J. Membrane Biol. 29, 1–22 (1976)

Electrolyte Transport by Gallbladders of Rabbit and Guinea Pig: Effect of Amphotericin B and Evidence of Rheogenic Na Transport

Richard C. Rose and David L. Nahrwold

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

Received 13 November 1975; revised 17 February 1976

Summary. Ion transport and electrical properties of rabbit and guinea pig gallbladders were investigated to gain further information about the active transport mechanism that mediates fluid absorption. The intracellular and transepithelial electrical potentials were measured simultaneously using the microelectrode technique. Exposure of the mucosal surface to Amphotericin B resulted in the prompt development of a serosa-positive electrical potential difference (*PD*) which could not be attributed to an alteration in ion diffusion potentials across either the cell membrane or across the tight junction. Because the Amphotericin B-induced *PD* was immediately dependent on warm temperatures and O_2, and was independent of NA and K concentration gradients across the cell membrane, it is suggested that active ion transport is directly responsible for the *PD*. Since the *PD* was abolished in the absence of Na in the bathing solutions, a rheogenic Na pump is postulated; this pump also appears to be operative in tissue not exposed to Amphotericin B. The specific tissue properties altered by Amphotericin B to produce a serosa-positive *PD* remain incompletely defined. The results of the present study indicate that ion transport by rabbit gallbladder *in vitro* is a consequence of a rheogenic active Na transport mechanism at the basolateral membranes which, in conjunction with a coupled NaCl influx process at the mucosal border, ultimately results in absorption of NaCl and water.

A model of electrolyte transport by gallbladder epithelium has been proposed primarily from the results of experiments on tissue from fish, guinea pig, and rabbit (Diamond, 1962*a*; Wheeler, 1963; Diamond, 1964). This model features coupled active transport of Na and Cl such that operation of the transport mechanism itself is electrically neutral. A later report attributed the small (-1.4 mV[1], serosa-negative) transmural *PD* frequently observed across rabbit gallbladder to an existing electrolyte concentration gradient across the cation-selective tight junction subsequent to NaCl absorption (Machen & Diamond, 1969). The recent

Address Correspondence to: Richard C. Rose, Ph.D., Department of Physiology, The Milton S. Hershey Medical Center, 500 University Drive, Hershey, Pennsylvania 17033.

1 The sign of the transmural *PD* refers to the serosal bathing solution.

study by Frizzell, Dugas and Schultz (1975) on unidirectional influxes of ions across the mucosal border of rabbit gallbladder has indicated that one site of Na-Cl coupling is at the mucosal membrane. The existing Na gradient across the mucosal membrane provides the energy to move Cl into the cell against an apparent electrochemical potential gradient. The resulting model of transport includes the possibility of coupled Na extrusion at the serosal membrane with K uptake by the cell since a ouabain-sensitive Na-K ATPase has been identified whose activity correlates with the rate of transepithelial NaCl transport (van Os & Sleegers, 1971). The degree of Na-K coupling was not specified in their model and thus the mechanism may be electrically neutral if one-for-one coupling is obligatory, or rheogenic[2] if Na extrusion exceeds K uptake. We suggested the possibility of a rheogenic ion pump[3] in gallbladders of goose, monkey and man because they develop spontaneous serosa-positive transepithelial *PD* of 1.5 to 7.6 mV which are quickly reduced by low temperatures or anaerobic conditions (Rose, Gelarden & Nahrwold, 1973; Gelarden & Rose, 1974).

Although the transepithelial *PD* of gallbladder has been extensively examined as an aid to understanding the active ion transport mechanism, measurement of the intracellular electrical potential has not been specifically considered as a means of distinguishing between rheogenic and electrically neutral transport. The present electrophysiologic study explores the possibility of rheogenic ion transport in rabbit and guinea pig gallbladders. Gallbladder mucosa was exposed to Amphotericin B in these experiments because of previous reports that this polyene antibiotic alters the anion permeability of biological tissues (Lichtenstein & Leaf, 1965) and elicits a serosa-positive *PD* in rabbit and guinea pig gallbladders (Cremaschi, Henin & Calvi, 1971*a*; Cremaschi, Montanari, Simonic & Lippe, 1971*b*). For purposes of comparison, Amphotericin B was also applied to goose and human gallbladders which differ

---

2 Rheogenic transport is used to describe a current-generating process as suggested by Schwartz (1971) and discussed in detail by Schultz, Frizzell and Nellans (1974).

3 Two mechanistically distinct types of active ion transport now appear to occur in rabbit gallbladder: (1) transport of Na at the basolateral membrane which is immediately dependent on cellular metabolic energy; and (2) intracellular accumulation of Cl against an electrochemical potential gradient which proceeds through the coupled NaCl influx process and is driven by the existing Na gradient. Convenient terminology to distinguish between the two types of active transport mechanisms would be helpful. The term "pump" has been used widely, but without formal definition, in the gallbladder literature to describe an active transport process which is considered to be *immediately* dependent on cellular metabolism; this paper will adhere to this terminology.

from the rabbit and guinea pig tissue by developing spontaneous serosa-positive *PDs* under control conditions.

The specific biological properties altered by Amphotericin B to elicit a serosa-positive *PD* remain incompletely identified; however, the present results are consistent with the presence of a rheogenic Na transport mechanism in rabbit and guinea pig gallbladders, both in the presence of Amphotericin B and under control conditions. Evidence which led to the proposal of a coupled NaCl pump mechanism is reconciled with a model in which NaCl enters the cell by a coupled mechanism at the mucosal membrane and Na is actively transported from the cell at the serosal border.

Materials and Methods

Gallbladders from rabbit, guinea pig and goose 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 bathing solution to remove bile. The serosa of human gallbladder was removed by blunt dissection, which causes only minor changes in electrical parameters (Rose *et al.*, 1973).

Transepithelial Ion Flux and Electrical Measurements

The bathing chamber and apparatus for measuring the transmural electrical potential difference (mucosa to serosa, ψms) and short-circuit current (*Isc*) were similar to those of Schultz and Zalusky (1964). Briefly, 1.13 cm^2 (or 0.30 cm^2 in the case of paired half-bladders) of tissue was held between the halves of a Lucite chamber and exposed on each surface to 15 ml buffered electrolyte solution at 37°C. The composition of control bathing solution was (in mM): NaCl, 142; $MgCl_2$, 1.2; $CaCl_2$, 0.9; K_2HPO_4, 1.2; KH_2PO_4, 0.2; $KHCO_3$, 10; and the initial pH was 7.2. The bathing solution was perfused across each surface of the tissue by means of a gas-lift circulating system driven by a water-saturated gas mixture containing 95% O_2 and 5% CO_2.

