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Microelectrode Studies of Fundic Gastric Mucosa: Cellular Coupling and Shunt Conductance

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Summary. *Necturus* fundic gastric mucosa was studied to define the magnitude of cellular and shunt conductance. Electrotonic coupling of surface epithelial cells by a low resistance pathway was shown by analysis of current spread within the epithelial sheet. From this analysis cellular conductance was found to be $4.18 \pm 1.57 \times 10^{-4} \Omega^{-1} \text{cm}^{-2}$, and transepithelial shunt conductance was $1.1 \times 10^{-4} \Omega^{-1} \text{cm}^{-2}$. The ratio of cell-to-shunt conductance was 3.80. These results are confirmed by a second technique in which the Δp.d. across the serosal membrane of surface cells was compared to the transepithelial Δp.d. resulting from a $\Delta[K^+]$ in the serosal solution. The data were analyzed using an electrical circuit equivalent to a mucosa composed of two highly coupled cell types. By this technique cell conductance was $3.07 \times 10^{-4} \Omega^{-1} \text{cm}^{-2}$, and transepithelial shunt conductance was $0.830 \times 10^{-4} \Omega^{-1} \text{cm}^{-2}$. The cell/shunt conductance ratio was 3.70. Thus, by two independent techniques the transepithelial shunt contributes only 1/5 of the conductance of *Necturus* fundic gastric mucosa.

Definition of the pathways of ion flow across epithelial tissues has presented problems not encountered in the study of individual cells. Until a decade ago (Ussing & Windhager, 1964) tissue conductance was thought to be the sum of conductance of its cells. The region of cell contact, the tight junction, was considered impermeable. The importance of the paracellular pathway has been confirmed in gallbladder (Diamond, Barry & Wright, 1971; Frömter, 1972), small intestine (Clarkson, 1967; Frizzell & Schultz, 1972), kidney proximal tubule (Windhager, Boulpaep & Giebisch, 1966; Hoshi & Sakai, 1967) and frog skin (Mandel & Curran, 1972). Epithelial conductance is the sum of cellular and paracellular (shunt) conductance.

Some criteria which predict the magnitude of the shunt conductance have been summarized by Diamond *et al.* (1971). A high transepithelial

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resistance, large potential difference (p.d.), asymmetry of p.d. responses to changes in ionic activity, high ion selectivity ratios, and maintenance of large concentration gradients would imply a small paracellular conductance. Consequently, fundic gastric mucosa appears to be a "tight" or nonleaky tissue, but quantitation of the two pathways remains important.

Blum, Hirschowitz, Helander and Sachs (1971) examined the magnitude of the shunt conductance of *Necturus* gastric mucosa by comparing the electrical resistance of the intact tissue with that of isolated cells. They concluded that the shunt conductance was no larger than cellular conductance. Treatment of the cells with proteolytic enzymes, the altered geometry, and the lack of polarization in the isolated system precluded more precise quantitation.

In this report cellular and shunt conductances are quantitated. Two methods for intact tissue will be described.

(1) A Δemf of the serosal cell membranes is induced by changing ionic activities in the serosal solution. The change of potential, across the serosal cell membrane, $\Delta\psi_{sc}$, and across the tissue, $\Delta\psi_{ms}$, is measured and analyzed using an equivalent circuit.

(2) Analysis of current spread within the epithelial sheet is a second, independent technique.

Experimental Methods

Adult *Necturus* were obtained commercially and maintained in an aquarium until use. Following decapitation the stomach was excised quickly and placed in amphibian Ringer's solution bubbled with 95% O_2 − 5% CO_2. After stripping the muscularis, the mucosa was stretched and mounted between two Lucite half-chambers. The upper half was open to expose the mucosal surface for microelectrode studies. The serosal and mucosal solutions were circulated from external reservoirs bubbled with 95% O_2 − 5% CO_2.

Fig. 1*A* is a microscopic section showing the cell types present in *Necturus* fundic gastric mucosa. The surface and neck cells comprise 37% of the cells; only the surface cells are accessible on the mucosal surface. Gastric pits branch into 3 to 5 gastric glands lined by oxyntic cells (64%). Micropuncture of oxyntic cells cannot be accomplished with visual control; iontophoresis after impalement can be used to identify the cell punctured (Shoemaker & Sachs, 1972). Thus, all micropunctures in these experiments are in surface epithelial cells.

Microelectrodes were prepared with a two-stage micropipette puller. They were examined with a phasecontrast microscope, filled with methanol in a vacuum, soaked in distilled water, and filled with 3 M KCl by diffusion during storage at 4 °C for 24 hr. Microelectrodes were used within 72 hr when the tip potential was less than 5 mV and the tip resistance was 10 to 40 MΩ.

Fig. 1B is a diagrammatic representation of equipment used to assess current spread within the mucosa. In the single microelectrode experiments the current-sending electrode and its current source, and recording connections were deleted from the circuit. Tissue resistance and the ratio of mucosal/serosal membrane resistance (R_m/R_s) were measured

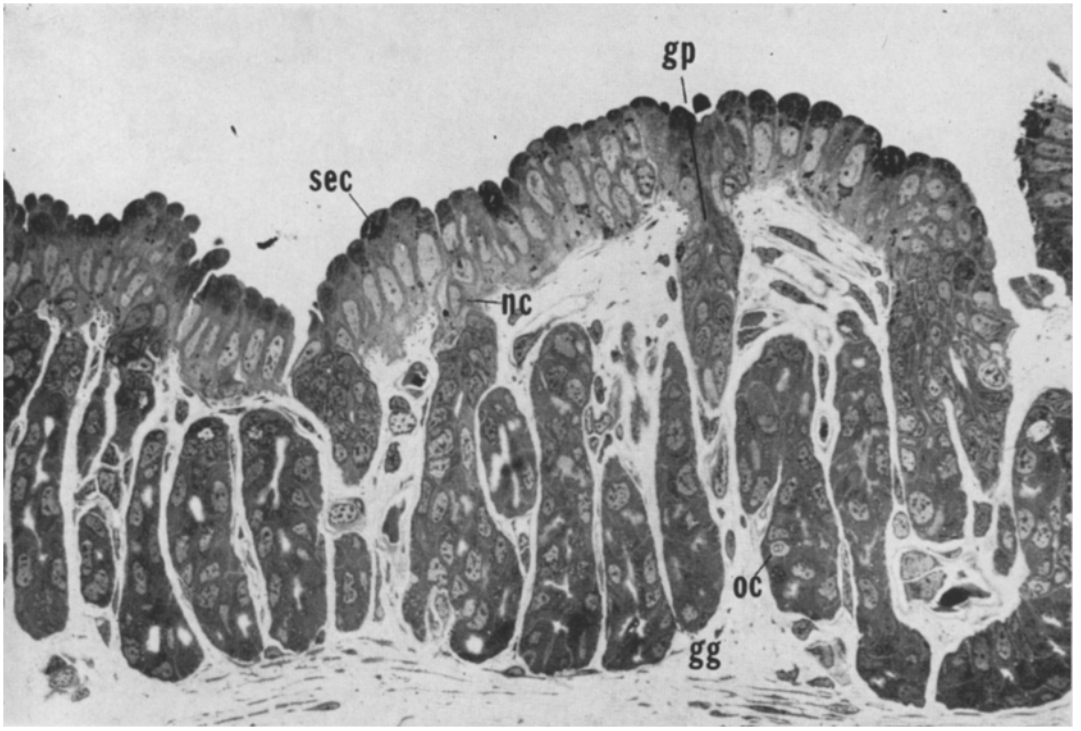

Fig. 1(A)

Fig. 1. (*A*) A photomicrograph of *Necturus* fundic gastric mucosa showing the complex geometry of the epithelial membrane. The mucosa is composed of (1) surface epithelial cells (*sec*), the only cells which can be directly visualized, (2) neck cells (*nc*) lining the gastric pits (*gp*), and (3) oxyntic cells (oc) which line the gastric glands (*gg*) 3 to 5 of which branch from each gastric pit. The muscular layer has been stripped from the mucosa but a layer of connective tissue restricts access to gastric glands from the serosal surface. (*B*) Diagram of microelectrode equipment designed for gastric mucosa experiments. M.E. 1 is a channel for voltage recording only. M.E.2 is a channel also equipped for current sending. The microelectrode channels may be referenced to either the serosal or mucosal macroelectrode C.E. In the diagram both are referenced to the mucosal macroelectrode. Provision is also made for recording the transpotential between electrodes C.E. and sending external current through Ag/AgCl electrodes from a current source on the left-hand side of the diagram. pH changes are monitored if necessary through a battery operated pH-meter, and the solutions are circulated by a gas lift system using 95% $O_2-5\%$ CO_2 or from Marriot bottles bubbled with 95% $O_2-5\%$ CO_2. The circulating solution is jetted across the surface of the mucosa by orifices directed at the mucosa

by pulses of 10 μamp/cm^2 through Ag-AgCl circular wires mounted in the chamber. The transepithelial p.d. was measured using a pair of saturated KCl-calomel electrodes with renewable junctions. Intracellular p.d. was measured with a microelectrode referenced to the serosal solution. All resistances were corrected for resistance of the solutions in the chamber. Current pulses were limited to less than 100 msec to minimize polarization effects.

