J. Membrane Biol. 37, 277–297 (1977)

Salt and Water Transport by Rabbit and Guinea Pig Gallbladder: Effect of Amphotericin B on NaCl Influx

Richard C. Rose and David L. Nahrwold

Departments of Physiology and Surgery, The Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

Received 6 December 1976; revised 27 April 1977

Summary. Previous studies have led to the suggestion that salt and water absorption by rabbit and guinea pig gallbladders exposed to Amphotericin B proceeds by a rheogenic Na pump at the basolateral cell membrane. The present study *in vitro* was designed to further characterize transport properties of rabbit and guinea pig gallbladders under control conditions and to identify the properties of gallbladder mucosa which are altered by Amphotericin B to allow for the induced serosa-positive electrical potential differences (PD). Potassium is required in the bathing solution at a low concentration to maintain normal tissue O_2 consumption, fluid absorption and the ability of the tissue to develop the maximum Amphotericin B-induced PD; the relative effectiveness of alkali metal cations in substituting for K is $K \geq Rb > Cs > Li > Na$. The carrier mechanism for coupled influx of Na and Cl across the mucosal border of gallbladder appears to be functional in the presence of Amphotericin B; in addition, the diffusional influx of chloride is not significantly altered by the antibiotic. The primary action of Amphotericin B which appears to modify rabbit and guinea pig gallbladders from having transmural PD's of less than ± 1 mV to having serosa-positive PD's of 5–30 mV is an increase in the mucosal cell membrane permeability to Na. This permeability change has the effect of partially uncoupling NaCl influx. A rheogenic Na pump mechanism at the basolateral membrane, presumably in operation under control conditions also, may account for the PD.

The gallbladders of rabbit and guinea pig *in vitro* develop no significant spontaneous transepithelial PD under control conditions, perhaps because the active transport mechanism is an electrically neutral NaCl pump (Diamond, 1962*a*) or because Na and Cl enter the cell across the mucosal border by a neutral, coupled process (Frizzell, Dugas & Schultz, 1975). Previous reports by Cremaschi, Henin and Calvi (1971) and Rose and Nahrwold (1976) have indicated that exposure of the mucosal surface of gallbladders from rabbit, guinea pig, tortoise, toad and frog to Amphotericin B results in the development of serosa-positive transepithelial PD's as great as 20 mV. The PD was characterized in the latter report as possibly being the result of an active rheogenic Na

pump at the serosal border, which was presumed also to function in net electrolyte absorption by the tissue. There are several apparent similarities between the electrical properties of rabbit and guinea pig gallbladders exposed to Amphotericin B and the properties under control conditions of intestine, gallbladders of goose, monkey and man, and certain other epithelial tissues. Thus, it was considered important to further characterize in gallbladder epithelium the dependence of the Amphotericin B-induced PD and the rate of fluid absorption on the ionic composition of the bathing solution; this may be helpful in identifying similarities between the ion transport mechanism of rabbit and guinea pig gallbladders and the corresponding properties of other epithelial tissues. The results of some studies on human gallbladder, which develops a spontaneous PD of 8 mV under control conditions, are included for comparison. Because the rates of most active transport processes are highly temperature sensitive, certain experiments performed on rabbit gallbladder by Cremaschi *et al.* (1971) at 27 °C were repeated in the present study at 37 °C.

Rose and Nahrwold (1976) mentioned several possible effects of Amphotericin B on rabbit and guinea pig gallbladders which could account for the induced serosa-positive transmural PD; three possibilities which currently deserve consideration are that Amphotericin B might A) increase the diffusional movement of Cl across the mucosal membrane, B) uncouple the mucosal membrane carrier-mediated NaCl transport mechanism, or C) increase the diffusional movement of Na across the mucosal membrane. In the present work the unidirectional influxes of Na and Cl across the mucosal membrane have been determined; possibility C appears to account for the Amphotericin B-induced PD.

Materials and Methods

Gallbladders from rabbit and guinea pig were removed shortly after sacrifice of the animal by Pentobarbital injection and placed immediately in control bathing solution at 0 °C. Human gallbladders were obtained at the time of cholecystectomy performed for cholelithiasis by members of the Department of Surgery. All patients were symptomatic. The fundus of human gallbladders was used for research and the remainder of the organ was sent to the Department of Pathology for histologic examination. Only results on human tissue having histologically normal mucosa are reported in this paper. Gallbladders were washed with cold Ringer's solution to remove bile. The serosa of human gallbladder was removed by blunt dissection, which causes only minor changes in electrical parameters (Rose, Gelarden & Nahrwold, 1973).

Transepithelial Electrical Measurements

The bathing chamber and apparatus for measuring the transmural electrical potential difference (mucosa to serosa) and short-circuit current (I_{sc}) were similar to those of Schultz and Zalusky (1964). Briefly, 1.13 cm^2 of tissue was held between the halves of a Lucite chamber and exposed on each surface to 15 ml of buffered electrolyte solution at 37 °C. The composition of control Ringer's was (in mM): NaCl, 142; $MgCl_2$, 1.2; $CaCl_2$, 0.9; K_2HPO_4, 0.2; $KHCO_3$, 10; and the initial pH was 7.2. Bicarbonate-free Ringer's contained (in mM): NaCl, 142; $MgCl_2$, 1.2; $CaCl_2$, 0.9; KH_2PO_4, 1.5; and K_2HPO_4, 4.2. Bathing solution was circulated across each surface of the tissue by means of a gas-lift circulating system driven by a water-saturated gas mixture of 95/5 O_2/CO_2 in the case of control Ringer's or 100% O_2 when HCO_3-free Ringer's was used. Na-free and K-free buffers were prepared by substituting Tris or choline for Na and by substituting Na for K. Chloride-free buffer was prepared from Ringer's by replacing Cl with SO_4; osmolarity was maintained by addition of mannitol. Both surfaces of the tissue were bathed by the same media unless otherwise noted.

Tips of Ringer-agar bridges were placed close to the membrane and the PD was measured using a pair of calomel electrodes leading to a high impedance electrometer. Tissue resistance was determined by recording the PD deflection in response to 50 μamp of direct current from an external battery source and correcting for fluid resistance. The sign of transmural PDs refers to the serosal solution.

Oxygen Consumption Measurements

Gallbladders were excised, opened and rinsed free of bile in control bathing solution at 0 °C. Since the $\dot{Q}_{O_2}$ of rabbit ileal mucosa was greatly depressed with the serosal musculature intact (Frizzell, Markscheid-Kaspi & Schultz, 1974), the muscle layers of gallbladder were removed using glass slides. Mucosal samples of 8–15 mg (wet weight) were placed in the test chamber of a YSI Biological Oxygen Monitor (Model 53). Oxygen tension (pO_2) of 3 ml of HCO_3-free bathing solution (containing mucosal sample) was measured with a Clark oxygen electrode whose current output is proportional to pO_2. The pO_2 was recorded on a Beckman Model 1005 recorder. The bathing solution was equilibrated with the atmosphere before the tissue sample and O_2 probe were inserted. The test solution was maintained at 37 °C with the use of a water pump (Lauda Model K-2) and a water jacket.

