Roles of Gastrin Release and Neural Reflexes during the Gastric Phase of Acid Secretion in Dogs[1]

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Abstract. The acid secretion from the main stomachs and from denervated Heidenhain pouches in response to peptone meals infused in the stomach was determined in 4 dogs. When expressed as percent of the maximal acid response to histamine, the acid secretion from the innervated and from the denervated fundic mucosa was similar, but the response from the Heidenhain pouch was larger when expressed as percent of the maximal response to pentagastrin. These studies indicate that, under the conditions used, short (gastrogastric) and long (vagovagal) reflexes during the gastric phase, are of minor importance in inducing the acid response to the meal.

Introduction

It has been known for many years that during the gastric phase of postprandial acid secretion the acid response is at least partly the result of gastrin release. More recently *Grossman's* [1–3, 7, 15] group showed that in dogs, distention of the stomach stimulates acid secretion through mechanisms which do not involve gastrin release but rather long and short vagal reflexes. Some data also indicate that contact of peptides with the fundic mucosa could lead to a marked increase in acid secretion through a local mechanism once

again independent from gastrin release [3]. However, the latter mechanisms have been demonstrated mainly in animals prepared with antral and/or fundic pouches, under a constant distending pressure. The relative importance of gastrin release, and of reflex and local mechanisms during the gastric phase in more physiological conditions, has not yet been assessed. The purpose of the present work is to make such an evaluation.

Material and Methods

Experiment 1

4 mongrel dogs with gastric fistulas (12–18 kg) were used. Preliminary experiments showed that the place of the cannula allowed the gastric content to be

easily mixed and drained. No experiments were started sooner than 3 weeks after surgery, and the time interval between the two experiments was at least 48 h. The dogs were deprived of food for at least 18 h before each test, but they could drink water freely. At the beginning of each experiment the gastric cannulas were opened, and basal secretions collected by gravity for 45 min before any stimulatory procedure was started. When residual food was found in the main stomach, or when the basal secretion exceeded 0.5 mEq/h, the experiment was cancelled. A rubber adapter was connected to the cannula through which the test meal could be instilled and the gastric content sampled. The test meals were solutions of Bactopeptone in water (Bactopeptone, Difco, Detroit, Mich.) in concentrations ranging from 0 to 30%. PVP iodine-125 (Radiochemical Center, Amersham, England) ($\pm$ 5,000 cpm/ml) as a tracer, and cold PVP (1 mg/ml) to prevent adsorption of the labeled PVP on the tubes and stomach walls, was added. The pH was ajusted to 7.4. The meal was injected through the gastric cannula with a peristaltic pump over a period of 90 s. The volume of each meal amounted to 295 ml. The gastric contents were mixed for 1 min every 10 min with a 50-ml syringe, and 5 ml were sampled. Immediately thereafter 10 ml of a solution containing PVP iodine-125 ($\pm$ 150,000 cpm/ml) and cold PVP (1 mg/ml) was injected into the stomach and carefully mixed with the gastric contents. A second sample of the gastric contents was then taken. The pH and acid content of gastric samples were determined with a titration assembly (Radiometer, Copenhagen, Denmark). The titration solution used was 0.05 M NaOH and the end point pH 7.4. Concentrations of PVP iodine-125 were determined in triplicate on 1-ml samples with a gamma counter (Minigamma, LKB, Sweden). Intragastric volumes and rates of acid secretion were determined according to the method described by *Dubois* et al. [5], using a Hewlett Packard MP97 computer (Palo Alto, Calif.). The use of this method has been validated for both water and Bactopeptone solutions [4, 5]. Moreover, in preliminary experiments, the 4 dogs used in the present study were treated with cimetidine (2.5 mg/kg/h) and an HCl solution (0.16 M) was infused at a constant rate into the stomach through a small catheter connected to the cannula. A 15% Bactopeptone meal was then introduced into the stomach and the HCl influx calculated by the above-mentioned method. Calculated influx ranged from 80 to 120% of the real influx.
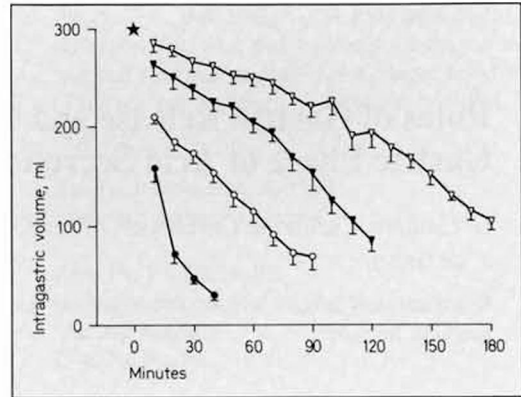
Fig. 1. Mean $\pm$ SEM intragastric volumes observed after introduction of water or Bactopeptone meals in the stomach of gastric fistula dogs. $\bullet$ = Bactopeptone 0%; $\circ$ = Bactopeptone 7.5%; $\blacktriangledown$ = Bactopeptone 15%; $\triangledown$ = Bactopeptone 30%. * = Theoretical value at the zero.

