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Influx Mechanisms for Na^+ and Cl^- Across the Brush Border Membrane of Leaky Epithelia: A Model and Microelectrode Study

H. Baerentsen, F. Giraldez* and T. Zeuthen

Institute of Medical Physiology A, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen N, Denmark, and
* The Physiological Laboratory, Cambridge, England

Summary. This paper presents a numerical model for the movement of Na^+, K^+, Cl^-, H^+ and HCO_3^- in a leaky epithelium. The model describes the active transport of Na^+ and K^+ at the serosal membrane and electrodiffusive permeation across the mucosal, serosal and junctional pathways. The model accounts for H^+ and HCO_3^- production in the cell. The influx of Na^+ and Cl^- is assumed to occur mainly via Na/H and Cl/HCO_3 exchange. The behavior of the cell, with this influx mechanism, is compared to a cell with an obligatory neutral coupled influx of Na^+ and Cl^-. All parameters are obtained from the literature, primarily from studies utilizing the *Necturus* gallbladder. The analysis shows (i) that it is virtually impossible in *steady-state* experiments to distinguish between cells with $Na/H-HCO_3/Cl$ transport and cells with Na/Cl transport mechanisms. (ii) That *nonsteady-state* experiments can decide whether $Na/H-HCO_3/Cl$ or Na/Cl transport mechanisms mediate the influx of salt. A comparison between studies with ion-selective microelectrodes and the model predictions indicates that the influx of Na^+ and Cl^- is mediated by $Na/H-HCO_3/Cl$ transport when the external solutions contain CO_2 and HCO_3. (iii) The model also explains the diuretic effects of furosemide and carbonic anhydrase inhibitor, as well as the stimulatory effects on salt transport of elevated levels of HCO_3^- at a constant pH. (iv) The model fails to explain some experiments performed in HCO_3/CO_2-free media and some experiments using inhibitors.

Key Words epithelia · Na/H exchange · Cl/HCO_3 exchange · ion-selective microelectrodes · transients · NaCl cotransport

Introduction

Epithelial cells possess special influx mechanisms for Na^+ and Cl^- ions in order to accommodate large transepithelial fluxes of these ions. Electrodiffusion is, within the physiological range of parameters, to slow.

It has been demonstrated in leaky epithelia that a large proportion of the influx of Na^+ and Cl^- across the luminal membrane is coupled in a ratio which approximates one. Recent estimates in *Necturus* gallbladder suggest that only 15% of Na^+ influx is electrodiffusive (Graf & Giebisch, 1979). The question discussed in this paper is whether the Na^+ and Cl^- influx is an obligatory coupling where a carrier translocates one Na^+ ion and one Cl^- ion from the lumen into the cell (Na/Cl transport) or whether Na^+ is exchanged neutrally with H^+ by one carrier and Cl^- exchanged neutrally with HCO_3^- by another carrier, the rate of transport of the two transport systems being coupled indirectly via the intracellular production of H^+ and HCO_3^- ($Na/H-HCO_3/Cl$ transport).

In a recent paper (Baerentsen, Christensen, Grove-Thomsen & Zeuthen, 1982), we derived a numerical model for the *Necturus* gallbladder which closely simulated many of the steady- and nonsteady-state experiments that have been performed. The model was based on electrodiffusion and simple kinetic descriptions of the active and passive carrier-mediated transport systems for Na^+, K^+ and Cl^- ions. Na^+ and Cl^- was assumed to enter neutrally. In the present paper we have extended this numerical model of the *Necturus* gallbladder to incorporate the HCO_3^- and H^+ ions and their intracellular production which is catalyzed by carbonic anhydrase (Fig. 1). Similar model considerations have been performed on the salivary gland (Cook & Young, 1981). We are studying the model when the transport of Na^+ and Cl^- are implemented by means of Na/H and Cl/HCO_3 countertransport and when it is implemented by Na/Cl cotransport. We have assumed the simplest transport kinetics and have selected the parameters in such a manner that the fluxes and concentration agree with observed values.

The analysis shows three things: (i) In var-

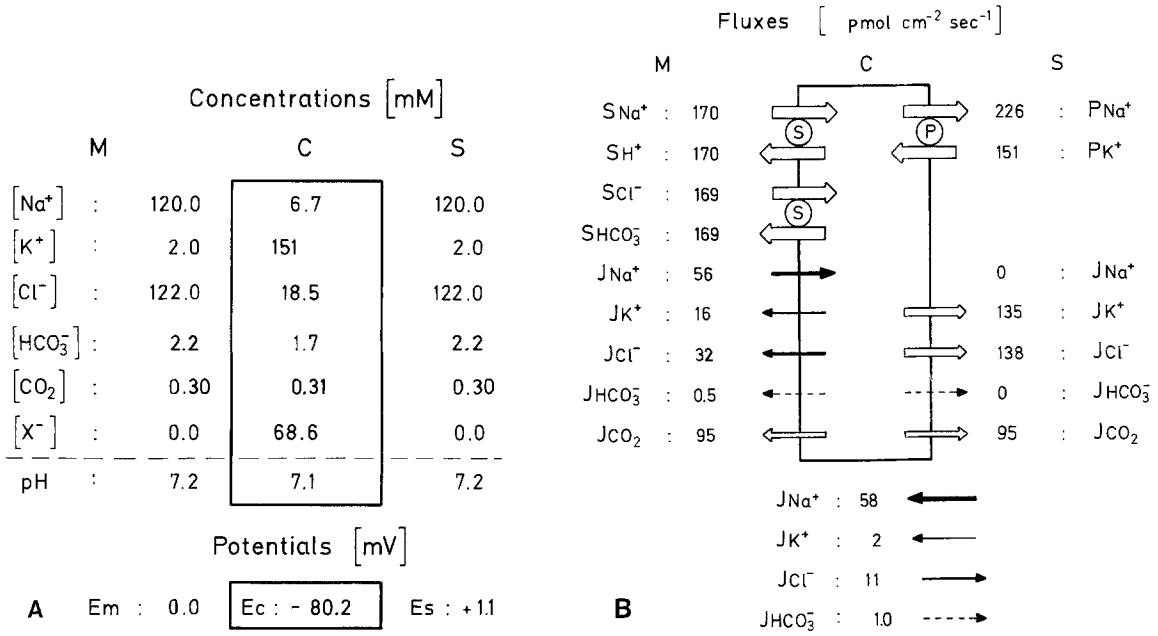

Fig. 1. Steady state of the model cell at an external pCO_2 of 1.3% (0.3 mM). *A* shows the concentrations and potentials in the mucosal (*m*), cellular (*c*), and serosal (*s*) compartment. *B* shows the ion fluxes across the membranes. *J* indicates electrodiffusive fluxes, *S* indicates the neutral countertransport systems of Na/H and of Cl/HCO_3. *P* indicates the Na^+ and K^+ pumped by the Na/K-ATPase. At the lower aspect of Fig. 1*B* the fluxes across the junctions are shown. *See* Table 1 and Materials and Methods for the description of parameters

ious steady states the influx of Na^+ mediated by the Na/H transport mechanism will be equal to the influx of Cl^- mediated by the Cl/HCO_3 countertransport mechanism within the limits of experimental precision. The model applies whether the transport rates are varied by changing the concentrations of Na^+ or Cl^-, changing kinetic parameters, changing CO_2 levels or by slowing the H/HCO_3 production by use of a carbonic anhydrase inhibitor. (ii) The transient changes in intracellular Na^+ and Cl^- concentrations effected by removal of either mucosal Na^+ ions or Cl^- ions can be explained by $Na/H-HCO_3/Cl$ transport but not by Na/Cl transport. (iii) Volume transport in the mode where mucosal transport is described by $Na/H-HCO_3/Cl$ should be reduced by a factor of three to four when $Na/H-HCO_3/Cl$ transport is abolished (*see* Fig. 4). This appears to be contradicted by some experiments using specific inhibitors.

