THE STUDY OF HUMORAL INHIBITION OF GASTRIC ACID SECRETION

BY

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Abstract

Part I Inhibition of Gastric Acid Secretion

Fat in the small bowel is a powerful inhibitor of gastric acid secretion. The gastric inhibitory agent(s) liberated from intestinal mucosa by the presence of fat has been named enterogastrone. Gastric inhibitory polypeptide (GIP), has been considered a candidate for enterogastrone. GIP is released into the circulation by infusion of fat into the proximal small bowel and inhibits gastric acid secretion under select experimental conditions. It has been proposed that the release of somatostatin, a potent inhibitor of acid secretion, may mediate the gastric inhibitory action of GIP. Recently, monoclonal antibodies raised to both GIP and somatostatin have been produced. The suitability of these antibodies for the study of the physiological roles proposed for their respective peptides is not known.

This study examined the inhibitory action of GIP and somatostatin on gastric acid secretion in the rat and in man. GIP was found to be a weak inhibitor of meal-stimulated gastric acid secretion in man when given in supraphysiological doses. When administered at a dose which produces less than the normal maximal physiological plasma level, GIP had little effect on the acid secretory response to the meal and no effect on either plasma gastrin or plasma SLI concentrations. In the rat, infusion of GIP produced a 60% reduction of meal-stimulated acid secretion, independant of changes in serum gastrin release.

Intraduodenal infusion of oleic acid in the rat reduced the gastric acid secretory response to a liver extract meal by 80% without affecting serum gastrin levels. A humoral gastric inhibitory agent, or "enterogastrone", was demonstrated in the portal blood of the rat following fat infusion.

Intravenous infusion of portal serum, which had been collected during an intraduodenal infusion of fat, reduced meal-stimulated acid secretion in a second animal.

A comparison of the inhibition of gastric acid secretion produced by intraduodenal infusion of either glucose or oleic acid with the release of IR-GIP in the portal serum was performed. The inhibitory effect of an intraduodenal fat infusion could not be explained by plasma IR-GIP. The release of GIP was not found to play a significant role in the mechanism for gastric inhibition by intestinal fat.

Part II

Monoclonal antibodies as Probes of Humoral Inhibitors of Gastric acid secretion

The ability of recently produced monoclonal antibodies to block in vivo the inhibitory action of exogenous GIP and somatostatin on gastric acid secretion was examined. Anti-GIP monoclonal antibody demonstrated a high affinity for GIP when compared to the polyclonal rabbit antiserum R07 in the ELISA. When administered either as an intravenous bolus, or after incubation with GIP for 1 hour at 37^{0}C, the antibody was unable to block the inhibitory effect of a GIP infusion on meal-stimulated gastric acid secretion in the rat. Monoclonal antibody 3.65H may not be suitable for the study of the role of endogenously released GIP.

Two anti-somatostatin monoclonal antibody clones S8 and S10, when given as intravenous boluses, blocked the inhibitory action of exogenous somatostatin on meal-stimulated gastric acid secretion in the rat. The antibody clone S10 however, had no effect on the inhibitory action of exogenous GIP on gastric acid secretion. Although both monoclonal

antibodies S8 and S10 effectively prevented the gastric inhibitory effect of infused somatostatin, the ability to block the physiological action of endogenously released gastric somatostatin remains to be determined.

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INTRODUCTION

Part I Inhibition of Gastric Acid Secretion

1. Effect of Fat on Gastric Acid Secretion : "Enterogastrone Concept"

Ewald and Boas in 1886, while studing carbohydrate digestion in the stomach, found that the addition of olive oil to a meal of starch paste inhibited secretion of gastric juice and delayed gastric emptying in their human subjects. The ingestion of fat was also shown to inhibit gastric secretions stimulated by sham feeding (Kasanski 1903). Initially it was thought that fat exerted this gastric inhibitory action while in the stomach. Sokolov in 1904, using dogs prepared with innervated gastric pouches and duodenal fistulae, demonstrated that fat acting in the duodenum and not the stomach, was responsible for the inhibition of gastric secretion. In addition, he found that the presense of HCl in the duodenum would also inhibit the gastric pouch response to a meal. A "nervous reflex" which originated primarily from the duodenum, was the mechanism first proposed to explain this inhibitory action of fat on the stomach, although there was no evidence to support the existence of such a neural mechanism (Pavlov 1910).

Farrel and Ivy in 1926, provided the first evidence of the existence of a humoral mechanism for gastric inhibition. They demonstrated in the dog, the ability of intestinal fat to inhibit the spontaneous hunger contractions of a denervated, autotransplanted gastric pouch. In 1929, Feng, Hou and Lim found that the inhibitory action of fat on gastric acid secretion also involved a humoral mechanism. They were able to show that secretions from similar autotransplanted gastric pouches in the dog were inhibited by a meal of fat. They also demonstrated that an intravenous infusion of

thoracic duct lymph, collected after ingestion of fat, did not alter gastric secretion, and that diversion of the thoracic duct had no effect on the gastric response to a fat meal. Their work suggested that a specific humoral factor or agent, and not a digestive product of fat absorbed from the duodenum, was responsible for fat inhibition of gastric secretion. They proposed that bile salts, reabsorbed from the intestine, might be the humoral agent. This proposition was based upon the fact that the ingestion of fat, in addition to gastric inhibition, stimulated contraction of the gallbladder with subsequent injection of bile into the duodenum. The influence of fat on the gallbladder was later shown by Ivy and Oldberg to be due to a humoral agent present in extracts of duodenal mucosa, which they named cystokinin (Ivy and Oldberg 1928).

Kosaka and Lim in 1930, using dogs prepared with denervated gastric pouches (Heidenhain) which were stimulated with meat meals, demonstrated that bile was not the humoral agent (Kosaka and Lim 1930a). They found that a large dose (5 $mg.kg.^{-1}$) of Ivy's cystokinin, injected subcutaneously, inhibited secretion from the Heidenhain pouch. In an attempt to recover the gastric inhibitory agent from the intestine, they prepared extracts of dog small bowel mucosa which had been previously exposed to fat. These extracts, when injected either intravenously or subcutaneously into dogs prepared with Heidenhain pouches, inhibited gastric acid secretion and motility. The degree of inhibition produced was comparable to the inhibition produced by the oral ingestion of fat. Extracts from segments of intestine not exposed to oil exhibited no inhibitory activity. They named this unknown inhibitory agent of gastric secretion and motility released from duodenal mucosa by fat, "enterogastrone" (Kosaka and Lim 1930b). In 1967, Gregory extended the definition of enterogastrone to include any gastric inhibitory agent(s) released from the

duodenum by fat, acid, and hypertonic solutions.

Several attempts were made to purify the mucosal extract and concentrate the active inhibitory principle further, but all met with little success (Kosaka et al. 1932, Kosaka and Lim 1933, Lim et al.1934, Grey et al. 1937, Greengard et al. 1946). Likewise, this gastric inhibitory agent, liberated by intestinal infusion of fat, has not been demonstrated in the circulation.

Fat in the small bowel is a potent inhibitor of pentagastrin-, histamine- and meal-stimulated gastric acid secretion in a variety of animal models (Schneider 1958, Konturek and Grossman 1965b, Halvorson et al. 1966, Johnson and Grossman 1969, Long and LaVigne 1969, Llanos et al. 1977, Yamagishi et al. 1980, Creutzfeldt et al. 1983) and in man (Johnson and Grossman 1969, Bochenek et al. 1971, Schmidt-Wilcke et al. 1975, Christiansen et al. 1976). The fat must be in a form which is absorbable from the small bowel and may be either a medium or long chain fatty acid or triglyceride (Sircus 1958, Konturek and Grossman 1965b, Longaand LaVigne 1969, Johnson and Grossman 1972, Schmidt-Wilcke et al. 1975). In man, Schmidt-Wilcke et al. found that an intraduodenal infusion of oleic acid produced the greatest degree of inhibition (50%) of pentagastrin-stimulated gastric acid secretion. Other medium and long chain fatty acids were examined and found to have a considerable, but lesser degree of inhibition (Schmidt-Wilcke et al. 1975).

Several investigators have looked specifically at the ability of medium chain fatty acids to inhibit gastric function. Such studies found that medium chain fatty acids did inhibit gastric emptying, but were not as effective as long chain fatty acids (Schneider 1958, Hunt and Knox 1962, Harkins et al. 1964). Long and LaVigne in 1969, found that medium chain triglycerides, compared with long chain triglycerides, were equally

as effective inhibitors of meal-stimulated acid secretion in both rats and dogs. This suggests that the incorporation of triglycerides into chylomicrons (which occurs with long chain but not medium chain triglycerides), rapid transport of the long chain triglycerides via the lymphatic channel, and the rapid entry of medium chain triglycerides into the portal vein do not play a critical role in the inhibition of gastric secretion by fat.

Konturek and Grossman, in 1965, used an elaborate chronic dog preparation to study the ability of various levels of the small bowel to inhibit gastric acid secretion after infusion with fat. The dogs were prepared with Heidenhain pouches, and had loops of either proximal and distal duodenum, jejunum, or ileum brought out to the abdominal wall as mucous fistulae. They infused a micellar fat mixture of oleic acid and bile into the various intestinal loops, and measured its effect on pentagastrin-stimulated acid secretion from the Heidenhain pouch. The fat, when infused into the small intestine at each level, inhibited acid secretion, with the greatest inhibition evoked by fat in the jejunal loop. They found a linear relationship to exist between fat absorption from the intestine and the degree of acid inhibition produced.

The possibility that an increase in the serum fat level may have an effect on gastric acid secretion was examined by Soon-Shiong et al. (Soon-Shiong et al. 1979a). They infused a 10% intralipid solution intravenously into dogs prepared with a gastric cannula, during pentagastrin stimulation of acid secretion. Serum levels achieved during the intralipid infusion were in excess of 1000 mg%. The hyperlipemic state had no effect on acid secretion.

The effect of intestinal fat on meal-stimulated acid secretion does not appear to be dependant on inhibition of gastrin release. Both

Christiansen et al., and Llanos et al., (in man and in the dog respectively), found that meal-stimulated serum gastrin levels were not affected by fat infusion (Christiansen et al. 1976, Llanos et al. 1977). Yamagishi and Debas however, demonstrated that oleic acid, infused intraduodenally at a rate capable of producing maximal pancreatic protein and bicarbonate secretion, resulted in a significant inhibition in the serum gastrin response to a liver extract meal (Yamagishi and Debas 1980).

2. Enterogastrone Candidates

The presence of fat in the small bowel has been shown to stimulate the release of several intestinal peptides, all capable of inhibiting gastric acid secretion. Three peptides, cholecystokinin, secretin and gastric inhibitory polypeptide (GIP) have been investigated as enterogastrone candidates.

Exogenous cholecystokinin is a weak stimulator of basal gastric acid secretion. It is a competitive inhibitor of gastrin, and in large doses may inhibit gastrin-stimulated acid secretion (Johnson and Grossman 1970a, Johnson and Grossman 1972, Baron 1976). This is likely a pharmacological action of cholecystokinin, related to its affinity for the gastrin receptors on the parietal cell. It is unlikely that cholecystokinin is the primary mediator of the gastric inhibitory effect of fat (Dockray 1981).

Since its discovery by Bayliss and Starling in 1902, the 27 amino acid polypeptide secretin has been known to be a powerful stimulator of bicarbonate rich pancreatic secretions. Acid entering the proximal duodenum is the major stimulant for secretin release. Ingestion of a meal leads to increases in plasma secretin levels, which coincide with decreases in pH in the proximal duodenum (Chey et al. 1981). Intraduodenal fat has also been found to increase plasma levels of secretin in the dog

(Meyer and Jones 1974, Faichney et al.1979), but not in man (Schaffalitzky de Muckadell and Fahrenkrug 1977).

The inhibitory effect of secretin on gastric acid secretion was first observed by Greenlee et al. in 1957. They found that an intravenous infusion of a secretin preparation inhibited both meal- and gastrin-stimulated gastric secretion, but not histamine-stimulated secretion from the Heidenhain pouch in the dog. The ability of secretin to inhibit gastrin- and meal-stimulated gastric acid secretion has been confirmed by others (Jordan and Peterson 1962, Wormsley and Grossman 1964, Chey et al. 1973, Rayford et al. 1976, Chey 1982). Secretin has also been shown to inhibit food-stimulated gastrin release in a non-competitive manner (Konturek et al. 1975, Chey 1982).

Secretin however, has little or no effect upon the gastric secretory response to histamine stimulation (Greenlee et al. 1957, Johnson and Duthie 1966, Chey et al. 1970), whereas the presence of fat in the duodenum is a powerful inhibitor of histamine-stimulated gastric acid secretion (Halvorson et al. 1966, Johnson and Grossman 1969). Llanos et al., in 1977, measured the release of plasma secretin in response to an intraduodenal infusion of sodium oleate during meal stimulation of gastric acid secretion in the dog. They found that plasma secretin was not elevated by an infusion of fat which was sufficient to produce a 70% inhibition of the gastric secretory response to the meal.

Secretin, as with cholecystokinin, is not likely to be the primary agent mediating the gastric inhibitory effect of intestinal fat.

3. Gastric Inhibitory Polypeptide

A. Discovery and Localization

Early crude preparations of Ivy and Oldberg's cystokinin (Ivy and

Oldberg 1928), when administered in large doses in the dog, inhibited meal-stimulated gastric acid secretion (Kosaka and Lim 1930a). Later preparations of duodenal mucosa which contained cholecystokinin activity, were shown to inhibit gastrin-stimulated acid secretion from a Heidenhain pouch in the dog (Gillespie and Grossman 1964).

Brown et al., in 1969, described the preparation of an "entero-gastrone" from a 10% pure preparation of porcine cholecystokinin. The preparation retained no cholecystokinin activity. An intravenous infusion of the preparation produced a 70% inhibition of gastrin-stimulated gastric acid secretion from the denervated fundic pouch, along with marked suppression of fundic and antral activity in the dog (Brown 1971, Brown and Dryburgh 1971).

In 1971, Brown and Dryburgh purified the fraction demonstrating this enterogastrone-like activity and discovered a distinct polypeptide which they named "gastric inhibitory polypeptide" (GIP), due to its gastric acid inhibitory property. The primary structure first published was a linear polypeptide of 43 amino acids (Brown 1971a). Jornvall et al. in 1981, reported a new sequence determination for GIP. Further analysis of the GIP preparation found the material to be heterogeneous. The main component is a linear peptide, 42 amino acid residues long, lacking the glutamine residue after position 29, as initially described. The secondary component, comprising of about 20% of the GIP preparation, appears identical to the main component except that it lacks the first two residues of the later. This shortened GIP peptide demonstrates little or no biological activity. (Jornvall et al. 1981)

Cells containing immunoreactive GIP (IR-GIP) were first identified by Polak et al. in 1973. The IR-GIP containing cells (K-cells), were found predominantly in the middle layer of the duodenal mucosa, and to a lesser

extent in the jejunal mucosa of both dog and man, distinct from other known endocrine cells. A radioimmunoassay was developed by Kuzio et al. in 1974, using a guinea pig antiserum raised to porcine GIP, which allowed the measurement of circulating levels of IR-GIP. Fasting serum levels of IR-GIP in man were found to average about 200 $pg.ml.^{-1}$, and to increase above 1200 $pg.ml,^{-1}$ within 45 minutes of the start of a meal (Kuzio et al. 1974).

A second physiological action has since been ascribed to GIP, the release of insulin under conditions of hyperglycemia. This insulinotropic effect and corresponding role in the glucoregulatory process, established GIP as a true hormone (Brown et al. 1981a).

B. Investigation of GIP as a Possible Enterogastrone

Early experiments examined the effect of an exogenous infusion of GIP on acid secretion in the denervated gastric pouch and innervated gastric remnant in the dog (Pederson and Brown 1972). GIP produced a 75% inhibition of the acid secretory response in the denervated gastric pouch stimulated by pentagastrin and histamine, and a 45% inhibition of the secretory response in the innervated gastric remnant stimulated by insulin hypoglycemia.

The nomination of GIP as the primary enterogastrone was strengthened with the discovery that fat in the proximal small bowel released IR-GIP into the circulation. In 1975, Pederson et al. characterized the serum IR-GIP response to oral fat and glucose in the dog. They found the release of IR-GIP to occur in a dose-related manner following graded oral loads of Lipomul (an oleic acid suspension) and glucose. O'Dorisio et al. in 1976, investigated other nutrients which might stimulate release of GIP in the dog. They found that an intraduodenal infusion of corn oil (long

chain triglycerides) evoked the greatest IR-GIP release, followed by glucose, amino acids, and medium chain triglycerides. The intraduodenal infusion of hydrochloric acid was also found to stimulate the release of IR-GIP, in both the rat and in man (Ebert et al. 1979)

Cleator and Gourley in 1975 examined the release of IR-GIP after oral ingestion of various food substances in man. They found that oral administration of Lipomul produced the largest increase in plasma IR-GIP, from a basal level of 250 $pg.ml.^{-1}$, to a peak post-prandial value of 1300 $pg.ml.^{-1}$. Oral glucose, galactose and leucine also stimulated IR-GIP release. Intraduodenal infusion of Lipomul produced a 90% inhibition of pentagastrin-stimulated gastric acid secretion in their human subjects, which correlated closely with the increase in serum IR-GIP observed.

In the innervated stomach preparations of the dog, and in man, GIP has been shown to be a much weaker inhibitor of pentagastrin-stimulated gastric acid secretion. This has raised doubts about the importance of GIP as the primary enterogastrone.

Debas and Yamagishi found exogenous GIP to be only a weak inhibitor of pentagastrin-stimulated gastric acid secretion in the innervated canine stomach (Debas and Yamagishi 1978). Similarly, Soon-Shiong et al. demonstrated that GIP was a poor inhibitor of pentagastrin-stimulated acid secretion in the innervated canine stomach under conditions of hyperlipemia and hyperglycemia (Soon-Shiong et al 1979a). Hyperlipemia augmented the inhibitory action of exogenous GIP, but only at the highest doses of pentagastrin stimulation when serum fat levels were over 1000 mg%.

Cleator and Gourley in 1975, found that an intravenous bolus of GIP (2 $\mu g.kg.^{-1}hr.^{-1}$) produced a significant (60%) inhibition of pentagastrin-stimulated acid secretion, but the serum IR-GIP levels measured after the

bolus were over twice the normal maximal physiological level (>4000 $pg.ml.^{-1}$).

Maxwell et al. studied the ability of exogenous GIP, administered as an infusion, to inhibit pentagastrin-stimulated gastric acid secretion in man (Maxwell et al. 1980). An infusion of GIP (2.0 $\mu g.kg.^{-1}hr.^{-1}$) produced negligible inhibition of the stimulated acid secretion, even though the dose of GIP infused produced serum levels of IR-GIP much greater than those seen after ingestion of a small meal (>6000 $pg.ml.^{-1}$).

Five previously vagotomized subjects were studied to assess whether or not vagal innervation might play a role in the gastric inhibitory action of GIP in man (Simmons et al. 1981). An insulin test (0.1 $unit.kg.^{-1}hr.^{-1}$) was performed on each subject to demonstrated that the vagotomy was complete. An intravenous infusion of GIP (2.0 $\mu g.kg.^{-1}hr.^{-1}$), which elevated plasma IR-GIP levels over 2500 $pg.ml.^{-1}$, failed to inhibit pentagastrin-stimulated gastric acid secretion. The inability of GIP to inhibit pentagastrin-stimulated acid secretion in vagotomized humans suggests that there may be a species difference between dog and man, for in the dog, GIP is a more effective inhibitor of the vagally denervated gastric pouch.

GIP has demonstrated only a weak inhibitory effect on meal-stimulated gastric acid secretion from the neurally intact canine stomach. Yamagishi and Debas in 1980, compared the inhibitory action of exogenous GIP on meal-stimulated acid secretion to that of GIP released endogenously by intraduodenal fat infusion in the dog. An intraduodenal infusion of oleic acid caused complete suppression of the acid response to a liver extract meal, with only a modest elevation of serum IR-GIP. An exogenous intravenous infusion of GIP produced a 40% inhibition of the

acid response to the meal while greatly elevating serum IR-GIP. The oleic acid infusion inhibited gastrin release in response to the meal. No changes in serum gastrin were seen during the GIP infusion.

Creutzfeldt et al. in 1983, performed duodenal infusions of glucose and fat and measured serum IR-GIP in the dog during intestinal stimulation of gastric acid secretion. They found that fat would significantly inhibit gastric acid secretion stimulated by 5% liver extract in the duodenum, when infused at low concentrations (0.5%), whereas much higher fat concentrations (4.0%) were required to obtain a significant increase in serum IR-GIP. A 5% duodenal infusion of glucose resulted in a significant elevation of IR-GIP, but failed to suppress gastric acid secretion.

To date, studies of the effect of GIP in man have all employed pentagastrin as the stimulus for gastric acid secretion. The effect of GIP in man during a meal, a more physiological stimulus for gastric acid secretion, remains unknown.

4. Somatostatin

A. Discovery and Localization

The tetradecapeptide somatostatin was originally isolated from extracts of sheep hypothalami and identified by its action as an inhibitor of growth hormone release (Brazeau et al. 1973). Subsequently, somatostatin has been shown to exert a potent inhibitory effect on several pituitary hormones (growth hormone, thyroid-stimulating hormone, prolactin, adrenocorticotropic hormone and thyrotropin-releasing hormone from the hypothalamus), and virtually all gastrointestinal and pancreatic endocrine and exocrine functions (Gerich et al. 1978, Patel et al. 1981, Schusdziarra 1983).

Somatostatin-like immunoreactivity (SLI) has been demonstrated

throughout the central nervous system, in nerve fibers, axons and neuronal cell bodies, using radioimmunoassay and immunohistochemical techniques (Elde et al.1978, Patel and Reichlin 1978). In the peripheral nervous system, SLI has been demonstrated in axons and nerve cell bodies of primary sensory nerves, vagus and siatic nerves, neuronal cell bodies of sympathetic ganglia and dorsal root ganglia, and in intrinsic neurons of the submucous and myenteric plexus of the gut (Hokfelt et al. 1975, Patel et al. 1981).

Immunoreactive somatostatin containing cells (D-cells) are present in the mucosa throughout the gastrointestinal tract. In the stomach, D-cells are found in the fundic and antral areas, with the highest concentration in the antrum. In the pancreas, the D-cells are located mainly in the periphery of the islets of Langerhans, in close proximity to glucagon-, pancreatic polypeptide- and insulin-producing cells (Arimura et al. 1975, Patel and Reichlin 1978, Patel et al. 1981, Schusdziarra 1983).

An extended molecular form of the tetradecapeptide somatostatin was isolated by Pradayrol et al. in 1978 from porcine intestine. It has also been found in the bovine hypothalamus (Brazeau et al. 1980) and rat pancreas (Benoit et al. 1982). The 28 amino acid polypeptide was characterized by Pradayrol et al., and found to contain the complete sequence of the tetradecapeptide at the C-terminal (Pradayrol et al. 1978). Studies of the biological activity of this larger form of somatostatin demonstrate that it possesses inhibitory effects similar to the smaller tetrapeptide, on all gastrointestinal and pancreatic exocrine and endocrine functions (Vaysse et al. 1981a, Schusdziarra 1983).

Neurocrine-, paracrine-, and endocrine-like pathways have all been proposed as modes of action for somatostatin. In the central nervous system, somatostatin is thought to function as a neurotransmitter or

neuromodulator, primarily because of its presence in nerve fibers and neuronal cell bodies (Yamada et al. 1980). Somatostatin is also found in nerve fibers and neuronal cell bodies of the gastrointestinal tract and forms part of an enteric peptidergic nervous system (Polak et al. 1981).

Somatostatin cells within the stomach and pancreas differ from the typical gut endocrine cell. They possess long cytoplasmic processes, which terminate close to neighbouring effector cells. In the stomach, these cell processes end near gastrin-producing cells, parietal cells and other epithelial cells. In the pancreas, the somatostatin cytoplasmic processes end close to glucagon- and insulin-producing cells and islet capillaries (Larsson et al. 1979, Larsson 1981). Such morphological evidence demonstrating the close proximity of somatostatin cell processes to putative target cells suggest a paracrine mode of action, with release of somatostatin directly onto the membrane of the neighbouring cells.

In the duodenum, somatostatin cells are more like typical endocrine cells. They are triangular in shaped, with extension to the luminal surface. It is possible that these D-cells may behave in the classical endocrine fashion, releasing somatostatin into the circulation in response to luminal stimuli to affect distal organs or cells (Shusdzarria et al. 1980a, Larsson 1981).

The co-existence of such pathways (neurotransmitter/modulator, paracrine, endocrine) would facilitate the selective inhibition by somatostatin of a wide variety of gastrointestinal and pancreatic endo -crine and exocrine functions.

B. Effect of Somatostatin on Gastric Acid Secretion

Somatostatin is one of the most potent inhibitors of gastric acid secretion in animals and man. Somatostatin has been shown to inhibit both

basal acid secretion, and gastric acid secretion stimulated by agents such as pentagastrin, histamine and urecholine (Bloom et al. 1974, Barros et al. 1975, Raptis et al. 1975, Konturek et al. 1976, Vant et al. 1977, Creutzfeldt and Arnold 1978, Thomas 1980a and 1980b, Lin et al. 1983), and has been found to completely abolish the acid secretory response to a meal (Gomez-Pan et al. 1975, Konturek et al. 1976, Seal et al. 1982). Histamine-stimulated acid secretion has been reported to be more resistant to inhibition by somatostatin, and only suppressed at low doses of histamine stimulation (Konturek et al. 1976, Creutzfeldt and Arnold 1978, Elde et al. 1978, Thomas 1980a and 1980b).

The mechanism for the inhibition of acid secretion by somatostatin remains unclear. Somatostatin may have a direct effect on the parietal cell. Somatostatin-containing D-cells in the gastric mucosa possess long cytoplasmic processes which terminate in close opposition to parietal cells. Their morphology suggests that they may deliver somatostatin directly on to the parietal cell membrane in a paracrine-like fashion (Larsson 1981). Somatostatin has been shown to reduce intracellular cyclic adenosine monophosphate (cyclic AMP) production in intestinal and gastric mucosal cells of the rat (Guandalini et al. 1980, Gespach et al. 1980), possibly through the inhibition of plasma membrane adenylate cyclase activity (Heisler et al. 1982). As histamine stimulation of acid secretion is mediated by the production of cyclic AMP in the parietal cell (Soll and Walsh 1979), it is possible that somatostatin may have a direct, inhibitory action on the parietal cell.

Prostaglandins are known potent inhibitors of acid secretion, and have been proposed as mediators of the inhibitory action of somatostatin on the parietal cell (Robert 1976, Ligumsky et al. 1983, Schusdziarra 1983). Prostaglandins appear to act by reducing cyclic AMP production

through the inhibition of parietal cell adenylate cyclase (Soll and Walsh 1979). Somatostatin has been shown to potentiate carbamylcholine-induced synthesis and release of prostaglandin E_2 from the isolated, perfused rat stomach. In addition, indomethacin (an inhibitor of prostaglandin synthesis) blocked the ability of somatostatin to inhibit bethanecol-stimulated acid secretion (Ligumsky et al.1983). Prostaglandin E_2 has also been shown to stimulate gastric somatostatin release, which would further amplify the acid inhibitory action of somatostatin (Saffouri et al. 1980, Schusdziarra et al. 1980b).

Reduction in gastric mucosal blood flow, with a subsequent decrease in acid secretion, does not appear to be the primary mechanism responsible for the inhibitory action of somatostatin. Inhibition of acid secretion by somatostatin, from the innervated stomach or from the denervated gastric pouch of the dog, was shown to be accompanied by a parallel reduction in gastric mucosal blood flow, as measured by the gastric clearance of plasma aminopyrine (Konturek et al. 1976, Lin et al. 1983). When the somatostatin infusion was stopped, acid secretion quickly returned to control levels while the gastric mucosal blood flow remained significantly depressed. This suggests that although mucosal blood flow is reduced during a somatostatin infusion, it is unrelated to the reduction in acid secretion.

It has been proposed that somatostatin may exert control over gastric acid secretion by regulation of gastrin release. D-cells in the antral mucosa have long "paracrine-like" processess which end near gastrin-producing cells (Larsson 1981, Buchan et al. 1985). Infusion of somatostatin has been shown to reduce basal gastrin secretion in both man and intact animals (Bloom et al. 1974, Raptis et al. 1975, Creutzfeldt and Arnold 1978), in the isolated perfused rat stomach (Saffouri et al. 1980),

and in rat antral organ culture (Harty et al. 1981). Other investigators however, failed to demonstate a change in gastrin levels after somatostatin infusion in man (Vatn et al. 1977, Fiddian-Green et al. 1980) or in the dog (Seal et al. 1982, Lin et al. 1983).

The isolated, perfused rat stomach has been used to further examine the interrelationship between somatostatin and gastrin secretion. In such preparations, stimulation with methacholine (Saffouri et al. 1980) and carbamyl choline (Martingdale et al. 1981) produced a dose-dependant inhibition of SLI secretion, demonstrating the existence of cholinergic inhibition of somatostatin release. Conversely, such stimulation also resulted in a dose-dependant increase in gastrin release into the circulation. Both of these effects were abolished by atropine. The reciprocal relationship observed between the inhibition of somatostatin and the secretion of gastrin supports the hypothesis that gastric somatostatin exerts a continuous restraint on basal gastrin secretion.

Somatostatin has also been shown to suppress meal- and insulin hypoglycemia-stimulated gastrin release (Bloom et al. 1974, Raptis et al. 1975, Creutzfeldt and Arnold 1978), and to inhibit carbachol-stimulated gastrin release from rat antral organ culture (Harty et al. 1981).

C. Release of Gastric Somatostatin

(i) Effect of Luminal Nutrients and HCl

Ingestion of a meal produces a significant elevation in the peripheral vein plasma levels of SLI, both in dogs and in man (Schusdziarra et al. 1978, Chayvialle et al. 1980, Wass et al. 1980, Penman et al. 1981). Acidification of the antral region of the stomach and the proximal small bowel has been shown to increase venous blood SLI levels in the dog (Schusdziarra et al. 1978). The degree to which the stomach, intestine and

pancreas contribute to the circulating somatostatin levels, remains unknown.

Penman et al., in 1981, found that dietary fat, protein, and to a lesser extent carbohydrate were potent stimuli for SLI release into the peripheral circulation in man. The mean fasting plasma SLI level, measured by radioimmunoassay, was 29 ± 5 $pg.ml.^{-1}$, and rose to a peak of 80 ± 9 $pg.ml.^{-1}$ after ingestion of fat. Sephadex chromatography was carried out on basal and stimulated plasma samples. Two main forms of somatostatin were found, the first with a molecular weight of 1600 which would correspond to the tetradecapeptide, and the second, a larger form of 3500 molecular weight. Feeding stimulated the release of both forms of somatostatin into the circulation (Penman et al. 1981).

(ii) Effect of the Autonomic Nervous System

Parasympathomimetics have been shown to exert an inhibitory effect on gastric somatostatin release (Saffouri et al. 1980, Martingdale et al. 1981, McIntosh et al. 1981a and 1981b, McIntosh and Pederson 1980c, Schubert and Makhlouf 1983). Studies on the effect of vagal stimulation on gastric somatostatin release have produced conflicting results. McIntosh et al. reported that stimulation of the vagus nerve produced an inhibition of SLI release from the isolated perfused rat stomach (McIntosh 1981a and 1981b). Chiba et al., using a similar model, found that vagal stimulation increased gastric SLI release (Chiba et al. 1980 and 1982). In vivo, vagal stimulation has resulted in both a reduction and an increase in portal vein SLI levels in the cat (Uvnas-Wallensten et al. 1978) and dog (Wasada et al. 1981) respectively.

Konturek et al. observed that somatostatin produced powerful inhibition of pentagastrin- and meal-stimulated acid secretion in both the

vagally innervated and denervated gastric pouch in the dog (Konturek et al. 1976). His findings suggest that vagal integrity does not play an essential role in the inhibitory mechanism of somatostatin.

Adrenergic mechanisms may also play a role in the control of somatostatin release from the stomach. Stimulation of splanchnic nerves during muscarinic blockade with atropine resulted in an increase of SLI released from the isolated perfused rat stomach (McIntosh et al. 1981b). In a similar model, the ß-adrenergic agonist isoproterenol produced a dose-dependant stimulation of gastric SLI release, whereas equivalent concentrations of ∂-adrenergic agonists had no effect. The ß-adrenergic stimulation of somatostatin secretion was abolished by the administration of the ß-antagonist propranolol (Koop et al. 1980, Goto et al. 1981).

(iii) Effect of Gastrointestinal Peptides

A number of gastrointestinal peptides are known to influence gastric release of somatostatin. Members of the structurally similar "secretin-glucagon" family of peptides, secretin, glucagon, GIP and vasoactive intestinal polypeptide (VIP), have all been shown to inhibit gastric acid secretion and gastrin release (Pederson and Brown 1972, Konturek et al. 1975, Villar et al. 1976a, Chiba et al. 1980c). They have also been shown to stimulate gastric SLI release (Chiba et al. 1980a and 1980c, Gespach et al. 1980, Rouiller et al. 1980, DuVale et al. 1981, McIntosh et al. 1981a, Wolfe et al. 1983b). This has led to the suggestion that somatostatin may mediate, at least in part, the inhibitory effects of the secretin-glucagon family of peptides on gastric acid secretion (Chiba et al. 1980c, DuVale et al. 1981, McIntosh et al. 1981a, Wolfe et al. 1983b).

Although GIP was shown to be a potent inhibitor of gastric acid secretion from the denervated gastric pouch (Pederson and Brown 1972), it

is only a weak inhibitor of acid secretion from the innervated stomach (Debas and Yamagishi 1978, Soon-Shiong et al. 1979a, Maxwell et al. 1980, Yamagishi and Debas 1980, Creutzfeldt et al. 1983). In addition, in the vagally denervated canine gastric pouch, the inhibitory action of GIP on pentagastrin-stimulated acid secretion was abolished by urecholine and bethanechol (Soon-Shiong et al. 1979b and 1984). In the isolated perfused rat stomach, McIntosh et al. found GIP to be a potent, dose-dependant stimulant of SLI release (McIntosh et al. 1981a). In this model, both vagal stimulation and the addition of acetylcholine to the perfusate, were shown to inhibit GIP-stimulated release of SLI. These inhibitory effects were blocked with the addition of atropine. They suggested that the local release of gastric SLI from the D-cell mediates the inhibitory action of GIP on the parietal cell. They proposed, therefore, that cholinergic inhibition of somatostatin release would explain the weak inhibitory action of GIP in the vagally innervated stomach. Confirmation of somatostatin as the local mediator of the inhibitory effect of GIP on the parietal cell remains to be demonstrated.

Other agents have also been shown to have an effect on gastric somatostatin release. Bombesin (Chiba et al. 1980a, Wolfe et al. 1983b), calcitonin (Chiba et al. 1980b), apomorphine (Uvnas-Wallensten et al. 1978), and CCK octapeptide (Rouiller et al. 1980) have all been found to stimulate the release of SLI. Substance P, an undecapeptide found in cell bodies and nerve fibers of the myenteric and submucosal plexi, and in nerve fibers penetrating the smooth muscle and lamina propria of the mucosa of gastrointestinal tract, has been shown to be a potent inhibitor of basal and both GIP- and isoproterenol-stimulated gastric SLI release (Chiba et al. 1980a, Kwok et al. 1985) and may have a role in the regulation of somatostatin release from the stomach.

Part II Antibodies as Probes of Humoral Inhibitors of Gastric Acid Secretion

Antibodies, in the form of polyclonal antisera, have been used in the investigation of physiological roles for the endogenously released peptides to which they have been raised (Gregor et al. 1963, Jaffe et al. 1969, Lipschutz et al. 1973, Villar et al. 1976). In theory, such antibodies, once infused into the circulation, bind to and thereby block the biological actions of the target peptides. A deficiency state of the specific peptide may thus be created and its effect on an animal model observed.

1. Investigations with GIP Antiserum

Ebert et al. have reported the in vivo use of a GIP antiserum in their investigation of the role endogenous GIP plays in glucose-induced insulin release (incretin effect) in the rat (Ebert et al. 1979, Ebert and Creutzfeldt 1982). The GIP antibodies were produced in rabbits by repeated immunization with pure porcine GIP. The IgG fraction from the polyclonal antiserum was prepared by ammonium sulfate precipitation of the rabbit serum.

To confirm the ability of the GIP antiserum to block in vivo the insulinotropic effect of GIP, they infused exogenous GIP and glucose intravenously into the rat after a bolus of GIP antiserum. The bolus of antiserum was shown to counteract the exogenous GIP-stimulated release of serum immunoreactive insulin (IRI). They found that an intravenous bolus of GIP antiserum, followed by a continuous infusion of dilute antiserum, completely abolished the release of IRI stimulated by intraduodenal HCl, but only partially eliminated IRI release in response to intraduodenal glucose.

They concluded that the insulin releasing effect of HCl in the

duodenum was due to its ability to release GIP. The incretin effect of duodenal HCl was prevented when the endogenously released GIP was blocked by the antiserum. However, because the antiserum could only partially eliminate the IRI response to intraduodenal glucose infusion, they suggested that the incretin effect of intraduodenal glucose was mediated not only by GIP, but by other gut factors as well.