Blood samples were obtained at regular intervals from a catheter placed in a leg vein, and perfused with saline solution containing heparin. Blood was allowed to clot at 4 °C, serum removed and stored at –20 °C until assayed for gastrin. Serum gastrin was measured by a previously described radioimmunoassay [17].

Experiment 2

4 other dogs (10–19 kg) were prepared with a denervated fundic pouch (Heidenhain pouch) and a gastric fistula. The pouch was made in such a way that the general shape of the stomach remained intact. The dogs were given the same conditions and controls as in experiment 1. The volumes of the meals were reduced to 250 ml. Acid secretion from the pouch was collected by gravity every 10 min, and the acid concentration determined with 0.05 M NaOH using a titration assembly.

In order to compare the rates of acid secretion in the 4 dogs, in which the gastric acid outputs were different, the results of the feeding tests were expressed as percentages of the maximal acid responses to pentagastrin and histamine. The maximal acid responses to histamine and pentagastrin were determined for the main stomach and the Heidenhain pouch in each of the dogs. Histamine (Merck, Darmstadt, FRG) and pentagastrin (ICI, Macclesfield, Cheshire, England)
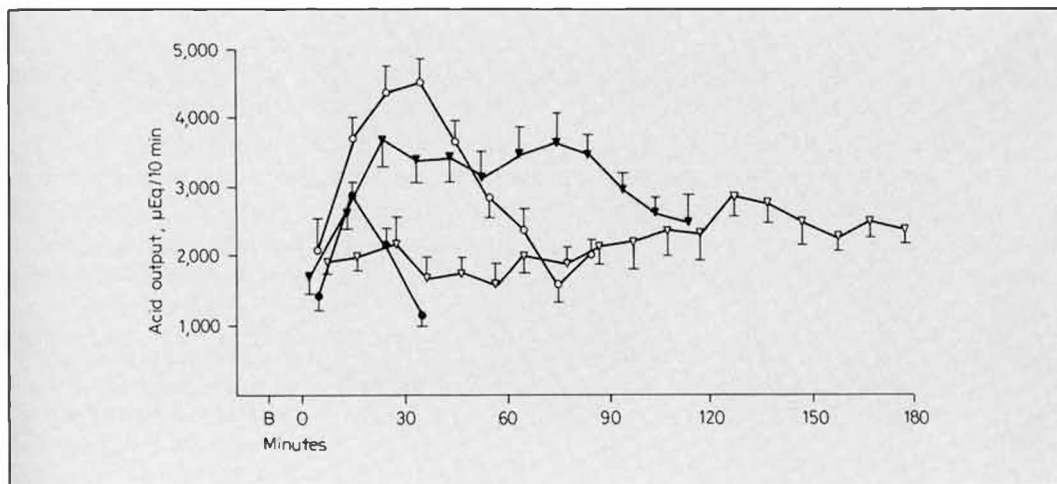
Fig. 2. Mean ± SEM acid outputs in response to Bactopeptone meals in gastric fistula dogs. Symbols as in figure 1. B = Basal values zero.

were infused intravenously for 45 min with a peristaltic pump, at doses ranging from 80 to 320 µg/kg/h and 2–8 µg/kg/h, respectively. Acid outputs were estimated during the last 15 min of each period. The highest secretion rate obtained was taken as the maximal acid output.

