Biochimica et Biophysica Acta, 291 (1973) 197–207

BBA 76127

PATH OF OSMOTIC WATER FLOW THROUGH RABBIT GALL BLADDER EPITHELIUM

C. H. VAN OS AND J. F. G. SLEGERS
Department of Physiology, University of Nijmegen, Nijmegen (The Netherlands)
(Received July 4th, 1972)

SUMMARY

Osmotic, P_f, and diffusive, P_d, water permeability of rabbit gall bladder epithelial membranes have been determined. The thickness of unstirred layers, or diffusion barriers, such as cell cytoplasm and the thick supporting layer of connective tissue, have been determined by butanol diffusion, assuming unstirred layers limited diffusion of this solute.

The contribution of these layers to the total resistance to ^{3}HHO diffusion was 85.7 ± 2.9% (S.E.). The measured ratio of the water permeabilities, P_f/P_d, decreased from 26.3 ± 4.3 to 3.1 ± 0.3 after making corrections of P_d for unstirred layer influences.

The temperature dependence of P_f has also been determined. Arrhenius plots of P_f show breaks at 10–15 and 20 °C at osmotic gradients of 100 and 200 mM sucrose, respectively. Below these temperatures, apparent activation energies amounted to 9.7 ± 1.5 kcal·mole^{-1}, values which are consistent with a solubility–diffusion mechanism in the water permeation process. Above the break temperature, apparent activation energies decreased to 3.5 ± 0.6 kcal·mole^{-1} which is consistent with values for water permeation through aqueous pores.

These results suggest that osmotic flow through gall bladder mucosa is mainly *via* the cellular membranes, *i.e.* the extracellular shunt, which is the main route for passive ion permeation, does not contribute significantly to the osmotic water flow in response to a sucrose gradient.

INTRODUCTION

During isotonic fluid reabsorption in gall bladder epithelium, a rapid osmotic water flow follows in response to active solute transport. In the local osmosis model of Diamond and Bossert[1], water enters the lateral intercellular spaces through the lateral cell membranes. Very recently, however, electrophysiological investigation on this epithelium by Barry *et al.*[2] and Frömter and Diamond[3] proved unambiguously that the main route of passive ion permeation is *via* an extracellular shunt residing in the tight junctions. Smulders and Wright[4] observed that sucrose and inulin diffuse across this tissue by the same extracellular pathway. If this shunt pathway also contributes predominantly to the osmotic water flow one has to expect that the

osmotic flow proceeds by bulk flow or viscous flow through aqueous pores of at least 12 Å, since inulin is a rod-shaped polymer with dimensions about 12 Å × 100 Å (ref. 5).

Viscous flow is characterized by values greater than one for the ratio of the osmotic, P_f, and the diffusional, P_d, water permeabilities, and determination of this ratio allows calculation of equivalent pore radius[6]. Therefore we determined both water permeabilities in order to find out if these parameters could give information about the route of water during osmotic flow. However, the magnitude of P_d can be greatly underestimated if unstirred layers are present. This has been shown in artificial[7] and biological membranes[8]. Hays[9] demonstrated for a reverse osmosis membrane, consisting of a thin but dense barrier in series with a thick but porous one, that the thin and dense barrier is rate limiting for flow but not for diffusion. Nearly the same situation is met with epithelial tissues where unstirred water layers (i) adjacent to the luminal membrane (ii) in the cell cytoplasm (iii) and in the thick supporting layer of connective tissue and smooth muscles, offer significant barriers to diffusion but not to flow[10,11]. Hays and Franki[11] determined the contribution of the thick supporting layer of toad bladder to the total resistance to ^{3}HHO diffusion by measuring ^{3}HHO diffusion through intact tissue and, after removal of epithelial cells, through the supporting layer. This procedure still gave underestimated P_d values, since the unstirred layer formed by the cell cytoplasm could not be taken into account. We tried to determine total unstirred layer thickness in intact tissue by measuring the diffusion of butanol, assuming that the diffusion of this solute is completely limited by the unstirred layers. Diffusive ^{3}HHO permeabilities, corrected for unstirred layer effects, closely approached P_f values determined in the same gall bladder.

For the same reason we determined the apparent activation energy of the water permeability since the temperature dependence of water permeation through a membrane can provide additional information about the mechanism underlying the water transport[12,13].

MATERIALS AND METHODS

Tissue preparation

Gall bladders were removed from albino rabbits of both sexes and cannulated as described previously[14]. During removal of bile and refilling of the bladder, care was taken to prevent overdistension of the tissue. Cannulated gall bladders were suspended in a beaker filled with Ringer solution of the following composition (mM) NaCl, 140; KCl, 7.0; $CaCl_2$, 1.0; $MgCl_2$, 1.0 NaH_2PO_4, 0.375; Na_2HPO_4, 2.125; pH 7.4; gassed with 100 % O_2 saturated with water vapor.

