J. Physiol. (1975), **244**, *pp.* 33–51
With 3 text-figures
Printed in Great Britain

TRANSPORT AND ELECTRICAL PHENOMENA IN RESTING AND SECRETING PIGLET GASTRIC MUCOSA

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(*Received 29 November 1973*)

SUMMARY

1. Gastric mucosae were isolated from piglets (0–5 days old) and mounted in a chamber where electrical properties and secretory function could be measured. Unlike many previously reported mammalian *in vitro* preparations, pig gastric mucosae were stable and physiologically responsive for many hours after isolation.

2. With similar Ringer solutions bathing both surfaces, the isolated piglet gastric mucosa maintained a p.d. with the mucosal surface 30–35 mV negative with respect to the serosal surface. Limitation of access of Na^+ from the mucosal bathing solution to the tissue (e.g. replacement of Na^+ on mucosal side with choline or treatment with 10^{-5} M amiloride) produced a decrease in p.d. and increase in mucosal resistance consistent with an hypothesis of Na^+ transport from mucosa to serosa.

3. Isotopic flux measurements (^{36}Cl and ^{24}Na) and net H^+ secretory rate were performed during open and short-circuit conditions, while the tissue was at rest and after stimulation of HCl secretion by 6×10^{-5} M histamine. Up to 90% of the respective short-circuit current for resting or secreting mucosae was accounted for as the algebraic sum of Cl^-, H^+ or Na^+ fluxes.

4. The net transport of Na^+ which occurred from mucosa to serosa during rest (*ca.* 4·7 μequiv/cm^2.hr) was somewhat reduced during HCl secretion (*ca.* 2·7 μequiv/cm^2.hr). This active transport of Na^+ was more resistant to anaerobiosis than was H^+ or Cl^- transport.

5. An active transport component of Cl^- from serosa to mucosa was clearly demonstrable in the non-secreting preparations (*ca.* 3·9 μequiv/cm^2.hr). Active Cl^- transport was stimulated three- to fourfold after H^+ secretion was stimulated by histamine. Anaerobiosis promptly reduced Cl^- and H^+ transport. An exchange diffusion component was demonstrated for Cl^- which appeared to be prominent during H^+ secretory activity and was considerably diminished in resting mucosae.

6. Large changes in mucosal resistance were associated with conditions of rest, histamine stimulation and anaerobic conditions; mean values were 113, 74 and 197 $\Omega.cm^2$, respectively. Electrical conductance of the isolated gastric mucosa was due primarily to partial ionic conductance of Cl^- (60–65%) and Na^+ (10–15%). The partial conductance of H^+ was extremely low. The observed increase in tissue conductance associated with H^+ secretory activity and the changes in the long-time constant p.d. transient to a current pulse are discussed in terms of the relative contribution of the serosal and mucosal plasma membrane surfaces.

INTRODUCTION

When a mammalian stomach or gastric mucosa is prepared *in vitro* with identical Ringer solutions bathing both surfaces, there is a spontaneous electrical potential difference (p.d.) with the mucosal solution negative with respect to the serosal solution. It has been established that this p.d. observed in isolated mammalian gastric mucosa is generated largely by active Na^+ transport from mucosal to serosal solutions and active Cl^- transport in the opposite direction. The relative rate of transport of the two ions varies in different species. For instance, in the dog Na^+ predominates, whereas in the fasted cat it is least significant (Kitahara, Fox & Hogben, 1969). Such isolated mammalian stomachs have also been shown to secrete acid at low but sustained rates of 1–2 μequiv/cm^2.hr (Cummins & Vaughan, 1965; Kitahara *et al.* 1969; Sernka & Hogben, 1969). However, these preparations could not be stimulated by secretagogues, thus it was not possible to study the interrelationships of the electrical and flux data with gastric H^+ secretory function.

Our previous paper shows that the gastric mucosa isolated from newborn pigs, up to about 5 days old, is stable for several hours *in vitro* and can readily be stimulated by histamine to secrete acid (Forte, Forte & Machen, 1975). For the present work we have used such preparations to evaluate the relative contribution of H^+, Cl^- and Na^+ transport to the electrophysiological activity of the tissue.

METHODS

Gastric mucosae from baby pigs (new-born to 5 days of age) were prepared in the manner described in the previous paper (Forte *et al.* 1975). Briefly, the stomach was removed and a 'blister' was formed between the outer muscle coats and the gastric mucosa in the fundic region. After the serosal muscle coat was dissected away, the remaining sheet of gastric mucosa was carefully tied on to the end of the mounting tube (1·4 cm^2 area) which was quickly assembled into the chamber (see Fig. 1 of Forte *et al.* 1975). Warmed bathing solutions were introduced into the chamber and

gassing with 95 % O_2 and 5 % CO_2 commenced immediately; recordings of electrical parameters were subsequently initiated. The bathing solutions were maintained at 37° C. The composition of various bathing solutions is given in Table 1.

The bridges for measurement of transmucosal electrical potential difference (p.d.) and for the passage of electric current through the mucosa were made of 1 M-NaCl in 4 % agar. The p.d. bridges were connected to a high-impedance electro-meter (Keithley) through calomel half cells. The output from the electrometer was connected to a recorder. The sign of all p.d. values is given for the mucosal side with respect to the serosal side.

TABLE 1. Composition of solutions used to measure electrophysiology and secretory parameters of isolated piglet gastric mucosa

	Cl^-–HCO_3^- solution	Cl^- mucosal solution	Ise^-–HCO_3^- solution	Ise^- mucosal solution	Choline mucosal solution
Na^+	147	147	147	147	—
K^+	5	5	5	5	5
Mg^{2+}	1·3	1·3	1·3	—	—
Ca^{2+}	1·3	—	1·3	—	—
Choline	—	—	—	—	147
Cl^-	129·6	127	—	—	152
HCO_3^-	25	—	25	—	—
$SO_4^=$	1·3	1·3	2·6	—	—
Isethionate	—	25	127	152	—
Glucose	11	—	11	—	—

All values are mM.
All solutions were aerated with 5 % CO_2 and 95 % O_2.

