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INTRAMUCOSAL GASTRIC ACID CONCENTRATION DETERMINED BY GLASS MICROELECTRODE TECHNIQUE

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SUMMARY

Penetrations of rat gastric mucosa with Pyrex or H^+-sensitive glass microelectrodes *in vivo* disclosed: (1) The potential difference within the mucosa is negative relative to serosa, the greatest values encountered in the mid-glandular zone; (2) regions of high acidity (H^+ activity to 170 mequiv/l) were encountered throughout the mucosa; (3) these highly acid areas are probably glandular lumina.

INTRODUCTION

The mechanism of gastric HCl production is not known; all the extant theories of HCl production, however, seem to hinge on the acidity of the fluid as it is secreted. We have therefore measured the pH within the mucosa of the secreting stomach and have recorded pH values as low as 0.77.

MATERIALS AND METHODS

Construction of microelectrodes

Glass microelectrodes of two types were constructed in a mechanical pipette puller. The first type, made of Pyrex capillary glass tubing, 1.0 ± 0.05 mm outer diameter, were used to measure electrical potential difference (PD). These electrodes were open tipped, with tip diameters of 0.5 μm or less. Suitable electrodes had a tip taper of 1:8 to 1:11 and were filled with 3 M KCl solution by boiling *in vacuo*. The electrodes subsequently chosen for use were those with tip potential of less than 10 mV and electrical resistance less than 50 MΩ (measured in 3 M KCl with a Beckman 41239 calomel half-cell as reference).

The second type of electrode was constructed from Corning No. 0150 pH-sensitive capillary glass tubing, 1.0 ± 0.05 mm outer diameter; these will subsequently be referred to as pH electrodes. Prior to pulling into electrodes the glass was coated with a compatible glaze, Pemco No. TR-514-A in No. 34 oil (Pemco, Division of Glidden Co., Baltimore) as described by Carter *et al.*[1]. Thus insulated, the capillaries were pulled into single-barrelled pipettes, open-tipped, with tip diameter less than 0.5 μm,

Abbreviation: PD, electrical potential difference.

and with tip taper 1:8 to 1:11. They were then filled with 0.5 M KCl by boiling *in vacuo*. The pH electrode was placed in a teflon electrode holder filled with 0.5 M KCl; an Ag–AgCl electrode in contact with the electrolyte solution in the holder was connected to a Beckman potentiometric recorder. A Beckman calomel half-cell electrode was used as reference.

The pH sensitivity of the electrodes was tested through a wide range of standards. These were sodium potassium phosphate (pH 7.00), National Bureau of Standards potassium tetraoxalate buffer (pH 1.679), and pure solutions of HCl of pH 0.68, pH 1.20, pH 3.32; the pH of each HCl standard was calculated by the formula $pH = -\log [H^+]$; the H^+ concentration used was that obtained by titration to electrical neutrality with 0.1 M NaOH.

Those with pH sensitivity of at least 25 mV per pH unit were selected for use. Our best electrodes had pH sensitivity of 45–50 mV per pH unit. Electrical resistance ranged between 150–1500 MΩ.

The pH response was not strictly linear through the entire range of standards tested. The least sensitivity was encountered between pH 0.68 and pH 1.20, intermediate sensitivity between pH 3.32 and pH 7.00, and greatest sensitivity between pH 1.20 and pH 3.32. A standard curve was therefore developed for each electrode used. Considerable variation between electrodes was noted; individual electrodes, however, exhibited reproducibility to within 0.1 pH unit on repeated testing over a period of several hours. These pH electrodes, if stored in the filling solution and refrigerated, were stable for 1–2 days after which the pH sensitivity deteriorated.

Lavallée[2] reported that the pH sensitivity of open-tipped electrodes of this type (but without the insulating glaze) was confined to the distal 1–2 μm of the electrode tip. In early experiments, however, we found that our electrodes were depth-sensitive and we therefore utilized the technique of glazing. The efficacy of this insulation was tested as described by Carter *et al.*[1]. Very thin latex membranes, approximately 1.0 μm thick, were prepared by placing a very small drop of undiluted latex injection compound over a 3-mm hole in a small square of parafilm. When dry, the thin latex film was laid over a small container of pH 2 buffer–agar into which had been placed a calomel half cell prior to hardening. The electrode tip was advanced through the latex into the agar under micrometer control and microscopic observation. At the desired depth of penetration, pH 6.8 buffer was overlaid around the shaft of the electrode. In well-insulated electrodes, no change in the electrometer reading occurred. The electrodes selected for use were effectively insulated such that the sensitivity was confined to the distal 10 μm or less of the tip.

