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Stimulation by HCO_3^- of Na^+ Transport in Rabbit Gallbladder

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Summary. Bicarbonate presence in the bathing media doubles Na^+ and fluid transepithelial transport and in parallel significantly increases Na^+ and Cl^- intracellular concentrations and contents, decreases K^+ cell concentration without changing its amount, and causes a large cell swelling. Na^+ and Cl^- lumen-to-cell influxes are significantly enhanced, Na^+ more so than Cl^-. The stimulation does not raise any immediate change in luminal membrane potential and cannot be due to a HCO_3^--ATPase in the brush border. The stimulation goes together with a large increase in a Na^+-dependent H^+ secretion into the lumen. All of these data suggests that HCO_3^- both activates $Na^+ - Cl^-$ cotransport and $H^+ - Na^+$ countertransport at the luminal barrier.

Thiocyanate inhibits Na^+ and fluid transepithelial transport without affecting H^+ secretion and HCO_3^--dependent Na^+ influx. It reduces Na^+ and Cl^- concentrations and contents, increases the same parameters for K^+, causes a cell shrinking, and abolishes the lumen-to-cell Cl^- influx. It enters the cell and is accumulated in the cytoplasm with a process which is Na^+-dependent and HCO_3^--activated. Thus, SCN^- is likely to compete for the Cl^- site on the cotransport carrier and to be slowly transferred by the cotransport system itself.

Key words: Gallbladder, bicarbonate transport, thiocyanate, HCO_3^--ATPase, NaCl cotransport, $H^+ - Na^+$ countertransport.

In a large number of epithelia HCO_3^- stimulates Na^+ transport [10, 12, 21, 30, 35, 37, 38, 48]. This effect has been particularly investigated in the kidney proximal tubule, where both a neutral $H^+ - Na^+$ exhange (H^+ secretion into the lumen, Na^+ absorption into the cell) and an electrogenic $H^+ - HCO_3^-$ exchange (H^+ secretion, HCO_3^- absorption), localized on the apical barrier, have been postulated [19, 20, 27, 28, 36, 37]. The former process also facilitates bicarbonate influx as CO_2 and H_2CO_3 into the cell by the luminal acidification; the latter drives bicarbonate directly and Na^+ indirectly by electrical coupling, and it should be supported by a HCO_3^--activated ATPase.

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Also in the gallbladder, a bicarbonate absorption and a stimulation by HCO_3^- of Na^+ transport have been shown [7, 8, 45, 46]. In this tissue Murphy and Martin [29] have revealed a carbamylsynthetase, which is able to synthetize carbamylphosphate from bicarbonate, ammonium, and ATP. Since carbamylphosphate can be used as an energetic substrate instead of ATP by the $Na^+ - K^+$-activated ATPase, Martin and Murphy [26] have suggested that the stimulation by bicarbonate is based on an energetic activation of the Na^+ pump at the basolateral barrier or, at least, that "bicarbonate affects metabolic events leading to mobilization of energy for transport" [25, 26].

On the other hand, in the gallbladder as well as in the kidney proximal tubule a process of acidification of the luminal solution has been demonstrated [33, 34, 47], and evidence has been provided of an inhibition of Na^+ transport by thiocyanate [33], an inhibitor of acid secretion, and of the HCO_3^--ATPase in the gastric mucosa [16, 17, 31]. Thus, Van Os [41] has suggested that a HCO_3^--dependent ATPase is present also in the gallbladder. The same electrogenic process, revealed in the kidney proximal tubule, should drive H^+ from the cell into the lumen in exchange with HCO_3^- and should cause a Na^+ absorption by increasing the negative intracellular potential. In this case Na^+ transport enhancement by bicarbonate should be due to a mechanism localized on the luminal membrane.

However, it is noteworthy that thiocyanate (in the absence of bicarbonate) does not inhibit H^+ secretion [33], although it inhibits Na^+ transport; this fact raises some doubt about the HCO_3^--ATPase hypothesis. Whitlock and Wheeler [47] and Sullivan and Berndt [33] have demonstrated that the luminal acidification is Na^+-dependent, ouabain-sensitive and that the luminal pCO_2 increases in the presence of bicarbonate; on the basis of these data, they have suggested the presence of a $H^+ - Na^+$ countertransport.

To sum up, bicarbonate action in the gallbladder epithelial cells is in turn ascribed to an energetic activation of the Na^+ pump on the basolateral barrier, to an electrogenic HCO_3^--dependent ATPase, or to a neutral countertransport on the brush border.

To shed some light on these problems, we have investigated the effects of bicarbonate and thiocyanate on cell ion concentrations (Na^+, K^+, Cl^-), cell volume, intracellular potential, Na^+ and Cl^- uptakes, and H^+ secretion; moreover, we are looking at the presence of a HCO_3^--dependent ATPase in the microvillar fraction of the epithelial cells. Here we report the first part of our research.

Materials and Methods

Gallbladder Preparation

New Zealand rabbits were killed by a blow on the neck. Gallbladders were excised and washed free from bile with Krebs-Henseleit solution (*Sol. 1* of Table 1).

Measurement of Water Net Transport

The organ (noneverted preparation) was cannulated, filled with Krebs-Henseleit solution, bathed with the same solution on the serosal side, and so incubated for half an hour; then, it was emptied, washed three times, filled with the test solution, and weighed. Water transport was measured gravimetrically after 30 and 60 min and expressed as $\mu l\ cm^{-2} hr^{-1}$. Only the second experimental period, in which possible changes of cell volume were probably absent, was taken into account.

Measurement of Cell Water and Ions

Gallbladders, opened flat, were incubated for 30 min in *Sol. 1*, then transferred in the test solution for 60 min. The latter was added with ^{3}H-sucrose (1 μCi/ml) to measure the extracellular space [2] and with $^{36}Cl^-$ or $^{22}Na^+$ (0.5 μCi/ml) to determine the rapid exchangeable pool of these ions. It has been demonstrated that $^{36}Cl^-$ and $^{22}Na^+$ enter the cell only from the mucosal side and equilibrate with cell Cl^- and Na^+ in 15–30 min [3]. In some experiments in which the bathing media were asymmetric, the noneverted cannulated preparation was used. In this case, ^{3}H-sucrose was added either to the mucosal

Table 1. Composition of the experimental solutions

Solution	Na^+	$Tris^+$	Choline$^+$	Cl^-	HCO_3^-	SO_4^{2-}	SCN^-	Mannitol
Krebs-Henseleit (Sol. 1)	143.0	–	–	127.7	25	1.2	–	–
HCO_3^- free (Sol. 2)	134.0	9.0	–	152.7	–	1.2	–	–
Sol. 3	134.0	9.0	–	127.7	–	13.7	–	12.5
Sol. 4	134.0	9.0	–	127.7	–	1.2	25	–
Sol. 5	143.0	–	–	102.7	25	13.7	–	12.5
Sol. 6	143.0	–	–	102.7	25	1.2	25	–
Sol. 7	143.0	–	–	–	25	65.0	–	63.8
Sol. 8	134.0	9.0	–	–	–	77.2	–	76.0
Sol. 9	143.0	–	–	–	25	52.6	25	51.4
Sol. 10	–	–	143.0	127.7	25	1.2	–	–
Sol. 11	134.0	9.0	–	102.7	–	13.7	25	12.5

Concentrations are given in mM. All the solutions contain (in mM): K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; $H_2PO_4^-$, 1.2.