Statistical Analysis

All experiments were performed twice on each of the 4 dogs. Statistical analysis of the data was performed using the analysis of variance. For each experiment, the total acid output was taken into consideration as recommended by *Elashoff* [6].

Results

Experiment 1

Water leaves the stomach very rapidly. Gastric emptying is slowed dose-dependently by adding increasing amounts of Bactopeptone to the meal (fig. 1).

An acid response was obtained with all meals. The peak acid output was lower with the 30% Bactopeptone meal than with the 7.5 or 15% Bactopeptone meals (fig. 2). Peak

gastrin concentrations were similar for the three meals containing Bactopeptone (fig. 3).

With the 0, 7.5 and 15% Bactopeptone meals, the importance and duration of the acid response was similar to the duration and importance of gastrin release. A linear correlation was found between the log gastrin level and acid output (fig. 4).

Experiment 2

A clearcut distention of the stomach was obtained with all of the Bactopeptone meals (fig. 5).

In the main stomach, the maximal acid outputs ranged from 3.04 to 6.54 mEq/10 min in response to histamine and from 2.02 to 4.83 mEq/10 min in response to pentagastrin. In the Heidenhain pouches these values ranged, respectively, from 0.47 to 1.21 mEq/10 min for histamine, and from 0.13 to 0.82 mEq/10 min for pentagastrin.

During the feeding tests, an acid response to all meals was obtained from both the fistula and the Heidenhain pouch. The highest
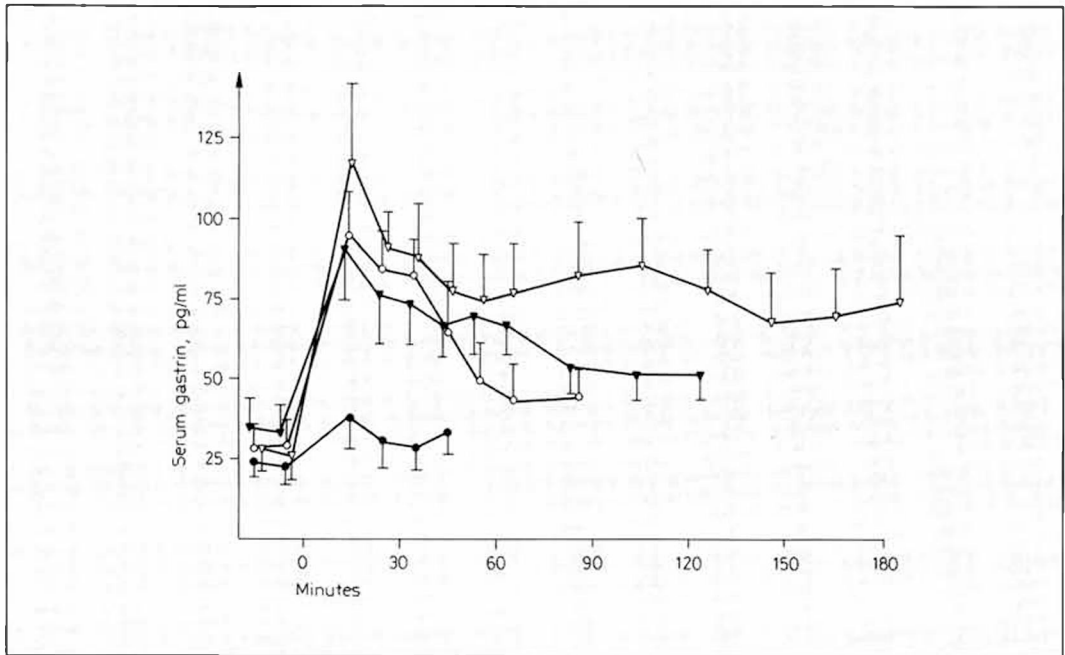
Fig. 3. Mean ± SEM serum gastrin concentrations in response to Bactopeptone meals in gastric fistula dogs. Symbols as in figure 1.
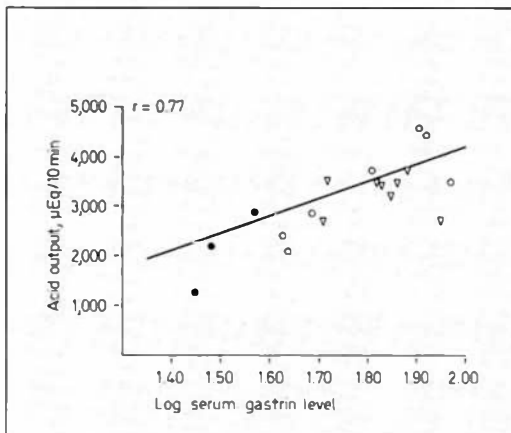