List of Symbols

m, c, s	index: mucosal, cellular and serosal compartment
V	cellular volume: $cm^3\ cm^{-2}$
Q_i	moles per cm^2 of the ion i: $mol\ cm^{-2}$
X^-	moles per cm^2 of intracellular fixed anions: $mol\ cm^{-2}$
z_x	average charge of intracellular anions
C^a	concentration of the ion i in the a compartment: mM
z_i	charge valency of the ion i
M_a	concentration of extracellular impermeable ions: mM
I_i^{ab}	current carried by the ion i through the ab membrane: $\mu A\ cm^{-2}$
I_T	total current through the epithelium: $A\ cm^{-2}$
E^{ab}	voltage across the ab membrane with respect to the a compartment: mV
J_i^{ab}	electrodiffusional flux of species i: $mol\ cm^{-2}\ sec^{-1}$, in case of H_2O: $cm^3\ cm^{-2}\ sec^{-1}$
P_i^{ab}	passive permeability of the ab membrane to the ion i: $cm\ sec^{-1}$
F_{Na}^{cs}, F_K^{cs}	ion fluxes of Na and K carried by the Na/K pump: $mol\ cm^{-2}\ sec^{-1}$
F_{Na}^{max}, F_K^{max}	saturation fluxes of the Na/K pump: $mol\ cm^{-2}\ sec^{-1}$
$K_{M,Na}, K_{M,K}$	affinity constants with respect to intracellular Na and extracellular K: mM
r	coupling ratio of the Na/K pump
$\frac{RT}{F}$	*ca.* 26 mV at room temperature
L_p^{ab}	hydraulic permeability of the membrane ab ($cm\ sec^{-1}\ osm^{-1}$)
D_{CO_2}	rate of endogeneous production of CO_2
S_{Na}^{mc}, S_H^{mc}	coupled influx across the mucosal membrane of Na^+ equal to the coupled efflux of H^+. The index mc is omitted in the Results and Discussion sections, because only transport across the brush border is considered
$S_{Cl}^{mc}, S_{HCO_3}^{mc}$	coupled influx of Cl^-, equal to the coupled

	influx of HCO_3^-. *mc* can be omitted, see above note
$K^s_{Na,H}$	proportionality constant: $cm^4\,mol^{-1}\,sec^{-1}$
K^s_{Cl,HCO_3}	proportionality constant: $cm^4\,mol^{-1}\,sec^{-1}$
$\Delta\tilde{\mu}_i^{ab}$	electrochemical potential difference for ion i across *ab* membrane
V_o	velocity constant for the formation of H^+ and HCO_3^-
T^{mc}_{Na}, T^{mc}_{Cl}	coupled NaCl entry fluxes: $mol\,cm^{-2}\,sec^{-1}$
K^T_{NaCl}	proportionality constant: $cm^4\,mol^{-1}\,sec^{-1}$
E^{ij}	potential difference across the tissue membrane *ij*: $E^i - E^j$: mV
Lp^{ij}	osmotic water permeability: $cm\,sec^{-1}\,osm^{-1}$
V^{Na}, V^{Cl}	potential recorded by the ion-selective microelectrode after subtraction of the electrical potential: mV

Materials and Methods

Model Description

This analysis is based upon the Koefoed-Johnson-Ussing three-compartment model of the epithelium. The cell layer (*c*) separates two infinite compartments, the mucosal (*m*) and the serosal (*s*) (Fig. 1). The cellular compartment is separated from the mucosal and the serosal compartment by the luminal (*mc*) and the basolateral membrane (*cs*). Ion and water transport are assumed to take place transcellularly through the cell membranes or through the cell junctions (*ms*). All compartments are assumed to be well stirred.

All electrodiffusion is described by the Goldman-Hodgkin-Katz constant-field equation

$$J_n^{ij} = P_n^{ij}\frac{Fz_n}{RT}E^{ij}\frac{C_n^i \exp\left(\frac{Fz_n}{RT}E^{ij}\right) - C_n^j}{\exp\left(\frac{Fz_n}{RT}E^{ij}\right) - 1} \tag{1}$$

where the indices i and j refer to specific compartments, the index n refers to the ion involved, P is the permeability, E is the potential difference across the membrane ($E^{ij} = E^i - E^j$), z is the charge valency of the ion, C is the concentration and R, T and F have their usual meanings.

Active ion transport mediated by Na/K-ATPase is described by the saturation kinetic scheme

$$F^{cs}_{Na} = F^{max}_{Na}\left(\frac{C^c_{Na}}{C^c_{Na} + K_{M,Na}}\right)^3\left(\frac{C^s_K}{C^s_K + K_{M,K}}\right)^2 \tag{2}$$

$$F^{cs}_K = -F^{cs}_{Na}/r$$

where F^{max} is the saturated active ion flux, K_M is the concentration at half-saturation and r is the coupling ratio between the active fluxes of Na^+ and K^+.

To include coupled, passive flows of ions we assume the existence of two types of coupled transport which are described by the first-order kinetics

$$S^{ij}_{n1} = K^S_{n1n2}(C^i_{n1}C^j_{n2} - C^j_{n1}C^i_{n2}), \tag{4}$$

$$S^{ij}_{n2} = -S^{ij}_{n1}. \tag{5}$$

These represent electrically neutral *countertransport* systems where the favorable gradient of one ion induces a counterflux of another ion.

$$T^{ij}_{n1} = K^T_{n1n2}(C^i_{n1}C^i_{n2} - C^j_{n1}C^j_{n2}), \tag{6}$$

$$T^{ij}_{n2} = T^{ij}_{n1}. \tag{7}$$

These equations represent an electrically neutral *cotransport* system where the gradient of one ion induces a coflux of another ion. The indices i and j refer to specific compartments, the indices $n1$ and $n2$ to specific ions, and K^S and K^T are rate constants.

The rate of the reaction $CO_2 + H_2O = H^+ + HCO_3^-$ inside the cell is enhanced by the enzyme carbonic anhydrase. The reaction is assumed to follow the rate law

$$-\frac{d[CO_2]}{dt} = \frac{d[H^+]}{dt} = \frac{d[HCO_3^-]}{dt} = V_o\left(CO_2 - \frac{[H^+][HCO_3^-]}{K_a}\right) \tag{8}$$

where V_o is a velocity constant for the reaction, and $K_a = 10^{-6.35}$ mol/liter at room temperature.

Water fluxes across the cell membranes are assumed to be proportional to the osmotic pressure across the membranes

$$J^{ij}_w = L^{ij}_p(\Sigma_n C^j_n - \Sigma_n C^i_n) \tag{9}$$

where L_p is the osmotic conductance of the membrane. The summations are carried out over all ion types present.

With the given assumptions it is now possible to write the following set of coupled differential equations

$$0 = C^c_{Na} + C^c_K - C^c_{Cl} - C^c_{HCO_3} + C^c_H + Z_x X^c/V, \tag{10}$$

$$\frac{dV}{dt} = J^{mc}_w - J^{cs}_w, \tag{11}$$

$$\frac{dQ_{Na}}{dt} = J^{mc}_{Na} - J^{cs}_{Na} + S^{mc}_{Na} - F^{cs}_{Na}, \tag{12}$$

$$\frac{dQ_K}{dt} = J^{mc}_K - J^{cs}_K + F^{cs}_K, \tag{13}$$

$$\frac{dQ_{Cl}}{dt} = J^{mc}_{Cl} - J^{cs}_{Cl} + S^{mc}_{Cl}, \tag{14}$$

$$\frac{dQ_{HCO_3}}{dt} = J^{mc}_{HCO_3} - J^{cs}_{HCO_3} + S^{mc}_{HCO_3} + V\cdot Y + C^c_{HCO_3}\frac{dV}{dt}, \tag{15}$$

$$\frac{dQ_H}{dt} = J^{mc}_H - J^{cs}_H + S^{mc}_H + V\cdot Y + C^c_H\frac{dV}{dt}, \tag{16}$$

$$\frac{dQ_{CO_2}}{dt} = J^{mc}_{CO_2} - J^{cs}_{CO_2} - V\cdot Y + C^c_{CO_2}\frac{dV}{dt} + D, \tag{17}$$

$$0 = E^{mc} + E^{cs} - E^{clamp}, \tag{18a}$$

$$0 = I^{mc} + I^{cs} - I^{clamp} \tag{18b}$$

where V is the cell volume, X^c is the amount of intracellular fixed ions with the average charge valency z_x, $Q_n = C^c_n V$ is the amount of intracellular ions of the type n, $Y = V_o([CO_2] - [H^+][HCO_3^-]/K_a$, $I^{ij} = \Sigma_n z_n J^{ij}_n$ is the total current carried through the membrane with indices *il*, E^{clamp} is the clamp potential and I^{clamp} is the clamp current. D is the rate of endogeneous production of CO_2. In