Since the electrodes had open tips, they could be expected to measure PD as well as pH. Each electrode was tested by imposition of a known voltage, from 0–90 mv, and the imposed voltage was faithfully registered.

Preparation of experimental animals

Sprague–Dawley rats, 300–500 g, were maintained on a standard laboratory chow diet with water *ad libitum*. Without prior fasting the animal was anesthetized by an intraperitoneal injection of sodium pentobarbital, at an initial dose of 30 mg/kg. The abdomen was widely incised, the stomach was delivered into the wound, incised along the greater curvature and emptied of contents. The animal was placed supine in a lucite box and the stomach was gently pulled up through a slot in a bakelite chamber

base and exposed, mucosa up, with blood supply intact. Particular care was taken not to damage or stretch the acid bearing portion of the stomach. A lucite chamber was then placed over the exposed stomach and secured to the chamber base, maintaining the gastric blood supply and forming a fluid-tight chamber opened at the top. The chamber base had been overlaid with a thin layer of Ringer's agar and the serosa of the stomach rested upon the agar. In this preparation the stomach continued to spontaneously secrete acid for 1–3 h. Viability was maintained for up to 6 h and gastric acid secretion could at any time be stimulated by the administration of reserpine or pentagastrin. We made no effort to quantitate gastric acid production, but simply monitored the mucosal chamber fluid at frequent intervals to determine that acid production was continuing.

Measurement of intramucosal PD and pH

Isotonic Ringer's solution, with HCl added to a specific pH when desired, was placed in the mucosal chamber. Calomel half-cell reference electrodes were in contact either with the serosa of the stomach, through a Ringer's agar bridge continuous with the thin agar layer upon which the serosa rested, or with the gastric mucosa, contacting the fluid in the secretory chamber. The microelectrode to be used was placed in the teflon electrode holder which in turn was filled with the appropriate solution filling the electrodes. The electrode holder was attached to a micromanipulator and the electrode tip was directed at the mucosa vertically from above. The electrode was inserted into the secretory fluid, then placed in contact with the mucosal surface (observed microscopically), then driven through the mucosa in 10 μm increments while the PD was recorded. No attempt was made to insert the electrode into gastric pits.

After penetration of the mucosa the electrode was withdrawn and checked for breakage as follows: for PD electrodes, tip potential and resistance were determined and the tip was observed microscopically. For pH electrodes, tip resistance and pH response to a strongly acid standard were checked. If any of these parameters were altered, the electrode and the data from the penetration were discarded.

Localization of electrode tip

We added 2 % lithium carmine, a positively charged dye, to the filling solution of several PD electrodes[3]. These electrodes were attached to a power supply; the circuit was completed by an Ag–AgCl wire rather than a calomel half-cell electrode. The dye-filled electrode was driven into the mucosa in a fashion identical to that described above. At a selected test depth of 100, 300, or 600 μm from the mucosal surface, a 2–3 μA direct current was applied for 1 s, then reversed for 1 s, after which the electrode was withdrawn, a full-thickness block of mucosa including the test site was removed, fixed in Bouin's solution, sectioned serially, and search was made microscopically for a dye spot in the unstained sections. In 12 such mucosal preparations the dye spot was located in 11 instances and was always within 10–30 μm of the recorded micrometer depth. The thickness of the acid-bearing mucosa was estimated from the 12 mucosal specimens to be 650–750 μm.

We assumed that all electrode penetration depths, PD and pH, corresponded similarly to the micrometer reading. No tip localization was ever attempted from the electrode actually recording PD or pH, however.

All electrode testing and electrical measurements were performed within a grounded screened cage. Electrical power to the cage was filtered alternating current.

RESULTS

PD measurement

116 penetrations with intact PD electrodes were carried out in the acid-bearing gastric mucosa of 51 rats. The transmucosal PD varied between 15–42 mV, mucosa always negative. Further, during all mucosal penetrations the electrode tip was always negative with reference to the serosa. The PD was not at all uniform as the electrode penetrated the mucosa but fluctuated widely. Although the recording from each penetration was unique and quite different from all others in many respects, a pattern or PD profile was easily recognizable in fully two-thirds of the recordings, was suggested in perhaps an additional 15 % of recordings, and no pattern was recognizable in the remaining 20 %. This PD profile is illustrated in Fig. 1a: after recording a mucosally negative transmucosal PD entry of the surface epithelium results in a small change toward less negativity, persisting as the electrode penetrates the more superficial portion of the mucosa. In the middle zone, a region of marked electrical negativity is encountered. In this zone, the PD of greatest magnitude was regularly observed, and was usually considerably greater than the transmucosal PD, reaching −60 to −80 mV. In the deep mucosal zone (in Fig. 1a, beyond 590 μm) the PD assumed less negativity but, with continued penetration, varied rather widely. Finally, with electrode tip through the mucosa (750 μm, Fig. 1a) the PD approached zero.