Na and Cl Influx Measurements

Unidirectional influxes of Na and Cl from the mucosal bathing solution into the epithelium were measured using a technique similar to that of Schultz, Curran, Chez and Fuisz (1967). Tissue samples were mounted in Lucite chambers which exposed the mucosal surface to a test solution of the desired composition; the serosal surface (0.29 cm^2) rested on moistened filter paper. The test solution contained [^{3}H] inulin and either ^{22}Na or ^{36}Cl. After a short exposure, the test solution was withdrawn and the exposed tissue was rinsed briefly with 0 °C isotonic mannitol. The tissue was cut out with a steel punch and extracted in 0.1 N HNO_3 for at least 2 hr. Tissue extracts and aliquots of the test solution were assayed simultaneously for ^{3}H and ^{22}Na or ^{36}Cl. Uptake of ^{22}Na or ^{36}Cl by the tissue across the mucosal surface was calculated after correction of adherent radioactive solution indicated by the inulin space. Influx studies were performed for 45 sec as justified in earlier experiments on rabbit gallbladder epithelium (Frizzell *et al.*, 1975).

Fluid Absorption Measurements

The technique for measuring fluid absorption by whole rabbit gallbladders was similar to the capillary technique previously described by Diamond (1962*b*). Gallbladders were everted on a glass rod, and polyethylene tube (1.2 mm, ID) was tied into the cystic duct. The gallbladder and tube were filled with control bathing solution taking care to eliminate air bubbles. The gallbladder was immersed in a beaker of bathing solution which was maintained at 37 °C and bubbled with 95% O_2–5% CO_2. After the luminal volume reached a steady-state level, the rate of progression of the meniscus along a meter stick laid beside the tube indicated the rate of fluid transport. Addition of the smooth muscle relaxant, Papaverine hydrochloride (5×10^{-4} M, final concentration) to the serosal bathing solution did not increase the apparent rate of fluid absorption, indicating that the meniscus movement is not attributed to changes in muscle tone.

Amphotericin B (40 μg/ml) was added to the mucosal bathing solution in studies on electrical properties and fluid absorption; in oxygen consumption studies, Amphotericin B was present at both tissue surfaces. Statistical significances have been evaluated with paired analyses using the student *t*-test.

Results

Effect of Na, Cl and K on the Amphotericin B-Induced PD

Replacement of Na in the mucosal and serosal bathing solutions by Tris has previously been shown to result in a lower steady value of the Amphotericin B-induced PD in rabbit gallbladder (Cremaschi, *et al.*, 1971; Rose & Nahrwold, 1976). The time course of the decrease in PD was currently investigated by rapidly replacing Na in the mucosal and serosal bathing solutions. In the three rabbit gallbladders tested, the PD approached zero within 5 min following Na removal (Fig. 1). A transient reversal of PD (2–7 mV) was noted in each experiment. Similar results have been observed on the spontaneous PDs of small intestine (Schultz & Zalusky, 1964) and human gallbladder (Rose *et al.*, 1973) following removal of Na. The reversal PD may be due either to a greater permeability of one surface of the cell membrane to Tris or Na than at the opposite cell surface (Schultz & Zalusky, 1964), or to an asymmetry of unstirred layers at the mucosal and serosal cell borders (Barry & Diamond, 1970).

Cremaschi *et al.* (1971) found that in the absence of Cl and HCO_3 in the bathing solution, the Amphotericin B-induced PD at 27 °C was nearly eliminated within 5 min. In the present experiments on three rabbit gallbladders, performed in HCO_3-free buffer at 37 °C, the Amphotericin B-induced PD in the absence of Cl was 53% of the peak value observed (13.2 mV) after control bathing solution (containing Amphotericin B)

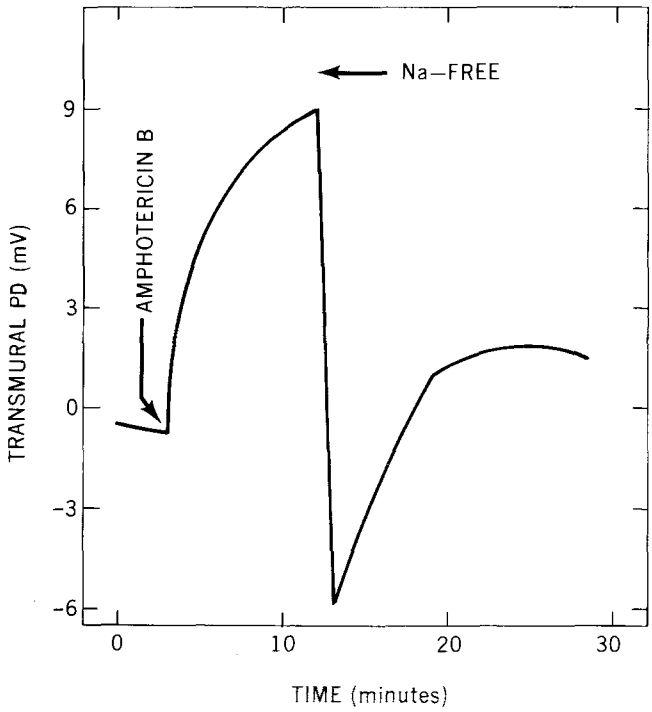

Fig. 1. Effect on the Amphotericin B-induced PD due to eliminating Na (Tris replacement) from the mucosal and serosal bathing solutions of rabbit gallbladder

was added to the chambers (Fig. 2). In four guinea pig gallbladders the PD induced by Amphotericin B in Cl-free buffer was 126% of the peak value (17.6 mV) in control bathing solution. Anaerobic conditions reduced the PD similar to the effect we observed on the spontaneous PD of gallbladders from goose, monkey and man (Rose *et al.*, 1973; Gelarden & Rose, 1974).

Cremaschi *et al.* (1971) reported that substitution of Na for K in the bathing solution results in no significant immediate effect on the Amphotericin B-induced PD in rabbit gallbladder; our preliminary experiments supported this finding. The possibility seemed to exist, however, that diffusion of K from muscle and epithelial cells could have temporarily maintained the extracellular K concentration high enough to meet any requirements of the pump mechanism.

