MICROELECTRODE STUDIES OF DOG'S GASTRIC MUCOSA

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ABSTRACT In anesthetized dogs, the potentials in the mucous coat and gastric cells were measured with microelectrodes. In the secreting stomach, with isotonic saline in contact with the mucosal surface, the orientation of the initial change in potential difference (PD) was often the same as that of the liquid junction potential between gastric juice and saline (the microelectrode became negative to a reference electrode in the saline) but the magnitude of the change was never more than 11 mv. On the basis of this finding an explanation is offered for the observation that in the secreting stomach replacing isotonic saline with isotonic HCl as the bathing fluid on the mucosal surface, results in a change in the serosal to mucosal PD of only 19 mv, which is 40% less than the liquid junction potential between gastric juice and saline. In the surface epithelial cells of both resting and secreting stomach, multiple levels of potentials were found. For the secreting stomach, the resistance between the interstitial fluid of the pit region and the fluid on the mucosal surface was 55 ohm cm^2, determined as the change in PD per unit of applied current across stomach. The implications of these findings are discussed with reference to the separate site theory of HCl formation.

INTRODUCTION

In previous work on the dog's stomach with macroelectrodes, it was found that the serosal surface is positive to the mucosal one and the PD between the submucosa and serosal surface is zero (9, 11). It was found for the resting stomach (H^+ rate = zero) with 0.16 M NaCl as the mucosal fluid that the average PD was about 65 mv while with 0.16 M HCl it was about 10 mv lower (19). With isotonic saline as the mucosal fluid, the establishment of secretion results in a decrease of the PD from a level of about 65 mv (serosa positive) to a level of about 35 mv, while with 0.16 M HCl as the secretory fluid, the establishment of secretion results in a transient increase in PD of about 5 mv (13, 19). During secretion, changing the secretory solution from 0.16 M NaCl to 0.16 M HCl and vice versa always resulted in a rapid change in the PD to a new level and the magnitude of this change was never more than 22 mv (average = 19 mv). If it be assumed that there is a uniform liquid junction between the gastric juice and the saline on the mucosal side then changing the

saline to HCl should abolish this liquid junction potential and the PD across the stomach should increase (serosal side should become more positive) by an amount equal to the magnitude of this junction potential. However, the liquid junction potential between gastric juice and 0.16 M NaCl was found to be 31 mv (4, 6, 18), i.e., substantially larger that the change in PD resulting from replacing the saline with HCl. We considered the possibility that under the in vivo conditions the liquid junction potential between gastric juice and saline might be only 19 mv. However on the basis of considerations to be described below we concluded that this possibility was untenable.

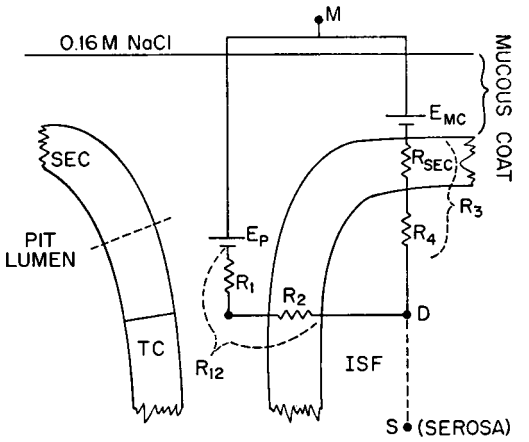

FIGURE 1 Schematic drawing of outer mucosa of secreting stomach with 0.16 M NaCl as the mucosal fluid. SEC = surface epithelial cells. M represents mucosal electrode, E_{MC} = liquid junction potential between bottom of mucous coat and saline, E_P is assumed to be equal to the liquid junction potential between gastric juice in pit lumen and saline (31 mv). R_1 = resistance in pit lumen, R_2 = resistance from lumen to interstitial fluid (ISF) via SEC and tubular cells, $R_{12} = R_1 + R_2$ (see text), R_3 = resistance via ISF and SEC ($R_3 = R_{SEC} + R_4$).

We formulated a hypothesis to explain the apparent discrepancy on the assumption that the liquid junction potential is not uniform. This hypothesis is illustrated by the equivalent circuit of Fig. 1. For simplicity, only the liquid junction emfs are shown since it is assumed that the only effect of changing from 0.16 M NaCl to 0.16 M HCl is the elimination of these junction potentials. The H^+ concentration of gastric juice in the gastric pits would be that of pure gastric juice since the composition of this fluid would be independent of the composition of the mucosal fluid because of the high velocity of bulk flow in the pit lumen (20). The liquid junction potential E_P between the pit fluid and the outer saline would be about 31 mv. The concentration of H^+ at the bottom of the mucous coat between pits, at the border between the mucous coat and the surface epithelial cells (SEC), might be only a fraction of that of pure gastric juice and would be a function of a number of factors such as the thickness of the mucous coat, the velocity of bulk flow in the mucous coat (about 5% of that in the pits), and the effect of gastric motility on the geometry of the coat. Gastric motility could produce a "to and fro" bulk flow in the mucous coat by rhythmically deforming it. If the liquid junction potential between the fluid at the bottom of the mucous coat and the saline solution (indicated by E_{MC}) is only a fraction of that of E_P then the change in PD in going from 0.16 M NaCl to 0.16 M HCl would

be less than the liquid junction potential between the solutions and would be given by

$$\Delta PD_{DM} = \Delta PD_{SM} = \frac{R_{12}}{R_{12} + R_3} E_{MC} + \frac{R_3}{R_{12} + R_3} E_P \qquad (1)$$

where $R_{12} = R_1 + R_2$ is the resistance between D and M via the limb with E_P in it and $R_3 = R_{SEC} + R_4$ and is the resistance via E_{MC}. It is assumed (vide infra) that

