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TRANSPORT OF SODIUM AND CHLORIDE ACROSS RAT GASTRIC MUCOSA *IN VITRO*

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SUMMARY

1. The effects of ion substitution, inhibitors and variations in transmural p.d. on the movements of sodium and chloride across an *in vitro* preparation of rat gastric mucosa have been studied.

2. The tissue maintained net steady-state transport of sodium in the mucosal-to-serosal direction in the absence of transmural gradients of electrochemical potential.

3. Sodium transport was independent of the presence of chloride, and was abolished by 1×10^{-5} M-amiloride. The inhibitor produced a decrease in short-circuit current equivalent to the depression of sodium transport, indicating that the sodium transport process was electrogenic.

4. Variations in transmural p.d. showed that the sodium transport process included two components: one that varied with p.d. and one that was independent of it.

5. These findings have been interpreted in terms of a system for sodium transport composed of three components: two rate-limiting entry mechanisms at the apical membrane, one of which can be represented as a conductive channel for sodium diffusion and the other as a neutral process possibly a sodium–hydrogen exchanger, and a voltage-independent pump at the basolateral membrane analogous to the constant-current pump models described in some other epithelia.

6. The tissue maintained a net secretory movement of chloride in the short-circuited condition.

7. The process responsible for net transport of chloride could be resolved into two components: one that was sodium dependent, electrogenic, and abolished by 8×10^{-3} M-acetazolamide, and one that was independent of the presence of sodium, electrically silent and abolished by 5×10^{-4} M-SITS (4-acetamido-4′-isothiocyano-2,2′-disulphonic acid stilbene). Both components of the chloride transport process varied with p.d.

8. These findings were interpreted in terms of a system of three components: two entry mechanisms at the basolateral membrane including a coupled sodium–chloride influx process and a chloride–bicarbonate exchanger in parallel, and a rate-limiting conductive channel at the apical membrane.

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9. In addition, the studies on the effects of variations in transmural p.d. on chloride fluxes revealed a symmetrical voltage-independent component, dependent on the presence of chloride in the *trans* compartment, and it was suggested that this component may reflect the presence of a chloride–chloride exchange mechanism.

INTRODUCTION

Much of the present understanding of gastric physiology has derived from studies on the isolated mucosa of amphibian species mounted in a flux chamber system. These studies have been facilitated by the demonstration of a simple arithmetic equivalence between the short-circuit current and the parallel secretory movements of hydrogen and chloride ions (Hogben, 1955). Less extensive study has been directed to analogous preparations derived from the stomachs of mammals, but such studies have demonstrated an important difference between mammalian and amphibian systems. In most preparations of mammalian stomach, a net absorptive movement of sodium has been observed in addition to the secretory movements of hydrogen and chloride ions (Kitahara, Fox & Hogben, 1969; Forte & Machen, 1975; Machen, Silen & Forte, 1978), and in some species the transport of sodium has been found to be a major determinant of the short-circuit current (Kitahara *et al.* 1969; Kuo & Shanbour, 1979). The existence of this additional, possibly independent transport process precludes application of the amphibian model to mammalian systems. The present study was directed to the characterization of the sodium and chloride transport processes in rat gastric mucosa. Our findings indicate that the sodium transport process included two components: one that varied with the transmural voltage, and one that was voltage-independent. Both components were inhibited by amiloride, and both contributed to the short-circuit current. The chloride secretion process also could be resolved into two components: a sodium-dependent electrogenic mechanism, and a sodium-independent electrically silent process that was inhibited by disulphonic stilbenes.

METHODS

Male, Sprague–Dawley rats, weighing 250–500 g, were allowed food and water *ad libitum* to the time of experiment, and were anaesthetized with sodium pentobarbitone (70 mg/kg, I.P.). The stomach was removed and the storage portion, which could be clearly distinguished by examination of the exterior surface, was cut free and discarded. The remaining portion was cut along the greater and lesser curvatures yielding a symmetrical pair of tissues, the ventral and dorsal walls, which were usually rinsed in 0·9% sodium chloride at room temperature. For experiments in which the effects of sodium or chloride removal were to be investigated, suitably substituted saline solutions were used to rinse the tissue. The external muscle coats over the lateral oxyntic gland region (McCabe, Kent & Hogben, 1969) were removed by a technique similar to that described by Forte, Forte & Machen (1975). The tissue was pinned, mucosal surface down, to a paraffin plate and covered with saline. Saline was injected through a fine hypodermic needle between the mucosa and the muscle coat to produce a blister, and the muscle coat was cut away with scissors.

For insertion into the flux chamber, each piece of tissue was gently stretched over an arrangement of pins mounted in a thin Lucite plate so that the exposed mucosa covered a circular hole (1 cm diameter) in the plate. A second plate was mated to the first, securing the tissue in place, and the two plates clamped between the halves of a Lucite flux chamber similar in design to that described by Schultz & Zalusky (1964). The elapsed time from removal of the stomach to mounting in the flux chamber was approximately 10 min.

Each half of the flux chamber was perfused with 30 ml incubation saline from a constant temperature (37 °C) water bath using a gas lift system.

The incubation salines were based on one of the following composition (mM); Na^+, 130; K^+, 5; Ca^{2+}, 1·3; Mg^{2+}, 1·3; Cl^-, 95; HCO_3^-, 25; SO_4^{2-}, 2·6; HPO_4^{2-}, 0·65; $H_2PO_4^-$, 0·14; 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonate (HEPES), 30; glucose, 10. In chloride-free experiments, NaCl and KCl were replaced by sodium isethionate and K_2SO_4, respectively. In sodium-free experiments, sodium was replaced by choline (113 mM) and Tris (15 mM). All solutions were adjusted to pH 7·5 after equilibration with gas (95% O_2/5% CO_2) and temperature.

4-acetamido-4′-isothiocyano-2,2′-disulphonic acid stilbene (SITS) (Calbiochem–Behring Corp.), amiloride (Merck, Sharp and Doehm), and acetazolamide (K and K Laboratories) solutions were freshly prepared for each experiment, and inhibitors were not stored in solution.

Unidirectional fluxes of sodium, chloride or mannitol were estimated using ^{22}Na, ^{36}Cl or [^{3}H]mannitol (New England Nuclear). At the beginning of a 1 h pre-incubation, tracer was added to one reservoir. Fluxes were calculated from the rate of appearance of tracer in the other reservoir during four or five consecutive 10 min periods in the second hour of incubation. At the end of any experiment, the specific activity of the tracer in the initially unlabelled reservoir never exceeded 0·5% of that in the solution to which the tracer was added.

Each stomach yielded two pieces of tissue, one from each of the ventral and dorsal surfaces, and in each experiment these two tissues were paired according to one of two experimental designs. In one design, the same unidirectional flux was estimated in both tissues of a pair, one member serving as a control while the other was subjected to some form of experimental manipulation. In this design, the effect of the manipulation was assessed as the difference between the values of the variable estimated in the two tissues, and this difference is given the symbol $\bar{d}$ in the text. A one-tailed Student's t test for the null hypothesis ($\bar{d} = 0$) was used to assess the significance of these differences. In the other design, paired tissues were used to estimate the opposed mucosal-to-serosal, and serosal-to-mucosal fluxes (J^i_{ms} and J^i_{sm} respectively) in the same condition of incubation. The difference between the paired estimates in this situation is the net flux, and is given the symbol J_{net} in the text.

