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Effects of Bicarbonate on Fluid and Electrolyte Transport by Guinea Pig and Rabbit Gallbladder: Stimulation of Absorption

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Summary. The effect of bicarbonate (HCO_3) on fluid absorption by guinea pig gallbladder was investigated *in vitro*. Stimulation of fluid absorption was concentration dependent resulting in a fourfold increase in transport over the range 1 to 50 mM. Phosphate, Tris, glycodiazine and glutamine buffers failed to substitute for HCO_3 in stimulating absorption. Unidirectional ^{22}Na fluxes were measured across short-circuited sheets of guinea pig and rabbit gallbladders mounted in Ussing-type chambers. In both species the net Na flux was unaffected by serosal HCO_3 alone but was stimulated by addition of HCO_3 to the mucosal bathing solution. Transepithelial electrical potential difference in rabbit gallbladder was about 1.4 mV (lumen positive) when HCO_3 was present in the mucosal or in both compartments. This fell to 0.2 mV under HCO_3-free conditions or when HCO_3 was present only in the serosal solution. The respective values for guinea pig gallbladder were -1.6 and -0.6 mV (lumen negative). HCO_3 stimulation of Na absorption by guinea pig gallbladder was abolished by increasing the bathing pH from 7.4 to 7.8, an effect resulting mainly from a reduction in J_{ms}^{Na}. Tris buffer (25 mM) inhibited HCO_3-dependent fluid absorption in this species completely at pH 8.5 and partially at 7.5. These results indicate that HCO_3 stimulates gallbladder transport in both species by an action from the mucosal side. This effect cannot be attributed to simple buffering of H^+ but may be explained by the participation of HCO_3 in the maintenance of intracellular H^+ for a Na/H-exchange.

Key words: gallbladder, NaCl absorption, HCO_3, Na/H-exchange, active transport

Evidence has recently been presented (Heintze, Petersen, Olles, Saverymuttu & Wood, 1979) for an essential role of exogenous HCO_3 in two transport processes in the guinea pig gallbladder:

a) a HCO_3 secretion

b) an absorption of NaCl stimulated by HCO_3.

A stimulatory effect of HCO_3 on the transport of Na has been observed in a number of other tissues such as rat proximal tubule (Ullrich, Radtke & Rumrich, 1971), rat jejunum (Podesta & Mettrick, 1977), rabbit ileum (Field, Fromm & McColl, 1971), cat pancreas (Schulz, 1971), frog choroid plexus (Wright, 1977) and toad bladder (Chen & Walser, 1977). In the case of the gallbladder most studies have been performed in the rabbit, where about half of the fluid absorption is HCO_3-dependent (Diamond, 1964). In contrast to the above-mentioned epithelia HCO_3 is absorbed only to a minor extent in this tissue (Wheeler, 1963). The stimulatory effect of HCO_3 on NaCl absorption in the guinea pig gallbladder occurs even in the presence of a net HCO_3 secretion into the lumen. Thus the latter epithelium appears particularly suitable for studies on HCO_3-dependent transport.

The present study described in this and a companion paper (Petersen, Wood, Schulze & Heintze, 1981) sought to examine the conditions and possible mechanism of the HCO_3 stimulatory effect in guinea pig and rabbit gallbladder.

Materials and Methods

All experiments were performed at 37 °C. Male guinea pigs (body weight 350–450 g) were killed by a blow on the neck and rabbits of either sex (body weight 3–4 kg) were killed by i.v. injection of pentobarbital (60 mg/kg). The gallbladder was quickly excised, rinsed several times with the appropriate solution and prepared for measurement of fluid transport or unidirectional fluxes.

