Pancreozymin Bioassay in Man Based on Pancreatic Enzyme Secretion: Potency of Specific Amino Acids and Other Digestive Products

VAY L. W. GO, ALAN F. HOFMANN, and W. H. J. SUMMERSKILL

From the Gastroenterology Unit, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

ABSTRACT The ability of products of digestion to stimulate pancreozymin secretion in man was investigated using a bioassay procedure, based on duodenal perfusion, which quantified the total outputs of pancreatic enzymes evoked by intraduodenal stimuli under steady-state conditions. Patterns of response resulting from physiologic intraduodenal concentrations of test material were basal output (with isotonic saline), washout of enzymes (with dextrose, micellar fatty acid, and amino acids), and sustained output of enzymes (with amino acids and micellar fatty acid). The sustained secretion of pancreatic enzymes found during the 2nd hr of perfusion and subsequently was characteristic of pancreozymin-induced secretion. The enzyme output in response to a mixture of essential and nonessential amino acids was significantly higher than that evoked by micellar fatty acid and was comparable with that resulting from the maximally tolerated dose of pancreozymin given by vein.

Perfusion with essential amino acids caused enzyme outputs comparable to those induced by perfusion with the original amino acid mixture, whereas perfusion with nonessential amino acids had no effect. When the essential amino acids were tested individually, only phenylalanine, methionine, and valine caused significant increases in pancreatic enzyme output; the effect of tryptophan was indeterminate. However, the pancreatic enzyme output was less in response to these three essential amino acids than to mixtures containing all of them.

This work was presented in part at the meeting of the Central Society for Clinical Research, Chicago, 31 October to 2 November 1968.

Received for publication 23 January 1970 and in revised form 13 April 1970.

INTRODUCTION

Pancreozymin, a hormone released from the mucosa of the upper part of the small intestine after ingestion of food, promotes secretion of pancreatic enzymes and evacuation of bile from the gallbladder, both of which are essential for normal digestion and absorption. In the dog, peptone, certain amino acids, and fatty acid soaps infused intraduodenally evoked secretion of pancreatic enzymes by this hormonal mechanism (1). The dietary components or derivatives causing secretion of pancreozymin in man are ill-defined, and earlier work, limited to the immediate response to temporary stimuli, fails to distinguish between "washout" of previously formed enzymes from the ducts (2) and specific secretion of enzymes from the acinar cells. Furthermore, the methods used allowed no meaningful comparison of the potencies of different stimuli.

McClure (3) found striking increases in intraduodenal concentrations of lipase, amylase, and proteolytic enzymes after intraduodenal instillation of triglycerides and beef peptone; dextrose was thought to give a smaller response. By a different technique, involving cannulation of the pancreatic duct during cholecystectomy, Doubilet and Fishman (4) found that hydrochloric acid or triglycerides placed in the duodenum stimulated a mixed secretion of enzymes, fluid, and bicarbonate in the postoperative period. In the amounts selected, glucose and protein exerted a similar but milder effect.

We report here the effects of physiologic intraduodenal concentrations of the major digestive products (5) on pancreozymin secretion as assessed by determining total pancreatic enzyme output in healthy man. Different patterns of enzyme secretion were defined, and the response due to pancreozymin release was identified. Since, under the circumstances of the study, amino acids were found

to have the greatest effect on pancreatic enzyme secretion, the potency, specificity, and magnitude of the responses to individual amino acids were characterized.

EXPERIMENTAL DESIGN AND ASSUMPTIONS

Pancreatic enzyme output in response to the intraduodenal perfusion of test materials was measured under steady-state conditions. The effects of saline (control), amino acids (either individually or in combinations), dextrose, and micellar fatty acid were compared with each other and with the response to the maximally tolerated dose of porcine pancreozymin given by vein.

