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Cl⁻ transport by gastric mucosa: cellular Cl⁻ activity and membrane permeability

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The mechanism of Cl⁻ secretion in the isolated, resting (i.e. cimetidine-treated) gastric mucosa of *Necturus* has been investigated with radioisotopic and electrophysiological techniques. Measurement of transepithelial ³⁶Cl⁻ fluxes (mucosal to serosal (M → S), $J_{ms}^{Cl^-}$; S → M, $J_{sm}^{Cl^-}$) during control conditions show that at open circuit, when the transepithelial potential difference $\psi_{ms} = 20$ mV (S ground), $J_{ms}^{Cl^-} = J_{sm}^{Cl^-}$, i.e. $J_{net}^{Cl^-} = 0$, but during short-circuit current conditions $J_{net}^{Cl^-} = I_{sc} = 2$ µequiv cm⁻² h. Experiments with low [Cl⁻] solutions indicate that Cl⁻ exchange diffusion does not contribute significantly to either $J_{ms}^{Cl^-}$ or $J_{sm}^{Cl^-}$. Double-barrelled, Cl⁻-selective microelectrodes showed that in open circuit, the cellular (C) chemical potential for Cl⁻, $\psi_c^{Cl^-} = 31$ mV (apparent [Cl⁻] = 29 mM), the electrical potential across the M membrane, $\psi_m = -34$ mV (mucosa ground) while that across the S membrane, $\psi_s = -52$ mV (serosa ground). During short-circuit current conditions, $\psi_m = \psi_s = -49$ mV and [Cl⁻]$_c$ = 30 mM. The permeability of the M membrane to Cl⁻ ($P_m^{Cl^-}$) was calculated both from the tracer experiments and the electrode measurements by using the constant-field equation. Short-term (45 s) uptake of ³⁶Cl⁻ at [Cl⁻]$_m$ = 96 mM during short circuit conditions gave $P_m^{Cl^-} = 2.6 \times 10^{-5}$ cm s⁻¹. Measurement of [Cl⁻]$_c$ by means of the electrodes when [Cl⁻]$_m$ was changed from 96 to 2 mM or from 2 to 96 mM gave $P_m^{Cl^-} = 2.9–5.7 \times 10^{-5}$ cm s⁻¹. Our results indicate that during open circuit conditions Cl⁻ is accumulated across the S membrane into gastric cells in an energy-requiring step, but since $J_{net}^{Cl^-} = 0$, Cl⁻ must leak back into the S solution at a rate equal to the entry rate. When the tissue is short-circuited, Cl⁻ secretion occurs ($J_{net}^{Cl^-} = I_{sc}$) owing to the same energy-requiring accumulation of Cl⁻ by the cells and a passive (apparently electrodiffusive) movement across the mucosal membrane.

Introduction

It is well known that Cl⁻ ions are essential for maintenance of maximal H⁺ secretory rates in the stimulated gastric mucosa. In addition to this Cl⁻ that accompanies H⁺ (so-called 'acidic Cl⁻' transport), this tissue can also secrete Cl⁻ in excess of H⁺, thereby giving rise to the lumen-negative potentials normally observed and to the fact that in the short-circuited state the (mucosally directed) negative short-circuit current is equal to the algebraic sum of the net Cl⁻ secretion minus net H⁺ secretion, i.e. $I_{sc} = J_{net}^{Cl^-} - J^{H^+}$ (Hogben 1955). It has also been found that the resting tissue (i.e. $J^{H^+} = 0$) actively transports Cl⁻ at rates equal to I_{sc} (Machen & McLennan 1980), and that this transport appears in foetal rabbit (Wright 1962) and meta-morphosing frog gastric mucosa (Forte *et al.* 1969) at times before oxyntic (H⁺-secreting) cells have even developed within the epithelium. The implication is, then, that this active Cl⁻ trans-port is at least partially generated by surface epithelial cells.

It has been proposed recently that this 'non-acidic Cl⁻' secretion is generated by a two-step, pump–leak process in which Cl⁻ is accumulated 'uphill' (i.e. energy-requiring) into gastric

cells by being coupled to the 'downhill' entry of Na^+ across the serosal membrane (Machen & McLennan 1980; Machen et al. 1980). This entry process may be neutral (McLennan et al. 1981), though the coupling between Na^+ and Cl^- (and, possibly, other ions like HCO_3^- (see Manning & Machen 1978, 1982)) is still very much an open question. The second step, the movement of Cl^- from cell to gastric lumen, may be a passive, conductive process whereby Cl^- is driven across the mucosal membrane 'up' a concentration gradient of some 30–70 mV (Spenney et al. 1974; Schettino & Curci 1980), lumen positive with respect to the cell.

TABLE 1. LIST OF SYMBOLS

symbol	units	definition
ψ_{ms}	mV	transepithelial electric potential, serosal solution ground
ψ_m or ψ_s	mV	cellular electrical potential across the mucosal (m) or serosal (s) membrane; potentials are referenced to m or s solution depending on circumstance (see text)
$\psi_m^{Cl^-}$ or $\psi_s^{Cl^-}$	mV	difference of chemical potential of Cl^- across the mucosal or serosal membrane. Equivalent to the equilibrium potential for Cl^-.
V^{Cl^-}	mV	potential recorded by the Cl^--sensitive electrode
$[Cl^-]$	mM	concentration of Cl^-; subscripts denote mucosal (m), serosal (s) or cellular (c) compartments
α	—	voltage divider ratio: ratio of resistances of mucosal and serosal membranes
R_t	$\Omega\ cm^2$	transepithelial resistance
J^{Cl^-}	$\mu equiv\ cm^{-2}\ h^{-1}$	flux of Cl^-; subscripts denote fluxes from mucosal solution to cell (mc), from mucosa to serosa (ms) or from serosa to mucosa (sm): $J_{net}^{Cl^-} = J_{sm}^{Cl^-} - J_{ms}^{Cl^-}$
I_{sc}	$\mu equiv\ cm^{-2}\ h^{-1}$	short-circuit current
$P_m^{Cl^-}$	$cm\ s^{-1}$	permeability of the mucosal membrane to Cl^-
$P_{ms}^{Cl^-}$	$cm\ s^{-1}$	permeability of the whole tissue to Cl^-, calculated from transepithelial flux measurements

The following data all support such a model.

1. Chemical and radioisotopic measurements suggest that $[Cl^-]$ in gastric cells, $[Cl^-]_c = 30$–40 mM (Harris & Edelman 1964; Davenport & Alzamora 1962; D. Culp and J. G. Forte, unpublished observations), and these values are severalfold larger than would be predicted for a passive distribution of this ion across a membrane with a potential of -50 mV (Spenney et al. 1974) to -75 mV (Schettino & Curci 1980), cell interior negative with respect to the serosal solution. The implication here is that Cl^- is out of equilibrium across this serosal membrane, and there must be some energy input to generate this accumulation of Cl^-.

