STUDIES ON THE REGULATION OF GASTRIN SECRETION

By

VINCENT ROBERT VILLAGRACIA CAMPOS

B.Sc., The University of British Columbia, 1984

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Department of Physiology

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 1989

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Physiology

The University of British Columbia
Vancouver, Canada

Date Oct. 2, 1989

ABSTRACT

The regulation of gastrin secretion *in vivo* is complex as a result of the multiplicity of nervous, luminal, endocrine and paracrine signals that are received by the G cell. The objective of these studies was to investigate the mechanisms that control G-cell function in normal and pathophysiological situations. Gastrin secretion was examined in lean and obese Zucker rats, in the dog and in the human using *in vivo* and *in vitro* methods.

Hypergastrinemia in the obese Zucker rat was found to be a progressive condition, which appears between 3 and 8 weeks after birth and increases in severity with age. Elevated gastrin levels persisted from the isolated, vascularly perfused stomach preparation with adult obese animals displaying secretory rates 3-fold higher than their lean littermates. Basal gastrin secretion was unaltered by atropine treatment in both groups indicating that post-ganglionic cholinergic inputs to the G cell of the obese rat were normal. This was supported by perfusion experiments in which electrical activation of the anterior vagus nerve augmented gastrin release equally (2-fold) from lean and obese stomachs, an effect that was abolished by the addition of atropine. Basal somatostatin secretion from the perfused stomachs of lean and obese rats was not different nor was the density of the antral D-cell population. This suggested that somatostatin was probably not involved in the hypergastrinemia of the obese animal. G-cell numbers in the antra of both groups of animals were similar at 3 weeks of age, but were doubled in 6 month-old obese rats compared to lean controls. It was hypothesized that G-cell hyperplasia secondary to increased food consumption in obese rats was responsible for hypergastrinemia. This possibility was investigated in pair-feeding experiments wherein the food intake of obese animals was restricted to match that of lean littermates from 5 to 8 weeks of age. Dietary restriction reduced the antral G-cell population of the obese rat to a similar level as that seen in lean animals, supporting the view that hyperphagia is the trigger for G-cell hyperplasia.

However, basal gastrin levels *in vivo* and from the *in vitro* stomach preparation of pair-fed obese animals were not significantly lower than those of obese animals fed *ad libitum*. Thus, abnormal feeding behaviour in the obese phenotype cannot account for gastrin hypersecretion and G-cell hyperplasia is not the primary cause of hypergastrinemia. The role of bombesin/gastrin releasing peptide (BN/GRP) in the hypergastrinemic condition of the obese rat was subsequently examined. GRP content in antral extracts of obese animals was markedly greater than their lean counterparts. Furthermore, the gastrin responses of perfused stomachs of the obese animal to exogenous BN were attenuated compared to the lean group. These results provided circumstantial support for the involvement of a BN/GRP drive in the hypergastrinemia of the obese Zucker rat. However, this conclusion could not be validated by perfusion experiments employing two putative BN/GRP antagonists, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P and [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin. Basal and BN-stimulated gastrin release from the perfused stomachs of both groups of animals were unaffected by treatment with either antagonist. Thus, a causal role for BN/GRP neurons in the hypergastrinemia of obese rats is suggested, but additional support awaits improvements in the synthesis of BN/GRP antagonists.

The control of gastrin secretion at the cellular level in the dog and human was investigated using isolated antral G cells in primary culture. Preferential enrichment for G cells by centrifugal elutriation and short-term culture resulted in preparations in which gastrin-containing cells accounted for 8.5% of the total viable adherent cell population. BN, from 0.01 to 100 pM, stimulated gastrin release from cultured canine G cells in a concentration-dependent manner. The gastrin response achieved with such low concentrations of BN suggested a direct stimulatory role for this peptide via high-affinity receptors. The BN analog, [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin, completely blocked BN-induced gastrin secretion from 0.01 to 1 pM and produced > 50% inhibition at the higher concentrations of BN tested. In comparison, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P augmented basal gastrin levels when given alone. Consequently, this

anatgonist elicited only modest suppression of BN-evoked gastrin secretion. Differential inhibition of BN-mediated gastrin release from cultured canine cells and from the isolated perfused stomachs of Zucker rats suggests the existence of a heterogeneous population of BN/GRP receptors among species.

In the final series of experiments, receptor-dependent and -independent regulation of gastrin secretion from cultured human antral G cells was investigated. With minor modifications, methods used in the canine preparation were successfully applied to produce cultures of human antral mucosal cells that were enriched for G cells. Gastrin-containing cells accounted for 15% of the total adhered cell population. Forskolin, A23187 and ß-PMA stimulated gastrin secretion from cultured human G cells in a concentration-dependent fashion. These results indicated that gastrin release in the human could be mediated by elevations in cytosolic cAMP levels, calcium influx or activation of protein kinase C. A direct stimulatory role for BN/GRP was supported by experiments demonstrating concentration-dependent enhancement of gastrin release by BN from 0.01 fM to 10 nM. In contrast to findings from the canine preparation, [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin augmented basal gastrin levels by itself and produced weak inhibition of BN-induced gastrin secretion from human antral G cells. This further supports the hypothesis that species differences exist with regard to gastric BN/GRP receptors of the Zucker rat, dog and human. Somatostatin potently suppressed forskolin- and BN-mediated gastrin release, but did not significantly alter basal gastrin levels. These results suggest that BN and somatostatin directly activate and inhibit G-cell function via specific and sensitive receptors. Furthermore, the adenylate cyclase and phosphatidyl inositide second messenger systems appear to be intracellular mediators of gastrin secretion from human antral G cells.

These investigations clearly demonstrated the complexity of the control of gastrin secretion in the Zucker rat, dog and human. Further clarification of the mechanisms that regulate G-cell function in the intact animal relies on improved methodology and the integration of information obtained from various experimental models.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I sincerely thank my advisor, Dr. Raymond A. Pederson, for his continual encouragement, perception and generosity. His contribution to my maturity as a student and as a person is beyond the scope of words and I will always value his friendship and trust.

I would also like to thank Dr. J.C. Brown whose constant prodding often masked his genuine concern for the students. I thank Dr. A.M.J. Buchan who willingly shared her time and expertise. Thanks are also in order for Dr. Y.N. Kwok for his constructive comments and unique sense of humour. I thank Dr. C.H.S. McIntosh for his seemingly boundless knowledge and assistance.

I wish to thank Karen Tubesing, Connie Chisholm, Petra Sanderson and Valerie McArthur, for all the assistance they so generously offered and for making the lab an enjoyable place to work. I wish to thank Mary Forsythe, Joe Tay and John Sanker for their encouragement and kindness.

I am grateful to my colleagues, Eric Accili, Sue Curtis, Bruce Verchere and Mike Wheeler for sharing with me their zest for work and play. I will especially remember the songs and the laughter. Thanks are also in order for Richard Senger and Piotr Czaykowski for their understanding and continued friendship.

I wish to acknowledge the financial support provided by the Medical Research Council of Canada Studentship program.

I would like to thank Maurizio Leschiutta, Paul Lasiuk and Sean Clarke for their enduring support. Time and distance apart only reaffirmed the value of their friendship.

I thank my parents for their genuine affection, especially my mother who taught me the importance of humility, perseverance and dedication. To my sisters, Marie and Jane and my brother, Steve, a special note of gratitude for always believing in my abilities.

To all those unmentioned who have in one form or another contributed to numerous memorable occasions during my academic training, I am most grateful.

Finally, a most hearfelt thank you to Anne whose patience and love fueled the successful completion of my studies. I will always remember and treasure the quality times.

This work is dedicated to Anne and my family.

INTRODUCTION

In the late 1800's, Pavlov's doctrine of "nervism" dominated the fields of cardiovascular and gastrointestinal physiology (Pavlov, 1883; Pavlov, 1897). This doctrine, which evolved during Pavlov's work under his mentor Botkin, was defined as " a physiological theory which tries to prove that the nervous system controls the greatest possible number of bodily activities". Indeed, this dogma was so ingrained that in 1902 Popielski, a student in Pavlov's laboratory, concluded that prevention of gastric secretion in response to food in the stomach could not be achieved by vagotomy and splanchnectomy because cell bodies controlling gastric function were located within the stomach wall itself. While this held true, no mention was given to the possibility of non-nervous stimuli acccounting for the observed acid production. In the same year, Bayliss and Starling (1902) discovered secretin. This discovery was particularly significant for it challenged the then accepted concept of nervism and represented an entirely novel mechanism of control of bodily functions. Bayliss and Starling went further to suggest the existence of a "gastric secretin" and in 1905, Starling adopted the word "hormone" to describe blood-borne "chemical messengers" that modulated cellular function at a site distant from their origin.

J. S. Edkins' inquiry into the mechanism of gastric secretion began as early as 1898 when, after citing the work of Heidenhain (1879) and Chischin, he theorized that the formation and absorption of foodstuffs, particularly peptone, in the stomach "in some way influence the epithelium so that secretion results". Seven year later, at the Royal Society Meeting in London, Edkins (1905) reported that intravenous administration of crude antral mucosal extracts in anesthetized cats resulted in acid production. He termed the active substance gastrin and suggested that it reached the mucosa via the circulation where it exerted hormonal actions on the acid secreting glands. Edkins (1906) further demonstrated that extracts from the pyloric region and not the fundic region of the stomach contained

gastrin. Despite such convincing evidence, Edkins' discovery was not universally accepted. In 1913, Sawitsch and Zeliony demonstrated that gastric secretion in response to instillation of Liebig's extract into isolated pyloric pouches could be blocked by prior treatment with atropine and concluded that vagal reflexes were responsible. Furthermore, the impurity of Edkins' antral preparations raised questions as to whether gastrin was indeed the active principle. For instance, Popielski and Panek (1909) proposed that gastric secretion was caused by vasodilatin, a hypotensive agent that was widely distributed in the body. Later, Dale and Laidlaw (1910) showed that histamine, a potent stimulant of acid secretion, was also widely distributed in the body. In succeeding years, additional evidence supporting a role for histamine as the gastric secretagogue emerged (Popielski, 1920; Sacks et al, 1932) and the gastrin theory came to be known as the gastrin hypothesis.

In 1938, Komarov reported the production of histamine-free extracts from canine antral mucosa containing a protein-like substance that was capable of potently stimulating gastric acid secretion when given intravenously. This observation persisted in spite of the addition of large doses of atropine. The presence of a histamine-free gastric secretagogue was confirmed by Uvnas in cats (1942), pigs (1945) and in man (1945), and led to the resurgence of gastrin as a physiological stimulant of acid secretion. Attempts to isolate and purify gastrin were unsuccessful until 1961 when Gregory and Tracy described a reliable and reproducible method of extracting gastrin from porcine antral mucosa that produced a copius secretion of acid when given intravenously, intramuscularly or subcutaneously to conscious dogs. Gregory and Tracy's work ultimately led to the isolation, purification and identification of two almost identical heptadecapeptides (Gregory and Tracy, 1964) which they termed porcine gastrin I and II. Determination of the structures of the two gastrin molecules (Gregory et al, 1964) revealed the only difference to be the presence of a sulfate moiety on the tyrosine of gastrin II (Figure 1), although sulfation had little or no effect on the physiological actions of the gastrin molecule. Synthesis of the gastrins soon followed (Anderson et al, 1964).

Figure 1: The amino acid composition of porcine gastrin I and II isolated from antral mucosa

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Glp-	Gly-	Pro-	Trp-	Met-	Glu-	Glu-	Glu-	Glu-	Glu-	Ala-	Tyr-	Gly-	Trp-	Met-	Asp-	Phe-NH_2

R = H or SO_3H

Subsequently, gastrin heptadecapeptides were isolated and sequenced from the antral mucosa of sheep, cow (Agarwal et al., 1968), dog (Agarwal et al., 1969a), cat (Agarwal et al., 1969b) and man (Bentley et al., 1966) and were found to differ only at one or two residues within the mid-region of the linear peptide chain (Figure 2). In the rat, substitutions have been revealed to occur in the amino-terminus rather than the mid portion (Reeve et al, 1981). This conservation of sequence among species represents the change of only one base of the codon triplet per substitution.

Figure 2: The amino acid composition of little gastrin I isolated from the antral mucosa of various species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Human	Glp-	Gly-	Pro-	Trp-	Leu-	Glu-	Glu-	Glu-	Glu-	Glu-	Ala-	Tyr-	Gly-	Trp-	Met-	Asp-	Phe-NH_2
Hog					-Met-												
Dog					-Met-			-Ala-									
Cat										-Ala-							
Cow & Sheep					-Val-					-Ala-							
Rat		-Arg-		-Pro-													

To date, three biologically active species of the gastrin molecule have been isolated in man. These include "big gastrin" or G-34, "little gastrin" or G-17 and "mini-gastrin"

Figure 3: Amino acid composition of human G-34, G-17 and G-14.

```
          1                                                                17
G-34      Glp-Leu-Gly-Pro-Gln-Gly-Pro-Pro-His-Leu-Val-Ala-Asp-Pro-Ser-Lys-Lys-
          18                                                               34
          Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe*

          1                                                                17
G-17      Glp-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe*

                    1                                                      14
G-14                Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe*
```

* represents an amidated carboxy terminus

or G-14 (Figure 3). Each variant has been designated according to its constituent number of amino acid residues and exists in the sulfated and nonsulfated forms. Big gastrin or G-34 was originally identified by Yalow and Berson (1970a) as the predominant circulating form of immunoreactive gastrin in plasma from patients with pernicious anemia or Zollinger-Ellison syndrome. G-34 has since been isolated from human gastrinoma extracts and porcine antral mucosa (Gregory and Tracy, 1972) and the amino acid sequence determined (Gregory and Tracy, 1975). G-14 was initially isolated from gastrinoma tissue extracts by Gregory and Tracy (1974) and later confirmed as a minor circulating form in man (Rehfeld et al, 1974). At least three other immunoreactive gastrin species with no known biological function have been identified. The first of these was reported to be a constituent of human serum and the jejunal mucosa and given the trivial name "big big gastrin" as it eluted in the void volume of Sephadex G-50 gel filtration chromatographs (Yalow and Berson, 1972; Yalow and Wu, 1973). Antibody absorption studies have indicated "big big gastrin" to be an artifact of serum and mucosal extracts resulting from non-specific protein interference of the radioimmunoassay (Rehfeld, 1974), although it

may represent a true component of some gastrinomas (Rehfeld et al, 1977). Secondly, Rehfeld and Stadil (1973) have identified a gastrin variant in the sera of Zollinger-Ellison syndrome patients that elutes just prior to G-34 from G-50 Sephadex columns and have designated it, "Component I" (Figure 4). Lastly, the NH_2-terminal tridecapeptide fragment of G-17 has been isolated in porcine antral extracts (Gregory, 1974) and in the sera of normal and gastrinoma patients (Dockray and Walsh, 1975). This finding predicted the concomitant release of the COOH-terminal tetrapeptide amide of G-17 and its existence has been identified immunochemically in antral extracts of man and pig (Rehfeld, 1978a). Rehfeld and Larsson (1979) reported large amounts of G-4 in porcine antral extracts. However, Gregory and his co-workers (1983) were unable to confirm these results and instead, isolated a hexapeptide that conformed to the C-terminal of gastrin.

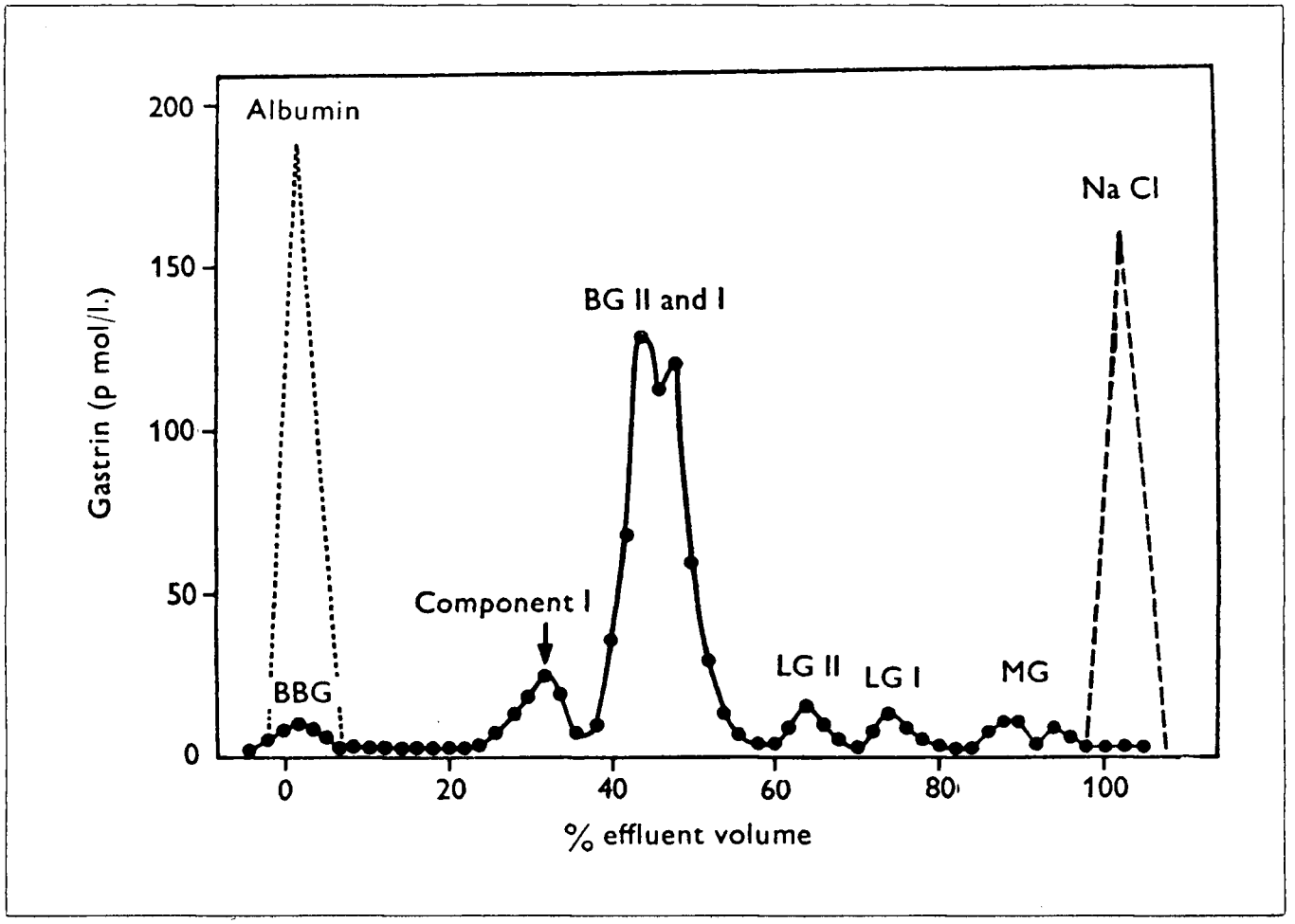


Figure 4 : Distribution of immunoreactive gastrins in human serum fractionated on a Sephadex G-50 column. (From Gregory, 1988).

Tracy and Gregory (1964) established that the C-terminal tetrapeptide amide, Trp-Met-Asp-Phe-NH_2, possessed the entire range of biological actions of G-17, although at one sixth the molar potency. They also demonstrated that removal of the C-terminal amide moiety or oxidation of the methionine residue results in almost complete loss of biological activity. Extensive studies of over 500 analogs of the tetrapeptide (Morley et al, 1965; Morley, 1968) confirmed these findings and further revealed that: a)N-acetylation of G-4 preserved or enhanced activity presumably by protecting the peptide from degradation by aminopeptidases; b)substitution of the L-amino acid residues by D-amino acids resulted in loss of function; c)the Met residue was most tolerant to replacement while the Asp residue was least tolerant; and d)substitution of Phe by Tyr preserved activity. Taken together, these observations suggested the involvement of the trytophan, methionine and phenylalanine residues in receptor binding whereas the aspartic acid and the amide group were functionally active sites. Subsequently, Lin and his colleagues (1976) showed that the C-terminal tripeptide amide possessed a small degree of secretagogue function. Currently, the gastrin variant that is in widest use is an analog of the C-terminal pentapeptide, BOC-ß-Ala-Trp-Met-Asp-Phe-NH_2, which has been termed "pentagastrin" and possesses the same degree of potency as the pentapeptide amide of gastrin (Morley et al, 1965).

Early studies concerned with the relative potency of the three biologically active gastrin species (Debas et al, 1974; Walsh et al, 1974; Walsh et al, 1976) indicated that based on exogenous doses needed to produce a specified level of a maximal response, molar potency increased with chain length, whereas when comparing circulating levels G-17 appeared to be more potent than G-14 or G-34. However, more recent studies have invited a reassessment of these findings. Exogenous infusions of equimolar amounts of G-14 and G-17 have been shown to result in similar rates of acid secretion with no difference in their clearance rates (Carter et al, 1979). In addition, infusion of synthetic human G-34 at a molar dose four-fold lower than that of G-17 produced similar acid secretion rates and

plasma gastrin levels in man (Eysselein et al, 1984). As the clearance of G-34 from the circulation had been shown to be four to six times slower than G-17 (Walsh et al, 1974; Walsh et al, 1976; Eysselein et al, 1984), the aforementioned observations suggested equipotency between G-34 and G-17 in stimulating acid secretion in humans.

Although the antrum is the greatest source of extractable gastrin, non-antral sites of gastrin exist in the body. Using radioimmunoassay, gastrin immunoreactivity was demonstrated in the duodenum of man, dog, cat, pig (Nilsson et al, 1973), monkey, rat, rabbit and guinea pig (Nilsson, 1976). In addition, human duodenal mucosa was reported to contain approximately the same amount of gastrin as the antral mucosa with very minute amounts of immunoreactivity along the remainder of the small intestine. Several studies have shown that gastrin content in the duodenum of the human fetus was greater than that in the adult (Larsson and Rehfeld, 1977; Larsson et al, 1977; Track, et al, 1977). In cats, dogs, rats and pigs, however, Nilsson and Brodin (1977) observed that duodenal gastrin content was markedly lower than that in the antrum. Trace amounts of gastrin immunoreactivity have also been found in the buccal mucosa, tongue, esophagus and corpus of man, dog, cat and pig (Nilsson et al, 1973). The existence of pancreatic gastrin in humans is yet unclear. Some investigators have reported its presence (Greider and McGuigan, 1971; Nilsson et al, 1973; Rehfeld and Iversen, 1973), whereas others have claimed the disappearance of pancreatic G cells early in postnatal life (Larsson et al, 1977; Stein et al, 1983). Furthermore, Andersen et al (1985) were unable to detect gastrin immunoreactivity in the fetal human pancreas. Gastrin-like material has been shown in the pancreas of pigs (Pointner and Flegel, 1976), mice (Dockray et al, 1977) and neonatal rats (Brand et al, 1984).

Gastrin immunoreactivity has also been identified in porcine neuro- and adenohypophysis (Rehfeld, 1978b). G-34 and Component I were the predominant forms in the corticotrophs of the anterior lobe, whereas G-17 was the major species in the melanotrophs of the intermediate lobe and the neural lobe (Rehfeld and Larsson, 1981).

Moreover, gastrin mRNA has been demonstrated in the porcine pituitary gland (Powell et al, 1985). Recently, the hypothalamo-hypophyseal neurones of the pig, cat, cow, rat and human have been shown to contain gastrin-like material (Rehfeld et al, 1984). The abdominal vagus nerves of dogs, cats and humans have also been reported to contain G-17 and G-34-like immunoreactivity (Uvnas-Wallensten et al, 1977). However, in a more recent study on dogs and cats, Dockray and his co-workers (1981) claimed that in most animals, the gastrin-like material consisted of cholecystokinin octapeptide, although a few animals exhibited some G-17-like immunoreactivity.

It has been demonstrated that approximately 90% of extractable antral gastrin in humans is G-17, whereas G-34 is in slightly greater abundance than G-17 in the duodenum (Berson and Yalow, 1971). Furthermore, about 45% of antral and duodenal gastrins are sulfated, while sulfation of jejunal gastrins is complete (Anderson, 1984). In contrast, several studies have established G-34 as the major circulating form of gastrin in Zollinger-Ellison syndrome patients as well as normal humans (Yalow and Berson, 1970a; Yalow and Berson, 1971; Rehfeld and Stadil, 1973; Rehfeld et al, 1974; Dockray and Walsh, 1975; Dockray et al, 1975; Taylor et al 1979). Using affinity chromatography, the ratio of circulating G-34 to G-17 during the fasting state has been determined to be 2:1 and 1:1 after a meal (Lamers et al, 1979). About 5% of the total circulating gastrin during basal and postprandial states is accounted for by G-14 and Component I. In addition, approximately half of the circulating G-34 and G-17 is sulfated (Lamers et al, 1982).

Identification of the gastrin-containing cell or G cell in the antral mucosa was originally achieved by McGuigan (1968) using immunofluorescence. In humans and dogs, these cells are located in the mid-portion of the gastric glands, whereas in rats, G cells are confined to the base of the glands. Ultrastructural examination of antral and duodenal G cells has revealed pyramidal-shaped cells in all species studied (Forssman et al, 1969; Solcia et al, 1969; Capella and Solcia, 1972, Greider et al, 1972; Rubin, 1972). Microvilli, which line the apical surface of these cells, project into the lumen, while gastrin-containing

granules predominate in the basal region adjacent to passing blood vessels. Such an arrangement is suited for the release of gastrin into the circulation in response to luminal stimuli. At rest, storage granules have been shown to be electron dense, about 200 nm in diameter and are largely comprised of G-34 (Forssman et al, 1980). Upon stimulation, enzymatic cleavage of the G-34 molecule resulted in storage granules that are less electron dense, 300 nm in diameter and consisted of G-17 and the amino-terminus of G-34. Human duodenal G cells, localized using antibodies directed towards the N-terminal of gastrin, have been shown to contain much smaller and denser storage granules than those of antral origin (Buchan et al, 1979). The greater proportion of G-34 to G-17 in the duodenum compared to the antrum was proposed to account for this distinction.

Biosynthesis of G-17 is believed to occur via enzymatic cleavage of G-34 (Dockray et al, 1978). Using antibodies specific for the amino-terminus of G-34, equal proportions of the N-terminal pentadecapeptide of G-34 and the C-terminal fragment of G-34, corresponding to G-17, were measured with a pair of lysine residues at positions 16 and 17 of G-34 as the cleavage site. Similarly, biosynthesis of G-34 is presumed to result from enzymatic digestion of two pairs of basic residues within the preprogastrin molecule (Dockray, 1980; Desmond et al, 1985).

The mode and sites of removal of gastrin from the circulation are not completely understood. Early studies implicating the kidney (Maxwell et al, 1971; Korman et al, 1972; Clendinnen et al, 1973) and the liver (Thompson et al, 1969; McGuigan et al, 1970) as key sites of gastrin clearance have lost support due to the finding that arterio-venous differences in gastrin content across the circulation of the head and limbs were similar to those across the renal and portal circulation during intravenous infusion of G-17 (Struntz et al, 1978a). Yet, nephrectomy resulted in reduced extraction of gastrin by peripheral tissues (Loly et al, 1982). Strunz and co-workers (1978) demonstrated that the hepatic circulation almost completely removed C-terminal gastrin fragments of eight or less residues, but did not preferentially metabolize G-17 or G-34 compared to other tissues. Furthermore, it has

been shown that circulating gastrin levels were identical after portal and peripheral infusion of G-17 in humans (Tranberg et al, 1982).

Little is known about specific enzymes involved in the degradation of gastrin. Walsh and Laster (1973) reported the presence of a deamidase in kidney homogenates that cleaves the C-terminal dipeptide amide from the tetrapeptide, but its effects *in vivo* and on larger gastrin forms are unknown. A similar deamidase was also found in liver homogenates and indeed, both C-terminal tetrapeptide and pentapeptide fragments of gastrin have been identified in bile as unchanged or in the deaminated form (Wyllie et al, 1974). However, the question still remains as to its significance *in vivo*. There is increasing evidence that the membrane-bound enzyme, endopeptidase 24.11 (enkephalinase), which has been isolated from various tissues including the liver, kidneys, brain, stomach and intestine is involved in the metabolism of gastrin. Several investigators have shown that infusion of G-17 in humans (Deschodt-Lanckman et al, 1987; Deschodt-Lanckman et al, 1988) and pigs (Power et al, 1987; Bunnett et al, 1988) *in vivo* resulted in the production of peptide fragments that corresponded to the *in vitro* products of endopeptidase 24.11 degradation of G-17.

A number of biological actions have been ascribed to gastrin (Table 1). However, caution must be taken when determining which effects are physiological or pharmacological. Walsh and Grossman (1975) proposed that only those actions that result from the forms and levels of gastrin released by a meal should be considered physiologically relevant. Gregory (1988) has extended this view by suggesting that consideration must be given to the modulatory actions of gastrin; actions that are facilitated by the interactions of the hormone with other circulating peptides and/or nervous inputs. Levels of gastrin that are sub-threshold for its own characteristic action may otherwise be sufficient to modulate the response of the same target cells to other stimuli (Grossman, 1970).

Table 1: Actions of Gastrin. (From Walsh and Grossman, 1975):

Water and electrolyte secretion	Stomach, pancreas, liver, small intestine, Brunner's glands
Enzyme secretion	Stomach, pancreas, small intestine
Inhibition of water, electrolyte and glucose absorption	Small intestine
Stimulation of smooth muscle	Lower esophageal sphincter, stomach, small intestine, colon, gallbladder
Inhibition of smooth muscle	Pyloric sphincter, ileocecal sphincter, sphincter of Oddi
Release of hormones	Pancreas (insulin), thyroid (calcitonin)
Increase in blood flow	Stomach, small intestine, pancreas
Trophic action	Gastric mucosa, small-bowel mucosa, pancreas

The primary physiological effect of gastrin is the stimulation of gastric acid secretion. This action has been well documented in numerous species *in vivo* (Edkins, 1905; Edkins, 1906; Komarov, 1938; Uvnas, 1942; Uvnas, 1945a; Uvnas, 1945b; Gregory and Tracy, 1964; Tracy and Gregory, 1964) as well as from the isolated parietal cell preparation (Soll, 1978a; Soll, 1978b; Chew and Hersey, 1982; Heldsinger and Vinik, 1984). Increased circulation within the oxyntic mucosa (Jacobson et al, 1966; Rudick et al, 1972) and stimulation of pepsinogen secretion (Emas and Grossman, 1967) have also been observed. However, it is unclear if increased pepsinogen secretion is secondary to acid secretion in normal humans. Johnson (1973) has shown that application of hydrochloric acid to denervated canine fundic pouches resulted in an augmentation of pepsinogen secretion that was unaltered by the addition of gastrin. Modest stimulation of intrinsic factor secretion by gastrin has been reported in man (Eysselein et al, 1984). Furthermore, increases in histidine decarboxylase activity (Aures et al, 1970) and in histamine release itself (Kahlson et al, 1964) have been associated with increased acid output, but a recent

study in dogs did not support a role for gastrin in histamine mobilization (Redfern et al, 1985).

Contraction of the lower esophageal sphincter by gastrin, an atropine-resistant response (Jensen et al, 1978), was initially believed to be a physiological effect (Cohen and Lipshutz, 1971). However, doses of gastrin needed to evoke sphincter contraction were greater than those required to elicit maximal gastric acid secretion in humans (Frank et al, 1973; Jensen et al, 1978). McCallum and Walsh (1979) have further demonstrated the absence of a relationship between serum gastrin concentrations and lower esophageal sphincter pressure in gastrinoma patients and patients with reduced sphincter tone.

Another physiologically relevant role of gastrin is its trophic action on the mucosa of the gastrointestinal tract, particularly the oxyntic or acid-secreting portion of the stomach (Johnson, 1976). This was first demonstrated by Crean et al (1969) who showed that pentagastrin treatment, but not histamine, resulted in hyperplasia of the oxyntic mucosa in rats and, conversely, that removal of the antrum led to gastric atrophy. It has also been shown that pentagastrin administration prevented the reduction in mucosal content of DNA and RNA in antrectomized rats (Johnson, 1974) and stimulated DNA synthesis in conscious dogs (Willems et al, 1972). Furthermore, in studies involving rats that are fed parenterally or starved, atrophy of the stomach, small intestine and pancreas associated with marked reductions in serum and antral gastrin content was reversed by pentagastrin and not histamine infusion (Johnson et al, 1974; Johnson et al, 1975a). In humans, atrophy of the oxyntic mucosa resulting from partial gastrectomy can be partly prevented by pentagastrin infusion (Olbe, 1974). In addition, hyperplasia of the parietal cell mass of Zollinger-Ellison syndrome patients has been reported along with an augmentation of their maximum secretory capacity of up to six-fold (Neuberger et al, 1972). Despite its profound capacity to stimulate growth in the oxyntic mucosa, gastrin has no trophic action on the antral mucosa (Johnson, 1977). Also, its trophic effects on the small bowel and the

pancreas have recently been questioned as elevated levels of gastrin in rats did not elicit hyperplasia (Stock-Damge et al, 1985; Hakanson et al, 1986).

The modulatory role of sub-threshold levels of gastrin is best understood by reviewing the complex interactions that exist among gastrin, histamine and acetylcholine in the control of parietal cell function. In cross-circulation experiments in cats, Uvnas (1942) first demonstrated that gastrin was a necessary component for successful vagal stimulation of the oxyntic cells. Similar findings were obtained in the dog, wherein vagal activation of the acid-secreting glands was restored after removal of the antrum and proximal duodenum by a dose of gastrin which by itself was insufficient to stimulate acid secretion (Olbe, 1964; Olbe et al, 1968). Knutson and Olbe (1974) also demonstrated that in contrast to cats and dogs, vagal stimulation of acid secretion in humans did not depend on the release of gastrin. They showed that resection of the antrum and duodenal bulb resulted in only a modest reduction of the acid secretory response to sham feeding and addition of sub-threshold levels of gastrin did not alter the response. However, it was also revealed that in humans, but not in dogs and cats, antral exclusion lowered the maximal acid output evoked by gastrin and histamine.

