Quantitation of Conductance Pathways in Antral Gastric Mucosa

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ABSTRACT The magnitude of cellular and shunt conductance of Necturus gastric antral mucosa was studied by (a) comparing the cellular PD response to transepithelial PD response during changes of ionic activity in the serosal bathing solution and (b) by measurement of current spread within the epithelial sheet. Using constant product KCl changes cellular resistance was 6,788 \( \Omega \text{cm}^2 \) and shunt resistance was 1,803 \( \Omega \text{cm}^2 \). Deletion of HCO\(_3^–\) from the serosal solution produced similar but quantitatively smaller changes in PD. Using HCO\(_3^–\) deletion cellular resistance was 7,338 \( \Omega \text{cm}^2 \) and shunt resistance was 1,973 \( \Omega \text{cm}^2 \). Measurement of current spread within the mucosa avoids changing ionic gradients yet gave very similar results; cellular resistance was 8,967 \( \Omega \text{cm}^2 \) and shunt resistance was 2,947 \( \Omega \text{cm}^2 \). The shunt contribution to transepithelial conductance ranged from 75.2 to 79.0%. Shunt selectivity was assessed using KCl dilution potentials, where mucosal dilution gave a small change in tissue PD compatible with an anion/cation selectivity ratio of 1.16 across the shunt, whereas serosal dilution effect was dominated by a PD change across the serosal membrane of the cell.

The magnitude of the cellular and transepithelial shunt conductance of a variety of epithelia has been quantitated. The proximal tubule (Windhager et al., 1966; Hoshi and Sakai, 1967), gall bladder (Diamond et al., 1971; Frömter, 1972), and intestine (Clarkson, 1967; Frizzell and Schultz, 1972) can be classified as "leaky" tissues with the cell:shunt conductance ratio being 1:10–20. These epithelia demonstrate low transepithelial potential difference (PD), symmetry of PD response to changes in ionic activity, low transepithelial resistance, and failure to support large concentration gradients. In contrast, frog skin, toad bladder, and fundic gastric mucosa develop large concentration gradients, have a high PD, high transepithelial resistance, and have an asymmetric response to changes in ionic activity. These epithelia have cell to shunt conductance ratios > 1 (Mandel and Curran, 1972; Reuss and Finn, 1974; Spenney et al., 1974).
Necturus antrum develops only a low PD dependent on mucosal to serosal Na\(^+\) transport, but has a relatively high resistance (Flemstrom and Sachs, 1974). Therefore quantitation of antral cellular and shunt conductance is of particular interest since antral mucosa is directly exposed to the large \((10^4)\) proton gradients generated by the adjacent fundic mucosa, a "tight" epithelium. We have quantitated these pathways using two techniques: (a) The cellular PD response is compared to transepithelial PD response after either a 10-fold constant product change in KCl or a change in \([\text{HCO}_3^-]\) in the serosal solution. (b) Analysis of current spread within the epithelium using a two-microelectrode technique.

**EXPERIMENTAL METHODS**

Adult Necturus were kept in an aquarium and fed minnows. Animals were killed by severing the spinal cord in the neck. The abdomen was quickly opened and the stomach removed and opened and placed in amphibian Ringer's solution bubbled with 95% O\(_2\)-5% CO\(_2\) at 23°C. The muscularis was stripped from the mucosa which was then stretched and mounted (mucosal surface upward) between two Lucite half chambers; the upper half had an open top to allow use of microelectrodes. The chambers were filled with mucosal and serosal solution (Table I) which were circulated from external reservoirs at 23°C by using 95% O\(_2\)-5% CO\(_2\) as an airlift system. Alternately the mucosa was perfused (15 ml/min) from Marriot bottles bubbled with 95% O\(_2\)-5% CO\(_2\). Fig. 1 shows the electrical arrangement for two microelectrodes. In the single-microelectrode experiments the current-sending electrode, manipulator, current source, and recording connections were deleted from