Water transport measurements

Water fluxes were determined in the direction from lumen to blood side. The sac preparations were filled with and suspended in Ringer solution in which NaCl was isosmotically replaced by Na_2SO_4 to block active fluid transport[15].

Osmotically induced water flows were measured gravimetrically as described by Diamond[16]. The serosal bathing solution was made hypertonic by the addition of 100 or 200 mosM·l^{-1} sucrose. The osmolarity of a solution was determined by measuring the freezing point depression (advanced osmometer type 3A). The tempera-

ture dependence of P_f was measured between 5 °C and 35 °C at intervals of 5 °C. The temperature of the bathing solution was controlled within 0.5 °C. Going from higher temperatures to lower or *vice versa* did not influence the results.

The rate of diffusion of ^{3}HHO and *n*-[1-^{14}C]butanol was determined as follows: The bladder was suspended in 12 ml sulphate Ringer stirred by O_2 bubbles and a magnetic fiea. ^{3}HHO (approx. 2.0 μCi) and [^{14}C]butanol (approx. 0.5 μCi) were added through the cannula to the luminal solution. Every 2 min, from 1 until 19 min, 20-μl samples were taken from the serosal solution. Before and after the isotopic experiment the bladder was weighed. After each experiment the gall bladder was opened and spread out on a Ringer-impregnated filter paper to determine the surface area and wet weight of the tissue. The isotope concentration in the lumen of the bladder, calculated from volume determinations by weighing, and the known added isotope concentration agreed with direct determinations from 10-μl samples taken out of the lumen 1 min after addition of isotope. Therefore, initial isotope concentrations were calculated and not measured. The radioactive samples were counted in a Packard liquid scintillation counter (Model 314 E) using Bray's[17] scintillation fluid. The radioisotopes were obtained from Radiochemical Centre, Amersham, England.

Calculation of permeability constants

The following equations and notations taken from Holtz and Finkelstein[18] were used:

(*A*) *Osmotic water permeability.* The water flux J_w in response to an impermeant solute is given by $J_w = -P_f \cdot A \cdot \phi \Delta C_s$, where J_w = flux of water in moles·s^{-1}; ΔC_s = concentration difference of impermeable solute in moles·l^{-1}; ϕ = osmotic coefficient; A = surface area of the bladder in cm^2; P_f = osmotic permeability coefficient in cm·s^{-1}.

(*B*) *Tracer permeability.* The flux J_i of a tracer "i" is given by $J_i = -(P_d)_i \cdot A (C_{im} - C_{is})$, where J_i = flux of tracer i in moles·s^{-1}; A = area of membrane in cm^2; $(C_{im} - C_{is})$ = concentration difference for tracer "i", between the mucosal and serosal solution, in moles·l^{-1}; $(P_d)_i$ = permeability coefficient for "i" in cm·s^{-1}.

The rate of appearance of radioactivity in the serosal bathing solution decreased exponentially with time. The measuring points were fitted with an exponential function by the method of least squares. The extrapolated zero time fluxes were calculated with S.E. values. The computer program was designed by Dr L. Hoofd. All errors are expressed as S.E. with the number of determinations in parentheses.

Unstirred layer corrections

In estimating the thickness of an unstirred water layer adjacent to a lipid bilayer, Holtz and Finkelstein[18] used butanol. They stated that for any molecule that is more soluble in hydrocarbon than in water, the observed permeability coefficient will merely be a measure of the thickness of the unstirred layers, *i.e.* $[(P_d)_{obs}]_i$ is approx. D_i/δ where D_i = free solution diffusion coefficient of solute i in cm^2·s^{-1}; δ = the sum of the unstirred layers in cm; $[(P_d)_{obs}]_i$ = observed permeability coefficient for i in cm·s^{-1}. Although an epithelial tissue is not so well defined as a lipid bilayer, the greatest part of the tissue may still be considered as an aqueous solution of mixed polymers. Therefore we tried butanol to see if it could give us information

about the effective thickness of intact gall bladder. The true isotopic water permeability of the luminal cell membrane, assuming that this membrane is rate limiting for water flow, is calculated from the expression for resistances in series:

$$\frac{1}{(P_d)_{obs}} = \frac{1}{(P_d)_m} + \frac{\delta}{D(^3HHO)}$$

where $(P_d)_{obs}$ = observed ^3HHO permeability of intact tissue in $cm \cdot s^{-1}$; $(P_d)_m$ = true cell membrane permeability in $cm \cdot s^{-1}$; δ = unstirred layer thickness calculated from butanol diffusion in cm; $D(^3HHO)$ = diffusion coefficient of ^3HHO in the unstirred regions in $cm^2 \cdot s^{-1}$. Instead of stripping the epithelial cell layer, we tried to destroy the resistance of the cell membrane for ^3HHO diffusion. This was done by 15 min exposure to chloroform, a treatment which reduces the electrical resistance to 4 % of its original value[19].

