*Suppression of Gastrin Release and Gastric Secretion by Gastric Inhibitory Polypeptide (GIP) and Vasoactive Intestinal Polypeptide (VIP)**

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Five dogs prepared with Heidenhain pouches received infusions of saline, GIP and VIP before and after a standard meat meal. Blood samples were obtained under basal conditions and at subsequent intervals for measurement of gastrin, insulin, GIP and VIP by radioimmunoassay. GIP and VIP infusions had no effect on basal levels of gastrin. GIP and VIP (in common with secretin and glucagon) were found to suppress food-stimulated release of gastrin and gastrin-stimulated acid secretion from the Heidenhain pouch. Insulin levels were significantly elevated during GIP and VIP infusions. Food released GIP (and perhaps VIP).

ALTHOUGH gastric inhibitory polypeptide (GIP) and vasoactive intestinal polypeptide (VIP) have not been awarded hormonal status, the similarities of these two polypeptides to the hormones secretin and glucagon have established them as members of the secretin-glucagon family.[4] Each of the four polypeptides has been isolated from small intestinal mucosal extracts[5,6,8,13,15,19,25] and each has many amino acid residues that occupy the same position; for example: secretin shares 14 amino acids with glucagon and 9 identities with VIP. In addition to their structural similarities, the common biologic action of all four polypeptides is their inhibitory effect on gastric secretion.[5–7,9–11,15,18,20,22–24]

Submitted for publication September 16, 1975.

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Supported by a grant from the National Institutes of Health (AM 15241).

* A preliminary report has appeared.[30]

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Previous studies conducted in our laboratory have shown that infusions of secretin[9,28] and glucagon[1] inhibit the release of gastrin in man and in dogs. We noted, furthermore, a paradoxical increase in gastrin levels after a secretin injection in patients with Zollinger-Ellison syndrome[28] and this observation has resulted in the use of the secretin challenge test for the diagnosis of the Zollinger-Ellison syndrome.[29] The present study was conducted, in dogs, to test the effect of GIP and VIP on basal and food-stimulated levels of gastrin and on gastric acid secretion.

Materials and Methods

Five healthy mongrel dogs weighing between 17 and 25 kg were prepared with standard Heidenhain pouches and were allowed to recover for three weeks. The dogs were studied in random fashion on three different days over a 7-day period. On random days during the 7-day test period, either normal saline, or GIP (2 μg/kg/hr) or VIP (1 μg/kg/hr) was infused for one hour, after which each dog was given a standard high protein meal. The infusion was then continued for an additional hour after feeding. Blood specimens were drawn at regular intervals before and after food, the serum was separated

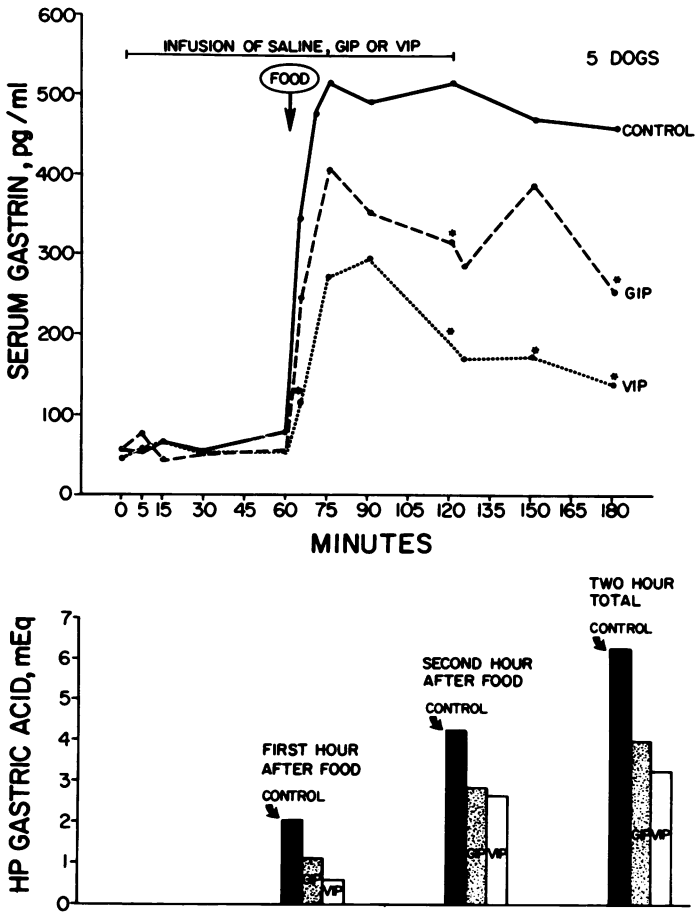

FIG. 1. Effect of GIP, VIP and normal saline on basal and stimulated serum gastrin levels and gastric acid secretions (mean data from five dogs). *Significantly diminished from control.

