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Effects of Bicarbonate on Fluid and Electrolyte Transport by the Guinea Pig Gallbladder: A Bicarbonate-Chloride Exchange

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Summary. Fluid transport and net fluxes of Na, K, Cl and HCO_3 by guinea pig gallbladder were investigated *in vitro*. A perfused gallbladder preparation was devised to simultaneously study unidirectional fluxes of ^{22}Na and ^{36}Cl. The net Cl flux exceeded the net Na flux during fluid absorption in the presence of HCO_3. This Cl excess was counterbalanced by a net HCO_3 secretion: a HCO_3-Cl exchange. PGE_1 reversed the direction of fluid transport and abolished the net Cl flux. The magnitude of the HCO_3 secretion remained unchanged, but shifted from a HCO_3-Cl exchange to a net secretion of $NaHCO_3$ and $KHCO_3$. Furosemide inhibited both the HCO_3-Cl exchange and HCO_3 secretion after PGE_1 without influencing fluid absorption. Ouabain inhibited the HCO_3-Cl exchange as well as fluid absorption; only the effect on the HCO_3 secretion was entirely reversible. Secreted HCO_3 appeared not to be derived from metabolic sources since HCO_3 secretion was abolished in a HCO_3-free bathing medium. HCO_3 secretion was also dependent on the Na concentration of the bathing fluid. Three lines of evidence are presented in favor of an active HCO_3 secretion in guinea pig gallbladder. HCO_3 is secreted against: (i) a chemical gradient, (ii) an electrical gradient and (iii) the direction of fluid movement under control conditions.

Bicarbonate is known to enhance the rate of fluid transport across gallbladder and a variety of other epithelia, e.g., rat proximal tubule (Ullrich, Radtke & Rumrich, 1971). The majority of previous studies relating to gallbladder fluid transport mechanisms in general and to HCO_3 in particular have confined themselves to studies of rabbit gallbladder. In this species HCO_3 both stimulates fluid absorption and is itself absorbed (Diamond, 1968).

Early in the present study, described in this and a companion paper (K. Heintze, K.-U. Petersen & J. R. Wood, *unpublished*), we observed, in contrast to

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the above mentioned results in rabbit gallbladder, a net HCO_3 secretion during fluid absorption by guinea pig gallbladder. This secretion was counterbalanced by Cl absorption in excess of that of Na. HCO_3 secretion has been observed not only under basal conditions but also during fluid secretion after serosal exposure to prostaglandin E_1 (PGE_1) (Heintze *et al.*, 1975). The fluid secreted contained HCO_3, Na, and K (Heintze *et al.*, 1976). Thus the guinea pig gallbladder is comparable to the rabbit ileum where HCO_3 secretion has been found under control conditions and after drug application (Field, 1974), but differs from the rat proximal tubule (Ullrich *et al.*, 1971), the rat jejunum (Hubel, 1973) and the rabbit gallbladder (Diamond, 1968), which show a net absorption of HCO_3.

The present paper deals with experiments designed to characterize the nature of the HCO_3-Cl exchange and its relation to the PGE_1-induced secretion of HCO_3.

Materials and Methods

Experiments were performed upon gallbladders from male guinea pigs (body weight 350–450 g). The animals were killed by a blow on the neck and the gallbladder was quickly excised. A 20-cm polyethylene cannula was tied into the cystic duct and the preparation was rinsed several times with Ringer's solution. All experiments were performed at 37°C.

Measurement of Fluid Transport and Net Ion Fluxes

Gallbladders were filled with about 1.5 ml of the test solution to a hydrostatic pressure of approximately 10 cm H_2O (Heintze, Petersen & Busch, 1978) and suspended in a bath containing 250 ml of the same solution. After a 40-min equilibration period fluid transport was measured by weighing the gallbladder on a precision balance at 10 to 15 min intervals (Diamond, 1962). Fluid transport was measured over 30 min under control conditions; thereafter drugs were applied to the serosal or mucosal side. In the latter case gallbladders were washed and refilled with the control solution containing the drug. To determine net ion fluxes, the gallbladder lumen was washed and refilled after the 40-min equilibration period. The preparation was weighed, reimmersed in the serosal bathing solution, and weighed again after a 90-min period. The luminal pH and pCO_2 were measured as described below, and the remaining luminal fluid was withdrawn for the determination of ion concentrations. Drugs, when used, were present at the appropriate side throughout the experiment, including the equilibration period and the washing and refilling procedures. Rates of fluid transport and net ion fluxes are expressed in $\mu l/cm^2$ hr and $\mu eq/cm^2$ hr, respectively, by calculating the surface area from the initial filling volume.

Net ion fluxes were calculated from the formula:

$$v_f \cdot c_f - v_i \cdot c_i / A \cdot t \tag{1}$$

where v = volume (ml); c = concentration (mM); A = surface area (cm^2); t = time (hr); i = initial; f = final.