RESULTS

Diffusive and osmotic water permeability

The observed permeability coefficient of butanol was independent of the concentration gradient between 0.5 and 5.0 mM. If butanol diffusion is completely unstirred layer limited, diffusion theory predicts that there should be an inverse correlation with medium viscosity. In the case of lipid bilayers, $(P_d)_{butanol}$ decreased by a factor of 2.8 when the viscosity was raised 2.8-fold by adding 2 M glucose to the medium at 25 °C (ref. 18). In intact gall bladders $(P_d)_{butanol}$ decreased by a factor of 2.28 and in chloroform treated bladders by 2.54 on addition of 2 M glucose to the luminal and serosal solution (three observations). The discrepancy between viscosity increase and $(P_d)_{butanol}$ decrease is likely to be caused by the fact that glucose, especially in intact tissue, can not penetrate 100 % of the tissue water.

In Table I mean tissue thickness is calculated from observed permeabilities of butanol and ^3HHO, assuming that diffusion of both species is completely unstirred layer limited. The mean value of intact tissue thickness based on butanol (892 ± 85 μm) is about the same (875 μm) as Smulders and Wright[4] reported for total unstirred layer thickness in like epithelium. These authors calculated unstirred layer thicknesses from time delay measurements of NaCl diffusion potentials and

TABLE I

MEAN EFFECTIVE THICKNESS ($\bar{\delta}$) CALCULATED FROM OBSERVED PERMEABILITIES AND FREE DIFFUSION COEFFICIENTS IN WATER, ASSUMING COMPLETELY UNSTIRRED LAYER LIMITED DIFFUSION OF BUTANOL AND ^3HHO

Diffusion coefficients of butanol in water are taken from Lyons and Sandquist[20] and free diffusion coefficients of ^3HHO are taken from Wang *et al.*[21].

	10^{-4} cm $\bar{\delta}$ (butanol)	10^{-4} cm $\bar{\delta}$ (^3HHO)	
Intact tissue	892 ± 85 (15)	1355 ± 117 (10)	$P < 0.01$
Chloroform-treated tissue	512 ± 69 (16)	664 ± 70 (10)	$P > 0.1$
	$P < 0.01$	$P < 0.001$	

sucrose induced streaming potentials. With these values unstirred layer limited permeability coefficients of butanol and propanol can be calculated. Their measured P_d values for both compounds proved to be identical to the calculated ones, which strongly supports our assumption of complete unstirred layer limited diffusion of butanol[4].

As shown in Table I, there is a significant difference between $\delta_{butanol}$ and $\delta(^3HHO)$ indicating an additional diffusion barrier for ^3HHO. After chloroform treatment, $\delta_{butanol}$ and $\delta(^3HHO)$ decrease significantly, compared with intact tissue but there is no significant difference beyond these values. From this we may conclude that chloroform has destroyed the extra resistance barrier to ^3HHO diffusion.

Histological examination of chloroform-treated bladders pointed out that the tissue is not uniformly effected. Areas where almost all epithelial cells have disappeared alternate with areas where almost no changes in the cell layer are apparent (Figs 1b and 1c). Visible changes in connective tissue structure could not

