J. Membrane Biol. 54, 89–102 (1980)

The Journal of
Membrane Biology

Unstirred Layer Effects in Osmotic Water Flow across Gallbladder Epithelium

T.J. Pedley and J. Fischbarg

Department of Applied Mathematics and Theoretical Physics, University of Cambridge, England and
Departments of Physiology and Ophthalmology, College of Physicians and Surgeons, Columbia University, New York

Summary. The standard one-dimensional model of the unstirred layer is applied in a re-examination of the experimental results of Wright, Smulders and Tormey (Wright, E.M., Smulders, A.P., Tormey, J. McD., 1972, *J. Membrane Biol.* **7**:198) who reported large transients in the osmotic flux of water from the serosal to the mucosal side of rabbit gallbladder epithelium. They initiated osmosis by the addition of sucrose to the mucosal bathing solution (initially, approximately 300 mOsm NaCl) and observed that the initial flux was more than ten times its eventual steady-state value; they interpreted this as a consequence of the piling-up of NaCl in the unstirred tissue layer on the serosal side of the epithelium. The present analysis (both steady-state and unsteady) shows that if measured values of layer thickness δ are used, together with reasonable values of the reduced diffusivity of NaCl in the tissue and of the fraction of tissue available for water flow, then one would predict a discrepancy of only about 10%, not tenfold, between the initial and final values of the flux. Thus the standard model is inconsistent with the observations. Furthermore, Wright et al's results cannot be used to infer that the osmotic permeability of epithelial cell membranes is much larger than steady-state measurements on whole epithelia would indicate. Mucosal-to-serosal flow is also analyzed, and in this case a considerably greater osmotic permeability is predicted; this result is consistent with the observed changes in structure of the lateral intercellular spaces when the direction of flow is reversed.

The main purpose of this paper is to point out the inability of the conventional model of "unstirred layers" to interpret the results of some experiments on osmotic water flux across epithelial cell layers. This will be done by a re-examination of the experimental results of Wright, Smulders and Tormey (1972) on rabbit gallbladder epithelium, with particular reference to those experiments in which the water flux was from the serosal to the mucosal side.

The experiments were performed with either everted or noneverted gallbladders, initially containing and surrounded by a salt solution (approximately 150 mM NaCl), the outer one stirred by a stream of gas bubbles. Osmotic water flux was initiated by adding sucrose to one or other of the solutions at a concentration of either 50 or 300 mM. The water flux (in or out) was measured by weighing the gallbladder at 5-min intervals. It was confirmed that actively driven water flux, in the absence of sucrose, was negligibly small because the experiments were carried out at room temperature (22–24 °C).

Wright et al. (1972) presented results both for the steady-state fluxes which eventually occur in each case and for the transient development of those steady-state fluxes. They gave the steady-state results in the form of the osmotic permeability (P_f, cm s^{-1}) which would be inferred from the measured water flux (J, cm s^{-1}) according to the equation

$$J = P_f \bar{V}_w C_b \tag{1}$$

where $\bar{V}_w$ is the molar volume of water ($=18.0$ cm^3 M^{-1}) and C_b is the applied sucrose concentration difference (M cm^{-3}). These steady-state results are given in Table 1; the suffixes $s-m$ and $m-s$ refer to water flux in the serosal-to-mucosal and mucosal-to-serosal directions, respectively. No difference was found between everted and noneverted gallbladders, with the implication that eversion did not damage the epithelium in any way. The quoted values of P_f are only apparent osmotic permeabilities because they are based on the assumptions that the epithelium is a uniform semipermeable membrane and that the concentration difference across it, driving

0022-2631/80/0054-0089 $02.80

Table 1. Steady-state values of the apparent osmotic permeability (P_f) of rabbit gallbladder[a]

C_b (mM)	P_{fs-m} (cm s^{-1})	P_{fm-s} (cm s^{-1})
50	2.4×10^{-3}	3.3×10^{-3}
300	1.4×10^{-3}	4.7×10^{-3}

[a] Inferred from measurements of osmotic water flux driven by two different concentrations of sucrose (C_b) in the bathing solution on either the serosal or the mucosal side of the epithelium.

the osmotic flux, is indeed C_b. As Wright et al. (1972) pointed out, "unstirred layer" effects make the latter assumption untenable, and their unsteady flux measurements were made in order to assess the importance of these effects.

They found that the steady-state fluxes in either direction took a time of the order of 30 min to develop. They also found, in the one case of $s-m$ flux in the everted gallbladder, that the initial flux (which could not be measured accurately because the first weighing could not take place until 5 min after the sucrose was mixed into the external, mucosal solution) was at least ten times the eventual steady-state flux.

Wright et al.'s interpretation of this observation is in terms of "unstirred layers" and may be stated as follows. When the (everted) gallbladder is placed in the sucrose-rich solution, the sucrose takes only a short time to diffuse to the mucosal surface of the epithelium across the mucosal unstirred layer[1]. The osmotic flow will begin and, assuming that the mucosal unstirred layer is sufficiently thin for the sucrose concentration at the mucosal surface not to be reduced significantly below C_b, the initial flux will be that corresponding to the concentration difference C_b, and will therefore accurately reflect the true permeability[2] of the epithelium. However, on the serosal side of the epithelium there is a relatively thick layer of connective tissue; its thickness varies with position on the gallbladder surface, but averages about 400 μm when the gallbladder is pumping and water flow is from the mucosa to the serosa (Tormey & Diamond, 1967), whereas it is only about half that value when the flow is in the other direction and may be even less (Smulders, Tormey & Wright, 1972). The fluid contained in this layer cannot be stirred, however vigorous the stirring in the serosal bathing solution. Therefore, after the initial high rate, the osmotic flow will progressively cause NaCl to pile up against the serosal surface of the epithelium (*see* Fig. 1*a*), tending to reduce the osmotic pressure difference across it and hence to reduce the flow. The experimental results show that reduction to be considerable.

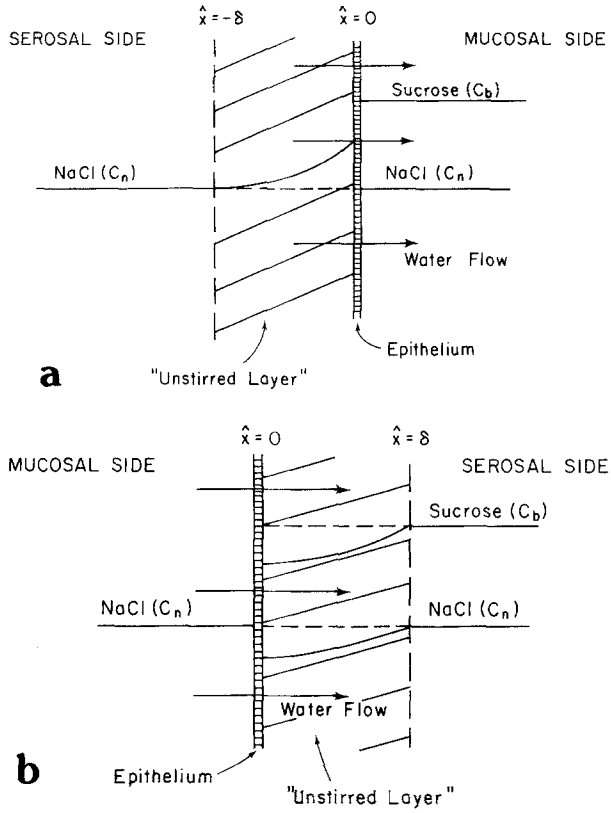

Fig. 1. Sketches of the one-dimensional model of the epithelium layer and the unstirred serosal tissue layer (thickness δ) on which it is mounted. (*a*): Serosal-to-mucosal osmotic flux of water driven by the sucrose osmolarity C_b; the water flow causes the NaCl osmolarity on the serosal side of the epithelium itself to increase above its value (C_n) in the bathing solution. (*b*): Mucosal-to-serosal osmotic flux causes the osmolarities of both sucrose and NaCl to fall on the serosal side of the epithelium

The reason why the considerable reduction in flux was not noticed during $s-m$ flow in a noneverted gallbladder was said to be that the initial flow takes water out of the serosal tissue layer (halving its thickness) and so could not be measured by the weighing technique. The fact that nothing similar was observed during $m-s$ flow in both everted and noneverted bladders can also be explained with reference to Fig. 1*b*. Here the osmotic flow will build up only slowly, because the time taken for the sucrose to diffuse across the tissue layer is considerable[3]. Fur-

[1] The unstirred layer was reported by Wright et al. to have a small thickness, δ_m, of about 100 μm, kept thin by the stirring, and the diffusivity of sucrose D_s is approximately 5.2×10^{-6} cm^2 s^{-1}, so the diffusion time-scale δ_m^2/D_s is about 19 sec.