| TABLE I | COMPOSITION OF SOLUTIONS |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Constant product KCl experiments | HCO\(_3^-\) experiments |
|                | Serosal control | ΔKCl | Mucosal control | Serosal control | ΔHCO\(_3^-\) depletion | Mucosal control |
| Na\(^+\)       | mM              | mM   | mM              | mM              | mM              | mM              |
| K\(^+\)        | 5               | 50   | 5               | 4.0             | 4.0             | 4.0             |
| Cl\(^-\)       | 80              | 8    | 80              | 91.4            | 91.4            | 91.4            |
| SO\(_4^-\)     | 36              | 8    | 8.8             | 7.2             | 7.2             | 7.2             |
| Mg\(^{2+}\)    | 1.0             | 1.0  | —               | 0.8             | 0.8             | 0.8             |
| Ca\(^{2+}\)    | 1.5             | 1.5  | —               | 1.8             | 1.8             | 1.8             |
| HCO\(_3^-\)    | 20              | 20   | 0               | 17.8            | —               | —               |
| H\(_2\)PO\(_4^-\) | 1.0             | 1.0  | —               | 0.8             | 0.8             | —               |
| HEPES          | —               | —    | —               | —               | 5.0             | —               |
| Glucose        | 5               | 5    | 5               | 2.0             | 2.0             | —               |
| Sucrose        | 36              | 15.5 | —               | —               | —               | —               |
| Mannitol       | —               | —    | 6.4             | 11.3            | —               | —               |

Composition of solutions (mM) used in the constant product KCl and Δ(HCO\(_3^-\)) experiments. Composition of solutions used for determination of dilution potential is given in Table V.
The design of the microelectrode chamber and electrical connections used in the dual microelectrode experiments. ME1 is used as the probing electrode while ME2 is used for current injection. Bathing solutions are circulated from external reservoirs; orifices at the edge of the chamber jet circulating fluid across the mucosal and serosal surfaces.

The tissue resistance and the ratio of mucosal:serosal membrane resistance ($R_m/R_s$) were measured by sending 10 μA/cm² through Ag-AgCl wires mounted around the edge of the chamber. The transepithelial PD was measured by a pair of saturated KCl-calomel electrodes (Radiometer K 4112, Radiometer, Copenhagen, Denmark) while the PD across the serosal cell membrane was measured between an intracellular electrode and one of the calomel electrodes. All resistances were corrected for the resistance of solutions. Current sending through the microelectrode or transepithelially was limited to less than 100 ms to minimize polarization effects.

Microelectrodes were prepared using a commercially available two-stage micropipette puller and microforge. Microelectrodes were examined under a microscope, filled with methanol in a vacuum, transferred to distilled water, and subsequently filled with 3 M KCl by diffusion during storage in 3 M KCl at 4°C for 24 h before use. Microelectrodes prepared in this manner were used within 72 h when the tip potential was less than 5 mV and the tip resistance was 5–40 MΩ.

Conduct of Experiments

The tissue mounted in the microelectrode chamber was bathed initially in mucosal solution and serosal solution (Table 1). In the two-microelectrode experiments these solutions alone were used. In experiments with changes of ionic activity, the serosal
solution was perfused from Marriot bottles at a rate of 15 ml/min. Solutions were changed by a stopcock arrangement so that flow rate and pressure were constant. After a change in ionic activity, transmucosal PD and the serosal-microelectrode PD were followed until stable for at least 3 min. During the course of one experiment several such changes can be done.

In the double microelectrode experiments the current-sending electrode was placed intracellularly and the second microelectrode was used to measure the ΔPD in a distant cell when $5.9 \times 10^{-9}$ μA was injected. The probing electrode was moved to cells progressively further from the current-sending electrode. Distance was measured with a micrometer eyepiece. In both types of experiments the relative resistance of the mucosal and serosal membranes ($R_m/R_s$) was measured by the ΔPD of the intracellular electrode (MIC) when current was sent transmucosally (Eq. 1).

$$\frac{R_m}{R_s} = \frac{\Delta PD_{mc}}{\Delta PD_{sc}}. \quad (1)$$

**RESULTS**

**Intracellular PD**

Micropuncture of antral epithelial cells was considered good if (a) there was a rapid negative change of PD which (b) was stable for at least 20 s and (c) the PD returned to the base line upon withdrawal of the microelectrode, (d) there was no change in tip resistance during or after the puncture. Fig. 2 displays the results of micropuncture of 85 cells in seven different *Necturus* antra. The PD across the serosal membrane was $-41.2 \pm 7.13$ mV (cell-solution) and the PD across the mucosal membrane was $-34.6 \pm 7.43$ mV (cell-solution). The mean transepithelial PD (mucosal-serosal) in these experiments was $-6.6$ mV.