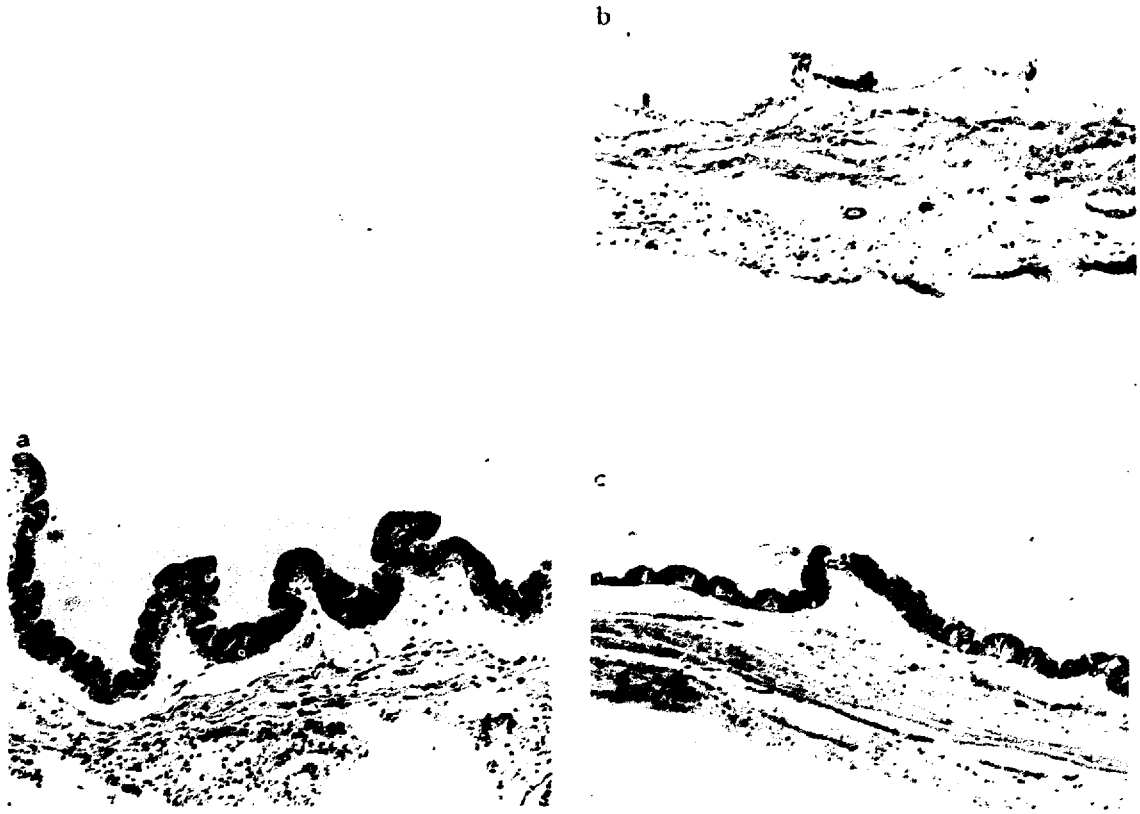

Fig. 1. Normal gall bladder (a) (Weigert stain) and chloroform-treated gall bladders (b and c) (HE stain) 128 ×. For further explanation see text.

be observed. A striking difference between untreated and treated bladders was in the number of folds on the luminal surface (Fig. 1). Folds were less pronounced in chloroform-treated species which may be due to change of smooth muscle tension. Probably this is the main reason for the decrease in thickness based on butanol diffusion from Table I. Other explanations for this observation are the extraction of the mucous layer at the luminal membrane, the removal of epithelial cells, and the possible extraction of lipophilic components of connective tissue. In order to calculate true isotopic water permeabilities we determined diffusive permeabilities of butanol

TABLE II

DETERMINATION OF $D(^3HHO)$ IN CHLOROFORM-TREATED TISSUE AND COMPARISON WITH FREE DIFFUSION COEFFICIENTS OF ^3HHO FROM Wang *et al.*[21]

Used values for $D_{butanol}$ in water were $0.54 \cdot 10^{-5}$, $0.88 \cdot 10^{-5}$ and $1.30 \cdot 10^{-5}$ $cm^2 \cdot s^{-1}$ at 5, 20 and 35 °C, respectively (Lyons and Sandquist[20]).

Temp. (°C)	*Chloroform-treated bladder*				*Water*
	10^{-5} $cm \cdot s^{-1}$ $(P_d)_{butanol}$	10^{-4} cm $\delta_{butanol}$	10^{-5} $cm \cdot s^{-1}$ $(P_d)(^3HHO)$	10^{-5} $cm^2 \cdot s^{-1}$ $D(^3HHO)$	10^{-5} $cm^2 \cdot s^{-1}$ $D(^3HHO)$
5	11.50	469	24.10	1.13	1.39
	9.91	544	18.09	0.98	
20	24.01	360	38.79	1.42	2.14
	8.45	1041	21.20	2.21	
	16.94	519	33.16	1.72	
	35.50	248	62.56	1.55	
35	28.13	462	40.77	1.88	3.04
	20.68	628	43.99	2.76	
	43.83	296	86.99	2.57	
	23.65	549	31.53	1.73	

and ^3HHO in intact gall bladder and again after chloroform exposure. In Table II diffusion coefficients of ^3HHO in chloroform-treated bladders are calculated and compared to self-diffusion coefficients of ^3HHO from Wang *et al.*[21]. In spite of using effective unstirred layer thickness from butanol diffusion, the mean value of $D(^3HHO)$ in chloroform treated tissue is 23% lower than the value of $D(^3HHO)$ in water which implies more hindering of ^3HHO than butanol in this layer. This difference is obscured in Table I by the variation of the thickness of individual bladders. The calculated $D(^3HHO)$ values of Table II are considered to represent diffusion coefficients of ^3HHO in the unstirred regions of intact gall bladder wall.

In Table III, true cell membrane permeabilities are given, calculated with values from butanol diffusion through intact tissue and $D(^3HHO)$ values from Table II. In this way, obtained $(P_d)_m$ values are compared to P_f values measured for the same bladder.