Tips of Ringer-agar bridges were placed close to the membrane and ψms was measured using a pair of calomel electrodes leading to a high impedance electrometer. Tissue resistance was determined by recording the *P.D.* deflection in response to 50 μA of direct current from an external battery source and correcting for fluid resistance. During ion flux experiments in the absence of Amphotericin B, ψms was always less than ± 1.5 mV, and no attempt was made to short-circuit the tissue; during exposure to Amphotericin B, ψms was nulled by the external battery (with appropriate corrections for fluid resistance) except for brief periods when the spontaneous ψms was recorded. Unidirectional transmural fluxes of ^{22}Na or ^{36}Cl were measured by sampling the initially unlabelled reservoir at 5 min.

intervals following introduction of the radioisotope. Steady-state fluxes were achieved by the third sampling period.

Transepithelial Diffusion Potentials

Prior to establishing diffusion potentials across rabbit gallbladders, the mucosal and serosal bathing solutions were changed from control bathing solution to an electrolyte solution of the following composition (in mM): NaCl, 142; $MgSO_4$, 1.2; $CaSO_4$, 0.9; and mannitol, 12. ψms of rabbit gallbladder bathed in this solution did not usually differ from that observed under control conditions. After ψms had stabilized, the solution bathing the mucosal surface of the tissue was replaced with one containing one-half the NaCl concentration present in the serosal bathing solution; osmolarity was maintained with mannitol. Upon establishment of this 2:1 NaCl gradient ψms reached a new, stable value within a few minutes. Diffusion between the salt-agar bridges and bathing solutions as previously discussed (Gelarden & Rose, 1974). Diffusion potentials established during exposure to Amphotericin B were also corrected for change in spontaneous ψms due to the lower Na concentration in the bathing solution by reference to Fig. 6[4]. The corrected diffusion potentials were then substituted into the Goldman-Hodgkin-Katz constant-field equation (Goldman, 1943; Hodgkin & Katz, 1949) to calculate the permeability ratio (P_{Cl}/P_{Na}) for each gallbladder.

Electrical Potential Profile Measurement

Simultaneous measurements of the transepithelial *PD* (ψms) and the intracellular electrical potential with reference to the mucosal solution (ψmc) were made with the technique described by Rose and Schultz (1971) for rabbit ileum. Microelectrodes were prepared from 1.5 mm OD borosilicate glass tubing, filled with 3M KCl and selected for a tip resistance of 5–15 Mohms and a tip potential of less than 5 mV. A KCl-agar bridge connected the microelectrode to a calomel half-cell which, in turn, was connected to the high-impedance probe of a negative capacitance Mediator A-35 amplifier. The Ringer-agar bridge in contact with the mucosal bathing solution was used as reference. ψms and ψmc were simultaneously recorded on a dual-channel recorder. The microelectrode was held by a micromanipulator (Eric Sobotka Co., Model MM-33) and driven by hand or by a stepping hydraulic drive unit (Kopf Instruments, Model 607) with a minimum advance of 1 micron. Cell impalements were judged successful by the criteria detailed by Rose and Schultz (1971) for mammalian ileum.

Oxygen Consumption Measurements

The apparatus for measuring O_2 consumption by rabbit gallbladder mucosa was quite similar to that used by Martin and Diamond (1966). Gallbladders were excised, opened and rinsed free of bile in control bathing solution at 0°C. Since the oxygen consumption ($\dot{Q}o_2$) of rabbit ileal mucosa was greatly depressed with the serosal musculature intact (Frizzell, Markscheid-Kaspi & Schultz, 1974), the muscle layers of gallbladder were removed

4 It is assumed from Fig. 6 that the spontaneous *PD* of each gallbladder is reduced by 25% due to reducing the Na concentration in the bathing solutions by 50% in the absence of a transmural ion gradient. The difference between the hypothetical value of ψms for individual tissues in the absence of a transmural ion gradient and the observed ψms is taken as the diffusion potential due to the 2:1 NaCl gradient. This correction is an approximation as indicated by the standard errors in Fig. 6.

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. Amphotericin B (50 μl) could be added to the test chamber without exposing the test fluid to the atmosphere.

Fluid Absorption Measurements

The technique for measuring water absorption by whole rabbit gallbladders was similar to that previously described by Diamond (1962*b*). Gallbladders were everted on a glass rod, and polyethylene tube (1.2 mm, I.D.) 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.

Results

Effect of Amphotericin B on Transepithelial PD

A recording of ψms from a rabbit gallbladder bathed on both surfaces by control bathing solution is shown in Fig. 1. Addition of Amphotericin B (40 μg/ml) to the serosal bathing solution resulted in no electrical change; subsequent addition to the mucosal solution caused the immediate development of a serosa-positive *PD* (8.1 ± 0.9 mV, n=12; short-circuit current, 260 ± 31 μA) which decayed to zero in 60–120 min. Mucosal Amphotericin B also induced an increase of ψms in gallbaldders from goose (5.3 ± 0.7 mV; n=8) guinea pig (22 ± 5 mV; n=8) and man (4.2 mV; n=2). The difference in bathing solution temperature between this study (37°C) and the previous study (27°C) possibly accounts for the lower *PD*s Cremaschi *et al.* (1971*a*) reported for rabbit (1.05 mV) and guinea pig (1.27 mV) gallbladders exposed to Amphotericin B.

Effect of Amphotericin B on Net Na Flux, O_2 Consumption and Volume Flow

The possibility was considered that development of a serosa-positive *PD* is associated with a greater rate of electrolyte transport from mucosa

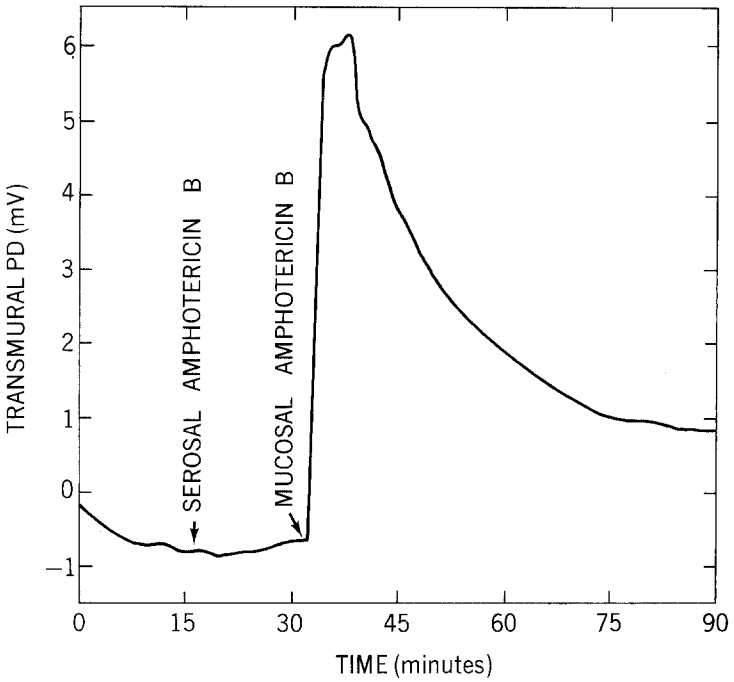

Fig. 1. The effect of Amphotericin B (40 μg/ml) in the serosal and mucosal bathing solutions on the transmural *PD* of rabbit gallbladder. Experiment performed in control bathing solution at 37°C

to serosa, as is the case in toad bladder (Bentley, 1968) and canine jejunum (Chen, Guerrant, Rhode & Casper, 1973). In rabbit gallbladder the rate of transport in the presence and absence of Amphotericin B was evaluated by independently measuring unidirectional transepithelial fluxes of ^{22}Na, net volume absorption, and O_2 consumption. In each of four experiments net transport of Na from mucosa to serosa was increased during the first five min period following Amphotericin B administration (Table 1). This increased rate of transport under short-circuit conditions corresponds with an increased rate of O_2 consumption immediately following exposure to Amphotericin B (Fig. 2)[5]. After 5–10 min exposure to Amphotericin B both net Na flux and O_2 consumption were lower than control values. Net volume absorption was measured in 3 everted rabbit gallbladders suspended in control bathing solution at 37°C. Following exposure of the mucosal surface to Amphotericin B the average rate of absorption was decreased from 90 mg/hr to 8 mg/hr within ten min.