![Figure 2. The frequency distribution of intracellular PDs is shown. The upper half of the figure shows the cell-mucosal PD while the lower half shows the cell-serosal PD. In this group of 85 micropunctures the mean transepithelial PD was $-6.6$ mV (mucosal-serosal).](image-url)
**Current Spread within the Mucosa**

In these experiments current ($I_o = 5.9 \times 10^{-9} \text{ A}$) was injected into a cell at $r = 0$. This current and the resulting potential is dissipated across the cell membrane of that cell, and, providing intercellular coupling exists, across the cell membranes of nearby cells. A second microelectrode was used to measure the $\Delta$PD in cells at varying distances from the cell of injection. Fig. 3 shows the $\Delta$PD in cells up to 500 $\mu$m from the site of current injection; electrical coupling

**Figure 3.** The PD is recorded in cells 25-500 $\mu$m from the cell at $r = 0$ into which $5.9 \times 10^{-9} \text{ A}$ was injected. The curve drawn is the Bessel function $V_r = AK_o(r/\lambda)$ generated from the experimentally derived parameters $A$ and $\lambda$. 
between cells of *Necturus* antral epithelium is indicated. Coupling was radial inasmuch as very similar APD's were recorded around a circle of any given radium from the cell of injection.

To further analyze the data we used the methods developed by Frömter (1972) for the gall bladder. In this model the mucosa is visualized as a flat sheet of thickness $L$ which is limited by membranes with resistivity, $R_z$. The resistivity to spread of current within the sheet is $R_s$. The spread of current is then described by the differential equation:

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

where $\lambda$ is the "space constant" defined by $(L R_s/R_c)^{1/2}$. The solution of the differential equation is the zero order modified Bessel function of the second kind:

$$V_r = AK_0(r/\lambda),$$

where $A = I_o R_s/2\pi L$. We quantitated $A$ and $\lambda$ using an IBM 370 computer (International Business Machines Corp., Armonk, N. Y.) programmed to generate the value of the Bessel function at the experimental $r$'s for values of $\lambda$ that are iterated from 10 to 1,000 $\mu$m. The solution was taken to be that value of $\lambda$ for which $A$ is most constant at all experimental $r$'s. Thus

$$\frac{V_r}{K_0(r/\lambda)} = A = \text{a constant}.$$

Membrane and shunt conductances were obtained by analysis of the electrical circuit (Fig. 4) equivalent to $R_z$, in these experiments. This circuit was modified from that used by Frömter (1972) in that current may flow from the cell across either the serosal or mucosal membrane. To complete the circuit, current flowing across the mucosal membrane must flow across the trans-

![Figure 4](image-url)

**Figure 4.** An electrical circuit equivalent to $R_z$ in the current spread experiments. $R_s$ is the resistivity of the serosal cell membranes and $R_m$ is the resistance of mucosal cell membranes. $R_{trans}$ is the transepithelial resistance. MIC represents the microelectrode used to inject current ($I_o = 5.9 \times 10^{-9}$ A).
epithelial resistance to the serosal (reference) side. \( R_s \) is the positive solution of the quadratic equation:

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

while the mucosal membrane resistance was obtained from the ratio \( R_m/R_s \):

\[
R_m = (R_m/R_s)R_s.
\]

The shunt resistance was obtained from:

\[
R_{\text{shunt}} = \frac{R_{\text{trans}}(R_m + R_s)}{R_m + R_s - R_{\text{trans}}}. \tag{6}
\]

And the cell/shunt resistance ratio was given by:

\[
\frac{R_{\text{cell}}}{R_{\text{shunt}}} = \frac{(R_m + R_s) - R_{\text{trans}}}{R_{\text{trans}}}. \tag{7}
\]

Table II gives the results of six experiments. The mean space constant, \( \lambda \), was 359 ± 53.3 \( \mu \text{m} \). The cellular component of transepithelial resistance was 8,967 ± 2,017 \( \Omega \text{cm}^2 \) while transepithelial shunt resistance was 2,947 ± 492 \( \Omega \text{cm}^2 \). Thus the ratio of cell resistance to shunt resistance was 3.04. In Necturus antrum the transepithelial shunt contributes 75% to total epithelial conductance.

Changes in Ionic Activity

To confirm the above quantitation of antral cellular and shunt conductance we chose a second technique which was independent of tissue geometry,

| Table II |
| Results of Five Experiments in Which Current Spread Within the Mucosa Was Studied |

| \( \lambda \) (\( \mu \text{m} \)) | \( R_{dl}/L \) (\( \Omega \text{cm}^2 \)) | \( R_{m}/R_s \) (\( \Omega \text{cm} \)) | \( R_{\text{trans}} \) (\( \Omega \text{cm} \)) | \( R_{\text{cell}} \) (\( R_m + R_s \)) (\( \Omega \text{cm}^2 \)) | \( R_{\text{shunt}} \) (\( \Omega \text{cm}^2 \)) | \( R_{\text{cell}}/R_{\text{shunt}} \) | \( X \pm \text{SD} \) |
|---|---|---|---|---|---|---|---|
| 410 | 1,576 | 0.66 | 1,351 | 8,650 | 2,519 | 3.43 | 359 ± 53.3 |
| 460 | 1,193 | 1.5 | 1,351 | 6,937 | 2,714 | 2.56 | 400 ± 584.5 |
| 410 | 2,236 | 0.65 | 2,887 | 12,157 | 3,761 | 3.21 | 416 ± 688.6 |
| 316 | 2,545 | 1.5 | 2,149 | 9,423 | 2,784 | 3.38 | 316 ± 2,017 |
| 310 | 2,428 | 1.0 | 2,121 | 7,672 | 2,931 | 2.62 | 359 ± 492.0 |