The mean value for the ratio $P_f/(P_d)_{obs}$ is 26.3 ± 4.3 and for the ratio $P_f/(P_d)_m$ 3.1 ± 0.4. This indicates that P_f/P_d ratio values after corrections as applied above give no reasons to postulate bulk flow of water through pores with radii greater than $\pm$ 4.5 Å, which is a situation simular to that met in the human red cell membrane[11,13].

If we compare $(P_d)_{obs}$ values (Table III) with $(P_d)_m$ values and express these values reciprocally as resistances, it turns out that the rate-limiting membrane for osmotic flow (which has been assumed to be the luminal membrane) constitutes only 14.3 ± 2.9% of the total tissue resistance to ^3HHO diffusion.

Temperature dependence of P_d and P_f

The mean of the P_f values obtained with 100 mM sucrose gradients was $5.2 \cdot 10^{-3} \pm 0.5 \cdot 10^{-3}$ $cm \cdot s^{-1}$ (18 observations). With 200 mM sucrose gradients, P_f values were significantly lower, *i.e.* 3.7 ± 0.4 (14 observations). This observation

TABLE III

CALCULATION OF TRUE CELL MEMBRANE PERMEABILITY $(P_d)_m$ FROM OBSERVED WATER PERMEABILITIES $(P_d)_{obs}$, AND UNSTIRRED LAYER CONTRIBUTIONS, $D(^3HHO)/\delta_{butanol}$.

$\delta_{butanol}$ is calculated from butanol diffusion through intact gall bladders and free diffusion coefficients of butanol (Table II).

Temp. (°C)	10^{-4} cm $\delta_{butanol}$	10^{-5} cm·s^{-1} $D(^3HHO)/\delta_{butanol}$	10^{-5} cm·s^{-1} $(P_d)_{obs}$	10^{-5} cm·s^{-1} $(P_d)_m$	10^{-5} cm·s^{-1} P_f	$P_f/(P_d)_{obs}$	$P_f/(P_d)_m$
5	870	12.98	11.28	85.8	257	22.8	3.0
	838	11.69	10.61	114.8	235	22.1	2.1
20	1000	14.20	12.19	86.1	320	26.3	3.7
	1301	16.98	16.34	432.9	476	29.1	1.1
	879	19.57	16.51	105.6	327	19.8	3.1
	260	59.60	38.82	111.1	403	10.4	3.6
35	967	19.44	18.36	330.0	1073	58.4	3.3
	1131	24.40	18.92	83.8	375	19.8	4.5
	758	33.90	28.78	188.6	495	17.2	2.6
	915	18.91	17.07	174.5	634	37.1	3.6

confirms the non-linear osmotic behaviour of this tissue described by Diamond[24]. Therefore, we measured the temperature dependence of both P_f determinations.

A break in the Arrhenius plot of P_f was found in all determinations. However, with 100 mM sucrose, the break appears between 10 and 15 °C while with 200 mM sucrose it appears at about 20 °C (Fig. 2). This suggests that lowering of the temperature augments the effect of increasing osmolarity resulting in a "phase transition" in the filtration rate controlling membrane.

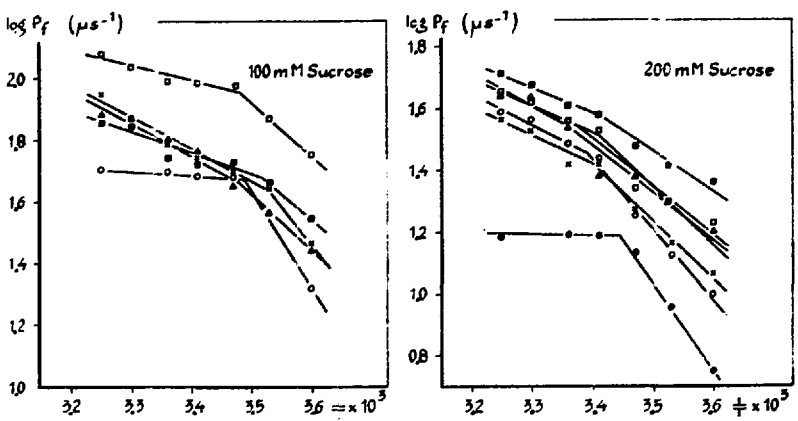

Fig. 2. Arrhenius plots of P_f. P_f is determined with an osmotic gradient of 100 mM (left) and 200 mM sucrose (right). Apparent activation energies are calculated from the slopes ⁄ 4.575 kcal·mole^{-1}.

The apparent activation energies of P_f and P_d are listed in Table IV in kcal·mole^{-1}. For both P_f values there is a significant decrease in apparent activation energy after the break in the Arrhenius plot. The apparent activation energy of ^3HHO diffusion is measured in intact gall bladders without unstirred layer corrections. The value 4.3 kcal·mole^{-1} probably reflects the activation energy of ^3HHO diffusion

in water, *in casu* the unstirred regions, because these regions comprise 85 % of the resistance to ^{3}HHO diffusion.