Measurement of Transepithelial Unidirectional Fluxes

A different preparation was used to measure transepithelial fluxes (Fig. 1). A second catheter was tied into the gallbladder fundus. This allowed continuous perfusion (1 ml/min) of both the mucosal and serosal sides. Before use, the mucosal perfusate was gassed with 95% oxygen + 5% carbon dioxide. Thus, this preparation differed from the sac preparation in that oxygen had access to both sides of the gallbladder. The volume of the serosal bath was 10 ml. Intraluminal pressure was maintained at about 10 cm H_2O. $^{22}NaCl$ and $Na^{36}Cl$ (final sp act 280 and 1600 cpm/μM, respectively) were added to one side and samples were collected from the opposite side at 5 min intervals for 40 min after a steady state had been reached. The mean of these fluxes represents the unidirectional flux. The isotope containing compartment was then rinsed several times and the opposite unidirectional flux was measured. Following the application of a drug, unidirectional fluxes were redetermined. When serosa to mucosa fluxes were measured, the serosal bath containing the isotopes was not perfused. No attempt was made to correct the specific activities of the labeled compounds for the loss due to transport and diffusion which represent a change in activity of less than 0.5%. Gallbladder surface area was calculated using the formula for a prolate spheroid:

$$2\pi b\left(b + a\,\frac{\arcsin\frac{1}{a}(a^2-b^2)^{\frac{1}{2}}}{\frac{1}{a}(a^2-b^2)^{\frac{1}{2}}}\right). \tag{2}$$

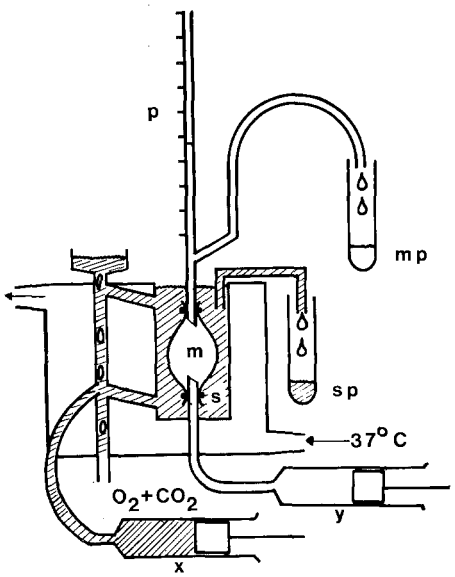

Fig. 1. Diagrammatic representation of the apparatus used to measure tracer fluxes across the gallbladder wall. The two sides of the mounted gallbladder are perfused at a constant rate from the two syringes *x* and *y*. The serosal compartment (hatched areas) was oxygenated by the gas lift shown. *m* = mucosal side; *s* = serosal side; *mp* = mucosal perfusate; *sp* = serosal perfusate; *p* = hydrostatic pressure

The major and minor axes a and b were measured from photographs taken during the control period of each individual experiment at a hydrostatic pressure of 10 cm H_2O.

^{22}Na was determined with a γ-scintillation counter (LKB Wallac), ^{36}Cl with a β liquid scintillation counter (Searle, Nederlands, B.V.) by using a cocktail of Triton x-100/Toluol with PPO and POPOP (purchased as Quickscint 402 from Zinsser, Frankfurt/M).

The serosa to mucosa flux (J_{sm}) and the mucosa to serosa flux (J_{ms}) of Na and Cl were calculated from the following formulae:

$$J_{sm}^{Na} = s_\gamma/\text{s.a.} \cdot t \cdot A \tag{3}$$

$$J_{ms}^{Na} = (s_\gamma \cdot V - R)/\text{s.a.} \cdot t \cdot A \tag{4}$$

$$J_{sm}^{Cl} = (s_\beta - s_\gamma \cdot F)/\text{s.a.} \cdot t \cdot A \tag{5}$$

$$J_{ms}^{Cl} = ((s_\beta - s_\gamma \cdot F)V - R)/\text{s.a.} \cdot t \cdot A. \tag{6}$$

$s_{\beta,\gamma} = \beta$ or γ activity of the sample (cpm); s.a. = specific activity (cpm/μM); t = time of the collecting period (hr); A = surface area (cm^2); V = volume factor (serosal bathing volume divided by sample volume); R = cpm in the serosal bathing volume at the beginning of the individual sample period; F = factor relating the β-emission of ^{22}Na to its γ-emission; the calibration curve obtained with varied concentrations of ^{22}Na and ^{36}Cl was linear in the range of the measured activities.

The net flux is given by $J_{ms} - J_{sm} = J_{net}$. Passive unidirectional fluxes were measured at 4°C as a check on the method. In 4 experiments performed at 4°C unidirectional fluxes were: $J_{ms}^{Na} = 3.11 \pm 0.34$; $J_{sm}^{Na} = 3.14 \pm 0.32$; $J_{ms}^{Cl} = 2.65 \pm 0.49$, and $J_{sm}^{Cl} = 2.71 \pm 0.55$ μeq/cm^2 hr.

Chemical Measurements

Na and K were determined by flame photometry (Eppendorf), Cl by a Cotlove chloridometer (Buchler), and pH and pCO_2 by means of an acid-base analyzer (Radiometer). HCO_3 was calculated by using the equation

$$\text{pH} = \text{pK}_1 + \log \text{HCO}_3/\text{pCO}_2 \cdot q' - 0.5\omega^{\frac{1}{2}}/(1 + \omega^{\frac{1}{2}}) \tag{7}[1]$$

(Edsal & Wyman, 1958). ($pK_1 = 6.352$; ω (ionic strength) = 0.160; q' (Henry's law coefficient for carbon dioxide) = 3.12×10^{-5}).