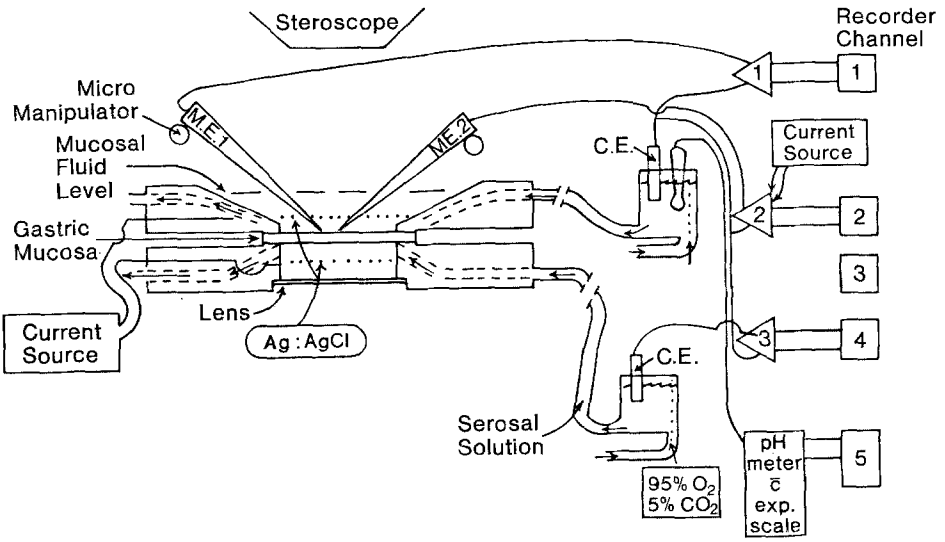

Fig. 1(B)

Conduct of Experiments

The composition of solutions is given in Table 1. Only mucosal and serosal solutions A were used in the current spread experiments. When ionic activities were changed, Marriott bottles and a stopcock arrangement were used to perfuse the solution. When

Table 1. Composition (mM) of solutions used

	Constant product KCl experiments					Constant serosal $[Cl^-]$ experiments	
	Serosal		Mucosal		Sulfate mucosal	5 mM K^+ Cl serosal	50 mM K^+ Cl serosal
	A	B	A	B			
Na^+	91	46	91	46	91	91	46
K^+	5	50	5	50	5	5	50
Cl^-	80	8	80	8	—	80	80
SO_4^{2-}		36	8	44	48	—	—
Mg^{++}	1.0	1.0	—	—	—	—	—
Ca^{++}	1.5	1.5	—	—	—	1.5	1.5
HCO_3^-	20	20	0	0	0	20	20
$H_2PO_4^-$	1.0	1.0	—	—	—	1	1
Glucose	5	5	5	5	5	5	5
Sucrose	—	36	15.5	51.5	55.5	—	—

Constant product KCl changes are performed with the 4 solutions at the far left. Solutions A are the starting solutions and B are the solutions giving the constant product KCl change. The remaining columns were used with $\Delta[K^+]$ while $[Cl^-]=80$ mM. The sulfate mucosal (column 5) was used in all these experiments. Sucrose is used to maintain osmolarity.

stable p.d.'s were obtained in solution A, perfusion (15 ml/min) with the alternate solution was started and continued until the transepithelial and intracellular p.d.'s were stable (about 5 min). Perfusion with solution A was restarted and continued until the p.d.'s were stable. Usually the p.d.'s returned to their original values. During the course of one experiment several such changes could be done.

Criteria of a "good" micropuncture are: (1) an abrupt change in p.d. when the cell is punctured, (2) stable p.d. for at least 20 sec, (3) return of microelectrode potential to control level upon withdrawal from the cell, and (4) no alteration in tip resistance. While a micropuncture must have a stable p.d. for at least 20 sec to be considered "good", stability over a much longer period was routinely achieved to allow measurements of p.d. during changes in ionic activity in the bathing solution.

In the current spread experiments one microelectrode was placed intracellularly to inject current pulses ($I_0 = 5.9 \times 10^{-9}$ amps). A second microelectrode was used to measure the resulting intracellular Δp.d. in other cells as a function of distance from the source cell. Distance was measured with a micrometer eyepiece in the stereomicroscope. In both types of experiments, the relative resistance of the mucosal and serosal membranes (R_m/R_s) was measured by the Δp.d. of the intracellular electrode when current was sent transmucosally. The mucosal (m) or serosal (s) solutions were used as reference.

$$\frac{R_m}{R_s} = \frac{\Delta \text{p.d.}_{mc}}{\Delta \text{p.d.}_{sc}}. \tag{1}$$

Results

Intracellular Potential Differences

Intracellular potential of surface epithelial cells was -30 to -65 mV referenced to the serosal solution. The value varied in different mucosae but in a single epithelium the range of values was usually quite small. A transient overshoot of the potential (about 1 sec) was occasionally recorded upon impalement; thereafter the potential was stable. If a leak around the electrode was responsible for this overshoot, it was obviously only transitory since a stable potential was obtained thereafter. Fig. 2 shows the distribution of intracellular p.d.'s recorded in fundic surface cells.

Effect of Constant Product KCl Changes[1]

These changes were used to minimize ion redistribution within the mucosa; similar techniques have been applied to muscle (Hodgkin & Horowicz, 1959). The *Necturus* fundic mucosa responds asymmetrically to constant product KCl changes. A mucosal constant product KCl change (Table 2) caused a 6.55 ± 3.54 mV increase in the transmucosal p.d.; a 5.5 ± 2.07 mV change was recorded across the mucosal membrane. In contrast, serosal

1 Constant product KCl change refers to a change in (K^+) and (Cl^-) such that $(K^+) \times (Cl^-)$ is 400 mM^2 at all times.

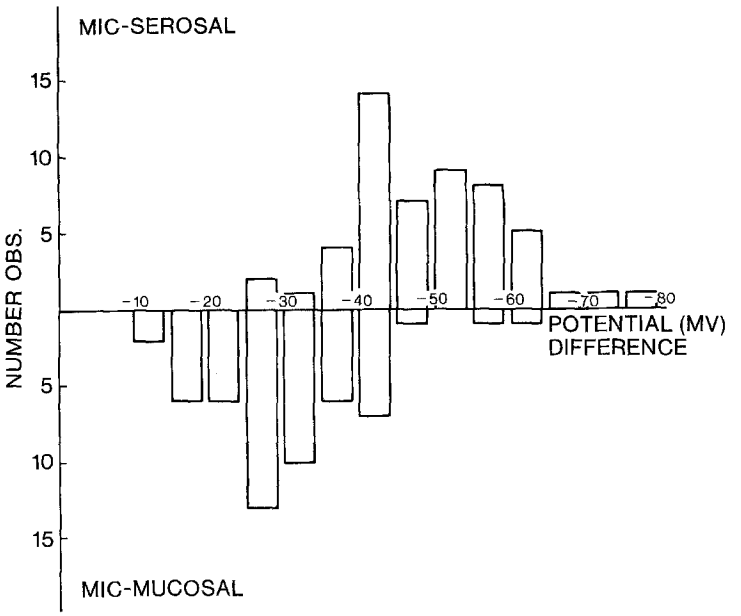

Fig. 2. The distribution of membrane potentials observed in 5 different experiments where a total of 50 cells were punctured. The upper part of the figure shows the potential measured between the microelectrode and serosal solution; the lower part is the potential between the microelectrode and mucosal solution, the difference being equal to the transtissue potential

Table 2. Changes in potentials with constant product changes in the mucosal solution

Exp. No.	$\Delta\psi_{mc}$	$\Delta\psi_{mc}/\Delta\psi_{ms}$
1	6	0.5
2	5	0.72
3	5	1.0
4	8	1.14
5	7	0.7
6	2	1.0
$\bar{X}\pm$SD	5.5±2.07	0.84±0.24

$\Delta\psi_{ms}$ designates the change in transepithelial p.d. when ionic concentration in the mucosal bathing solution is changed. $\Delta\psi_{mc}$ designates the corresponding change in p.d. across the mucosal cell membranes

constant product changes caused a transmucosal Δp.d. of 54 ± 6.2 mV. A 42 ± 9.8 mV change occurred across the serosal membrane and a 12 ± 7.2mV change was measured across the mucosal membrane of the surface epithelial cells. Fig. 3 shows a typical experiment in which the constant product change is made in the serosal solution. Table 3 summarizes the results of five experiments.