Table 1. Permeabilities of the cellular and paracellular pathways

	mc	*ms*	*cs*
Na	0.1	10.8	0.0
K	1.1	21.2	10.0
Cl	0.7	2.0	3.0
HCO_3	0.1	10.0	0.1
H	0.01	0.01	0.01
CO_2	10000	10000	10000

Unit: 10^{-6} cm sec^{-1}; *mc:* mucosal membrane; *cs:* serosal membrane; *ms:* paracellular pathway.
For the choice of parameters for Na^+, Cl^- and K^+ *see* Baerentsen et al. (1982). The choices of the other parameters are discussed on p. 208.

Eqs. (10)-(18) we use the fluxes marked *S* (Eq. 4 and 5). These can be interchanged with the fluxes marked *T* (Eq. 6 and 7).

Equation (10) describes electroneutrality. Equations (11) and (12) to (17) describe mass balance. Equations (18a) and (18b) are mutually exclusive and describe the experimental setup. If the specific experiment is performed by voltage clamp, Eq. (18a) should be used, and if the experiment is performed by current clamp, (18b) should be used. Most situations investigated in this paper are current clamp, $I^{clamp}=0$. Equation (18b) takes into account the paracellular fluxes.

The system of equations describes the relationship between the variables: V, Q_{Na}, Q_K, Q_{Cl}, Q_{HCO_3}, Q_H, E^{mc} and E^{cs}. Steady states are obtained when all time derivatives are zero. The response of the model cell to a given perturbation is obtained by integration.

We obtained a steady-state solution to the mathematical model by using a modified version of the Gauss-Newton method for unconstrained minimization. Finite difference approximations of third order was used to integrate the derivatives. All computations were carried out on a UNIVAC 1100 Computer at RECKU (Regional Computer Center at the University of Copenhagen).

Microelectrodes

The microelectrode techniques were essentially the same as those described previously (Zeuthen, 1982). *Necturus* gallbladder cells were probed by double-barrelled ion-selective microelectrodes (Zeuthen, 1980) and either intracellular Na^+ or Cl^- concentration was recorded. The initial rate of change in concentration of these ions was examined when the mucosal solution was rendered Cl^--free by replacement with gluconate of Na^+-free by replacement with TRIS.

Solutions

The choice of solution presented a practical problem. For reasons of comparison with other microelectrode experiments (e.g. Ericson & Spring, 1982*a*, *b*) it was useful to use (in mM): Na^+ 100, K^+ 2.5, Ca^{++} 1, Mg^{++} 1, Cl^- 96, HCO_3^- 10, PO_4^{3-} 0.5, bubbled with 99% O_2/1% CO_2, pH ~7.6. On the other hand this solution is not well-defined mathematically because pH is not defined by the HCO_3^-/CO_2 ratio alone, and the final pH equilibration will involve changes in concentrations. In the model considerations we therefore used solutions without PO_4^{3-}. Furthermore, in the model the effects of varying Na^+, Cl^-, pCO_2 and HCO_3^- concentrations was to be tested (*see*, e.g., Fig. 4). Na^+ and Cl^- concentrations were therefore decreased from a maximally 120 mM while different concentrations of HCO_3^-/CO_2 were used.

Choice of Parameters

For compatibility we have used the same parameters for the permeation of Na^+, K^+ and Cl^- as in our previous publication (Table 1 in Baerentsen et al., 1982), except for P_{Cl}^{cs} which is 3×10^{-5} cm sec^{-1} in this study, compared to 5×10^{-5} cm sec^{-1} in the previous study.

The Na/K pump is assumed to be electrogenic with $F_{Na}^{max}=-\frac{3}{2}F_K^{max}=1200$ pmol cm^{-2} sec^{-1}; $K_{M,Na}=5$ mM and $K_{M,K}=0.1$ mM. The amount of fixed intracellular anions is $X^-=55\times10^{-3}$ mol/cm^2 and the average charge $z_X=-2$. The water permeabilities $L_p^{mc}=0.5$ L_p^{cs} are about 10^{-3} cm $osmol^{-1}$ sec^{-1} (Persson & Spring, 1982; Zeuthen, 1982; *see also* Table 1).

In this study we varied $P_{HCO_3}^{mc}=P_{HCO_3}^{cs}$ from 5×10^{-6} to 0.1×10^{-6} cm sec^{-1}; $P_{HCO_3}^{ms}$ is constant at 10.0×10^{-6} cm sec^{-1}; $P_H^{mc}=P_H^{cs}=P_H^{ms}=0.01\times10^{-6}$ cm sec^{-1}. The choices are somewhat arbitrary, but the effects of varying $P_{HCO_3}^{mc}$ and $P_{HCO_3}^{cs}$ will be investigated. As previously reported P_{HCO_3} is likely smaller than our choice for this parameter (Hunter, 1977; Sachs, Faller & Rabon, 1982).

The passage of CO_2 through the membrane has not been found to be rate limiting and it is difficult to assign a number to its permeability. It must be several orders of magnitude larger than that for any ion. We use the values $P_{CO_2}^{mc}=P_{CO_2}^{cs}=P_{CO_2}^{ms}=0.01$ cm sec^{-1}.

We assume that the reaction $CO_2+H_2=H_2CO_3=H^+ +HCO_3^-$ can be described by first-order kinetics. This means the intermediate reaction (H_2CO_3 formation and dissociation) is instantaneous and the overall reaction can be described by the rate law, Eq. (8). The magnitude of the velocity constant V_0 is dependent upon the availability of the enzyme carbonic anhydrase. Unfortunately, there is no estimate of the velocity constant for the gallbladder epithelia. We have assumed the constant is the same as in the red blood cell, $V_0=10^4$ sec^{-1} (Roughton, 1964).

The parameter D_{CO_2} describes the rate of metabolic production of CO_2 in the gallbladder cell, a part of which arises from the activity of the Na/K-ATPases. This part is proportional to the active Na flux. We have assumed $D_{CO_2}=360$ pmol cm^{-2} sec^{-1}, independent of the active Na flux. This simplification would approximate the production of CO_2 from the Na/K-ATPase alone if the efficiency were 5%.

The parameter $K_{Na/H}^S$ in the expression for the Na/H countertransport in the mucosal membrane is chosen to ensure the carrier-mediated Na flux contributes at least 60% to the total Na flux at normal steady states. The corresponding parameter for the Cl/HCO_3 countertransport is determined by the requirements of equal influxes of Na^+ and Cl^- across the mucosal membrane at the given concentrations. We use the values $K_{Na/H}^S=2.0\times10^4$ cm^4 mol^{-1} sec^{-1}, and $K_{Cl/HCO_3}^S=1.4$ cm^4 mol^{-1} sec^{-1}. The ratio $K_{Na/H}^S$ to K_{Cl/HCO_3}^S agrees with values determined by Kinsella and Aronson (1980) in brush border membranes of kidney proximal tubules.

Results

Concentrations and Fluxes During Steady States in Solutions Containing HCO_3^-/CO_2

The steady-state concentrations and potentials which correspond to a cell with the permeabilities listed in Table 1 and bathed in a solution containing 2.2 mM HCO_3^- and 0.3 mM CO_2 (1.3%) is given in Fig. 1*A*, the corresponding fluxes are shown in Fig. 1*B*. The concentrations and fluxes of Na^+, K^+ and Cl^- agree with existing data as discussed previously (Baerentsen et al., 1982). The intracellular electrical potential E^{mc} is -80 mV. If P_K^{cs} were reduced by 40% to 6×10^{-6} cm sec^{-1}, E^{mc} was -71 mV.