The sustained pancreatic enzyme output measured in response to some but not all intraduodenal stimuli presumably reflected secretion of pancreozymin, the hormonal basis of the relationship having been established in the dog (1). We use the term "pancreozymin" because of its historic association with pancreatic enzyme secretion, but we recognize that pancreozymin and cholecystokinin are probably identical (6). In estimating pancreozymin secretion, we have made the assumption, customary in bioassay techniques, that proportional relationships exist between the magnitudes of the stimuli, amounts of hormone released, and quantities of enzyme secreted. Release of secretin may have occurred simultaneously (1) in our model, but volume and bicarbonate output were not measured. By contrast, it is improbable that gastrin release was evoked and unlikely that it could influence pancreatic enzyme output in these studies. Gastric pH was less than 2.0 in all but 6% of instances and only once exceeded 4.0; reflux of duodenal contents was minimal (see below); and the physiologic effect of gastrin on pancreatic enzyme secretion is small compared with that of pancreozymin (7, 8). Furthermore, glycine was found not to affect enzyme output although it is a potent stimulant to gastrin release in the dog (9). And, finally, pancreatic enzyme outputs of the same magnitude as those we attribute to pancreozymin were found previously (10) when the distal jejunum, a site far removed from gastrin release, was perfused with amino acids.

METHODS

45 healthy volunteers (43 men and 2 women, ages 22–46 yr) fasted overnight before participating in these studies. Total outputs of pancreatic lipase, trypsin, and amylase were determined by a method, detailed elsewhere (11), involving simultaneous perfusion and aspiration of both the stomach and the duodenum. In brief, a double-lumen polyvinyl tube (I.D., 2 mm) was positioned fluoroscopically in the duodenum. Test solutions, containing polyethylene glycol (PEG) (5 g/liter) as a duodenal marker, were instilled into the second portion of the duodenum at 10 ml/min, and duodenal contents were recovered by siphonage at the ligament of Treitz. These specimens were collected over ice and pooled at 20-min intervals. The gastric tube, consisting of a polyvinyl tube (I.D., 2 mm) cemented to a Levin tube by tetrahydrofuran, was positioned so that the perfusion site was in the fundus and the sampling site was in the antrum. ^{51}Cr-labeled CrCl (2 μCi/ml) was used as a gastric marker. The stomach was perfused at 5 ml/min, and the gastric contents were aspirated distally through the Levin tube by continuous mechanical and intermittent hand suction.

Concentrations of PEG and ^{51}Cr were determined in both gastric and duodenal aspirates. Concentrations of lipase, trypsin, and amylase were measured in the duodenal samples. From the concentrations and recoveries of markers and enzymes when steady-state conditions had been established, as reflected by PEG concentrations in the duodenal aspirates (12), total pancreatic enzyme output was calculated (11). Under these conditions, the recovery of gastric contents is 83.3% (SE 1.3), while 11.6% (SE 1.4) of gastric contents passes to the duodenum. Simultaneously, 8.5% (SE 1.0) of duodenal contents refluxes to the stomach and 36.7% (SE 3.3) of duodenal marker is recovered at the ligament of Treitz (11).

The perfusates (Table I) contained physiologic intraduodenal concentrations of the test substances (5). All perfusates were warmed to 37°C and adjusted to pH 6.0; they were made isotonic with sodium chloride. All solutions of amino acids, micellar fatty acid, and dextrose were instilled for 2-hr periods. The isotonic saline was used as a control, being instilled for the initial 60 min of every study and also for a 60-min period after every test solution. Steady-state conditions, as reflected by stable concentrations of PEG (11, 12), were achieved after 20 min of each perfusion.

The standard amino acid mixture (182 mmoles/liter) was isonitrogenous with 30 g of beef muscle hydrolysate per

TABLE I

Composition of Perfusates (mM)

Isotonic saline	150.0	
Dextrose	277.5	
Micellar fatty acid	20.0	
Sodium taurocholate		7.0
Sodium taurodeoxycholate		3.0
Monoolein		10.0
Standard amino acid mixture*	182.6	
Essential amino acid mixture	78.4	
1. Isoleucine		11.3
2. Leucine		17.0
3. Methionine		5.2
4. Phenylalanine		6.7
5. Threonine		10.6
6. Tryptophan		1.4
7. Valine		12.8
8. Lysine		13.4
Nonessential amino acid mixture	104.2	
1. Arginine		10.5
2. Aspartic acid		20.1
3. Cystine		1.6
4. Glutamic acid		28.4
5. Glycine		21.4
6. Histidine		4.7
7. Proline		12.8
8. Tyrosine		4.7

* Mixture of essential and nonessential amino acid mixtures.

liter (13). The standard solution of micellar fatty acid was prepared by dissolving monoolein (chiefly 1-monoolein) in sodium taurocholate and sodium taurodeoxycholate. This monoglyceride is rapidly hydrolyzed to fatty acid in the proximal small intestine in man, and we confirmed this by thin-layer chromatography of the aspirates, which showed the predominant lipid present to be fatty acid.