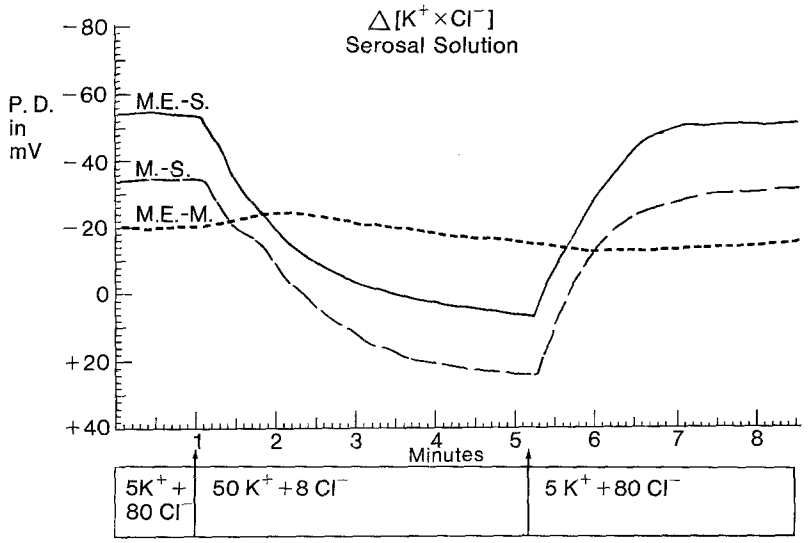

Fig. 3. The effect of a constant product KCl change (serosal solution) on the transepithelial potential and the potential across the serosal cell membrane and across the mucosal cell membrane. In the experiment shown, the Δp.d. across the serosal membrane is almost equivalent to the transepithelial Δp.d. Table 2 gives the means for all experiments

Table 3. Change in electrical parameters during constant product KCl experiments

Exp. No.	Trans resistance Sol. A	Trans resistance Sol. B	R_m/R_s Sol. A	R_m/R_s Sol. B	$\Delta\psi_{ms}$	$\Delta\psi_{sc}$	$\Delta\psi_{mc}$	$\Delta\psi_{sc}/\Delta\psi_{ms}$
1	1395	1350	0.93	0.52	59	55.5	3.5	0.94
2	2124	1844	1.47	1.47	50.75	43.25	7.5	0.85
3	1687	1485	1.2	1.0	55	32.5	22.5	0.59
4	2160	2160	1.28	1.0	60	46	14	0.77
5	1350	1215	1.0	1.11	45	32.5	12.5	0.72
Avg. ±	1743±	1610±	1.18±	1.02±	54±	42.0±	12.0±	0.77±
SD	387	386	0.22	0.34	6.2	9.8	7.2	0.13

The mucosa, initially bathed by mucosal and serosal solution A, is perturbed by changing the serosal solution to solution B. Serosal to mucosal ionic gradients (mM) are Na^+, 46:91; K^+, 50:5, Cl^-, 8:80; SO_4^{2-}, 36:8. Other ions remain in the same proportion as in solution A. Transresistance is calculated from the transepithelial Δp.d. when 10 $\mu A/cm^2$ is sent transepithelially. R_m/R_s is the Δp.d. across the mucosal cell membrane: Δp.d. across the serosal cell membrane when 10 $\mu A/cm^2$ is sent transepithelially. $\Delta\psi_{ms}$ is the change in transepithelial p.d. resulting from the change in ionic activity. $\Delta\psi_{sc}$ is the corresponding change across the serosal membrane of a surface epithelial cell.

Rose and Schultz (1971) described the analysis of a similar problem resulting from transport of sugars or amino acids across the mucosal membrane of the intestinal epithelial cell. The model could not be used directly

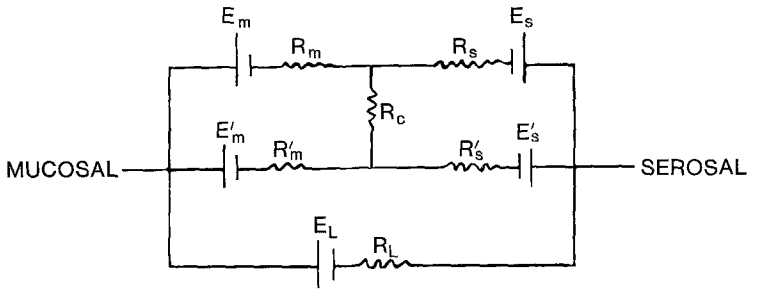

Fig. 4. An electrical circuit equivalent to *Necturus* gastric mucosa. The subscript *s* stands for serosal; *m* for mucosal, *L* for paracellular shunt. Primed letters designate oxyntic cell, nonprimed surface cell. *E* designates an emf and *R* a resistance. R_c is a coupling resistance between surface cells, oxyntic cells, and between the two cell types. In this diagram it designates coupling between surface and oxyntic cells. It is considered equal in magnitude within and between the two cell systems

since gastric fundic mucosa is composed of at least two cell types. We extended their model to include two cell types. Fig. 4 is the equivalent circuit for this model. E_s and E_s' represent the emf's, and R_s and R_s' represent the resistivities of the serosal membranes of surface epithelial and oxyntic cells (primed letter), respectively. E_m and E_m' are the emf's, and R_m and R_m' are the resistivities of the mucosal membranes of surface epithelial and oxyntic cells, respectively. E_L and R_L represents an emf and a resistivity of a transepithelial shunt pathway. R_c represents an intercellular coupling resistance of the type described by Loewenstein and Kanno (1964). Coupling between cells in isolated gastric tubules has been demonstrated previously (Blum *et al.*, 1971) and is quantitated for surface cells (vide infra). In the model we have assumed that the surface cell system and the oxyntic cell system of the gastric glands, as well as the two cell systems, are connected by the coupling resistance. Further, we have assumed that R_c is equal in the two cell systems. Experimentally the emf's are changed by altering ionic composition; in the analysis we have assumed that ΔE_s or $\Delta E_s' \gg \Delta E_L$. For simplicity ΔE_L is taken as 0. For validation of these assumptions please refer to the Discussion.

The equivalent circuit is used to predict the response, $\Delta\psi_{sc}$ and $\Delta\psi_{ms}$, if the Δemf arises in (1) E_s or (2) E_s' or (3) $E_s + E_s'$. In each instance the intercellular coupling resistance is either (a) zero or (b) infinite. In the first case (ΔE_s) the ratio $\Delta\psi_{sc}/\Delta\psi_{ms}$, the Δp.d. across the serosal membrane/Δp.d. across the tissue resulting from the change in ionic activity, is given in Eq. (2) if $R_c \to 0$ or Eq. (3) if $R_c \to \infty$. (For further derivation *see* Appendix.)

$$\Delta\psi_{sc}/\Delta\psi_{ms}=1+\frac{R_m R'_m}{R_L(R_m+R'_m)} \tag{2}$$

$$\Delta\psi_{sc}/\Delta\psi_{ms}=1+\frac{R_m}{R_L}+\frac{R_m}{R'_s+R'_m}. \tag{3}$$

If the response is due to a change in E'_s the ratio of $\Delta\psi_{sc}/\Delta\psi_{ms}$ is given in Eq. (2) if $R_c\to 0$ or Eq. (4) if $R_c\to\infty$:

$$\Delta\psi_{sc}/\Delta\psi_{ms}=\frac{R_s}{R_s+R_m}. \tag{4}$$

The third case is that the change in ionic composition affects a change of emf of the serosal membranes of both the surface epithelial and the oxyntic cells. Under these conditions the relationship of Eq. (2) still holds if $R_c\to 0$. Eq. (2) is valid for *any* change in either E_s or E'_s. If $R_c\to\infty$, a more complicated relationship exists:

$$\Delta\psi_{sc}/\Delta\psi_{ms}=\frac{R_m}{R_L\left[1+\frac{\Delta E'_s}{\Delta E_s}\left(\frac{R_s+R_m}{R'_s+R'_m}\right)\right]}+\frac{1+\frac{R_m+\left(\frac{\Delta E'_s}{\Delta E_s}\right)R_s}{R'_m+R'_s}}{1+\frac{\Delta E'_s}{\Delta E_s}\left(\frac{R_s+R_m}{R'_s+R'_m}\right)}. \tag{5}$$

The range of each function may be helpful in analysis of experimental results. Eqs. (2) and (3) will produce values greater than unity. In contrast, Eq. (4) will produce values from 0 to 1, but none larger. Only Eq. (5) will produce values for $\Delta\psi_{sc}/\Delta\psi_{ms}$ ranging above zero but not limited at one. Clearly, Eqs. (3) and (4) are only special cases of Eq. (5) where $\Delta E_s \gg \Delta E'_s$ and $\Delta E'_s \gg \Delta E_s$, respectively.