Measurement of Fluid Transport

Gallbladders were cannulated and filled with the test solution to a hydrostatic pressure of approximately 10 cm H_2O (Heintze, Pe-

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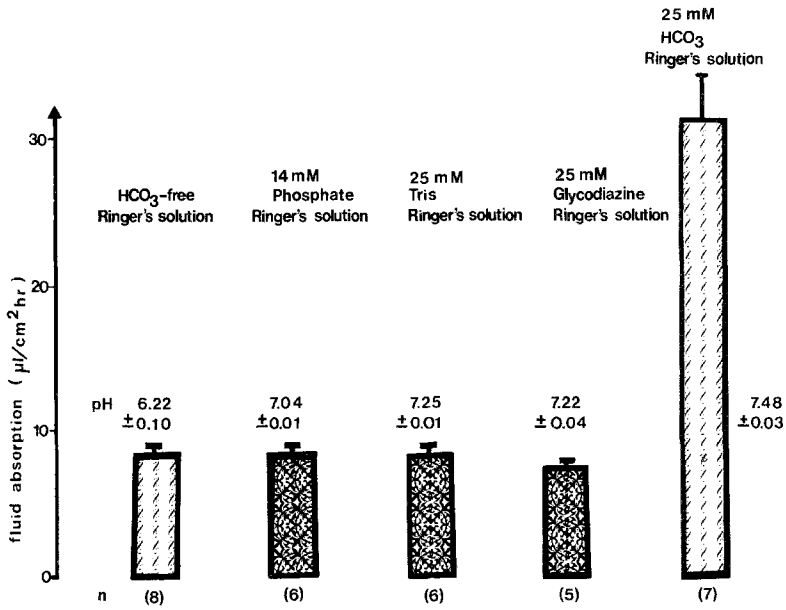

Fig. 1. Effect of substitution of HCO_3 by various buffers on fluid absorption by guinea pig gallbladder. Solutions are of identical osmolarity (obtained by addition of NaCl). Final intraluminal pH values after the 90-min transport period are shown. Each value represents the mean ±SEM of 5–8 experiments

tersen & Busch, 1978) and suspended in an organ bath. They were refilled after a 40-min equilibration period and fluid transport was measured gravimetrically (Diamond, 1962). At the end of each experiment intraluminal pH and in some instances pCO_2 were measured by means of a glass capillary introduced into the gallbladder lumen. Samples of luminal fluid were directly aspirated to an acid-base analyzer (Radiometer).

Surface area was calculated from the initial filling volume assuming a sphere.

Electrical and Transepithelial Flux Measurements

For electrical measurements the gallbladder was mounted as a flat sheet between the halves of a symmetrical Lucite chamber as described by Schultz and Zalusky (1964). The area of tissue exposed to the bathing solutions was 0.6 cm^2.

A circle of high viscosity silicone gel (Bayer AG) was applied to the bevelled inner rim of the interface between the two chamber halves to minimize edge damage. Each half-chamber (volume 1 ml) was directly attached to a vertical reservoir (volume 9 ml). The contained solution was mixed and oxygenated by a gas lift and maintained at 37 °C.

Transepithelial potential difference (V_{ms}) was recorded using agar bridges (prepared with Ringer's solution) positioned within 2 mm of each surface of the tissue and connected through matched calomel half-cells to a Keithley 179 TRMS digital multimeter.

To measure tissue resistance an external current of 50 μA was passed through silver-silver chloride electrodes in saturated KCl connected to Ringer's-agar bridges in contact with the bathing solution at the end of each chamber. Transepithelial Na fluxes were measured in paired tissues under steady-state conditions using the above chambers. ^{22}Na was added to one side of the chamber and duplicate samples (1-ml samples replaced by an equal volume of unlabeled solution) were collected from the opposite side at 10-min intervals during a 30-min period. The tissues were short-circuited throughout the experiment except when V_{ms} readings were being taken. In each tissue fluxes were measured over three or four 30-min periods employing different experimental conditions arranged in different sequences to minimize bias.

Since unidirectional fluxes were measured in short-circuited tissues, permeabilities could readily be calculated from J_{sm} assuming that J_{sm} is passive (Frizzell & Heintze, 1980).

^{22}Na was measured using a γ-scintillation counter (LKB Wallac).