Methods of determination of \( R_{\text{trans}} \) and \( R_m/R_s \) are given in the text and calculation of \( R_m \), \( R_s \), and \( R_{\text{shunt}} \) used Eqs. 5, 4, and 6, respectively.
and depended on a different set of assumptions for quantitative analysis. In this way agreement between the two techniques substantiated the assumptions inherent in each analytical model.

Changing the \([K^+]\) and \([Cl^-]\) in the serosal bathing fluid caused a change in both the transepithelial PD, \(\Delta \psi_{mz}\), and the PD across the serosal, \(\Delta \psi_{sc}\), and mucosal membrane, \(\Delta \psi_{mc}\). Table I gives the solutions used and Fig. 5 shows the change in transepithelial PD, \(\Delta \psi_{mz}\), and the change across the serosal membrane, \(\Delta \psi_{sc}\), in a typical experiment. Table III gives the results of four mucosae subjected to a constant product KCl change in the serosal bathing fluid. In these experiments the \(\Delta \psi_{mz}\) was 9.20 ± 0.89 mV and \(\Delta \psi_{sc}\) was 28.9 ± 3.00 mV.

The mean transepithelial resistance was 1,424 Ω cm² and did not change during the experiments. The resistance of the serosal and mucosal membrane was monitored by the ratio \(R_m/R_s\) which was 1.32 ± 0.60 before and 2.02 ± 0.77 during the constant product change and returned to 1.39 ± 0.60 on replacing the original solutions. The return of the ratio as well as \(\psi_{sc}\), indicates that there was satisfactory stability of the midropuncture within the time periods used.

To further analyze these data we used an electrical circuit (Fig. 6) equivalent to *Necturus* antral mucosa, composed largely of one cell type. This cell was given a lumped emf on the serosal \((E_s)\) and mucosal \((E_m)\) membrane. The resistances of the serosal and mucosal membranes were \(R_s\) and \(R_m\), respectively. A transepithelial shunt was given a resistance \(R_z\) and an emf \((E_z)\). Rose and Schultz (1971) used a similar circuit to examine the PD response of intestinal epithelial cells to transport of sugars and amino acids across the mucosal membrane. We included their shunt resistance at the serosal and mucosal membranes in the lumped emf and resistance of those membranes. The emfs' are absolute values and in a particular solution the sign as well as magnitude must be inserted.

According to this model the transepithelial PD \(\psi_{mz}\) is:

\[
\psi_{mz} = \frac{R_z(E_s + E_m) + E_z(R_s + R_m)}{R_s + R_m + R_z},
\]

and the PD \(\psi_{sc}\) across the serosal cell membrane is given by:

\[
\psi_{sc} = \frac{E_s(R_m + R_z) + E_z(R_s + E_m)}{R_s + R_m + R_z}.
\]

In these experiments we assumed that the ΔPD resulted from a change in emf

1 In these experiments a constant product KCl change means a change in \([K^+]\) and \([Cl^-]\) such that the product \([K^+] \times [Cl^-] = 400 \text{ mM}^2\) at all times.
Figure 5. $\psi_{sc}$, $\psi_{me}$, $\psi_{ma}$ were recorded during a constant product KCl change in the serosal solution. Large changes in $\psi_{sc}$ and $\psi_{me}$ are recorded while $\psi_{ma}$ changes much less. Analysis of these changes is given in the text.

