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Stimulation of Gallbladder Fluid and Electrolyte Absorption by Butyrate

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Summary. Gallbladder fluid and electrolyte transport was investigated *in vitro*. In guinea pig gallbladder, equimolar substitution of acetate, propionate, butyrate or valerate for HCO_3 was increasingly effective in stimulating fluid absorption. The stimulatory potency of these compounds was a function of their chloroform water partition coefficients. The stimulatory effects of the isomers isobutyrate and isovalerate were less than predicted from their partition coefficients. Acidification of the gallbladder lumen, however, was strictly dependent on the partition coefficients for all of the above fatty acids. Unidirectional ^{22}Na fluxes were measured in rabbit and guinea pig gallbladders under short-circuit conditions. In the presence of butyrate stimulation of net Na flux was due entirely to an increase in the mucosal-to-serosal Na flux. Stimulation by butyrate was abolished by its omission from the mucosal bathing solution. The transepithelial electrical potential difference in both rabbit and guinea pig gallbladder became more lumen positive following mucosal but not serosal addition of butyrate. Net ^{14}C-butyrate fluxes were too small to account for stimulation of Na absorption in either species. Butyrate stimulation of Na absorption by guinea pig gallbladder was abolished by increasing the bathing pH from 7.4 to 8.1. Tris buffer (25 mM) partially inhibited butyrate-dependent gallbladder fluid absorption by rabbit and guinea pig at pH 6.4 and 7.0, respectively, and completely at pH 8.4. These results reveal a marked similarity between butyrate and HCO_3 stimulation of gallbladder NaCl and fluid absorption. The results are best explained by a double ion-exchange model, in which butyrate (HCO_3) in the mucosal solution acts to maintain the intracellular supply of H^+ and butyrate (HCO_3) for countertransport of Na and Cl, respectively.

Key words: gallbladder, NaCl absorption, HCO_3, short-chain fatty acids, Na/H-exchange, HCO_3/Cl-Exchange, active transport

Bicarbonate stimulates fluid and electrolyte absorption by guinea pig and rabbit gallbladder. This effect is dose-dependent, requires HCO_3 on the mucosal side and is abolished by an elevation in the bathing pH from 7.4 to 7.8 (Heintze, Petersen & Wood, 1981). The mechanism of HCO_3-stimulated absorption is difficult to examine directly in view of rapid interconversion of the components of the HCO_3-CO_2 system. Due to this difficulty we sought a substance with similar biological effects to HCO_3 but lacking a volatile component. The finding that short-chain fatty acids can substitute for HCO_3 in stimulation of Na transport by rat proximal tubule (Ullrich, Radtke & Rumrich, 1971) was therefore of special interest.

Of the fatty acids examined in the present study butyrate proved to be as effective as HCO_3 in stimulating absorption. This paper examines some aspects of butyrate-stimulated transport in an attempt to gain insight into the mechanism by which butyrate and HCO_3 stimulate NaCl and fluid absorption.

Materials and Methods

Experiments were performed using gallbladders from male guinea pigs (350–450 g) or rabbits (3–4 kg) of either sex.

Measurement of Fluid Transport, Ion Fluxes and Electrical Parameters

The methods employed to measure fluid absorption, unidirectional fluxes, transepithelial electrical potential difference (V_{ms}) and tissue conductance (G_t) are described in the preceding paper (Heintze et al., 1981). Unidirectional fluxes of ^{22}Na and ^{14}C-butyrate were

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measured simultaneously. ^{22}Na was determined with a γ-scintillation counter (LKB Wallac), ^{14}C-butyrate with a β-liquid scintillation counter (Searle, Netherlands, B.V.) using a cocktail of Triton X-100/Toluol with PPO and POPOP (purchased as Quickscint 402 from Zinsser, Frankfurt/M).

The β-emission of butyrate was obtained by subtraction of the sodium contribution from the β-emission of the sample.

To determine the net flux of a solute s (J^s_{net}) across the sac preparation, the gallbladder lumen was rinsed and refilled after a 40-min equilibration period. This was followed by a 90-min period during which fluid absorption was measured. Luminal concentrations of Na and K were determined by flame photometry (Eppendorf) and of Cl using a Cotlove chloridometer (Buchler). Net solute fluxes ($\mu Eq/cm^2$ hr) were calculated using the equation:

$$J^s_{net} = V_f \cdot C^s_f - V_i \cdot C^s_i / A \cdot t \quad (1)$$

where V=volume (cm^3); C=concentration (mM); A=surface area (cm^2); t=time (hr); i=initial; f=final and s=solute.

Net fluxes of butyrate were measured using ^{14}C-butyrate added to the butyrate containing Ringer's solution. Butyrate concentrations were calculated from the activities of the samples. To evaluate a possible contribution of $^{14}CO_2$ to the activity of the luminal fluid obtained during the 90-min period, samples were counted for their activities before and after removal of HCO_3/CO_2 by addition of $BaCl_2$ at pH 11 to form $BaCO_3$. There was no significant difference between the values obtained before and after this treatment.

Solutions, Chemicals and Statistics

The Ringer's solution contained (in mM): Na 142.2, K 5.0, Ca 1.2, Mg 1.2, Cl 122.0, glucose 5.0, pyruvate 5.0 and 25 of either HCO_3 or individual fatty acids as indicated. The terms butyrate-free and HCO_3-free are used interchangeably to describe a modified Ringer's solution containing Cl in place of butyrate or HCO_3. Solutions without HCO_3 were titrated to pH 7.4 with NaOH and gassed with 100% O_2. HCO_3 Ringer's solution was gassed with 5% CO_2 in O_2 to a pH of 7.5. In some solutions 25 mM Tris was added at the expense of Na.

Chemicals: ^{14}C-butyrate and ^{22}Na were obtained from Amersham Buchler. All other reagents were purchased from Merck AG.

Means and their standard errors are presented throughout.

Parallel control experiments were performed with each experimental group. Statistical analyses were performed using the paired or unpaired t-test as appropriate.

Results

Effects of HCO_3 Replacement by Fatty Acids

To determine whether the stimulation of fluid transport by HCO_3 is specific to this anion, the effect of HCO_3 replacement by short-chain fatty acids was examined. Fluid transport rates were measured in guinea pig gallbladders bathed in solutions where HCO_3 was replaced by equimolar concentrations of various fatty acids. Formate (not shown in Fig. 1 because of its different pK value; fluid absorption 10.3 ± 0.6 $\mu l/cm^2$ hr; final luminal pH 5.93 ± 0.04; $n=6$) had no effect on fluid transport. In contrast, butyrate completely restored the reduced fluid absorption (Fig. 1). Its effect remained constant over more than