Fig. 4. Correlation between the log serum gastrin levels and acid outputs in gastric fistula dogs stimulated with 0, 7.5 adn 15% Bactopeptone meals. Symbols as in figure 1.
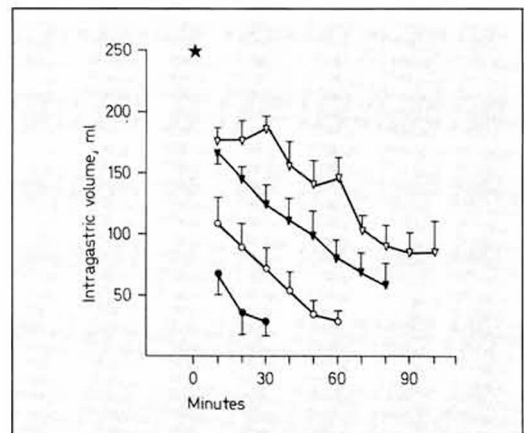

Fig. 5. Mean ± SEM intragastric volumes observed after introduction of water or Bactopeptone meals in the stomach of gastric fistula dogs provided with Heidenhain pouches. Symbols as in figure 1. * = Theoretical value at the zero.

Fig. 6. Mean ± SEM acid outputs from gastric fistulas (---) and the Heidenhain pouches (——) in response to liquid meals expressed as percent of the maximal acid response to histamine. **a** Bactopeptone 0%. **b** Bactopeptone 7.5%. **c** Bactopeptone 15%. **d** Bactopeptone 30%.
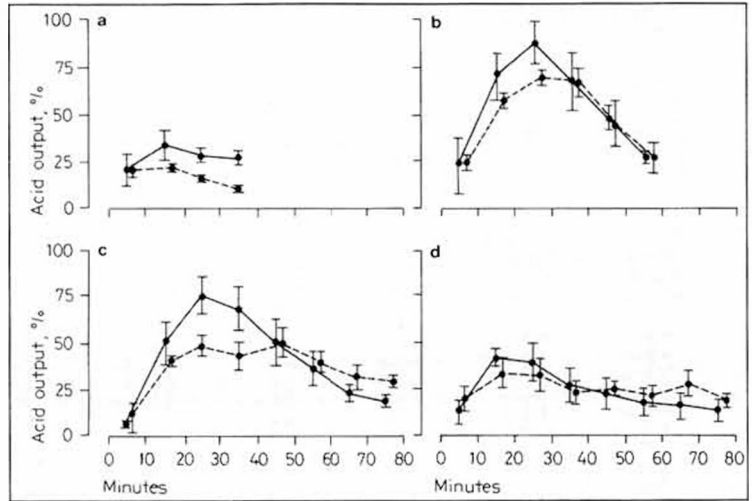
Fig. 7. Mean ± SEM acid outputs from gastric fistulas (---) and the Heidenhain pouches (——) in response to liquid meals expressed as percent of the maximal acid response to pentagastrin. **a** Bactopeptone 0%. **b** Bactopeptone 7.5%. **c** Bactopeptone 15%. **d** Bactopeptone 30%.
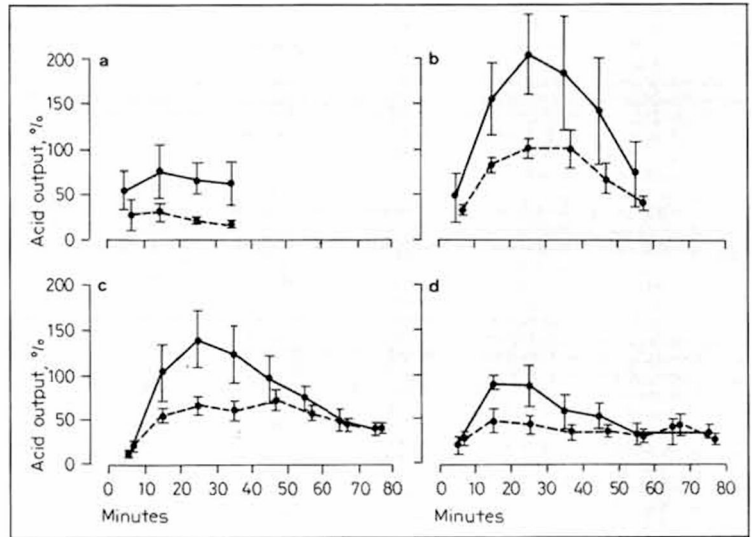
acid output was observed with the 7.5% peptone meal (fig. 6, 7).