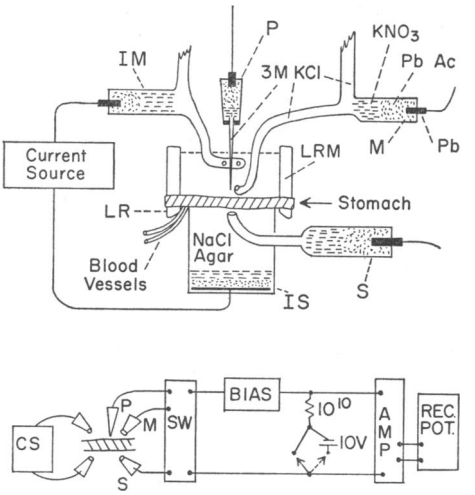

FIGURE 2 Schematic diagram of experimental arrangement. Pb-Pb acetate nonpolarizable electrodes used for mucosal electrode M, for serosal electrode S, for microelectrode P, and for current sending electrodes IM and IS. LR = lucite ring on serosal side. This ring had an oblique cut in it so that the vessels could be slipped inside in order to maintain an intact blood supply. LRM = lucite mucosal ring. Long screws (not shown) with their bases coated with rubber were used to clamp the stomach between LR and LRM. In the lower part of this fig, a block diagram of the circuit is shown. CS = current source, including 20 v battery, variable resistors, microammeter, and appropriate switches. Output could be sent across stomach or to internal circuit with same resistance as stomach circuit so that the magnitude of the current could be adjusted before sending current across the stomach. See text.

the PD between the serosal electrode and point D is zero and therefore that $PD_{SM} = PD_{DM}$ (see Fig. 2). If E_{MC} is less than E_P, then an increase in PD in going from 0.16 M NaCl to 0.16 M HCl of less than the liquid junction potential between gastric juice and saline could be explained. This hypothesis could be tested by exploring the mucous coat with a microelectrode. The results of such explorations are reported in this paper.

We anticipated, that apart from a liquid junction potential in the mucous coat, that punctures of the SEC would result in two jumps in PD, one upon entering the cells and the second upon entering the interstitial fluid (ISF). We found, contrary

to expectations, multiple levels of potential and these findings will also be presented. Another purpose of this paper is the presentation of measurements of the resistance between the ISF and the mucosal and serosal surfaces. These experiments were performed in order to test a particular version of the separate site theory of gastric secretion (12, 14, 15).

METHODS

As illustrated in Fig. 2, a segment of the stomach of dogs anesthetized with amobarbital was placed between two rings of lucite in such a way as to maintain an intact blood supply. The details of the chambered-gastric segment technique have been described in detail previously (9, 10) so that only a brief description will be given here. A large isotonic saline agar surface (area = 14 cm^2) made contact with the serosal surface and either 0.16 M NaCl or 0.16 M HCl was placed on the mucosal side. A microelectrode P and two pairs of macroscopic electrodes were used, one pair for sending current and the other pair for PD measurements. M refers to the mucosal and S to the serosal PD electrodes. The microelectrodes were the conventional glass capillary type filled with 3 M KCl and had a resistance of from 5–15 MΩ. All of the electrodes were connected to the measuring circuit via Pb-Pb acetate electrodes.

Measurements were attempted with the microelectrode held in a micromanipulator but owing to the vigorous motility of the stomach (which could not be inhibited by smooth muscle inhibitors), the tips usually broke within a few seconds after a puncture. For the work reported in this paper, a modification of the hanging microelectrode method of Woodbury and Brady (22) was used with microelectrodes of conventional length but suspended by a thin wire (0.001 inches in diameter) from a micromanipulator. With this technique it was possible to obtain penetrations of over 1 mm (as determined by observations of marks on the shaft) before the tip broke.

However, for penetrations of shorter depths it was difficult to know how far the tip of a regular microelectrode had penetrated so that some experiments were performed with microelectrodes in which a small glob of dental wax was placed on the shaft; the wax acted as a "stop" and limited the depth of penetration.

In the lower portion of Fig. 2, a diagram of the circuit is shown. By means of a selector switch Sw, the PD could be measured between P and M, S and P, or S and M. An ink-writing recording potentiometer with a 1 sec full scale deflection was connected to the output of a battery operated amplifier (input impedance 10^{14} ohms). In order to have a frequent check on the integrity of the microelectrode tip, the resistance of the microelectrode was measured at frequent intervals by switching in a battery (up to 10 v) through a 10^{10} ohm resistor placed across the input to the amplifier. Breakage of the tip was associated with a marked drop in resistance. For the sake of clarity, the resistance measurements of the microelectrodes are omitted from the figures. A bias voltage, placed in one of the leads to the amplifier, was used for calibrations of the potentiometer during measurements with a microelectrode. The whole dog table was enclosed in a large wire mesh cage from which AC current was excluded.