[2] The standard one-dimensional theory suggests that the reduction would be less than 2% (Dainty, 1963; Pedley & Fischbarg, 1978).

[3] If the thickness of the serosal layer, δ_s, is 400 μm, the diffusion time scale δ_m^2/D_s is about 5 min.

thermore, as the osmotic flow builds up it will tend to sweep both sucrose and salt away from the epithelium, and is at no time likely to exceed its eventual steady-state value.

In this paper we set out the conventional one-dimensional theory of the unstirred layer, providing analytic solutions for the steady-state flux in the cases of both $s-m$ and $m-s$ flux. We also examine the unsteady development of the flux in the former case, the one in which Wright et al. (1972) observed significant transient behavior, by the same technique as we have used for a simpler "sweeping away" problem (Pedley & Fischbarg, 1978). On using numerical values appropriate to Wright et al.'s experiments, our main conclusion is that, according to this one-dimensional model, the steady-state and transient observations for $s-m$ flow are incompatible. In particular, the steady-state results suggest that the measured values of P_f need to be corrected by only a few percent, while the unsteady results (as described above) require a correction of at least tenfold. The implications of this incompatibility will be discussed.

Schafer, Patlak and Andreoli (1974) gave a numerical solution to the mathematical problem set out below for various values of the parameters which arise. Their main application was to experiments on kidney proximal tubule; in a brief discussion of the gallbladder experiments of Wright et al. (1972) they obtained unsteady results similar to our own, but they did not notice the incompatibility with the steady-state results. It is hoped that the present analytic treatment will make it easier to pick out particular conclusions.

1. Theory

(a) Serosal-to-Mucosal Flow

According to the one-dimensional model, the epithelial cell layer is modelled as a thin semi-permeable membrane occupying the plane $\hat{x}=0$ (Fig. 1*a*). The "unstirred" layer of porous serosal tissue occupies the region $-\delta<\hat{x}<0$. We assume that the serosal fluid in $\hat{x}<-\delta$ contains NaCl at uniform concentration, corresponding to an osmolarity C_n (in a 150 mM solution of NaCl, C_n would be somewhat less than 300 mOsmol liter^{-1}; when numerical values are required, we shall assume that C_n has this value). We also assume that the mucosal fluid in $\hat{x}>0$ contains NaCl with uniform osmolarity C_n and, at time $t=0$, sucrose is introduced with uniform osmolarity C_b. These assumptions mean that we are neglecting the effect of unstirred layers in the two fluid compartments compared with that of the relatively thick layer of connective tissue. This agrees with the assumption of Wright et al. (1972) and with the computed results of Schafer et al. (1974). The more significant of these layers would be that on the mucosal side, where sucrose and NaCl would tend to be swept away. However, the thickness and hence the effect of a diffusion layer in a fluid depends on the rate of stirring (*see*, e.g., Ginzburg & Katchalsky, 1963). Since in the present case the steady-state osmotic flux is the same whether the mucosal surface is bathed by the unstirred inner fluid or the stirred outer fluid, the mucosal unstirred layer (and its effect) can be neglected for our purposes.

The reflection coefficient of gallbladder epithelium for sucrose is effectively unity, and Smulders et al. (1972) have shown that its diffusional permeability to sucrose is very small (especially when there is $s-m$ osmotic flux), so we may suppose that only a negligible amount of sucrose crosses into the serosal unstirred layer. We are therefore concerned solely with the distribution of NaCl there (strictly speaking we should treat the sodium and chloride ions separately, but we assume that electrical neutrality is preserved and that they diffuse together). When the NaCl concentration at the serosal side of the epithelium starts to rise, the salt will tend to diffuse across the epithelium. Also, and probably more importantly, some will be carried across the epithelium with the water flow, because the reflection coefficient to NaCl is less than 1. However, both these factors will tend to reduce the piling-up effect and hence to reduce the correction which must be made to measured permeabilities. In order to maximize the predicted correction, therefore, we shall assume that the epithelium is impermeable to salt.

Not all the space in the tissue layer is available for passage of water or NaCl; we follow Schafer et al. (1974) and suppose that the layer is homogeneous and that a fraction α of it is available. Wright et al. (1972) suggested that $\alpha\simeq 0.5$.

The effective diffusivity of NaCl in the space of the porous medium is likely to be somewhat less than its value in free solution (1.5×10^{-5} cm^2 s^{-1}). There is little data on diffusion through such tissue layers. Maurice (1969) has measured effective diffusivities for various solutes across the corneal stroma and reports that the value for small molecules such as NaCl is approximately half its value in free solution. Hoshiko, Lindley and Edwards (1964), and Winn et al. (1964) measured the diffusivity of radioactive sodium in the corium of frog skin, obtaining values of about 3.3×10^{-6} cm^2 s^{-1} and 4.2×10^{-6} cm^2 s^{-1}, respectively. Diamond (1972) has given an estimate of 6×10^{-6} cm^2 s^{-1} for the diffusivity of bromide in the serosa of fish gallbladder, also about a quarter of its

value in free solution. We denote the NaCl diffusivity by D_n and as a standard value in the following calculations we therefore take $D_n = 0.38 \times 10^{-5}$ $cm^2 s^{-1}$.

If the NaCl osmolarity is denoted by $C(x,t)$, then the convection-diffusion equation governing the distribution of C in the layer $-\delta < \hat{x} < 0$ is

$$\frac{\partial C}{\partial \hat{t}} + V\frac{\partial C}{\partial \hat{x}} = D_n \frac{\partial^2 C}{\partial \hat{x}^2} \tag{2}$$

where V is the effective convection velocity, greater than the measured velocity J because of the limited area available for flow across the tissue:

$$V = J/\alpha. \tag{3}$$

The boundary conditions on C are that it is equal to the bulk osmolarity, C_n, at $\hat{x} = -\delta$, and that there is zero flux across the epithelium at $\hat{x} = 0$:

$$C(-\delta, \hat{t}) = C_n \tag{4}$$

$$VC - D\frac{\partial C}{\partial \hat{x}} = 0 \quad \text{at } \hat{x} = 0. \tag{5}$$

The initial condition is

$$C(\hat{x}, 0) = C_n \tag{6}$$

and the flux J across the epithelium is proportional to the difference in osmolarity across it:

$$J = P_f \bar{V}_w [C_b + C_n - C(0, \hat{t})] \tag{7}$$

where P_f is the true permeability of the epithelium, modelled as a homogeneous membrane.

We begin by putting the problem into dimensionless form, using the variables introduced by Pedley and Fischbarg (1978):

$$x = \hat{x}/\delta; \quad t = D_n \hat{t}/\delta^2; \quad \theta = C/C_b \tag{8}$$

and introducing the two dimensionless parameters

$$\beta = P_f \bar{V}_w C_b \delta / \alpha D_n; \quad \theta_n = C_n/C_b. \tag{9}$$

In terms of these, the equation and boundary conditions (2)–(7) become

$$\frac{\partial \theta}{\partial t} + \beta[1 + \theta_n - \theta(0,t)]\frac{\partial \theta}{\partial x} = \frac{\partial^2 \theta}{\partial x^2} \tag{10}$$

$$\theta(-1, t) = \theta_n \tag{11}$$

$$\beta[1 + \theta_n - \theta(0,t)]\,\theta(0,t) = \frac{\partial \theta}{\partial x}(0,t) \tag{12}$$

$$\theta(x, 0) = \theta_n. \tag{13}$$

Steady-state solution. The object of the steady-state theory is to calculate the actual value of P_f corresponding to a measured value of J and to see how large a correction is needed to the value derived from Eq. (1). If we denote the dimensionless steady-state concentration by $\theta_s(x)$ and write

$$\gamma = 1 + \theta_n - \theta_s(0) \tag{14}$$

Eqs. (7) and (9) give the relation

$$J = \beta\gamma \cdot \alpha D_n/\delta. \tag{15}$$

Thus, if α, D_n and δ are known, a measurement of J is equivalent to a measurement of $\beta\gamma$. If the theory yields a relationship between β and γ, β and hence P_f can then be inferred from the steady-state measurement. Note that, from Eqs. (7) and (14), γ represents the ratio between the actual osmotic flux corresponding to a particular value of β and the flux which would be present in the absence of an unstirred layer.