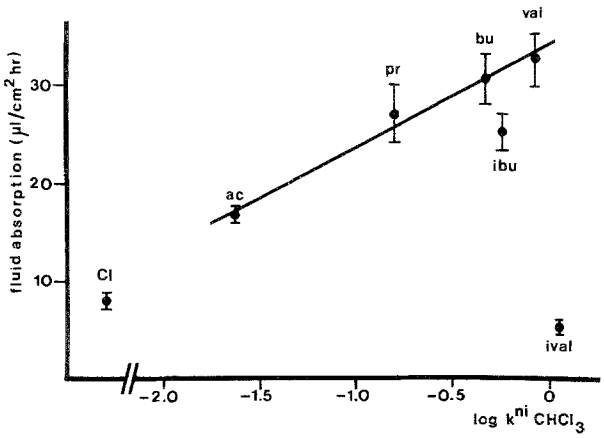

Fig. 1. Effect of equimolar substitution of HCO_3 by short-chain fatty acids on fluid absorption by guinea pig gallbladder. Rates of fluid absorption are plotted against the chloroform water partition coefficients of the fatty acids determined by Jackson et al. (1978). Each value represents the mean ± SEM of 6–7 experiments. *Cl*=chloride, *ac*=acetate, *pr*=propionate, *bu*=butyrate, *ibu*=isobutyrate, *val*=valerate, *ival*=isovalerate

240 min. The stimulatory potencies of the straight-chain fatty acids tested paralleled their chloroform-water partition coefficients (values from Jackson, Williamson, Dombrowski and Garner, 1978). The isomers isobutyrate and isovalerate had less marked effects than those predicted from their partition coefficients (Fig. 1). In contrast to isovalerate, pivalate, another isomer of valerate, partially stimulated fluid absorption (20.6 ± 1.7 $\mu l/cm^2$ hr, $n=8$, in comparison to 8.2 ± 1.6 $\mu l/cm^2$ hr, $n=6$, measured in controls in HCO_3-free solution, $p<0.0005$). Caproate was tested at the concentration of only 5 mM in view of its water solubility. Its stimulation of fluid absorption (19.8 ± 0.8 $\mu l/cm^2$ hr, $n=6$) was significantly greater ($p<0.025$) than that with 5 mM valerate (16.8 ± 0.9 $\mu l/cm^2$ hr, $n=6$).

The intraluminal pH fell over the 90-min test period with all fatty acids examined. This acidification was inversely related to the lipid solubility of the straight- and branched-chain fatty acids tested (Fig. 2). Intraluminal pCO_2 was measured in a further group of six guinea pig gallbladders exposed to 25 mM butyrate Ringer's solution (fluid absorption 32.0 ± 4.7 $\mu l/cm^2$ hr) and found after 90 min to be less than 8 mm Hg (threshold value of the acid-base analyzer).

Stimulation of fluid absorption by butyrate was concentration-dependent. The concentration-response curve for butyrate was almost identical to that obtained with HCO_3 (Fig. 3). To determine the extent to which butyrate might also substitute for Cl, fluid absorption was measured in a solution where Cl and HCO_3 were entirely replaced by butyrate (137.0 mM) and the respective sulfate salts of Ca, Mg and K.

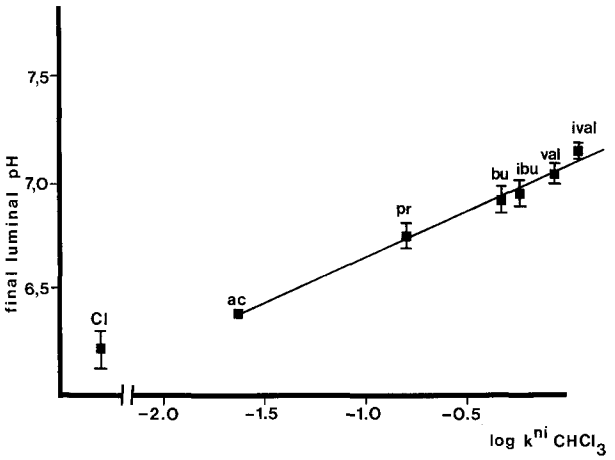

Fig. 2. Effect of equimolar substitution of HCO_3 by short-chain fatty acids on luminal pH measured after a 90-min period in guinea pig gallbladders (sac preparation). pH values are plotted versus the chloroform water partition coefficients as determined by Jackson et al. (1978). Data are from the same experiments as in Fig. 1. (For abbreviations, *see* Fig. 1.)

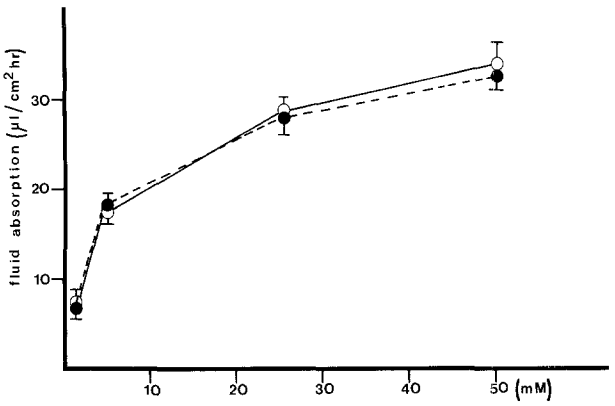

Fig. 3. Concentration dependency of butyrate-stimulated fluid absorption (open circles). The response to HCO_3 (solid circles) is shown for comparison (data from Heintze et al., 1981). Each value represents the mean ± SEM of 5–6 experiments

Absorption under these conditions was 13.5±1.1 µl/cm² hr with a final luminal pH of 7.05±0.06 ($n=6$).

Fluid absorption by rabbit gallbladder was also reduced in the HCO_3-free solution. This reduction in fluid absorption was completely reversed by equimolar substitution of HCO_3 by butyrate. The final luminal pH with butyrate was not different from that observed in HCO_3-free Ringer's solution (Fig. 4).

Net ion fluxes in the presence of 25 mM HCO_3 were measured over a 60-min period ($n=4$). The resulting J_{net} values for Na, K, Cl and HCO_3 were 15.5±0.7; −0.2±0.3; 13.8±0.7; 1.9±0.4 µEq/cm² hr, respectively (a negative sign indicating secretion).

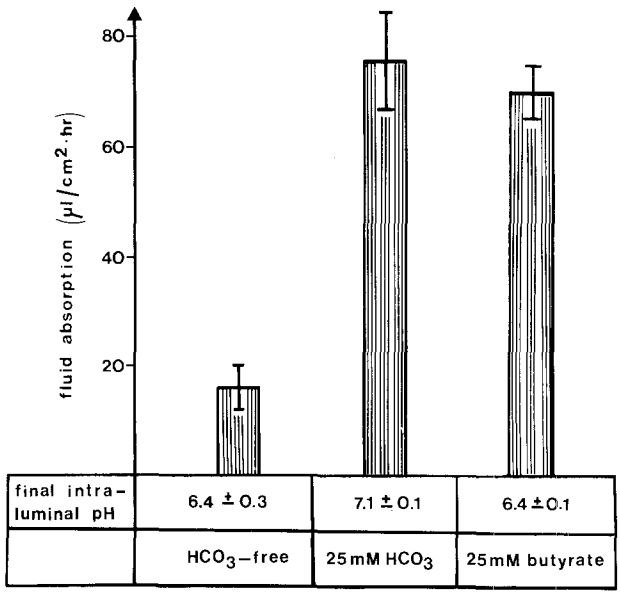

Fig. 4. Effects of butyrate on intraluminal pH and fluid absorption in rabbit gallbladder (sac preparation). Each value represents the mean ± SEM of 4–6 experiments

Side Specificity of the Stimulatory Effect of Butyrate

The above experiments using the sac preparation do not permit conclusions on the sidedness of the butyrate stimulatory effect. For this reason, unidirectional Na fluxes were measured in guinea pig and rabbit gallbladders under short-circuit conditions (Table 1). A stimulation of Na absorption was observed when butyrate was present at both sides of the gallbladder wall. In contrast, no effect was seen with serosal butyrate alone.