The resistance between the microelectrode and either the serosal or mucosal surface was obtained by measuring the resistance between the microelectrode and either electrode S or M and then correcting for the resistance of the agar or mucosal fluid between the surfaces and the corresponding electrode. The resistance between the microelectrode and either electrode S or M was determined by measuring the change in PD resulting from sending current via the current sending electrodes (IM and IS of Fig. 2).

The pH of the secretory fluid changes from about 7.0 to less than 1.0 and it was important to perform the following controls. Liquid junctions were formed in a tube several centimeters

in diameter with a 0.16 M NaCl solution layered on top of a 0.16 M HCl–5% sucrose mixture (or with 0.016 M HCl above 0.16 M HCl). Two reference electrodes were used, making contact with the two solutions via readily renewable saturated KCl junctions. The PD between the microelectrode and one of the reference electrodes was compared to that between the two reference electrodes. It was found that moving the microelectrode rapidly from one solution to the other resulted in an immediate change in the PD of a magnitude equal to that between the reference electrodes. When a tip was deliberately broken, determined by a marked drop in the resistance of the microelectrode, it was found that movement of the microelectrode from the saline to the HCl and vice versa gave rise to artifacts of the type previously described for macroelectrodes (17).

The possibility was considered that under the in vivo condition the liquid junction potential between gastric juice and saline might be only about 19 mv. On the basis of the following findings this possibility was considered to be untenable. It was found that (*a*) the mucosa coat is a weak gel in which the ionic mobilities are the same as in aqueous solutions, (*b*) the magnitude of the liquid junction potential between gastric mucus equilibrated with 0.16 M NaCl and gastric mucus equilibrated with gastric juice (or artificial gastric juice or 0.16 M HCl) was in the range of 29 to 31 mv, (*c*) that the magnitude of the liquid junction potential was not dependent on the "thickness" of the junction, and (*d*) the magnitude of the potential was not a function of the area of the junction (4, 16, 18, 19). Secretion was stimulated by subcutaneous injections of histamine and methacholine (19). Secretory rates were determined by aspirating the mucosal fluid and determining its titratable acidity. Nine dogs were used in these experiments.

In previous work (2) in which the stomach was frozen in liquid nitrogen and then serially sectioned at −20°C it was found (*a*) that the ISF area in the pit region was about 50% of the total area, (*b*) that the first tubular cells were at least 200 μ from the surface, and (*c*) that at a distance of about 500 μ from the surface there were no SEC and the ISF area was only about 10% of the total area at that level. Penetration of the microelectrode to a depth of 1 mm should result, with a certainty of almost 100%, in punctures of the cells of the tubules.

RESULTS

Resting and Secreting Stomach Explored with Hanging Microelectrodes with "Stops"

When microelectrodes with "stops" 30–80 μ from the tip were lowered into the mucosa the PD between P and M increased to a final steady-state level equal to the PD between S and M (see Fig. 6). Since in a resting stomach there is very little net transport of ions across the mucosa (1), the ion gradients in the mucous coat should be essentially zero and the change in PD should be due to a puncture of a SEC. Upon rapid lowering of a hanging microelectrode with a "stop," the PD does not increase as rapidly as it does with a microelectrode without a "stop" undoubtedly owing to the viscous drag of the mucus on the "stop." The difference between these responses is illustrated by comparing Figs. 6 and 7. That is, the PD with a microelectrode with a "stop" increased in steps with intermittent pauses. When the first change in PD was opposite to the over-all PD, its magnitude with either saline or HCl as the mucosal fluid was never greater than 11 mv for either the resting or secreting stomach.

There are certain objections to the use of microelectrodes with "stops." The glob of wax is about 1 mm in diameter, i.e. many times the diameter of the microelectrode shaft, and the mucous coat is undoubtedly deformed during a puncture so that the ionic composition of the mucous coat could be changed by bulk movement of mucosal fluid into it. For example, in the secreting stomach, with isotonic saline as the mucosal fluid the $[H^+]$ in the mucous coat could be decreased by bulk movement of saline with the consequence that the liquid junction potential between the fluid in the mucous coat and the saline would be decreased. It is therefore necessary to confirm the finding that the magnitude of the inverted initial change in PD is never over 11 mv with microelectrodes without "stops."

Resting Stomach Potentials with Hanging Microelectrodes without "Stops"

Punctures of short duration were obtained by lowering the hanging microelectrode to a point where the inherent movements of the stomach resulted in a rhythmic penetration of the mucosa by the microelectrode. In Fig. 3, the PD between the microelectrode and the mucosal electrode is shown for seven consecutive spontaneous punctures (1–7*R*) with 0.16 M NaCl as the mucosal fluid. It was found as illustrated in this figure that a number of levels of potential were observed. In punctures 1 and 7*R* the highest level was 59 mv which was equal to the over-all PD measurement between electrode S and M. In some punctures the PD remained