In a similar study, Lauritsen et al. found the incretin effect after oral glucose in conscious rats was completely abolished after treatment with GIP antiserum (Lauritsen et al. 1981). The differences in results reported by the two investigators may be explained by the antisera used, as discussed by Ebert and Creutzfeldt (1982). Whereas Ebert and Creutzfeldt used pure porcine GIP, the antiserum used by Lauritsen et al. was raised against 40% pure porcine GIP. It is possible that this antiserum may exhibit a different affinity for GIP in vivo, or may crossreact with other as yet unknown peptides.

Antibodies raised to GIP have also been used to examine the role which endogenously released GIP plays during meal-stimulation of gastric acid secretion. Wolfe et al. in 1983, performed peptone meal-stimulated acid secretion studies on dogs with innervated stomachs prepared with a gastric fistula (Wolfe et al. 1983a). After infusion of the meal, they noticed immediate increases in serum IR-GIP and gastrin in both the portal and peripheral circulations. In a similar study, prior to the start of the meal, rabbit serum containing antibodies raised to GIP was intravenously infused in an attempt to bind to and inhibit the endogenously released GIP. This resulted in an increase in both meal-stimulated acid secretion and serum gastrin response. They concluded that GIP functions as a physiological inhibitor of meal-stimulated gastric acid secretion, and appears to exert this effect, at lease in part, through its effect on gastrin

release. However, these observations have not yet been confirmed by others.

The potentiation of meal-stimulated gastric acid secretion, after inactivation of endogenously released GIP with a specific antibody, provides indirect support of a role for GIP in the inhibition of acid secretion under physiological conditions. However, studies confirming the ability of such antibodies raised to GIP, to block the inhibitory action of an exogenous infusion of GIP on acid secretion, have not been performed.

2. Investigations with Somatostatin Antiserum

Antisera raised to somatostatin have been used to investigate the following physiological actions attributed to somatostatin: inhibition of the release of growth hormone, thyrotropin, thyrotropin-releasing hormone and prolactin (Arimura et al. 1976, Ferland et al. 1976, Chihara et al. 1978); inhibition of pancreatic exocrine and endocrine secretion (Schusdziarra et al. 1980a); and inhibitory control over gastrointestinal function (Saffouri et al. 1979, Schusdziarra et al. 1980, Chiba et al. 1981, Duvale et al. 1981, Wolfe et al. 1983b, Short et al. 1985).

Both Saffouri et al. in 1979, and Short et al. in 1985, reported that in the isolated perfused rat stomach, an infusion of rabbit somatostatin antiserum produced a significant increase in gastrin release into portal blood. This, they suggested, was confirmation that gastric somatostatin exerts a continuous restraint on gastrin secretion in the basal state. Infusion of the antiserum blocked the somatostatin effect, thereby facilitating the increase in gastrin release.

Similarily, Duval et al. in 1981, found that an infusion of sheep somatostatin antiserum augmented bombesin stimulated gastrin release in the isolated perfused rat stomach. The authors postulate that during the

bombesin infusion, the concomittant release of antral somatostatin antagonizes bombesin-stimulated gastrin release. The addition of the antiserum blocked the effect of the locally released somatostatin, thus allowing the increase in gastrin secretion.

In order for an infusion of antiserum to block the local "paracrine" action of endogenous somatostatin, the antibodies must be able to reach the interstitial space of the target organ. To determine if injected antibodies could quickly reach the gastric interstitial space in the dog, Schusdziarra et al. compared gastric mucosal concentrations of ^{125}I-labelled gamma-globulin after intra-arterial injection, to ^{125}I (as NaI), which becomes widely distributed thoughout the extracellular space (Schusdziarra et al. 1980). One hour after the ^{125}I injection, the radioactive count of the antral mucosa was greater than 50% of the activity detected in the serum. However, one hour after the injection of labelled gamma -globulin, no radioactivity could be detected in the antral mucosa. They concluded it was unlikely that injected antiserum could rapidly reach interstitial spaces to block the "paracrine" action of somatostatin.

To further elucidate the pathway for control of gastrin release by somatostatin, Chiba et al. administered antisomatostatin rabbit immuno -gammaglobulin (IgG) to the intact anaesthetized rat, isolated perfused rat stomach, and to cultured rat antral mucosa (Chiba et al. 1981c). In both the intact animal and the isolated perfused rat stomach, infusion of the IgG did not affect gastrin release. However, in the incubated rat antral mucosa, addition of the antibody significantly increased basal gastrin release. A similar finding was reported by Wolfe et al. in 1983. They added somatostatin antiserum to rat antral mucosa cultures and found an enhancement of basal gastrin release into the culture medium (Wolfe et al.

1983b). The investigators concluded that the inhibitory effect of somatostatin on gastrin release was primarily through a paracrine rather than an endocrine pathway.

3. Monoclonal Antibodies

The use of polyclonal antisera, as probes to investigate the physiological roles of peptides, has certain drawbacks. Although the antisera have been raised to specific antigens, they remain heterogenous antibody preparations. The antisera, once in circulation, may affect not only the target peptide but may also interact with other unknown agents. In addition, the quantity of any single antiserum produced is limited and finite.

The development of hybridoma technology, and its application to the production of antibodies, by Kohler and Milstein in 1975 have solved these problems (Kohler and Milstein 1975). Hybrid cells, formed by the fusion of antibody- producing cells to myeloma cells, can be grown in vivo and will continue to produce large quantities of monoclonal antibodies specific for the antigen against which they were raised.

A mid to C-terminal monoclonal antibody with a high affinity to GIP has been described (Buchan et al. 1982). The monoclonal antibody, designated 3.65H, when compared with rabbit and guinea-pig antisera to GIP, was found to be the most sensitive antibody for the detection of intestinal IR-GIP containing cells. The ability of this monoclonal antibody to act in vivo and block a biological action of infused GIP, the inhibition of gastric acid secretion, is unknown. Such in vivo activity must be verified before the antibody may be used in the investigation of proposed physiological roles for endogenously released GIP.

Four monoclonal antibodies raised to somatostatin have recently been

developed (Buchan et al. 1985). All 4 antibodies were found, by both immuno- cytochemical methods and in the enyzme-linked immunosorbent assay (ELISA), to bind to both the 28, 14 cyclic, and 14 linear amino acid forms of somatostatin. In addition, 2 of the antibodies, designated S8 and S20, were capable of binding to fragments of somatostatin (figure 1).

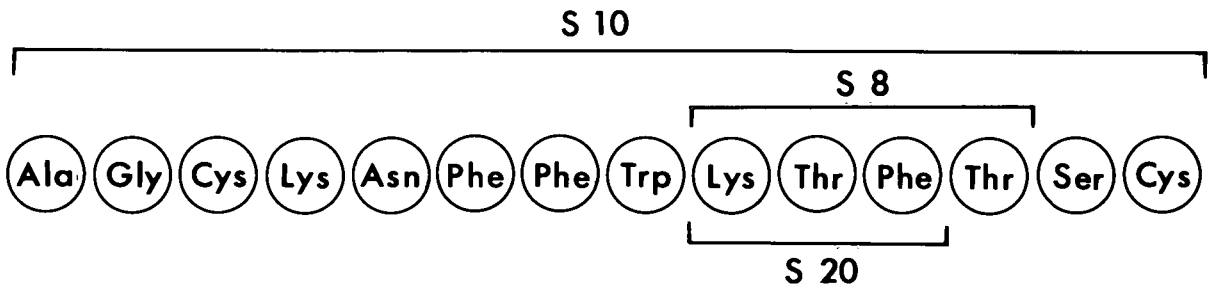


Figure 1. Aminoacid composition of somatostatin fragments which were bound by somatostatin antibody clones S8, S10 and S20

The ability of these somatostatin monoclonal antibodies, to block in vivo the inhibitory effect of somatostatin on gastric acid secretion has not been determined.

PURPOSE

Part I Inhibition of Gastric Acid Secretion

1. To study the effect of exogenous GIP on meal-stimulated gastric acid secretion, plasma gastrin, and plasma SLI in man

2. To study the effect of exogenous GIP and somatostatin on meal- and pentagastrin-stimulated acid secretion and meal-stimulated gastrin release in the rat.

3. To demonstrate the "enterogastrone effect" produced by an intra-duodenal infusion of oleic acid or HCl on meal-stimulated gastric acid secretion

4. To demonstrate the presence of the unknown agent "enterogastrone" in portal blood and to assess the role of endogenous GIP in the inhibition of gastric acid secretion after an intraduodenal infusion of fat.

Part II

Monoclonal Antibodies as Probes of Humoral Inhibitors of Gastric Acid Secretion

1. To assess the ability of GIP monoclonal antibody 3.65H, to block in vivo the inhibitory action of exogenous GIP on gastric acid secretion

2. To assess the ability of somatostatin monoclonal antibody clones S8, S10 and S20, to block in vivo the inhibitory action of exogenous somatostatin on gastric acid secretion

3. To assess the role of endogenous somatostatin in the inhibition of gastric acid secretion by exogenous GIP

METHOD

Part I Inhibition of Gastric Acid Secretion

1. Human Study

Effect of intravenous GIP infusion on meal-stimulated gastric acid secretion

A. Meal stimulation and measurement of gastric acid secretion

The study consisted of 6 volunteers, 3 males and 3 females, with an age range from 20 to 32 years. The study was approved by a Human Research Review Committee, and all the subjects gave full, informed, writen consent. All the subjects were without apparent medical disease, and did not take antacid or other acid reducing drugs. The study involved the testing of each subject on 2 separate days, with each subject acting as his or her own control.

After an overnight fast, a double-lumen, radiopaque, nasogastric tube (An-10, H.W. Anderson Products Inc., New York) was passed into the stomach. The tube was fluoroscopically positioned into the most dependant part of the stomach. Gastric contents were emptied by hand aspiration, followed by collection of basal gastric secretions over two 15 minute periods. Acid output of the basal secretions was determined by titration with 0.2 M NaOH to a pH of 7.0. Titration equipment consisted of a PHM 84 Research pH Meter, TTT 80 Titrator, ABU 80 Autoburette, and a TTA 80 Titration Assembly (Radiometer, Copenhagen).

Meal-stimulated gastric acid secretion was measured by intragastric titration, as described by Richardson et al. in 1976 and Lam et al. in 1980.

The test meal consisted of an 8% solution of peptone (Bactopeptone, Difco Lab., Detroit, Michigan) in distilled water, warmed to 37^0C and pH adjusted to 5.5. Following the collection of basal secretion, the first of 4 consecutive 400 ml meals was instilled through the nasogastric tube into the stomach. Each meal lasted for 45 minutes, after which it was aspirated and the next meal instilled.

Acid secretion was measured throughout the meal, using the intragastric titration technique. A peristaltic pump (Brewer Automatic Pipeting Machine, Scientific Equipment Products, Baltimore, Maryland) with a motor-driven glass syringe, was used to continuously sample 40 ml volumes of the peptone meal through the nasogastric tube. Each sample was drawn through a small chamber into which was fitted a combined glass electrode (GK2401C Radiometer, Copenhagen). The intragastric pH of the meal was maintained at 5.5 by infusion of 0.5 M NaOH through the small polyethylene side channel of the nasogastric tube. The volume of 0.5 M NaOH infused, which corresponded directly to the amount of acid secreted, was recorded throughout (REC Space 80 Servogragh Radiometer, Copenhagen).

B. Exogenous administration of GIP

Pure porcine GIP was provided by Dr. J.C. Brown, Department of Physiology, Universtiy of British Columbia. The GIP was prepared in a solution of 0.15 M NaCl and 2% by volume of the subjects own serum (to minimize the non-specific adsorption of GIP to glasswear and infusion apparatus), then passed through a 0.22 µm Millex-GS filter (Millipore Corp., Bedford, Mass.). On the day of the GIP study, the GIP solution was infused through a *22 intravenous catheter positioned in an arm vein, by means of a Harvard Infusion Pump (Southnatick, Mass.) at increasing doses

of 0, 0.5, 1.0 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$, given sequentially with each meal. On the day of the control study, intravenous infusion of a 2% solution of the subjects own serum in normal saline replaced the GIP solution.

C. Blood collection for RIA of plasma gastrin, IR-GIP and SLI

At the start of each study, a #20 intravenous catheter was positioned in an arm vein for the collection of blood samples. The catheter was kept patent with heparinized saline. Five ml of blood was immediately collected in a sterile fashion, centrifuged at 3000 RPM for 10 minutes (Silencer model 103FRS, Tokyo, Japan), and the serum used for the preparation of the serum-saline solution.

Blood samples for RIA were collected in 7 ml Vacutainer evacuated glasstubes containing 0.125 mg of EDTA (Becton Dickson Canada, Mississauga, Ontario), with 500 KIU of aprotinin added (Trasylol, Miles Pharmaceuticals, Rexdale, Ontario). The blood samples were drawn 30 minutes before, and immediately prior to the start of the first peptone meal, and at 15 minute intervals during all 4 meals. After collection, the blood was centrifuged at 3000 RPM for 10 minutes and plasma frozen for subsequent RIA of gastrin, GIP and somatostatin.

D. Statistical analysis

Statistical analysis was performed between the test groups and their respective controls using Student's t-test for paired values. Differences were considered significant if $p < 0.05$.

2. Animal Model for Gastric Acid Secretion Studies

A. Surgical preparation

Male Sprague-Dawley rats, weighing between 150 and 200 gms, were used in all gastric acid secretion studies. After an overnight fast with water ad libitum, the animals were anaesthetized with an intraperitoneal injection of 25% urethane (Sigma Chemical Company, St. Louis, MO.), 1.25 $mg.kg.^{-1}$ body weight. A midline abdominal incision was made, and a double-lumen polyethylene gastric cannula was placed, through a small opening, into the non-secretory part of the stomach (figure 2). The duodenum was ligated approximately 3 to 4 mm distal to the pylorus, and the abdomen closed.

Through a neck incision, the cervical esophagus was ligated and a tracheostomy performed, using a 15 mm length of #14 angiocatheter cannula. The right external jugular vein was cannulated with PE-50 polyethylene tubing (Becton, Dicksin and Company, New Jersy) and flushed with a heparinized saline solution (20 $U.ml.^{-1}$) to keep the intravenous line patent.

Upon completion of the surgical procedure, the stomach was carefully flushed with normal saline to remove gastric contents, and to assure proper function of the cannula. Basal gastric secretions were collected over 10 minute periods for a minimum of 30 minutes. At the end of each 10 minute period, the stomach was flushed with 10 ml of normal saline, followed by 10 ml of air. The gastric effluent was collected and basal acid output determined by titration with 0.1 M NaOH (Radiometer apparatus, as previously described) to a pH of 7.0.

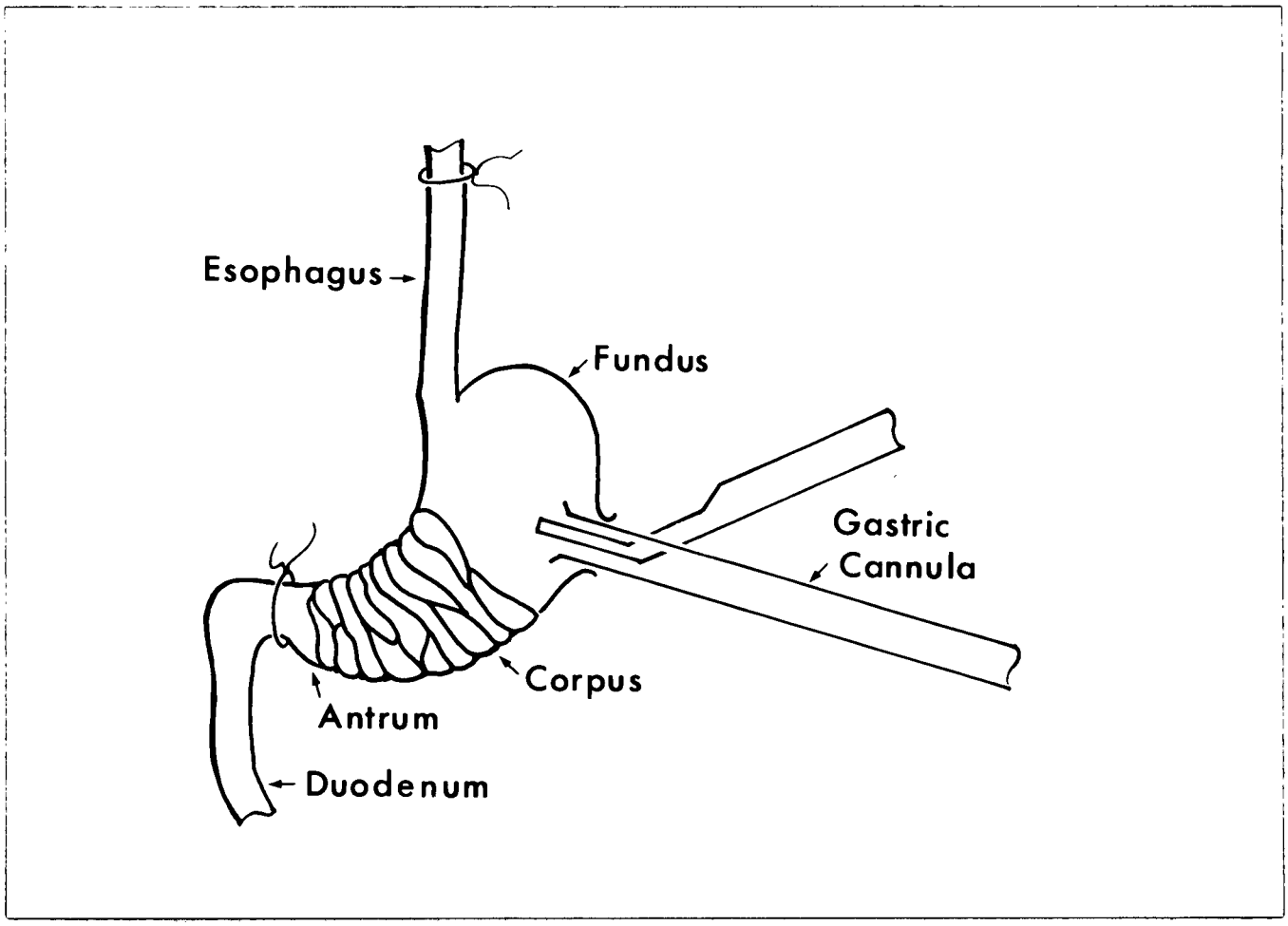


Figure 2. Double-lumen gastric cannula position in the non-secretory part of the stomach

B. Liver extract meal stimulation of gastric acid secretion

Gastric acid secretion was stimulated by a meal which consisted of a 5% liver extract solution (T.E.C. Chemical Inc. Myerstown, P.A.) in normal saline. The liver extract meal was filtered through #1 Whatman filter paper (Whatman, England), and the pH adjusted to 5.5 by the addition of HCl. A 10 ml meal was instilled into the stomach via the gastric cannula, and at 10 minute intervals the meal was flushed from the stomach by a new meal, which replaced it. Gastric stimulation continued for a total of 90 minutes. Each meal was collected and acid output determined by back titration with 0.1 M NaOH to pH 5.5. In a separate control study, 10 ml of normal saline was instilled into the stomach in place of the liver extract meal. The saline was flushed from the stomach at 10 minute intervals, and acid output determined by titration with 0.1 M NaOH to pH 7.0.

Upon completion of the study, 1 ml of blood was drawn by cardiac puncture prior to sacrifice, centrifuged at 4000 RPM for 10 minutes, and the serum immediately frozen for subsequent RIA of gastrin (or GIP).

C. Pentagastrin stimulation of gastric acid secretion

(i) Pentagastrin dose response

Following a 30 minute basal gastric collection period, pentagastrin (Ayerst Lab., Montreal), was diluted in 0.15 M NaCl to give doses of 0.5, 1, 2, 4, 8, 16, 32, and 64 $\mu g.kg.^{-1}hr.^{-1}$, and infused intravenously at a rate of 0.6 $ml.hr.^{-1}$ by means of a Harvard Infusion Pump (Southnatick, Mass.) through the jugular catheter of the animal preparation. Each dose was infused for 30 minutes, and given consecutively in ascending order during the experiment. Gastric acid collection was performed with 10 ml meals of normal saline which were flushed from the stomach at 10 minute intervals, and acid output determined as previously described.

Dowd-Riggs linear transformation of the Michaelis-Menten equation (Dowd 1965), was used to determine the calculated maximal response (CMR) and the dose required for half maximal acid secretion response (D_{50}).

(ii) Infusion of pentagastrin at 16 µg.kg.$^{-1}$hr.$^{-1}$

Following basal gastric collection in the animal preparation, pentagastrin, diluted in 0.15 M NaCl, was intravenously infused, by means of a Harvard Infusion Pump, at a dose of 16 µg.kg.$^{-1}$hr.$^{-1}$. Gastric acid collection and measurement was performed, as previously described, for 60 minutes.

D. Statistical analysis

All statistical analysis was performed between the test groups and their respective controls using the Student's t-test for unpaired values.

3. Demonstration of an 'enterogastrone effect'

A. Intraduodenal Infusion of Oleic Acid during Liver Extract Meal Stimulation of Gastric Acid Secretion

(i) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared as described in section 2A. In addition, a #18 angiocatheter cannula was inserted through a small opening into the proximal duodenum. The hub of the cannula was brought out of the abdomen and the abdominal incision closed.

(ii) Intraduodenal infusion of oleic acid during meal stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, purified oleic acid ($C_{18}H_{34}O_2$, Fisher Scientific Company, Fairlawn, New Jeresy) was infused, by means of a Harvard Infusion Pump, through the duodenal catheter at a rate of 1.2 ml.hr.$^{-1}$. Liver extract meal-stimulation and measurement of gastric acid secretion were performed, as previously described, simultaneous with the oleic acid infusion. Upon completion of the meal, blood was collected by cardiac puncture, for subsequent RIA of serum gastrin.

B. Intraduodenal infusion of HCl during Liver Extract Stimulation of Gastric Acid Secretion

The anaesthetized rat model for gastric acid secretion was prepared with an intraduodenal cannula as described in section 3A. Following a 30 minute basal gastric collection period, 0.5 M HCl (Fisher Scientific, New Jersy) was infused, by means of a Harvard Infusion Pump, through the duodenal catheter at a rate of 1.2 ml.hr^{-1}, during meal stimulation and gastric acid secretion measurement as previously described.

C. Demonstration of "Enterogastrone" in Portal Blood

(i) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared with an intraduodenal cannula, as described in section 3A. In addition, a fine polyethylene catheter (PE-50 tubing) was introduced into the distal superior mesenteric vein via a cecal venous branch. The tip of the catheter

was advanced proximally through the superior mesenteric vein, and positioned within the portal vein (figure 3). The catheter was flushed with a heparinized saline solution to keep the lumen patent prior to the collection of portal blood.

(ii) Portal blood collection

Upon completion of the surgical procedure, an intrajugular infusion of normal saline was given, by means of a Harvard Infusion Pump, at a rate of 2.4 ml.hr.$^{-1}$. Basal gastric secretions were collected for 30 minutes and acid output determined as previously described. Any animal preparation with basal acid secretion > 5.0 µEq. per 10 minute period was excluded from the study. Following the basal gastric collection period, oleic acid was infused, by means of a Harvard Infusion Pump, through the duodenal catheter at a rate of 1.2 ml.hr.$^{-1}$ for 30 minutes. Portal blood (5 ml.) was then collected from the portal vein catheter over a 5 minute period, and the animals sacrificed.

The blood was centrifuged at 3000 RPM for 10 minutes and the serum either frozen for subsequent RIA of GIP, or lyophilized (Edwards High Vacuum pump, Sussex, England) and stored at -20^{0}C.

A second group of animals, prepared in an identical fashion, acted as the control for the intraduodenal oleic acid infusion. After basal gastric collection, they were held for 30 minutes without the intraduodenal oleic acid infusion, before portal blood collection, as described above.

(iii) Infusion of lyophilized portal serum during a saline meal

a) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared

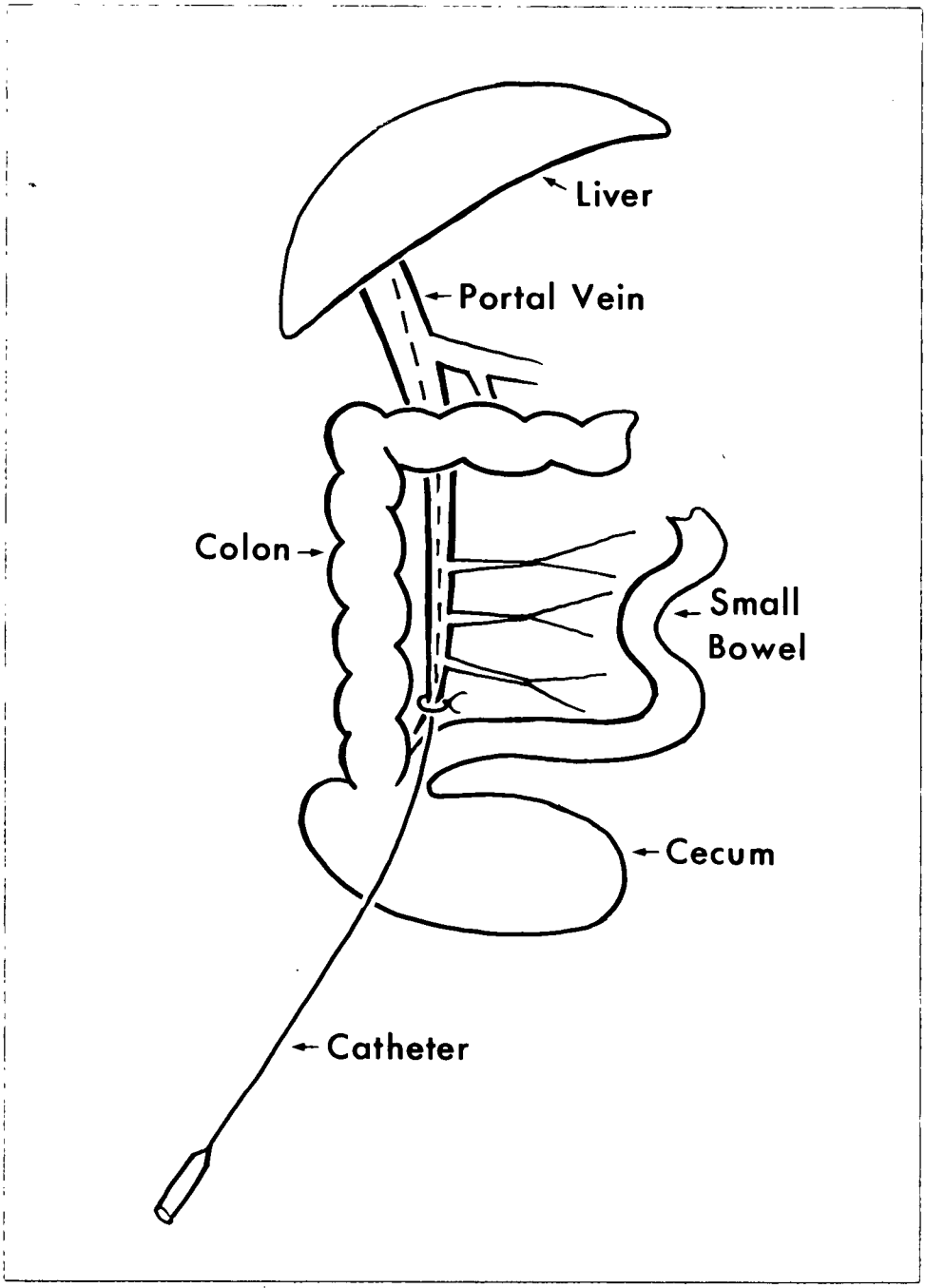


Figure 3. Schematic diagram of portal vein cannula

as described in section 2A.

b) Intravenous infusion of portal serum during saline meal

Following a 30 minute basal gastric collection period, a saline meal was given for 90 minutes and gastric acid secretion measured as described in section 2C. Lyophilized portal serum, previously collected after 30 minutes of intraduodenal oleic acid infusion, (as described above), was dissolved in 0.5 ml. of 0.15 M NaCl and infused, by means of a Harvard Infusion Pump, into the jugular vein over 1 minute at the start of the meal.

(iv) Infusion of lyophilized portal serum during liver extract meal stimulation of gastric acid secretion

a) Surgical preparation

In a separate study, the anaesthetized rat model for gastric acid secretion was prepared as described in section 2A.

b) Intravenous infusion of portal serum during liver extract meal

Following a 30 minute basal gastric collection period, a liver extract meal was given and gastric acid secretion measured as described in section 2B.

When a plateau of gastric acid secretion had been established 40 minutes after the start of the meal, lyophilized portal serum, previously collected as described above, was dissolved in 0.5 ml. of 0.15 M NaCl and infused, by means of a Harvard Infusion Pump, into the jugular vein over 1 minute. Meal stimulation and measurement of gastric acid secretion was

performed for a further 50 minutes. Upon the completion of the meal, blood was collected by cardiac puncture for subsequent RIA of serum gastrin.

D. Effect of an Intraduodenal Infusion of Oleic Acid, or Glucose, on Gastric Acid Secretion and Portal IR-GIP Release

(i) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared with intraduodenal and portal vein cannula as described in section 3C. Upon completion of the surgical preparation, 800 µl of blood was taken from the portal catheter for subsequent RIA of IR-GIP.

(ii) Intraduodenal infusion of oleic acid, or glucose, during liver extract meal stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, a 5% liver extract meal was given for 60 minutes and gastric acid secretion measured as previously described. An intraduodenal infusion of either: 1. purified oleic acid, or 2. a 10% glucose solution was performed, by means of a Harvard Infusion Pump, at 1.2 ml.hr.$^{-1}$ throughout the meal stimulus. A third group of animals, prepared in a simular fashion, did not receive an intraduodenal infusion and acted as the control.

Portal vein blood samples (800 µl) were taken 10, 20, 30 and 60 minutes from the start of the meal, for subsequent RIA of serum IR-GIP.

4. Gastrointestinal peptides

A. Effect of Exogenous GIP on Gastric Acid Secretion

(i) GIP infusion during a saline meal

a) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared as described in section 2A.

b) Intravenous infusion of GIP during saline meal

Following a 30 minute basal gastric collection period, a saline meal was given for 90 minutes and gastric acid secretion measured as described in section 2C. Pure porcine GIP, prepared in a solution consisting of 0.15 M NaCl and 0.01% RIA grade bovine serum albumin (BSA, Sigma Chemical Company, St. Louis, M.O.), was intravenously infused, by means of a Harvard Infusion Pump, at a dose of 0.2 $\mu g.kg.^{-1}hr.^{-1}$ throughout the saline meal.

(ii) GIP infusion during pentagastrin stimulation of gastric acid secretion

a) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared, as described in section 2A. In addition, both of the external jugular veins were cannulated with PE-50 polyethylene tubing, and flushed with a heparinized saline solution.

b) Intravenous infusion of GIP during pentagastrin stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, pentagastrin, diluted in 0.15 M NaCl, was intravenously infused by means of a Harvard Infusion Pump, at a dose of 16 $\mu g.kg.^{-1}hr.^{-1}$ for 60 minutes, during which time a saline meal was given and gastric acid secretion measured as described in section 2C.

Pure porcine GIP was prepared in a solution of 0.15 M NaCl and 0.01% BSA. The GIP solution was intravenously infused, by means of a Harvard Infusion Pump, at a dose of 2.0 $\mu g.kg.^{-1}hr.^{-1}$ throughout the pentagastrin stimulus.

A control study was performed in a similar fashion, in which the 0.01% BSA solution was infused alone throughout the meal stimulus.

(iii) High Pressure Liquid Chromatography (HPLC) purified GIP infusion during pentagastrin stimulation of gastric acid secretion

HPLC-purified GIP was provided by Dr. J.C. Brown, Department of Physiology, University of British Columbia, and prepared in a solution of 0.15 M NaCl and 0.01% BSA.

Following a 30 minute basal gastric collection period, pentagastrin stimulation was performed for 60 minutes and gastric acid secretion measured, as described above. The HPLC-purified GIP solution was infused intravenously at a dose of 2.0 $\mu g.kg.^{-1}hr.^{-1}$ throughout the pentagastrin stimulus.

(iv) GIP infusion during meal stimulation of gastric acid secretion

a) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared, as described in section 2A. Upon completion of the surgical preparation, 800 µl of blood was taken from the jugular catheter for subsequent RIA of IR-GIP.

b) Intravenous infusion of GIP during meal stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, a 5% liver extract meal was given for 90 minutes and gastric acid secretion measured, as described in section 2B. Pure porcine GIP was prepared in a solution of 0.15 M NaCl and 0.01% BSA. The GIP solution was intravenously infused, by means of a Harvard Infusion Pump, at doses of 0.2 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$ throughout the meal stimulus.

Upon completion of the meal, blood was collected by cardiac puncture for subsequent RIA of serum gastrin and IR-GIP.

B. Effect of exogenous somatostatin on gastric acid secretion

(i) Somatostatin infusion during saline meal

a) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared as described in section 3A.

b) Intravenous infusion of somatostatin during saline meal

Following a 30 minute basal gastric collection period, a saline meal was given for 90 minutes and gastric acid secretion measured, as described in section 2C. Somatostatin (Somatostatin-14, Sigma Chemical Company, St. Louis, M.O.) prepared in a solution of 0.15 M NaCl and 0.01% BSA, was intravenously infused, by means of a Harvard Infusion Pump, at a dose of 2.0 $\mu g.kg.^{-1}hr.^{-1}$ throughout the saline meal.

(ii) Somatostatin infusion during pentagastrin stimulation of gastric acid secretion

a) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared with 2 external jugular vein catheters as described in section 4A.

b) Intravenous infusion of somatostatin during pentagastrin stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, pentagastrin, diluted in 0.15 M NaCl, was intravenously infused by means of a Harvard Infusion Pump, at a dose of 16 $\mu g.kg.^{-1}hr.^{-1}$ for 60 minutes, during which time a saline meal was given and gastric acid secretion measured, as described in section 2C.

Somatostatin, prepared in a solution of 0.15 M NaCl and 0.01% BSA, was infused intravenously, by means of a Harvard Infusion Pump, at a dose of 2.0 $\mu g.kg.^{-1}hr.^{-1}$ throughout the pentagastrin stimulus.

(iii) Somatostatin infusion during meal stimulation of gastric acid secretion

a) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared, as described in section 2A.

b) Intravenous infusion of somatostatin during meal stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, a 5% liver extract meal was given for 90 minutes and gastric acid secretion measured, as described in section 2B. Somatostatin, prepared in a solution of 0.15 M NaCl and 0.01% BSA, was intravenously infused, by means of a Harvard Infusion Pump, at doses of 0.2 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$ throughout the meal stimulus.

Upon completion of the meal, blood was collected by cardiac puncture for subsequent RIA of serum gastrin.

5. Radioimmunoassay

A. Gastrin

Radioimmunoassays (RIA) for serum gastrin were performed as described by Walsh (Walsh 1974).

(i) Preparation of buffer, labelled gastrin, antiserum and standards

Assay buffer

The stock buffer used in the gastrin assay consisted of 0.02 M sodium barbital with 0.5% RIA grade bovine serum albumin (Sigma Chemical

Company, St. Louis, MO.), pH adjusted with HCl to 8.4.

Gastrin label

Iodine isotope ^{125}I-labelled gastrin was obtained from Dr. J.C. Brown, Department of Physiology, University of British Columbia, Vancouver, and was diluted with stock buffer to give approximately 2000 CPM per 100 µl.

Antiserum

The rabbit antiserum employed, 1611 pool 4A (kindly donated by Dr. J. Walsh, VA Wadsworth Hospital Center, Los Angeles), detects both small (G-17) and large (G-34) molecular forms of gastrin. The antiserum was diluted with stock buffer to an initial dilution of 1 in $2x10^3$, and stored in 1 ml aliquots. The final dilution of antiserum used was 1 in $6x10^4$, which yielded 30 to 40% binding of ^{125}I-gastrin in the absence of unlabelled gastrin. At this concentration, the antiserum was sensitive to serum concentrations of gastrin between 5 and 400 pg.ml.$^{-1}$.

Standards

Synthetic human gastrin G-17 was diluted with stock buffer to a concentration of 800 pg.ml.$^{-1}$ and stored at -20^{0}C. It was serially diluted with buffer to produce gastrin standards of 400, 200, 100, 50, 25, 12.5, and 6 pg.ml.$^{-1}$ for the standard curve.

(ii) Assay procedure

The gastrin standard or serum sample (100 µl), previously stored at -20^{0}C, were added with 100 µl of antiserum and 100 µl of gastrin label, to 700 µl of stock buffer solution, for a total assay volume of 1 ml. The

assay was then incubated for 48 hours at 4^0C. All serum samples were assayed in triplicate.

Charcoal separation

Bound and free ^{125}I-gastrin were separated using 1.25% activated charcoal (carbon decolorizing Norit, Fisher Scientific Company, Fairlawn, New Jersy), and 0.25% dextran T-70 made up in 0.04 M PO_4 buffer, pH 6.5. Prior to use, 8% charcoal extracted human plasma (prepared by filtering outdated blood bank plasma, then mixing with 1% carbon decolorizing Norit for 1 hour at 4^0C, followed by centifugation and filtration of the supernatant) was added to the charcoal solution and mixed for 1 hour at 4^0C. The charcoal buffer was added in 400 µl aliquots to each assay tube, the tubes vortexed, then centifuged at 3000 RPM for 30 minutes. The supernatant was discarded and the pellet (free gastrin) counted using a Searle automatic gamma counter, model 1185 (Searle Analytic Inc., Des Plaines, Illinois).