2. Cl^- transport is critically dependent on the $[Na^+]$ of the serosal, but not the mucosal, solution (Machen & McLennan 1980). This dependence on Na^+ was rapid and reversible and not due to non-specific inhibition of metabolic processes. The simplest explanation was that Na^+ was directly 'activating' the Cl^- transport system.

3. Ouabain in the serosal solution reduces net Cl^- transport to zero (Forte & Machen 1975).

4. The effects of high $[K^+]$ (more than 10 mM) in the mucosal (Hogben 1968) or serosal solution, or both (Hogben 1968; Machen & McLennan 1980) and of 1 mM Ba^{2+} (McLennan et al. 1980) have also been shown to be most easily interpreted in terms of this model. Thus, high $[K^+]$ or $[Ba^{2+}] > 10$ mM tend to depolarize the cellular membrane potential, thereby reducing the driving force for Cl^- exit from the cell to the mucosal solution.

In the work described in this paper we have used electrophysiological and radioisotopic techniques to test directly two aspects of this model. With double-barrelled Cl^--selective micro-

electrodes we have measured intracellular electrical potential and Cl⁻ activity of surface epithelial cells to determine whether Cl⁻ was indeed 'actively' accumulated within cells across the serosal membrane. We have also used these techniques as well as short-term (45 s) uptake of ^{36}Cl⁻ from the mucosal solution into gastric cells to measure the Cl⁻ permeability of the mucosal membrane and thereby assess the mechanism by which Cl⁻ permeates this membrane. The symbols used throughout the text are listed in table 1.

A portion of this work (Machen & Zeuthen 1980; Machen *et al.* 1980) has been presented previously.

METHODS AND MATERIALS

Dissection, chambers and solutions

Stomachs were removed from double-pithed mudpuppies, *Necturus maculosus*, and gastric mucosas were stripped from underlying muscle layers by blunt dissection. Tissues were then mounted in chambers for measurements of transepithelial flux, electrophysiology, or ion uptake. Edge damage was minimized by using silicone grease around the chamber's apertures. Both sides of the tissue were bathed with a Ringer solution containing 87 mM NaCl, 18 mM NaHCO₃, 4 mM KCl, 1 mM KH₂PO₄, 1 mM MgCl₂ and 1 mM CaCl₂ (pH 7.4 when gassed with 95 % $O_2 + 5 \% CO_2$). In some experiments [Cl⁻] was altered by replacement with gluconate. All tissues were rendered resting at the start of the experiment by washing the serosal surface several times with Ringer's and then adding 10^{-5} M cimetidine (a gift from Smith, Kline and French) to the serosal solution.

Transepithelial flux and electrophysiological experiments

Potential difference (ψ_{ms}, serosa ground) resistance (R_t) and short-circuit current (I_{sc}) were measured by using 2 M KCl–agar bridges, calomel electrodes, a high-impedance electrometer, a voltage-clamping device and strip-chart recorders. The area of the chamber apertures was 1.13 cm², and circulation of the bathing fluids was accomplished by using a standard gas lift. Unidirectional fluxes of ^{36}Cl⁻ were measured during both open-circuit and short-circuit current conditions by methods described previously (Forte & Machen 1975).

Tissue uptake of ^{36}Cl⁻

Unidirectional uptake of Cl⁻ across the mucosal membrane into the cells ($J_{mc}^{Cl^-}$) was measured with techniques similar to those of Schultz *et al.* (1967). Briefly, the gastric mucosa was isolated and then placed in a beaker containing oxygenated Ringer solution with 10^{-4} M cimetidine to ensure that H⁺ secretion had been abolished. Then the tissue was cut into pieces and mounted in modified Ussing chambers (aperture = 0.62 cm²), which allowed easy access from the mucosal side. The serosal side of the tissue was perfused with a gas lift system while the mucosal side was bubbled with the use of syringe needles. During a 30 min preincubation period in the chamber, the tissue was bathed on the serosal side with standard Cl⁻–HCO₃⁻ Ringer solution and on the mucosal side with either Ringer solution or with one in which [Cl⁻] was reduced to either 48 or 10 mM by replacement with gluconate. The tissues were continuously short-circuited during the pre-incubation period.

Uptakes of ^{36}Cl⁻ were measured in the short-circuited state with [Cl⁻] = 96, 48 or 10 mM by using [³H]polyethylene glycol (PEG), $M_r = 900$, for correction of extracellular space as described by others (Schultz *et al.* 1967). At the end of the pre-incubation period, the mucosal

solution was removed; the tissue was then gently blotted to remove excess 'cold' solution, and the test solution ($^{36}Cl^-$ activity $= 0.5 \,\mu Ci \, ml^{-1}$; [$^3H$]PEG activity $= 1 \,\mu Ci \, ml^{-1}$) was rapidly perfused onto the mucosal surface. After 45 s the radioactive solution was rapidly removed and saved for counting to obtain the $^{36}Cl^-$ and ^3H specific activities for each individual sample. This 45 s time was chosen because preliminary measurements showed that uptake reached a steady state at 60 s. Durbin (1977) had previously shown for bullfrog gastric mucosa that Cl^- movement across the whole epithelium reached a steady state within 2 min. The tissue was washed once with ice-cold isotonic mannitol solution and then punched out and placed directly into a test tube containing 0.1 M HNO_3. After an 8 h shaking the tissues were dissolved, and a 1 ml sample was taken for counting by liquid scintillation. Extracellular correction for cellular $^{36}Cl^-$ uptake amounted to about 5% of total uptake. All results have been converted into units of microequivalents per square centimetre per hour for comparison with the transepithelial flux and electrophysiological experiments. In four control experiments with the use of [^3H]PEG and [^{14}C]mannitol as the test molecules, it was found that mannitol and PEG gave identical values for extracellular space correction after 45 s equilibrium. We used [^3H]PEG for all experiments reported here.

Microelectrode methods: chamber and tissue

The tissues were stripped of their underlying muscles and mounted in a modified Ussing chamber with an exposed area of $0.6 \, mm^2$. The chamber exerted a minimum of pressure on the tissue, and the insulation was ensured by silicone grease. This small area was advantageous because peristaltic movements were reduced. The tissue was bathed on both sides by the solutions described above. The ψ_{ms} was recorded by means of two Ag/AgCl electrodes connected to the bath via two agar bridges containing 2 M or saturated KCl. The tip of the serosal electrode was placed near the serosa, which includes connective tissue and some smooth muscle; the tip of the mucosal electrode was positioned 100–200 μm from the mucosal surface. Current (not more than 70 $\mu A \, cm^{-2}$) could be passed transmurally via two ring-shaped Ag/AgCl wires. The rate of fluid perfusion at the mucosal surface was fast: the 0.6 ml was renewed every 2 s; the 3 ml of serosal solution was not perfused during the course of the experiments.

We attempted to assess damage (edge damage, etc.) done to the tissue by stripping off the muscle layer and mounting it in the small Ussing chamber in the following manner. The intact, isolated stomach was first tied as a sac with one reference electrode inside the lumen and another outside in the beaker into which the sac was immersed. The ψ_{ms} was between 17 and 23% (four tissues) lower in the chamber compared with the sac.