When expressed as percentages of the maximal acid response to histamine, the acid outputs from the main stomach and the Heidenhain pouch were almost identical. When expressed as percentages of the maximal response to pentagastrin, the acid responses from the Heidenhain pouches were significantly greater than those from the fistulas for the 0, 7.5 and 15% bactopeptone meals (p < 0.01 in all three cases).

When the whole set of data is taken into consideration, a striking parallelism can be observed between the responses from the main stomach and those from the denervated pouch.

Discussion

The different meals used in the present study all elicited a gastric distention, and a clear cut gastrin and acid response. The duration of distention increased with the concentration of peptone in the meal. This observation is explained by the presence in the gut of osmoreceptors activating inhibitory feedback mechanisms slowing gastric emptying. Gastric emptying of similar solutions was faster in dogs provided with Heidenhain pouches than in dogs with a simple gastric fistula. This difference is probably related to the acceleration of gastric emptying occurring after fundectomy [16]. The peak acid response, but not the peak serum gastrin response, was lower with the 30% Bactopeptone meal than with the less concentrated meals. This inhibitory effect can be explained by the high osmolarity of the most concentrated meal. In fact, *Teichmann* et al. [14] observed that infusion of hypertonic solutions in the duodenum caused greater suppression of acid secretion than of gastrin release. For the 0, 7.5 and 15% Bactopeptone meals, correlation was found between log serum gastrin levels and acid response, indicating that gastrin could be the most important mediator of the acid response in the conditions used. The correlation is, however, weak, and other mechanisms (stimulating and inhibiting) are thus probably also involved.

Several reflex mechanisms originating from the stomach and inducing an acid secretion from the parietal cells have been demonstrated in dogs [1–3]. Distention of an innervated antral pouch with a 100 mM HCl solution induces an important acid response from a vagally innervated stomach, without any gastrin release. Vagal denervation of the antral pouch abolishes this effect. Thus, this response is probably a vagovagal reflex called the pyloro-oxyntic reflex. Distention of a vagally innervated antrectomized stomach, or of a denervated Heidenhain pouch, causes a modest increase in acid secretion but potentiates the responses to exogenous stimuli, such as pentagastrin or histamine [1, 7]. These responses are presumed to be mediated by vagovagal and intramural reflexes. It has also been stated that contact of peptides with the oxyntic gland area could induce an acid response through a chemical agent acting topically [3]. In this case, however, it has recently been reported that the increase in titratable acidity could partly be due to carbonic acid rather than solely to HCl [7]. *Peters* et al. [11] recently reported that, in man, propranolol reduced distention-induced gastrin release but not gastric acid secretion. This is another example of a nongastrin-mediated reflex acid response.