Table 2. Na^+ and Cl^- influxes measured at different incubation periods

	30 sec	45 sec	60 sec	75 sec
Na^+ ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)	41.0 ± 2.1 (4)	38.2 ± 1.5 (5)	36.8 ± 2.1 (4)	38.5 ± 4.0 (4)
Cl^- ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$	30.0 ± 5.2 (4)	30.2 ± 2.3 (7)	–	29.1 ± 4.0 (4)

Influxes are not corrected for the paracellular fraction. Experiments were carried out in *Sol.1.* Data are reported as mean ± SEM, with the number of experiments in parenthesis.

or to the serosal saline, and mean values of the serosal and mucosal extracellular spaces were determined.

At the end of the experiment, gallbladders were processed as reported in ref. 3 to determine cell water and Na^+, K^+, Cl^- cell concentrations and amounts. K^+ was measured only spectrophotometrically; Na^+ and Cl^- were determined both spectrophotometrically [3] and radiochemically to have a value of total concentration and of the rapid exchangeable pool.

Measurement of Cl^- and Na^+ Uptakes

Gallbladders, opened flat, were mounted on a nylon mesh between two Lucite chambers with the luminal surface facing upwards, exposed (0.61 cm^2) within the upper chamber, filled with 1 ml saline. After a preincubation period of 30 min in *Sol. 1,* the tissue was allowed to equilibrate in the test solution for 45 min and then exposed on the luminal side for 45 sec to the same test solution, added with ^3H-sucrose (10 μCi/ml) and with $^{36}Cl^-$ and $^{22}Na^+$ (4 μCi/ml). The influx ($\mu eq\ cm^{-2} hr^{-1}$) was constant at least for 75 sec exposure (*see* Table 2); so, by the use of 45-sec periods unidirectional influxes are measured. The fluid stirring was obtained by bubbling the suitable gas mixture in the luminal saline and by continuous perfusion of the serosal chamber with gassed saline moved by a peristaltic pump. At the end of the experiment the medium was withdrawn, the tissue squeezed with ice-cold isotonic solution of mannitol and punched off, then processed as described in ref. 3.

Measurement of Na^+ and Cl^- Transepithelial Fluxes

Gallbladders, opened flat, were mounted between two Lucite chambers, each filled with 6 ml incubation saline. The area exposed was 0.63 cm^2. After a 30-min period of preincubation in *Sol. 1,* the tissue was incubated in the test solution for 2 periods of 30 min each, with $^{22}Na^+$ or $^{36}Cl^-$ added to the serosal fluid (0.5 μCi/ml). Stirring was vigorously carried out both by a magnetic stirrer and by the usual bubbling with the adequate prehumidified gas mixture. At the end of each experimental period the luminal solution was withdrawn and replaced, if necessary, by fresh saline. The radioactivity of the luminal fluid was never greater then $1/200^{th}$ of the serosal radioactivity, so that unidirectional fluxes were measured. Samples in duplicate were analyzed by a liquid scintillation spectrometer (Tri-Carb Packard Instrument Co. model 3385).

Electrical Measurements

These measurements were carried out with the apparatus and the procedure described elsewhere [14, 15].

Measurement of Luminal Acidification

Gallbladders, opened flat, were mounted between two Lucite chambers with an exposed area of 0.63 cm^2. The serosal chamber contained 6 ml fluid; conversely, the luminal chamber had a very small volume and was filled with only 0.5 ml fluid, vigorously stirred by the bubbling of the suitable gas. After a preincubation period of 30 min with *Sol. 1,* the gallbladder was incubated for 1 hr with the test solution and the luminal pH was continuously monitored by a combined electrode (EA 125 Metrohm, Herisau) connected to an E-388 Compensator (Metrohm, Herisau, Switzerland); pH was kept constant by addition of NaOH (3×10^{-2} N), and this way the H^+ produced was calculated.

Salines

The solutions used are reported in Table 1: they were bubbled with 5% CO_2 and 95% O_2, when HCO_3^- was present, with 100% O_2 in its absence.

Results

Site of Bicarbonate Action

The effect of bicarbonate on water and Na^+ transport is reported in Table 3. Both transports are nearly doubled when 25 mM bicarbonate

Table 3. Effects of bicarbonate on water and Na^+ net transport

	Net fluid transport ($\mu l \cdot cm^{-2} \cdot hr^{-1}$)	Net Na^+ transport ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)
Without HCO_3^- $Cl^- = 152.7$ mM (Sol. 2)	45.7 ± 2.9 (33)	7.0 ± 0.4 (33)
With HCO_3^- $Cl^- = 127.7$ mM $HCO_3^- = 25$ mM (Sol. 1)	89.4 ± 5.1 (21)	13.8 ± 0.8 (21)
P%	< 0.1	< 0.1

Data are reported as mean $\pm$SEM, with the number of experiments in parenthesis. Na^+ transport is calculated from water transport, knowing that the transferred solution is a Na^+ salt isotonic saline. *P* = statistic probability.

is substituted for an equivalent amount of Cl^-. In parallel with such an increase, cell Na^+ and Cl^- concentrations (spectrophotometrically or radiochemically determined) significantly rise, as well as the amounts of the two ions and cell water. Conversely, K^+ cell concentration drops in spite of the fact that its total quantity remains constant (Table 4*a* and *b*).

These overall data clearly demonstrate that bicarbonate stimulates Na^+ and Cl^- net uptakes across the apical barrier of the cell so that

Table 4a. Effect of bicarbonate on cell ion concentrations[a]

		Na	*Na[b]	K	Cl	*Cl[b]
A)	Without HCO_3^- $Cl^- = 152.7$ mM (Sol. 2)	69.0±2.5 (89)	42.2±1.8 (66)	128.7±1.4 (88)	79.6±3.4 (27)	56.7±4.9 (9)
B)	With HCO_3^- $Cl^- = 127.7$ mM (Sol. 1)	88.0±2.6 (38)	76.5±3.9 (27)	77.6±2.3 (39)	91.2±3.2 (35)	76.2±6.7 (4)
P%	(*A–B*)	<0.1	<0.1	<1	<2	<5

[a] Concentrations are expressed as meq·liter^{-1}.
[b] Radiochemically determined.

All the data are reported as mean ±SEM, with the number of experiments in parenthesis, *P*=statistic probability.

Table 4b. Effect of bicarbonate on cell water and ion quantities[a]

		Na dw	*Na[b] dw	K dw	Cl dw	*Cl[b] dw	Cell water dw
A)	Without HCO_3^- $Cl^- = 152.7$ mM (Sol. 2)	345 ±12 (89)	211 ±9 (67)	661 ±30 (55)	398 ±41 (27)	283 ±25 (9)	5.0 ±0.2 (61)
B)	With HCO_3^- (25 mM) $Cl^- = 127.7$ mM (Sol. 1)	684 ±35 (32)	573 ±29 (27)	580 ±29 (34)	705 ±48 (30)	571 ±50 (4)	7.5 ±0.3 (34)
P%	(*A–B*)	<0.1	<0.1	NS	<0.1	<0.1	<0.1

[a] Ion quantities are reported as μeq·g^{-1} of mucosa dry wt; cell water is expressed in ml·g^{-1} of mucosa dry wt.
[b] Radiochemically determined.