From Eq. (10), the equation satisfied by $\theta_s(x)$ is

$$\beta\gamma\frac{d\theta_s}{dx} = \frac{d^2\theta_s}{dx^2}$$

and the solution which satisfies boundary conditions (11) and (12) is

$$\theta_s = \theta_n e^{\beta\gamma(x+1)}. \tag{16}$$

[In dimensional terms this is, of course, the same as the familiar "unstirred layer" solution:

$$C = C_n e^{\frac{V}{D_n}(\hat{x}+\delta)}.]$$

The relation (14) now yields

$$e^{\beta\gamma} = 1 + \frac{1-\gamma}{\theta_n} \tag{17}$$

which is the required relation between β and γ. Indeed, since the product $\beta\gamma$ can be inferred directly from the measurement of J, this relation immediately defines a value for γ. Equation (17) will be used in §2*a* to determine the value of P_f in Wright et al.'s (1972) preparation.

Unsteady solution. The object of unsteady theory is to predict the variation of the flux J with time for a given value of the governing parameter β. However, a complete analytical solution of the nonlinear problem specified by Eqs. (10)–(13) is not possible, and we have restricted our analysis to (i) small times, in order to see how the flux begins to depart from its initial value, and (ii) large times, in order to see how it approaches its steady-state value and to estimate the

time it takes to do so. We define the dimensionless flux

$$j(t)=J/(P_f \bar{V}_w C_b)=1+\theta_n-\theta(0,t) \tag{18}$$

so that $j(0)=1$ and $j\to\gamma$ as $t\to\infty$.

The details of the unsteady analysis and the approximations needed for it are given in the Appendix; here we merely quote the conclusions. First, for small values of t,

$$j(t)\approx 1-2\beta\theta_n(t/\pi)^{\frac{1}{2}}; \tag{19}$$

this is valid as long as

$$t\ll \mathrm{Min}\,(1,\beta^{-2}). \tag{20}$$

Equation (19) shows how the flux falls rapidly from its initial value; there is no question, e.g., of an initial increase in flux, followed by a later fall (except during the short delay associated with the small mucosal unstirred layer). Second, for large values of t,

$$j(t)\approx\gamma+be^{-kt} \tag{21}$$

where b is an undetermined constant, and k is the root with the smallest real part of Eq. (A19), which is reproduced here for convenience:

$$\cos q(\gamma+\alpha_1 e^{\beta_1})+\beta_1\frac{\sin q}{q}(1+\theta_n-\tfrac{3}{2}\gamma-\tfrac{1}{2}\alpha_1 e^{\beta_1})$$
$$-\alpha_1 e^{\frac{1}{2}\beta_1}=0 \tag{22}$$

where

$$\beta_1=\beta\gamma;\quad \alpha_1=\beta_1^2\theta_n/k;\quad q=(k-\tfrac{1}{4}\beta_1^2)^{\frac{1}{2}}. \tag{23}$$

In the appendix we quote an expansion for k in powers of β_1, useful when β_1 is small (Eq. (A23)), but in general Eq. (22) must be solved numerically. Computation of k will give an estimate for the time-scale τ for eventual decay of the unsteady part of the flux; this can be defined as the value of t at which $|e^{-kt}|$ becomes equal to e^{-3} or 0.05, so that

$$\tau=3\delta^2/(D_n\,\mathrm{Re}(k)). \tag{24}$$

(b) Mucosal-to-Serosal Flow

This is the case depicted in Fig. 1*b* in which both NaCl and sucrose are swept away from the epithelium by the osmotic flux; we perform only the steady-state analysis in this case. We let the unstirred tissue layer occupy the region $0<\hat{x}<\delta$, so that the flux is again in the positive $\hat{x}$ direction. We again ignore the unstirred layer on the mucosal side, where the NaCl osmolarity is C_n; in the bulk fluid on the serosal side, the osmolarity of NaCl is C_n and that of sucrose is C_b.

This time we require an equation for each of the solutes. If $C_1(\hat{x})$ is the steady-state distribution of NaCl osmolarity, with diffusivity D_n in the porous tissue, and if $C_2(\hat{x})$ is the corresponding distribution for sucrose (with diffusivity D_s), the governing equations are

$$\text{for NaCl:}\quad \frac{J}{\alpha}\frac{dC_1}{d\hat{x}}=D_n\frac{d^2C_1}{d\hat{x}^2}$$
$$\text{for sucrose:}\quad \frac{J}{\alpha}\frac{dC_2}{d\hat{x}}=D_s\frac{d^2C_2}{d\hat{x}^2}. \tag{25}$$

The boundary conditions are

$$\text{at } \hat{x}=\delta\text{: } C_1=C_n,\ C_2=C_b; \tag{26}$$

$$\text{at } \hat{x}=0\text{: } \frac{J}{\alpha}C_1-D_n\frac{dC_1}{d\hat{x}}=\frac{J}{\alpha}C_2-D_s\frac{dC_2}{d\hat{x}}=0 \tag{27}$$

while the osmotic flux J is given by

$$J=P_f\bar{V}_w[C_1(0)+C_2(0)-C_n]. \tag{28}$$

The solutions of Eq. (25) which satisfy the boundary conditions (26) and (27) are

$$C_1=C_n\exp\left[\frac{J\delta}{\alpha D_n}(x-1)\right];$$
$$C_2=C_b\exp\left[\frac{J\delta}{\alpha D_s}(x-1)\right] \tag{29}$$

where $x=\hat{x}/\delta$. Substitution into Equation (28) then gives the following dimensionless equation for γ $(=J/P_f\bar{V}_w C_b)$ in terms of β and θ_n which are defined by Eq. (9):

$$\gamma=e^{-\lambda\beta\gamma}-\theta_n(1-e^{-\beta\gamma}). \tag{30}$$

Here

$$\lambda=D_n/D_s \tag{31}$$

and γ is again the ratio of the actual osmotic flux to that which there would be in the absence of the unstirred layer.

2. Results

(a) Serosal-to-Mucosal Flow: Steady State

The dimensional parameters corresponding to Wright et al.'s (1972) experiments have all been given

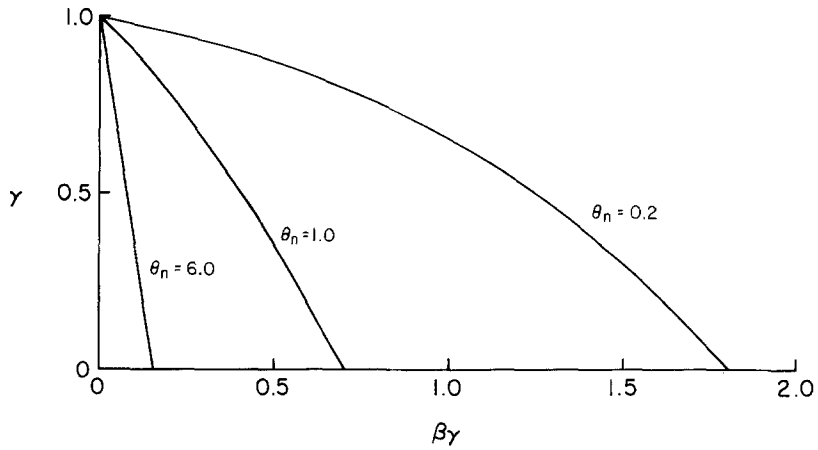

Fig. 2. Graphs of the ratio of apparent osmotic permeability in steady-state experiments to actual permeability (γ) against the measurable dimensionless osmotic water flux ($\beta\gamma$) for the case of serosal-to-mucosal flow and for three different values of the ratio of initial NaCl osmolarity and applied sucrose osmolarity ($\theta_n = C_n/C_b$)

above; they are

$\bar{V}_w = 18.0\,\mathrm{cm^3\,M^{-1}}$; $\alpha = 0.5$;

$\delta = 200\,\mu\mathrm{m}$; $C_n = 300 \times 10^{-6}\,\mathrm{M\,cm^{-3}}$;

$D_n = 0.38 \times 10^{-5}\,\mathrm{cm^2\,s^{-1}}$.