When bathing solutions were asymmetric, e.g. with Cl^-–HCO_3^- solution and choline mucosal solution on opposite sides of the tissue, liquid junction p.d.s arise at the tips of the agar–1 M-NaCl bridges (see Barry & Diamond, 1970), Junction p.d.s were measured in the chamber with the appropriate solutions separated physically by parafilm but connected electrically by a saturated KCl–agar bridge. All reported values for transepithelial p.d. have been corrected for these junction potentials.

Resistance was measured as previously described (Forte *et al.* 1975), with current being delivered through the saline bridges from a variable d.c. source via Ag/AgCl electrodes. In addition, short-circuit current was measured in traditional fashion by decreasing the p.d. to zero. Compensation was made for the resistance of the bathing solutions between the tips of the potential measuring bridges.

The rate of H^+ secretion was measured with a Radiometer pH stat. The solution bathing the mucosal surface was maintained at pH 5·0 by the addition of 0·1 N-NaOH. The amount of base added was recorded every 2·5 or 5 min and the secretory rates calculated accordingly. The initial 5 min period following a solution change was excluded from calculations in order to allow for equilibration with the gas phase. Stimulation of H^+ secretion was achieved by adding histamine–HCl in a final concentration of 6×10^{-5} M to the serosal side.

Unidirectional fluxes of Cl^- were measured with ^{36}Cl separately or in double isotope experiments with ^{24}Na. With ^{36}Cl alone aliquots of bathing solution were removed every 15–20 min and the isotope was measured by liquid scintillation; Cl^- flux was calculated according to a method described previously (Forte, 1969). When ^{24}Na and

^{36}Cl were used simultaneously an aliquot of the experimental solution was placed into scintillation vials and first monitored for ^{24}Na by Cerenkov radiation (Haberer, 1965) with a scintillation spectrometer. The instrument gain was adjusted so that ^{36}Cl efficiencies were negligible ($< < 0{\cdot}1\%$). After a suitable period had elapsed for decay of ^{24}Na (10–14 days) 1 ml. Bray solution was added to each vial and the ^{36}Cl assayed by the conventional liquid-scintillation procedures.

Where appropriately noted, the mean of individual values has been calculated and reported along with ± S.E. of mean; the number in the sample is given in parentheses. Since there was some variability from mucosa to mucosa in the absolute value of p.d., resistance and H^+ secretion, a more rigorous test of significance for induced changes was often applied. Thus after experimental manipulation, such as solution changes or addition of histamine, parametric differences were measured on individual tissues. These changes were treated as paired differences and their significance for the population was subsequently evaluated. The significance of a mean was evaluated with Student's *t* test.

RESULTS

General electrical characteristics. Some of the typical characteristics of the isolated piglet gastric mucosas have been shown in the previous paper (Forte *et al.* 1975) and are extended here. A typical experiment is shown in Fig. 1. Shortly after the preparation was mounted in the chamber with NaCl Ringer solutions on both sides, the measured p.d. was small and the resistance was very low, but both of these parameters continued to increase and eventually stabilized over a period of about an hour following mounting. After 1–1·5 hr in the chamber the p.d. was about −30 mV (range −12 to −46 mV), mucosa negative with respect to serosa, and the transmucosal resistance stabilized at about 125 $\Omega.cm^2$ (range 65–240 $\Omega.cm^2$). Typically, the rate of H^+ secretion in these freshly isolated preparations was zero or was low (0–1 $\mu equiv/cm^2.hr$).

As pointed out previously (Forte *et al.* 1975), histamine produced an increase in p.d. and a decrease in tissue resistance (Table 2). These changes signify an increase in electrical power output capability of the mucosa. Along with the electrical changes, H^+ secretion was markedly stimulated by histamine to secretory rates ranging from 3·7 to 14 $\mu equiv/cm^2.hr$. Typical responses to ion substitution in the mucosal solution are shown in Fig. 1 and are summarized for various resting and histamine-stimulated preparations in Table 2. When choline replaced Na^+ in the mucosal solution there was a consistent small drop in p.d. and/or a rise in tissue resistance. The net effect was a decrease in current that could be delivered by the tissue (see later) and was virtually the same for resting or secreting preparations.

Replacing mucosal Cl^- with isethionate produced an increase in both p.d. and tissue resistance (Fig. 1 and Table 2). The effects were qualitatively similar for resting and secreting preparations, although the absolute changes were more pronounced in the former. Treating the piglet gastric

mucosa as a black box, we can say that the mucosal border has a significant Cl^- permeability; the p.d. observed with isethionate solutions bathing the mucosal surface is shunted by Cl^-. The p.d. and resistance response to replacing Cl^- of the mucosal solution with the more impermeant isethionate anion is similar to that observed in the frog skin when SO_4^- or other impermeant anions replace Cl^- in the external bathing solution (Koefoed-Johnsen & Ussing, 1951; Ferreira, 1968). It is noteworthy that, as demonstrated in Fig. 1, the rate of H^+ secretion by piglet gastric mucosa was not significantly changed by Na^+ or Cl^- replacement on the mucosal side.

Also shown in Fig. 1 are the results of passing an external electrical current through the tissue bathed in Cl^- Ringer solution (see also Table 3). When relatively low current densities (6–7 μequiv current/cm^2.hr) were passed in either direction, the changes in H^+ secretion were only a small fraction of the total secretory rate (less than 10%). Thus, most of the