As seen in a dissection microscope, roughly 90% of the mucosal surface consisted of surface epithelium and less than 10% of openings of gastric pits. This, together with the finding of a narrow distribution of intracellularly measured potentials (see below and figure 2), indicates that only surface cells were penetrated with the microelectrodes.

Microelectrodes

Double-barrelled, Cl^--sensitive microelectrodes were produced as described by Zeuthen et al. (1974) and Zeuthen (1980). The reference barrel was pulled from a glass tube with an outer diameter of 1 mm and an inner diameter of 0.5 mm. The tube contained an internal fibre for easy filling. The ion-selective barrel was pulled from a glass with an outer diameter of 1.8 mm and an inner diameter of 1.2 mm. Electrodes pulled from these glasses had an impedance of the small barrel of 40–100 MΩ and of the large barrel of 20–60 MΩ when filled with 2 M KCl. This

corresponds to tip diameters of 0.1–0.3 μm. The total tip diameter was less than 0.5 μm. The inside of the tip of the large barrel was rendered hydrophobic by exposure to the vapour of dimethyldichlorosilane. At 70 % humidity 5 s exposure was optimal; at lower humidities, longer exposures were used. The ion exchanger was Corning code 477315. The reference barrel was usually filled with 2 M KCl and in some cases with saturated Na_2SO_4 or Na^+-isethionate. The electrodes had a sensitivity of 41.5 mV for a tenfold change in Cl⁻ activity at [Cl⁻] > 20 mM and a selectivity of between 1:4 and 1:15 for Cl⁻ over HCO_3^- (figure 1).
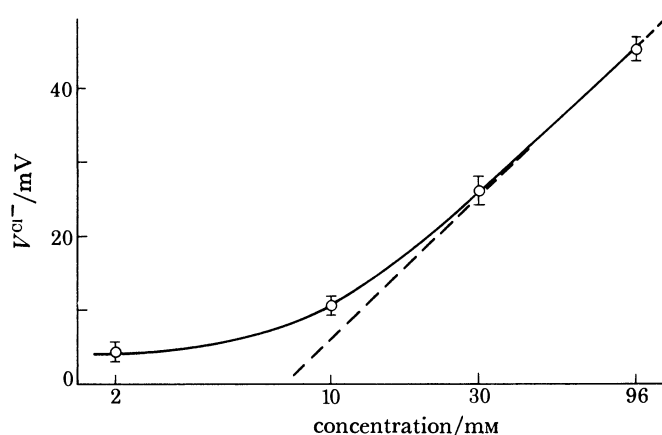

FIGURE 1. The calibration of five double-barrelled Cl⁻-sensitive microelectrodes given by the electrode potential V^{Cl^-}. The [Cl⁻] (abscissa) was varied by replacing Cl⁻ with gluconate and keeping the concentration of HCO_3^- constant at 18 mM. The vertical bars indicate the standard error.
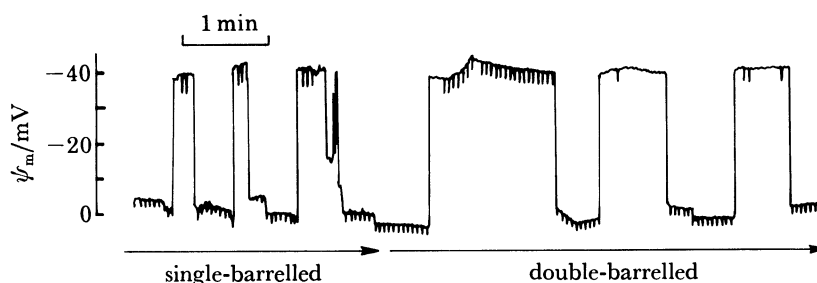

FIGURE 2. A tracing of ψ_m recordings in one tissue by one single-barrelled (80 MΩ) and one double-barrelled electrode with an impedance of the reference barrel of 65 MΩ. The impedance of the electrode was monitored every 4 s by passing a current pulse through the electrode.

Quality of recordings

When using double-barrelled microelectrodes, one must be concerned with leaks around the electrode tips. We therefore performed control experiments in which we compared the intracellular electrical potential (ψ_m) and voltage divider ratio (α) measurements with single-barrelled and double-barrelled electrodes.

The recordings of ψ_m were stable and uniform (figure 2), and there was no difference in this potential whether the double-barrelled or single-barrelled electrodes were employed. In one tissue three double-barrelled electrodes (with 70, 80 and 80 MΩ impedances of the reference barrel) recorded in 13 cells a potential of -36 ± 4.9 mV and $\alpha = 5.28 \pm 1.6$, whereas two single-barrelled electrodes (70 and 40 MΩ) recorded in 13 cells a potential of -37.9 ± 5.8 mV and

$\alpha = 4.43 \pm 0.3$. It is therefore clear that single and double-barrelled electrodes were measuringly nearly identical ψ_m and α. We conclude that double-barrelled electrodes induce no more damage than single-barrelled electrodes.

One recurrent problem was that the tip of the electrode got plugged with mucus. This was observed as a marked (tenfold) increase in the impedance of the reference barrel. Thus, an additional criterion for a valid recording was that the electrode impedance did not increase by more than threefold when the tip was positioned intracellularly, and it returned to baseline during withdrawal (see figure 2). Other criteria for valid recordings are listed elsewhere (Zeuthen 1980).

The impedance of the ion-selective barrel was about 10^{10}–10^{11} Ω, and this barrel had a time constant of between 0.5 and 1.0 s. The electrodes were calibrated in solutions similar to that in which the tissue was bathed but with various amounts of the Cl^- replaced by gluconate. The apparent intracellular $[Cl^-]$ was found to be around 29 mM (see Results section), assuming an intracellular activity coefficient similar to that in free solution, 0.76. The electrode was therefore calibrated both before and after a successful cellular penetration in solutions with Cl^- concentrations around 30 mM. This procedure minimized errors due to extrapolation between different calibration points.

RESULTS

Tracer measurements

Unidirectional Cl^- fluxes: open-circuit and short-circuit current conditions

Results from experiments in which paired halves of the same tissues were used for flux measurements during short-circuit conditions are shown in table 2; $J_{net}^{Cl^-} = J_{sm}^{Cl^-} - J_{ms}^{Cl^-}$ was calculated for each individual experiment and compared with the I_{sc}. Obviously $I_{sc} = J_{net}^{Cl^-}$ in these resting tissues, as has been previously found for frog gastric mucosa (Machen & McLennan 1980).

Because separate tissues were used for unidirectional flux measurements at open circuit, $J_{net}^{Cl^-}$ was calculated by subtracting the average $J_{ms}^{Cl^-}$ from $J_{sm}^{Cl^-}$. For these six experiments the average $\psi_{ms} = -20.5 \pm 2.0$ mV.