The elegant experiments of Soll (1978a; 1978b; 1982) have demonstrated the interdependency that exists among gastrin, histamine and acetylcholine on parietal cell function *in vivo*. Using the isolated canine parietal cell preparation, he demonstrated the presence of specific receptors for each secretagogue. Moreover, potentiation of the parietal cell response was achieved when any pair or all three secretagogues were used and the potentiating interactions were abolished by histamine antagonists and anticholinergic agents. The existence of a specific gastrin receptor has been further demonstrated by the work of Soll and his co-workers (1984). Again, utilizing isolated canine parietal cells and ^{125}I-[leu^{15}]-gastrin, they estimated the presence of 44,000 receptors per parietal cell and found that gastrin binding correlated with gastrin stimulation of parietal cell activity. Gastrin binding was unaffected by the addition of atropine, cimetidine (H_2-receptor

antagonist), secretin and somatostatin, but was markedly inhibited by the introduction of a gastrin receptor antagonist, proglumide.

The complexity of the regulation of gastrin secretion can be attributed to the presence of nervous, luminal, endocrine and possibly paracrine influences controlling G-cell function. Before the advent of radioimmunoassay methods, assessment of stimulation and inhibition of gastrin secretion was based upon fluctuations in gastric acid secretion. The involvement of the parasympathetic nervous system, specifically the vagus nerve, in the control of G-cell activity was originally demonstrated by Uvnas (1942) in anesthetized cats. He showed that the gastric acid secretory response to vagal stimulation was abolished by prior elimination of antral function either by resection or local anesthetics and that the response could be restored by intravenous administration of antral extracts. He concluded that the vagus evoked the release of a pyloric hormone, which he presumed to be gastrin, and that simultaneous action of vagally-released gastrin and of the vagus itself on the parietal cell were required for optimal acid secretory response. Similarly, Olbe (1964) demonstrated that the acid secretory response to vagal stimulation by sham-feeding or insulin hypoglycemia in dogs was poor after combined resection of the antrum and the duodenal bulb. Again, administration of low doses of exogenous gastrin restored the acid response to sham-feeding. Further support for vagal release of gastrin was presented by Fyro (1967) who showed that electrical stimulation of the vagus resulted in reduced gastrin-like activity in the antrum and duodenum of cats.

Direct demonstration of gastrin release caused by vagally-mediated pathways was eventually achieved with the introduction of the radioimmunoassay for gastrin (McGuigan and Trudeau, 1968; Yalow and Berson, 1970b). Subsequently, stimulation of gastrin secretion in response to sham feeding was demonstrated in dogs and this effect was abolished by vagotomy (Tepperman et al, 1972) or by high doses (> 50 μg/kg) of atropine (Nilsson et al, 1972). In humans, a small but significant rise in serum gastrin levels was demonstrated in sham feeding experiments wherein the pH of the gastric contents was

maintained at 5 and the acid secretory response measured by intragastric titration (Richardson et al, 1977; Feldman et al, 1979). Further evidence for vagally-induced gastrin secretion was shown in the dog in experiments in which insulin hypoglycemia augmented circulating gastrin levels, a response which was blocked by vagotomy (Tepperman et al, 1972) and by high doses of atropine (Csendes et al, 1972). In contrast, vagotomy did not abolish the gastrin response to insulin hypoglycemia in humans, indicating that it is not a vagally-mediated event (Stadil and Rehfeld, 1974). Large doses of atropine have also been shown to reduce gastrin levels released by a test meal in cats (Svensson et al, 1976) and recently, gastrin release induced by electrical stimulation of the vagus was observed from the isolated vascularly perfused porcine antrum (Holst et al, 1983; Olesen et al, 1987). Collectively, these studies implied that the cholinergic system is involved in the stimulation of gastrin secretion not only at the pre-ganglionic stage but also at the post-ganglionic level. However, there is an abundance of information to suggest otherwise.

For instance, it has been proposed that specific blockade of cholinergic muscarinic receptors on the G cell cannot be deduced from studies demonstrating inhibition of the gastrin response to sham feeding, insulin hypoglycemia or a test meal by atropine because of the large amounts of atropine used (Grossman, 1979). Furthermore, gastrin release evoked by electrical stimulation of the vagus has been shown to be resistant to prior administration of atropine in dogs (Smith et al, 1975), cats (Uvnas et al, 1975; Uvnas-Wallensten and Andersson, 1977) and pigs (Holst et al, 1987). Also, Dockray and Tracy (1980) have reported that sham feeding in dogs elevated gastrin levels and that this was unaffected by atropine treatment. Atropine-resistant release of gastrin by electrical stimulation of the subdiaphragmatic vagal trunks has been demonstrated in the isolated, vascularly perfused rat stomach preparation (Nishi et al, 1985). However, Pederson and his colleagues (1981) showed that this effect could be partially reduced by atropine and completely abolished by hexamethonium treatment. In addition, gastrin release evoked by

transmural stimulation (Schubert et al, 1982) or by the nicotinic receptor agonist, 1,1-dimethyl-4-phenylpiperazinium (DMPP) (Schubert and Makhlouf, 1982) was inhibited by 15% and 35%, respectively, with the addition of atropine. The latter studies indicated that in the rat, cholinergic and non-cholinergic postganglionic neurons mediate intramural activation of gastrin secretion.

Studies investigating the effects of cholinomimetic agents on gastrin secretion have produced mixed results. Arterial administration of bethanechol (Krejs et al, 1977) and topical application of acetylcholine (Tepperman et al, 1972) stimulated gastrin release from canine antral pouches. However, these studies have been criticized for their application of large doses to elicit the gastrin response. In particular, the amounts of acetylcholine used to induce gastrin release have also been shown to contribute to a breakdown of the gastric mucosal barrier (Davenport and Kauffman, 1975). Since a breakdown in the barrier can result in gastrin secretion (Bedi at al, 1971), the gastrin releasing action of cholinomimetics may represent a non-specific effect (Grossman, 1979). Intravenous infusion of cholinergic agonists produced weak or no augmentation of the circulating gastrin levels in the dog (Sjodin and Nilsson, 1973; Sjodin and Nilsson, 1974; Hirschowitz and Gibson, 1978). Similarly, infusion of carbachol in man evoked the release of gastric acid but not gastrin (Vatn et al, 1975). In comparison, cholinergic stimulation of gastrin secretion from the isolated perfused rat stomach is well documented in that acetylcholine (Koop et al, 1982), methacholine (Saffouri et al, 1980; Schubert et al, 1982) and carbachol (Martindale et al, 1982; Sue et al, 1985) have all been shown to elicit dose-dependent increases in gastrin output that were blocked by atropine. Moreover, carbachol-induced gastrin secretion was abolished by the inclusion of pirenzepine (Sue et al, 1985), a selective class M_1 muscarinic receptor antagonist (Hammer and Giachetti, 1982). These data suggested that the cholinergic system may play a more crucial role in the stimulation of gastrin release at the level of the G cell in rodents than in other mammals.

Vagotomy and cholinergic blockade studies have further reinforced the hypothesis that, in most mammals (except the rat), acetylcholine does not mediate vagal stimulation of gastrin secretion at postganglionic sites. In humans, small doses of atropine augmented basal serum gastrin levels (Feldman et al, 1979) as well as the gastrin responses stimulated by a test meal (Korman et al, 1971; Walsh, 1971; Becker et al, 1974a), sham feeding (Feldman et al, 1979) and insulin hypoglycemia (Farooq and Walsh, 1975). In addition, the increased gastrin response produced by atropine treatment did not appear to be secondary to reduced acidity, as it persisted even when intragastric pH was held constant (Farooq and Walsh, 1975). Similarly, small doses of atropine elevated the serum gastrin response of dogs to food whereas large amounts of atropine were without effect (Impicciatore et al, 1977). Also, augmentation of basal and food-stimulated gastrin outputs has been demonstrated after vagotomy in humans (Korman et al, 1972; McGuigan and Trudeau, 1972; Kronborg et al, 1973, Stern and Walsh, 1973; Jaffe et al, 1974; Stadil and Rehfeld, 1974) and dogs (Korman et al, 1972; Hollinshead et al, 1985). Although there is some evidence to suggest that the gastrin response to food in vagotomized dogs is mediated by cholinergic pathways (Debas et al, 1976), the mechanisms underlying basal hypergastrinemia in these animals are not known. The elevation in gastrin levels after vagotomy did not appear to be the result of reduced feedback inhibition of gastric acid on the G cell as acute alkalinization of the gastric contents did not stimulate gastrin secretion (Farooq and Walsh, 1975). It was probably not a consequence of G-cell hyperplasia because of the rapid onset of hypergastrinemia in vagotomized dogs (Hollinshead et al, 1985). Rather, results from these studies indicated the presence of a vagal inhibitory tone that is mediated by cholinergic neurons and its removal by vagotomy or atropine pre-treatment results in augmented gastrin release. Direct evidence for this has been shown in the dog where intravenous administration of bethanechol inhibited bombesin-stimulated gastrin secretion (Taylor et al, 1979).

Much information has been gained from vagotomy studies as to the origin of the vagal inhibitory tone on gastrin cell function. The augmented gastrin output to food was observed even after selective denervation of vagal fibres to the human proximal stomach (Stadil and Rehfeld, 1974), suggesting that the vagotomy effect was indirect. Lam et al (1978) demonstrated that the increase in circulating gastrin levels in duodenal ulcer patients was not different after truncal or selective parietal cell vagotomy. They also revealed that the gastrin response to truncal vagotomy was abolished by antral resection. These results suggested that, in humans, cholinergic vagal fibres present in the fundus and corpus exert a continuous restraint on gastrin release and that the antrum is the source of gastrin evoked by vagotomy. Similar studies in the dog wherein selective resection of vagal trunks to the antrum or the proximal stomach was performed also indicated that antral vagal fibres are stimulatory whereas vagal branches that run along the proximal stomach are inhibitory (Schafmayer et al, 1978; Hirschowitz and Gibson, 1979; Debas et al, 1984).

There is a growing body of research that supports a physiological role for bombesin-like peptides as the stimulatory postganglionic neurotransmitters of vagally-induced gastrin secretion. Bombesin (BN), a tetradecapeptide, was originally isolated and sequenced from the skin of the frog, *Bombina bombina* (Erspamer et al, 1970; Anastasi et al, 1971), and its potent secretagogue effect on gastrin-containing cells was first shown in the dog (Bertaccini et al, 1974). Erspamer and Melchiorri (1975) reported the presence of bombesin-like immunoreactivity in tissue extracts of the gastrointestinal tract of mammals and subsequently, a bombesin-like peptide was identified from porcine non-antral gastric tissue (McDonald et al, 1978). The mammalian bombesin was termed gastrin releasing peptide (GRP) after its ability to markedly augment plasma gastrin concentrations in dogs. Isolation and structure determination of porcine gastric (McDonald et al, 1979) and intestinal (McDonald et al, 1983) GRP revealed it to consist of 27 amino acids, the C-terminal decapeptide amide sequence of which was homologous to that of BN except for a single substitution at the 8th position from the carboxy-terminus (Figure 5). Bombesin-like

Figure 5: The amino acid composition of bombesin and gastrin releasing peptide from several species.

Bombesin

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Glp	Gln	Arg	Leu	Gly	Asn	Gln	Trp	Ala	Val	Gly	His	Leu	Met*

Porcine GRP

1			5					10						15						20						25		
Ala	Pro	Val	Ser	Val	Gly	Gly	Gly	Thr	Val	Leu	Ala	Lys	Met	Tyr	Pro	Arg	Gly	Asn	His	Trp	Ala	Val	Gly	His	Leu	Met*		

Canine GRP

			Pro	Gly			Gln							Asp														

Human GRP

Val			Leu	Pro	Ala									Thr														

*represents an amidated carboxy-terminus.

immunoreactivity (BLI) (Polak et al, 1978; Dockray et al, 1979) and gastrin releasing peptide-like immunoreactivity (GRPLI) (Yanaihara et al, 1981; Buffa et al, 1982; Iwanaga, 1983; Moghimzadeh et al, 1983; Ekblad et al, 1984; Costa et al, 1984; Leander et al, 1984) have been localized in neuronal structures of the gastrointestinal tract of mammals including the dog, rat, human, pig, mouse, cat and guinea pig. In addition, GRP/BN-containing nerve fibers have been observed to course high up into the glandular areas of the the corpus and antrum thus providing an anatomical basis for its secretagogue function in the stomach. The origin of these nerve fibers is largely believed to be the intrinsic nervous system of the gut (McDonald, 1988), although GRPLI/BLI has been identified in the vagus (Moghimzadeh et al, 1983).

Early studies revealed that intravenous administration of BN in the dog potently stimulated gastric acid secretion and plasma gastrin levels (Bertaccini et al, 1974). The gastrin response to BN was resistant to antral denervation and atropine pre-treatment, was partly refractory to acidification of the antrum and was completely blocked by antral

resection (Bertaccini et al, 1974; Impicciatore et al, 1974; Erspamer and Melchiorri, 1975). GRP-mediated gastrin release has also been shown to be atropine resistant (McDonald et al, 1978) and independent of vagal integrity (Greenberg et al, 1985) in dogs. In humans, intravenous infusion of BN (Basso et al, 1977; Bloom et al, 1979; Varner et al, 1981) or GRP (Fletcher et al, 1983; Wood et al, 1983) produced copius secretion of gastrin. Comparative studies in the dog showed that GRP and BN possesssed identical biological effects and were equipotent on a molar basis in their abilities to evoke release of gastroenteropancreatic hormones (McDonald et al, 1981; McDonald et al, 1983b; Lambert et al, 1984) and gastric acid (Lambert et al, 1984).

As GRPLI/BLI has only been identified in neural structures, it is generally believed that GRP exerts its effects directly on the G cell via local release rather than in a neuroendocrine manner. In addition to the *in vivo* studies already mentioned, Richelsen et al (1983) demonstrated that BN-stimulated gastrin release from antropyloric glands of human and rat was resistant to hexamethonium and atropine treatment. Furthermore, in the anesthetized dog, local intra-arterial injections of tetrodotoxin (TTX) failed to inhibit the gastrin response to exogenous GRP suggesting a mode of action independent of neural pathways (McDonald and Fox, 1984). Recently, convincing evidence for the direct stimulation of G-cell function by GRP/BN was demonstrated in isolated canine G cells in primary culture (Giraud et al, 1987; Sugano et al, 1987). Indeed, the presence of high affinity GRP/BN receptors has been shown on sections of canine antral mucosa (Vigna et al, 1987) and recently, on isolated canine antral G cells (Vigna et al, 1988).

Much support for GRP as the mediator of vagally-induced gastrin secretion has been obtained from investigations in the pig and rat. In the *in vivo* anesthetized pig model, electrical stimulation of the vagus resulted in elevated GRP output from the corpus and the antrum (Knuhtsen et al, 1984). Vagal stimulation also evoked concomitant increases in gastrin and GRP release and inhibition of somatostatin secretion from the isolated vascularly perfused stomach of the pig (Holst et al, 1983) and rat (Nishi et al, 1985).

Furthermore, addition of atropine further augmented GRP levels, converted the inhibition of somatostatin to stimulation and did not alter the gastrin response to vagal activation in the rat (Nishi et al, 1985). Results from this and other studies (DuVal et al, 1981; Schubert et al, 1982; Schubert and Makhlouf, 1982) predict a model wherein vagal control of gastrin secretion in the rat is mediated by two intramural neurons: a postganglionic cholinergic neuron which facilitated gastrin release by reducing the continuous restraint exerted by somatostatin on the G cell and a postganglionic peptidergic neuron that directly activated the G cell via release of GRP. Substantial support for this hypothesis was obtained by Schubert and his colleagues (1985) who demonstrated complete inhibition of the gastrin responses to field stimulation or DMPP by atropine in combination with BN antiserum.

There is somewhat less information available regarding the mechanisms responsible for vagal inhibition of gastrin release. Although it appears that postganglionic cholinergic pathways are involved, it is not known if acetylcholine is the final mediator. As selective proximal vagotomy resulted in augmented plasma gastrin levels in the human (Stadil and Rehfeld, 1974; Lam et al, 1978) and dog (Schafmayer et al, 1978; Hirschowitz and Gibson, 1979; Debas et al, 1984), it is reasonable to assume that cholinergic inhibition of gastrin secretion is indirect. Grossman (1979) proposed that the vagally-induced inhibitor of gastrin release may be the same agent as that responsible for vagal suppression of gastric acid secretion, ie. the vagogastrone. Several candidates have been shown to antagonize gastrin release as well as the secretagogue action of gastrin on parietal cells. These include secretin (Thompson et al, 1972), glucagon (Becker et al, 1973), vasoactive intestinal polypeptide (VIP) (Rayford et al, 1974), gastric inhibitory polypeptide (GIP) (Rayford et al, 1974), calcitonin (Becker et al, 1974) and somatostatin (Raptis et al, 1975). However, the levels of secretin, glucagon, VIP, GIP and calcitonin needed to elicit inhibition of gastrin release are beyond those observed under physiological conditions (Walsh and Grossman, 1975). There is some evidence to suggest that somatostatin secretion is regulated by the parasympathetic system in some mammals. In humans, plasma

somatostatin levels were augmented by insulin hypoglycemia via a cholinergic-dependent pathway (Wass et al, 1980; Glaser et al, 1981). Furthermore, electrical stimulation of the vagus elevated circulating somatostatin concentrations in the dog, although the peak response occurred after cessation of stimulation, suggesting an indirect effect (Guzman et al, 1979). In contrast, vagal stimulation reduced somatostatin secretion in pigs (Holst et al, 1983), cats (Uvnas-Wallensten et al, 1980) and rats (McIntosh et al, 1981; Nishi et al, 1985). Such scant and conflicting evidence underscores the lack of concrete information implicating the involvement of any one particular peptide or neurotransmitter in the vagal inhibition of gastrin secretion.

Apart from the parasympathetic nervous system, there is some indication that the sympathetic nervous sytem may take part in the regulation of gastrin secretion in some mammals. Intravenous administration of epinephrine elevated circulating gastrin levels in humans and the response was inhibited by a ß-adrenergic blocker (Stadil and Rehfeld, 1973). The possible involvement of the ß-adrenergic system was further supported by the findings that the gastrin responses to insulin-induced hypoglycemia (Christensen, 1984) and gastric distention after atropine (Peters et al, 1982) were suppressed by propranolol, a ß-adrenergic receptor antagonist, in human subjects. In addition, the plasma gastrin response to distention after atropine was augmented by α-adrenergic receptor blockade with phentolamine (Peters et al, 1982). Lucey et al (1985) also demonstrated moderate increases in postprandial gastrin concentrations with the administration of thymoxamine, an α-adrenergic antagonist. Taken together, these data suggested the participation of stimulatory ß- and inhibitory α-adrenergic mechanisms in the control of gastrin release in humans. Similar results have been found in the rat wherein ß-adrenergic agonists elicited potent release of gastrin *in vivo* and *in vitro* which was inhibited by ß-receptor blockers (Lundell et al, 1976; Hsu and Cooper, 1977; DeSchryver-Kecskemeti et al, 1981; Short et al, 1985; Harty et al, 1988). Conversely, α-receptor activation reduced basal gastrin secretion which was normalized by the addition of phentolamine (DeSchryver-Kecskemeti

et al, 1981). Short and his co-workers (1985) further demonstrated that ß-adrenergic-induced release of gastrin from the isolated perfused rat stomach was abolished by inclusion of antibodies to GRP indicating that the ß-adrenergic effect in this species is mediated by GRP intramural neurons. In one study, however, neither isoproterenol nor the specific $ß_2$-receptor agonist, salbutamol, could evoke significant gastrin secretion from the *in vitro* rat stomach preparation (Koop et al, 1983).

Results from investigations into the gastrin releasing property of gastric distention have been mixed. In isolated canine antral and fundic pouches, distension produced gastrin secretion and the response was inhibited by parasympathetic denervation or by atropine treatment (Debas et al, 1974; Debas et al, 1975). On the other hand, baloon or saline distention of the intact canine stomach did not result in significant elevations of gastrin concentrations (Forrest et al, 1978). Likewise, in human subjects, distention of the intact stomach failed to significantly augment circulating gastrin levels (Schrumpf and Stradaas, 1974; Soares et al, 1977; Forrest et al, 1978; Schiller et al, 1980), but stimulated gastric acid output (Soares et al, 1977; Schiller et al, 1980). However, the gastrin response to distention was markedly enhanced by atropine administration (Schiller et al, 1980), implying that inhibitory cholinergic and stimulatory noncholinergic pathways are involved. Evidence exists to suggest that the stimulatory noncholinergic mechanism is mediated by ß-adrenergic receptors (Peters et al, 1982).

Digestion products of protein, in particular amino acids and small peptides, have been shown to evoke gastrin release (Elwin, 1974; Strunz et al, 1978b). It was also revealed that the accessibility and position of the amino group was critical for the gastrin releasing activity (Elwin, 1974). For instance, the secretory effects of glycine and alanine were diminished when the amino group was methylated or in the α-configuration, respectively. Tryptophan and phenylalanine have been found to be the most potent gastrin secretagogues (Taylor et al, 1982) and the gastrin response requires luminal contact as intravenous administration of amino acids failed to stimulate gastrin secretion (Isenberg and

Maxwell, 1978; McArthur et al, 1983). Peptone solutions given intragastrically produced concentration-dependent increases in circulating plasma levels in humans (Lam et al, 1980), whereas glucose or fat did not cause appreciable release of gastrin (Richardson et al, 1976). It has been proposed that activation of gastrin secretion by dietary amino acids is dependent on their entry into the G cell and their subsequent conversion into amines by decarboxylation (Lichtenberger et al, 1982). Schiller and his colleagues (1982) observed that low doses of atropine inhibited the gastrin response to an intragastric infusion of amino acids and concluded that unlike sham feeding or distention-stimulated gastrin secretion, food-stimulated gastrin release in humans may be regulated by a stimulatory cholinergic mechanism. Decaffeinated coffee (Feldman et al, 1981) and wine (Lenz et al, 1983) have also been shown to be potent stimulants of gastrin release in the human, and ethanol elevated gastrin concentrations in the dog (Elwin, 1969; Becker et al, 1974b) but not in humans (Becker et al, 1974b; Lenz et al, 1983). Finally, calcium ions augmented gastrin release when given orally (Levant et al, 1973) and intravenously (Reeder et al, 1974).

The primary physiological inhibitor of gastrin secretion during a meal is acid in the gastric lumen. This effect was first discovered by the indirect method of measuring acid output from canine Heidenhain pouches as an index of gastrin release. Several investigators determined that antral acidification produced marked reduction of the gastrin responses to local stimulation (Oberhelman et al, 1952; Longhi et al, 1957), insulin-induced hypoglycemia (Oberhelman et al, 1957) or sham feeding (Pe Thein and Schofield, 1959). Nilsson et al (1972) gave direct evidence of the inhibitory nature of acid on gastrin release using radioimmunoassay. They showed that plasma gastrin concentrations stimulated by sham feeding in dogs were augmented when secreted acid was removed via a gastric fistula. Significant inhibition of gastrin release was observed below pH 3 with maximal suppression at pH 1 (Walsh et al, 1975). In normal humans, the gastrin response to an amino acid test meal was diminished by 80% at pH 2.5. This feedback inhibiton, initially believed to be a direct effect of acid on the G cell (Redford and Schofield, 1965),

may be mediated by cholinergic pathways since low doses of atropine blocked the inhibitory action of low intragastric pH on sham feeding-stimulated gastrin secretion (Feldman and Walsh, 1980). Also, there is some indication that acid does not suppress all stimulants of gastrin. Schiller and his co-workers (1980) demonstrated that gastrin output evoked by distention in human subjects was identical at pH 2.5 and 5. Although acute administration of acid or alkali did not alter basal gastrin levels (Debas et al, 1974; Higgs et al, 1974), extended periods of hypoacidity (hypochlorhydria) or lack of acid (achlorhydria), as seen in patients with atrophic gastritis, resulted in basal hypergastrinemia (Yalow and Berson, 1971; Creutzfeldt et al, 1971; Peters et al, 1983) which may be secondary to accompanying G-cell hyperplasia (Creutzfeldt et al, 1971; Peters et al, 1983).

An adequate analysis of the physiological regulators of gastrin secretion necessitates a more detailed scrutiny of the relationship between somatostatin and gastrin. Immunocytochemical studies have established that the majority of the somatostatin-like immunoreactivity (SLI) in the mammalian stomach is found in endocrine-like cells, termed D cells (Hokfelt et al, 1975; Polak et al, 1975; Rufener et al, 1975), although SLI-containing neurons have been identified in antral muscle layers (Keast et al, 1984). The antrum and corpus contain the highest density of D cells and their occurrence here is of particular interest because of their close association with antral G cells and corporeal parietal cells. In addition to the possible endocrine control of gastrin secretion by somatostatin released into the circulation, D cells project tail-like processes that often contact on G cells thus providing an anatomical basis for potential paracrine control of G-cell activity via somatostatin secreted into the interstitial spaces (Alumets et al, 1979; Larsson et al, 1979). Larsson (1984) further substantiated the paracrine concept by demonstrating anterograde transmission of SLI granules along the length of the cytoplasmic extensions of the D cell.

Infusion of exogenous somatostatin has clearly been shown to result in profound inhibition of gastrin release during basal and stimulated conditions (Bloom et al, 1974;

Raptis et al, 1975; Vatn et al, 1977; Schusdziarra, 1983; Koop et al, 1988). Furthermore, a substantial amount of evidence exists to suggest that a functional and reciprocal relationship occurs between gastrin and somatostatin secretion. Gustavsson and Lundquist (1978) demonstrated simultaneous stimulation of gastrin and reduction of somatostatin levels in the antral portal circulation of anesthetized pigs after instillation of a meal or alkaline substances into the stomach. Similar observations have been reported using the isolated vascularly perfused rat stomach preparation, wherein cholinergic agonists (Saffouri et al, 1980; Martindale et al, 1982), electrical stimulation of the vagus (McIntosh et al, 1981) and field stimulation (Schubert et al, 1982) increased gastrin secretion and suppressed somatostatin output. In contrast, concomitant inhibition of gastrin and augmentation of somatostatin were observed during the infusion of secretin, glucagon, VIP (Chiba et al, 1980) and prostaglandin E_2 (Saffouri et al, 1980). In addition, inclusion of somatostatin antiserum in the perfusate produced significant elevation of basal gastrin levels suggesting that somatostatin exerts a continuous restraint on the G cell (Saffouri et al, 1979; Short et al, 1985; Koop et al, 1988; Tang et al, 1988). It has been proposed that suppression of this somatostatinergic tone is partly responsible for the observed gastrin response to cholinomimetics and vagal stimulation from the *in vitro* rat stomach. In spite of such evidence, it appears that the functional linkage is not permanent. Bombesin infusion has been shown to result in increased concentrations of both peptides (DuVal et al, 1981; Martindale et al, 1982), whereas the opposite was observed with methionine-enkephalin (Chiba et al, 1979). Also, post-vagotomy hypergastrinemia in rats has been shown to be independent of changes in SLI secretion (Pederson et al, 1981), but rather a consequence of augmented cholinergic activation of a normal G-cell population (Pederson et al, 1984). Isoproterenol (Koop et al, 1982; Koop et al, 1983) and GIP (Pederson et al 1981) produced marked stimulation of somatostatin concentrations without altering gastrin levels.

The routes by which somatostatin exerts its suppressive action on gastrin secretion are not completely understood. Immunoneutralization studies support an endocrine mode

of action (Saffouri et al, 1979; Short et al, 1985; Koop et al, 1988; Tang, 1988) and this has been reinforced by the finding that physiological concentrations of somatostatin significantly lowered acetylcholine-induced gastrin release from the perfused rat stomach (Koop et al, 1988). Contrary to these reports, Chiba and his colleagues (1981) were unable to potentiate gastrin secretion using antibodies to somatostatin in anesthetized rats as well as from the *in vitro* rat stomach. Augmented gastrin release was only exhibited upon the addition of somatostatin antiserum to the incubation medium containing scrapings of rat antral mucosa. Using a similar preparation, Wolfe et al (1984) demonstrated an elevated gastrin response to carbachol-stimulation with somatostatin immunoneutralization. Taken together, these latter studies lend support to a paracrine pathway for the local regulation of G-cell function by somatostatin.

There is little information available regarding the intracellular mechanisms that mediate stimulus-secretion coupling in gastrin cells. This has been largely due to methodological limitations in obtaining a single cell preparation sufficiently enriched for G cells. Recent technical advances have permitted the isolation and enrichment of gastric endocrine cells of the dog and subsequently, the study of the 2nd messenger systems involved in gastrin secretion in these animals (Giraud et al, 1987; Sugano et al, 1987). These investigators observed potent stimulation of IR-G release from cultured canine G cells after a 2 h exposure to dibutyryl cAMP and the adenylate cyclase activator, forskolin. Furthermore, concentration-dependent release of gastrin was demonstrated with the addition of the calcium ionophore, A23187, and the phorbol ester, ß-PMA, a protein kinase C activator (Giraud et al, 1987). Conversely, depletion of Ca^{2+} from the medium resulted in marked attenuation of the gastrin response to bombesin (Sugano et al, 1987). Collectively, these results suggested that bombesin-induced gastrin release from canine G cells is partially mediated by Ca^{2+}-dependent pathways and that cAMP as well as Ca^{2+}-protein kinase C mechanisms are involved in G-cell stimulation.

The significant biological actions of gastrin, particularly its secretagogue and mitosis-promoting functions within the gastrointestinal tract, have provided the impetus for research leading to the accumulation of a voluminous amount of data concerning the hypo- and hypersecretion of this hormone in normal and pathophysiological states in experimental animals and humans. As there are no known diseases of G-cell hypoplasia and hyposecretion, researchers have relied upon food restriction and total parenteral nutrition models in order to examine the development and pathogenesis of gastrin deficiency. Prolonged food deprivation has been shown to result in a marked reduction of both serum and antral gastrin content in rats (Lichtenberger et al, 1975; Bertrand and Willems, 1980; Koop et al, 1982; Schwarting et al, 1986), an effect believed to be, at least in part, caused by a decrease in the G-cell population (Lichtenberger et al, 1975; Bertrand and Willems, 1980; Schwarting et al, 1986). These authors concluded that luminal stimulation of the gastrointestinal tract is necessary for the preservation of G-cell activity and numbers. Although this may be true, the multitude of metabolic changes that accompany total food restriction cannot be ignored when interpreting observations from starvation studies. To circumvent these difficulties, the model of intravenous feeding or total parenteral nutrition has been developed.

Total parenteral nutrition (TPN) facilitates the study of changes in gut morphology and hormone release in the absence of luminal stimulation but with adequate nutritional support. TPN has been shown to result in hypoplasia of the stomach, small bowel and pancreas of rats and pigs (Johnson et al, 1975a; Johnson et al, 1975b; Goldstein et al, 1985). In addition, a dramatic reduction in antral and serum gastrin levels was demonstrated in parenterally-fed rats (Johnson et al; 1975a; Johnson et al, 1975b; Track, 1980; Campos et al, 1987). TPN-induced gastrin hyposecretion was also observed under stimulated conditions *in vivo* and persisted in the isolated perfused stomach preparation and was associated with antral G-cell hypoplasia (Campos et al, 1987). Furthermore, the re-introduction of oral feeding with a solid rat chow diet normalized the gastrin

concentrations and the G-cell population of animals after TPN treatment (Wheeler, 1988). Collectively, these results suggested that oral intake of food is a necessary requirement for the maintenance of normal G-cell numbers, secretory function and gastrointestinal growth.

On the other hand, gastrin overproduction has received much attention because of its implications for gastic acid secretion. Gastrin plays a crucial role in the pathogenesis of peptic ulcer disease found in Zollinger-Ellison syndrome, isolated retained antrum and antral G-cell hyperfunction syndromes (Walsh and Lam, 1980; Lamers, 1988). Zollinger and Ellison (1955) were the first to correctly hypothesize that islet cell tumors present in patients with recurrent aggressive peptic ulcer were the source of a stimulant of gastric acid secretion. Their proposal was confirmed with the isolation of gastrin-like substances from tumor tissue the sequences of which were compatible with G-17 and G-34 (Gregory et al, 1969; Gregory and Tracy, 1972). Gastrinomas most frequently occur in the pancreas, although duodenal (Goodman, 1964; Oberhelman and Nelson, 1964; Thompson et al, 1968) and gastric (Royston et al, 1972; Larsson et al, 1973) sites have been reported. In contrast, antral G-cell hyperfunction is characterized by basal hypergastrinemia, an exaggerated gastrin response to a test meal that is antral in origin and persists in spite of significantly high levels of gastric acid (Berson and Yalow, 1972; Berson et al, 1973; Lamers et al, 1978; Lewin et al, 1984; Lamers et al, 1988). G-cell hyperfunction may (Lewin et al, 1984) or may not (Lamers et al, 1978) be accompanied by an increase in antral G-cell population. Thirdly, elevated circulating gastrin levels have been demonstrated in patients who have undergone combined antrectomy and gastrojejunostomy where the antrum is retained at the duodenal stump (Walsh and Lam, 1980; Lamers et al, 1988). The underlying mechanism for gastrin hypersecretion is believed to be the lack of feedback inhibition on the G cell by acid because of its isolation from the parietal cell mass. Differential diagnosis of these three disorders is possible by measuring the gastrin responses to secretin and a test meal (Lamers, 1981). Intravenous administration of secretin promotes paradoxical release of gastrin from gastrinomas but not from antral

tissue, whereas food produces dramatic increases in gastrin secretion in patients with antral G-cell hyperfunction only.