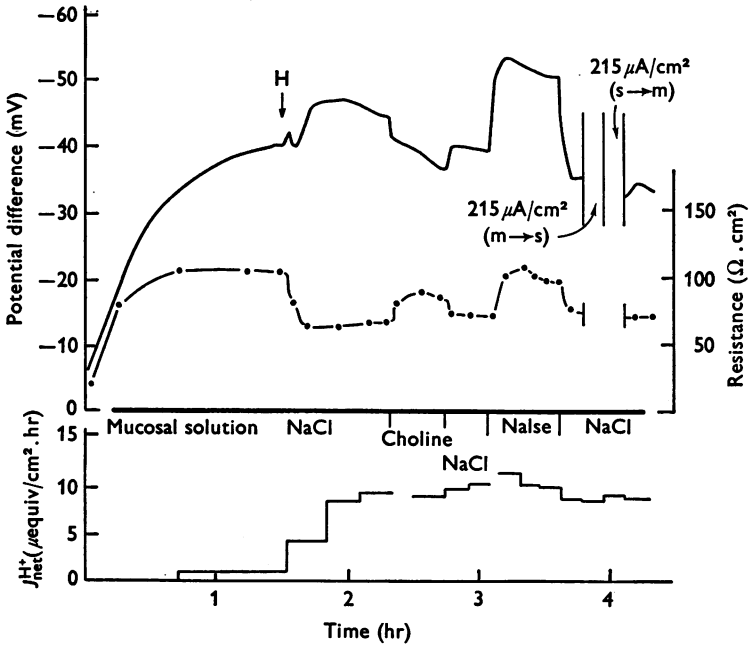

Fig. 1. Electrophysiological responses of isolated piglet gastric mucosa to histamine and to changes in the major ionic constituents of the mucosal solution. At the time indicated by the arrow (H) 6×10^{-5} M histamine was added to the serosal side. During the indicated time periods the mucosal solutions were changed; compositional details are given in Table 1. Late in the experiment 215 μA/cm^2 was passed from an external circuit; first a positive current was passed from mucosa to serosa (m → s), then from serosa to mucosa (s → m), as indicated. Upper graph: continuous line, p.d.; interrupted line, resistance.

current passed from external sources through the piglet gastric mucosa must be traversing pathways of conductance other than (e.g. parallel to) those directly associated with H^+ transport.

TABLE 2. P.d. and resistance characteristics of resting and histamine-stimulated piglet gastric mucosa with various ionic substituents in the mucosal solution

Mucosal solution*	p.d. (mV)	R_0 ($\Omega.cm^2$)	Histamine (6×10^{-5} M)
NaCl	-32.5 ± 1.9 (17)	117 ± 12 (16)	0
NaCl	-36.6 ± 1.8 (17)	66 ± 4 (16)	+
Δ**	$+4.1 \pm 1.1$	-51 ± 10	—
NaCl	-30.9 ± 2.4 (6)	100 ± 16 (6)	0
Choline Cl	-25.3 ± 1.4 (6)	112 ± 17 (6)	0
Δ**	-5.6 ± 2.7	$+12 \pm 3.2$	—
NaCl	-32.5 ± 2.7 (9)	65 ± 6 (9)	+
Choline Cl	-27.9 ± 3.5 (9)	77 ± 6 (9)	+
Δ**	-4.6 ± 1.7	$+12 \pm 2.4$	—
NaCl	-30.9 ± 2.4 (6)	137 ± 21 (6)	0
NaIse	-50.3 ± 2.5 (6)	164 ± 20 (6)	0
Δ**	$+19.5 \pm 3.5$	$+27 \pm 5.9$	—
NaCl	-29.9 ± 4.1 (8)	78 ± 5 (8)	+
NaIse	-36.6 ± 4.2 (8)	96 ± 7 (8)	+
Δ**	$+6.8 \pm 1.5$	$+18 \pm 4.5$	—

* Refers to major ionic constituents of solution on mucosal side. Specific compositional details are given in Table 1. Values are reported as the mean ± S.E. with the total number of preparations shown in parentheses.

** Δ's refer to absolute change, regardless of sign, induced by the indicated treatment for the individual preparations. Changes for a given mucosa were treated as paired differences; the changes were averaged and shown as the means ± S.E. All values for the changes reached the 5% level of significance.

When Cl^- was removed from the serosal bathing solution (replaced with isethionate), the resistance increased and the p.d. usually decreased by a small amount, although the steady-state level of p.d. was somewhat variable. For instance, with isethionate Ringer solution on both sides the mean p.d. and resistance values were (1) for resting preparation: -28.3 ± 8.6 (6) mV and 192 ± 44 (5) $\Omega.cm^2$; and (2) for histamine-stimulated preparations: -18.8 ± 5.8 (10) mV and 135 ± 19 (10).cm^2. In a few of the latter preparations (2 out of 10) the resulting p.d. with isethionate solution on both sides reversed in sign (mucosal surface became positive to the serosal side by 3 and 9 mV). Removal of Cl^- from the serosal bathing medium effected a marked reduction in H^+ secretion to zero or very low values (mean 0.6 ± 0.3 (6) μequiv/cm^2.hr) even in histamine-stimulated preparations. A graphical representation of p.d. and H^+ secretion, when Cl^- was altered in the bathing solutions, is shown in Fig. 2, for a single experiment. It is of interest that addition of histamine to the Cl^--free

mucosa caused a decrease in p.d., in contrast to the increase which was ordinarily observed when Cl^- was present in the serosal solution. In the absence of Cl^-, passage of electrical current from serosal to mucosal solutions ($\approx$ 4·5 μequiv current/cm^2.hr) brought about a slight increment in H^+ secretion ($\approx$ 0·9 μequiv H^+/cm^2.hr), but even in these special conditions of Cl^--free bathing solutions it appears that most of the current must be carried through pathways other than the mechanism of H^+ translocation.