Chemical Measurements, Solutions and Chemicals

pH and pCO_2 were determined and HCO_3 concentration calculated as described previously (Heintze et al., 1979). The Ringer's solution contained (in mM): Na 143.4, K 5.0, Ca 1.2, Mg 1.2, Cl 122.0, HCO_3 25.0, H_2PO_4 1.2, glucose 5.0, pyruvate 5.0. It was gassed with 5% CO_2 in O_2 to yield a pH of 7.4–7.5. Under HCO_3-free conditions NaH_2PO_4 was omitted and HCO_3 replaced with 25 mM Cl (subsequently termed HCO_3-free solution) or various buffers: 14 mM Na_2HPO_4/NaH_2PO_4, 25 mM Tris (osmolarity maintained by addition of sodium chloride), 25 mM glutamine or 25 mM glycodiazine. All HCO_3-free solutions were titrated to a pH of 7.4 and bubbled with 100% O_2. Unless otherwise stated, the solutions at both faces of the tissue were identical.

Chemicals and Drugs: Glycodiazine was a gift from Dr. D.E. Schultze, Schering AG, Berlin; isotopes were purchased from Amersham Buchler; glutamine and all other reagents were obtained from Merck AG.

Means and their standard errors (SEM) are presented throughout. Parallel control experiments were performed with each experimental group. Statistical analysis of the data was performed using the paired or unpaired *t*-test as appropriate.

Results

Effect of HCO_3 Replacement by other Buffers

The rate of fluid absorption by guinea pig gallbladder is substantially reduced by omission of HCO_3 from the bathing medium. To determine whether the stimulation of NaCl absorption by HCO_3 reflects its buffering property, fluid absorption was measured in solutions where HCO_3 was replaced by phosphate, Tris buffer or glycodiazine. Transport rates with these buffers did not differ significantly from those obtained under unbuffered conditions where HCO_3 had been replaced by Cl (Fig. 1). The initial intraluminal pH for each test solution was 7.4. In HCO_3 Ringer's

Table 1. Side specificity of the HCO_3 stimulation of Na absorption by guinea pig and rabbit gallbladder and effect of CO_2 on Na absorption by guinea pig gallbladder

Species	Test solution	n	J_{Na} (μEq/cm² hr)			P_{Na} (cm sec$^{-1}\cdot 10^{-6}$)
			J_{ms}	J_{sm}	J_{net}	
Guinea pig	HCO_3-free	7	6.7±0.7[a]	5.6±1.3	1.1±0.3[a]	11.2±2.3
	HCO_3 serosal	7	7.7±0.8[b]	5.7±1.1	2.0±0.7[a]	11.6±1.8
	HCO_3 both sides	7	11.2±0.7	5.4±0.6	5.8±0.7	11.1±1.2
	HCO_3-free+5% CO_2	4	8.6±0.7[e]	5.0±0.6	3.6±0.3[b,f]	9.8±0.9
Rabbit	HCO_3-free	4	22.7±3.4[d]	15.7±1.4	6.9±2.6[c]	29.7±2.6
	HCO_3 serosal	4	23.4±2.4[b]	17.8±1.3	5.6±1.4[a]	33.5±0.9
	HCO_3 both sides	4	33.4±1.1	17.5±1.3	15.9±0.8	32.4±2.3

Values represent means ±SEM.

[a-e] Statistically different from HCO_3 both sides at $p<0.0005$; 0.005; 0.01; 0.0125; 0.025, respectively.

[f] Statistically different from HCO_3-free at $p<0.0005$.

solution the intraluminal pH increased by 0.08±0.03 units. In the unbuffered HCO_3-free solution the pH fell to 6.22±0.1. A less marked decrease occurred with each of the three substitute buffers (Fig. 1).

An additional difference between the HCO_3 Ringer's solution and solutions containing other buffers was the gassing procedure (95% O_2:5% CO_2 in the former and 100% O_2 in the latter). We therefore examined the effect of these different gas mixtures on Na and fluid transport by guinea pig gallbladder. HCO_3-free solution was gassed with 5% CO_2. By addition of NaOH a pH of 6.8 was achieved which remained constant during the 30-min experimental period. J_{net}^{Na} was 3.6 μEq/cm² hr, a value significantly higher than that obtained with 100% O_2 ($p<0.0005$) but lower than with 25 mM HCO_3 ($p<0.005$) (Table 1). These changes in J_{net}^{Na} are in agreement with the corresponding changes in fluid absorption. In a further group of experiments ($n=6$) the rate of fluid transport in a HCO_3-free solution increased from 10.9±0.9 to 20.4±1.2 μl/cm² hr ($p<0.0005$) when the bathing medium was bubbled with 95% O_2 and 5% CO_2 in place of 100% O_2. In these experiments the extent of spontaneous HCO_3 formation from CO_2 was approximately 3 mM in the serosal bath and 2 mM in the luminal fluid. Final pH was 6.44±0.01 in the lumen and 6.65 in the serosal bathing solution.