Two different cases are considered, as follows and as listed in Table 1:

Case I. "low gradient": $C_b = 50 \times 10^{-6}\,\mathrm{M\,cm^{-3}}$ and apparent $P_f = 2.4 \times 10^{-3}\,\mathrm{cm\,s^{-1}}$.

Case II. "high gradient": $C_b = 300 \times 10^{-6}\,\mathrm{M\,cm^{-3}}$ and apparent $P_f = 1.4 \times 10^{-3}\,\mathrm{cm\,s^{-1}}$.

In each case the recorded value of P_f is used to infer the measured value of J from Eq. (1), and then Eq. (15) is used to derive the corresponding value of $\beta\gamma$. Finally the quantity γ is deduced from Eq. (17); γ is the ratio of the actual flux to the flux which would have occurred in the absence of the unstirred layer, and hence represents the factor by which the quoted values of P_f are underestimates of the "true" values of osmotic permeability for the epithelium.

Case I: Here $\theta_n = 6.0$ and $\beta\gamma$ turns out to be 0.023, so Eq. (17) gives $\gamma = 0.86$. Thus the actual value of hydraulic permeability is only 16% above that quoted by Wright et al. (1972).

Case II: Here $\theta_n = 1.0$ and $\beta\gamma = 0.080$, so $\gamma = 0.92$; the underestimate in this case is only 9%.

It may be that the thickness of the tissue layer is larger than the quoted thickness, that the fraction of space available for flow is smaller than 0.5, and that the diffusivity of solute in the layer is smaller than one quarter of that in free solution. In any of these cases the quantity $\delta/\alpha D_n$ would be increased; that would mean an increase in $\beta\gamma$ (from Eq. (15)) and hence a reduction in γ (from Eq. (17)). Indeed, Eq. (17) shows that γ decreases nonlinearly as $\beta\gamma$ is increased, to such an extent that γ falls to zero (implying that β, and hence P_f, are infinite) when $\beta\gamma$ rises to the critical value $\ln(1+1/\theta_n)$, equal to 0.154 in Case I and 0.693 in Case II. This means that there is an upper limit on the measurable osmotic flux however large the hydraulic permeability of the epithelium is taken to be. The limiting values of $\beta\gamma$ are demonstrated graphically in Fig. 2 where the roots of Eq. (17) are displayed for three values of θ_n. Two of these values have already been used (6.0 and 1.0) and the third is much smaller (0.2), corresponding to a case in which the background NaCl osmolarity is five times smaller than C_b.

These results might suggest that it is not unreasonable to expect a tenfold underestimate in hydraulic permeability ($\gamma = 0.1$) for attainable values of $\beta\gamma$. However, in case I this would not be achieved until $\beta\gamma = 0.14$, while in case II it would require $\beta\gamma = 0.64$. For these values to obtain, it would be necessary for the quantity $\delta/\alpha D_n$ to exceed the value used above by a factor of approximately 6 and 8 in the two cases, respectively. As discussed below, this is highly improbable.

(b) Serosal-to-Mucosal Flow: Unsteady

The only unsteady results to be presented are the values of k, and hence of the decay time τ, which are determined for different parameter values from Eq. (22). These have been computed for three values of θ_n (= 6.0, 1.0, 0.2) and for a range of $\beta\gamma$ between zero and the value at which γ becomes zero. It turns out that k is real in all these cases (and presumably for intermediate values of θ_n also), indicating that the final approach to the steady state is purely monotonic, not oscillatory as happens for large enough β in the example analyzed by Pedley and Fischbarg (1978).

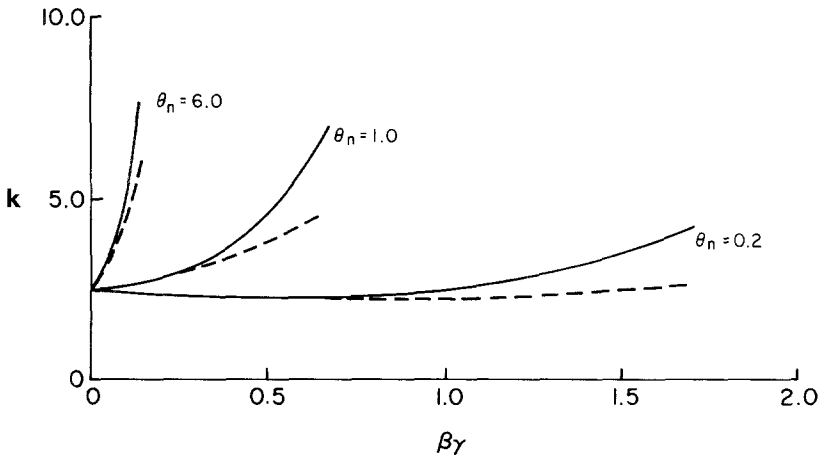

Fig. 3. Graphs of k, inversely proportional to the time scale for setting up the steady state, against $\beta\gamma$. *Solid curves:* exact solution of Eq. (22). *Broken curves:* approximate solution for small $\beta\gamma$ (Eq. (A23))

The computed values of k are plotted against $\beta\gamma$ in Fig. 3, with the small $\beta\gamma$ approximation (Eq. (A23)) given by the broken curves. For the larger values of θ_n, k increases as $\beta\gamma$ increases, indicating that the time to a steady state, τ (Eq. (24)), decreases as the osmotic flux increases; this was also found in a different case by Pedley and Fischbarg (1978). For $\theta_n = 0.2$, however, k at first decreases, indicating an increase in τ; examination of Eqs. (A23) and (A21) shows that this effect will always be observed if $\theta_n < 0.5$. However, really small values of θ_n are of no interest, since the piling-up effect would not be significant in that case.

For the parameter values of cases I and II, β_1 is quite small, and k remains close to the value $\pi^2/4$ ($=2.47$); in detail we have

Case I: $\theta_n = 6.0$; $\beta_1 = 0.023$; $k = 2.78$; $\tau = 1.1\,\delta^2/D_n \approx 1.9$ min.

Case II: $\theta_n = 1.0$; $\beta_1 = 0.080$; $k = 2.57$; $\tau = 1.2\,\delta^2/D_n \approx 2.0$ min.

Thus the predicted values of τ are considerably less than the observed 30-min transient in Wright et al.'s experiments. In order for τ to be as large as 30 min, either k would have to be much smaller (and we have already seen that that is impossible) or δ^2/D_n would have to be 15 times larger. The latter could be achieved by an increase in δ or a decrease in D_n or both. However, since either would mean an increase in k (Fig. 3*a*), in fact δ^2/D_n would have to become larger still.

Let us consider case I, the case for which the unsteady observations were reported, and seek to estimate the factors by which the various parameters would have to be changed for consistency with both steady and unsteady results. We saw in (*a*) above that, for $\gamma = 0.1$, $\beta\gamma$ would have to equal 0.14, a sixfold increase. Figure 3 then gives $k = 7.7$, so that for $\tau = 30$ min, δ^2/D_n must be increased by a factor of about 44. Even if δ were increased by a factor of 4, to 800 μm, as has been suggested by Smulders and Wright (1971), this would still require D_n to be reduced by a factor of 2.8 below the chosen value, already a factor of 4 below its value in free solution. Then for $\delta/\alpha D_n$ to be increased by sixfold, α would have to be 0.93 instead of 0.5. Thus it is possible to make the theory agree with the observations only by assigning unrealistic values to the parameters δ and D_n; this point is taken up further in the discussion.