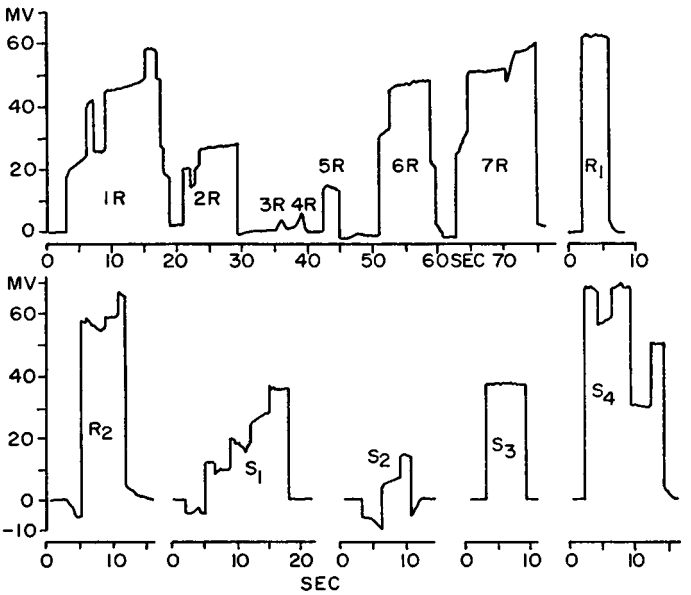

FIGURE 3 PD between hanging microelectrode P and mucosal fluid electrode M in mv vs. time in seconds. Upward deflection means P positive to M. R refers to resting stomach and S to secreting stomach. Mucosal bathing fluid 0.16 M NaCl.

relatively constant for 4 or 5 sec at a given level while in other punctures it gradually changed between jumps. For example in 2*R* (Fig. 3), a level of 27–28 mv was maintained for 6 sec while in puncture 7*R* the PD progressively increased from 26–32 mv in about 1.5 sec. Following a series of spontaneous punctures like 1 and 7*R*, it was found that by lowering the microelectrode a short distance, spontaneous punctures could be obtained in which the PD would increase in one step to the level of the over-all PD as illustrated by R_1 of Fig. 3. A further lowering of the microelectrode would usually result in a sustained puncture as illustrated in Fig. 7. It was also found that, after a series of punctures like 1–7*R*, raising the microelectrode a short distance would result in only an occasional puncture. With this technique it seems reasonable to believe that if there were emfs in the mucous coat oriented oppositely to the over-all PD, one should by appropriate positioning of the microelectrode, be able to frequently record them and determine their magnitude.

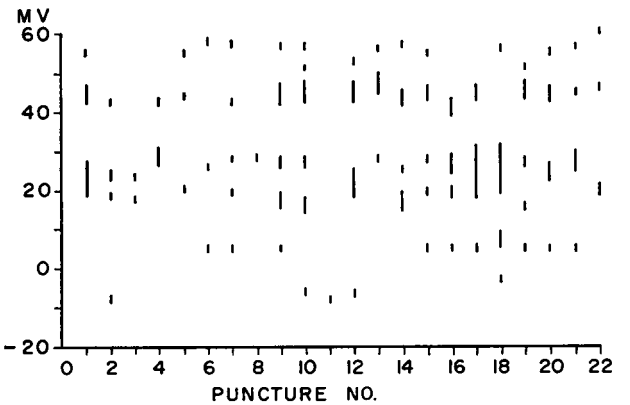

FIGURE 4 Levels of PD in mv between microelectrode P and mucosal electrode M in a resting stomach with 0.16 M NaCl as mucosal fluid: Serosal electrode S was positive to mucosal electrode M by 59 mv. 22 consecutive punctures.

Fig. 4 shows the levels in 22 consecutive spontaneous punctures with 0.16 M NaCl as the mucosal fluid. The length of the lines represents the magnitude of the gradual changes in PD between jumps. In some punctures as illustrated in R_2 in Fig. 3, the initial change in PD was in the opposite direction from the over-all PD. It was found that 11% of the punctures showed an initial deflection oriented oppositely to that of the over-all PD and except for one puncture (in which the $\Delta PD = -14$ mv) the magnitude of the change was not greater than 12 mv. A series of experiments were performed with 0.16 M HCl as the secretory fluid and the results were not distinguishable from those with 0.16 M NaCl as the secretory fluid, nor from those described in the following section in the secreting stomach with 0.16 M HCl as the secretory fluid.

For the secreting stomach, the PD is a function of the H^+ concentration of the secretory fluid (19). With 0.16 M NaCl, the average PD is about 35 mv while with 0.16 M HCl it is about 55 mv. When saline is present initially and secretion is allowed to accumulate, the PD increases from 35 mv towards 55 mv as the pH approaches that of pure gastric juice. This was confirmed in the present experiments. Fig. 3 shows three punctures of the secreting stomach with fresh saline present (S_1, S_2, and S_3) and one (S_4) with 0.16 M HCl. Fig. 5 shows the results of consecutive spontaneous punctures for the secreting stomach with 0.16 M NaCl as the secretory fluid (Fig. 5 *A*) and with 0.16 M HCl as the secretory fluid (Fig. 5 *B*). Inspection of the data shows that there were no obvious differences in the number of potential levels between the resting and secreting stomach. Occasionally as illustrated in the series