The transmural electric potential difference (p.d.) was estimated with an Orion Research digital millivoltmeter (model 701, input impedance $> 10^{12}\ \Omega$) connected to the flux chamber through calomel half-cells and polyethylene salt bridges filled with 4% agar saturated with KCl. Electric current, from external custom-made sources, was applied to the tissue through Pb/$PbCl_2$ electrodes and KCl/agar bridges. The latter bridges were inserted into the chamber approximately 5 cm from the surfaces of the tissue so that a uniform density of current flow could be assumed. The resistance of the saline between the tips of the p.d.-sensing bridges (R_s) was determined before the tissue was prepared and mounted, and subsequent estimates of the tissue resistance (R_t) were corrected for this quantity. Values of R_t were normalized to 1 cm^2 of exposed tissue, and were converted to conductance (G_t) for presentation. Because R_s was a significant component of the resistance of the system, the value shown on the voltmeter was not equal to the voltage drop across the tissue when current was passed from the external source (Rehm, 1968). Accordingly, the conditions used to establish a clamped condition were calculated using formulae based on the considerations given by Rehm (1968).

In studies on the effects of variations in p.d. on ion fluxes, the experimental period was usually divided into two parts. In the first part the transmural p.d. was clamped at 0 mV and the ion flux estimated during four 10 min periods. The p.d. was then changed to some other value in the range ± 50 mV. A 10 min period was allowed for the flux to stabilize in the new steady state, and fluxes estimated in the next three 10 min periods.

The mathematical model used to examine the effect of p.d. on ion fluxes was based on the parallel-channel system described by Frizzell & Schultz (1972). It is assumed that the flux of an ion may include two components: one that varies with p.d. and one that is independent of it. The relation between the components and the observed flux is described by the following expression:

$$J^i_{12} = {}_vJ^i_{12}\xi + j^i_{12}. \tag{1}$$

In this expression J^i_{12} represents the flux of an ion i from compartment 1 to compartment 2, and j^i_{12} represents the component that is independent of p.d. ${}_vJ^i_{12}\xi$ is the component of the flux that varies with the electric driving force ξ, which is defined by:

$$\xi = \frac{zFV_t}{RT\,(\exp(zFV_t/RT) - 1)},$$

where V_t is the p.d. across the tissue ($V_2 - V_1$), and z, F, R and T have the usual electrochemical significance.

Eqn. (1) indicates that a plot of J^1_{12} against ξ will yield a straight line of slope ${}_vJ^1_{12}$, and of intercept j^1_{12}. The validity of the model and the values of the parameters were estimated by least-squares linear regression analysis of the variation in an observed flux when p.d. was varied in the range ± 50 mV.

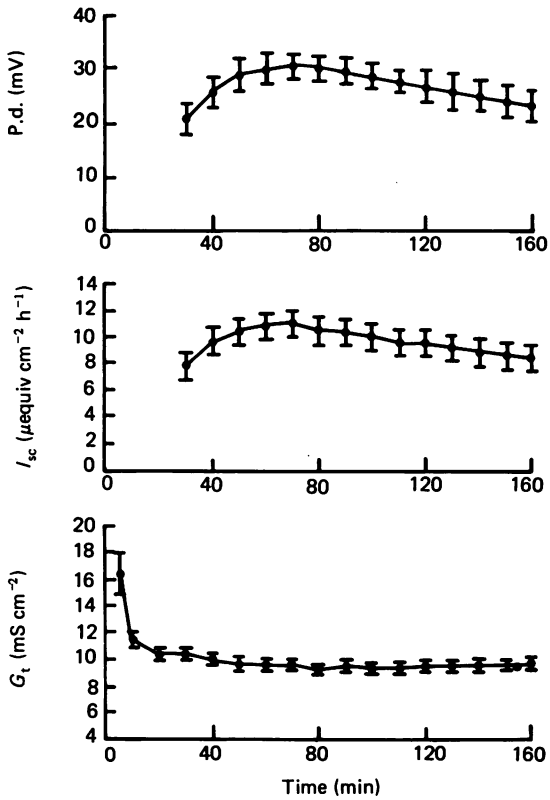

Fig. 1. Variations in electrical variables with time. Tissues were mounted in flux chambers and incubated in the unmodified incubation saline under open-circuited conditions. Estimates of p.d. and tissue resistance were made at 10 min intervals, and values of short-circuit current calculated from these estimates. Each data point is the mean of ten observations $\pm$S.E. of mean.

RESULTS

General properties

Fig. 1 shows that, during the first hour of incubation, p.d. and short-circuit current (I_{sc}) increased toward maximum values of 30 ± 3 mV (serosal side positive) and 11 ± 1 μequiv cm^{-2} h^{-1}, respectively ($n = 10$). Following a brief plateau, the values of p.d. and I_{sc} commenced a linear decay with time, and decreased by approximately 15% of their peak values during the second hour of incubation. G_t decreased rapidly during the first 20 min of incubation, but remained constant through the remainder of the experiment. After 60 min of incubation the mean value of G_t was

$9{\cdot}7 \pm 0{\cdot}3$ mS cm^{-2}. Accordingly, pre-incubation for 1 h was used before ion fluxes were determined. Ion fluxes estimated during the second hour showed only random variation with time, and values estimated in four or five consecutive 10 min periods were usually averaged.

TABLE 1. Electrical and transport characteristics of isolated rat gastric mucosa. Tissue was prepared as described in the Methods and incubated in the unmodified incubation saline. The open-circuit p.d., tissue resistance and short-circuit current (I_{sc}) were estimated at the end of a 1 h pre-incubation; the tissue was then clamped at 0 mV and ion fluxes were estimated during four or five consecutive 10 min periods. Tissues were paired to allow estimation of net fluxes. Values of J^{i}_{net} may differ slightly from arithmetic difference between unidirectional fluxes due to rounding errors. Values given for I_{sc} are averages of the current required to maintain the short-circuited condition during the period of flux determination. All values are means ± S.E. of means, with the number of tissues studied given in parentheses. For the electrical variables, data from observations of paired tissues were averaged. Numbers of tissues studied are given in parentheses

P.d. (mV)		33 ± 2 (15)
G_t (mS cm^{-2})		$8{\cdot}7 \pm 0{\cdot}3$ (15)
I_{sc} (μequiv cm^{-2} h^{-1})		$8{\cdot}9 \pm 0{\cdot}5$ (15)
Ion fluxes (μequiv cm^{-2} h^{-1}):	J^{Na}_{ms}	$7{\cdot}3 \pm 0{\cdot}4$ (8)
	J^{Na}_{sm}	$1{\cdot}8 \pm 0{\cdot}1$ (8)
	J^{Na}_{net}	$5{\cdot}4 \pm 0{\cdot}4$ (8)
	J^{Cl}_{ms}	$4{\cdot}8 \pm 0{\cdot}2$ (7)
	J^{Cl}_{sm}	$7{\cdot}0 \pm 0{\cdot}4$ (7)
	J^{Cl}_{net}	$-2{\cdot}2 \pm 0{\cdot}3$ (7)

When tissue is mounted in a flux chamber by compression, the edge of the exposed tissue may suffer damage, and it has been shown that this edge damage may influence estimates of tissue conductance (Dobson & Kidder, 1968). If it is assumed that the width of the zone of damaged tissue is independent of the total area of exposed tissue, the fraction of exposed surface influenced by edge damage will be proportional to the ratio of circumference to area (c/a). In a study of eight pairs of tissues, one member of each pair was mounted in a chamber fitted with a large aperture (diameter = 1 cm: $c/a = 4$ cm^{-1}), and the other member of the pair in a chamber with a small aperture (diameter = 0·5 cm: $c/a = 8$ cm^{-1}). The values of G_t were $8{\cdot}1 \pm 0{\cdot}3$ and $8{\cdot}8 \pm 0{\cdot}5$ mS cm^{-2} respectively for the large and small apertures. The mean paired differences for these observations, $0{\cdot}7 \pm 0{\cdot}5$ mS cm^{-2}, did not differ significantly from zero ($P > 0{\cdot}1$), indicating that the preparation was minimally influenced by edge damage.