| TABLE III |
| RESULTS OF CONSTANT PRODUCT KCl CHANGES IN FOUR MUCOSAE |

| $\psi_{sc}$ | $\psi_{me}$ | $R_{ma/R_{ma}}$ |
|------------|-------------|----------------|
| $K^+ = 5$  | $K^+ = 5$   | $K^+ = 5$     |
| mV         | mV          | mV            |
| 62.2±3.5   | 32.2        | 14.9          | 10.3          | 3.21 | 1.41 | 2.14 | 1,538 |
| 32.2       | 14.9        | 10.3          | 3.21          | 1.41 | 2.14 | 1,538 |
| 48.0±4.4   | 29.7        | 9.1           | 8.7           | 3.41 | 1.88 | 1.90 | 1,869 |
| 2.7        | 0.7         | 0.5           | 0.41          | 0.81 | 0.85 | 38   |
| 34.1±1.9   | 25.1        | 8.9           | 8.3           | 2.97 | 1.52 | 2.94 | 850   |
| 2.2        | 2.5         | 2.0           | 0.27          | 0.33 | 0.97 | 16   |
| 33.0±11.0  | 28.4        | 9.3           | 9.5           | 2.98 | 0.47 | 1.08 | 1,438 |
| 1.5        | 2.9         | 0.6           | 0.24          | 0.53 | 1.18 | 23   |
| Mean ±SD   | 28.9        | 9.2           | 3.14          | 1.32 | 2.02 | 1,424 |
| 3.0        | 0.89        | 0.21          | 0.60          | 0.77 | 25   |

In one column, the intracellular and transmucosal potentials before the constant product KCl change are given and then the change in PD after the change is in the adjacent column. After the changes the potential returns to its control value. Further analysis of $\Delta \psi_{sc}/\Delta \psi_{me}$ is given in the text.
at the serosal cell membrane ($\Delta E_s$) and that no change occurred in membrane or shunt resistance. Thus for a change in $E_s$:

$$\Delta \psi_{ms} = \frac{\Delta E_s R_L}{R_s + R_m + R_L}.$$  \hspace{1cm} (10)

$$\Delta \psi_{se} = \frac{\Delta E_s (R_m + R_L)}{R_s + R_m + R_L}.$$  \hspace{1cm} (11)

$$\Delta \psi_{sc}/\Delta \psi_{ms} = 1 + R_m/R_L.$$  \hspace{1cm} (12)

From Table III the ratio $\Delta \psi_{se}/\Delta \psi_{ms} = 3.14$; hence $R_m/R_L = 2.14$. From this relationship and $R_m/R_s = 1.32$ (Table III), the serosal and mucosal membrane resistances and the transepithelial shunt resistance can be obtained by substitution into

$$R_{\text{transe}} = \frac{(R_m + R_s)R_L}{R_m + R_s + R_L}.$$  \hspace{1cm} (13)

The transepithelial shunt resistance was 1,803 $\Omega \text{cm}^2$ while the serosal and mucosal membrane resistances were 2,931 and 3,857 $\Omega \text{cm}^2$, respectively. Thus the cellular resistance was 6,788 $\Omega \text{cm}^2$ and the cell/shunt resistance ratio was 3.77.

Identical experiments were also performed using deletion of $\text{HCO}_3^-$ from the serosal bathing solution; thus the $\Delta(\text{HCO}_3^-)$ was 17.8 mM. Fig. 7 shows typical PD changes which were recorded and Table IV gives the results of five experiments. The mean $\Delta \psi_{se}$ was 12.9 $\pm$ 6.6 mV while the mean $\Delta \psi_{ms}$ was 3.9 $\pm$ 2.1 mV. The ratio $\Delta \psi_{sc}/\Delta \psi_{ms}$ was 3.31 $\pm$ 0.18 which was very close to that recorded using the constant product KCl changes. With the exception of the first experiment the potentials returned to basal level when $\text{HCO}_3^-$ was returned to the serosal solution. The transmucosal resistance did not change significantly during the course of the ion changes.

Applying the same equations to this perturbation gave a ratio $R_m/R_L = $
2.31. Mucosal membrane resistance was 4,558 \( \Omega \text{cm}^2 \) and serosal membrane resistance was 2,780 \( \Omega \text{cm}^2 \). Thus cellular resistance \((R_m + R_s)\) was 7,338 \( \Omega \text{cm}^2 \) while transepithelial shunt resistance was 1,973 \( \Omega \text{cm}^2 \). The cell to shunt resistance ratio was 3.71 or the shunt contributed 79\% of total epithelial conductance.
In these experiments the transepithelial shunt $\Delta emf$ was assumed to be small compared to the cell membrane response. To investigate anion-cation selectivity of the shunt pathway, dilution potentials for KCl were measured using a 10-fold dilution of KCl; the dilute solution was placed on the mucosal side in some experiments and on the serosal side in other experiments. Table V gives the composition of the control and dilute solutions used in these experiments.

The control solution bathed both sides of the epithelium initially. A microelectrode was inserted into a surface cell and when the PD was stable, one solution was replaced with the 10-fold dilution of KCl. After the PD had stabilized the control conditions were reestablished. In any one mucosa only mucosal or serosal changes were performed. Transepithelial resistance and the ratio $R_m/R_s$ was measured as described above. Table VI gives the results of dilution of the mucosal solution and Table VII gives the results of dilution of the serosal solution.