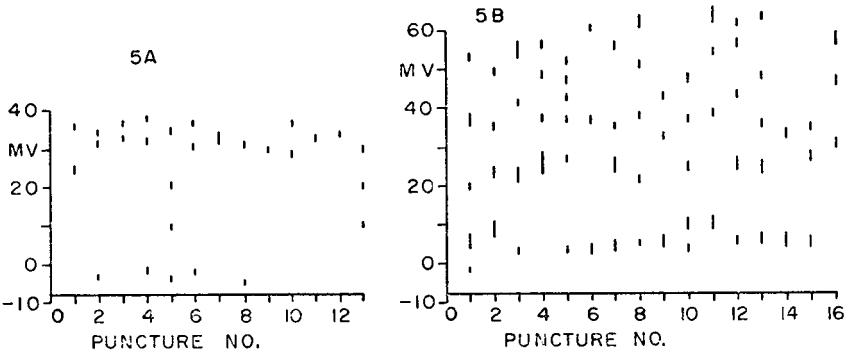

FIGURE 5 Levels of PD between P and M in mv for consecutive punctures in secreting stomach. Fig. 5 *A* with 0.16 M NaCl and Fig. 5 *B* with 0.16 M HCl as mucosal fluid. In Fig. 5 *A*, S positive to M by 36 mv, and in Fig. 5 *B*, S is positive to M by 58 mv.

of punctures of Fig. 5 *A* there would be series of punctures of only one or two potential jumps. However raising the microelectrode a short distance would then usually result in a series of potential jumps similar to those obtained in the fifth puncture in Fig. 5 *A* and in S_1 of Fig. 3.

In both the resting and secreting stomach with 0.16 M HCl as the secretory fluid, the initial change in PD was opposite to the over-all PD in less than 4% of the punctures and the magnitude was less than 11 mv. In the secreting stomach with fresh 0.16 M NaCl as the secretory fluid, the initial change in PD was opposite to the over-all change in 31% of the punctures and the magnitude of change was 11 mv or less.

In most of the punctures the maximal PD between P and M was equal to that between S and M. However, occasionally with regular microelectrodes, the PD between P and M was greater than that between S and M. Out of hundreds of punctures in both the resting and secreting stomach, the maximal PD observed between the microelectrode and the mucosal electrode was 92 mv.

The data were examined from the point of view of finding ways to delineate any differences in the number of potential levels that might exist between the results in the resting and secreting stomach with saline or HCl as the secretory fluids. Apart from the per cent of the initial changes with inverted orientations, there were no obvious differences and since we are dealing with a moving target, we decided that further analysis would not be warranted.

Resistance Measurements

Fig. 6 shows a representative experiment on the secreting stomach in which a microelectrode was used with a "stop" 40 μ from the tip. The microelectrode was lowered

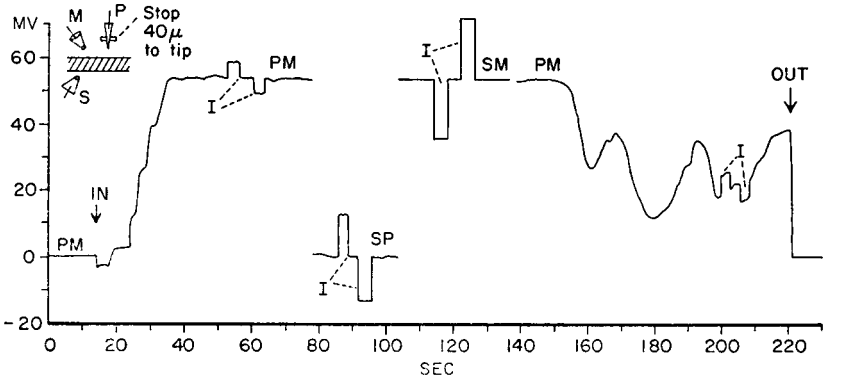

FIGURE 6 Resistance measurements during puncture with microelectrode with "stop" 40 μ from tip. pH of mucosal fluid was 1.0 (secretion allowed to accumlate). "In" denotes beginning of puncture and "out" end of puncture. During puncture PM, SP, SM, and then PM again were measured. Ordinate in mv and positivity means P + to M, S + to P, and S + to M. Step changes in PD (indicated by *I*) due to sending current of 1 ma across stomach via current sending electrodes IM and IS of Fig. 2. The rhythmic variations of the PD during the latter part of the puncture were common when microelectrodes were used with "stops." The hanging microelectrode was often displaced from a vertical position by the motility of the stomach and with a "stop" this displacement would be expected to result in an outward movement of the tip.

at the time indicated by the arrow "in" and rapidly raised at the time indicated by "out." The first change in PM was negative and the PD increased relatively slowly to a level essentially equal to the over-all PD. While the microelectrode was "in," the PD was also measured between S and P, then between S and M, and finally between P and M again. The step changes (marked *I*) seen in the records of Fig. 6 and 7 are due to the sending of 1 ma first in one direction and then in the opposite direction. The sum of the step changes in PD of PM and SP is equal to that of SM. The average value of the change in PD between P and M due to the sending of 1 ma for the experiments on the secreting stomach was 3.9 mv (SD $\pm$ 1.02, no. of experiments = 37). About one third of these experiments were performed with microelectrodes with "stops" and there was no significant difference between the