and then stored at −20° until assayed for gastrin, glucose, insulin, GIP and VIP. In addition, gastric secretions were collected at 30-minute intervals for gastric acid measurement. The number of studies was limited by the small available supply of GIP and VIP.

Gastrin concentrations in serum were measured by use of a double antibody technique.[12,21] The antigastrin antibody used in the method was generated in New Zealand white rabbits using synthetic human gastrin I (amino acid residues 2–17) conjugated to bovine serum albumin as an immunogen. The antibody was used in a final dilution of 1:80,000 and is immunologically reactive with all known molecular forms of gastrin.[27]

GIP assay was generously performed by Professor John Brown and colleagues. The validation and specificity of the GIP radioimmunoassay has been described previously.[7,16]

VIP was measured in the laboratory of Dr. Bloom and colleagues.[2,3] Antibodies were raised to pure porcine VIP conjugated to bovine serum albumin (3 mg total protein injected at 3-month intervals into rabbits). The antibody was harvested after one year. At a titer of 1:320,000, the antibody achieved 50% binding. Pure porcine VIP (supplied by Professor Viktor Mutt, Karolinska Institute) was iodinated using lactoperoxidase and was purified by ion-exchange chromatography. The assay was set up with 20% plasma; in the standard curve, VIP-free plasma (made by affinity chromatography) was added to each assay tube. The reference standard used was pure porcine VIP and the sensitivity of the assay was such that it was possible to detect changes between individual plasma samples of 10 pg/ml with 95% confidence. In studies on the specificity of the assay, no cross-reaction was found with glucagon, GIP or secretin. Interassay variation is within 15% to 20%.

Glucose was determined by the ortho-toluidine method. Insulin levels were determined by radioimmunoassay with a commercial kit (Schwarz-Mann). Blood samples for glucose and insulin were lost for one dog; data are reported for 4 dogs. Gastric acid secretions were titrated with 0.1 N NaOH to an end point, with phenol red used as indicator.

Results are expressed as the mean ± one standard error. The Student's "t" test was used to analyze the data for statistical significance of differences between means. When analyzing results with different basal levels, integrated analysis was used.[28] For all analyses, differences with a P value of less than 0.05 were considered significant.

Results

Gastrin Levels

The mean basal serum gastrin levels before saline, GIP or VIP infusions were 59 ± 4.6 pg/ml, 58 ± 19 pg/ml and 46 ± 7.9 pg/ml, respectively. These levels did not change significantly throughout the one-hour time interval during the infusion of saline, GIP, or VIP (Fig. 1).

We found postprandial gastrin levels in two of the 5 dogs to be unusually high in *all* studies; we found no reason for this and we assume it to be the result of individual variation among dogs. The degree of suppression of gastrin concentration by GIP and VIP, however, was similar in dogs with high and with normal gastrin values.

In control dogs that received saline, mean serum gastrin levels increased to 517 ± 97 pg/ml 15 minutes after food and remained on a plateau above 450 pg/ml until the end of the study (Fig. 1).

During the GIP infusion, mean serum gastrin levels increased after food in values of 409 ± 158 pg/ml at 15 minutes and then declined progressively for the next 2 hours except for a second peak at 150 minutes. Mean serum gastrin concentration was 319 ± 155 pg/ml at two hours (one hour after food) and at three hours (two

TABLE 1. *Mean Glucose and Insulin Values for Four Dogs Under Basal Conditions and During Infusions of Normal Saline (Control), GIP or VIP (before and after food)*

		Infusions								
					Postcibal					
	Basal	15	30	60	75	90	120	135	150	180
Glucose (mg/100 ml)										
Control	62	67	64	64	78	82	72	84	73	79
GIP	75	78	77	79	81	86	129	130	86	91
VIP	65	73	77	81	84	85	76	72	75	82
Insulin (μU/ml)										
Control	13.9	12.3	10.4	14.3	18.3	26.6	17.6	19.3	18.1	16.4
GIP	15.5	17.0	19.1	14.8	19.3	22.1	24.8	15.3	18.8	16.6
VIP	16.7	21.8	17.4	16.0	31.7	37.5	24.8	22.5	21.4	21.4

hours after food) was 258 ± 107 pg/ml. These values, denoted by asterisks, were significantly lower ($P < 0.05$) than those of saline control dogs (Fig. 1).