In both species, V_{ms} was dependent on the NaCl absorptive process. Gallbladders were exposed on each occasion for 10–15 min to different butyrate conditions as shown in Table 2. The low V_{ms} of 0.2 mV recorded in rabbit gallbladder exposed only to serosal butyrate alone showed a marked increase on addition of butyrate to the mucosal bathing solution. This increase in V_{ms} was sustained and uninfluenced by withdrawal of butyrate from the serosal bathing medium (Table 2). V_{ms} in the guinea pig gallbladder responded similarly to addition of butyrate with a sharp drop in its lumen negative potential; i.e. with a shift of V_{ms} towards a more positive lumen (Table 2).

Table 1. Side specificity of the butyrate stimulation of Na absorption and butyrate fluxes in guinea pig and rabbit gallbladder

	Na (μEq/cm² hr)			Butyrate (μEq/cm² hr)			P_{Na}
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	(cm sec^{-1} × 10^{-6})
Guinea pig (n=4)							
Butyrate-free	4.8±0.1[a]	3.8±0.1	1.0±0.1[b]				7.7±0.5
Butyrate serosal (25 mM)	4.8±0.3[b]	3.6±0.3	1.2±0.3[b]				7.6±0.5
Butyrate both sides (25 mM)	9.5±1.1	3.8±0.5	5.7±1.2	3.4±0.2	3.1±0.2	0.3±0.1	8.2±0.8
Rabbit (n=4)							
Butyrate-free	17.2±2.3[c]	13.8±1.7	3.4±0.8[b]				25.5±2.2
Butyrate serosal (25 mM)	19.1±2.2	15.0±2.0	4.1±0.7[b]				29.8±3.7
Butyrate both sides (25 mM)	24.5±0.8	14.1±2.3	10.4±1.7	5.0±0.7	2.5±0.4	2.5±0.4	29.0±3.6

Values represent means ± SEM. J_{Na} and J_{But} were simultaneously measured.
[a,b,c] Statistically different from butyrate both sides at $p<0.0025$; 0.01; 0.0125, respectively.

Table 2. Effect of butyrate (25 mM) on transepithelial electrical potential difference (V_{ms}) and tissue conductance (G_t) of guinea pig and rabbit gallbladder

	Butyrate-free	Butyrate serosal	Butyrate mucosal	Butyrate both sides
Guinea pig (n=4)				
V_{ms} (mV)	−1.6±0.3	−1.9±0.4	−0.7±0.2[b]	−0.6±0.2[a,d]
G_t (mmhos/cm²)	8.3±0.5	8.5±0.4	8.5±0.3	8.2±0.3
Rabbit (n=4)				
V_{ms} (mV)	+0.2±0.1	+0.2±0.03	+1.7±0.1[c]	+1.4±0.04[c]
G_t (mmhos/cm²)	34.3±1.6	33.6±2.7	35.1±1.4	32.4±2.1

Values represent means ± SEM. V_{ms} referred to serosal solution.
[a] Statistically different from butyrate-free at $p<0.01$ and from butyrate serosal at $p<0.0125$.
[b,c] Statistically different from butyrate-free and butyrate serosal at $p<0.025$ and at $p<0.0005$, respectively.
[d] Statistically different from butyrate mucosal at $p<0.025$.

Table 3. Fluid absorption and net ion fluxes in guinea pig gallbladder bathed by butyrate (25 mM) Ringer's solution (n=18)

Fluid absorption (μl/cm² hr)	J_{net} (μEq/cm² hr)			
	Na	K	Cl	^{14}C-butyrate
−30.4±2.0	+4.9±0.5	−0.2±0.1	+4.4±0.2	+0.4±0.04

Values represent means ± SEM. A negative sign (−) indicates secretion, a positive sign (+) absorption.

Net Butyrate Fluxes

Unidirectional fluxes of ^{14}C butyrate were measured to investigate the contribution of net butyrate absorption to the stimulation of Na transport (Table 1). The J_{net}^{But} values of 0.3 in the guinea pig and of 2.5 μEq/cm² hr in the rabbit are too small to account for the marked stimulation of Na absorption. The value for guinea pig gallbladder was confirmed by measurement of the net butyrate flux using the sac preparation with ^{14}C-butyrate present on both sides of the gallbladder wall (Table 3). The net ion fluxes in the guinea pig gallbladder (Table 3) indicate that in the presence of 25 mM butyrate cation absorption exceeded anion absorption by 0.3 μEq/cm² hr. This value is not different from the net butyrate absorption.

pH Dependency of Butyrate Stimulated Transport

To examine whether or not protonation of butyrate is involved in its stimulatory action, transport rates were measured in butyrate Ringer's solution at different pH values. In the first series, 25 mM Tris buffer was added to a 25 mM butyrate Ringer's solution (at the expense of Na) to attempt to stabilize the pH

Table 4. Effect of pH and Tris buffer on butyrate-stimulated fluid absorption by guinea pig and rabbit gallbladder

Test solution	n	Initial pH	Final luminal pH	Fluid absorption ($\mu l/cm^2$ hr)
Guinea pig				
Butyrate (25 mM)	18	7.4	6.99 ± 0.04	30.4 ± 2.0
Butyrate (25 mM) + Tris (25 mM)	4	7.0	6.88 ± 0.01	20.3 ± 2.3 [c]
	4	8.4	8.4 ± 0.01	6.4 ± 1.2 [a,d]
Rabbit				
Butyrate (25 mM)	6	7.4	6.41 ± 0.12	70.2 ± 4.8
Butyrate (25 mM) + Tris (25 mM)	4	6.4	6.30 ± 0.01	34.5 ± 4.6 [b]
	4	8.4	8.17 ± 0.03	13.9 ± 2.6 [a,e]

Values represent means ± SEM. They were measured over a 90-min period using the sac preparation.
[a, b, c] Statistically different from Tris-free butyrate (pH 7.4) at $p <$ 0.0005; 0.0025; 0.025, respectively.
[d, e] Statistically different from the low pH Tris group at $p <$ 0.0005; 0.01, respectively.