For preliminary studies, each standard solution (amino acid mixture, micellar fatty acid, or dextrose) was perfused for 2 hr in each of eight healthy male volunteers, with complete randomization of period sequences. Because, by the criteria applied, amino acids were found to be the most potent in stimulating pancreatic enzyme output, additional studies were performed to determine specifically the nature of the stimulus. Solutions of the essential (78 mmoles/liter) and nonessential (104 mmoles/liter) amino acids were prepared in the same concentrations as in the original mixture. Each mixture was perfused in the eight volunteers for a 2 hr period. Subsequently, solutions of the individual essential amino acids (20 mmoles/liter) were perfused into the duodenum randomly in 12 volunteers for periods of 2 hr. An incomplete block design, with blocks of two plots, was used (14). To determine sensitivity of pancreozymin release in relation to the magnitude of the stimulus, additional studies with perfusates containing amino acids at 18 and 270 mmoles/liter were made in six volunteers, the individual amino acids being present in the same proportion as in the standard mixture.

To determine "maximal" pancreatic enzyme output, cholecystokinin-pancreozymin (kindly supplied by Dr. E. Jorpes, Karolinska Institutet) was administered by vein at an initial dose of 0.125 CHR U/kg per min and increased to the maximally tolerated dose (0.25–0.35 CHR U/kg per min) in eight volunteers while intraduodenal perfusion with isotonic saline was maintained. The highest enzyme output before the development, if any, of side-effects (abdominal pain, nausea, vomiting, diarrhea, or fever) was characterized and validated (11, 15) as the maximal response. To study the possible systemic effect of amino acids on pancreatic enzyme output, the standard amino acid solution was given intravenously to three volunteers for 2 hr while simultaneous intraduodenal perfusion with isotonic saline was maintained.

All amino acids were purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Sodium taurocholate and sodium taurodeoxycholate were obtained from Maybridge Research Chemicals (U.K.), and monoolein (>95% monoglyceride) was purchased from Eastman Organic Chemicals (Rochester, N. Y.). ^{51}Cr was counted in a sodium iodide well-counter (Gamma Spectrometer, Picker Corp., White Plains, N. Y.) and PEG was determined by the method of Hyden (16). Pancreatic lipase and trypsin concentrations in duodenal aspirates were determined in an automatic titrater using Lipomul (Upjohn Co., Kalamazoo, Mich.) and tosyl-L-arginine methyl ester as a substrate (17). The amylase content was measured by the method of Smith and Roe (18). In 15 duodenal samples analyzed in triplicate, the mean coefficients of variation in the determination of PEG, amylase, lipase, and trypsin were 1.2, 2.6, 2.8, and 0.2%, respectively. The reproducibility of the perfusion technique was tested by measuring outputs of pancreatic enzymes on 12 occasions in the same individuals at 8:30 a.m. and 1:00 p.m. on the same day and at a week later; the coefficient of variation was $<6\%$ on 8 occasions and ranged from 9.1 to 13.8% on the remainder.

RESULTS

The patterns of pancreatic enzyme output in response to intraduodenal perfusion with isotonic saline or standard solutions, represented by trypsin outputs (Fig. 1), included basal, washout, and sustained secretions. Outputs of trypsin, lipase, and amylase did not differ in proportion to each other during this study (Fig. 2) or under other test circumstances (Table II). Basal output of enzymes occurred continuously during perfusion with isotonic saline and after the washout period when dextrose was used (Fig. 1). With each test solution there was a rapid and significant ($P < 0.01$) increase in enzyme output during the first 20 min. The response to

