Correlation of the Surface pH, Histology, and Gastrin Concentration of Gastric Mucosa

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Gastrin is assumed to come from the mucosa of the gastric antrum, but gastrin-like activity has been reported from extra-antral sites. There have been occasional reports of isolation of gastrin-like activity from extracts of fundic mucosa, especially mucosa taken from the area of the gastric cardia. Although gastrin has been assumed to arise in the stomach nearly exclusively from antral mucosa, no studies have been made to correlate the gastrin content of mucosa with the histologic architecture at the line of transition between antrum and fundus. Capper and colleagues carefully mapped the surface of the human gastric mucosa with a pH probe at operation and attempted to correlate the alkaline pH of the antral mucosa with the concentrations of gastrin that were determined by bioassay of sections of mucosa on either side of the antral-fundic junction. The gastrin content was significantly less on the acid side of the pH demarcation line in 10 of 18 specimens, but in eight of these only a slight difference could be demonstrated. They suggested that the antral-fundic junction, as determined by gastrin content, may be a wide border zone containing both antral and fundic mucosa.

In an attempt to define better the extra-antral areas of gastrin synthesis or storage and to relate histologic structure to gastrin content, we have measured by radioimmunoassay the gastrin concentration in adjacent sites over the entire gastric mucosa of the dog, and we have correlated these measurements with mucosal histology and surface pH.

Materials and Methods

Acute studies were performed in five healthy adult mongrel dogs weighing from 20–25 Kg. Each dog was anesthetized with intravenous pentobarbital sodium (15 Mg./Kg.), and 100 mg. betazole hydrochloride (Histalog—Lilly) was administered intramuscularly to stimulate gastric secretion. The abdomen was opened through a midline incision and the duodenum was divided 1 cm. distal to the pylorus. The stomach was then opened widely through a longitudinal incision into the anterior walls 2 cm. from the greater curvature which extended through the junction with both esophagus and pylorus. Hemostasis was meticulous, and the cut edges of the stomach were sutured tautly to the abdominal wall so that the entire mucosa of the stomach presented as a flat surface.

One hour after the administration of Histalog, the gastric mucosa was carefully sponged dry, at which time the mucosa was observed to be actively secreting. With the esophagus, pylorus, and lesser curvature of the stomach as reference points, the pH of the fluid emerging from the mucosa was determined at 2-cm. intervals over the surface of the gastric mucosa with a pH meter (Radiometer—Copenhagen) and combination glass-reference electrode (Beckman model 39030). Care was taken to avoid pressure on the gastric mucosa except for the weight of the electrode. The points at
Fig. 1. Diagram of the mucosal surface of the canine stomach showing average mucosal gastrin concentration, pH levels and histologic demarcation between antrum and fundus in five dogs. E denotes esophagus. The dotted line between the esophagus and the pylorus represents the lesser curvature of the stomach. The mean gastrin concentration (and the range of gastrin content) is given beneath each square as nanograms of gastrin per gram of mucosa (ng/g). Individual pH determinations for all five dogs are given within the squares. The shading represents the area that was histologically typical antrum.

which pH determinations were made (Fig. 1) were marked with a minute droplet of toluidin blue. Full thickness biopsy specimens of the gastric wall, 1 cm. in diameter and centered on the dye mark, were taken with a sharp punch and were placed in a fixing solution (10% formalin-pH 7.6) for later histologic evaluation. For later measurement of gastrin content, sections of gastric mucosa (2 cm. × 2 cm.) were dissected from the area surrounding each biopsy site. These mucosal specimens were gently sponged free of blood, placed in previously weighed containers, and thoroughly minced. The specimens were then diluted 1:10 in distilled water and immediately placed in a boiling water bath for 30 minutes. After centrifugation at 20,000 rpm for 20 minutes, the supernatant solution was removed and frozen for later measurement of gastrin concentration by radioimmunoassay. In early studies gastrin was measured in paired tissue specimens that were treated either by initial boiling and later freezing or by freezing alone.

The radioimmunoassay utilizes antigastrin serum produced in rabbits immunized with the 2–17 residues of synthetic human gastrin I conjugated to bovine serum albumin. Our current assay technique11 is a modification of our initial method.6 All samples, after appropriate dilution, were assayed twice.

The determinations of the pH of the mucosal surface, interpretations of histology, and the measurements of gastrin concentrations were each performed by different investigators working separately and (in the case of the latter two studies) without knowledge of the origin of the tissue in order to prevent prejudicial interpretation of the data.

Results

The measurements of surface pH showed good reproducibility, but to minimize error the first reading at each site was taken for record. The results of the radioimmunoassay of unboiled specimens were found to be erratic, with gastrin concentrations about 1/1000 that of boiled specimens. All results in this study are from mucosal specimens that were boiled immediately on removal.