Table 1 shows the results of experiments in which the opposed fluxes of sodium and chloride were estimated under short-circuit conditions in the unmodified incubation saline. The tissues were paired to allow evaluation of net fluxes. Net transport of sodium occurred in the mucosal-to-serosal direction, and J^{Na}_{net} was numerically equivalent to approximately 60% of the short-circuit current. The net movement of chloride occurred in the serosal-to-mucosal direction, and was equivalent to approximately 25% of I_{sc}.

Effects of ion substitution

Table 2 shows the results of experiments in which choline was substituted for sodium, or chloride was replaced by isethionate and sulphate.

In the chloride-free condition the tissue conductance and short-circuit current were significantly decreased, but the fluxes of sodium were not changed.

TABLE 2. Effect of ion substitution on electrical properties, and on ion and mannitol fluxes across rat gastric mucosa *in vitro*. Conditions of experiments and expression of results generally similar to those described in Table 1, but in some experiments choline was substituted for sodium, or chloride was replaced by isethionate and sulphate. Tissues were paired to allow effects of ion substitution on unidirectional fluxes to be examined, and values of $\bar{d}$ represent mean differences from paired controls

	Control	Chloride-free	$\bar{d}$	n
G_t (mS cm^{-2})	8·8 ± 0·4	5·4 ± 0·2	−3·4 ± 0·4***	14
I_{sc} (μequiv cm^{-2} h^{-1})	7·3 ± 0·4	5·2 ± 0·4	−2·1 ± 0·5***	14
J_{ms}^{Na} (μequiv cm^{-2} h^{-1})	6·7 ± 0·5	6·7 ± 0·3	−0·1 ± 0·4	8
J_{sm}^{Na} (μequiv cm^{-2} h^{-1})	2·6 ± 0·3	2·6 ± 0·2	0·0 ± 0·3	6
	Control	Sodium-free	$\bar{d}$	n
G_t (mS cm^{-2})	9·1 ± 0·2	10·8 ± 0·3	1·7 ± 0·2***	24
I_{sc} (μequiv cm^{-2} h^{-1})	6·8 ± 0·4	−0·1 ± 0·1	−6·8 ± 0·4***	24
J_{ms}^{Cl} (μequiv cm^{-2} h^{-1})	4·8 ± 0·1	5·8 ± 0·3	1·0 ± 0·2**	4
J_{sm}^{Cl} (μequiv cm^{-2} h^{-1})	7·7 ± 0·6	6·6 ± 0·3	−1·1 ± 0·5*	8
J_{ms}^{man} (nmol cm^{-2} h^{-1})	50 ± 5	61 ± 6	11 ± 4*	6
J_{sm}^{man} (nmol cm^{-2} h^{-1})	56 ± 2	75 ± 8	19 ± 9*	6

* Denotes $\bar{d}$ different from zero at $P < 0·05$.
** Denotes $\bar{d}$ different from zero at $P < 0·01$.
*** Denotes $\bar{d}$ different from zero at $P < 0·001$.

Removal of sodium from the incubation saline decreased J_{sm}^{Cl} and increased J_{ms}^{Cl}. Tissue conductance was increased by approximately 20% and I_{sc} was abolished in the absence of sodium. In an additional series of experiments it was found that the fluxes of the passively transported solute, mannitol, were increased when sodium was omitted from the incubation saline. This observation, together with the increase in G_t observed in this condition, suggested that the permeability of the tissue was generally increased in the absence of sodium, and that the increased value of J_{ms}^{Cl} could be ascribed to this change.

The experiments included in Table 2 were designed to allow examination of the effects of ion substitution on the individual ion fluxes, and tissues were paired for comparison of corresponding fluxes in the presence and absence of an ion. Accordingly, the data in Table 2 do not allow a conclusion to be drawn concerning the level of chloride transport that was maintained in the absence of sodium. This question was addressed in additional experiments in which J_{ms}^{Cl} and J_{sm}^{Cl} were estimated in paired tissues, both incubated in sodium-free conditions. For eight pairs of tissues, the mean paired difference (J_{net}^{Cl}) was $-1·4 \pm 0·4$ μequiv cm^{-2} h^{-1}, and was significantly different than zero ($P < 0·01$). It was concluded that net transport of chloride was inhibited but not abolished in the absence of sodium.

Effects of variations in transmural p.d.

Fig. 2 shows the effects of variations in transmural p.d. on the fluxes of sodium estimated in the unmodified incubation saline, and plotted in the format suggested by eqn. (1). In these studies, J_{ms}^{Na} and J_{sm}^{Na} were determined in paired experiments to allow comparison of the effects of p.d. on the opposed fluxes. In each case the flux

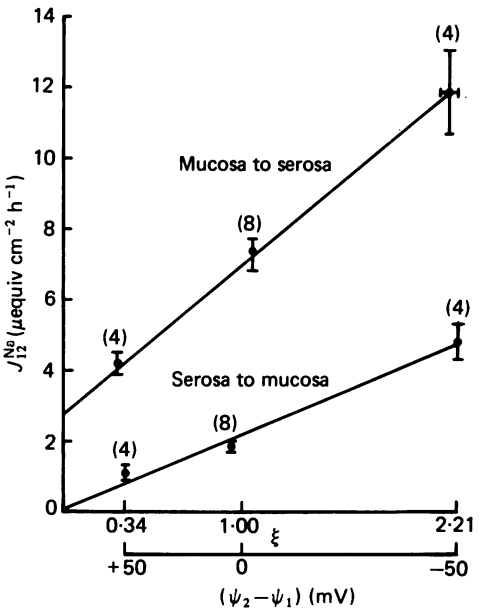

Fig. 2. Effects of variations in p.d. on sodium fluxes. Tissues (n = 8 pairs) were incubated in the unmodified incubation saline, and a 1 h period was allowed for the tracer to achieve a steady state. The tissue was then short-circuited and the sodium flux estimated during four consecutive 10 min periods. The transmural p.d. was then clamped at some other value in the range ±50 mV, a 10 min period allowed to stabilize in the new steady state, and the sodium flux estimated in the next three consecutive 10 min periods. Linear regressions for the two fluxes were calculated as follows:

$$J_{ms}^{Na} = 4{\cdot}2\ (\pm 0{\cdot}5)\ \xi + 2{\cdot}7\ (\pm 0{\cdot}7) \quad (r = 0{\cdot}91)$$
$$J_{sm}^{Na} = 2{\cdot}1\ (\pm 0{\cdot}2)\ \xi + 0{\cdot}1\ (\pm 0{\cdot}3) \quad (r = 0{\cdot}93).$$