The promise of a cure for peptic ulcer disease with the isolation of gastrin by Gregory and Tracy (1964) has not materialized. In duodenal ulcer patients, fasting serum gastrin concentrations were similar to those in normal controls although their gastrin responses to food were somewhat higher than normal (Walsh, 1978). These results, taken together with the fact that about 50% of duodenal ulcer patients have elevated gastric acid outputs (Lamers, 1988), suggest an impairment in the feedback inhibition exerted by acid on gastrin secretion. Furthermore, postprandial gastrin secretion in duodenal ulcer subjects was significantly lower than normals during an acidified meal test (Walsh et al, 1975). To date, the underlying mechanism of abnormal autoregulation is not known. The elevated circulating gastrin levels observed after a meal in duodenal ulcer patients may bear some clinical significance, as hypersensitivity of the parietal cell mass to exogenous (Isenberg et al, 1975) and endogenous (Lam et al, 1980) gastrin has been observed. In comparison, patients with gastric ulcers generally display lower rates of acid secretion than normal individuals (Wormsley and Grossman, 1965). Reduced acid secretion leading to an attenuated inhibitory signal on the G cell may account for the higher fasting and meal-stimulated serum gastrin levels of gastric ulcer patients (Trudeau and McGuigan, 1971; Gedde-Dahl, 1974).

Studies investigating gastrin secretion in obesity have yielded mixed results. The gastrin responses to a meal (Besterman et al, 1978; Schrumpf et al, 1981) and to epinephrine (Atkinson et al, 1979) were reported to be similar in lean and obese subjects. In contrast, fasting and meal-stimulated hypergastrinemia were demonstrated in obese nondiabetic human patients (Schusdziarra et al, 1985), as well as obese patients with non-insulin-dependent diabetes (Type II or maturity-onset diabetes) (Sasaki et al, 1983; Kanatsuka et al, 1984). Increased serum and antral gastrin concentrations have also been shown in 10 week-old genetically obese (ob/ob) mice (Morton et al, 1985) and in the

obese, genetically diabetic (db/db) mouse (Lichtenberger and Ramaswamy, 1979), both of which are animal models of maturity-onset diabetes. Similar observations have been found in rats made obese by lesions of the ventromedial hypothalamus (VMH) (Chikamori et al, 1982). As pair feeding did not ameliorate gastrin responses in VMH-lesioned rats, it was further suggested that gastrin hypersecretion was probably secondary to a disturbance of the parasympathetic nervous system which plays a crucial role in the development of obesity and hyperinsulinemia in these animals (Powley and Opsahl, 1974; Chikamori et al, 1977; Inoue and Bray, 1977).

The Zucker "fatty" rat (Figure 6) has been widely used as a model of obesity. The ""fatty"" rat originally developed from a spontaneous mutation resulting from a Merck Stock M rat and Sherman rat breeding cross (Zucker and Zucker, 1961). Obesity in this animal is monogenic and transmitted as a recessive Mendelian characteristic. Increased adiposity is expressed as a hyperplasia (Johnson et al, 1971) and hypertrophy (Bray, 1969) of adipocytes, particularly in subcutaneous sites (Johnson et al, 1971). In addition, hyperlipidemia in the form of greatly elevated cholesterol (Zucker and Zucker, 1962), triglyceride (Barry and Bray, 1969; Bremond et al, 1982) and lipoprotein (Zucker, 1965; Schonfeld et al, 1974) levels has been well documented. It is generally believed that hyperinsulinemia in the ""fatty"" rat is intimately involved in the manifestation of obesity (Zucker and Antoniades, 1972; Bryce et al, 1977; Godbole and York 1978). There is some debate, however, over its exact role in the pathogenesis of the obese state. Some investigators claim that hyperinsulinemia precedes and, in turn, causes increased hepatic lipogenesis (Bryce et al, 1977; Godbole and York, 1978), whereas others speculate that the primary defect is an elevation in lipogenic enzyme activity resulting in preferential deposition of nutrients in fat stores with insulin hypersecretion arising from hyperphagia (Greenwood et al, 1983).

Although the etiology of hyperinsulinemia in the obese rat is not fully understood, some evidence exists to suggest that this defect is mediated, at least in part, by the vagus

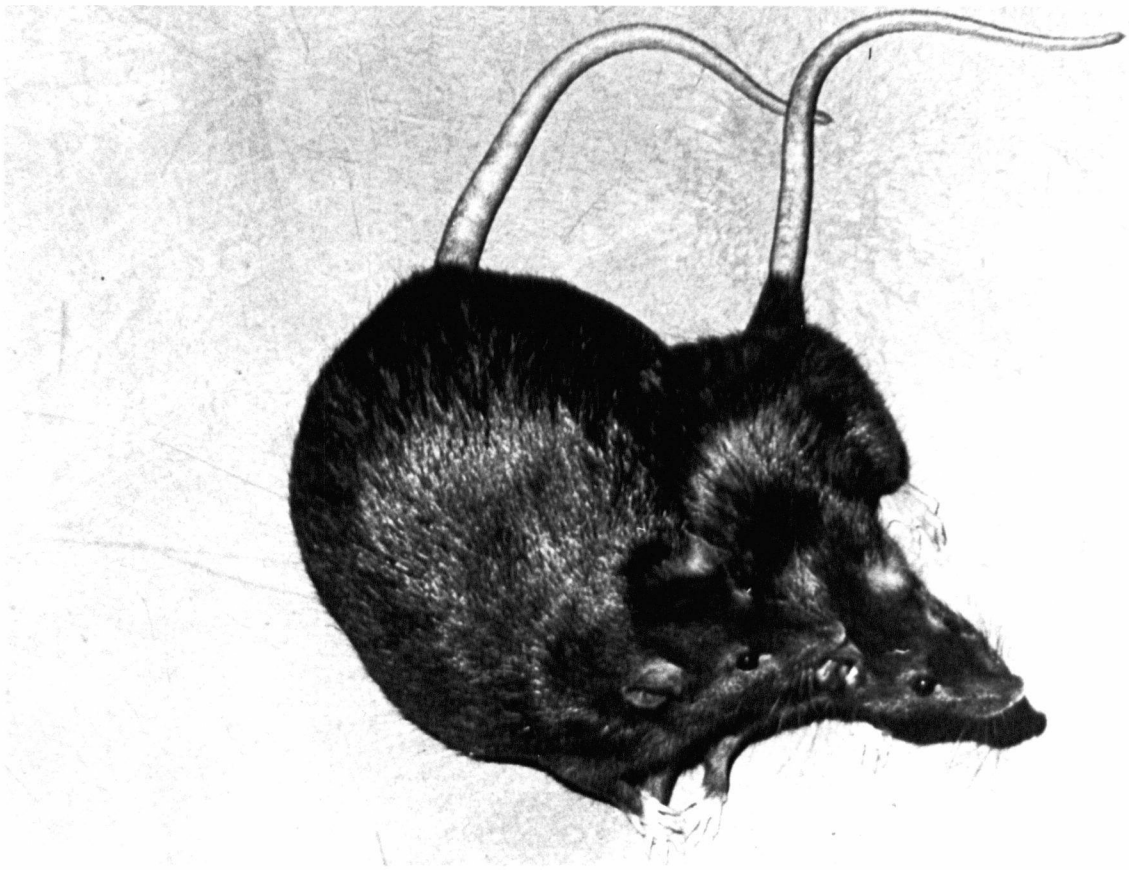


Figure 6 : The Zucker rat strain: obese (left) and lean (right) littermates.

nerve. Rohner-Jeanrenaud et al (1983) demonstrated atropine-sensitive arginine-stimulated insulin release from the isolated perfused stomach of young obese animals. Furthermore, vagotomy and atropine treatment have been shown to reduce the augmented insulin response of obese rats to a glucose load (Rohner-Jeanrenaud et al, 1983; Fletcher and McKenzie, 1988).

The aim of this thesis was to systematically study the mechanisms that control G-cell function in the lean and obese Zucker rat, dog and human. The availability of the Zucker rat strain and the presence of multiple imbalances in the endocrine profile of the obese rat (Bray, 1970; Bray and York 1971; Bray et al, 1974), particularly hyperinsulinemia, prompted an investigation into the circulating gastrin levels of this animal. A preliminary report indicated that the Zucker "fatty" rat possessed plasma gastrin concentrations that were higher than those of lean littermates (Pederson et al, 1985). Thus, the unique opportunity was available to study abnormal gastrin secretion superimposed upon the pathophysiological syndrome of obesity . Furthermore, local breeding and housing of the Zucker rat colony facilitated observations on early development of the hypergastrinemic condition and its relationship to the onset of obesity.

Studies investigating the onset, severity and the regulation of hypergastrinemia of the obese animal are reported in the first part of this thesis. Because of possible vagal dysfunction in obese rats, early studies focussed on the cholinergic control of gastrin secretion in lean and obese animals using the isolated vascularly perfused stomach preparation. The effects of atropine treatment on gastrin secretion under basal conditions and during electrical stimulation of the vagus nerve were examined. The potential for functional and anatomical linkage between somatostatin and gastrin cells was also investigated by measuring immunoreactive-somatostatin release from the *in vitro* stomachs of lean and obese rats as well as the antral D-cell density of both groups of animals. Gastrin-containing cells in antral sections of 3 week-old and 6 month-old animals were quantified to determine if G-cell hyperplasia could account for gastrin excess in the obese

rat. Furthermore, hyperphagia as a signal for gastrin hypersecretion was examined in pair-feeding experiments. Obese animals were given food rations equal to the daily food intake of lean animals from 5-8 weeks of age. Subsequently, fasting plasma gastrin concentrations, basal gastrin secretion from the perfused stomach preparation and the antral G-cell population of lean controls, freely-feeding obese animals and diet-restricted obese rats were determined. Finally, the involvement of bombesin/gastrin releasing peptide in the hypergastrinemia of the obese Zucker rat was assessed. Antral GRP content was measured and the *in vitro* responses of lean and obese animals to exogenous BN in the presence and absence of two putative BN antagonists, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P and [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN, were investigated.

Whole animal and isolated organ studies have provided much insight into how multiple signals acting directly or indirectly on the G cell are integrated to regulate gastrin secretion *in vivo*. Observations from these studies, however, do not allow precise assessment of the actions of gastrin secretagogues and inhibitors because of the potential for interactions among paracrine, endocrine, neural and luminal influences prior to and/or at the G cell. Accurate determination of the actions of secretagogues and inhibitors can only be achieved by investigating the determinants of gastrin release at the cellular level. Recent advances in methodology involving preferential enrichment of gastric endocrine cell preparations by centifugal elutriation and short-term tissue culture have permitted the study of the local regulation of peptide secretion from isolated canine D and G cells (Soll et al, 1984; Yamada et al, 1984; Giraud et al, 1987; Sugano et al, 1987). These investigators provided convincing evidence of direct inhibition and stimulation of antral G cells by somatostatin and BN/GRP, respectively (Giraud et al, 1987; Sugano et al, 1987). Recently, the existence of high affinity BN/GRP receptors on isolated canine G cells was reported (Vigna et al, 1988). In addition, intracellular events leading to the release of gastrin were shown to involve the adenylate cylase and phosphatidyl inositide systems (Giraud et al, 1987; Sugano et al, 1987).

To date, three classes of BN/GRP-receptor antagonists have been described. One class behaved as substance P (SP) analogs and were also SP antagonists (Jensen et al, 1984; Jensen et al, 1988). The second class was [D-Phe^{12}] BN analogs (Heinz-Erian et al, 1987; Merali et al, 1988) and the third class included BN analogs with reduced peptide bonds (Coy et al, 1988). The latter group of compounds featured the replacement of the peptide bond between amino acids with a methyl amide (CH_2NH) moiety. These three classes of BN/GRP-receptor antagonists have been shown to be competitive inhibitors of BN-stimulated amylase secretion from dispersed pancreatic acini (Jensen et al, 1984; Heinz-Erain et al, 1987; Coy et al, 1988; Jensen et al, 1988), but their effectiveness in reducing BN-evoked gastrin release remains equivocal. The antagonist [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] SP attenuated the gastrin response to exogenous GRP and blocked vagally-mediated gastrin release in pigs (Holst et al, 1987). However, another SP analog, [D-Arg^{1}, D-Trp^{7,9}, Leu^{11}] substance P (spantide), failed to alter BN-stimulated gastrin secretion from isolated canine G cells in primary culture even at spantide concentrations six orders greater than that of BN (Giraud et al, 1987). Because the ability to demonstrate inhibition of effect at the level of the G cell is fundamentally important in the assignment of a physiological role for BN in the stimulation of gastrin secretion, an investigation of the efficacy of two putative bombesin antagonists, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P and [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN, in suppressing BN-stimulated gastrin release was undertaken. To facilitate the interpretation of these studies with respect to work already described in the dog, isolated and cultured canine cells were used as the experimental model. Furthermore, since these antagonists were also employed in experiments examining the role of BN/GRP in the hypergastrinemia of the obese Zucker rat, the opportunity for qualitative characterization of BN/GRP receptors between species was provided.

The availability of gastric tissue from healthy organ donor transplant patients provided the impetus for the investigation of the local regulation of peptide secretion from

isolated and cultured human gastric endocrine cells described in the remainder of this thesis. Major differences exist between mammalian species in the control of gastrin secretion as studied in the intact organism. Although studies similar to those described here using human cells have been reported in canine cells (Giraud et al, 1987; Sugano et al, 1987), it cannot be assumed that canine G-cell control mechanisms are identical to those found in the human gastrin-secreting cell. Thus, a method for the isolation and culture of human antral mucosal cells enriched for G cells is described in this paper. The secretory responses of these cells to stimulators and inhibitors were taken as a measure of their functional integrity. The intracellular mechanisms that mediate stimulus-secretion coupling in human G cells were investigated employing post-receptor activators of the cAMP and Ca^{2+}-protein kinase C second messenger systems. In addition, peptidergic control of G-cell function was examined using bombesin and the putative bombesin antagonist, [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN. Finally, the involvement of somatostatin in G-cell function was examined in experiments in which somatostatin was added singly or in combination with G-cell activators. Immunoneutralization experiments using somatostatin-specific monoclonal antibodies were also undertaken.

Clearly, the benefits of using an isolated and cultured cell preparation in the study of the control of gastrin secretion cannot be overstated. However, this approach is by itself not sufficient to explain all the enigmas that abound in regulatory peptide physiology. In conjuction with results obtained from investigations using cellular preparations of increasing structural integrity, information obtained from single cell studies can provide invaluable corroborating evidence in the proper elucidation of the complex physiological mechanisms that control peptide secretion in the intact animal. Further to the study of the regulation of gastrin release in the Zucker rat, the dog and the human, the goal of this thesis is to illustrate the importance of the integration of a broad range of techniques in the study of a single biological process.

METHODS AND MATERIALS

I. RADIOIMMUNOASSAY (RIA):

A. Rationale:

The radioimmunoassay (RIA) method was originally developed by Yalow and Berson (1960). It is a competitive binding assay based upon the principles that govern antigen-antibody reactions. Peptide quantification of unfractionated plasma and perfusate samples was performed using the RIA because of its high sensitivity, specificity and reproducibility.

B. Calculations:

In all assays, segregation of bound and free ^{125}I-peptide was achieved by charcoal separation and only the free material retained in the charcoal pellet was counted. Consequently, the percentage of ^{125}I-peptide bound was determined by difference according to the following formula:

$$\% \text{ Bound} = \frac{(C_{total} - C_{sample})}{C_{total}} - \frac{(C_{total} - C_{NSB})}{C_{total}} \times 100$$

where C = counts per min and NSB = non-specific binding. NSB tubes contained every incubation component used in the sample tubes except for the antiserum in order to measure the amount of non-specific binding of labelled peptide to organic and inorganic components of the incubation mixture. A standard curve was then constructed by plotting % specific binding versus the standard concentrations (Figure 7a). Peptide concentrations of the samples were determined by the orthogonal point on the standard curve for any given % bound. Alternatively, a data reduction software system was used to transform the standard plot into a logit-log plot and the peptide concentrations were calculated according to the raw

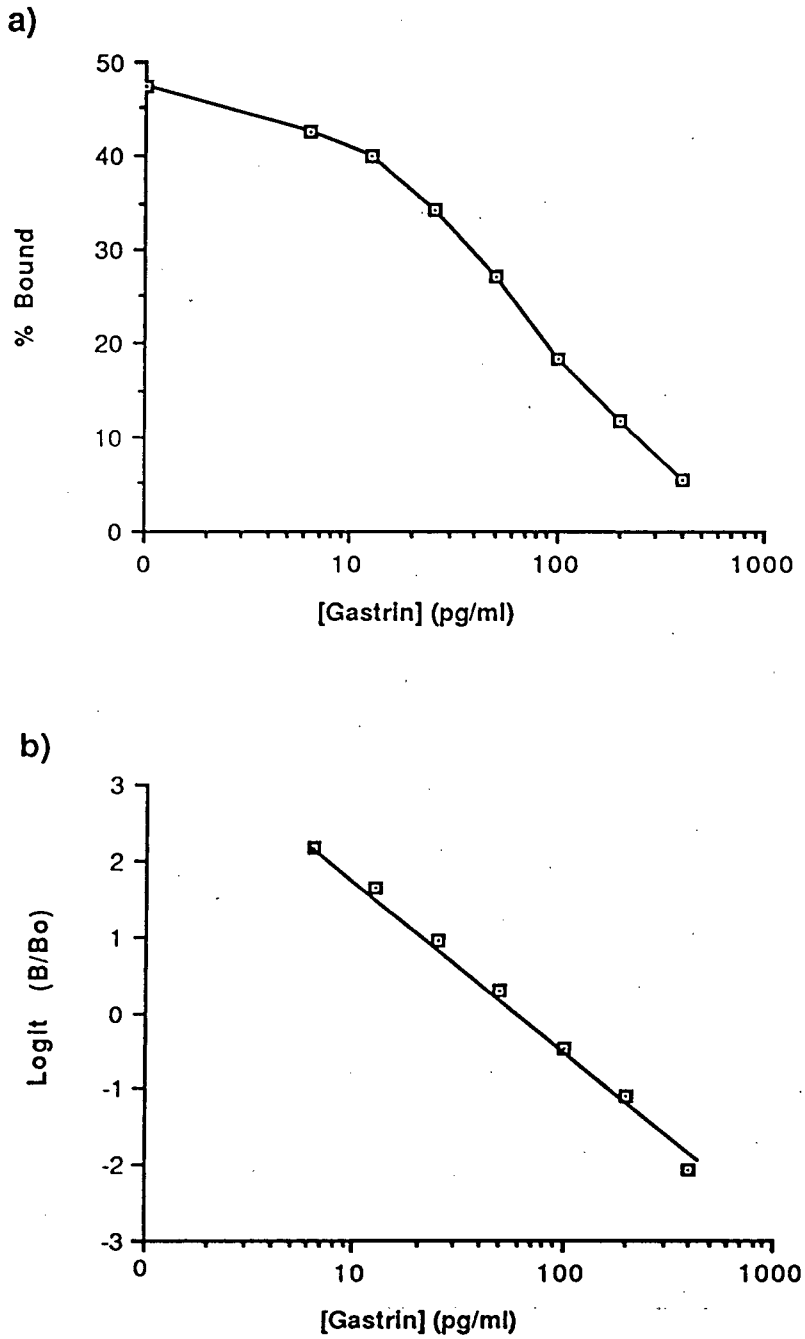


Figure 7 : a) Immunoreactive-gastrin standard curve: % Bound of ^{125}I-labelled gastrin vs. [Gastrin] in pg/ml.
b) Immunoreactive-gastrin standard curve: Logit [B/Bo] vs. [Gastrin] in pg/ml.

counts (Figure 7b). Multiplication of the peptide concentrations by the dilution factor and/or the perfusion flow rate gave the final peptide quantities.

C. Gastrin:

Immunoreactive-gastrin (IR-G) was measured using a RIA protocol originally described by Jaffe and Walsh (1978).

1. Iodination:

The iodination procedure was a modification of the method described by Stadil and Rehfeld (1972). Five micrograms of synthetic human gastrin I were dissolved in 10 µl 0.4 M phosphate buffer (0.4 M Na_2HPO_4, titrated to pH 7.4 with 0.4 M NaH_2PO_4). This mixture was incubated with 0.2 mCi $Na^{125}I$ and 10 µl chloramine T (0.5 mg/ml in 0.04 M phosphate buffer) for 60 s. The oxidation reaction was terminated by the addition of 10 µl sodium metabisulphite (0.5 mg/ml in 0.04 M phosphate buffer) and buffered with 0.5 ml imidazole buffer (0.05 M, pH 7.5). A 1.5 ml polypropylene tube (Eppendorf®, Brinkmann Instruments, Westbury, New York) was used as the iodination vessel.

Purification of the labelled hormone was achieved using a modification of the procedure described by Brown et al. (1976). Fractionation of the iodination mixture was accomplished by anion exchange chromatography. One gram of Sephadex DEAE A-25 was washed and swollen in 0.05 M imidazole buffer overnight, and equilibrated on a 0.9 cm x 13 cm column with the imidazole buffer. The column was connected to a peristaltic pump which was fed by a gradient vessel containing 50 ml of imidazole buffer in the proximal arm and 55 ml of 1 M NaCl in the distal arm. The iodination mixture was added to the column and 2 ml fractions were collected at an elution flow rate of 1 ml/min. Ten microliter aliquots were counted and an elution profile plotted. The 4 fractions after the second radioactive peak containing the bulk of the monoiodinated gastrin were tested in an RIA. Fractions that displayed the best standard curves and the most accurate control values were pooled, diluted in RIA buffer, dispensed in 1 ml aliquots (900,000 counts/tube;

sufficient for 3 assays) and stored at -20°C. The iodinated gastrin was used in the assay at a final concentration of 2000 cpm/100 µl/tube in RIA buffer.

2. Assay Buffer:

The assay buffer consisted of 0.02 M (4.12 g/L) sodium barbital and 0.5% BSA (5 g/L, RIA grade, Sigma) in distilled H_2O. The solution was titrated to pH 8.4 with concentrated HCl.

3. Antisera:

a) Zucker Rat Studies:

Preliminary studies in the Zucker rat employed antiserum L2 (a kind gift from Dr. G.J. Dockray, Liverpool, U.K.) at a final dilution of 1:300,000. Antiserum L2 contains a C-terminal directed antibody which binds human G-17-I and -II in approximately equimolar amounts, and G-34 to a lesser degree (Dockray et al, 1977). L2 does not recognize cholecystokinin variants. Due to the limited source of this antiserum, a second antiserum 1611 (a generous gift from Dr. J.H. Walsh, CURE, Los Angeles, CA.) was used in the assay at a final dilution of 1:250,000. This antibody binds to C-terminal residues, recognizes G-34 and G-17 equally well and possesses a very low affinity for cholecystokinin peptides (0.6% cross-reactivity; Walsh et al, 1982). Antisera L2 and 1611 were initially dissolved in distilled H_2O, then diluted in assay buffer to a 1:500 dilution and stored in 1 ml aliquots at -20°C until used in the assay. The range of sensitivity of both antisera was between 10 pg/ml and 250 pg/ml. A comparison of sample gastrin levels obtained with antisera L2 and 1611 is shown in Figure 8. The nearly identical profiles were taken as validation of their use interchangeably in the Zucker rat studies. As a further control, all assays measuring the effect of a particular treatment (eg. atropine) were done with either L2 or 1611 only and not a combination of both antisera.

b) Canine Studies:

IR-G levels in the supernatants and cell extracts were measured using antiserum 1611.

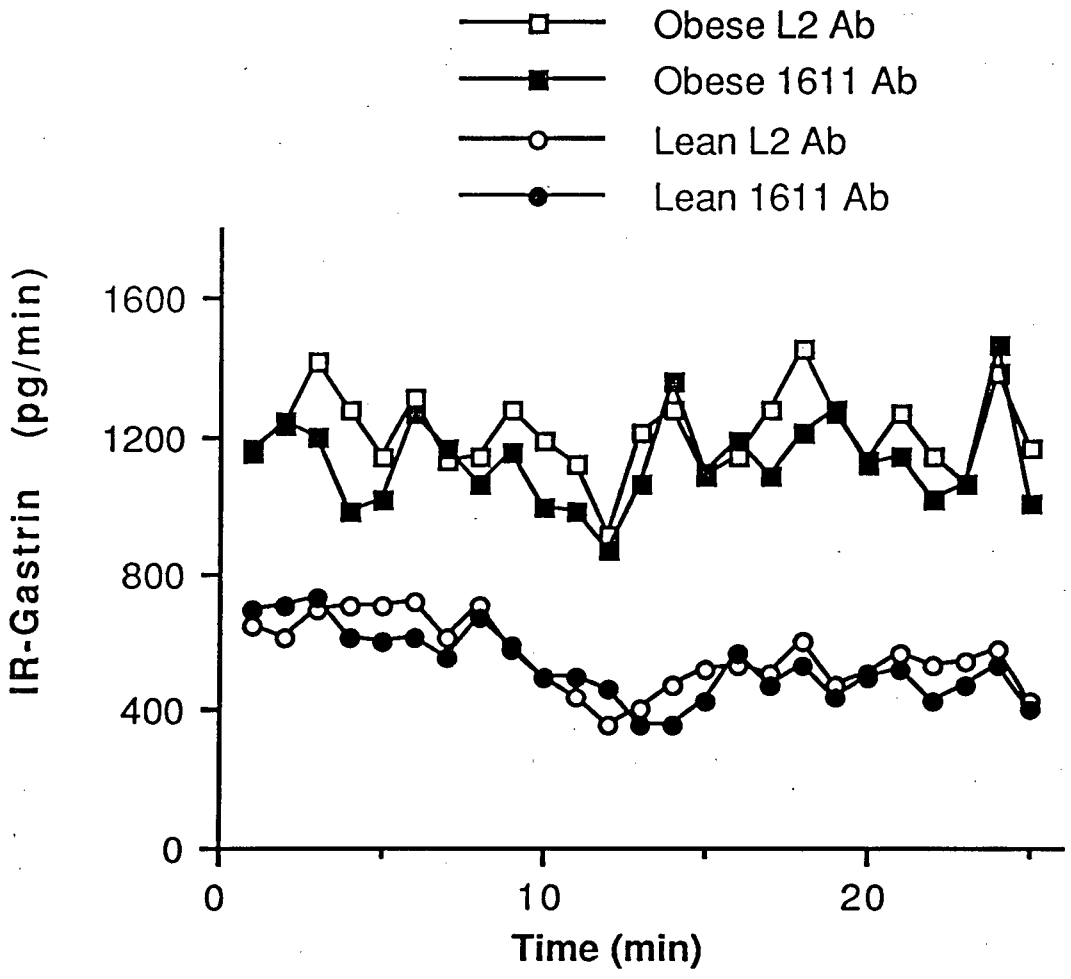


Figure 8 : A comparison of basal IR-G levels obtained by radioimmunoassay using antisera L2 and 1611.

c) Human Studies:

IR-G levels in the supernatants and cell extracts were measured with an antiserum raised in guinea pig against synthetic human gastrin I, antiserum CKG2 (Kwok et al, MRC Regulatory Peptide Group, unpublished results). Antiserum CKG2 was stored at 1:100 dilution in 0.9% saline containing 0.1% NaN_3 and 0.5% BSA (RIA grade) at -20°C and used in the assay at a final dilution of 1:2,500,000. CKG2 recognizes gastrin-17 only and does not cross-react with cholecystokinin octapeptide. The useable range of the assay is from 25 pg/ml to 800 pg/ml.

4. Standards:

Synthetic human gastrin I was stored in 200 µl aliquots of 100 ng/ml assay buffer at -20°C. For use in the assay, one aliquot was diluted to 50 ml of assay buffer and then serially diluted to generate standards ranging from 6.25 to 400 pg/ml. The standards were stored as 1 ml fractions at -20°C. In assays of samples from the isolated human G-cell cultures, the standard curve was extended to include 800 and 1600 pg/ml.

5. Controls:

Synthetic human gastrin I was stored in 1, 2 and 3 ml aliquots of 100 pg/ml assay buffer at -20°C. Control samples were included in each assay in duplicate in order to monitor intra- and inter-assay variability. A deviation of $\pm$ 25 pg/ml from the expected control value invalidated the assay.

6. Sample Preparation:

Samples with IR-G levels that fell beyond the steep portion of the standard curve plot (ie. greater than 250 pg/ml with antisera L2 and 1611 or 800 pg/ml with antiserum CKG2) were diluted with RIA buffer manually or by an automatic diluting system (LKB Model 2075, Sweden).

7. Protocol:

Assays were performed using 12 x 75 mm glass tubes (Maple Leaf Brand, MacMillan Bathurst, Canada) on a refrigerated table. The incubation mixture in each tube

consisted of: a) 100 µl of either standard, control or sample; b) 100 µl of antibody (except in NSB tubes) and c) 100 µl of label. The total volume was made up to 1 ml with assay buffer. Total counts, NSB and standards were tested in triplicate whereas samples and controls were in duplicate in each assay. The assays were incubated for 48 h at 4°C prior to separation.

8. Charcoal Extracted Plasma (CEP):

Outdated human plasma (Canadian Red Cross, Vancouver) was filtered (Size 4 filter paper, Whatman®, W. & R. Balston, England) and then stirred with 1% activated charcoal for 1 h at 4°C. This mixture was subsequently centrifuged at 10,000 rpm for 30 min at 4°C and the supernatant collected after 2 filter passes. The CEP was aliquoted into 10 ml fractions and stored at -20°C for use in the RIA.

9. Separation:

Separation of free iodinated and noniodinated (cold) gastrin from bound peptide was accomplished using dextran coated, activated charcoal (12.5 g activated charcoal + 2.5 g dextran T-70 were made up to 1 L with 0.04 M phosphate buffer, pH 7.5, and stirred overnight at 4°C). On the day of separation, 7% CEP was added to the gastrin charcoal 1 h prior to use, with continued stirring. Each assay tube, except the total counts tubes, received 200 µl of the mixture, followed by vortexing and by centrifugation at 3000 rpm for 30 min at 4°C. After centrifugation, the supernatant containing bound gastrin was decanted and the remaining charcoal pellet containing free labelled and cold peptide was allowed to dry over absorbent paper overnight. The assays were then counted for 3 min/tube on a gamma counter (Model 1285, Searle Analytic, Illinois or Gammatrac®, Model 1290, Tracor Analytic, Illinois).

D. Somatostatin:

Somatostatin-like immunoreactivity (SLI) was measured by a RIA protocol originally described by McIntosh et al (1978).

1. Iodination:

Five micrograms of synthetic Tyr^1-somatostatin were dissolved in 10 µl distilled H_2O. This mixture was incubated wtih 10 µl 0.5 M phosphate buffer (pH 7.5), 1 mCi $Na^{125}I$ and 10 µl chloramine T (2 mg/ml in 0.05 M phosphate buffer) for 30 s. The oxidation reaction was stopped by the addition of 10 µl sodium metabisulphite (5 mg/ml in 0.05 M phosphate buffer). After vortexing, 1 ml CEP and 20 mg QUSO microfine silica (G-32) were added to the iodination mixture, vortexed and centrifuged for 3 min. The supernatant was discarded, and the pellet was washed in 1 ml distilled H_2O and centrifuged for 3 min (2 x). The resulting pellet was resuspended in 1ml of an acetic acid/acetone mixture (100 µl glacial acetic acid, 3.9 ml acetone and 4 ml distilled H_2O) and centrifuged for 15-20 min. The supernatant was collected and diluted to 1,000,000 cpm/10 µl in 0.1 M acetic acid containing 0.1% BSA. Aliquots of 100 µl were lyophilized and stored at -20°C.

On the day of the assay, the ^{125}I-somatostatin was further purified on a Sephadex CM-52 column (0.9 cm x 10 cm) previously equilibrated with 0.002 M ammonium acetate buffer, pH 4.6. Each aliquot of label was dissolved in the equilibration buffer, applied to the column and washed with the same buffer at a flow rate of 1 ml/min for 20 min. Two milliliter fractions were collected. The ^{125}I-somatostatin was then eluted from the column with 0.2 M ammonium acetate buffer, pH 4.6, at a flow rate of 1 ml/min. Ten microliter aliquots were counted and an elution profile plotted. For the assay, the peak fraction was neutralized in 2 M NaOH and subsequently diluted to 3500 cpm/100 µl in assay buffer.

2. Assay Buffer:

The assay buffer consisted of 23.8 mM (4.9 g/L) sodium barbital, 3.9 mM (0.32 g/L) sodium acetate, 0.25 mM (0.1 g/L) sodium merthiolate (ethylmercurithiosylicylic acid sodium salt) and 43.6 mM (2.55 g/L) sodium chloride in distilled H_2O. The solution was titrated to pH 7.4 with HCl and stored at 4°C. BSA (0.5%, RIA grade, Pentex®, Miles) and aprotinin (1%) were added to the assay buffer prior to use in the assay.