Thus, dependence of the PD on extracellular K was further evaluated on gallbladder mucosa preincubated 25–40 min at 0 °C under anaerobic conditions in K-free, HCO_3-free buffer to lower the tissue K content. The PDs of rabbit and guinea pig gallbladder bathed in K-free, HCO_3-

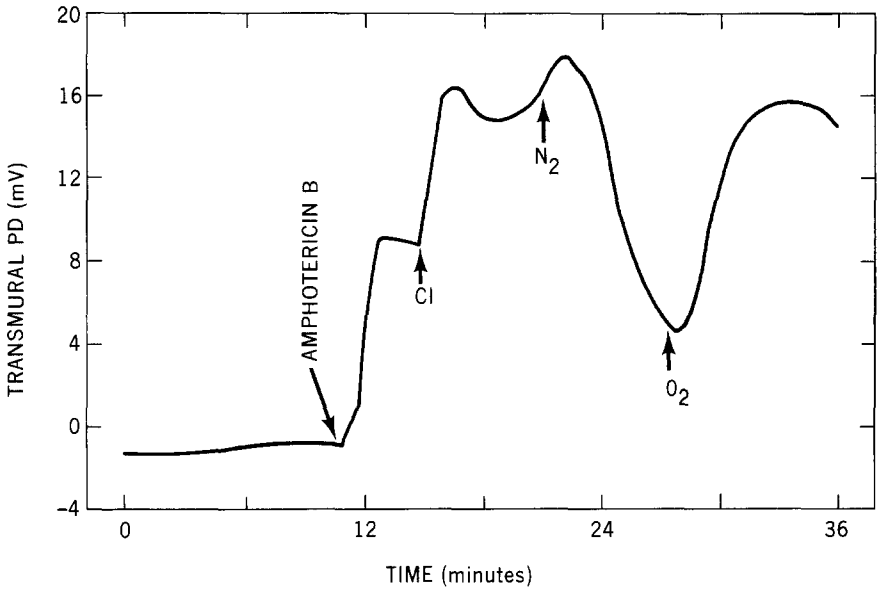

Fig. 2. Amphotericin B-induced PD in rabbit gallbladder bathed in Cl-free buffer. Control Ringer's ([Cl] = 145 meq/liter) bathed the tissue at time = 15 min. Anaerobic conditions lasted from 21 to 27 min

free buffer are given in Fig. 3 and Table 1. Addition of Amphotericin B resulted in an increased PD in each tissue. KCl (10 mM) was then added either first to the mucosal and then to the serosal bathing solution, or in the reverse sequence. The gallbladder of both species had a greater response to mucosal K than to serosal K. Raising the mucosal bathing solution K concentration of rabbit gallbladder to 20, 30 and 40 meq/liter resulted in additional slight increases in the PD (1.5 mV/10 meq/liter increment) and raising the serosal K concentration resulted in slight decreases in the PD (0.7 mV/10 meq/liter). These minor PD changes may represent diffusion potentials established by transepithelial K gradients since rabbit gallbladders are more permeable to K than to Na (Diamond & Harrison, 1966).

The possibility that a K diffusion potential accounts for the relatively large PD induced by 10 mM KCl was evaluated in guinea pig gallbladders which were preincubated 30–40 min at 0 °C in K-free buffer. The intracellular concentration of K in the muscle-free mucosa, as determined by standard chemical techniques (Schultz, Fuisz & Curran, 1966), was less than 20 meq/liter, as compared to 85 meq/liter under control conditions (Frizzell *et al.*, 1975). When bathed anaerobically at 37 °C in K-free HCO_3-free buffer, the PD was near zero (Fig. 4). Addition of Amphotericin B resulted in a PD of 1–3 mV; addition of KCl (10 mM) to the

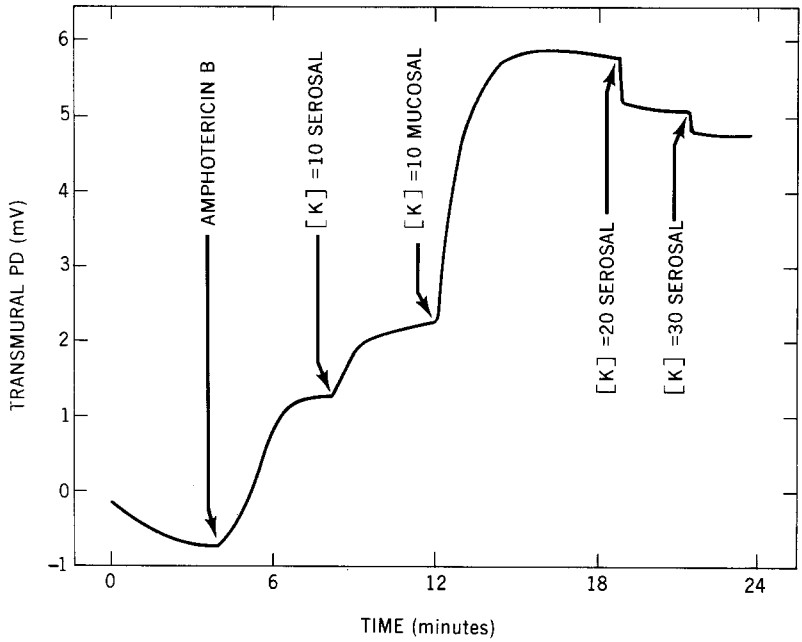

Fig. 3. Rabbit gallbladders were preincubated 30 min at 0 °C in K-free, HCO_3-free buffer bubbled with 100% N_2. Gallbladders were then bathed in the same buffer at 37 °C with 100% O_2 while the transmural PD was measured. Amphotericin B (40 μg/ml) was added to the mucosal solution. Subsequent K concentrations in the serosal and mucosal solutions are given in meq/liter

Table 1. Dependence of Amphotericin B-induced PD on mucosal and serosal K

	K-free		Amphotericin B		Mucosal K		Serosal K	
	PD	*R*	PD	*R*	PD	*R*	PD	*R*
Rabbit (4)	−0.1±0.1	17	1.2±0.1	16	7.9±2.0	15	8.7±1.5	14
Guinea pig (4)	−0.8±0.4	60	4.0±1.4	36	7.1±2.0	31	8.0±1.6	30
	K-free		Amphotericin B		Serosal K		Mucosal K	
	PD	*R*	PD	*R*	PD	*R*	PD	*R*
Rabbit (4)	−0.5±0.1	19	1.3±0.3	18	2.5±0.5	17	6.0±1.1	16
Guinea pig (6)	0.5±0.2	81	4.1±1.1	55	6.3±1.2	42	9.6±2.2	36

Gallbladders were preincubated 25–40 min at 0 °C in K-free, HCO_3-free buffer bubbled with 100% N_2. They were then mounted in chambers and bathed aerobically in the same buffer at 37 °C until a steady PD was measured. Amphotericin B (40 μg/ml) was added to the mucosal solution; as steady PD's were achieved, K (10 mM, final conc.) was added first to the mucosal solution and then to the serosal solution (upper), or in the reverse sequence (lower). Values for PD are mean ±SE in mV and for resistance (*R*) are in Ω cm^2. Number of observations in parentheses.