Effect of HCO_3 Replacement by Glutamine

A previous report indicated a stimulatory effect of glutamine on fluid absorption by the rabbit gallbladder (Martin & Murphy, 1974). In contrast, glutamine (25 mM) failed to influence fluid absorption in the guinea pig gallbladder. The absorption rate was 9.9±0.7 μl/cm² hr over a 90-min period with a final intraluminal pH of 6.67±0.04 ($n=6$).

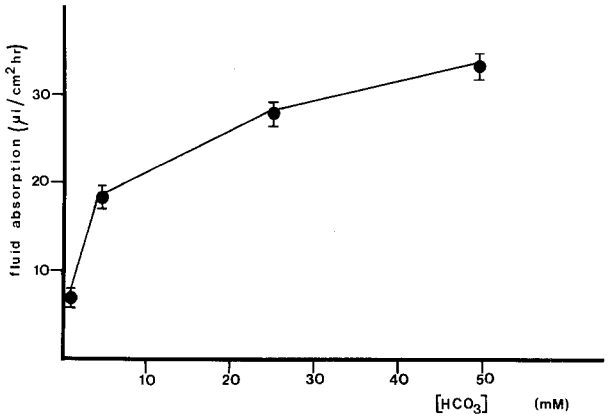

Fig. 2. Concentration dependency of HCO_3-stimulated fluid absorption. Gas composition and resulting pH were: 100% O_2, 7.3 (1 mM); 1% CO_2, 99% O_2, 7.45 (5 mM); 5% CO_2, 95% O_2, 7.45 (25 mM); 10% CO_2, 90% O_2, 7.5 (50 mM). Each value represents the mean ±SEM of 6 experiments

Side Specificity and Concentration Dependency of the Stimulatory Effect of HCO_3

The stimulatory effect of HCO_3 on fluid absorption by guinea pig gallbladder was concentration-dependent in the range 1 to 50 mM (Fig. 2). To localize any side specificity of this effect undirectional fluxes of Na were measured. J_{net}^{Na} with HCO_3 present only in the serosal bathing medium was not different from that observed in the HCO_3 free solution. In contrast J_{ms}^{Na} was stimulated when HCO_3 was present on both sides of the epithelium. This finding was noted in both guinea pig and rabbit gallbladder (Table 1). Experiments in guinea pig gallbladder with HCO_3 at the mucosal side only ($n=2$) yielded a stimulated J_{net}^{Na} ($J_{ms}^{Na}=11.8$, $J_{sm}^{Na}=5.0$ μEq/cm² hr).

Table 2 summarizes the electrical parameters measured with 25 mM HCO_3 at one side, both sides or

absent. In both species a stimulated Na absorption was concomitant with a considerable shift of V_{ms} resulting in a more positive lumen with respect to the serosal solution. Tissue conductances (G_t) (Table 2) and the transepithelial permeability of Na (P_{Na}) (Table 1) remained unchanged under all conditions examined.

Table 2. Effect of HCO_3 on transepithelial electrical potential difference (V_{ms}) and tissue conductance (G_t) of guinea pig and rabbit gallbladder

	HCO_3-free	HCO_3 serosal	HCO_3 mucosal	HCO_3 both sides
Guinea pig ($n=4$)				
V_{ms} (mV)	-1.6 ± 0.3	-1.5 ± 0.3	-0.6 ± 0.2[a]	-0.6 ± 0.2[a]
G_t (mmhos/cm^2)	8.3 ± 0.5	8.2 ± 0.7	8.5 ± 0.8	8.5 ± 0.7
Rabbit ($n=4$)				
V_{ms} (mV)	$+0.2\pm0.1$	$+0.3\pm0.1$	$+1.5\pm0.2$[b]	$+1.5\pm0.2$[b]
G_t (mmhos/cm^2)	34.3 ± 1.6	35.1 ± 1.4	32.4 ± 3.0	32.3 ± 3.7

Values represent means $\pm$ SEM. V_{ms} is referred to the serosal solution.
[a] Statistically different from HCO_3-free and HCO_3 serosal at $p<0.01$ and $p<0.025$, respectively.
[b] Statistically different from HCO_3-free and HCO_3 serosal at $p<0.0025$.