3. Antibody:

A monoclonal antibody raised against cyclic somatostatin-14 (S-14) (Buchan et al, 1985), SOMA 03, was used in the assay at a final dilution of 1:4 x 10^6 as previously described (McIntosh et al, 1987). Crude ascites fluid containing SOMA 03 was normally stored at -20°C. A fraction of the ascites was thawed, filtered through a sterile 0.22 μm filter unit (Millipore, Bedford, Massachusetts), diluted with an equal volume of 0.9% saline containing 0.1% NaN_3 and 0.5% BSA (saline-azide-BSA) and stored at -70°C. When needed, frozen 20 μl aliquots were thawed and added to 980 μl saline-azide-BSA to comprise the stock antibody solution. This was stored at 4°C. Ten microliters of the stock antibody solution were diluted in 100 ml of assay buffer prior to use in the assay. SOMA 03 recognizes all somatostatin variants (McIntosh et al, 1987) and is directed against the central region of the S-14 molecule. It does not cross-react with GIP, motilin or gastrin (Buchan et al, 1985).

4. Standards:

Synthetic cyclic somatostatin was dissolved in 0.1 M acetic acid containing 0.05% BSA (Pentex®, Miles) to a concentration of 0.2 mg/ml and 50 μl aliquots were lyophilized and stored at -20°C. On the day of the assay, one aliquot was dissolved in 1 ml assay buffer (10 μg/ml) and then serially diluted in assay buffer to generate standards ranging from 3.9 pg/ml to 500 pg/ml.

5. Sample Preparation:

All perfusate samples collected for SLI determination were added to tubes containing aprotinin to yield a final concentration of 1% and then stored at -20°C. On the day of the assay, samples were thawed at 4°C.

6. Protocol:

Assays were performed using 12 x 75 mm glass tubes (Maple Leaf Brand, MacMillan Bathurst, Canada) on a refrigerated table. The incubation mixture in each tube consisted of: a) 100 μl of either standard or sample; b) 100 μl of antibody (except in NSB

tubes) and c) 100 µl of label. The total volume was made up to 0.4 ml with assay buffer. Total counts, NSB and standards were tested in triplicate whereas samples and controls were in duplicate in each assay. The assays were incubated for 72 h at 4°C prior to separation.

7. Separation:

Separation of free iodinated and cold somatostatin from bound peptide was accomplished using dextran coated, activated charcoal (1.25% activated charcoal + 0.25% dextran T-70 were dissolved in 0.05 M phosphate buffer, pH 7.5, and stirred for at least 1 h at 4°C). Thirty minutes prior to use, 0.1% CEP was added to the somatostatin charcoal with continued stirring. Each assay tube, except those for total counts, received 1 ml of the mixture, and were vortexed and centrifuged at 3000 rpm for 30 min at 4°C. After centrifugation, the supernatant containing bound somatostatin was decanted and the remaining charcoal pellet containing free iodinated and cold peptide was allowed to dry over absorbent paper overnight. The tubes were then counted for 3 min on a gamma counter.

II. ANIMAL STUDIES:

A. Isolated Perfused Stomach Preparation:

The isolated vascularly perfused stomach preparation provided an *in vitro* system for assessing gastric hormone release in lean and obese animals in response to basal conditions and the actions of various agents.

1. Solutions and Reagents:

a) Krebs concentrate:

A concentrated stock solution (10x) consisting of the following reagents was prepared in distilled H_2O and stored at 4°C:

285 ml of 154 mM KCl

243 ml of 103 mM $CaCl_2$

78 ml of 154 mM $MgSO_4 \cdot 7H_2O$

97 ml of 154 mM KH_2PO_4.

b) Krebs-Ringer Bicarbonate Perfusate:

The perfusate consisted of a 0.9% saline solution in which 0.2% BSA and 3% dextran were dissolved overnight and maintained at 4°C. On the day of perfusion, $NaHCO_3$ (6.5 g dissolved in 500 ml of distilled H_2O), Krebs concentrate and glucose (55 ml of a 50% commercial dextrose solution in 500 ml of distilled H_2O) were added in appropriate volumes to give the following desired final concentrations: 4.4mM KCl, 2.5mM $CaCl_2$, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.5 mM KH_2PO_4, 25mM $NaHCO_3$, 120 mM NaCl and 80 mg/dl glucose. Saline (0.9%) was added to the perfusate to its final volume. The glucose concentration of the perfusate was checked prior to every experiment with a Beckman Glucose Analyzer II (Beckman Instrument Inc., Fullerton, Ca.). The osmolality and pH of the perfusate were also measured periodically and were found to be 280-285 mOsm/kg and 7.4, respectively.

c) Peptides and Drugs:

Peptides and pharmacological agents were introduced into the perfusion medium immediately prior to entry into the organ preparation. This was achieved by sidearm infusion into the aortal cannula. Peptides were initially dissolved in 100μl of 10 mM acetic acid and concentrated stock solutions were prepared in distilled H_2O containing 0.1% BSA. Stock solutions of drugs were directly dissolved in the perfusion medium. These preparations were kept on ice during the course of an experiment and added in appropriate volumes to the perfusate to achieve the desired final concentration with consideration given to the infusion (5 or 10 ml/h) and perfusion (3 ml/min) flow rates. Sidearm infusions were performed using a Harvard syringe infusion pump (Ealing Scientific, St. Laurent, PQ).

In experiments designed to produce a linear gradient of delivery of a particular agent, a calculated volume of the concentrated material was added to the distal flask of a dual identical flask set-up connected in series.

d) Heparin-Saline:

Stock heparin solution (10,000 U/ml) was diluted in 0.9% saline to a concentration of 2 U/ml.

2. Perfusion Procedure:

a) Apparatus (Figure 9):

The perfusion medium was maintained at 37.5°C and at a pH of 7.4 by a heating block regulated by a thermostatic feedback control unit, and by continuous stirring and gassing with 95% O_2 and 5% CO_2. The perfusate was delivered to the heating block using a peristaltic pump (Holter, model 921) and the perfusion pressure monitored and maintained between 40 and 60 mm Hg. Preparations in which the pressure remained elevated above 60 mm Hg were not used. A filter bubble trap immediately preceded the arterial cannula in order to remove air bubbles and unwanted particles from the perfusate. During the experiment, the external portion of the perfused stomach was kept warm with a 60 watt desk lamp and covered with plastic wrap to prevent temperature disturbances due to moisture loss and/or circulating air.

b) Surgical Protocol:

Following an overnight fast, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg, Somnitol®) and the stomach isolated according to a modification of the method of Lefebvre and Luyckx (1977). The abdominal viscera were exposed following a midline incision from the pelvis to the sternum and paired lateral incisions to the body wall. The left kidney and adrenal gland vessels were doubly ligated and sectioned to facilitate access to the aorta. Preparation of the aorta for cannulation involved the placement of single, loose ligatures rostral to the celiac artery, immediately distal to the superior mesenteric artery and 1-2 cm caudal to the left renal artery

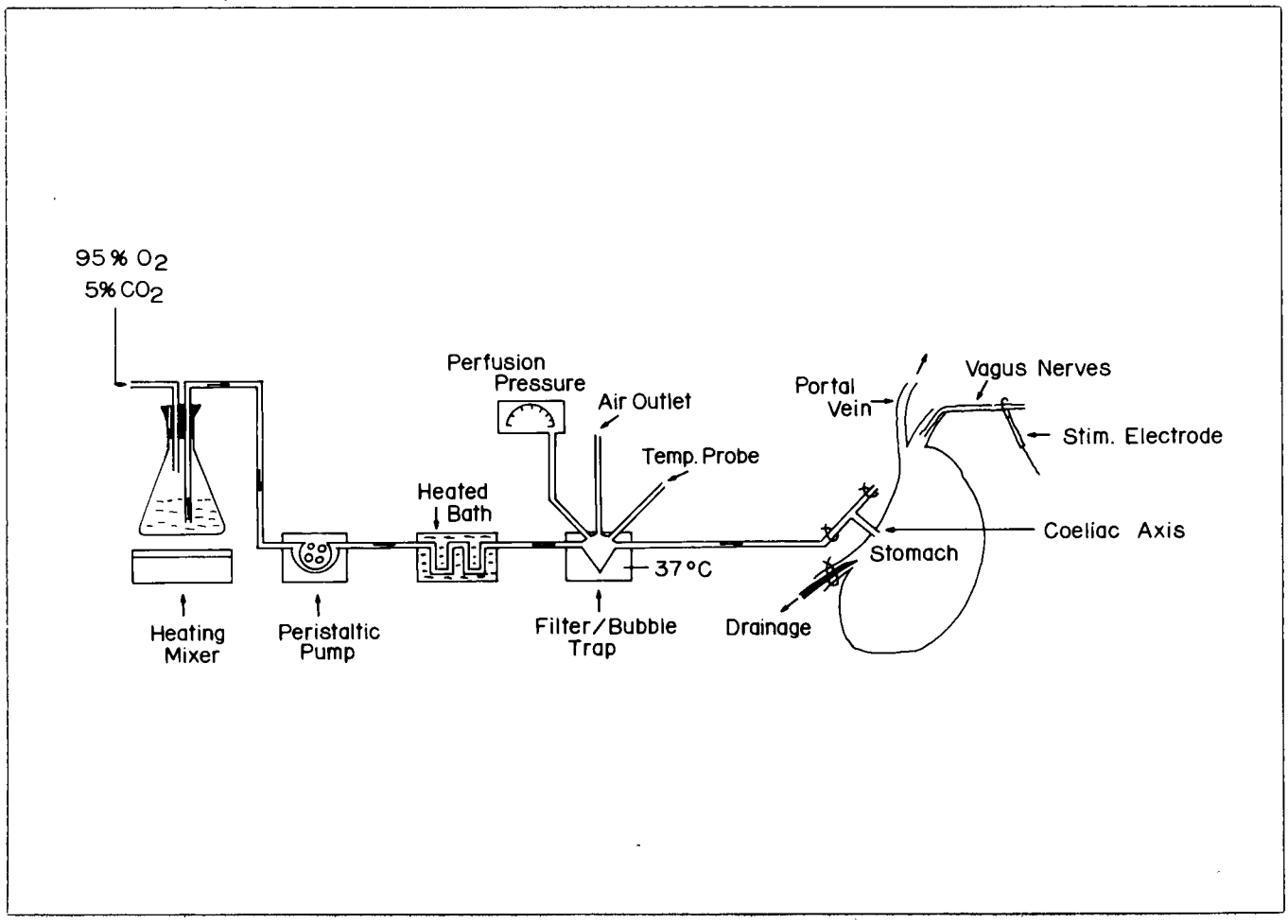


Figure 9 : The isolated vascularly perfused rat stomach preparation.

stump. Preparation for portal cannulation consisted of placing one single loose ligature around the portal vessels and the bile duct leading into the base of the liver and another single loose ligature around the portal vein. The descending colon was doubly ligated and freed from the underlying connective tissue to the level of the superior mesenteric artery. The superior mesenteric artery was doubly ligated and sectioned. Double ligatures were carefully positioned between the stomach and the pancreas and spleen ensuring the preservation of the right gastric and right gastroepiploic arteries. The proximal duodenum was sectioned and a drainage tube inserted and secured to the pylorus. The small bowel, pancreas, spleen and large bowel were subsequently removed. The right kidney and adrenal gland vessels were single ligated and left intact. In experiments requiring electrical stimulation of the vagus nerve, the anterior vagus was isolated subdiaphragmatically on the surface of the esophagus and marked with a loose ligature.

The abdominal aorta was then cannulated with polyethylene tubing (P.E. 160, Clay Adams, New Jersey) and positioned adjacent to the superior mesenteric artery. Cannulation was followed by injection of 2 ml of heparin-saline to prevent clotting. The animal was hemi-sectioned at the level of the diaphragm and a second cannula was placed into the portal vein to permit collection of the venous effluent. Perfusion via the celiac artery was maintained at 3 ml/min and, following a 20 min equilibration period, portal vein effluent was collected at 1 or 5 min intervals using an automated fraction collector. Aliquots were stored at -20°C prior to peptide determination. If required, the vagus nerve was placed gently on a miniature electrode connected to a stimulator at the start of the perfusion. Stimulation parameters were 7V, 10 Hz, 5 ms.

B. Amino Acid Tolerance Test:

Overnight fasted animals were administered a 10% peptone solution (0.5 ml/100 g body weight) by a feeding tube. Blood samples (400 μl) were collected into heparinized capillary tubes from the tail vein prior to (basal) and at 10, 20, 30, 60, 90 and 120 min after

the oral amino acid challenge. Plasma samples were stored at -20°C for subsequent IR-gastrin determination.

C. Zucker Rat Colony:

1. Breeding:

Original breeding pairs were supplied by Dr. P. Bechtel of the Animal Sciences Laboratory of the University of Illinois. The most common pairing used was of lean female heterozygotes (Fa/fa) with obese males (fa/fa) as fatty females are sterile. The resulting progeny were approximately 50 % obese.

2. Housing:

All animals were housed in polyethylene cages lined with commercial rodent bedding. Pelleted food and water were available *ad libitum*. Animal rooms were supplied with a 12 h light/dark cycle and a re-circulation ventilation system. Each cage housed either a single breeding pair, or a lactating female plus her litter or 3-4 adults of mixed phenotype. Zucker pups were weaned 21 days after birth and separated according to sex at about 5 weeks of age. Male Zucker rats were used in all experiments as male and female animals exhibited different secretory profiles with males displaying markedly higher basal gastrin levels *in vivo* and *in vitro*.

3. Identification:

Each litter was numbered according to the date of birth and each animal was uniquely identified by an ear marking scheme. Obese rats of weaning age were not visually distinguishable from their lean littermates. Identification of lean and obese phenotypes was achieved by histological examination of pancreatic sections as hypertrophic islets have been demonstrated in obese animals as early as 1 week of age (Chan et al, 1985).

4. Pair Feeding Studies:

At 4 weeks of age, Zucker pups were housed individually in wire-screen cages specially designed to monitor daily food intake. These cages were equipped with a feeding channel through which access to a food pan was provided. Food was in the form of

ground rat chow to prevent losses associated with movement of food pellets from the feeding area to the main cage unit. Animals were given a period of 7 days to acclimatize to their new environment during which food and water were provided *ad libitum.* During this period of adjustment, identification of lean and obese phenotypes became possible using visual and tactile cues. The weights of the food pan, the food allotment and the animals were measured each day at around 5 p.m. and at the same time the next day to determine the daily food consumption and weight gain. The food portions were removed at 10 p.m. on the eve of the start of the pair feeding regimen and fasting blood samples were taken from the tail vein the next morning. For the next 21 days (from 5-8 weeks of age), lean animals continued to receive food *ad libitum* (designated L),whereas the obese animals were divided into two groups: one group fed *ad libitum* (designated O-AL) and the second group (designated O-PF) given a food allotment equal to the mean intake of the L group calculated over the previous 24 h. To ensure that the O-PF group received their full complement of rations, food particles that may have accumulated on the removable metal tray lining the underside of the feeding channel were returned to the food pan twice daily, at 8 a.m. and at 4 p.m. Without exception, the O-PF animals completely exhausted their food allocation. Three weeks after the start of the pair feeding schedule, animals were fasted overnight and fasting plasma samples were obtained prior to isolation and perfusion of the stomach. Immediately following the perfusion experiment, antral tissue samples were obtained for immunocytochemical examination.

III. IMMUNOCYTOCHEMISTRY:

A. Rationale:

Immunocytochemical methods were employed in order to visually localize specific antigens in tissue sections and cell cultures. The reliability of these techniques is dependent

upon highly selective antigen-antibody reactions and subsequent identification of these reaction sites using flourochromes or enzyme-activated chromophores.

B. Tissue/Cell Fixation and Preparation:

1. Tissue Samples:

a) Fixation:

Antral tissue samples were obtained from 6 month-old lean and obese Zucker rats immediately following perfusion and immersed in Bouin's solution (75 ml saturated picric acid+ 25 ml formaldehyde) for 2 h at room temperature. Antral tissue from 3-week old Zucker pups was collected after an overnight fast and treated identically. Fixed tissue was subsequently washed in 70% ethanol and stored in this medium at room temperature.

b) Dehydration:

Tissue samples, carefully cut to yield the appropriate orientation and size, were then loaded into cassettes (Fisher Scientific) for automatic processing (Histomatic® tissue processor, Model 166, Fisher Scientific). Dehydration was accomplished by sequential exposure to increasing concentrations of ethanol, 80% for 30 min, 90% for 30 min and 100% for 30 min (3 distinct washes), followed by xylene treatment for 30 min (2 x). The final stage involved two 1 h steps under vacuum in molten paraffin wax at 60°C to ensure complete infiltration of the tissue samples.

c) Embedding:

Samples were removed from the automatic processor and carefully embedded in paraffin at 60°C with the aid of a tissue embedding centre (Model 166, Sybron Canada, St. Laurent, PQ). The paraffin blocks were allowed to set at 4°C and subsequently stored at -20°C.

d) Cutting Tissue Sections:

Frozen paraffin blocks were trimmed and set in a rotary microtome (Model 113, Reichert-Jung) at room temperature. Five micron sections were cut, floated on 50% ethanol and transferred into a heated water bath (50°C) for flattening. Suitable sections

were then mounted on glass slides, 2-3 sections/slide, and dried on a hot plate set at 37°C overnight.

2. Cell Cultures:

Immediately after release studies, the cells in the culture wells were fixed in Bouin's solution for 10-15 min at room temperature, washed twice in 70% ethanol, twice in phosphate-buffered saline-azide (PBS-azide) solution and stored at 4°C. The PBS-azide stock solution consisted of 20.5 g NaH_2PO_4, 95.5 g Na_2HPO_4, 701.3 g NaCl and 5 g NaN_3 in 8 L distilled H_2O, pH 7.2. The stock solution was diluted 1:10 in distilled H_2O for routine use.

C. Blocking:

Tissue sections were deparaffinized by soaking in xylene for 5 min (2x) and then the xylene residues removed by immersion in petroleum ether for 1.5 min. To prevent non-specific staining due to the presence of endogenous peroxidase activity, tissue and cell samples were immersed in PBS-azide containing 0.3% H_2O_2 for 30 min at room temperature and then washed 3x in PBS-azide.

D. Immunostaining:

1. First and Second Antibody Layers:

Gastrin-containing and somatostatin-containing cells were localized with the monoclonal antibodies 109-21 (a gift from Dr. J. H. Walsh, CURE) at a dilution of 1:100 and S8 at a dilution of 1:1000 (Buchan et al, 1985), respectively. These first antibody layers were added to separate sections or culture wells overnight at 4°C. Slides were stored in fiberglass chambers containing moist paper to prevent evaporation of the antibody layer. Slides and culture wells were then washed 3x with PBS-azide for 5 min each and the second antibody layer, consisting of biotinylated horse anti-mouse immunoglobulins (Vector) at a dilution of 1:200, was added for 1 h at room temperature. For tissue sections, antibody layers were diluted in PBS-azide, whereas for cultured cells, PBS-azide containing 0.25% triton X-100 was used.

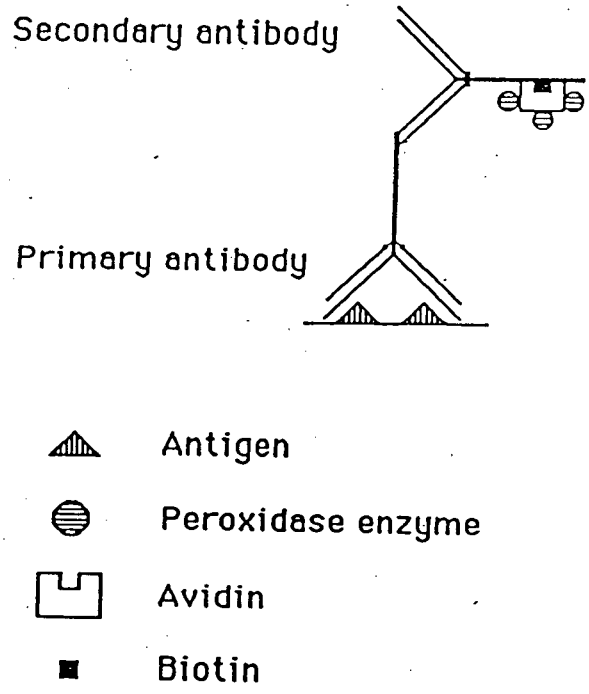


Figure 10 : Diagrammatic representation of the avidin-biotin-peroxidase staining method.

2. Avidin-Biotin-Peroxidase Complex Method:

After the second antibody layer had been completely removed with PBS-azide (3x), a third layer comprised of a complex of biotinylated peroxidase with avidin (Vector) was added at a dilution of 1:200 for 1 h at room temperature (Figure 10). Avidin is an egg-white glycoprotein possessing four binding sites for biotin of extremely high affinity (Hsu et al, 1981).

3. Peroxidase Reaction:

a) Tissue Samples:

Slides were washed with PBS-azide (3x) and developed in diaminobenzidine (DAB 25 μg/100 ml PBS-azide) containing 0.025% H_2O_2. The reaction proceeded for 8-10 min and the slides then washed in PBS-azide and running tap water for at least 10 min.

b) Cell Cultures:

Culture wells were washed with PBS-azide (3x) and developed in DAB (25 μg/100 ml of 0.1 M Tris buffer, pH 7.6) containing 40 mg NH_4Cl, 200 mg D-glucose and 0.3 mg glucose oxidase. The reaction continued for 15-20 min and the culture wells then washed in PBS-azide followed by running tap water.

E. Counterstaining:

1. Tissue Sections:

Slides were counterstained, dehydrated and cleared according to the following procedure:

Hematoxylin	1 s, rinsed in tap water until clear
Acid ethanol (10% HCl + 70% ethanol in distilled H_2O)	1 s, rinsed in tap water
Lithium chloride	1 s, rinsed in tap water
70% ethanol	1 min
90% ethanol	1 min
100% ethanol	5 min

Xylene I	5 min
Xylene II	5 min

Slides were coverslipped with Permamount® immediately after removal from xylene and allowed to air dry.

2. Cell Cultures:

Hematoxylin was added to the culture wells for 1-2 s and cleared in running tap water. Stained cells were stored in PBS-azide at room tempertaure.

F. Quantification:

1. Tissue sections:

Immunoperoxidase-positive cells were quantified using a computerized morphometrics system (Videoplan MOP40, Zeiss, Canada) linked directly to a research light microscope (Zeiss, Canada) via a videocamera. The total number of immunoreactive-G and -D cells, the total mucosal area and the mean epithelial height were determined in at least 10 areas of the antral mucosa. The number of G or D cells per mm mucosa was calculated according to the following formula:

$$\#\,\text{cells/mm mucosa} = \#\,\text{cells/total mucosal area (mm}^2\text{)} \cdot \text{mean epithelial height (mm).}$$

2. Cell Cultures:

The total number of immunoreactive-G or -D cells were counted in at least 1000 adhered cells/well and the percentage calculated.

IV. ISOLATED G-CELL STUDIES:

A. Rationale:

The isolated G-cell preparation provided a useful model with which the specificity of action of regulatory peptides and various pharmacological agents could be studied.

B. Solutions and Reagents:

1. Hanks' Balanced Salt Solution (HBSS-BSA):

A concentrated stock solution (5x) was prepared in 2 L of distilled H_2O as follows:

$NaHCO_3$	3.5 g
HEPES buffer (N'-2-Hydroxyethylpiperazine-N'-Ethanesulfonic Acid)	23.8 g
HBSS powder	1 package

The stock solution was adjusted to pH 7.2 with 10 M NaOH and stored at 4°C. On the day of the experiment, the Hanks' stock solution was diluted to 1x concentration and made up to 0.1% BSA.

2. Basal Medium Eagle Solution (BME):

Fresh BME solution consisting of the following reagents was prepared in distilled H_20 on the day of the experiment:

$NaHCO_3$ (3.5% stock solution)	63 ml/L
HEPES buffer (1 M stock solution)	10 ml/L
Glutamine	0.29 g/L
BME powder	1 package/L
BSA	1 g/L

The mixture was adjusted to pH 7.2 prior to final volume and stored at 4°C.

3. Dulbecco's Modified Eagle's Medium (DMEM):

DMEM was purchased directly from the Cancer Control Agency of British Columbia. In this form, the medium contained the following:

D-glucose	1000 mg/L D-glucose (5.5mM)
Sodium pyruvate	110 mg/L
$NaHCO_3$	3.7 g/L
HEPES buffer	25 mM
Phenol red	15 mg/L

Glutamine 290 mg/L

Various antibiotics

In instances when a lower glucose concentration was needed, DMEM was prepared from powder in distilled H_2O and supplemented with the reagents listed above. The appropriate amount of glucose was added from a 50% commercial dextrose solution and the medium adjusted to pH 7.2.

4. Endocrine Cell Culture Medium:

Culture medium was prepared fresh on the day of the experiment and consisted of DMEM containing 5.5 mM glucose. This solution was supplemented with the following:

5% dog serum (for canine cells) or fetal calf serum (for human cells)

2 ng/ml nerve growth factor

8 μg/ml insulin

1 μg/ml hydrocortisone

50 μg/ml gentamycin sulfate.

In human cell cultures, the culture medium was further complemented with amphotericin B (Fungizone®, final concentration of 0.25 μg/ml) and cytosine ß-D-arabinofuranoside (final concentration of 20 μM) to reduce fungus growth and fibroblast proliferation, respectively.

5. Release Medium:

Release medium consisted of DMEM containing 0.1% BSA (RIA grade) and 1% aprotinin (Trasylol®, 500 KIU/ml). In canine and human studies, the glucose concentration of the medium was 2.7 mM and 4.4 mM, respectively.

C. Secretagogues:

The sources of somatostatin, bombesin, [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}] substance P, forskolin, ß-phorbol 12-myristate 13-acetate (ß-PMA), 4α-phorbol 12-myristate 13-acetate (α-PMA) and A23187 are listed in Appendix I. S10 antibody was developed at the MRC Regulatory Peptide Group (Buchan et al, 1985). Synthesis of the

[Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin analog was undertaken as previously described (Coy et al 1988).

Peptides were initially dissolved in 100 μl of 10 mM acetic acid and made up to a 40-fold concentration of that required for the experiment in release medium. Aliquots of concentrated peptide were stored at -70°C. Forskolin was initially dissolved in ethanol and a stock solution of 40 x 10^{-4} M was stored at -20°C. ß-PMA, A23187 and α-PMA were initially dissolved in dimethylsulfoxide (DMSO) and stock solutions of 40 x 10^{-5} M were stored at -20°C. Further dilutions of receptor-dependent and post-receptor agents were made in release medium.

D. Cell Isolation, Enrichment and Culture:

1. Canine Model (Table II):

The antrum was dissected from the canine stomach and washed in HBSS-BSA. The mucosa was carefully dissected from the submucosa, weighed and minced into small fragments (about 4 mm^3) using a pair of scalpel blades. Antral tissue (8-10 g/50 ml) was exposed to an initial collagenase (Type I) digestion of 300 U/ml in BME for 15 min at 37°C with shaking. The resulting dispersed cells were largely comprised of mucous cells and were discarded. The remaining tissue was further dispersed by sequential collagenase treatment, 300 U/ml for 1 h then 600 U/ml for 1 h, with each step followed by the addition of 300 μl of 0.5 M disodium ethylenediaminetetraacetate (EDTA, final concentration about 3 mM) for 15 min at 37°C. The cell suspensions resulting from the 2nd and 3rd collagenase digests were filtered through Nitex mesh (240 μm, B. & S. H. Thompson, Scarborough, Ont.) and washed with HBSS-BSA, then with HBSS-BSA containing 0.01% dithiothreitol (HBSS-BSA-DTT) and resuspended in 20 ml of HBSS-BSA-DTT. Dithiothreitol was utilized as a reducing agent thereby breaking the sulfide bonds present in mucin complexes and allowing its removal by washing. Prior to elutriation, the cell suspensions were pooled and filtered through finer Nitex mesh (62 μm, B. & S. H. Thompson).

Table II: Method for the isolation of gastric endocrine cells of the dog and human.

Canine or human antrum

Collect and mince mucosa

Disperse with collagenase and EDTA

Canine cells	Human cells
1) 300 U/ml for 15 min, decant	1) 300 U/ml for 15 min, decant
2) 300 U/ml for 1 h, then 3 mM EDTA for 15 min	2) 600 U/ml for 1 h, then 5 mM EDTA for 15 min
3) 600 U/ml for 1 h, then 3 mM EDTA	3) Repeat step (2)
	4) 900 U/ml for 1 h, then 5 mM EDTA for 15 min

Filter all cell suspensions (except from digest 1, 240 μm Nitex mesh)

Wash cells twice

Resuspend cells

Canine cells	Human cells
In 20 ml of HBSS-BSA-DTT	In 20 ml of HBSS-BSA-DTT containing 0.001% deoxyribonuclease

Pool all cell suspensions and filter (62 μm)

Counterflow elutriation

Canine cells	Human cells
1) Load at 2500 rpm, 25 ml/min	1) Load at 2500, 25 ml/min
2) Wash at 2500 rpm, 25 ml/min for 3 min	2) Wash at 2500 rpm, 25 ml/min for 3 min
3) Collect 100 ml at 2100 rpm, 25 ml/min (Fraction 1 = D cells)	3) Collect 100 ml at 2100 rpm, 40 ml/min (Fraction 1 = G cells)
4) Collect 100 ml at 2000 rpm, 55 ml/min (Fraction 2 = G cells)	4) Collect 100 ml at 1800 rpm, 55 ml/min (Fraction 2 = D cells)

Culture for 40-48 h at 37°C

Further enrichment for G cell content was achieved by counterflow elutriation using a Beckman elutriator rotor. This process allowed separation of cell types on the basis of their sedimentation rates by varying the rotor speed and/or the elution flow rate. The elutriation tubing and rotor were sterilized with H_2O_2 followed by 70% ethanol and washed with sterile water. Cells were collected under sterile conditions using HBSS-BSA as eluant at a loading density of 2 x 10^8 cells/run. Cells were loaded at a rotor speed of 2500 rpm and a flow rate of 25 ml/min and washed for 3 min under the same conditions. Maintaining the same flow rate, the rotor speed was reduced to 2100 rpm and a 100 ml fraction was collected (Fraction 1). The rotor speed and flow rate were then adjusted to 2000 rpm and 55 ml/min, respectively, and a 100 ml fraction was collected (Fraction 2). Each run was followed by reducing the rotor speed to 700 rpm and increasing the elution flow rate to >100 ml/min in order to remove any remaining debris in the elutriation chamber. Immunocytochemical and radioimmunoassay results later showed that the majority of immunoreactive-G cells eluted in Fraction 2 whereas the greater proportion of immunoreactive-D cells eluted in Fraction 1. Consequently, 12-well collagen-coated plates (Costar, Cambrige, Massachusetts) were seeded at a density of 2 x 10^6 cells/ml of culture medium in each well with cells from Fraction 2. The cultures were maintained at 37°C in a humidified atmosphere of 95% 0_2-5% CO_2 for 2 days.

2. Human Model (Table II):

In cooperation with the British Columbia Pacific Organ Retrieval and Transplant Program, human gastrointestinal tissue was obtained from male and female organ transplant donors with consent from next of kin for use in research purposes. The antrum was carefully dissected from the accompanying corpus and small bowel and washed in HBSS-BSA. The mucosa was carefully dissected from the submucosa, weighed (14.9 $\pm$ 1.5 g, mean $\pm$ SEM, n=17) and minced into small fragments (about 4 mm^3) using a pair of scalpel blades. Antral tissue (8-10 g/50ml) was initially digested in 300 U/ml collagenase (Type I) in BME for 15 min in an agitating water bath at 37° C. The resulting dispersed

cells consisted mainly of mucous cells and were discarded. The remaining tissue was further digested by sequential collagenase treatment, 600 U/ml (Type I) for 1 h (2x) followed by 900 U/ml (Type XI) for 1h, with each step followed by the addition of 500 μl of 0.5M EDTA (final concentration about 5 mM) for 15 min. Type I and XI collagenase differ in terms of purity as the former contains other proteases such as trypsin, whereas the latter does not. The cell suspension resulting from each collagenase and EDTA digest was filtered through Nitex mesh (240 μm, B. & S. H. Thompson) and washed with HBSS-BSA-DTT, then with HBSS-BSA-DTT containing 0.001% deoxyribonuclease and resuspended in 20 ml of the latter medium. Deoxyribonuclease was employed to degrade any DNA that may have been liberated during the digestion process and consequently, reducing the viscosity of the cell pellet. Prior to elutriation, filtrates from the 2nd, 3rd and 4th digests were pooled and filtered through finer Nitex mesh (62 μM, B. & S. H. Thompson).

Further enrichment for G cell content was also achieved by counterflow elutriation. The elutriation tubing and chamber were sterilized as before. Cells were collected under sterile conditions using HBSS-BSA as eluant at a loading density of 1 x 10^8 cells/run. Cells were loaded at a rotor speed of 2500 rpm and a flow rate of 25 ml/min and washed for 3 min under identical conditions. The rotor speed and flow rate were adjusted to 2100 rpm and 40 ml/min, respectively, and a 100 ml fraction was collected (Fraction 1). A second 100 ml fraction was obtained (Fraction 2) at a rotor speed of 1800 rpm and a flow rate of 55 ml/min. Immunocytochemical and radioimmunoassay results later showed that the majority of immunoreactive-G cells eluted in Fraction 1 whereas the greater proportion of immunoreactive-D cells eluted in Fraction 2. Consequently, 24-well collagen-coated culture plates (Costar) were seeded with cells from Fraction 1 at a density of 1 x 10^6 cells/well in a total volume of 0.5 ml of culture medium. The cultures were maintained at 37°C in a humidified atmosphere of 95% O_2-5% CO_2 for 40-48 h.