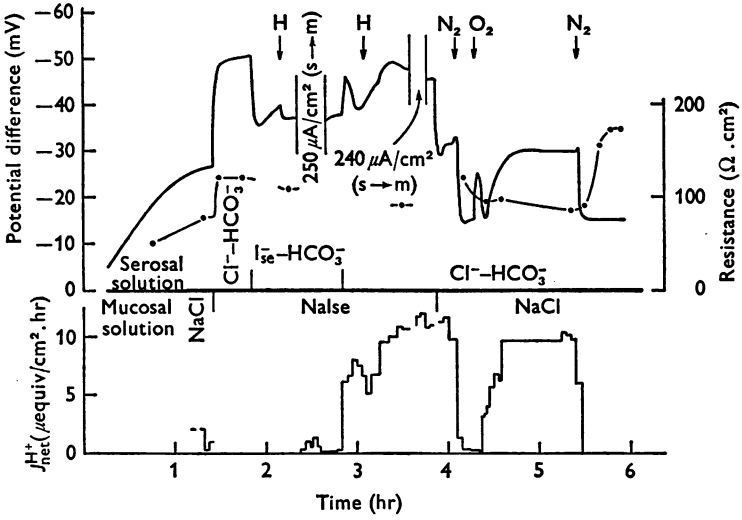

Fig. 2. Electrophysiological and secretory responses of isolated piglet gastric mucosa to Cl^- replacement in both bathing solutions and to deprivation of a supply of O_2. Histamine (H, 6×10^{-5} M to serosal solution) was added at the times indicated. Cl^- was removed from the mucosal and/or serosal solutions by substituting with the appropriate isethionate solution (Ise^--HCO_3^- or NaIse; see Table 1 for compositional details). During the two designated periods a positive current was passed from serosal to mucosal side. Removal of O_2 by replacing the gas phase with 95 % N_2-5 % CO_2 (N_2 arrow) brought about prompt electrical and secretory changes which were reversible if oxygen was reinstituted (O_2 arrow) to the system shortly after anaerobiosis. Upper graph: continuous line, p.d., interrupted line, resistance.

The changes in electrical and secretory parameters which occurred when oxygen was removed or added back to the bathing solutions (Fig. 2) show an important and immediate dependency on oxidative metabolism. After switching the gassing to 95 % N_2 and 5 % CO_2, H^+ secretion was rapidly and reversibly (providing the anaerobic period was not too long) reduced to zero. The p.d. fell during anaerobiosis, but not to zero (average $-13{\cdot}3 \pm 1{\cdot}5$ (10) mV), while the transmucosal resistance increased

two- to threefold (average $187 \pm 16{\cdot}5$ (10) $\Omega.cm^2$). The p.d. which was typically maintained at -10 to -15 mV during anaerobiosis was reduced to zero by the further addition of inhibitors, such as fluoride (10^{-2} M), to the bathing solutions.

Transepithelial ionic fluxes. Unidirectional fluxes of Na^+ and Cl^- from serosal to mucosal solution ($J_{sm}^{Na^+}$, $J_{sm}^{Cl^-}$) and from mucosal to serosal solution ($J_{ms}^{Na^+}$, $J_{ms}^{Cl^-}$) are reported in Table 3. Also given are the measurement of H^+ secretory rate ($J_{net}^{H^+}$, short-circuit current (I_{sc}) and the calculated net Na^+ ($J_{net}^{Na^+}$) and Cl^- ($J_{net}^{Cl^-}$) fluxes. In the basal, resting state on open circuit there was a movement from mucosa to serosa of both Na^+

TABLE 3. Fluxes of Cl^- and Na^+ during open- and short-circuited conditions for resting and histamine-stimulated piglet gastric mucosa

	μequiv/cm².hr			
	Resting		Histamine-stimulated	
	Open circuit	Short-circuit	Open circuit	Short-circuit
$J_{sm}^{Cl^-}$	$+8{\cdot}3 \pm 0{\cdot}4$ (8, *31*)	$+12{\cdot}4 \pm 1{\cdot}3$ (3, *6*)	$+23{\cdot}3 \pm 1{\cdot}2$ (5, *9*)	$+26{\cdot}3 \pm 2{\cdot}5$ (3, *6*)
$J_{ms}^{Cl^-}$	$-9{\cdot}1 \pm 0{\cdot}5$ (6, *21*)	$-8{\cdot}5 \pm 0{\cdot}6$ (3, *6*)	$-15{\cdot}8 \pm 1{\cdot}1$ (4, *9*)	$-13{\cdot}3 \pm 0{\cdot}9$ (4, *9*)
$J_{sm}^{Na^+}$	$-1{\cdot}0 \pm 0{\cdot}05$ (2, *8*)	$-0{\cdot}7 \pm 0{\cdot}04$ (2, *5*)	$-0{\cdot}8 \pm 0{\cdot}1$ (2, *5*)	$-0{\cdot}7 \pm 0{\cdot}1$ (2, *4*)
$J_{ms}^{Na^+}$	$+4{\cdot}6 \pm 0{\cdot}5$ (2, *8*)	$+5{\cdot}4 \pm 0{\cdot}7$ (2, *4*)	$+2{\cdot}8 \pm 0{\cdot}7$ (2, *5*)	$+3{\cdot}4 \pm 1{\cdot}0$ (2, *4*)
$J_{net}^{Cl^+}$	$-0{\cdot}8$	$+3{\cdot}9$	$+7{\cdot}5$	$+13{\cdot}0$
$J_{net}^{Na^+}$	$+3{\cdot}6$	$+4{\cdot}7$	$+2{\cdot}0$	$+2{\cdot}7$
$J_{net}^{H^+}$	$-0{\cdot}4 \pm 0{\cdot}2$ (7, *7*)	$-0{\cdot}6 \pm 0{\cdot}2$ (5, *10*)	$-7{\cdot}9 \pm 0{\cdot}5$ (5, *11*)	$-7{\cdot}8 \pm 0{\cdot}9$ (3,*7*)
Σ^{Cl^-, Na^+, H^+}	$+2{\cdot}4$	$+8{\cdot}0$	$+1{\cdot}6$	$+7{\cdot}9$
I_{sc}	—	$+7{\cdot}2 \pm 0{\cdot}5$ (6, *12*)	—	$+8{\cdot}7 \pm 0{\cdot}3$ (6, *13*)

The sign indicates the direction of charge transfer across the mucosa, being given as positive for an electron flow from serosa to mucosa. J, flux; superscript denotes ion; sm, serosa to mucosa; ms, mucosa to serosa; net, (ms + sm); I_{sc}, short-circuit current. Values given as the mean ± S.E. Numbers in parentheses are the number of preparations and, in italics, the number of separate measurements.

and Cl^-, while a low level of H^+ secretion occurred. It is clear that histamine induced a marked increased in H^+ secretion and Cl^- fluxes. For the latter, $J_{sm}^{Cl^-}$ was increased about threefold while $J_{ms}^{Cl^-}$ was more modestly elevated, thus the stimulation by histamine of $J_{net}^{Cl^-}$ and $J_{net}^{H^+}$ were nearly equivalent on open circuit. On the other hand, $J_{net}^{Na^+}$ was reduced after histamine stimulation. For both the resting and histamine-stimulated preparations the algebraic sum of Na^+, Cl^- and H^+ fluxes was small, but not zero as would be predicted if these were the only mobile ions in the system. Possible reasons to account for this discrepancy could be (*a*) the limited sample size or (*b*) the contribution of an additional ionic species (e.g. K^+ moving from serosa to mucosa down its electrical gradient).