During VIP infusion, postprandial mean serum gastrin levels rose to 298 ± 175 pg/ml at 90 minutes, declined to 193 ± 92 pg/ml at 120 minutes and at 240 minutes was 142 ± 62 pg/ml. After food, serum gastrin values during VIP infusion were significantly lower than in the control dogs at 65, 120, 150 and 180 minutes (Fig. 1).

Gastric Secretion

The mean total amount of acid secreted in the first postcibal hour during infusion of saline (control), GIP and VIP was 2.0, 1.0 and 0.6 mEq/hr, respectively (Fig. 1). The total amount of acid during the second hour of infusion was 4.3 mEq/hr (control), 2.9 mEq/hr (GIP) and 2.7 mEq/hr (VIP). The total two-hour gastric acid output during the infusion of saline, GIP and VIP was 6.3, 4.0 and 3.3 mEq. The suppression of gastric acid secretion by GIP and VIP infusions, although impressive, was not significant.

Blood Glucose Levels

Fasting blood glucose levels were 62 ± 13 mg/100 ml, 75 ± 8 mg/100 ml and 65 ± 7 mg/100 ml in the control, GIP and VIP groups, respectively (Table 1). Neither infusions of saline nor GIP had an effect on basal blood glucose. VIP infusion increased basal blood glu-

