Routes of Nonelectrolyte Permeability in Gallbladder

Effects of 2,4,6-Triaminopyrimidinium (TAP)

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ABSTRACT   The organic cation 2,4,6-triaminopyrimidinium (TAP), which blocks the tight junction channels for cation permeability across gallbladder, also inhibits gallbladder permeability (P) to urea and glycerol without significantly affecting P to Cl^-, sucrose, 1,7-heptanediol, or water (osmotic or diffusional permeabilities). These effects together with the comparisons of P's in frog with P's in rabbit gallbladder suggest that sucrose migrates exclusively through the leakage pathway (through where Cl^- permeates), and that urea and glycerol permeate in addition through both, the tight junction channels for cations and a polar pathway at the cell membranes. Water and 1,7-heptanediol probably permeate mainly through the epithelial cell membranes.

INTRODUCTION

Once it was evident that the main pathway for electrolyte permeation across the gallbladder is paracellular, the possibility that this pathway could also be an important route of nonelectrolyte permeation was foreseen immediately (Frömter and Diamond, 1972). This possibility, however, has not yet been experimentally tested. In a previous paper (Moreno, 1975) it was concluded that the paracellular ionic pathway has two parallel components: *first* the main pathway of current, cation selective and blocked by 2,4,6-triaminopyrimidinium (TAP), called tight junction cation channels or simply cation channels, and *second*, the main pathway of Cl^- permeability, which appears to have free solution characteristics, is insensitive to TAP, and was referred to as the leakage pathway or Cl shunt. Consequently, in addition to the cell membrane routes common to most cells and epithelia, nonelectrolytes can migrate across gallbladder through these two paracellular routes, and the distinction between the routes of nonelectrolyte permeation in gallbladder becomes rather complicated.

I have attacked this problem following two lines: (a) by studying the effect of TAP on nonelectrolyte permeability, and (b) by comparing nonelectrolyte permeabilities (with and without TAP) of frog with that of rabbit gallbladder. This is because Hingson and Diamond (1972), in a study of nonelectrolyte reflection coefficients (σ's), concluded that the polar route which facilitates permeability of small nonelectrolytes in most cell membranes was present in rabbit but not in frog gallbladder epithelial cell membranes.

METHODS

The general methods for dissecting and mounting rabbit and bullfrog gallbladders in chambers, and measuring their ionic permeabilities (P_i) and conductances (G_i) were described previously (Moreno and Diamond, 1974, 1975 b; Moreno, 1975).

Measurements of Tracer Fluxes

A tracer quantity (1–10 μCi/ml depending on the experiment) of the radioactive isotope was added to one of the solutions, called side 1 (generally serosal)[1] and the rate of appearance of the tracer on the other side, called side 2, was determined by withdrawing two 100-λ samples from side 2 at intervals ranging from 5 to 40 min (5 min for water and 1,7-heptanediol, 40 min for sucrose, 20 min for all the other solutes studied). After the aliquots were withdrawn, 200 λ of "cold" saline was added to side 2 to maintain its volume constant. This procedure was repeated for four to five periods, the averaged value of the fluxes calculated in those periods was taken as the flux of that particular gallbladder. Aliquots of solution were withdrawn from side 1 at the beginning and end of each experiment to determine its isotope activity. All radioactive samples were assayed and counted by conventional liquid scintillation techniques. Water ^{3}H, urea ^{14}C, sucrose ^{14}C, and 1,7-heptanediol ^{14}C were obtained from ICN Pharmaceuticals Inc., Cleveland, Ohio, and methylamine ^{3}H·HCl, glycerol ^{3}H, and glycerol ^{14}C from New England Nuclear, Boston, Mass. 2,4,6-triaminopyrimidine was obtained from Eastman Organic Chemicals, Rochester, N. Y.

The control and TAP inhibition measurements were always done in the same gallbladder. In most cases two halves of the same gallbladder were studied simultaneously, as described previously (Moreno, 1975), one serving as control. In a few experiments the control and experimental measurements were performed on the same piece of gallbladder: first the control fluxes were measured, then the solutions were replaced by cold solutions, and the new experimental conditions were set, and after 30–60 min the fluxes (experimental period) were measured again.