Table 3. Effect of pH and Tris buffer on HCO_3-stimulated fluid absorption by guinea pig gallbladder

Test solution	n	Initial pH	Final luminal pH	Fluid absorption (μl/cm^2 hr)
HCO_3 (25 mM)	5	7.45	7.52 ± 0.03	30.8 ± 1.8
HCO_3 (25 mM) + Tris (25 mM)	4	8.50	8.14 ± 0.07	7.1 ± 1.9[a]
	4	7.50	7.59 ± 0.04	24.3 ± 2.1[b,c]

Values represent means $\pm$ SEM. Tris was added at the expense of Na, all solutions were gassed by 5% CO_2 in O_2.
[a, b] Statistically different from Tris-free HCO_3 at $p<0.0005$; 0.025, respectively.
[c] Statistically different from Tris/HCO_3 (pH 8.5) at $p<0.0005$.

pH Dependency of HCO_3-Stimulated Transport

In the presence of a nonvolatile buffer, addition of H^+ to the gallbladder lumen will decrease the concentration of the buffer anion and simultaneously elevate that of its protonated form. In contrast, after H^+ addition to a HCO_3/CO_2 buffer part of the formed H_2CO_3 will leave the lumen as CO_2. If this reaction is relevant to HCO_3 stimulation of transport the latter must be sensitive to pH elevation and presence of an additional buffer which competes with HCO_3 for H^+. In HCO_3 Ringer's solution buffered with Tris at pH 8.5,[1] fluid absorption by guinea pig gallbladder was not different from that measured under HCO_3-free conditions. At pH 7.5 fluid absorption was partially stimulated but was significantly lower than that in HCO_3 Ringer's solution without Tris (Table 3). To investigate the pH effect in the absence of Tris, unidirectional Na fluxes were measured in 25 mM HCO_3 Ringer's solution which had been titrated to a pH of 7.8 using NaOH. The J^{Na}_{net} (Table 4) did not differ from that obtained under HCO_3-free conditions. In accordance with Table 2, inhibition of Na absorption by pH elevation caused a significant hyperpolarization of V_{ms} (Table 4).

Discussion

The present study demonstrates that HCO_3 stimulates net Na fluxes and net fluid absorption by guinea pig and rabbit gallbladder. This occurs despite the secretion of HCO_3 into the guinea pig gallbladder lumen and only a small rate of HCO_3 absorption by rabbit

[1] In the sac preparation, the luminal fluid is subject to pH changes due to epithelial transport of HCO_3 or H^+ ions. For this reason examination of pH influence on NaCl absorption requires the presence of a buffer in this preparation, while in the Ussing chamber the volume of the mucosal bathing solution is too big to be changed in its pH by epithelial function.

Table 4. Effect of pH on HCO_3-stimulated Na absorption, transepithelial potential difference (V_{ms}) and tissue conductance (G_t) of guinea pig gallbladder

pH	n	J_{Na} (μEq/cm^2 hr)			V_{ms} (mV)	G_t (mmhos/cm^2)
		J_{ms}	J_{sm}	J_{net}		
7.45	4	11.4 ± 0.6	5.5 ± 0.7	6.0 ± 0.3[a]	-0.4 ± 0.3[a]	9.1 ± 1.7
7.80	4	8.6 ± 1.4	6.8 ± 1.3	1.8 ± 0.6	-1.9 ± 0.4	10.8 ± 1.0

Values represent means $\pm$ SEM. V_{ms} referred to serosal solution. Gassing was 5% CO_2 in O_2. In the high pH experiments, pH was 8.0 at the beginning and 7.78 ± 0.03 at the end of the 30 min flux period.
[a] Statistically different from pH 7.8 at $p<0.0005$.

gallbladder. These findings eliminate the possibility that the stimulatory effect of HCO_3 may be due to net HCO_3 absorption.