When the mucosal solution was diluted (osmolality maintained with mannitol) a $3.1 \pm 0.22$-mV change in potential occurs transmucosally. Across the mucosal membrane a $1.9 \pm 0.52$-mV change is recorded and $1.2 \pm 0.39$ mV is recorded across the serosal membrane (Table VI).

The ratio $\Delta\psi_{m\text{c}}/\Delta\psi_{m\text{s}}$ is 0.61 which was very different from the ratios obtained with the constant product KCl and (HCO$_3^-$) changes in the serosal solution. According to equations developed for a $\Delta E_M$

$$\frac{\Delta\psi_{m\text{c}}}{\Delta\psi_{m\text{s}}} = 1 + \frac{R_s}{R_m}$$

a value of 0.61 was incompatible with a $\Delta E_M$ being the origin of the $\Delta PD$. Solution of the equations for $\Delta E_L$ revealed that:

$$\frac{\Delta\psi_{m\text{c}}}{\Delta\psi_{m\text{s}}} = \frac{R_m}{R_m + R_s} = \frac{1}{1 + \frac{R_s}{R_m}}$$

**Table V**

| COMPOSITION OF SOLUTIONS USED FOR MEASUREMENT OF KCl DILUTION POTENTIAL |
|-----------------------------|-----------------------------|
| **Control solution**       | **10-fold KCl dilution**    |
| mM                          | mM                          |
| K$^+$                       | 95                          | 9.5                          |
| Cl$^-$                      | 80                          | 8.0                          |
| Mg$^{2+}$                   | 1.5                         | 1.5                          |
| Ca$^{2+}$                   | 1.0                         | 1.0                          |
| HCO$_3^-$                   | 19                          | 19                           |
| H$_2$PO$_4^-$               | 1.0                         | 1.0                          |
| Choline                     | --                          | 13.5                         |
| Mannitol                    | --                          | 146.5                        |
TABLE VI
TRANSMUCOSAL AND INTRACELLULAR PD IN SYMMETRICAL CONTROL SOLUTION AND THE CHANGE IN PD DURING DILUTION OF THE MUCOSAL SOLUTION

| Control | Δψ_m | Control | Δψ_m | Control | Δψ_m | Control | Δψ_m | Control | Dilution |
|---------|-------|---------|-------|---------|-------|---------|-------|---------|----------|
| 7       | 3     | 27      | 2     | 20      | 1     | 1,400   | 2,150 |
| 6       | 3.5   | 30      | 1     | 24      | 2.5   | 1,525   | 2,250 |
| 8       | 3     | 31      | 1     | 23      | 2     | 650     | 1,050 |
| 7.5     | 2.8   | 30      | 1.5   | 22.5    | 1.3   | 700     | 1,200 |
| 7       | 3.3   | 32      | 0.8   | 25      | 2.5   | 700     | 1,250 |
| 7       | 3     | 26      | 1     | 19      | 2     | 850     | 1,250 |
| 8       | 3     | 26      | 1     | 18      | 2.0   | 850     | 1,250 |
| 8.5     | 3     | 26      | 1     | 17.5    | 2.0   | 900     | 1,325 |

Mean ±SD 7.4 3.1 28.5 1.2 21.1 1.9 946 1,465 0.79 0.22 2.5 0.39 2.9 0.32 332 461

Changes in $R_{trans}$ are recorded in the two columns at the right. Return to control conditions reestablishes the prior PD.

TABLE VII
TRANSMUCOSAL AND INTRACELLULAR PD IN SYMMETRICAL CONTROL SOLUTIONS, AND THE CHANGE IN PD DURING DILUTION OF THE SEROSAL SOLUTION

| Control | Δψ_m | Control | Δψ_m | Control | Δψ_m | Control | Δψ_m | Control | Dilution |
|---------|-------|---------|-------|---------|-------|---------|-------|---------|----------|
| 4       | 4     | 23      | 12    | 19      | 8.0   | 1,350   | 1,900 |
| 4       | 4     | 23      | 10    | 19      | 6     | 1,450   | 1,850 |
| 3       | 4     | 30      | 10    | 27      | 6     | 1,500   | 1,900 |
| 5       | 5     | 32      | 10.5  | 27      | 5.5   | 1,300   | 1,725 |

Mean ±SD 4 4.3 27.0 10.6 23.0 6.4 1,400 1,844 0.8 0.5 4.7 0.95 4.6 1.1 91.3 82.6

Return to control conditions reestablishes prior PD. Changes in $R_{trans}$ are recorded in the two columns at the right.