Just before and after tracer permeability measurements, two 2:1 NaCl dilution potentials bracketing one K:Na biionic potential were measured. During each experiment, the membrane resistance was measured every 10 min. Because of the careful

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[1] Smulders and Wright (1971) found no difference in the two unidirectional fluxes serosa to mucosa and mucosa to serosa for sucrose, urea, acetamide, 1,4-butanediol, 1,7-heptanediol, and nicotinamide in rabbit gallbladder. This finding was confirmed here for urea in rabbit gallbladder and extended to urea and glycerol in frog gallbladder (two preliminary experiments).

check of the state of the preparation before, during, and after the flux measurements, I was sure of not including damaged preparations in the averages.[2]

The ionic permeabilities for Na^+ and Cl^- were obtained from the partial ionic conductances of these ions (G_X's) through the relation $P_X = (G_X RT)/(F^2 C_X)$, where R, T, and F have the usual meaning, and C_X is the concentration of the ion X. The values of the partial Na^+ conductance (G_{Na} or G_{Na}(total) depending on whether it was or was not, respectively, corrected by the Cl shunt) and G_{Cl} were obtained as described previously (Moreno and Diamond, 1974; Moreno, 1975).

The diffusional permeability coefficients experimentally observed (P_{obs}) were corrected for unstirred-layer effects as described by Smulders and Wright (1971). On the average, the thickness of the total unstirred layer estimated from the time-course of sucrose streaming potentials (as described by Diamond, 1966), was 434 ± 21 μm (15) in frog and 418 ± 32 μm (8) in rabbit gallbladder. The correction factors P/P_{obs} introduced by these unstirred layer corrections in the tracer permeabilities measured during the control period were, in frog gallbladder: 2.12 for water, 1.84 for 1,7-heptanediol, 1.03 for urea and $H_3CNH_3^+$, and 1.00 for glycerol and sucrose; and in rabbit gallbladder: 1.23 for urea, 1.06 for $H_3CNH_3^+$ and glycerol, and 1.01 for sucrose.

The solution compositions were as described in the previous paper (Moreno, 1975). TAP was added to both sides of the preparation at a concentration of 12.0 mM (concentration of the active or charged form of 2,4,6-triaminopyrimidine, $pK_1 = 6.74$, pH of the solutions $= 6.1$). The measurements were done at 23°C.

Measurements of the Steady-State Osmotic Water Permeability

These measurements were done in noneverted sacs, by the gravimetric method described in detail by Diamond (1962, 1964). Briefly, the noneverted gallbladder was cannulated, and the lumen filled with Ringer's solution before suspending the preparation in a beaker of saline stirred by bubbling with pure oxygen. The steady-state osmotic water permeability (P_f) was calculated from the change in the water flow when 100 mM sucrose was added to the serosal saline.

In these experiments TAP was tested at a concentration of 10 mM in both mucosal and serosal sides. When TAP was not present 10 mM $(H_3C)_4N \cdot Cl$ was added to the Ringer's to compensate for the osmotic effects (10 mM $(H_3C)_4N \cdot Cl$ has no effect on the electrical properties of frog gallbladder [Moreno, 1975]).

RESULTS

The transepithelial permeability coefficients P's, obtained for frog and rabbit gallbladders during the control period and after 12 mM TAP was added to

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[2] This is probably the reason why the permeability values for sucrose in rabbit gallbladder reported here (Table I) are more than two times smaller than those reported by Smulders and Wright (1971) and van Os et al. (1974). The value of the permeability coefficient for sucrose is, however, similar to the one obtained from Fig. 1 of Smulders et al., 1972 ($P = 2.3 \times 10^{-6}$ cm/s) when a more careful check of the preparations was made. The greater permeability coefficients for sucrose obtained by van Os et al. (1974) could be due to the use of sac preparations by these authors.

both sides of the preparation are summarized in Table I. Except for 1,7-heptanediol and water, the P's during the control period are higher in rabbit than in frog gallbladder. TAP has, however, qualitatively the same effect in both species: TAP significantly decreases the P's of Na^+, $H_3CNH_3^+$, urea, and glycerol, and does not affect significantly the P's of Cl^- and sucrose in frog and rabbit gallbladder. In frog gallbladder, P_{water} and $P_{heptanediol}$ are not affected by TAP (the statistical significance refers to paired data).