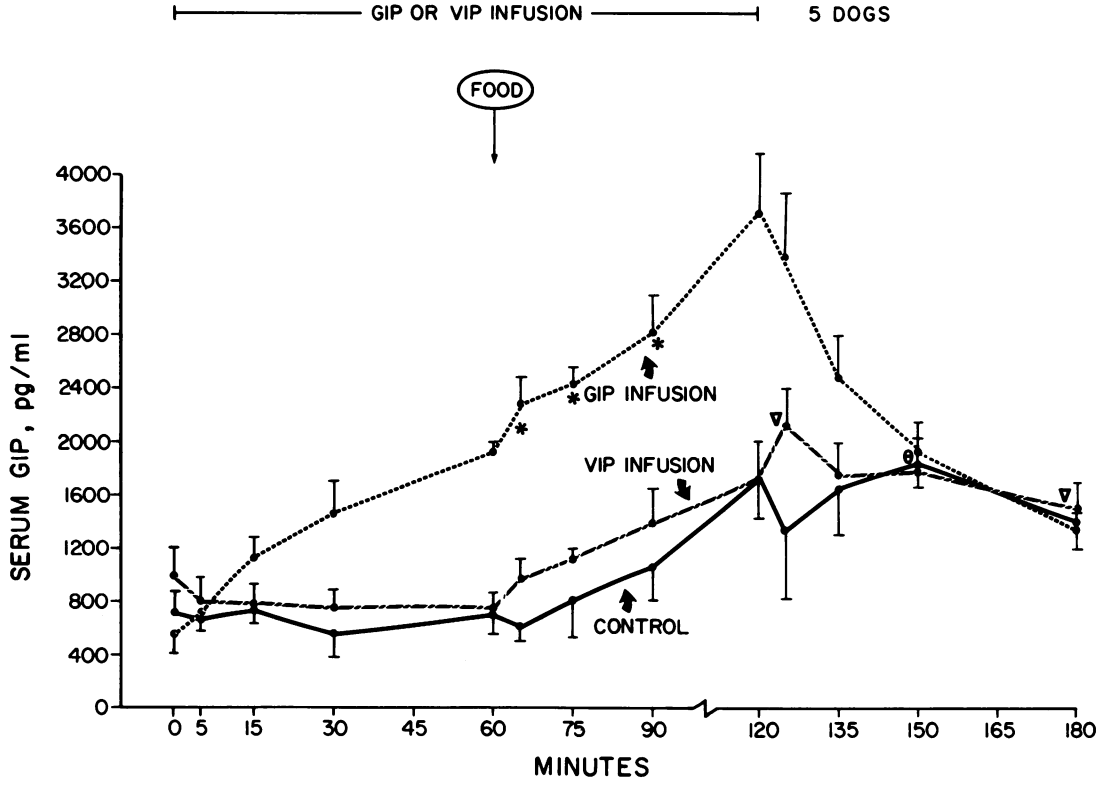

FIG. 2. Serum GIP levels before and after feeding during infusion of GIP, VIP or saline (control). (Assay performed by Professor Brown.)* Significant increase above control during GIP infusion; ∇Significant increase above baseline during VIP infusion; ΘSignificant increase above baseline during control infusion.

cose levels from 65 ± 7 mg/100 ml to 81 ± 4 mg/100 ml at 60 minutes ($P < 0.05$). As expected, feeding increased blood glucose levels significantly above basal in all three groups. Integrated analysis revealed no significant differences in glucose levels between the GIP and control infusions. With VIP, however, there was a significant increase of integrated glucose output in the 60 to 90-minute period, postcibal.

Insulin Levels

Basal insulin values were 13.9 ± 1.6 μU/ml, 15.5 ± 0.7 μU/ml and 16.7 ± 0.8 μU/ml in the control, GIP and VIP groups, respectively (Table 1). Serum insulin levels did not change significantly during saline control infusion and the postcibal elevation in this group was not statistically significant. When compared to basal levels, GIP caused significant elevations of insulin at 30 minutes. GIP plus food caused significant elevations above basal at 90 and 120 minutes. When compared to control values, GIP alone caused a significant increase in insulin at 30 minutes, and GIP plus food caused a significantly greater insulin level than did saline plus food at 120 minutes. The *integrated* insulin level was significantly increased above control value during the first 30 minutes of the GIP infusion.

VIP plus food resulted in significant elevations of insulin levels above baseline at 90, 150 and 180 minutes. Compared to the postcibal control infusion, VIP caused significant increases in insulin at 150 and 180 minutes.

GIP Levels

Basal GIP levels were 710 ± 211 pg/ml in the control group, 554 ± 155 pg/ml and 988 ± 225 pg/ml in the GIP and VIP groups, respectively (Fig. 2). GIP infusion resulted in a rapid and sustained increase in serum GIP concentrations. Mean serum GIP continued to increase after feeding and by 120 minutes was 3720 ± 426 pg/ml. After the GIP infusion was stopped, serum GIP levels dropped and at 150 minutes was greater than baseline but not different from VIP or saline control. GIP levels did not increase during infusions of VIP and saline alone. However, VIP plus food resulted in significant elevations of GIP at 125 and 180 minutes; food caused a significant rise in GIP at 150 minutes during control infusion. As expected, GIP plus food caused significantly greater GIP levels than did saline plus food at 65, 75 and 90 minutes.

VIP Levels

Basal levels of VIP were 29 ± 8 pg/ml in the control group, 38 ± 11 pg/ml and 52 ± 7 pg/ml in the GIP and VIP groups, respectively (Fig. 3). During VIP infusion, serum VIP concentrations increased to 91 ± 11 pg/ml at 60 minutes and to 105 ± 15 pg/ml at 65 minutes,