(*c*) *Mucosal-to-Serosal Flow: Steady State*

For flow in this direction the thickness of the serosal tissue layer (δ) is increased by water imbibition to 400 μm (Smulders et al., 1972), which suggests that the fraction of space available for flow (α) is increased from 0.5 to 0.75. We suppose that D_n is unchanged, although in view of the wider interstitial spaces in this case it may well be increased somewhat. We once more denote the two values of C_b (Table 1) as cases I and II; in this case the quoted values P_f are somewhat larger than for $s-m$ flow: 3.3×10^{-3} cm s^{-1} and 4.7×10^{-3} cm s^{-1}, respectively. We calculate the relevant value of $\beta\gamma$ in the same way as in § 2*a*, and then use Eq. (30) to calculate the value of γ in each case. We need to know the value of one additional parameter, the ratio of diffusivities (Eq. (31)). In free solution this has the value 2.9; in porous tissue it may be somewhat larger, since the mobility of sucrose molecules may well be reduced more than that of the smaller Na^+ and Cl^+ ions. However, in the absence of further information we assume that the ratio has the same value of 2.9 in the tissue layer.

Case I. $\theta_n = 6.0$ and $\beta\gamma$ takes the value 0.042. Equation (30) gives $\gamma = 0.64$, which means that the quoted osmotic permeability is a 56% underestimate.

Case II. $\theta_n = 1.0$ and $\beta\gamma = 0.36$; then $\gamma = 0.05$, and the actual osmotic permeability is about 20 times higher than the value quoted.

These results show that $m-s$ flow experiments will give a much more serious underestimate of the osmotic permeability of an epithelium than $s-m$ flow experiments. This is because in the unstirred layer both sucrose and NaCl are swept away by $m-s$ osmotic flow, while in the case of $s-m$ flow it is only NaCl which is piled up against the epithelium. Now the apparent values of P_f for $m-s$ flow are comparable with but slightly larger than those for $s-m$ flow (Table 1). The present results show that the "true" P_f for $m-s$ flow, especially for larger values of C_b, may in fact be *much* larger than for $s-m$ flow.

Equation (3) shows that again there is an upper limit above which $\beta\gamma$ cannot rise because γ falls to zero. In this case, however, the critical value is much smaller than in the case of $s-m$ flow, showing that the maximum measurable osmotic flux J (Eq. (15)) is also much lower since $\alpha D_n/\delta$ is not much reduced. In Case I, with $\theta_n = 6.0$, the critical value of $\beta\gamma$ is 0.12, while in case II ($\theta_n = 1.0$) it is 0.39.

3. Discussion

It is our contention that the above results concerning $s-m$ osmotic flux demonstrate that Wright et al.'s (1972) experimental observations cannot be interpreted in terms of the conventional unstirred layer model. This conclusion is based principally on the steady-state results (§ 2*a*) which show, using values of the parameters appropriate to the experiments, that the ratio of apparent to actual osmotic permeability is greater than 0.85, and therefore the measurements give an underestimate of less than 16%. This should be contrasted with the 10-fold underestimate deduced by Wright et al. (1972) from their unsteady experiment. The conclusion is reinforced by the unsteady analysis (§ 2*b*) which predicts that the steady state should be set up after a time of about 2 min, not the observed 30 min.

On the other hand, it was demonstrated that the model could be forced into consistency with the observations if different values of the parameters were used, i.e., if the ratio $\delta/\alpha D_n$ were 6 (Case I) or 8 (Case II) times greater than the value chosen by us, and if δ^2/D_n were about 44 times larger (Case I). It is incumbent upon us, therefore, to consider carefully whether our parameter values were correctly chosen or whether they could be adjusted realistically to make the model work.

Layer thickness, δ. Tormey and Diamond (1967) stated that the thickness of the serosal tissue layer in whole rabbit gallbladder was not uniform (because of the folds in the mucosal surface), but that it had an average value of about 375 μm in the natural, pumping state (i.e., with $m-s$ water flow). Smulders et al. (1972) found thicknesses of about half this value, explained by the fact that excised segments of gallbladder were stretched in their apparatus. Since Wright et al. (1972) used whole gallbladders, the former, larger value is more appropriate. However, Smulders et al. (1972) also showed that the thickness of the tissue layer in their preparation was more than halved when the flow direction was reversed, in the $s-m$ direction. There is no reason to doubt that a similar reduction occurs in the sac preparation, so choosing a value of δ of 200 μm in the case of $s-m$ flow seems quite justified; at most, it might imply a slight overestimate.

It is important here to emphasize that δ as defined here is an anatomically measurable quantity, the thickness of tissue layer in which stirring cannot take place. We have assumed that the NaCl concentration at the far side of the layer from the epithelium is exactly C_n, whereas in fact there will also be a slight piling-up effect in the serosal bathing solution close to the edge of the tissue layer. However, in the presence of even gentle stirring, the additional unstirred layer in the bathing fluid will be very thin, especially when, as here, the osmotic flow is directed towards the solid boundary; House (1974, p. 108) quotes values of around 60 μm for its thickness. As an outside estimate perhaps 100 μm should be added to δ for this additional effect, although since D_n in the fluid is twice as great as that in the tissue layer this will grossly overestimate the effect. The value of δ for $s-m$ flow is thus brought up to 300 μm.

Now, Wright et al. (1972) quoted Smulders and Wright (1971) as reporting that the serosal unstirred layer should be given a thickness of 800 μm. However, this was an "effective" value, obtained on the assumption of free solution diffusivity by interpreting measurements of the half-time for development of steady-state concentration distributions (inferred from NaCl diffusion potentials), in terms of a theoretical model of unsteady diffusion in the absence of osmotic flow or of stirring. We prefer to use instead a measured value of tissue layer thickness.

Diffusivity D_n. We clearly recognize that diffusion is inhibited in a porous medium because of the tortuosity of the pathways for the solute molecules and the reduction in cross-sectional area available for transport. As far as we can ascertain, there is little information available concerning this process, either experimental or theoretical, and it is clearly an area where much work needs to be done. We chose a

value for D_n, equal to one quarter of its value in free solution, based on the data reported above by Maurice (1969), Hoshiko et al. (1964), Winn et al. (1964) and Diamond (1972). It may be that the presence of the muscle layer in gallbladder wall, with rather small gaps between extensive smooth muscle cells, cuts down the rate of diffusion still further, although that would presumably be accounted for, at least in Diamond's measurements. To analyze this effect would require a complicated two- or three-dimensional theory; all we can say in advance is that the reduction in diffusive transport by such a layer will be significantly less than the reduction in cross-sectional area available for transport, because the concentration gradients near the gaps would be considerably enhanced (Nir & Pfeffer, 1979). In the present one-dimensional model the effect must be averaged out and expressed in terms of an effective diffusivity for the layer as a whole. However, it does not seem reasonable to reduce D_n below about one-fifth of its value in free solution, $0.3 \times 10^{-5}\,\mathrm{cm^2\,s^{-1}}$.

Fraction of volume available for flow, α. Since the depth of the tissue layer varies according to the direction of water flux, so must the fraction of volume occupied by water. If we accept that about three-quarters of the volume normally consists of extracellular water, in the absence of water flow or during $m-s$ flow (i.e., $\alpha=0.75$), then when the width is halved during $s-m$ flow the value of α must fall to 0.5, the value used by Wright et al. (1972). We are not justified in using a smaller value. Smulders et al. (1972), however, showed that the extracellular volume is nonuniformly distributed during $s-m$ flow. The submucosal tissue, between the epithelium and the muscle layer, is extremely compressed, and the muscle layer itself is also significantly compressed, while the subserosal tissue, between the muscle layer and the serosal bathing solution, is not significantly compressed. The present model cannot elucidate the effects of such differential compression; what is required is a model in which α varies with $\hat{x}$. Presumably D_n must also be allowed to vary with $\hat{x}$, being lowest in the most compressed region; a knowledge of the relationship between D_n and α would, however, be needed before such a model could be used quantitatively.

The above remarks suggest that δ may be as large as 300 μm for $s-m$ flow and D_n may be as small as $0.3 \times 10^{-5}\,\mathrm{cm^2\,s^{-1}}$. In this extreme case, $\delta/\alpha D_n$ would be increased by a factor of less than 2 over the value used above, and δ^2/D_n by a factor of less than 3. These numbers fall well short of the values (6 and 44, respectively) required to make the model consistent with the observations.