TABLE 2. UNIDIRECTIONAL ^{36}Cl FLUXES DURING OPEN-CIRCUIT AND SHORT-CIRCUIT CONDITIONS

(All values are in microequivalents per square centimetre per hour. Average + s.e.m.; numbers in parentheses are the numbers of experiments.)

flux	open circuit	short circuit
$J_{sm}^{Cl^-}$	2.42 ± 0.61 (3)	3.31 ± 1.16 (4)
$J_{ms}^{Cl^-}$	2.72 ± 1.84 (3)	1.19 ± 0.31 (4)
$J_{net}^{Cl^-}$	-0.30†	2.12 ± 0.50 (4)
I_{sc}	—	2.12 ± 0.53 (4)

† $J_{net}^{Cl^-}$ was calculated from the difference between the average unidirectional fluxes.

Estimation of epithelial chloride permeability

In the actively secreting frog (Forte 1969) and piglet (Forte & Machen 1975) gastric mucosa, a substantial portion of the unidirectional fluxes can be attributed to exchange diffusion. We have used measurements of $J_{ms}^{Cl^-}$ to obtain estimates of the magnitude of such Cl^- exchange diffusion

for the resting *Necturus* gastric mucosa. The protocol was first to measure $J_{ms}^{Cl^-}$ during control, short-circuit conditions when [Cl⁻] in both solutions was 96 mM. The the solutions were changed to 3.5 mM Cl⁻ on the mucosal side and Cl⁻-free on the serosal side, and the measurements were repeated. During these latter conditions ψ_{ms} and I_{sc} were approximately zero (within 1 mV). Cl⁻ permeability, $P_{ms}^{Cl^-}$, was then calculated from the ratio $P_{ms}^{Cl^-} = J_{ms}^{Cl^-}/[Cl^-]_m$. The reasoning here was that if exchange diffusion was contributing a significant fraction to $J_{ms}^{Cl^-}$, then $P_{ms}^{Cl^-}$ would be larger when Cl⁻ bathed both sides of the epithelium compared with the low-[Cl⁻] experiments. Results from four separate experiments in which $P_{ms}^{Cl^-}$ was compared in the same tissues are shown in table 3. $P_{ms}^{Cl^-}$ measurements were not statistically different from each other in the two conditions, and the conclusion is that Cl⁻ exchange diffusion cannot be detected in these resting tissues.

TABLE 3. TRANSEPITHELIAL Cl⁻ PERMEABILITY MEASUREMENTS

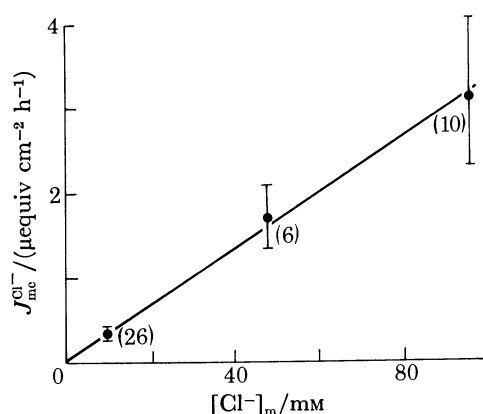| $[Cl^-]_m$	$[Cl^-]_s$	$P_{ms}^{Cl^-}/(10^{-5}\ cm\ s^{-1})$
96	96	0.6 ± 0.3 (4)
3.5	0	1.2 ± 0.2 (4)

FIGURE 3. Unidirectional ³⁶Cl⁻ uptake by gastric mucosa at different $[Cl^-]_m$. $J_{mc}^{Cl^-}$ was measured at 45 s with three different $[Cl^-]_m$ during short-circuit conditions with normal Ringer solution on the serosal side.

Cellular uptake of ³⁶Cl⁻

We have used 45 s uptakes of ³⁶Cl⁻ ($J_{ms}^{Cl^-}$) to estimate the tracer permeability of the mucosal membrane to Cl⁻. All tissues were short-circuited during the uptake measurements. Results of experiments in which we measured $J_{ms}^{Cl^-}$ at 10, 48 and 96 mM are shown in figure 3. The intercept of the line is very close to zero, and the slope is linear over the range of concentrations tested. There was no correlation (not shown) between $J_{mc}^{Cl^-}$ and either I_{sc} or R_t, the transepithelial resistance.

Electrophysiological measurements

We have measured electrophysiological parameters with normal Cl⁻–HCO₃⁻ Ringer solution bathing the serosal side of the tissue while the mucosal solution contained either this same Ringer solution or one in which all but 2 mM Cl⁻ was replaced with gluconate or isethionate. A typical experiment is shown in figure 4, where we measured ψ_{ms}, ψ_s and V^{Cl^-} during control and low-
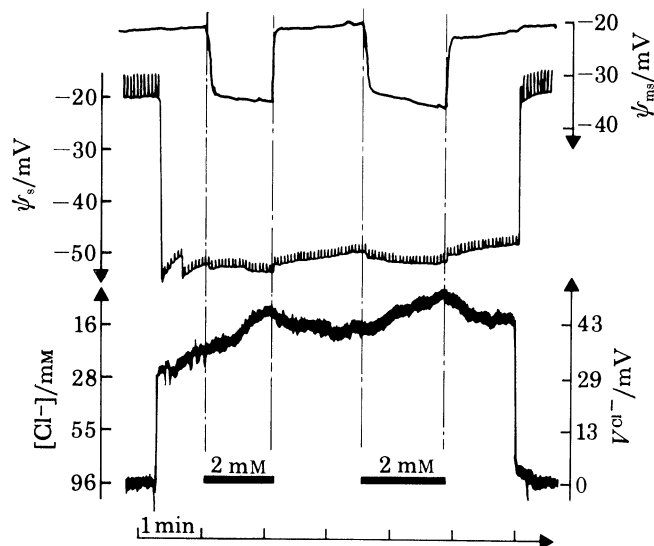
FIGURE 4. Transepithelial electrical potential difference, ψ_{ms} (serosa ground), the cellular electrical potential ψ_s (also relative to the potential of the serosal solution), the potential of the Cl^- electrode, V^{Cl^-}, and the apparent $[Cl^-]_c$ as a function of time, recorded by a double-barrelled microelectrode. At $t = 0$, the microelectrode was advanced into the cell from the mucosal solution, and ψ_s was about -53 mV. V^{Cl^-} in the cell was initially about 35 mV relative to V^{Cl^-} of 0 mV in the mucosal solution where $[Cl^-]_m = 96$ mM. This corresponded initially to $[Cl^-]_c \approx 23$ mM. After 1 min and approximately 4 min, $[Cl^-]_m$ was reduced to 2 mM, and the resulting changes in cellular and transepithelial parameters were recorded. Note that low $[Cl^-]_m$ solutions caused ψ_{ms} to hyperpolarize by about 15 mV while ψ_s depolarized by only 2 mV. V^{Cl^-} increased owing to the drop in $[Cl^-]_c$ during the low-$[Cl^-]_m$ treatment.
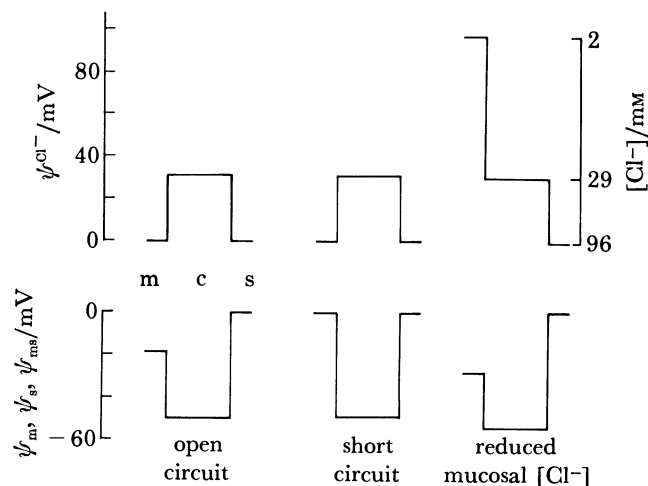

FIGURE 5. The average electrical and chemical potentials for Cl^- as well as apparent $[Cl^-]$ in the mucosal (m), cellular (c) and serosal (s) compartments during conditions of open circuit and short circuit. At low $[Cl^-]_m$ these are initial values, before reaching the steady state.