The luminal pH and pCO_2 were measured by using a glass capillary introduced into the gallbladder lumen. Samples of luminal fluid were aspirated directly to the acid-base analyzer, thus avoiding contact of the sample with the atmosphere. The gallbladder remained in its bathing solution during this procedure.

Solutions, Chemicals, and Statistics

The Ringer's solution contained (in mM): Na, 142.1; K, 7.0; Ca, 2.0; Mg, 1.2; Cl, 124.3; HCO_3, 25.0; H_2PO_4, 1.2; glucose, 5.5; pyruvate, 5.0. This solution was gassed with a mixture of 95% O_2 and 5% CO_2 to yield a pH of 7.5. In experiments where HCO_3 was omitted,

[1] $0.5\omega^{\frac{1}{2}}/(1 + \omega^{\frac{1}{2}})$ is a term relating pK_1 to pK_1', i.e., the pK value in a saline.

it was substituted by an equimolar amount of Cl and gassed with 100% O_2. The pH was titrated to 7.4; owing to the pH lability of the unbuffered solution, the gallbladders were exposed to pH values varying from 7.1 to 7.5. In some experiments Na was partially replaced by choline. The solutions bathing both sides of the gallbladder were always of equal composition.

Chemicals: Na-furosemide was obtained from Hoechst AG; PGE_1 was a gift from Dr. J. Pike, the Upjohn Company, Kalamazoo, Michigan. ^{22}Na and ^{36}Cl were obtained from Amersham-Buchler; all other reagents were purchased from Merck AG.

Statistics: Parallel control experiments were performed with each experimental group. Throughout, mean values of n experiments with standard error of the mean (SEM) are presented. Statistical analysis was performed using the t test.

Results

Fluid and Electrolyte Transport under Control Conditions (25 mM HCO_3)

The rate of fluid absorption under control conditions was 31.1 ± 3.1 μl/cm² hr (n = 7). A different group is represented in Fig. 2*a* depicting net ion fluxes for Na, K, Cl and HCO_3 as measured over the 90 min transport period.

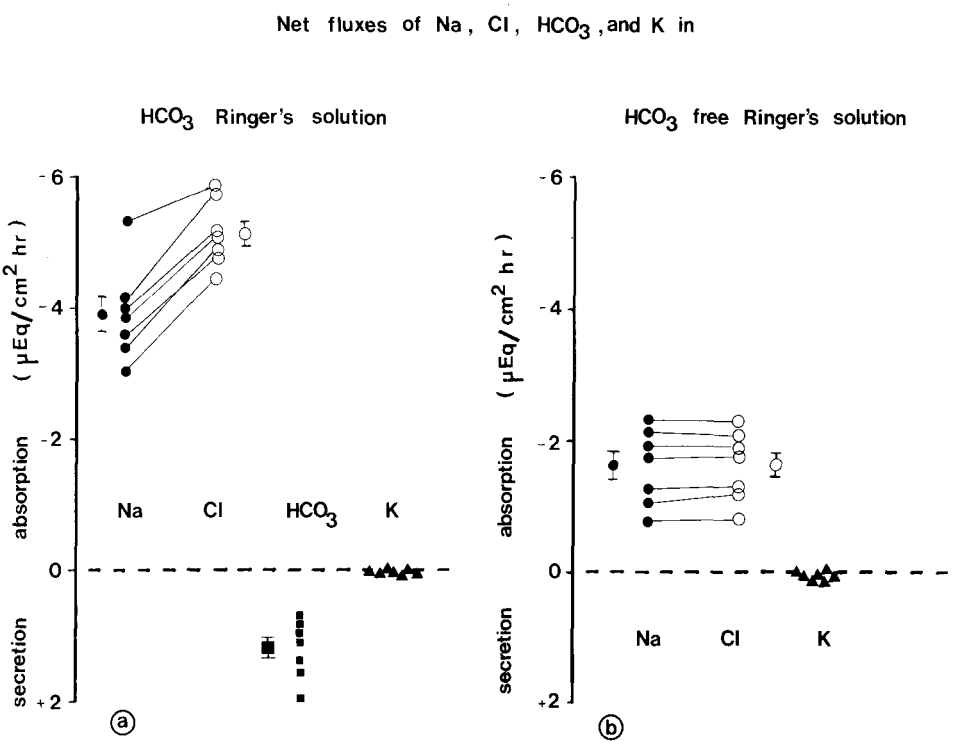

Fig. 2. (*a*): Net fluxes of Na, Cl, HCO_3 and K in HCO_3 Ringer's solution in 7 gallbladders. (*b*): Net fluxes of Na, Cl, HCO_3 and K in HCO_3-free Ringer's solution in a second group of 7 gallbladders. Values for Na and Cl in individual experiments are connected by a line.

In each experiment the net chloride flux (J^{Cl}_{net}) exceeded the net sodium flux (J^{Na}_{net}). The mean J^{Cl}_{net} of 5.1 ± 0.2 μeq/cm² hr was significantly greater ($p < 0.005$) than the mean J^{Na}_{net} of 3.9 ± 0.24 μeq/cm²hr. This difference ($J^{Cl}_{net} - J^{Na}_{net}$) was equal in magnitude to an opposing HCO_3 flux ($J^{HCO_3}_{net}$). The net K flux was zero. Over the 90-min period, the average increases in the luminal pH and pCO_2 were 0.054 ± 0.005 and 5.77 ± 0.57 mm Hg, respectively. The calculated increase in HCO_3 concentration was 7.94 ± 0.38 mM.