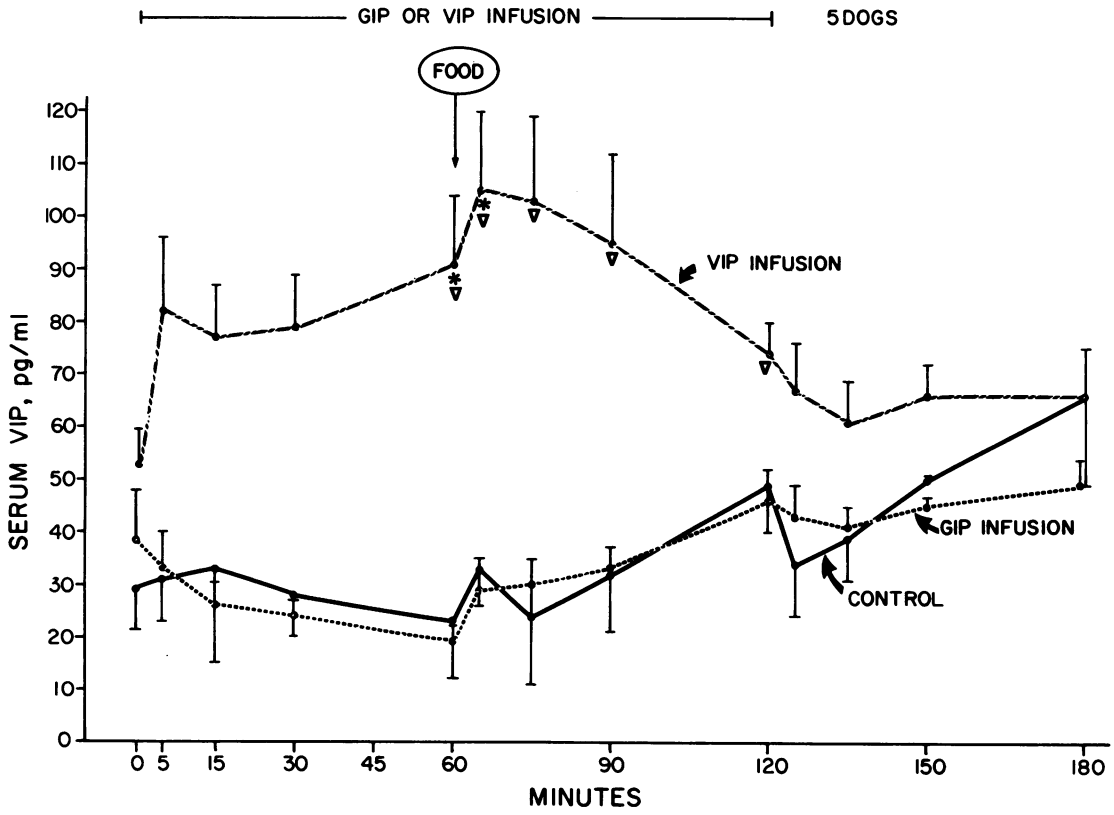

FIG. 3. Serum VIP levels before and after feeding during infusion of GIP, VIP or saline (control). * Significant increase above control during VIP infusion; ∇Significant increase above baseline during VIP infusion.

5 minutes after feeding. These values were significantly greater than that found in the saline control group. The VIP levels at 60, 65, 75, 90 and 120 minutes were significantly elevated above baseline. In dogs infused with GIP and saline, serum VIP concentrations did not increase significantly above basal levels.

Discussion

Secretin, glucagon, vasoactive intestinal polypeptide and gastric inhibitory polypeptide are closely-related members of the same family of hormones. These hormones have remarkably similar amino acid sequences.[26] Secretin has a molecular weight of 3055 and has 27 amino acid residues. Glucagon has a molecular weight of 3485 and 14 of the 29 amino acids are in identical positions with secretin. VIP has a molecular weight of 3326 and has 28 amino acid residues, 9 of which are in positions identical with secretin. GIP is a larger peptide with a molecular weight of 5105 and has 43 amino acids, of which 9 are in positions common with secretin.

Our present studies demonstrated that at the concentrations used in these experiments, neither GIP nor VIP infusions had an effect on basal gastrin levels. When the dogs were fed and the infusions continued, serum gastrin concentrations increased rapidly above baseline levels. Fifteen minutes after feeding, serum gastrin levels had attained peak values in each group of dogs. Serum gastrin concentrations in dogs that received GIP were significantly lower than concentrations in dogs that received saline at two time periods (120 and 180 minutes). The serum gastrin response to food was significantly lower at 65, 120, 150 and 180 minutes in the dogs that received VIP infusions than in the dogs that received saline.