Possible Invalidity of the One-Dimensional Model

Since adjustment of the model parameters, within reasonable limits, does not seem to yield an explanation of the experimental findings, we must ask whether the whole idea of a one-dimensional system may not be invalid. It is quite clear that the epithelium and the serosal tissue layer are not uniform in the transverse direction. First the tissue layer: the folds in the mucosa have a height of about 100 μm and a transverse width varying between 100 μm during $m-s$ flow and as little as 1 μm during $s-m$ flow (Smulders et al. 1972, Fig. 7). In the latter case it may be that the part of the epithelium occupying the folds ceases to contribute to water flux, so that the effective area of epithelium is the same as that of the unfolded muscle layer beneath; other things being equal, this could still be treated one-dimensionally. During $m-s$ flow, however, the epithelial area is greater than that of the muscle layer (by a factor of about 1.5), it varies over a length scale of 100 μm, and ought properly to be treated two-dimensionally. This is a problem to be tackled in the future. Even so, with the effective area and the effective layer width δ varying by factors of less than 2, it is difficult to see how tenfold flux changes could result from the breakdown of one-dimensionality.

But then there is the fact that the epithelium itself is not a uniform semi-permeable membrane, but consists of cells with long and (in the case of $s-m$ flow) tortuous lateral intercellular spaces between them. If much of the water flow at any stage passes through the intercellular spaces rather than across the basal membranes of the cells (*see* below), then the funnelling of this flow through them and the distribution of solute concentration in the tissue near them cannot be treated one-dimensionally (Fig. 4). Once more the one-dimensional theory breaks down and a rather difficult two-dimensional theory, such as that of Goldgraben and Weinbaum (1973), must be performed instead.

If, therefore, the lack of agreement between experiment and theory were a consequence not of experimental artefact but of the breakdown of the conventional one-dimensional model of the unstirred layer, then the implications would be far-reaching. It would mean that the standard theory should henceforth be abandoned for all epithelial studies. Past interpretations based on it could no longer be relied on. However, there are arguments against this rather drastic conclusion, which stem from experiments on epithelia from which the tissue layer has been stripped. For both a leaky epithelium (corneal endothelium; *see* Fischbarg, Warshavsky & Lim, 1977) and a tight one (frog skin; *see* Lau et al., 1979),

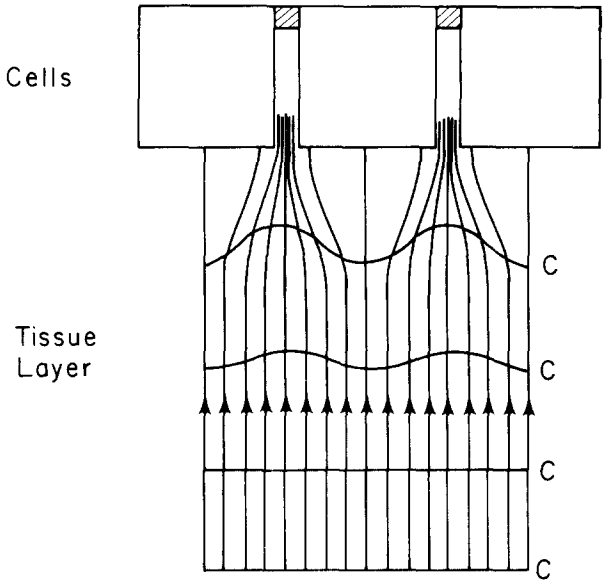

Fig. 4. Schematic diagram of flow streamlines (arrowed) and lines of constant salt concentration (C) in the tissue layer when much of the flow enters the lateral intercellular spaces

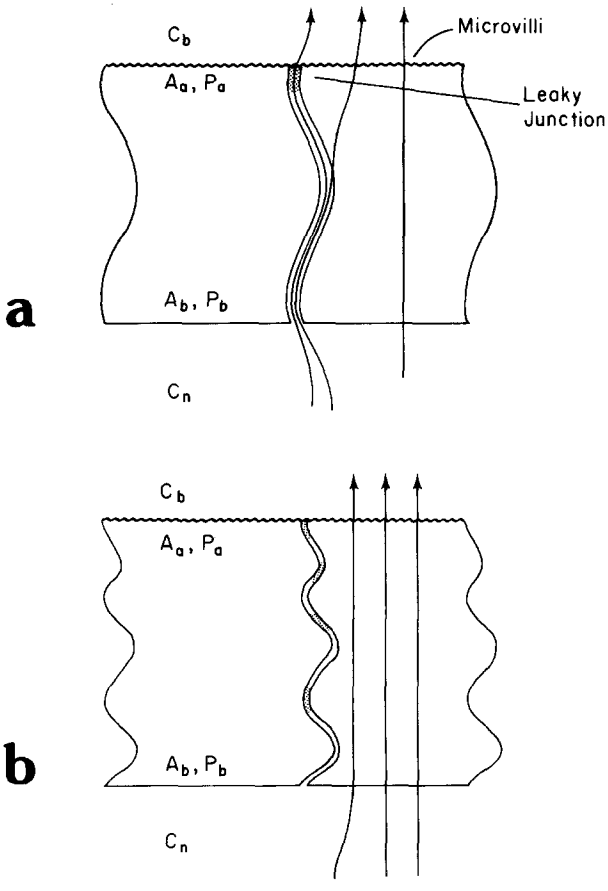

Fig. 5. Sketch of possible routes of serosal-to-mucosal water flow (*a*) initially, when the lateral intercellular spaces are open, and (*b*) after they have closed. Also marked are the concentrations in the different compartments (C_b, C_n) and the areas (A) and permeabilities (P) of the different membranes (*see* text)

absence of the tissue layer makes only a small difference to osmotic permeability and to pumping performance. For a tight epithelium this is to be expected; the result for a leaky one, however, suggests that, after all, solute polarization in the tissue (unstirred) layer has very little effect, that the apparent osmotic permeability of the epithelium is close to its actual permeability, and that this is determined by the membrane of the epithelial cells and the junctions and spaces between them, not by the neighboring tissue.

Wider Implications

A further possible reason for the model failing to explain the observations is that the observed osmotic transients are not a consequence of solute polarization effects at all, but result from some quite different physical mechanism. The only mechanisms which seem to us to allow the possibility of a 30-min transient are cell volume changes or tissue retraction. We have already seen that the tissue layer thickness is reduced when $s-m$ flow begins with a simultaneous marked narrowing of many of the intercellular spaces (Smulders et al., 1972). Much of this process would appear to take place in a time-scale of about 20 sec, however, as suggested by the transient electrical resistance measurements of Wright et al. (1972). Nevertheless, it is not inconceivable that further tissue retraction, or cell swelling, could continue to occur in Wright et al.'s sac preparation over a longer time-scale. This would have the effect of gradually causing the intercellular spaces and the cell junctions to become more and more firmly closed over the observed 30-min period (this idea has now been confirmed by van Os, Wiedner and Wright (1979): *see* below).

Such a mechanism, of course, would not affect the osmotic water flow unless the route of water movement were normally directed largely through the intercellular spaces, rather than crossing the basal cell membrane. Let us suppose the first to be the case. Water flowing into these spaces could in principle pass out both across the cell via the lateral and the apical cell membranes and through the leaky junction between the cells (Fig. 5*a*). If due to tissue retraction the spaces became completely blocked so that neither of these pathways was available (Fig. 5*b*), then it is obvious that the apparent osmotic permeability of the epithelium as a whole would fall. It could easily fall by a factor of 10 if, normally, 90% of the water flow passed into the intercellular spaces.

Thus if the osmotic transients are a consequence of tissue retraction and if solute polarization effects are not very significant (as we have argued above)

then we may tentatively draw three conclusions: (i) most of the $s-m$ osmotic water flow normally passes through the lateral intercellular spaces, whether or not it traverses the cell junctions; (ii) the initial value of the osmotic permeability recorded by Wright et al. (1972) is more likely to be representative of the normal epithelium than is the final one; and (iii) the final value is representative of transport across the basal and apical cell membranes in series. This final value, P_f, would be simply related to the osmotic permeabilities of the basal and apical cell membranes (P_b and P_a, respectively) as follows. We assume on the basis of the ultrastructural studies of Smulders et al. (1972) that the area of basal membrane available for transport is the same as that of the epithelium as a whole, but that the area of the apical surface is rather larger on account of the microvilli: say A_a per unit area of epithelium. Then it follows at once, from equating the water flux across the two membranes on the assumption that the osmolarity of the cell contents is uniform across the cell, that

$$\frac{1}{P_f}=\frac{1}{P_a A_a}+\frac{1}{P_b}. \tag{32}$$

Thus if A_a is large and if P_a and P_b have similar values, P_f will be close to P_b. Only if P_a were small compared with P_b, and A_a were not too large, could P_f be much lower than P_b, and this seems unlikely. Thus it does not seem unreasonable to conclude that the final value of P_f measured by Wright et al. (1972) is quite close to the osmotic permeability of the basal cell membrane.