Determination of serum gastrin

Standard curves were prepared by plotting the percentage of total radioactivity bound (% bound) against gastrin standard concentrations. The gastrin concentration of the serum samples were then found, by reading from the standard curve the gastrin concentration which corresponded to the % bound of the serum sample.

B. Gastric Inhibitory Polypeptide

Radioimmunoassays for serum GIP were kindly performed by Dr. R. Pederson, of the Department of Physiology, University of British Columbia.

C. Somatostatin

Radioimmunoassays for serum somatostatin were kindly performed by Dr. Tadataka Yamada, of the Center for Ulcer Research and Education, Medical and Research Services, VA Wadsworth Hospital Center, Los Angeles, California.

Part II

Monoclonal Antibodies as Probes of Humoral Inhibitors of Gastric Acid Secretion

1. GIP Monoclonal Antibody (3.65H)

The mid to C-terminal specific GIP monoclonal antibody, designated 3.65H, was provided by Dr. J.C. Brown, Department of Physiology, University of British Columbia.

A. Binding affinity of monoclonal antibody 3.65H for GIP in vitro

An in vitro comparison of GIP binding between the monoclonal antibody and a polyclonal rabbit antiserum, R07 (provided by Dr. J.C. Brown, Department of Physiology, University of British Columbia) was performed using the Enzyme-Linked Immunosorbent Assay (ELISA) (Voller 1976). GIP (50 nmoles per well) was bound to the 96-well assay plate to act as the solid phase antigen. Either the antibody, or the antiserum R07 as purified IgG, was then added to each well at increasing concentrations from 0.09 to 50 $\mu g.ml.^{-1}$. The end reaction product was quantified using a MR 580 Micro ELISA reader (Dynatech Labs, Virginia) to determine the relative degree of binding to GIP.

B. Effect of monoclonal antibody 3.65H on the inhibition of meal-stimulated gastric acid secretion by exogenous GIP

(i) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared, as described in part I, section 2A.

(ii) Intravenous infusion of GIP during meal-stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, a 5% liver extract meal was given for 90 minutes and gastric acid secretion measured, as described in part I, section 2B. Pure porcine GIP was prepared in a solution of 0.15 M NaCl and 0.01% BSA. The GIP solution was infused intravenously throughout the meal stimulus at 0.2 $\mu g.kg.^{-1}hr.^{-1}$, by means of a Harvard Infusion Pump, either immediately following preparation, or after incubation for 18 hours at 4^0C.

Upon completion of the meal, blood was collected by cardiac puncture prior to sacrifice, for subsequent RIA of serum gastrin.

(iii) Intravenous infusion of monoclonal antibody 3.56H

The monoclonal antibody 3.65H, partially purified by 50% ammonium sulfate percipitation from mouse ascites fluid, was diluted in 0.15 M NaCl. The antibody was administered through the jugular catheter either as :

a) a 100 µg. bolus, 1 hour prior to the liver extract meal stimulus, or

b) an infusion at 6 $\mu g.kg.^{-1}hr.^{-1}$, by means of a Harvard Infusion Pump, after incubation with GIP either for 1 hour at 37^0C, or 18 hours at 4^0C, followed by charcoal separation.

(iv) Charcoal separation and measurement of GIP in the incubated preparation

Charcoal separation was performed on the incubated preparations of antibody 3.65H and GIP just prior to intravenous infusion, to remove any unbound GIP which may be present in the solution (Kuzio 1974). The incubated preparations (monoclonal antibody incubated with GIP for either 1 hour at 37^0C, or 18 hours at 4^0C) were vortexed with 500 µl of the

charcoal solution in 5 ml. assay tubes, then centrifuged at 3000 RPM for 30 minutes, and the supernatant collected for subsequent infusion.

The concentration of GIP present in the incubated preparation after charcoal separation was determined using ^{125}I-labelled GIP (provided by Dr. J.C. Brown). The monoclonal antibody was incubated with ^{125}I-labelled GIP for 1 hour at 37^{0}C, or 18 hours at 4^{0}C, followed by charcoal separation as described above. The radioactivity of the solution before charcoal separation, and of the supernatant after centrifugation, was counted, using a Searle automatic gamma counter, as previously described. The calculated percentage of radioactive GIP present after charcoal separation, permitted the adjustment of the initial concentration of GIP incubated with the antibody, to produce a final infusate dose of 0.2 $\mu g.kg.^{-1}hr.^{-1}$.

(v) Verification of GIP binding to the monoclonal antibody in the incubated preparation

The verification of GIP binding to the monoclonal antibody in the incubated preparations was performed by Dr. A.M.J. Buchan, Department of Physiology and Medicine, University of British Columbia. Aliquots of all antibody infusates, whether incubated alone or with GIP (for 1 hour at 37^{0}C or 18 hours at 4^{0}C) were centrifuged with activated charcoal as described above. They were then applied as the first layer antibody on 5 micron sections of Bouin's fixed rat small bowel mucosa previously shown to contain IR-GIP cells. The sections were then immunostained with peroxidase conjugated rabbit-antimouse IgG, 1:300 for 1 hour at 20^{0}C. The peroxidase reaction was developed with diaminobenzidene with 0.001% hydrogen peroxidase and the sections were counterstained with haematoxylin.

To verify the presence of the monoclonal antibody in the preparation

incubated with GIP after charcoal separation, a sample of this solution was acidified with 0.5 ml. of 0.1 M HCl, to free any bound GIP from the antibody (Rouslahi 1976). The solution was shaken for 30 minutes, neutralized with 0.1 M NaOH, and then used as the first layer antibody in the immunocytochemical technique described above.

2. Somatostatin Monoclonal Antibodies

Somatostatin monoclonal antibodies, designated clone S8, S10 and S20, were provided by Dr. J.C. Brown, Department of Physiology, Univ. of British Columbia. The region of the somatostatin 14 molecule detected by the antibodies is shown in figure 1. The monoclonal antibody clone S10 has been shown to bind only to the intact somatostatin 14 molecule. The antibody clone S20 binds to the mid to C-terminal sequence of somatostatin, whereas clone S8 is C-terminal specific (Buchan 1985).

A. Effect of monoclonal antibody clones S8, S10 and S20 on the inhibition of meal-stimulated gastric acid secretion by somatostatin

(i) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared, as described in part I, section 2A.

(ii) Meal stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, a 5% liver extract meal was given for 90 minutes and gastric acid secretion measured, as described in part I, section 2B. Upon completion of the meal,

blood was collected by cardiac puncture prior to sacrific, for subsequent RIA of serum gastrin.

(iii) Intravenous infusion of somatostatin monoclonal antibody during a liver extract meal

The monoclonal antibody clones S8, S10 and S20, partially purified by 50% ammonium sulfate percipitation from mouse ascites fluid, were diluted in 0.15 M NaCl. Each antibody was individually administered, as a 100 µg. bolus through the jugular catheter, 1 hour prior to the start of a liver extract meal.

(iv) Intravenous infusion of somatostatin monoclonal antibody during a liver extract meal and somatostatin infusion

Somatostatin was prepared in a solution of 0.15 M NaCl and 0.01% BSA. The monoclonal antibody clones S8, S10 and S20, were individually administered, as a 100 µg. bolus through the jugular catheter, 1 hour prior to the start of a liver extract meal and intravenously infused of somatostatin, at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, by means of a Harvard Infusion Pump..

B. Effect of monoclonal antibody clone S10 on the inhibition of meal-stimulated gastric acid secretion by GIP

(i) Surgical preparation

The anaesthetized rat model for gastric acid secretion was prepared, as described in part I, section 2A.

(ii) Intravenous infusion of GIP during meal stimulation of gastric acid secretion

Following a 30 minute basal gastric collection period, a 5% liver extract meal was given and gastric acid secretion measured, as described in part I, section 2B. Pure porcine GIP was prepared in a solution of 0.15 M NaCl and 0.01% BSA. The GIP solution was intravenously infused throughout the meal stimulus at 0.2 $\mu g.kg.^{-1}hr.^{-1}$, by means of a Harvard Infusion Pump.

(iii) Intravenous infusion of somatostatin monoclonal antibody

The monoclonal antibody clone S10, as purified IgG, was diluted in 0.15 M NaCl and administered as a 100 µg. bolus through the jugular catheter, 1 hour prior to the meal stimulus and GIP infusion.

RESULTS

Part I Inhibition of Gastric Acid Secretion

1. Human Study

A. Peptone meal stimulation of gastric acid secretion

Acid secretion was measured during the last 30 minutes of each 45 minute peptone meal. To eliminate the wide variability observed between subjects, acid secretion has been expressed, as a percentage of the maximal acid output (MAO) obtained in response to a meal during the course of each corresponding study (Figure 4). In the control study, an 8% peptone meal produced a 4 to 5 fold increase in acid secretion over basal, which was maintained throughout the meal stimulus.

B. Effect of GIP on meal-stimulated gastric acid secretion.

Intravenous infusion of GIP at 1.0 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$ produced a 30% reduction of the gastric acid secretory response to the peptone meal (Figure 4). In 2 subjects, infusion of GIP at 2.0 $\mu g.kg^{-1}hr.^{-1}$ during the meal, reduced acid secretion to less than 50% of the maximal secretion observed (Figure 5).

C. Measurement of plasma IR-GIP

Plasma IR-GIP was not affected by the intragastric instillation of 8% peptone, and remained at basal levels throughout the meal. Intravenous infusion of GIP during the peptone meal produced a dose-related increase in plasma IR-GIP levels (Figure 6).

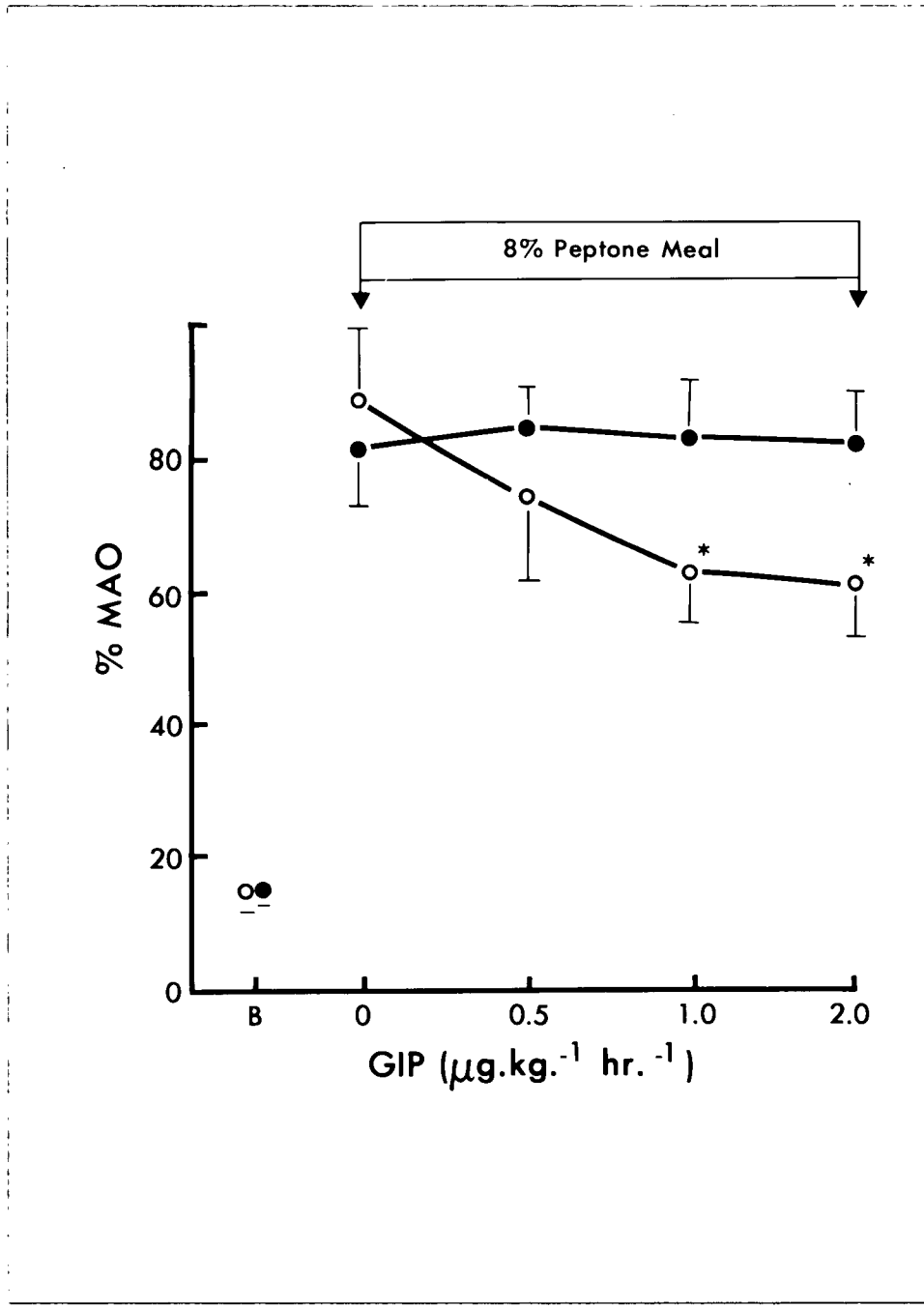


Figure 4. Effect of a peptone meal and intravenous GIP infusion on gastric acid secretion in man

closed circles : peptone meal alone (n=5)

open circles : peptone meal and intravenous GIP infusion (n=5)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

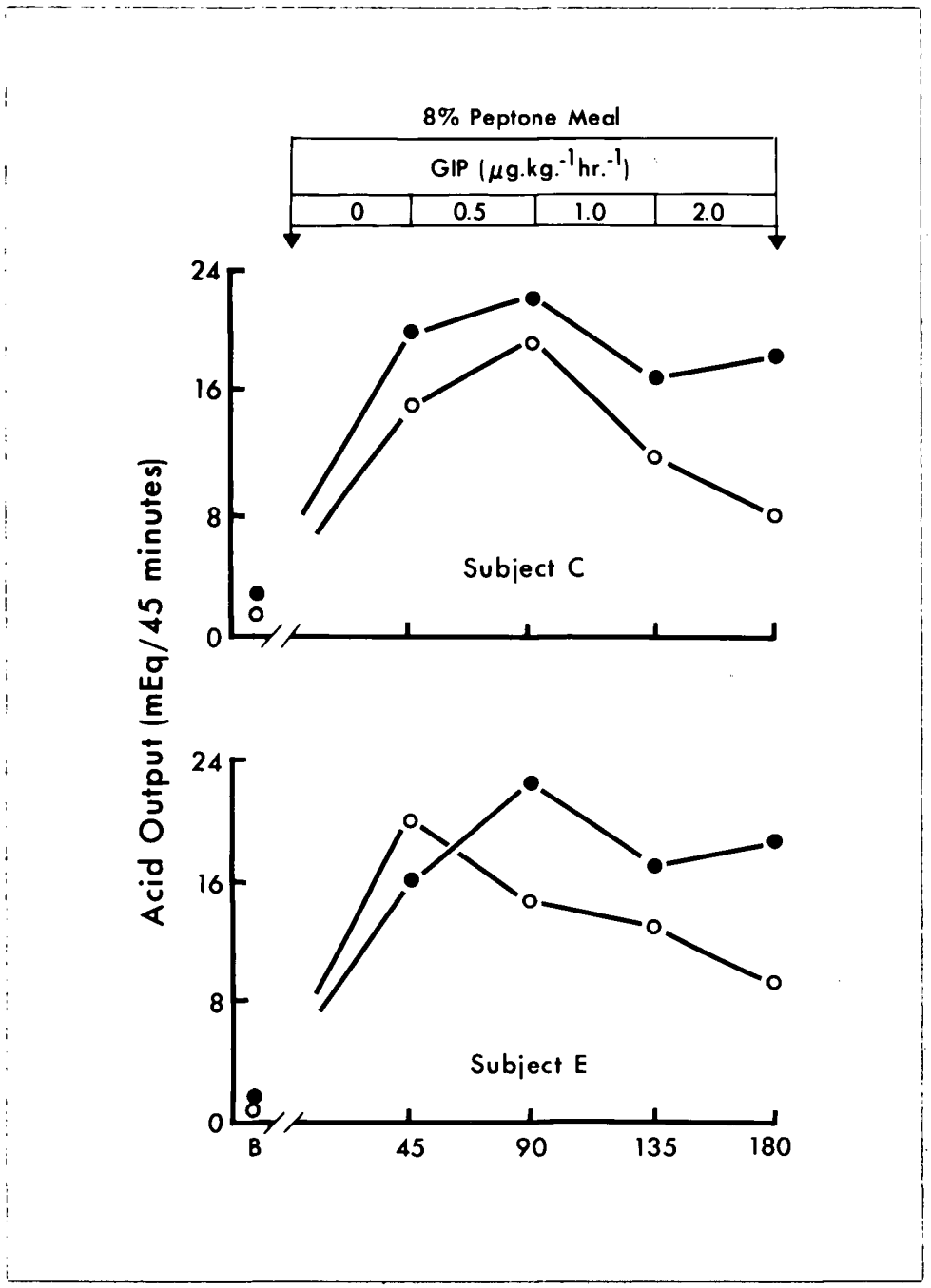


Figure 5. Effect of an intravenous infusion of GIP on the gastric acid secretory response to a peptone meal in 2 subjects

closed circles : peptone meal alone

open circles : peptone meal and intravenous GIP infusion

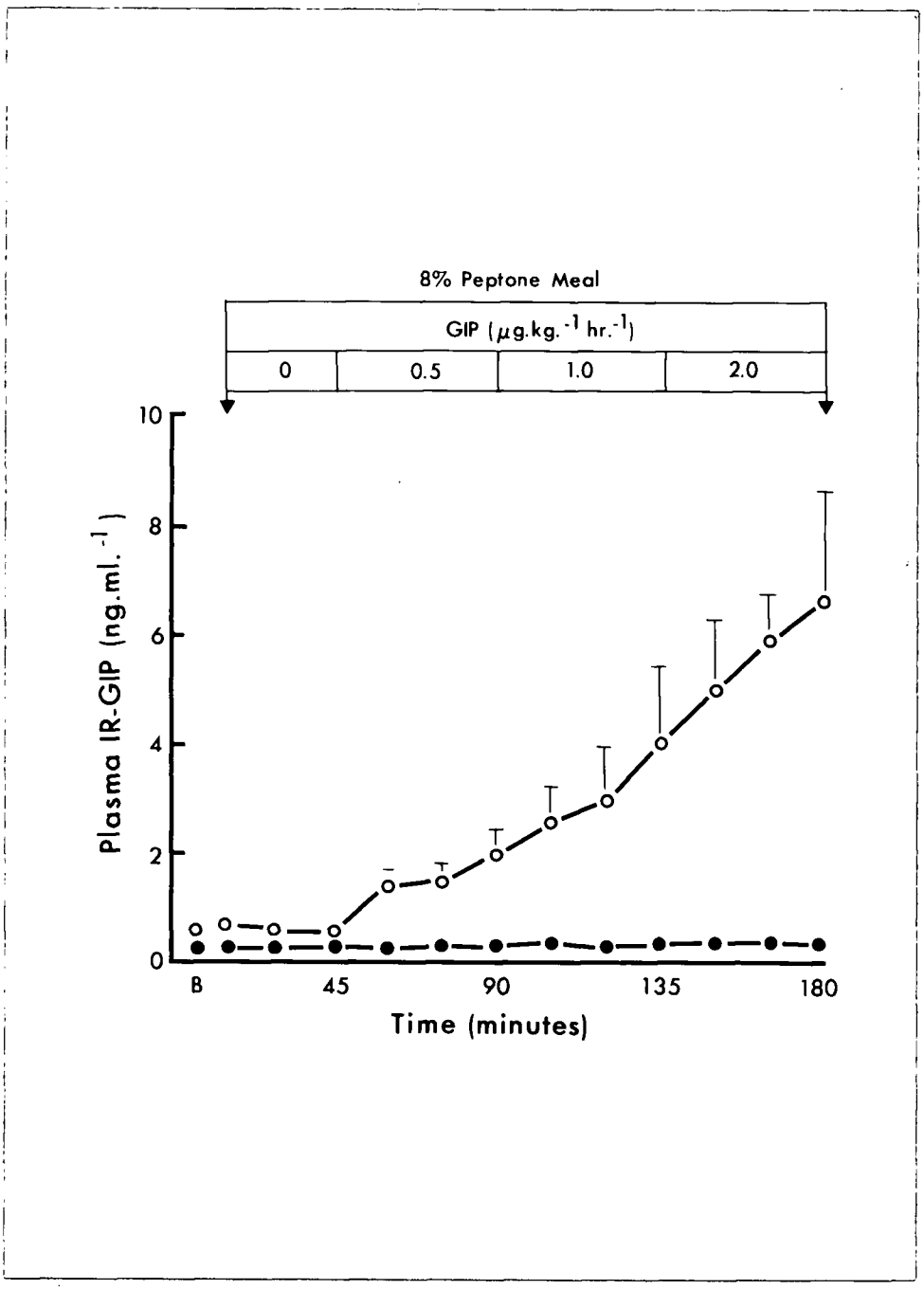


Figure 6. Effect of a peptone meal and intravenous GIP infusion on plasma IR-GIP

closed circles : peptone meal alone (n=5)

open circles : peptone meal and intravenous GIP infusion (n=6)

each point represents mean ± SEM

D. Effect of GIP infusion on plasma gastrin

Intravenous infusion of GIP had no effect on meal-stimulated plasma gastrin levels (Figure 7).

E. Effect of GIP infusion on plasma SLI

Plasma SLI remained at basal levels throughout the peptone meal, and was not effected by the intravenous infusion of GIP (Figure 8).

2. Animal Model for Gastric Acid Secretion Studies

A. Liver extract meal stimulation of gastric acid secretion

The 5% liver extract meal produced a 3 fold increase in acid secretion over basal by 30 minutes, as shown in figure 9. The increased rate of acid secretion was maintained throughout the meal stimulus. The control meal of normal saline did not stimulate acid secretion above basal levels.

B. Pentagastrin stimulation of gastric acid secretion

(i) Pentagastrin dose response

Intravenous infusion of graded doses of pentagastrin, produced a linear, dose-dependant increase in gastric acid secretion (figure 10).

(ii) Linear regression analysis

Linear transformation analysis of the pentagastrin dose response, tabulated from mean absolute responses, is shown in figure 11. The CMR, determined by the y-intercept, is 25.6 µEq./10 minute period. The D_{50}, determined by the negative slope of the regression line, is 12.2 $\mu g.kg.^{-1}hr.^{-1}$.

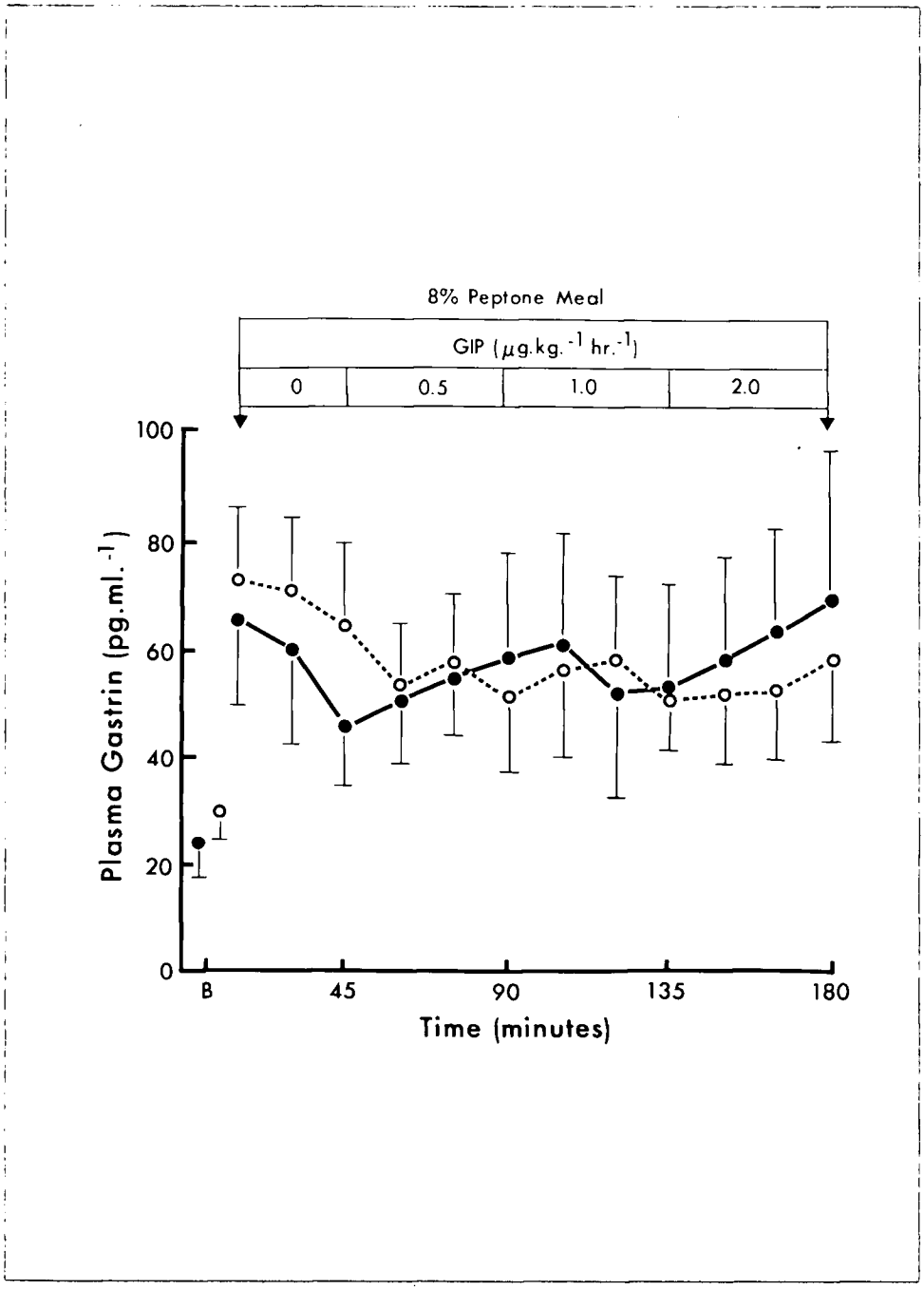


Figure 7. Effect of intravenous GIP infusion on meal-stimulated plasma gastrin

closed circles : peptone meal alone (n=5)

open circles : peptone meal and intravenous GIP infusion (n=5)

each point represents mean ± SEM

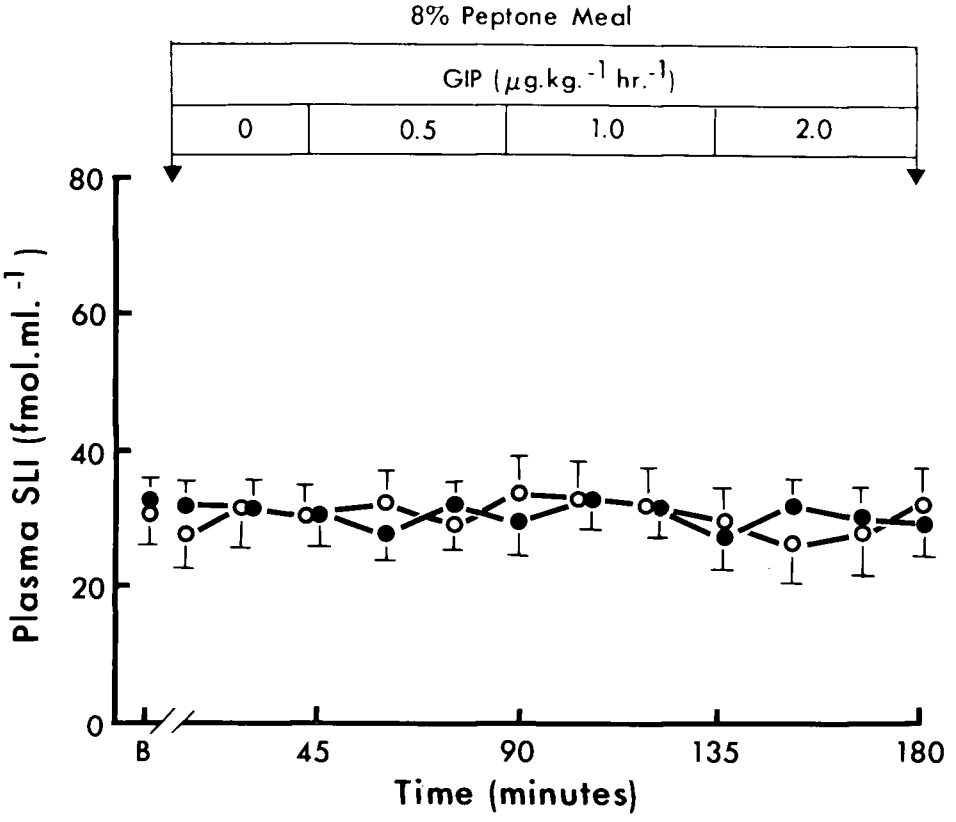


Figure 8. Effect of a peptone meal and intravenous GIP infusion on plasma somatostatin

closed circles : peptone meal alone (n=5)

open circles : peptone meal and intravenous GIP infusion (n=5)

each point represents mean ± SEM

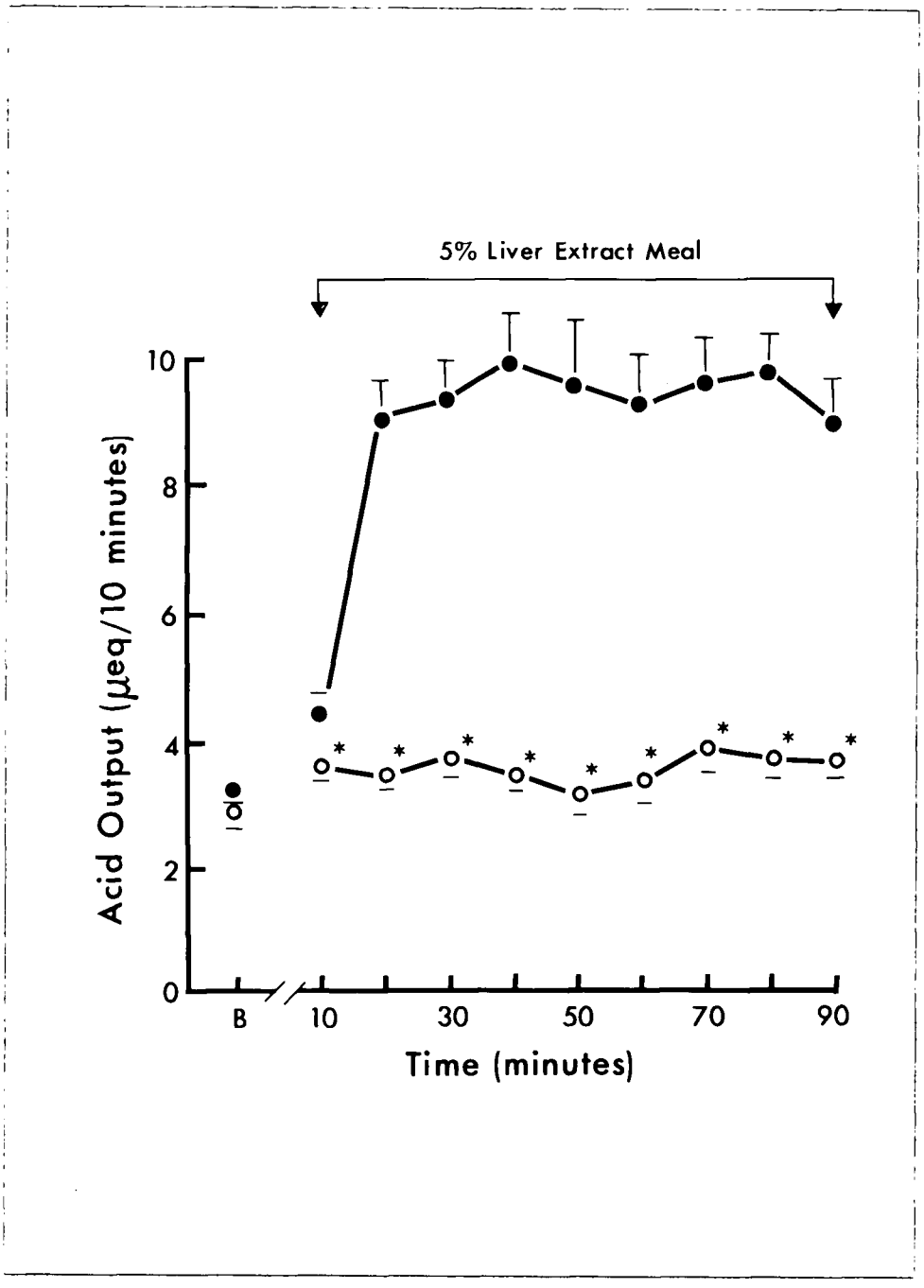


Figure 9. Effect of a 5% liver extract meal on gastric acid secretion

closed circles : 5% liver extract meal (n=16)

open circles : saline meal (n=6)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

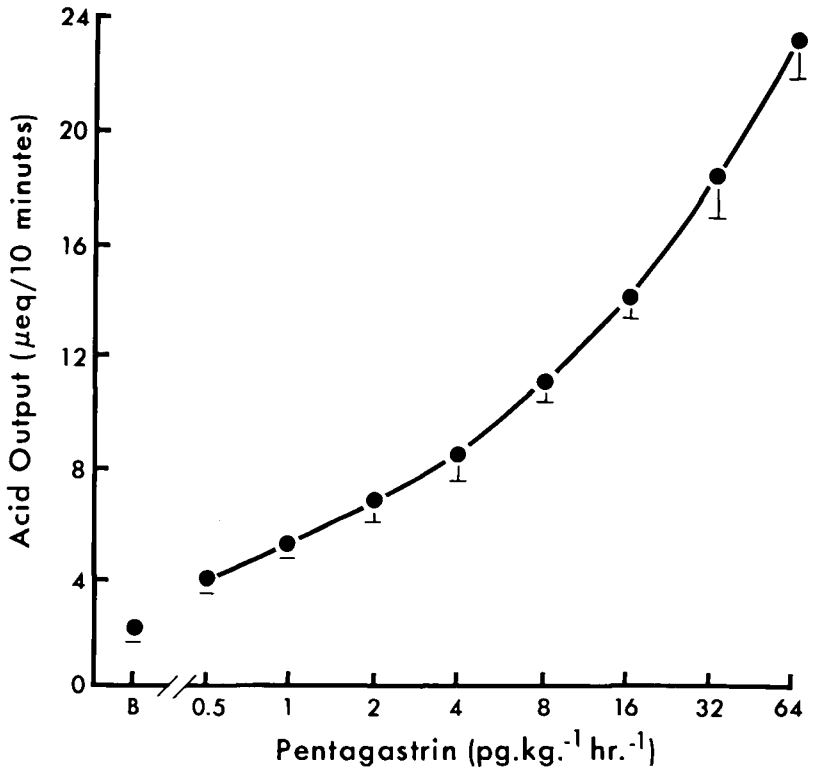


Figure 10. Pentagastrin dose-response stimulation of gastric acid secretion

closed circles : intravenous pentagastrin infusion

each point represents the mean ± SEM acid output (average of the 2nd and 3rd 10 minute periods) during each dose of pentagastrin infused (n=8)

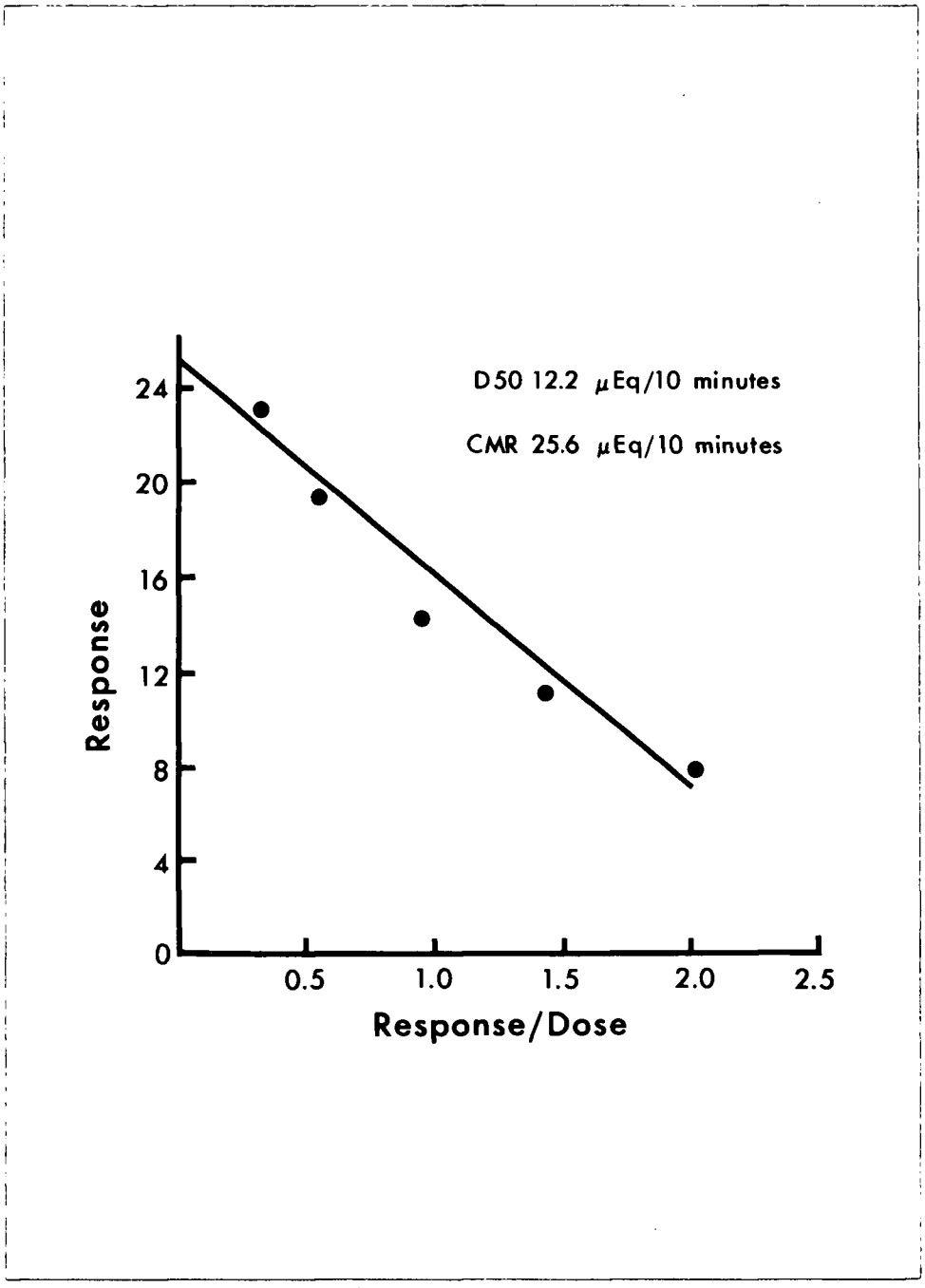


Figure 11. Linear regression analysis of pentagastrin dose response

Data from figure 8 plotted as response/dose on the x-axis vs response on the y-axis

the y-intercept estimates the CMR

the negative slope estimates the D_{50}

(iii) Effect of an infusion of pentagastrin at 16 $\mu g.kg.^{-1}hr.^{-1}$

Intravenous infusion of pentagastrin at 16 $\mu g.kg.^{-1}hr.^{-1}$ produced a 3 fold increase in acid secretion over basal (figure 12). The increase in gastric acid secretion was similar to the increase observed during a 5% liver extract meal (figure 9), and continued throughout the pentagastrin infusion.