E. Release Studies:

After a 40-48 h incubation period, the culture medium was aspirated and the adherent cells were washed twice with 0.5 ml (human cells) or 1.0 ml (canine cells) of release medium. Stock solutions of reagents were added to duplicate wells in 25 μl aliquots to achieve the desired final concentration in a total volume of 1 ml. After a 2 h incubation period, the release medium was collected and centrifuged for 2 min in a Beckman microfuge (Microfuge B, Beckman Instruments). The supernatant was collected and stored at -70°C for future radioimmunoassay. Adherent cells from control wells were extracted in 1 ml of distilled H_2O or 2 M acetic acid, boiled for 10 min, centrifuged and the supernatant collected for determination of total cell content of immunoreactive-gastrin (IR-G) and somatostatin-like immunoreactivity (SLI), respectively.

V. EXPRESSION OF RESULTS:

Data was presented as Mean ± standard error of the mean (X ± SEM). The integrated response was calculated by the following formula:

$$IR = [(H_1 + H_2)/2] \cdot (t_2 - t_1) + [(H_2 + H_3)/2] \cdot (t_3 - t_2) + \ldots + [(H_{N-1} + H_N)/2] \cdot (t_N - t_{N-1})$$

where IR is the integrated response to a stimulus, H is the peptide concentration and t is the time. The subscripted values represent the time periods of sample collection where N is the final time period. In cell culture studies, peptide release was further expressed as % of total cell content (%TCC) due to considerable inter-animal variation. In studies investigating the effect of BN antagonists on BN-stimulated gastrin release, % inhibition was calculated as follows:

$$\%I = \frac{H(\text{control}, + BN) - H(+\text{antagonist}, + BN)}{H(\text{control}, + BN) - H(\text{control}, \text{no } BN)} \cdot 100$$

where %I is the % of inhibition by the BN antagonist and H is the peptide concentration. This equation is accurate when the gastrin response to BN is greater than that to BN plus the antagonist. When the reverse is true (ie. when the gastrin response to BN is less than that to BN plus the antagonist), the denominator term of the formula above is reduced to $H_{(control, + BN)}$ only. The % inhibition of the antagonist on basal gastrin release in the absence of any BN is determined as follows:

$$\%I = \frac{H(basal) - H(+antagonist)}{H(basal)} \cdot 100$$

VI. STATISTICAL INTERPRETATION:

In all rat studies, statistical significance was determined using Student's t-test for paired and unpaired data. In all isolated cell studies, statistical significance was determined using the Mann-Whitney U-test for independent groups due to the heterogeneity of the variance values. In both tests, the significance level was set at $P < 0.05$.

RESULTS

I. STUDIES IN LEAN AND OBESE ZUCKER RATS:

A. Plasma Gastrin Determination:

1. Fasting Levels (Figure 11):

Obese 6 month-old and 3 month-old animals displayed fasting plasma gastrin levels that were 3-fold and 2-fold higher, respectively, than those of lean littermates (331 $\pm$ 64 pg/ml vs. 110 $\pm$ 13 pg/ml and 197 $\pm$ 23 pg/ml vs. 107 $\pm$ 15 pg/ml, $P < 0.05$, Figure 11). In contrast, 3 week-old obese pups were not hypergastrinemic compared to their lean counterparts (81 $\pm$ 20 pg/ml vs. 84 $\pm$ 19 pg/ml).

2. Peptone-stimulated Levels (Figure 12):

After an overnight fast, 10% peptone was administered orally to lean and obese animals (0.5 ml/100 g body weight) . Peak gastrin levels were obtained 120 min after the peptone challenge and represented approximately a 2-fold increase in the circulating gastrin concentrations of both groups of animals (from 99 $\pm$ 10 pg/ml to 243 $\pm$ 37 pg/ml in the lean group and from 266 $\pm$ 58 pg/ml to 526 $\pm$ 92 pg/ml in the obese group, Figure 12a). At all time periods sampled, the plasma gastrin content of obese rats was significantly greater than lean ones ($P < 0.05$). Furthermore, the gastrin response of the obese group appeared to still be rising 2 h after peptone administration, whereas the gastrin response of lean animals reached a plateau at this point. The integrated gastrin output of lean rats was 50% less than that of obese animals (23.4 $\pm$ 2.6 ng vs. 50.3 $\pm$ 7.7 ng, $P < 0.05$, Figure 12b).

B. Gastrin Secretion from the Isolated Vascularly Perfused Rat Stomach: The Involvement of the Cholinergic Nervous System and Somatostatin:

1. Basal Gastrin Secretion (Figure 13):

Basal gastrin release from the isolated perfused stomachs of 6 month-old obese rats was approximately three times greater than that from lean stomachs (525 pg/min vs. 150 pg/min, $P < 0.05$, Figure 13).

2. Effect of Atropine on Basal Gastrin Secretion (Figure 14):

The possible involvement of a hyperactive post-ganglionic cholinergic drive mediating basal gastrin hypersecretion was investigated by infusing atropine (10 μM) into the *in vitro* stomach preparation from 11-30 min of a 40 min perfusion. Atropine treatment did not alter basal gastrin levels in both lean and obese animals (Figure 14).

3. Effect of Atropine on Vagally-Mediated Gastrin Secretion (Figure 15):

Electrical stimulation of the anterior vagus (7V, 10 Hz, 5 ms) was performed from 11-30 min of a 40 min perfusion experiment. Vagal stimulation resulted in a prompt and significant increase in gastrin secretory rates from the stomachs of lean and obese rats ($P < 0.05$, Figure 15). Although peak values were markedly greater in obese rats compared to lean animals (2800 $\pm$ 950 pg/min vs. 450 $\pm$ 100 pg/min), the relative gastrin response to vagal activation was twice the basal output in both groups. The infusion of atropine (10 μM) during vagal stimulation (from 20-30 min) produced an immediate reduction to basal rates in gastrin secretion from lean and obese stomachs.

4. Basal Somatostatin Secretion (Figure 16):

The possibility that hypergastrinemia may be a consequence of somatostatin hyposecretion in obese animals was examined by measuring gastric SLI release during basal conditions. Basal somatostatin secretion was not significantly different in lean and obese rats (Figure 16).

C. Antral Endocrine Cell Morphology (Figures 17-19):

Antral tissue sections were immunostained for gastrin-containing G cells and somatostatin-containing D cells using sequence-specific monoclonal antibodies. Quantification of antral mucosal sections (Figure 17 a-d) revealed a doubling in the G-cell number of obese animals compared to lean controls in 6 month-old adults (175 $\pm$ 8 cells/mm mucosa vs. 79 $\pm$ 6 cells/mm mucosa, $P < 0.05$, Figure 19), but not in 3 week-old weanling pups (106 $\pm$ 9 cells/mm mucosa vs. 103 $\pm$ 8 cells/mm mucosa). However, 21 day-old lean animals displayed a slightly higher number of G cells than lean adult rats.

In comparison, quantification of the D-cell population (Figure 18 a & b) demonstrated no significant difference in the number of antral D cells of lean and obese animals either at 3 weeks (69 $\pm$ 8 cells/mm mucosa vs. 60 $\pm$ 5 cells/mm mucosa, Figure 19) or at 6 months of age (26 $\pm$ 4 cells/mm mucosa vs. 29 $\pm$ 5 cells/mm mucosa).

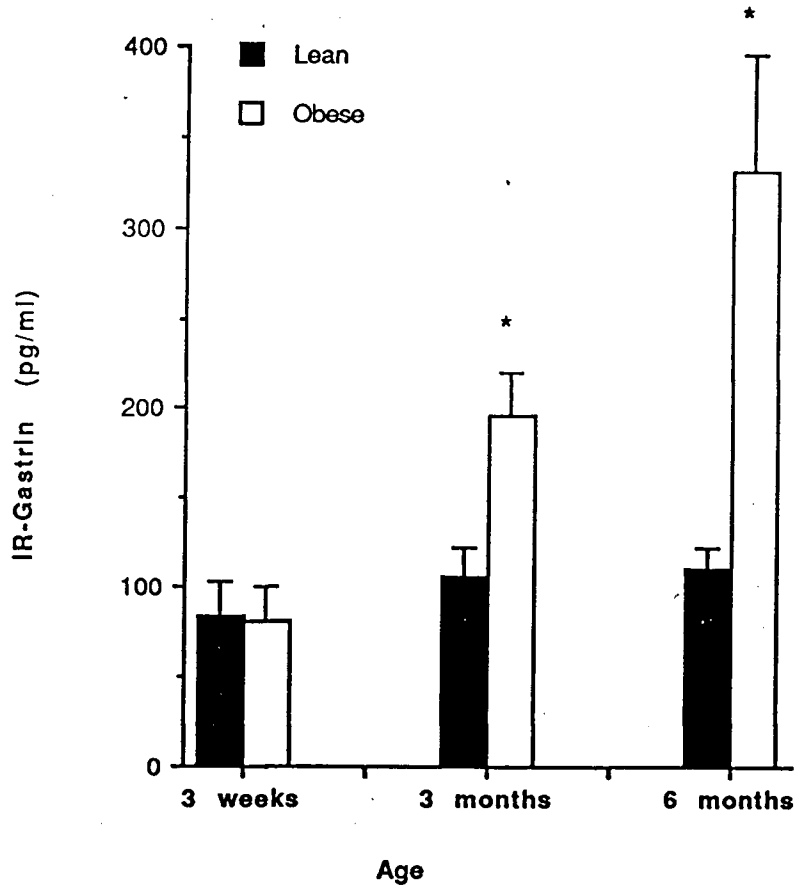


Figure 11 : Fasting plasma gastrin concentrations in lean and obese Zucker rats at 3 weeks (n=10 and n=5, respectively), 3 months (n=6 and n=8, respectively) and 6 months of age (n=14 and n=12, respectively). Levels are expressed as mean ± standard error of the mean in pg/ml. In this and subsequent figures, * denotes significance to at least $P < 0.05$.

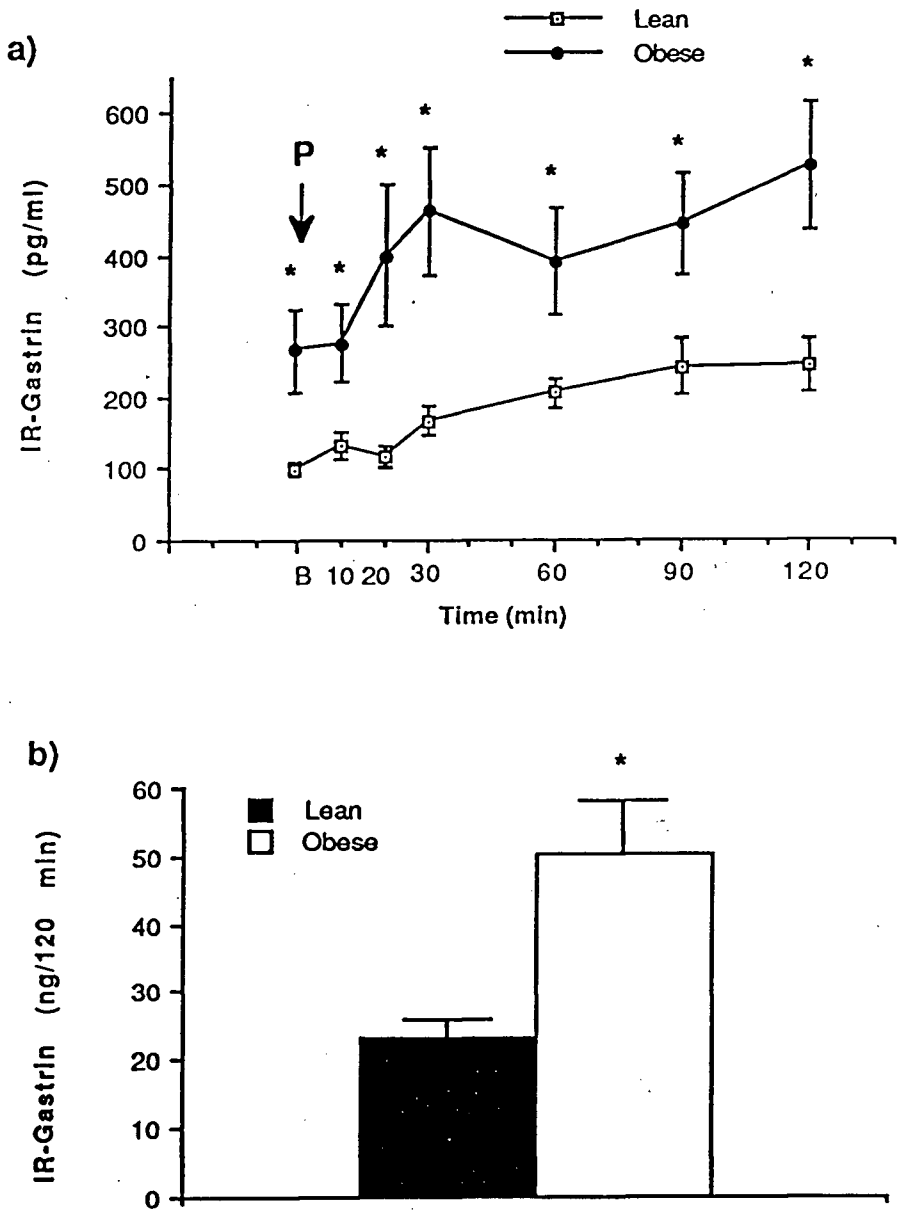


Figure 12 : a) Plasma gastrin levels in lean (n=10) and obese (n=7) adult Zucker rats after oral administration of 10% peptone (500 mg/kg). Levels are expressed as mean ± standard error of the mean in pg/ml. P represents the point of peptone administration at 0 min. In this series of experiments, the Mann-Whitney U-test was used to compare gastrin release from lean rats to that from obese animals at each sampling period due to the considerable difference in variance values.
b) Integrated gastrin response to oral peptone. Levels are expressed as mean ± standard error of the mean in ng over the 2 h test period.

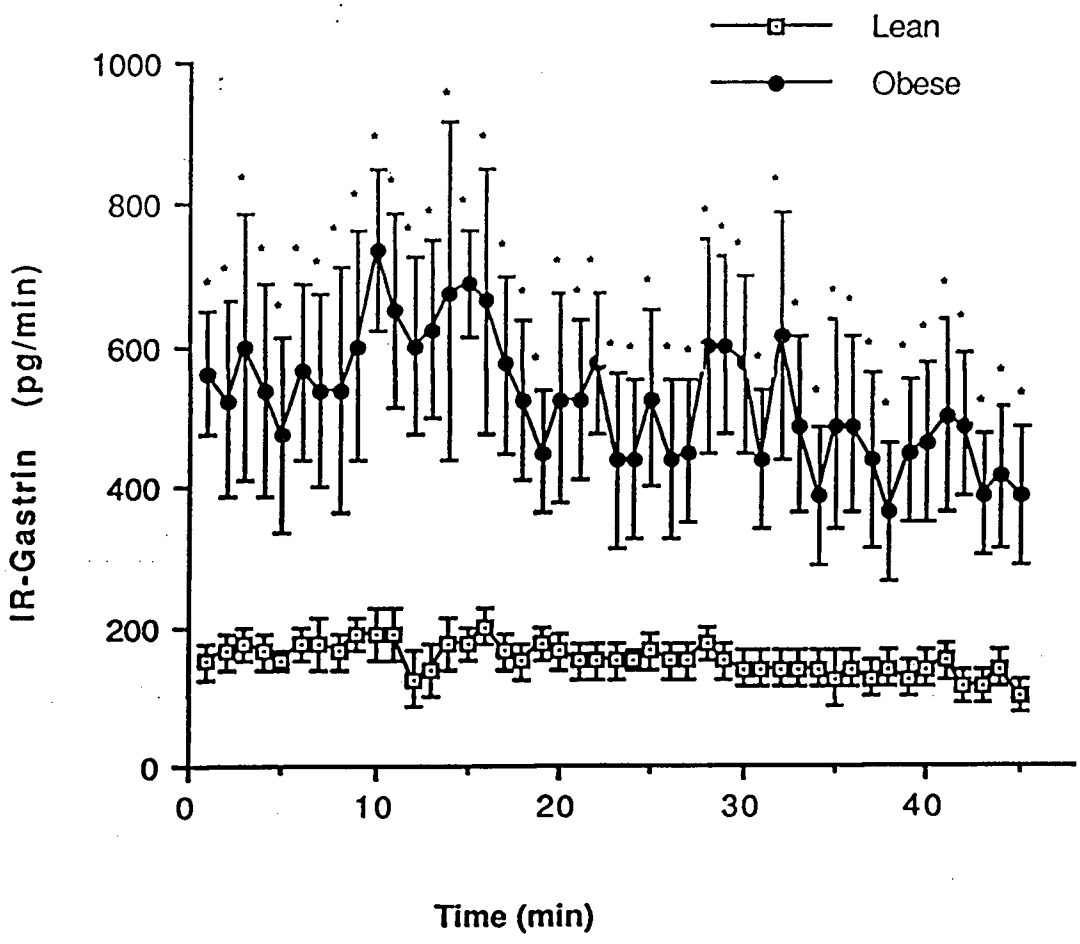


Figure 13 : Gastrin secretion from the isolated perfused stomach of lean (n=7) and obese (n=5) adult Zucker rats during basal conditions. In this and subsequent figures, levels are expressed as mean ± standard error of the mean in pg/min. In this series of experiments, gastrin release from lean stomachs was compared to release from obese stomachs using the Mann-Whitney U-test.

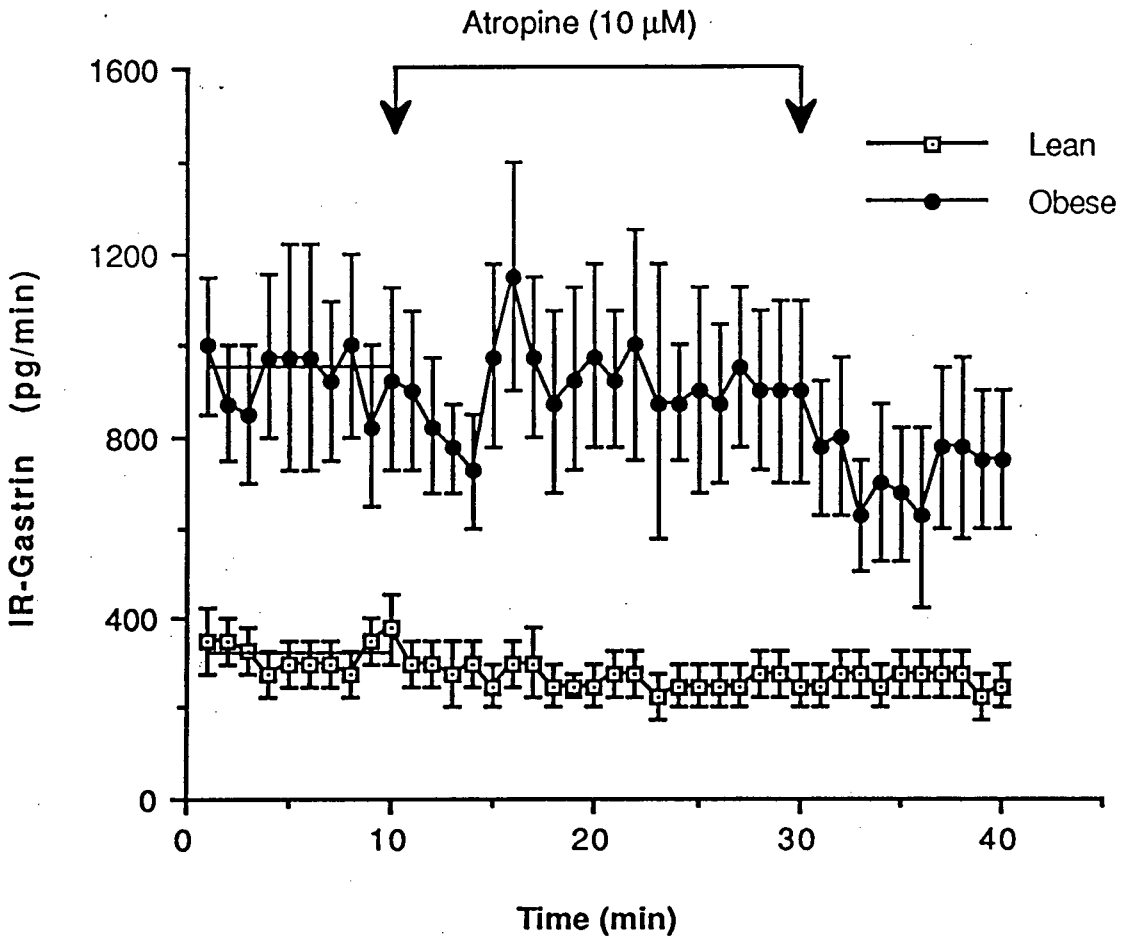


Figure 14 : The effect of atropine (10 µM) on basal gastrin secretion from the isolated perfused stomach of lean (n=7) and obese (n=8) Zucker rats. The gastrin response during atropine treatment was compared to the mean basal output prior to atropine infusion and no significant difference was found. The mean basal output is designated with a bar from 1-10 min.

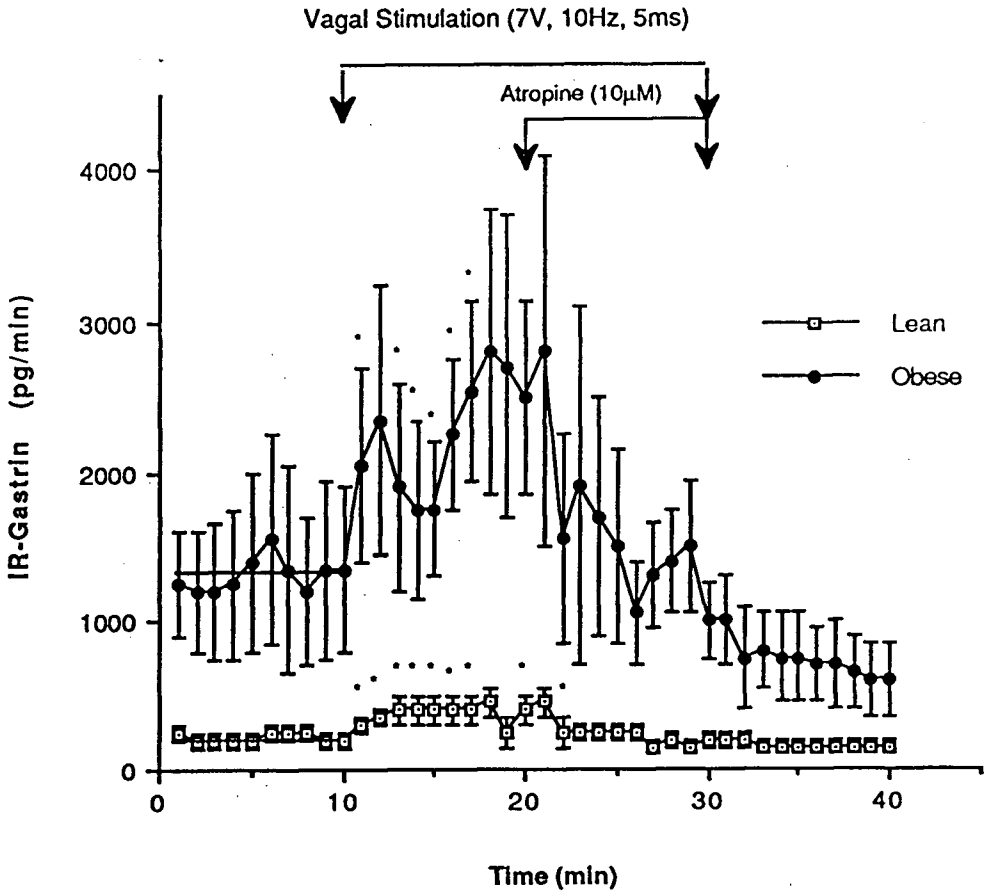


Figure 15 : The effect of atropine (10 μM) on gastrin secretion evoked by electrical stimulation of the anterior vagus (7 V, 10 Hz, 5 ms) from the isolated perfused stomach of lean (n=4) and obese (n=6) Zucker rats. The gastrin response during vagal stimulation was compared to the mean basal output prior to vagal stimulation. The mean basal ouput is designated with a bar from 1-10 min.

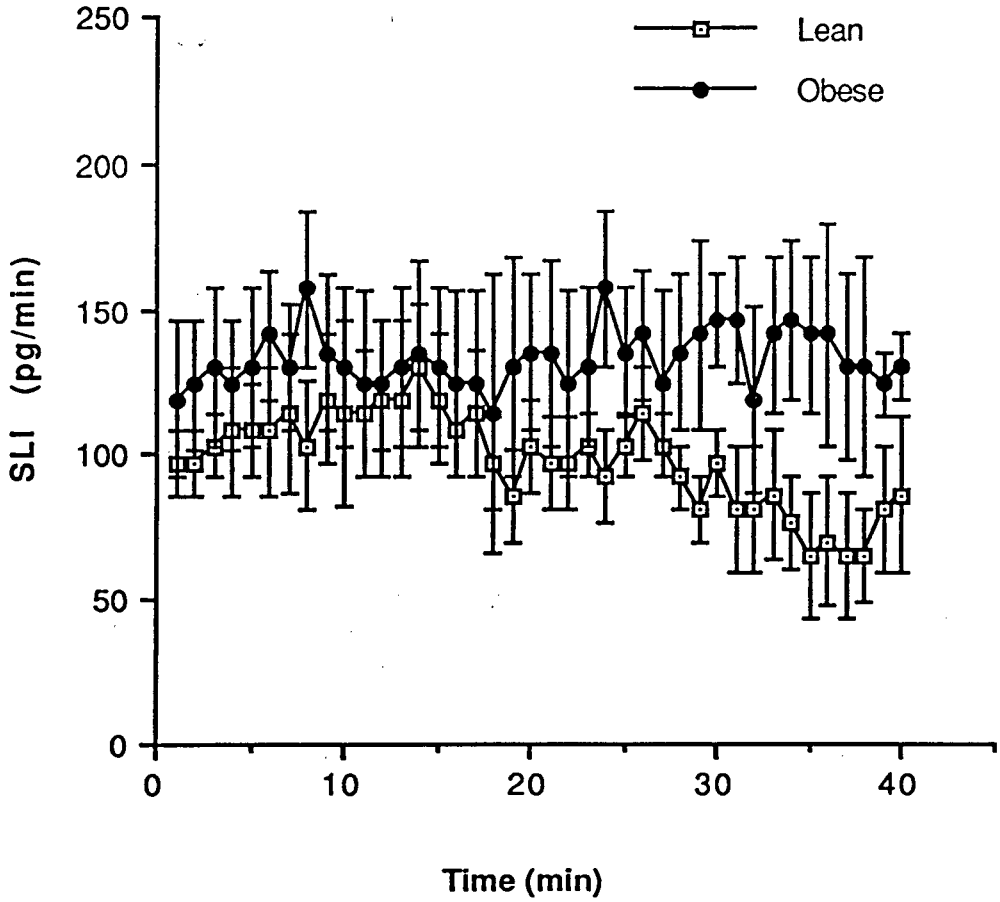


Figure 16 : Somatostatin secretion from the isolated perfused stomach of lean (n=4) and obese (n=4) Zucker rats during basal conditions. Somatostatin release from lean stomachs was compared to that from obese stomachs and no significant difference was observed.

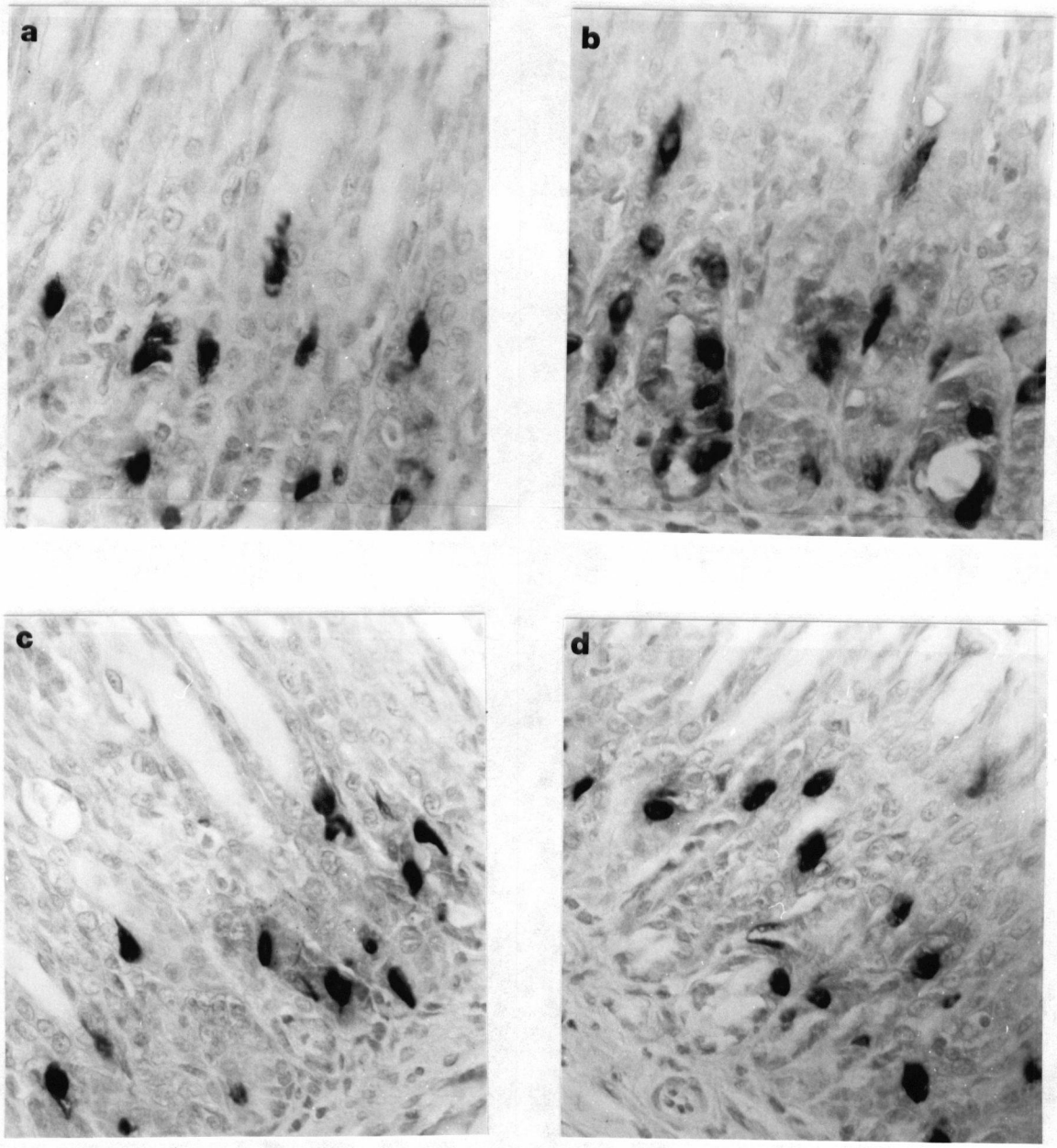


Figure 17 : Immunocytochemical localization of antral gastrin-containing cells. Magnification, x400.
a) Lean, 6 months of age.
b) Obese, 6 months of age.
c) Lean, 21 days old.
d) Obese, 21 days old.

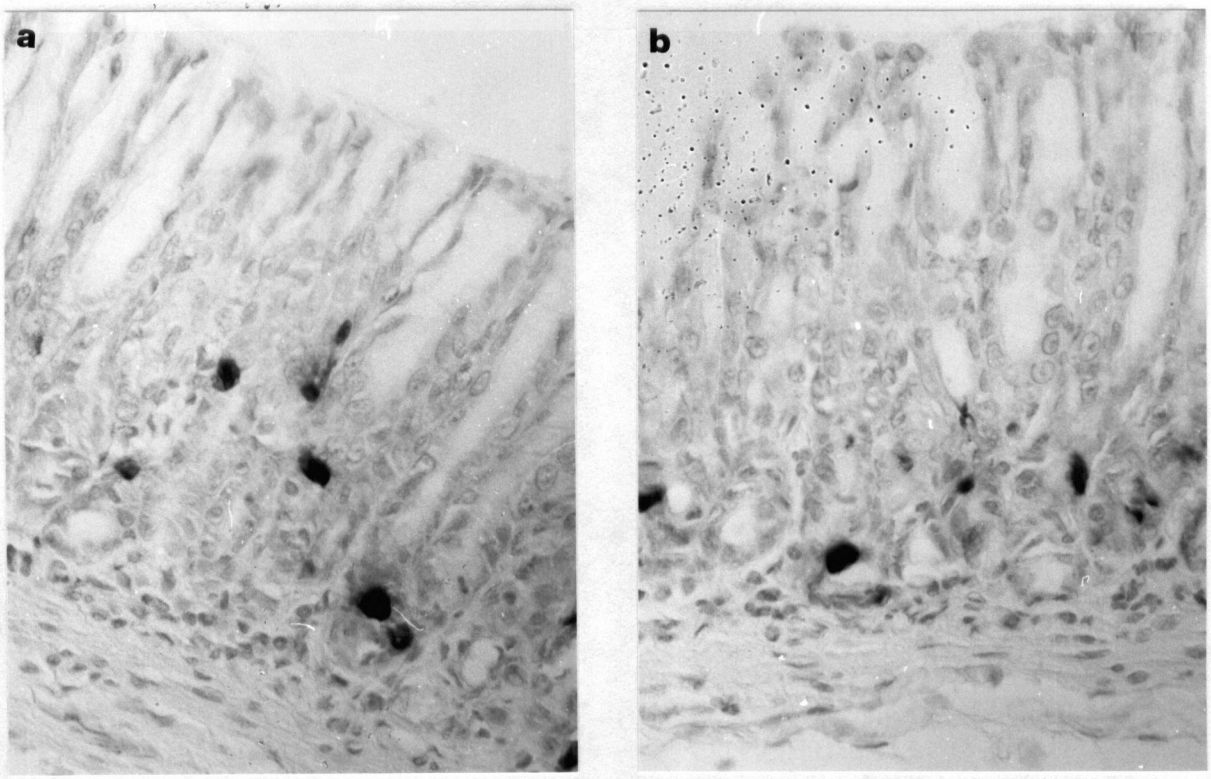


Figure 18 : Immunocytochemical localization of antral somatostatin-containing cells. Magnification, x400.
a) Lean.
b) Obese.