Table 5. Effect of pH on butyrate-stimulated Na absorption by guinea pig gallbladder ($n = 5$)

pH	Na ($\mu Eq/cm^2$ hr)		
	J_{ms}	J_{sm}	J_{net}
8.1	7.3 ± 1.5	5.5 ± 1.1	1.8 ± 1.3 [a]
7.4	10.8 ± 1.4	5.2 ± 1.2	5.5 ± 1.2

Values represent means ± SEM.
[a] Statistically different from pH 7.4 at $p < 0.025$.

at the final luminal pH (7.0 in guinea pig, 6.4 in rabbit) or pH 8.4. The former pH values were selected to simulate the pH conditions present in the gallbladder lumen in the absence of Tris buffer. With a pH of 8.4 at both sides of the gallbladder wall the rates of fluid absorption were not different from those in HCO_3-free unbuffered solution. At pH 7.0 or 6.4 fluid absorption was significantly stimulated in both species (Table 4), though this stimulation was significantly smaller than that observed in butyrate Ringer's solution without Tris buffer. A serosal pH of 8.4 cannot per se explain the lack of stimulation as fluid absorption was fully stimulated (34.5 ± 2.5 $\mu l/cm^2$ hr) when Tris-free butyrate Ringer's solution at pH 8.4 bathed both sides of guinea pig gallbladder ($n = 4$). Under these conditions, the gallbladder lumen was acidified to a pH of 7.03 ± 0.03, which was not different from that observed at a starting pH of 7.4.

The Ussing chamber[1] was used to examine the high pH effect in the absence of a buffer (Table 5). Elevation of the bathing pH from 7.4 to 8.1 abolished butyrate-dependent Na absorption.

[1] The higher mucosal bathing volume in the Ussing chamber compared to that in the sac preparation ensures stable pH values in the solutions even in the absence of a buffer.

Discussion

The finding that butyrate and other short-chain fatty acids can substitute for HCO_3 in stimulation of NaCl transport is in agreement with similar results in other epithelia, e.g. rat proximal tubule (Ullrich et al., 1971) and choroid plexus (Wright, 1977). Like HCO_3 (formation of CO_2) these substances (A^-) possess the ability both to buffer H-ions and by this reaction to form a lipid soluble product (AH). It is of considerable interest that the stimulatory potency of the fatty acids tested paralleled their chloroform water partition coefficients. Jackson et al. (1978) reported a direct correlation between the chain length and lipid solubility of the above acids. Their uptake into intestinal epithelial cells from the mucosal bathing solution increased with chain length, probably due to nonionic diffusion of the protonated form (AH). This finding suggests that the ability of both CO_2 and protonated fatty acid anion (AH) to gain access to the cell interior may be involved in their stimulation of NaCl and fluid absorption.

Side Specificity of the Butyrate Stimulatory Effect and Changes in V_{ms}

The dependence of butyrate-stimulated NaCl absorption on its mucosal presence is shown in the substitution experiments, where butyrate was effective when present in both mucosal and serosal compartments but ineffective with serosal presence alone. Butyrate is not different from HCO_3 in this respect (Heintze et al., 1981). The notion that HCO_3 and butyrate act from the mucosal side is also supported by the concomitant changes in V_{ms}, which were similar for butyrate to those observed under various HCO_3 conditions, in both guinea pig and rabbit. Thus, V_{ms} appears to be a suitable parameter to distinguish between stimulated and unstimulated NaCl absorption. The observed shift in V_{ms} towards a more positive lumen on enhanced NaCl absorption may well be due to an increased backflux of NaCl from the intercellular spaces into the lumen, as suggested by Machen and Diamond (1969).

Interaction of Fatty Acids with H^+-Secretion

As reported in the preceding paper (Heintze et al., 1981) omission of HCO_3 from the bathing medium revealed an acidification of the luminal fluid. A decline in intraluminal pH was also observed when fluid

transport was stimulated by fatty acids, but was less in the presence of butyrate than in the butyrate-free solution. This occurred despite a pK value for butyrate of only 4.8. The buffering effect of butyrate may result from the ability of its lipid-soluble undissociated form (AH) to leave the gallbladder lumen by nonionic diffusion. The higher acidification rate observed with equimolar concentrations of acetate or propionate supports the view that buffering by various fatty acids is a function of the lipid solubility rather than pK. Jackson et al. (1978) found no difference in influx rates between straight and branched short-chain fatty acids. This finding is in accordance with a reduction in luminal acidification by isobutyrate and isovalerate as expected from their chloroform water partition coefficients (Fig. 2). The reduced or absent stimulatory effect of both branched compounds, however, suggests that besides lipid solubility certain structural requirements must be met for stimulation of NaCl absorption (*see* p. 189).

In parallel with our findings (Heintze et al., 1981) regarding HCO_3-dependent fluid and NaCl absorption, the corresponding butyrate-stimulated processes were inhibited by pH elevation in the bathing solutions with and without Tris buffer. The flux experiments examining the effect of elevation of pH (Table 5) indicate that luminal pH may be critical for NaCl absorption. Jackson et al. (1978) have reported that a pH elevation from 7.4 to 8.4 significantly inhibited octanate influx into intestinal cells. The inhibition of butyrate-dependent fluid absorption by Tris may be due to competition between Tris and butyrate for H^+ ions. A similar Tris effect on HCO_3-dependent fluid absorption has been discussed in detail in the preceding paper (Heintze et al., 1981). These findings suggest that interaction between fatty acids and secreted H^+ is of importance in their stimulation of transport.

Role of Metabolism of SCFA in Stimulation of NaCl Absorption

It is likely that short-chain fatty acids (SCFA) are metabolized by gallbladder epithelial cells. Their catabolism may provide an additional energy substrate and thereby augment the energy available for NaCl absorption. For this to provide an explanation for their stimulation of NaCl transport in HCO_3-free solutions it is necessary to assume that the energy supply is rate-limiting under these conditions. The glucose and pyruvate content of the experimental solutions and the use of freshly excised rather than substrate depleted preparations make this unlikely. Furthermore if degradation of SCFA were to be responsible for their stimulatory effects then such a stimulation should be dependent on the number of molecules of ATP available for cellular metabolism from degradation of the respective SCFA.

The possible energy gain from butyrate is 7 times higher than that from propionate[2]. Since permeability also is in favor of cellular accumulation of butyrate, stimulation of fluid absorption by butyrate should be much higher than by propionate. The butyrate effect is, however, only 1.2 times greater than that of propionate. This might be attributed to a decreasing ratio of absorbed NaCl over consumed ATP; in this case a much larger quantum of additional energy would be required for a further increase in absorption rates. Fluid absorption in the presence of valerate is 1.1 times greater than with butyrate; however, the possible energy gain from valerate is only 75% of that from butyrate[2]. Thus, stimulation of fluid absorption is unlikely to result from energy derived from SCFA metabolism. It also seems improbable that the branched chain fatty acids cannot be used for ATP synthesis. Isobutyrate and isovalerate are metabolites of leucine and valine, respectively, and undergo oxidative metabolism at least in kidney, heart and liver mitochondria (Wakil, 1970; Tischler & Goldberg, 1980). If SCFA stimulate transport by serving as an energy source, they should not be inferior to, e.g., propionate in this respect. Energy gain from degradation of either of the branched SCFA is 3 times that from propionate metabolism[2]. In contrast to isovalerate, no known metabolic pathway is available to degrade pivalate (another isomer of valerate). It is thus improbable, that stimulation of fluid absorption by this compound can be explained by an increase in the energy available to drive NaCl absorption.