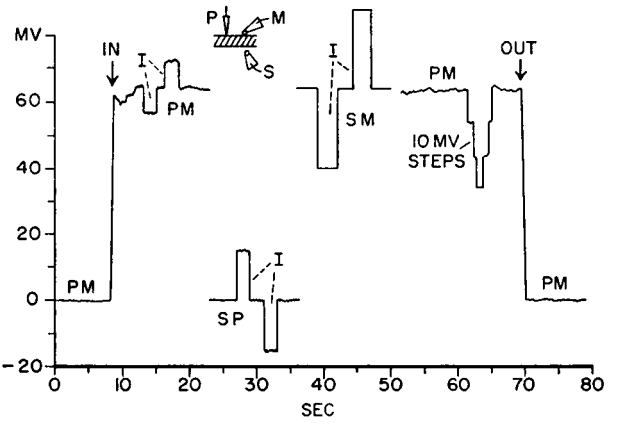

FIGURE 7 Resistance measurements during puncture of resting stomach with 0.16 M NaCl as mucosal fluid. Microelectrode without "stop" used. "In" denotes beginning of puncture and "out" end of puncture. During puncture PM, SP, SM, and then PM again were measured. Ordinate in mv and positivity means P + to M, S + to P, and S + to M. Step changes of PD (indicated by *I*) in PM, SP, and SM due to sending 1 ma across stomach via current sending electrodes first in one direction and then in the opposite direction. A voltage calibration shown in latter part of puncture.

results with the two types of electrodes. As would be predicted from previous studies with macroelectrodes (13, 16), there was no significant difference for the secreting stomach between the resistance with fresh 0.16 M NaCl and that with 0.16 M HCl as the mucosal fluid and the data were pooled for the above calculation. The average value for the change in PD between S and P due to the sending of 1 ma was 13.1 mv (SD $\pm$ 2.3).

The above values are from those experiments in which the PD between P and M was equal to that between S and M. In some of the experiments with microelectrodes with "stops," resistance measurements were obtained when the PD between P and M was definitely less than the PD between S and M. In these experiments in both resting and secreting stomachs the change in PD between P and M with the application of 1 ma was essentially equal to that obtained when the PD between P and M was equal to that between S and M. In five such experiments in the secreting stomach (one shown in Fig. 6) with PD_{PM} less than one half of PD_{SM}, the value of $\Delta PD_{PM}/1$ ma was from 80–100% of the value when $PD_{PM} = PD_{SM}$. On the basis of the assumptions that for a microelectrode with a "stop" (*a*) the tip of the microelectrode has penetrated into the interstitial fluid when the PD between P and M is equal to that between S and M, and (*b*) the tip is inside a SEC when the PD between P and M is less than that between S and M, it would seem that the resistance across the interstitial fluid border of the SEC is a small fraction of the total resistance across the SEC (see below).

Fig. 7 is an illustrative experiment on the resting stomach in which a microelectrode without a "stop" was used. It is comparable to Fig. 6. The average of the

change in PM due to 1 ma for all of the experiments on the resting stomach with 0.16 M NaCl as the mucosal fluid was 6.5 mv (SD ± 2.08, no. of experiments = 19) which is significantly greater than the corresponding value for the secreting stomach ($P < 0.001$, Students t test). The average change in PD between S and P due to 1 ma for the resting stomach was 14.8 mv (SD ± 2.8).

DISCUSSION

Liquid Junction Potential and the PD

It will be recalled that in the secreting stomach with 0.16 M NaCl as the mucosal fluid, when the initial jump in PD is oriented oppositely to the over-all PD, the magnitude is never more than 11 mv. This means that E_{MC} of Fig. 1 is 11 mv or less. It might be argued that during penetration of the mucous coat the microelectrode is moving so fast that it enters the cell before the PD due to E_{MC} could be recorded. This is negated by the findings: (*a*) that the PD of a liquid junction (in a test tube) is recorded with our technique within one second following the movement of the microelectrode through the boundary, (*b*) that with microelectrodes with "stops" it requires about 10 sec for a steady-state PD equal to the over-all PD to be reached, and (*c*) that in the exploration with microelectrodes without "stops" a deliberate attempt was made to position the microelectrode so as to measure the junction potentials in the mucous coat. We are not arguing that the per cent of punctures with an initial inverted orientation is important; our argument is that if E_{MC} (of Fig. 1) is larger than 11 mv then we should frequently measure an initial ΔPD greater than 11 mv.

The question may be raised as to why the microelectrode did not occasionally become negative by more than 11 mv since there would appear to be a finite probability of the tip penetrating into the depths of a pit. However, during studies on the in vitro frog stomach with microscopic visualization it was found that as long as there was the slightest movement of the mucosa it was impossible to lower the tip an appreciable distance into a pit without puncturing a SEC cell (unpublished observations). The spontaneous movements of the frog's mucosa are less vigorous than those of the dog's stomach so that it was feasible to hold the microelectrode with a micromanipulator without an almost immediate breakage of the tip. In only five cases, out of many hundreds of microelectrode explorations in the secreting frog stomach, was it possible to lower the microelectrode into the bottom of a pit and withdraw it without puncturing a SEC. These were only accomplished on the very rare occasions when motility was absent. Randomly lowering a microelectrode in the frog's gastric mucosa convinced us that, as long as there was motility, the probability of descending into a pit for even a short distance without puncturing a SEC was very close to zero.