During conditions when transmucosal p.d. was reduced to zero (i.e. short-circuit) it is clear that the net transport of Cl^- occurred in the direction from serosa to mucosa while net Na^+ transport was from mucosa to serosa. These net ion-translocation processes are designated as active transport on the basis of criteria suggested by Ussing & Zerahn (1951). Stimulation of active H^+ transport, $J^{H^+}_{net}$, from serosa to mucosa by addition of histamine effected a large increase in $J^{Cl^-}_{net}$ and a smaller decrement in $J^{Na^+}_{net}$. The short-circuit current is nearly equivalent (within 10%) to the algebraic sum of the net transport of H^+, Cl^- and Na^+ (Table 3), so it is likely that these are the principal species of ionic transport components.

The changes in ion flux which occurred upon application of short-circuit current are instructive (Table 3). Reduction of the p.d. to zero resulted in enhanced $J^{Na^+}_{net}$ and $J^{Cl^-}_{net}$ for both resting and secreting preparations while, as pointed out above, $J^{H^+}_{net}$ was very little altered by passage of current. This occurred by virtue of increased unidirectional fluxes of Cl^- and Na^+ in the direction of the respective active transport process (i.e. $J^{Cl^-}_{sm}$ and $J^{Na^+}_{ms}$), as well as a decrease in the unidirectional 'back-fluxes' ($J^{Cl^-}_{ms}$ and $J^{Na^+}_{sm}$). However, it may be concluded from the relative magnitude of the flux changes that Cl^- carries a much greater fraction (> 60%) of the current passing through the tissue than either Na^+ or H^+; thus Cl^- movement clearly makes the greatest contribution to transmucosal conductance.

Exchange diffusion of Cl^-. Cl^- flux from the serosal to the mucosal side was dependent upon the concentration of Cl^- in the trans, or mucosal, solution. The changes in $J^{Cl^-}_{sm}$ when Cl^- of the mucosal solution was replaced by isethionate are shown in Fig. 3. This trans-dependent Cl^- flux has been shown to be an exchange diffusion component of the total ionic flux (Heinz & Durbin, 1957; Forte, 1969). As previously determined for the frog, we found that the magnitude of Cl^- exchange diffusion in the isolated piglet gastric mucosa was related to secretory activity of the tissue.

The average of $J^{Cl^-}_{sm}$ in the resting mucosa decreased from $6{\cdot}85 \pm 0{\cdot}19$ (2) to $4{\cdot}50 \pm 0{\cdot}14$ (2) μequiv/cm^2.hr when Cl^- of the secretory solution was replaced by isethionate. During maximal histamine stimulation of acid secretion, isethionate substitution caused this $J^{Cl^-}_{sm}$ to decrease from $19{\cdot}0 \pm 1{\cdot}7$ (5) to $11{\cdot}2 \pm 1{\cdot}3$ (5) μequiv/cm^2.hr. In one experiment where the histamine-stimulated epithelium was made anaerobic (N_2 replaced O_2), replacing Cl^- of the mucosal solution with isethionate caused a relatively small decrease in $J^{Cl^-}_{sm}$, i.e. from 5·6 to 2·6 μequiv/cm^2.hr. Thus, during maximal histamine stimulation, the exchange diffusion component of Cl^- was considerably greater than during the more basal state prior to histamine or after secretion was inhibited with anaerobiosis.

Effects of transport inhibitors. Thiocyanate (SCN^-) and related compounds are well-known inhibitors of gastric H^+ secretion, *in vitro* and *in vivo*. The effects of adding SCN^- (5·5 mM) to the serosal bathing solution of the isolated piglet mucosa are shown in Fig. 3. As typically observed in frog (Forte & Davies, 1964; LeFevre *et al.* 1964; Forte, 1968), a pronounced decrease in H^+ secretion and $J_{sm}^{Cl^-}$ occurred, while R_0 was elevated; however, contrary to what has been observed with amphibians, the p.d. decreased after SCN^- treatment. Also shown in Fig. 3 is that the subsequent removal of oxygen from the bathing solutions produced the typical response of a reduction in $J_{net}^{H^+}$, $J_{sm}^{Cl^-}$ and p.d., and a further elevation in tissue resistance.

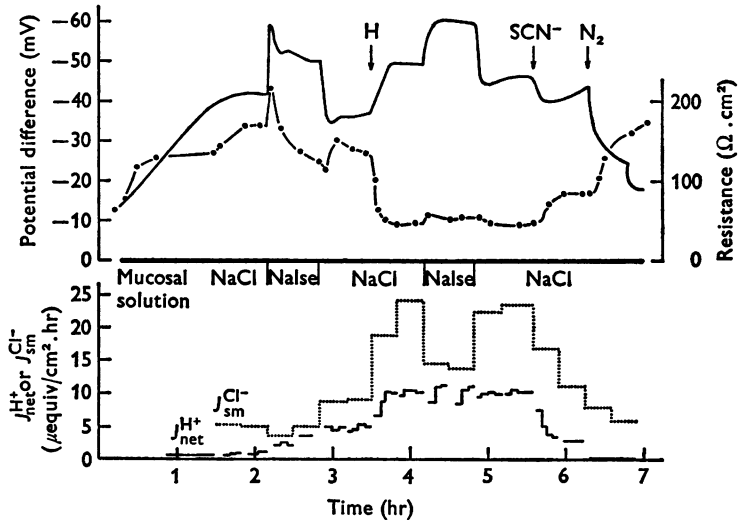

Fig. 3. Electrical parameters and H^+ and Cl^- fluxes before and after histamine (H). Rate of H^+ secretion is denoted by $J_{net}^{H^+}$, Cl^- flux from serosal to mucosal solution is denoted by $J_{sm}^{Cl^-}$. The degree of Cl^- exchange diffusion was assessed by measuring $J_{sm}^{Cl^-}$ with (NaCl) and without (NaIse) Cl^- present in the mucosal solution. At the indicated time (SCN^-) 10 mM NaSCN was added to the serosal solution. Finally, removal of O_2 was accomplished by bubbling the bathing solutions with 95 % N_2–5 % CO_2. Upper graph: continuous line, p.d.; interrupted line, resistance.