The influxes of S_{Na} and S_{Cl}[1] are almost equal in the chosen steady state. This equality also holds if the luminal concentration of Cl^- is varied. At very small values of S_{Cl} (Fig. 2*A*) there is a deviation from the one-to-one relation because the electrodiffusive flux of HCO_3^- becomes significant. This deviation becomes more pronounced if a cell is considered in which $P_{HCO_3}^{mc}$ and $P_{HCO_3}^{cs}$ are a factor of 20 larger, 2×10^{-6} cm sec^{-1}. In this case S_{Na} is on average 40 pmol cm^{-2} sec^{-1} larger than S_{Cl} (Fig. 2*A*, filled circles) and S_{Na}/S_{Cl} averages 1.2. Thus, any experimentally induced decrement in S_{Na} will be accompanied by an equally large decrease in S_{Cl}.

The equality between S_{Na} and S_{Cl} holds whether the fluxes are decreased by substitution of mucosal Cl^- or Na^+ ions (Fig. 2*B*). In this Figure the parameters and concentrations are similar to those given in Table 1 and Fig. 1 apart from the external HCO_3^- concentrations which are increased to 22 mM and the CO_2 which is increased to 3 mM (pH constant 7.2). This choice of parameters allows for a larger range of fluxes to be investigated.

The equality between S_{Na} and S_{Cl} also holds if either the rate constant for the Na/H transport *or* the Cl/HCO_3 transport are decreased (Fig. 3*A*). Thus, inhibiting one leg of the Na/H $-HCO_3/Cl$ transport system will inhibit both transport systems. This situation is analogous to poisoning either entry mechanism by a specific inhibitor.

Since the coupling between the Na^+ and Cl^- transport depends on the availability of H^+ and HCO_3^- in the cell and therefore on the rate at which CO_2 and H_2O combine and dissociate, it is of interest to see how the model reacts to decreased hydrolysis of CO_2, i.e., the effect of the carbonic anhydrase inhibitors. This is demonstrated in Fig. 3*B*. Carbonic anhydrase inhibitor decreases the fluxed of Na^+ and Cl^- in equal amounts. Thus, the rate of S_{Na} and S_{Cl} are equally dependent on the availability of H^+ and HCO_3^-.

It is the total fluxes across the brush border, mediated plus electrodiffusive ($S_{Na} + J_{Na}^{mc}$), which are experimentally detectable. A decrement in the total flux of Na^+ will be accompanied by an equal decrease in the total flux of Cl^-. This is shown in Fig. 3*B*. We have studied the case where decrements in fluxes are implemented by a carbonic anhydrase inhibitor. The identical

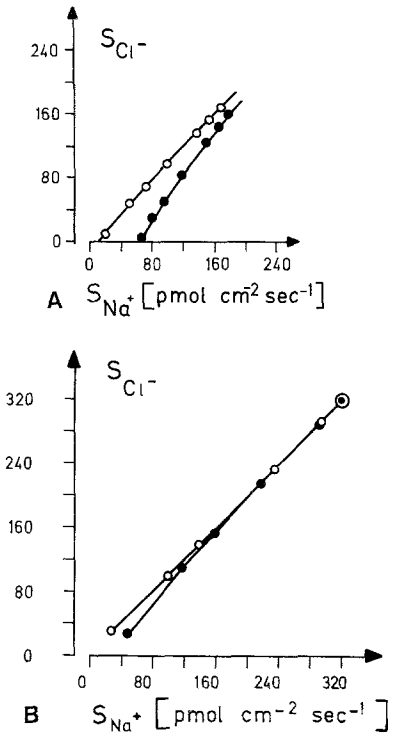

Fig. 2. *A.* The relationship between the carrier-mediated fluxes S_{Cl} and S_{Na} (*see* Fig. 1) when the fluxes are reduced by substituting the chloride in the mucosal solution with an impermeant ion. Open circles represents the results obtained with the model cell represented by the parameters in Table 1, $P_{CO_2} = 1.3\%$, $P_{HCO_3}^{mc} = 0.1 \times 10^{-6}$ cm sec^{-1}. The upper point represents $C_{Cl}^m = 120$ mM, i.e. the steady state given in Fig. 1 *A & B*. The other points are the steady states obtained with $C_{Cl}^m = 100$, 80, 40, 20, 10 and 1 mM. The closed circles represent the same values when $P_{HCO_3}^{mc}$ is 2×10^{-6} cm sec^{-1}. *B.* The relationship between S_{Cl} and S_{Na} when the fluxes are decreased by substituting either chloride (closed circles) or Na^+ (open circles) with impermeant ions. The parameters are the same as in Table 1 and Fig. 1 except that $pCO_2 = 13\%$, $HCO_3^m = 22$ mM. The closed circles (Cl^- substitution) represent steady states with C_{Cl}^m in mM = 120, 80, 40, 20, 10 and 1. The open circles represent steady states with C_{Na}^m in mM = 120, 100, 60, 20, 10 and 1.

[1] The index *mc* is omitted; *see* note in list of symbols.

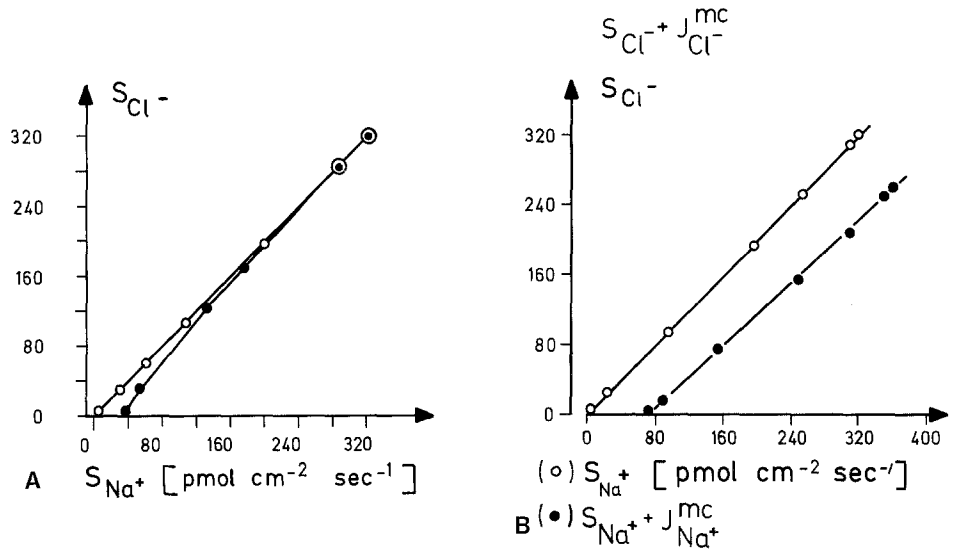

Fig. 3. *A.* The relationship between S_{Cl} and S_{Na} when the fluxes are decreased by reducing the rate constant for the Na/H countertransport (open circles) and by reducing the rate constant for the Cl/HCO_3 countertransport (closed circles). The open circles represent (from the upper right corner) steady states with $K^s_{Na,H}=2\times10^4$, 7×10^3, 2×10^3, 7×10^2, 2×10^2, 20. The closed circles represent steady states with K^s_{Cl,HCO_3} 1, 0.5, 0.1, 0.05, 5×10^{-3}, 5×10^{-4}. Other parameters as in Fig. 2*B*. *B.* The relationship between S_{Cl} and S_{Na} (open circles) when the fluxes are decreased by gradual inhibition of the intracellular production of H^+ and HCO_3^- (Eq. 8). The open circles represent steady states obtained for the rate constant V_o (sec^{-1})$=10^4$, 10^3, 2×10^2, 10^2, 40, 10, 1. The closed circles represent the *total* fluxes $S_{Cl}+J_{Cl}$, $S_{Na}+J_{Na}$ across the mucosal membrane. Parameters as in Fig. 2

decreases in Na^+ and Cl^- fluxes occur if the decrements are brought about by changes in either external concentrations of Na^+ or Cl^-, or by decreasing the rate constants.