TABLE IV

APPARENT ACTIVATION ENERGIES IN kcal·mole^{-1} OF OSMOTIC AND DIFFUSIVE WATER PERMEABILITY OF RABBIT GALL BLADDER

P_f	100 mM sucrose	(5–10 °C)	9.7 ± 1.5 (5)	(15–35 °C)	3.1 ± 1.0 (5)
	200 mM sucrose	(5–20 °C)	9.4 ± 0.5 (6)	(20–35 °C)	3.5 ± 0.6 (6)
P_d	intact gall bladder	(5–35 °C)	4.3 (2)	^{3}HHO in water	4.6 ± 0.1 (ref. 21)

DISCUSSION

We have to consider the question of whether the ratio of flow and diffusion, after corrections for unstirred layer effects as applied in this study, may be used to estimate pore dimensions. In well-defined artificial membranes[18,25], and red cell membranes[16], these parameters have led to pore radii which were in agreement with radii obtained from diffusion of small molecules of known radius. In more complex systems, with several membranes in series as epithelial tissues, for example, estimation of pore radii has often led to erroneous results due to underestimation of the diffusional flow caused by unstirred layers[8,26]. Hays and Franki[11] demonstrated clearly that the supporting layer of toad bladder constituted 51 % of the resistance to diffusion but offered no significant barrier to flow. Unstirred layers in opposition to the luminal cell membrane and in the cell cytoplasm were not taken into account. In this study we estimated the total unstirred layer thickness from butanol diffusion and corrected the observed water permeabilities with values for $D(^3\text{HHO})$ in the unstirred regions determined in chloroform-treated gall bladders. Three assumptions underlying this correction have to be considered more closely.

Firstly, the assumption that butanol diffusion is completely unstirred layer limited which is based on a simplistic epithelial structure model *i.e.* two thin hydrophobic cell membranes in series with compartments consisting of aqueous solutions of polyelectrolytes. In this model the thickness of the aqueous compartments is a dominating factor in the diffusion of hydrophobic solutes through the epithelium. Indeed, Smulders and Wright[4] found for butanol no difference between measured P_d values and those calculated from unstirred layer thickness values of rabbit gall bladders. Moreover, they reported a reflection coefficient for butanol indistinguishable from zero. The results of Table I show clearly that in intact tissue there is an additional barrier to ^{3}HHO diffusion besides the barrier which is rate limiting for butanol. After chloroform treatment the barriers for butanol and ^{3}HHO become equal, which supports the assumption of unstirred layer limited butanol permeability.

Secondly, we assumed that the luminal cell membrane is rate limiting for flow. No direct evidence is available for this assumption. In epithelia where antidiuretic hormone regulates the water flow through the tissue, it is generally accepted that the rate-limiting barrier for water passage is at the luminal membrane[27–29]. In *Necturus* proximal tubule, functionally comparative to gall bladder, Bentzel

et al.[30] provided evidence for a luminal rate-limiting membrane. Cremaschi *et al.*[31] reported that Amphotericin B, a polyene antibiotic which interacts specifically with sterol-containing membranes[32], only effects the luminal membrane but not the basal or lateral cell membranes of gall-bladder epithelium. Since cholesterol lowers the water permeability of thin lipid membranes[33], this observation strongly supports our assumption that the luminal membrane is less permeable to water than the basal or lateral ones.

Thirdly, we assumed that ^{3}HHO diffuses in the unstirred regions as have been determined in chloroform-treated bladders. This assumption may lead to underestimation of the unstirred layer effect, since $D(^3\text{HHO})$ in the unstirred regions of the intact tissue can only be smaller than the used $D(^3\text{HHO})$ value. Chloroform treatment might have loosened the connective tissue structure by extraction of lipophilic components.

In calculating true permeabilities (Table III), the contribution of unstirred layers to the total resistance to ^{3}HHO diffusion turned out to be about 85 %. This situation makes the accuracy of $(P_d)_{obs}$ determinations extremely important, as has been emphasized by Everitt *et al.*[34]. Therefore, we subsequently measured $P(^3\text{HHO})$ in the same bladder six times and found standard errors of $\pm$ 3 %. The same relative errors, but independent from each other, occur in determinations of δ and $D(^3\text{HHO})$. This leads to a relative standard error of about 8 % in the calculated $(P_d)_m$ values of Table III, which is still sufficiently low to maintain confidence in these values.