All the data are reported as mean ±SEM, with the number of experiments in parenthesis. *P*=statistic probability.

water is driven in and the cell swells. No K^+ movement is revealed (its intracellular amount is not modified) with a consequent dilution of the cation.

Na^+ and Cl^- Influxes across the Luminal Barrier

The parallel increase in Na^+ and Cl^- net uptakes across the luminal barrier could be caused by enhanced lumen-cell influxes or by inhibited cell-lumen outfluxes. A tentative calculation of the latter is shown in Fig. 1, where one can observe that outfluxes increase. A direct measurement of the former is reported in Table 5, in which highly significant increases in both Na^+ and Cl^- influxes are evident: the enhancement

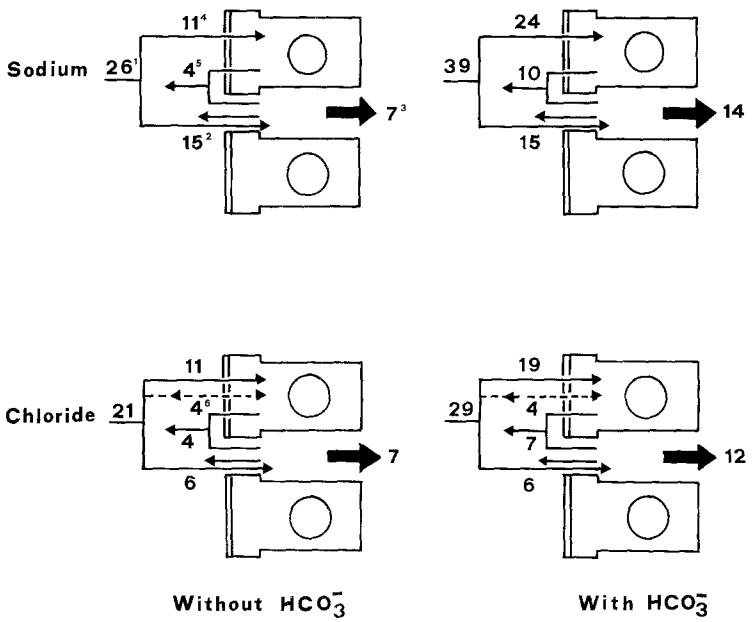

Fig. 1. Cellular and paracellular fluxes of Na^+ and Cl^- in the absence and presence of HCO_3^-. (*1*): Total lumen-cell influxes, taken from Table 5. (*2*): Paracellular fractions, derived from transepithelial (serosa-mucosa) fluxes (Table 6) and from the residual influxes measured in Cl^--free and Na^+-free solutions (Table 9 and pages 157, 158). (*3*): Net transepithelial fluxes of Na^+ (taken from Tables 3 and 9) and of Cl^- (calculated on p. 162). (*4*): Corrected lumen-cell influxes, calculated as the difference between *1* and *2*. (*5*): Backflux: it is comprehensive of the cell to lumen fraction and of the fraction due to the ions accumulated in the channels. It is calculated as the difference between *4* and *3*. (*6*) Cl^- exchange diffusion (*see* ref. 11), calculated as the difference between the residual Cl^- influx in Na^+-free solution (Table 9) and the transepithelial serosa to mucosa flux (Table 6). All the data are expressed as $\mu eq \cdot cm^{-2} \cdot hr^{-1}$

Table 5. Effects of bicarbonate on Na^+ and Cl^- influxes

		Na^+ ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)	Cl^- ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)
A)	Without HCO_3^- $Na^+ = 134$ mM $Cl^- = 127.7$ mM (Sol. 3)	25.9 ± 1.2 (14)	21.3 ± 1.8 (20)
B)	With HCO_3^- $Na^+ = 143$ mM $Cl^- = 127.7$ mM (Sol. 1)	38.6 ± 1.2 (17)	29.8 ± 1.9 (15)
$P\%$	$(A-B)$	<0.1	<1

Influxes are not corrected for the paracellular fraction. Data are given as mean $\pm$ SEM, with the number of experiments in parenthesis. P = statistic probability.

Table 6. Effect of bicarbonate on Na^+ and Cl^- transepithelial fluxes and permeability (serosa to mucosa)

		Na^+		Cl^-	
		J_{Na} [a]	P_{Na} [b]	J_{Cl} [a]	P_{Cl} [b]
A)	Without HCO_3^- $Na^+ = 134$ mM $Cl^- = 127.7$ mM (Sol. 3)	17.6 ± 1.5 (4)	37.2 ± 3.7 (4)	6.9 ± 1.1 (6)	14.9 ± 2.3 (6)
B)	With HCO_3^- $Na^+ = 143$ mM $Cl^- = 127.7$ mM (Sol. 1)	16.7 ± 3.5 (5)	32.5 ± 6.8 (5)	6.0 ± 1.5 (8)	12.8 ± 3.2 (8)
$P\%$	$(A-B)$	NS	NS	NS	NS

[a] Fluxes are expressed in $\mu eq \cdot cm^{-2} \cdot hr^{-1}$.

[b] Permeability coefficients are expressed in $cm \cdot sec^{-1} \cdot 10^{-6}$; they were calculated using the Nernst-Planck equation.

All the data are given as mean $\pm$ SEM, with the number of experiments in parenthesis. P = statistic probability.

of Na^+ influx is about 13 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$, whereas that of Cl^- is much lower (about 7 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$).

Influxes measured with the different incubation fluids are comparable to each other as Cl^- concentration is kept constant (Na_2SO_4) and manni-

tol are substituted for $NaHCO_3$ when it is absent, *see Sol. 3)*; also, Na^+ concentration is varied only to a very limited extent.

The measured influxes are not corrected for the fraction which enters the paracellular route; so the observed increments could be ascribed to an increased permeability of the tight-junction and lateral space pathway or to a solvent drag effect related to the doubled fluid transport. To investigate these possibilities, transepithelial serosa-mucosa fluxes of both Na^+ and Cl^-, in the presence and in the absence of bicarbonate, were determined. These fluxes are measurements of the passage through the paracellular route only [3]. Experiments were carried out in the same solutions used for influx measurements. The results are reported in Table 6. They clearly demonstrate that bicarbonate does not significantly modify the paracellular pathway. The mean ratio between Na^+ and Cl^- permeability is about 2.5, which nearly corresponds to that reported by other authors on the basis of electrical experiments [1].