5 The $\dot{Q}o_2$ of rabbit gallbladder mucosa under control conditions in the present study was 1.68 μl/g wet wt (n=7). Assuming a dry wt/wet wt ratio of 0.126 (Frizzell *et al.*, 1975) this corresponds to a $\dot{Q}o_2$ of 13.3 μl/g dry wt which is within the range of the values Martin and Diamond (1966) reported for whole gallbladders with intact musculature.

Table 1. Effect of Amphotericin B on transmural sodium flux in rabbit gallbladder[a]

Period	J^{ms}	J^{sm}	J^{net}
3	3.60±0.24	1.29±0.17	2.31±0.20
4	3.74±0.34	1.33±0.17	2.41±0.34
5	3.67±0.37	1.36±0.14	2.31±0.27
6	3.23±0.17	1.26±0.14	1.94±0.20
7	3.57±0.27	1.33±0.10	2.24±0.20
8	6.56±0.31	2.96±0.51	3.60±0.34
9	2.82±0.54	1.46±0.20	1.39±0.48
10	3.50±0.20	1.60±0.31	1.94±0.48
11	2.79±0.44	1.56±0.17	1.22±0.61
12	2.82±0.37	1.56±0.20	1.29±0.37
13	2.11±0.24	1.36±0.27	0.75±0.41
14	2.31±0.34	1.33±0.20	0.95±0.41

[a] m and s designate the mucosal and serosal solutions, respectively; J^{jk} designates the unidirectional flux of Na from the jth compartment to the kth compartment. $J^{net}=J^{ms}-J^{sm}$. The fluxes are given in micromoles per square centimeter for each 5 min flux period. Values are mean±SEM on paired determinations in four gallbladders. Amphotericin B was added to the mucosal bathing solution after the 7th flux period. The open-circuit p.d. during periods 3–7 was 1.1±0.3 mV. Periods 8–14 were performed under short-circuit conditions.

The significant result of these experiments is that a substantial serosa-positive Amphotericin B-induced *PD* was measured after net Na flux, O_2 consumption and fluid absorption were reduced below control levels. This supports the earlier report (Cremaschi *et al.*, 1971*a*) that the serosa-positive *PD* is not the result of an increased rate of active ion transport.

Diffusion Potentials at the Cell Membrane

Cremaschi *et al.*, (1971*a*) suggested that the Amphotericin B-induced *PD* is the result of diffusion of Na salts across the mucosal membrane into the epithelial cells. They did not specify which biological property is altered by Amphotericin B to elicit the *PD*. Their theory on the origin of the *PD* may be evaluated by altering the Na concentration gradient across the cell membrane which would be expected to change the magnitude of the Amphotericin B-induced *PD*. Gallbladders from rabbit, guinea pig and goose were preincubated 50–70 min at 0°C in K-free buffer to increase the intracellular Na concentration. From preliminary experiments on intracellular ion concentrations in rabbit gallbladder mucosa (serosal musculature removed) under these conditions and from similar experiments performed on rabbit ileal mucosa (Rose *et al.*, *unpublished*

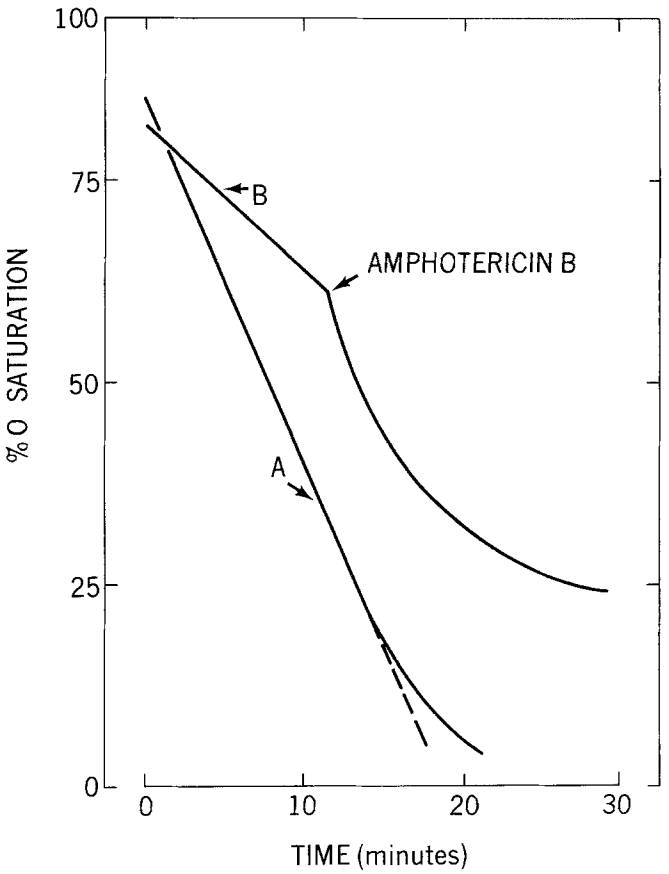

Fig. 2. Oxygen consumption by rabbit gallbladder in the absence (Experiment *A*) or presence (Experiment *B*) of Amphotericin B. The ordinate is % oxygen saturation in a closed vessel containing a piece of gallbladder stripped of musculature. The rate of oxygen consumption is independent of % saturation above 25% (traced from the original recording)

observation) it appears likely that this preincubation increases the intracellular Na concentration from an initial value of 66 mEquiv/liter (Frizzell *et al.*, 1975) to approximately the concentration in the bathing solution (142 mEquiv/liter). They were mounted in Ussing-type chambers and bathed at 37°C on both surfaces by buffer which had the Na concentration adjusted to 50 mEquiv/liter (Tris replacement). Although uncertainties exist concerning the activity of Na in the cellular environment, it seems likely that the preincubation procedure, followed by bathing the tissue in a solution having only 35% of the Na concentration in control bathing solution, would markedly reduce, or possibly reverse, the Na activity gradient across the cell membrane as compared with control conditions. The initial *PD* (Fig. 3) was quite low in gallbladders