Figure 1 is a schematic drawing of the mucosal surface of the stomach opened longitudinally through the anterior wall near the greater curvature. E denotes the esophagus, and a dotted line is drawn along the lesser curve. The squares represent the sites where mucosal pH, histology and gastrin content were determined in the five dogs. The pH determinations for all five dogs are given within the squares, and the average gastrin content (and range of gastrin content) in nanograms of gastrin per gram of mucosa is given beneath the squares. The average proportion of each mucosal sample that was identified histologically as antrum (determined by multiple sections at each site) is depicted by the proportionate shading of each square. Those mucosal sites that were on histologic evaluation entirely fundus in all dogs, contained (with one exception at the antral-fundic junction) concentrations of gastrin from 2–72 nanograms per gram. In sharp contrast, the mucosa which was histologically antrum contained between 10,100 and 42,500 ng/gm. (10.1–42.5 μg/Gm.) of gastrin.

There was good correlation between higher pH values and the higher concentrations of mucosal gastrin at the histologic antral-fundic junction. Several paraesophageal or high cardiac biopsy sites were found to contain concentrations of gastrin that were 2 to 3 times higher than the average concentration in fundic mucosa. There were also scattered sites that gave high pH readings, but there was poor correlation in the paraesophageal region between pH and gastrin content. In each case at histologic examination all mucosal biopsies from this area (cardia) were identified as fundus, although the mucosa was thin and the concentration of parietal cells was low.
Histologic examination of the pyloric region shows pyloric glands occupying the deeper one-half of the thickness of the mucous membrane (Fig. 2a). The pyloric gland cells are identified by the presence of basilar compressed quarter-moon-shaped nuclei. Pits lined by surface mucous cells constitute the superficial half of the pyloric mucous membrane. Figure 2b is a photomicrograph of typical fundic mucosa. The light basophilic chief cells with centrally-placed round nuclei and the eosinophilic granular parietal cells are prominent; basal glands are absent. In Figure 2c, the histologic transition zone between the pylorus and fundus is shown to be well demarcated and is characterized by the presence of glands containing a mixture of pyloric gland cells and parietal cells with a few scattered chief cells. In the narrow line at the antral-fundic junction, elements of both fundus and antrum were present in sections that were only 1 cm in diameter, whereas biopsy specimens on either side of the line yielded either typical fundus or antrum.

**Discussion**

These studies demonstrate the necessity for immediate inactivation of tissue autolysis in order to halt the rapid loss of gastrin after excision of the tissue. There was a thousand-fold loss of gastrin in those tissues not subjected to boiling immediately on removal. This observation is consistent with the studies of Berson and Yalow who found that quick-freezing of mucosal specimens with dry ice, followed by boiling at a later time, gave much higher gastrin values than did simple initial freezing. It seems imperative to inactivate tissue autolysis quickly, either by immediate boiling or by quick freezing. If tissues are allowed to stand at room temperature or even in the refrigerator, gastrin values will fall.

The importance of the gastric antrum as the primary source of gastrin is well established. Although the histologically defined antrum has been found to correlate with the area of gastric mucosa that has a high surface pH, Capper and co-workers were the first to
attempt to correlate mucosal pH with gastrin concentration. They found a wide antral-fundic transition zone in peptic ulcer patients. In our study we measured immunochemically recognizable gastrin in normal dog stomachs and found a high degree of correlation between nonacid-secreting gastric mucosa and the area of mucosa containing high concentrations of gastrin. The transition zone at the antral-fundic junction as determined by histologic observation and as determined by measurement of surface pH and mucosal gastrin content, seems to be narrow. There were several areas of high pH and of relatively high gastrin content adjacent to the cardia, but there was not good correlation between the two in this area.

Gastrin-like activity has been reported in mucosa from the gastric cardia,8,11,12,15 and Elwin and Uvnäs9 have emphasized the similarity between the mucosa of the cardia and antrum, but no gastrin has been found previ-}

ously in the central gastric fundus.13 The concentrations of gastrin in the antral mucosa as measured by radioimmunoassay in our study are in the order of magnitude of several micrograms per gram of mucosa which is in agreement with previous studies.2,10,21 Fundus gastrin levels are approximately 1/1000 the levels noted in antral mucosa, i.e. nanograms/gram. This relationship between antral and fundic gastrin concentration is consistent with the observations of McGuigan who reported 500 times more gastrin-containing cells in the antrum than in the fundus of pigs, as shown by immunofluorescent studies.18

More important extra-antral sources of gastrin are probably the duodenum and jejunum.9,23 Significant amounts of gastrin may be produced by the duodenum in man26 and it is apparent that standard resection procedures for peptic ulcer do not excise all gastrin-bearing tissue. We have found diminished but still detectable levels of serum gastrin in dogs after total gastrectomy.19

Summary

In this study we measured the gastrin concentration and surface pH at intervals over the entire gastric mucosa of five dogs and correlated these levels with the histologic anatomy of gastric mucosa. Maximum extraction of gastrin from mucosa was affected by boiling the tissues immediately after procurement. An increasing pH and a high gastrin concentration characterized the mucosal area designated histologically as antrum. There was a sharp demarcation of gastrin content and histology at the antral-fundic junction. Although antral gastrin concentrations were about 1000 times higher than fundic concentration, significant amounts of gastrin were consistently measured in fundic mucosa; this study provides additional evidence for the existence of extra-antral sites for gastrin production.

References

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