Concentrations and Fluxes During Steady States in Solutions with Low HCO_3^- *and* CO_2 *Concentrations*

Many experiments have been performed using HCO_3^-- and CO_2-free solutions. The system is not lacking CO_2 or HCO_3^- since there is always an endogeneous production of CO_2. We have investigated how the Na/H and Cl/HCO_3 transport systems will function when CO_2 and HCO_3^- are reduced in the outer medium. Figure 4 shows how S_{Na} and S_{Cl} are affected when CO_2 and HCO_3^- are reduced in the outer medium while keeping pH constant at 7.2 (parameters as in Table 1 and Fig. 1). Whatever the CO_2 level, S_{Na} is always equal to S_{Cl} to within 1%. Working with CO_2-free solutions and finding $S_{Cl}=S_{Na}$ is not necessarily indicative of an obligatory neutral coupling of Na^+ and Cl^- influxes. The mediated fluxes of Na^+ and Cl^- remain relatively high down to very low CO_2 levels. Only at a CO_2 of 0.15 mM (0.5%) will the fluxes of Na^+ and Cl^- be reduced to one-third of the maximum. A reduction of the CO_2 level by a factor 30 only reduces the ion fluxes by a factor of 3.

The Influxes of Na^+ *and* Cl^- *in Nonsteady States*

The behavior of the model varies greatly under certain nonsteady-state conditions, depending on the choice of the Na^+ and Cl^- entry mechanism. Replacing either Na^+ or Cl^- from the mucosal compartment leads to transient readjustments in the mediated fluxes until a new steady state is reached. In this type of experiment, a close match between the Na^+- and Cl^--mediated fluxes can be predicted at any time for an obligatory NaCl-coupled entry (Eqs. 4 and 5). This will be so irrespective of whether Na^+ ions or Cl^- ions are replaced. This is far from the case if a $Na/H-Cl/HCO_3$ system is assumed to be operative as shown in Fig. 5. Because the link between the entry of Na^+ and Cl^- is the intracellular availability of H^+ and HCO_3^-, the mediated fluxes (S_{Na} and S_{Cl}) at $t=0$ are relatively independent of each other. It is not until the intracellular H^+ and HCO_3^- concentrations change that the Na influx is dependent on C^m_{Cl} and the Cl influx on C^m_{Na}.

The initial rates of change of C^c_{Na} and C^c_{Cl} predicted by the model when C^m_{Cl} or C^m_{Na} are

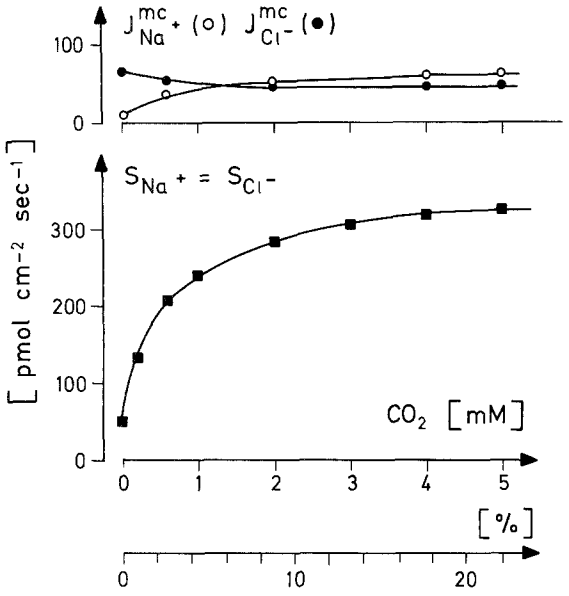

Fig. 4. The effects of decreased external CO_2 concentration on the electrodiffusive fluxes J_{Na} and J_{Cl}, and the mediated fluxes S_{Na} and S_{Cl} across the brush border membrane. The parameters are the same as in Fig. 1. The points represent steady states obtained by reducing the concentration of CO_2 and HCO_3^-, while maintaining pH constant at 7.2. S_{Na} equals S_{Cl} to within 1%

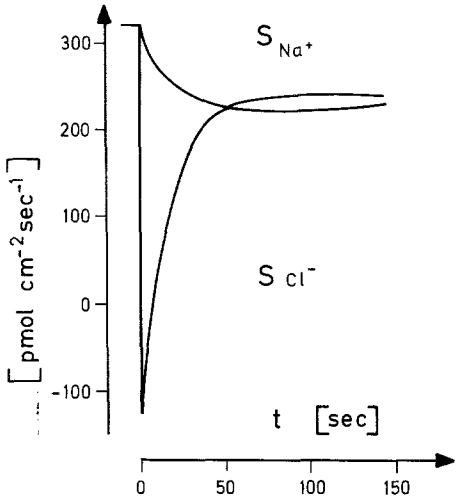

Fig. 5. The mediated fluxes S_{Na} and S_{Cl} across the mucosal membrane as a function of time when *half* the mucosal Cl^- ions were replaced abruptly by an impermeant ion. The initial steady state was the same as the one shown in Fig. 2

Table 2. Transient states of the *Necturus* gallbladder. Comparisons between model predictions and experiments

		dC^c_{Na}/dt (mM/sec)	$t_{0\,Na/0\,Cl}$ [d]	dC^c_{Cl}/dt (mM/sec)	$t_{0\,Cl/0\,Na}$
Na/Cl model[a]	0Na	0.38	1.2	0.36	0.9
	0Cl	0.33		0.39	
Na/H−HCO_3/Cl model[b]	0Na	0.33	6.0	0.05	20.2
	0Cl	0.06		1.07	
Experiments[c]	0Na	0.24±0.06	4.1	0.03±0.01	14.0
	0Cl	0.06±0.04		0.42±0.22	

[a] Na/Cl transport: $C^c_{Na}=10.4$ and $C^c_{Cl}=37.1$ (mM).
[b] Na/H−HCO_3/Cl transport: $C^c_{Na}=10.9$ and $C^c_{Na}=39.4$ (mM).
[c] The values are mean ±SD of four to six cells. C^c_{Na} was 18.9±1.9 and 19.4±4.1 and C^c_{Cl} was 28.6±2.3 and 29.0±1.9 (mM). The average membrane potential was 51.8±5.8 mV ($n=20$).
[d] $t_{0\,Na/0\,Cl}$ is the ratio $(dC^c_{Na}/dt)_{0\,Na}/(dC^c_{Na}/dt)_{0\,Cl}$ and $t_{0\,Cl/0\,Na}$ is $(dC^c_{Cl}/dt)_{0\,Cl}/(dC^c_{Cl}/dt)_{0\,Na}$.

substituted, are compiled in Table 2. The first part of the Table shows the initial rates when Na/Cl transport is assumed to be operative. The rates of changes in C^c_{Cl} and C^c_{Na} are roughly equal (between 0.3 and 0.4 mM/sec) whether C^m_{Cl} or C^m_{Na} are replaced. If the Na/H−HCO_3/Cl is assumed to operate (Eqs. 6 and 7), the rate of change in C^c_{Na} is about 6 times larger when C^m_{Na} is replaced than when C^m_{Cl} is replaced. Similarly, the initial rate for C^c_{Cl} is about 20 times larger for the C^m_{Cl} replacement than for the C^m_{Na} replacement (second part of Table 2). It should be pointed out, that when computing the initial rates of change in C^c_{Na} and C^c_{Cl}, the effect of the electrodiffusional fluxes and the changes in cell volume are both taken into acount. Even so, the differences in the medicated fluxes for the two entry mechanisms are clearly reflected in the initial rates of change in the Na and Cl concentrations in the cell.