3. Demonstration of "Enterogastrone Effect"

A. Effect of an Intraduodenal Infusion of Oleic Acid on Meal-Stimulated Gastric Acid Secretion

(i) Meal-stimulated gastric acid secretion

Intraduodenal infusion of oleic acid produced an 80% inhibition of the gastric acid secretory response to the liver extract meal (figure 13).

(ii) Serum gastrin concentration

The intraduodenal infusion of oleic acid during the meal, did not produce a significant difference in serum gastrin concentration, when compared to the meal stimulus alone (figure 14).

B. Effect of an Intraduodenal Infusion of 0.5 M HCl on Meal-Stimulated Gastric Acid Secretion

Intraduodenal infusion of 0.5 M HCl produced a 60% inhibition of the gastric acid secretory response to the liver extract meal (figure 15).

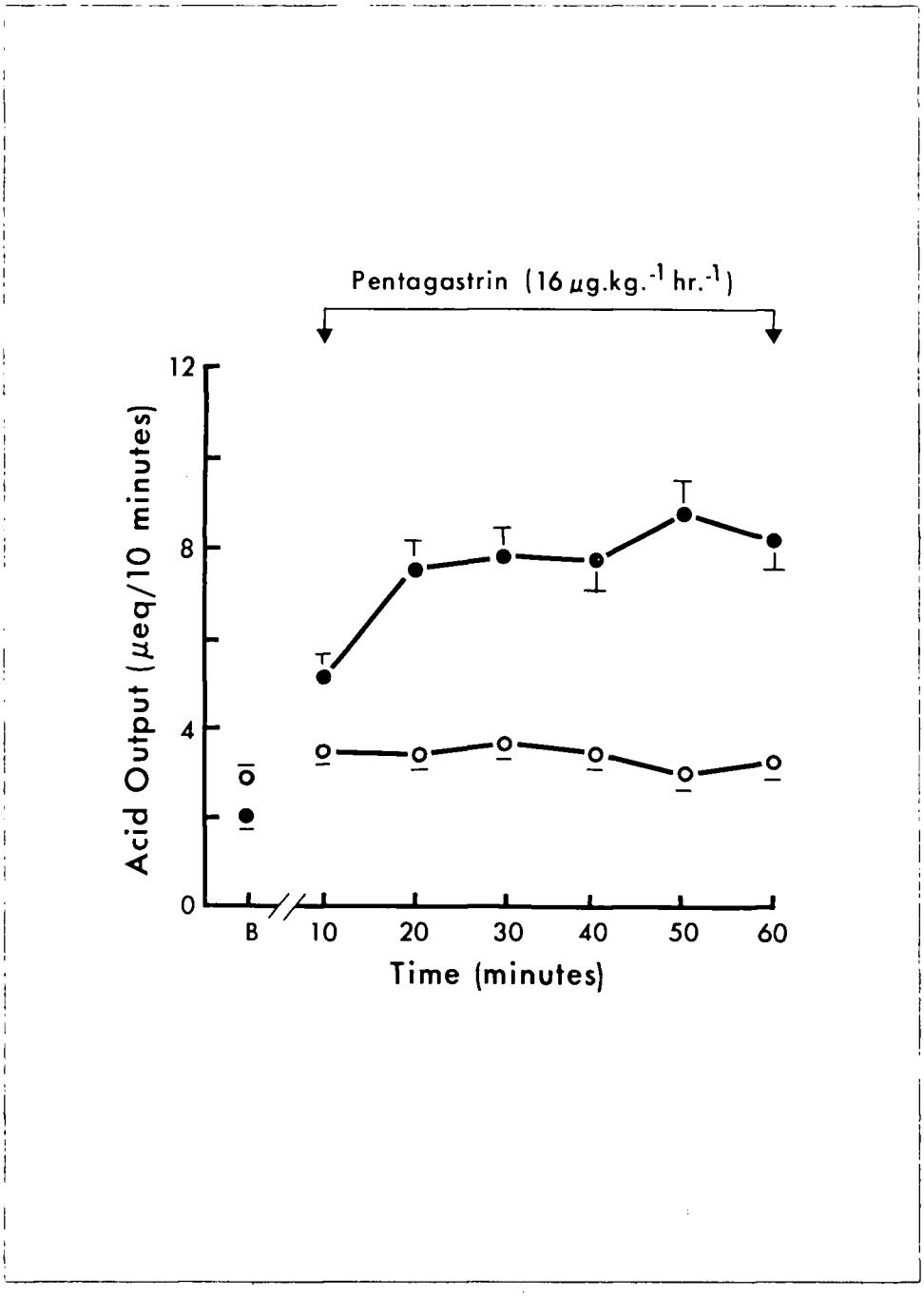


Figure 12. Effect of an intravenous pentagastrin infusion on gastric acid secretion

closed circles : intravenous pentagastrin infusion (n=30)

open circles : saline meal alone (n=6)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

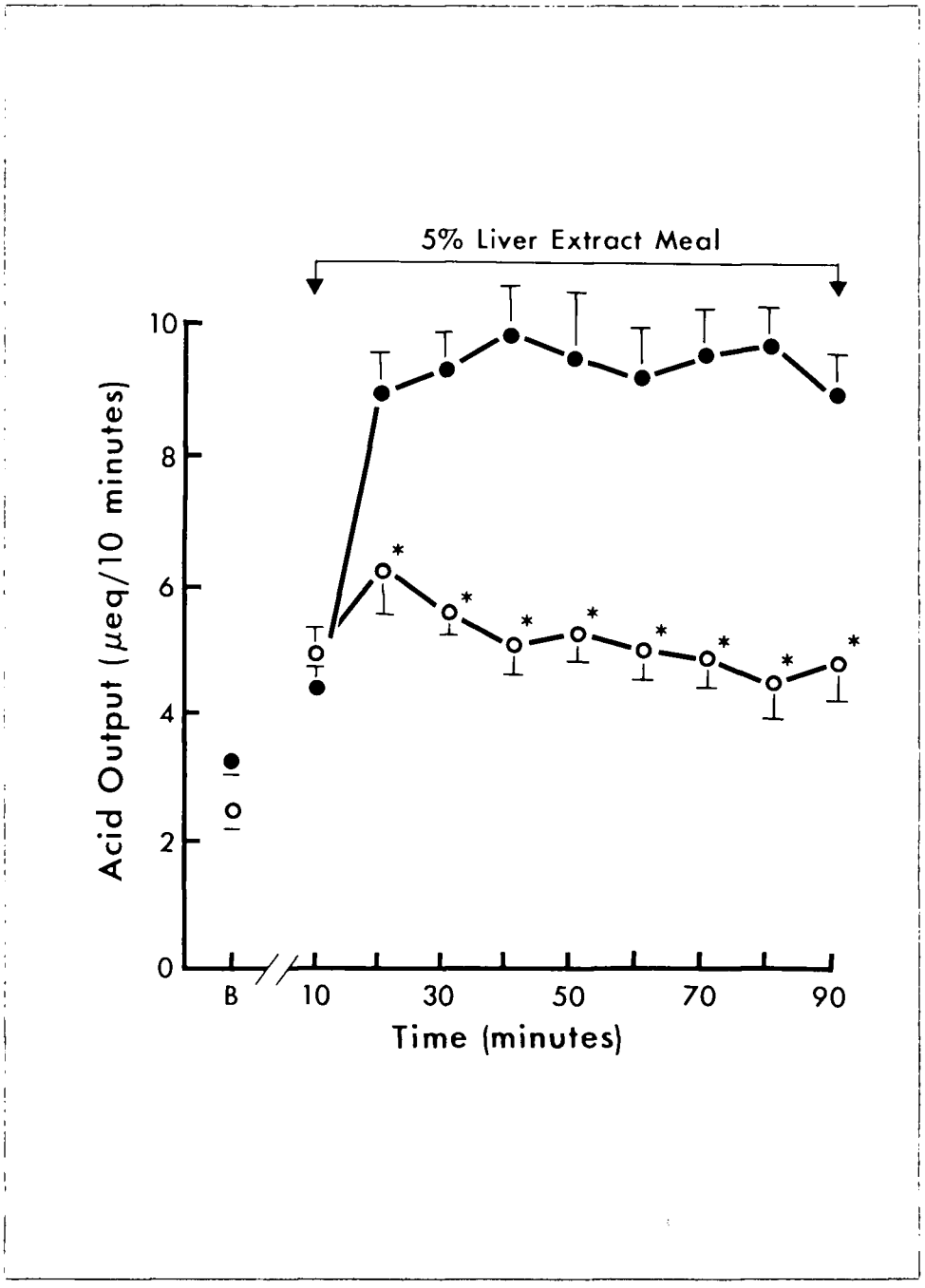


Figure 13. Effect of an intraduodenal infusion of oleic acid on gastric acid secretion

closed circles : 5% liver extract meal alone (n=16)

open circles : intraduodenal infusion of oleic acid during a 5% liver extract meal (n=8)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

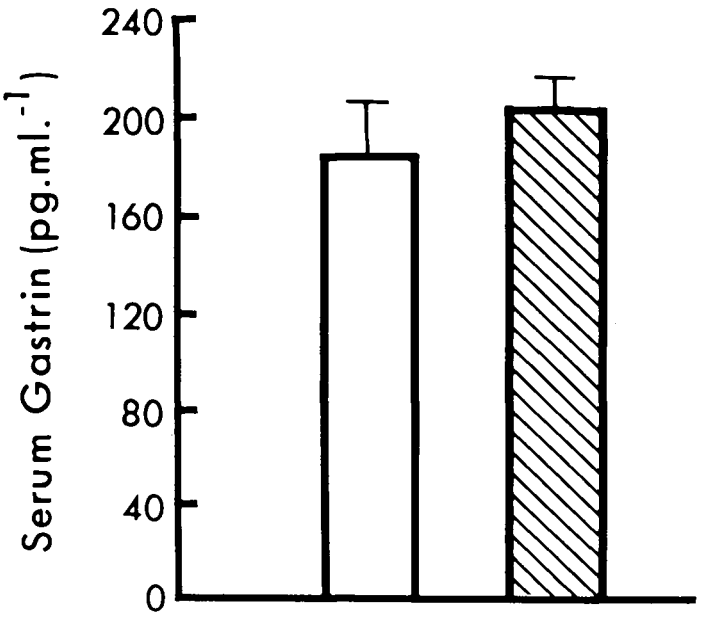


Figure 14. Effect of an intraduodenal infusion of oleic acid on meal-stimulated release of gastrin

open bar : liver extract meal alone (n=6)

hatched bar : liver extract meal and intraduodenal infusion of oleic acid (n=5)

each bar represents mean ± SEM

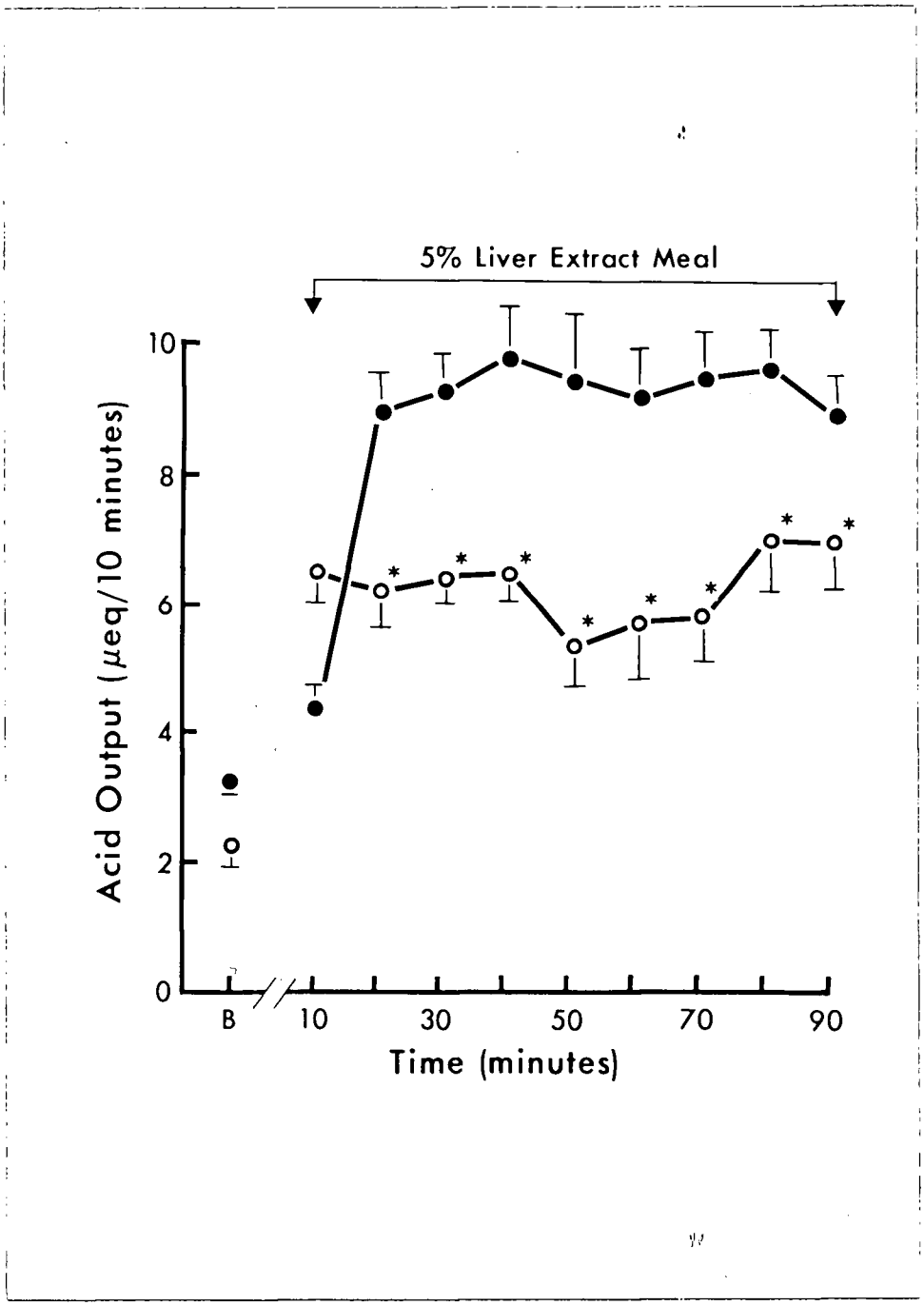


Figure 15. Effect of an intraduodenal infusion of 0.5 M HCL on gastric acid secretion

closed circles : 5% liver extract meal alone (n=16)

open circles : intraduodenal infusion of 0.5 M HCl during a 5% liver extract meal (n=11)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

C. Effect of Intravenous Infusion of Lyophilized Portal Serum

(i) Portal serum infusion during saline meal

Intravenous infusion of portal serum (collected after an intraduodenal oleic acid infusion) during a saline meal, had no effect on gastric acid secretion (figure 16).

(ii) Meal-stimulated gastric acid secretion

Gastric acid secretion after 40 minutes of liver extract meal-stimulation, is shown in figure 17. The "plateau acid output", expressed in µeq per 10 minute period, is the average of the acid secretion for the 3^{rd} and 4^{th} 10 minute period from the start of the meal.

Gastric acid output, following intravenous infusion of lyophilized portal serum 40 minutes after the start of a liver extract meal, is shown in figure 18. The acid output of each animal per 10 minute period, after infusion of the portal serum, has been expressed as a percentage of each respective plateau acid output. The infusion of lyophilized portal serum, collected from a separate group of animals after an intraduodenal infusion of oleic acid, significantly reduced meal-stimulated acid secretion. Infusion of lyophilized portal serum, which had been collected from a control group of animals without intraduodenal oleic acid, produced a small increase in meal-stimulated acid secretion.

(iii) Peripheral serum gastrin concentration

The infusion of lyophilized portal serum collected after intraduodenal oleic acid, did not produce a significant difference in peripheral serum gastrin concentration in the meal-stimulated animal preparation, when compared to the infusion of portal serum collected without prior

Figure 16. Effect of an intravenous bolus of portal serum on a normal saline meal

closed circles : saline meal alone (n=6)

open circles : saline meal with intravenous bolus of portal serum collected after intraduodenal oleic acid infusion (n=6)

triangles : saline meal with intravenous bolus of control portal serum (n=4)

each point represents mean ± SEM

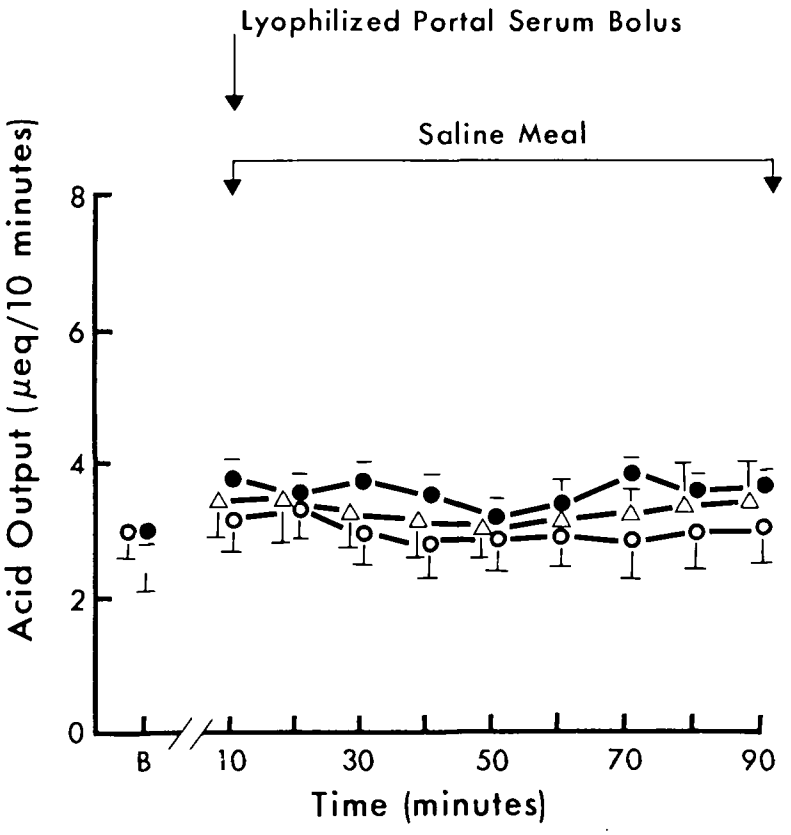

Lyophilized Portal Serum Bolus
Saline Meal
Acid Output (μeq/10 minutes)
8
6
4
2
0
B
10
30
50
70
90
Time (minutes)

Figure 17. "Plateau acid output" after 40 minutes of liver extract meal-stimulation

the plateau acid output is the average of the acid secretion for the 3rd and 4th 10 minute period from the start of the meal

open bar : group which will receive control portal serum (n=7)

hatched bar : group which will receive portal serum collected after intraduodenal fat infusion (n=7)

each bar represents mean ± SEM

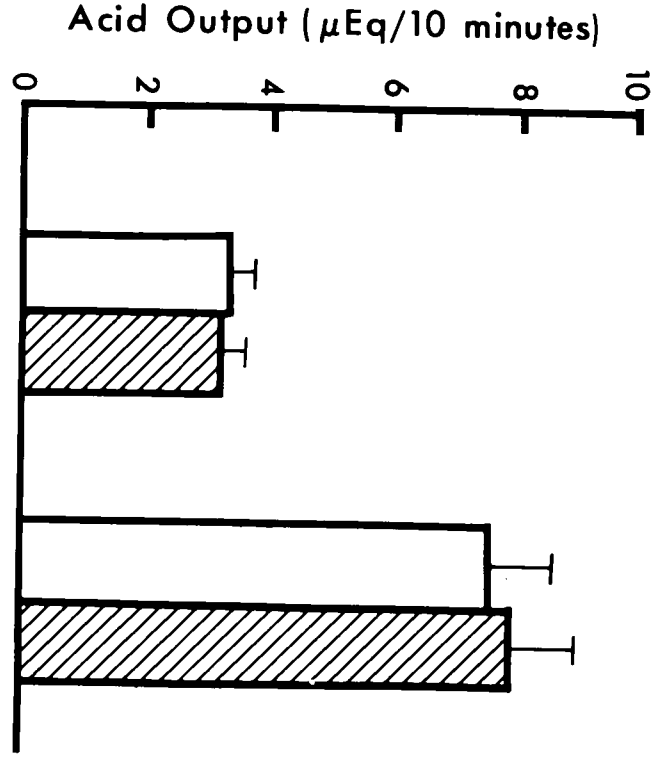

Acid Output (μEq/10 minutes)
0
2
4
6
8
10

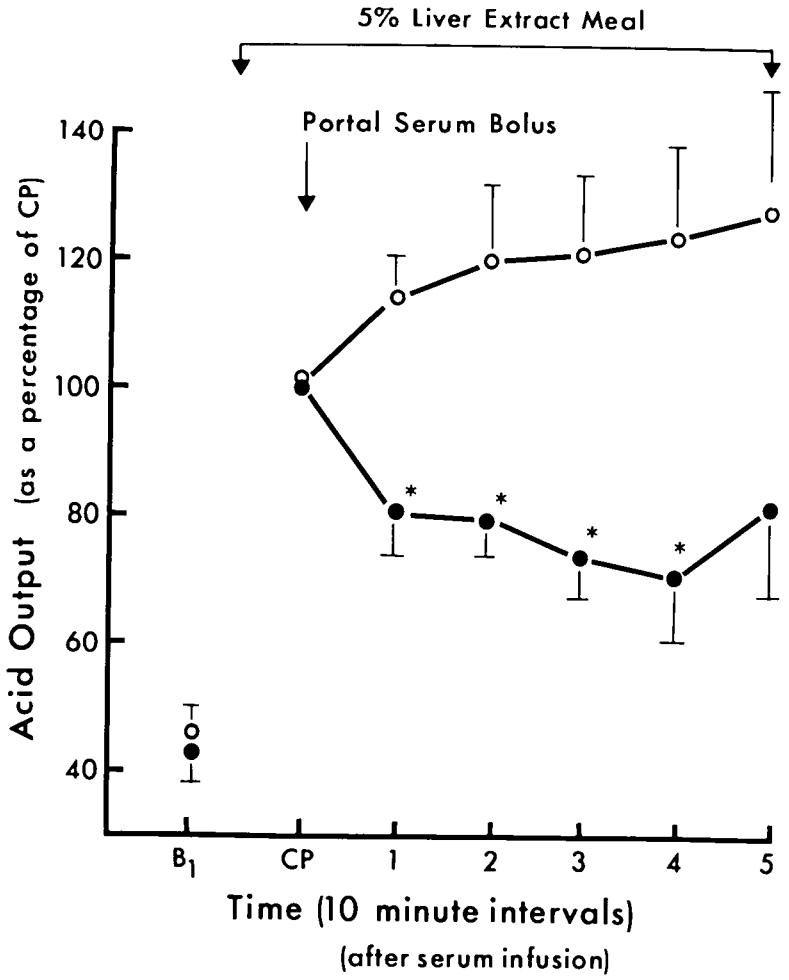
Figure 18. Effect of an intravenous infusion of lyophilized portal serum on meal-stimulated gastric acid output

acid output of each animal after infusion of the portal serum, is expressed as a percentage of each respective plateau acid output

open circles : group which received portal serum collected after intraduodenal fat infusion (n=7)

closed circles : group which received control portal serum (n=7)

* denotes significant difference ($p < 0.01$)


5% Liver Extract Meal
Portal Serum Bolus
140
120
100
80
60
40
Acid Output (as a percentage of CP)
B1
CP
1
2
3
4
5
Time (10 minute intervals)
(after serum infusion)

intraduodenal oleic acid (figure 19).

(iv) Portal serum IR-GIP concentration

The concentration of IR-GIP in the portal serum did not significantly differ, when collected either with or without prior intraduodenal infusion of oleic acid, (figure 20).

D. Effect of an Intraduodenal Infusion of Oleic Acid or Glucose on Gastric Acid Secretion and Portal IR-GIP Release

(i) Meal stimulation of gastric acid secretion

Intraduodenal infusion of oleic acid produced a significant reduction of the gastric acid secretory response to the liver extract meal (figure 21). The reduction in acid secretion was observed by the 1st 10 minute period after the start of the meal, and was maintained throughout the meal stimulus.

Intraduodenal infusion of a 10% glucose solution did not significantly effect the gastric acid secretory response to the liver extract meal, when compared to the control (figure 21).

(ii) Measurement of portal serum IR-GIP

Ten minutes after the start of the intraduodenal glucose infusion, portal serum IR-GIP concentrations were significantly elevated above control levels, and remained elevated throughout the study (figure 21). Increased portal serum IR-GIP concentrations were observed 30 minutes after the start of the intraduodenal infusion of oleic acid, but only found to be significantly elevated above control IR-GIP levels after 60 minutes of oleic acid infusion (figure 21).

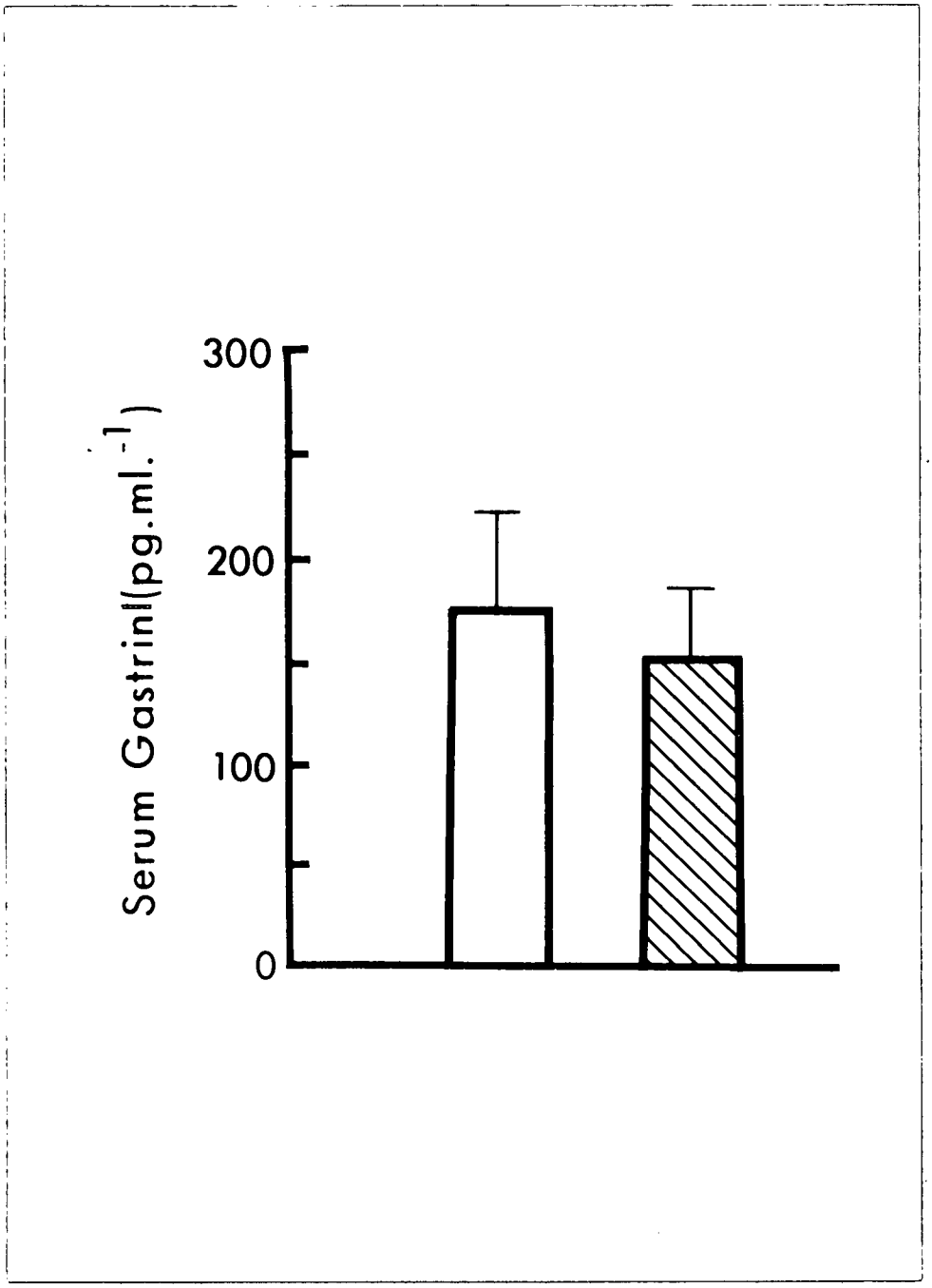


Figure 19. Effect of lyophilized portal serum infusion on peripheral serum gastrin concentration in the meal-stimulated animal preparation

open bars : group which received control portal serum (n=6)

hatched bars : group which received portal serum collected after intraduodenal fat infusion (n=6)

each bar represents mean ± SEM

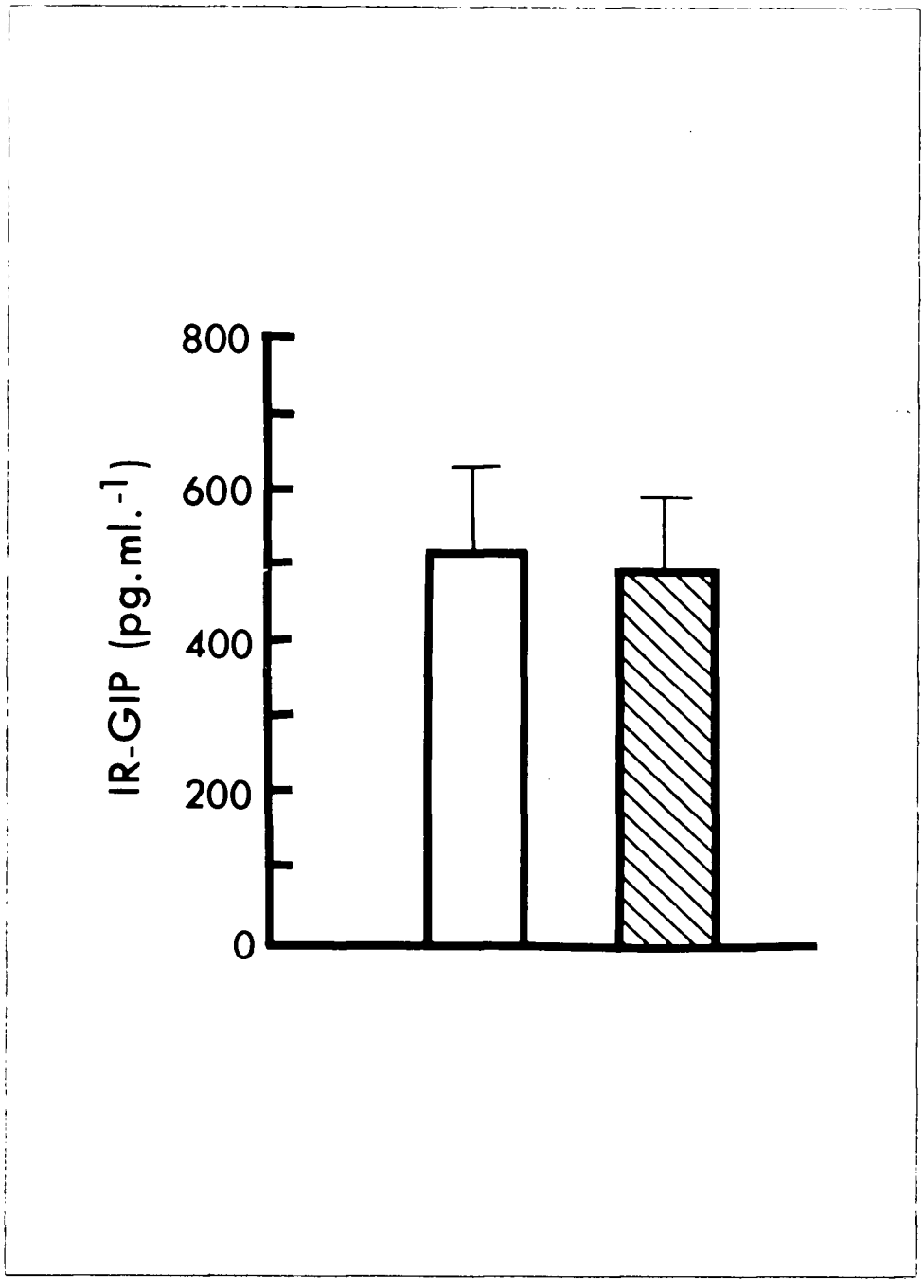


Figure 20. Portal serum IR-GIP concentration

open bar : portal serum collected after intraduodenal infusion of oleic acid (n=17)

hatched bar : portal serum collected without prior intraduodenal oleic acid (n=20)

each bar represents mean ± SEM

Figure 21. Effect of an intraduodenal infusion of oleic acid or glucose on gastric acid secretion and portal release of IR-GIP

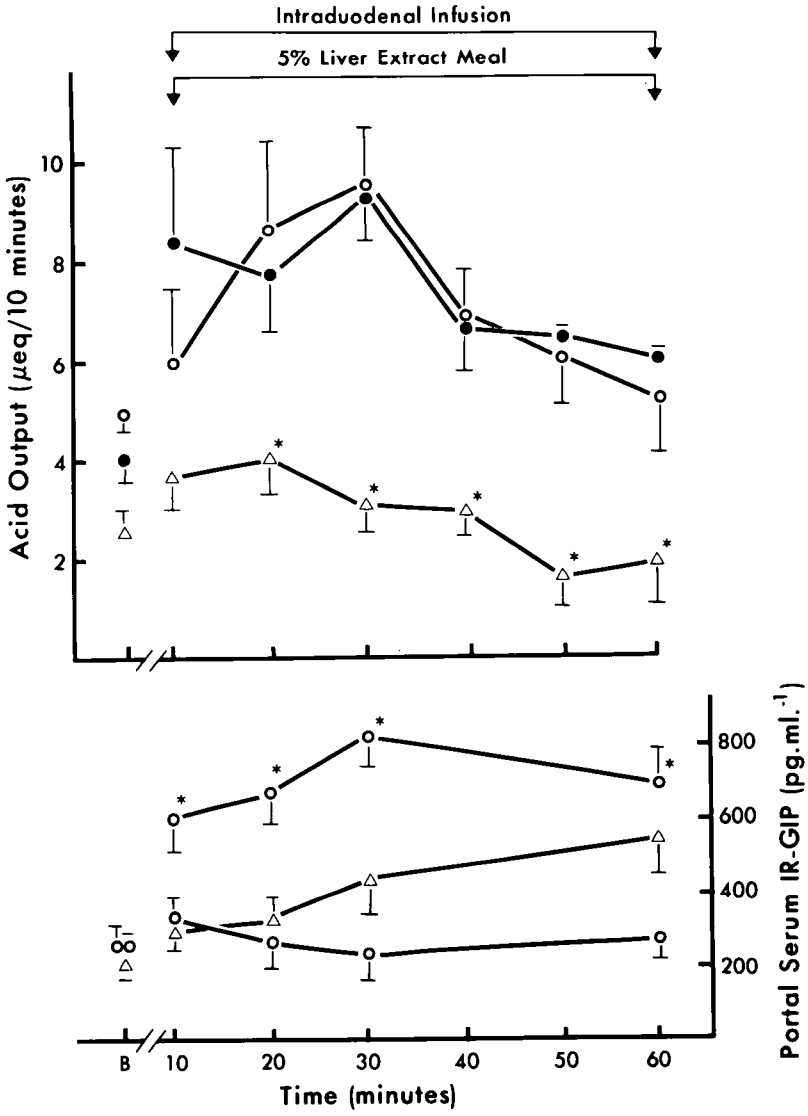
closed circles : liver extract meal alone (n=4)

open circles : liver extract meal with intraduodenal infusion of 10% glucose (n=4)

triangles : liver extract meal with intraduodenal infusion of oleic acid (n=5)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)


Intraduodenal Infusion
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
10
8
6
4
2
800
600
400
200
Portal Serum IR-GIP (pg.ml.-1)
B
10
20
30
40
50
60
Time (minutes)

(iii) Comparison of portal serum IR-GIP with % inhibition of meal-stimulated acid secretion

In figure 22, the change in portal serum concentrations of IR-GIP, measured during the first 30 minutes of a liver extract meal, are plotted against the percent inhibition of gastric acid secretion produced by either an intraduodenal infusion of glucose or oleic acid. Intraduodenal infusion of glucose produced a marked elevation of IR-GIP levels with little effect on acid secretion.