$[Cl^-]_m$ conditions. A summary is shown in figure 5. We shall report the average electrical potential measurements first and then deal with V^{Cl^-} and $[Cl^-]_c$ measurements.

Membrane potentials and resistances: control and low $[Cl^-]_m$

As shown in table 4, with normal saline and under open circuit conditions, ψ_m was -34 mV and ψ_{ms} was -18 mV, mucosa negative. Thus, ψ_s was -52 mV (cell with respect to serosal

solution). Since $\alpha = R_m/R_s$ was much larger than unity, it is clear that the 80% of R_t – 230 Ω cm² was localized to the mucosal membrane. The current–voltage relation of the tissue was linear in the range of currents tested 0–70 μA cm⁻² (not shown). During short-circuit conditions the electrical potential difference across the two membranes was $-49+3.2$ mV ($n = 7$).

When Cl⁻ in the mucosal solution was replaced by the impermeable ion gluconate the potentials and resistances changes. The ψ_{ms} increased by 9.3 ± 1.0 mV ($n = 11$), now totalling

TABLE 4. ELECTROPHYSIOLOGICAL PARAMETERS: CONTROL AND LOW-[Cl⁻] MUCOSAL SOLUTIONS

(Averages ± s.e.m. for steady-state measurements. Numbers in parentheses represent numbers of different animals, with intracellular measurements based on 5–10 cells for each animal. $\psi_m^{Cl^-}$, $\psi_s^{Cl^-}$ and [Cl⁻]$_c$ were not determined during the steady state in low-[Cl⁻]$_m$ conditions.)

	control	low-[Cl⁻] mucosal
ψ_{ms}/mV	-18 ± 1.1 (11)	-27 ± 3.1 (11)
ψ_m/mV	-34 ± 2.3 (10)	-26 ± 3.1 (11)
ψ_s/mV	$-52\dagger$	$-54\dagger$
$\psi_m^{Cl^-}(= \psi_s^{Cl^-})/mV$	31 ± 3.7 (10)	—
[Cl⁻]$_c$/mM	29 ± 3.0 (10)	—
$R_t/(\Omega$ cm²)	230 ± 35 (13)	435 ± 50 (6)
α	4.5 ± 1.0 (6)	5.2 ± 1.6 (6)

† Calculated from ψ_m and ψ_{ms}.

-27 mV, caused by a depolarization of ψ_m (now -26 mV) and a hyperpolarization of ψ_s (to -54 mV). The voltage divider ratio α increased by a factor of 1.15 ± 0.04 ($n = 6$) to 5.2 and R_t by a factor of 1.89 ± 0.22 ($n = 6$) to 435 Ω cm².

Apparent [Cl⁻]$_c$: *control and with low* [Cl⁻]$_m$

A typical experiment in which [Cl⁻]$_c$, ψ_s and ψ_{ms} were measured is shown in figure 4. With normal [Cl⁻] on both mucosal and serosal sides of the tissue and in open-circuit conditions, the difference in chemical potential for Cl⁻ across the mucosal membrane was initially about 35 mV, which corresponded to a [Cl⁻]$_c$ of about 23 mM. As shown in table 4 and figure 5, with Ringer solution on both sides of the tissue the average difference of chemical potential for Cl⁻ across the mucosal and serosal membranes was 31 mV, which corresponds to an intracellular [Cl⁻]$_c$ = 29 mM.

In order to estimate the influence of other ions on the measurements of the intracellular [Cl⁻], three tissues were depleted of Cl⁻ by incubation in a Cl⁻-free Ringer. Under these conditions, chemical potential for Cl⁻ was $-0.73+1.01$ mV (15 cells) relative to a solution of 2 mM Cl⁻ and 18 mM HCO$_3^-$. With a selectivity of no worse than 1:4 of Cl⁻ over HCO$_3^-$, this is equivalent to an apparent [Cl⁻]$_c$ no larger than 6 mM. The reference barrels in these experiments were filled with 2 M KCl. That the contamination from this filling solution is small is supported by the fact that when the reference barrel of the electrodes was filled with either 2 M KCl or with saturated Na⁺ isethionate similar results were found. Thus intracellular ions other than Cl⁻ may simulate up to 6 mM of Cl⁻, at least in the situation where the external [Cl⁻] is low. If these ions were present during control situations, intracellular [Cl⁻] could be about 23 mM. It is, though, questionable whether this correction applies. It should be made if the following two conditions hold: (i) the perturbing anions should be present intracellularly both in control conditions and in conditions with low external [Cl⁻]; (ii) the selectivity of the electrode for Cl⁻ over these

anions should be the same at high and low $[Cl^-]$. In the following we have chosen not to subtract the 6 mM. This has no consequences for the conclusions we reach.

The relations between electric and chemical potential differences across the cell membrane in open-circuit and short-circuit situations and in the case of reduced mucosal $[Cl^-]$ are summarized in figure 5 and table 4. During open-circuit conditions $\psi_m^{Cl^-}$, the chemical potential for Cl^- across the mucosal membrane, is equal to ψ_m, and Cl^- ions were in approximate equilibrium across the mucosal membrane. However, since $\psi_s = -52$ mV $> \psi_s^{Cl^-}$, Cl^- is more concentrated across the serosal membrane than would be expected for a passive distribution. There was no significant change in $[Cl^-]_c$ when the short-circuiting currents were passed. Thus in the short-circuited state, $\psi_m = -\psi_s > \psi_s^{Cl^-} = \psi_m^{Cl^-}$, i.e. Cl^- is distributed out of equilibrium across both membranes.