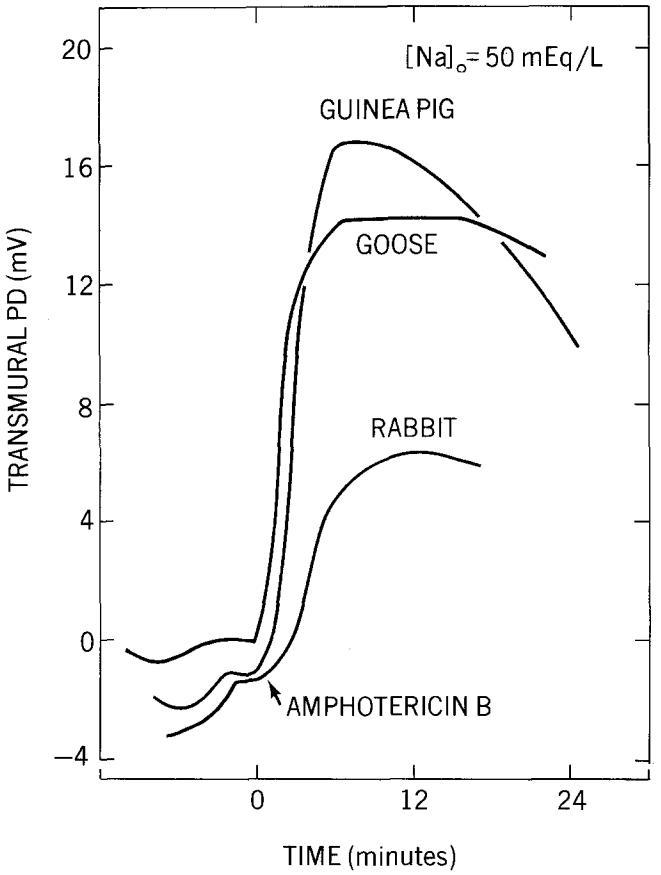

Fig. 3. Effect of Amphotericin B on the transmural *PD* of gallbladders with high $[Na]_i$. Rabbit, goose and guinea pig gallbladders were preincubated 50–70 min at 0°C in K-free buffer to increase $[Na]_i$. The bathing solution had [Na] = 50 mEquiv/liter (Tris replacement). Amphotericin B (40 μg/ml) was added to the mucosal bathing solution. Experiment performed at 37°C

of rabbit, goose and guinea pig. Addition of Amphotericin B resulted in an immediate increase in *PD* of gallbladder from each species (rabbit: $\Delta\psi ms = 0.6 \pm 2.3$ mV, n = 3; goose: $\Delta\psi ms = 10.3 \pm 2.7$ mV, n = 3) without a significant change in tissue resistance. The finding that the Amphotericin B-induced *PD* was normal when the Na concentration gradient across the cell membrane was markedly altered suggests that the *PD* does not result from a Na diffusion potential. The possibility of a K diffusion potential contributing to the *PD* was evaluated in guinea pig gallbladders preincubated in the same way and then bathed in buffer having [Na] = 50, [K] = 35 and [Tris] = 65 mEquiv/liter. This three-fold increase in the external K concentration did not significantly change the Amphotericin-B induced *PD* (maximum response, 15.6 mV; n = 3). Thus, it may be con-

cluded that the transepithelial *PD* is not established by Na or K diffusion potentials across the cell membrane.

Diffusion Potentials Across the Tight Junction

The possibility has previously been discussed (Gelarden & Rose, 1974) that gallbladder epithelium might have at least two important emfs which contribute to the transmural *PD*. A serosa-negative emf may exist in the ionic diffusion gradient which is present across the tight junction during absorption as proposed by Machen and Diamond (1969), and rheogenic active Na transport at the serosal cell border might represent a serosa-positive emf. One effect of Amphotericin B might be to reduce the contribution of the diffusion potential generated across the tight junction by making the tissue less cation-selective. This would unmask the serosa-positive emf of active Na transport and result in a more serosa-positive *PD*. The effect of Amphotericin-B on the relative permeability of rabbit gallbladder to Cl and Na was investigated using artificially established 2:1 NaCl concentration gradients as an index of cation-anion selectivity. In seven samples of rabbit gallbladder P_{Cl}/P_{Na} was 0.11 ± 0.03 under control conditions and increased to 0.21 ± 0.03 ($P < 0.01$) at the peak response to Amphotericin B.

The Amphotericin B effect on Na and Cl diffusion across rabbit gallbladder was further evaluated by measuring serosa-to-mucosa fluxes of ^{22}Na and ^{36}Cl on short-circuited tissue in control bathing solution. The results of these experiments indicate that during the initial four 5-min test periods there is a gradual decrease in Na and Cl conductance which is associated with a progressive increase in tissue resistance measured electrically (Fig. 4)[6]. Addition of Amphotericin B resulted in an increase in Cl conductance and no change in Na conductance. These findings agree with the results of electrical measurements in indicating an increase in P_{Cl}/P_{Na}.

The present results suggest that Amphotericin B reduces the contribution of a serosa-negative emf at the tight junction; however, since this emf is unlikely to contribute more than approximately 2 mV to the transmural *PD* of rabbit gallbladder under control conditions (Machen & Diamond, 1969), this effect does not entirely account for the observed

6 Wright, Barry and Diamond (1971) found a progressive decrease in rabbit gallbladder resistance measured electrically during the same time period. The difference in findings might be attributed to the different experimental contitions; the gallbladders of Wright *et al.* were bathed in 150 mM NaCl whereas ours were bathed in a balanced buffer solution.

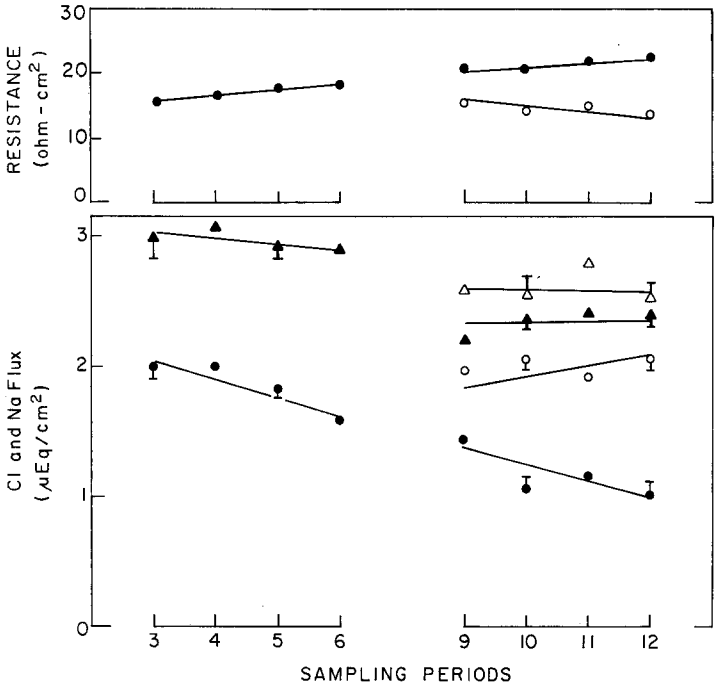

Fig. 4. Effect of Amphotericin B on Na and Cl diffusion from serosa to mucosa and on tissue reistance. Periods 3–6 represent control values for resistance, Na flux (triangles) and Cl flux (circles) on eight gallbladders. Periods 9–12 represent four gallbladders under continued control conditions (filled symbols) and four gallbladders which were exposed to Amphotericin B (open symbols)

8 mV change in *PD*, and another, yet unidentified, response is anticipated. Although the specific changes in tissues properties induced by Amphotericin B have not been completely identified, additional studies were performed to determine what properties of Amphotericin B-treated tissue might contribute to the *PD*.