Effects of HCO_3-Free Media on Transport

Symmetrical removal of $NaHCO_3$ from both bathing solutions (replacing it with 25 mM NaCl) decreased the net fluid absorption by 65.3 ± 6.25% to 10.8 ± 1.0 μl/cm² hr. Transfer of these gallbladders into a solution with 25 mM HCO_3 restored fluid absorption to 29.5 ± 4.0 μl/cm² hr, demonstrating the reversibility of the decline of fluid transport in HCO_3-free media. Under HCO_3-free conditions the net K flux remained zero, the net fluxes of Na and Cl were reduced, and the excess absorption of Cl over Na was abolished (Fig. 2*b*).

From the above results three transport processes may be postulated, namely: (i) HCO_3-dependent Na and Cl absorption since removal of HCO_3 results in an inhibition of net NaCl and fluid absorption by approximately two thirds; (ii) HCO_3-independent Na and Cl absorption, defined as the absorption rate remaining after removal of HCO_3; (iii) HCO_3-Cl exchange as reflected by the excess in net Cl absorption over that of Na and the opposing HCO_3 flux. The following experiments were designed to investigate various aspects of the HCO_3-Cl exchange.

Na Requirement for HCO_3-Cl Exchange

HCO_3 secretion was found to be dependent on the Na concentration of the bathing solution. As shown in Fig. 3, the HCO_3 secretion increased with increasing Na concentration. The rate of HCO_3 secretion in a solution containing 144 mM Na was found to be independent of the rate of Na absorption (Fig. 4).

Effects of Inhibitors on Net Ion Fluxes

Added to the serosal bathing fluid 10^{-4}M furosemide had no effect on the net fluxes, whereas 10^{-3} M applied to either side virtually abolished the HCO_3-Cl exchange but was without effect on the net fluxes of K (0.05 ± 0.01 μeq/cm² hr under all four conditions investigated in Fig. 5) and Na and on net fluid absorption (Fig. 5 and Table 1).

In contrast, serosal application of 10^{-6} M PGE_1 reversed the net Na flux and the direction of net fluid transport. Though the net Cl flux was markedly inhibited during this secretion, the net HCO_3 secretion remained unchanged and a net K secretion was observed (Table 1). 10^{-3} M ouabain applied to the serosal side, inhibited fluid absorption as well as the HCO_3-Cl exchange (Table 2). The inhibi-

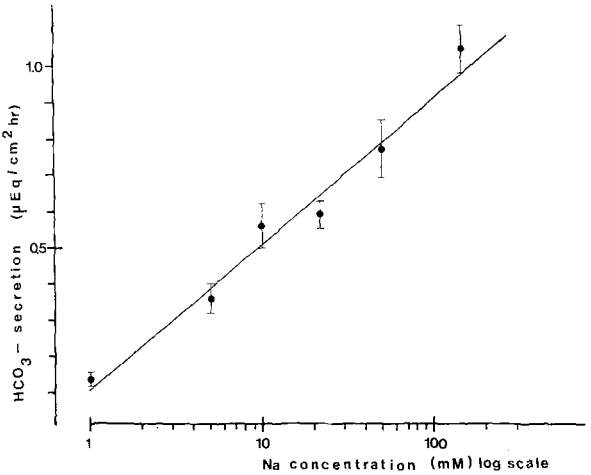

Fig. 3. Relation between HCO_3 secretion and Na concentration. Each value represents the mean ± SEM of 5-7 experiments where Na was substituted for by choline in a HCO_3 Ringer's solution (25 mM)

tion of Na and Cl fluxes and of fluid absorption was only slightly reversible when the serosal surface was washed by replacing the external bathing solution with fresh Ringer's solution at 5 min intervals for 20 min. In contrast, washing completely restored the net HCO_3 secretion and the corresponding Cl excess over the net Na absorption (Table 2). Fluid transport and net ion fluxes determined in the three groups of control experiments did not differ significantly from each other.

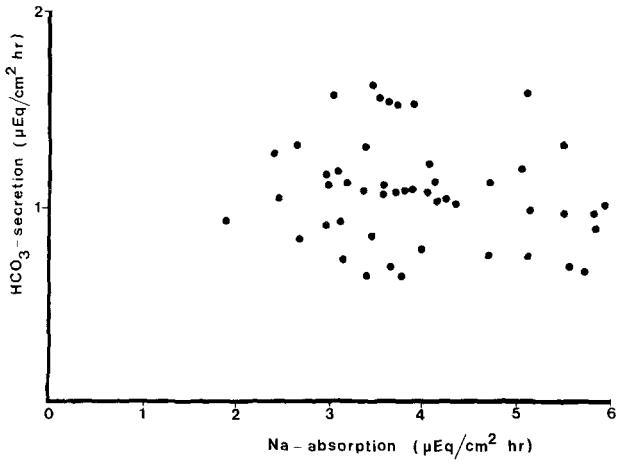

Fig. 4. Relation between HCO_3 secretion and net Na absorption in Ringer's solution containing 142 mM Na and 25 mM HCO_3