On a molar basis, VIP appears to be a much more potent inhibitor of gastrin release than is GIP. For example, at 90 minutes, the control gastrin concentration of 494 ± 113 pg/ml was diminished by approximately 30% (355 ± 175 pg/ml) by GIP and by 40% (298 ± 175 pg/ml) by VIP (Fig. 1). The relative molar concentration of GIP to VIP at 90 minutes was 19:1. This ratio was calculated by comparing the concentration (in pg/ml at 90 min) of GIP (Fig. 2) and VIP (Fig. 3), divided by their respective molecular weights

$$\text{i.e., } \left(\frac{2800}{5104} \; vs \; \frac{95}{3326} \right)$$

The gastric acid output follows a similar pattern. VIP produced a greater suppression of gastric acid output in response to food than GIP. Acid output in the control group was approximately 50% more than that of dogs who received either GIP or VIP. This suppression, however, was not statistically different. Pederson and Brown[20] have shown inhibition of histamine and pentagastrin-induced gastric secretion with GIP doses ranging from 0.5 to 4 μg/kg/hr (in the same range as in this experiment). It is logical to assume that suppression of gastrin will have as a consequence the suppression of gastrin-induced gastric secretion.

Brown and colleagues[7] have shown that GIP in doses as high as 10 mg/kg has no effect on basal glucose levels. Our results confirm their findings. We found an increase in glucose after VIP, which confirmed the observation of Kerins and Said.[14]

Insulin levels rose after GIP infusion. Since there was no associated release of glucose, it may be assumed that GIP is insulinotropic. This corroborates a recent demonstration of the direct release of insulin from pancreas by GIP.[6] VIP, like glucagon, stimulates both lipolysis and glycogenolysis.[14] It is not possible from our experiments to determine the cause of the hyperinsulinemia observed during VIP infusion: is it brought about by a direct action of VIP or does it result from the elevation of glucose? VIP has been shown to release insulin directly from pancreatic tissue *in vitro*,[17] so that insulin may have been increased by VIP in our study by both routes.

Human plasma GIP concentrations, measured by radioimmunoassay, have been reported to increase after ordinary meals.[7] Our results in dogs confirm this finding. There was a suggestion of release of VIP after feeding in the control group. The latter values, however, were not elevated significantly above baseline.

Measurements of GIP and VIP by specific radioimmunoassays show that infusion of VIP has no effect on GIP plasma levels and *vice versa*. As expected, GIP plasma levels increased during GIP infusions and VIP plasma levels increased during VIP infusions, indicating that these polypeptides are immunologically recognizable in circulation.

References

1. Becker, H. D., Reeder, D. D. and Thompson, J. C.: Effect of Glucagon on Circulating Gastrin. Gastroenterology, 65:28–35, 1973.
2. Bloom, S. R. and Polak, J. M.: The Role of VIP in Pancreatic Cholera. *In* Gastrointestinal Hormones, J. C. Thompson (ed), Austin, University of Texas Press, 1975; pp. 635–642.
3. Bloom, S. R., Polak, J. M. and Pearse, A. G.: Vasoactive Intestinal Peptide and Watery-Diarrhea Syndrome. Lancet, 11:14–16, 1973.
4. Bodanszky, M.: The Secretin Family and Evolution. *In* Gastrointestinal Hormones. J. C. Thompson (ed), Austin, University of Texas Press, 1975; pp. 507–518.
5. Brown, J. C. and Dryburgh, J. R.: A Gastric Inhibitory Polypeptide II. The Complete Amino-Acid Sequence. Can. J. Biochem., 49:867–872, 1971.
6. Brown, J. C., Dryburgh, J. R., Moccia, P. and Pederson, R. A.: The Current Status of GIP. *In* Gastrointestinal Hormones, J. C. Thompson (ed.), Austin, University of Texas Press, 1975, pp. 537–547.