Now one of the main reasons for performing osmotic experiments with an epithelium is the hope that they will shed some light on the properties of the epithelial cell membranes and hence on the mechanics of coupled salt and water transport across the epithelium. Diamond and Bossert (1967) proposed the now famous standing gradient model to explain this mechanism, based on the active pumping of sodium into the lateral intercellular spaces with water flowing osmotically. These authors (and subsequently Segel (1970)), showed that the model was consistent with experiment in that it does predict approximately isotonic transport (the osmolarity of the serosal exudate being equal to that of the mucosal bathing solution) for certain values of the relevant parameters. However, Hill (1975) asserted that if correct values were used for the dimensions of the lateral intercellular spaces, then isotonic transport would not be predicted by the model unless the osmotic permeability of the lateral cell membranes were 10–100 times greater than the generally accepted value of $10\text{–}20\times10^{-4}\,\mathrm{cm\,s^{-1}}$; this latter value is the same as the final measured osmotic permeability of the epithelium as a whole (Table 1). Diamond (1977) has subsequently used Wright et al.'s (1972) results as evidence that the actual osmotic permeability of the epithelium as a whole, *and hence of the cell membrane*, is much greater than previously supposed. However, if we assume that the osmotic permeability of the lateral and basal cell membranes are comparable, then our results, combined with the above discussion of the effects of tissue retraction, suggest that this conclusion may not be justified, at least in so far as it concerns the osmotic permeability of the cell membrane.

Finally, we may remark, as did Smulders et al. (1972) and Wright et al. (1972), that the closure of the lateral intercellular spaces (and hence of the transjunctional pathway) is a perfectly sound explanation for the osmotic permeability of the epithelium being lower for $s-m$ flow than for $m-s$ flow. Indeed, the results quoted in §2*c* above, showing that the unstirred layer effect does cause a significant underestimate of osmotic permeability for $m-s$ flow, show that the discrepancy between the osmotic permeabilities for flow in the two directions is probably much greater than that indicated in Table 1.

Postscript

After this paper was submitted, the paper by van Os et al. (1979) was published. In it the experiments of Wright et al. (1972) were repeated in a chamber preparation instead of a sac preparation. Osmotic transients were not observed during $s-m$ flow (after the first 5 sec), and these authors also attributed the long time-scale transients of the earlier work to tissue shrinkage. A reduction in wet weight of the gallbladder tissue of 22.5%, over a time-scale of 10–15 min, was reported, and this explanation of the transients is quite self-consistent. The osmotic permeability of the gallbladder epithelium for $s-m$ flow was found by van Os et al. (1979) to be $9.3\times10^{-3}\,\mathrm{cm\,s^{-1}}$, a factor of about four greater than the eventual steady-state value of Wright et al. (1972). If this observation is put into the theory of §2(*a*), case I, with the chosen values of δ and D_n, then the value of γ derived from Eq. (17) is 0.45, so that these experiments do suggest that the "true" osmotic permeability of the epithelium is approximately 2.2 times the measured value as a result of the serosal "unstirred layer". On the other hand, van Os et al. report that the steady-state osmotic flow develops after only 5 sec, which is much *shorter* than it should be for these values of δ and D_n (*see* §2(*b*)). Furthermore, van Os et al. also made streaming potential measure-

ments which suggest that the true osmotic permeability is more than five times the apparent value, not twice as deduced here (this, too, is based on a one-dimensional interpretation). Thus it still cannot be said that the experimental results can be fully explained by the one-dimensional unstirred layer model.

This work was supported by the U.S. Public Health Service, Grant EY01080 and RCDA EY00006 to J.F. T.J.P. gratefully acknowledges the receipt of a Travel Grant from the Wellcome Trust. An Abstract outlining some of the results of this paper has been published elsewhere (Fischbarg & Pedley, 1979).

Appendix
Details of the Unsteady Analysis for Serosal-to-Mucosal Flow

(a) Small t

Initially, $\theta=\theta_n$ everywhere in the layer. After a very short time, NaCl will have begun to accumulate near $x=0$, but there will have been no time for the effect of this accumulation to diffuse any significant distance from $x=0$. Thus, the presence of another boundary at $x=-1$ will have no influence on the NaCl distribution at first, and the initial departure from a uniform concentration will be the same as if the layer were semi-infinite, with the other boundary at $x=-\infty$. Furthermore, the concentration distribution represents only a small departure from its initial value, and we can define $\theta'(x,t)$ so that

$$\theta(x,t)=\theta_n+\theta'(x,t)$$

where $|\theta'|\ll\theta_n$ everywhere but $|\partial\theta'/\partial x|$ is not necessarily small. Substituting into Eq. (10) and boundary conditions (11)–(13), and linearizing, we obtain the following problem for θ':

$$\frac{\partial\theta'}{\partial t}+\beta\frac{\partial\theta'}{\partial x}=\frac{\partial^2\theta'}{\partial x^2} \tag{A1}$$

$$\theta'(-\infty,t)=0 \tag{A2}$$

$$\frac{\partial\theta'}{\partial x}(0,t)=\beta\theta_n \tag{A3}$$

$$\theta'(x,0)=0. \tag{A4}$$

We seek the initial time dependence of θ' at $x=0$ since the dimensionless flux (Eq. (18)) is $j(t)=1-\theta'(0,t)$.

The above can be transformed into a standard diffusion problem by the substitution

$$\theta'=e^{\frac{1}{2}\beta x}\theta'' \tag{A5}$$

so that Eq. (A1) becomes

$$\frac{\partial\theta''}{\partial t}+\frac{\beta^2}{4}\theta''=\frac{\partial^2\theta''}{\partial x^2}.$$

The second term can now be neglected because of the assumption that θ', and hence θ'', is small compared with its derivatives, so the final equation is:

$$\frac{\partial\theta''}{\partial t}=\frac{\partial^2\theta''}{\partial x^2}.$$

A similar approximation shows that none of the boundary conditions is altered by the substitution. The solution of this diffusion problem is

$$\theta''(x,t)=2\beta\theta_n t^{\frac{1}{2}}\{\xi(1+\operatorname{erf}\xi)+\pi^{-\frac{1}{2}}e^{-\xi^2}\} \tag{A6}$$

where $\xi=x/2t$ (Carslaw & Jaeger, 1959, p. 75). Using Eq. (A5) and putting $x=0$ we obtain

$$j(t)=1-\theta'(0,t)=1-2\beta\theta_n(t/\pi)^{\frac{1}{2}} \tag{A7}$$

at small values of t.

The length of time for which the various approximations are valid can be estimated (i) by substituting Eq. (A6) into the neglected terms in the equation and boundary conditions: these are small compared with the terms retained as long as $\beta t^{\frac{1}{2}}\ll 1$; (ii) by putting $x=-1$ in Eq. (A6), and noting that θ' remains negligibly small there as long as $t^{\frac{1}{2}}\ll 1$. Thus the estimate (A7) is accurate for small times such that

$$t\ll\min(1,\beta^{-2}); \tag{A8}$$

when β is large this is a very short time indeed.

(b) Large t

Here we follow Pedley and Fischbarg (1978) and use a combined analytical and numerical approach to calculate the rate at which $\theta(x,t)$ approaches its steady state form $\theta_s(x)$. We suppose that for large time we may write

$$\theta(x,t)=\theta_s(x)-\phi(x,t)$$

where $|\phi|\ll|\theta_s|$ for all x. Substituting this into equation (10) and boundary conditions (11) and (12), and using Eq. (16) for θ_s, we obtain the following linear problem for ϕ:

$$\frac{\partial^2\phi}{\partial x^2}-\beta\gamma\frac{\partial\phi}{\partial x}-\frac{\partial\phi}{\partial t}=-\beta^2\gamma\theta_n e^{\beta\gamma(x+1)}\phi(0,t) \tag{A9}$$

$$\phi(-1,t)=0 \tag{A10}$$

$$\frac{\partial\phi}{\partial x}(0,t)=\beta(2\gamma-1-\theta_n)\phi(0,t). \tag{A11}$$

The initial condition (13) is irrelevant because the linearization is valid only in the final stages of the approach to the steady state.