4. Gastrointestinal Peptides

A. Effect of Exogenous GIP Infusion on Gastric Acid Secretion

(i) GIP infusion during a saline meal

Intravenous infusion of GIP at 0.2 $\mu g.kg.^{-1}hr.^{-1}$ during a normal saline meal, had no effect on gastric acid secretion (figure 23).

(ii) GIP infusion during pentagastrin stimulation of gastric acid secretion

Intravenous infusion of GIP at 2.0 $\mu g.kg.^{-1}hr.^{-1}$ produced a significant increase in pentagastrin-stimulated acid secretion, when compared to pentagastrin infusion alone (figure 24). The increase in acid secretion was observed by the 2^{nd} 10 minute period after the start of the pentagastrin and GIP infusions, and was maintained throughout the gastric stimulation.

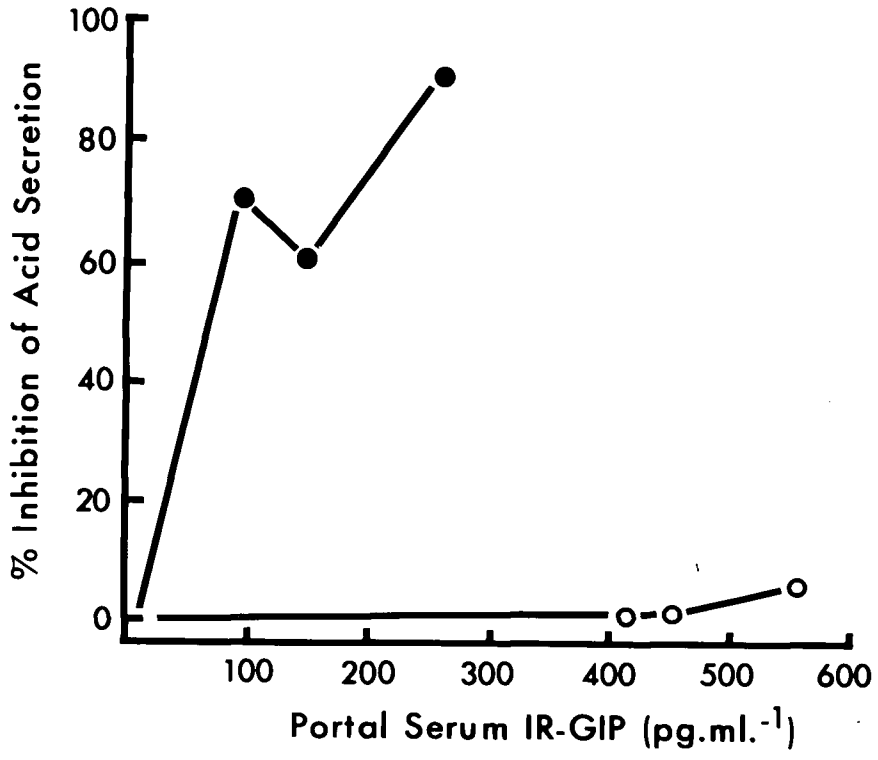


Figure 22. Comparison of portal serum IR-GIP with % inhibition of meal-stimulated acid secretion, after intraduodenal infusion of either glucose or oleic acid

closed circles : intraduodenal glucose

open circles : intraduodenal oleic acid

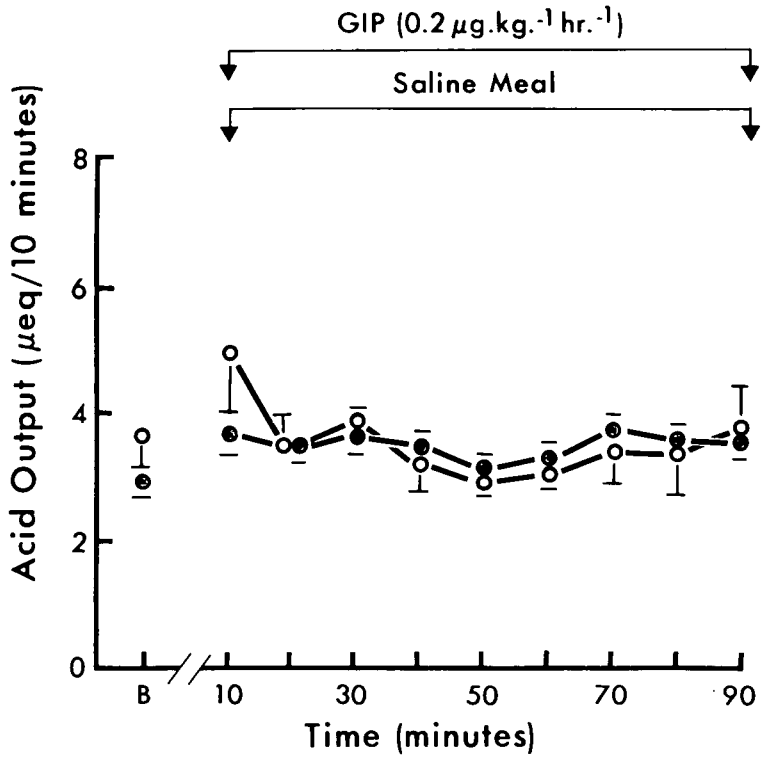


Figure 23. Effect of an intravenous infusion of GIP on acid secretion during a normal saline meal :

closed circles : saline meal alone (n=6)

open circles : saline meal with GIP infusion at 2.0 $\mu g.kg.^{-1}hr.^{-1}$ (n=6)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

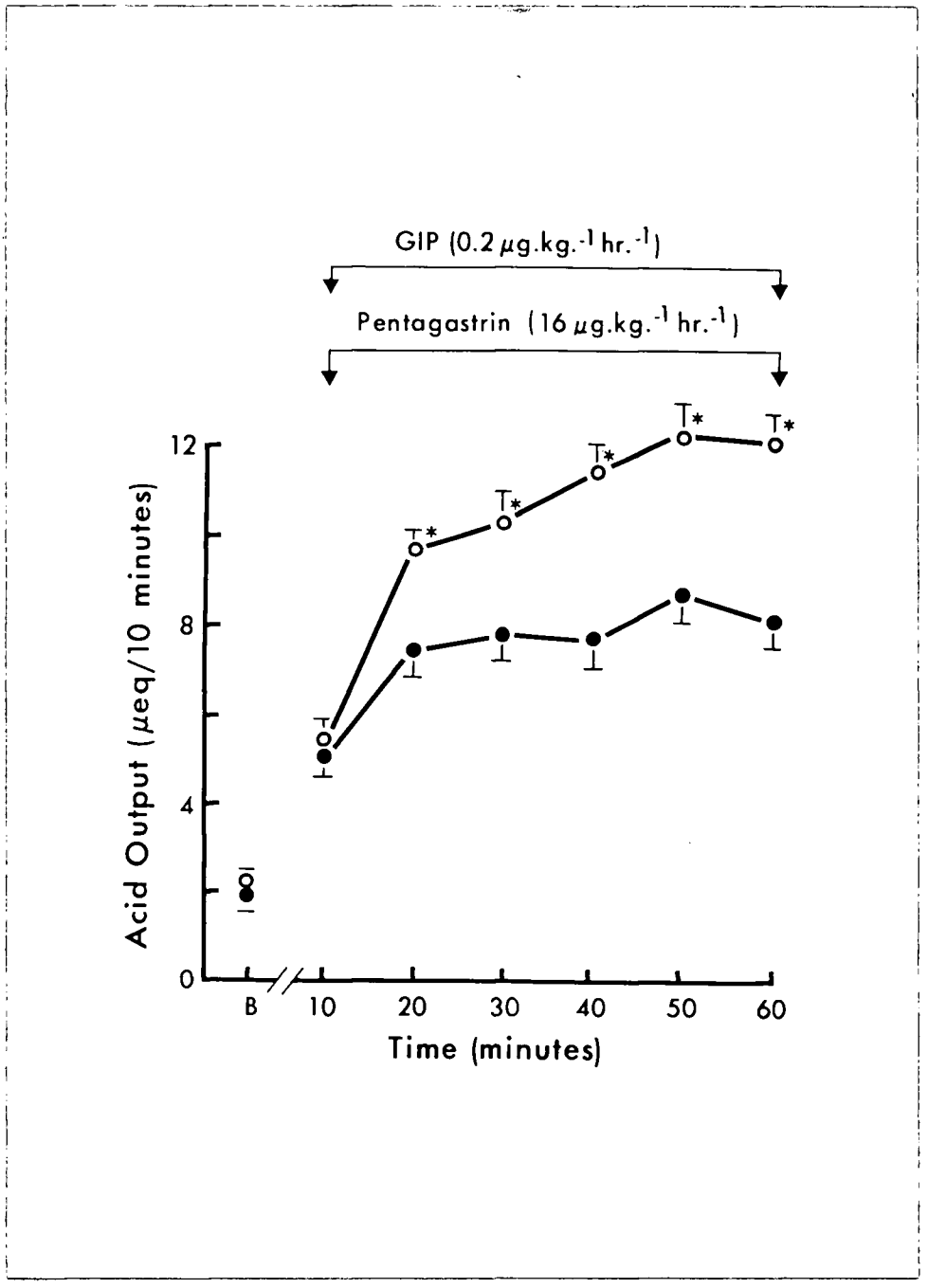


Figure 24. Effect of an intravenous infusion of GIP on pentagastrin-stimulated gastric acid secretion

closed circles : pentagastrin infusion alone (n=30)

open circles : pentagastrin infusion with GIP infusion at 2.0 $\mu g.kg.^{-1}hr.^{-1}$ (n=29)

each point represents mean ± SEM

* denotes significant difference ($p < 0.01$)

(iii) HPLC-purified GIP infusion during pentagastrin stimulation of gastric acid secretion

Intravenous infusion of HPLC-purified GIP at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, had no effect on pentagastrin-stimulated gastric acid secretion (figure 25).

(iv) GIP infusion during meal stimulation of gastric acid secretion

Intravenous infusion of GIP at 0.2 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$, produced a 60% reduction of the gastric acid secretory response to the liver extract meal (figure 26).

GIP infusion at 0.2 $\mu g.kg.^{-1}hr.^{-1}$ during the liver extract meal, had no significant effect on the serum gastrin concentration, when compared to the meal stimulus alone (figure 27).

Serum IR-GIP concentrations were significantly elevated above basal by the GIP infusion at 0.2 $\mu g.kg.^{-1}hr.^{-1}$during the liver extract meal. They were not effected by the meal stimulus alone, and remained at basal levels (figure 28).

B. Effect of Exogenous Somatostatin Infusion on Gastric Acid Secretion

(i) Somatostatin infusion during saline meal

Intravenous infusion of somatostatin at 2.0 $\mu g.kg.^{-1}hr.^{-1}$ during a normal saline meal, had no effect on gastric acid secretion (figure 29).

(ii) Somatostatin infusion during pentagastrin stimulation of gastric acid secretion

Intravenous infusion of somatostatin at 2.0 $\mu g.kg.^{-1}hr^{-1}$ produced a

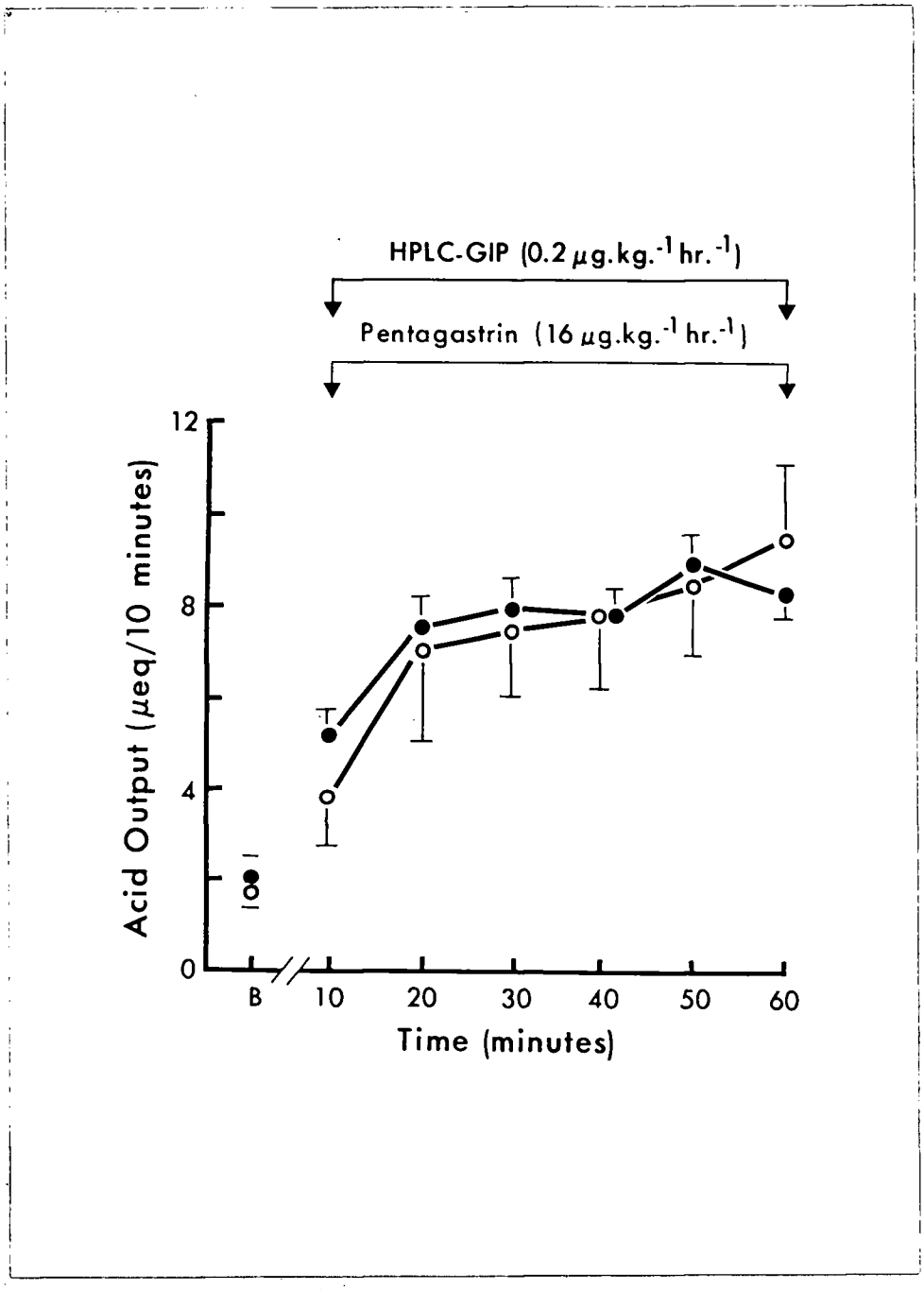


Figure 25. Effect of an intravenous infusion of HPLC-purified GIP on pentagastrin-stimulated gastric acid secretion

closed circles : pentagastrin infusion alone (n=30)

open circles : pentagastrin infusion with HPLC-purified GIP infusion at 2.0 µg.kg.$^{-1}$hr.$^{-1}$ (n=7)

each point represents mean ± SEM

Figure 26. Effect of an intravenous infusion of GIP on liver extract meal-stimulated gastric acid secretion

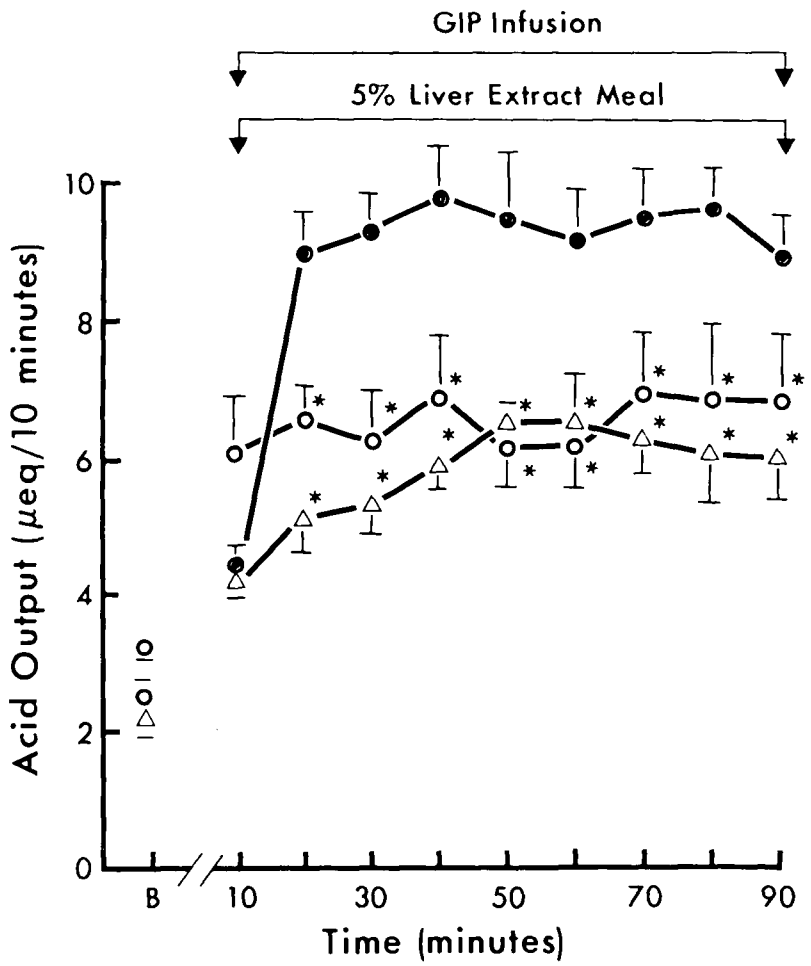
closed circles : liver extract meal alone (n=16)

open circles : liver extract meal and GIP infusion at 2.0 $\mu g.kg.^{-1}hr.^{-1}$ (n=8)

triangles : liver extract meal and GIP infusion at 0.2 $\mu g.kg^{-1}hr.^{-1}$ (n=8)

each point represents mean ± SEM

* denotes significant difference ($p < 0.01$)


GIP Infusion
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
10
8
6
4
2
0
B
10
30
50
70
90
Time (minutes)

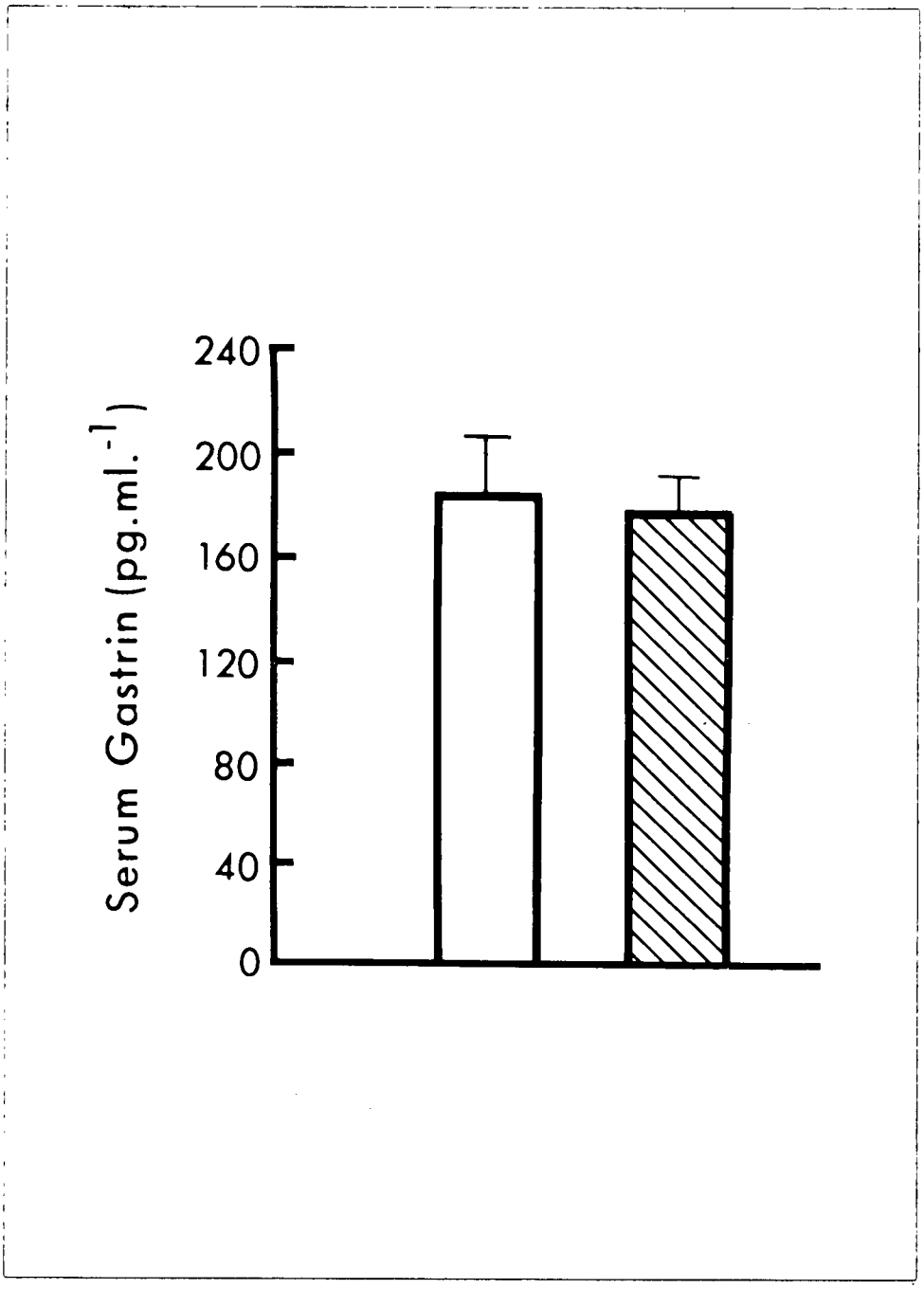


Figure 27. Effect of intravenous GIP infusion (0.2 µg.kg.$^{-1}$hr.$^{-1}$) on meal-stimulated release of gastrin

open bar : liver extract meal alone (n=6)

hatched bar : liver extract meal with GIP infusion (n=6)

each bar represents mean ± SEM

Figure 28. Serum IR-GIP concentrations, after GIP infusion during a liver extract meal

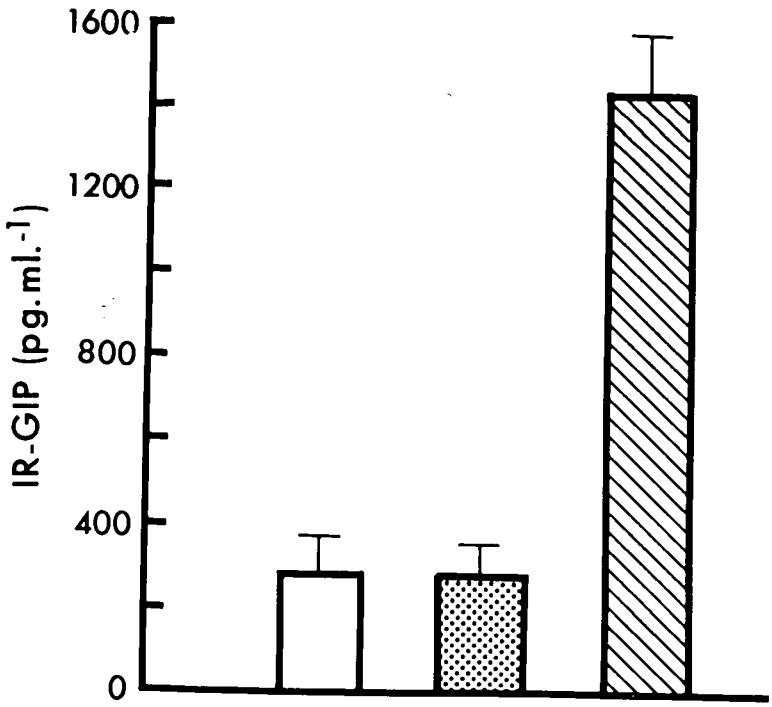
open bar : basal (n=4)

shaded bar : liver extract meal alone (n=4)

hatched bar : liver extract meal with GIP infusion ($0.2\ \mu g.kg.^{-1}hr.^{-1}$) (n=6)

each bar represents mean ± SEM

* denotes significant difference ($p < 0.05$)


1600
1200
800
400
0
IR-GIP (pg.ml.-1)

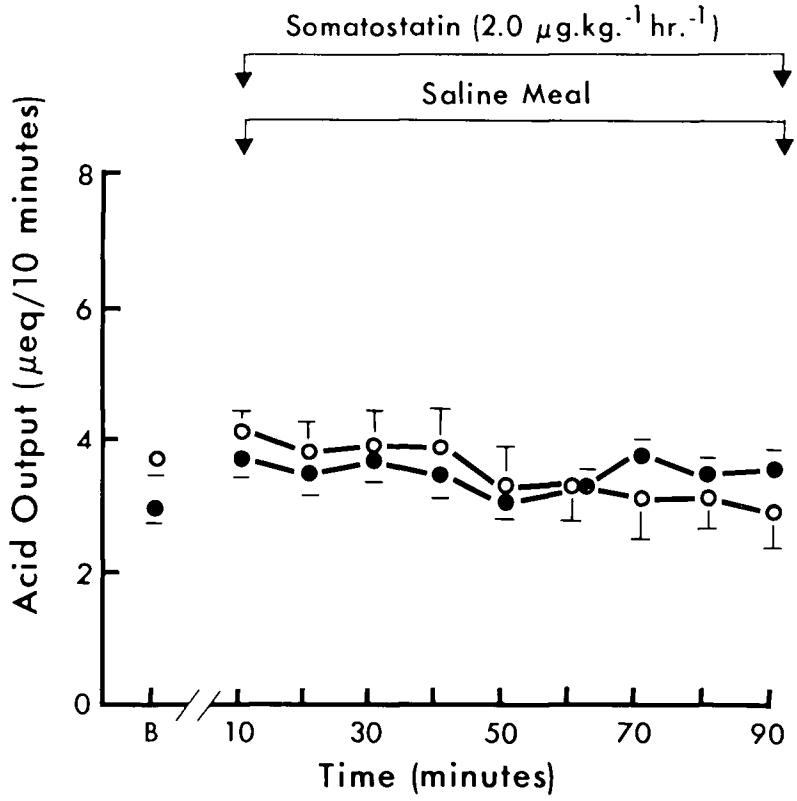


Figure 29. Effect of an intravenous infusion of somatostatin on acid secretion during a normal saline meal

closed circles : saline meal alone (n=6)

open circles : saline meal with somatostatin infusion at 2.0 $\mu g.kg.^{-1}hr.^{-1}$ (n=6)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

small increase in pentagastrin stimulated acid secretion, when compared to pentagastrin infusion alone (figure 30). The increase in acid secretion was statistically significant at the 5^{th} and 6^{th} 10 minute period after the start of the pentagastrin and somatostatin infusion.

(iii) Somatostatin infusion during meal stimulation of gastric acid secretion

Intravenous infusion of somatostatin at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, produced an 80% reduction of the gastric acid secretory response to the liver extract meal (figure 31).

Somatostatin infusion during the liver extract meal significantly reduced the serum gastrin concentration, when compared to the meal stimulus alone (figure 32).

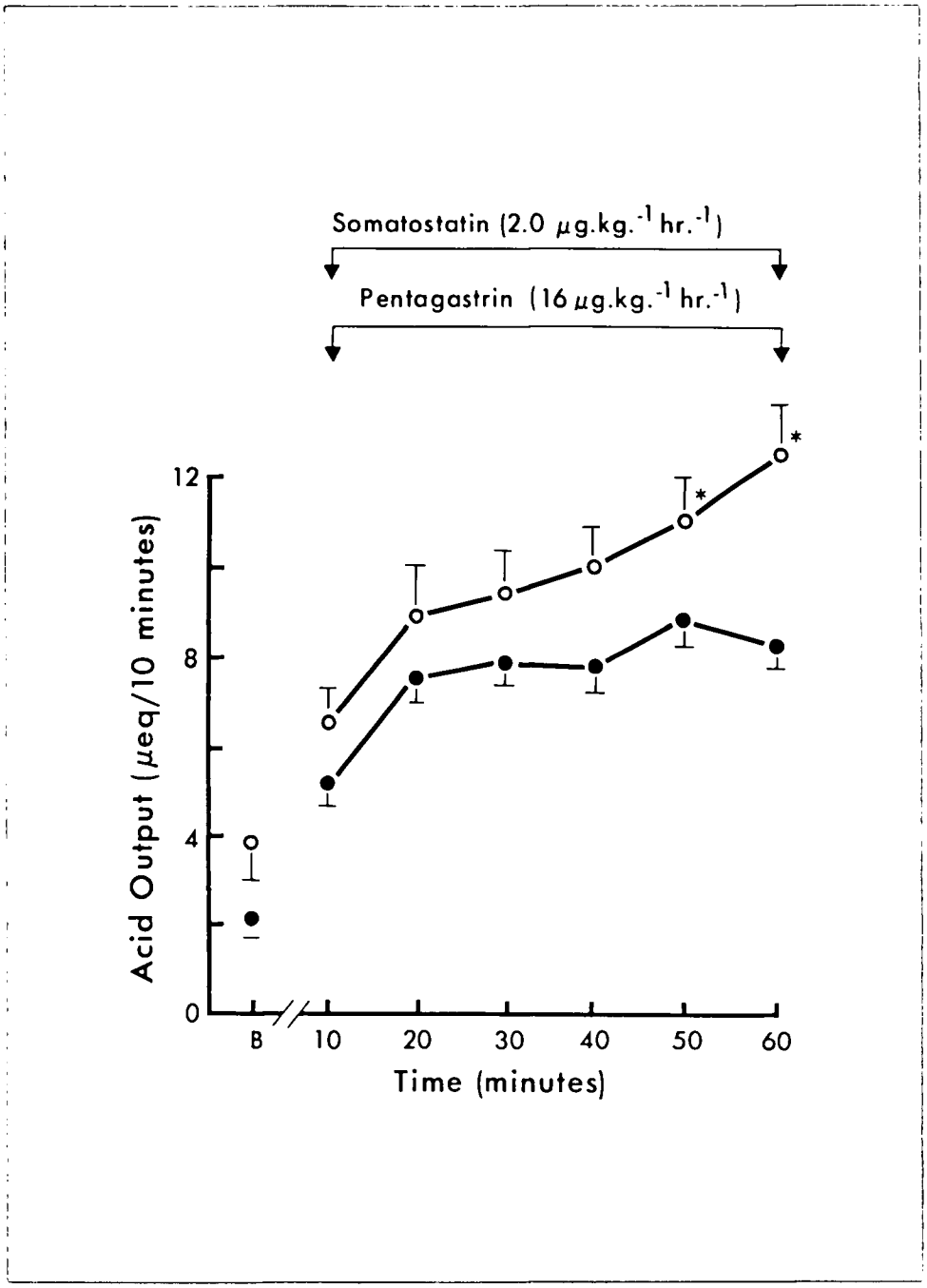


Figure 30. Effect of an intravenous infusion of somatostatin on pentagastrin-stimulated gastric acid secretion

closed circles : pentagastrin infusion alone (n=30)

open circles : pentagastrin infusion with somatostatin infusion at 2.0 $\mu g.kg.^{-1}hr.^{-1}$ (n=6)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

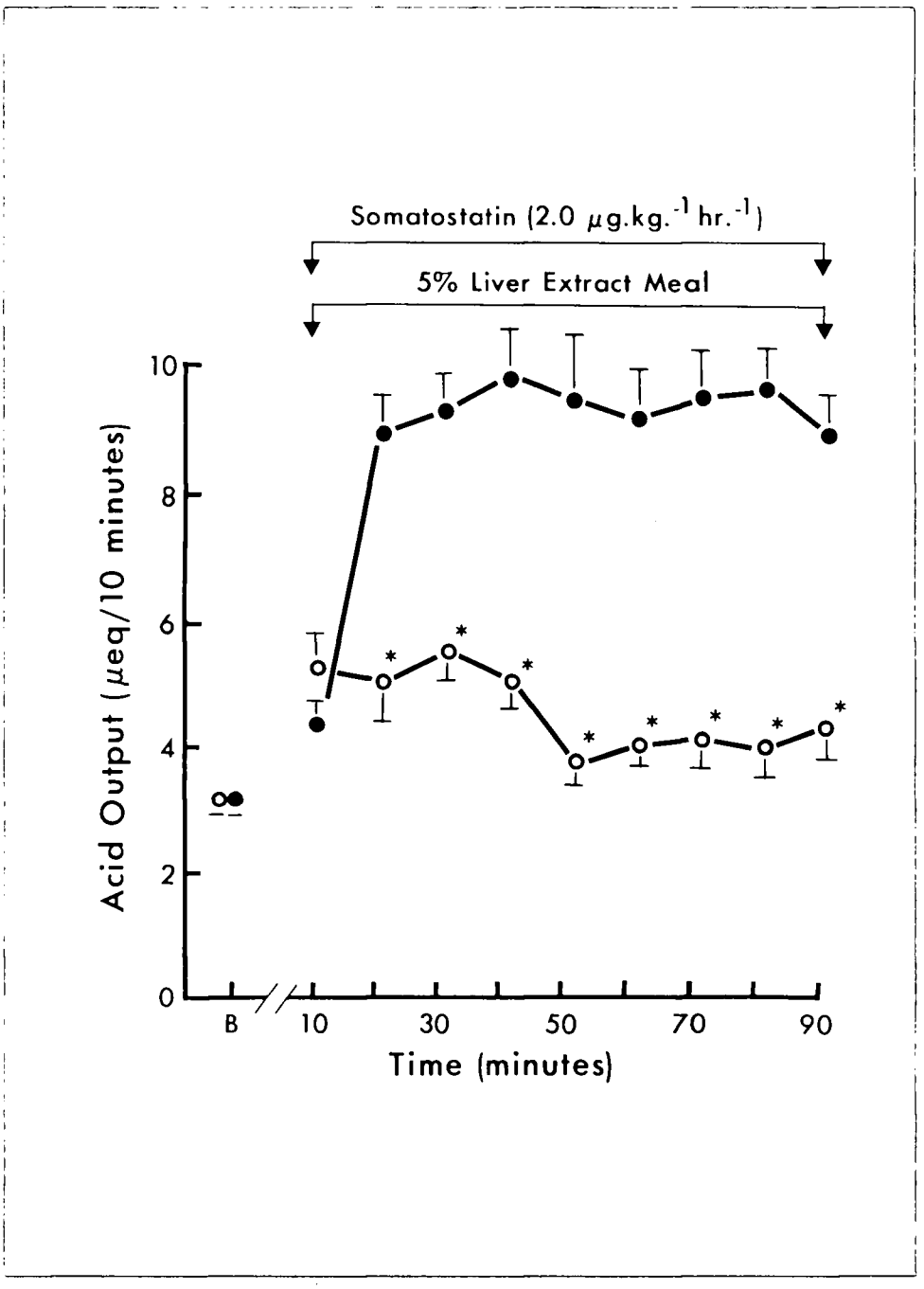


Figure 31. Effect of an intravenous infusion of somatostatin on liver extract meal-stimulation of gastric acid secretion

closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with somatostatin infusion at 2.0 μg.kg.$^{-1}$hr.$^{-1}$ (n=10)

each point represents mean ± SEM

* denotes significant difference ($p < 0.01$)

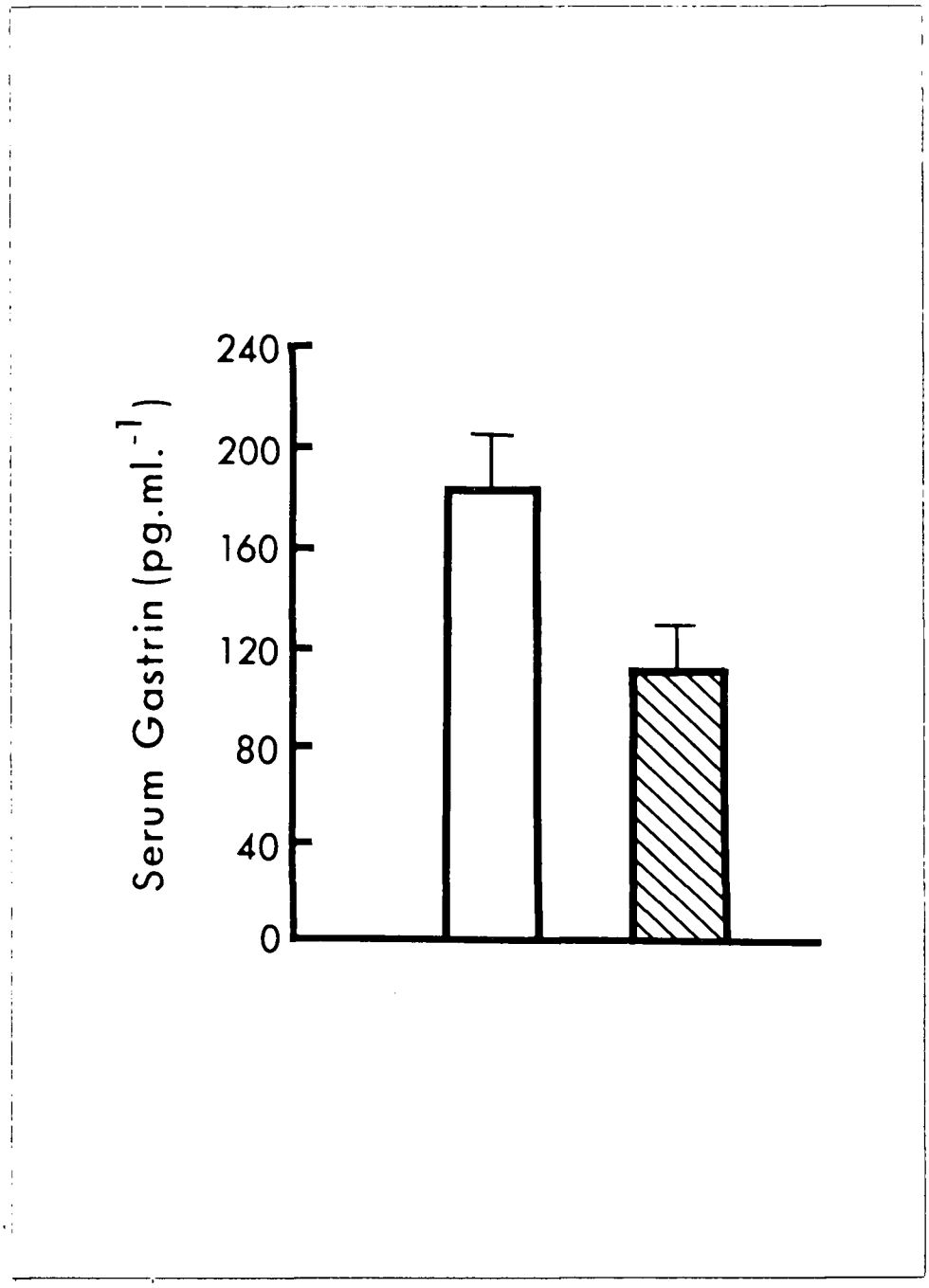


Figure 32. Effect of an intravenous infusion of somatostatin on meal-stimulated release of serum gastrin

open bar : liver extract meal alone (n=6)

hatched bar : liver extract meal with somatostatin infusion at 2.0 µg.kg.$^{-1}$hr.$^{-1}$ (n=7)

each bar represents mean ± SEM

* denotes significant difference ($p < 0.01$)

Part II

Monoclonal Antibodies as Probes of Humoral Inhibitors of Gastric Acid Secretion

1. GIP Monoclonal Antibody (3.65H)

A. Binding affinity of monoclonal antibody 3.56H for GIP in vitro

Antibody 3.65H, in vitro demonstrated a greater degree of binding to GIP than did the polyclonal rabbit antiserum R07 (Figure 33).