\[
\frac{\Delta \psi_{sc}}{\Delta \psi_{mc}} = \frac{R_s}{R_m + R_s} \quad \text{(16)}
\]

\[
\frac{\Delta \psi_{mc}}{\Delta \psi_{sc}} = \frac{R_m}{R_s} \quad \text{(17)}
\]

Thus the ratio $\Delta \psi_{mc}/\Delta \psi_{sc}$ should be very close to the voltage divider ratio, $R_m/R_s$. $\Delta \psi_{mc}/\Delta \psi_{sc}$ was 1.58 and the ratio $R_m/R_s$ in these experiments was 1.55.

Upon dilution of the serosal solution very different results were obtained.
(Table VII). In this case the transepithelial ΔPD was 4.3 ± 0.5 mV while the
ΔPD across the serosal cell membrane was 10.6 mV. The ratio Δψ_{ser}/Δψ_{m,}, was
2.44 which is somewhat less than the same ratio obtained with constant product
KCl (3.14) or Δ[HCO₃⁻] (3.31). The ratio Δψ_{ser}/Δψ_{m,} was 0.60 which was
very different from the voltage divider ratio 1.55 and likewise Δψ_{ser}/Δψ_{m,} is
very different from \( \frac{R_{s}}{R_{m} + R_{e}} \) and was not compatible with a ΔPD caused
solely by a \( \Delta E_{L} \).

**DISCUSSION**

The gastric antrum has not received the interest that has been focused on the
gastric fundus. Recently the electrophysiologic properties of *Necturus* gastric
antrum have been studied by Flemstrom and Sachs (1974). They found that
*Necturus* antrum developed a small potential difference, 4–10 mV lumen nega-
tive. In symmetrical solutions the short circuit current could be attributed to a
mucosal to serosal Na⁺ flux. Ouabain abolished PD when added to the serosal
solution; amiloride, in the mucosal solution abolished the PD in the absence of
a HCO₃⁻ gradient. The effect of amiloride was reversible.

Previously the PD of antrum has been studied in vivo in humans (Andersson
and Grossman, 1965) and dogs (Dennis et al., 1959). Dyke et al. (1969) used
H⁺, Na⁺, K⁺, and H₂O flux into fundic or antral pouches to assess permea-
bility. In those experiments where surface area was measured Na⁺ and H⁺ flux
was 7–15 times greater in antral pouches than in fundic pouches. Himal et al.
(1970) found that Na⁺ and H⁺ flux was less in canine antral than in duodenal
mucosa by a ratio of \( \frac{1}{2} \) to \( \frac{1}{4} \). No in vitro studies have attempted quantitation
of the cellular and transepithelial shunt conductance in antral mucosa.

In this study we have quantitated the cellular and transepithelial shunt re-
stance in antral mucosa by two different techniques. Table VIII summarizes
the results obtained by each technique.

In these experiments the mean cellular resistance was 7,697 Ωcm², while the

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**Table VIII**

**Summary of Results of the Three Types of Experiments Used to
Quantitate Cellular and Transepithelial Shunt Resistance**

| Type             | Trans. resistance | Cellular resistance | Shunt resistance | Shunt contribution to epithelial conductance |
|------------------|-------------------|---------------------|------------------|--------------------------------------------|
| Constant product KCl | 1,424             | 6,788               | 1,803            | 79.0                                       |
| Δ[HCO₃⁻]         | 1,554             | 7,338               | 1,973            | 78.8                                       |
| Current spread   | 2,211             | 8,967               | 2,947            | 75.2                                       |
| Mean             | 1,730             | 7,697               | 2,241            | 77.7                                       |

Note the very close agreement of percent contribution of the shunt in the three types of experiments.
mean shunt resistance was 2,241 Ωcm². The mean cell/shunt resistance ratio
was 3.43. The cellular pathway accounted for 22.3% and the shunt pathway
accounted for 77.7% of tissue conductance. The mean transepithelial resist-
ance was 1,730 Ωcm².

Not only is the magnitude of the shunt conductance important, but so is the
selectivity. This can be deduced from the KCl and HCO₃⁻ experiments or can
be measured directly using dilution potentials. In the case of KCl dilution
under our conditions, the maximum potential which should develop due to
differing mobilities of K⁺ and Cl⁻ is less than 1 mV. We have also argued that
the shunt should be symmetric, i.e. an equal potential should be obtained re-
gardless of which side is diluted, and the obtained selectivities should be the
same.