7. Brown, J. C., Dryburgh, J. R. and Pederson, R. A.: Gastric Inhibitory Peptide. In Endocrinology of the Gut. W. Y. Chey and F. P. Brooks (eds.), Thorofare, Charles B. Slack, Inc., 1974; pp. 76–82.
8. Brown, J. C. and Pederson, R. A.: A Multiparameter Study on the Action of Preparations Containing Cholecystokinin-Pancreozymin. Scand. J. Gastroenterol., 5:537–541, 1970.
9. Bunchman, H. H., Reeder, D. D. and Thompson, J. C.: Effect of Secretin on the Serum Gastrin Response to a Meal in Man and in Dog. Surg. Forum, 22:303–305, 1971.
10. Dreiling, D. A. and Janowitz, H. D.: The Effect of Glucagon on Gastric Secretion in Man. Gastroenterology, 36:580–581, 1959.
11. Greenlee, H. B., Longhi, E. H., Guerrero, J. C. et al.: Inhibitory Effect of Pancreatic Secretin on Gastric Secretion. Am. J. Physiol., 190:396–402, 1957.
12. Jackson, B. M., Reeder, D. D. and Thompson, J. C.: Dynamic Characteristics of Gastrin Release. Am. J. Surg., 123:137–142, 1972.
13. Jorpes, E. and Mutt, V.: On the Biological Activity and Amino Acid Composition of Secretin. Acta Chem. Scand., 15:1790–1791, 1961.
14. Kerins, C. and Said, S. I.: Hyperglycemic and Glycogenolytic Effects of Vasoactive Intestinal Polypeptide. Proc. Soc. Exp. Biol. Med., 142:1014–1017, 1973.
15. Konturek, S. J., Piotr, T., Dembinski, A. and Krol, R.: Vasoactive Intestinal Peptide: Comparison with Secretin for Potency and Spectrum of Physiologic Action. *In* Gastrointestinal Hormones. J. C. Thompson (ed.), Austin, University of Texas Press, 1975; pp. 611–633.
16. Kuzio, M., Dryburgh, J. R., Malloy, K. M. and Brown, J. C.: Radioimmunoassay of Gastric Inhibitory Polypeptide. Gastroenterology, 66:357–364, 1974.
17. Makhlouf, G. M. and Said, S. I.: The Effect of Vasoactive Intestinal Peptide (VIP) on Digestive and Hormonal Function. *In* Gastrointestinal Hormones. J. C. Thompson (ed.), Austin, University of Texas Press, 1975; pp. 599–610.
18. Melrose, A. G.: Effect of Glucagon on Gastric Secretion in Man. Gut, 1:142–145, 1960.
19. Orci, L., Pictet, R., Forssman, W. G. et al.: Structural Evidence for Glucagon Producing Cells in the Intestinal Mucosa of the Rat. Diabetologia, 4:56–67, 1968.
20. Pederson, R. A. and Brown, S. R.: Inhibition of Histamine, Pentagastrin and Insulin-stimulated Canine Gastric Secretion by Pure "Gastric Inhibitory Polypeptide". Gastroenterology, 62:393–400, 1972.
21. Rayford, P. L., Reeder, D. D. and Thompson, J. C.: Interlaboratory Reproducibility of Gastrin Measurements by Radioimmunoassay. J. Lab. Clin. Med., 86:521–528, 1975.
22. Robinson, R. M., Harris, K. and Head, C. I.: Effect of Glucagon on Gastric Secretion. Proc. Soc. Exp. Biol. Med., 96:518–520, 1957.
23. Said, S. I. and Mutt, V.: Polypeptide with Broad Biological Activity: Isolation from Small Intestine. Science, 169:1217–1218, 1970.
24. Said, S. I. and Mutt, V.: Potent Peripheral and Splanchnic Vasodilator Peptide from Normal Gut. Nature, 225:863–864, 1970.
25. Sutherland, E. W. and DeDuve, L.: Origin and Distribution of the Hypoglycemia-Glycogenolytic Factor of the Pancreas. J. Biol. Chem., 175:663–664, 1948.
26. Thompson, J. C. (ed.): Gastrointestinal Hormones. Austin, University of Texas Press, 1975, Appendix, p. 654.
27. Thompson, J. C., Rayford, P. L., Ramus, N. I. et al.: Pattern of release and uptake of heterogeneous forms of gastrin. *In* Gastrointestinal Hormones. J. C. Thompson (ed.), Austin, University of Texas Press, 1975; pp. 635–642.
28. Thompson, J. C., Reeder, D. D., Bunchman, H. H. et al.: Effect of Secretin on Circulating Gastrin. Ann. Surg., 176: 384–392, 1972.
29. Thompson, J. C., Reeder, D. D., Villar, H. V. and Fender, H. R.: Natural History and Experience with Diagnosis and Treatment of the Zollinger-Ellison Syndrome. Surg. Gynecol. Obstet., 140:721–739, 1975.
30. Villar, H. V., Fender, H. R., Rayford, P. L., et al.: Inhibition of gastrin release and gastric secretion by GIP and VIP. *In* Gastrointestinal Hormones. J. C. Thompson (ed.), Austin, University of Texas Press, 1975; pp. 467–472.