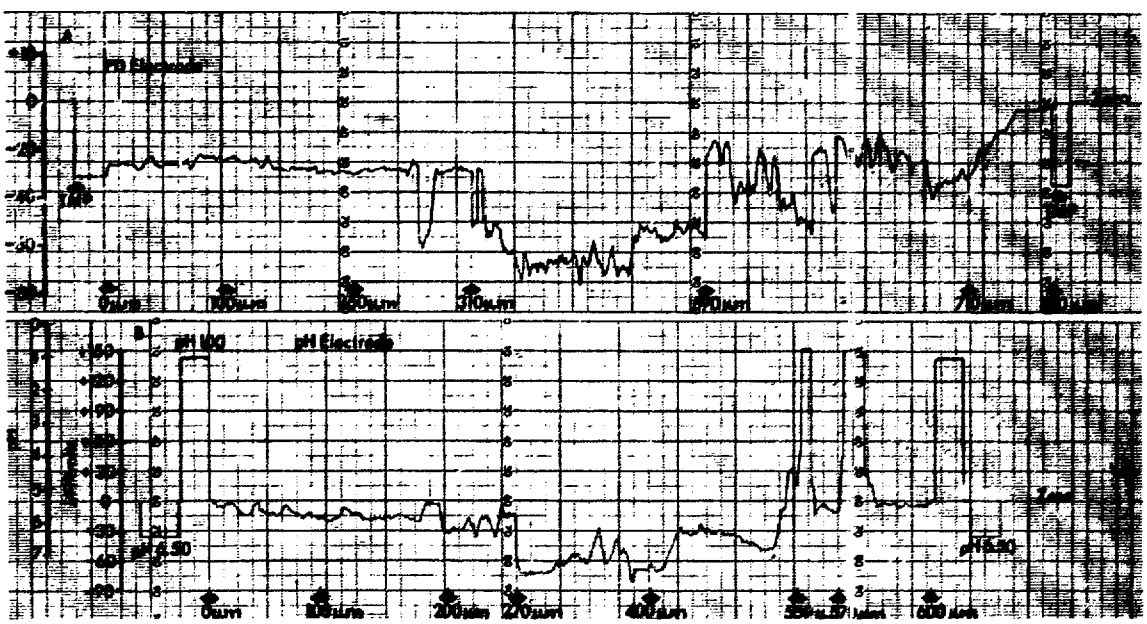

Fig. 1. A. Mucosal penetration with PD electrode. The transmucosal PD is 31.3 mV (mucosa negative). During penetration the electrode tip is always negative. The heavy lines perpendicular to the abscissa represent 1-min intervals. The numbers along the abscissa refer to depth of electrode penetration from the mucosal surface. See text for description and interpretation of the PD profile. B. Mucosal penetration with pH electrode. After standardization with pH 1.00 and 6.50 solutions overlaid on the mucosal surface, mucosal penetration produces a pattern similar to that with PD electrode. At 550 μm and again at 570 μm from mucosal surface, regions of high positive PD are encountered, representing areas of high acidity. TMP = transmembrane potential.

pH measurement

The pH electrodes responded to PD as well as to H^+ activity. Increasing acidity (decreasing pH) resulted in increasing electrical positivity registered by the pH electrodes; therefore, since the PD profile of the mucosa was always electrically negative as shown with the PD electrodes, and since highly acid solutions evoked electrically positive deflection from the pH electrodes, penetration of the mucosa by the pH electrodes could be expected to result in recordings similar to those obtained with PD electrodes, provided no regions of high acidity were encountered. If such regions were encountered, however, the high electrical positivity evoked by the highly acid solution could be expected to contrast sharply with the negative PD arising from the mucosa *per se*.

Thus, 112 mucosal penetrations were performed with pH electrodes in the 51 rats; these penetrations resulted in recordings similar to those obtained with PD electrodes, except that in sharply defined regions within the mucosa, sudden high positive PD deflections occurred, indicating the presence of acid in high concentration (Fig. 1b, 550 μm, 570 μm). 34 such regions were encountered in successful penetrations in 29 of the rats. The regions were 10–40 μm in dimension and were encountered at depths between 100 and 640 μm from the mucosal surface.