Alternatively, metabolism of butyrate may lead to enhanced CO_2 release into the lumen and thus to formation of HCO_3. However, at a pH of 7.0 and a pCO_2 of, at most, 8 mm Hg no more than about 2 mM HCO_3 can be produced. Extrapolation from the concentration-response curve (Fig. 3) indicates only a minor effect at this HCO_3 concentration. HCO_3 formation cannot therefore account for the observed stimulation of NaCl absorption by butyrate.

Route of Buffer Anion

The above considerations regarding the involvement of H^+ secretion in butyrate-dependent transport

[2] Molecules ATP gained from degradation of 1 molecule propionate: 4; butyrate: 28; valerate: 21; isobutyrate: 12; isovalerate: 12, without degradation of the resulting acetoacetate (for description of metabolic degradation, *see* Dagley & Nicholson, 1970).

might be explained in terms of a Na/H-exchange leading to uptake of undissociated buffer by the epithelial cells. This implies that the flux of AH from lumen to cell equals the rate of the butyrate-dependent Na/H-exchange, e.g. 3.5 μEq/cm² hr in guinea pig gallbladder. Bicarbonate- and SCFA-stimulated Na transport are associated with a significant net flux of the respective buffer anion in other tissues (Ullrich et al., 1971; Wright, 1977). This net transport results from entry of the protonated buffer anion into the cell and after dissociation its transport across the basolateral cell membrane. While the first of these two steps seems to apply to the gallbladder, the second takes place only to a very limited extent since HCO_3 and butyrate absorption from the gallbladder lumen are only 2–2.5 μEq/cm² hr in the rabbit; in the guinea pig net butyrate absorption is only 0.3 μEq/cm² hr. The system mediating Na and anion exit at the basolateral cell membrane seems to possess only a low affinity for fatty acids as concluded from the low fluid absorption rates obtained in solutions containing propionate (Whitlock & Wheeler, 1967) or butyrate (p. 184) as the only anion available for absorption. This poses the question as to the further fate of buffer anion after its entry into the cell. Since complete consumption by cellular metabolism or intracellular accumulation of A^- is unlikely and exit across the basolateral membrane of minor importance, recycling of butyrate for luminal Cl remains an attractive hypothesis. In the rat jejunum a carrier has been described which mediates the anionic countertransport of acetate, propionate, butyrate and HCO_3 (Lamers & Hülsmann, 1975). In such a counter-transport system, specific structure requirements are expected. Its presence also in the gallbladder would explain the reduced stimulatory effect of isobutyrate versus butyrate and absent effect of isovalerate. The same explanation may apply to the failure of glycodiazine to substitute for HCO_3 (Heintze et al., 1981) in contrast to its effects in other epithelia (Ullrich et al., 1971; Wright, 1977). The protonated form of glycodiazine is lipid soluble, but its chemical structure markedly differs from that of the fatty acids.

Under steady-state conditions a model featuring Na/H- and Cl/A^--exchanges demands identical secretion rates of H^+ and butyrate ions into the gallbladder lumen. Consequently after an initial period of acidification (sac preparation) where no cellular butyrate is available to accompany H^+, a pH is reached beyond which no further decrease in luminal acidity occurs. Net absorption of buffer anion, however, must also result in acidification of the mucosal compartment under steady-state conditions. Butyrate absorption by rabbit exceeded that by guinea pig gallbladder by more than 2 μEq/cm² hr. This may partially account[3]) for the observation that luminal acidification in the presence of butyrate was higher in rabbit than in guinea pig gallbladder.

HCO_3-and Butyrate-Stimulated Transport

The above considerations imply that HCO_3 and fatty acids, in particular butyrate, increase NaCl absorption by the same mechanism. The following findings provide some support for this assumption:

1. both HCO_3 and butyrate must be present in the mucosal solution for transport stimulation;
2. butyrate- and HCO_3-dependent fluid absorption are reduced in the presence of Tris buffer;
3. butyrate- and HCO_3-stimulated fluid and electrolyte absorption are prevented by pH elevation;
4. HCO_3- and butyrate-dependent fluid transport are inhibited by the disulfonic stilbene SITS (Heintze, Olles, Petersen & Wood, 1978).

The effects of SITS and Tris buffer appear selective in that these agents did not affect fluid transport in the HCO_3-free solution.

Working Model of Butyrate Stimulation of NaCl Absorption

The above findings are summarized in a model to describe butyrate-stimulated NaCl and fluid absorption (Fig. 5). The similarities between butyrate and HCO_3-dependent transport, discussed previously, suggest that such a model may also apply to HCO_3 stimulation of transport. The essential feature of this model is a double exchange system mediating parallel countertransport of Na/H and Cl/butyrate (A^-) in the apical membrane. Considering the limited cellular supply of H^+ ions recirculation of H^+ is essential for such a system. In the model, fatty acids (AH) serve this function. After entry into the cell, AH readily dissociates due to the low pK value (about 4.8) of the fatty acids, thereby delivering not only H^+ but also A^- to the cell interior. Exchange of A^- for Cl couples Cl influx to that of Na. As a net phenomenon, the resulting double exchange system displays the properties of a coupled NaCl transport process as described by Frizzell, Dugas and Schultz

[3] Butyrate absorption was 0.3 and 2.5 μEq/cm² hr in guinea pig and rabbit gallbladder, respectively. In a gallbladder (gb) (surface area 5 cm²) filled with 1 ml of 25 mM butyrate Ringer's solution, these rates equal 1.5 and 12.5 μEq/gb hr, respectively. Using the buffer equation for a volatile buffer, it may be shown that these rates are sufficient for a pH reduction by 0.03 in the guinea pig and 0.3 pH units in the rabbit gallbladder.

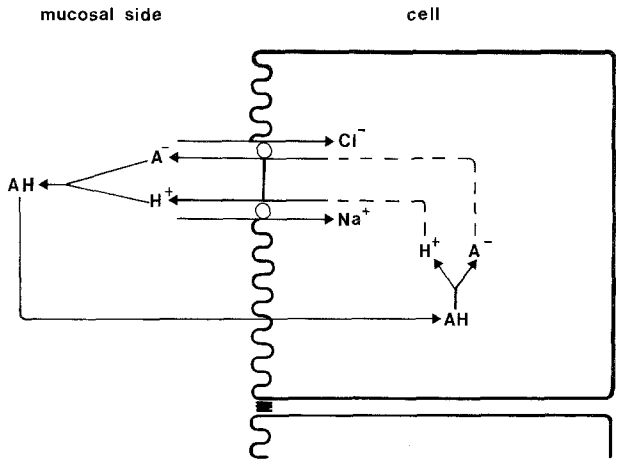

Fig. 5. Double ion exchange model proposed for the coupled uptake of Na and Cl across the apical membrane of gallbladder epithelial cells

(1975). With respect to the HCO_3-CO_2 system, A^- also stands for HCO_3 and AH for CO_2. It should be noted that this model refers only to that part of NaCl absorption which depends on exogenous butyrate or HCO_3. Dependence of the remaining part on endogenous HCO_3/CO_2 is an attractive posibility which remains to be substantiated.