As was discussed previously (Moreno and Diamond, 1975 a, 1975 b; Moreno, 1975, pp. 99, 110) there is compelling evidence that Cl^- permeates through the leakage pathway, whose characteristics resemble those of a free solution shunt. Consequently all solutes listed in Table I, regardless of whether they use other routes will, presumably, permeate through the leakage pathway in a ratio close to their free solution diffusion coefficients ratio. I, therefore, corrected their P's by the equation:

$$P_X \text{ (corrected)} = P_X - P_{Cl}\left(\frac{D_X}{D_{Cl}}\right), \tag{1}$$

where D_X and D_{Cl} are the free solution diffusion coefficients of the solute X and Cl^-, respectively. Table II shows the values of P_X's corrected by Eq. 1.

Sucrose

The predicted values of $P_{sucrose}$ across the leakage pathway ($= P_{Cl} \times D_{sucrose}/D_{Cl}$) are 0.86×10^{-6} cm/s in frog gallbladder and 2.28×10^{-6} cm/s in rabbit gallbladder. These predicted values are in fact *larger* than the measured $P_{sucrose}$ values reported in Table I (by a factor of 1.3 in rabbit and by 3.0 in frog gallbladder), therefore $P_{sucrose}$ (corrected) gives artifactual negative numbers. The fact that actually the predicted leakage sucrose permeability exceeds the measured permeability suggests that the leakage pathway can account for all the sucrose permeability observed in frog and rabbit gallbladders, and that the leakage pathway poses more restriction to the diffusion of bigger molecules like sucrose vs. Cl^- than does the free solution. This restriction is more marked in frog than in rabbit gallbladder ($D_{sucrose}/D_{Cl} = 0.30$, while $P_{sucrose}/P_{Cl}$ is 0.23 ± 0.03 (6) in rabbit and 0.09 ± 0.02 (6) in frog).

Since for all the solutes except water $D_{Cl} > D_X$, their corrected P values obtained through Eq. 1 and listed in Table II are probably underestimations, especially in frog gallbladder. However, since $D_X > D_{sucrose}$ these corrections should involve less error than for $P_{sucrose}$.

Urea and Glycerol

Table I shows that rabbit gallbladder is, respectively, eight and five times more permeable to urea and glycerol than frog gallbladder. This is in ac-

TABLE I

EFFECT OF TAP ON THE PERMEABILITY OF THE SOLUTES ACROSS FROG
AND RABBIT GALLBLADDER

Compound	Frog		Rabbit	
	Control	TAP	Control	TAP
	10^{-6} cm/s	10^{-6} cm/s	10^{-6} cm/s	10^{-6} cm/s
Water	683±125 (3)	684±64 (3)	202*	
1,7-Heptanediol	135±25 (3)	125±25 (3)	68*	
Urea	8.2±1.0 (5)	3.2±0.3 (5)	65±17 (3)	36±11 (3)
Glycerol	2.8±0.5 (5)	1.6±0.2 (5)	13.4±1.8 (3)	6.8±1.7 (3)
Sucrose	0.26±0.09 (3)	0.29±0.06 (3)	1.8±0.2 (3)	1.7±0.4 (3)
$H_3CNH_3^+$	9.5±1.7 (4)	3.7±0.5 (4)	20.8±2.8 (4)	6.5±1.5 (4)
Na^+	13.5±1.3 (17)	3.7±0.5 (17)	32.6±5.0 (8)	11.1±1.8 (8)
Cl^-	3.2±0.2 (17)	3.7±0.5 (17)	7.5±0.9 (8)	8.1±1.1 (8)

The number of cases is taken as the number of gallbladders or individual experiments. The
control and TAP P measurements were done in the same gallbladder. All the values have been
corrected by the unstirred layer effects (p. 119). The values of Na^+ and Cl permeabilities were
obtained from electrical measurements (G_{Na} (total) and G_{Cl}) as described in Methods.
* From Wright and Pietras (1975).