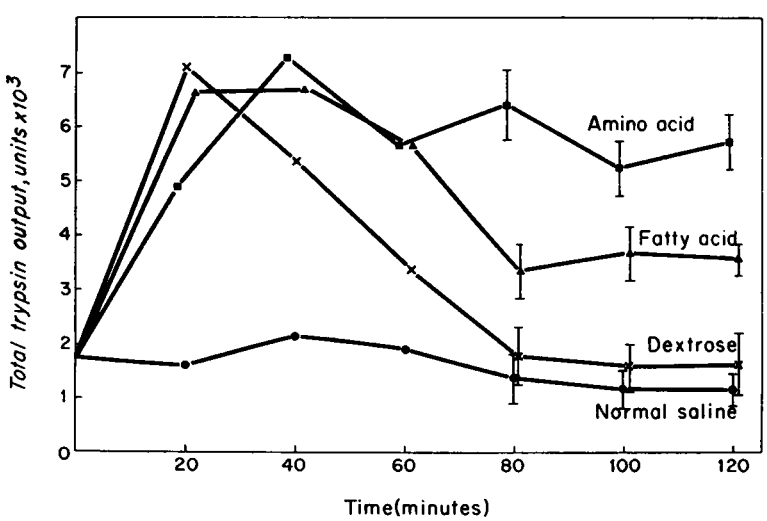

FIGURE 1 Trypsin output (mean ±SE) during intraduodenal perfusion with amino acid mixture, micellar fatty acid, dextrose, or isotonic saline.

dextrose was temporary, lasting 40 min, and it had the characteristics of a washout effect. The amino acid mixture and micellar fatty acid caused sustained outputs of all enzymes. Therefore, to distinguish the sustained enzyme output attributable to pancreozymin release from basal and washout secretions, the enzyme output during the 2nd hr of perfusion has been taken as representative; all subsequent data reported are from this period.

Outputs of enzymes in response to perfusion with the standard amino acid mixture during this 2nd hr were significantly higher than those in response to micellar fatty acid ($P < 0.05$), and the responses to micellar fatty acid were greater than those to dextrose or isotonic saline ($P < 0.05$). The enzyme outputs in response to isotonic saline and dextrose did not differ significantly (Fig. 2).

When essential amino acids were used, pancreatic enzyme outputs did not differ significantly from those evoked by the standard mixture or by the maximally tolerated dose of pancreozymin given by vein (Table II). By contrast, the enzyme outputs after perfusion with nonessential amino acids were no greater than those during perfusion with isotonic saline. When each essential amino acid was used individually, the highest enzyme outputs occurred with phenylalanine, valine, and methionine (Fig. 3). The responses were comparable to each other, lower ($P < 0.05$) than outputs evoked by the essential amino acid mixture or pancreozymin given by vein, and greater ($P < 0.05$) than the response to isotonic saline (Table II). Pancreatic enzyme output in response to isoleucine, leucine, lysine, or threonine did not differ from the basal output in response to isotonic saline (Fig. 3). The pancreatic enzyme output in response to tryptophan (Fig. 3) was equivocally increased ($P = 0.05$) in relation to the response to isotonic saline.

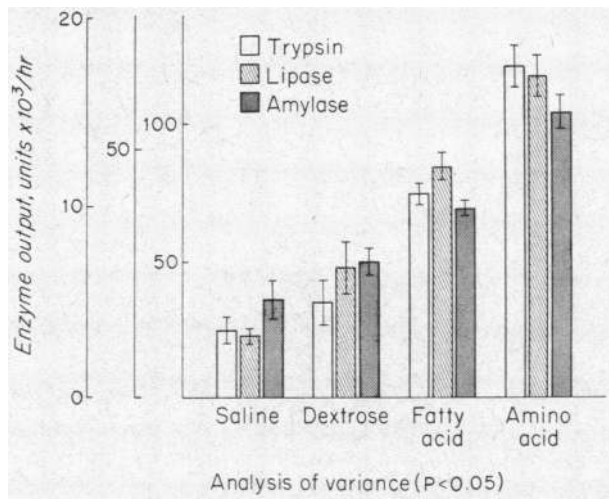

FIGURE 2 Outputs of trypsin, lipase, and amylase (mean ±SE) during 2nd hr of intraduodenal perfusion with saline or digestive products.