Necturus fundic mucosae give a spectrum of $\Delta\psi_{sc}/\Delta\psi_{ms}$ (Table 3) in response to the constant product KCl change. Values from 0.59 to 0.94 are obtained. Clearly, with these values (mean 0.77), Eqs. (2) and (3) do not apply, yet the surface cells are not responding passively to a $\Delta E'_s$. If the surface cell $\Delta\psi_{sc}$ is only a function of a $\Delta E'_s$ (Eq. 4), $\Delta\psi_{sc}/\Delta\psi_{ms}$ will be given by the voltage divider ratio:

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}}=\frac{R_s}{R_m+R_s}=\frac{1}{\frac{R_m}{R_s}+1}=0.46.$$

In fact, not even one mucosal response is predicted by its voltage divider ratio. Therefore, the surface cell must have a ΔE_s, and the correct analytical

model would have to include a $\Delta E_s + \Delta E'_s$ and R_c would have to be large to give a ratio < 1, hence Eq. (5). This requirement, that R_c is large, is not in agreement with intercellular coupling found in this tissue (*see below*).

Eq. (5) predicts that $\Delta\psi_{sc}/\Delta\psi_{ms}$ measured in a surface cell will be less than unity only when $\Delta E_s \ll \Delta E'_s$. ΔE_s can be quantitated if R_c is large. $\Delta\psi_{mc}$ results from a change in current flow across R_m (12.0 mV) (Table 3). From the voltage divider ratio, R_m/R_s, ΔIR_s is 10.2 mV. Thus ΔE_s, due to the constant product change, is 31.8 mV. It has not been possible to directly measure the potential changes in an oxyntic cell, but assuming even a maximum change of 58 mV (the Nernst potential) ΔE_s is not small relative to $\Delta E'_s$.

Inconsistencies, that R_c is large and that ΔE_s is small compared to $\Delta E'_s$, lead to the conclusion that E_m or E'_m may be undergoing some change – specifically an electrogenic mechanism (the Cl^- pump, a component of E'_m) is being inhibited by the low serosal $[Cl^-]$.

For a $\Delta E_s + \Delta E'_s + \Delta E'_m$, $\Delta\psi_{sc}/\Delta\psi_{ms}$ is given by Eq. (6), if $R_c \to 0$:

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}} = \frac{(\Delta E_s R'_s + \Delta E'_s R_s)[R_L(R_m + R'_m) + R_m R'_m] + R_s R'_s R_m \Delta E'_m}{R_L(R_m + R'_m)(\Delta E_s R'_s + \Delta E'_s R_s) + R_L R_m (R_s + R'_s)\Delta E'_m}; \tag{6}$$

If $R_c \to \infty$,

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}} = \frac{R_m}{R_L\left[1 + \dfrac{\Delta E'_m + \Delta E'_s}{\Delta E_s}\left(\dfrac{R_s + R_m}{R'_s + R'_m}\right)\right]} + \frac{1 + \dfrac{R_m + R_s\left(\dfrac{\Delta E'_m + \Delta E'_s}{\Delta E_s}\right)}{R'_s + R'_m}}{1 + \dfrac{\Delta E'_m + \Delta E'_s}{\Delta E_s}\left(\dfrac{R_s + R_m}{R'_s + R'_m}\right)}. \tag{7}$$

Eqs. (6) and (7) predict that inhibition of anion (Cl^-) transport ($\Delta E'_m$), and an increase in E'_s due to $\Delta[K^+]$ would be additive. ΔE_s could appear small compared to the sum $\Delta E'_s + \Delta E'_m$.

To examine the involvement of E'_m we have performed similar experiments utilizing a 10-fold change of $[K^+]$ from 5 to 50 mM; presumably, Cl^- emf is constant under these conditions. In these experiments sulfate replaces Cl^- in the mucosal solution while $[Cl^-]$ is 80 mM in the serosal solution. Table 4 summarizes the data from six experiments in which the serosal solution $[K^+]$ is changed. Fig. 5 shows similar data obtained when Cl^- is replaced by SO_4^{2-} in both serosal and mucosal solutions.

When $[Cl^-]$ is constant, $\Delta\psi_{sc}$ is 34.7 ± 5.79 mV and $\Delta\psi_{ms}$ is 30.3 ± 7.11 mV. The ratio $\Delta\psi_{sc}/\Delta\psi_{ms}$ is 1.17. All experiments (Table 4) give $\Delta\psi_{sc}/\Delta\psi_{ms} > 1$. With $[Cl^-]$ constant these results are very different from

Table 4. Change in electrical parameters with change in serosal $[K^+]$

	R_{trans} (before ΔK^+)	R_{trans} (after ΔK^+)	R_m/R_s (before ΔK^+)	R_m/R_s (after ΔK^+)	$\Delta\Psi_{ms}$	$\Delta\Psi_{sc}$	$\Delta\Psi_{mc}$	$\Delta\Psi_{sc}/\Delta\Psi_{ms}$
	2025	1822	1.62	1.58	21	32	−11	1.52
	2767	2700	1.61	1.44	39	42	− 3	1.08
	2160	1755	1.73	1.64	22.5	25	− 2.5	1.11
	2497	2430	1.72	1.5	35	38	− 3	1.09
	2835	2295	1.63	1.48	33	36	− 3	1.09
	3105	2902	1.88	1.44	31	35	− 4	1.13
$\bar{X}\pm$	2565±	2317±	1.70±	1.51±	30.3±	34.7±	− 4.42±	1.17±
SD	416	461	0.10	0.08	7.11	5.79	3.26	0.17

R_{trans} designates the transepithelial resistance determined from the transepithelial Δ p.d. when 10 μA/cm² is sent transepithelially. R_m/R_s is the Δ p.d. across the mucosal membrane: Δ p.d. across the serosal membrane of a surface epithelial cell when 10 μA/cm² is sent transepithelially. $\Delta\Psi_{ms}$ is the transepithelial change in p.d. and $\Delta\Psi_{sc}$ is the change in p.d. across the serosal membrane of a surface epithelial cell resulting from the $\Delta[K^+]$ from 5 to 50 mM. All experiments are done with SO_4^{2-} replacing Cl^- in the mucosal solution while $[Cl^-]=80$ mEquiv/liter in the serosal solution

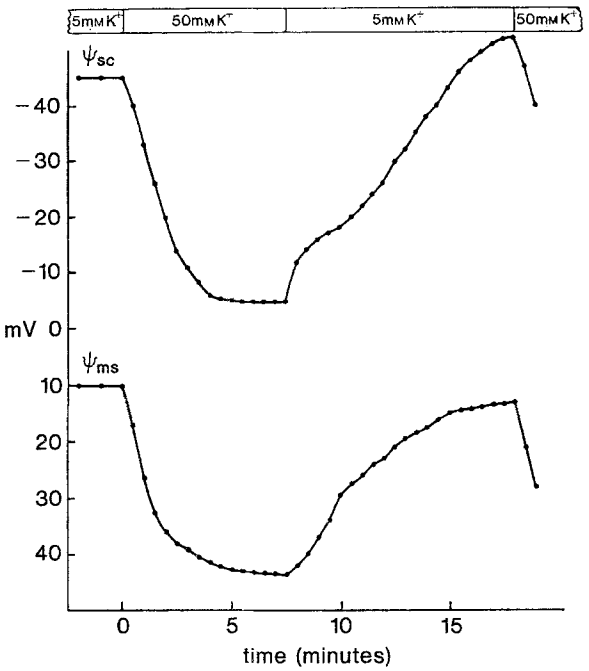

Fig. 5. This figure demonstrates the effect of a 10-fold change in $[K^+]$ in the serosal bathing solution. The transepithelial potential Ψ_{ms}, and the potential from the serosal solution to intracellular electrodes, Ψ_{sc}, are recorded. $[K^+]$ in the serosal solution is recorded at the top of the figure, and sulfate replaces Cl^- in both serosal and mucosal solutions. Note that in these experiments, in contrast to the constant product KCl changes, the $\Delta\Psi_{sc}$ is greater than the $\Delta\Psi_{ms}$

those obtained using constant product KCl changes. Constant [Cl^-] seems to avoid the $\Delta E'_m$ which occurs in changing to [Cl^-] serosal solution.