Effect of Bicarbonate on Membrane Potential

A contribution to the enhanced influxes may be imputed to changes in luminal membrane potential. For this reason we have continuously monitored, with microelectrode technique, the brush border PD of a cell during the change of the luminal solution from a bicarbonate-free to a bicarbonate-containing saline. Figure 2 shows that no effect was

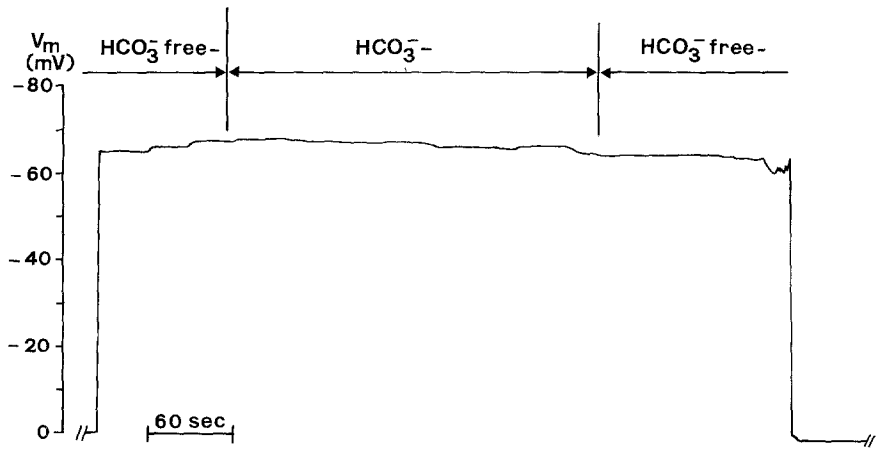

Fig. 2. Recording of brush border membrane potential (V_m) during luminal solution change. Serosal medium was a bicarbonate free medium (*Sol. 2*) in any case. Luminal medium was bicarbonate free (*Sol. 2*) or a bicarbonate containing (*Sol. 1*) saline

Table 7. Effect of bicarbonate on the luminal membrane potential (V_m)

Time (min)	Incubation media	V_m (mV)	P%
0–45	Without HCO_3^- (Sol. 2)	70.2 ± 0.4 (137) (6)	<0.1
45–90	With HCO_3^- (Sol. 1)	66.1 ± 0.5 (127) (6)	<0.1
90–125	Without HCO_3^- (Sol. 2)	70.0 ± 0.5 (100) (6)	

Data are given as mean ± SEM, with the number of impalements and gallbladders in parenthesis. P = statistic probability.

recorded for a 4–5 min exposure to HCO_3^-. A small and reversible depolarization appears only after 45 min from the replacement of the bicarbonate-free with the bicarbonate-containing saline on both sides of the tissue (Table 7). This slow modification is likely to be associated with the decrease of K^+ cell concentration consequent to the exposure to a bicarbonate-containing solution (Table 4a). The depolarization can contribute to vary Na^+ and Cl^- influxes by nearly 8%. As later shown (*see also* Fig. 1), the cellular component of the influxes is about 11 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$ in the absence of bicarbonate; thus, the variation of membrane potential could reduce Na^+ and increase Cl^- influx by only 0.9 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$. This value is negligible with respect to the determined variations which, one has to emphasize, are both positive.

Site of Action of Thiocyanate

In Table 9 (lines *A* and *B*) thiocyanate is shown to halve water and Na^+ transepithelial transport when bicarbonate-containing media are used. Its action seems in some way opposite to that of bicarbonate. Also the effect exerted on cell ion concentrations and amounts and on cell water moves in an opposite direction (Table 8*a* and *b*, lines *A* and *B*): cell Na^+, Cl^- and water are lowered, whereas K^+ is increased. The overall pattern indicates that Na^+ and Cl^- net uptakes through the apical membrane are inhibited.

It is noteworthy that the effect is much more pronounced on Cl^- than on Na^+: Cl^- cell concentration is reduced by about 75%, whereas

Table 8a. Effect of thiocyanate on cell ion concentrations[a]

		Na	*Na[b]	K	*Cl[b]
A)	With HCO_3^-	93.6	75.6	84.9	76.2
	Without SCN^-	±6.4	±13.0	±7.1	±6.7
	(Sol. 5)	(11)	(5)	(11)	(4)
B)	With HCO_3^-	60.3	41.5	146.3	22.3
	With SCN^-	±4.2	±7.6	±5.9	±2.4
	(Sol. 6)	(15)	(5)	(17)	(4)
P%	(*A–B*)	<0.1	<5	<0.1	<0.1
C)	Without HCO_3^-	39.1	17.3	157.8	13.3
	With SCN^-	±8.8	±2.6	±5.8	±9.3
	(Sol. 4)	(10)	(4)	(10)	(6)
P%	(*B–C*)	<5	<5	NS	NS

[a] Concentrations are expressed as meq·liter^{-1}.

[b] Radiochemically determined.

All the data are reported as mean ±SEM, with the number of experiments in parenthesis. *P*=statistic probability.

Table 8b. Effect of thiocyanate on cell water and ion content[a]

		Na / dw	*Na[b] / dw	K / dw	*Cl[b] / dw	Cell water / dw
A)	With HCO_3^-	723	582	622	662	7.7
	Without SCN^-	±73	±100	±67	±54	±0.3
	(Sol. 5)	(9)	(5)	(9)	(9)	(9)
B)	With HCO_3^-	333	245	861	165	5.9
	With SCN^-	±46	±45	±68	±18	±0.6
	(Sol. 6)	(14)	(5)	(16)	(4)	(17)
P%	(*A–B*)	<0.1	<2	<5	<0.1	<5
C)	Without HCO_3^-	206	86	801	60	5.0
	With SCN^-	±46	±13	±79	±47	±0.4
	(Sol. 4)	(10)	(4)	(10)	(6)	(10)
P%	(*B–C*)	NS	<2	NS	NS	NS

[a] Ion quantities are expressed as μeq·g^{-1} of mucosa dry wt; cell water is expressed as ml·g^{-1} of mucosa dry wt.

[b] Radiochemically measured.

All the data are reported as mean ±SEM, with the number of experiments in parenthesis. *P*=statistic probability.

Table 9. Lumen to cell Na^+ and Cl^- influxes (J_{Na}, J_{Cl})[a], Fluid net transport (J_w) and Na^+ net transepithelial transport ($J_{Na(net)}$)[b]

		J_{Na}	J_{Cl}	J_w	$J_{Na(net)}$
A)	With HCO_3^- With Cl^- (Sol. 5)	39.9 ±1.0 (8)	28.0 ±2.5 (12)	90.0 ±7.8 (9)	13.9 ±1.2 (9)
B)	With HCO_3^- With Cl^- With SCN^- (Sol. 6)	25.4 ±2.3 (5)	9.7 ±2.0 (8)	44.3 ±2.6 (8)	6.8 ±0.4 (8)
P%	(*A–B*)	<0.1	<0.1	<0.1	<0.1
C)	With HCO_3^- Without Cl^- (Sol. 7)	26.0 ±3.1 (4)	–	37.9 ±4.2 (6)	5.8 ±0.6 (6)
P%	(*B–C*)	NS	–	NS	NS
D)	Without HCO_3^- With Cl^- With SCN^- (Sol. 11)	14.1 ±3.0 (5)	9.8 ±1.9 (5)	6.7 ±2.6 (9)	1.0 ±0.4 (9)
P%	(*C–D*)	<5	–	<0.1	<0.1
E)	Without HCO_3^- Without Cl^- (Sol. 8)	12.7 ±2.6 (6)	–	1.5 ±1.4 (20)	0.2 ±0.2 (20)
P%	(*D–E*)	NS	–	NS	NS
F)	With HCO_3^- With Cl^- With SCN^- (Sol. 6) +0.5 mM Ouabain	–	–	4.2 ±4.3 (3)	0.6 ±0.7 (3)
P%	(*E–F*)	–	–	NS	NS
G)	With HCO_3^- Without Cl^- With SCN^- (Sol. 9)	–	–	40.1 ±6.2 (4)	6.2 ±1.0 (4)

[a] Influxes are not corrected for the paracellular fraction.