This model is based principally on five observations:

1. the marked congruity between the stimulatory effects of butyrate and HCO_3 on NaCl and fluid absorption (*cf.* to Heintze et al., 1981);
2. the dependence of the stimulatory effects of SCFA on lipid solubility (Fig. 1);
3. the correlation between the lipid solubility of SCFA and their reduction of luminal acidification (Fig. 2);
4. the mucosal requirement for butyrate in its stimulation of Na absorption (Table 1);
5. the inability of butyrate absorption to account for butyrate-stimulated Na absorption (Tables 1 and 3).

Support for the above model emerges also from studies in other epithelia. A similar model postulating transepithelial exchange of Na/H and HCO_3/Cl has been previously proposed to explain electrolyte transport by the human ileum (Turnberg, Bieberdorf, Morawski & Fordtran, 1970). Murer, Hopfer and Kinne (1975) have described a Na/H antiport in brush-border membrane vesicles from rat small intestine. In an extension of their study to anion transport, Liedtke and Hopfer (1977) and Liedtke (1980) suggested that a Na/H countertransport coupled to a Cl/OH or Cl/HCO_3 countertransport may be responsible for the neutral NaCl uptake reported by Nellans, Frizzell and Schultz (1973).

Stimulated Double Ion Exchange Versus Activation of a Regulatory Carrier Site

The above model ascribes transport stimulation by lipophilic buffers to an augmented cellular uptake of NaCl across the mucosal membrane. Such a mechanism promoting Na entry into the cell is consistent with the finding of Cremaschi, Henin and Meyer (1979) that HCO_3 stimulates the coupled influx of NaCl in the rabbit gallbladder. The above authors postulated two simultaneously operating mechanisms:

1. a Na/H exchange responsible for net HCO_3 absorption;
2. activation by HCO_3 of a regulatory site on the NaCl carrier.

Since in the guinea pig gallbladder no net HCO_3 absorption occurs only the latter mechanism needs consideration. Even without net HCO_3 absorption all observations presented in this study are compatible with a double ion exchange while some of them are not easily understood in terms of a carrier model with a HCO_3-sensitive regulatory site, e.g. the inhibition of NaCl absorption by Tris buffer and elevation of the pH of the bathing solution. The above results indicate that butyrate and HCO_3 may act by a common mechanism. In terms of the above carrier model it would be expected that the capacity of fatty acids to stimulate fluid absorption would increase in the sequence valerate < butyrate < propionate < acetate < formate, i.e. with increasing molecular similarity to HCO_3. Since the opposite is the case it seems more reasonable that HCO_3 and butyrate stimulate transport by accepting secreted H^+ and delivering it to the cell interior by nonionic diffusion to maintain intracellular H^+ for Na/H-exchange.

Intracellular pH as Coupling Factor between Na/H and Cl/A−(HCO_3) Countertransports

Consideration of the possible driving forces for the above model may provide insight into the coupling between the two countertransport systems. On the basis of the present data, the participation of a primary active H^+ transport cannot be excluded. The chemical Na gradient across the apical membrane may, however, be sufficient to drive the Na/H exchange. A possible contribution of a primary active anion translocation cannot however be excluded. Humphreys and Chou (1979) have recently revived the discussion on anion-stimulated ATPase in intestinal brush border membrane. The anion exchange may also be driven by ion gradients. In an electrically neutral ion exchange only the chemical potential differences must be considered as driving forces influencing ion move-

ment. Under this condition, Cl will enter the cell downhill due to its concentration gradient. Exit of A^- would be driven by the Cl influx. The advantage of such a mechanism lies in cancellation of the adverse electrical potential differences rendering the chemical potential difference the only driving force.

Since the Cl conductances of both the apical and basolateral membranes have been reported to be negligible or absent in rabbit (Henin & Cremaschi, 1975; van Os & Slegers, 1975) and *Necturus* gallbladder (Reuss & Finn, 1975; van Os & Slegers, 1975) the chemical rather than the electrochemical potential differences must be used for evaluation of Cl equilibrium. Hence the arising question is not why intracellular Cl is above its electrochemical equilibrium but why it is below its chemical equilibrium. Clearly, intracellular Cl activity is the result of its influx and exit. Cl uptake by gallbladder cells depends on a sufficient intracellular supply of exchangeable anion, the latter being secondary to the rate of Na entry via the Na/H-exchange, which in turn is dependent on the activity of the Na pump. Ultimately Cl influx is regulated by the Na pump. Since the baso-lateral Cl conductance is negligible or absent, Cl exit across this membrane must overcome its chemical gradient ($Cl_{ec}/Cl_{ic}=122/35=3.5$, value for intracellular Cl (Cl_{ic}) from Duffey, Turnheim, Frizzell & Schultz, 1978). This could be accomplished by Cl coupling to the movement of other solutes. An intracellular pH not higher than 7.0 ($HCO_{3ec}/HCO_{3i}=25/7.1=3.5$) would be needed for a basolateral Cl/HCO_3 exchange. Since this would require a 100% efficiency of coupling and intracellular pH seems to be more alkaline than 7.0, cotransport with K seems to be a more likely possibility. The intracellular K activities measured in *Necturus* (Garcia-Diaz & Armstrong, 1980; Reuss, Weinmann & Grady, 1980) and rabbit gallbladder (Gunther-Smith, Duffey & Schultz, 1980) constitute chemical gradients which by far are sufficient to drive electroneutral KCl cotransport out of the cell. Such a mechanism was favored in the electrophysiological studies of Reuss et al. (1980) in *Necturus* gallbladder.

Since Cl entry depends on the intracellular availability of HCO_3 (A^-) the intracellular pH is of particular interest. Its central role in butyrate and HCO_3 movement is shown in Fig. 6. A pCO_2 isobar has been calculated from the Henderson-Hasselbalch equation with pCO_2 held constant at 35 mm Hg using a pK of 6.21 (Heintze, Petersen, Olles, Saverymuttu & Wood, 1979). While equal magnitude of intracellular and extracellular pCO_2 appears to be a reasonable assumption, it is not certain whether the permeability of the apical membrane for AH permits numerical adjustment of internal and external AH. Therefore, intracellular A^- has been calculated from the buffer