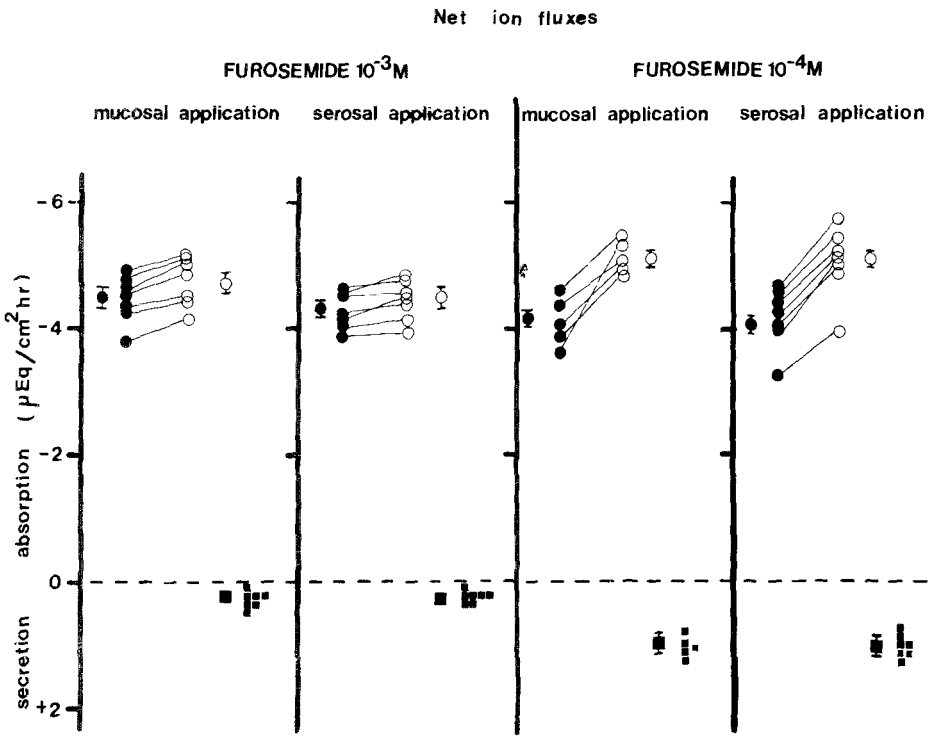

Fig. 5. Net fluxes of Na (●), Cl (○), and HCO_3 (■) in the presence of furosemide 10^{-3} or 10^{-4} M applied to the serosal or mucosal solution. Values for Na and Cl in individual experiments are connected by a line. Mean ± SEM values are shown. The SEM's of the means of the HCO_3 fluxes in the presence of 10^{-3} M furosemide were too small for graphic representation. Net fluxes in the presence of 10^{-4} M furosemide were not different from control values.

Unidirectional Fluxes of ^{22}Na and ^{36}Cl

The Na dependence of the HCO_3-Cl exchange raises the question whether Na ions are required to accompany the secreted HCO_3 or whether this dependence reflects some other facilitatory role. To examine this question, we measured unidirectional ^{22}Na and ^{36}Cl fluxes under control conditions and in the presence of furosemide, using the perfused gallbladder preparation. Fig. 6 shows the serosal-mucosal and mucosal-serosal fluxes of Na and Cl in a typical control experiment and demonstrates the stability of the preparation for flux determinations over a 3-hr period. Calculation of the net Na and Cl fluxes confirms the above finding of an excess Cl absorption over that of Na using non-isotopic methods (Table 3). Absolute values for the net Na and Cl fluxes were somewhat higher than those found by using the sac preparation (Fig. 2a). This difference may result from the better oxygen availability to the mucosa in the perfused preparation. 10^{-3} M furosemide, applied to the serosal side, virtually abolished the excess in Cl absorption (Table 3); these findings are in close

Table 1. Fluid absorption and net ion fluxes under control conditions and after serosal application of furosemide or PGE_1

Treatment		Fluid absorption (μl/cm^2 hr)	Net ion fluxes (μeq/cm^2 hr)			
			Na	K	Cl	HCO_3
Controls	$n = 8$	-26.83 ± 1.52	-4.31 ± 0.22	$+0.03 \pm 0.04$	-5.22 ± 0.15^{d}	$+1.17 \pm 0.28$
Furosemide (10^{-3}M)	$n = 7$	-26.80 ± 1.65	-4.30 ± 0.13	$+0.05 \pm 0.02$	-4.56 ± 0.12^{b}	$+0.22 \pm 0.03^{c}$
PGE_1 (10^{-6} M)	$n = 8$	$+6.32 \pm 0.85^{a}$	$+0.66 \pm 0.11^{a}$	$+0.34 \pm 0.07^{b}$	-0.31 ± 0.10^{a}	$+1.25 \pm 0.12$

Values represent mean ± SEM. A negative sign (−) indicates absorption (net transport from mucosa to serosa) and a positive sign (+) secretion (net transport from serosa to mucosa).

[a,b,c,d] Statistically different from control at $P < 0.0005$; $P < 0.0025$; $P < 0.005$; from control Na absorption at $P < 0.0025$, respectively.