TABLE II

EFFECT OF TAP ON THE PERMEABILITY OF THE SOLUTE ACROSS FROG AND
RABBIT GALLBLADDER CORRECTED BY THE LEAKAGE PATHWAY

Compound	Frog		Rabbit	
	Control	TAP	Control	TAP
	10^{-6} cm/s	10^{-6} cm/s	10^{-6} cm/s	10^{-6} cm/s
Water	678±123 (3)	681±64 (3)	129*	
1,7-Heptanediol	132±25 (3)	122±21 (3)	65*	
Urea	5.6±0.1 (5)	0.9±0.3 (5)	59±13 (3)	30±10 (3)
Glycerol	1.1±0.5 (5)	0.1±0.2 (5)	8.1±2.3 (3)	1.6±1.0 (3)
Sucrose	—	—	—	—
$H_3CNH_3^+$	7.2±1.5 (4)	1.5±0.9 (4)	15.2±2.3 (4)	1.7±0.9 (a4)
Na^+	11.2±1.1 (17)	1.6±0.2 (17)	27.9±4.6 (8)	6.0±0.9 (a8)
Cl^-	—	—	—	—

Each entry is the average of the P's corrected (in each individual experiment) by Eq. 1. Since
the leakage pathway probably discriminates against bigger solutes more than free solution, the
permeability of solutes larger than Cl^- is presumably an underestimate, especially for frog gall-
bladder (see comments in the text).
* Calculated from Wright and Pietras (1975).

cordance with the idea of rabbit having more or "more effective" specific
polar pathways which facilitate the permeation of urea and glycerol (Hingson
and Diamond, 1972). After correcting for the leakage pathway (Table II),
the ratio P_x(rabbit)/P_x(frog) becomes 10.4 for urea and 7.4 for glycerol,
indicating that the interspecies difference in P_{urea} and $P_{glycerol}$ is not due to
the leakage pathway. P_{urea} and $P_{glycerol}$ are inhibited by TAP in both species,

but the interspecies ratios become even larger: $P_X(\text{rabbit})/P_X(\text{frog}) = 30$ for urea and 20 for glycerol.

The simplest interpretation of these data is that urea and glycerol can permeate the gallbladder through two pathways (besides the leakage pathway): a polar pathway at the epithelial cell membranes, insensitive to TAP, much more effective in rabbit than in frog gallbladder, and responsible for the σ_{urea} differences seen by Hingson and Diamond, (1972), and the tight junction cation channels, sensitive to TAP and present in both species but proportionately more important in frog gallbladder.

This interpretation, however, relies on the assumption that TAP does not affect the polar pathway at the cell membranes, and the only evidence for this is the apparent specificity of TAP for the cation channels. Nevertheless, should it turn out that TAP affects the pathway for small nonelectrolytes in the cell membrane, the last paragraph simply may involve the sensitive and insensitive fractions of the flux of urea and glycerol through the polar pathways of the cell membrane.

Water and 1,7-Heptanediol

The values of P_{water} and $P_{\text{heptanediol}}$ are the largest given in Tables I and II. The similarity of the values of Table I with those of Table II, and the lack of effect of TAP on P_{water} and $P_{\text{heptanediol}}$ in frog gallbladder suggest that neither the leakage pathway nor the cation channels are the main route of water or heptanediol permeation. The main route of their permeation is probably across the epithelial cell membranes. This idea is supported by the following: 1,7-heptanediol is a highly lipid soluble nonelectrolyte ($K_{\text{oil}} = 3.1 \times 10^{-2}$); most single cell membranes (see House, 1974) and artificial lipid bilayers (Cass and Finkelstein, 1967) have P_{water} equal or larger than those reported here; and P_{water} and $P_{\text{heptanediol}}$ (Table II) are one to two orders of magnitude larger than P_{Na} or $P_{\text{H}_3\text{CNH}_3}$ which permeate through the cation channels.