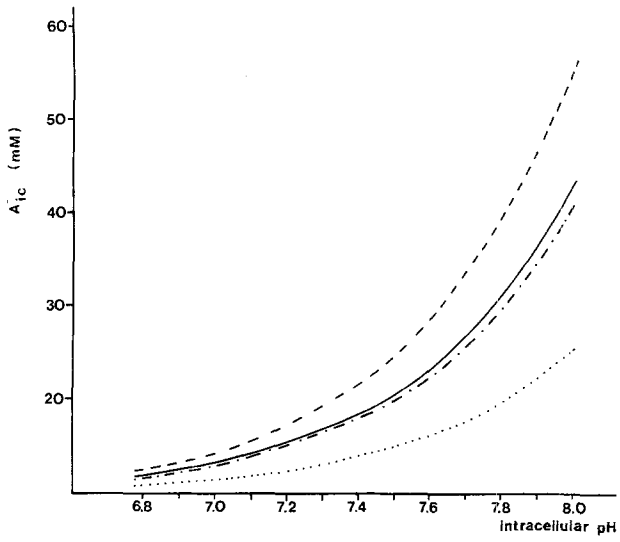

Fig. 6. Concentrations of intracellular HCO_3 and butyrate anion (A_{ic}^-) as a function of the intracellular pH. Curves were calculated for HCO_3 (——, $pCO_2=35$ mm Hg) and three different intracellular concentrations of the undissociated form of butyrate (AH_{ic}): 0.02 mM (· · · · ·), 0.04 mM (–·–·–) and 0.06 mM (– – –). *See text* for further explanation

equation for three different conditions: intracellular AH of 0.02, 0.04 and 0.06 mM, where 0.06 mM equals the AH concentration in a 25-mM butyrate Ringer's solution at pH 7.4 ($pK=4.81$). The disappearance of intracellular H^+ ions exchanged for external Na momentarily causes an increase in intracellular pH and subsequently dissociation of AH or formation of HCO_3 from CO_2. At a fixed intracellular AH or pCO_2, intracellular A^- or HCO_3 increases with increase in pH. With AH = 0.04 mM, increase of intracellular butyrate with pH is described by the same function as elevation of intracellular HCO_3. Of course, Fig. 6 does not imply that intracellular pH really increases to 7.4 or higher values. It may be calculated, however, that at an intracellular pH of 7.2 HCO_3 (A^-) concentration in the cell amounts to 10.7 mM. This may be sufficient for neutral exchange against Cl. Such values do not appear unreasonable considering findings in other tissues. There is general agreement that in muscle cells intracellular HCO_3 is about one order of magnitude higher than expected from the membrane potential and external concentration (Woodbury, 1965). Using HCO_3 selective microelectrodes, Khuri, Agulian, Bogharian, Nassar and Wise (1974) measured an intracellular HCO_3 activity of 11.1 mM in cells of *Necturus* proximal tubule. This value is more than ten times higher than that predicted for passive distribution. The intracellular pH was calculated as 7.44 in this study.

Recently it was considered inconceivable that two apparently independent but interacting exchange pro-

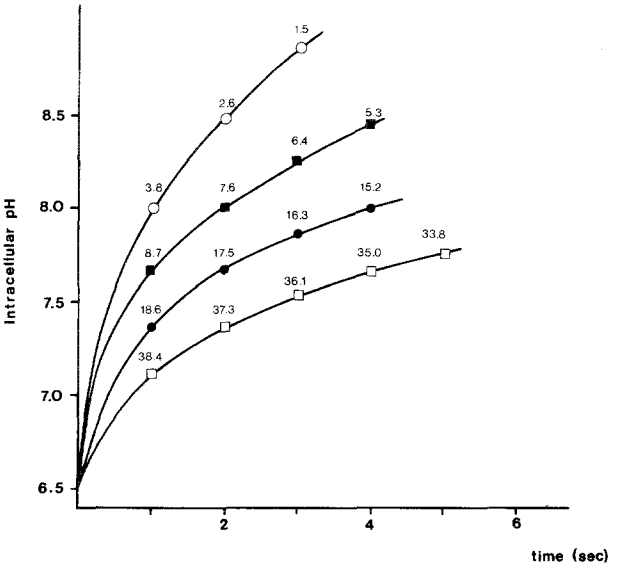

Fig. 7. Elevation of intracellular pH and exhaustion of intracellular acid (BH) as a function of time assuming a H^+ secretion rate of 1.16 nEq/μl sec. Values have been calculated for a buffer (B + BH) with pK = 8.5 and concentrations of 5 mM (○—○), 10 mM (■—■), 20 mM (●—●) or 40 mM (□---□). The remaining concentration of BH is indicated for each second

cesses could account for the fact that omission of Na inhibits Cl entry into rabbit gallbladder cells (Duffey, Thompson, Frizzell & Schultz, 1979). However, as reported by Cremaschi et al. (1979), omission of Na from the bathing solution eliminated H^+ secretion by rabbit gallbladder. In terms of the proposed model, this would result in dissipation of accumulated intracellular HCO_3 favoring HCO_3 countertransport with Cl. Thus, Cl influx into the cell is tightly coupled to that of Na by the intracellular pH. Fig. 7 illustrates the rapid exhaustion of intracellular H^+ ions available in the form of protonated buffer molecules (BH). In rabbit gallbladder, the model predicts a H^+ secretion of about 10 μEq/cm² hr in butyrate-dependent Na absorption. Related to the intracellular space, this value equals 1.16 nEq/μl sec, since 1 cm² of epithelial surface represents an intracellular volume of about 2.4 μl ($10^4 \times 10^4 \times 30$ μm = 3.0 μl, about 80% of which belong to the intracellular space). Treating the cell as a closed system this relation permits a direct evaluation of the changes in intracellular pH for a given buffer concentration and pK. The intracellular buffer value of gallbladder epithelial cells is unknown. In muscle, the estimated contribution of protein and phosphate to the buffer value is about two times higher than that of the HCO_3 buffer (Woodbury, 1965). Hence the range of 5–40 mM intracellular buffer covered by Fig. 7 appears reasonable. As shown in this Figure, H^+ secretion at the indicated rate leads to rapid dissipation of the intracellular supply of BH. From an initial pH of 6.5, a pH of 7.75 is reached within 5 sec even with a hypothetical 40 mM buffer at the favorable pK of 8.5. The contributions of the fatty acid and HCO_3/CO_2 buffer systems may be neglected under such conditions because of their much lower pK values. Hence Na/H-exchange without recycling of H^+ leads to a reduction of intracellular H^+ ions and an increase in pH which is ultimately likely to adversely affect cellular function[4].

In this context, the anionic countertransport of Cl and HCO_3 (A^-) serves two functions: stabilization of the intracellular pH by removal of HCO_3 (A^-) and translocation of buffer anion from the intracellular compartment to the mucosal solution, where formation of CO_2 (AH) ensures recycling of H^+ ions to the cell interior. In the absence of Cl, removal of buffer anions from the cell interior and H^+ recycling are compromised. This must lead to a decrease in cellular uptake of Na. If in the absence of Cl, HCO_3 is still present in the bathing solution, H^+ recycling would still be possible to a certain degree and Na influx in exchange for H^+ would continue at a reduced rate. Omission of both HCO_3 and Cl would then result in a further reduction of Na influx. These considerations are in accordance with findings of Cremaschi et al. (1979) who reported 93% inhibition of H^+ secretion by rabbit gallbladder when HCO_3 was absent from the bathing solution. Na influx from lumen to cell was reduced from 39.9 to 26 μEq/cm² hr by omission of Cl. In the absence of both HCO_3 and Cl, Na influx was further reduced to 12.7 μEq/cm² hr, a value which may represent the paracellular fraction. Thus, coupling of Na influx to that of Cl depends directly on intracellular pH.