Table 2. Fluid absorption and net ion fluxes under control conditions, in the presence of ouabain and after removal of ouabain

Treatment		Fluid absorption ($\mu l/cm^2$ hr)	Net ion fluxes ($\mu eq/cm^2$ hr)			
			Na	K	Cl	HCO_3
Controls	$n = 6$	-29.4 ± 1.25	-4.81 ± 0.25	$+0.02 \pm 0.02$	-5.73 ± 0.22^{c}	$+0.97 \pm 0.03$
Ouabain (10^{-3} M)	$n = 6$	-1.17 ± 0.32^{a}	-0.15 ± 0.04^{a}	$+0.03 \pm 0.02$	-0.16 ± 0.10^{a}	$+0.21 \pm 0.06^{a}$
Ouabain (10^{-3} M)	$n = 7$	$-6.71 \pm 0.31^{a,b}$	$-1.24 \pm 0.10^{a,b}$	$+0.05 \pm 0.01$	$-2.23 \pm 0.06^{a,b}$	$+0.83 \pm 0.05$

Values represent means ± SEM. A negative sign (−) indicates absorption (net transport from mucosa to serosa) and a positive sign (+) secretion. In the last group of experiments 7 gallbladders were pretreated with 10^{-3} M ouabain. After a complete inhibition of fluid absorption had been obtained, gallbladders were refilled and transferred 4 times to a ouabain-free solution in 5 min intervals.

[a,b,c] Statistically different from control at $P < 0.0005$; from ouabain at $P < 0.0005$; from control sodium absorption at $P < 0.01$, respectively.

Table 3. Unidirectional fluxes of ^{22}Na and ^{36}Cl under control conditions and in the presence of furosemide

	Na (μeq/cm^2 hr)			Cl (μeq/cm^2 hr)			
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	J^R_{net}
Control period 1	11.84 ± 0.63	6.76 ± 0.40	5.08 ± 0.11	12.38 ± 0.24	6.07 ± 0.24	6.31 ± 0.24	1.07 ± 0.17
Control period 2	12.02 ± 0.48	7.18 ± 0.45	4.84 ± 0.24	12.25 ± 0.40	6.08 ± 0.37	6.17 ± 0.40	1.07 ± 0.13
$n = 5$							
Control period 1	10.72 ± 0.38	5.58 ± 0.34	5.14 ± 0.15	12.02 ± 0.37	5.72 ± 0.38	6.30 ± 0.16	1.16 ± 0.10
Furosemide (10^{-3} M) (serosally appl.)	9.85 ± 0.30	5.36 ± 0.23	4.49 ± 0.10	10.13 ± 0.23[a]	5.43 ± 0.23	4.70 ± 0.11[b]	0.22 ± 0.03[b]
$n = 5$							

J^R_{net} is calculated from $J^{Cl}_{net} - J^{Na}_{net}$; it represents either net cation movement from mucosa to serosa or net anion movement from serosa to mucosa. [a,b] Statistically different from controls at $P < 0.0025$ and $P < 0.0005$ respectively.

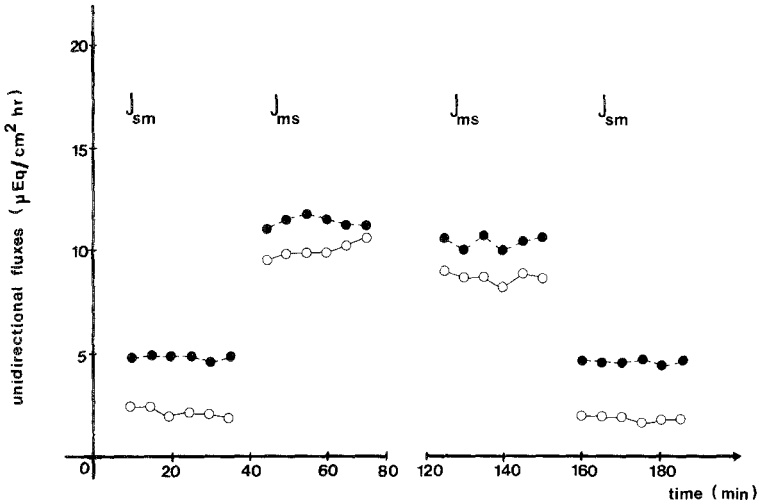

Fig. 6. Unidirectional fluxes of ^{22}Na (●———●) and ^{36}Cl (○———○) in a typical control experiment. To assess the stability of the preparation, fluxes were redetermined after a 40-min period had elapsed.

agreement with the results obtained in the net ion flux determinations (Table 1). J_{sm}^{Na} was not changed by furosemide.

Effects of Furosemide on PGE_1 Induced Net Fluid Secretion

As shown above, PGE_1 can reverse the direction of fluid transport from net absorption to net secretion. Under these conditions the net HCO_3 secretion, as observed under control conditions, remained unchanged. This raises the possibility that the PGE_1-induced net fluid secretion may result from a net movement of HCO_3. If this were true, furosemide, as an inhibitor of the HCO_3-Cl exchange, might be expected to inhibit net fluid secretion. In order to test this possibility 10^{-3} M furosemide was applied to the serosal bathing solution. Net fluid secretion was rapidly inhibited. This effect persisted as long as furosemide was present but was rapidly reversible on transfer of the preparation to Ringer's solution containing only PGE_1 (Fig. 7).