Estimation of the pH of these regions demands an accurate knowledge of the contribution to the recorded PD from sources other than H^+ activity. We made two assumptions: first, we assumed the regions reflecting high acid concentrations were actually lumina of the mucosal pits or glands and, second, that PD measured between glandular lumen and serosa was identical to the transmucosal PD measured between mucosal surface and serosa. With these assumptions, the absolute value of the transmucosal PD was added to that obtained during the high positive deflection. The resultant value, in mV, was then converted to pH from the calibration data of the electrode involved. Fig. 2 shows the calculated pH values for the 34 areas encountered (abscissa) plotted against the depth of electrode penetration from the mucosal surface (ordinate). The pH was 0.77 in 11 instances; in 25, it was below 1.00. The pH 0.77 regions were distributed throughout the mucosal thickness to a depth of 640 μm from the surface.

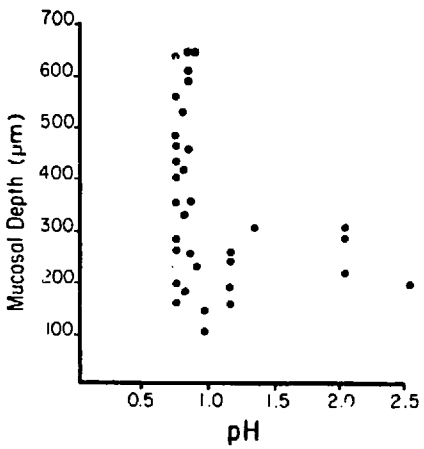

Fig. 2. Distribution of regions of high acidity encountered in penetrations with pH electrodes. Each point represents the calculated peak acidity (abscissa) of such an area plotted against the depth of penetration from mucosal surface (ordinate) at which the region was encountered

Multiple penetrations into forestomach, liver, duodenum and muscle of several rats never resulted in positive PD deflections as observed in the acid-bearing gastric mucosa.

DISCUSSION

Analysis of the PD profile on the basis of the data presented permits no firm conclusions. Of interest is the finding that in the mid-zone of the mucosa the PD measurements were regularly of much greater magnitude than the transmucosal potential. This finding may suggest that the transmucosal PD is generated in that region which corresponds roughly to the area of maximum parietal cell population. If this tentative interpretation is correct, reconciliation of the observed electrical data would seem to require either that, (1) the cell(s) responsible for the large magnitude PD be in electrical series with the other cells functioning as resistances; or, alternatively, that, (2) the cells responsible for the high magnitude PD are electrically asymmetrical, as suggested by Villegas[3], with the transmucosal PD representing the difference between the mucosally directed and serosally directed electrical forces.

It is likely that the regions of high acidity are actually the lumina of the glands and not, for example, the interior of a certain type of cell. First, the rarity of encountering the region during any one penetration, together with the almost universal distribution of the high acidity regions throughout the mucosa is best explained by the distribution of glandular lumina. Second, the dimensions of the regions are appropriate for glandular lumina. The acid-producing glands of the mammalian stomach are coiled. A perpendicular penetration should thus randomly enter and traverse short segments of the glandular lumina then re-enter glandular cells. The abrupt onset and end of the high positive electrical deflections and the 10–40 μm transversed during the deflection support this interpretation. Finally, it seems far more reasonable that acid of the concentrations encountered be present in glandular lumina than in the interior of any cell.

The lowest pH observed, pH p.77, reflects H^+ concentration of 170 mequiv/l, which approximates the theoretical maximum acid concentration which can be secreted by the gastric mucosa, according to Hollander[4].

The distribution of the pH 0.77 acid throughout the depth of the gland casts serious doubt on any theory of HCl formation which demands an acid concentration gradient in the glandular lumen. Hirschowitz[5], for example, postulated an active secretion of all components of gastric juice except H^+ from the chief cell region of the gastric gland, and ar $Na^+ : H^+$ exchange mechanism in the parietal cell area to account for the final acidity of the secretion. Rehm and co-workers[6] proposed the separate site theory, in which Cl^- is actively secreted and H^+ at a different site (perhaps even from the mucosal surface) in response to the net negative charge of the chloride ion. Each of these theories seem to require a region of low acidity deep in the gland, the secretion becoming more acid toward the mucosal surface. Our observations fail to confirm this possibility.

On the other hand, the observed values permit the interpretation that acid is secreted in varying concentrations up to 170 mequiv/l, or is mixed with varying quantities of a neutral secretion as proposed by Hollander[4]. It is even possible that once the acid is secreted, its concentration is altered by ionic exchange, diffusion or absorption

as the fluid traverses the gland[7]. The fact that we often found lower acidities at more superifical levels lends support to the latter possibility.

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