When $[Cl^-]$ in the mucosal solution was changed abruptly for a period of about 1 min (figure 4) from 95 to 2 mM by replacement by isethionate or gluconate, $[Cl^-]_c$ changed transiently. During the first 30 s, $[Cl^-]_c$ decreased at a rate (dc/dt) of 0.16 mM s^{-1}. When $[Cl^-]_m$ was returned to 96 mM, $[Cl^-]_c$ increased at a rate of 0.32 mM s^{-1}. By using the measured intracellular and extracellular electrical and chemical potentials and assuming a cell height h (= volume:surface ratio) of 40 μm then an electrodiffusive permeability can be calculated from:

$$J_{mc}^{Cl^-} = h\frac{dc}{dt} = P_m^{Cl^-}\frac{\psi_m zF}{RT}\frac{[Cl^-]_m - [Cl^-]_c \exp(\psi_m zF/RT)}{1 - \exp(\psi_m zF/RT)}, \qquad (1)$$

where $RT/zF = -25.3$ mV and subscripts m and c indicate the mucosal and the cellular compartment. From 23 similar recordings (9 animals) $P_m^{Cl^-}$ was calculated to be $2.9 \pm 1.5 \times 10^{-5}$ cm s^{-1} for the case where Cl^- ions were leaving the cell. In the case where $[Cl^-]_m$ was increased and there was an influx of Cl^- into the cell, $P_m^{Cl^-}$ was calculated to be $5.7 \pm 1.6 \times 10^{-5}$ cm s^{-1}. With pooled influx and efflux data $P_m^{Cl^-}$ is calculated to be 4.3×10^{-5} cm s^{-1}. If the volume:surface ratio is only 20 μm, e.g. because half of the intracellular volume is inaccessible to rapid exchange of Cl^- ions, then the permeabilities are only half as large.

DISCUSSION

Electrophysiological measurements in gastric mucosa

During open-circuit conditions the intracellular potentials measured across the mucosal membrane of surface epithelial cells averaged -34 mV (table 4, figure 5). This value is somewhat larger than that reported previously for the *Necturus* gastric mucosa (Spenney *et al.* 1974), and the difference may be due to the fact that higher-resistance (i.e. finer-tipped) microelectrodes were used in the present study. Schettino & Curci (1980) have observed even larger cellular potentials (average -54.5 mV) in surface cells of frog (*Rana esculenta*) gastric mucosa. We believe that we have minimized membrane damage caused by microelectrode penetration for the following reasons. (1) With the use of single-barrelled electrodes with resistances of 40–80 MΩ, cellular penetration gave abrupt changes in potential that were stable and showed little or no overshoots, which are typical of damaged, leaky cells (figure 2). Furthermore, single-barrelled and double-barrelled electrodes yielded very similar values for ψ_m. (2) In experiments in which $[Cl^-]_m$ was altered, ψ_m changed by about 10 mV while ψ_s changed by only a small amount (table 4, figure 4), indicating that there was little shunting of the potentials around the microelectrode tip. (3) Large values of α indicated that the resistance of the mucosal

membrane was 4.5 times larger than that of the serosal membrane. T. Schettino (personal communication) has measured $\alpha = 5$–7 in surface cells of frog gastric mucosa by using micro-electrodes with resistances of 60–130 MΩ. (4) In unpublished experiments we have found the K$^+$ activity of surface cells to be 85 mM, which is close to the value of 95 mM measured for frog gastric mucosa by Schettino & Curci (1980). We also found an apparent cellular concentration of Na$^+$ of about 10 mM. Surface cells of *Necturus* gastric mucosa are thus rather similar to cells of other epithelia in that K$^+$ activity is high, Na$^+$ activity is low, and Cl$^-$ activity ≈ 30 mM (e.g. gall-bladder (Zeuthen 1978; Reuss & Grady 1979); kidney proximal tubule (Spring & Kimura 1978); small intestine (Zeuthen *et al.* 1978; White 1977) (see also review by Zeuthen 1981)).

We attribute the relatively small differences between our potential and Cl$^-$ measurements and those of Schettino (personal communication) and Schettino & Curci (1980) to the following factors. (1) Species differences, i.e. *Rana esculenta* compared with *Necturus maculosus*. (2) Different *in vitro* techniques: we incubated the gastric mucosa in cimetidine to reduce sponaneously histamine-induced H$^+$ secretion and attendant changes in electrophysiology. Apparently Schettino & Curci (1980) just mounted the tissues directly in chambers without a pre-incubation period. (3) The higher K$^+$ activity of frog compared with *Necturis* surface cells (see above). However, we should point out that whatever caused the differences between their results and our own, these differences are small, and we conclude that our experimental results are not significantly contaminated by leakage artefacts around the microelectrodes.

Cellular Cl⁻ activity and site of active Cl⁻ transport

During both open-circuit and short-circuit conditions, $[\text{Cl}^-]_c \approx 29$ mM (figures 4 and 5). This value is somewhat lower than the concentrations measured for the whole tissue by chemical (30–40 mM (Davenport & Alzamora 1962)) and radioisotopic (40–60 mM (Harris & Edelman 1964)) techniques. However, in recent unpublished experiments with the use of isotopic analysis of frozen, sectioned frog (*Rana catesbiana*) gastric mucosa, Culp & Forte (personal communication) have found $[\text{Cl}^-]_c = 30$ mM in surface epithelial cells. A similar value for $[\text{Cl}^-]_c$ has been obtained for surface cells of *Rana esculenta* by using the electron microprobe analysis technique on freeze-dried sections of resting gastric mucosa (A. Dorge, R. Rick & T. E. Machen, unpublished observations)

During both open-circuit and short-circuit current conditions, cellular Cl$^-$ activity is too high to be explained by a simple, passive distribution of this ion across the serosal membrane (figures 4 and 5). Our measurements offer proof that Cl$^-$ is accumulated within surface cells across the serosal membrane in an energy-requiring process. During open-circuit conditions Cl$^-$ is distributed across the mucosal membrane passively (figures 4 and 5) and $J_{\text{net}}^{\text{Cl}^-} = 0$ (table 1; Forte & Machen 1975). Under these conditions, there is an 'active' accumulation of Cl$^-$ within the cells, but the Cl$^-$ must leak back into the serosal solution at a rate equal to the entry rate.

When the tissue is short-circuited, net Cl$^-$ secretion now occurs and $J_{\text{net}}^{\text{Cl}^-} = I_{\text{sc}}$ (table 1) (Machen & McLennan 1980). Under these conditions cellular Cl$^-$ activity did not change significantly (compared with open-circuit conditions), and there was an increase in ψ_m and decrease in ψ_s (figure 5). The increase in ψ_m would increase the driving force of the movement of Cl$^-$ from the cell into the mucosal solution. There would also be a slightly reduced driving force for Cl$^-$ to leak back into the serosal solution from the cell. This small depolarization of ψ_s

would not be expected to decrease this backflux out of the cell very much, however, and there must therefore have been a concomitant increase in Cl^- entry from the serosal solution into the cell to match the increase in net Cl^- movement across the mucosal membrane into the lumen.

Chloride permeability of the mucosal membrane

We have used both tracer and electrophysiological techniques to assess $P_m^{Cl^-}$. From the average $J_{mc}^{Cl^-} = 3.15\ \mu equiv\ cm^{-2}\ h^{-1}$ obtained by tracers, $\psi_m = -49\ mV$, $[Cl^-]_m = 96\ mM$, and $[Cl^-]_c = 0\ mM$ (i.e. unidirectional Cl^- uptake was measured), we have used the constant field equation (equation (1)) to calculate Cl^- permeability of the mucosal membrane. The tracer results (figure 3) indicate that at normal external Cl^- concentration $P_m^{Cl^-} = 2.8 \times 10^{-5}\ cm\ s^{-1}$. This represents the permeability of the entire mucosal surface of the gastric mucosa to Cl^-.