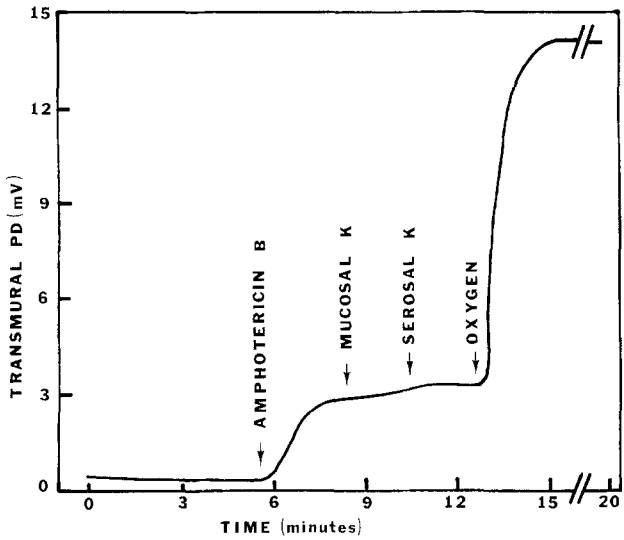

Fig. 4. The transmural PD of a guinea pig gallbladder preincubated 30 min at 0 °C in K-free, HCO_3-free buffer and bathed initially in the same buffer at 37 °C with 100% N_2. Amphotericin B (40 μg/ml) and KCl (10 mM) were added as indicated under anaerobic conditions. The major increase in PD began within 10 sec after bubbling the bathing media with 100% O_2

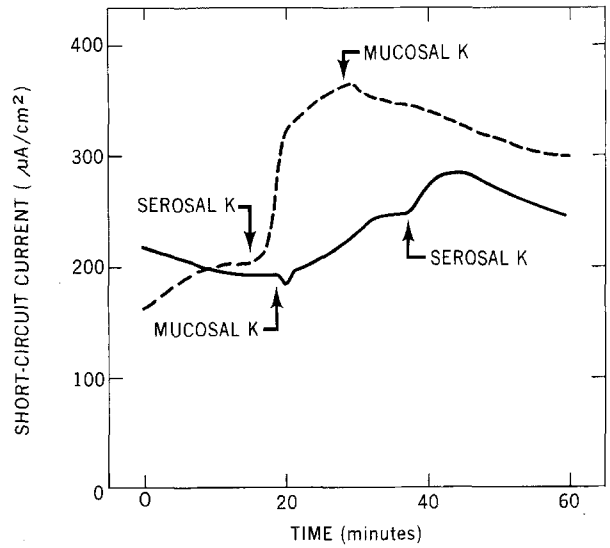

Fig. 5. Short-circuit current in paired samples of human gallbladder preincubated 30 min at 0 °C in K-free buffer and bathed in the same buffer at 37 °C. Mucosal or serosal addition of K produced a final K concentration of 10 meq/liter

mucosal or serosal bathing solution had little effect on the PD; when O_2 was made available to the tissue a prompt increase in PD to 13–15 mV resulted. Thus, the major component of PD appears to be closely linked to cellular metabolism but not to a transmembrane K gradient.

For purposes of comparison, four samples of human gallbladder, which develops a spontaneous serosa-positive PD in the absence of Amphotericin B, were investigated for an effect of K on the electrical properties. The tissue was preincubated and mounted aerobically as described above for rabbit and guinea pig gallbladder. Of the maximum PD and short-circuit current (212 $\mu A/cm^2$) observed when K was present in both bathing solutions, a mean of 11% was dependent on mucosal K and 28% was dependent on serosal K (Fig. 5).

Effect of Rb, Cs and Li on the Amphotericin B-Induced PD

The frog skin also requires K in the bathing solution to have normal electrical properties and electrolyte absorption (Koefoed-Johnsen & Ussing, 1958); Rb partially substitutes for K (Huf & Wills, 1951), perhaps because it also has a strong affinity for the transport system (Curran & Cereijido, 1965). The effectiveness of Rb, Li and Cs in maintaining the transport properties of gallbladder mucosa has not previously been determined.

Rabbit gallbladders were preincubated as described above to reduce the tissue K content. Following a brief control period in K-free, HCO_3-free buffer, sequential additions were made of Amphotericin B to the mucosal solution, a substitute cation (10 meq/liter) to the mucosal and serosal solutions, and finally K was added to both bathing solutions to develop the maximum Amphotericin B-induced PD under these conditions. As seen in Table 2, Li was relatively ineffective, Cs was somewhat effective, and Rb was nearly as effective as K in developing the PD.

Ionic Stimulation of Oxygen Consumption

Martin and Diamond (1966) reported that O_2 consumption by whole rabbit gallbladders is reduced upon removal of Na from the bathing solution. Because their results could have been influenced by the muscle layers present in their preparation either consuming O_2 or limiting availability of O_2 to the mucosa (Frizzell *et al.*, 1974), these experiments

Table 2. Effect of Cs, Rb and Li on the Amphotericin B-induced PD

	Initial	Amphotericin B	Cation addition	K addition
Li	−0.1	1.2±0.1	1.7±0.1	4.8±0.7
Cs	0.5	0.9±0.2	2.3±0.5	4.3±1.1
Rb	−0.6	1.5±0.2	3.7±0.1	3.8±0.7

Values are means ±SE of PD in three rabbit gallbladders. Gallbladders were preincubated 30 min at 0 °C in K-free, HCO_3-free buffer. The initial PD was determined at 37 °C in the same buffer. Amphotericin B was added to the mucosal solution. The replacement cation was added to the mucosal and serosal solutions. K was added to both solutions at a final concentration of 10 meq/liter.

were repeated in rabbit gallbladders stripped of musculature. As seen in Table 3, O_2 consumption was reduced in the absence of Na but not in the absence of Cl.

Samples of mucosa from five rabbit gallbladders were preincubated 50 min in K-free buffer at 0 °C to reduce the tissue K content. O_2 consumption was then determined with the tissue incubated in K-free buffer; subsequent addition of K to attain the K concentration in control Ringer's increased O_2 consumption approximately threefold. In tissue samples similarly preincubated, addition of Rb (10 meq/liter) resulted in a 240% increase in O_2 consumption.