The possibility was considered that the stimulatory effect of HCO_3 may be due not to the HCO_3 ion itself, but to the gassing of HCO_3-containing solutions by CO_2. The experiments examining the effect of pCO_2 on transport in the HCO_3-free solution (Table 1) indicate that CO_2 alone cannot completely restore the effect of 25 mM HCO_3. The partial effect seen may be ascribed to an action of the CO_2 alone, to the 2–3 mM HCO_3 spontaneously formed or a combination of these. With reference to the concentration-response curve (Fig. 2), the calculated HCO_3 concentration would only partially account for the observed stimulation of transport by CO_2.

Side Specificity of the HCO_3 Effect

Little attention has been paid to the side specificity of HCO_3 stimulation of NaCl absorption. To our knowledge the solutions bathing both surfaces of the gallbladder wall were of equal composition in all related studies. Our data conclusively show that HCO_3 must be present in the mucosal solution to stimulate NaCl absorption by both guinea pig and rabbit gallbladder (Tables 1 and 2). Experiments with HCO_3 at the mucosal side only permit no definite interpretation since under these conditions HCO_3 will also be present at the basolateral cell membrane due to the presence of a substantial unstirred compartment in the subserosal space. In rabbit gallbladder the transepithelial p.d. has been found to correlate with NaCl absorption (Machen & Diamond, 1969). Under conditions of reduced or abolished NaCl absorption, e.g. HCO_3 replacement with Cl, V_{ms} was about 0.2 mV compared to a mean of 1.4 mV under control conditions. This is in excellent agreement with the present study where V_{ms} was about 1.5 mV when HCO_3 was present on both sides or only on the mucosal side of the rabbit gallbladder. In contrast a low value (0.3 mV) was recorded with the HCO_3-free solution on both sides or with HCO_3 only in the serosal bathing medium. Similarly, the lumen negative V_{ms} in the guinea pig gallbladder was reduced from about -1.6 mV under unstimulated conditions to -0.6 mV in the presence of mucosal or bilateral HCO_3; i.e., the lumen became more positive as in the rabbit gallbladder. Therefore, measurements of V_{ms} also support a mucosal side of action and clearly show that HCO_3 present only at the serosal side is insufficient to restore HCO_3-dependent NaCl and fluid absorption.

A number of authors (Cremaschi & Hènin, 1975; Hènin & Cremaschi, 1975; van Os & Slegers, 1975) have concluded that the total conductance of the basolateral membrane is insufficient to permit passive Cl exit. To explain this finding, an exchange of cellular Cl for serosal HCO_3 has been proposed (Duffey, Turnheim, Frizzell & Schultz, 1978). Our data do not exclude the possibility that an additional effect of HCO_3 may be exerted at the basolateral membrane. However, evidence is as yet not available to substantiate the unpublished observation of Dugas, Frizzell and Schultz (Duffey et al., 1978) that serosal HCO_3 *alone* is sufficient to stimulate NaCl absorption in rabbit gallbladder.

Replacement of HCO_3 by Buffers in the Guinea Pig Gallbladder

In marked contrast to the small, but significant increase in intraluminal pH observed in HCO_3 Ringer's solution, acidification of the luminal fluid was found with all HCO_3-free solutions used. HCO_3 secretion appears to mask hydrogen ion secretion which is revealed under HCO_3-free conditions. Although capable of buffering the secreted H^+,[2] neither Tris, phosphate nor glycodiazine could substitute for the stimulatory effect of HCO_3. This failure demonstrates that buffering alone cannot account for the stimulatory effect of HCO_3.

Interaction of HCO_3 and H^+-Secretion

The conclusion that HCO_3 does not act to stimulate Na absorption by simple buffering of secreted H^+ does not necessarily conflict with the concept that H^+-secretion may be central to Na absorption. Evidence for a Na/H-exchange in gallbladder has been previously presented (Sullivan & Berndt, 1973; Cremaschi et al., 1979). H^+-secretion appears to be masked in the guinea pig gallbladder by the HCO_3/Cl-exchange mechanism (Heintze et al., 1979).