Almost all of the above-mentioned experiments were performed on animals prepared with pouches, under rather large and constant intragastric pressures. One can therefore wonder whether these mechanisms do intervene under more physiological conditions. If this were the case, and under the conditions used in our experiments, a larger response from the main stomach, rather than from the denervated pouch, could have been expected, since only the main stomach is innervated, distended and filled with the peptone solution. On the contrary, the acid responses were similar when expressed as percentages of the maximal acid response to histamine, and the responses from the pouches were larger when expressed as percentages of the maximal acid response to pentagastrin.

Under the conditions used in our experiments, one could have imagined that the intragastric volumes were such that virtually no distention occurred. But this was not the case,

at least with the 15 and 30% Bactopeptone meals. During these experiments, the intragastric volumes exceeded 100 ml for at least 50 min, and it must be taken into consideration that in Heidenhain pouch dogs the volume of the main stomach was decreased by the construction of the pouch. In the experiments of *Debas* et al. [1], distention of an antral pouch with an acid solution infused at a pressure of 15 cm initiated a marked acid secretion from the innervated fundic mucosa. In *Strunz's and Grossman's* [13] experiments, distention of the intact stomach at a pressure of 15 cm resulted in an intragastric volume of about 125 ml.

Some of the above-mentioned reflexes could have been blocked by an acidification of the contents, of the main stomach, but in our experiments the intragastric pH was monitored, and fell below pH 4 only at the very end of the experiments.

In the Heidenhain pouches, the acid response to 7.5% Bactopeptone meal was larger than the maximal response to pentagastrin. This can be explained by the release of a stimulatory hormone different from gastrin (entero-oxyntin) [7]. If this assumption is correct it might be surprising that such a response was not observed in the main stomach. However, intragastric administration of a peptone meal induces both stimulatory and inhibitory mechanisms [7]. The inhibitory mechanisms act probably more strongly on the innervated stomach than on the denervated one. In fact, some of the inhibitory mechanisms are probably reflexes, and most of the hormonal inhibitors act more strongly on the innervated than on the denervated fundic mucosa. This could explain why the response from the pouch is larger than the one from the main stomach when both are expressed as percentages of the maximal responses to pentagastrin.

An alternative explanation could be offered to the experiments of *Debas* et al. [1]. These authors concluded that the gastrin-independent stimulation of acid secretion obtained after antral distention with an acid solution is a pyloro-oxyntic reflex, because the acid response occurred only in the innervated fundic mucosa and was completely abolished by antral denervation. But this stimulation may be hormonal provided that it is assumed that the antrum is able to release a nongastrinic hormone able to stimulate acid secretion, and the release and action of which are weakly inhibited by acidification of the antrum but strongly dependent on the vagal tone. Interestingly enough, in the experiments of *Debas* et al. [1–3], gastrin release by antral distention was almost completely abolished by antral denervation, but distention of the denervated antrum with bicarbonate still induced a large acid secretion from the innervated and denervated stomach. On the other hand, indirect evidence indicates that the antrum could release a nongastrinic stimulant of acid secretion [10].

Thus, our data indicate, under the conditions used (namely in the absence of cephalic phase, and with a liquid meal of 250 ml), either that stimulatory reflexes acting on the oxyntic mucosa are of minor importance in inducing the acid response, or that reflex inhibition overcomes reflex stimulation in the innervated stomach.

In a recent study [12] performed on humans, and using peptone meals injected into the stomach, a strong correlation between a rise in serum gastrin and in acid secretion was observed. In the present experiments similar observations were made. In such conditions, and in both species, it seems that the main mechanisms which stimulate acid secretion are hormonal and humoral. How-

ever, the vagal tone is able to modulate the responses. The similarity of the inhibitory effects of hypertonic peptone meals on the innervated and on the vagally denervated stomach indicates that during the gastric and intestinal phases, vagal impulses are as unimportant for the inhibition of acid secretion as they are in the stimulation of this secretion.