[b] Fluxes are expressed in $\mu eq \cdot cm^{-2} \cdot hr^{-1}$ for ions, and $\mu l \cdot cm^{-2} \cdot hr^{-1}$ for fluid.

All the data are reported as mean ±SEM with the number of experiments in parenthesis. *P*=statistic probability.

Na^+ is lowered by only 45%. Only when HCO_3^- is omitted from the bathing media containing SCN^-, do water and Na^+ transepithelial transport decrease towards zero (Table 9, line *D*). In this case Na^+ cell

concentration is reduced by a further 32%, whereas Cl^- is not further modified to a significant extent (Table 8*a* and *b*, line *C*).

A parallel pattern is displayed by the analysis of Na^+ and Cl^- unidirectional influxes (lumen-cell) (Table 9). Na^+ influx, when media contain bicarbonate (lines *A* and *B*), is inhibited by thiocyanate from 39.9 to 25.4 μeq $cm^{-2}hr^{-1}$ ($\Delta = -14.5$ μeq $cm^{-2}hr^{-1}$) and Cl^- influx, under the same conditions, is inhibited from 28.0 to 9.7 μeq $cm^{-2}hr^{-1}$ ($\Delta = -18.3$ μeq $cm^{-2}hr^{-1}$), equivalent to the value measured when Cl^- entry into the cell via cotransport is completely blocked [3]; the bicarbonate omission (line *D*) does not further inhibit Cl^- influx which remains equal to 9.8 μeq $cm^{-2}hr^{-1}$. This minimal value represents Cl^-, which moves through the paracellular pathway [3] and perhaps an exchange diffusion process [6, 11]. The SCN^--dependent decrease in Na^+ influx is nearly equal to that of Cl^- in accordance with an abolishment of NaCl cotransport. It is impossible to state if the small difference between the two decreases (about 4 μeq $cm^{-2}hr^{-1}$) is statistically significant, as our analysis could not be carried out on paired data.

At all events the inhibition of Na^+ influx caused by SCN^- (from 39.9 to 25.4 μeq $cm^{-2}hr^{-1}$) is equivalent to that obtained by omitting Cl^- from the bathing fluids (from 39.9 to 26.0 μeq $cm^{-2}hr^{-1}$, *see* line *C*) and is noticeable that also water and Na^+ transepithelial transports are lowered to the same extent by SCN^--containing or by Cl^--free media (lines *B* and *C*).

In the HCO_3^- presence, thiocyanate reduces, but does not abolish Na^+ entry into the cell, in spite of the fact that Cl^- entry is completely stopped. In a parallel manner, fluid and Na^+ transepithelial transports are also reduced but not abolished. Only when HCO_3^- is omitted from the incubation media containing SCN^- (line D), are the two transepithelial transports nearly completely blocked and is Na^+ influx decreased to 14.1 μeq $cm^{-2}hr^{-1}$, which is not significantly different from 12.7 μeq $cm^{-2}\,hr^{-1}$, i.e., the value obtained with media free from transportable anions[1] (*see* line *E*).

Thus the fraction of Na^+ influx between 39.9 and 25.4 (26.0) seems coupled with Cl^- and the fraction between 25.4 (26.0) and 14.1 (12.7) is in some way linked with HCO_3^-. The first fraction is affected by thiocyanate, the second is thiocyanate-independent. The same conclusion is

[1] This residual influx represents, in a minimal fraction, Na^+ which enters the cell through independent and electrogenic channels [3, 4, 15, 44], Na^+ which perhaps exchanges with H^+ [33, 47 and this paper] and, as a major part, extracellular Na^+ which enters the paracellular pathway [3].

achieved by examining transepithelial transports of fluid and Na^+ measured with Cl^--free media containing HCO_3^-. Their values are not affected by thiocyanate (lines *C* and *G*).

Thiocyanate Accumulation into the Cell

In Table 10, transepithelial (V_{ms}) and luminal (V_m) membrane potentials are reported under control conditions and after a 30–60 min exposure to thiocyanate. Transepithelial PD varies from a slightly negative to a slightly positive value in accordance with a reduction of Na^+ salt transport and of the consequent negative backdiffusion potential from the lateral spaces to the lumen [24]. Luminal membrane potential increases to -66.1 mV, and this is expected on the basis of the raised K^+ cell concentration (Table 8*a*) and of the dependence of V_m on K^+ [15, 44].

Due to its liposolubility, thiocyanate is likely to cross cell membranes and enter the cytoplasm in spite of the fact that other anions (e.g., Cl^-) are not permeant by such a simple diffusion [3, 15]. To test this point, we have radiochemically measured SCN^- concentration in the cell by exposing the tissue to the ^{14}C-labeled molecule for 1 hr. Thiocyanate cell concentration was 8.5 mM when the exposure was effected on both sides. If a purely passive diffusional permeability is involved, we can predict that the cell concentration should be 1.9 mM (considering a $V_m = -66.1$ mV; *see* Table 10, line *B*), a value significantly different from the determined 8.5 mM. Thus, thiocyanate seems to be accumulated into the cell against its electrochemical potential difference.

On the other hand, the SCN^- cellular concentration (2.2 mM), measured after exposing only the serosal side to the anion, is nearly equal to the predicted equilibrium concentration (1.9 mM). Then, within the limits of errors introduced by the possible loss of SCN^- through the luminal membrane, only a passive diffusional movement seems to take place through the basolateral membrane, so that the accumulation process should be located on the brush border.

Table 10 also reports that, in the absence of bicarbonate, SCN^- cell concentration is largely decreased (3.4 mM) and that, with Na^+-free incubation media[2], it tends towards the equilibrium concentration. These

[2] In this experiment NaSCN in the bathing media was reduced from 25 to 0.5 mM to bring Na^+ near to zero. Since this SCN^- level is without effect on transport and intracellular potential, the equilibrium concentration of the drug was calculated based on -58.7 mV instead of -66.1 mV.