These considerations for water and 1,7-heptanediol, do not mean, however, that the cation channels are impermeant to them. A permeability of 1×10^{-5} cm/s for water or 1,7-heptanediol through these channels sensitive to TAP would probably not have been detected in these experiments, since the P's of these solutes are more than one order of magnitude higher. The steady-state osmotic water permeability in frog gallbladder was not affected significantly by TAP either: $P_f = 1.5 \pm 0.3 \times 10^{-2}$ cm/s ($n = 3$) during the control period vs. $P_f = 1.5 \pm 0.1 \times 10^{-2}$ cm/s ($n = 3$) after TAP.[3]

[3] The value of the steady-state P_f reported here for frog gallbladder is close to that found under similar circumstances for rabbit gallbladder (1.7 × 10⁻² cm/s, Wright et al., 1972; van Os and Sleegers, 1973). These values of steady-state P_f are probably gross underestimations of the instantaneous, real one (Wright et al., 1972; van Os, 1974). The reason for that is the solute polarization due to the "sweeping away effect" (Dainty, 1963) which led to actual steady-state osmotic gradients

Na^+ and $H_3CNH_3^+$

Both P_{Na} and $P_{H_3CNH_3}$ are strongly inhibited by TAP in frog and rabbit gallbladder, as it is shown in Table I and II. This is apparently a common feature of most monovalent hydrophilic cations (Moreno, 1975).

Table III shows the values of $P_{H_3CNH_3}$ (corrected by the leakage pathway) obtained from two completely different methods: (a) measurement with tracer techniques, and (b) calculation from the ratio $(P_{H_3CNH_3}/P_{Na})$ obtained from H_3CNH_3:Na biionic potential and values of G_{Na} obtained from conductances and dilution potential measurements (as discussed on p. 119).

TABLE III

FROG AND RABBIT PERMEABILITY TO $H_3CHN_3^+$

Method employed to obtain $P_{H_3CN H_3^+}$	Frog			Rabbit		
	$P_{H_3CN H_3^+}$		$\alpha_{H_3CN H_3}$	$P_{H_3CN H_3^+}$		$\alpha_{H_3CN H_3}$
	Control	TAP		Control	TAP	
	10^{-6} cm/s	10^{-6} cm/s	10^{-6} cm/s	10^{-6} cm/s	10^{-6} cm/s	10^{-6}cm/s
Tracer fluxes	7.2 ± 1.5	1.5 ± 0.9	0.79	15.2 ± 2.3	1.7 ± 0.8	0.89
Electrical measurements	9.0 ± 1.7	1.3 ± 0.6	0.86	14.8 ± 4.6	3.1 ± 1.0	0.79

Each value is the average of four individual gallbladders. $P_{H_3CNH_3^+}$ (corrected for the Cl shunt) was calculated in two ways: (a) with tracers as described in Methods, and (b) from parameters obtained from electrical methods, $P_{H_3CNH_3}/P_{Na}$ and G_{Na} as described on p. 119. $\alpha_{H_3CNH_3}$ is the fractional inhibition of $P_{H_3CNH_3}$ (Moreno, 1975, Eq. 7).

Although both techniques for calculating $P_{H_3CNH_3}$ are completely independent their results are the same within experimental error: both in the control period and after addition of 12 mM TAP the ratio of $H_3CNH_3^+$ and Na^+ P's obtained from biionic potentials is similar to the ratio of their tracer fluxes, and the values of the fractional inhibition of $P_{H_3CNH_3}$, $\alpha_{H_3CNH_3}$ (Moreno, 1975, Eq. 7), obtained from both techniques and displayed in Table III (average $\alpha_{H_3CNH_3}$ = 0.83 in frog and 0.84 in rabbit gallbladder) are close to the values of α_{Na} obtained at these TAP concentrations (α_{Na} = 0.82 in frog and 0.80 in rabbit gallbladder). These findings indicate that both in the control period and under 12 mM TAP, $H_3CNH_3^+$ and Na^+ cross mainly

much smaller than the instantaneous gradient. Therefore, the lack of effect of TAP on the steady-state P_f could, in principle, be due to a compensatory balance between a hypothetical inhibition of the instantaneous P_f by TAP and the enhancement of the steady-state osmotic gradient by the reduction of P_f. However, such compensatory balance is unlikely since while the osmotic water flux at a fixed osmotic gradient is linearly dependent on P_f, the dissipation of the osmotic gradient by the sweeping away effect is an exponential function of P_f (Dainty, 1963; Wright et al., 1972).

through the same extracellular pathway, and that $H_3CNH_3^+$ and not H_3CNH_2 is the permeant species (see also Moreno and Diamond, 1975 b).

