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Electrical Properties and Diffusion Potentials in the Gallbladder of Man, Monkey, Dog, Goose and Rabbit

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Summary. Electrical properties of gallbladders from several species were investigated *in vitro*. Gallbladders were mounted as a flat sheet in a Lucite Ussing-type chamber and bathed on each surface with buffered HCO_3-free Ringer's solution. Rabbit and dog gallbladders developed a mean transmural electrical potential difference (p.d.), not significantly different from zero, whereas gallbladders of goose, monkey and man developed serosa-positive p.d.'s which were dependent on the availability of O_2 and were abolished by ouabain and low temperatures. The short-circuit current (SCC) was dependent on the presence of Na in the bathing medium; choline and K were ineffective substitutes. Bromide replacement for Cl reduced SCC by less than 15% in goose and monkey gallbladders; sulfate replacement eliminated SCC. Isethionate replacement maintained some SCC in gallbladder of monkey but not of goose. The presence of HCO_3 in the bathing medium abolished SCC in goose gallbladder but increased slightly SCC in gallbladders of monkey and man. To determine whether the anion-cation selectivity of gallbladders from each species correlated with the magnitude and orientation of the spontaneous p.d., diffusion potentials were artificially established by diluting the mucosal solution 50% with isotonic mannitol. Gallbladders from all species studied are cation-selective. Epithelial removal demonstrated that the primary site of tissue resistance and the ability to discriminate between cations and anions resides in the epithelium. In view of the cation selectivity of these tissues it is unlikely that local osmotic gradients, established in the lateral intercellular spaces during NaCl absorption, are the cause of serosa-positive p.d.'s seen in the goose, monkey and human gallbladders.

Until recently, most studies of absorption of electrolyte solutions by gallbladder epithelia *in vitro* have used tissue from fish (Diamond, 1962*a*, *b*, *c*), rabbit (Diamond, 1964; Dietschy, 1964; Whitlock & Wheeler, 1964; Machen & Diamond, 1969), and guinea pig (Diamond, 1964). Gallbladders of these species are capable of the basic task of electrolyte and water absorption. This absorptive process is characterized by active transport of NaCl from mucosa to serosa; water apparently follows passively on the basis of an osmotic gradient.

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The transmural electrical potential difference (p.d.) across gallbladders of fish, rabbit and guinea pig *in vitro* is normally very close to zero. Thus, Diamond (1962*b*) suggested that the active transport process involves an electrically neutral, coupled movement of NaCl out of the epithelial cell into the lateral intercellular space. In a later study, Machen and Diamond (1969) observed a -1.4 mV[1] p.d. across maximally transporting rabbit gallbladder. As a coupled NaCl pump would not directly generate an electrical potential, this small serosa-negative p.d. was postulated to be the result of diffusion potentials established within the lateral intercellular spaces across the cation-selective tight junction. Coupled NaCl pumps located at the lateral cell membrane are expected to create a slightly hypertonic solution of NaCl within the lateral intercellular space (Diamond & Bossert, 1967). Sodium would tend to diffuse back into the mucosal solution across the cation-selective tight junction faster than Cl, thereby creating the observed serosa-negative p.d.

A recent study of human gallbladder (Rose, Gelarden & Nahrwold, 1973) showed that electrolyte absorption across this tissue occurs in association with a 7.6 mV serosa-positive p.d. In the present paper we report that most dog gallbladders have a spontaneous p.d. near zero and gallbladders of goose and three species of monkey have spontaneous serosa-positive p.d.'s of 1.5 to 3.6 mV. Diamond's model of electrically neutral NaCl transdort could accommodate the continuum of transmural p.d.'s from -1.4 to $+7.6$ mV in gallbladders of various species if it were known that those gallbladders with serosa-positive p.d.'s have anion-selective properties at the tight junction rather than cation-selective tight junctions as in rabbit gallbladder.

In the present investigation we examined several properties of the spontaneous p.d.'s of goose, dog and monkey gallbladders. In addition, diffusion potentials were artificially established across the gallbladders of man, goose, monkey, dog and rabbit to determine if their individual spontaneous p.d.'s correlate with different anion-cation selectivity properties of the tight junction. It is concluded that the anion-cation selectivity of the tight junction is of the wrong orientation to account for the spontaneous serosa-positive p.d. observed in gallbladders of man, goose and monkey. However, local osmotic gradients established across the tight junction during absorption might create diffusion potentials which modify the serosa-positive p.d. by reducing its magnitude. Some of these data have been published in preliminary form (Gelarden & Rose, 1973; Rose, 1974).

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[1] p.d.'s are reported as serosal surface with respect to mucosal surface.

Materials and Methods

Gallbladders from rabbit, dog, goose and monkey were removed immediately after sacrifice of the animal by Pentabarbital 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 gallbladder 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 dog and human tissue was removed by blunt dissection.[2] We have previously found that removal of the serosa causes only minor changes in electrical parameters (Rose *et al.*, 1973).