Guldvog [8] and *Guldvog and Gedde-Dahl* [9] have performed experiments on dogs provided with both innervated and denervated pouches. The animals were fed with a normal meal. The early acid response was larger in the innervated pouch than in the denervated pouch, but the late acid response was similar in both pouches. These data are in agreement with the above-mentioned conclusion, and also indicate that, at least in dogs, vagal impulses do play a role in the stimulation of acid secretion during the cephalic phase.

References

1 Debas, H.T.; Konturek, S.J.; Walsh, J.H.; Grossman, M.I.: Proof of a pyloro-oxyntic reflex for stimulation of acid secretion. Gastroenterology 66: 526–532 (1974).

2 Debas, H.T.; Walsh, J.H.; Grossman, M.I.: Evidence for oxynto-pyloric reflex for release of antral gastrin. Gastroenterology 68: 687–690 (1975).

3 Debas, H.T.; Grossman, M.I.: Chemical bathing the oxyntic gland area stimulates acid secretion in dog. Gastroenterology 69: 654–659 (1981).

4 Druart, M.L.; Hestermans, Y.; Woussen-Colle, M.C.; De Graef, J.: Effets de la somatostatine sur la vidange gastrique, la sécrétion acide et la libération de gastrine post-prandiale chez le chien. Gastroentérol. clin. Biol. 5: 719–727 (1981).

5 Dubois, A.; Van Eerdewegh, P.; Gardner, J.D.: Gastric emptying and secretion in Zollinger-Ellison syndrome. J. clin. Invest. 59: 255–263 (1977).

6 Elashoff, J.D.: Down with multiple t-tests. Gastroenterology 80: 615–620 (1981).

7 Grossman, M.L.: Regulation of gastric acid secretion; in Johnson, Physiology of the gastrointestinal tract. p. 659 (Raven Press, New York 1981).

8 Guldvog, I.: Vagally innervated and denervated gastric pouch in one and the same dog. A new model. Scand. J. Gastroent. 15: 921–928 (1980).

9 Guldvog, I.; Gedde-Dahl, D.: Comparison of physiological and pharmacological stimulation of acid secretion in vagally innervated and denervated gastric pouches in the same dog. Scand. J. Gastroent. 15: 929 (1980).

10 Keuppens, F.; Bremen, J.; Woussen-Colle, M.C.; De Graef, J.: Failure of pentagastrin administration to restore postprandial acid secretion from Heidenhain pouches after antrectomy in dogs. Surgery, St Louis 80: 586–690 (1976).

11 Peters, M.N.; Walsh, J.H.; Ferrari, J.; Feldman, M.: Adrenergic regulation of distention induced gastrin release in humans. Gastroenterology 82: 659–663 (1982).

12 Shiu Kum Lam.; Isenberg, J.L.; Grossman, M.I.; Lane, W.H.; Walsh, J.H.: Gastric acid secretion in abnormally sensitive to endogenous gastrin released after peptone test meals in duodenal ulcer patients. J. clin. Invest. 65: 555–562 (1980).

13 Strunz, U.T.; Grossman, M.I.: Effect of intragastric pressure on gastric emptying and secretion. Am. J. Physiol. 235: E552–E555 (1978).

14 Teichmann, R.K.; Swierczek, J.S.; Rayford, P.L.; Thompson, J.C.: Effect of duodenal osmolarity on gastrin and secretion release and on gastric and pancreatic secretion. World J. Surg. 3: 623–630 (1979).

15 Vagne, M.; Grossman, M.I.: Gastric and pancreatic secretion in response to gastric distention in dogs. Gastroenterology 57: 300–310 (1969).

16 Wilbur, B.G.; Kelly, K.A.; Code, C.F.: Effect of gastric fundectomy on canine electrical and motor activity. Am. J. Physiol. 226: 1145–1149 (1974).

17 Woussen-Colle, M.C.; Willems, G.; De Graef, J.: Relationship of the gastrin response to the amount of food ingested in normal subjects. Digestion 15: 322–328 (1977).

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