Though several features of the double ion exchange model remain to be tested experimentally, it adequately explains the current findings. Under all conditions examined, butyrate was indistinguishable from HCO_3 in stimulation of NaCl and fluid absorption. This substance may provide a valuable tool for further investigation of the mechanism of bicarbonate stimulation of NaCl transport.

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[4] Formation of H^+ from dissociation of H_2O would rapidly elevate intracellular OH to the same extent and thus pH. Exit of OH across the apical border in exchange for Cl is not possible as evinced by the inhibition of NaCl transport in the absence of HCO_3 or butyrate. Efflux across the basolateral membrane would require an anionic conductivity of the same magnitude as net Cl absorption. However, as discussed by Duffey et al. (1978), even the total conductance of this barrier is too low to permit exit of Cl in a conductive form.

References

Cremaschi, D., Henin, S., Meyer, G. 1979. Stimulation by HCO_3 of Na^+ transport in rabbit gallbladder. *J. Membrane Biol.* **47**:145–170

Dagley, S., Nicholson, D.E. 1970. An Introduction to Metabolic Pathways. Blackwell Scientific Publications, Oxford and Edinburgh

Duffey, M.E., Thompson, S.M., Frizzell, R.A., Schultz, S.G. 1979. Intracellular chloride activities and active chloride absorption in the intestinal epithelium of the winter flounder. *J. Membrane Biol.* **50**:331–341

Duffey, M.E., Turnheim, K., Frizzell, R.A., Schultz, S.G. 1978. Intracellular chloride activities in rabbit gallbladder. Direct evidence for the role of the sodium gradient in energizing "uphill" chloride transport. *J. Membrane Biol.* **42**:229–245

Frizzell, R.A., Dugas, M.C., Schultz, S.G. 1975. Sodium chloride transport by rabbit gallbladder: Direct evidence for a coupled NaCl influx process. *J. Gen. Physiol.* **65**:769–795

Garcia-Diaz, J.F., Armstrong, W. McD. 1980. The steady-state relationship between sodium and chloride transmembrane electrochemical potential differences in *Necturus* gallbladder. *J. Membrane Biol.* **55**:213–222

Gunther-Smith, P.J., Duffey, M.E., Schultz, S.G. 1980. Intracellular potassium activities in rabbit gallbladder. *Fed. Proc.* **39**:1080

Heintze, K., Olles, P., Petersen, K.-U., Wood, J.R. 1978. Effects of a disulphonic stilbene on fluid and electrolyte transport in guinea pig isolated gallbladder. *J. Physiol. (London)* **284**:152P–153P

Heintze, K., Petersen, K.-U., Olles, P., Saverymuttu, S.H., Wood, J.R. 1979. Effects of bicarbonate on fluid and electrolyte transport by the guinea pig gallbladder: A bicarbonate-chloride exchange. *J. Membrane Biol.* **45**:43–59

Heintze, K., Petersen, K.-U., Wood, J.R. 1981. Effects of bicarbonate on fluid and electrolyte transport by the guinea pig and rabbit gallbladder: Stimulation of absorption. *J. Membrane Biol.* **62**:175–181

Henin, S., Cremaschi, D. 1975. Transcellular ion route in rabbit gallbladder. Electric properties of the epithelial cells. *Pfluegers Arch.* **355**:125–139

Humphreys, M.H., Chou, L.Y.N. 1979. Anion stimulated ATPase activity of brush border from rat small intestine. *Am J. Physiol.* **236**:E70–E76

Jackson, M.J., Williamson, A.M., Dombrowski, W.A., Garner, D.E. 1978. Intestinal transport of weak electrolytes. Determinants of influx at the luminal surface. *J. Gen. Physiol.* **71**:301–327

Khuri, R.N., Agulian, S.K., Bogharian, K., Nassar, R., Wise, W. 1974. Intracellular bicarbonate in single cells of *Necturus* kidney proximal tubule. *Pfluegers Arch.* **349**:295–299

Lamers, J.M.J., Hülsmann, W.C. 1975. Inhibition of pyruvate transport by fatty acids in isolated cells from rat small intestine. *Biochim. Biophys. Acta* **394**:31–45

Liedtke, C.M. 1980. Mechanisms of Chloride Translocation Across the Intestinal Microvillus Membrane. Ph. D. Thesis. Case Western Reserve University, Cleveland, Ohio

Liedtke, C.M., Hopfer, U. 1977. Anion transport in brush border membranes isolated from rat small intestine. *Biochem. Biophys. Res. Commun.* **76**:579–585

Machen, T.E., Diamond, J.M. 1969. An estimate of the salt concentration in the lateral intercellular spaces of rabbit gallbladder during maximal fluid transport. *J. Membrane Biol.* **1**:194–213

Murer, H., Hopfer, U., Kinne, R. 1975. Sodium/proton antiport in brush border membrane vesicles isolated from rat small intestine and kidney. *Biochem. J.* **154**:597–604

Nellans, H.N., Frizzell, R.A., Schultz, S.G. 1973. Coupled sodium chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* **225**:467–475

Os, C.H. van, Slegers, J.F.G. 1975. The electrical potential profile of gallbladder epithelium. *J. Membrane Biol.* **24**:341–363

Reuss, L., Finn, A.L. 1975. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. II. Ionic permeability of the apical cell membrane. *J. Membrane Biol.* **25**:141–161

Reuss, L., Weinman, S.A., Grady, T.P. 1980. Intracellular K activity and its relation to basolateral membrane ion transport in *Necturus* gallbladder epithelium. *J. Gen. Physiol.* **76**:33–57

Tischler, M.E., Goldberg, A.L. 1980. Amino acid degradation and effect of leucine on pyruvate oxidation in rat atrial muscle. *Am. J. Physiol.* **238**:E480–E486

Turnberg, L.A., Bieberdorf, F.A., Morawski, S.G., Fordtran, J.S. 1970. Interrelationships of chloride, bicarbonate, sodium and hydrogen transport in the human ileum. *J. Clin. Invest.* **49**:557–567

Ullrich, K.J., Radtke, H.W., Rumrich, G. 1971. The role of bicarbonate and other buffers on isotonic fluid absorption in the proximal convolution of the rat kidney. *Pfluegers Arch.* **330**:149–161

Wakil, S.J. 1970. Fatty acid metabolism. *In:* Lipid metabolism. S.J. Wakil, editor. pp. 1–48. Academic Press, New York and London

Whitlock, R.T., Wheeler, H.O. 1967. Anion transport by isolated rabbit gallbladder. *Am. J. Physiol.* **213**:1199–1204

Woodbury, J.W. 1965. Regulation of pH. *In:* Physiology and Biophysics. T.C. Ruch and H.J. Patton, editors, pp. 899–934. W.B. Saunders Company, Philadelphia and London

Wright, E.M. 1977. Effect of bicarbonate and other buffers on choroid plexus Na^+/K^+ pump. *Biochim. Biophys. Acta* **468**:486–489

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