The bathing chamber and apparatus for measuring the p.d. and short-circuit current (SCC) have already been described (Rose *et al.*, 1973). 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 the control bathing solution was (in mM): NaCl, 142; $MgCl_2$, 1.2; $CaCl_2$, 0.9; K_2HPO_4, 4.2; KH_2PO_4, 1.5; and glucose, 5.5. The initial pH of the bathing solution was 7.2.

The bathing solution was perfused across each surface of the tissue by means of a gas-lift circulating system driven by water-saturated 100% O_2. When the bathing solution contained HCO_3, 95% O_2-5% CO_2 was used.

The tips of Ringer's agar bridges were positioned close to each surface of the membrane and the p.d. 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 50μamp of direct current from an external battery source and correcting for fluid resistance.

Diffusion potentials were artificially established across gallbladders of each species after the spontaneous p.d. had stabilized. The mucosal and serosal bathing solutions were changed from the 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. This solution differs from the control bathing solution in that Na concentration is equal to Cl concentration and there is no K or buffer present. The p.d. of tissues bathed in this solution did not usually differ from that observed in control conditions. The pH measured during diffusion potential studies was found never to fall below a value of 6.7. Wright and Diamond (1968) demonstrated in rabbit gallbladder that a drop in pH did not alter the cation-anion selectivity of the tissue until the pH fell below approximately 6.0. After the p.d. 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. Equal osmolarities were maintained by the addition of mannitol to the mucosal solution. Upon establishment of this 2:1 NaCl gradient the p.d. reached a new, stable value within a few minutes and remained there for 3 to 5 min. This diffusion potential was corrected for junction potentials between the NaCl-agar bridges and bathing solution using the Planck equation modified to use activity coefficients which were obtained from Robinson and Stokes (1965) and it was assumed that the activity coefficients of Na and Cl were equal at a given concentration of NaCl (Guggenheim assumption).

Diffusion potentials were also corrected for the spontaneous p.d.'s generated by goose, monkey and human gallbladders. Due to the linear relationship between Na concentra-

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[2] Although the tissue was mounted with the serosa removed, the blood side will, for convenience, be referred to as the serosal surface.

Table 1. Transmural p.d., short-circuit current and tissue resistance of gallbladders

Species	p.d. (mV)	SCC (μamp/cm^2)	R (Ω cm^2)
Rabbit (9)	-0.2 ± 0.1	-9	23
Dog (12)	$+0.2 \pm 0.1$	4	46
Stumptail (7)	$+1.5 \pm 0.2$	43	35
Rhesus (5)	$+2.5 \pm 0.5$	93	27
African Green (11)	$+2.7 \pm 0.2$	77	35
Goose (21)	$+3.6 \pm 0.3$	120	30
Man[a] (46)	$+7.6 \pm 0.6$	136	52

[a] Previously published observations (Rose *et al.*, 1973). Sign of the p.d. refers to serosal surface; number of observations in parentheses.

tion and the spontaneous p.d. (*see* Results), it was possible to estimate what p.d. would have existed in the presence of the lower mucosal Na concentration without the establishment of a diffusion potential. This p.d. was then used to calculate the magnitude of the diffusion potential. These values were substituted into the Goldman-Hodgkin-Katz constant-field equation (Goldman, 1943; Hodgkin & Katz, 1949) to calculate the Cl/Na permeability ratio for each gallbladder. All errors are reported as standard errors of the mean.

Results

The mean values of spontaneous p.d., SCC and tissue resistance for the gallbladders of rabbit, dog, monkey, goose, and man are shown in Table 1. The gallbladders of goose and three species of monkey are seen to develop serosa-positive p.d.'s in common with the previous report on human gallbladder (Rose *et al.*, 1973). Maximum p.d.'s in the gallbladder of goose ranged from 1.6 to 7.0 mV and in monkey from 1.1 mV for the *Macaca arctoides* (Stumptail) to 4.2 mV for *Macaca mulatta* (Rhesus). In rabbit gallbladder the maximum p.d. ranged from -0.6 to $+0.2$ mV, and in mongrel dog gallbladders from -0.3 to $+1.2$ mV. The gallbladder of one Dalmation developed a serosa-positive p.d. of 2.6 mV and, as will be discussed later, this relatively substantial p.d. was O_2 and ouabain sensitive. The p.d.'s reported here were observed after the tissue had been bathed for 5 to 15 min in HCO_3-free control Ringer's.