We were also interested in the effects inhibitors of Na^+ transport would have on the histamine-stimulated mucosa. Amiloride, a diuretic, blocks Na^+ transport in frog skin (Crabbé & Ehrlich, 1968; Salako & Smith, 1970; Nagel & Dörge, 1970), toad bladder (Bentley, 1968) and renal tubule (Baer, Jones, Spitzer & Russo, 1967); ouabain is a well-known inhibitor of the Na^+ pump.

The electrophysiological effects of adding amiloride (10^{-5} M) to the

mucosal solution of piglet gastric mucosa were directly compared with the effects of replacing all the Na^+ in the same solution with choline. Amiloride rapidly (within 20 sec) decreased the p.d. and increased the resistance. The results of four separate experiments are shown in Table 4. Note that amiloride affected the electrical parameters to nearly the same extent as removal of all the Na^+ from the solution. Neither amiloride nor choline affected H^+ secretion.

TABLE 4. The effects of Na^+ replacement compared with the addition of amiloride to the mucosal solution of histamine-stimulated isolated piglet gastric mucosa

	Normal bathing solutions		Choline mucosal solutions		Normal bathing solutions		10^{-5}M amiloride to mucosal side	
Expt.	p.d.	R_o	p.d.	R_o	p.d.	R_o	p.d.	R_o
1	−23·4	62·9	−20·3	65·5	−21·5	61·6	−19·7	67·3
2	−21·9	48·2	−11·7	61·6	−18·5	57·9	−10·7	70·6
3	−26·1	66·4	−17·9	71·3	−22·6	71·1	−15·6	79·7
4	−27·6	48·0	−27·0	53·1	−28·1	46·7	−28·6	51·8
Mean	−24·7	56·3	−19·2	62·8	−22·6	59·3	−18·6	67·3

Values for p.d. expressed in mV with the serosal side as reference. Resistance, R_o, expressed in $\Omega.cm^2$.

TABLE 5. Effects of 10^{-5} M ouabain on p.d., resistance and H^+ secretion of histamine-stimulated piglet gastric mucosa

	Initial			Final		
Expt.	p.d.	R_o	$J^{H^+}_{net}$	p.d.	R_o	$J^{H^+}_{net}$
1	−14·3	114·4	4·9	+3·4	111·0	2·6
2	−36·9	71·4	1·4	+0·8	38·6	0·6
3	−11·3	64·5	7·0	+3·2	54·0	4·3
4	−22·6	251·0	1·7	+5·0	129·8	1·0
5	−27·4	74·8	10·0	+3·7	47·6	0·0
Mean	−22·5	115·2	5·0	+3·2	76·2	1·7

'Initial' refers to steady values before ouabain. 'Final' refers to values 40–60 min after adding ouabain to serosal solution.

Table 5 shows results of five experiments in which ouabain (10^{-5} M) was added to the serosal solution of the piglet gastric mucosa. The onset of the observed change occurred within 3 min and usually achieved a new steady-state value by 30 min after adding ouabain. Specifically, p.d. always decreased and became positive (inverting after 15–30 min) and the H^+ secretion decreased, but usually not to zero. Contrary to what was observed for other modes of inhibition, transmucosal resistance was decreased by ouabain.

DISCUSSION

The isolated piglet gastric mucosa actively transports H^+, Cl^- and Na^+; moreover, these processes are closely associated with the electrical activity of the tissue. In general, the present findings are similar to those reported for frog gastric mucosa and also for other *in vitro* mammalian gastric preparations, with the notable exception that piglet gastric mucosa is quite responsive to addition of histamine.

Cl^- transport. Absolute values of Cl^- flux were clearly higher in piglet than in frog, but for both preparations proportionate increases in active Cl^- transport and exchange diffusion were associated with stimulation by histamine (cf. Forte, 1969). Such observations have been interpreted in terms of an increase in the number of available Cl^- transport and exchange sites which accompanies active HCl secretion. An increase in available apical membrane surface area is also known to occur in stimulated oxyntic cells of various species including piglet (Sedar, 1965; Kasbekar, Forte & Forte, 1968; Helander *et al.* 1972; Machen & Forte, 1973). Furthermore, the large increase in transmucosal ionic conductance associated with HCl secretory activity would also be a consistent result of an increase in surface area of some limiting membrane.

Active Na^+ transport. The piglet mucosa actively transports Na^+ from lumen to blood, and this function is present in both resting and secreting tissue. However, upon stimulation of HCl secretion by histamine a significant reduction ($P < 0{\cdot}05$) in $J_{ms}^{Na^+}$ occurred while $J_{sm}^{Na^+}$ remained essentially unchanged ($P > 0{\cdot}1$). Thus net Na^+ transport measured during short-circuit conditions was markedly reduced (by about 42 %) during maximal H^+ secretion. Considered along with the fact that unidirectional Na^+ fluxes were only 30–50 % of the net H^+ transport after histamine stimulation, these results clearly invalidate any proposed mechanism whereby a Na^+/H^+ exchange would be the fundamental source of gastric H^+ (Hirschowitz, 1961), as has been suggested for some other epithelia (e.g. Pitts, 1968; Schulz, 1972; Rector, Fordtran & Turnberg, 1972). Kitahara *et al.* (1969) noted that $J_{ms}^{Na^+}$ was reduced when they experimentally lowered the pH of the mucosal bathing solutions of several mammalian *in vitro* stomach preparations. These authors proposed that H^+ and Na^+ might be competing for the same cationic permeation sites at the luminal border. Perhaps a similar situation obtains in the case of the piglet gastric mucosa stimulated by histamine. That is to say, the increased H^+ secretory rate might provide a local concentration of protons sufficient to produce the observed reduction in $J_{ms}^{Na^+}$. Bulk flow associated with HCl secretion in the present case might also impede the access of isotopic Na^+ to permeation sites.