K Stimulation of Fluid Absorption

Because some models of transepithelial electrolyte transport include a K-dependence of the pump mechanism, and since K was necessary for rabbit gallbladder to develop the maximum Amphotericin B-induced PD and O_2 consumption, it was considered important to confirm the earlier report of Frederikson and Leyssac (1969) that K must be present also in order to have maximum fluid absorption by the tissue. Absorption was evaluated with the capillary technique by initially filling four rabbit gallbladders and the associated tubing with K-free buffer. When suspended in the same solution at 37 °C fluid absorption was 75±7 µl/hr. Addition of K (10 meq/liter) to the serosal bathing solution resulted in increased absorption (167±9 µl/hr; $P<0.01$; Fig. 6). The effect of K added to the mucosal solution was evaluated in six everted gallbladders with the same technique. The initial rate of absorption was 47±7 µl/hr and after addition of K it increased to 77±8 µl/hr ($P<0.01$). Thus,

Table 3. Effect of Na, Cl, K and Rb on O_2 consumption of rabbit gallbladder mucosa

	Q_{O_2}	n	P
Na-free buffer	9.4± 1.4	3	<0.05
Control Ringer's	17.7± 1.6		
Cl-free buffer	15.9± 1.6	6	>0.2
Control Ringer's	16.6± 1.5		
K-free buffer	14.4± 3.5	5	<0.01
Control Ringer's	43.0±15.6		
K-free buffer	8.4± 1.1	7	<0.01
Rb addition	29.0± 5.2		

Values represent Q_{O_2} in $\mu l/O_2/mg$ hr from 3–5 min of O_2 consumption determination on gallbladder mucosa. Rb was added to K-free buffer at a final concentration of 10 meq/liter. All experiments were performed in the absence of Amphotericin B.

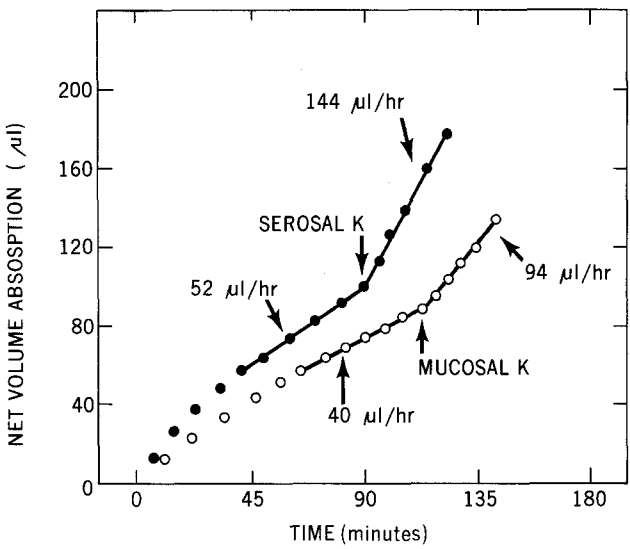

Fig. 6. Net volume absorption in two rabbit gallbladders exposed initially on both surfaces to K-free buffer. K was added at the times indicated to the mucosal or serosal solution at a final concentration of 10 meq/liter. Rates of absorption have been calculated from the slope of the linear plot and are expressed in µl/hr

it may be concluded that fluid transport by rabbit gallbladder is stimulated by K at either the mucosal or the serosal surface.

Effect of Amphotericin-B on Na and Cl Influx

The possibility has previously been discussed (Frizzell *et al.*, 1975) that the spontaneous PD of gallbladder and intestinal mucosa might

Table 4. Unidirectional influxes of Na and Cl in absence of Amphotericin B

	Na influx			Cl influx		
	Control	Cl-free	*P*	Control	Na-free	*P*
Rabbit	37.3±5.4 (7)	25.5±2.4 (7)	<0.01	25.2±3.0 (11)	21.0±2.7 (12)	<0.05
Guinea pig	9.6±1.4 (15)	7.3±1.7 (15)	<0.01	11.6±0.7 (12)	8.2±0.7 (15)	<0.01[a]

Number of determinations are given in parentheses. Values are μmoles/cm² hr ±SE. Statistical evaluations were performed using paired analyses.

[a] Studies were performed with Cl=50 meq/liter in the bathing solution to reduce diffusional influx of Cl.

be influenced by the mechanism of sodium chloride entry across the mucosal cell membrane into absorptive cells. Thus, coupled electrically neutral entry of NaCl in series with a rheogenic Na pump at the serosal membrane could provide for equal rates of Na and Cl absorption without the presence of a serosa-positive transmural PD. The possibility exists that Amphotericin-B could elicit a PD by effectively uncoupling Na and Cl influx. This might occur either directly by altering the diffusional entry of Na or of Cl, or by affecting the NaCl carrier mechanism. To evaluate these three possibilities, influxes of Na and Cl were measured in gallbladders of rabbit and guinea pig.

In the absence of Amphotericin B, Na influx into rabbit gallbladder was reduced by 41% when a Cl-free test solution was used (Table 4). Also, Cl influx was reduced when Na-free test media was used. Thus, our information supports the report of Frizzell *et al.* (1975) that a transport mechanism at the mucosal border mediates coupled entry of NaCl. Since Na influx into guinea pig gallbladder mucosa was reduced in Cl-free media, and Cl influx was reduced in Na-free media (Table 4), it appears that NaCl influx is coupled in this tissue also.

In rabbit gallbladder exposed to Amphotericin B, Na influx was reduced in the absence of mucosal Cl (control: 35.2±3.2; test: 30.2±0.6 μeq/cm²hr, $P<0.05$); thus, coupling between Na and Cl influx is demonstrable in the presence of Amphotericin B although the extent of coupling might be reduced. Chloride influx into rabbit and guinea pig gallbladder was not significantly different following tissue exposure to Amphotericin B (Table 5) but Na influx was increased by 41–46% in each tissue. Thus, the primary immediate action of Amphotericin

Table 5. Unidirectional influxes of Na and Cl in presence of Amphotericin B

	Na influx			Cl influx		
	Control	Ampho-tericin B	*P*	Control	Ampho-tericin B	*P*
Rabbit	45.9±4.4 (6)	64.6±9.2 (6)	<0.01	17.7±3.4 (8)	21.4±6.1 (6)	>0.2
Guinea pig	14.9±1.0 (13)	21.8±1.0 (11)	<0.01	14.2±1.2 (13)	12.8±1.3 (13)	>0.2

Number of determinations is given in parentheses. Values are μmoles/cm^2 hr ±SE. Statistical evaluations were performed using paired analyses.

B on gallbladder mucosa appears to be an increase in mucosal membrane permeability to Na.

Discussion

The simultaneous absorption of Na and Cl in the absence of a significant transepithelial PD is a well-documented feature of electrolyte transport by rabbit gallbladders. In early studies NaCl absorption was attributed to an electrically neutral NaCl transport mechanism at the serosal cell membrane since, among other characteristics, removal of either Na or Cl from the bathing solution eliminated fluid absorption without affecting the transepithelial PD (comprehensive reviews by Diamond, 1968 and Dietschy, 1966).

An alternate concept that Na extrusion at the serosal membrane is coupled to cellular K uptake, but is not coupled to Cl transport, is supported by the observation that the activity of a ouabain-sensitive, [Na+K]-ATPase in rabbit gallbladder is correlated with the rate of transepithelial Na transport (van Os & Slegers, 1971). It has been suggested that the active transport mechanism in those gallbladders which develop spontaneous serosa-positive transepithelial PDs (goose, monkey and man) is a rheogenic (or electrogenic) Na pump (Rose *et al.*, 1973; Gelarden & Rose, 1974). We have suggested also (Rose & Nahrwold, 1976) that the active transport mechanism in rabbit and guinea pig gallbladders exposed to Amphotericin B is a rheogenic Na pump. Reuss and Finn (1975*a*, *b*) discussed the possibility that a rheogenic pump contributes to the electrical potential profile of *Necturus* gallbladder, but the available information did not allow firm conclusions to be drawn.