Glucose Requirement

The spontaneous p.d.'s observed in freshly mounted monkey and goose gallbladders were not dependent upon the addition of glucose, although the p.d. of small intestine from these species was found to rapidly increase following glucose addition (*unpublished observation*) as in other species (Schultz & Zalusky, 1964). However, two goose gallbladders did show a glucose response under the following conditions: both of these gallbladders had a spontaneous p.d. of approximately 3.6 mV immediately after mounting, and this p.d. did not respond to the addition of 5 mM glucose. After approximately 1 hr in Ringer's which contained no glucose the p.d. had fallen to 1.5 mV. Upon addition of 5 mM glucose at this time to the mucosal solution the p.d. of one gallbladder increased from 1.5 to 4.1 mV (SCC increased from 55 to 145 μamp/cm^2) and the p.d. of the other gallbladder increased from 1.4 to 3.7 mV (SCC increased from 50 to 130 μamp/cm^2). These changes took place within 15 sec and were not observed when glucose was added to only the serosal bathing solution. This electrical response may result from increased rates of cellular metabolism providing additional energy supplies to the transport machinery since gallbladder mucosa is not known to have coupled Na-sugar transport capability as in small intestine (Goldner, Schultz & Curran, 1969).

Cation Replacement Experiments

Ion substitution experiments were performed on goose and monkey gallbladders to determine the ionic requirements for maintenance of SCC and spontaneous p.d. The Na requirement was studied by sequentially reducing the Na concentration (choline substitution) in the solution bathing both sides of the tissue from 142 mEquiv/liter to zero in four steps. Following each dilution the SCC was allowed to stabilize before the next dilution was made. In the three goose gallbladders studied SCC and p.d. were linearly dependent upon the Na concentration (Fig. 1), as previously demonstrated in human gallbladder (Rose *et al.*, 1973). In the absence of Na the p.d. was not significantly different from zero. Three monkey gallbladders, two *Cercopithecus aethiops* (African Green), and one Rhesus, also had a linear relationship between SCC and Na concentration. The linear relationship between SCC and Na concentration indicates that the active transport mechanism in these gallbladders does not become saturated by Na at external Na concentrations up to 142 mM. Because this relationship occurs with a constant Cl concentration in the bathing solution, it seems likely that Na transport from mucosa to serosa is the primary generator of the p.d. with Cl having little contribution. Diamond (1962*a*) found that removal of Na from the bathing solution eliminated fluid absorption by fish gallbladder. In gallbladders of goose

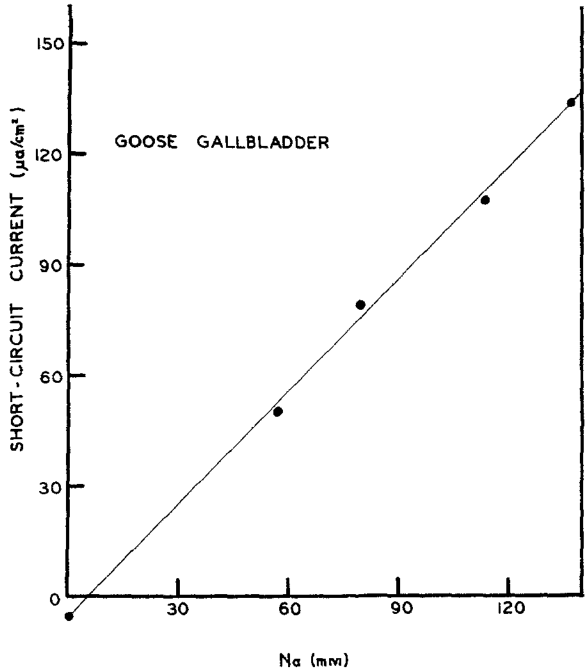

Fig. 1. The effect on short-circuit current of reducing the Na concentration in the mucosal and serosal bathing solutions of a representative goose gallbladder. Choline replaced Na

and monkey, complete substitution of Na by choline resulted in a transitory reversal in polarity of the transmural p.d. ranging from −7.0 to −19.0 mV, which is similar to the earlier report on human gallbladder. Opposite transients of 0.6 to 2.0 mV were recorded when control bathing solution again bathed the tissue.

The Na concentration in the mucosal and serosal bathing solutions was also reduced to zero by abrupt, complete replacement by K. In seven observations on gallbladders of goose this resulted in a transitory reversal in polarity of the p.d. such that the serosal solution became negative with respect to the mucosal solution by 4 mV. After 15 to 30 min in Na-free solution, the p.d. stabilized near zero. Complete replacement of Na by K in the solution bathing the gallbladder of African Green monkeys resulted in a reversal of p.d. polarity in two of five observations. Possible causes of the transitory reversal of the polarity include differing permeabilities to K or Na at opposite surfaces of the cell membrane, (Schultz & Zalusky, 1964), asymmetry of unstirred layers at the mucosal and serosal cell borders (Barry & Diamond, 1970) or the so-called polarization p.d. (Wedner & Diamond, 1969).

Table 2. Anion replacement studies

Bathing medium	Goose		Monkey	
	SCC (μamp/cm^2)	ΔSCC (%)	SCC (μamp/cm^2)	ΔSCC (%)
Control	(21) 120±10	–	(23) 70±6	–
Br for Cl	(3) 110	– 12	(4) 58	– 5
SO_4 for Cl	(6) – 11	–112	(4) 0	–100
ISE for Cl	(4) – 5	–106	(5) 37	– 59
HCO_3[a]	(3) – 10	–110	(2) 101	+ 6

[a] 25 mM $NaHCO_3$ substituted for 25 mM NaCl. ISE, isethionate. A positive SCC indicates a serosa-positive spontaneous p.d.