TABLE II

Pancreatic Enzyme Output in Response to Different Stimuli

Intraduodenal perfusate	Enzyme output, mean ±SE		
	Trypsin	Lipase	Amylase
		U × 10³/hr	
Isotonic saline	5.1 ±2.0	24.7 ±4.2	30.0 ±10.5
Standard amino acid mixture			
18 mM	16.4 ±7.5	64.5 ±6.2	122.1 ±1.6
180 mM	17.4 ±7.1	73.8 ±8.4	134.3 ±6.2
272 mM	16.5 ±2.5	93.6 ±6.8	147.0 ±11.3
Essential amino acid mixture	24.2 ±2.1	93.6 ±6.8	147.9 ±11.3
Nonessential amino acid mixture	7.8 ±2.3	41.5 ±5.7	42.6 ±11.3
Individual amino acids, 20 mM			
Methionine	8.1 ±0.4	50.1 ±0.9	63.2 ±18.7
Phenylalanine	12.3 ±2.6	50.4 ±1.3	80.5 ±20.9
Valine	8.4 ±2.4	53.5 ±1.4	87.3 ±17.9
Tryptophan	5.6 +1.0	30.4 ±8.9	38.8 ±16.0
Isotonic saline + pancreozymin by vein	28.0 ±1.2	117.1 ±12.1	166.1 ±22.6
Isotonic saline + standard amino acid mixture by vein	8.5 ±3.2	19.3 ±7.2	39.6 ±21.4

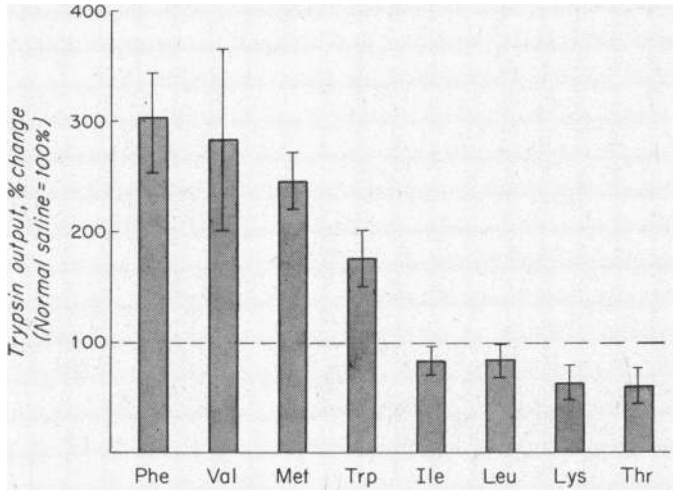

FIGURE 3 Changes in trypsin output during intraduodenal perfusion with individual essential amino acids, compared with perfusion with isotonic saline. By analysis of variance, $P < 0.02$ for difference between isotonic saline and valine and methionine.

Perfusions with amino acids in concentrations of 18, 182, and 272 mmoles/liter all gave similar outputs of enzymes (Table II), which did not differ significantly from the maximal output in response to pancreozymin given by vein. Intravenous infusion of the amino acid mixture, by contrast, produced no change in outputs of pancreatic enzymes compared to those during the control periods with isotonic saline.

DISCUSSION

Three distinct patterns of pancreatic enzyme secretion in man were delineated. First, a steady, relatively small, basal output of enzymes occurred when isotonic saline was the perfusate. Second, all hydrolytic products of digestion produced an immediate washout effect. Third, amino acids and micellar fatty acid both evoked a sustained secretion of enzymes which, in the case of amino acids, did not differ from the enzyme output, considered to be maximal, in response to pancreozymin infused by vein (11, 15).

Continuous basal secretion of pancreatic juice has been reported to occur with pancreatic fistula in man (19) but may then partially reflect release of secretin due to acid in the duodenum. Our results confirm those of Ågren and Lagerlöf (20) by showing continuous basal secretion of pancreatic juice in healthy man, even when gastric contents are aspirated. Continuous basal secretion of pancreatic juice from the isolated perfused canine pancreas has also been documented (21).

The rapid discharge of pancreatic enzymes evoked by all three digestive products but subsiding after approximately 40 min when dextrose was used is consistent with washout of preformed enzymes from the pancreatic ducts (2). The temporary action of dextrose may reflect stimulation of secretin release (22), especially because similarly transient enzyme secretion follows the continuous intravenous administration of secretin (23). By contrast, continuing outputs of enzymes occurred when amino acids or fatty acid was used and presumably (1) reflected augmentation or replacement of the initial washout response by endogenously released pancreozymin.

