln F/dT = 0$, $d\ln m_{Na}/dT = 0$, and $\alpha_D = 3.2 \times 10^{-3}$ K⁻¹ ($T = 310$ K). Parenthetically, this finding limits the usefulness of only measuring the thermal dependence of stromal charge F , as the coefficient of thermal expansion α_l should also be considered as shown above. For the case of $d\ln m_{Na}/dT = 0$, Eq. 10 predicts that $d\ln\sigma/dT < 0$ for α_D in the range -7×10^{-3} K⁻¹ to 4×10^{-3} K⁻¹ (see Fig. 2).

The idealized Donnan model (Eq. 10) can be compared with predictions of the microcontinuum approach described above. Given P , α_p , and $d\ln\sigma/dT$, n_f is found from Eq. 6, and Eq. 10 solved for the Donnan $d\ln\sigma/dT$, with $P_D = P$, $F = n_f$, $\alpha_D = \alpha_p$. In the present idealized Donnan formalism (Hodson, 1971; Elliott et al., 1980), $d\ln m_{Na}/dT$ can be shown to be proportional to the temperature dependency of stromal charge. Over the expected range in $d\ln\sigma/dT$ shown in Fig. 2, a weighted

calculation suggests $\text{dln } m_{\text{Na}}/\text{dT} \approx 0.5 \times \text{dln}\sigma/\text{dT}$, with a lower bound of $\text{dln } m_{\text{Na}}/\text{dT} = 0$. The solutions of Eq. 10 for various α_p are in good agreement with the microcontinuum electrostatic model, with a difference of <5% at $\alpha_D = 0$ as shown in Fig. 2. Even at the lower bound $\text{dln } m_{\text{Na}}/\text{dT} = 0$, $\text{dln}\sigma/\text{dT}$ remains negative (Fig. 2). Thus, the idealized Donnan model (Eq. 10) confirms the trend $\text{dln}\sigma/\text{dT} < 0$ predicted by the electrostatic analogy (Eq. 6). This consensus from macro- and microcontinuum modeling is both remarkable (given the different initial perspectives) and reassuring, with the two predictions converging. Again, we recognize that an extended, nonidealized (and more complex) Donnan formalism is possible (Grodzinsky, 1983; Stell and Joslin, 1986), but the present concordance of predictions seems to rule out an excessive error in the calculated trends.

Whereas Eq. 1 holds for the case of constant surface charge at a given temperature, it is possible that σ will change with a perturbation to the temperature T . In the present formalism, σ equals (total number of sites/area), so σ can increase (as T falls) either through an increase in the total number of sites, or through a decrease in the area over which the charge operates. The physical basis for such a coulombic mechanism is unknown, but a thermally induced conformational change in the corneal stromal proteoglycans, or their microenvironment, could possibly affect stromal charge.

The present results support the notion that the thermal response of corneal stromal swelling pressure P can be usefully modeled on the basis of diffuse double layer electrostatic interactions. An earlier model (Hart and Farrell, 1971) used statistical thermodynamic arguments to suggest a major role for electrostatic forces in the origin of P . However, that formalism relied heavily on unproven values for various phenomenological parameters, and was unable to predict the thermal dependence of P . In this paper, consideration of the temperature dependency of swelling pressure with a macrocontinuum approach (viz., the Donnan pressure P_D) led to the same conclusion reached with a microcontinuum model. That is, if stromal charge is allowed to change with temperature (without imposing direction), then a negative temperature dependency of σ is predicted over the range of likely values of α_p , the temperature coefficient for pressure. Therefore, a single underlying mechanism is capable of giving P a thermal dependence which can be theoretically positive or negative. Thus, the contrasting conditions of α_p being negative (Hara and Maurice, 1972), or zero (Hedbys and Dohlman, 1963) are not necessarily contradictory, but may merely reflect the different values of $\text{dln}\sigma/\text{dT} < 0$ generated by the same mechanism as shown here. Moreover, the present agreement between microcontinuum modeling (Eq. 6) and predictions using Donnan swelling theory (Eq. 10) support the Donnan osmotic effect as the

principal component in a macrocontinuum description of corneal stromal swelling pressure.

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