Anion Replacement Experiments

Complete replacement of Cl in the bathing solutions by Br for approximately 20 min reduced SCC by 12% in three goose gallbladders and by 5% in monkey gallbladders (two African Green, two Stumptail) (Table 2). There was no change in tissue resistance. Upon return to control conditions SCC returned to near its original value within 20 min.

With SO_4 as the replacement anion, goose and monkey gallbladders had no significant p.d. Transitory serosa-negative p.d.'s of 2 to 6 mV were observed in both species stabilizing near zero after 20 to 40 min. In contrast, human gallbladder maintained 70% of its original SCC when SO_4 replaced Cl (Rose *et al.*, 1973). Tissue resistance in goose and monkey gallbladders increased 10 to 53 Ω cm^2 with sulfate replacement.

Isethionate (ISE) replacement for Cl in the goose gallbladder resulted in a transitory 3- to 4-mV reversal of polarity of the p.d. which then gradually approached zero. However, in three of five monkey gallbladders tested, ISE replacement for Cl reduced SCC by only 40%. The gallbladder of one Rhesus monkey maintained an SCC of 94 μamp/cm^2 for 30 min. The other two monkey gallbladders behaved in a manner similar to that observed with SO_4 replacement. Tissue resistance in goose and monkey gallbladders increased by 16 to 38 Ω cm^2. Diamond (1962*a*) found that replacement of Cl by Br had little effect on fluid absorption but replacement by SO_4 in the mucosal solution nearly eliminated absorption. Maximal rates of fluid absorption in rabbit gallbladder depended on the presence of Na and Cl in the mucosal bathing solution (Wheeler, 1963).

In rabbit gallbladder, the presence of HCO_3 has been shown to double the rate of NaCl transport (Diamond, 1964) and make the tissue more serosa-negative (Machen & Diamond, 1969). The p.d. we report in Table 1 for rabbit gallbladder (–0.2 mV) is less serosa-negative than that reported by Machen and Diamond (1969; −1.4 mV) because the bathing solution in the present study contained no HCO_3. In other experiments (Rose & Long, *unpublished observations*) the addition of 25 mM HCO_3 resulted in a p.d. closer to the previous report. Three goose gallbladders bathed in buffered electrolyte containing 25 mM HCO_3 had a lower SCC than under control conditions. In contrast, two gallbladders from monkey and one from man had small increases in SCC when HCO_3 was added. One observation in dog gallbladder showed the SCC increasing from −16 μamp/cm^2 to −32 μamp/cm^2 upon HCO_3 addition along with the development of a more serosa-negative p.d.

Ouabain Effect

In experiments in which ouabain (5×10^{-4} M) was added to the serosal bathing solution of goose gallbladder SCC was reduced by 50% in 17 min (n = 3); addition of ouabain to the mucosal solution reduced SCC by 50% in 30 min (n = 2). In monkey gallbladder 50% inhibition took 15 min when ouabain was applied to the serosal surface (n = 3) and 22 min when applied to the mucosal surface (n = 2, Fig. 2). Thus ouabain is somewhat

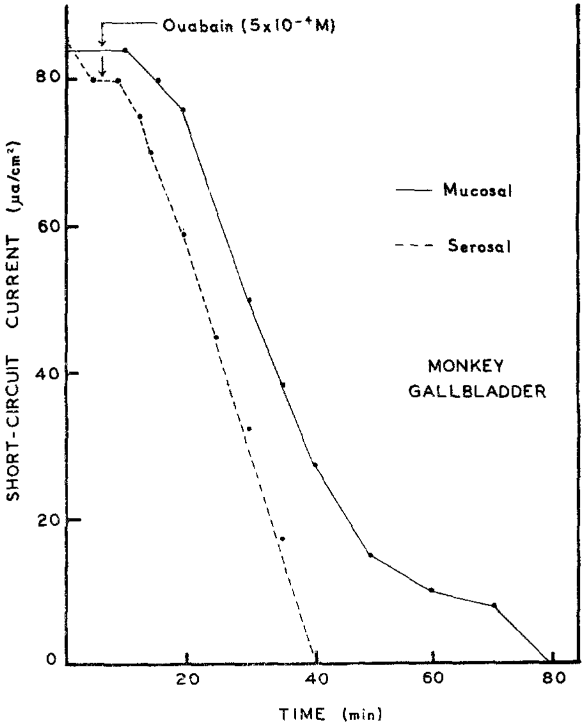

Fig. 2. The effect of ouabain in the mucosal or serosal bathing solution on the short-circuit current of gallbladders from two African Green monkeys

more effective in inhibiting electrical properties when exposed to the serosal, rather than the mucosal, surface which is consistent with the probable effect of ouabain on the Na-K stimulated ATPase located at the serosal or lateral border of the cell membrane (Post, Merritt, Kinsolving & Albright, 1960). Ouabain was also effective in reducing the p.d. of dog gallbladder, in one case from the initial value of −1.8 mV to zero in 20 min.