Discussion

In contrast to a number of other epithelia, the gallbladder is believed to possess only a single solute transport mechanism (Diamond, 1968). Previous studies support the concept of a coupled NaCl transport in rabbit gallbladder with a less pronounced anion specificity, as this epithelium is known also to absorb HCO_3 (Wheeler, 1963; Diamond, 1964). A mechanism for HCO_3 secretion seems to be lacking in the rabbit gallbladder. Neither HCO_3 secretion under control condi-

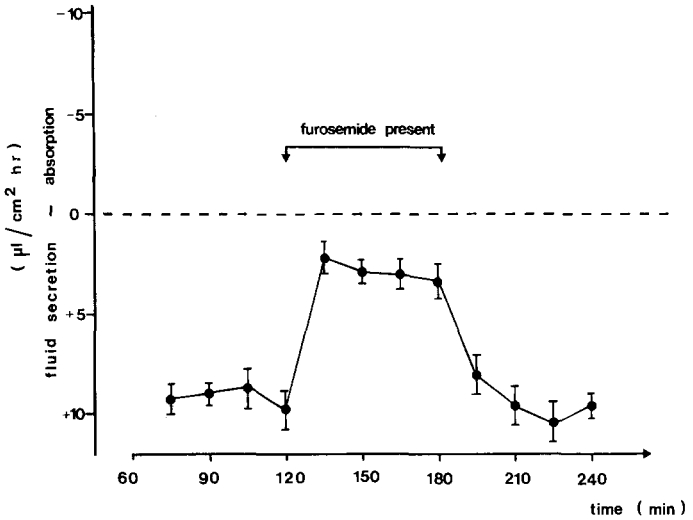

Fig. 7. Effect of 10^{-3} M furosemide on fluid secretion induced by 10^{-6} M PGE_1. Both drugs were applied to the serosal solution. PGE_1 was present throughout the entire experiment. Each point represents the mean ± SEM of 6 experiments

tions (Diamond, 1968), nor fluid secretion in the presence of PGE_1, PGE_2, or $PGF_{2\alpha}$ (Leyssac, Bukhave & Frederiksen, 1974) have been observed in this species.

The present results demonstrate, however, by two independent methods that Cl absorption by the guinea pig gallbladder exceeds that of Na when bathed in Ringer's solution containing HCO_3. This excess absorption of Cl is counterbalanced by a net HCO_3 secretion into the gallbladder lumen (Tables 1–3). HCO_3 secretion into the gallbladder lumen has been previously reported in guinea pigs (Herman, Wilson & Kazyak, 1958). These early findings are, however, difficult to interpret due to a large discrepancy between the reported anion and cation net fluxes.

In HCO_3-free Ringer's solution the HCO_3-Cl exchange was abolished and the NaCl absorption fell to about one third of the value seen in the presence of 25 mM HCO_3. Thus, the secreted HCO_3 was not derived from metabolic sources, and exogenous HCO_3 seems to be required for two transport processes, namely, a HCO_3-stimulated NaCl absorption, which will be discussed in the companion paper (Heintze *et al.*, *unpublished*) and the HCO_3 secretion.

It is difficult to determine whether the alkalinization of the gallbladder lumen reflects a HCO_3 secretion or a H absorption. In the latter case a decreased or, at best, a constant pCO_2 would be expected in the lumen. The increase in luminal pCO_2 suggests a net HCO_3 secretion. Moreover, in HCO_3-free solutions a net secretion of H is observed, as will be demonstrated in the following paper. It seems unlikely that this H secretion will be reversed during transport in a HCO_3-containing medium. Furthermore, the measurements of pH and pCO_2 could be mis-

leading due to a disequilibrium pH[2]. The good agreement between the difference in the net fluxes of Na and Cl and the HCO_3 net flux calculated from the measurements of pH and pCO_2 indicates that any existing disequilibrium pH is likely to be insignificant.

The stoichiometric exchange of HCO_3 and Cl does not indicate an obligatory coupling. When the NaCl absorption was abolished by PGE_1, the HCO_3 secretion was unchanged and comparable in its magnitude to the control values (Table 1). This finding demonstrates that HCO_3 secretion can occur in the absence of Cl absorption, and implies that we are dealing with the same HCO_3 transport mechanism under both conditions. Such a view is supported by the ability of furosemide to inhibit the HCO_3 secretion in either case.

Furosemide (10^{-3} M) was equally effective at inhibiting the HCO_3-Cl exchange when applied to either side of the gallbladder but did not affect net ion fluxes at 10^{-4} M (Fig. 5). Therefore, it cannot be decided whether it inhibits two different transport mechanisms located at the apical and basolateral membrane or an intracellular mechanism involved in the secretion of HCO_3. Findings obtained in ox red blood cells suggest that furosemide can act directly on the cell membrane to inhibit anion transport (Cousin & Motais, 1976).