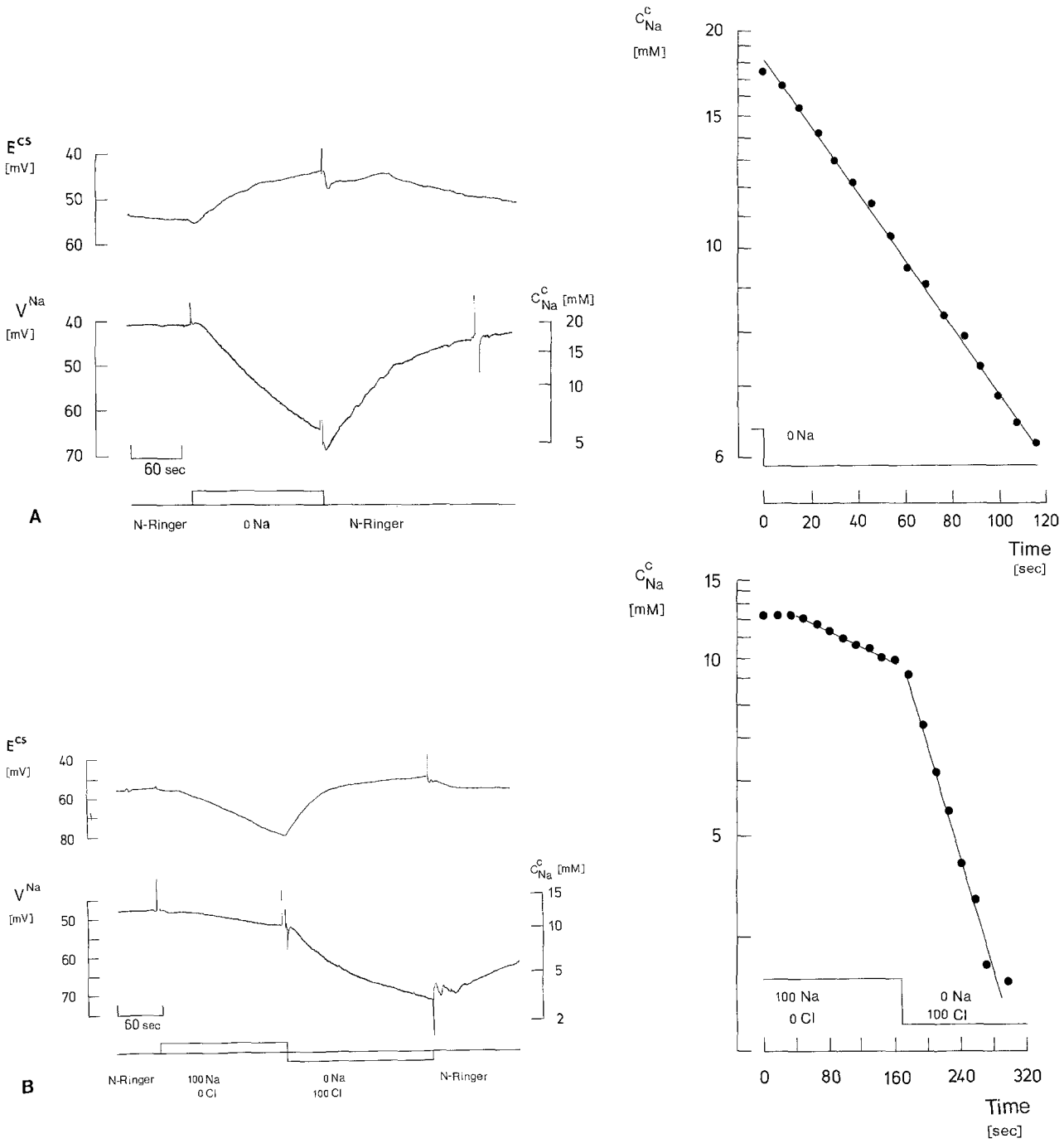

Fig. 6. *A*) Change of intracellular Na concentration, C^c_{Na}, after the removal of Na from the mucosal solution. The upper trace shows the cell membrane potential and the lower trace shows the Na-sensitive electrode potential minus the cell membrane potential (V^{Na}) recorded with a double-barrel microelectrode; both potentials were measured relative to the serosa. The change from the normal solution (100 mM Na) to a nominally Na-free solution in the mucosal perfusate is indicated by the bar. This caused the mucosal solution to become 20 mV positive relative to the serosal bath (not shown). The transient change in C^c_{Na} is plotted on the right on log-linear coordinates as the percent change of C^c_{Na} *vs.* time. The initial rate of change of Na concentration was computed from $(dNa^c/dt)_{t=0} = \Delta C^c_{Na} \cdot k$ and in this case is 0.190 mM sec^{-1} for $(C^c_{Na})_{t=0} = 17.4$ mM, $(Na^c)_{t=\infty} = 3.0$ mM and $k = 0.013\,sec^{-1}$. *B*) Effect of the replacement of luminal Na or Cl on C^c_{Na}. Replacement of Cl^- caused the mucosal solution to become a few mV positive relative to the serosal bath (not shown). On the left the simultaneous recording of E^{cs} and V^{Na} are shown as in *A*. Initially the mucosa is bathed in the normal *Necturus*-Ringer's solution. The mucosal bathing solution is then changed first to a 0Cl solution (100 mM Na + 0 mM Cl), next to 0Na solution (0 mM Na + 96 mM Cl) and finally back to the normal solution as indicated by the bars below the recording. The data are plotted on the right as in *A*. The rate constants for the 0Cl and the 0Na changes are 0.003 and 0.019 sec^{-1} [$(C^c_{Na})_{t=0} = 12.3$ mM and $(C^c_{Na})_{t=\infty} = 3.0$ mM)]. The points were placed by best visual fit

Experiments with Ion-Sensitive Microelectrodes

The type of nonsteady-state experiments described above were performed in *Necturus* gallbladder epithelium by recording the transients in C^c_{Na} and C^c_{Cl} after replacements of C^m_{Na} and C^m_{Cl}. An example of the changes in C^c_{Na} recorded with double-barrelled ion-sensitive microelectrodes after Na or Cl replacements is shown in Fig. 6. The average values of dC^c_{Na}/dt and dC^c_{Cl}/dt at $t=0$ are given in the third part of Table 2. As shown, the rate of change in C^c_{Na} is about four times larger when C^m_{Na} is replaced than when it is C^m_{Cl}. The rate of change in C^c_{Cl} is fourteen times larger for the Cl^- replacement than for the Na^+ replacement. These differences cannot be explained solely by electrodiffusive fluxes or cell volume changes unless unreasonable permeability coefficients are assumed.

These results, showing a clear departure from 1:1 ratio predicted by the Na/Cl cotransport system, strongly suggest the presence of another transport mechanism. In fact, they are consistent with the $Na/H-Cl/HCO_3$ countertransport system assumed in this study (*see* Table 2).

Discussion

The values for the concentrations and fluxes of Na^+, K^+ and Cl^- are in agreement with existing data as discussed previously (Baerentsen et al., 1982; *see also* Fig. 1*A* and *B*). The model assumes that most of the Na^+ transport is transcellular. This has recently been corroborated (Giraldez, 1982). The intracellular concentrations of H^+ and HCO_3^- are also consistent with existing data. *Necturus* gallbladder cells have an intracellular pH of 7.1 to 7.5, measured with electrodes (Zeuthen, 1978; Weinman & Reuss, 1982). HCO_3^- is predicted to be accumulated against its electrochemical gradient in accordance with previous measurement in other epithelia (Khuri, Bogharian & Agulian, 1974).

Comparison between Na/Cl *and* $Na/H-Cl/HCO_3$ *Transport in Steady States*

This paper shows that in steady states the mediated flux of Na^+ will be equal to the mediated flux of Cl^-, within experimental error. This applies to i) steady states which are obtained by reducing either mucosal Na^+ or Cl^- concentrations (Fig. 2*A* and *B*), ii) to steady states which are obtained by inhibiting either the Na/H or the Cl/HCO_3 transport (Fig. 3*A*), iii) to steady states in which the countertransport are slowed due to reduced supply of H^+ and HCO_3^-, i.e. by the application of carbonic anhydrase inhibitor (Fig. 3*B*) or by iv) reduced supply of exogenous CO_2 (Fig. 4). The equality of the mediated fluxes in steady states results from the intracellular production of one H^+ ion and one HCO_3^- via the hydrolysis of CO_2. Thus, in steady states one H^+ ion has to be removed from the cell for each HCO_3^- ion removed. The removal of these ions can only take place to any significant degree by mediated transport systems. Within the range of the physiological values for the intracellular concentrations of HCO_3^- and H^+ the electrodiffusive fluxes of these ions will be insignificant.