The form of this problem suggests that ϕ can be written as a sum of terms in each of which the time variation is exponential. Writing

$$\phi = e^{-kt} g(x) \tag{A12}$$

where $Re(k)$ is expected to be positive, we obtain

$$g'' - \beta\gamma g' + kg = -\beta^2 \gamma \theta_n e^{\beta\gamma(x+1)} g(0), \tag{A13}$$

$$g(-1) = 0, \quad g'(0) = \beta(2\gamma - 1 - \theta_n) g(0) \tag{A14}$$

where a prime refers to differentiation with respect to x. Since the system is linear, g will be undetermined to within an arbitrary constant (in principle, determinable from the initial conditions), so without loss of generality we can set

$$g(0) = -1. \tag{A15}$$

This is an eigenvalue problem which has a nontrivial solution only for certain values of k. The eigenvalue with the lowest real part will correspond to that solution of the form of Eq. (A12) which decays most slowly as $t \to \infty$, and hence determines the approach to the steady state.

The solution of the problem defined by Eq. (A13) to (A15) can be found by the methods used by Pedley and Fischbarg (1978). The function $g(x)$ takes the form:

$$g(x) = \alpha' e^{\beta\gamma(x+1)} + e^{\frac{1}{2}\beta\gamma x} (A \cos qx + B \sin qx)$$

where

$$\alpha' = \beta^2 \gamma \theta_n / k \tag{A16}$$

$$q = (k - \tfrac{1}{4}\beta^2\gamma^2)^{\frac{1}{2}} \tag{A17}$$

and A, B are constants which must be chosen to satisfy the boundary conditions (A14) and (A15). These conditions give three homogeneous equations for the two unknowns A and B, elimination of which shows that the problem has a solution of the form (A12) if and only if k satisfies the following transcendental equation:

$$\cos q(\gamma + \alpha_1 e^{\beta_1}) + \beta_1 \frac{\sin q}{q} (1 + \theta_n - \tfrac{3}{2}\gamma - \tfrac{1}{2}\alpha_1 e^{\beta_1}) - \alpha_1 e^{\frac{1}{2}\beta_1} = 0 \tag{A18}$$

where

$$\beta_1 = \beta\gamma \quad \text{and} \quad \alpha_1 = \alpha'\gamma = \beta_1^2 \theta_n / k. \tag{A19}$$

Equation (A18) has to be solved numerically for general values of β (or equivalently, of β_1). However, when β_1 is small, it can readily be shown, using Eqs. (17), (A17), (A18) and (A19), that q can be expanded in powers of β_1 as follows:

$$q = \frac{\pi}{2} + q_1 \beta_1 + q_2 \beta_1^2 + O(\beta_1^3) \tag{A20}$$

where

$$q_1 = \frac{2}{\pi}(\theta_n - \tfrac{1}{2}) \tag{A21}$$

and

$$q_2 = \frac{2}{\pi}\left[\theta_n(\theta_n + 1) - \frac{2}{\pi}\theta_n - \frac{4}{\pi^2}(\theta_n - \tfrac{1}{2})^2\right]. \tag{A22}$$

Hence

$$k = \frac{\pi^2}{4} + \pi q_1 \beta_1 + \beta_1^2 (q_1^2 + \pi q_2 + \tfrac{1}{4}) + \ldots. \tag{A23}$$

Thus, for small β_1, k is real and positive; as β_1 increases from zero, k increases if $\theta_n > \frac{1}{2}$, but decreases if $\theta_n < \frac{1}{2}$.

Finally we note that the dimensionless flux $j(t)$ (Eq. A7) will for large t take the form

$$j(t) = \gamma + be^{-kt} \tag{A24}$$

where b is an undetermined constant. If k is real, the approach to the final steady state will be monotonic, but if k is complex it will be oscillatory.

References

Carslaw, H.S., Jaeger, J.C. 1959. Conduction of Heat in Solids. (2nd ed.) Clarendon Press, Oxford

Dainty, J. 1963. Water relations of plant cells. *Adv. Bot. Res.* **1**:279

Diamond, J.M. 1962. The mechanism of solute transport by the gall-bladder. *J. Physiol. (London)* **161**:474

Diamond, J.M. 1977. The epithelial junction: Bridge, gate and fence. *Physiologist* **20**:10

Diamond, J.M., Bossert, W.H. 1967. Standing-gradient osmotic flow. A mechanism for coupling water and solute transport in epithelia. *J. Gen Physiol.* **50**:2061

Fischbarg, J., Lim, J.J., Bourguet, J. 1977. Adenosine stimulation of fluid transport across rabbit corneal endothelium. *J. Membrane Biol.* **35**:95

Fischbarg, J., Pedley, T.J. 1979. Inadequacy of the standard unstirred layer model in accounting for osmotic water flow across epithelia. *J. Physiol. (London)* **292**:73*P*

Fischbarg, J., Warshavsky, C.R., Lim, J.J. 1977. Pathways for hydraulically and osmotically-induced water flows across epithelia. *Nature (London)* **266**:7

Ginzburg, B.Z., Katchalsky, A. 1963. The frictional coefficients of the flows of non-electrolytes through artificial membranes. *J. Gen. Physiol.* **47**:403

Goldgraben, J.R., Weinbaum, S. 1973. On the mixing of a low Reynolds number biological jet with a quiescent outer bathing solution. *J. Fluid Mechan.* **59**:159

Hill, A.E. 1975. Solute-solvent coupling in epithelia: A critical examination of the standing-gradient osmotic flow theory. *Proc. R. Soc. Lond. B* **190**:99

Hoshiko, T., Lindley, B.D., Edwards, C. 1964. Diffusion delay in frog skin connective tissue: A source of error in tracer investigations. *Nature (London)* **201**:932

House, C.R. 1974. Water transport in cells and tissues. E. Arnold, London

Lau, Y.T., Parsons, R.H., Brady, R.J., Feeney, G.A. 1979. Water movement across split frog skin. *Biochim. Biophys. Acta* **551**:448

Maurice, D.M. 1969. In: The Eye. H. Davson, editor. chap. 7 in Vol. 1, p. 489. Academic Press, New York—London

Nir, A., Pfeffer, R. 1979. Transport of macromolecules across arterial wall in the presence of local endothelial injury. *J. Theor. Biol.* **81**:685

Os, C.H. van, Wiedner, G., Wright, E.M. 1979. Volume flows across gallbladder epithelium induced by small hydrostatic and osmotic gradients. *J. Membrane Biol.* **49**:1

Pedley, T.J., Fischbarg, J. 1978. The development of osmotic flow through an unstirred layer. *J. Theor. Biol.* **70**:427

Schafer, J.A., Patlak, C.S., Andreoli, T.E. 1974. Osmosis in cortical collecting tubules. *J. Gen. Phyisol.* **64**:201

Segel, L.A. 1970. Standing-gradient flows driven by active solute transport. *J. Theor. Biol.* **29**:233

Smulders, A.P., Tormey, J. McD., Wright, E.M. 1972. The effect of osmotically induced water flows on the permeability and ultrastructure of the rabbit gallbladder. *J. Membrane Biol.* **7**:164

Smulders, A.P., Wright, E.M. 1971. The magnitude of nonelectrolyte selectivity in the gallbladder epithelium. *J. Membrane Biol.* **5**:297

Tormey, J. McD., Diamond, J.M. 1967. The ultrastructural route of fluid transport in rabbit gall bladder. *J. Gen. Physiol.* **50**:2031

Winn, P.M., Smith, T.E., Campbell, A.D., Huf, E.G. 1964. Sodium diffusion in epidermis and corium of frog skin and in Ringer-agar gel. *J. Cell Comp. Physiol.* **64**:371

Wright, E.M., Smulders, A.P., Tormey, J. McD. 1972. The role of the lateral intercellular spaces and solute polarization effects in the passive flow of water across the rabbit gall bladder. *J. Membrane Biol.* **7**:198

Received 7 June 1979; revised 12 November 1979