We have also used rates of change of intracellular Cl^- activity measured electrophysiologically (figure 4) and ψ_m and the same constant field assumption to make another estimate of the $P_m^{Cl^-}$ of the surface cells (see figure 4 and the Results section). These calculations are limited somewhat by unstirred layer effects, i.e. the concentration at the membrane surface may be slightly different from that in the bulk solution. This probably explains why the calculated permeability was larger when the $[Cl^-]_m$ is returned to 95 from 2 mM Cl^- than when it is reduced from 96 to 2 mM Cl^-. Also the volume:surface ratio or assumed cell height of 40 μm may be somewhat inaccurate: the 'effective' cell height will be affected by the amount of cytoplasm occupied by cellular organelles that do not equilibrate readily with Cl^-. With these reservations in mind, the estimate obtained for $P_m^{Cl^-}$ with electrodes (2.9–$5.7 \times 10^{-5}\ cm\ s^{-1}$) agrees quite well with the $P_m^{Cl^-}$ data obtained from the flux measurements.

This permeability has the characteristics of a purely passive process. Thus, in short-circuited gastric mucosa, Cl^- uptake across the mucosal membrane was a linear function of $[Cl^-]_m$. We should point out, though, that if Cl^- movement across the mucosal membrane is through a conductive 'pore', then it might be expected that as $[Cl^-]_m$ is increased, ψ_m should hyperpolarize; $J_{mc}^{Cl^-}$ would tend to plateau from this effect alone. The absence of saturation as $[Cl^-]_m$ is increased in the present experiments may be explained by the fact that $J_{mc}^{Cl^-}$ is occurring across two different cell types (in parallel) that may have different permeabilities to Cl^-. Also, since the tissue was short-circuited during uptake measurements, changes in ψ_m due to changes in $[Cl^-]_m$ would be reduced compared with those expected if uptakes were measured in open circuit.

The linear function of $J_{mc}^{Cl^-}$ with $[Cl^-]_m$ is consistent with the fact that exchange diffusion (usually attributed to 'carriers' that saturate) does not seem to contribute markedly to Cl^- permeability in these resting gastric mucosas. Thus measurements of $J_{ms}^{Cl^-}$ at high and low $[Cl^-]$ showed that the Cl^- permeability does not differ in the two situations (table 3). If Cl^- exchange diffusion were contributing significantly to the Cl^- permeability of the tissue, then $P_{ms}^{Cl^-}$ would have been reduced in the low-$[Cl^-]$ and Cl^--free bathing solution conditions.

Using the value of $P_m^{Cl^-}$ calculated from uptake or from electrophysiological experiments and the same $[Cl^-]_m$, $[Cl^-]_c$ and ψ_m used above, we calculate that net Cl^- flux from cell to lumen should range from 2.3 to 5.2 $\mu equiv\ cm^{-2}\ h^{-1}$, values close to the average of 2.12 $\mu equiv\ cm^{-2}\ h^{-1}$ measured in the flux experiments (table 2). The implication is that in the short-circuited state, $[Cl^-]_c$, ψ_m and $P_m^{Cl^-}$ are large enough to drive Cl^- passively across the mucosal membrane at a rate equal to that of active Cl^- secretion. An active step for Cl^- transport at the mucosal membrane need not be invoked.

Conclusions and uncertainties

In many ways the resting gastric mucosa behaves as a typical electrogenic system that has an active Cl⁻ transporting mechanism and very little parallel conductance for other ions. Thus in the short-circuited state $I_{sc} = J_{net}^{Cl^-}$; in the open-circuited state this 'pump' generates a mucosa negative potential (table 1) (Machen & McLennan 1980), which opposes net Cl⁻ transport, and $J_{net}^{Cl^-}$ is reduced (table 1; see also Hogben 1955). Reductions in I_{sc} by removal of either Na⁺ or Cl⁻ from bathing solutions are associated with reductions in tissue conductance (Machen & McLennan 1980). Because Cl⁻ is out of equilibrium (too high) by about 21 mV (short circuit) to 24 mV (open circuit) across the serosal membrane (figure 5), there must be an 'active' step for Cl⁻ transport at this membrane by some cells of the tissue. Owing to the heterogenous nature of the epithelium, it is difficult to localize absolutely this transport to the surface cells. We note, though, that the frog gastric mucosa is capable of actively secreting Cl⁻ before oxyntic cells have developed (Forte *et al.* 1969). This active Cl⁻ transport may be a basic quality of all cell types of the epithelium.

It is known that active Cl⁻ transport is dependent on the presence of Na⁺ in the serosal, but not the mucosal, solution (Machen & McLennan 1980). This Cl⁻ transport is also inhibited by ouabain (Forte & Machen 1975) and K⁺-free serosal solutions (Machen *et al.* 1978). By analogy with other Cl⁻-transporting epithelia (Frizzell *et al.* 1979), we have previously proposed that the electrochemical gradient for Na⁺ between serosal solutions and cells is used to drive Cl⁻ into gastric mucosal cells (Machen & McLennan 1980; Machen *et al.* 1980), though we have no definite proof that the entry step is neutral or even that Na⁺ and Cl⁻ movements are directly coupled. Cl⁻ transport by resting gastric mucosa is also dependent on the $[HCO_3^-]$ in the serosal solution (Manning & Machen 1978, 1982; Schiessel *et al.* 1980).

The exit of Cl⁻ from the cell into the mucosal solution exhibits some characteristics that one would expect for conductive movements of this ion. Thus, when the tissue is short-circuited, ψ_m increases by 15 mV and $J_{net}^{Cl^-}$ movement across the mucosal membrane increases from zero to a value equal to I_{sc}. Also, the calculations of $P_m^{Cl^-}$ are consistent with the idea that Cl⁻ movement across the mucosal membrane is a passive, electrodiffusive process. Finally, Cl⁻-free mucosal solution caused ψ_m to depolarize by about 10 mV (figure 5).

The fact that this epithelium is a composite one, consisting of two major types of cells in a mosaic manner, makes it difficult to make a unified interpretation of all the results. For example, when $[Cl^-]_m$ was reduced from 96 to 2 mM, R_t increased by only 36 % and ψ_m depolarized by only 10 mV. These changes contrast with those predicted from the constant field equation and assuming that the whole epithelium consisted of surface cells with $P_m^{Cl^-} = 4 \times 10^{-5}$ cm s⁻¹ $\gg$ $P_m^{K^+} = P_m^{Na^+} = P_m^{HCO_3} = 0$: R_t should have changed by a factor of 2.7 and ψ_m should have depolarized by about 80 mV instead of the recorded 10 mV. These types of effect may be caused by the parallel combination of surface cells and tubular oxyntic cells, which may have different permeability and transport properties. Also, it is not known whether surface cells and oxyntic cells are coupled electrically. These types of effects might tend to cause a circulating current between the surface and oxyntic cells, thereby reducing changes in ψ_m and R_t compared with those expected for an epithelium with a perfectly Cl⁻-selective mucosal membrane. Finally, the presence of unstirred layers at the surface of the epithelium and within gastric pits may cause problems in these types of experiments in which $[Cl^-]$ in the bulk solution is changed drastically, but $[Cl^-]$ at the membrane interfaces may change by smaller amounts.