Several reports suggest an obligatory coupling of the transport of Na^+ and Cl^- across the brush border membrane of leaky epithelia (Diamond, 1962; Nellans, Frizzell & Schultz, 1973; Cremaschi & Henin, 1975; Duffey et al., 1978, 1979; Field, 1978; Spring & Kimura, 1978; Armstrong et al., 1979; Frizzell et al., 1979*a*, *b*; Reuss & Weinmann, 1979; Ramos & Ellory, 1981). None of these papers could determine whether the coupling is obligatory (Na/Cl) or mediated via two transport systems ($Na/H-Cl/HCO_3$) because they consider mainly steady states. As demonstrated in the present paper there may be no large difference in the behavior of a Na/Cl and a $Na/H-HCO_3/Cl$ transport system during steady states[2].

High Membrane Permeabilities for HCO_3

If the passive permeability for HCO_3^- is high (4 to 10×10^{-6} cm sec^{-1}), the Na/H transport system and the Cl/HCO_3 system will not be forced to transport at exactly the same rate because some HCO_3^- can escape by electrodiffusion. At normal external concentrations (Fig. 1) and with a total passive permeability for HCO_3^- of

[2] In experiments on *Necturus* gallbladder in which luminal Na^+ was replaced by TRIS, Garzia-Diaz and Armstrong (1980) recorded a linear relationship between the electrochemical potential difference for Na^+ and that for Cl^- across the mucosal membrane: The point needs further experimental investigations. In the present study and in the study by Weinman and Reuss (1982) the intracellular potential depolarized when Na^+ was replaced by an inert ion; in the study by Garzia-Diaz and Armstrong (1980) the potential remained constant.

the same order as the permeability to K^+, the following values are calculated: $S_{Na/H}=192$ pmol cm^{-2} sec^{-1}, $S_{Cl/HCO_3}=146$ pmol cm^{-2} sec^{-1} and the electrodiffusive flux of HCO_3^-, $J^{mc}_{HCO_3}=46$ pmol cm^{-2} sec^{-1}. However, the major pathway for ion translocation is still the mediated pathway, and if the transport via one leg of $Na/H-HCO_3/Cl$ transport system is slowed down then the transport via the other leg will be slowed by almost the same amount (Fig. 2).

Transport at Low (Zero) CO_2 *and* HCO_3 *Concentrations*

One method of deciding whether the transport of NaCl is via Na/Cl or $Na/H-Cl/HCO_3$ transport would be to study the transport at zero CO_2 and HCO_3 concentrations. Absent intracellular HCO_3^- is not guaranteed even if the external solution is HCO_3^-- and CO_2-free; there will always be an endogenous production of CO_2 and HCO_3^- and the intracellular levels will be determined by the permeabilities of the membranes.

The equality between the Na/H and Cl/HCO_3-mediated fluxes is maintained even at low values of CO_2 (Fig. 4). With endogenous CO_2 production the $Na/H-HCO_3/Cl$ transport system is still operative, although at a slow rate. Any experimentally provoked reduction in intracellular Na^+ concentration could be accompanied by a reduction of the intracellular Cl^- concentration, mediated by the $Na/H-HCO_3/Cl$ transport system[3].

The model also predicts, at least qualitatively, which fraction of the transepithelial flux of Na^+ enters via electrodiffusion, and which fraction enters via the neutral $Na/H-HCO_3/Cl$ transport mechanism. Graf and Giebisch (1979) used 10 mM HCO_3^- and 1% CO_2 in their perfusion solution. They suggest that only 15% of the total influx of Na^+ is electrodiffusive. Van Os and Slegers (1975) used CO_2-free solution and found that up to 64% of the Na^+ influx could be electrodiffusive. This is in accordance with our model (Fig. 4); the lower the CO_2 the larger the fraction of the Na^+ influx carried by electrodiffusion.

On the other hand if the transepithelial volume transport is unaffected by a reduction in HCO_3/CO_2 concentration as claimed by Ericson and Spring (1982*a*) then the model can only explain this if the electrodiffuse permeability for Na^+ is increased across the mucosal membrane or if another transport mechanism for NaCl was activated. The model as such predicts that the salt and water transport would go down by a factor of four (Fig. 4).

It is in accordance with the model (Fig. 1) that removal of HCO_3^- and CO_2 from the media abolish the short-circuit current and Cl^- absorption. This is found in, for example, *Amphiuma* intestine (White, 1980), but not consistently in all tissues (*see*, e.g., Ramos & Ellory, 1981).

Separation of the Na/H *and* Cl/HCO_3 *Fluxes in Nonsteady States*

Figure 5 shows that Na/H-mediated flux and Cl/HCO_3 fluxes are different during the transient period when either mucosal Na^+ or mucosal Cl^- concentrations are reduced abruptly. These differences in fluxes of Na^+ and Cl^- can be sensed by intracellular microelectrodes. These differences cannot be accounted for by the effect of passive electrodiffusional fluxes or cell volume changes if the cell parameters are kept within physiological values. If the influx of Na^+ and Cl^- were mediated by an obligatory coupled influx of Na/Cl, then the initial rates of change in C^c_{Na} or C^c_{Cl} would be largely independent of whether C^m_{Cl} or C^m_{Na} were replaced.

The experiments show large differences between the ratios of the initial rates of change for intracellular sodium and for chloride. These nonsteady-state experiments are evidence against an obligatory coupled NaCl entry. In fact, the initial changes in C^c_{Na} and C^c_{Cl} reported here are in close agreement with those predicted by a $Na/H-HCO_3/Cl$ mechanism (*see* Table 2). Similar results were obtained in floun-

[3] *Volume regulation.* When the osmolarity of the external medium of epithelia is changed the volume of the cells is subsequently regulated. Initially epithelial cells respond as perfect osmometers either by swelling or shrinking but this reference is followed by a volume regulatory phase in which the volume of the cells return towards the original volume. Volume regulatory swelling in epithelial cells may be mediated by the stimulation of an *additional* entry mechanism of Na^+ and Cl^- (*Necturus* gallbladder, Fisher, Persson & Spring, 1981; Ericson & Spring, 1982*b*; frog skin, Ussing, 1982). Some evidence indicates this additional influx is mediated by $Na/H-HCO_3/Cl$ transport (Fisher et al., 1981; Ericson & Spring, 1982*b*). This mechanism of influx is in accordance with the observation (not reported) that the present model volume regulates rather poorly since its capacity for ion flux remains constant during abrupt changes in external osmolarities. Other cell systems (ascites tumor cells, Hofmann, Sjoholm & Simonsen, 1981) appear to incorporate an obligatory neutral influx of Cl^- ion (with K^+) during volume regulation.

der intestine measuring intracellular Cl^- (Ellory, Ramos & Zeuthen, 1978). They obtained a ratio of six between the rate of change of C^c_{Cl} in zero C^m_{Cl} to the rate of change in zero C^m_{Na}.

Recently, intracellular H^+ concentrations have been recorded in the replacement experiments discussed above (Machen & Zeuthen, 1983). In the CO_2-containing solutions the rate of acidification when Na^+ ions were replaced in the luminal solution and the rate of alkalinization when Cl^- ions were removed were in good agreement with the expected rates of Na/H and Cl/HCO_3 exchange if these mechanisms maintained transepithelial transport of NaCl. If the solutions had been equilibrated with atmospheric air (no HCO_3^-) the data were in agreement with those of Weinman and Reuss (1982); the rate of Na/H (and Cl/HCO_3) exchange were at least one order of magnitude too small to explain transepithelial transport.