The changes in electrical parameters when Na^+ in the mucosal solution was replaced by choline were similar to those which occurred when amiloride was added to the mucosal surface (cf. Table 4). Both procedures had about the same effects on decreasing current. The site of action for amiloride appears to be at specific Na^+ entry ports in various epithelial systems (Crabbé & Ehrlich, 1968; Bentley, 1968; Salako & Smith, 1970). The observed decrease in p.d. and increase in resistance when this diuretic reagent was added to piglet gastric mucosa is also consistent with a restriction of Na^+ permeation from mucosal solution to pump sites within gastric epithelial cells.

Although active Na^+ transport from mucosa to serosa has been demonstrated here and in other isolated mammalian gastric preparations (Cummins & Vaughan, 1965; Kitahara *et al.* 1969), there has been some question as to the operation or function of this process *in vivo*. It has been suggested that the Na^+ transport observed for *in vitro* mammalian gastric mucosa might be the artifactual result of a hypoxic epithelial preparation. In reference to this point we have noted that within 20 min of reducing P_{O_2}, either to 140 torr or complete anaerobiosis, H^+ secretion by isolated piglet gastric mucosa was clearly inhibited but Na^+ transport function remained relatively intact; the latter was judged by the changes in p.d. and I_{sc} after amiloride treatment or choline replacement on the mucosal side. Thus, as would appear for frog (Flemström, 1971), H^+ and Cl^- transport by piglet gastric mucosa is more sensitive to hypoxia than is Na^+ transport.

An active Na^+ pump from epithelial cell interior to serosal side appears to be an important mechanism for ionic homoeostasis in developing (Wright, 1962; Forte *et al.* 1969) and adult (Davenport, 1962; Kitahara *et al.* 1969) gastric mucosa. The restricted transepithelial Na^+ transport in stomach compared with an absorptive epithelium like the gall-bladder or intestine might suggest a more local regulatory function for the Na^+ pump, such as maintenance of the ionic milieu of gastric epithelial cells. Davenport (1962) showed that while ouabain, the classic inhibitor of the Na^+ pump, reduced H^+ secretion by frog gastric mucosa there was a concomitant rise in tissue Na^+ and fall in K^+. Furthermore, the inhibitory effects of ouabain were prevented by elevating $[K^+]$ in the serosal bathing fluid. The requirement for K^+ in HCl secretion is well known, and it is possible that the restriction of this cation to a critical enzymic site is the mode by which ouabain operates. The complete reversal of the transepithelial p.d. after ouabain treatment of the piglet gastric mucosa represents at least one apparent point of difference with isolated amphibian preparations. Several possibilities may be proposed for the observed p.d. reversal within the framework of Na^+ pump inhibition. These

include the direct abolition of an electrogenic Na^+ pump or the indirect diminution of an ion gradient e.m.f. (e.g. K^+) at the serosal surface. In addition, disturbance of the ionic milieu could in turn alter other potential generating systems.

Ion substitution. When both sides of piglet gastric mucosa are bathed with Ringer solution, ion gradients of Na^+ and Cl^- at the mucosal interface are likely to contribute to the total trans-tissue p.d. For frog gastric mucosa the very low permeability of the mucosal surface to Na^+ precludes a significant contribution of this cation to the p.d. Suggestive evidence has been presented here that the apical plasma membrane of the piglet oxyntic cell is not the principal site for Na^+ permeation and access to the pump. In contrast to other cell types, very clear increases in oxyntic cell apical membrane surface area are associated with H^+ secretory activity induced by histamine for the piglet (Machen & Forte, 1973) as well as other species (Helander *et al.* 1972). Yet changes in p.d. and resistance after Na^+ removal from the mucosal solution were virtually identical for resting or secreting piglet preparations (cf. Table 2). Thus it is more likely that the Na^+-responsive membrane was the apical surface of another epithelial cell type, such as the mucous cells or the mammalian chief cell.

A similar set of arguments may be developed for passive Cl^- permeation at the oxyntic cell apical surface, since p.d. and resistance changes occurring after mucosal replacement of Cl^- were more attenuated in actively secreting preparations than in resting ones. On the other hand, the large increases in Cl^- exchange diffusion and active Cl^- transport are temporally correlated with the increased apical surface of oxyntic cells (Machen & Forte, 1973), thus suggesting this membranous site for these transport activities.

Removal of Cl^- from the serosal solution of piglet mucosa caused changes in p.d. and resistance which were similar to those observed in frog (Forte, Adams & Davies, 1963) but not nearly so pronounced. It appears that Cl^- gradients and conductance at the serosal interface contribute relatively less to the electrical characteristics of the porcine preparation than of the frog.

In addition, the H^+ secretory mechanism of piglet gastric mucosa seems to be very dependent on the presence of Cl^- in the serosal solution. When serosal Cl^- was replaced by isethionate, H^+ secretion was abolished or reduced to very low rates. This dependency of the H^+ secretory mechanism upon the presence of serosal Cl^- is of some interest. It is unlikely to be due to a more restricted permeability of the piglet mucosa to isethionate since the relative resistance change after Cl^- removal was even less than typically observed in frog. Perhaps the H^+ secretory mechanism, especially at the more brisk rates observed here in the

mammal, is more directly dependent upon the presence of Cl^- or cannot be so easily 'uncoupled' from Cl^- transport. For instance, toward the latter possibility a very tight, almost obligatory stoicheiometry between H^+ and Cl^- transport occurs in species such as marine elasmobranchs (Hogben, Brandes & Stevens, 1972) or even in amphibia under specially treated conditions (Solberg & Forte, 1971*a*). It is significant that even with the very large changes in unidirectional fluxes both H^+ and Cl^- transport by short-circuited gastric mucosa were stimulated to nearly the same extent by histamine (cf. Table 3: $\Delta J_{net}^{H^+}$ is about 80% of $\Delta J_{net}^{Cl^-}$). This adds a measure of support to the notion that some degree of coupling between H^+ and Cl^- occurs, but without fixed stoicheiometry, through a common site mechanism in this preparation. Moreover, the slight excess of $\Delta J_{net}^{Cl^-}$ over $\Delta J_{net}^{H^+}$ after histamine stimulation is consistent with the net elevated I_{sc} and p.d. in the piglet, in contrast to the typical decrease in p.d. observed in *in situ* dog or *in vitro* frog preparations (Rehm, 1953; Crane, Davies & Longmuir, 1948).