It is surprising that a transepithelial current, i.e. the short-circuit current, has no or little

effect on the apparent $[Cl^-]_c$. One explanation may be that the current effects equally large Cl^- fluxes across both the serosal and mucosal membrane, despite the different Cl^--transport mechanisms on the two membranes. That this is quite possible can be predicted from a recent numerical model of epithelia (Baerentsen *et al.* 1982), which uses the Goldman equation and reasonable assumptions about active and passive carriers. Another possible explanation is that the gastric pits may carry a large fraction of the current.

REFERENCES

Baerentsen, H., Christensen, O., Grovethomson, P. & Zeuthen, T. 1982 A numerical model of cellular epithelial ion-transport. *J. Membrane Biol.* (In the press.)

Davenport, H. W. & Alzamora, F. 1962 Sodium, potassium, chloride and water in frog gastric mucosa. *Am. J. Physiol.* **202**, 711–715.

Durbin, R. P. 1977 Chloride transport and acid secretion in stomach. *Gastroenterology*, **73**, 927–930.

Forte, J. G. 1969 Three components of Cl^- flux across isolated bullfrog gastric mucosa. *Am. J. Physiol.* **216**, 167–174.

Forte, J. G., Limlomwongse, L. & Kasbekar, D. K. 1969 Ion transport and the development of hydrogen ion secretion in the stomach of the metamorphosing bullfrog tadpole. *J. gen. Physiol.* **54**, 79–95.

Forte, J. G. & Machen, T. E. 1975 Transport and electrical phenomena in resting and secreting piglet gastric mucosa. *J. Physiol. Lond.* **244**, 33–51.

Frizzell, R. A., Field, M. & Schultz, S. G. 1979 Sodium-coupled chloride transport in epithelial tissues. *Am. J. Physiol.* **236**, F1–F8.

Harris, J. B. & Edelman, I. S. 1964 Chemical concentration gradients and electrical properties of gastric mucosa. *Am. J. Physiol.* **206**, 769–782.

Hogben, C. A. M. 1955 Active transport of chloride by isolated frog gastric epithelium. Origin of the gastric mucosal potential. *Am. J. Physiol.* **180**, 641–649.

Hogben, C. A. M. 1968 Observations on ionic movements through the gastric mucosa. *J. gen. Physiol.* **51**, 2485–2495.

Machen, T. E. & McLennan, W. L. 1980 Na^+-dependent H^+ and Cl^- transport in *in vitro* frog gastric mucosa. *Am. J. Physiol.* **238**, G403–G413.

Machen, T. E., McLennan W. L. & Zeuthen, T. 1980 Electrogenic Cl^- secretion by resting gastric mucosa: Na–Cl co-transport model. In *Hydrogen ion transport in epithelia* (ed. I. Schultz, G. Sachs, J. G. Forte & K. J. Ullrich), pp. 379–390.

Machen, T. E., Silen, W. & Forte, J. G. 1978 Na^+ transport by mammalian stomach. *Am. J. Physiol.* **234**, E228–E235.

Machen, T. E. & Zeuthen, T. 1980 Electrophysiology of Cl^- secretion in the stomach. *J. Physiol., Lond.* **301**, 48P. [Abstract.]

McLennan, W. L., Machen, T. E. & Zeuthen, T. 1981 Ba^{2+} inhibition of electrogenic Cl^- secretion by *in vitro* piglet and bullfrog gastric mucosa. *Am. J. Physiol.* **239**, G151–G160.

Manning, E. C. & Machen, T. E. 1978 HCO_3^- stimulation of Cl^- transport in *in vitro* gastric mucosa. *Fedn Proc. Fedn. Am. Socs exp. Biol.* **38**, 1060. [Abstract.]

Manning, E. C. & Machen, T. E. 1982 Effects of bicarbonate and pH on chloride transport by gastric mucosa. *Am. J. Physiol.* **243**, 660–668.

Reuss, L. & Grady, T. P. 1979 Effects of external sodium and cell membrane potential on intracellular chloride activity in gall bladder epithelium. *J. Membrane Biol.* **51**, 15–31.

Schettino, T. & Curci, S. 1980 Intracellular potassium activity in epithelial cells of frog fundic gastric mucosa. *Pflügers Arch. Eur. J. Physiol.* **383**, 99–103.

Schiessel, R., Merhav, A., Mathews, J. B., Fleischer, L. A., Barzilai, A. & Silen, W. 1980 Role of nutrient HCO_3^- in protection of amphibian gastric mucosa. *Am. J. Physiol.* **239**, G536–G542.

Schultz, S. G., Curran, P. F., Chez, R. A. & Fuisz, R. E. 1967 Kinetic relations of the Na-amino acid interaction at the mucosal border of the intestine. *J. gen. Physiol.* **50**, 1261–1286.

Spenney, J. G., Shoemaker, R. L. & Sachs, G. 1974 Microelectrode studies of fundic gastric mucosa: cellular coupling and shunt conductance. *J. Membrane Biol.* **19**, 105–128.

Spring, K. R. & Kimura, G. 1978 Chloride reabsorption by renal proximal tubules of *Necturus*. *J. Membrane Biol.* **38**, 233–254.

White, J. F. 1977 Activity of chloride in absorptive cells of *Amphiuma* small intestine. *Am. J. Physiol.* **232**, E553–E559.

Wright, G. H. 1962 Transfers of water, sodium, chloride and hydrogen ions across the gastric mucosa of the rabbit foetus. *J. Physiol., Lond.* **163**, 281–293.

Zeuthen, T. 1978 Intracellular gradients of ion activities in the epithelial cells of the *Necturus* gall bladder recorded with ion-selective microelectrodes. *J. Membrane Biol.* **39**, 185–218.

Zeuthen, T. 1980 How to make and use double-barrelled ion-selective microelectrodes. In *Current topics in membranes and transport*, vol. 13 (ed. E. Boulpaep), pp. 31–47. New York: Academic Press.

Zeuthen, T. 1981 Ion transport in leaky epithelia studied with ion-selective microelectrodes. In *The application of ion-selective microelectrodes* (ed. T. Zeuthen), pp. 27–46. Amsterdam: North-Holland/Elsevier.

Zeuthen, T., Hiam, R. C. & Silver, I. A. 1974 Recording of ion activities in the brain. In *Ion selective microelectrodes* (ed. H. Berman & N. Herbert), pp. 145–156. London: Plenum Press.

Zeuthen, T., Ramos, M. & Ellory, J. C. 1978 Inhibition of active chloride transport by piretanide. *Nature, Lond.* **273**, 678–680.