PD Dependence on O_2, Na and Warm Temperature

Rheogenic Na transport at the serosal cell membrane was suggested as a possible emf to account for the spontaneous serosa-positive *PD* observed in gallbladders of goose, monkey and man (Rose *et al.*, 1973; Gelarden & Rose, 1974), but the occurance of rheogenic ion transport in rabbit gallbladder was not indicated by previous experiments. An emf associated with such transport would be characterized by close dependence on metabolic energy supplies. Thus, the Amphotericin B-induced

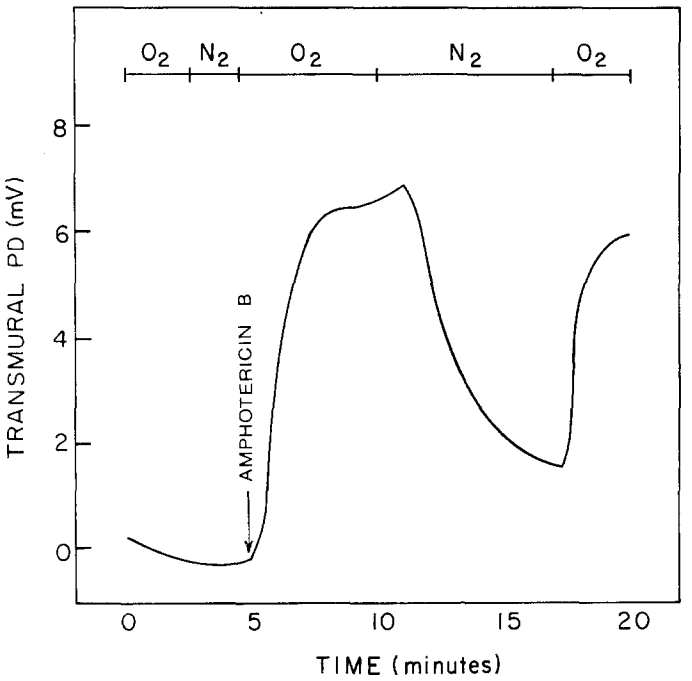

Fig. 5. Effect of anaerobiosis on the transmural *PD* of a rabbit gallbladder before and after Amphotericin B administration

PD was elicited under control conditions, and at the peak response N_2 was substituted for O_2 (Fig. 5). Anaerobic conditions resulted in a prompt fall in *PD* with no immediate change in tissue resistance; the *PD* was reestablished upon return to aerobic conditions. Similar results have been reported for tissues which have spontaneous transepithelial *PD*s under control conditions, such as goose and monkey gallbladders (Gelarden & Rose, 1974), human gallbladder (Rose *et al.*, 1973), rat intestine (Barry, Dikstein, Matthews, Smyth & Wright, 1964) and toad bladder (Essig, 1965).

The dependence of the Amphotericin B-induced *PD* on the presence of Na was evaluated by bathing the tissue in buffer solutions which had Na concentrations of 150, 100, 50 or 0 mEquiv/liter (Tris substitution). Because the Amphotericin B-induced *PD* is transient, the order of application of buffer solutions was randomized on individual tissue samples. A nonlinear relationship resulted between *PD* and Na concentration with approximately 0 mV existing in the absence of Na (Fig. 6). The results of this experiment are consistent with the concept of rheogenic Na absorption in rabbit gallbladders exposed to Amphotericin B; the

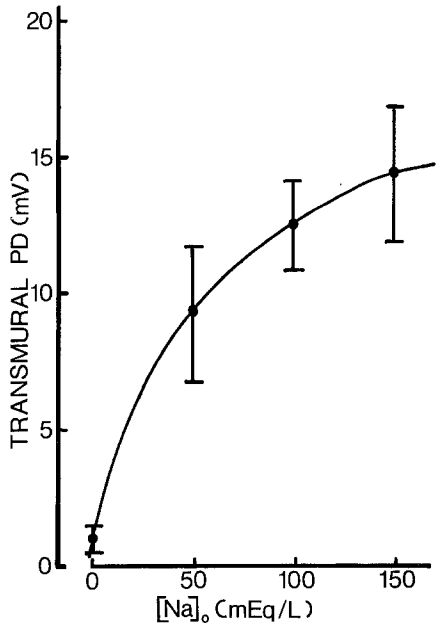

Fig. 6. Effect on transmural *PD* of reducing the Na concentration in the mucosal and serosal bathing solutions in the presence of Amphotericin B. Na replaced by Tris

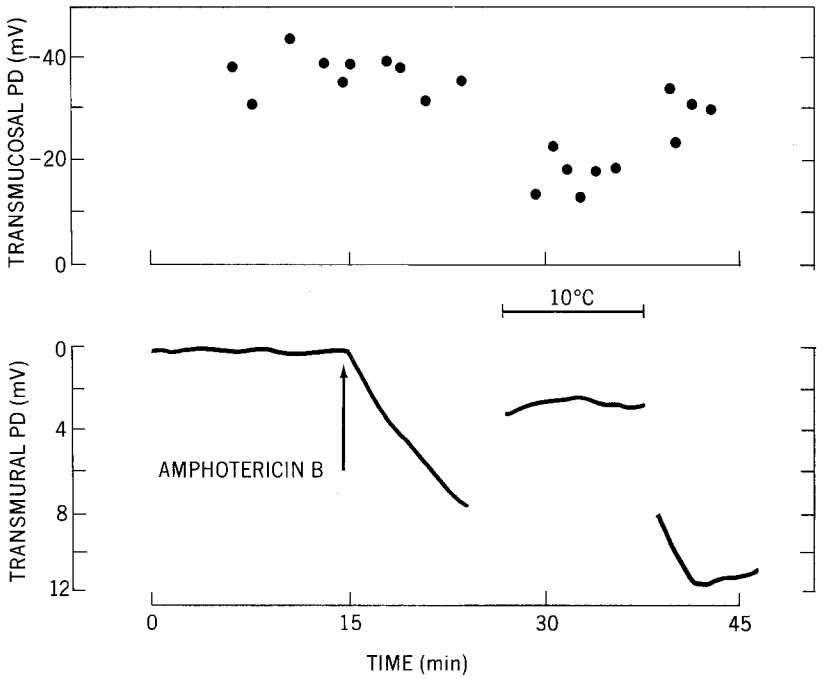

Fig. 7. Simultaneous measurements of ψmc and ψms in rabbit gallbladder. Tissue in control bathing solution at 37°C except for a brief cooling to approximately 10°C where indicated. Data points for ψmc represent individual cell impalements. ψms is a tracing of the original recording. Amphotericin B was added to the mucosal and serosal bathing solutions (40 μg/ml) where indicated