was linearly correlated with the function ξ ($r > 0{\cdot}91$: $P < 0{\cdot}001$ in both cases), indicating that the two-channel model used to examine the effects of p.d. was satisfactory. The relation of J_{ms}^{Na} and ξ indicated that this flux included both p.d.-sensitive ($_vJ_{ms}^{Na}$ = slope = 4·2 ± 0·5 μequiv cm^{-2} h^{-1}) and p.d.-independent (j_{ms}^{Na} = intercept = 2·7 ± 0·7 μequiv cm^{-2} h^{-1}) components. In contrast, the intercept for the relation of J_{sm}^{Na} and ξ was not significantly different from zero (j_{sm}^{Na} = 0·1 ± 0·3 μequiv cm^{-2} h^{-1}), indicating that the backflux of sodium included only a p.d.-dependent component ($_vJ_{sm}^{Na}$ = 2·1 ± 0·2 μequiv cm^{-2} g^{-1}). The slopes of the two fluxes differed significantly ($P < 0{\cdot}001$), and this difference will be termed rectification of the p.d.-sensitive movements. Thus, the net transport of sodium that occurred in the short-circuited condition ($\xi = 1$) could be ascribed to the presence

of a p.d.-independent component of the mucosal-to-serosal flux, and to rectification of the p.d.-sensitive movements.

Fig. 3 shows the results of analogous studies on the fluxes of chloride. In these experiments also both fluxes were found to vary linearly with ξ ($r > 0{\cdot}94$: $P < 0{\cdot}001$ in both cases), again supporting use of a two-channel model. However, in the case

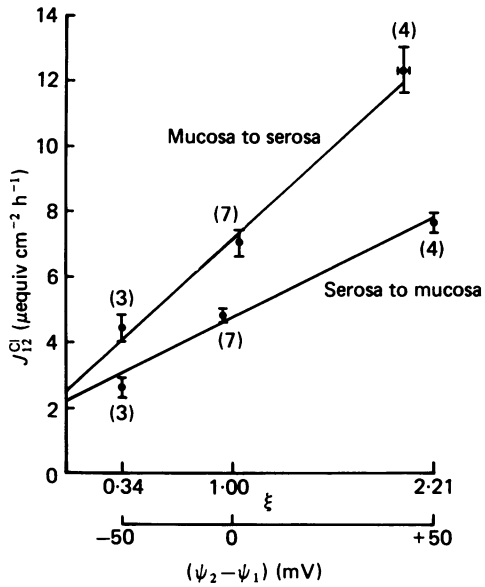

Fig. 3. Effects of variations in p.d. on chloride fluxes. Conditions and design of experiments were similar to those described in Fig. 2, but chloride fluxes were estimated. Regression analysis of these data ($n = 7$ tissue pairs) yielded the following relations:

$$J_{ms}^{Cl} = 2{\cdot}5\ (\pm 0{\cdot}3)\ \xi + 2{\cdot}2\ (\pm 0{\cdot}4) \quad (r = 0{\cdot}94)$$
$$J_{sm}^{Cl} = 4{\cdot}6\ (\pm 0{\cdot}4)\ \xi + 2{\cdot}5\ (\pm 0{\cdot}6) \quad (r = 0{\cdot}95).$$

of chloride both transmural fluxes included significant voltage-independent components ($j_{ms}^{Cl} = 2{\cdot}2 \pm 0{\cdot}4$ μequiv cm^{-2} h^{-1} and $j_{sm}^{Cl} = 2{\cdot}5 \pm 0{\cdot}6$ μequiv cm^{-2} h^{-1}). The values of these intercepts did not differ significantly, but the slope of the relation between J_{sm}^{Cl} and ξ ($_vJ_{sm}^{Cl} = 4{\cdot}6 \pm 0{\cdot}4$ μequiv cm^{-2} h^{-1}) was significantly larger than that of the relation between J_{ms}^{Cl} and ξ ($_vJ_{ms}^{Cl} = 2{\cdot}5 \pm 0{\cdot}3$ μequiv cm^{-2} h^{-1}). This means that the net movement of chloride that occurred in the short-circuited condition could be attributed solely to rectification of the p.d.-dependent movements.

Table 3 shows the results of a voltage-clamp experiment in which the effects of omission of chloride from the serosal fluid on J_{ms}^{Cl} were examined. The absence of chloride from the serosal fluid decreased J_{ms}^{Cl} and abolished the intercept of the relation between the flux and ξ, but did not change the slope of this relation significantly. These findings indicated that the p.d.-independent component of chloride transport was dependent on the presence of chloride in the *trans* compartment.

Effects of ion transport inhibitors

Preliminary experiments showed that addition of amiloride to the mucosal fluid produced a dose-dependent decrease in I_{sc}, and that this response was maximal when the concentration of the inhibitor was 1×10^{-5} M. Table 4 shows the effects of this concentration of amiloride on the transport of sodium, and on G_t and I_{sc}.

TABLE 3. Effect of unilateral chloride omission on chloride flux. Data are taken from a voltage-clamp experiment similar to that described in Fig. 2. Values of J_{ms}^{Cl} given in the Table were estimated during period in which tissue was short-circuited. Values of slope and intercept were estimated by regression analysis of the variation of J_{ms}^{Cl} with p.d. In some experiments isethionate and sulphate were substituted for chloride in the fluid used on the serosal side of the tissue. Tissues were paired to allow comparison of chloride fluxes in this condition with those observed when both mucosal and serosal fluids contained chloride

	J_{ms}^{Cl} (μequiv cm^{-2} h^{-1})	Slope ($_vJ_{ms}^{Cl}$) (μequiv cm^{-2} h^{-1})	Intercept (j_{ms}^{Cl}) (μequiv cm^{-2} h^{-1})
Control (14)	4·3 ± 0·2	2·8 ± 0·2	1·5 ± 0·3
Cl-free serosa (14)	2·4 ± 0·2***	2·6 ± 0·2	0·0 ± 0·3***

*** Denotes difference from control significant at $P < 0.001$.

TABLE 4. Effects of amiloride on sodium transport and electrical correlates. Data in the upper part of the Table are taken from studies on short-circuited tissue similar to those described in Table 1. Data in the lower part are derived from voltage-clamp experiments similar to those described in Fig. 2. Tissues were paired to allow examination of effects of amiloride. When present, the inhibitor was added only to the mucosal fluid at a concentration of 1×10^{-5} M

Short-circuit experiments		Control	Amiloride
G_t (mS cm^{-2})		9·3 ± 0·3 (20)	8·8 ± 0·2 (20)*
I_{sc} (μequiv cm^{-2} h^{-1})		8·7 ± 0·4 (20)	4·2 ± 0·3 (20)***
J_{ms}^{Na} (μequiv cm^{-2} h^{-1})		6·8 ± 0·4 (12)	3·3 ± 0·1 (12)***
J_{sm}^{Na} (μequiv cm^{-2} h^{-1})		2·8 ± 0·3 (8)	2·9 ± 0·2 (8)
Voltage-clamp experiments			
J_{ms}^{Na} (μequiv cm^{-2} h^{-1}):	slope	4·2 ± 0·4 (12)	3·3 ± 0·2 (12)*
	intercept	2·3 ± 0·5 (12)	0·2 ± 0·2 (12)***
J_{sm}^{Na} (μequiv cm^{-2} h^{-1}):	slope	3·0 ± 0·3 (8)	2·8 ± 0·3 (8)
	intercept	0·0 ± 0·4 (8)	0·4 ± 0·4 (8)

* Denotes difference from corresponding control significant at $P < 0.05$.
*** Denotes difference from corresponding control significant at $P < 0.001$.