With the intercellular coupling demonstrated below, the equivalent circuit should include an R_c which is small and can be taken to approach zero. If $R_c \to 0$, Eq. (2) is valid for a change in E_s, E'_s, or both. Hence, Eq. (2) is used for further analysis.

$$0.17 = \frac{R_m R'_m}{R_L(R_m + R'_m)}.$$

Under the conditions that $R_c \to 0$, the voltage divider ratio R_m/R_s actually reflects the mucosal membranes in parallel and serosal membranes in parallel.

$$\text{Voltage divider ratio} = \frac{\dfrac{R_m R'_m}{R_m + R'_m}}{\dfrac{R_s R'_s}{R_s + R'_s}}.$$

Thus, from Table 4

$$\frac{R_m R'_m}{R_m + R'_m} = 1.70 \frac{R_s R'_s}{R_s + R'_s} = 0.17 R_L.$$

Utilizing the equivalent electrical circuit where $R_c \to 0$

$$R_L = 4.70 R_{\text{trans}} = 12{,}055\ \Omega\,\text{cm}^2$$

whereas cellular resistance in the mucosa is:

$$\frac{R_m R'_m}{R_m + R'_m} + \frac{R_s R'_s}{R_s + R'_s} = R_{\text{cell}} = 3{,}258\ \Omega\,\text{cm}^2.$$

Thus the cellular and paracellular shunt conductance of fundic gastric mucosa can be estimated utilizing a coupling resistance which approaches zero. The paracellular shunt resistance is 3.70 times the cellular resistance.

Double Microelectrode Experiments

When pulses of 5.9×10^{-9} amps are injected intracellularly, radial coupling can be demonstrated by measuring the resulting intracellular Δ p.d. as a function of distance from the current source. Fig. 6 shows the voltage recorded at several points 33 μ and 66 μ from the current-sending electrode. Radial coupling is shown. The site or molecular anatomy of the low resis-

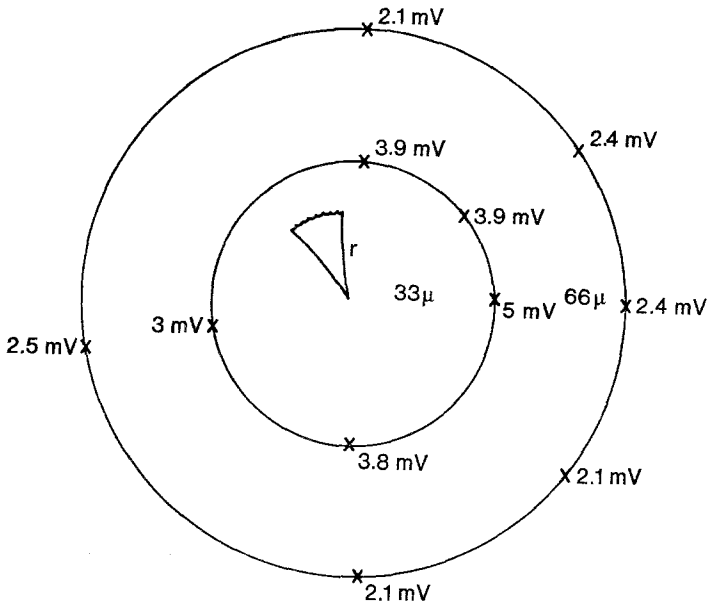

Fig. 6. This figure demonstrates the Δp.d. recorded in several cells at 33 and 66 μ from a surface epithelial cell at $r=0$ when 5.9×10^{-9} amps is injected in the cell. Coupling is demonstrated to be radial

tance pathway is, at present, unknown, but "tight junctions" have been demonstrated in gastric mucosa.

Fig. 7 shows the Δp.d. recorded in cells at various distances from the current-sending electrode. To analyze this curve we utilize the model developed by Frömter (1972) for the gallbladder. According to this model the mucosa is a flat epithelial sheet defined by its thickness (L) and resistance (R_c) to current flow radially within the sheet and the resistance (R_Z) to flow of current across the limiting membrane into the bathing solution. The differential equation defining the potential as a function of distance (r) is:

$$\frac{d^2V}{dr^2}+\frac{1}{r}\frac{dV}{dr}-\frac{1}{\lambda^2}V=0. \tag{8}$$

λ, the space constant, is defined by $\lambda^2=(L\,R_Z)/R_c$. The solution of the differential equation is the zero order modified Bessel function (K_0) of the second kind.

$$V_r=AK_0(r/\lambda) \quad \text{where } A=(I_0\,R_c)/2\pi L. \tag{9}$$

A numeric solution is obtained using an iterative technique with an IBM 370 computer. The solution is that value of λ for which A is most constant at the experimental r's.

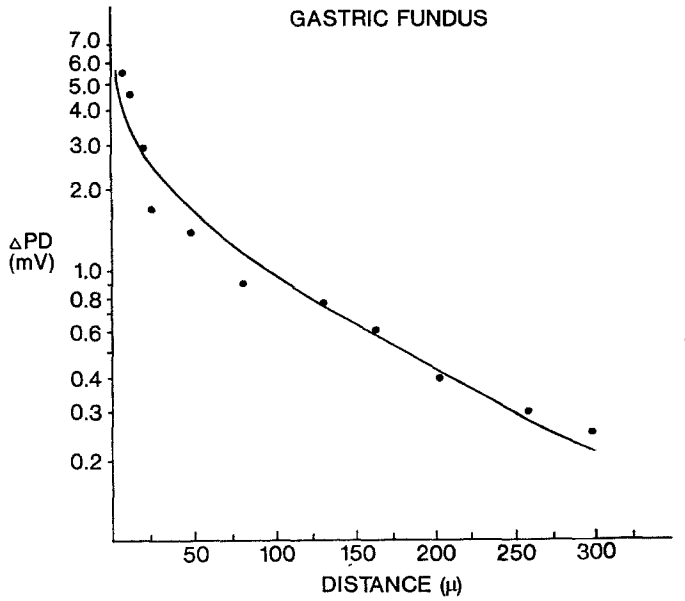

Fig. 7. The voltage recorded intracellularly at a distance r from the current-sending electrode in the gastric fundus. The curve drawn is the Bessel function, $V=AK_0(r/\lambda)$, generated from the experimentally derived parameters A and λ

The results for 11 different experiments are given in Table 5. A space constant λ ranges from 196 to 960 μ. The mean λ is 408 μ and standard deviation is 217 μ.

The computed value of λ and the electrical circuit equivalent to R_Z (Fig. 8) provide an alternate quantitation of the cellular and shunt resistance in the intact mucosa. This circuit differs from that used by Frömter (1972) in that current leaving surface cells via the mucosal membranes must return to the reference side by crossing the transepithelial resistance. Frömter (1972) used only the shunt conductance in the return limb presumably because transepithelial reistance in gallbladder is dominated by the shunt resistance.

R_s, the serosal cell membrane resistivity, is the positive solution of the quadratic equation:

$$-[R_m/R_s]\,R_s^2+[R_Z+(R_m/R_s)\,R_Z-R_{\text{trans}}]\,R_s+R_{\text{trans}}\,R_Z=0. \tag{10}$$

R_M, the mucosal cell membrane resistivity, is

$$R_M=(R_m/R_s)\,R_s \tag{11}$$

and the shunt resistivity R_L is

$$R_L=\frac{R_{\text{trans}}(R_m+R_s)}{(R_m+R_s)-R_{\text{trans}}} \tag{12}$$

Table 5. Gastric fundus

	Space constant λ (microns)	g_{cell} ($\times 10^{-4}\,\Omega^{-1}\,cm^{-2}$)	g_{trans} ($\times 10^{-4}\,\Omega^{-1}\,cm^{-2}$)
	300	5.72	4.36
	344	5.59	4.36
	196	5.59	6.73
	344	5.42	4.36
	340	5.40	4.36
	242	5.18	6.73
	960	3.24	8.72
	604	3.51	4.36
	256	2.24	6.17
	410	2.08	3.90
	492	2.03	3.98
Mean$\pm$SD	408$\pm$217	4.18$\pm$1.57	5.28$\pm$1.57

λ is the "space constant" determined experimentally. g_{trans} is $1/R_{trans}$ determined by sending 10 $\mu A/cm^2$ transepithelially. R_{trans} used for calculation is $2.7 \times R_{trans}$ measured to account for surface area of gastric glands (*see text*). $g_{cell}(1/R_{cell})$ is calculated as given in the text