Anaerobiosis

Replacement of O_2 by N_2 for 5 min resulted in a 73% reduction of SCC in three goose gallbladders (Fig. 3) and a 70% SCC reduction in three monkey gallbladders. Upon restoration of O_2 at this time SCC was re-established nearly to its original level. Continued exposure to N_2 resulted in a gradual decline in SCC to zero. Re-establishment of O_2 after 40 min of anaerobic conditions resulted in no increase in SCC. The plateau noticed in all experiments upon O_2 deprivation (Fig. 3) could be

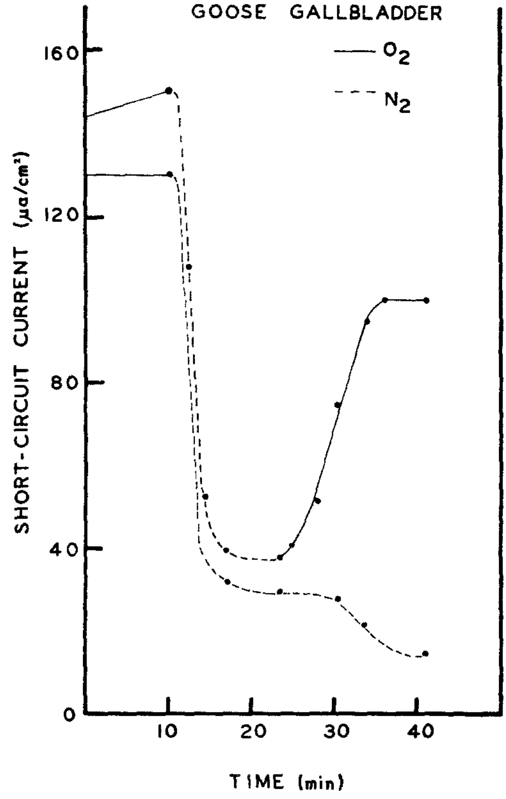

Fig. 3. The effect of anaerobiosis on short-circuit current of paired samples of a goose gallbladder. The effect is partially reversible if O_2 depletion lasts 15 min

due to stores of metabolic energy capable of maintaining minimal transport until they are gradually depleted. Anaerobic conditions did not result in an increase in tissue resistance in gallbladders of goose and monkey as it did in human gallbladder (Rose *et al.*, 1973). Approximately 45 sec of anaerobic conditions were required for an initial tissue response; upon restoration of O_2 following anaerobic conditions an effect was evident in 25 sec. The p.d. of gallbladders from two mongrel dogs (initial values 0.6, 0.7 mV) and one Dalmatian (initial value 2.6 mV) also decreased by approximately 50% under anaerobic conditions and returned upon restoration of O_2. No N_2 response was detected in three dog gallbladders with spontaneous p.d.'s ranging from -0.2 mV to $+0.2$ mV.

Temperature Effects

A temperature sensitivity of the electrogenic Na pump has been reported in neuron (Carpenter & Alving, 1968), frog skeletal muscle (Cross, Keynes & Rybova, 1965), cat heart muscle (Page & Storm, 1965), and rat smooth muscle (Taylor, Paton & Daniel, 1969). Raising the temperature of the bathing solution from 37 to 42 °C resulted in an SCC increase in the goose gallbladder (Fig. 4) of 35 μamp/cm² ($Q_{10} = 2.2$) and a 17% decrease in

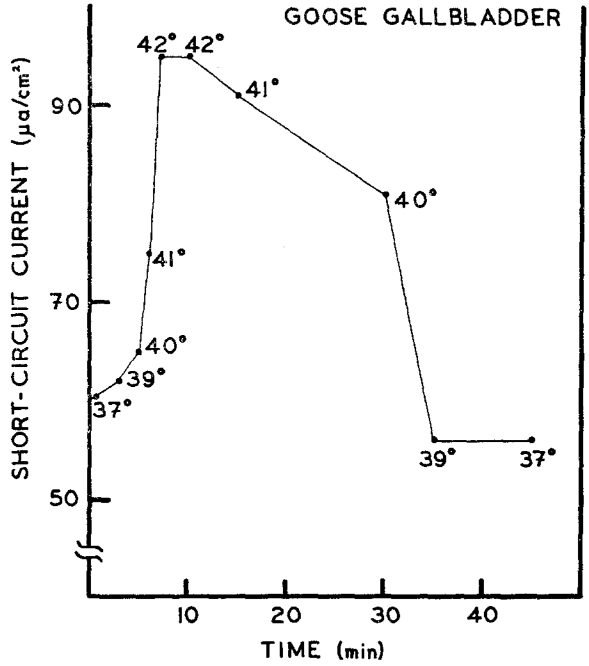

Fig. 4. The effect on the short-circuit current of goose gallbladder of raising the bathing solution temperature to 42 °C

tissue resistance. Upon cooling to 37 °C, SCC and resistance returned to near their initial value. Warming the monkey gallbladder beyond 42 °C resulted in an irreversible decrease of SCC to zero (Fig. 5).