The data given in the upper part of the Table are derived from observations made in the short-circuited condition. The presence of amiloride was associated with a decrease of approximately 50% in the short-circuit current, and a small, but significant decrease in the conductance of the tissue. J_{ms}^{Na} was also markedly decreased in the presence of amiloride, but J_{sm}^{Na} was not changed in this condition. Comparison of the unpaired estimates of J_{ms}^{Na} and J_{sm}^{Na} obtained in the presence of amiloride suggested that the inhibitor abolished the net transport of sodium.

The data given in the lower part of Table 4 are taken from voltage-clamp studies designed to examine the effects of amiloride on the p.d.-sensitive, and p.d.-independent

components of sodium transport. These experiments showed that amiloride did not change the parameters of the relation between J_{sm}^{Na} and ξ, but decreased both the slope and the intercept of the corresponding relation for J_{ms}^{Na}. In fact, the estimate of j_{ms}^{Na} obtained in the presence of amiloride was not significantly different from zero,

TABLE 5. Effect of SITS on chloride fluxes. Conditions of experiments and expression of results generally similar to those described in Table 1. Tissues were paired to allow examination of the effects of SITS (0·5 mM) on chloride fluxes and electrical variables

	Control	0·5 mM-SITS	$\bar{d}$
G_t (mS cm^{-2})	7·7±0·3	6·9±0·2	−0·9±0·2 (23)***
I_{sc} (μequiv cm^{-2} h^{-1})	7·7±0·4	7·3±0·3	−0·4±0·3 (23)
J_{ms}^{Cl} (μequiv cm^{-2} h^{-1})	4·5±0·6	4·2±0·3	−0·3±0·4 (7)
J_{sm}^{Cl} (μequiv cm^{-2} h^{-1})	8·1±0·5	6·8±0·4	−1·3±0·4 (16)**

** Denotes $\bar{d}$ different from zero at $P < 0·01$.
*** Denotes $\bar{d}$ different from zero at $P < 0·001$.

TABLE 6. Effects of acetazolamide on chloride fluxes. Conditions of experiments and expression of results generally similar to those described in Table 5, but the inhibitor used was 8 mM-acetazolamide, and tissues were paired to allow examination of the effects of the inhibitor on unidirectional fluxes in all cases

Saline		Control	Acetazolamide	$\bar{d}$
Control	G_t (mS cm^{-2})	8·4±0·4	7·4±0·5	−1·0±0·4 (12)*
	I_{sc} (μequiv cm^{-2} h^{-1})	7·1±0·5	5·0±0·3	−2·1±0·3 (12)***
	J_{ms}^{Cl} (μequiv cm^{-2} h^{-1})	4·5±0·4	4·7±0·4	0·2±0·5 (4)
	J_{sm}^{Cl} (μequiv cm^{-2} h^{-1})	7·7±0·7	6·1±0·4	−1·6±0·7 (8)*
Na-free	G_t (mS cm^{-2})	10·8±0·3	10·1±0·4	−0·8±0·3 (12)*
	I_{sc} (μequiv cm^{-2} h^{-1})	0·0±0·1	0·2±0·1	0·2±0·1 (12)
	J_{ms}^{Cl} (μequiv cm^{-2} h^{-1})	5·5±0·4	5·9±0·5	0·4±0·4 (6)
	J_{sm}^{Cl} (μequiv cm^{-2} h^{-1})	6·6±0·3	7·1±0·3	0·5±0·3 (6)

* Denotes $\bar{d}$ different from zero at $P < 0·05$.
*** Denotes $\bar{d}$ different from zero at $P < 0·001$.

indicating that the inhibitor abolished the voltage-independent component of sodium transport. Comparison of the unpaired estimates of ${}_vJ_{ms}^{Na}$ and ${}_vJ_{sm}^{Na}$ obtained in the presence of amiloride suggested that the inhibitor also abolished the rectification of the p.d.-sensitive movements, and this was confirmed in a separate series of voltage-clamp experiments in which J_{ms}^{Na} and J_{sm}^{Na} were determined in paired tissues, both in the presence of amiloride. In experiments on ten pairs of tissues, ${}_vJ_{ms}^{Na} = 2·6 \pm 0·1$ μequiv cm^{-2} h^{-1} and ${}_vJ_{sm}^{Na} = 2·3 \pm 0·3$ μequiv cm^{-2} h^{-1}, and these values did not differ significantly. Thus, these experiments supported the suggestion, based on the observations on short-circuited tissue, that amiloride abolished the net transport of sodium, and demonstrated further that this effect was associated with changes in both p.d.-sensitive and p.d.-independent components of the mucosal-to-serosal flux.

Table 5 includes the results of experiments directed to the effects of the anion transport inhibitor, SITS, on the transport of chloride. The tissues were paired to allow

examination of the effects of SITS on the individual chloride fluxes and on the electrical properties of the tissue. These studies showed that addition of 0·5 mM-SITS to the incubation saline significantly decreased J^{Cl}_{sm} and tissue conductance, but did not change J^{Cl}_{ms} or short-circuit current. Comparison of the unpaired estimates of J^{Cl}_{ms} and J^{Cl}_{sm} observed in the presence of SITS suggests that the net transport of chloride was not abolished in this condition. The observations that SITS did not alter the short-circuit current, and that the component of chloride transport that occurred in the absence of sodium was electrically silent (Table 2), suggested that SITS inhibited the sodium-independent component of chloride transport. To test this possibility, a second series of experiments was conducted in which tissues were paired to allow evaluation of net chloride transport, and the effects of SITS examined in a sodium-free saline. In a series of nine experiments, all conducted in a sodium-free saline, the net movement of chloride observed in the absence of SITS was $1{\cdot}4 \pm 0{\cdot}3$ μmol cm^{-2} h^{-1}, and in the presence of 0·5 mM-SITS was $-0{\cdot}1 \pm 0{\cdot}2$ μmol cm^{-2} h^{-1}. Thus these experiments demonstrated that SITS abolished the net transport of chloride that occurred in the absence of sodium.

Table 6 shows the results of similar experiments directed to the effects of acetazolamide on chloride transport. The data shown in the upper part of the Table were taken from experiments conducted in the unmodified incubation saline, and show that in the presence of sodium addition of acetazolamide decreased J^{Cl}_{sm}, short-circuit current and tissue conductance, but did not change J^{Cl}_{ms}. In contrast, addition of acetazolamide to the sodium-free saline did not significantly change either of the chloride fluxes or the electrical correlates of transport.

DISCUSSION

In confirmation of previous work (Kitahara *et al.* 1969; Sernka & Hogben, 1969) our experiments showed that, in the absence of transmural gradients of electrochemical potential, the isolated gastric mucosa of the rat maintained net steady-state movements of sodium in the mucosal-to-serosal direction, and of chloride from serosa to mucosa. Fig. 4 summarizes the conclusions drawn from the three approaches used to characterize these transport processes.

Sodium transport

Removal of chloride from the incubation saline did not influence the fluxes of sodium, indicating that the process responsible for the net transmural movement of sodium did not include a step which coupled the movements of sodium and chloride analogous to the mechanisms described in studies on gall-bladder and intestine (Frizzell, Koch & Schultz, 1976; Nellans, Frizzell & Schultz, 1975).