B. Effect of monoclonal antibody 3.65H on the inhibition of meal-stimulated gastric acid secretion by exogenous GIP

(i) Inhibition of meal-stimulated gastric acid secretion by GIP

An intravenous infusion of GIP at 0.2 $\mu g.kg.^{-1}hr.^{-1}$ produced a 60% reduction of the gastric acid secretory response to the meal. Infusion of GIP, both shortly after preparation, and after 18 hours at $4^{0}C$, produced similar reductions of acid secretion (Figure 34).

(ii) Effect of a 100 µg bolus of monoclonal antibody 3.65H on the inhibition of gastric acid secretion by GIP.

The antibody, when given alone as an intravenous 100 µg bolus 1 hour prior to a liver extract meal, had no effect on the gastric acid secretory response to the meal. When given as a 100 µg bolus, one hour prior to the start of the meal and an infusion of GIP at 0.2 $\mu g.kg.^{-1}hr.^{-1}$, the antibody had no effect on the inhibition of gastric acid secretion by GIP (Figure 35).

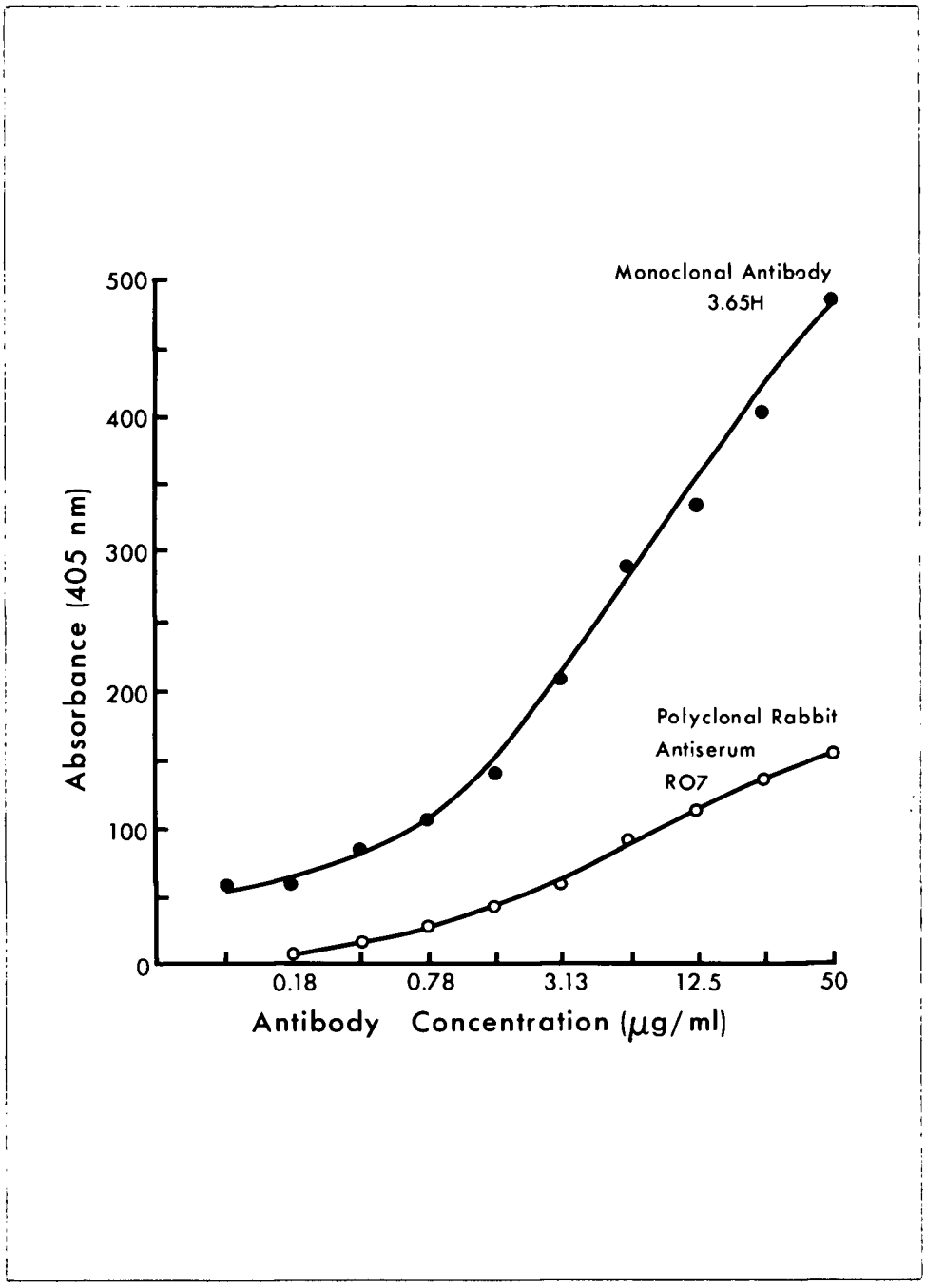


Figure 33. In vitro comparison of GIP binding, between monoclonal antibody 3.65H and polyclonal rabbit antiserum R07, using the Enzyme-Linked Immunosorbent Assay (ELISA)

closed circles : monoclonal antibody 3.65H

open circles : polyclonal rabbit antiserum R07

Figure 34. Effect of an intravenous infusion of GIP on liver extract meal-stimulated gastric acid secretion

closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with GIP infused shortly after preparation (n=8)

triangles : liver extract meal with GIP infused after incubation for 18 hours at $4^{0}C$ (n=4)

each point represents mean ± SEM

* denotes significant difference ($p < 0.01$)

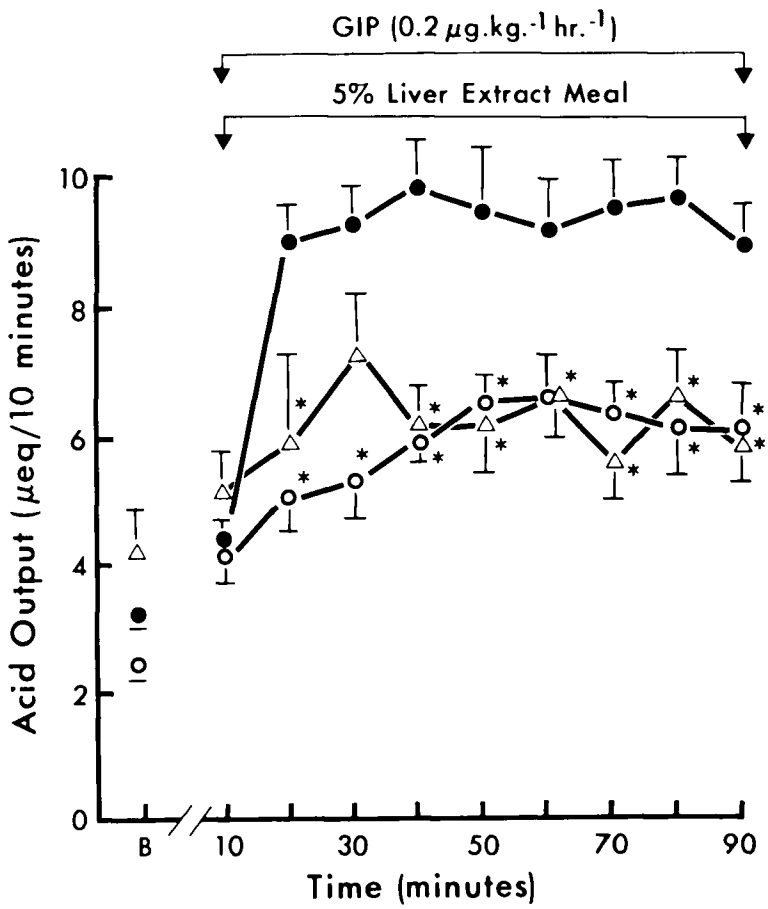

GIP (0.2 μg.kg.-1 hr.-1)
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
10
8
6
4
2
0
B
10
30
50
70
90
Time (minutes)

Figure 35. Effect of a 100 µg bolus of monoclonal antibody 3.65H on the inhibition of gastric acid secretion by GIP

The antibody was given as an intravenous bolus 1 hour prior to the start of a liver extract meal and GIP infusion

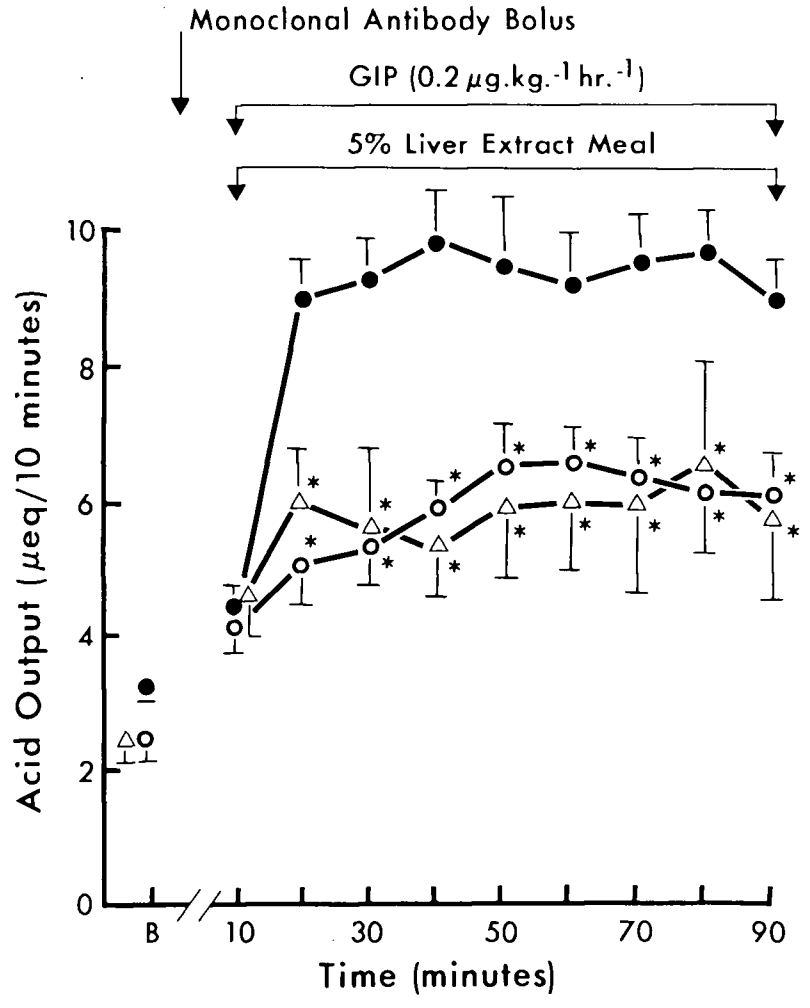
closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with GIP infusion (n=8)

triangles : liver extract meal with monoclonal antibody bolus and GIP infusion (n=6)

each point represents mean ± SEM

* denotes significant difference ($p < 0.01$)


Monoclonal Antibody Bolus
GIP (0.2 μg.kg.-1 hr.-1)
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
10
8
6
4
2
0
B
10
30
50
70
90
Time (minutes)

(iii) Verification of GIP binding to the monoclonal antibody in the incubated preparation

The antibody, after incubation alone for 1 hour at 37°C or 18 hours at 4^0C, was able to detect IR-GIP cells present in the stained sections of the small bowel mucosa. When preincubated with GIP for 1 hour at 37^0C or 18 hours at 4^0C, the antibody 3.65H did not detect these cells. After acidification of this pre-incubated solution, the IR-GIP cells were again detected (figure 36).

(iv) Effect of incubation of GIP with antibody 3.65H, on GIP inhibition of gastric acid secretion

During the liver extract meal, an intravenous infusion of GIP (0.2 $\mu g.kg.^{-1}hr.^{-1}$) incubated with 3.65H (6.0 $\mu g.kg.^{-1}hr.^{-1}$) for one hour at 37^0C, then centrifuged in activated charcoal, produced a 60% inhibition of acid secretion, which was similar to the inhibition produced by a GIP infusion alone. The infusion of GIP incubated with 3.65H at 4^0C for 18 hours followed by charcoal separation, did not inhibit meal-stimulated gastric acid secretion (Figure 37).

(v) Serum Gastrin Measurements

There was no significant difference in serum gastrin concentration, after an intravenous 100 µg bolus of monoclonal antibody 3.65H 1 hour prior to the liver extract meal, when compared to the meal stimulus alone (Figure 38).

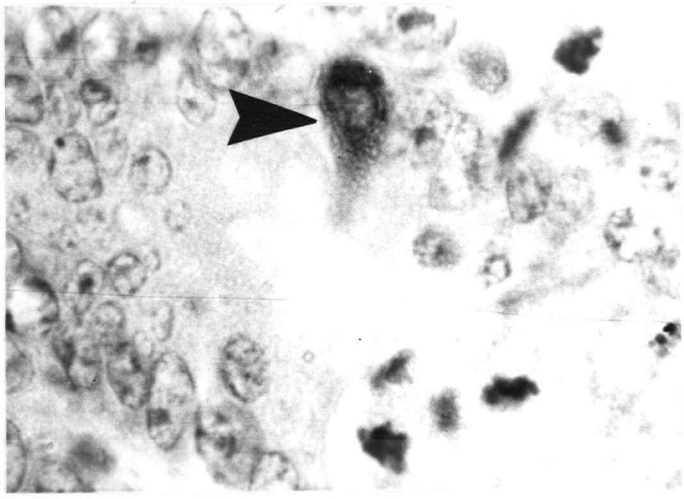
Figure 36. Verification of GIP binding to the monoclonal antibody 3.65H in the incubated preparation

a) IR-GIP cell (arrow) in a section of rat jejunum, immunostained with the monoclonal antibody (x100)

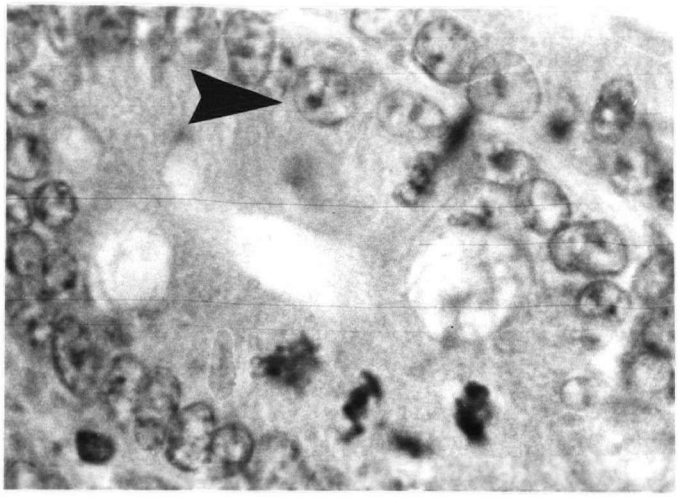
b) a section immunostained with the monoclonal antibody after preincubation with GIP for 18 hours at 4^0C and charcoal separation (x100)
note : absence of immunoreactivity

c) IR-GIP cell (arrow) in a section immunostained with the preincubated monoclonal antibody after charcoal separation and acid treatment (x100)

A



B



C

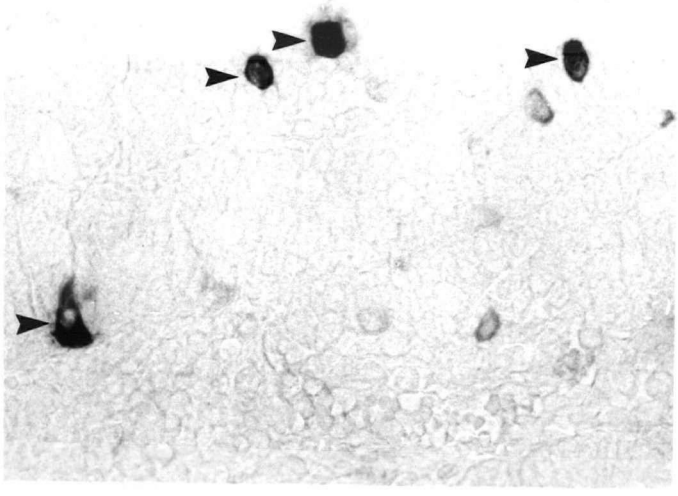


Figure 37. Effect of incubation of monoclonal antibody 3.65H with GIP, on GIP inhibition of gastric acid secretion

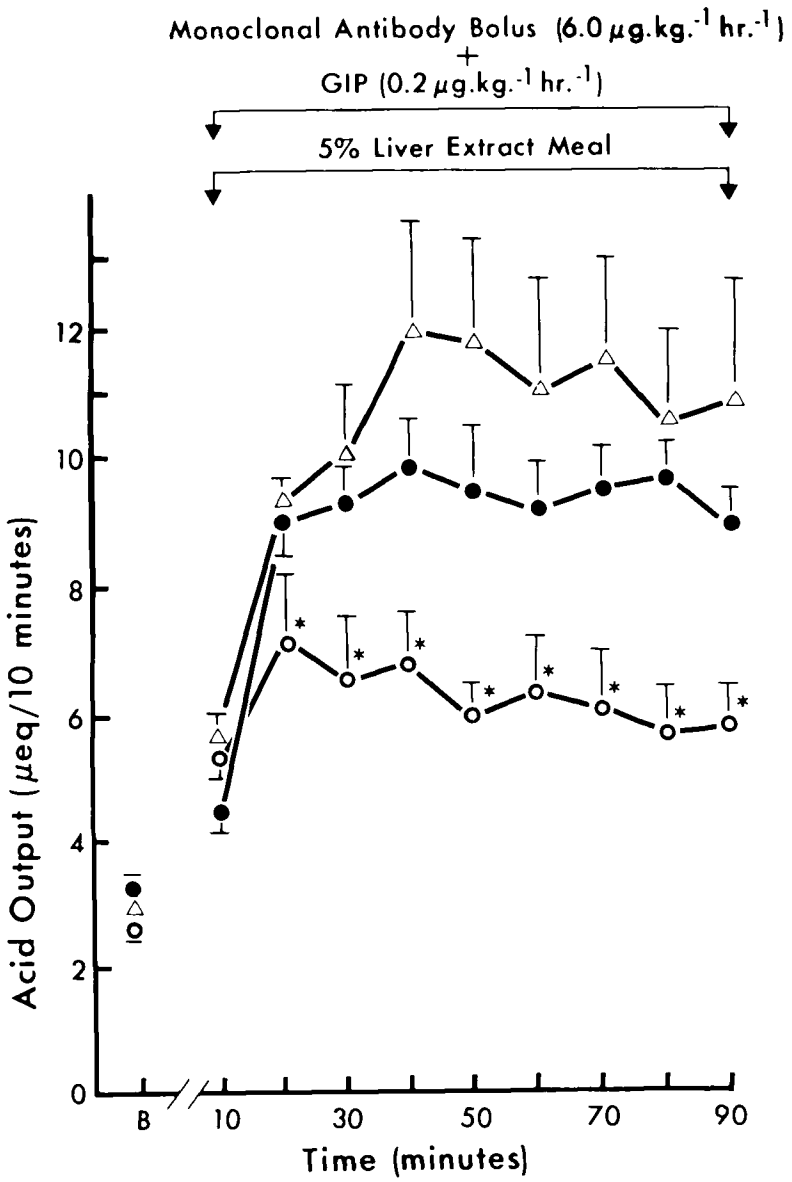
closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with infusion of GIP incubated with the monoclonal antibody for 1 hour at 37^{0}C (n=6)

triangles : liver extract meal with infusion of GIP incubated with the monoclonal antibody for 18 hours at 4^{0}C (n=11)

each point represents mean ± SEM

* denotes significant difference ($p < 0.01$)


Monoclonal Antibody Bolus (6.0 μg.kg.-1 hr.-1)
+
GIP (0.2 μg.kg.-1 hr.-1)
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
0
2
4
6
8
10
12
B
10
30
50
70
90
Time (minutes)
*

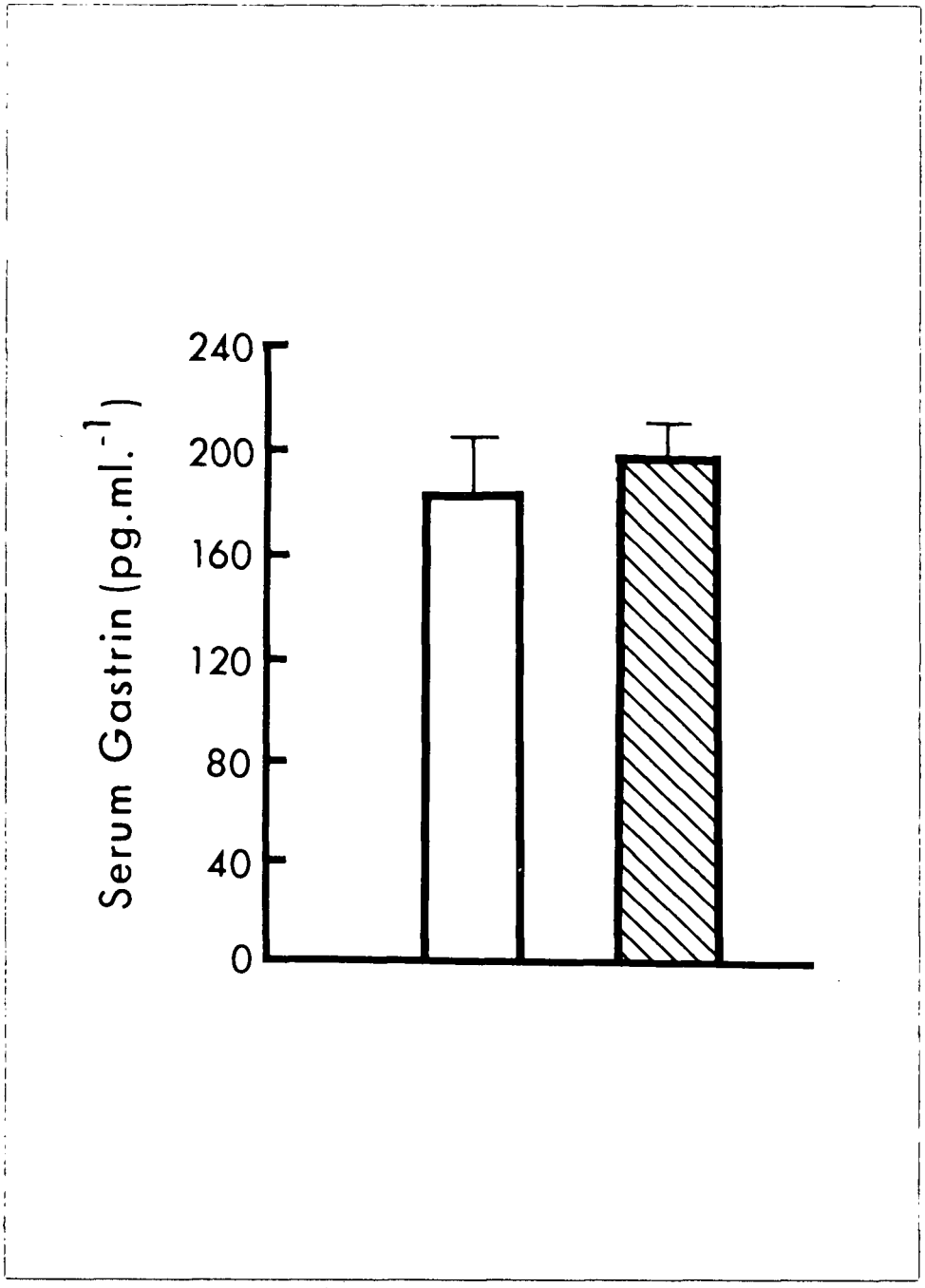


Figure 38. Effect of intravenous bolus infusion of monoclonal antibody 3.65H on meal-stimulated release of gastrin

open bar : liver extract meal alone (n=6)

hatched bar : liver extract meal plus monoclonal antibody bolus (n=9)

each bar represents mean ± SEM

2. Somatostatin Monoclonal Antibody Clones S8, S10 and S20

A. Effect of a 100 µg bolus of monoclonal antibody clones S8, S10 and S20 on meal-stimulated gastric acid secretion

The antibody clones S8 and S10, when given alone as an intravenous 100 µg bolus, 1 hour prior to a liver extract meal, produced a 30% reduction of the gastric acid secretory response to the meal (figure 39).

The antibody clone S20, when administered in a similar fashion, did not significantly change meal-stimulated acid secretion (figure 40).

B. Effect of a 100 µg bolus of monoclonal antibody clones S8, S10 and S20 on the inhibition of meal-stimulated gastric acid secretion by somatostatin

Intravenous infusion of somatostatin at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, produced an 80% reduction of the gastric acid secretory response to the liver extract meal (figure 31).

When the antibody clones S8 and S10 were given as an intravenous 100 µg bolus, 1 hour prior to the start of a liver extract meal and intravenous infusion of somatostatin at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, the gastric acid inhibitory effect of the somatostatin infusion was abolished (figure 41). In the group which received the bolus of clone S10, a sharp, significant reduction in acid secretion was observed at the 8^{th} and 9^{th} 10 minute period from the start of the meal.

The antibody clone S20, when given as an intravenous 100 µg bolus, 1 hour prior to the start of a liver extract meal and intravenous infusion of somatostatin at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, had no effect on the inhibition of gastric acid secretion by the somatostatin infusion (figure 42).

Figure 39. Effect of a 100 µg bolus of monoclonal antibody clone S8 or S10 on meal-stimulated gastric acid secretion

The antibody was given as an intravenous bolus 1 hour prior to the start of a liver extract meal

closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with monoclonal antibody clone S8 bolus (n=7)

triangles : liver extract meal with monoclonal antibody clone S10 bolus (n=14)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

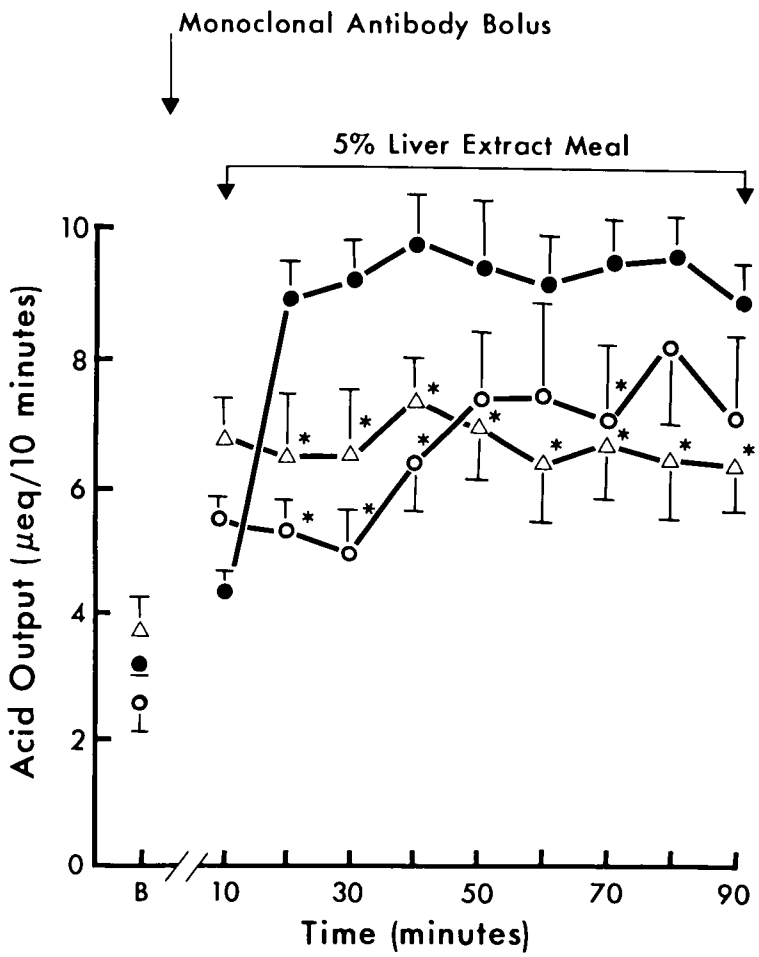

Monoclonal Antibody Bolus
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
0
2
4
6
8
10
B
10
30
50
70
90
Time (minutes)

Figure 40. Effect of a 100 µg bolus of monoclonal antibody clone S20 on meal-stimulated gastric acid secretion

The antibody was given as an intravenous bolus 1 hour prior to the start of a liver extract meal

closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with monoclonal antibody clone S20 bolus (n=6)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

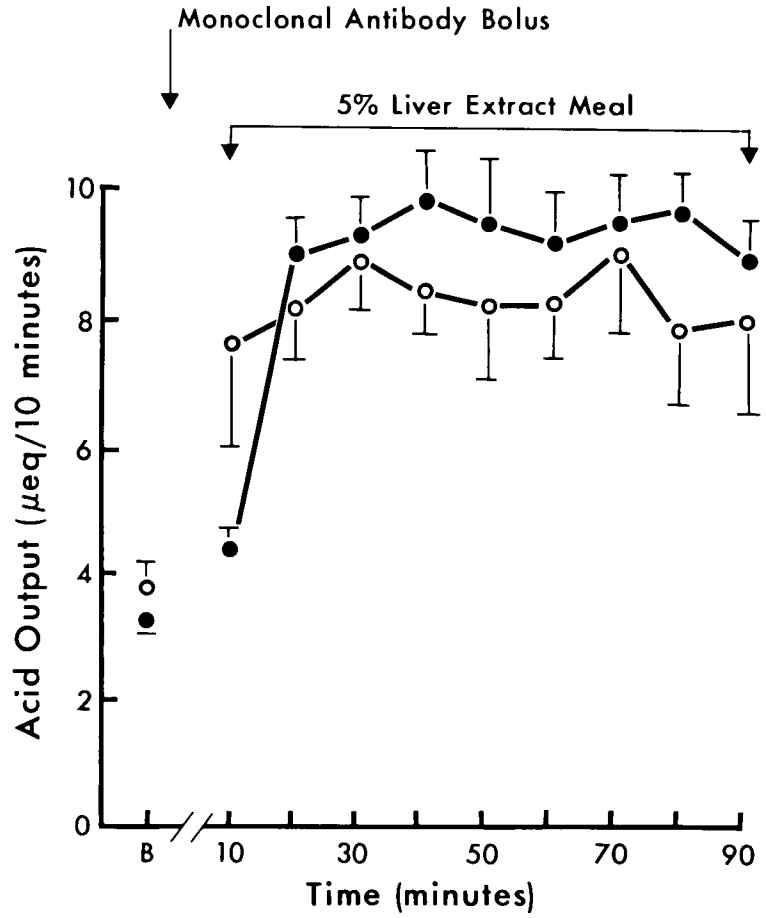

Monoclonal Antibody Bolus
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
10
8
6
4
2
0
B
10
30
50
70
90
Time (minutes)

Figure 41. Effect of a 100 µg bolus of monoclonal antibody clone S8 or S10 on the inhibition of gastric acid secretion by somatostatin

The antibody was given as an intravenous bolus 1 hour prior to the start of a liver extract meal and somatostatin infusion

closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with somatostatin infusion (n=10)

closed triangles : liver extract meal with monoclonal antibody clone S8 bolus and somatostatin infusion (n=5)

open triangles : liver extract meal with monoclonal antibody clone S10 bolus and somatostatin infusion (n=12)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)

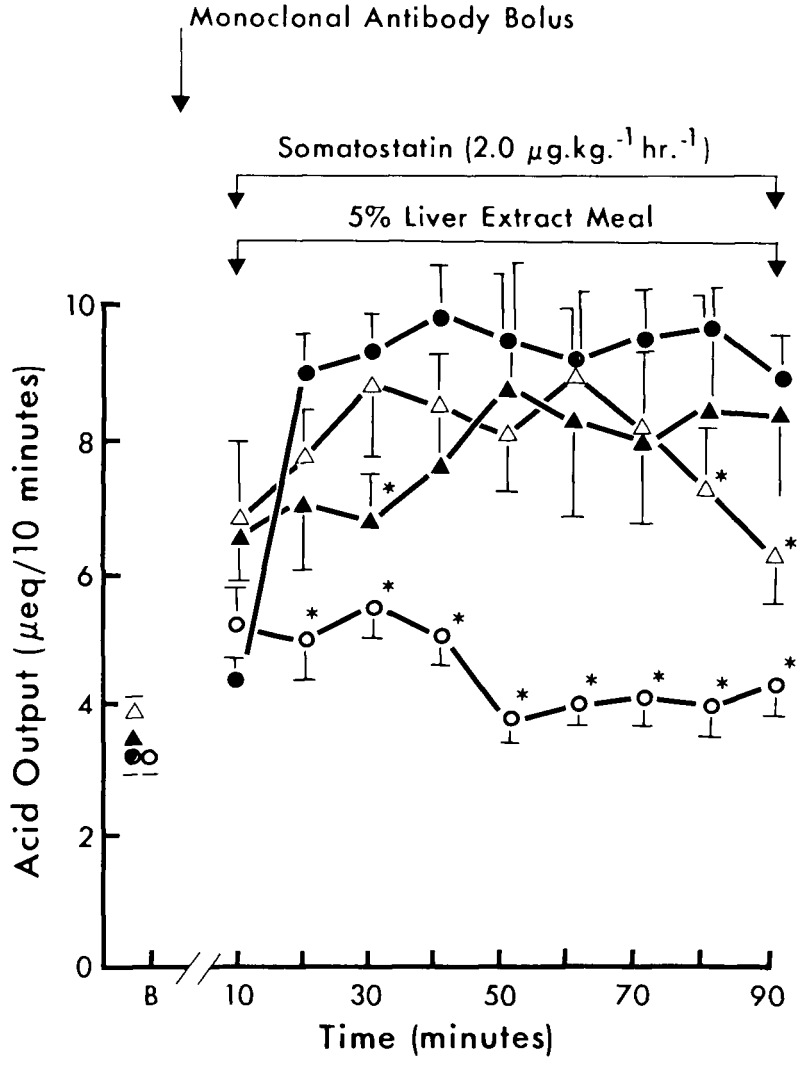

Monoclonal Antibody Bolus
Somatostatin (2.0 μg.kg.-1 hr.-1)
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
10
8
6
4
2
0
B
10
30
50
70
90
Time (minutes)

Figure 42. Effect of a 100 µg bolus of monoclonal antibody clone S20 on the inhibition of gastric acid secretion by somatostatin

The antibody was given as an intravenous bolus 1 hour prior to the start of a liver extract meal and somatostatin infusion

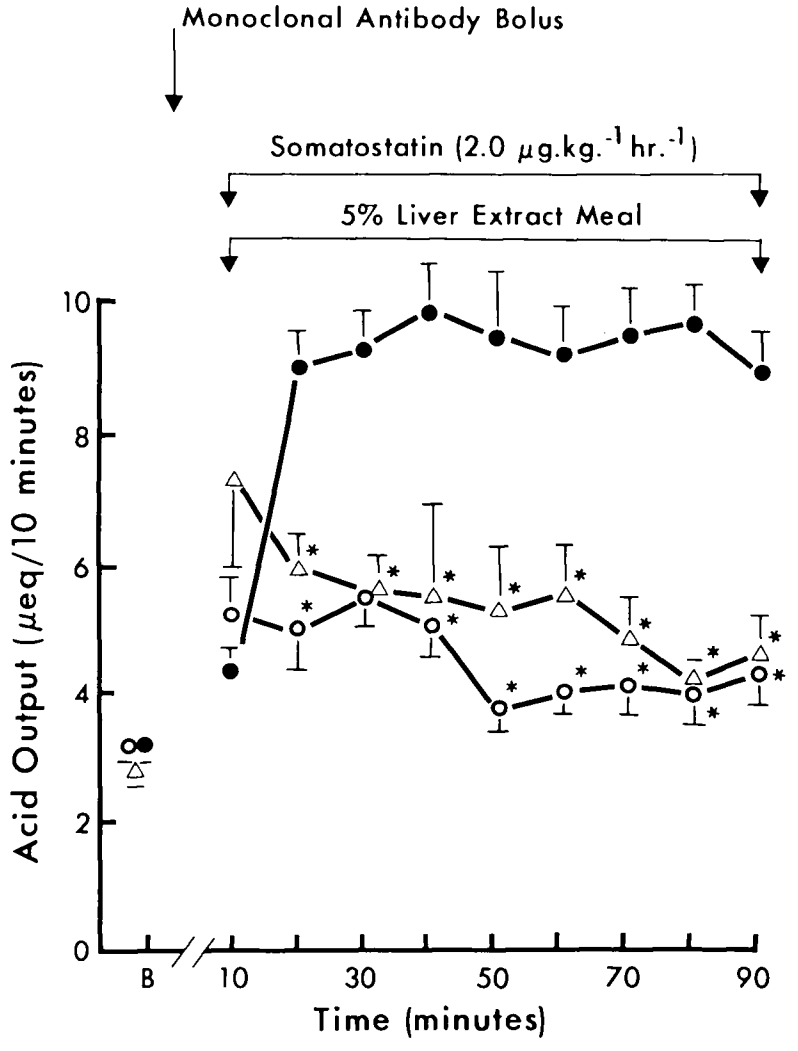
closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with somatostatin infusion (n=10)

triangles : liver extract meal with monoclonal antibody clone S20 bolus and somatostatin infusion (n=7)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)


Monoclonal Antibody Bolus
Somatostatin (2.0 μg.kg.-1 hr.-1)
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
10
8
6
4
2
0
B
10
30
50
70
90
Time (minutes)

C. Effect of a 100 µg bolus of monoclonal antibody clone S10 on the inhibition of meal-stimulated gastric acid secretion by GIP

Intravenous infusion of GIP at 0.2 $\mu g.kg.^{-1}hr.^{-1}$, produced an 60% reduction of the gastric acid secretory response to the liver extract meal (figure 26).