A number of findings favor the assumption of HCO_3 secretion as an independent transport mechanism separate from that for NaCl absorption. The rate of the HCO_3 secretion was independent of the magnitude of the NaCl absorption (Fig. 4) and HCO_3 secretion also occurred in the absence of NaCl absorption as shown in the presence of PGE_1. Moreover, furosemide acted selectively to inhibit HCO_3-Cl exchange leaving Na and fluid absorption unchanged. The inhibitory effect of ouabain on HCO_3-Cl exchange could be dissociated from the effect on fluid absorption by its different reversibility, and finally fluid absorption was ten times more sensitive to SITS than the HCO_3-Cl exchange, as will be reported in the following paper.

The ileum of a number of species has been shown *in vivo* to secrete HCO_3 (Hubel, 1969; Powell, Malawer & Plotkin, 1968; Turnberg *et al.*, 1970), as does the guinea pig gallbladder. In the intestinal epithelium, however, the question remains unsolved whether secretion and absorption are functions of separate secretory and absorptive cells (Field, 1974). Since in the guinea pig gallbladder 95% of the epithelium are formed by a single cell type X. (L. Busch, *unpublished observation*), it seems to be justified to ascribe both absorptive and secretory function to only one cell type. Therefore, any hypothesis should take into account that both processes, absorption and secretion, are brought about by the same cell.

As in rabbit ileum (Powell, Binder & Curran, 1973) anion secretion in the guinea pig gallbladder depends on the presence of Na in the bathing solutions. These authors suggested a transepithelial $NaHCO_3$ secretion. Such a conclusion, however, is not likely to apply to the guinea pig gallbladder. Were a transcellular movement of Na to accompany the secretion of HCO_3, two events would be ex-

[2] Disequilibrium pH is observed when the change of the concentrations of H or HCO_3 is fast enough to exceed the rate at which H_2CO_3 reaches equilibrium with CO_2 (*cf.* Brodsky & Schilb, 1974).

pected: an increase of the net fluid absorption in the presence of furosemide and a reduction in the J_{sm}^{Na} under conditions where the HCO_3 secretion was inhibited by furosemide (Tables 1 and 3). The absence of such changes makes it unlikely that the Na dependence of the HCO_3 secretion (Fig. 3) reflects such a transcellular cotransport of Na and HCO_3.

The nature of the driving force of HCO_3 secretion is as yet obscure. Nonetheless, it meets several criteria for designation as an active transport process. HCO_3 secretion into the lumen is directed against its electrochemical gradient (guinea pig gallbladder has a mucosal negative transepithelial potential difference [Henin *et al.,* 1977]) and cannot be explained by solvent drag. It is further characterized by its dependence on external sodium and its inhibition by ouabain.

There are at least two alternative explanations of active HCO_3 secretion. Firstly, the guinea pig gallbladder might possess a secretory system for HCO_3 directly dependent on metabolic energy, most probably on ATPase. In fact, Kinne-Saffran and Kinne (1974) described a HCO_3-stimulated ATPase in the rat proximal tubule, but found insensitivity to ouabain to be one important feature of this enzyme. Secondly, Frizzell, Field and Schultz (1978) recently suggested that Cl secretion by the rabbit colon might be attributable to a Na-coupled Cl entry into the secretory cell across the basolateral membrane. Active transcellular secretion would thus be provided with energy by the Na gradient across this membrane. An analogous interaction of Na and HCO_3 might occur in the guinea pig gallbladder and energize HCO_3 secretion. Frizzell *et al.* (1978) referred to such a process as a secondary active transport. The site of $NaHCO_3$ cotransport, however, cannot be localized at the present time. Hence a coupled secretion of $NaHCO_3$ across the apical membrane cannot be excluded.

In summary, the transport processes most consistent with our findings are a coupled $NaHCO_3$ entry at the basolateral membrane and, alternatively, a coupled $NaHCO_3$ exit across the mucosal border. The movement of HCO_3 across the opposite cell membrane would involve an exchange of HCO_3 for Cl. Recycling of Na across the membrane where it participates in HCO_3 cotransport would account for the excess in net Cl absorption over that of Na under control conditions. Accordingly, transepithelial HCO_3 transport would display the characteristics of a Na-dependent HCO_3-Cl exchange.

It is of interest that Na-dependent HCO_3 secretion has recently been observed also in perfused cortical collecting tubules of rabbit kidney. This transport was independent of Na absorption and of the transepithelial voltage and continued in the absence of Cl in the bathing solution (McKinney & Burg, 1978).

In the presence of PGE_1 the magnitude of HCO_3 secretion remained unchanged but shifted from a HCO_3-Cl exchange to a net secretion of $NaHCO_3$ and $KHCO_3$ (Table 1). The prostaglandin effects might be explained on the basis of observations in other epithelia, where they are accompanied by an increase in intracellular cAMP levels in the intestinal mucosa (Kimberg *et al.*, 1974) and in the guinea pig gallbladder (Heintze, 1977); cAMP in turn inhibits the NaCl influx across the luminal membrane in rabbit ileum (Nellans, Frizzell & Schultz, 1973) and rabbit gallbladder (Frizzell, Dugas & Schultz, 1975). These findings are likely to account for the inhibition of the NaCl and fluid absorption in the guinea

pig gallbladder in response to PGE_1. However, the nature of coupling between HCO_3 and the accompanying cations cannot be decided on the basis of the present data and deserves further investigation.

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