Addition of amiloride to the mucosal fluid abolished the net transport of sodium and produced an equivalent decrease in I_{sc}, suggesting that the sodium transport process was electrogenic.

Two cellular loci have been identified as possible sites of action of amiloride in inhibiting epithelial sodium transport. At low concentrations (usually $< 1 \times 10^{-5}$ M) amiloride is a potent, competitive inhibitor of entry processes at the apical membrane (Benos, 1982), and at high concentrations ($> 5 \times 10^{-4}$ M) a depression of sodium pump

function at the basolateral membrane may be observed (Soltoff & Mandel, 1983). In the present experiments, amiloride was found to abolish the net steady-state transport of sodium at a concentration of 1×10^{-5} M. This concentration is substantially below the range in which direct effects on pump activity have been

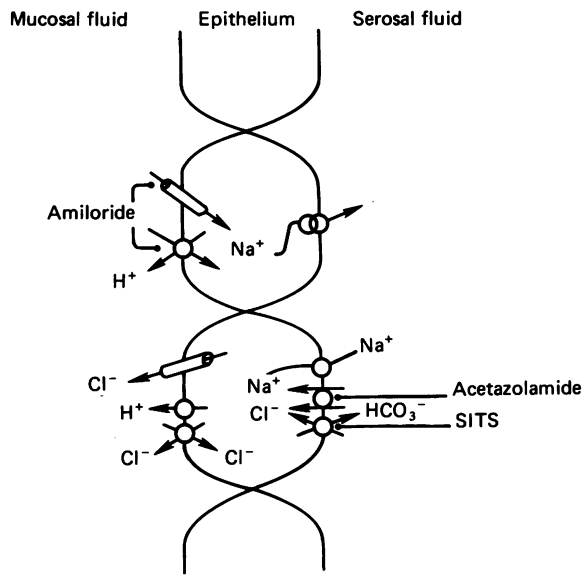

Fig. 4. Model for sodium and chloride transport processes in rat grastric mucosa *in vitro*. The sodium transport system is considered to include three components: two rate-limiting entry mechanisms at the apical membrane, one conductive channel in which sodium movement varies with p.d., and an electrically neutral mechanism, possibly a sodium–hydrogen exchanger, in which the movement of sodium is independent of p.d.; and a pump process at the basolateral membrane in which the movement of sodium is independent of p.d., but which is electrogenic, and which may be represented as a constant-current source. The system responsible for the net transport of chloride also includes three components: two entry mechanisms at the basolateral membrane, one sodium-coupled, and a chloride–bicarbonate exchanger; and a conductive chloride channel at the apical membrane in which the movement of chloride varies with p.d. Part of the chloride movement in this channel is compensated by a parallel movement of hydrogen ions, with the result that this component of chloride transport is electrically silent. In contrast, because the sodium pump process is electrogenic, the sodium-dependent component of chloride transport contributes to the short-circuit current. In addition, the transmural fluxes of chloride include components mediated by a chloride–chloride exchange mechanism, but these movements do not contribute to the net transport process. Open circles represent the sites of action of the inhibitors amiloride, acetazolamide and SITS.

observed in previous work, and is consistent with an effect on entry processes at the luminal aspect of an epithelial cell.

In studies on frog skin it has been shown that a significant component of the sodium blackflux utilized the transcellular channel, and that this component of the backflux was inhibited by amiloride (Biber & Mullen, 1976). In the present experiments J_{sm}^{Na} was not affected by a concentration of amiloride that abolished net transport of sodium, suggesting that in the rat gastric mucosa the backflux of sodium utilized a

shunt pathway independent of the sodium transport system, and it may be that the paracellular channel constituted a major component of this pathway. This suggestion was supported by the finding in the voltage-clamp experiments that J_{sm}^{Na} increased linearly with the function ξ, and exhibited an extrapolated intercept at the origin. This pattern of relation is consistent with the proposal that J_{sm}^{Na} was a simple diffusive movement (Frizzel & Schultz, 1972).

The finding that the extrapolated intercept of the relation between J_{ms}^{Na} and ξ was greater than zero indicated that a component of the sodium transport process was independent of p.d. This observation could not be explained by proposing that only one membrane of the sodium transporting epithelial cell included a mechanism in which the flow of sodium was independent of p.d. In the latter situation the p.d.-dependent flow at the other membrane would cease in the presence of a large adverse p.d. ($\xi = 0$), and the role of the p.d.-independent mechanism in the transcellular movement would be obscured. Thus the finding of a p.d.-independent component in the transmural flux carries the implication that both apical and basal membranes included mechanisms in which the flow of sodium was independent of p.d. However, the finding that the slope of the relation between J_{ms}^{Na} and ξ was greater than that of the corresponding relation for J_{sm}^{Na} indicated that the sodium transport system included also a channel in which sodium movement was voltage-dependent, and which was accessible to sodium moving in the mucosal-to-serosal direction, but was inaccessible to sodium moving from serosa to mucosa. The least complex system that is consistent with these findings includes three components:

(i) a conductive mechanism for sodium entry at the apical membrane in which the flux of sodium varies with p.d., and which constitutes the rate-limiting step for the voltage-dependent component of sodium transport,

(ii) a parallel, electrically neutral entry mechanism at the apical membrane which facilitates access of the p.d.-independent component of sodium transport to the pump process, and which is the rate-limiting step for the voltage independent component, and

(iii) a pump mechanism located in the basolateral membrane of the sodium transporting epithelial cell.

Sodium absorption from the resting stomach has been suggested to be coupled to hydrogen ion secretion (Bagajski, Code & Schlegel, 1972), and it may be that the electrically neutral entry mechanism is represented by a sodium–hydrogen exchanger. It has been shown that the inhibitory actions of amiloride on sodium entry mechanisms may be associated both with blockage of conductive sodium channels, and with inhibition of a neutral sodium–hydrogen exchanger (Benos, 1982). Thus the proposal of two parallel processes for sodium entry at the apical membrane is not inconsistent with the observation that the inhibitor completely abolished the transport of sodium.

Although the pump process is not considered to constitute a rate-limiting step in transcellular sodium movement, it is clear that the pump mechanism must be independent of p.d. If this step was p.d.-sensitive, sodium movement through the pump would cease in the presence of a large adverse p.d. ($\xi = 0$), and the influence of the electrically neutral entry mechanism would not be observed. In contrast, because the conductive entry process is considered to be a rate-limiting step, the

transcellular movement of sodium will exhibit a p.d.-dependent character even if the pump process is independent of p.d. It is of interest to note that studies on some other sodium transporting epithelial systems have suggested that the pump process may be described in terms of an infinite resistance, constant-current source, rather than a low resistance, constant-voltage system (Boulpaep, 1976).

Chloride transport

In studies on guinea-pig stomach, Klemperer, Lelchuk & Caplan (1983) found that the secretory movement of chloride ceased when sodium was omitted from the incubation saline. In contrast, our experiments on the rat gastric mucosa showed that substitution of choline for sodium in the incubation saline decreased but did not abolish the net movement of chloride, indicating that the chloride transport process included both sodium-dependent, and sodium-independent components. The observation that p.d. and I_{sc} were abolished when sodium was omitted from the incubation saline showed that the sodium-independent component of chloride transport in rat gastric mucosa was electrically silent.