When the antibody clone S10 was given as an intravenous 100 µg bolus, 1 hour prior to the start of a liver extract meal and intravenous infusion of GIP at 0.2 $\mu g.kg.^{-1}hr.^{-1}$, the antibody had no effect on the inhibition of gastric acid secretion by GIP (figure 43).

Figure 43. Effect of a 100 µg bolus of monoclonal antibody clone S10 on the inhibition of gastric acid secretion by GIP

The antibody was given as an intravenous bolus 1 hour prior to the start of a liver extract meal and GIP infusion

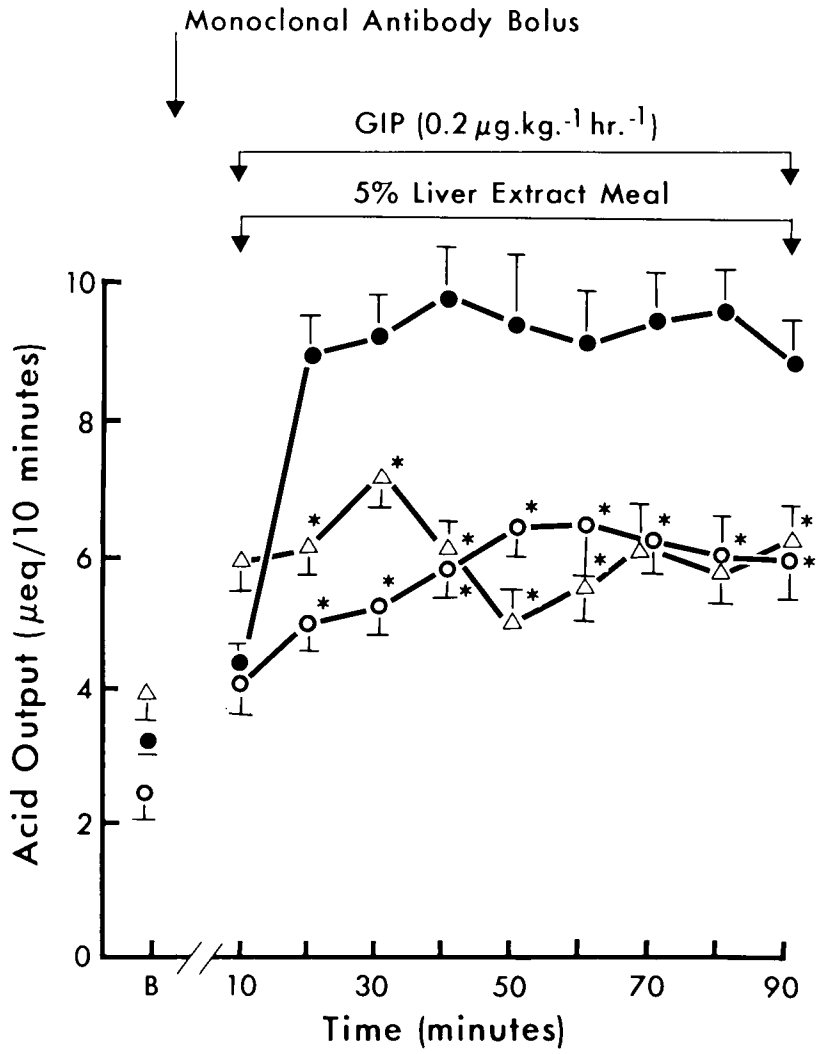
closed circles : liver extract meal alone (n=16)

open circles : liver extract meal with GIP infusion (n=8)

triangles : liver extract meal with monoclonal antibody clone S10 bolus and GIP infusion (n=7)

each point represents mean ± SEM

* denotes significant difference ($p < 0.05$)


Monoclonal Antibody Bolus
GIP (0.2 μg.kg.-1 hr.-1)
5% Liver Extract Meal
Acid Output (μeq/10 minutes)
0
2
4
6
8
10
B
10
30
50
70
90
Time (minutes)

DISCUSSION

Part I Inhibition of Gastric Acid Secretion

1. Effect of GIP on meal-stimulated gastric acid secretion in man

The role of GIP as an inhibitor of pentagastrin-stimulated gastric acid secretion in man has been assessed by several investigators (Cleator and Gourley 1975, Maxwell et al. 1980, Simmons et al. 1981). In 1975, Cleator and Gourley found that an intravenous bolus of GIP (2.0 $\mu g.kg.^{-1}$) produced a 60% inhibition of pentagastrin-stimulated gastric acid secretion in human subjects. However, after the bolus, the serum IR-GIP concentration were measured and found to be over twice the maximal stimulated level (>4000 $pg.ml.^{-1}$).

Maxwell et al. (1980) demonstrated that an intravenous infusion of GIP at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, during pentagastrin stimulation in man, produced negligible inhibition of gastric acid secretion, although serum IR-GIP levels were elevated above 6000 $pg.ml.^{-1}$. In a similar study performed on previously vagotomized patients, a GIP infusion of 2.0 $\mu g.kg.^{-1}hr.^{-1}$ which elevated IR-GIP levels above 2500 $pg.ml.^{-1}$, also failed to inhibit pentagastrin-stimulated acid secretion (Simmons et al. 1981).

In humans, with either intact stomachs or after vagal denervation, GIP appears to have little or no inhibitory effect on pentagastrin-stimulated acid secretion, unless infused at a supraphysiological dose. The effect of GIP on meal-stimulated acid secretion in man however, was unknown.

In this study, an 8% peptone meal was used as the stimulus for acid secretion in man. Instillation of the peptone meal into the stomach

stimulates acid secretion by : 1. gastric distension (a. of the antrum, resulting in the release of gastrin, b. of the fundus and body of the stomach, stimulating acid secretion independant of gastrin release, mediated by short intramural and long vagovagal neural reflexes) and 2. direct chemical interaction (stimulation of gastrin release, and a possible direct stimulation of the parietal cell) (Grossman 1967 and 1981).

The dose of 8% peptone chosen for the meal had previously been shown to be the dose of peptone capable of producing maximal or near maximal gastric acid secretion in humans (Lam et al. 1980).

The acid secretory response to the meal varied greatly between test subjects. In order to compare acid secretion, the acid output for a single study, which comprised of 4 sequential 45 minute peptone meals, was expressed as a percentage of the maximal acid output obtained for any 1 meal during that study. This normalization allowed the tabulation of acid output data from all subjects, and subsequent assessment of the effect of an intravenous infusion of GIP on acid secretion.

In the control study, the peptone meal produced a 4 to 5 fold increase in acid output over basal during the first 45 minute period, which continued throughout the meal stimulus. Infusion of GIP at doses of 1.0 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$during the peptone meal, produced a significant 30% reduction in acid output. However, infusion of GIP at 1.0 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$ produce plasma IR-GIP concentrations which were elevated above the normal maximal IR-GIP levels measured in humans during a meal or intraduodenal fat stimulus (Kuzio et al. 1974, Cleator and Gourley 1975).

Infusion of GIP at 0.5 $\mu g.kg.^{-1}hr.^{-1}$ produced plasma IR-GIP levels within the normal physiological range, but did not significantly inhibit the gastric acid secretory response to the meal.

This study demonstrates that GIP is a weak inhibitor of meal-stimulated gastric acid secretion in humans when given in supraphysiological doses. When administered at a dose which produces less than the normal maximal physiological plasma level, GIP has little effect on the acid secretory response to the meal.

The effect of GIP on meal-stimulated gastrin release remains unclear. Villar et al. (1976a), demonstrated that an exogenous GIP infusion inhibited meal- stimulated gastrin release in the dog. In a similar model, Yamagishi and Debas (1980) found that exogenously administered GIP was without significant effect on the gastrin response.

Peptone meal stimulation of gastric acid secretion, allowed the examination of the effect of GIP on meal-stimulated plasma gastrin release in humans. Gastric instillation of the meal produced a 3 fold increase in plasma gastrin levels over basal. Intravenous infusion of GIP, at 0.5, 1.0 and 2.0 $\mu g.kg.^{-1}hr.^{-1}$, had no effect on plasma gastrin levels during the peptone meal stimulus. GIP does not appear therefore, to have a significant effect on meal-stimulated release of plasma gastrin in man.

GIP has been shown to stimulate the gastric release of somatostatin in the isolated perfused rat stomach (McIntosh et al. 1981a). This has led to the proposal that somatostatin may mediate, at least in part, the inhibitory effect of GIP on gastric acid secretion. In this human study, plasma SLI concentrations remained at basal levels throughout the meal stimulus, and were not affected by the intravenous infusion of GIP.

It has been postulated that somatostatin may act directly on the parietal cell, in a paracrine-like fashion, to inhibit gastric acid secretion (Larsson 1981). Such a local action of somatostatin, may explain the failure to observe an increase in peripheral plasma SLI concentration during the infusion of GIP.

2. Animal Model for Gastric Acid Secretion Studies

An anaesthetized rat model has been developed to study meal-stimulated gastric acid secretion. Liver extract meal stimulation, and measurement of acid secretion by extragastric titration of base, is complicated by the unstable nature of the meal. Accurate measurement of gastric H^+ secretion depends upon the stability of the pH of the meal solution.

When meal stimulation of acid secretion is performed using a "closed" system, with continuous, recirculating perfusion of the stomach and a meal reservoir where changes in pH are measured, any spontaneous change or drift in the pH of the meal results in the fictitious measurement of acid secretion. To eliminate the possibility of such a "pH drift" of the meal reserve, individual meals were collected over 10 minute periods, followed by the measurement of H^+ secretion by back titration with NaOH.

In addition, the use of 10 minute meals allowed the stimulation and measurement of acid secretion in several animal preparations simultaneously.

Gastric instillation of the meal resulted in a 3 fold increase in acid secretion over basal, which continued throughout the meal stimulus. The secretion of acid in response to the meal was similar to the acid output produced by an infusion of pentagastrin at 16 $\mu g.kg.^{-1}hr.^{-1}$ (calculated D_{50} for pentagastrin is 12.2 $\mu g.kg.^{-1}hr.^{-1}$).

The establishment of this model for meal-stimulated gastric acid secretion provided a sensitive, physiological bioassay, in which the effects of gastrointestinal peptides or intestinal perfusions could be studied.

3. Demonstration of an Enterogastrone Effect

Enterogastrone remains a poorly defined factor, which is liberated from intestinal mucosa by the presence of fat, acid or hypertonic solutions, and inhibits gastric secretion and motility. Infusion of fat into the proximal small bowel has proven to be a potent inhibitor of gastric acid secretion in a wide variety of animal models, and in man (Schneider 1958, Konturek and Grossman 1965b, Halvorson et al. 1966, Johnson and Grossman 1969, Long and LaVigne 1969, Bochenek et al. 1971, Schmidt-Wilcke 1975, Christiansen et al. 1976, Llanos et al. 1977, Yamagishi and Debas 1980, Creutzfeldt et al. 1983).

In this study, an intraduodenal infusion of oleic acid produced an 80% inhibition of the gastric acid secretory response to a liver extract meal. This inhibition of acid secretion by fat was independant of changes in meal-stimulated serum gastrin. There remains conflicting evidence with regards to the role which inhibition of gastrin release plays in fat induced inhibition of meal-stimulated gastric acid secretion. Yamagishi and Debas demonstrated in the dog, that an intraduodenal perfusion of fat completely suppressed meal-stimulated gastric acid secretion and significantly reduced gastrin release in response to the meal (Yamagishi and Debas 1980). Others however, have found that meal-stimulated plasma gastrin levels were not affected by intraduodenal infusion of fat, and have concluded that the inhibition of acid secretion by fat is not mediated by suppression of gastrin release (Christiansen et al. 1976, Llanos et al. 1977, Creutzfeldt et al. 1983).

Inhibition of gastric acid secretion by the presence of intraduodenal HCl has been well established (Anderson 1960, Wormsley and Grossman 1964, Bochenek et al. 1971, Konturek and Grossman 1965a and 1965b). In

this study, an intraduodenal infusion of hydrochloric acid (0.5 M HCl) produced a 60% inhibition of meal-stimulated acid secretion. Intraduodenal infusion of HCl was found to be less potent an inhibitor of gastric acid secretion than a similar infusion of fat. The differing degree of inhibition produced by HCl and by fat may be explained, in part, by the differences in the mechanisms which have been proposed for their actions.

Grossman has proposed, that the inhibition of acid secretion by HCL is likely mediated mainly by secretin released from the proximal small intestine. He supports this conclusion with the following points: 1. both acid in the intestine and exogenous secretin inhibit gastrin-stimulated acid secretion, but are almost completely ineffective against histamine-stimulated secretion; 2. graded doses of acid infused into the duodenum produce graded increases in pancreatic bicarbonate secretion and parallel graded increases in inhibition of gastrin stimulated acid secretion, both of which can be mimicked by graded doses of exogenous secretin; and 3. the kinetics of inhibition by acid in the duodenum and by exogenous secretin are both noncompetitive (Grossman 1981).

The mechanism for the inhibition of gastric acid secretion by intraduodenal fat remains unknown. Although intraduodenal fat is a known stimulus for secretin release into the circulation (Meyer and Jones 1974, Faichney et al. 1979), secretin does not appear to be the primary agent mediating the gastric inhibitory effect. Llanos et. al. (1977) found that they were able to infuse sodium oleate intraduodenally in the dog, at a rate capable of producing a 70% inhibition of meal-stimulated gastric acid secretion, but insufficient to produce a release of plasma secretin. In addition, intraduodenal infusion of fat has been shown to be a powerful inhibitor of histamine-stimulated gastric acid secretion (Halvorson et al. 1966, Johnson and Grossman 1969), whereas exogenous secretin infusion

has had little effect (Greenlee et al. 1957, Johnson and Duthie 1966, Chey et al. 1970).

The unknown principle, enterogastrone, is presumed to be a humoral agent (Gregory et al. 1967). As such, in order to affect acid secretion, it must be released into the circulation upon presentation of fat to the proximal small bowel mucosa. To date, such an inhibitory factor had not been demonstrated in the portal blood.

Cannulation of the portal vein in the rat, has allowed the collection of portal blood during an intraduodenal infusion of oleic acid, an infusion which produces almost total inhibition of meal-stimulated acid secretion. Intravenous bolus infusion of this portal serum (dissolved in 0.15 M NaCl after previous lyophilization) inhibited meal-stimulated acid secretion, independant of changes in peripheral gastrin release. This study confirms that following an intraduodenal infusion of fat, a humoral gastric acid inhibitory agent, or "enterogastrone", is released into the portal blood.

The degree of inhibition produced by this unknown factor, was less than the near total inhibition of meal-stimulated acid secretion seen during intraduodenal infusion of oleic acid. Failure of the portal serum to abolish the acid secretory response to a meal, may be due to the inability of the single intravenous bolus to create the serum concentration of the "inhibitory factor" produced by an intraduodenal fat infusion. Or perhaps, in the intact animal under physiological conditions, the gastric inhibition produced by intraduodenal fat may be mediated by both humoral and neural mechanisms in a synergistic fashion. Such a neural mechanism would not be operant during the inhibition of gastric acid secretion produced by infusion of portal serum. The existance of such neural and humoral synergy for the mediation of intestinal inhibition of gastric acid secretion remains to be determined.

The possibility that GIP may participate in the inhibitory action of intestinal fat on gastric acid has been investigated ever since its isolation by Brown et al. in 1969. Both ingestion, and intraduodenal infusion of fat have been shown to be potent stimuli for IR-GIP release (Pederson et al. 1975, Cleator and Gourley 1975, O'Dorisio et al. 1976). However, in this study a 30 minute intraduodenal infusion of oleic acid had little effect on portal serum IR-GIP concentrations. The gastric inhibitory effect of the portal serum collected after intraduodenal oleic acid, could not be explained by IR-GIP release.

Yamagishi and Debas (1980) measured simultaneously the effects of an intraduodenal infusion of oleic acid on meal-stimulated gastric acid secretion and IR-GIP release in the dog. They found that an intraduodenal infusion of oleic acid sufficient to give maximal or near maximal pancreatic protein secretion, produced significant inhibition of the acid secretory response to a liver extract meal by 20 minutes, and total inhibition by 1 hour. IR-GIP, measured in the peripheral serum throughout the study, rose only slightly above basal after the start of the intraduodenal oleic acid, and was found to be significantly increased only after 50 minutes of fat infusion. It appears that the pattern of the release of GIP in response to an intraduodenal infusion of fat, as measured in the peripheral serum of the dog, occurs too late to account for the early inhibitory action of fat on gastric acid secretion.

To examine further the effect of endogenously released GIP on gastric acid secretion, infusions of either oleic acid or glucose were performed during simultaneous liver extract meal stimulation of gastric acid secretion and collection of portal blood for RIA of IR-GIP. Numerous studies have demonstrated release of IR-GIP after either ingestion or intraduodenal infusion of glucose (Cataland et al.1974, Cleator and Gourley

1975, Pederson et al. 1975, Ebert et al. 1982, Creutzfeldt et al. 1983). Intraduodenal infusion of glucose has also been shown to reduce gastric acid secretion, but it is not as potent an inhibitor of acid secretion as fat (Konturek and Grossman 1965b, Creutzfeldt et al. 1983).

In this study, the intraduodenal infusion of oleic acid produced an early, significant reduction of the gastric acid secretory response to the meal, but only significantly elevated portal serum IR-GIP levels after 60 minutes of infusion. Intraduodenal glucose however, had little effect on gastric acid secretion, but produced a significant increase in portal serum IR-GIP 10 minutes after the start of the infusion. A comparison of the inhibition of meal-stimulated acid secretion produced by either intraduodenal infusion of glucose or oleic acid, with the release of IR-GIP in the portal serum, suggests that it is unlikely the release of IR-GIP plays a significant role in the mechanism for gastric inhibition by intestinal fat.

4. Gastrointestinal Peptides

Intravenous infusion of the gastrointestinal peptide GIP in innervated stomach preparations in the dog, and in man, has produced only weak inhibition of pentagastrin-stimulated gastric acid secretion (Debas and Yamagishi 1978, Soon-Shiong et al. 1979a, Maxwell et al. 1980, Simmons et al. 1981). In this study, an exogenous administration of pure porcine GIP in the rat prepared with an innervated stomach, resulted in an unexpected increase in the gastric acid secretory response to a pentagastrin infusion. A similar infusion of HPLC-purified GIP did not demonstrate this gastric stimulatory effect, and resulted in a normal pentagastrin-stimulated acid

secretory response. Administration of the pure GIP preparation during a saline meal without pentagastrin infusion, had no effect on acid secretion.

It would appear therefore, that a gastric stimulatory agent is present in the pure porcine GIP preparation, and removed during the process of HPLC-purification. The "unknown agent" seemed to enhance acid secretion already stimulated by pentagastrin, but did not stimulate acid secretion in the basal state. The presence of such a stimulant may obscure attempts to identify distinct gastric inhibitory actions for GIP. The existance of such a gastric stimulatory agent, present in pure GIP preparations and removed by HPLC-purification, remains to be confirmed.

Pure porcine GIP is an inhibitor of physiologically-stimulated gastric acid secretion from the innervated stomach. Exogenous administration of GIP in the intact rat model, which elevated serum IR-GIP 5 fold over basal levels, produced a 60% inhibition of the meal-stimulated acid secretory response. Other studies, performed in man (as described earlier in this work) and in the dog (Yamagishi and Debas 1980), confirm the inhibitory effect of GIP on meal-stimulated acid secretion in the innervated stomach, although IR-GIP concentrations measured during the GIP infusion in both of these studies were in the supraphysiological range.

The inhibitory effect on gastric acid secretion produced by an infusion of GIP was independant of meal-stimulated gastrin release. This was consistant with similar findings in studies performed in man (this work) and in the dog (Yamagishi and Debas 1980).

Exogenous GIP infused during a meal stimulus, demonstrates only a moderate degree of inhibition of acid secretion. Although producing maximal or supramaximal physiological levels of serum IR-GIP, GIP infusion has not inhibited the acid secretory response to the meal to the

same degree seen after intraduodenal infusion of oleic acid.

It is possible that the powerful inhibitory effect of intestinal fat may be mediated by several gastrointestinal peptides acting together. Similarily, potentiation of the action of one or more such peptides by a neural inhibitory mechanism may be required in order to abolish the acid secretory response to a meal. Such an inhibitory neural action would be absent during the infusion of a candidate "enterogastrone".

GIP, although capable of producing modest inhibition of meal-stimulated gastric acid secretion, is not likely to be the primary agent mediating the gastric acid inhibitory effect of intestinal fat.

Somatostatin has been shown to be one of the most potent inhibitors of gastric acid secretion in animals, and in man (Bloom et al. 1974, Barros et al. 1975, Gomez-Pan et al. 1975, Raptis et al. 1975, Konturek et al. 1976, Vant et al. 1977, Thomas 1980a and 1980b, Seal et al. 1982, Lin et al. 1983). In this study, an infusion of somatostatin at 2.0 $\mu g.kg.^{-1}hr.^{-1}$, failed to inhibit pentagastrin-stimulated gastric acid secretion. It is possible that the dose of pentagastrin chosen to stimulate acid secretion (the pentagastrin dose infused was 16 $\mu g.kg.^{-1}hr.^{-1}$, the calculated pentagastrin D_{50} was 12.2 $\mu g.kg.^{-1}hr.^{-1}$) may be responsible for the inability of somatostatin to inhibit acid secretion, as has been observed by others. Somatostatin infusion may inhibit acid secretion stimulated with a lower dose of pentagastrin in this model, but these experiments remain to be performed.

Exogenous somatostatin was a potent inhibitor of meal-stimulated acid secretion in this study. The role of somatostatin, in the mechanism of gastric acid secretion by intestinal fat, remains to be determined.

Part II Monoclonal Antibodies as Probes of Humoral Inhibitors of Gastric Acid Secretion

Both GIP and somatostatin antisera have been used as in vivo probes in the investigation of the physiological roles attributed to the endogenously released peptide to which they have been raised (Arimura et al. 1976, Ferland et al. 1976, Chihara et al. 1978, Ebert et al. 1979, Saffouri et al. 1979, Schusdziarra et al. 1980a, Chiba et al. 1981, Duvale et al. 1981, Lauritsen et al. 1981, Ebert and Creutzfeldt 1982, Wolfe et al. 1983a and 1983b, Short et al. 1985). Recently, monoclonal antibodies with a high affinity for either GIP or somatostatin, have been developed (Buchan et al. 1982 and 1985). In this study, demonstration of the inhibitory effect of exogenous GIP and somatostatin on meal-stimulated acid secretion in the rat model, permitted investigation of the ability of these new monoclonal antibodies to block in vivo the gastric inhibitory action of their respective peptides.

The monoclonal antibody 3.65H, demonstrated a high affinity for GIP when compared in vitro to the polyclonal rabbit antiserum R07 in the ELISA. When administered as a large intravenous bolus one hour prior to the meal stimulus in the rat, the antibody was unable to block the inhibitory effect of a GIP infusion on gastric acid secretion.

In an attempt to facilitate binding of the antibody to exogenous GIP, both the antibody and GIP were incubated together. Unbound GIP was then removed by charcoal adsorption, and the incubated preparation intravenously infused throughout the meal. An immunocytochemical technique was employed to verify the presence of GIP, and its binding to the antibody, in the infusate after charcoal separation. When the antibody preparation alone was applied to sections of small bowel, IR-GIP cells were demonstrated in the mucosa. However, when the antibody was

incubated with GIP prior to application on the sections, immunostaining of the IR-GIP cells was inhibited. This confirmed that GIP was present in the infusate and occupied antibody binding sites, which prevented detection of the IR-GIP cells. After acidification to free the GIP bound to the antibody, this solution once again successfully detected IR-GIP cells.

The monoclonal antibody, after incubation with GIP for 1 hour, was unable to block the inhibitory effect of the GIP on meal-stimulated gastric acid secretion. However, after incubation with the GIP for 18 hours prior to infusion, the antibody was able to successfully block exogenous GIP's inhibitory action.

The immunocytochemical study verified, that the GIP in the perfusate after a 1 hour incubation, was bound to the antibody at the time of the infusion. The mechanism by which the GIP was able to produce a gastric inhibitory effect once in the circulation, is not known. Dissociation (partial or complete) of the GIP from the antibody may have occurred. It is possible that 18 hours of incubation may stabilize the antibody-to-antigen binding, and once in the circulation prevent the dissociation and subsequent inhibitory effect of the GIP. Alternatively, binding of GIP to the antibody over the 18 hour incubation period may have produced steric hinderence of the peptide, with subsequent loss of biological activity.

The requirement of a prolonged period of incubation of the antibody with GIP, indicates that monoclonal antibody 3.65H would not be suitable for the study of the role of endogenously released GIP as an enterogastrone. It also suggests, that although an antibody may demonstrate a high affinity for the target peptide in vitro, the ability of the antibody to bind to, and subsequently block the biological action of the peptide in vivo must be verified.

Three somatostatin monoclonal antibodies, designated clones S8, S10

and S20, were investigated in the rat. Two of the antibodies, clones S8 and S10, which bind to the C-terminal sequence and to the whole somatostatin 14 molecule respectively, demonstrated in vivo the ability to bind to and subsequently block the inhibitory action of exogenously infused somatostatin on gastric acid secretion.

The 2 antibodies, S8 and S10, when administered intravenously as a bolus prior to the start of a liver extract meal, were found to produce a 30% inhibition of the acid secretory response to the meal. When given prior to the start of a meal and a somatostatin infusion, the inhibitory effect of the somatostatin on gastric acid secretion was prevented.

The mechanism for the small, but significant reduction in meal-stimulated acid secretion after bolus infusion of either S8 or S10 is not known. When the antibody, clone S20, was infused in a similar fashion prior to a meal, it did not significantly affect the acid secretory response, nor did it block the inhibitory effect of a somatostatin infusion on meal-stimulated acid secretion. The inhibitory effect of S8 and S10 does not therefore, appear to be a non-specific response in the animal to the infusion of a monoclonal antibody. This inhibition, seen only with the antibodies which were able to block the gastric action of somatostatin, may be related to the binding activity that is retained by the antibodies when in the circulation.

GIP, along with other members of the secretin-glucagon family of gastrointestinal peptides, have been shown to stimulate the release of gastric SLI (Chiba et al 1980a and 1980b, Gespach et al. 1980, Rouiller et al. 1980, Duvale et al. 1981, McIntosh et al. 1981a, Wolfe et al. 1983b). It has been suggested that the local release of SLI from the gastric D-cell may mediate the inhibitory effects of GIP on the parietal cell (McIntosh et al. 1981a). The demonstration of the ability of a somatostatin monoclonal

antibody to block in vivo the inhibitory effect of a somatostatin infusion on gastric acid secretion, provided a probe with which to investigate the role that somatostatin may have as a mediator of the inhibitory effect of GIP.

When given as a bolus infusion 1 hour prior to a liver extract meal and a GIP infusion, the somatostatin antibody S10 had no effect on the inhibitory action of the GIP on gastric acid secretion. One explanation for the failure of the somatostatin antibody to block the inhibitory effect produced by a GIP infusion, is that somatostatin may not be an intermediate step in the pathway for acid inhibition by GIP. It has been proposed however, that endogenous somatostatin may be acting directly at the parietal cell, being released into the interstitial space in a paracrine-like fashion (Larsson 1981). The antibody S10, has demonstrated that it is capable of blocking the action of somatostatin which is present in the circulation after infusion. In order for the antibody to block the local paracrine action proposed for endogenous somatostatin, the antibody must be able to reach the interstitial space of the gastric mucosa. The interstitial distribution of S10 shortly after infusion is not known. It has been proposed however, that it is unlikely such an infusion of antibody could rapidly reach the interstitial spaces to block the paracrine action of somatostatin (Schusdziarra et al 1980a). The ability of somatostatin antibody clone S10 to block the biological action of endogenously released gastric somatostatin remains to be determined.

The development of new monoclonal antibodies raised to specific gastrointestinal peptides will be followed by their increasing deployment in the investigation of proposed physiological roles for these peptides. The ability of the antibodies, however, to bind to corresponding peptides in vivo and block their physiological actions should first be verified.

Table i. Effect of a peptone meal on gastric acid secretion in man

a. time in minutes from start of peptone meal

	Acid secretion (mEq/15 minute period)				
a	A	B	C	D	E
-30	0.6	0.4	1.0	1.5	0.5
0	0.5	0.3	0.6	0.2	0.4
15	1.3	0.0	5.0	0.8	4.1
30	1.6	0.0	8.8	1.8	5.5
45	1.1	1.7	6.0	1.1	6.4
60	0.6	2.6	8.2	1.8	6.0
75	1.1	3.2	7.1	1.8	8.4
90	1.1	1.2	7.7	1.8	9.1
105	0.7	0.9	3.3	2.3	4.0
120	0.8	1.3	7.3	2.4	6.7
135	0.7	5.5	6.6	1.9	6.6
150	0.7	0.9	3.5	1.4	5.8
165	0.7	3.1	8.4	2.2	5.5
180	0.9	2.3	6.8	2.2	7.5

Table ii. Effect of a peptone meal and GIP infusion on gastric acid secretion in man

a. time in minutes from start of peptone meal

b. dose of GIP infused intravenously ($\mu g.kg.^{-1}hr^{-1}$)

		Acid secretion (mEq/15 minute period)				
a	b	A	B	C	D	E
-30		0.4	0.1	0.2	0.1	0.3
0		0.4	0.1	0.5	3.6	0.5
15		0.5	1.2	3.1	2.1	3.9
30	0.0	3.0	3.8	5.7	1.8	8.7
45		2.0	4.6	6.5	1.6	7.8
60		1.7	3.2	6.2	2.4	4.8
75	0.5	0.3	3.8	7.2	2.8	4.7
90		1.0	3.6	5.9	3.9	6.0
105		1.0	3.1	4.3	2.3	5.8
120	1.0	1.3	3.6	5.6	2.9	3.8
135		1.1	3.4	2.9	2.3	4.2
150		1.1	2.4	2.6	2.5	2.7
165	2.0	1.3	4.3	3.0	3.2	2.8
180		1.4	2.6	3.1	2.1	5.0

Table iii. Plasma IR-GIP concentration during a peptone meal in man

a. time in minutes from start of peptone meal

	Plasma IR-GIP (pg.100ml^{-1})				
	A	B	C	D	E
a.					
-30	22	46	59	50	20
0	34	30	49	19	0
15	37	30	44	38	13
30	22	32	30	18	8
45	15	100	43	42	8
60	22	29	40	36	0
75	15	27	23	28	0
90	24	19	37	21	13
105	26	19	28	33	18
120	14	35	62	56	17
135	5	19	29	45	0
150	24	31	22	22	0
165	20	27	40	43	8
180	10	32	38	36	23

Table iv. Plasma IR-GIP concentration during a peptone meal and GIP infusion in man

a. time in minutes from start of peptone meal

b. dose of GIP infused intravenously ($\mu g.kg.^{-1}hr^{-1}$)

		Plasma IR-GIP ($pg.100ml^{-1}$)					
a	b	A	B	C	D	E	F
-30		37	70	58	15	76	100
0		50	65	48	40	51	154
15		35	60	76	28	72	152
30	0.0	21	57	74	31	57	108
45		53	63	54	22	64	90
60		95	108	230	62	130	210
75	0.5	57	133	185	82	200	240
90		64	168	285	86	240	350
105		125	205	320	96	370	460
120	1.0	75	320	>400	90	470	525
135		95	360	>400	185	525	875
150		120	525	>400	255	700	825
165	2.0	300	775	>400	390	710	750
180		190	870	>400	260	710	1350

Table v. Plasma gastrin response to a peptone meal in man

a. time in minutes from start of peptone meal

	Plasma Gastrin ($pg.ml^{-1}$)				
	A	B	C	D	E
a.					
-30	29	46	85	16	35
0	28	27	81	72	30
15	85	112	38	22	74
30	74	118	25	16	65
45	51	78	19	21	65
60	53	88	25	23	66
75	54	89	36	30	64
90	54	130	42	17	53
105	51	135	43	27	52
120	34	126	25	18	58
135	36	123	31	22	52
150	39	123	48	28	58
165	48	124	51	19	76
180	49	170	42	32	56

Table vi. Plasma gastrin response to a peptone meal and GIP infusion in man

a. time in minutes from start of peptone meal

b. dose of GIP infused intravenously ($\mu g.kg.^{-1}hr^{-1}$)

		Plasma Gastrin ($pg.ml^{-1}$)				
a	b	A	B	C	D	E
-30		33	25	18	27	33
0		40	46	12	18	46
15		83	128	21	31	72
30	0.0	85	117	24	29	74
45		105	91	31	25	72
60		60	88	32	27	64
75	0.5	57	100	27	37	62
90		51	106	25	28	52
105		63	116	22	27	55
120	1.0	42	112	30	57	56
135		56	90	33	28	48
150		48	106	15	21	72
165	2.0	49	106	29	27	52
180		42	125	47	23	60

Table vii. Plasma SLI concentration during a peptone meal in man

a. time in minutes from start of peptone meal

	Plasma SLI ($fmol.ml^{-1}$)				
	A	B	C	D	E
a.					
-30	24	31	37	30	29
0	28	36	34	27	36
15	31	31	32	30	38
30	36	31	30	26	30
45	40	39	29	22	22
60	19	33	28	26	30
75	34	33	30	28	31
90	26	38	29	15	39
105	—	36	28	23	40
120	23	28	30	30	42
135	18	34	18	26	38
150	27	35	31	33	34
165	27	21	35	29	34
180	19	31	24	28	41

Table viii. Plasma SLI concentration during a peptone meal and GIP infusion in man

a. time in minutes from start of peptone meal

b. dose of GIP infused intravenously ($\mu g.kg.^{-1}hr^{-1}$)