DISCUSSION

Route of Sucrose Permeability

Smulders and Wright (1971) and Smulders et al. (1972) identified the route of sucrose permeation with the tight junction pathway, where Frömter and Diamond (1972) also localized the main route of ion permeation. They based this conclusion on the following experimental observations in rabbit gallbladder: (a) the sucrose permeability that they found was relatively large (see footnote 2, however), (b) the apparent activation energy of $P_{sucrose}$ was not different from that of its free solution diffusion coefficient, $D_{sucrose}$, (c) the ratio $P_{sucrose}/P_{inulin}$ (=6) across rabbit gallbladder was close to the free solution ratio $D_{sucrose}/D_{inulin}$ (=4), and (d) when the mucosal solution was made hypertonic with sucrose or mannitol, and therefore the lateral intercellular spaces collapsed (as did all the extracellular spaces including the subserosa muscularis mucosa and submucosa), the sucrose permeability was reduced (as well as the permeability of any other solute so far measured under such circumstances).

More recently van Os et al. (1974), studying the nonelectrolyte permeability of rabbit gallbladder sac preparations further showed that dextran (mol wt = 16.000, a molecule of nearly 30 × 30 × 200 Å) was still permeant, but that albumin (molecular radius $\simeq$ 40 Å) was impermeant in most of their preparations. They also interpreted this permeability as due to the paracellular route of ion permeation through where Na^+ crosses the gallbladder, and concluded that this route is formed by $\simeq$80-Å diameter pores.

In contrast with the interpretation of these authors, the evidence presented in this paper indicates that sucrose *does not* permeate through the tight junction cation channels where most of the ionic current crosses, but through the leakage pathway. The evidence presented here supporting this view is: (a) the leakage pathway or Cl shunt can account for all the sucrose permeability observed, and (b) TAP does not affect $P_{sucrose}$ or P_{Cl} in frog or rabbit gallbladder, but can block the tight junction cation channels for Na^+, other cations, and apparently also urea and glycerol. Furthermore the equivalent cylindrical radii of the cation channels obtained from previous studies (Moreno and Diamond, 1974, 1975 a, 1975 b) is $\simeq$4 Å for rabbit and $\simeq$8 Å for frog gallbladder. Taking this value literally it is difficult to imagine a molecule of sucrose or dextran (minimal radii 5 and 15 Å, respectively) permeating through the rabbit cation channels. Even if one should not rely on the absolute value of these equivalent channel radii (Moreno and Diamond, 1975 b) interspecies comparisons between steric restrictions or equivalent

pore radii are valid. For instance, for an ion X of radius $= 4$ Å the empirical and theoretical predictions for the ratio $(P_X/P_{Na})_{frog}/(P_X/P_{Na})_{rabbit}$ is 20, while this experimental ratio for $X =$ sucrose is 0.36. Since all our evidence indicates that frog and rabbit cation channels do not differ much in their chemical specificity (Moreno and Diamond, 1974, 1975 *b*), I conclude that the cation channels are not the pathway for sucrose.

An illustration to the point comes from observing that from studies on large hydrophilic nonelectrolyte permeability in rabbit gallbladder, van Os et al. (1974) concluded that the route of their permeation has an equivalent radius of $\simeq 40$ Å, i.e. 10 times larger than the equivalent radius of the cation channels (Moreno and Diamond, 1975 *b*), forcing the conclusion that the two routes are not the same.

Anatomical Location of the Pathways

Since the conductance across the gallbladder is mainly cationic ($P_{Na}/P_{Cl} \simeq$ 5), the cation-specific channels should be at the tight junction, where Frömter and Diamond (1972) found that most of the current crosses the gallbladder. The anatomical location and identity of the Cl shunt, on the other hand, remains obscure. At first glance three good candidates for the leakage pathway are: (*a*) damage produced experimentally at the edge of the chamber window ("edge damage"), (*b*) damaged, dying, or dead cells, whose permeability resembles that of free solution, and (*c*) especially leaky, nonselective channels at the tight junctions (separated from the cation-selective channels and parallel with them).