Although acetazolamide has been used as a specific inhibitor of carbonic anhydrase (Maren, 1967), this agent has also been shown to inhibit chloride transport in some epithelia (Hogben, 1967; Kitahara, Fox & Hogben, 1967; Nellans *et al.* 1975). The present experiments showed that acetazolamide depressed gastric chloride transport in the presence of sodium, but did not alter chloride transport in the sodium-free condition. Accordingly, it was concluded that acetazolamide inhibited the sodium-dependent component of the chloride transport process, but did not influence the sodium-independent component. In this context it is of interest to note that Nellans *et al.* (1975) found that acetazolamide inhibited a sodium-coupled component of chloride influx in the small intestine.

The observations that acetazolamide elicited equivalent decreases in J^{Cl}_{net} and I_{sc}, and that omission of chloride from the incubation saline decreased I_{sc} by an amount equivalent in magnitude to the sodium-dependent, acetazolamide-sensitive component of chloride transport, indicated that the sodium-dependent component of chloride transport was electrogenic. An electrogenic, sodium-dependent component of chloride secretion has also been demonstrated in studies on frog gastric mucosa (Machen & McLennan, 1980).

The disulphonic stilbenes, such as SITS, have been shown to act as inhibitors of some chloride transport processes in both non-polar cells (Cabantchik, Knauf & Rothstein, 1978) and epithelial systems (Brodsky, Durham & Ehrenspeck, 1979). In the present experiments, SITS was found to depress the net transport of chloride observed in the presence of sodium, and to abolish the residual chloride transport seen in its absence. These findings indicated that SITS inhibited the sodium-independent component of the chloride transport process, but did not allow any conclusion concerning the possible effect of the inhibitor on the sodium-dependent component. Two arguments suggest that SITS did not affect the sodium-dependent component of chloride transport. First, the magnitude of the SITS-induced depression of net chloride transport seen in the presence of sodium, was equivalent to the rate of chloride transport observed in the absence of sodium. Secondly, because the sodium-independent component of chloride transport was electrically silent, the

observation that SITS did not alter the short-circuit current supported the suggestion that the inhibitor did not affect the electrogenic sodium-dependent component.

Both J_{ms}^{Cl} and J_{sm}^{Cl} exhibited significant positive intercepts in their relations with ξ. The finding that the p.d.-independent component of a chloride flux was abolished when chloride was absent from the *trans* compartment was consistent with the proposal that these p.d.-independent components reflected the chloride–chloride exchange mechanism described in previous studies on gastric mucosa (Heinz & Durbin, 1957; Forte, 1969). Because the p.d.-independent components of the two chloride fluxes were of comparable magnitudes, it is clear that the exchange mechanism did not contribute to the net transport of chloride observed in the short-circuited condition, and that the observed net movement resulted from rectification of the voltage-sensitive flows. By analogy with the discussion of the corresponding experiments on sodium transport, the observation of rectification in chloride movements implies that either (1) the flux of chloride across one membrane of the chloride-transporting cell was p.d.-dependent and rate-limiting, or (2) the movements of chloride across both apical and basolateral membranes were p.d.-dependent.

A mechanism for sodium-dependent chloride secretion in epithelial systems has been described by Frizzell, Field & Schultz (1979). The system includes three components:

(i) a process that couples the diffusive movement of sodium across the basolateral membrane of an epithelial cell with a parallel movement of chloride, and which leads to accumulation of chloride in the intracellular compartment to an extent that is determined by the magnitude of the sodium gradient,

(ii) a sodium pump which extrudes sodium through the basolateral membrane, which recycles sodium entering the cell through the chloride-coupled mechanism, and which maintains the diffusion gradient for sodium, and

(iii) a chloride channel at the apical membrane that allows the anion to diffuse from the intracellular compartment into the mucosal fluid.

The following observations are consistent with the suggestion that a component of the chloride transport process in rat gastric mucosa may be described in terms of the model of Frizzell *et al.* (1979): (a) part of the chloride transport process was sodium-dependent but did not include coupling between the transmural movements of the two ions, (b) the chloride transport process varied with p.d., consistent with the proposal of a conductive chloride channel in the apical membrane, and (c) the process was inhibited by acetazolamide which has been shown to block a coupled sodium–chloride influx process in the small intestine (Nellans *et al.* 1975).

The finding that the sodium-dependent component of chloride transport across rat gastric mucosa was electrogenic does not necessarily imply that the stoicheiometry of sodium and chloride movements at the basolateral membrane was not 1:1. If the pump process responsible for maintaining the sodium gradient was electrogenic, the chloride flux through this process would be associated with a net flow of charge.

The finding that a component of the chloride transport process was independent of the presence of sodium requires the proposal of a second entry mechanism for chloride at the basolateral membrane in parallel with the sodium-coupled movement. Rehm (1967) has presented evidence for the existence of a chloride–bicarbonate

exchanger at the basolateral membrane of chloride-secreting cells in frog gastric mucosa, and it was of interest to find that the sodium-independent component of chloride transport in rat gastric mucosa was inhibited by SITS, which has been shown to interfere with chloride–bicarbonate exchange mechanisms in other systems (Cabantchik *et al.* 1978).

The sodium-independent component of gastric chloride transport varied with p.d., suggesting that a chloride-permeable channel also contributed as a rate-limiting step to this movement. However, the sodium-independent transport of chloride did not contribute to the short-circuit current, indicating that movement of the anion through the conductive channel was associated with movements of other species that conserved electroneutrality. In this connexion it is of interest to note that studies on frog gastric mucosa (Durbin & Heinz, 1958) suggested that the non-electrogenic component of chloride transport was associated with acid secretion. Because the mucosal solution used in our experiments was well buffered, the rate of acid secretion in these studies could not be estimated, but the data of Hogben & Karal (1973) indicated that the rate of acid secretion in unstimulated rat gastric mucosa *in vitro* was of comparable magnitude to the sodium-independent component of chloride transport observed in the present studies. Accordingly, it may be suggested that the electroneutrality of the sodium-independent component of gastric chloride transport was maintained by a parallel movement of hydrogen ions at the apical membrane.

Arguments analogous to those summarized in the discussion of the sodium transport mechanism, indicate that the chloride–chloride exchange mechanism must provide a pathway in parallel with the conductive chloride channel in the apical membrane, and in series with the neutral mechanisms in the basolateral membrane, if the exchange process is to contribute an observable p.d.-independent component to the transmural flux. The proposal of an apical membrane locus for the chloride–chloride exchange mechanism is consistent with previous work on amphibian gastric mucosa (Forte, 1969).

Conclusions

Fig. 4 summarizes a model for transport of sodium and chloride across rat gastric mucosa *in vitro* based on the present experiments. The model suggests that the sodium and chloride transport processes represent activities of two different cell populations, and two arguments may be cited in support of this proposal. First, Wright (1962) showed, in studies on fetal rabbit stomach, that sodium transport could be demonstrated at an early stage of development when only surface epithelial cells were present, and that chloride transport was ontogenically related with the appearance of glandular elements at a later stage of development. Secondly, if both sodium channels in the apical membrane and the coupled sodium–chloride influx process in the basolateral membrane were present in the same cell population, an interaction between the transmural serosal-to-mucosal movements of sodium and chloride may have been expected, but the present experiments showed that J_{sm}^{Na} was independent of the presence of chloride.