The sustained secretion of pancreatic enzymes after perfusion with the amino acid mixture or fatty acid during the 2nd hr parallels the pattern of response which has been attributed to secretion of pancreozymin in animals (1) and validated by numerous studies using porcine pancreozymin in man. The potency of amino acids was greater than that of fatty acid or dextrose at the physiologic intraduodenal concentrations used. Even at much lower concentration (18 mmoles/liter), amino acids caused outputs of pancreatic enzymes indistinguishable from those considered to be maximal in response to the administration of pancreozymin by vein (18). The possibility was explored that, after absorption, the amino acids used might increase pancreatic enzyme synthesis, and hence secretion, directly; such an effect has been demonstrated in vitro (24). However, because pancreatic enzyme output remained basal when amino acids were given by vein, our results are unlikely to have been influenced through this mechanism. Micellar fatty acid was also found to stimulate pancreatic enzyme secretion, and various other lipids, including triglyceride

and diethyl ether, produce a similar effect in several species, probably by releasing pancreozymin from the small intestine (1). Fatty acids are the simplest derivatives with these properties but their mechanism of action is unknown and was not pursued by us because the amino acids were more potent by the criteria applied.

To investigate the specificity of the response to amino acids, it was first established that only essential amino acids stimulated pancreatic enzyme output. Because the effect of essential amino acids was identical with that of the mixture of essential and nonessential amino acids, no inhibitory action can be attributed to nonessential amino acids. When the essential amino acids were examined individually, stimulation of pancreatic enzyme output was found only with phenylalanine, methionine, valine, and possibly tryptophan. However, the responses to these amino acids individually were less than those evoked by the mixture of essential amino acids. Thus, two or more amino acids may complement each other in the metabolic pathways involving synthesis or release of pancreozymin, perhaps by acting preferentially or cumulatively at different sites. The effects of individual amino acids on pancreatic enzyme secretion and pancreozymin release have not previously been investigated in man. Wang and Grossman (1) found that phenylalanine, tryptophan, and leucine were effective in short-term studies in dogs. Others (25) have reported that addition of methionine, isoleucine, and phenylalanine to the diet of dogs with chronic fistulas produced increased concentrations of lipase and "protease" in pancreatic juice. The discrepancies between these results may reflect differences in species and methods.

The intermediary steps relating the intraduodenal action of certain essential amino acids to the secretion of pancreozymin cannot be specified from our experiments. All of those amino acids effective in man are α-amino monocarboxylic essential acids with an uncharged side chain, are believed to be absorbed by the same transport mechanism, and are present in the polypeptide chain of pancreozymin (26, 27). It lately has been recognized that specific amino acids may stimulate synthesis or secretion, or both, of other polypeptides and protein. Leucine stimulates insulin release (28), arginine stimulates growth hormone secretion (29), and tryptophan and isoleucine stimulate albumin synthesis (30). Amino acids with these properties all are of the "essential" variety, a connotation which may come to include an essential function in metabolic control as well as in the regulation of normal growth.

ACKNOWLEDGMENT

This investigation was supported in part by Research Grant AM-6908 from the National Institutes of Health, Public Health Service.