Frizzell *et al.* (1975) have presented convincing evidence that NaCl influx across the mucosal membrane of rabbit gallbladder proceeds by a coupled carrier-mediated mechanism which operates independent of cellular metabolic energy supplies. Since that study did not have direct bearing on the possibility of a coupled NaCl pump at the serosal membrane, the transport model they presented would be consistent with either concept of the pump. Interpretation of the present experiments might lend additional support to one of the alternate views of the active transport mechanism.

Effect of Amphotericin B on Gallbladder

Several possible actions of Amphotericin B on rabbit and guinea pig gallbladder mucosa which might account for the induced serosa-positive PD have previously been discussed (Rose & Nahrwold, 1976). Three of the most promising of these possibilities are evaluated in the present study; each has to do with an alteration of Na and/or Cl flux across the mucosal membrane.

A) Amphotericin B may increase the mucosal membrane permeability to Cl. Since Cl is at a higher electrochemical potential in cells of rabbit gallbladder mucosa than in the bathing fluid (Frizzell *et al.*, 1975), an increase in the mucosal membrane permeability to Cl could induce a serosa-positive PD by resulting in a Cl diffusion potential between the cell interior and the mucosal bathing fluid. It now seems unlikely that this is the origin of the PD since no increase in mucosal permeability to Cl was detected. In addition, a substantial decrease (or reversal) of the electrochemical potential difference of Cl across the mucosal membrane due to raising the mucosal solution Cl concentration (Fig. 2) resulted in only a 25% decrease of the PD in guinea pig gallbladders and a 90% increase in the PD of rabbit gallbladders. Also, the immediate dependence of the PD on aerobic conditions and 10 meq/liter K in the bathing media is not characteristic of a diffusion potential.

B) Amphotericin B may uncouple the carrier-mediated NaCl influx mechanism. A transmural PD could then result from an existing rheogenic Na pump since Cl would not be simultaneously transported by an obligatory mechanism. The present results suggest that this possibility may account for part of the observed PD; coupling between Na and Cl influx was still evident in the presence of the antibiotic, but the extent of coupling may have been reduced.

C) Amphotericin B may increase mucosal membrane permeability to Na. This could result in a transmural PD since the fraction of net Na flux which is not associated with a coupled NaCl influx mechanism may be balanced by net Cl flux driven by a serosa-positive PD. Influx studies on both rabbit and guinea pig gallbladders indicate a substantial increase in mucosal membrane permeability to Na.

This action of the antibiotic on gallbladder mucosa is similar to the proposed effect on canine jejunum (Chen, Guerrant, Rohde & Casper, 1973) and toad bladder (Lichtenstein & Leaf, 1965; Bentley, 1968) which respond with an increase in PD, short-circuit current and net Na transport.

The PD induced by Amphotericin B in gallbladder mucosa was not dependent on the normal concentration gradients between the intracellular and extracellular fluids of either K (Fig. 4) or Na (Rose & Nahrwold, 1976) but was immediately dependent on O_2. Thus, it appears that the PD cannot be attributed to cation diffusion potentials. The induced PD in rabbit and guinea pig gallbladders may be somewhat analogous to the increased PD in mammalian ileum following addition of actively transported sugars to the mucosal bathing solution (Schultz & Zalusky, 1964); in both cases additional Na from the mucosal solution is brought into the cell membrane without an equal forced entry of Cl. As Na is actively transported toward the serosal solution, a transmural PD develops which accounts for part of the net Cl absorption that presumably takes place through extracellular pathways (Wright, Barry & Diamond, 1971; Frömter, 1972; Frizzell *et al.*, 1975). It appears that under these conditions the Na pump in gallbladder is rheogenic, as is thought to be the case under control conditions in both the toad bladder (Finn, 1974) and mammalian ileum (Rose & Schultz, 1971; Frizzell & Schultz, 1972). Since no immediate action of Amphotericin B is postulated on the pump itself, it follows that the Na pump in gallbladder mucosa may be rheogenic under control conditions also, as previously suggested (Rose & Nahrwold, 1976).

Our earlier report indicated that Amphotericin B causes an increase in tissue conductance measured electrically and in transmural Cl diffusion, but no change in Na diffusion. These results may be reconciled with the present findings by recognizing that the first measurements of transmural fluxes were made 10 min after addition of Amphotericin B and the effect becomes increasingly evident with time. The present measurements on influx were complete within 4 min after addition of Amphotericin B. Thus, it appears that the antibiotic has an immediate

effect on mucosal permeability which increases entry of Na into the cell, net Na transport, the PD and O_2 consumption in addition to an undefined delayed effect which increases tissue conductance and reduces net Na transport, the PD and O_2 consumption. Mendoza, Handler and Orloff (1967) also detected a temporal dissociation between the effect of Amphotericin B on the permeability of toad bladder to urea and water and the effect on Na transport.

The Role of Ions in Fluid Absorption

It is well documented that Na is required in the bathing solutions to have a maximum rate of fluid absorption by rabbit gallbladder and a normal transepithelial PD in gallbladders of goose, monkey and man. Thus, active transport of Na from mucosa to serosa is likely to be a primary event in electrolyte absorption by these tissues. In rabbit gallbladder mucosa, removal of Na from the bathing solution eliminated the Amphotericin B-induced PD. Removal of Na reduced O_2 consumption in whole rabbit gallbladders by 45% (Martin & Diamond, 1966) and in rabbit gallbladder mucosa by 47% (Table 3). The conclusion drawn from both studies is that a substantial fraction of O_2 consumption by gallbladder mucosa supports active Na transport. It appears that Na is actively extruded from the cells of gallbladder mucosa, similar to intestinal mucosa, and the resulting transmembrane electrochemical gradient of Na may be used to bring Cl into the cell on the coupled NaCl transport mechanism (Frizzell *et al.*, 1975).

Removal of Cl from the bathing solution reduces net absorption of Na and water by gallbladder epithelium, presumably due to the consequent reduction of Na influx across the mucosal membrane on the NaCl carrier mechanism. Martin and Diamond (1966) reported that $\dot{Q}_{O_2}$ of rabbit gallbladder was reduced by 7–9% when the mucosal bathing solution Cl was replaced by isethionate or SO_4; serosal replacement of Cl by SO_4 was without effect on $\dot{Q}_{O_2}$. In the present preparation, replacement of Cl by SO_4 at both surfaces had no significant effect on $\dot{Q}_{O_2}$. Our results are difficult to reconcile with the model of a coupled NaCl pump at the serosal membrane, since elimination of Cl would be expected to reduce O_2 consumption associated with active NaCl transport to the same extent as elimination of Na. One can speculate that this finding fits with the model of coupled NaCl influx if this stripped preparation has significant entry of sodium occurring across either the brush border membrane independent of chloride, or across the basolateral membrane,

which may become leaky due to the stripping procedure. In the latter case, sodium would be recycling across the serosal membrane through the pump, resulting in high O_2 consumption, but little net volume absorption.