Both the sodium and chloride transport systems included parallel elements. Thus our findings suggested that the sodium transport process included parallel processes in the apical membrane, and the studies on chloride suggested parallel mechanisms

in both apical and basolateral membranes. It may be that these parallel processes represent the activities of different subpopulations of sodium- and chloride-transporting cells, but no evidence has been obtained to support this proposal, and for this reason the model presented in Fig. 4 includes only two cell types.

Our studies showed that both the sodium transport process and the sodium-dependent chloride movement contributed to the short-circuit current, and that the sodium-independent chloride transport was electrically silent. It was noted that the sum of the net sodium movement and the sodium-independent chloride movement was always less than the short-circuit current, indicating that other species also contributed to the electrical properties of the tissue. The nature of these additional processes is not known, but they may include transport of a cation in the mucosal-to-serosal direction, or of an anion from serosa to mucosa. Because the short-circuit current was abolished when sodium was omitted from the incubation saline, it may be suggested that the additional processes were sodium dependent.

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REFERENCES

BAGAJSKI, J., CODE, C. F. & SCHLEGEL, J. F. (1972). Sodium–hydrogen-ion exchange across canine resting gastric mucosa. *American Journal of Physiology* **222**, 858–863.

BENOS, D. J. (1982). Amiloride: a molecular probe of sodium transport in tissues and cells. *American Journal of Physiology* **242**, C131–C145.

BIBER, T. U. L. & MULLEN, T. L. (1976). Saturation kinetics of sodium efflux across isolated frog skin. *Journal of Membrane Biology* **231**, 995–1001.

BOULPAEP, E. L. (1976). Electrical phenomena in the nephron. *Kidney International* **9**, 88–102.

BRODSKY, W. A., DURHAM, J. & EHRENSPECK, G. (1979). The effects of a disulphonic stilbene on chloride and bicarbonate transport in the turtle bladder. *Journal of Physiology* **287**, 559–573.

CABANTCHIK, Z. I., KNAUF, P. A. & ROTHSTEIN, A. (1978). The anion transport system of the red blood cell. The role of membrane protein evaluated by the use of 'probes'. *Biochimica et biophysica acta* **515**, 239–302.

DOBSON, J. G. & KIDDER, G. W. (1968). Edge damage effect in *in vitro* frog skin preparations. *American Journal of Physiology* **214**, 719–724.

DURBIN, R. P. & HEINZ, E. (1958). Electromotive chloride transport and gastric acid secretion in the frog. *Journal of General Physiology* **41**, 1035–1047.

FORTE, J. G. (1969). Three components of Cl^- flux across isolated bullfrog gastric mucosa. *American Journal of Physiology* **216**, 167–174.

FORTE, J. G., FORTE, T. M. & MACHEN, T. E. (1975). Histamine-stimulated hydrogen ion secretion by *in vitro* piglet gastric mucosa. *Journal of Physiology* **244**, 15–31.

FORTE, J. G. & MACHEN, T. E. (1975). Transport and electrical phenomena in resting and secreting piglet gastric mucosa. *Journal of Physiology* **244**, 33–51.

FRIZZELL, R. A., FIELD, M. & SCHULTZ, S. G. (1979). Sodium-coupled chloride transport by epithelial tissues. *American Journal of Physiology* **236**, F1–8.

FRIZZELL, R. A., KOCH, M. J. & SCHULTZ, S. G. (1976). Ion transport by rabbit colon. I. Active and passive components. *Journal of Membrane Biology* **27**, 297–346.

FRIZZELL, R. A. & SCHULTZ, S. G. (1972). Ionic conductances of extracellular shunt pathway in rabbit ileum. *Journal of General Physiology* **59**, 318–346.

HEINZ, E. & DURBIN, R. P. (1957). Studies of chloride transport in the gastric mucosa of the frog. *Journal of General Physiology* **41**, 101–117.

HOGBEN, C. A. M. (1955). Active transport of chloride by isolated frog gastric epithelium: origin of the gastric mucosa potential. *American Journal of Physiology* **180**, 641–649.

HOGBEN, C. A. M. (1967). The chloride effect of carbonic anhydrase inhibitors. *Molecular Pharmacology* **3**, 318–326.

HOGBEN, C. A. M. & KARAL, D. R. (1973). Further studies on the isolated rat gastric mucosa. In *Alfred Benzon Symposium V: Transport Mechanisms in Epithelia*, ed. USSING, H. H. & THORN, N. A., pp. 236–253. New York: Academic Press.

KITAHARA, S., FOX, K. R. & HOGBEN, C. A. M. (1967). Depression of chloride transport by carbonic anhydrase inhibitors in the absence of carbonic anhydrase. *Nature* **214**, 836–837.

KITAHARA, S., FOX, K. R. & HOGBEN, C. A. M. (1969). Acid secretion, Na^+ absorption, and the origin of the potential difference across isolated mammalian stomachs. *American Journal of Digestive Diseases and Science* **14**, 221–238.

KLEMPERER, G., LELCHUK, S. & CAPLAN, S. R. (1963). Na^+-coupled Cl^- transport in the gastric mucosa of the guinea pig. *Journal of Bioenergetics and Biomembranes* **15**, 121–134.

KUO, Y. J. & SHANBOUR, L. L. (1979). Chloride, sodium, potassium, and hydrogen ion transport in isolated canine gastric mucosa. *Journal of Physiology* **291**, 367–380.

MACHEN, T. E. & MCLENNAN, W. L. (1980). Na^+-dependent H^+ and Cl^- transport in *in vitro* frog gastric mucosa. *American Journal of Physiology* **238**, G403–413.

MACHEN, T. E., SILEN, W. & FORTE, J. G. (1978). Na^+ transport by mammalian stomach. *American Journal of Physiology* **234**, E228–235.

MAREN, T. H. (1967). Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiological Reviews* **47**, 595–781.

MCCABE, D. R., KENT, T. H. & HOGBEN, C. A. M. (1969). Distribution and weights of various cell types in the rat stomach. *Anatomical Record* **163**, 555–561.

NELLANS, H. N., FRIZZELL, R. A. & SCHULTZ, S. G. (1975). Effect of acetazolamide on sodium and chloride transport by the *in vitro* rabbit ileum. *American Journal of Physiology* **238**, 1808–1814.

REHM, W. S. (1967). Ion permeability and electrical resistance of the frog's gastric mucosa. *Federation Proceedings* **26**, 1303–1313.

REHM, W. S. (1968). An analysis of the short-circuiting technique applied to *in vivo* tissues. *Journal of Theoretical Biology* **20**, 341–354.

SCHULTZ, S. G. & ZALUSKY, R. (1964). Ion transport in isolated rabbit ileum. I. Short circuit current and Na fluxes. *Journal of General Physiology* **47**, 567–584.

SERNKA, T. J. & HOGBEN, C. A. M. (1969). Active ion transport by isolated gastric mucosae of rat and guinea pig. *American Journal of Physiology* **217**, 1419–1424.

SOLTOFF, S. P. & MANDEL, L. J. (1983). Amiloride directly inhibits the Na, K-ATPase activity of rabbit kidney proximal tubules. *Science* **220**, 957–959.

WRIGHT, G. H. (1962). Net transfers of water, sodium, chloride, and hydrogen ions across the gastric mucosa of the rabbit foetus. *Journal of Physiology* **163**, 281–293.