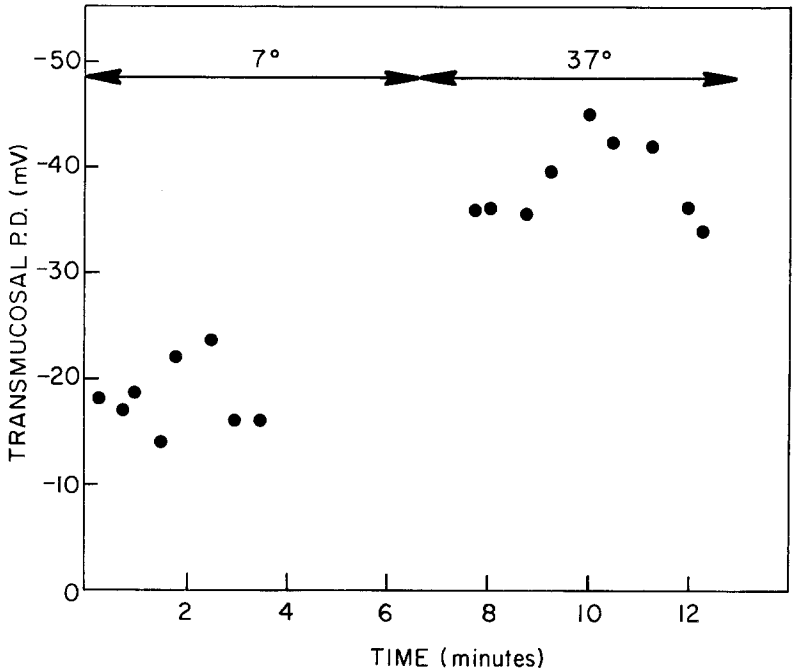

Fig. 8. Values of ψmc in a rabbit gallbladder in control bathing solution (without Amphotericin B) at 7°C and at 37°C. Data points represent individual cell impalements

availability of Na to the transport mechanism is one factor which determines the maximum transmural *PD*.

Simultaneous measurements of ψms and ψmc were made at 37°C and at 10°C to determine if sudden inhibition of active transport processes by low temperatures affected the electrical potential profile of rabbit gallbladder exposed to Amphotericin B. As seen in Fig. 7, cooling the tissue quickly reduced both ψms and ψmc. This immediate dependence of the electrical properties on warm temperatures is a characteristic of rheogenic transport mechanisms.

The occurrence of rheogenic active Na transport might also contribute to the electrical potential profile of rabbit gallbladder in the absence of Amphotericin B. Thus, ψmc and ψms were measured simultaneously using the microelectrode technique. Tissue samples were mounted in the chambers while ψmc and ψms were measured first for 5 min at 7°C and then for 10 min at 37°C (Fig. 8). At 7°C ψms in each of three tissue samples was near zero (+0.3 mV) and ψmc was -15 ± 4 mV. Following warming ψms fluctuated between -1.0 and $+1.0$ mV and ψmc increased to -35 ± 7 mV. The immediate change in ψmc upon return of the tissue to conditions which promote absorption suggests that rheogenic ion transport contributes to the *PD*.

Discussion

The results of most early studies on electrolyte transport by rabbit, guinea pig and fish gallbladder epithelium supported the concept that an electrically neutral NaCl pump is the driving force for fluid absorption (Diamond, 1968). In contrast, it was suggested that gallbladders of monkeys, goose and man, which have serosa-positive transepithelial *PD*s of 1.5 to 7.6 mV might have rheogenic ion pump mechanisms (Rose *et al.*, 1973; Gelarden & Rose, 1974). We suggested that the coupling between Na and Cl transport in the latter group of tissues could be electrical in nature, rather than occurring at the pump site as in the neutral pump model. Very recently Frizzell *et al.*, (1975) have stressed that the ion transport properties of rabbit gallbladder are such that the distinction between neutral transport of NaCl and electrical coupling may not be meaningful. They proposed a model for the rabbit gallbladder which features an obligatory coupled influx of Na and Cl across the mucosal border. Na is actively transported from the cell into the serosal solution; since the apparent electrochemical potential of Cl is higher in the cell than in the serosal solution, Cl exit from the cell may be diffusional. Although the details of the active Na transport mechanism were not specified in the model, the possibility was included that Na extrusion at the serosal membrane may be partially coupled to K transport into the cell. This possibility seems likely in view of the findings of Frederiksen and Leyssac (1969) and us (*unpublished observations*) that maximal rates of NaCl and fluid absorption by rabbit gallbladder depend on the presence of low concentrations of K in the bathing media. Incomplete coupling between Na and K transport would render the pump rheogenic, in which case it would not differ electrically from the mechanism suggested to be responsible for the serosa-positive *PD* in gallbladders of monkey, goose and man.

The possibility that rheogenic transport of some ion does, in fact, occur in rabbit gallbladder is supported by the present findings that in gallbladders exposed to Amphotericin B the transmural and transmucosal *PD*s are quickly reduced by conditions which inhibit cellular metabolism. Rabbit gallbladder epithelium has previously been reported to actively absorb Na, Cl and HCO_3 (Wheeler, 1963) and possibly OH (Dietschy, 1966), and to secrete H (Whitlock & Wheeler, 1969). Active transport of Cl, HCO_3, OH and H are in the wrong direction to account for the serosa-positive *PD* measured in the present experiments. Sodium-dependence of the Amphotericin B-induced *PD* suggests that Na absorp-

tion may proceed by a rheogenic pump mechanism. In gallbladder epithelium not exposed to Amphotericin B ψmc immediately hyperpolarized as ion transport processes were activated by warming the tissue from 7 to 37°C. This observation indicates that rheogenic Na transport may contribute to the intracellular electrical negativity in rabbit gallbladder even when the tissue is not treated with Amphotericin B.

The absence of a significant transmural *PD* across rabbit gallbladder epithelum under control conditions *in vitro* distinguishes this tissue from small intestine (Schultz & Zalusky, 1964), large intestine (Ussing & Andersen, 1955), stomach (Rehm, 1950), gallbladders of other species (Rose *et al.*, 1973; Gelarden & Rose, 1974) and other transporting epithelia. Evidence is accumulating that rheogenic active ion transport directly contributes to the transepithelial *PD* in ileum (Rose & Schultz, 1971; Frizzell & Schultz, 1972), gallbladders of man, goose and monkey (Rose *et al.*, 1973; Gelarden & Rose, 1974), amphibian skin (Bricker, Biber & Ussing, 1963) and bladder (Herrera, 1968). With the current evidence that rheogenic Na transport may occur in rabbit and guinea pig gallbladders, the possibility must be considered that epithelial tissues with a wide variety of spontaneous transepithelial electrical *PD*s possess active Na (or Na-K) transport mechanisms with properties more similar than previously recognized.