Table 10. Thiocyanate cell concentration and effect of thiocyanate on the transepithelial (V_{ms}) and luminal (V_m) membrane potentials

		SCN^- (meq·liter^{-1}) determined	predicted[a]	V_{ms} (mV)	V_m
A)	With HCO_3^- Without SCN^- (Sol. 1)	–	–	−0.2 ±0.2 (5)	−58.7 ±0.7 (72-5)
B)	With HCO_3^- With SCN^- (25 mM) (Sol. 6)	8.5 ±0.7 (7)	1.9	+0.8 ±0.1 (5)	−66.1[b] ±1.0 (63-5)
C)	Without HCO_3^- With SCN^- (25 mM) (Sol. 4)	3.4 ±0.6	1.9	–	–
D)	Without HCO_3^- With SCN^- (0.5 mM) (Sol. 2 +0.5 mM NaSCN)	0.27 ±0.02 (4)	0.05	–	–
E)	With HCO_3^- With SCN^- (0.5 mM) Na^+ = 0.5 mM (Sol. 10 +0.5 mM NaSCN)	0.08 ±0.03 (4)	0.05	–	–
F)	Serosal side: With HCO_3^-, with SCN^- (Sol. 6) Mucosal side: With HCO_3^-, without SCN^- (Sol. 1)	2.2 ±1.6 (4)	1.9	–	–

[a] Predicted on the basis of the Nernst equation, considering $V_m = -66.1$ and -58.7 mV for SCN^- = 25 and 0.5 mM, respectively.

[b] Measured after 30 min of incubation in SCN^- medium.

All the data are reported as mean ± SEM, with the number of experiments in parenthesis. In the case of V_m, the first number in parenthesis indicates the impalements, the second one the gallbladders.

data indicate that the accumulation process is enhanced by HCO_3^- and is Na^+ dependent.

Metabolic Effects of Thiocyanate?

In those experiments in which thiocyanate (25 mM) was present only in the serosal media, fluid transport and Na^+, K^+, and Cl^- cell concentrations and contents were also determined. The results were not signifi-

cantly different from the controls reported in Table 8*a–b* (line *A*). Then, in spite of its presence in the cytoplasm, SCN^- does not change the intracellular environment: it is clear that it does not act in a metabolic way from the inner side of the brush border. A direct and significant action on cell respiration also seems to be ruled out on the observation that, after thiocyanate treatment on both sides of the epithelium, cell Na^+ decreases and cell K^+ increases. An inhibition of ATP production should cause the opposite effect. For instance , 10^{-4} M dinitrophenol and 3×10^{-3} M monojodoacetate bring cell Na^+ to 137.1 ± 1.6 mM (4 expts.) and K^+ to 9.9 ± 7.2 mM (4 expts.).

Hydrogen Ion Secretion and Bicarbonate Transport

It has been reported that HCO_3^- does not increase H^+ secretion [34]. Then, the HCO_3^--dependent Na^+ influx (*see* page 154) cannot be due to an increased $H^+ - Na^+$ countertransport induced by HCO_3^-. To further examine this crucial point, we have carried out luminal pH measurements, in the presence and in the absence of HCO_3^-, with the same bathing solutions and with the same gas mixture bubbled on both sides of the tissue; pH was kept constant by addition of NaOH (3×10^{-2} N).

The results obtained are reported in Table 11. When the bathing solution is a HCO_3^--free saline (*Sol. 2*), the secretion is equal to 0.23 ± 0.03 μeq $cm^{-2}hr^{-1}$ (6 expts.); it largely increases when the bathing solutions contain bicarbonate (3.37 ± 0.65 μeq $cm^{-2}hr^{-1}$, 10 expts.), but in spite of the presence of bicarbonate, it decreases to zero when Na^+ is absent.

This last datum, concerning Na^+ relevance in the process, completely confirms the previous results of other authors [33, 47] and supports the validity of the method used. On the contrary, a significant increase in secretion, due to the anion, is in disagreement with the data of Sullivan and Berndt, quoted above. The explanation of this fact may be based on the sac preparation used by these authors, with gas mixture bubbled only on the serosal side. In the absence of bicarbonate, in spite of 100% O_2 bubbling, metabolic CO_2 accumulates in the lumen of the sac; this can create a HCO_3^- amount sufficient to enhance H^+ secretion. Even if the increase is only partial, the large variance between animals can make the differences between the two averages not significant.

Table 11. H^+ secretion measured in different bathing media[a]

		J_{H^+} ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)
A)	Without HCO_3^- (Sol. 2)	0.23 ± 0.03 (6)
B)	With HCO_3^- (Sol. 1)	3.37 ± 0.65 (10)
P%	(*A–B*)	< 1
C)	With HCO_3^- With SCN^- (Sol. 6)	3.34 ± 0.40 (9)
P%	(*B–C*)	NS
D)	With HCO_3^- Without Na^+ (Sol. 10)	0.0 ± 0.0 (2)
P%	(*C–D*)	< 0.1

[a] H^+ secretion was measured over a 60-min period and after a 30-min preincubation period.

All the data are reported as mean $\pm$ SEM, with the number of experiments in parenthesis. *P* = statistic probability.

In Table 11 (line *C*), H^+ secretion is shown not to be affected by thiocyanate, also in the presence of bicarbonate. Since Sullivan and Berndt have reported that the drug does not act in the absence of bicarbonate [33], H^+ secretion is clearly independent from it in any case.

Discussion

Enhancement of $Na^+ - Cl^-$ Cotransport by Bicarbonate

All the three hypotheses reported in the Introduction concerning the site and the pattern of bicarbonate action exhibit clear internal complications.

First, the hypothesis about the energetic action of HCO_3^- on Na^+ pump by the synthesis of carbamylphosphate (CP) [26] seems contradictory. Since 2 ATP are needed to synthetize 1 CP, but 1 CP substitutes for 1 ATP on the pump, HCO_3^- seems to reduce instead of favoring the energetic supply.

Second, the hypothesis concerning a SCN^--sensitive HCO_3^--ATPase [41] is complicated by the fact that thiocyanate inhibits NaCl transport, but not H^+ secretion [33].

Third, concerning the hypothesis of the $H^+ - Na^+$ countertransport [33, 47], it requires that, in the presence of bicarbonate, the increased Na^+ entry is accompanied by an enhanced H^+ secretion; on the contrary, under these conditions, Sullivan and Berndt [34] did not observe any increase in luminal acidification.

Moreover, the second and the third hypotheses suppose that the entire increase in Na^+ transport is caused by an induced $NaHCO_3$ transfer. As a matter of fact, it is well documented [8, 45, 46] that when bicarbonate is added to the bathing fluids a $NaHCO_3$ transepithelial transport is involved. However, it does account for only a minor part of the overall increase in Na^+ transport; this may be easily calculated from our data and from the data reported by other authors.

Under basal conditions, when bicarbonate is absent, Cl^- net flux is equal to Na^+ net flux [46]. In the presence of bicarbonate, fluid transport increases from 45.7 to 89.4 $\mu l\ cm^{-2}hr^{-1}$ (*see* Table 3 and ref. 7), from which data it is possible to calculate that Na^+ transport increases from 7.0 to 13.8 $\mu eq\ cm^{-2}hr^{-1}$ (knowing that the fluid transfer is approximately an isotonic solution of Na^+ salts, *see* ref. 8). When 25 mM $NaHCO_3$ is present in the incubation media, the HCO_3^- concentration in the transported fluid is 18 mM [8], which indicates a HCO_3^- transfer equal to 1.6 $\mu eq\ cm^{-2}hr^{-1}$ ($13.8\ \frac{18}{154} = 1.6$) and a Cl^- transfer equal to 12.2 $\mu eq\ cm^{-2}hr^{-1}$ (13.8–1.6 = 12.2). If the enhanced fluid and Na^+ transports were caused only by an induced $NaHCO_3$ transfer the HCO_3^- net flux should be equal to 6.8 $\mu eq\ cm^{-2}hr^{-1}$ (13.8 − 7.0 = 6.8) instead of 1.6 $\mu eq\ cm^{-2}hr^{-1}$ and the Cl^- net flux should remain equal to 7.0 $\mu eq\ cm^{-2}hr^{-1}$. All these calculations clearly emphasize that the presence of bicarbonate not only induces a $NaHCO_3$ transfer, but also enhances NaCl transport.