Other Evidence for $Na/H-HCO_3/Cl$ *Transport*

Turnberg et al. (1970) have suggested the double exchange of Cl/HCO_3 and Na/H as the overall regulatory mechanism for acid-base balance in human ileum. A similar model has been proposed in rat kidney proximal tubule and small intestine (Pitts, Ayer & Schiess, 1949; Pitts, 1961; Murer, Hopfer & Kinne, 1976; Liedtke & Hopfer, 1977, 1982*a*, *b*) as well as frog skin *in vitro* (Garcia-Romeu & Ehrenfeld, 1975; Alvarado, Dietz & Mullen, 1975), gills (Motais & Garcia-Romeu, 1972), gallbladder (Whitlock & Wheeler, 1969), and urinary bladder of the toad (Frazier & Vanatta, 1971) and turtle (Green, Steinmetz & Frazier, 1968; *see also* Sachs, et al., 1982). In choroid plexus Wright (1977) found that the buffer glycodiazine was about 50% more effective than HCO_3^- in stimulating the transepithelial Na^+ transport. In the gallbladder (Petersen & Heintze, 1982), butyrate, the anion of butyric acid, could mimic the effects of HCO_3^-. In both reports it was concluded that the experimental results were best explained if the transport of Na^+ and Cl^- was mediated by a $Na/H-Cl/HCO_3$ system and that the glycodiazine ion and the butyrate ion permeate the brush border via the HCO_3 pathway. For a slightly different view on Na^+ and Cl^- influx, *see* White (1980).

There is also direct evidence of an acidification of the lumen of the *Necturus* gallbladder. In some experiments (Zeuthen, 1980) a pH microelectrode with a recessed tip was pressed lightly against the brush border of a *Necturus* gallbladder. The pH-sensitive glass had a distance of 30 to 40 μm from the mucosal membrane and the efflux of H^+ ions into the dead space (200 μm^3) of the electrode was recorded. The dead space was acidified by the tissue to pH 5 within 10 to 20 min. This acidification was reversibly abolished by carbonic anhydrase inhibitor, quite in agreement with the model presented here (Figs. 1 and 3*B*). Recently an amiloride-sensitive Na/H exchange has been described for the mucosal membranes (Kinsella & Aronson, 1981; Weinmann & Reuss, 1982, *see also* discussion below).

The loop diuretics: furosemide, piretanide and bumetanide (Zeuthen, Ramos & Ellory, 1978; Frizzell, Smith, Vosburgh & Field, 1979*b*; Ramos & Ellory, 1981; Ericson & Spring, 1982*a*) reduce the uptake of ions into epithelial cells to the same extent as obtained by removing either Na^+ or Cl^- from the mucosal solution; this is usually more than 50%.

Furosemide and bumetanide inhibit Cl^- transport in red blood cells (for references, *see* Wieth & Brahm, 1982) where the transport is mediated by Cl/HCO_3 exchange (Brazy & Gunn, 1976). If the effects of the inhibitor can be extrapolated from one type of tissue to another, an assumption that needs careful examination (Wieth & Brahm, 1982), then the effects in the epithelial cell could be obtained by inhibiting the HCO_3/Cl exchange only.

Carbonic anhydrase inhibitor acts as a diuretic. This agrees with the properties of this inhibitor described by our model: It abolishes the formation of H^+ and HCO_3^- and therefore inhibits the Na/H and the Cl/HCO_3 transport systems. The transepithelial fluxes of Na^+ and Cl^- are diminished and consequently so is the fluid transport. In disagreement with our model, carbonic anhydrase inhibitor does not cause Cl^- to attain equilibrium concentrations in all tissues (White, 1980). Furthermore carbonic anhydrase may be located on the cell membrane as well, not only in the cytoplasm (Frömter & Ullrich, 1980), as was assumed in our model.

Evidence Against $Na/H-HCO_3/Cl$ *Transport*

When ouabain is administrated to the serosal face of the *Necturus* gallbladder, the cells begin

to swell, initially at a rate of 5.4 µl hr^{-1} cm^{-2} (Ericson & Spring, 1982*a*). This is about half of earlier estimates of the rate of volume transport and NaCl uptake, which were equivalent to 10 to 12 µl hr^{-1} cm^{-2} (van Os & Slegers, 1975; Reuss & Weinman, 1979; Spring et al., 1981; Zeuthen, 1982). This ouabain-induced swelling was unaffected by amiloride, SITS and the removal of HCO_3^-; and it was concluded that this volume transport could not be effected by $Na/H-HCO_3/Cl$ transport.

The effects of amiloride (10^{-3} M) are ambiguous. In *Necturus* gallbladder it is reported to abolish Na/H exchange (Weinman & Reuss, 1982) but not, as mentioned above, the ouabain-induced cell swelling (Ericson & Spring, 1982*a*) which is a consequence of NaCl influx into the cell. On the other hand it is reported that amiloride in rabbit gallbladder abolished transepithelial salt and volume transport (Frederiksen, 1973; Frederiksen & Eldrup, 1981).

Although it has been demonstrated that Na/H does exist on the mucosal membrane of *Necturus* gallbladder (Weinmann & Reuss, 1982; *see above*), the rate at which H^+ appears in the luminal solution is smaller than expected to arise from a Na/H exchange powerful enough to mediate a Na^+ influx equivalent to the transepithelial transport (Weinmann & Reuss, 1982); the rate of H^+ extrusion was only 7% of the net Na^+ transport. It could be argued, however, that H^+ and HCO_3^- combine at membrane level and that all the transported H^+ ions do not appear in the mucosal lumen.

Li^+ could replace Na^+ in the Na/H transport (Weinmann & Reuss, 1982), but not in the transepithelial transport (Ericson & Spring, 1982*a*).

The effects of SITS are also ambiguous. In some reports it had an effect on transport when applied from the serosal side only: kidney proximal tubule (Frömter & Ullrich, 1980) and intestine (White, 1980). This would indicate that HCO_3/Cl exchange is not located on the luminal side because SITS is a potent inhibitor of this countertransport system in red blood cell. In some reports on brush border vesicles, however, SITS did block Cl/HCO_3 exchange at the brush border (Liedtke & Hopfer, 1982*b*). In other cell types SITS blocks Cl/HCO_3 exchange, turtle bladder (Ehrenspeck & Brodsky, 1976) and in muscle fiber (Boron, Russell & Brodwick, 1978).

Energetics

The source of energy for an obligatory coupled entry of Na/Cl derives from the Na/K-ATPase. This Na^+ pump allows a large potential energy to be contained in the Na^+ gradient across the mucosal membrane. This gradient suffices to move Cl against its electrochemical gradient into the cell.

The source of energy for the countertransport system, $Na/H-HCO_3/Cl$ derives from two sources: the Na^+ gradient discussed above, and from the gradients of HCO_3^- and H^+ and therefore indirectly from the production of H_2CO_3 inside the cell. That the latter is the case can be seen from Fig. 3*B*; if the production of H_2CO_3 ($H^+ + HCO_3^-$) is decreased to the same rate as extracellularly by the application of inhibitors of carbonic anhydrase, then the $Na/H-HCO_3/Cl$ transport system ceases to transport despite the existence of a Na^+ gradient. With no H_2CO_3 in the system, transepithelial Na^+ transport would be mediated by electrodiffusion across the mucosal membrane and pumping across the serosal membrane. This Na^+ transport would be accompanied by a Cl^- flux and some backflux of Na^+ across the junctions.

A Working Hypothesis

This paper presents and analyzes evidence for and against $Na/H-HCO_3/Cl$ transport as the mediator of neutral NaCl transport across the mucosal membrane of *Necturus* gallbladder. In view of the quality of both kinds of evidence we find it appropriate to suggest as a working hypothesis a model which would reconcile these opposing views:

In HCO_3/CO_2-containing solutions Na^+ and Cl^- influx is mediated by $Na/H-HCO_3/Cl$ transport. When the external solutions contain no or little HCO_3/CO_2, Na^+ and Cl^- influx is maintained by a mechanism which is still neutral but which does not manifest itself by pronounced luminal or cellular acidification when Na^+ or Cl^- in the medium is replaced by inert ions.

Resolution of these questions will require measurements of rates of volume transport, rates of luminal H^+, Na^+, HCO_3^- and Cl^- transport.

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