Possible Actions of Amphotericin B

The present experiments give some information regarding the several possibilities by which Amphotericin B may cause a serosa-positive *PD* to develop across gallbladder epithelia.

(a) Cremaschi *et al.* (1971*a*) suggested that the *PD* results from establishment of a Na diffusion potential across the mucosal cell membrane. This possibility now seems untenable since reduction or reversal of the Na gradient across the membrane did not prevent development of a normal Amphotericin B-induced *PD*.

(b) If the Na pump mechanism is rheogenic, the *PD* might be due to a marked increase in the rate of Na transport from mucosa to serosa as postulated to be the case in toad bladder (Bentley, 1968) and canine jejunum (Chen *et al.*, 1973). This possibility may be ruled out in rabbit gallbladder since experiments on fluid absorption, O_2 consumption and transmural fluxes of Na indicate that the rate of active Na transport from mucosa to serosa is depressed when the Amphotericin B-induced *PD* is still quite high.

(c) Amphotericin B might cause the ion pump to change from a coupled NaCl mechanism to a rheogenic Na transport system. Although the recent work of Frizzell *et al.* (1975) demonstrated that Na and Cl enter the cell across the mucosal border by a coupled mechanism which is not directly dependent on cellular metabolism, their experiments did not specifically eliminate the possibility proposed by Diamond (1962*a*) that active transport of Na and Cl out of the cell at the serosal border is also coupled. An effect of Amphotericin B on uncoupling the active transport mechanism cannot be ruled out from the present experiments; it seems somewhat unlikely, however, that mucosal application of Amphotericin B, but not serosal application, would have this effect. In addition, the present experiments suggest that the Na transport mechanism is rheogenic even in the absence of Amphotericin B.

(d) Two opposing emfs might exist in gallbladder or rabbit and guinea pig which may tend to nearly cancel the effects of each other under control conditions. Thus, Amphotericin B might reduce or reverse a serosa-negative diffusion potential established by the presence of an electrolyte concentration gradient across the tight junction subsequent to fluid absorption (Machen & Diamond, 1969) and thereby unmask the effect of rheogenic Na transport from mucosa to serosa. Although the present experiments do, in fact, indicate that Amphotericin B reduces the cation selectivity of the tight junction, this effect alone is too small to account entirely for the development of a serosa-positive *PD* of 6–12 mV in rabbit gallbladder.

(e) Amphotericin B might uncouple Na and Cl entry across the mucosal border by directly affecting the carrier mechanism. This might allow Na to enter the cell independent of Cl and could, therefore, result in electrical coupling between Na and Cl transport; a transepithelial *PD* would necessarily develop to promote a rate of Cl absorption equal to the rate of Na absorption mediated by the pump.

(f) Amphotericin B might increase the permeability of the mucosal membrane to Cl. Because Cl is apparently at a higher electrochemical potential in the cell than in the bathing solution (Frizzell *et al.*, 1975), some Cl which enters the cell through the coupled NaCl mechanism would exit the cell into the mucosal solution. Rheogenic Na transport would develop a serosa-positive *PD* of sufficient magnitude to result in net Cl flux from mucosa to serosa through the extracellular shunt and transcellular pathways. Thus, the *PD* might result from a Cl diffusion potential and/or rheogenic Na transport.

(g) Amphotericin B might increase the mucosal membrane permeabili-

ty to Na which would allow increased diffusional entry of Na. This is similar to uncoupling the entry of NaCl across the mucosal border, and could account for the serosa-positive *PD* if a rheogenic Na pump mechanism is operative. Little information is currently available to assess points (*e*), (*f*) and (*g*) as possible mechanisms of the action of Amphotericin-B, and direct measurement of Na and Cl influx at the mucosal border is warranted.

The possibility may be evaluated that the Amphotericin B-induced *PD* is great enough that Cl absorption may be attributed exclusively to electrical coupling as predicted under the extreme conditions of points (*e*), (*f*) and (*g*) above. For small values of ψms (<25 mV) when the concentration of an ion, designated [*i*] in the mucosal (*m*) and serosal (*s*) bathing solutions is equal, the net diffusional flow of the ion through transepithelial shunt and passive conductance pathways is given by the equation (Schultz, 1974)

$$_{d}J_i = -P_i[i] \qquad Z_i F \psi ms/RT$$

where P_i is the permeability of the diffusional pathway to *i*. When ψms is zero (as under the short-circuit conditions of the experiment in Fig. 4) $P_i[i] = J_i^{ms} = J_i^{ms}$ which expressed in μmoles/cm^{2}hr is numerically equal to the partial ionic conductance of *i* (G_i) expressed in mmhos/cm^2. During the 15 min period when the Amphotericin B-induced *PD* is at its peak J_{Na}^{net} is 28 μmol/cm^{2}hr (Table 1) and $J_{Cl}^{sm} = G_{Cl} = 24$ mmho/cm^2 (Fig. 4). Thus, in order for net Cl transport to equal active Na transport through pure electrical coupling, ψms would have to be 30 mV. Since the mean observed value of ψms was only about 5 mV it appears that an intermediate situation might exist whereby Cl transport is coupled to active Na transport partially through electrical coupling and partially through the neutral NaCl influx process at the mucosal membrane.

Reevaluation of Evidence Which Led to Coupled Pump Model

Several lines of evidence led to the hypothesis that an active NaCl transport mechanism is located along the basolateral membrane of rabbit and guinea pig gallbladders (comprehensive reviews by Diamond (1968) and Dietschy (1966)). This O_2-dependent pump was postulated to consist of a carrier molecule having a Na transport site and a Cl transport site. Since neither Na nor Cl could be actively transported alone by the gallbladder, electrically neutral absorption of NaCl resulted. If the present proposal of a rheogenic active transport mechanism is an alterna-

tive to the coupled NaCl transport model, the experimental observations which led to the original model will have to be reconciled.

One of the primary observations leading to the neutral model was that during net electrolyte absorption *in vitro* when identical solutions bathed both surfaces of rabbit or fish gallbladder the transepithelial *PD* was always near zero. However, as discussed in detail by Frizzell *et al.* (1975), rheogenic Na transport may occur in an epithelial preparation and yet the coupling between Na and Cl transport may be through a neutral coupled transport mechanism, such as the NaCl entry step across the mucosal border of rabbit gallbladder. Thus, separation of charge (Na^+ from Cl^-) during transport may occur exclusively across the serosal membrane (although the cell interior may become electrically negative with respect to the mucosal as well as the serosal bathing solution due to a low resistance shunt path between the two solutions). If, for example, the serosal membrane is quite permeable to Cl but the mucosal membrane is not, entry of Cl into the cell will be by the neutral coupled NaCl influx process, and exit of Cl across the serosal border will be diffusional. A transepithelial *PD* need not develop to ensure equal rates of Na and Cl absorption.