A role of extracellular K in stimulating net fluid absorption by rabbit gallbladder was first indicated by the work of Frederiksen and Leyssac (1969) and is supported by the present findings (Figs. 3, 5 and 6). The rapid stimulation of O_2 consumption, fluid absorption and the Amphotericin B-induced PD by K applied extracellularly are consistent with a direct role of this ion at the pump site. The observation that mucosal K was at least as effective as serosal K in stimulating the PD in human and rabbit gallbladder and in stimulating fluid absorption in rabbit gallbladder is not predicted by a model of Na-K exchange at the serosal membrane; this also differs from the observation in rabbit ileum where only serosal K was effective in producing the spontaneous transepithelial PD (Rose, 1976). However, due to the leaky nature of the tight junctions in rabbit gallbladder to ion diffusion, and due to the apparent location of the ATPase activity along the lateral intercellular space, and the bulk flow of fluid through the lateral intercellular space from the pump site toward the serosal solution (Kaye, Wheeler, Whitlock & Lane, 1966), K might approach the pump more easily from the mucosal solution than from the serosal solution. Frömter (1972) has discussed in detail the possibility that the lateral spaces make significant contributions to the total transepithelial resistance of *Necturus* gallbladder. The effectiveness of alkali metal cations in supporting the PD by substituting for K (Rb > Cs > Li > Na) was the same order as Lindley and Hoshiko (1964) found in K substitution studies at the inside border of frog skin.

The specific role of K at the pump site has not been determined. The Koefoed-Johnsen and Ussing (1958) model of electrolyte transport by amphibian skin proposes electrically neutral exchange of intracellular Na for extracellular K. The indication that the Na pump in gallbladder mucosa of several species is rheogenic is incompatible with this concept. Coupled transport of 3 Na for 2 K as proposed for red cell membranes (Sen & Post, 1964) would account for K stimulation of net Na transport and allow for a rheogenic pump. It has not yet been determined, however, that the stimulatory effect of K is exerted as this cation is transported into the cell, or alternatively, if transport of K and its stimulatory effect on Na transport are independent events.

The interaction between active transport of Na and K in other epithelial cell types is also unclear. In rabbit ileum, extracellular K stimulated

active Na transport from the cells, and cellular accumulation of K was stimulated by the presence of Na in the bathing solution (Rose, 1976); however, the presence of mucosal Na did not stimulate uptake of K across the serosal border (Nellans & Schultz, 1976) and thus, tightly coupled Na-K exchange appears unlikely. In toad urinary bladder, the dependence of normal short-circuit current on serosal K was attributed to this cation increasing the entry of Na into the cell across the mucosal border through an alteration of permeability properties (Essig & Leaf, 1963); however, Robinson and Macknight (1976), favored an intracellular requirement for K. Thus, additional studies on the role of K are needed, and the possibility must specifically be considered that in gallbladder epithelium, extracellular K stimulates the ATPase involved in Na transport independent of its own transport into the cell.

Frizzell *et al.* (1975) have used ion flux measurements and theoretical considerations to evaluate the possibility that tissue conductance of rabbit gallbladder is sufficiently high to prevent development of a transmural PD greater than the ± 1 mV observed in studies at several laboratories. They calculated that the PD would have to be greater than ± 10 mV for pure electrical coupling to account for passive movement of either Na or Cl to the active movement of the other ion. They concluded that the low observed PD cannot be due to the low resistance shunt alone, and suggested that the coupling between Na and Cl fluxes across the mucosal membrane obviates the need of a substantial transmural PD to drive absorption of the passively transported ion (Cl). The finding that significant PDs develop in rabbit and guinea pig gallbladders exposed to Amphotericin B, in spite of concomitant increases in tissue conductance, gives empirical support to the concept that gallbladder conductance is not so great as to prevent development of a significant transepithelial PD.

The information currently available supports a mechanism of electrolyte transport by rabbit gallbladder epithelium which differs in certain important respects from the earlier model (Diamond, 1962*a*). The active transport mechanism appears to be a rheogenic pump which extrudes Na from the cell. The well-documented co-transport of Na and Cl across the tissue in the absence of a transmural electrical gradient is apparently not due to a property of the pump itself, but is mediated by an energy-independent carrier mechanism within the mucosal membrane. The coupling of Na and Cl transport at this site under control conditions prevents the rheogenic Na transport mechanism from developing a transepithelial PD (Fig. 7). Following exposure of the tissue to Amphotericin B, the

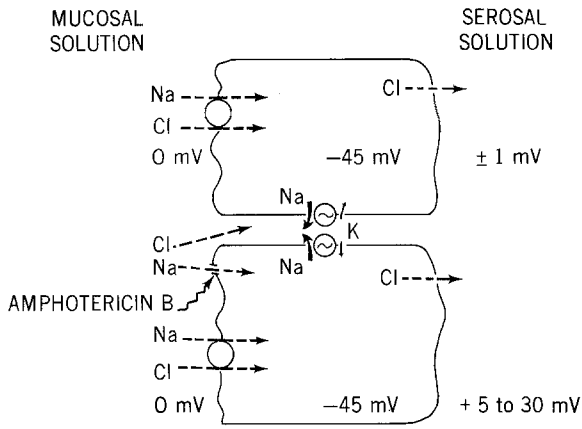

Fig. 7. Working models for Na and Cl transport by rabbit and guinea pig gallbladder under control conditions (upper) and upon exposure to Amphotericin B (lower). *See text* for discussion

diffusional flux of Na from the mucosal solution into the cell becomes significant and coupling between transepithelial Na and Cl transport may be due in part to the neutral entry mechanism at the mucosal membrane and in part to the serosa-positive PD.

The proposed models of transport by gallbladder (Fig. 7) are incomplete in several important respects and the following possibilities should be considered: a) separate active transport mechanisms may be involved in transepithelial electrolyte transport and maintenance of the cellular volume and ionic composition as previously proposed for rabbit gallbladder (Martin & Diamond, 1966), urinary bladder (Macknight, Civan & Leaf, 1975), and ileum (Nellans & Schultz, 1976); b) in addition to the coupled Na-Cl entry observed in rabbit gallbladders, those gallbladders which develop spontaneous transepithelial PDs may also have Cl-independent pathways for Na entry; and c) Cl exit from the transporting cells may be diffusional and brought about, in part, by the electrical potential difference across the serosal membrane.