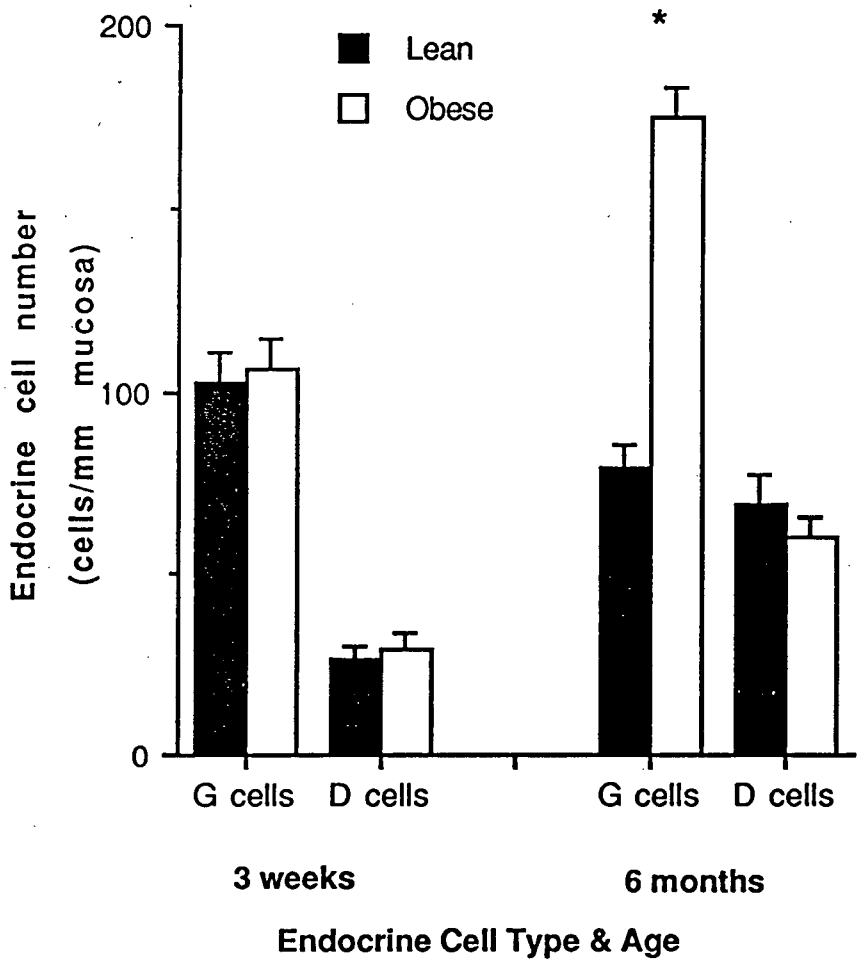


Figure 19 : Quantification of the antral endocrine cell population in lean and obese Zucker rats at 3 weeks (n=6 and n=5, respectively) and 6 months of age (n=7 and n=8, respectively). Quantities are expressed as mean ± standard error of the mean in cells/mm mucosa.

D. The Effect of Pair Feeding on the Gastrin Cell Population and Secretion:

1. Daily Weight Gain (Figure 20 & Table III):

Lean and obese animals were housed in individual cages at 4 weeks after birth, an age when their mean body weights were not significantly different (44 ± 1 g vs. 43 ± 9 g, Figure 20 & Table III). After a 7 day acclimatization period, animals were phenotypically distinguishable and a group of obese rats was introduced to a feeding schedule that matched the intake of the lean rats. At 5 weeks of age, the mean body weight of obese rats was slightly greater than their lean littermates (73 ± 6 g vs. 65 ± 2 g), although this difference was not statistically significant. In addition, fasting plasma gastrin concentrations at 35 days were not different in the two groups (156 ± 13 pg/ml, n=32 in lean animals vs. 182 ± 33 pg/ml, n=11 in obese litttermates). During the 21 day test period, food intake was carefully monitored and daily weights recorded (Figure 20 and Table III).

2. Fasting Plasma Gastrin Levels after Pair Feeding (Figure 21):

Eight week-old obese Zucker rats that received food *ad libitum* displayed fasting plasma gastrin levels that were two-fold greater than their lean counterparts (243 ± 26 pg/ml vs. 129 ± 16 pg/ml, $P < 0.05$, Figure 21). Similarly, pair-fed obese pups were hypergastrinemic compared to lean animals (218 ± 29 pg/ml vs. 129 ± 16 pg/ml, $P < 0.05$), but exhibited similar circulating gastrin concentrations as the obese*ad libitum* group.

3. Basal Gastrin Secretion after Pair Feeding (Figure 22):

Basal gastrin release from the isolated perfused stomachs of obese rats that were fed *ad libitum* was approximately two-fold greater than from lean animals (400 pg/min vs. 200 pg/min, Figure 22a). Basal gastrin secretion from diet-restricted rats was also greater than from lean littermates (300 pg/min vs. 200 pg/min). The integrated gastrin outputs of the obese *ad libitum* group and the obese pair-fed group were 84% and 43% greater than that of lean animals, respectively (16.7 ± 3.4 ng and 13.0 ± 2.3 ng, respectively, vs. 9.1 ± 1.0 ng, $P < 0.05$, Figure 22b). The integrated gastrin responses of the two obese groups were not significantly different.

4. Quantification of the Antral G-cell Population after Pair Feeding (Figure 23):

The number of gastrin-containing cells was significantly greater in the obese *ad libitum* group compared to their lean counterparts (125 $\pm$ 11 cells/mm mucosa vs. 93 $\pm$ 6 cells/mm mucosa, $P < 0.05$, Figure 23). In contrast to findings *in vivo* and from the *in vitro* stomach preparation, obese animals that were pair fed did not exhibit antral G-cell hyperplasia compared to lean rats (81 $\pm$ 6 cells/mm mucosa vs. 93 $\pm$ 6 cells/mm mucosa), but rather, possessed a G-cell population that was significantly less than that of freely-feeding obese rats ($P < 0.05$).

Table III : Daily body weights of lean rats (n=16) and obese animals that are on restricted (obese pair fed, n=12) and unrestricted (obese ad lib, n=10) diets from 28-56 days of age. Levels are expressed as mean ± standard error of the mean in g. Pair feeding was initiated on day 35.

Age (days)	Lean	Obese *Ad Libitum*	Obese Pair Fed
28	44 ± 1	43 ± 9	49 ± 3
29	44 ± 1	41 ± 6	48 ± 4
30	44 ± 2*†	54 ± 4	57 ± 3
31	50 ± 1*†	56 ± 4	58 ± 3
32	56 ± 2†	60 ± 5	63 ± 3
33	56 ± 1*†	65 ± 3	66 ± 3
34	60 ± 1*†	67 ± 3	70 ± 2
35	65 ± 2†	73 ± 6	73 ± 3
36	67 ± 2	74 ± 7	71 ± 3
37	67 ± 3	77 ± 9	72 ± 4
38	77 ± 3	84 ± 7	74 ± 3
39	78 ± 2*	87 ± 5†	78 ± 2*
40	85 ± 3	91 ± 9	78 ± 3
41	86 ± 3	95 ± 6†	84 ± 2*
42	83 ± 2	95 ± 9	85 ± 3
43	92 ± 3	101 ± 9	87 ± 3
44	96 ± 3	104 ± 9†	89 ± 3*
45	100 ± 3†	111 ± 7†	92 ± 2*
46	108 ± 4†	114 ± 7†	96 ± 2*
47	95 ± 3*	116 ± 8†	96 ± 3*
48	109 ± 6	127 ± 11†	100 ± 4*
49	112 ± 4*†	127 ± 8†	103 ± 3*
50	116 ± 4*†	131 ± 8†	105 ± 3*
51	121 ± 4*†	135 ± 9†	108 ± 2*
52	125 ± 4†	137 ± 9†	111 ± 3*
53	127 ± 4†	138 ± 8†	112 ± 3*
54	128 ± 4	143 ± 14	120 ± 4
55	135 ± 4*†	152 ± 9†	121 ± 3*
56	140 ± 4†	155 ± 10†	125 ± 4*

* significantly different from Obese *Ad Libitum* Group at $P < 0.05$

† significantly different from Obese Pair Fed Group at $P < 0.05$

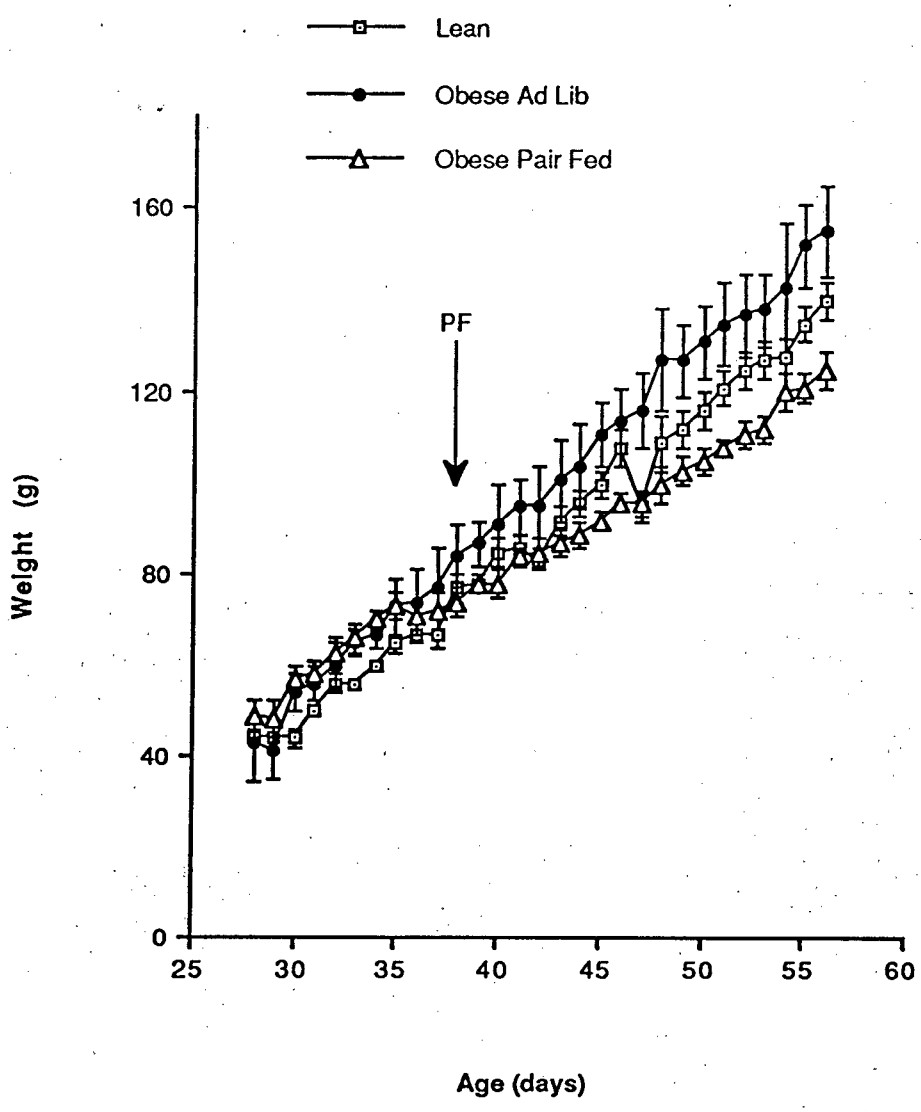


Figure 20 : Daily body weights of lean rats (n=16) and obese animals that are on restricted (obese pair fed, n=12) and unrestricted (obese ad lib, n=10) diets from 28-56 days of age. PF represents the start of the pair feeding regimen. Levels are expressed as mean ± standard error of the mean in g. Significant differences are shown in Table III.

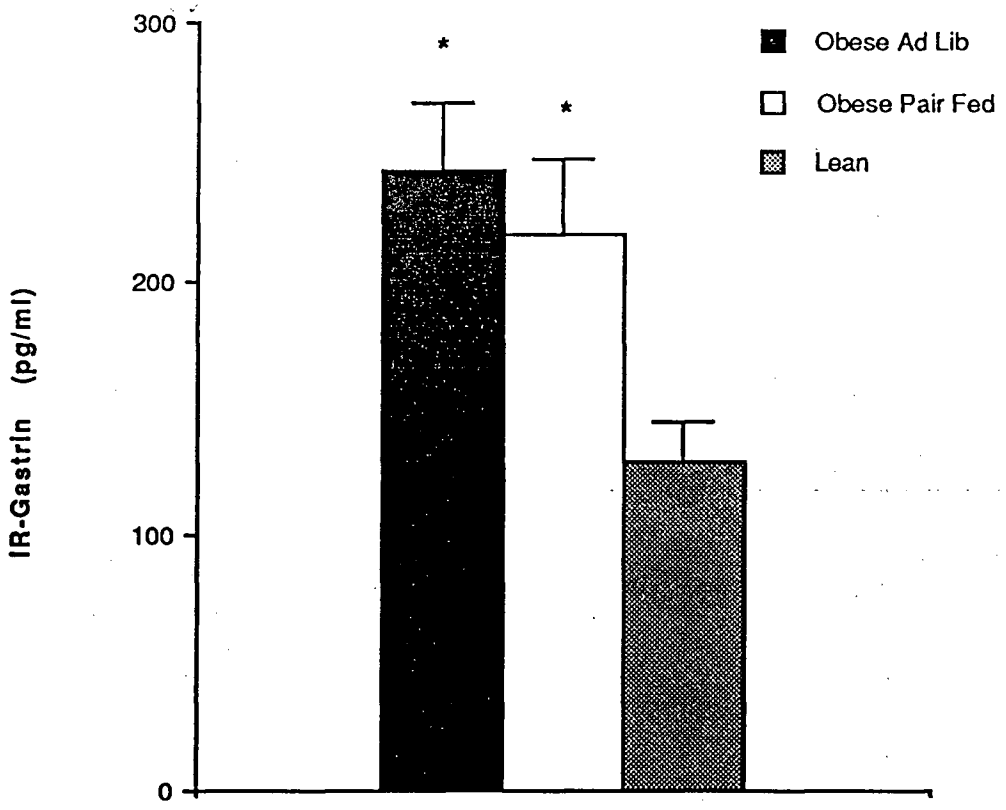


Figure 21 : Fasting plasma gastrin concentrations in 8 week-old lean (n=22), obese pair fed (n=12) and obese *ad libitum* (n=14) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/ml. * denotes significantly greater levels than lean animals at $P < 0.05$. The plasma gastrin concentrations of the two groups of obese rats were not significantly different.

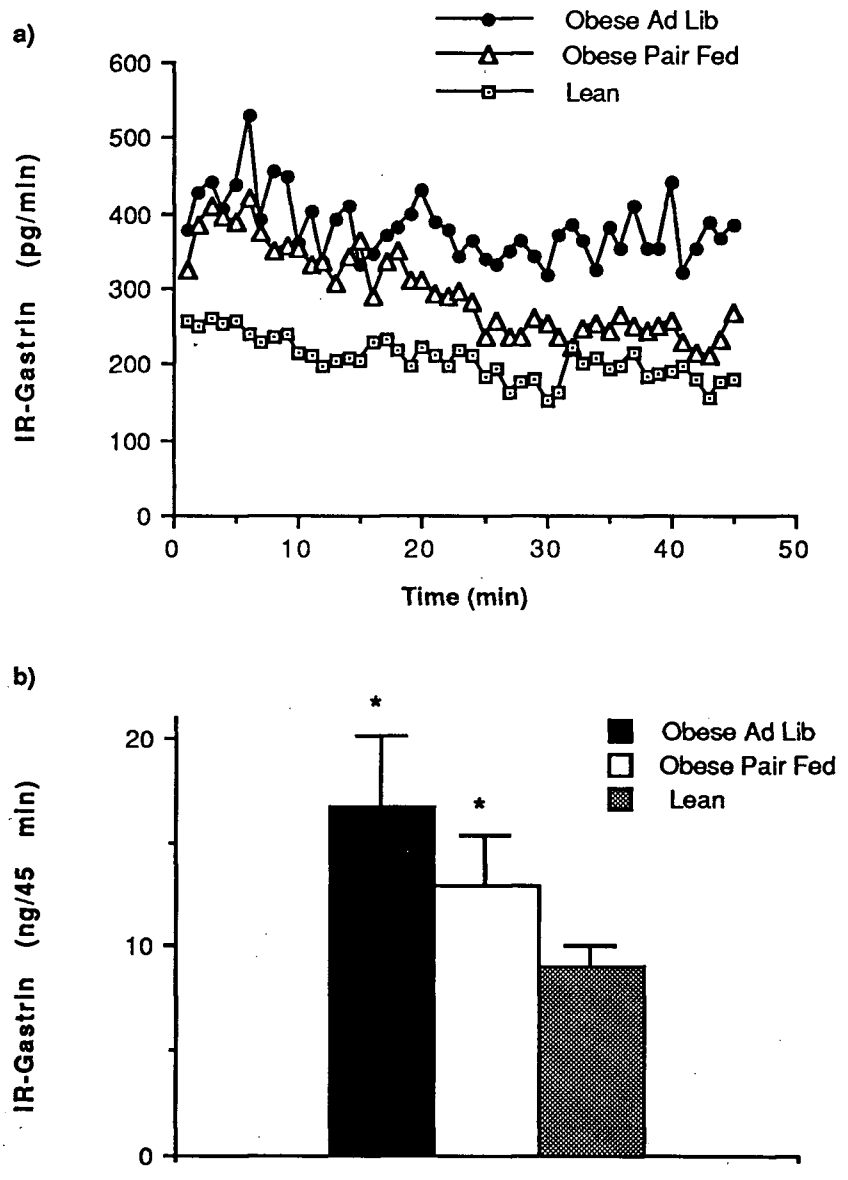


Figure 22 : a) Gastrin secretion from the isolated perfused stomach of 8 week-old lean (n=25), obese pair fed (n=15) and obese *ad libitum* (n=13) Zucker rats during basal conditions. Levels are expressed as the mean in pg/min. Standard error bars were not added to maintain clarity.
b) Integrated gastrin output during the 45 min perfusion period. Levels are expressed as mean ± standard error of the mean in ng/45 min. * denotes significantly greater levels than lean animals at $P < 0.05$. The integrated gastrin outputs of the two groups of obese rats were not significantly different.

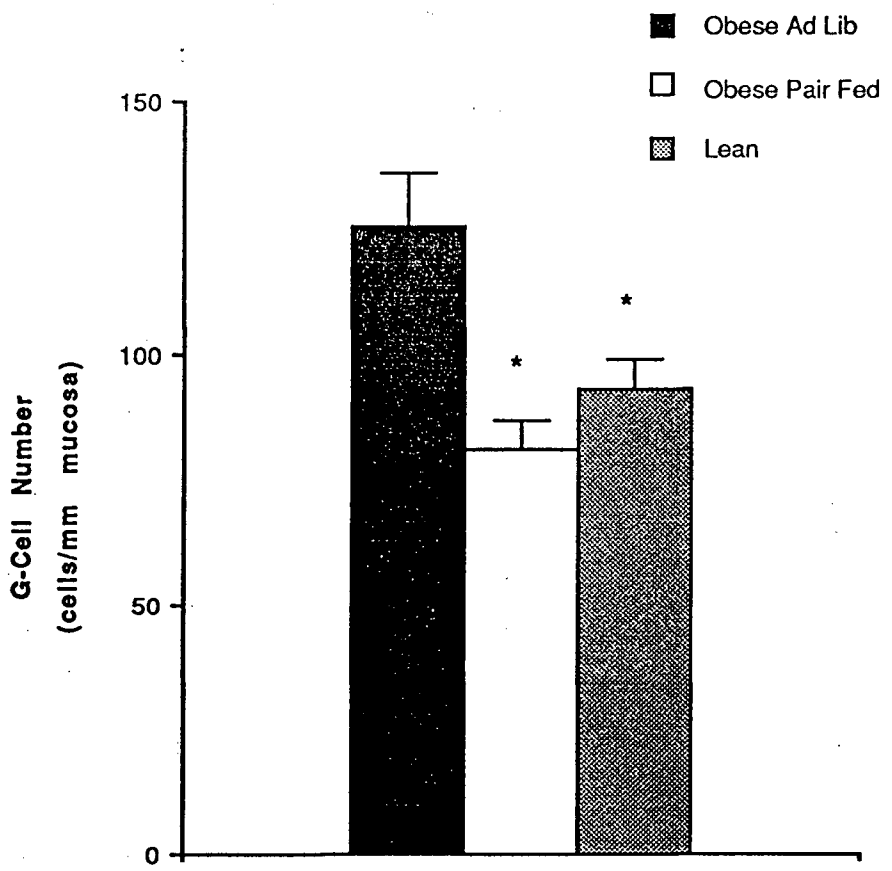


Figure 23 : Quantification of the antral G-cell population of 8 week-old lean (n=24), obese pair fed (n=12) and obese *ad libitum* (n=11) Zucker rats. Levels are expressed as mean ± standard error of the mean in cells/mm mucosa. * denotes significantly lower levels than obese ad libitum animals at $P < 0.05$. The G-cell numbers of lean and obese pair fed rats were not significantly different.

E. The Role of Bombesin/Gastrin Releasing Peptide in the Hypergastrinemia of the Obese Zucker Rat:

1. GRP Content of Gastric Tissue Extracts (Table IV):

After an overnight fast, whole stomachs of lean and obese adult Zucker rats were collected, washed in saline and frozen in liquid nitrogen. Radioimmunoassay of frozen tissue for GRP content was performed in the laboratory of Dr. J. H. Walsh (CURE) according to a previously described method (Walsh and Wong, 1979).

The GRP content of the corpus of lean and obese animals was not significantly different (Table IV). However, the GRP concentration of antral tissue extracts of obese rats was at least 3.5-fold greater than that of lean animals ($P < 0.05$).

Table IV : GRP content of gastric tissue extracts of lean (n=4) and obese (n=4) adult Zucker rats.

Tissue	Phenotype	IR-GRP (fmol/mg tissue)
Corpus	Lean	4.6 ± 0.8
	Obese	3.8 ± 0.7
Antrum	Lean	< 1.0
	Obese	3.6 ± 0.7 *

* $P < 0.05$

2. The Effect of Exogenous Bombesin on Hormone Release from the Isolated Vascularly Perfused Stomach (Figure 24-27):

a) The Effect of 0-10 nM Bombesin Gradient on Basal Gastrin Release (Figure 24):

Using a dual flask system wherein the peptide was introduced into the distal flask, a 0-10 nM bombesin gradient was produced during basal conditions. The BN gradient is depicted as a straight diagonal line beginning at 0 nM at the start of the experiment and ending at 10 nM at its cessation. The approximate BN concentration at any point during the perfusion period was then determined by extrapolation. There was an immediate rise in IR-G levels of lean rats reaching a peak value that was 337% greater than the initial secretion rate (from 304 $\pm$ 43 pg/ml at 2 min to 1330 $\pm$ 151 pg/ml at 6 min, Figure 24) at 1.7 nM BN. Gastrin release during the remainder of the gradient was sustained at a level 3-fold greater than that of controls (900 pg/min vs. 300 pg/min, $P < 0.05$). In contrast, significantly greater release of gastrin from BN-treated obese stomachs compared to controls (1653 $\pm$ 278 pg/ml vs. 914 $\pm$ 151 pg/min, $P < 0.05$, Figure 24) was not observed until 14 min after the start of the gradient at 4.0 nM BN. The gastrin response of obese stomachs to the BN gradient was concentration dependent. The maximal output was achieved at 9.4 nM BN (at the 34th min) with IR-G levels rising 324% above the intial secretion rate (2671 $\pm$ 593 pg/min vs. 630 $\pm$ 149 pg/min).

b) The Effect of 0-10 nM Bombesin Gradient on Basal Somatostatin Release (Figure 25):

SLI release from BN-treated lean stomachs rose to a peak value that was 50% greater than the initial rate (234 $\pm$ 95 pg/min vs. 157 $\pm$ 53 pg/min, Figure 25) at 1.8 nM BN, 6 min after the start of the gradient. However, this response was not significantly higher than the control level (150 $\pm$ 26 pg/min). The somatostatin secretion rate of control lean stomachs during the remainder of the gradient was not different from that of stomachs that received BN (150 pg/min vs. 200 pg/min). Similarly, maximum SLI levels from

obese stomachs, observed at 2.8 nM BN, were 80% higher than the initial output (295 ± 92 pg/min vs. 165 ± 78 pg/min, Figure 25) at 10 min, but this was not significantly different from control values (222 ± 49 pg/min). SLI secretion from the perfused stomachs of the two obese groups was identical throughout the duration of the gradient.

c) Bombesin Concentration-Response Curve: Effect on Gastrin Release (Figure 26:

After a 10 min basal period, a single concentration of BN was infused into the isolated perfused stomach preparation from 11-20 min. These experiments were performed to circumvent difficulties that may be encountered in interpreting BN-stimulated hormone release during a continuous gradient; for instance, desensitization. BN stimulated gastrin release in both groups of animals in a concentration-dependent fashion from 0.1 nM to 100 nM ($P < 0.05$, Figure 26). In terms of a percentage of basal release, IR-G secretion from the *in vitro* stomachs of lean animals was greater than that of obese rats at all concentrations of BN tested. Although this difference was statistically significant ($P < 0.05$) at 1 and 100 nM BN only, the trend of greater responsiveness to BN stimulation was clearly demonstrated by lean stomachs compared to obese stomachs. Maximal stimulation in obese stomachs was observed at 10 nM BN (270 ± 71 % of basal) with comparable release at 100 nM BN (273 ± 50 % of basal). In comparison, gastrin output from lean stomachs was already 366 ± 15 % of basal at 1 nM BN and the gastrin response was still rising at 100 nM BN (499 ± 47 % of basal).

d) Bombesin Concentration-Response Curve: Effect on Somatostatin Release (Figure 27):

BN elicited modest increases in SLI secretion at 10 and 100 nM BN in lean animals (149 ± 19 % of basal and 154 ± 11 % of basal, respectively, $P < 0.05$, Figure 27) and at 100 nM BN in obese rats (126 ± 10 % of basal, $P < 0.05$). The somatostatin response to BN was not significantly different between the two phenotypes at all concentrations of BN tested.

3. The Effect of Substance P on Basal Gastrin Release (Figure 28):

This series of experiments was performed to examine the effect of substance P on G-cell function. As subsequent experiments employed a substance P analog as a bombesin antagonist, it was necessary to determine whether inhibition of gastrin release produced by treatment with the substance P analog, if any, could be attributed to its antagonistic action against substance P or bombesin or both.

After a 10 min basal period, substance P (10 nM) was introduced via side arm infusion for an additional 10 min. Substance P did not alter basal gastrin secretion from obese stomachs (Figure 28). In lean animals, basal IR-G levels were slightly reduced during the 2nd 5-minute collection period (from 155 $\pm$ 15 pg/min to 124 $\pm$ 14 pg/min, $P < 0.05$) only.

4. The Effect of Bombesin Antagonists on Basal Gastrin Release (Figures 29 & 30):

a) The Effect of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}] Substance P (Figure 29):

The isolated stomachs of lean and obese animals were perfused under basal conditions for 15 min and then infused with 1.4 μM [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}] SP from 16-30 min of the 45 min experiment. Basal gastrin secretion was unaltered in both groups during the infusion period (Figure 29 a & b). The integrated gastrin ouputs of obese stomachs during and subsequent to administration of the SP analog were not significantly different from the initial basal response (15.7 $\pm$ 5.4 ng/15 min from 16-30 min and 12.5 $\pm$ 5.1 ng/15 min from 31-45 min vs. 14.6 $\pm$ 3.4 ng/15 min from 1-15 min, Figure 29b). In lean animals, the integrated gastrin yield during SP analog treatment was similar to the basal value (3.7 $\pm$ 0.5 ng/15 min from 16-30 min vs. 3.5 $\pm$ 0.4 ng/15 min from 1-15 min), but both responses were significantly greater than that observed after removal of the infusate (2.1 $\pm$ 0.4 ng/15 min from 31-45 min, $P < 0.05$).

b) The Effect of [Leu^{13}-ψ-CH_2NH-Leu^{14}] Bombesin (Figure 30):

After a 15 min basal period, 1 μM [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin was introduced into the *in vitro* stomach preparation via sidearm infusion from 16-30 min of the 45 min test period. In lean and obese stomachs, the BN analog had no effect on basal gastrin secretion (Figure 30 a & b). The integrated gastrin outputs of obese rats before, during and subsequent to the infusion of the BN analog were not significantly different (16.8 + 4.9 ng/15 min, 15.7 + 4.6 ng/15 min and 11.3 + 3.4 ng/15 min, respectively, Figure 30b). Similar results were obtained from lean littermates.

5. The Effect of Bombesin Antagonists on Bombesin-stimulated Gastrin Release (Figures 31-34):

a) The Effect of [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] Substance P (Figures 31 & 32):

Lean and obese animals were divided into two groups: one received 1 nM BN from 21-30 min of a 45 min experiment (control group) whereas another was given 1 nM BN from 21-30 min plus 1.4 μM [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] Substance P during the entire test period (test group). IR-G release from the stomachs of lean and obese test animals was not significantly different from that of corresponding control animals at all 5-min sampling times (Figure 31 a & b). The integrated gastrin responses of SP analog-treated lean rats 10 min prior to and during the BN infusion were not significantly lower than lean controls (2.7 ± 0.5 ng/10 min vs. 2.7 ± 0.3 ng/10 min from 11-20 min and 5.4 ± 0.9 ng/10min vs. 7.7 ± 1.2 ng/10 min from 21-30 min, Figure 32). Likewise, the integrated gastrin outputs of obese stomachs receiving the SP analog were not different from those of control stomachs (9.3 ± 2.2 ng/10min vs. 8.7 ± 1.7 ng/10min from 11-20 min and 16.0 ± 3.5 ng/10min vs. 17.3 ± 3.4 ng/10min).

b) The Effect of [Leu^{13}-ψ-CH_2NH-Leu^{14}] Bombesin (Figures 33 & 34):

Using the same experimental design as (a), test animals were infused with 1 μM [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin from 1-45 min, with 1 nM BN superimposed from

21-30 min whereas controls were not treated with the BN analog. Significantly lower IR-G levels were not observed from lean and obese stomachs receiving [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin compared to the control group (Figure 33). In addition, the integrated gastrin responses of test animals of both phenotypes 10 min prior to and during BN infusion were not different from those of corresponding control rats (in the lean group: 2.9 ± 0.5 ng/10min vs. 2.8 ± 0.3 ng/10min from 11-20 min and 5.2 ± 0.9 ng/10min vs. 7.7 ± 1.4 ng/10min from 21-30 min; in the obese group: 9.7 ± 0.8 ng/10min vs. 8.6 ± 1.9 ng/10min from 11-20 min and 14.1 ± 1.7 ng/10min vs. 16.4 ± 3.8 ng/10min from 21-30 min, Figure 34).