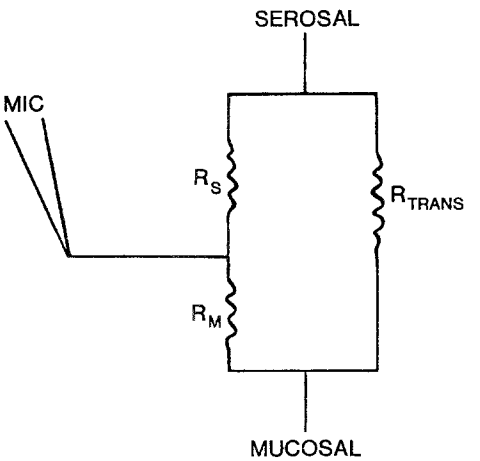

Fig. 8. A simplified circuit equivalent to R_Z as measured in the two microelectrode experiments. Current may flow across the serosal cell membrane represented by R_S or alternatively across the mucosal membrane R_M and back across the tissue, where R_{trans} is the lumped tissue resistance to transepithelial current flow

and the shunt-to-cell resistivity ratio is

$$\frac{R_L}{R_m+R_s}=\frac{R_{trans}}{(R_m+R_s)-R_{trans}}. \tag{13}$$

These equations are directly applicable to a flat tissue such as the gallbladder. In contrast, *Necturus* fundic mucosa is multiply indented with glands

which greatly increase the surface area subjected to measurements. Blum *et al.* (1971) have shown that for each cm^2 of surface area there is 1.7 cm^2 of tubular surface area. Thus, the transepithelial resistivity is calculated from the surface plus glandular surface area assuming that conductance per unit area is identical for the two cell types, and thus, transepithelial resistance is corrected for this increased surface area (2.7 × measured R_{trans}). Because some injected current must flow into the tubular glands, the distance of current spread may be greater than the measured r. λ is also altered by this glandular conductance but no model could be found which considers current spread into the gastric glands.

Table 5 shows the results of 11 experiments in which the cellular conductance and transepithelial conductance are computed. The 11 mucosae demonstrate a mean space constant λ of 408 ± 217 μ. The mean cellular conductance is $4.18 \pm 1.57 \times 10^{-4} \Omega^{-1}\,cm^{-2}$ and mean transepithelial conductance is $5.28 \pm 1.57 \times 10^{-4} \Omega^{-1}\,cm^{-2}$. The shunt conductance is $1.10 \times 10^{-4} \Omega^{-1}\,cm^{-2}$. The ratio of cell-to-shunt conductance is 3.80. In terms of resistance the ratio of shunt resistance to cell resistance is also 3.80. This value is very close to that obtained using the cellular response to changes in ionic activity (3.70).

In four of 11 experiments (Table 5) the calculated shunt conductance is a small negative value. The gastric pits which form a mosaic of surface discontinuities may provide the explanation for this finding. In *Necturus* these pits are not visible using the stereomicroscope. The probing microelectrode may traverse a pit or avoid them altogether. The gastric glands will act as resistances in parallel to the surface cells surrounding the pit. If the microelectrode traverses near a pit, a lower space constant and an overestimate of cell membrane conductance will result. In fact, the cellular conductance of this group is larger than the cellular conductance of the others. Thus, when this value is subtracted from the transepithelial conductance, the shunt conductance may have a negative value.

Discussion

Data derived from chambered gastric fundic mucosa suggest that gastric fundic mucosa is quite tight. It develops a p.d. of 30 to 40 mV, supports a 10^6-fold H^+ gradient, and reacts asymmetrically to changes in ion concentration. *In vivo* studies also suggest that fundic mucosa has conductance properties quite different from other areas of the gastrointestinal tract. Dyck, Werther and Rudick (1969) and Himal, Young, Rudick, Werther and Janowitz (1970) studied the loss of ions from the lumen of the GI tract

and found that "back flux of ions" in the antrum was 15 times, in the duodenum 45 times and in the small intestine 60 times larger than that in gastric fundus. With this supporting evidence it is essential to quantitate the magnitude of the cellular and transepithelial shunt resistance in fundic gastric mucosa.

Using two techniques we find that the shunt conductance is small compared to tissues such as intestine, gallbladder, or proximal tubule. A change of $[K^+]$ in the serosal solution causes a transepithelial Δp.d. and a Δp.d. across the serosal membrane of the surface epithelial cell. These changes result from a Δemf of the serosal membrane of both the surface and oxyntic cells. Estimation of the shunt resistance requires analysis of an electrical circuit equivalent to the gastric mucosa (Fig. 4). The analysis is greatly simplified if one or two elements can be perturbed while the others are held constant. Specifically, we assume that (1) the transepithelial shunt emf incurs no significant ΔE_L and that the diffusion potential due to change in $[K^+]$ and $[Cl^-]$ is small compared to the Δp.d. measured; (2) the changes in p.d. are a result of a change in an emf across the serosal membrane of the surface or oxyntic cells; and (3) that the resistance of surface and oxyntic cell membranes and the transepithelial shunt undergoes negligible change.

To quantitate cellular and shunt resistance from experiments utilizing changes in ionic composition we assume that $\Delta E_L \ll \Delta E_{\text{cell}}$. The shunt pathway may be considered to be a single "thick" membrane, and its response to ion changes is symmetric. A 10-fold constant product change in the mucosal solution causes a tissue response of 6.5 mV. If we assume that this is all due to a change in E_L, an identical ΔE_L occurs with a serosal constant product change. At most, this is 12% of the cell membrane change. This is clearly a maximal value, since we neglected any possible cell membrane change.

Similarly, changes of $[K^+]$ in mucosal and serosal solutions, show a highly asymmetric response; the mucosal response is only a small fraction of the serosal response (Sachs *et al.*, 1971). Thus, the shunt in gastric mucosa, like gallbladder (Diamond *et al.*, 1971), does not discriminate significantly between K^+ and Na^+.

The model allows prediction of $\Delta\psi_{sc}/\Delta\psi_{ms}$ if the Δemf occurs solely in the paracellular shunt. Eqs. (14) and (15) give the $\Delta\psi_{sc}/\Delta\psi_{ms}$ if the changes were due only to a ΔE_L while all other emf's are constant. If $R_c \to 0$

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}} = \frac{\dfrac{R_s R_s'}{R_s + R_s'}}{\dfrac{R_s R_s'}{R_s + R_s'} + \dfrac{R_m R_m'}{R_m + R_m'}} \tag{14}$$

while if $R_c \to \infty$

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}} = \frac{R_s}{R_s + R_m}. \tag{15}$$

$\Delta\psi_{sc}/\Delta\psi_{ms}$ must be less than 1 and must reflect the voltage divider ratio. From Table 4, $\Delta\psi_{sc}/\Delta\psi_{ms}$ would be 0.39 if the change were due only to ΔE_L. In contrast, $\Delta\psi_{sc}/\Delta\psi_{ms}$ was 1.17. According to this model for gastric mucosa, the Δemf in these experiments does not arise from the paracellular shunt. Comparing the predicted $\Delta\psi_{sc}/\Delta\psi_{ms}$ to the actual results, the shunt would appear to contribute minimally to the Δemf.

Further, ΔE_L can be quantitated in the $\Delta[K^+]$ experiments. Calculated ΔI_{sc} is 12 μamps cm^{-2} in these experiments. R_{trans} and R_m/R_s are the mean values from Table 4. Cell resistance and shunt resistance are taken from Table 5 because these values do not depend on ionic gradients. ΔIR is calculated for both membranes giving ΔE_L of -1.08 mV. In contrast, ΔE_s is calculated to be 37.3 mV. Thus, by this method ΔE_L is estimated to be only 2% of ΔE_s. By these three criteria we find $\Delta E_L \ll \Delta E_s$ and consider that the experimental design conforms adequately to the requirements of the model.

The origin of the Δemf within the cell is also crucial to analysis of these experiments. Based on the equivalent circuit (Fig. 4) and the assumption that the constant product KCl change affects only E_s and E'_s, a $\Delta\psi_{sc}/\Delta\psi_{ms} < 1$ can only arise if $\Delta E_s \ll \Delta E'_s$, and only if R_c is large. It can be calculated, however, that ΔE_s is 31.8 mV. The obvious explanation for this discrepancy is that another E is changing (i.e. E'_M, the electrogenic Cl^- pump emf) due to the reduction in $[Cl^-]$ from 80 to 8 mM. Thus when $[Cl^-]$ is kept constant, and only $[K^+]$ is changed from 5 to 50 mM, the ratio $\Delta\psi_{sc}/\Delta\psi_{ms}$ is invariably greater than 1. With a small coupling resistance the serosal or mucosal membrane of the surface and oxyntic cells are parallel resistances. $\Delta\psi_{mc} = -4.42$ mV is the ΔIR across $R_m R'_m/(R_m + R'_m)$. ΔIR across $R_s R'_s/(R_s + R'_s)$ is -2.60 mV and the Δemf across the serosal membranes is 37.3 mV. It is not possible to quantitate ΔE_s and $\Delta E'_s$ since the relative magnitude of R_s and R'_s remain unknown. With this information, and the low value for R_c derived from the two electrode experiments we can calculate the shunt conductance contribution to tissue conductance.