References

Barry, P.H., Diamond, J.M. 1970. Junction potentials, electrode standard potentials and other problems in interpreting electrical properties of membranes. *J. Membrane. Biol.* **3**:93

Bentley, P. 1968. Action of Amphotericin B on the toad bladder: Evidence for sodium transport along two pathways. *J. Physiol. (London)* **196**:703

Chen, L.C., Guerrant, R.L., Rohde, J.E., Casper, A.G.T. 1973. Effect of Amphotericin B on sodium and water movement across normal and cholera toxin-challenged canine jejunum. *Gastroenterology* **65**:252

Cremaschi, D., Henin, S., Calvi, M. 1971. Transepithelial potential difference induced by Amphotericin B and NaCl-$NaHCO_3$ pump localization in gallbladder. *Arch. Int. Physiol. Biochim.* **79**:889

Curran, P.F., Cereijido, M. 1965. K fluxes in frog skin. *J. Gen. Physiol.* **48**:1011

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

Diamond, J.M. 1962*b*. The mechanism of water transport by the gallbladder. *J. Physiol. (London)* **161**:503

Diamond, J.M. 1968. Transport mechanisms in the gallbladder. *In*: Handbook of Physiology: Alimentary Canal, Vol. 5. p. 2451. American Physiological Society, Washington

Diamond, J.M., Harrison, S.C. 1966. The effect of membrane fixed charges on diffusion potentials and streaming potentials. *J. Physiol. (London)* **183**:37

Dietschy, J.M. 1966. Recent developments in solute and water transport across the gall bladder epithelium. *Gastroenterology* **47**:395

Essig, A., Leaf, A. 1963. The role of potassium in active transport of sodium by the toad bladder. *J. Gen. Physiol.* **46**:505

Finn, A.L. 1974. Transepithelial potential difference in toad urinary bladder is not due to ionic diffusion. *Nature (London)* **250**:495

Frederiksen, O., Leyssac, P.P. 1969. Transcellular transport of isosmotic volumes by the rabbit gallbladder in vitro. *J. Physiol. (London)* **201**:201

Frizzell, R.A., Dugas, M.C., Schultz, S.G. 1975. Sodium chloride transport by rabbit gallbladder: Direct evidence for a coupled NaCl influx process. *J. Gen. Physiol.* **65**:769

Frizzell, R.A., Markscheid-Kaspi, L., Schultz, S.G. 1974. Oxidative metabolism of rabbit ileal mucosa. *Am. J. Physiol.* **226**:1142

Frizzell, R.A., Schultz, S.G. 1972. Ionic conductances of extracellular shunt pathways in rabbit ileum: Influence of shunt on transmural sodium transport and electrical potential differences. *J. Gen. Physiol.* **59**:318

Frömter, E. 1972. The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**:259

Gelarden, R.T., Rose, R.C. 1974. Electrical properties and diffusion potentials in the gallbladder of man, monkey, dog, goose and rabbit. *J. Membrane Biol.* **19**:37

Huf, E.G., Wills, J. 1951. Influence of some inorganic cations on active salt and water uptake by isolated frog skin. *Am. J. Physiol.* **167**:255

Kaye, G.I., Wheeler, H.O., Whitlock, R.T., Lane, N. 1966. Fluid transport in the rabbit gallbladder. *J. Cell Biol.* **30**:237

Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298

Lichtenstein, N.S., Leaf, A. 1965. Effect of Amphotericin B on the permeability of the toad bladder. *J. Clin. Invest.* **44**:1328

Lindley, B.D., Hoshiko, T. 1964. The effects of alkali metal cations and common anions on the frog skin potential. *J. Gen. Physiol.* **47**:749

Macknight, A.D.C., Civan, M.M., Leaf, A. 1975. Some effects of ouabain on cellular ions and water in epithelial cells of toad urinary bladder. *J. Membrane Biol.* **20**:387

Martin, D.W., Diamond, J.M. 1966. Energetics of coupled active transport of sodium and chloride. *J. Gen. Physiol.* **50**:295

Mendoza, S.A., Handler, J.S., Orloff, J. 1967. Effect of Amphotericin B on permeability and short-circuit current in toad bladder. *Am. J. Physiol.* **213**:1263

Nellans, H.N., Schultz, S.G. 1976. Relations among transepithelial sodium transport, potassium exchange, and cell volume in rabbit ileum. *J. Gen. Physiol.* **68**:441

Reuss, L., Finn, A.L. 1975*a*. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. I. Circuit analysis and steady-state effects of mucosal solution ionic substitutions. *J. Membrane Biol.* **25**:115

Reuss, L., Finn, A.L. 1975*b*. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. II. Ionic permeability of the apical cell membrane. *J. Membrane Biol.* **25**:141

Robinson, B.A., Macknight, A.D.C. 1976. Relationships between serosal medium potassium concentration and sodium transport in toad urinary bladder. II. Effects of different medium potassium concentrations on epithelial cell composition. *J. Membrane Biol.* **26**:239

Rose, R.C. 1976. Transport of sodium and potassium in intestinal epithelial cells. *Life Sci.* **18**:1229

Rose, R.C., Gelarden, R.T., Nahrwold, D.L. 1973. Electrical properties of isolated human gallbladder. *Am. J. Physiol.* **224**:1320

Rose, R.C., Nahrwold, D.L. 1976. Electrolyte transport by gallbladders of rabbit and guinea pig: Effect of Amphotericin B and evidence of rheogenic Na transport. *J. Membrane Biol.* **29**:1

Rose, R.C., Schultz, S.G. 1971. Studies on the electrical potential profile across rabbit ileum: Effects of sugars and amino acids on transmural and transmucosal electrical potential differences. *J. Gen. Physiol.* **57**:639

Schultz, S.G., Curran, P.F., Chez, R.A., Fuisz, R.E. 1967. Alanine and sodium fluxes across the mucosal border of rabbit ileum. *J. Gen. Physiol.* **50**:1241

Schultz, S.G., Fuisz, R.E., Curran, P.F. 1966. Amino acid and sugar transport in rabbit ileum. *J. Gen. Physiol.* **49**:849

Schultz, S.G., Zalusky, R. 1964. Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**:567

Sen, A.K., Post, R.L. 1964. Stoichiometry and localization of adenosine triphosphate-dependent sodium and potassium transport in the erythrocyte. *J. Biol. Chem.* **239**:345

van Os, C.H., Slegers, J.F.G. 1971. Correlation between (Na^+-K^+) activated ATPase activities and the rate of isotonic fluid transport of gallbladder epithelium. *Biochim. Biophys. Acta* **241**:89

Wright, E.M., Barry, P.H., Diamond, J.M. 1971. The mechanism of cation permeation in rabbit gallbladder; conductances, the current-voltage relation, the concentration dependence on anion-cation discrimination, and the calcium competition effect. *J. Membrane Biol.* **4**:331