The role of K^+ in contributing to interfacial gastric e.m.f.s has been extensively studied in amphibian preparations (Harris & Edelman, 1964; Spangler & Rehm, 1968), but we have not as yet investigated the role of this important cation in the piglet mucosa.

Long time-constant transient. When an external pulse of current (ΔI) is passed through the isolated piglet gastric mucosa there is a rapid (0·5–1·0 sec) change in transmembrane potential ($\Delta p.d._0$), followed by a slower change in potential which eventually achieves a steady value within 1 to 2 min ($\Delta p.d._\infty$). Such long time-constant transients, beyond the feasible limit for membrane dielectric capacitance, have been noted in several epithelial tissues (Janacek, 1963; Rehm *et al.* 1973), but are especially prominent in gastric mucosa (Noyes & Rehm, 1971). One plausible explanation holds that the long time-constant transient is the result of a polarization of e.m.f.s at the various membrane interfaces of the epithelial cells (Rehm, 1967). However, the mechanistic basis and cellular loci for the polarization phenomena are uncertain (Kidder & Rehm, 1970; Barry & Hope, 1969; Forte, 1971; Noyes & Rehm, 1971). The true electrical resistance (R_0) or conductance (G_0) of the system must be obtained from the nearly instantaneous (after capacitative discharge) values, thus

$$G_0 = 1/R_0 = \Delta I/\Delta p.d._0.$$

As long as there is a significant long time-constant transient in the tissue, G_0 will be greater than the so-called steady-state conductance given by

$$G_\infty = \Delta I/\Delta p.d._\infty.$$

Values for membrane potential, resistance, short-circuit current and

calculations of G_0 and G_∞ are summarized in Table 6 for resting and histamine-stimulated piglet gastric mucosae. Stimulation of secretion is associated with an increase in G_0/G_∞. Twenty to 30 min after switching the gas phase to N_2, secretory rates for the piglet preparation were clearly zero and the electrical transient was virtually non-existent. For these anoxic tissues a pulse of current produced a nearly square voltage response, thus as shown in Table 6 G_0/G_∞ tends to approach unity.

TABLE 6. Electrical parameters and long time-constant electrical transient of isolated piglet gastric mucosa during various states of secretory activity

	Resting mucosae	Histamine-stimulated	Anaerobic
p.d. (nV)	$-32{\cdot}4 \pm 2{\cdot}5$ (6)	$-34{\cdot}9 \pm 2{\cdot}6$ (6)	$-13{\cdot}8 \pm 1{\cdot}6$ (6)
R_0 (Ω.cm^2)	113 ± 11 (5)	74 ± 7 (6)	197 ± 20 (6)
I_{sc} (μA/cm^2)	199 ± 20 (6)	238 ± 11 (6)	76 ± 5 (6)
G_0 (mmho/cm^2)	$9{\cdot}2 \pm 0{\cdot}9$ (5)	$13{\cdot}9 \pm 1{\cdot}2$ (5)	$5{\cdot}4 \pm 0{\cdot}6$ (6)
G_∞ (mmho/cm^2)	$6{\cdot}1 \pm 0{\cdot}3$ (6)	$6{\cdot}9 \pm 0{\cdot}5$ (6)	$5{\cdot}3 \pm 0{\cdot}6$ (6)
G_0/G_∞	1·5	2·0	1·02
$J_{sm}^{H^+}$ (μequiv/cm^2.hr)	$0{\cdot}4 \pm 0{\cdot}3$ (6)	$7{\cdot}9 \pm 1{\cdot}2$ (5)	0·0 (8)

Numbers in parentheses represent the number of preparations from which information was available to compute the mean ± S.E. See text for definition of the parameters.

In these experiments when conditions for H^+ secretion were widely variant (e.g. resting *vs.* histamine-stimulated *vs.* anaerobic) there were concomitant gross changes in total issue conductance as well as in the magnitude of the long time-constant transient. It may be recalled that marked ultrastructural changes in surface membranes occur during various states of secretion (see above). Thus the relative contribution to tissue resistance of the limiting serosal and mucosal membranes would be expected to change with secretion, and the model of differential permeabilities for the generation of a polarization of e.m.f.s (Kidder & Rehm, 1970) might still apply.

Ionic conductances. Since there are relatively slow time-dependent changes in gastric membrane potential during a pulse of current, it is not feasible to correlate ionic fluxes, measured over periods of 10–20 min, with the conductance measured 'instantaneously' (i.e. within 1 sec). We have therefore evaluated the contribution of specific ions to total membrane conductance by calculating from the steady-state measurements the relative changes in ion flux associated with current alterations. The short-circuit current and the change in steady-state ion flux values between open and short-circuited conditions were compared. On this basis and from the data of Table 3 it appears that Cl^- accounts for about 60–65% of the steady-state ion current flowing through piglet gastric

mucosa, and that this is approximately the same for resting or secreting preparations. Only about 15% of the current can be accounted for by change in $J_{net}^{Na^+}$ in resting mucosae and even less ($\approx 8\%$) in secreting mucosae. Current-induced changes in $J_{net}^{H^+}$ were relatively small indicating that H^+, OH^- or HCO_3^- do not carry much of the current (1–2% of total). This apparent low H^+ conductivity is energetically useful for a tissue which must secrete volumes of acid against large H^+ gradients. Approximately 20% for resting and 30% for histamine-stimulated tissues of the steady state in flow, or conductance, must be accounted for by ion species other than Na^+ or Cl^-. The most likely possibility for the remaining conductive species is K^+.

We thank Professor R. D. Keynes, F.R.S., for helpful discussions, support and hospitality during our stay at the Agricultural Research Council. T. E. Machen was the recipient of a Bank of America–Gianinni Foundation postdoctoral fellowship. This work was supported in part by USPHS Grant no. AM 10141.

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