Substitution of a nonabsorbed anion, such as SO_4, or a nonabsorbed cation, such as choline, in the bathing solution of rabbit gallbladder resulted in the development of no significant transepithelial *PD* (Wheeler, 1963; Dietschy, 1964). This was taken to mean that there could not be independent active transport of Na or Cl at the serosal membrane and supported the concept of coupled active transport. The same conclusion was derived from the observation that removal of either Na or Cl from the bathing solution reduced absorption of the other ion and of water. However, these observations are not inconsistent with the active transport mechanism being a rheogenic Na pump since the influx of Na and Cl across the mucosal membrane is a coupled process; thus, it appears likely that removal of Cl from the bathing solution restricts entry of Na into the cell and thereby limits availability of substrate at the pump site. Likewise, removal of Na from the bathing solution limits pump activity and the associated water flux.

Oxygen consumption in a variety of transporting epithelia increases upon stimulation of the tissue's active transport mechanism. It is necessary to have both Na and Cl in the mucosal bathing solution to have maximum O_2 consumption (Martin & Diamond, 1966) or fluid transport (Dietschy, 1964) in rabbit gallbladder. These findigs also seemed to indicate that both ions were necessary at the pump site; again, however,

this is consistent with the presence of a rheogenic Na pump in series with obligatory, coupled NaCl influx at the mucosal border which supplies the Na necessary to activate the pump mechanism.

The observation that Cl is transported from the mucosal to the serosal bathing solution against an electrochemical gradient (Wheeler, 1963; Dietschy, 1964) is consistent with the presence of a rheogenic Na pump as well as with a neutral NaCl pump. Operation of the Na pump renders the intracellular Na concentration low; thus, cotransport of Cl with Na is a "downhill" process for Na but results in accumulation of Cl inside the cell against an electrochemical gradient (Frizzell *et al.,* 1975). Exit of Cl across the serosal border could then be diffusional but still proceed against a concentration greater than that in the mucosal solution.

The observation that ouabain inhibits electrolyte transport in gallbladders which develop little or no transmural *PD* (Dietschy, 1964) and reduces the *PD* of goose, monkey and human gallbladders (Rose *et al.,* 1973; Gelarden & Rose, 1974) seems to favor a common action of the glycoside on each tissue, which may be similar to its action on other transporting epithelia, such as intestine.

The present experiments suggest that an O_2-dependent, rheogenic active transport mechanism for Na exists at the serosal membrane of rabbit and guinea pig gallbladders. This pump normally contributes to the intracellular electrical negativity and, within the model of Frizzell *et al.* (1975), is ultimately responsible for transport of Cl across the mucosal membrane against an electrochemical potential difference by maintaining the low intracellular electrochemical potential of Na.

Experiments have not yet been performed to determine if coupled entry of NaCl exists at the mucosal membrane of gallbladders which develop spontaneous serosa-positive *PD*s (goose, monkey and man). From previous determinations of J_{Na}^{net} and J_{Cl}^{sm} on human gallbladder (Rose *et al.,* 1973) we can calculate that a *PD* of 18 mV would be necessary for pure electrical coupling to drive net Cl transport from mucosa to serosa at a rate equal to active Na transport. In contrast, a *PD* of only 7.6 mV was observed. Since Amphotericin B elicits a greater ψms in goose and human gallbladders, the possibility appears likely that in these tissues the rate of net anion transport under control conditions equals net Na transport partly through the mechanism of coupled NaCl influx at the mucosal border and partly through electrical coupling. If future experiments support this concept, the previous evidence suggesting that mechanistically different active ion transport processes exist in electrically polarized gallbladders, nonpolarized gall-

bladders, and small intestine will be largely negated, and a unified model of the pump may be attractive.

This work was supported by a Public Health Service Research Grant from the National Institute of Arthritis, Metabolism and Digestive Diseases (AM 15926). The expert technical assistance of Marilyn Koch is appreciated.

References

Barry, R.J.C., Dikstein, S., Matthews, J., Smyth, D.H., Wright, E.M. 1964. Electrical potentials associated with intestinal sugar transfer. *J. Physiol. (London)* **171**:316

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

Bricker, N.S., Biber, T., Ussing, H.H. 1963. Exposure of the isolated frog skin to high potassium concentrations at the internal surface. I. Bioelectric phenomena and sodium transport. *J. Clin. Invest.* **42**:88

Chen, L.C., Guerrant, R.L., Rhode, 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*a*. Transepithelial potential difference induced by Amphotericin B and NaCl-$NaHCO_3$ pump localization in gallbladder. *Arch. Int. Physiol. Biochim.* **79**:889

Cremaschi, D., Montanari, C., Simonic, T., Lippe, C. 1971*b*. Cholesterol in plasma membranes or rabbit gallbladder epithelium tested with Amphotericin B. *Arch. Int. Physiol. Biochim.* **79**:33

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

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

Diamond, J.M. 1964. Transport of salt and water in rabbit and guinea pig gall bladder. *J. Gen. Physiol.* **48**:1

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

Dietschy, J.M. 1964. Water and solute movement across the wall of the everted rabbit gall bladder. *Gastroenterology* **47**:395

Dietschy, J.M. 1966. Recent developments in solute and water transport across the gall bladder epithelium. *Gastroenterology* **50**:692

Essig, A. 1965. Active sodium transport in toad bladder despite removal of serosal potassium. *Am. J. Physiol.* **208**:401

Frederikson, O., and Leyssac, P.P. 1969. Transcellular transport of isosmotic volumes by the rabbit gall-bladder *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

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

Goldman, D.E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27**:37

Herrera, F.C. 1968. Action of ouabain on bioelectric properties and ion content in toad urinary bladder. *Am. J. Physiol.* **215**:183

Hodgkin, A.L., Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London)* **108**:37

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

Machen, T.E., Diamond, J.M. 1969. An estimate of the salt concentration in the lateral intercellular spaces of rabbit gall-bladder during maximal fluid transport. *J. Membrane Biol.* **1**:194

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

Rehm, W.S. 1950. A theory of the formation of hydrochloric acid by the stomach. *Gastroenterology.* **14**:401

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

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. 1974. Shunt pathway, sodium transport and electrical potential profile across rabbit ileum. *In*: Transport Mechanisms in Epithelia. Munksgaard, Cophenhagen

Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1974. Ion transport by mammalian small intestine. *Annu. Rev. Physiol.* **36**:51

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

Schwartz, T.L. 1971. Direct effects on the membrane potential due to "pumps" that transfer no net charge. *Biophys. J.* **11**:944

Ussing, H.H., Andersen, G. 1955. The relation between solvent drag and active transport of ions. *Proc. Int. Congr. Biochem.* (3rd) Brussels. p. 434

van Os, C.H., Sleegers, 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

Wheeler, H.O. 1963. Transport of electrolytes and water across wall of rabbit gall bladder. *Am. J. Physiol.* **205**:427

Whitlock, R.T., Wheeler, H.O. 1969. Hydrogen ion transport by isolated rabbit gallbladder. *Am. J. Physiol.* **217**:310

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 of anion-cation discrimination, and the calcium competition effect. *J. Membrane Biol.* **4**:331