		Plasma SLI ($fmol.ml^{-1}$)				
a	b	A	B	C	D	E
-30		46	22	19	30	44
0		46	25	13	28	34
15		35	13	23	23	48
30	0.0	25	35	20	35	40
45		28	23	21	33	44
60		23	27	12	35	54
75	0.5	18	33	28	30	38
90		29	27	19	36	49
105		31	32	20	31	45
120	1.0	20	29	33	54	15
135		19	16	33	34	39
150		21	23	10	33	43
165	2.0	28	27	12	34	40
180		21	26	21	42	50

Table ix. Effect of a saline meal on gastric acid secretion

		Acid output (μEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	2.4	3.6	3.6	4.6	4.6	3.9	4.0	4.9	3.8	3.6
2.	2.6	3.0	2.7	2.8	2.5	2.7	3.0	4.1	4.7	4.8
3.	2.6	4.4	3.2	3.5	3.3	2.8	3.4	3.1	3.3	3.4
4.	3.3	3.8	3.8	3.8	3.4	2.9	2.1	2.6	2.4	2.1
5.	2.4	2.4	3.1	3.8	3.5	3.6	3.7	4.1	3.7	3.7
6.	3.6	2.7	3.7	3.4	3.0	2.6	3.4	4.0	3.7	3.7

Table x. Pentagastrin dose-response stimulation of gastric acid secretion

		Acid output (μEq/10 minute period)							
		pentagastrin infusion ($\mu g.kg.^{-1}hr.^{-1}$)							
no.	B	0.5	1.0	2.0	4.0	8.0	16	32	64
1.	3.5	4.8	5.6	8.1	10.1	14.7	15.8	11.9	19.1
		5.4	6.9	9.0	13.0	14.8	16.2	13.8	24.0
		4.9	8.0	9.7	13.0	14.3	16.0	13.8	26.5
2.	2.3	3.7	3.6	3.3	4.4	9.6	14.1	11.5	21.8
		3.7	3.4	3.0	5.3	11.5	16.3	7.3	25.4
		3.3	3.3	4.9	6.0	7.8	14.0	10.9	29.1
3.	1.9	3.2	3.5	6.4	5.7	11.4	12.6	9.9	15.3
		3.3	4.9	7.9	7.2	11.6	12.7	6.8	20.5
		3.0	6.2	8.5	9.6	10.0	13.5	11.0	9.6
4.	2.7	3.8	4.3	7.9	10.8	10.6	17.2	18.3	28.5
		4.0	5.8	8.7	10.8	14.7	13.4	13.4	27.5
		4.0	7.5	10.8	10.8	14.2	17.6	12.3	31.7
5.	3.5	3.0	4.5	6.0	8.0	9.5	11.0	16.5	23.0
		3.0	6.0	7.0	8.5	10.0	13.0	23.0	23.0
		3.0	6.0	8.0	9.0	11.0	14.0	23.0	26.0
6.	2.0	2.5	4.0	4.0	5.5	9.5	12.0	15.0	19.5
		3.0	4.0	5.5	7.5	10.5	14.5	16.5	20.0
		3.5	4.0	6.0	8.5	10.5	14.5	17.0	20.0

Table x. continued

Acid output (μEq/10 minute period)

		pentagastrin infusion ($\mu g.kg.^{-1}hr.^{-1}$)							
no.	B	0.5	1.0	2.0	4.0	8.0	16	32	64
7.	1.5	2.0	4.0	3.5	4.5	8.5	11.5	17.0	21.0
		3.0	4.0	4.0	5.5	9.5	14.0	17.0	25.0
		3.5	4.0	4.0	7.0	11.0	14.5	22.0	25.0
8.	1.0	2.0	5.0	5.0	6.0	6.5	9.5	12.0	15.5
		3.5	4.5	5.0	6.0	8.0	11.0	14.0	16.5
		5.5	5.0	5.5	6.0	9.0	12.0	14.5	16.0

Table xi. Effect of an intravenous infusion of pentagastrin (16 $\mu g.kg.^{-1}hr.^{-1}$) on gastric acid secretion

		Acid output (µEq/10 minute period)					
no.	B	1	2	3	4	5	6
1.	1.9	5.3	10.5	8.0	10.3	7.5	8.1
2.	1.2	5.3	7.6	8.1	7.4	7.3	8.3
3.	1.2	1.2	3.1	3.4	3.6	6.6	7.4
4.	2.3	6.6	12.0	9.7	12.8	13.0	14.9
5.	1.4	4.6	5.0	6.9	5.7	7.2	8.3
6.	3.2	6.9	6.3	6.8	8.6	7.7	7.5
7.	3.2	7.3	11.8	9.6	11.9	11.9	10.8
8.	2.2	5.3	8.5	7.6	8.5	8.9	8.8
9.	2.8	3.8	6.0	14.1	11.6	5.8	7.5
10.	2.1	6.3	9.0	9.8	8.4	8.2	8.5
11.	3.6	5.1	7.4	8.1	10.4	18.9	17.3
12.	2.0	8.4	7.9	7.4	7.2	8.1	8.8
13.	1.3	2.9	4.4	5.6	8.2	9.0	6.6
14.	1.0	4.3	8.9	8.3	10.3	15.9	20.5
15.	3.3	5.9	5.0	5.2	4.2	4.6	3.8
16.	1.8	4.0	6.5	6.9	5.3	6.4	6.9
17.	0.9	1.8	3.7	4.0	4.6	5.5	6.2
18.	0.7	3.0	4.6	4.5	7.0	7.6	3.9
19.	3.3	9.0	12.2	13.1	15.6	15.4	14.6
20.	1.5	5.3	10.5	16.3	14.4	15.1	12.1
21.	2.4	5.6	10.5	13.0	14.3	17.3	14.1

Table xi. continued

		Acid output (µEq/10 minute period)					
no.	B	1	2	3	4	5	6
22.	2.3	2.1	4.3	5.4	4.1	6.6	5.8
23.	1.4	6.6	9.4	7.9	7.2	7.9	7.7
24.	1.7	3.4	4.9	5.1	5.4	4.6	4.2
25.	0.8	1.5	3.6	4.7	5.1	4.4	3.5
26.	1.8	5.6	7.1	6.2	6.5	7.0	6.4
27.	1.1	5.8	8.8	7.6	9.0	7.4	2.3
28.	2.4	5.1	6.1	6.0	9.2	6.1	5.2
29.	3.0	4.7	6.2	5.8	6.4	6.5	7.0
30.	1.8	3.1	5.5	4.6	4.8	4.3	5.1

Table xii. Effect of a 5% liver extract meal on gastric acid secretion

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	0.7	2.6	7.4	9.0	10.2	10.5	7.5	9.1	12.4	8.8
2.	2.9	4.2	6.5	4.9	8.0	7.3	11.2	9.5	6.6	9.2
3.	3.1	7.1	10.6	8.0	6.9	7.8	6.7	7.7	7.9	7.4
4.	3.1	4.0	5.2	8.6	9.8	13.2	12.6	14.5	13.4	14.5
5.	3.1	6.0	10.0	8.2	9.1	10.4	8.3	8.8	11.4	12.0
6.	3.8	6.9	7.7	8.4	6.4	6.0	4.9	6.0	7.0	8.2
7.	3.3	5.4	10.5	13.0	11.1	13.8	8.0	12.3	8.4	8.5
8.	3.7	4.4	10.2	9.7	10.8	11.2	10.6	9.3	11.5	9.5
9.	3.6	5.9	10.7	11.7	10.2	10.3	9.2	7.4	8.0	4.6
10.	1.2	3.3	7.6	8.0	8.1	6.2	8.1	7.6	7.3	4.1
11.	3.1	5.0	12.0	11.4	9.6	6.9	10.1	9.8	8.8	6.2
12.	3.2	—	10.1	11.9	18.7	21.0	16.6	15.3	13.6	10.6
13.	2.1	1.7	6.5	9.0	11.4	9.9	11.7	11.0	12.0	10.7
14.	2.3	1.4	9.4	10.7	11.3	7.7	9.7	10.5	10.7	10.1
15.	1.6	3.2	6.2	6.6	6.4	5.2	6.1	5.2	5.7	7.6
16.	3.0	4.6	14.0	9.6	10.2	4.5	5.2	9.1	10.5	10.8

Table xiii. Effect of an intraduodenal infusion of oleic acid on gastric acid secretion

		Acid output (μEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	3.2	5.0	6.3	5.4	3.7	3.7	3.6	3.3	3.2	3.0
2.	1.4	4.2	5.8	5.8	4.0	4.8	3.3	5.5	5.2	4.1
3.	1.9	5.2	6.3	6.6	5.5	5.1	5.2	4.2	3.5	3.3
4.	3.3	6.8	6.4	6.9	6.4	6.4	5.3	6.6	6.2	6.1
5.	3.2	3.7	4.1	5.0	5.2	3.3	4.1	3.0	4.2	5.1
6.	3.2	6.4	10.4	4.8	3.4	5.7	5.0	4.0	2.2	2.9
7.	2.4	2.8	4.5	4.6	6.0	6.3	6.8	6.5	5.5	7.4
8.	1.7	5.4	5.6	5.2	5.8	6.0	6.0	5.4	6.5	5.9

Table xiv. Effect of an intraduodenal infusion of 0.5 M HCl on gastric acid secretion

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	2.8	6.0	8.0	6.8	6.1	6.5	8.0	3.1	6.9	7.0
2.	1.2	8.5	7.2	6.8	7.8	3.7	7.5	7.5	7.6	5.7
3.	1.8	5.8	5.7	7.5	7.1	6.8	5.2	6.0	5.6	5.7
4.	2.7	7.0	6.4	6.7	7.3	5.9	7.4	7.4	10.8	6.0
5.	2.7	6.9	6.6	6.2	5.2	5.1	3.8	5.8	5.7	6.6
6.	1.9	4.9	3.4	4.6	5.2	3.5	2.4	5.2	5.4	10.8
7.	3.6	2.2	4.1	6.6	6.0	7.0	6.2	7.2	5.2	6.6
8.	3.6	6.8	6.8	7.1	6.8	7.7	6.3	7.0	6.8	8.3
9.	2.8	5.5	5.7	6.4	5.3	3.5	3.7	5.8	6.1	5.1
10.	3.5	5.6	8.5	7.8	9.2	2.4	9.1	8.9	8.5	9.1
11.	4.8	11.7	7.5	7.4	6.9	7.9	5.7	5.6	8.5	8.9

Table xv. Effect of an intravenous bolus of portal serum on a normal saline meal

a. infusion of portal serum collected after intraduodenal infusion of oleic acid

Acid output (μEq/10 minute period)

no.	B	1	2	3	4	5	6	7	8	9
1.	2.3	2.5	2.3	1.9	1.2	1.3	1.6	1.5	1.8	2.3
2.	2.7	2.3	2.1	2.3	1.6	1.7	2.0	1.9	2.2	2.1
3.	3.2	4.6	4.6	2.9	4.6	3.9	3.8	3.6	4.5	4.5
4.	4.1	3.1	4.8	4.5	3.5	4.5	4.1	3.7	3.1	3.3
5.	2.6	2.4	2.6	2.6	2.4	2.5	2.4	2.2	2.1	2.1
6.	2.7	3.2	3.5	2.5	2.0	2.5	2.3	2.7	2.0	2.5

b. infusion of control portal serum (no intraduodenal oleic acid)

Acid output (μEq/10 minute period)

no.	B	1	2	3	4	5	6	7	8	9
1.	1.4	1.7	1.2	1.1	1.1	1.3	1.1	1.4	1.6	1.2
2.	3.4	3.5	3.3	3.0	3.2	3.1	3.2	2.8	2.8	2.7
3.	2.3	2.6	2.1	2.1	2.3	2.6	2.8	3.5	3.7	3.7
4.	2.5	5.7	5.5	6.0	5.6	4.8	4.9	5.3	6.1	6.7

Table xvi. Effect of an intravenous bolus of portal serum (collected after intraduodenal oleic acid infusion) on meal-stimulated acid secretion

Portal serum bolus given after 40 minutes of meal stimulation

		Acid output (μEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.4	6.8	8.0	7.1	9.5	6.4	7.8	6.1	4.7	6.8
2.	2.2	6.1	6.7	6.5	7.8	6.5	6.0	7.0	6.7	5.0
3.	2.7	5.8	6.5	6.5	6.5	5.2	5.4	4.9	5.5	4.9
4.	3.2	5.9	6.0	4.4	5.3	2.7	3.3	4.4	2.4	7.6
5.	5.1	—	11.1	11.5	14.9	12.2	7.0	6.5	6.6	5.4
6.	1.9	5.5	7.6	8.6	8.6	5.7	6.6	4.7	6.0	5.4
7.	3.9	5.5	6.1	—	6.8	6.8	6.4	5.1	5.6	5.7

Table xvii. Effect of an intravenous bolus of portal serum (without prior intraduodenal oleic acid infusion) on meal-stimulated acid secretion

Portal serum bolus given after 40 minutes of meal stimulation

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	2.8	4.0	5.0	5.8	4.5	6.9	6.5	6.0	8.1	8.0
2.	2.0	6.0	8.7	11.0	9.4	8.8	8.4	9.6	10.9	10.0
3.	2.3	2.5	7.1	9.4	10.6	13.3	18.5	17.6	19.3	22.6
4.	3.8	3.3	6.0	6.8	5.5	7.0	7.0	5.0	5.0	5.2
5.	4.3	7.4	7.4	5.6	8.4	8.2	7.8	8.7	7.7	6.7
6.	2.9	2.3	3.5	4.4	5.4	4.7	5.4	5.4	4.5	4.6
7.	3.8	5.6	9.3	9.6	9.5	11.7	10.9	13.9	11.7	13.1

Table xviii. Portal serum IR-GIP levels

a. portal serum collected after intraduodenal oleic acid infusion

b. portal serum collected without prior intraduodenal oleic acid infusion

	a	b		a	b
1.	300	370	11.	195	1200
2.	315	280	12.	130	120
3.	830	130	13.	750	2400
4.	340	68	14.	290	340
5.	150	320	15.	1400	210
6.	260	120	16.	400	640
7.	900	500	17.	700	800
8.	580	360	18.		680
9.	82	700	19.		460
10.	580	250	20.		215

Table xix. Effect of an intraduodenal infusion of oleic acid or glucose on gastric acid secretion and portal release of IR-GIP

a. liver extract meal alone

Acid output (μEq/10 minute period)

no.	B	1	2	3	4	5	6
1.	4.6	8.3	11.0	11.6	8.3	6.6	5.9
2.	4.1	8.6	6.6	10.2	7.3	6.4	5.6
3.	5.1	3.5	5.5	8.3	6.3	6.2	6.1
4.	2.6	13.1	8.0	7.5	4.7	6.8	6.0

Portal IR-GIP ($pg.ml.^{-1}$)

no.	B	1	2	3	6
1.	185	280	280	480	420
2.	215	170	450	150	320
3.	240	250	185	165	175
4.	290	380	—	170	280
5	300	425	90	180	180

table xix. continued

b. liver extract meal with intraduodenal glucose infusion

Acid output (μEq/10 minute period)

no.	B	1	2	3	4	5	6
1.	5.1	3.5	5.5	8.3	6.3	6.2	6.1
2.	4.2	7.8	8.2	8.1	5.0	3.7	2.8
3.	3.9	3.2	7.2	8.6	6.4	6.4	5.4
4.	4.8	8.9	13.7	13.2	9.8	7.9	7.4

Portal IR-GIP ($pg.ml.^{-1}$)

no.	B	1	2	3	6
1.	370	330	380	700	630
2.	220	900	660	660	900
3.	280	1000	920	950	410
4.	130	590	690	930	410

table xix. continued

c. liver extract meal with intraduodenal oleic acid infusion

Acid output (μEq/10 minute period)

no.	B	1	2	3	4	5	6
1.	1.0	2.3	2.8	2.2	2.1	1.2	0.2
2.	1.5	4.3	4.9	3.0	2.7	1.2	1.2
3.	2.9	2.3	3.8	3.5	2.5	0.9	1.4
4.	3.9	5.3	5.6	4.7	4.7	3.4	4.8
5.	3.4	4.5	2.7	2.1	3.2	—	2.5

Portal IR-GIP ($pg.ml.^{-1}$)

no.	B	1	2	3	6
1.	—	450	320	370	960
2.	210	120	530	680	410
3.	120	490	380	630	550
4.	230	230	235	320	240
5.	190	115	165	230	550

Table xx. Effect of an intravenous infusion of GIP (0.2 $\mu g.kg.^{-1}hr.^{-1}$) on acid secretion during a normal saline meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	3.6	3.5	3.3	3.4	2.7	4.1	3.4	2.1	3.1	2.5
2.	3.6	7.2	4.5	3.5	3.5	2.8	3.1	2.6	2.1	2.3
3.	5.7	7.0	4.4	3.9	3.0	2.7	2.6	2.0	2.0	2.4
4.	5.0	5.2	4.1	5.0	4.8	3.5	4.2	5.0	4.8	4.6
5.	2.7	4.4	2.6	3.2	2.3	2.5	3.0	4.4	3.1	3.7
6.	2.2	2.7	2.3	3.7	2.5	1.8	1.8	4.3	5.3	6.5

Table xxi. Effect of an intravenous infusion of GIP (2.0 $\mu g.kg.^{-1}hr.^{-1}$) on pentagastrin-stimulated (16 $\mu g.kg.^{-1}hr.^{-1}$) gastric acid secretion

		Acid output (µEq/10 minute period)					
no.	B	1	2	3	4	5	6
1.	0.9	3.6	9.2	14.0	17.0	16.1	16.6
2.	2.7	6.0	8.8	7.1	8.5	8.8	9.2
3.	2.3	6.3	7.4	6.7	7.8	8.1	9.6
4.	3.6	11.8	19.1	18.7	18.7	19.9	18.7
5.	1.2	4.6	5.7	6.1	8.5	11.6	10.5
6.	2.0	4.6	10.0	10.3	12.2	11.9	12.3
7.	0.2	9.1	19.1	13.7	13.1	14.4	10.5
8.	1.8	5.5	8.0	8.8	7.2	8.0	8.6
9.	3.6	8.2	11.2	11.0	10.6	14.1	23.2
10.	2.2	4.6	5.9	7.2	9.7	9.9	7.8
11.	4.0	5.3	9.8	12.4	15.1	12.8	14.0
12.	1.6	1.6	5.9	7.0	5.6	5.3	5.4
13.	2.6	3.7	6.6	8.5	7.3	8.2	7.5
14.	1.4	3.8	7.4	8.9	8.8	8.1	7.5
15.	1.8	7.0	6.7	9.7	9.6	9.3	9.8
16.	3.1	9.1	12.0	13.6	13.0	14.6	14.5
17.	2.2	7.9	11.8	13.5	14.4	18.7	19.4
18.	1.9	4.4	10.6	12.9	12.6	13.8	14.2
19.	1.3	4.7	8.6	10.0	10.6	11.5	10.9
20.	0.4	3.2	3.1	4.2	5.8	6.0	6.7
21.	2.6	7.1	14.8	16.5	19.2	21.8	20.3

Table xxi. continued

		Acid output (µEq/10 minute period)					
no.	B	1	2	3	4	5	6
22.	4.0	12.1	14.3	11.2	15.8	18.6	17.6
23.	2.0	6.4	9.2	8.8	9.0	9.7	9.1
24.	1.0	2.4	7.3	8.6	10.7	13.2	13.7
25.	1.0	1.9	7.3	10.0	12.4	13.6	12.8
26.	1.0	1.1	4.6	10.6	10.9	11.5	9.0
27.	1.2	7.9	9.5	10.5	12.2	13.0	10.5
28.	1.1	5.0	6.2	7.0	6.1	6.9	7.8
29.	2.7	7.0	9.5	7.7	12.3	11.6	9.2

Table xxii. Effect of an intravenous infusion of HPLC purified-GIP ($2.0\ \mu g.kg.^{-1}hr.^{-1}$) on pentagastrin-stimulated ($16\ \mu g.kg.^{-1}hr.^{-1}$) gastric acid secretion

		Acid output (µEq/10 minute period)					
no.	B	1	2	3	4	5	6
1.	1.8	11.9	13.2	13.2	14.9	15.6	17.7
2.	2.0	2.2	9.2	12.8	10.6	11.9	11.9
3.	1.3	1.9	3.0	3.8	3.4	3.6	4.5
4.	1.7	2.9	12.2	10.2	9.5	10.1	10.4
5.	1.7	0.5	1.0	2.4	3.7	3.3	5.3
6.	0.7	—	4.9	5.2	8.2	8.2	10.2
7.	1.9	2.3	4.1	5.0	4.9	5.6	6.1

Table xxiii. Effect of an intravenous infusion of GIP (2.0 $\mu g.kg.^{-1}hr.^{-1}$) on meal-stimulated gastric acid secretion

		Acid output (μEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	1.4	5.0	7.5	10.2	7.5	8.1	7.5	12.4	14.8	13.5
2.	1.2	2.2	5.8	5.5	4.6	6.7	5.8	5.2	5.9	4.3
3.	3.6	8.0	6.7	5.1	9.1	6.6	7.0	6.9	8.4	8.5
4.	1.8	11.0	9.1	6.2	5.4	3.9	5.2	5.6	4.5	6.0
5.	1.8	5.9	4.1	2.8	3.4	3.4	2.9	3.4	3.7	3.3
6.	2.8	9.1	7.3	7.7	10.0	7.9	7.1	6.5	7.7	8.4
7.	2.1	3.1	6.9	6.9	10.4	7.3	8.9	9.6	7.4	7.2
8.	1.7	4.6	5.7	5.9	4.8	5.5	5.1	6.5	3.1	3.8

Table xxiv. Effect of an intravenous infusion of GIP (0.2 µg.kg.$^{-1}$hr.$^{-1}$) on meal-stimulated gastric acid secretion

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	2.8	4.4	4.5	6.7	5.6	6.4	7.2	5.6	5.1	7.6
2.	1.7	3.0	3.1	3.1	4.6	6.2	5.0	5.1	4.7	4.2
3.	3.0	4.1	4.5	4.8	5.0	6.0	5.1	5.2	5.3	4.9
4.	1.9	3.7	5.5	5.0	6.1	6.1	4.4	5.0	5.7	5.7
5.	2.2	4.3	4.4	5.5	7.5	8.6	8.7	7.3	8.6	5.7
6.	4.0	6.0	6.8	7.2	6.4	6.8	9.2	8.9	8.1	8.6
7.	1.7	3.6	4.9	4.8	5.4	4.7	3.4	5.7	2.5	4.0
8.	2.3	5.2	7.3	5.5	6.2	7.3	9.0	7.5	8.6	7.5

liver extract meal with GIP (0.2 µg.kg.$^{-1}$hr.$^{-1}$), after incubation for 18 hours at 4^{0}C

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	5.4	3.9	5.8	8.4	4.8	6.5	7.3	6.5	7.1	6.3
2.	5.5	6.4	8.9	8.8	7.4	7.4	7.5	6.2	6.1	6.2
3.	3.8	5.6	6.2	7.2	6.5	6.7	6.8	5.8	8.3	6.3
4.	2.4	5.0	2.3	5.1	5.4	3.6	4.7	2.9	4.8	4.5

Table xxv. Serum IR-GIP concentrations

a. basal levels

b. liver extract meal alone

c. liver extract meal with infusion of GIP (0.2 $\mu g.kg.^{-1}hr.^{-1}$)

	a	b	c
no.			
1.	100	450	1200
2.	470	265	1170
3.	310	120	2100
4.	240	265	1600
5.			1330
6.			1220

Table xxvi. Effect of an intravenous infusion of somatostatin ($2.0\ \mu g.kg.^{-1}hr.^{-1}$) on acid secretion during a normal saline meal

	Acid output (μEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.4	4.9	5.8	6.8	7.2	6.7	5.0	5.9	5.5	5.8
2.	4.0	3.3	3.3	2.8	2.8	2.1	1.9	1.9	2.0	1.3
3.	2.7	2.7	3.5	2.8	2.8	1.9	2.7	2.1	2.0	1.8
4.	3.2	4.3	2.5	3.5	3.5	1.7	3.2	2.0	2.7	2.2
5.	3.8	4.3	3.3	2.3	2.4	2.5	2.2	1.9	2.2	2.2
6.	3.8	4.8	4.4	5.0	4.4	3.9	4.0	4.1	3.6	3.6

Table xxvii. Effect of an intravenous infusion of somatostatin (2.0 $\mu g.kg.^{-1}hr.^{-1}$) on pentagastrin-stimulated (16 $\mu g.kg.^{-1}hr.^{-1}$) gastric acid secretion

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.2	8.4	12.2	10.3	11.2	10.7	11.9	12.1	11.1	13.7
2.	4.4	5.4	5.9	8.3	11.8	13.5	16.4	18.4	16.0	15.0
3.	3.0	4.8	5.3	9.8	9.9	9.9	12.9	15.8	15.1	14.7
4.	4.6	7.5	8.0	8.4	8.4	9.7	10.4	10.9	10.7	10.6
5.	3.2	4.8	7.0	6.8	10.0	7.5	9.2	9.6	11.0	13.0
6.	4.4	8.7	13.8	13.4	13.4	15.0	14.8	18.2	19.3	20.9
7.	4.8	9.4	13.7	17.9	23.7	20.2	18.7	20.2	22.1	20.8

Table xxviii. Effect of an intravenous infusion of somatostatin (2.0 $\mu g.kg.^{-1}hr.^{-1}$) on meal-stimulated gastric acid secretion

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	3.1	3.2	3.5	3.4	3.9	3.8	4.0	3.9	3.8	3.9
2.	3.7	4.7	2.9	8.3	9.4	4.4	2.6	1.9	2.3	4.7
3.	2.5	4.6	6.9	4.7	3.7	3.5	2.9	2.8	4.2	8.3
4.	4.9	7.1	5.2	—	4.9	3.5	4.7	5.1	5.4	4.3
5.	2.4	2.4	3.5	5.5	6.0	3.6	4.7	5.7	1.4	1.0
6.	1.8	7.7	10.7	6.6	6.3	6.1	5.9	4.4	4.2	3.9
7.	4.4	6.5	4.9	6.2	3.8	3.4	3.9	4.6	3.8	5.6
8.	3.1	9.5	4.6	6.9	4.3	4.6	4.2	5.7	5.7	4.9
9.	2.9	4.7	4.3	3.4	3.7	1.4	2.0	4.2	5.4	—
10.	3.5	3.0	3.5	4.7	5.1	4.0	4.9	2.8	2.8	2.6

Table xxix. Effect of a 100 µg intravenous bolus of monoclonal antibody 3.65H on the inhibition of gastric acid secretion by GIP (0.2 $\mu g.kg.^{-1}hr.^{-1}$)

The antibody was given 1 hour prior to the start of a liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.1	6.0	8.7	10.4	8.4	8.6	8.7	11.7	14.0	12.6
2.	0.9	2.5	4.5	7.1	5.5	6.2	6.3	5.6	7.8	—
3.	2.3	6.5	4.3	2.0	3.0	2.0	2.5	1.7	2.7	3.5
4.	1.5	4.5	6.8	4.2	5.7	6.7	7.7	6.2	4.0	3.4
5.	2.4	3.2	4.1	4.1	3.1	3.6	3.4	3.6	3.7	3.1
6.	2.3	5.5	7.7	5.8	5.9	8.3	7.2	6.3	6.9	5.7

Table xxx. Effect of incubation of monoclonal antibody 3.65H with GIP, on GIP (0.2 µg.kg.$^{-1}$hr.$^{-1}$) inhibition of gastric acid secretion

a. infusion of GIP incubated with 3.65H for 1 hour at 37^{0}C during meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	1.6	3.7	5.5	3.6	5.2	3.3	2.9	4.0	3.7	3.4
2.	2.0	1.7	1.5	2.1	—	1.1	2.4	2.3	3.1	3.2
3.	2.9	6.1	6.7	9.2	11.8	10.8	10.5	8.7	9.8	11.2
4.	2.9	5.4	5.6	4.9	4.6	3.2	4.6	4.7	4.3	3.0
5.	2.8	0.9	5.1	7.2	4.8	4.8	6.8	7.5	6.4	6.1
6.	2.0	2.3	4.5	4.4	3.7	3.6	3.7	4.2	3.2	2.2

b. infusion of GIP incubated with 3.65H for 18 hour at 4^{0}C during meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.1	10.1	13.5	18.0	24.2	19.7	21.3	19.5	17.4	21.3
2.	3.7	5.1	9.6	14.7	19.1	14.3	15.1	15.7	15.2	21.3
3.	4.2	6.3	9.1	9.0	12.2	12.0	8.4	10.4	10.6	11.1
4.	0.6	6.8	6.4	6.0	6.0	5.8	4.2	3.7	4.3	4.3
5.	3.0	4.3	8.8	9.3	7.1	7.5	6.4	6.7	8.9	5.5
6.	2.5	3.5	8.1	7.7	16.2	20.8	15.8	19.2	17.2	15.8
7.	1.2	1.0	6.6	5.3	6.8	5.1	4.9	5.4	4.1	4.3
8.	3.6	4.7	9.5	8.5	6.8	10.5	10.3	11.9	10.1	10.5
9.	1.1	2.8	9.1	8.1	9.6	10.8	14.3	14.6	11.0	11.2
10.	2.7	8.1	15.8	15.8	18.9	17.5	16.0	15.4	12.8	10.9
11.	2.4	3.7	5.0	6.3	5.9	6.4	6.0	5.3	4.7	4.3

Table xxxi. Effect of a 100 µg intravenous bolus of monoclonal antibody clone S8 on meal-stimulated gastric acid secretion

The antibody was given 1 hour prior to the start of a liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.1	6.3	6.8	5.7	8.3	8.1	8.5	8.6	8.4	8.0
2.	2.5	—	3.2	2.8	3.1	4.8	1.9	3.9	4.9	4.4
3.	2.7	4.5	5.5	5.3	5.4	6.3	6.3	6.4	7.8	7.8
4.	2.6	7.1	7.0	8.9	10.0	14.1	16.4	12.8	16.7	13.1
5.	2.2	6.7	5.9	4.5	—	5.2	5.1	4.1	5.0	4.6
6.	3.5	3.8	5.4	5.4	5.0	5.0	4.9	4.4	6.4	2.7
7.	1.4	4.3	3.3	2.7	6.8	8.8	9.3	9.2	9.2	—

Table xxxii. Effect of a 100 µg intravenous bolus of monoclonal antibody clone S8 on the inhibition of gastric acid secretion by somatostatin (2.0 $\mu g.kg.^{-1}hr.^{-1}$)

The antibody was given 1 hour prior to the start of the liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	3.4	8.5	4.7	5.2	4.7	3.1	2.1	2.3	1.7	3.1
2.	3.3	10.2	—	—	8.4	9.4	10.2	11.1	10.7	10.5
3.	2.1	2.8	6.5	8.2	9.6	9.3	7.3	9.4	10.1	8.0
4.	4.0	8.2	7.1	5.7	7.1	6.3	10.4	7.6	8.7	10.1
5.	4.1	4.4	9.5	7.5	8.4	16.0	12.0	10.0	11.6	11.0

Table xxxiii. Effect of a 100 µg intravenous bolus of monoclonal antibody clone 510 on meal-stimulated gastric acid secretion

The antibody was given 1 hour prior to the start of a liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.3	7.9	7.4	4.6	5.8	7.4	—	4.3	4.0	5.2
2.	4.7	11.9	18.5	11.5	14.0	14.0	14.7	13.8	14.8	12.4
3.	4.2	12.2	8.5	8.9	10.2	7.0	5.1	5.2	4.0	3.7
4.	2.7	—	3.3	8.6	5.0	4.0	6.6	5.0	4.6	5.0
5.	3.3	6.3	6.0	3.1	4.3	6.4	5.8	3.5	5.5	6.9
6.	4.7	8.2	8.7	13.6	10.8	13.8	7.7	13.0	8.5	11.7
7.	3.1	4.2	5.8	4.2	10.8	5.6	6.2	4.8	5.6	5.3
8.	3.6	7.3	5.5	4.6	5.9	3.2	2.9	3.5	2.8	4.3
9.	4.3	4.9	4.7	3.8	4.7	3.7	4.1	3.7	2.9	2.5
10.	2.4	6.3	5.8	6.3	7.5	6.1	7.0	8.8	7.8	7.2
11.	4.1	4.9	5.0	4.1	5.6	4.3	5.0	6.3	4.1	2.8
12.	1.7	3.8	4.3	6.0	6.4	7.6	6.3	6.7	7.3	7.7
13.	2.6	4.7	4.6	7.3	6.7	7.9	9.5	8.5	9.8	8.3
14.	3.2	5.9	4.3	4.7	6.2	6.3	6.0	6.0	7.7	5.0

Table xxxiv. Effect of a 100 µg intravenous bolus of monoclonal antibody clone S10 on the inhibition of gastric acid secretion by somatostatin (2.0 $\mu g.kg.^{-1}hr.^{-1}$)

The antibody was given 1 hour prior to the start of a liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	3.0	14.0	11.4	10.5	10.0	8.5	8.9	8.8	9.2	9.2
2.	2.1	7.0	13.4	17.9	17.4	9.9	9.5	17.3	20.5	16.6
3.	1.1	3.5	10.5	17.0	9.7	9.2	10.6	8.4	7.6	5.0
4.	4.8	8.7	13.7	10.1	12.4	10.8	13.5	11.8	12.0	7.7
5.	3.8	3.4	6.4	10.3	9.9	13.2	13.0	10.5	—	10.0
6.	4.3	5.3	4.9	7.4	7.6	7.4	8.4	5.9	5.2	5.6
7.	3.9	—	9.6	11.2	9.4	9.1	—	—	5.8	5.3
8.	3.0	—	6.2	3.8	6.9	8.5	6.1	10.8	5.1	—
9.	3.3	9.5	—	8.3	10.5	6.8	8.7	6.7	6.6	4.5
10.	4.5	1.9	6.3	9.2	6.6	7.5	6.9	7.7	8.4	6.5
11.	1.9	7.0	6.4	5.6	5.4	3.0	4.1	2.8	4.0	3.0
12.	3.8	5.3	5.1	4.8	5.3	3.5	7.8	5.1	4.9	4.2

Table xxxv. Effect of a 100 µg intravenous bolus of monoclonal antibody clone S20 on meal-stimulated gastric acid secretion

The antibody was given 1 hour prior to the start of a liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	4.0	5.7	4.9	—	6.7	6.0	6.5	8.0	7.1	5.3
2.	4.6	5.3	9.2	7.2	7.7	7.3	6.2	8.5	6.2	5.4
3.	4.9	13.7	10.5	7.0	10.2	11.7	7.7	10.3	6.3	9.6
4.	3.8	9.2	—	17.2	—	11.2	12.2	16.0	13.8	14.5
5.	1.7	—	7.8	7.0	8.8	5.6	6.9	3.3	4.7	5.3
6.	4.6	5.0	8.6	6.6	9.1	8.0	10.3	8.3	9.4	—

Table xxxvi. Effect of a 100 µg intravenous bolus of monoclonal antibody clone S20 on the inhibition of gastric acid secretion by somatostatin (2.0 $\mu g.kg.^{-1}hr.^{-1}$)

The antibody was given 1 hour prior to the start of a liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	2.0	9.8	7.3	3.2	3.1	3.1	3.6	4.1	4.1	3.9
2.	3.9	6.4	4.2	4.4	2.5	3.3	4.1	3.1	4.0	4.1
3.	2.6	13.8	4.7	5.3	2.5	4.1	4.8	4.2	3.3	3.6
4.	2.4	5.9	5.9	4.5	7.0	4.5	5.6	3.7	4.5	3.9
5.	4.3	8.0	7.3	6.4	7.0	8.0	6.1	6.0	5.0	6.3
6.	3.0	1.3	5.9	3.3	4.0	4.1	—	4.5	3.4	4.4
7.	3.0	6.6	—	10.7	12.5	10.3	9.2	8.3	—	5.0

Table xxxvii. Effect of a 100 µg intravenous bolus of monoclonal antibody clone S10 on the inhibition of gastric acid secretion by GIP (0.2 $\mu g.kg.^{-1}hr.^{-1}$)

The antibody was given 1 hour prior to the start of the liver extract meal

		Acid output (µEq/10 minute period)								
no.	B	1	2	3	4	5	6	7	8	9
1.	3.7	6.7	7.0	7.6	7.7	4.8	5.2	5.5	7.6	7.4
2.	4.6	6.0	5.8	8.8	6.3	5.2	8.0	8.2	5.5	7.5
3.	3.9	5.8	7.8	6.1	6.4	6.4	7.8	8.9	5.5	5.7
4.	2.1	5.3	4.6	6.4	5.9	3.0	4.1	4.0	7.3	8.0
5.	4.9	6.9	5.6	6.6	5.2	5.4	5.6	6.9	6.1	5.7
6.	5.0	—	7.5	9.0	4.9	5.0	3.7	4.9	3.1	4.2

Table xxxviii. Serum gastrin concentrations

a. liver extract meal alone

b. liver extract meal with GIP infusion (0.2 $\mu g.kg.^{-1}hr.^{-1}$)

c. liver extract meal with somatostatin infusion (2.0 $\mu g.kg.^{-1}hr.^{-1}$)

d. liver extract meal with intraduodenal oleic acid infusion

e. liver extract meal with monoclonal antibody 3.65H (100 µ bolus)

f. meal with portal serum infusion (with intraduodenal oleic acid)

g. meal with portal serum infusion (without intraduodenal oleic acid)

	a	b	c	d	e	f	g
no.							
1.	230	185	100	240	189	215	215
2.	125	163	150	230	109	86	400
3.	215	240	50	195	220	135	157
4.	260	152	170	290	400	320	80
5.	152	163	70	178	143	108	117
6.	127	178	150		135	93	125
7.			70		315		
8.					83		

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