However, in the absence of HCO_3 net H^+-secretion was of a similar magnitude in guinea pig and rabbit.[3] Values measured in the sac preparation are a function of H^+-secretion and H^+-backflux and as such only reflect the ability of the epithelium to establish and maintain a pH gradient. The true H^+-secre-

[2] Acidification of the lumen could be accounted for by the addition of H^+ or the removal of HCO_3 or OH from the luminal fluid. There is reasonable evidence for hydrogen ion secretion in the rabbit gallbladder (Sullivan & Berndt, 1973; Cremaschi, Henin & Meyer, 1979). For reasons of convenience the acidification of the lumen is regarded as H^+-secretion in this study.

[3] Guinea pig: 204 nEq/cm² hr and 66 pEq/cm² hr in phosphate-buffered and unbuffered solutions, respectively, as calculated from Fig. 1.

Rabbit: 519 nEq/cm² hr and 65 pEq/cm² hr in phosphate-buffered and unbuffered solution, respectively; data calculated from Sullivan and Berndt (1973) assuming a surface area of 7 cm².

tion from the cell to the mucosal solution (J_{cm}) is therefore likely to be grossly underestimated in the above calculations. By titration of the acid secreted into the rabbit gallbladder lumen (pH clamping), Cremaschi et al. (1979) measured a H^+-secretion of 3.4 μEq/cm² hr. HCO_3-stimulated Na absorption was inhibited by a small elevation of the bathing pH and may therefore critically depend upon the availability of intraluminal H^+ ions. This is supported by the inhibitory effect of Tris on HCO_3-stimulated fluid absorption which was complete at pH 8.5 and partial at pH 7.5. The inhibitory effect of Tris at the lower pH may therefore result from competition of Tris with HCO_3 for luminal or intracellular H^+. Though a possible nonspecific inhibitory effect of Tris cannot be excluded, no such effect was observed under HCO_3-free conditions.

Possible Mechanisms of HCO_3-Stimulated Fluid Absorption

The mechanism by which HCO_3 stimulates Na absorption by various epithelia is ill understood. Martin (1974) proposed that HCO_3 acts to stimulate gallbladder fluid absorption by increasing transport-related energy metabolism. His study, however, deals more with the abilities of HCO_3 and glucose to preserve transport over long periods than with their ability to stimulate fluid transport. Owing to several inconsistencies in this paper a rigorous evaluation of the reported data is not possible. The hypothesis that HCO_3 exerts its stimulatory effect by serving as a substrate for synthesis of carbamyl phosphate (Martin & Murphy, 1974) has been rejected on energetic considerations (Frizzell & Heintze, 1980).

The above findings indicate the importance of an interaction between mucosally applied HCO_3 and H^+ for the HCO_3 stimulatory effect. This effect cannot be ascribed to a simple buffering of luminal acidification. Neither can stimulation be attributed to changes in P_{Na} and G_t since HCO_3 solely increased the J_{ms}^{Na}. This observation and the mucosal side specificity of the HCO_3 effect are in agreement with HCO_3 stimulation of NaCl influx in rabbit gallbladder (Cremaschi et al., 1979). The latter study postulated a HCO_3-sensitive site on the carrier which accomplishes the coupled NaCl uptake across the apical membrane. Such a model, however, does not explain the critical dependence of the HCO_3 stimulating effect on the luminal H^+ concentration. As discussed by these authors, the central issue remains, as to whether HCO_3 only modifies the carrier or is itself subject to carrier-mediated translocation in the mucosal membrane.

In view of the inherent difficulties in studying transport of the HCO_3–CO_2 system due to its volatile component (CO_2) further investigations of the mechanism of HCO_3-stimulated transport have been performed using butyrate as a HCO_3 substitute. This attempt to understand HCO_3-dependent fluid absorption, discussed in detail in the following paper (Petersen, Wood, Schulze & Heintze, 1981) includes the participation of butyrate and, by analogy, HCO_3 in the maintenance of intracellular H^+ supply for a Na/H-exchange and its recirculation to the lumen in exchange for luminal Cl. Thus, a double ion exchange (Na/H and HCO_3/Cl) is postulated, resulting in apparently coupled influx of NaCl into gallbladder cells.

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