The effect of $\Delta[K^+]$ and constant product KCl changes on membrane resistance is assessed by the voltage divider ratio and by transepithelial resistance. There is a small (9.6%) but statistically significant change in transepithelial resistance ($t = 3.20$, $0.01 < p < 0.025$). The change in the ratio

R_m/R_s ($t = 3.24$, $0.01 < p < 0.025$) as a result of change in ionic activity is statistically significant, but is only 10.8%.

Identical conclusions are derived from current spread and changes of ionic activity methods, although the assumptions of the analysis are different. The ratio of shunt resistance to cell resistance is 3.70 when assessed by change in ionic activity and 3.80 when assessed by radial spread of current injected intracellularly in a surface cell.

By this latter technique, however, 4/11 mucosae gave small negative values for the shunt conductance. The remainder gave small positive values. We have felt this represents experimental error which is most manifest in mucosae with large shunt resistance (small shunt conductance);

$$g_{\text{tissue}} - g_{\text{cell}} \to 0.$$

Analysis of current spread in mucosa such as proximal tubule gallbladder, or intestine with a large tissue conductance compared to cellular conductance can tolerate small errors in cellular conductance. Mucosae where g_{tissue} approaches g_{cell} cannot. Alternatively, the model of a flat epithelial sheet does not account for tubular indentations (gastric pits) which can be considered high conductance discontinuities in the plane of the tissue. Unfortunately, a mathematical expression to describe a mucosa multiply indented by tubular glands could not be found.

It would appear from these studies that techniques independent of tissue geometry are most applicable to gastric fundic mucosa; but by using an adequately large sample of mucosae, techniques dependent on mucosal geometry can be used. Whichever technique is used, the shunt conductance of the *Necturus* fundic gastric mucosa is only 1/5 of tissue conductance.

Appendix

The fundic mucosa of *Necturus* is composed of two major cell types – the surface epithelial and oxyntic cells. A low resistance pathway has been demonstrated between cells of the same type and between the different types. The electrical circuit equivalent to a mucosa with these characteristics is shown in Fig. 4. This circuit was analyzed by first obtaining an expression for the current flow I_c across the coupling resistance:

$$I_c R_c = (E_s - E_s') + I_s R_s - I_s' R_s'$$

where the subscripts s and m refer to the serosal and mucosal membrane of the surface cell system and s' and m' refer to the same membranes of the

Table 6. Formulas

	$R_c \to 0$
Change due to ΔE_s	$1+\dfrac{R_m R'_m}{R_L(R_m+R'_m)}$
Change due to $\Delta E'_s$	$1+\dfrac{R_m R'_m}{R_L(R_m+R'_m)}$
Change due to $\Delta E_s+\Delta E'_s$	$1+\dfrac{R_m R'_m}{R_L(R_m+R'_m)}$
Change due to $\Delta E_s+\Delta E'_s+\Delta E'_m$	$\dfrac{[R_L(R_m+R'_m)+R_m R'_m](\Delta E_s R'_s+\Delta E'_s R_s)+\Delta E'_m R_m R_s R'_s}{R_L(R_m+R'_m)(\Delta E_s R'_s+\Delta E'_s R_s)+\Delta E'_m R_m R_L(R_s+R'_s)}$

Formulas relating change in p.d. across the serosal membrane of a surface cell to the change in p.d. measured transepithelially when there is a change in one or more emf in the surface, oxyntic cell, or both. To obtain these formulas the coupling resistance is

oxyntic cell system. I_s and I'_s can then be found from the expression for the p.d. drop (ψ_{ms}) transmucosally and by substitution:

$$I_s=\frac{\psi_{ms}[R'_s(R_m+R'_m)-R_c(R'_s+R'_m)]-E_s[R'_s(R_m+R'_m)+R_m R'_m-R_c(R'_s+R'_m)]+E_m[R_c(R'_s+R'_m)-R'_m R'_s]+R_m R'_m E'_s-R_m R'_s E'_m}{R_m R_s(R'_s+R'_m)+R'_m R'_s(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)}$$

$$I'_s=\frac{\psi_{ms}[R_s(R_m+R'_m)-R_c(R_s+R_m)]+R_m R'_m E_s-R_s R'_m E_m-E'_s[R_s(R_m+R'_m)+R_m R'_m-R_c(R_s+R_m)]+E'_m[R_c(R'_s+R'_m)-R_s R_m]}{R_m R_s(R'_s+R'_m)+R'_m R'_s(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)}.$$

By appling Kirchhoff's law ψ_{ms} can be found to be

$$\psi_{ms}=\frac{\begin{aligned}&E_s R_L[R'_s(R_m+R'_m)-R_c(R'_s+R'_m)]+E_m R_L[R'_m(R_s+R'_s)-R_c(R'_s+R'_m)]\\&+E'_s R_L[R_s(R_m+R'_m)-R_c(R_s+R_m)]+E'_m R_L[R_m(R_s+R'_s)-R_c(R_s+R_m)]\\&+E_L[R_m R_s(R'_m+R'_s)+R'_m R'_s(R_m+R_s)-R_c(R_s+R'_s)(R_m+R'_m)]\end{aligned}}{\begin{aligned}&R_L(R_m+R'_m)(R_s+R'_s)+R_m R_s(R'_m+R'_s)+R'_m R'_s(R_m+R_s)\\&-R_c R_L(R_m+R_s+R'_m+R'_s)-R_c(R_s+R_m)(R'_s+R'_m)\end{aligned}}$$

relating $\Delta\psi_{sc}/\Delta\psi_{ms}$
$R_c \to \infty$
$1+\dfrac{R_m}{R_L}+\dfrac{R_m}{R'_m+R'_s}$
$\dfrac{R_s}{R_m+R_s}$
$\dfrac{R_m}{R_L\left[1+\dfrac{\Delta E'_s}{\Delta E_s}\left(\dfrac{R_s+R_m}{R'_s+R'_m}\right)\right]}+\dfrac{1+\dfrac{R_m+\left(\dfrac{\Delta E'_s}{\Delta E_s}\right)R_s}{R'_m+R'_s}}{1+\dfrac{\Delta E'_s}{\Delta E_s}\left(\dfrac{R_s+R_m}{R'_s+R'_m}\right)}$
$\dfrac{R_m}{R_L\left[1+\dfrac{\Delta E'_m+\Delta E'_s}{\Delta E_s}\left(\dfrac{R_s+R_m}{R'_s+R'_m}\right)\right]}+\dfrac{1+\dfrac{R_m+R_s\left(\dfrac{\Delta E'_m+\Delta E'_s}{\Delta E_s}\right)}{R'_m+R'_s}}{1+\dfrac{\Delta E'_m+\Delta E'_s}{\Delta E_s}\left(\dfrac{R_s+R_m}{R'_s+R'_m}\right)}$

taken to be small compared to membrane resistances and is visualized to approach zero. Alternatively, R_c is visualized as large compared to membrane resistance and is visualized to approach infinite size

and ψ_{sc}, the voltage drop across the serosal membrane of the surface epithelial cell, is found by substitution into

$$\psi_{sc}=E_s+I_sR_s$$

$$\psi_{sc}=\frac{\begin{array}{l}\{R_L(R_m+R'_m)(R_s+R'_s)+R_mR_s(R'_m+R'_s)+R'_mR'_s(R_m+R_s)\\ -R_c[R_L(R_m+R_s+R'_m+R'_s)+(R_s+R_m)(R'_s+R'_m)]\}\{E_s[R_mR'_mR'_s\\ -R_cR_m(R'_m+R'_s)]+E_m[R_sR'_mR'_s-R_cR_s(R'_s+R'_m)]+E'_sR_mR_sR'_m\\ +E'_mR_mR_sR'_s\}+R_L[R_sR'_s(R_m+R'_m)-R_cR_s(R'_s+R'_m)]\{E_s[R'_s(R_m+R'_m)\\ -R_c(R'_s+R'_m)]+E_m[R_c(R'_s+R'_m)-R'_m(R_s+R'_s)]+E'_s[R_s(R_m+R'_m)\\ -R_c(R_s+R_m)]+E'_m[R_c(R_s+R_m)-R_m(R_s+R'_s)]\}+E_L[R_sR'_s(R_m+R'_m)\\ -R_cR_s(R'_s+R'_m)][R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)]\end{array}}{\begin{array}{l}[R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)][R_L(R_m+R'_m)\\ \cdot(R_s+R'_s)+R_mR_s(R'_m+R'_s)+R'_mR'_s(R_m+R_s)-R_cR_L(R_m+R_s+R'_m+R'_s)\\ -R_c(R_s+R_m)(R'_s+R'_m)]\end{array}}.$$