Cooling the bathing solution to 0 to 4 °C reversibly abolished p.d. and SCC in gallbladders of goose, monkey and man (Fig. 6). Resistance increased to 250 to 300% of initial values, reaching as high as 145 Ω cm^2 in one human gallbladder. Warming the solutions abruptly back to 37 °C resulted in a slight hyperpolarization in all tissues followed by return to the original SCC and p.d. The time required to re-establish the original SCC and p.d. appeared to depend only on how fast the bathing solution could be rewarmed to 37 °C. In one goose gallbladder, the rise from an SCC of essentially zero at 4 °C to near the original value of 150 μamp/cm^2 took only 60 sec. Similar results were obtained in gallbladders of monkey and man. The rapidity of these SCC and p.d. changes indicates that they are not due to changes in intracellular ion concentrations. Rather, these results are consistent with a more direct coupling of electrolyte transport and electrical properties, such as a temperature-sensitive electrogenic pump.

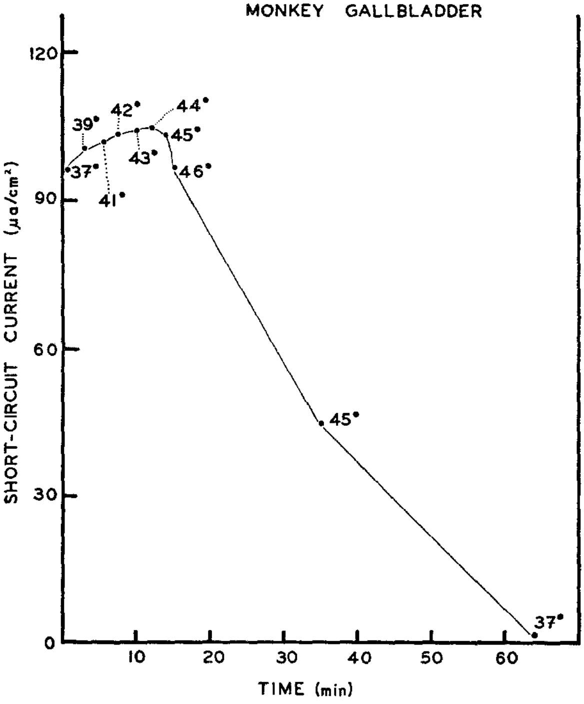

Fig. 5. The effect of temperatures higher than 42 °C on short-circuit current of African Green monkey gallbladder

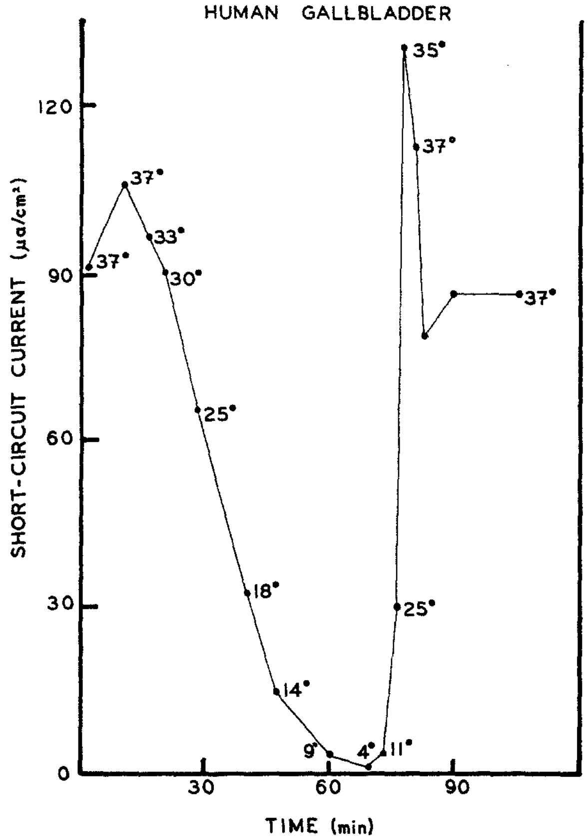

Fig. 6. The effect on short-circuit current of lowering to 4 °C the temperature of the solution bathing human gallbladder

Diffusion Potentials

Diffusion potentials were artificially established across the gallbladders of rabbit, dog, goose, monkey and man by replacing the mucosal bathing solution with one containing one-half the NaCl concentration present at the serosal surface; mannitol was added to maintain osmotic equilibrium. The resulting diffusion potentials remained stable for 3 to 5 min and then declined toward zero, similar to the previous report in rabbit gallbladder (Barry, Diamond & Wright, 1971). Diffusion potentials measured in goose, monkey and human gallbladders were corrected for the lower spontaneous serosa-positive p.d. which occurs when Na concentration is reduced in the absence of an ion gradient (Fig. 1). The corrected potentials were then used with the constant-field equation to calculate the permeability ratio of Cl/Na. The P_{Cl}/P_{Na} values at 37 °C are given in Table 3.