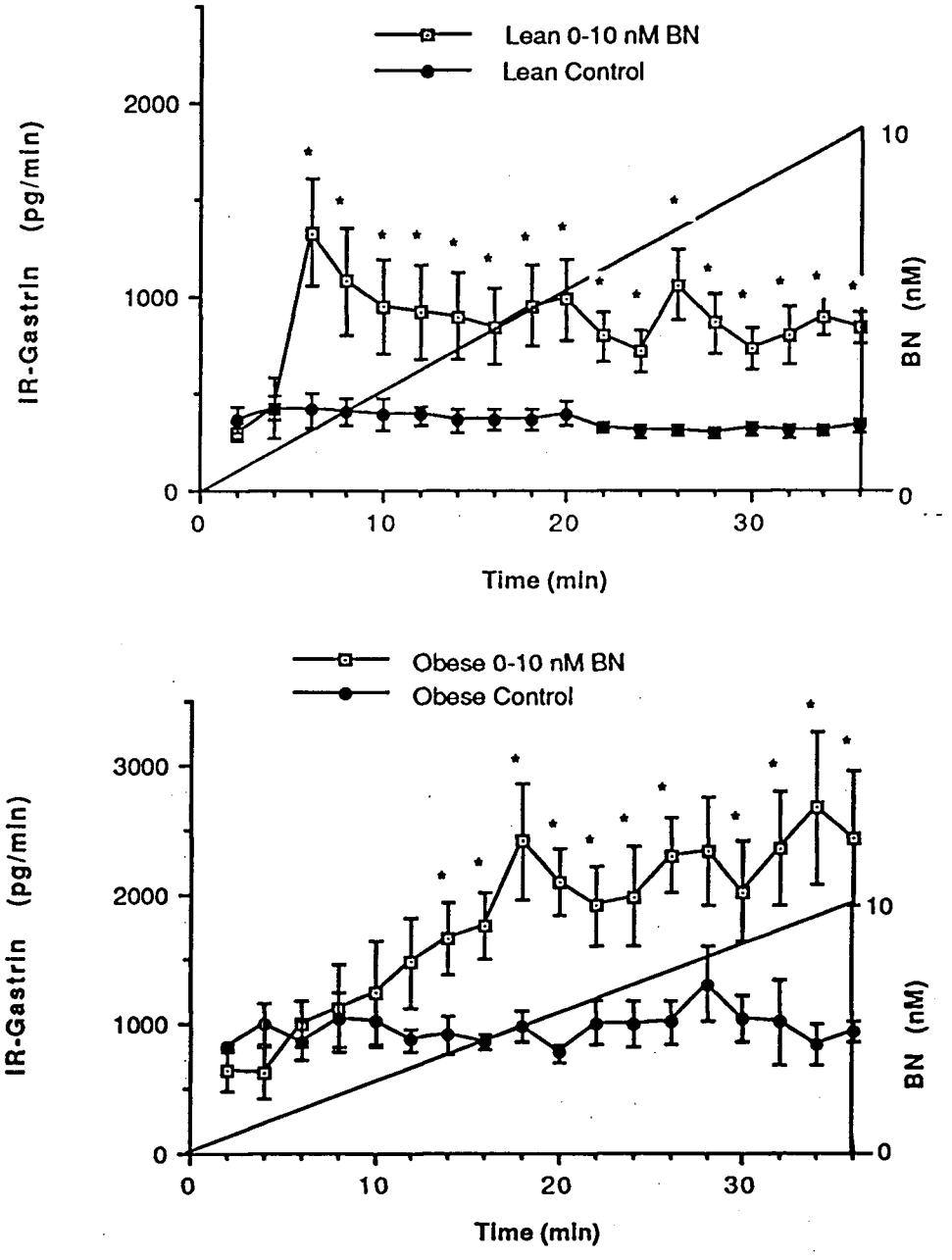
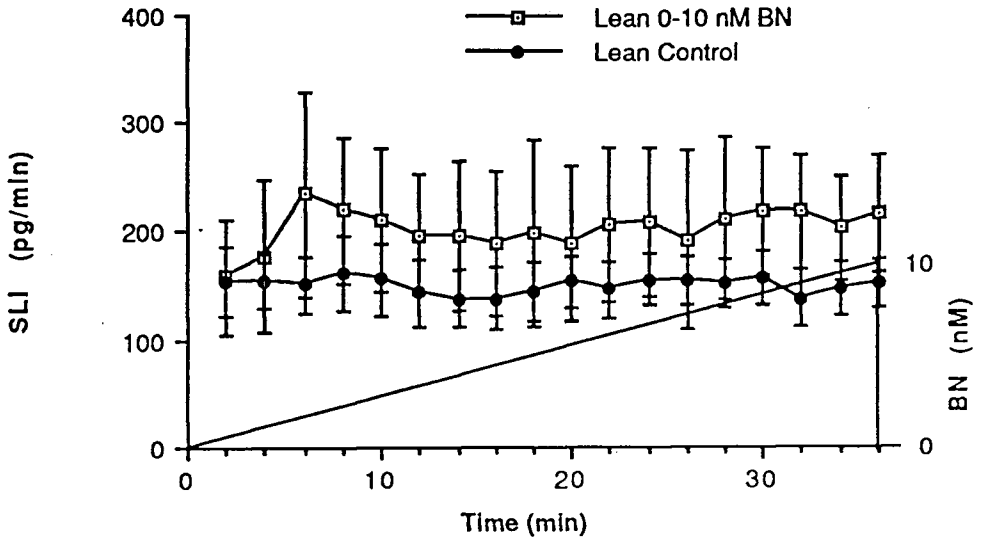


Figure 24 : The effect of a 0-10 nM bombesin gradient on basal gastrin secretion from the isolated perfused stomach of lean (n= 5) and obese (n=4) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/min and * denotes significantly greater values compared to lean (n=5) and obese (n=3) control animals to at least $P < 0.05$. The gradient duration was 36 min with samples taken every 2 min.



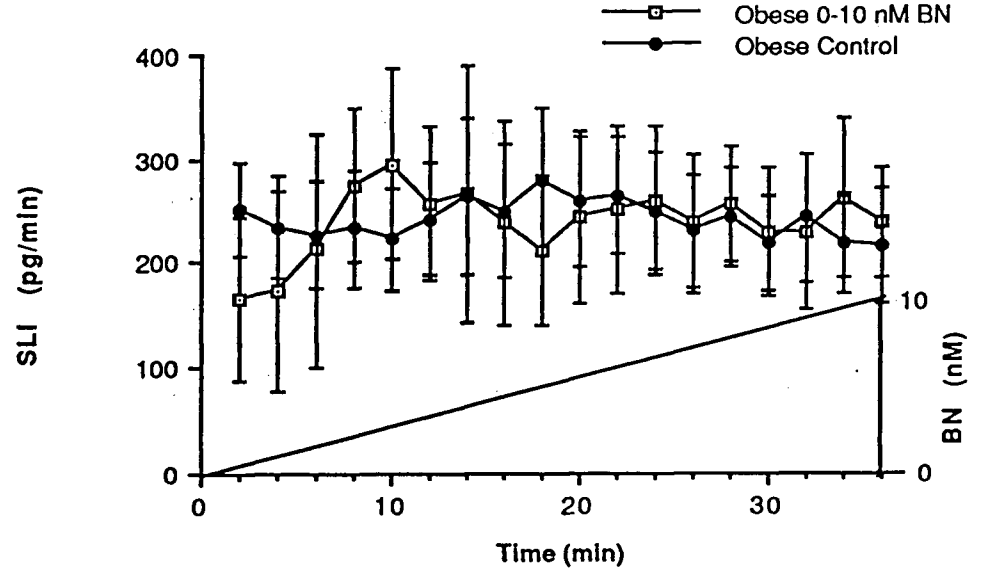


Figure 25 : The effect of a 0-10 nM bombesin gradient on basal somatostatin secretion from the isolated perfused stomach of lean (n=5) and obese (n=5) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/min. The response to BN was compared to control values in lean (n=5) and obese (n=5) animals at each 2 min sampling period and no significant difference was found.

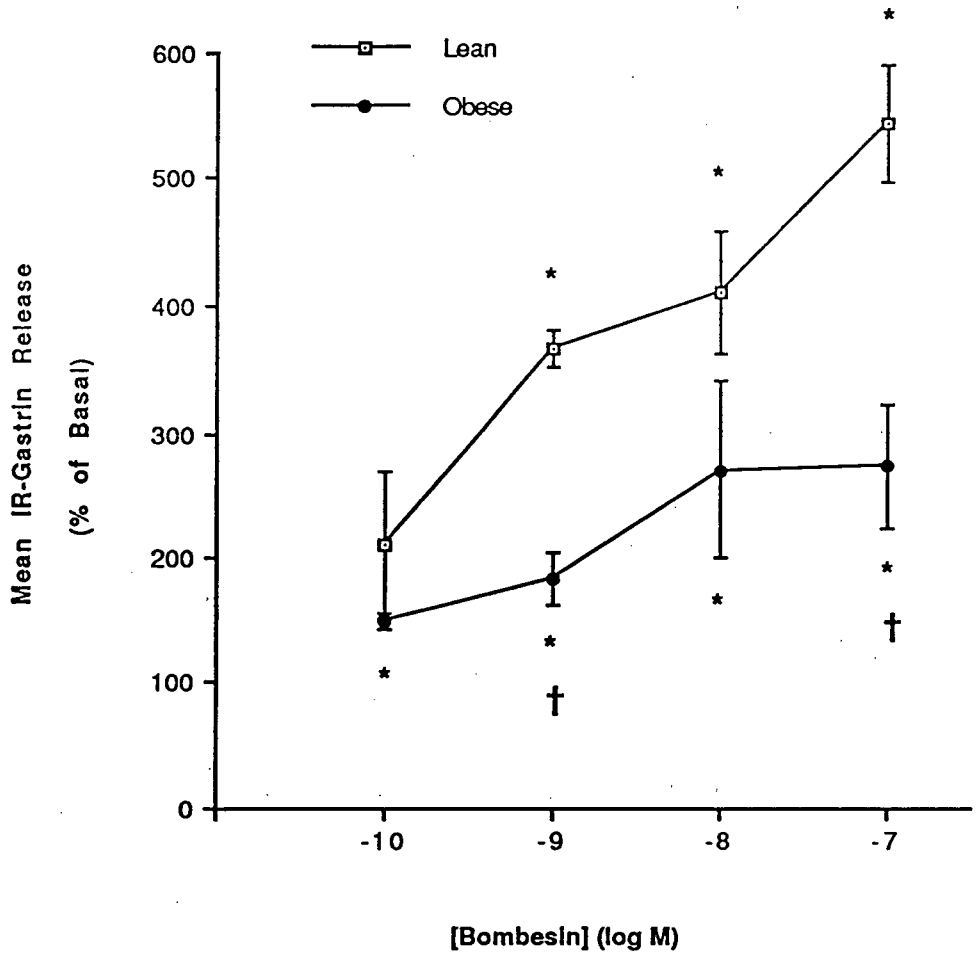


Figure 26 : Bombesin concentration-response curve: effect on basal gastrin release. BN from 0.1 nM to 100 nM was infused into lean (n=4 each) and obese (n=4 each) stomachs for 10 min after an initial 10 min basal period. Levels are expressed as mean ± standard error of the mean in % of basal gastrin release (determined from 1-10 min) and * denotes significant increases over basal at $P < 0.05$. The responses to BN in lean rats were compared to those in obese animals and † denotes statistical significance to at least $P < 0.05$.

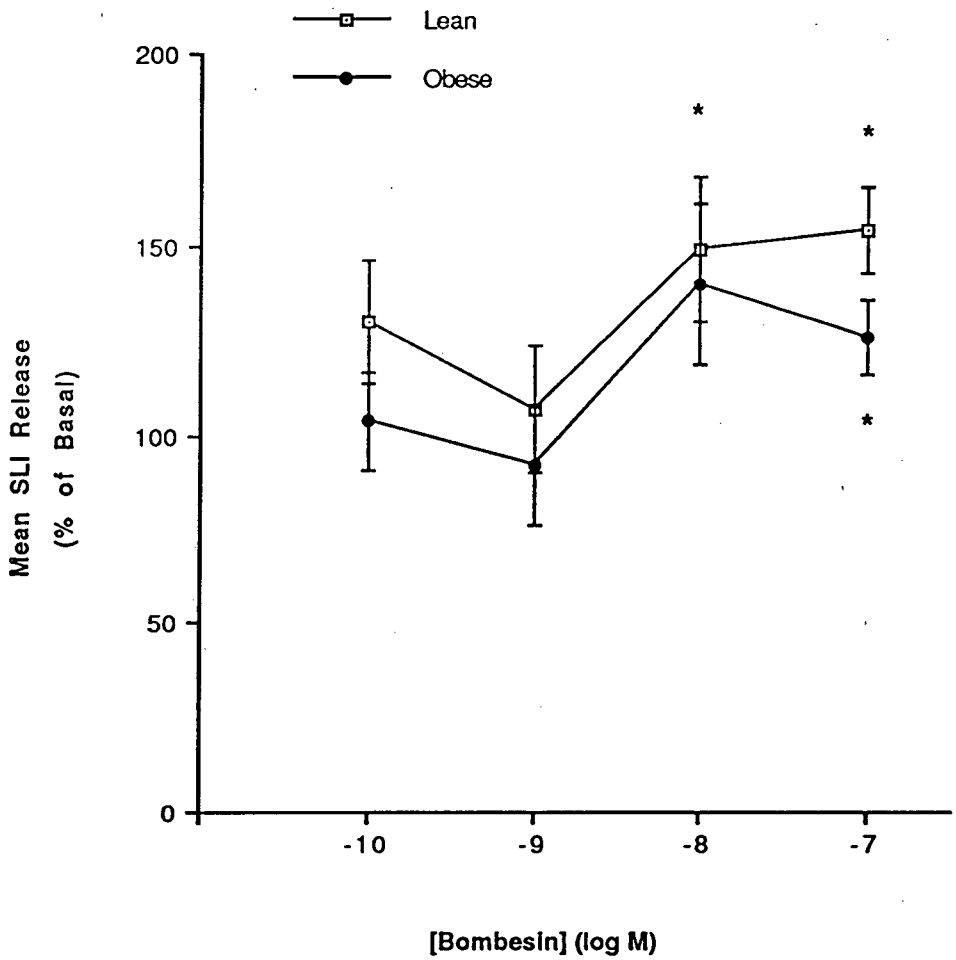


Figure 27 : Bombesin concentration-response curve: effect on basal somatostatin release. BN from 0.1 nM to 100 nM was infused into lean (n=4 each) and obese (n=4 each) for 10 min after an initial 10 min basal period. Levels are expressed as mean ± standard error of the mean in % of basal somatostatin release (determined from 1-10 min) and * denotes significant increases over basal at $P < 0.05$. The responses to BN in lean rats were not different from those in obese animals.

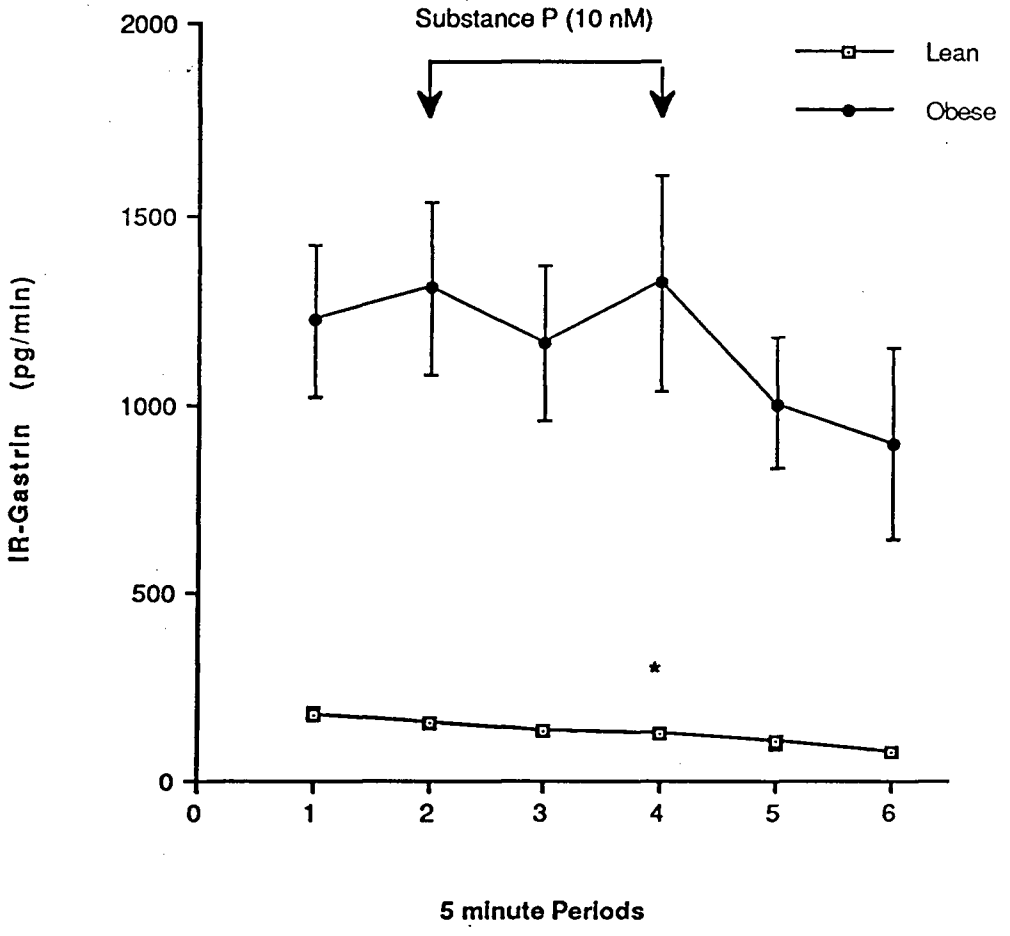


Figure 28 : The effect of substance P (10 nM) on basal gastrin release from the isolated perfused stomach of lean (n=7) and obese (n=6) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/min. IR-G levels during substance P infusion were compared to the basal 5-minute period immediately prior to the start of the infusion and * denotes statistical significance to at least $P < 0.05$.

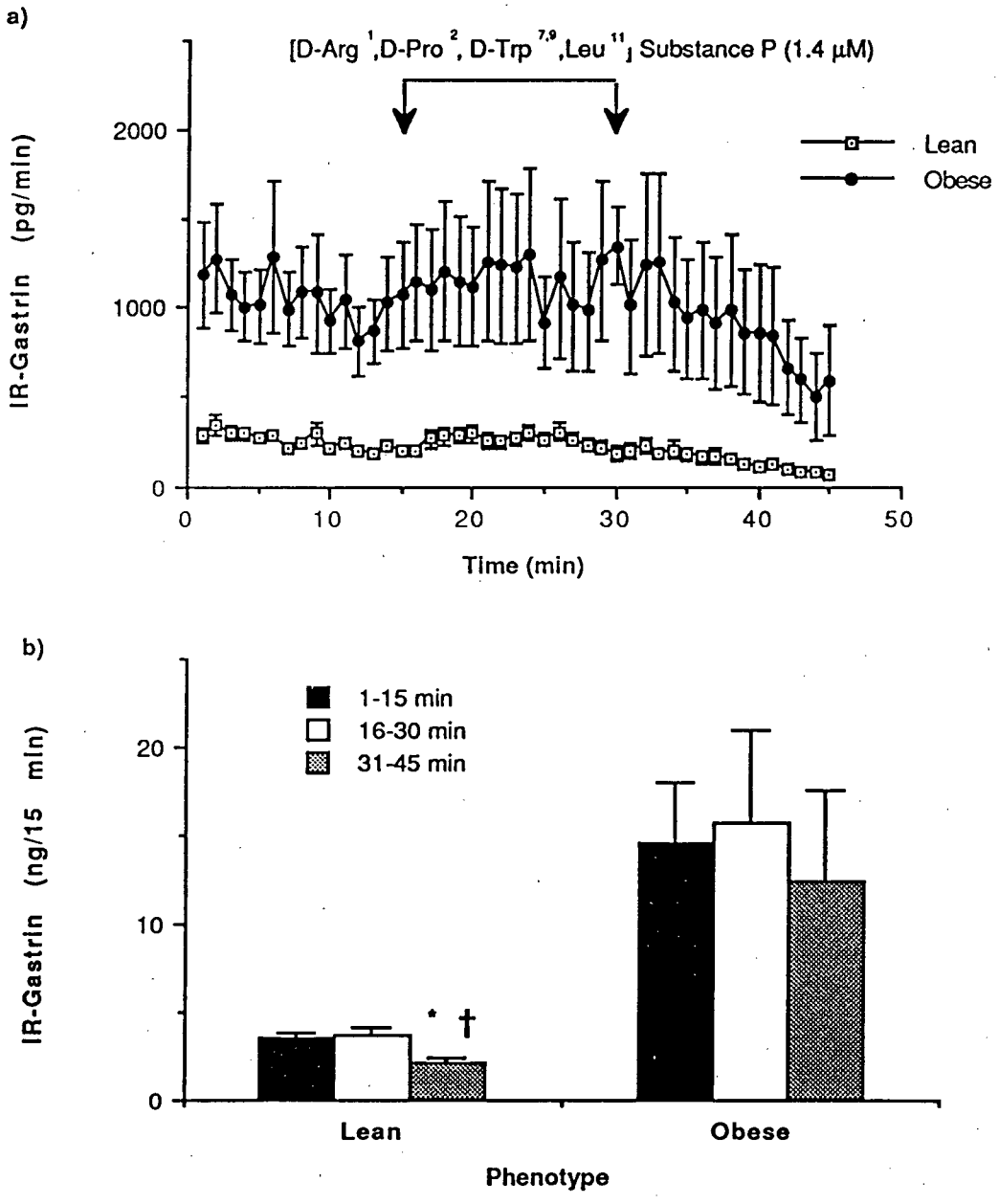


Figure 29 : a) The effect of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}] substance P (1.4 µM) on basal gastrin release from the isolated perfused stomach of lean (n=6) and obese (n=5) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/min.
b) Integrated gastrin responses before, during and after infusion of the SP analog. Levels are expressed as mean ± standard error of the mean in ng/15 min. The integrated outputs before and during the infusion were not significantly different in both groups of animals. * and † denotes significantly lower levels compared to the responses obtained from 1-15 min and 16-30 min, respectively.

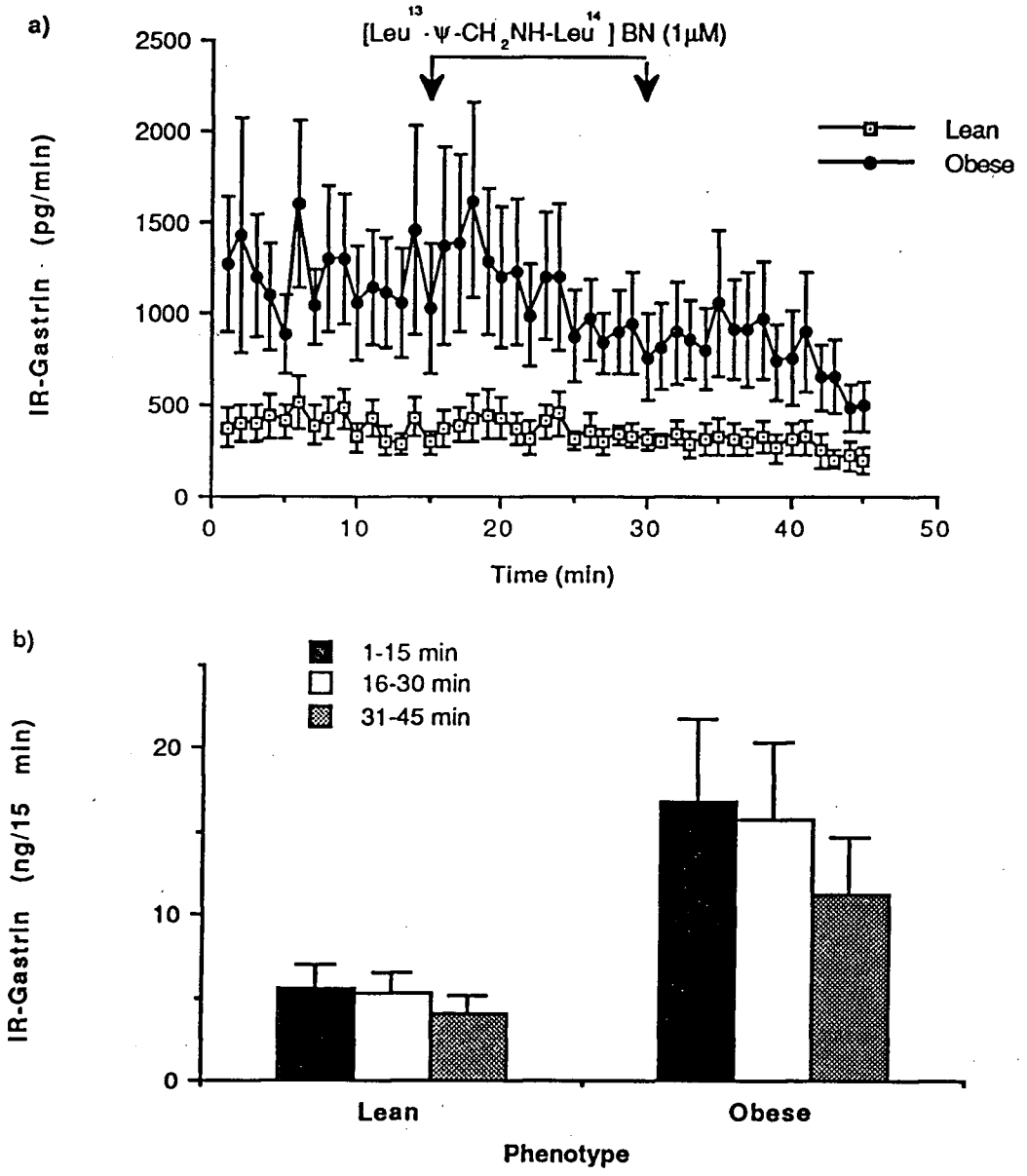
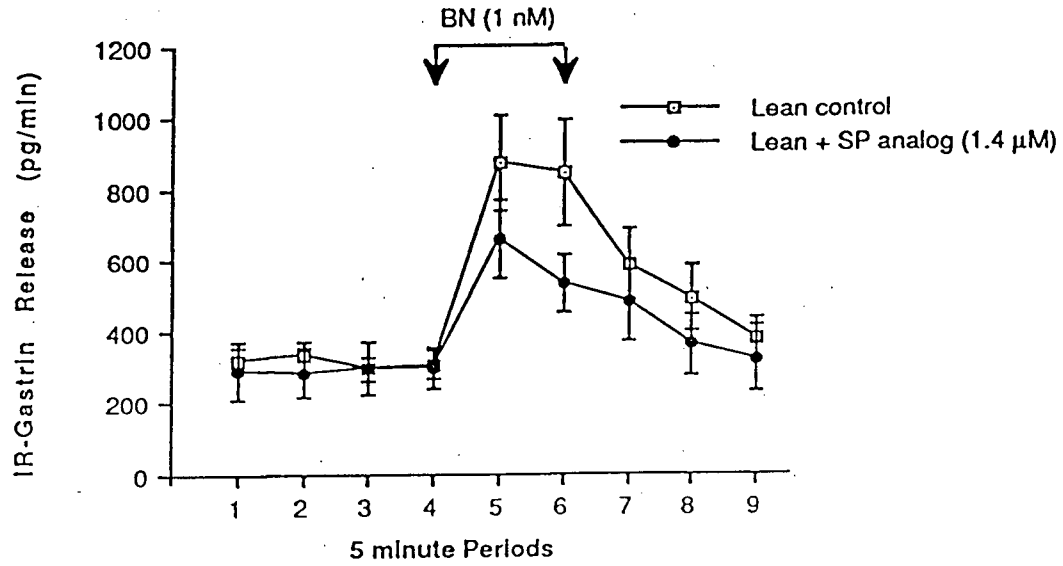


Figure 30 : a) The effect of [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin (1 μM) on basal gastrin release from the isolated perfused stomach of lean (n=4) and obese (n=3) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/min.
b) Integrated gastrin responses before, during and after infusion of the BN analog. Levels are expressed as mean ± standard error of the mean in ng/15 min. The integrated outputs before, during and subsequent to the infusion were not significantly different from one another in lean and obese animals.



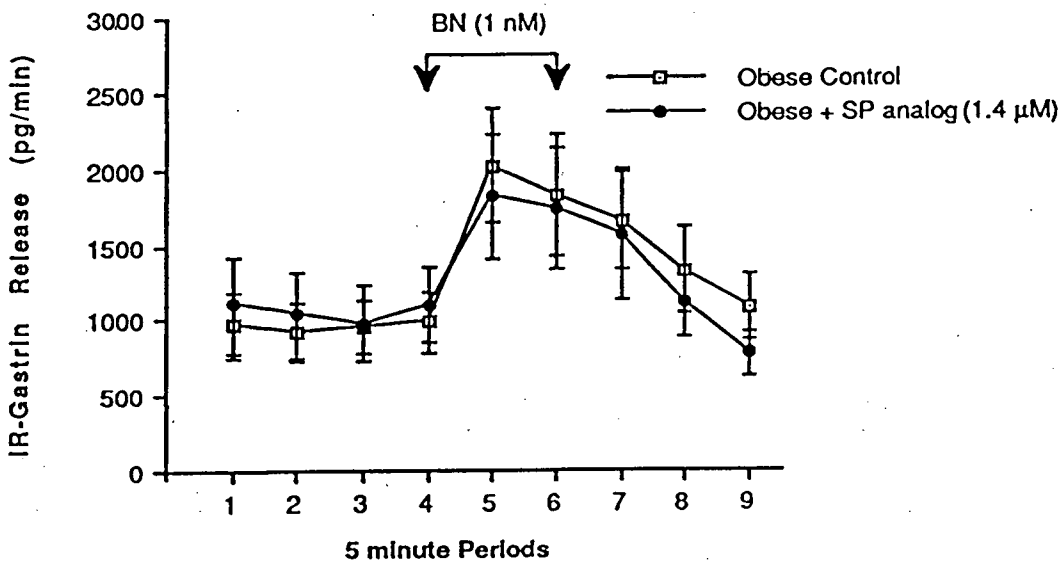


Figure 31 : The effect of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}] substance P (1.4 μM) on bombesin-stimulated (1 nM) gastrin release from the isolated perfused stomach of lean (n=6) and obese (n=7) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/min. IR-G secretion from test animals was not significantly different from lean (n=9) and obese (n=9) controls.

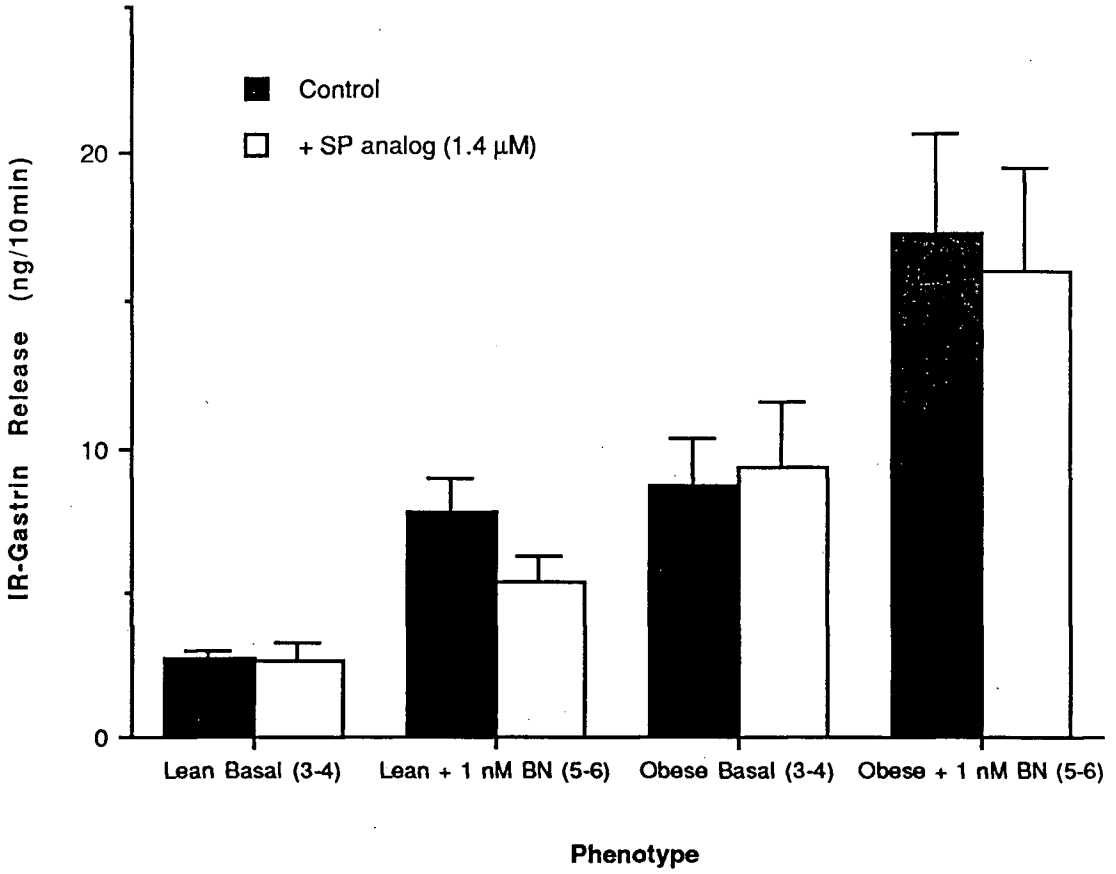
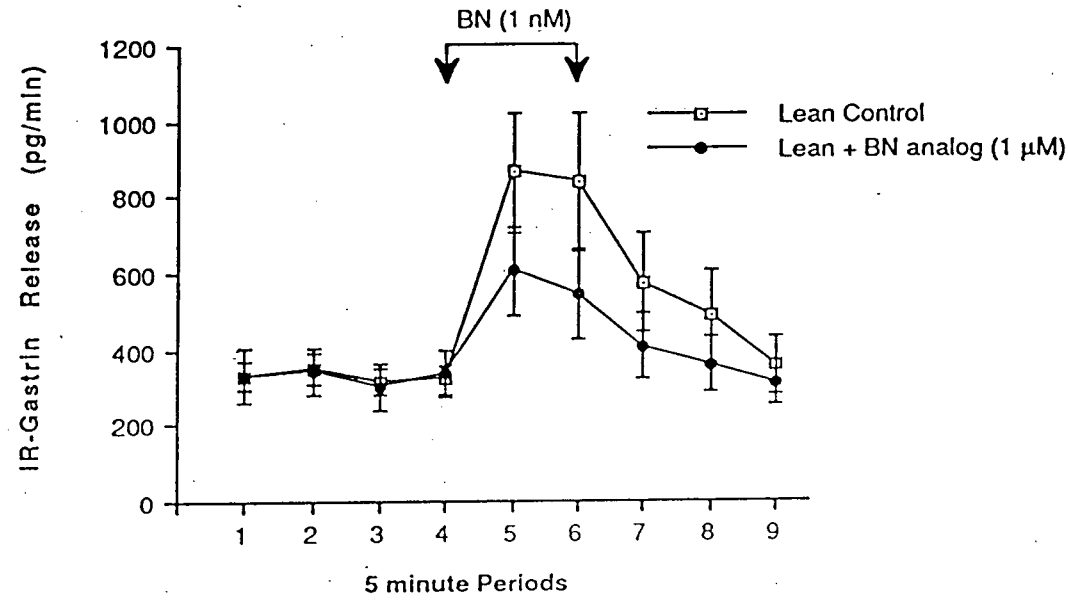


Figure 32 : The integrated gastrin responses of the isolated perfused stomachs of lean (n=6) and obese (n=7) Zucker rats to BN (1nM) plus [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P (1.4 μM). Levels are expressed as mean ± standard error of the mean in ng/10 min. The integrated outputs of test animals 10 min before and during BN infusion were not significantly different from lean (n=9) and obese (n=9) controls.