In the comparison of $(P_d)_m$ with P_f, we assumed that unstirred layer effects are trivial in determining P_f. In frog skin[8], toad bladder[11] and red blood cell[35], unstirred layers had no effect on the magnitude of P_f. Very recently, Rajerison *et al.*[36] demonstrated that P_f of isolated frog skin epithelium was identical to P_f of intact skin. However, a significant correction for P_f values arises from the P_f dependence on medium osmolarity. The difference in mean values for P_f determined with 100 and 200 mM sucrose gradients is 30 % and extrapolation to isotonic condition gives P_f values at least 30 % greater than those used in Table III. By taking into account this osmolarity influence the mean value for the ratio P_f/P_d *i.e.* 3.1 $\pm$ 0.4, increases to 4.0. From this we may conclude that if water permeation through rabbit gall bladder proceeds by bulk flow, the equivalent pore radii will not exceed the value 5.4 Å. This implies that the main route of water flow is through the epithelial cells and that the extracellular shunt, the passive pathway for ions and inulin[3,4] does not contribute significantly to this flow. For predominant permeation of water *via* the shunt pathway, one can calculate that the ratio $P_f/(P_d)_m$ has to exceed the value 14 in order to arrive to pore radii greater than 12 Å, which dimension is needed for inulin permeation.

The following considerations are in support of the conclusion reached from the P_f/P_d ratio. The osmotic permeability is determined with sucrose gradients although gall bladder is not completely impermeable for this solute. Since both sucrose and inulin diffuse through the extracellular shunt pathway the reflection coefficient of sucrose in this shunt will be closer to zero than to one which has been pointed out by Smulders and Wright[4]. For this reason sucrose must be rather ineffective in pulling water through this extracellular shunt.

The P_f value of gall bladder is not extraordinary high compared to the

relatively high conductance. In Table V resistance and osmotic water permeabilities of several membrane species are listed. It is obvious that there is no simple correlation between both parameters. An unmodified blackfilm is as permeable to water as most biological membranes. For this reason it is very unlikely that the small junctional area, responsible for the high conductance, contribute significantly to the osmotic water flow, despite the fact that this pathway might be highly permeable to water. Therefore P_f determinations with sucrose gradients provide information about the cellular membranes.

TABLE V

COMPARISON BETWEEN ELECTRICAL RESISTANCE AND OSMOTIC WATER PERMEABILITY IN SEVERAL MEMBRANE SPECIES

	$R(\Omega \cdot cm^2)$	$P_f(10^{-3} \cdot cm \cdot s^{-1})$	*Refs.*
Rabbit gall bladder	28	5.2	This paper
Proximal tubule *Necturus*	80	2.3	ref. 37
Frog skin	2000	0.9	ref. 8
Toad bladder + antidiuretic hormone	800	19.3	ref. 26
Nitella translucens	26400		ref. 38
		4.3	ref. 39
Black film	10^8	1.0	ref. 7
Black film + nystatin	100	4.0	ref. 18

From apparent activation energies of P_f from Table IV we reach the same conclusion. The measured activation energies 3.5 ± 0.6 (200 mM sucrose) and 3.1 ± 1.0 (100 mM sucrose) kcal·mole^{-3} between 20–35 °C and 10–35 °C, respectively, are comparable to activation energies of P_f in human and dog red cell membranes, 3.3 ± 0.4 and 3.7 ± 0.4 kcal·mole^{-1}, respectively, as reported by Viera *et al.*[12]. These values are not significantly lower than 4.2 kcal·mole^{-1}, which is the inverse value of the activation energy for bulk water viscosity[13]. According to Gary-Bobo and Solomon[13] this is a second criterion for classical viscous flow. This second criterion for viscous flow is not fullfilled at any temperature. Below 10–15 °C with a gradient of 100 mmoles sucrose and below 20 °C with a gradient of 200 mmoles, the activation energy rises to 9.7 ± 1.5 kcal·mole^{-1} and 9.4 ± 0.5 kcal·mole^{-1}, respectively. These values are not significantly lower than activation energies expected for a simple solubility–diffusion mechanism in the water permeation process. For such a mechanism apparent activation energies are predicted between 10.5 and 12.4 kcal·mole^{-1} (ref. 40). Hence a possible explanation for the break in the Arrhenius plots is a phase transition from a rather hydrated state, with areas in the membranes in which water possess bulk water properties, to a relatively dehydrated state without such areas. A second explanation can be a gradual decrease in pore size, which causes increasing "structure" of water in the aqueous pore. In that case activation energies are expected between 4 and 16 kcal·mole^{-1}, *i.e.* the lower limit referring to diffusion in water, and the upper limit to diffusion in ice-like water or single ice crystals[40]. Apparent activation energies of P_f from rabbit gall bladder reflects typical values

for lipid membranes which supports the conclusion that the osmotic flow through the tissue is merely controlled by the cellular membrane and not by the extracellular shunt pathway.

ACKNOWLEDGEMENT

Our thanks are due to Dr W. M. C. Eling, Department of Cytology and Histology, for histological preparation and examination of gall bladders; Dr J. H. Veerkamp, Department of Biochemistry, for counting service of radioactive samples. Mr M. D. de Jong is gratefully acknowledged for his skillful technical assistance.