Edge damage is probably the least likely candidate for this pathway: P_{Cl} does not increase when the area of the tissue exposed in a chamber is reduced from 113 to 10 mm² and therefore the edge/area relation altered by a factor of 3.4 (Moreno and Diamond, 1974), and neither does the value of $P_{sucrose}$ under similar circumstances (Smulders and Wright, 1971). Furthermore, sac preparations which should be free of edge damage show P_{Na}/P_{Cl} ratios similar to chamber preparations, indicating similar P_{Cl} in both types of preparations (unpublished observations), and van Os et al. (1974), using sac preparations of rabbit gallbladder, report $P_{sucrose} = 7.2 \times 10^{-6}$ cm/s, i.e., four times *larger* than the values reported here (see footnote 2).

The knowledge we have so far on the characteristics of the leakage pathway is not enough for distinguishing between the remaining possibilities *b* and *c*. Two experimental observations are, however, better explained by the third possibility, i.e. leakage channels at the tight junctions as opposed to damaged or dead cells (the comments between brackets indicate why I think that they remain inconclusive). First, hypertonic mucosal solutions decrease the sucrose permeability while they collapse the lateral intercellular spaces (Smulders

et al., 1972). This could be taken as a hint favoring leaky junction channels instead of damaged cells. [However, under such experimental conditions, not only the lateral intercellular spaces but all the extracellular spaces of the gall-bladder collapse and the whole structure of the gallbladder changes: see, for instance Fig. 11 of Smulders et al. (1972). Furthermore the permeability of all solutes tested including 1,7-heptanediol, 1,4-butanediol, water, and Na^+ is reduced by this treatment, and therefore, the sucrose permeability changes cannot unequivocally be associated with a particular pathway.] And second, the fact that for frog and rabbit gallbladder $(P_{sucrose}/P_{Cl}) < (D_{sucrose}/D_{Cl})$ is in agreement with the findings of van Os et al. (1974) on the mannitol-sucrose-inuline-dextran series, and the albumin impermeability in some rabbit gall-bladder preparations, indicating that a steric-type size restriction through the leakage pathway does exist. This would be explained more easily by a pore or channel of relatively rigid walls than by a missing or dead cell. [However a damaged area or a dying cell could have under some circumstances, charac-teristics which will restrain the passage of bigger solutes.]

Pathways of Permeation

Three parallel pathways of passive permeation across frog and rabbit gall-bladder must be distinguished: the cation channels at the tight junction, the leakage pathway, and a cellular pathway, across the epithelial cell mem-branes. The characteristics of the cation permeation through the tight junc-tion cation channels have been described previously (Moreno and Diamond, 1974, 1975 a, 1975 b; Moreno, 1975). The results presented here suggest that the cation channels account also for a significant portion of P_{urea} and $P_{glycerol}$ in rabbit gallbladder, and probably most of them in frog gallbladder. How-ever Cl^-, sucrose, water, and 1,7-heptanediol do not permeate significantly through this pathway.

The second pathway, called leakage pathway or Cl shunt, has in a first approximation characteristics of a free solution parallel shunt (see Moreno, 1975; Moreno and Diamond, 1975 a, b) with some extra restriction (probably steric) to the movement of larger solutes, especially in frog gallbladder, and is not affected by TAP. It is responsible for all the Cl^- and probably all sucrose permeabilities and presumably that of other big, polar solutes. In normal Ringer's solutions it is only responsible for a minor fraction of the trans-epithelial conductance.

The cellular pathway has the characteristics of nonelectrolyte permeation through most cell membranes (Collander, 1954; Diamond and Wright, 1969; Sha'afi et al., 1971). It is probably responsible for the lipid soluble nonelectro-lyte permeability, the active Na^+ transport, and the osmotic and diffusional water permeability. The small hydrophilic nonelectrolytes (urea and glyc-

erol studied here) are probably partially crossing through the cell membrane, bypassing the lipid barriers through a polar pathway (e.g. pores or carriers). The data on this paper suggest that this facilitated diffusion is almost (or totally) absent in frog gallbladder as compared to rabbit gallbladder, as was previously suggested by Hingson and Diamond (1972).

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