Table 3. Established diffusion potentials and Cl/Na permeability ratios at 37 °C

Species	2:1 NaCl Diffusion potential (mV)	P_{Cl}/P_{Na}
Rabbit (9)	− 8.4±0.7	0.32
Dog (11)	− 3.7±1.1	0.63
Stumptail (4)	−12.0±0.7	0.16
Rhesus (4)	− 8.3±1.2	0.32
African Green (6)	− 9.1±0.7	0.29
Goose (14)	− 8.9±0.4	0.30
Man (9)	− 2.2±0.5	0.76

Sign of the diffusion potential refers to the serosal surface. Number of observations in parentheses.

Table 4. Electrical properties of gallbladders at 22 °C

Species	p.d. (mV)	R (Ω cm^2)	2:1 NaCl Diffusion potential (mV)	P_{Cl}/P_{Na}
Rabbit (4)	−0.1±0.1	34	−4.2±0.9	0.57
Dog (4)	+0.1±0.2	65	−0.4±0.3	0.94
African Green (5)	+0.6±0.1	59	−4.3±1.0	0.57
Goose (12)	+0.4±0.1	41	−4.8±0.4	0.52
Man (5)	+1.6±0.2	59	−0.2±0.5	0.98

In all tissues studied, including the ileum of rabbit and man (*unpublished observation*), the P_{Cl}/P_{Na} ratio was less than 1.00 indicating that these tissues are more permeable to Na than to Cl. The P_{Cl}/P_{Na} value of 0.32 for rabbit gallbladder is consistent with the value (0.33) obtained by Dietschy (1964). Similar experiments were carried out at 22 °C (Table 4). The spontaneous p.d. measured in HCO_3-free control Ringer's at 22 °C remains essentially unchanged in rabbit and dog gallbladders. However, the spontaneous p.d.'s of goose, monkey and human gallbladders are markedly reduced at 22 °C. The tissues increased in resistance 30 to 50% at 22 °C and became less cation-selective, as previously noted by Machen and Diamond (1969). These permeability changes represent a higher Q_{10} for Na permeation than for Cl, as would be expected if most Cl permeation were in a shunt. Alternatively, to the extent that gallbladder epithelial cells might imbibe water and swell at low temperatures, the resulting occlusion of lateral intercellular spaces could restrict NaCl diffusion through this extracellular pathway accounting for the increase in tissue resistance and decrease in cation selectivity.

Table 5. Properties of gallbladders after removal of epithelium

Species	Tissue resistance (Ω cm^2)			Diffusion potential[a] (mV)
	Initial	Following epithelial removal	% loss	
Rabbit (5)	23	6	74	+1.1
Dog (6)	49	26	47	+1.3
Stumptail (2)	23	12	48	+1.5
Rhesus (2)	29	11	62	+2.4
African Green (2)	33	13	61	+0.9
Goose (6)	38	10	74	+1.5
Human (3)	36	11	69	+2.3

[a] 2:1 NaCl diffusion potential after removing the epithelium with a scalpel blade.

Epithelial Removal

Wright and Diamond (1968) stated that the submucosal and serosal tissue resistance accounted for less than 6% of the total tissue resistance in rabbit gallbladder. To determine the contribution of the epithelium to total tissue resistance and to the measured diffusion potentials we removed the epithelial layer of gallbladder from five species by scraping with a scalpel and by exposure to chloroform (Table 5). In rabbit gallbladder an average of 26% of the total resistance still remained after scraping. Histological examination of the epithelial layer revealed it to have been effectively removed by the combined scraping and exposure to chloroform. These results indicate that the conductance of gallbladders is controlled primarily by the epithelium, but to varying degrees in different species. Diffusion potentials established across these tissues after scraping were small in magnitude with a serosa-positive orientation. The serosal tissue remaining after scraping apparently has no cation-anion selectivity and the small serosa-positive potential probably results from the greater mobility of Cl in free solution. This indicates that the epithelium is responsible for the gallbladder's ability to discriminate between cations and anions, as previously concluded by Wright and Diamond (1968).

Discussion

Most epithelial tissues studied have been found to develop a serosa-positive transmural p.d. which is dependent either directly or indirectly on active transport of cations. In contrast, studies *in vitro* on the gallbladders of fish, guinea pig and rabbit have shown p.d.'s close to zero.

On the basis of these and other observations, a model of electrolyte absorption by gallbladder has been presented which includes electrically neutral transport of NaCl (Diamond, 1962*b*; Wheeler, 1963; Dietschy, 1964) across the lateral cell membrane into the lateral intercellular space (Diamond & Bossert, 1967). The transport mechanism is thought to create a slightly hyperosmotic NaCl solution in the lateral intercellular space near the tight junction. Thus, the −1.4 mV serosa-negative p.d. across maximally transporting rabbit gallbladder was postulated to be a consequence of Na tending to diffuse slightly faster than Cl from the hypertonic solution across the cation-selective tight junction to the mucosal bathing solution (Machen & Diamond, 1969).