The finding that E_{MC} in Fig. 1 is 11 mv or less confirms the explanation presented in the Introduction for the finding that in the secreting stomach, replacing isotonic

saline with isotonic HCl results in a change in PD which is about 40% less than the liquid junction potential between gastric juice and saline. The finding that the value of E_{MC} is less than 11 mv enables us to obtain some idea of the resistance via the outer SEC relative to that via the pits. It is obvious from equation 1 that the resistance via the SEC cannot be orders of magnitude greater than that via the pits since as R_{SEC} approaches infinity, ΔPD_{DM} approaches E_P. Assuming E_P = 31 mv, and ΔPD_{DM} = 19 mv, it follows from equation 1 that when E_{MC} = 11 mv, R_3 = 0.67 R_{12} and when E_{MC} = 0, R_3 = 1.6 R_{12}. In other words for the secreting stomach the effective resistance via the pits is about equal to that of the outer SEC which means that about half of an applied current would pass through the outer SEC and the

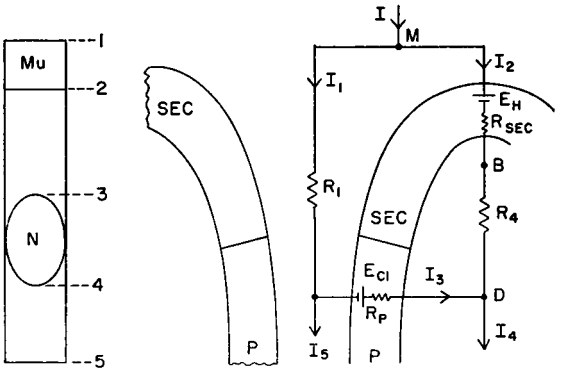

FIGURE 8 On left schematic drawing of a surface epithelial cell (SEC), MU refers to mucus in apical end of cell, N = nucleus, five levels of morphological discontinuities are indicated. On right a lumped parameter equivalent circuit shown for analysis of a particular formulation of the separate site theory on the basis of the resistance data. P = parietal cells, E_H = emf of electrogenic H^+ mechanism assumed on the basis of this particular formulation of the separate site theory to be in SEC, E_{Cl} = emf of electrogenic Cl^- mechanism in parietal or other tubular cells, R_P = resistance of parietal cells, R_{SEC} = resistance of SEC. D and B regions in extracellular fluid. I's = changes in the magnitudes of currents in the various parts of the circuit due to the sending of 1 ma across the stomach from the external source.

other half via the pit lumina. Therefore a knowledge of the ΔPD_{DM} (see Fig. 8) in response to an applied current would enable an estimate to be made of the actual resistance of the outer SEC ($\Delta PD_{DM}/0.5\ I$).

Resistance Measurements

The main purpose of the resistance measurements was to determine the resistance in the secreting stomach between the interstitial fluid of the pit region and the secretory fluid in order to test a particular formulation of the separate site theory (15).

Fig. 8 illustrates this particular formulation that we wish to examine. It is assumed that the H^+ mechanism is located in the SEC and that the Cl^- mechanism is located in the parietal cells. It is further assumed that a lumped parameter circuit is adequate

for this analysis. In Fig. 8 E_H, E_{Cl}, R_{SEC}, and R_P represent the emfs of the H^+ and Cl^- mechanisms and the resistances across the SEC and parietal cells. Previous studies have shown that the extracellular volume in the pit and upper tubular region is about 50% of the total volume of this portion of the mucosa (2), and therefore the resistance in this part of the ISF should be relatively small. The change in PD between points B and M of Fig. 8 due to the sending of current across the stomach would be less than that between D and M, i.e.,

$$\Delta PD_{BM} < \Delta PD_{DM}. \tag{2}$$

We define R_{DM} as

$$R_{DM} \equiv \frac{(R_1 + R_P)(R_{SEC} + R_4)}{R_1 + R_P + R_{SEC} + R_4}. \tag{3}$$

It follows from equations 2 and 3

$$\frac{\Delta PD_{BM}}{I} < \frac{\Delta PD_{DM}}{I} \leqq R_{DM} \tag{4}$$

where I is the total current ($I = I_1 + I_2$). The equality sign in equation 4 applies only if I_5 of Fig. 8 equals zero and since I_5 would obviously not be zero the inequality would hold. It follows from the foregoing that regardless of whether the microelectrode tip was at D or B that the change in PD between the microelectrode and M divided by the total current would be less than R_{DM}, i.e.,

$$\frac{\Delta PD_{PM}}{I} < R_{DM}. \tag{5}$$

The average value of $\Delta PD_{PM}/I$ for the secreting stomach was 3.9 mv per ma or 55 ohm cm² (area = 14 cm²). Now we would like to known the total IR drop around the loop linking the parietal and SEC, i.e. the sum of R_1, R_P, R_{SEC}, and R_4. For a given value of R_{DM} the minimum resistance around this loop would occur when the resistance of the two limbs between D and M are equal, i.e. when $R_{SEC} + R_4 = R_1 + R_P$. With 55 ohm cm² as the resistance across the loop, the minimum resistance around the loop would be four times this value or 220 ohm cm². Now in order to calculate the IR drop around the loop we need to know the H^+ rate calculated as current. The H^+ rate in our preparation ranged from 3.0–8.0 $\times$ 10^{-8} Eq sec^{-1} cm^{-2} which expressed as current is 2.9–7.7 ma cm^{-2}. Using 2.9 ma cm^{-2}, the minimum value for the H^+ rate and the minimum resistance around the loop (220 ohm cm²), the total IR drop would equal 638 mv which would represent a minimum value for the sum of E_{Cl} and E_H. We believe that it is highly unlikely that the sum of these emfs would be this high and conclude that the above form of the separate site theory is untenable.