Using these expressions we can obtain an expression for the ratio $\Delta\psi_{sc}/\Delta\psi_{ms}$ over some increment in ΔE_s, $\Delta E'_s$ or $\Delta E_s+\Delta E'_s$. When there is only a change

ΔE_s, while E'_s remains constant

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}} = \frac{\begin{array}{l}\{[R_L(R_m+R'_m)(R_s+R'_s)+R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)]\\ -R_c[R_L(R_s+R_m+R'_s+R'_m)+(R_s+R_m)(R'_s+R'_m)]\}[R_mR'_sR'_m\\ -R_c(R_s+R_m))R'_s+R'_m)+R_cR_s(R'_s+R'_m)]+[R_LR_sR'_s(R_m+R'_m)\\ -R_cR_LR_s(R'_s+R'_m)][R'_s(R_m+R'_m)-R_c(R'_s+R'_m)]\end{array}}{\begin{array}{l}R_L[R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)]\\ \cdot[R'_s(R_m+R'_m)-R_c(R'_s+R'_m)\end{array}}.$$

Alternatively for some increment in E'_s, $\Delta E'_s$, while E_s is constant, the function relating the change measured across the serosal membranes of surface epithelial cells to the transmucosal change is

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}} = \frac{\begin{array}{l}R_mR_sR'_m\{R_L(R_m+R'_m)(R_s+R'_s)+R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)\\ -R_c[R_L(R'_s+R'_m+R_s+R_m)+(R_s+R_m)(R'_s+R'_m)]\}+[R_LR_sR'_s(R_m+R'_m)\\ -R_cR_LR_s(R'_s+R'_m)][R_s(R_m+R'_m)-R_c(R_s+R_m)]\end{array}}{\begin{array}{l}R_L[R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)]\\ \cdot[R_s(R_m+R'_m)-R_c(R_s+R_m)]\end{array}}.$$

The change in transmucosal and intracellular p.d. could result from a change in both E_s and E'_s. Thus, the expression relating the potential drop, $\Delta\psi_{sc}$, across the serosal membrane of the surface epithelial cells to the transmucosal potential change, $\Delta\psi_{ms}$

$$\frac{\Delta\psi_{sc}}{\Delta\psi_{ms}} = \frac{\begin{array}{l}\{R_L(R_m+R'_m)(R_s+R'_s)+R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)\\ -R_c[R_L(R_s+R_m+R'_s+R'_m)+(R_s+R_m)(R'_s+R'_m)]\}\\ \cdot\left[R_mR'_m\left(R_s+\left(\frac{\Delta E'_s}{\Delta E_s}\right)R_s\right)-R_c(R_s+R_m)-R_cR_s(R'_s+R'_m)\right]\\ +[R_LR_sR'_s(R_m+R'_m)-R_cR_LR_s(R'_s+R'_m)]\\ \cdot\left\{\left(\frac{\Delta E'_s}{\Delta E_s}\right)[R_s(R_m+R'_m)-R_c(R_s+R_m)]+R'_s(R_m+R'_m)-R_c(R'_s+R'_m)\right\}\end{array}}{\begin{array}{l}R_L[R_sR_m(R'_s+R'_m)+R'_sR'_m(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)]\\ \cdot\left\{R'_s(R_m+R'_m)-R_c(R'_s+R'_m)+\left(\frac{\Delta E'_s}{\Delta E_s}\right)[R_s(R_m+R'_m)-R_c(R_s+R_m)]\right\}\end{array}}.$$

Lastly, the change in potential $\Delta\psi_{sc}$ and $\Delta\psi_{ms}$ could result from a change in E'_m as well as in $\Delta E_s+\Delta E'_s$. In this case the relation is still more complex.

$$\frac{\Delta\psi_{cs}}{\Delta\psi_{ms}} = \frac{\begin{aligned}&\{R_L(R_m+R'_m)(R_s+R'_s)+R_m R_s(R'_m+R'_s)+R'_m R'_s(R_m+R_s)\\&-R_c[R_L(R_m+R_s+R'_m+R'_s)+(R_s+R_m)(R'_s+R'_m)]\}\{\Delta E_s[R_m R'_m R'_s\\&-R_c R_m(R'_m+R'_s)]+\Delta E'_s R_m R_s R'_m+\Delta E'_m R_m R_s R'_s\}\\&+R_L[R_s R'_s(R_m+R'_m)-R_c R_s(R'_s+R'_m)]\{\Delta E'_s[R'_s(R_m+R'_m)-R_c(R'_s+R'_m)]\\&+\Delta E'_s[R_s(R_m+R'_m)-R_c(R_s+R_m)]+\Delta E'_m[R_c(R_s+R_m)-R_m(R_s+R'_s)]\}\end{aligned}}{\begin{aligned}&R_L[R_s R_m(R'_s+R'_m)+R'_s R'_m(R_s+R_m)-R_c(R_s+R_m)(R'_s+R'_m)]\\&\cdot\{\Delta E_s[R'_s(R_m+R'_m)-R_c(R'_s+R'_m)]+\Delta E'_s[R_s(R_m+R'_m)-R_c(R_s+R_m)]\\&+\Delta E'_m[R_m(R_s+R'_s)-R_c(R_s+R_m)]\}\end{aligned}}.$$

Since in the gastric fundic mucosa the individual membrane resistivities have not been quantitated, some further simplifications are necessary. There are two alternative assumptions: (1) that R_c is much less than all membrane resistances and can be taken to approach zero, or (2) that R_c is large compared to cell membrane resistance and can be taken to approach infinite size. Table 6 gives the formulas which result from these alternative assumptions. Application of these formulas to the gastric fundic mucosa is given in the previous pages.

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References

Blum, A. L., Hirschowitz, B. I., Helander, H. F., Sachs, G. 1971. Electrical properties of isolated cells of *Necturus* gastric mucosa. *Biochim. Biophys. Acta* **241**:261

Clarkson, T. W. 1967. The transport of salt and water across isolated rat ileum. Evidence for at least two distinct pathways. *J. Gen. Physiol.* **50**:695

Diamond, J. M., Barry, P. H., Wright, E. M. 1971. Route of transepithelial ion permeation in the gall bladder. *In*: Electrophysiology of Epithelial Cells. G. Giebisch, editor. p. 23. Schattauer Verlag

Dyck, W. P., Werther, J. L., Rudick, J. 1969. Electrolyte movement across canine antral and fundic mucosa. *Gastroenterology* **56**:488

Frizzell, R. A., Schultz, S. G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum. Influence of shunt on transmucosal Na transport and electrical potential differences. *J. Gen. Physiol.* **29**:318

Frömter, E. 1972. Route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**:259

Himal, H. S., Young, S. B., Rudick, J., Werther, J. L., Janowitz, H. D. 1970. Ionic flux across the duodenal mucosa: Effects of varying concentrations of acid. *Gastroenterology* **58**:959 (Abstr.)

Hodgkin, A. L., Horowicz, P. 1959. The influence of K and Cl ions on membrane potential of single muscle fibers. *J. Physiol.* **148**:127

Hoshi, T., Sakai, F. 1967. Comparison of electrical resistance of surface cell membrane and cellular wall in proximal tubule of Newt kidney. *Jap. J. Physiol.* **17**:627

Loewenstein, W. R., Kanno, Y. 1964. Studies on epithelial (gland) cell junction. *J. Cell Biol.* **22**:65

Mandel, L. J., Curran, P. F. 1972. Response of frog skin to steady state voltage clamping. I. The shunt pathway. *J. Gen. Physiol.* **59**:503

Rose, R. C., Schultz, S. G. 1971. Studies on the electrical potential profile across rabbit ileum: The effect of sugars and amino acids on transmural and transmucosal electrical potential differences. *J. Gen. Physiol.* **57**:639

Sachs, G., Shoemaker, R. L., Blum, A. L., Helander, H. F., Makhlouf, G. M., Hirschowitz, B. I. 1971. Microelectrode studies of gastric mucosa and isolated gastric cells. pp. 257–279. Symp. Med. Hoechst. *In*: Electrophysiology of Epithelial Cells

Shoemaker, R. L., Sachs, G. 1972. Microelectrode studies of *Necturus* gastric mucosa. *In.*: Gastric Secretion. G. Sachs, E. Heinz and K. J. Ullrich, editors. pp. 147–164. Academic Press, New York

Ussing, H. H., Windhager, E. E. 1964. Nature of shunt path and active sodium transport through frog skin epithelium. *Acta Physiol. Scand.* **61**:484

Windhager, E. E., Boulpaep, E. L., Giebisch, G. 1966. Electrophysiological studies on single nephrons. *Proc. 3rd Int. Cong. Nephrol.*, Karger, Basel. Vol. 1, p. 35