With the discovery of gallbladders having serosa-positive p.d.'s it was necessary to investigate the possibility that local osmotic gradients create diffusion potentials which account for the observed transmural p.d.'s. Thus, the model of electrically neutral NaCl transport could apply to the gallbladders of goose, monkey and man; the conditions would have to be that these gallbladders are anion-selective rather than cation-selective so that preferential diffusion of Cl from a hyperosmotic solution in the lateral intercellular space across the tight junction could account for the serosa-positive p.d.

At 37 °C the artificially established diffusion potentials across all gallbladder epithelia were serosa-negative; therefore these gallbladders are cation-selective. The P_{Cl}/P_{Na} ratios range from the most discriminatory value of 0.16 for gallbladder of Stumptail monkey to 0.76 for gallbladder of man. At 22 °C the permeability ratios for all tissues increased significantly, with P_{Cl}/P_{Na} ratios for dog and man approaching unity. But, under no condition did the P_{Cl}/P_{Na} ratio exceed unity; these tissues were consistently cation-selective. In order for the serosa-positive p.d.'s of human, goose and monkey gallbladders to result from a local osmotic gradient as in Diamond's model, these gallbladders would have to be anion-selective so that diffusion of Cl down its concentration gradient from the lateral intercellular space to the mucosal solution would result in a serosa-positive p.d. The major route for ion permeation is believed to be the high conductance pathway across the epithelium consisting of the lateral intercellular space and the tight junction, by-passing the cell interior (Wright, Barry & Diamond, 1971).

Although the anion-cation selectivity properties of gallbladders from man, goose and monkey are of the wrong orientation to account for the observed spontaneous transmural p.d.'s, the possibility exists that the local osmotic gradient described by Machen and Diamond (1969) does produce an electromotive force, in this case tending to reduce the magnitude of the serosa-positive p.d.

The source of the electromotive force responsible for creating the serosa-positive p.d. in gallbladders of man, goose and monkey remains unknown. The immediate dependence of the p.d. on Na in the bathing solution and on temperatures near 37 °C is consistent with electrogenic transport of Na from mucosa to serosa. Total dependence of the p.d. on a concentration gradient of K between intracellular and extracellular fluids does not seem likely because these gradients would not be expected to change rapidly enough to account for changes in p.d. seen with anaerobiosis and temperature changes. The definitive experiments to demonstrate electrogenic Na transport in gallbladder, however, have not yet been performed.

The most difficult finding to reconcile with an electrogenic Na pump in gallbladders of goose and monkey is the observation that replacement of Cl by SO_4 or ISE reduces the p.d. Since the membrane is more permeable to Cl than to SO_4 or ISE it would be expected that the replacement anions would be less able to follow actively transported Na and a greater charge separation would result. A possible explanation for these observations may tentatively be offered on the basis of the recent report by Dugas and Frizzell (1974) that part of the Na flux across the mucosal membrane of rabbit gallbladder is coupled to Cl flux in the same direction. If the influx of Na and Cl across the mucosal border of goose and monkey gallbladders is also coupled, removal of Cl from the bathing solution would limit the amount of Na available to the active transport mechanism at the serosal and/or lateral cell membrane. The p.d. would then be smaller due to a lower rate of Na absorption. The dependence of Na influx across the mucosal border of goose and monkey gallbladders on the presence of Cl has not yet been evaluated; nor has the rate of fluid absorption been compared in the presence and absence of Cl in the bathing solution. However, fluid absorption by the gallbladder of rabbit was found to be lower when Cl in the bath was replaced by SO_4 (Diamond, 1962*a*) or by ISE (Wheeler, 1963).

The mean spontaneous p.d. of dog gallbladder is near zero, but values of individual tissues range from −1.8 to +2.6 mV. One dog gallbladder with a p.d. of −0.6 mV was stimulated by HCO_3 to a more serosa-negative value of −1.8 mV. This p.d. was subsequently abolished by the addition of ouabain. Serosa-positive p.d.'s in three dog gallbladders were substantially reduced by elimination of O_2 from the bathing solution; they subsequently recovered in aerobic conditions. Thus, it appears that individual gallbladders, even within a single species, may develop serosa-positive or serosa-negative p.d.'s during absorption.

To reconcile the data on electrical properties of gallbladders from all species we must consider the possibility that each gallbladder has two important electromotive forces associated with absorption: one emf may result from electrogenic transport of Na from mucosa to serosa tending to make the tissue serosa-positive and the other emf could result from the presence of a local NaCl gradient across the cation-selective tight junction tending to develop a serosa-negative p.d. The observed spontaneous p.d. across a gallbladder would then be the net effect of these two opposing emf's modified, to an undetermined extent, by tissue conductance ion concentration gradients between intracellular and extracellular fluids and possibly by other tissue properties.

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