A second indication concerning an activation on NaCl transepithelial transport may be derived from the paper of Wheeler et al. [45]. These authors report that ethoxzolamide, a potent inhibitor of carbonic anhydrase, reduces the fluid absorption without any alteration of its ionic composition. This means that the produced H_2CO_3 not only is useful for the bicarbonate transfer, but also activates NaCl transport. If the H_2CO_3 produced was only a substrate for a $NaHCO_3$ transfer, the inhibition of its production should alter the Cl^-/HCO_3^- ratio in the transported fluid in favor of Cl^-.

Finally, our results directly demonstrate an activation by bicarbonate of both Na^+ and Cl^- entry at the apical membrane of the cell: in the presence of bicarbonate, not only Na^+ cell concentration and content and not only Na^+ influx (lumen-cell), but also the corresponding parameters for Cl^- increase. Since in rabbit gallbladder the cotransport with Na^+ is the only pathway through which Cl^- enters the cell at the apical barrier [3, 9, 11, 15], this cotransport seems to be the target of the HCO_3^- action.

The activation by HCO_3^- and the inhibition by SCN^- seem to be among the main features of NaCl cotransport.

Sullivan and Berndt [33] reported that fluid transport is inhibited by 25 mM thiocyanate in the absence of bicarbonate; Van Os et al. [42] described a partial inhibition by SCN^- also when the bathing media contain bicarbonate. Our data completely confirm these results and shed some light on the site of action of the drug in the gallbladder. Its action on the HCO_3^--ATPase [17, 31], on acid secretion in the stomach [16], on the $Cl^- - HCO_3^-$ exchange in the gills [5], and on cytochrome *c* [18] is well demonstrated. In gallbladder epithelium it reduces Na^+ and Cl^- cell concentrations simultaneously with the inhibition of water and Na^+ transport. This suggests an action at the brush border level on the cotransport of the two ions. The more pronounced effect on Cl^- cell concentration, the elimination of the cellular fraction of Cl^- influx, the correspondence of the inhibition of Na^+ influx, water and Na^+ transepithelial transport obtained with SCN^- and with Cl^--free media, the lack of any HCO_3^- action on Cl^- influx in the presence of SCN^-, all emphasize that Cl^- entry into the cell is completely blocked by SCN^-. Consequently, the fraction of Na^+ influx linked to Cl^- by the cotransport system is nearly completely eliminated. The residual influx of Na^+ (4 μeq $cm^{-2}hr^{-1}$, difference between the decreases in Na^+ and Cl^- influxes), if statistically significant, could be ascribed to a persistent slight cotransport of Na^+ with a competing anion, i.e., with SCN^- itself. The process of accumulation of SCN^- into the cell exhibits all the features of the cotransport system (i.e., Na^+-dependence, activation by HCO_3^-). Thus, SCN^- seems to compete with Cl^- for the cotransport with Na^+, with the consequent abolition of Cl^- entry into the cell and the formation of a poorly mobile complex (carrier-$Na^+ - SCN^-$) which slowly permeates and accumulates the drug into the cell. On the basis of our data, any metabolic effect of SCN^-, due to its ability to bind cytochrome *c*, is ruled out.

How Can Bicarbonate Activate $Na^+ - Cl^-$ Cotransport?

A possibility is that bicarbonate activates a regulatory site present on the carrier. This site seems partially stimulated by HCO_3^- also when the anion is absent from the bathing solutions; in fact, the inhibitors of carbonic anhydrase also reduce NaCl transport under these conditions [33]. The hydration of metabolic CO_2 then seems to be important. This regulatory site should remain active also when SCN^- is linked to the Cl^- site, as SCN^- accumulation is strongly reduced when HCO_3^--free bathing media are used.

The second possibility is that the two carrier sites for Na^+ and Cl^- exhibit a higher affinity, respectively, for H^+ and HCO_3^- when the carrier is on the side of the plasma membrane facing the cytoplasm. In such a way, Na^+ and Cl^- would be the main absorbed ions from the lumen to the cell, whereas H^+ and HCO_3^-, at least in part, would occupy the sites of the carrier coming back to the lumen. Thus, $Na^+ - Cl^-$ cotransport could exchange Na^+/H^+ and Cl^-/HCO_3^-. When HCO_3^- is absent from the bathing media metabolic H_2CO_3 would be utilized, but when HCO_3^- is present in the media this fraction too would contribute so that a larger $H^+ - HCO_3^-$ backflux is built up corresponding to a larger loading of the carrier. Since the mobility of the loaded carrier is higher than that of the unloaded one [3], larger amounts of molecules could return and face outwards; this could enhance NaCl influx.

It is difficult to choose between the two possibilities only on the basis of the known data. However, some considerations suggest that the second explanation is less sound. As a matter of fact, it needs a H_2CO_3 secretion via the cotransport both when HCO_3^- is present and when it is absent from the bathing media. It has been reported [33] that inhibitors of carbonic anhydrase can reduce NaCl transport, but not acid secretion, in the absence of bicarbonate, whereas they reduce both parameters in the presence of bicarbonate. Moreover, our data show that, with bicarbonate-containing media also, H^+ secretion is independent from SCN^-, whereas the cotransport is inhibited by the drug. On this basis, the hypothesis of an allosteric activation of the carrier is the most probable one.

$NaHCO_3$ Transepithelial Transfer and $Na^+ - H^+$ Countertransport

We have previously calculated that in the presence of HCO_3^-, 1.6 μeq $cm^{-2}hr^{-1}$ of $NaHCO_3$ should be transepithelially transferred. Our results

confirm that a $NaHCO_3$ transport takes place in the gallbladder. In fact, bicarbonate induces an increase in Cl^- influx which is not so large as that of Na^+, so that also another anion, i.e., HCO_3^- itself, should be transported. Moreover, a fraction of Na^+ cellular concentration, of Na^+ influx, of water and Na^+ transepithelial transport is HCO_3^--dependent, but SCN^-- and Cl^--independent.

The $NaHCO_3$ transfer could be carried out by the cotransport system itself. In fact, a competition between HCO_3^- and Cl^- for the same site of the cotransporting carrier is not excluded, but:

1) It must be largely exceeded by the activation of the cotransport itself, as Cl^- influx even increases.

2) The cotransported HCO_3^- can account for only a minor part of HCO_3^- influx as thiocyanate does not inhibit the HCO_3^--dependent Na^+ influx, whereas it nearly completely inhibits that fraction which is linked to Cl^-.