In the case of mucosal dilution of KCl, there was a 3.1 ± 0.22 mV increase
in transepithelial potential. This per se is not sufficient evidence that the shunt
was the source of the ΔPD. However, Eq. 15 predicts that the Δψₑₑ/Δψₑₑ will be
in the ratio of $R_m/R_s$ if current flow is altered by a change in shunt emf.
Since this in fact happened (Table VI) it is legitimate to conclude that the
shunt selectivity for Cl⁻ over K⁺ was 1.16, i.e. the shunt is weakly anion selec-
tive.

Serosal dilution of KCl gave a PD change of 4.3 ± 0.5 mV (Table VII).
But since $Δψₑₑ/Δψₑₑ = 2.47$ and the predicted ratio $R_s/R_m$ was 0.65, this
change was largely due to a change in cell potential, in contrast to what is ob-
tained with mucosal dilution. These considerations demonstrate that it is im-
portant to determine the suitability of a given ion for dilution potential as-
essment of shunt selectivity.

The constant product KCl experiments or the ΔHCO₃⁻ experiments also
allow assessment of the change in shunt emf under these conditions ($ΔE_L$).
Thus if the ΔPD arises only from $ΔE_L$, from Eqs. 2 and 3 we have

$$Δψₑₑ/Δψₑₑ = \frac{R_s}{R_m + R_s} = \frac{1}{R_m/R_s + 1},$$

and for the ΔKCl the predicted value was 0.43, the value obtained was 3.14,
and for ΔHCO₃⁻ the predicted value was 0.38 and the value obtained was
3.31; hence $ΔE_L$ was small as would be predicted from the dilution potentials.

If the change in potential is due to both a $ΔE_s$ and a $ΔE_L$ then again from
Eqs. 2 and 3 we have

$$Δψₑₑ/Δψₑₑ = \frac{ΔE_s(R_m + R_L) + ΔE_L R_s}{ΔE_s R_L + ΔE_L (R_s + R_m)},$$

and using either the mean resistance from all experiments or the resistance
from the current spread experiments, it is possible to calculate $ΔE_L$, deriving
$ΔE_s$ from $Δψₑₑ + ΔIR_s$. Table IX shows that in the case of the KCl experi-
TABLE IX
SUMMARY OF CALCULATED VALUES FOR IR DROPS AND ΔEₜ ENABLING CALCULATION OF THE MAGNITUDE OF ΔEₜ

| Experiment | ΔIRₑ | ΔRIₑ | ΔΔEₜ | ΔEₜ¹ | ΔEₜ² |
|------------|------|------|------|------|------|
| Constant Product KCl | 19.7 | 14.9 | 43.8 | -0.81 | -3.1 |
| Δ(HCO₃⁻)² | 9.0  | 5.45 | 18.4 | -0.64 | -1.58 |

ΔEₜ¹ is the value calculated using the mean resistance values for all experiments. ΔEₜ² is the value calculated using resistance values from only the current spread experiments since those experiments are not dependent on ionic gradients.

The use of ionic changes also rests on several explicit assumptions. The assumption of low shunt selectivity compared to membrane selectivity has been dealt with above. It is also assumed that the changes in emf occur only across one membrane. This follows in the antrum from consideration of the ΔIₑ in the ΔKCl and ΔHCO₃⁻ experiments which is 6.46 and 2.51 μA, respectively. This allows prediction of the Δψₑₑ due to current flow alone, being 24.9 and 11.4 mV in the two cases, which compare well to the measured values of 19.7 and 9.0 mV. Finally, constancy of resistance parameters is assumed in utilizing the equations developed. Transmucosal resistance did not change during the experiments, whereas membrane resistance ratio Rₑ/Rₑ increased by 53% (t = 2.43, P < 0.05) during the KCl change and fell by 25% (t = 5.19, P < 0.005) during HCO₃⁻ deletion. However, there is close agreement between the two
ionic change conditions, and between those and the current spread technique (Table VIII).

We can therefore conclude that in antral mucosa cellular conductance contributes 22.3% of tissue conductance whereas in fundic mucosa cellular conductance accounts for at least 80% of tissue conductance. Antral conductance is relatively anion selective at neutral pH, and progressively becomes more anion selective with fall in pH (Bajaj and Sachs, unpublished). The antrum is exposed to the same proton gradient as the fundus, with a shunt conductance about four times greater than the fundus. The physiologic significance of this finding is not established; it may be of importance in control of gastrin release by antral "G" cells (Berkowitz et al., 1971; Andersson and Elwin, 1971). The distribution of shunt conductance however correlates well with the known distribution of ulcer in man.

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