In the foregoing the problem of electrical coupling between the SEC and the parietal cells was analyzed on the basis of coupling via the ISF and the lumen fluids. On the basis of Lowenstein's work (7) in which it has been shown that the electrical resistance between adjacent epithelial cells in a wide variety of tissue is very low, one might raise the question as to whether coupling would occur between the SEC and parietal cells via the bridges between cells and the lumen. On the basis of the finding that the major part of the resistance across the SEC is located at the luminal surface of the SEC, the same objection would apply to this type of coupling as for the coupling via the ISF.

It should be pointed out, on the basis of the analysis in the previous section, that R_{SEC} is approximately equal to the resistance via the pits and that the value of R_{SEC} would be approximately equl to $\Delta PD_{PM}/0.5\ I$ or 119 ohm cm^2 (3.9 mv/0.5 ma $\times$ 14 cm^2).

Are the PDs in the Mucosa of a Magnitude Substantially Higher than the Transmucosal PD?

One of the possibilities for H^+ secretion that has been considered (5, 10) is that the PD across the luminal membrane of the cell secreting the H^+ is large enough for the transport of this ion. If the pH of the cytoplasm of the acid secreting cell adjacent to the luminal membrane was about 7.4, then about 400 mv would be needed to transport the H^+ across the luminal membrane into the luminal fluid [60 mv $\times$ log ($10^{-0.8}/10^{-7.4}$)]. The possibility has been considered that a higher H^+ concentration than $10^{-7.4}$ M may be present in the cytoplasm adjacent to the secretory membrane (5, 10). For example, if the pH was 3.8 ($10^{-3.8}$ M) in this region and the H^+ concentration in the lumen was $10^{-0.8}$ M, then the H^+ would have to be transported up only a 1000-fold gradient instead of a 1,000,000-fold gradient and a PD of about 180 mv would represent the equilibrium value of the emf. Therefore it is possible that a PD of about 200 mv, assuming a high membrane permeability to H^+, could transport the H^+ at the observed rates. There are a large number of conceptual difficulties implicit in this suggestion such as the problem of the origin of an emf giving rise to a PD of this magnitude and also the problem of the PD at the opposite interface which would have to be about 145 mv (since the over-all PD of 55 mv has to be accounted for). Since we made numerous punctures to a depth of at least 1 mm and never observed a PD greater than 92 mv, we conclude that the magnitude of the PD between the cells and the lumen fluid or the ISF is not greater than 92 mv. Therefore the electric field could produce a concentration gradient of no more than about 30-fold.

We should point out that in explorations of the frog gastric mucosa we never observed a PD in the frog stomach greater than about 65 mv (unpublished observations). The maximum value of the PD reported by Villegas (21) for the frog mucosa was given as 49.2 mv ($\pm$0.5 SE). Villegas attempted to identify the site of his punctures by electrophoretic deposition of a dye from his microelectrode. Since we found

that the frog's mucosa is in constant movement, it is possible that the microelectrode tip during the deposition of dye might be some distance from its position during the last measurement of the PD.

Multiple Levels Of Potential

The above results indicate that during movement of the microelectrode through the SEC there are about five potential jumps in both the resting and secreting stomach. One might anticipate only two potential jumps on the assumption that the potential gradient inside the SEC is negligible. However, as illustrated in Fig. 8, the SEC has certain unusual histological features (2.8). The nuclei of these cells are quite large and cytoplasm between the nuclei and the cell border at the level of maximal nuclear width is practically nonexistent. Five regions of morphological discontinuity are indicated in this figure and these provide a possible histological basis for the finding of multiple potential levels. It is apparent that there are potential gradients inside the cells. The work of Choudhury and Snell (3) on the frog skin is of interest in this connection since they found an appreciable potential gradient inside the cells of this tissue. The frog skin potential increased more gradually than did ours but this difference may be related to the difference in the histology of the two tissues. The mucosal cell layer of the stomach is a single cell layer while the frog skin contains a multiple cell layer with cytoplasmic bridges between the cells. However, since our tissue was constantly moving, we are not able to relate the potential change with distance. All we can say is that there are multiple jumps of potential during penetration of the SEC.

One might be tempted to assume that the potential jumps are associated with local emfs. However, it must be recalled that there are at least four types of cells in the mucosal cell layer (8). The possibility cannot be eliminated that the algebraic sum of the emfs in one type of cell is not equal to that of the other types, with the consequence that there is current flow from one cell type to another. Obviously, the magnitude of this current would be less than that demanded for electrical coupling between the H^+ and Cl^- mechanisms. The potential jumps could be due to either *IR* drops or local emfs or both. In other words, it is possible that there are no emfs in the surface cells when the potential jumps are all in the same direction or that all of the potential jumps are primarily due to local emfs. However, when a potential jump in the opposite direction to the over-all PD it is possible to conclude that at least one emf is present in these cells. For example, it one assumes that current flows through the SEC from the ISF to the lumen, then a jump in the direction opposite to the over-all PD must be due to a local emf. On the other hand, if one assumes that current flows in the opposite direction, then all the *IR* drops would be oppositely oriented to the over-all PD, and every potential jump in the direction of the over-all PD would indicate the presence of a local emf.

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