REFERENCES

1 J. M. Diamond and W. H. Bossert, *J. Gen. Physiol.*, 50 (1967) 2061.
2 P. H. Barry, J. M. Diamond and E. M. Wright, *J. Membrane Biol.*, 4 (1971) 358.
3 E. Frömter and J. M. Diamond, *Nature*, 235 (1972) 9.
4 A. P. Smulders and E. M. Wright, *J. Membrane Biol.*, 5 (1971) 297.
5 C. F. Phelps, *Biochem. J.*, 95 (1965) 41.
6 A. K. Solomon, *J. Gen. Physiol.*, 51 (1968) 335s.
7 A. Cass and A. Finkelstein, *J. Gen. Physiol.*, 50 (1967) 1765.
8 J. Dainty and C. R. House, *J. Physiol.*, 185 (1966) 172.
9 R. M. Hays, *J. Gen. Physiol.*, 51 (1968) 387.
10 T. Hosh'ko, B. D. Lindley and C. Edwards, *Nature*, 201 (1964) 932.
11 R. M. Hays and N. Franki, *J. Membrane Biol.*, 2 (1970) 263.
12 F. L. Vieira, R. I. Sha'Afi and A. K. Solomon, *J. Gen. Physiol.*, 55 (1970) 451.
13 C. M. Gary-Bobo and A. K. Solomon, *J. Gen. Physiol.*, 57 (1971) 610.
14 C. H. Van Os and J. F. G. Slegers, *Biochim. Biophys. Acta*, 241 (1971) 89.
15 J. Mc. D. Tormey and J. M. Diamond, *J. Gen. Physiol.*, 50 (1967) 2031.
16 J. M. Diamond, *J. Gen. Physiol.*, 48 (1964) 1.
17 G. Bray, *Anal. Biochem.*, 1 (1960) 279.
18 R. Holtz and A. Finkelstein, *J. Gen. Physiol.*, 56 (1970) 125.
19 F. M. Wright and J. M. Diamond, *Biochim. Biophys. Acta*, 163 (1968) 57.
20 P. A. Lyons and C. L. Sandquist, *J. Am. Chem. Soc.*, 75 (1953) 3896.
21 J. W. Wang, C. V. Robinson and J. S. J. Edelman, *J. Am. Chem. Soc.*, 75 (1953) 466.
22 V. W. Sidel and A. K. Solomon, *J. Gen. Physiol.*, 41 (1957) 243.
23 C. V. Paganelli and A. K. Solomon, *J. Gen. Physiol.*, 41 (1957) 259.
24 J. M. Diamond, *J. Physiol.*, 183 (1966) 58.
25 E. R. Renkin, *J. Gen. Physiol.*, 38 (1955) 225.
26 R. M. Hays and A. Leaf, *J. Gen. Physiol.*, 45 (1962) 905.
27 M. S. Lichtenstein and A. Leaf, *Ann. N.Y. Acad. Sci.*, 137 (1966) 556.
28 J. J. Grantham, C. E. Ganotte, M. B. Burg and J. Orloff, *J. Cell. Biol.*, 41 (1969) 562.
29 S. Jard, J. Bourquet, P. Favard and N. Carasso, *J. Membrane Biol.*, 4 (1971) 124.
30 C. J. Bentzel, B. Parsa and D. K. Hare, *J. Am. Physiol.*, 217 (1969) 570.
31 D. Cremaschi, C. Montanari, T. Simonic and C. Lippe, *Arch. Int. Physiol. Biochim.*, 79 (1971) 33.
32 S. C. Kinsky, S. A. Luse and L. L. M. van Deenen, *Fed. Proc.*, 25 (1966) 1503.
33 A. Finkelstein and A. Cass, *Nature*, 216 (1967) 717.
34 C. T. Everitt, W. R. Redwood and D. A. Haydon, *J. Theor. Biol.*, 22 (1969) 20.
35 R. I. Sha'Afi G. T. Rich, V. W. Sidel, W. Bossert and A. K. Solomon, *J. Gen. Physiol.*, 50 (1967) 1377.
36 R. J. Rajerison, M. Montegut, S. Jard and F. Morel, *Pflügers Arch. Ges. Physiol*, 332 (1972) 302.
37 C. J., Bentzel, M. Davies, W. N. Scott, M. Zatzmann and A. K. Solomon, *J. Gen. Physiol.*, 51 (1968) 517.
38 J. Hogg, E. J. Williams and R. J. Johnston, *Biochim. Biophys. Acta*, 150 (1968) 518.
39 J. Dainty and B. Z. Ginzburg, *Biochim. Biophys.* Acta, 79 (1964) 102.
40 H. D. Price and T. E. Thompson, *J. Mol. Biol.*, 41 (1969) 443.