3) It is not possible to state that the sequence of affinity for the anion site of the cotransport is $HCO_3^- > SCN^- > Cl^-$ (so that SCN^- can stop Cl^-, but not HCO_3^- influx) as HCO_3^- affinity for the transport system has been demonstrated to be lower than that of Cl^- [8].

All these observations lead us to conceive NaCl cotransport and $NaHCO_3$ transfer as distinct processes so that the stimulation by HCO_3^- of Na^+ transport seems to be due both to an enhancement of $Na^+ - Cl^-$ cotransport and to an induction of a $NaHCO_3$ transfer.

The next problem to deal with is the nature of this independent $NaHCO_3$ transfer. It is noticeable that it is not inhibited by thiocyanate and it is electrically silent. These two features are not in accordance with the transport sustained by a HCO_3^--ATPase, so that this enzyme is quite unlikely to be of some relevance. Also the slow and slight depolarization observed with time after incubation with bicarbonate-containing media is against the presence of this ATPase which *per se* should cause hyperpolarization. Moreover, a preliminary direct assay of it, which we are carrying out on the isolated brush borders of the gallbladder, is providing a negative outcome (*unpublished results*). On the other hand, recent studies on plasma membranes of different epithelial cells, in which this ATPase was previously revealed, indicate that its finding was only due to contamination by mitochondria [39, 40]. Van Os himself, in collaboration with Mircheff and Wright, has excluded that the HCO_3^--ATPase is present in the brush borders of intestinal epithelial cells [43].

Thus, it seems that $NaHCO_3$ transfer may be attributed either to a $Na^+ - HCO_3^-$ independent cotransport, not affected by SCN^-, or to

a neutral $H^+ - Na^+$ countertransport. Both processes should cause a luminal acidification, which in fact occurs, but with the former process the acidification is the derived phenomenon, whereas with the latter it is the primary event. The presence of a secretion of hydrogen ions, derived from fixed acids, also when HCO_3^- is absent [33, 47 and this paper] demonstrates that the proton extrusion is a primary event. A second proof, which leads to the same conclusion, is given by the fact that in the presence of HCO_3^- the luminal pCO_2 increases [34], whereas a primary HCO_3^- absorption should cause its decrease [32]. Moreover, under the same conditions the luminal acidification becomes sensitive to inhibitors of carbonic anhydrase [34]; this enzyme is localized in the gallbladder beneath the brush border and along the basolateral membranes [13]. Also, the fact that SCN^- does not inhibit the HCO_3^--dependent fraction of Na^+ influx is in favor of a $Na^+ - H^+$ countertransport, rather than of a $Na^+ - HCO_3^-$ cotransport. Finally, our data indicate a significant increase in H^+ secretion in the presence of HCO_3^-, and this observation eliminates any internal discrepancy in the countertransport hypothesis. All these data suggest that H^+ derived from H_2CO_3 synthetized in the cytoplasm can be secreted, when bicarbonate is present, in exchange with Na^+ which enters the cell "downhill". Our data confirm that, with Na^+-free bathing solutions, the secretion is stopped.

In conclusion, the activated $Na^+ - Cl^-$ cotransport and the activated $H^+ - Na^+$ countertransport seem to account for the bicarbonate stimulation of the transepithelial transfer of Na^+ salts and fluid[3].

How Can Bicarbonate Activate the Countertransport?

To explain HCO_3^- entry into the cell by the mechanism of $H^+ - Na^+$ countertransport, it is necessary that the luminal solution be more acid than the cell. Only in this way does HCO_3^- tend to bind H^+ in the lumen and to dissociate it in the cytoplasm. Zeuthen [49] has observed in the *Necturus* gallbladder that the pH is 6.5–7.2 in the proximity of the brush border and 6.9 at the apical end of the cell, also when in the bulk solution is 7.5. Similar observations have been made on small intestine [22, 23]. They demonstrate that an acid microenvironment does exist near the brush border; it can also explain HCO_3^- absorption when

[3] It has been observed that bicarbonate stimulates Na^+ transport mainly when it is present on the serosal side [9]. It should be very difficult to explain this outcome if only a $NaHCO_3$ absorption was induced, whereas no internal contradiction is given by a NaCl cotransport stimulated by bicarbonate on the cytoplasm side.

the secretion is not yet sufficient to adequately decrease the pH of the bulk solution.

The activation of $Na^+ - Cl^-$ cotransport raises Na^+ cell concentration and indirectly depolarizes intracellular electrical potential, so that the lumen-cell gradient of Na^+ electrochemical potential diminishes: the countertransport work should then be depressed. This way then seems inadequate to explain the countertransport stimulation by bicarbonate. On the contrary, it is conceivable that H^+ secretion, with the consequent entry into the cell of H_2CO_3 and CO_2, causes a slight decrease in cellular pH which can enhance the countertransport and stimulate Na^+ influx. This is possible only if the "downhill" Na^+ entry is regulated by a limiting pool of exchangeable H^+.

An allosteric activation of the countertransport by HCO_3^- may be an alternative explanation of the observed data.

Enhanced HCO_3^- Transport in the Absence of Cl^-

A last point to discuss is the following: the block of $Na^+ - Cl^-$ cotransport, due to the absence of Cl^- in the bathing media, stimulates $NaHCO_3$ transport. This fact is clear if one considers that HCO_3^- transepithelial transfer is equal to 1.6 $\mu eq\ cm^{-2}hr^{-1}$ when $Na^+ - Cl^-$ cotransport functions (*see* page 162), but rises to 5.8 $\mu eq\ cm^{-2}hr^{-1}$ when Cl^--free bathing solutions are used. This may be deduced from Table 9, line *C*: in the absence of Cl^- the residual Na^+ transport is equal to 5.8 $\mu eq\ cm^{-2}hr^{-1}$ and should be followed by HCO_3^-, the only permeant anion present. The most likely explanation is that, under these conditions, HCO_3^- occupies the Cl^- site on the cotransporting carrier.

An equivalent net movement of Na^+ (6.8 $\mu eq\ cm^{-2}hr^{-1}$, *see* Table 9, line *B*) is observed when media containing HCO_3^-, Cl^- and SCN^- are used. Since, under these conditions, Cl^- transport is abolished by the strong SCN^- competition and the Cl^- site is presumably occupied by SCN^-, it is difficult to conceive that in this case HCO_3^- can be cotransported with Na^+. On the other hand, Table 11 shows that H^+ secretion is SCN^--independent and that the possible HCO_3^- transport through that route is unchanged. It is, then, to be concluded that SCN^- follows the transferred Na^+. This anion is in fact accumulated into the cell by the cotransport system (*see* page 158) and has a large gradient of electrochemical potential favorable to bring it out of the cell towards the subepithelial spaces; moreover, it can freely permeate through the basolateral membrane of the cell (*see* page 158).

In conclusion, when the cotransport system has the Cl^- site free from this anion or occupied by SCN^-, the level of Na^+ transport can be larger than predicted, as HCO_3^- or SCN^- themselves can, at least in part, substitute for Cl^- in the transport.

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