REFERENCES

1. Wang, C. C., and M. I. Grossman. 1951. Physiological determination of release of secretin and pancreozymin from intestine of dogs with transplanted pancreas. *Amer. J. Physiol.* **164**: 527.
2. Lagerlöf, H. O. 1942. Pancreatic function and pancreatic disease: studied by means of secretin. *Acta Med. Scand. Suppl.* **128**: 1.
3. McClure, C. W. 1937. The exocrine functions of the pancreas. *Ann. Intern. Med.* **10**: 1848.
4. Doubilet, H., and L. Fishman. 1961. Human biliary-pancreatic secretion. *Amer. J. Gastroenterol.* **35**: 499.
5. Borgström, B., A. Dahlqvist, G. Lundh, and J. Sjövall. 1957. Studies of intestinal digestion and absorption in the human. *J. Clin. Invest.* **36**: 1521.
6. Jorpes, J. E. 1968. The isolation and chemistry of secretin and cholecystokinin. *Gastroenterology.* **55**: 157.
7. Valenzuela, J. E., M. Petermann, and C. Ugarte. 1968. Pancreatic secretion stimulated by a gastrin-like pentapeptide. *Amer. J. Dig. Dis.* **13**: 767.
8. Stening, G. F., and M. I. Grossman. 1969. Gastrin-related peptides as stimulants of pancreatic and gastric secretion. *Amer. J. Physiol.* **217**: 262.
9. Elwin, C.-E., and B. Uvnäs. 1966. Distribution and local release of gastrin. *In* Gastrin. M. I. Grossman, editor. University of California Press, Berkeley. 69.
10. Go, V. L. W., A. F. Hofmann, and W. H. J. Summerskill. 1969. Pancreozymin: sites of secretion and effects on pancreatic enzyme output in man. *J. Clin. Invest.* **48**: 29a. (Abstr.)
11. Go, V. L. W., A. F. Hofmann, and W. H. J. Summerskill. 1970. Simultaneous measurements of total pancreatic, biliary, and gastric outputs in man using a perfusion technique. *Gastroenterology.* **58**: 321.
12. Soergel, K. H. 1968. Inert markers. *Gastroenterology.* **54**: 449.
13. Spector, W. S., editor. 1956. Handbook of Biological Data. W. B. Saunders Company, Philadelphia. 199.
14. Zoellner, J. A., and O. Kampthrone. 1954. Incomplete block designs with blocks of two plots. Research Bulletin of the Iowa Agricultural Experimental Station. 418.
15. Banwell, J. G., B. E. Northam, and W. T. Cooke. 1967. Secretory response of the human pancreas to continuous intravenous infusion of pancreozymin-cholecystokinin (Cecekin). *Gut.* **8**: 380.
16. Hyden, S. A. 1955. Turbidometric method for the determination of higher polyethylene glycols in biological materials. *Ann. Agr. Coll. Swed.* **22**: 139.
17. Pelot, D., and M. I. Grossman. 1962. Distribution and fate of pancreatic enzymes in small intestine of the rat. *Amer. J. Physiol.* **202**: 285.
18. Smith, B. W., and J. H. Roe. 1949. A photometric method for the determination of α-amylase in blood and urine, with use of the starch-iodine color. *J. Biol. Chem.* **179**: 53.
19. Babkin, B. P. 1950. Secretory Mechanism of the Digestive Glands. Hoeber Medical Division, Harper & Row, Publishers, New York. 2nd edition. 199.
20. Ågren, G., and H. Lagerlöf. 1936. The pancreatic secretion in man after intravenous administration of secretin. *Acta Med. Scand.* **90**: 1.

21. Hermon-Taylor, J. 1968. A technique for perfusion of the isolated canine pancreas: responses to secretin and gastrin. *Gastroenterology.* **55**: 488.
22. Young, J. D., L. Lazarus, and D. J. Chisholm. 1968. Radioimmunoassay of secretin in human serum. *J. Nucl. Med.* **9**: 641.
23. Wormsley, K. G. 1968. The action of secretin on the secretion of enzymes by the human pancreas. *Scand. J. Gastroenterol.* **3**: 183.
24. Hokin, L. E. 1951. Amino-acid requirements of amylase synthesis by pigeon-pancreas slices. *Biochem. J.* **50**: 216.
25. Magee, D. F., and S. S. Hong. 1959. Daily output of pancreatic juice and some dietary factors which influence it. *Amer. J. Physiol.* **197**: 27.
26. Matthews, D. M., and L. Laster. 1965. Absorption of protein digestion products: a review. *Gut.* **6**: 411.
27. Mutt, V., and J. E. Jorpes. 1968. Structure of porcine cholecystokinin-pancreozymin. I. Cleavage with thrombin and with trypsin. *Eur. J. Biochem.* **6**: 156.
28. Milner, R. D. G. 1969. Stimulation of insulin secretion *in vitro* by essential aminoacids. *Lancet.* **1**: 1075.
29. Cremer, G. M., J. M. Bilstad, C. Faiman, and Karen E. Moxness. 1968. Circulating levels of anterior pituitary hormones and insulin after arginine infusion. *Mayo Clin. Proc.* **43**: 776.
30. Rothschild, M. A., M. Oratz, J. Mongelli, L. Fishman, and S. S. Schreiber. 1969. Amino acid regulation of albumin synthesis. *J. Nutr.* **98**: 395.