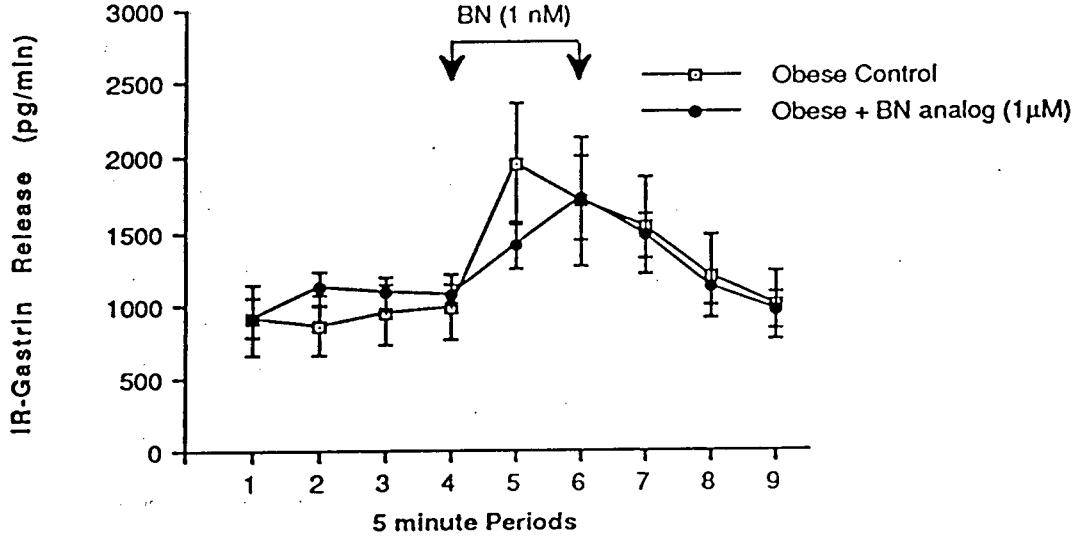


Figure 33 : The effect of [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin (1 μM) on bombesin-stimulated (1 nM) gastrin release from the isolated perfused stomach of lean (n=6) and obese (n=4) Zucker rats. Levels are expressed as mean ± standard error of the mean in pg/min. IR-G secretion from test animals was not significantly different from lean (n=7) and obese (n=8) controls.

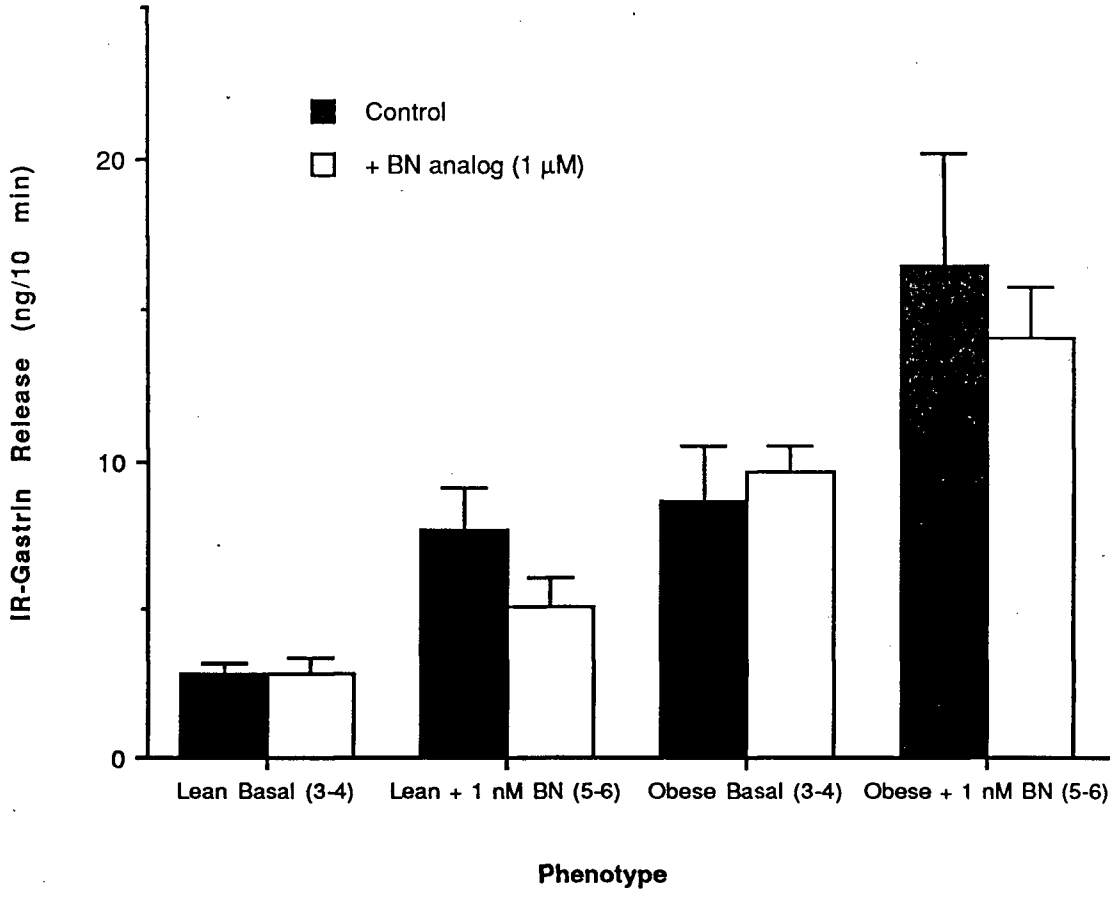


Figure 34 : The integrated gastrin responses of the isolated perfused stomachs of lean (n=6) and obese (n=4) Zucker rats to BN (1nM) plus [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin (1 μM). Levels are expressed as mean ± standard error of the mean in ng/10 min. The integrated outputs of test animals 10 min before and during BN infusion were not significantly different from lean (n=7) and obese (n=8) controls.

II. STUDIES IN ISOLATED CANINE G CELLS:

A. Culture of Canine Antral Gastrin Cells (Figure 35):

Radioimmunoassay of cell extracts from post-elutriation fractions 1 and 2 revealed that IR-G levels from fraction 2 were approximately 2 to 3-fold greater than fraction 1. Consequently, cells from fraction 2 were cultured for 48 h at 37°C. Immunocytochemical examination of the culture plates demonstrated that gastrin-containing G cells and somatostatin-containing D cells accounted for 8.5% (n=2) and 1% (n=2), respectively, of the total viable adherent cells. Mucin cells comprised the remainder of the adherent population (Figure 35).

B. Pattern of Immunoreactive-Gastrin Release During Basal Conditions (Figure 36):

Basal gastrin release was found to be 1.91 ± 0.48 %TCC (Figure 36). BN, from 0.01 to 100 pM, stimulated gastrin release from cultured G cells in a concentration-dependent fashion (Figure 36). Maximal gastrin response was achieved at 100 pM (4.52 ± 1.32 %TCC) with comparable stimulation at 10 pM (4.37 ± 1.12 %TCC).

C. Effect of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}] Substance P on Basal and Bombesin-stimulated Gastrin Release (Figures 37 & 39):

Addition of [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}] SP, at 1 μM significantly increased basal gastrin levels by 25.4 ± 10.4% (from 1.91 ± 0.48 %TCC to 2.38 ± 0.60 %TCC, $P<0.05$, Figures 37 & 39). Bombesin-stimulated gastrin release was partially inhibited by the SP analog (1 μM) over the BN concentration range of 0.1 pM to 100 pM. However, maximal inhibition at 1 pM BN was only 71.4 ± 11.9% (3.86 ± 1.15 %TCC with 1 pM BN alone compared to 2.72 ± 0.91 %TCC in the presence of the antagonist, Figures 37 & 39).

D. Effect of [Leu^{13}-ψ-CH_2NH-Leu^{14}] Bombesin on Basal and Bombesin-stimulated Gastrin Release (Figures 38 & 39):

In contrast, [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN, at 1 μM did not alter basal gastrin release (Figures 38 & 39). Furthermore, the BN analog (1 μM) completely blocked BN-activated gastrin secretion over the BN concentration range of 0.01 pM to 1 pM. Inhibition of gastrin released by 10 and 100 pM BN was 74.8 ± 12.9% and 56.2 ± 15.4%, respectively ($P<0.05$, Figures 38 & 39). Figure 39 demonstrates the relative ability of each antagonist at 1 μM to suppress gastrin release produced by BN treatment. Inhibition by the BN analog from 0.01 to 1 pM BN was significantly greater than inhibition by the SP analog (93.0 ± 10.2% vs. 22.1 ± 20.0%, 96.7 ± 12.6% vs. 45.3 ± 5.7% and 107.7 ± 12.0% vs. 71.4 ± 11.9%, respectively, $P<0.05$).

E. Pattern of Release of Somatostatin-like Immunoreactivity:

Somatostatin levels were measured from cell extracts and averaged 434 ± 237 pg/ml (n=4). Radioimmunoassay of the release medium indicated that somatostatin levels were below the detection limit of the assay (< 12-15 pg/ml).

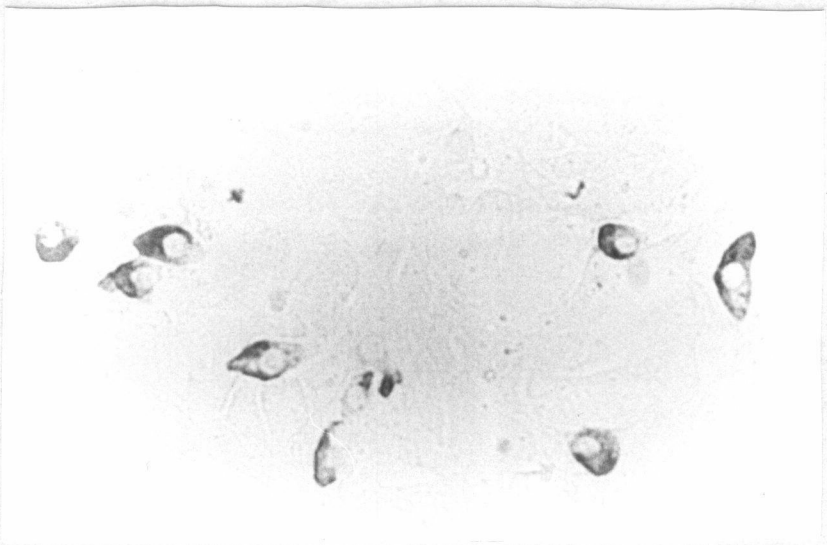


Figure 35 : Light micrograph of a representative group of immunoreactive-gastrin cells after a 2 day culture. Magnification, x400.

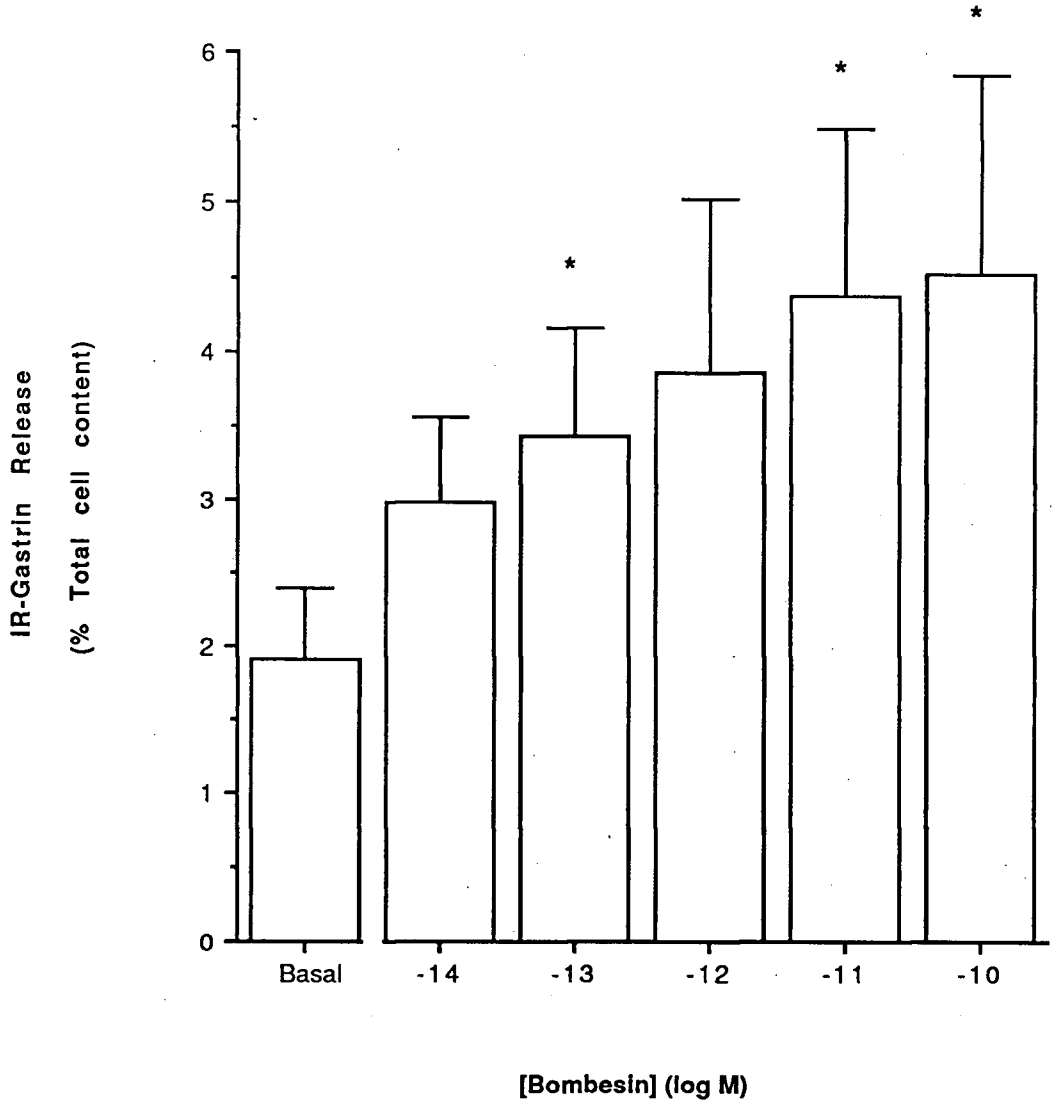


Figure 36 : Immunoreactive-gastrin release in response to 0.01 to 100 pM bombesin (n=6) after a 2 h incubation. Levels are expressed as mean ± standard error of the mean in % of total cell content. BN-stimulated gastrin release was compared to basal gastrin release. In this and subsequent figures, * denotes significance to at least $P < 0.05$.

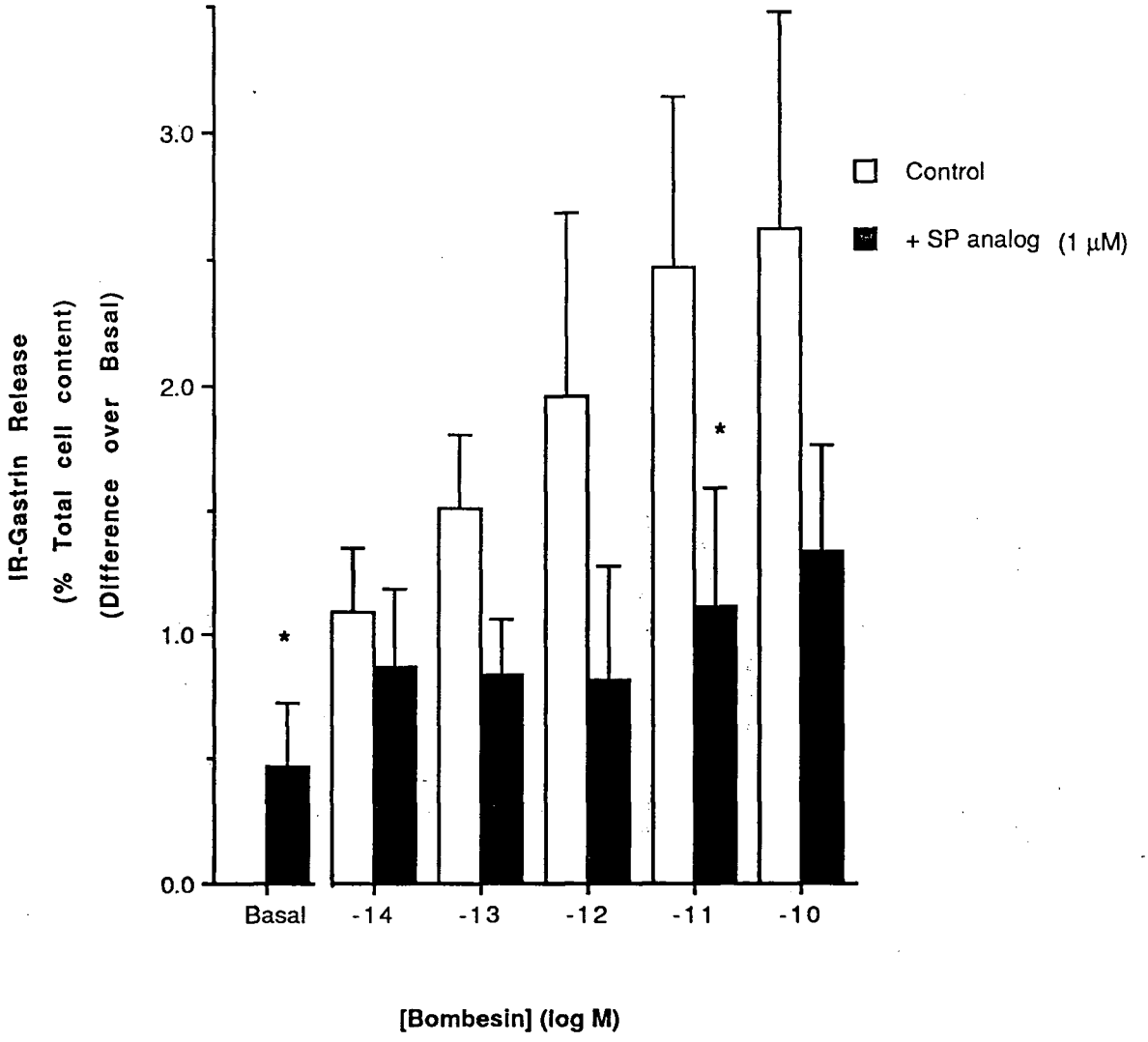


Figure 37 : The effect of 1 μM [D-Arg[1], D-Pro[2], D-Trp[7,9], Leu[11]] substance P on basal and bombesin-stimulated gastrin release (n=6) after a 2 h incubation. Gastrin levels are expressed as mean ± standard error of the mean in % total cell content over basal values. The gastrin responses to bombesin were compared in the presence and absence of the SP analog.

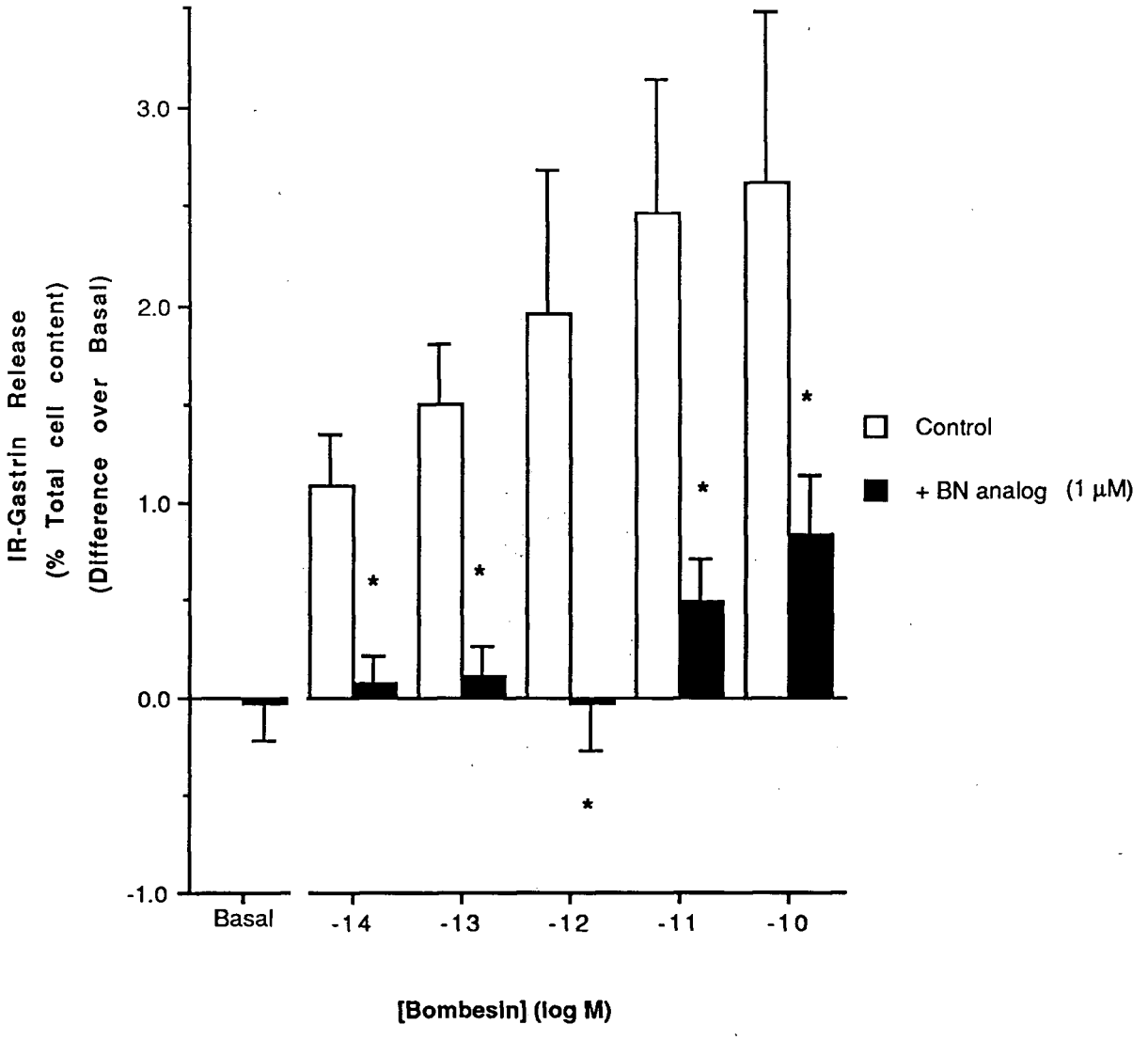


Figure 38 : The effect of 1 μM [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin on basal and bombesin-stimulated gastrin release (n=6) after a 2 h incubation. Gastrin levels are expressed as mean ± standard error of the mean in % total cell content over basal values. The gastrin responses to bombesin were compared in the presence and absence of the BN analog.

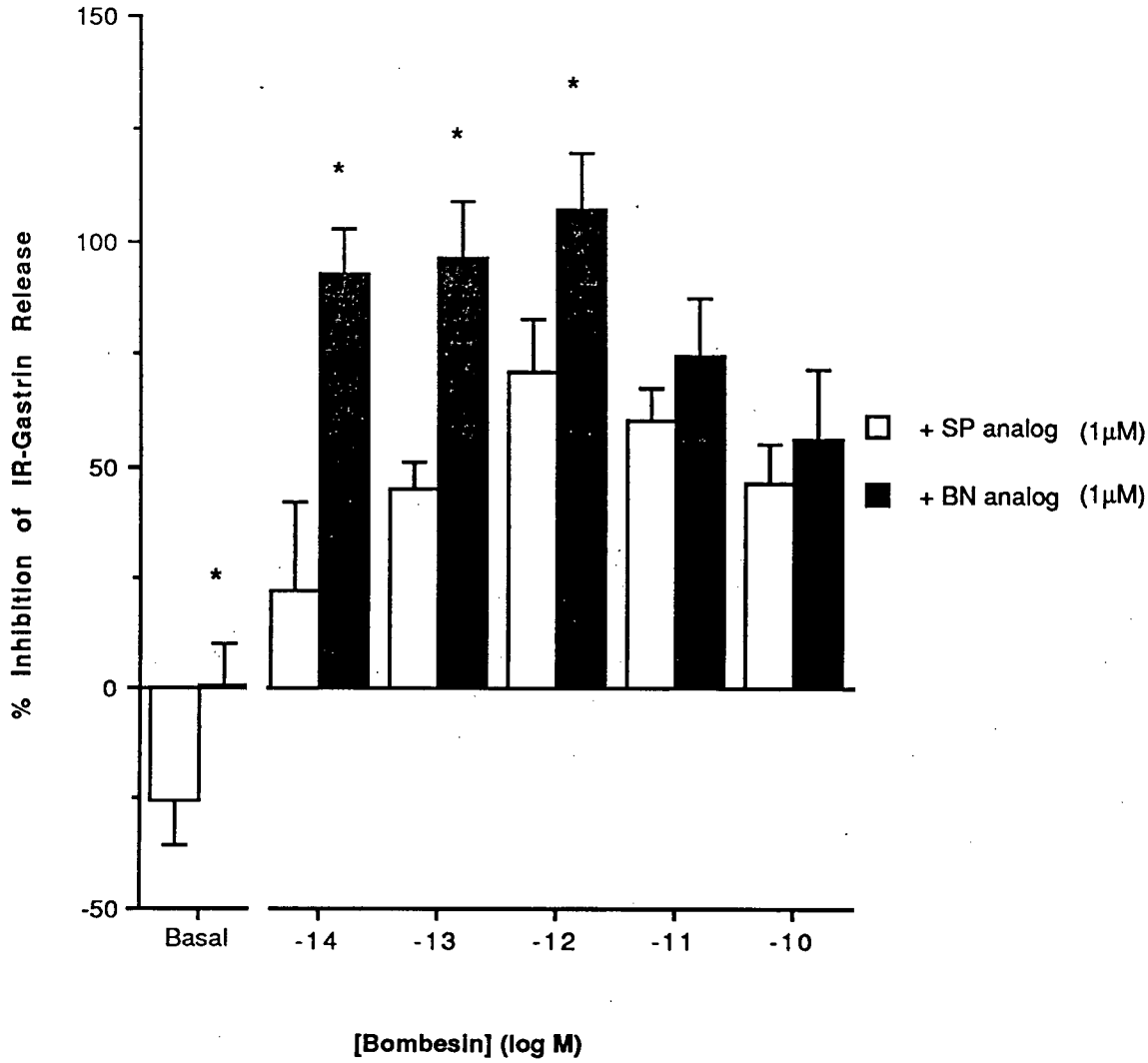


Figure 39 : Degree of inhibition of basal and bombesin-stimulated gastrin release by the substance P and bombesin analogs (n=6). The degree of inhibition elicited by the SP analog was compared to that produced by the BN analog.

III. STUDIES IN ISOLATED HUMAN G CELLS:

A. Isolation and Culture of Human Antral G Cells (Figures 40-42):

IR-gastrin levels of cell extracts from each digestion step are shown in Figure 40. A progressive increase in gastrin content was observed after each digest with the greatest concentration appearing in dispersed cells after digest 4 (52.6 $\pm$ 25.2 pmol/10^6 cells, n=4). Radioimmunoassay of cell extracts from pooled digests and post-elutriation samples revealed that IR-G content was enriched 2-fold in Fraction 1 whereas a decrease was found in Fraction 2 (from 19.9 $\pm$ 6.6 pmol/10^6 cells in pooled pre-elutriation cells to 41.6 $\pm$ 11.0 pmol/10^6 cells and 13.4 $\pm$ 7.2 pmol/10^6 cells in Fractions 1 and 2, respectively, n=11, Figure 41). Consequently, cells from Fraction 1 were cultured for 48 h at 37°C. Immunocytochemical staining of the culture plates demonstrated that gastrin-containing G cells and somatostatin-containing D cells accounted for 14.8 $\pm$ 1.5% and 5.4 $\pm$ 0.7% (n= 9), respectively, of the total viable adherent cells (Figure 42 a & b). Mucin cells comprised the remainder of the adherent population. Serotonin-immunoreactive cells were not detected in these preparations.

B. Post-receptor Activation of Gastrin Release:

1. Effect of Forskolin $\pm$ Somatostatin on Basal Gastrin Release (Figure 43):

Basal gastrin release was found to be 1.59 $\pm$ 0.20 %TCC (Figure 43). Forskolin, which increases intracellular cAMP levels by direct activation of adenylate cyclase, significantly stimulated basal gastrin secretion in a concentration-dependent manner from 1 to 50 μM ($P < 0.05$, Figure 43). Maximal gastrin response was achieved at 50 μM (3.78 $\pm$ 0.32 %TCC) with comparable stimulation at 10 μM (3.63 $\pm$ 0.78 %TCC). The addition of 10 nM somatostatin did not alter basal gastrin levels (1.56 + 0.32 %TCC), but completely

blocked the gastrin response to 1, 5 and 10 μM forskolin ($P < 0.05$, Figure 43). Even at 50 μM forskolin, inhibition by somatostatin was 57% ($P < 0.05$).

2. Effect of S10 Antibody on Forskolin-mediated Gastrin and Somatostatin Release (Figure 44):

The role of endogenous somatostatin on forskolin-mediated gastrin release was investigated using a monoclonal antibody to somatostatin, S10 (Buchan et al, 1985). S10 antibody (10 μg/ml) did not alter basal gastrin secretion (1.40 ± 0.11 %TCC without S10 vs. 1.67 ± 0.15 %TCC with S10, Figure 44), but potently increased the gastrin response to forskolin over the concentration range of 1 to 50 μM. Maximal augmentation of the gastrin output was 241% (from 2.75 ± 0.50 %TCC to 6.00 ± 1.26 %TCC, $P < 0.05$) at 5 μM forskolin. Parallel determination of SLI release by radioimmunoassay revealed that forskolin produced concomitant concentration-dependent elevations in SLI levels (Figure 44). Basal somatostatin secretion was maximally increased by 372% (from 0.64 ± 0.16 %TCC to 3.02 ± 0.93 %TCC, $P < 0.05$) at 50 μM forskolin. SLI levels in S10-treated wells could not be determined with the available SLI radioimmunoassay due to the competition for immunoreactive-somatostatin between the antibody used in the assay and the S10 monoclonal antibody.

3. Effect of A23187 on Basal Gastrin Release (Figure 45):

The involvement of Ca^{2+} in the release of gastrin was examined using the calcium ionophore, A23187. Addition of A23187 from 0.1 to 5 μM evoked concentration-dependent increases in gastrin secretion ($P < 0.05$, Figure 45). At 5 μM A23187, the gastrin output rose 685% (from 1.60 ± 0.16 %TCC to 12.56 ± 1.90 %TCC).

4. Effect of Phorbol Esters, α- and ß-PMA, on Basal Gastrin Release (Figure 46):

The phorbol ester, ß-PMA, which activates protein kinase C, also stimulated gastrin secretion in a concentration-dependent fashion from 1 nM to 1 μM ($P < 0.05$, Figure 46). The maximal response was achieved at 1 μM ß-PMA with gastrin levels rising 1250%

above basal values (from 1.57 ± 0.15 %TCC to 21.20 ± 3.80 %TCC). To investigate the possibility of non-specific stimulation by phorbol esters, the α isomer of PMA which does not activate protein kinase C was introduced into the culture wells. Over the concentration range of 1 nM to 1 μM, α-PMA did not modify basal gastrin release (Figure 46).

C. Receptor-Dependent Regulation of Gastrin Release:

1. Effect of Bombesin on Basal Gastrin and Somatostatin Release (Figure 47):

Bombesin from 0.01 fM to 10 nM significantly stimulated gastrin secretion ($P < 0.05$, Figure 47). The gastrin response was concentration-dependent and peaked at 10 nM BN wherein gastrin levels increased 257% over basal (from 1.68 ± 0.35 %TCC to 6.00 ± 0.77 %TCC). In contrast, somatostatin secretion was unaltered by BN treatment over the entire concentration range tested.

2. Effect of [Leu^{13}-ψ-CH_2NH-Leu^{14}] Bombesin on Bombesin-stimulated Gastrin Release (Figures 48 & 49):

Bombesinergic control of gastrin secretion was further investigated using a putative bombesin antagonist, [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin. Addition of the BN analog, at 1 μM, significantly augmented basal gastrin release (from 1.87 ± 0.17 %TCC to 2.76 ± 0.15 %TCC, $P < 0.05$, Figure 48), but did not alter the gastrin response to 0.1 fM BN (3.16 ± 0.43 %TCC vs. 2.93 ± 0.50 %TCC). Modest inhibition of BN-mediated gastrin release was observed between 10 fM and 10 nM BN, however, the reduction in gastrin output was only significant at 0.1 pM BN (from 3.70 ± 0.36 %TCC to 2.84 ± 0.09 %TCC, $P < 0.05$). Figure 49 illustrates the degree of inhibition of BN-evoked gastrin release produced by the BN analog. Maximal inhibition of 60.6% ± 30.9% was seen with 10 fM BN, but the gastrin response observed at this level was not significantly different from that seen with the BN analog alone (2.35 ± 0.22 %TCC vs. 2.76 ± 0.15 %TCC).

3. Effect of Somatostatin on Bombesin-stimulated Gastrin Release (Figure 50):

Further examination of the peptidergic control of gastrin secretion showed that somatostatin at 10 nM produced significant inhibition of the gastrin response to bombesin over the concentration range of 10 fM to 10 nM ($P < 0.05$, Figure 50). Gastrin release elicited by 0.1 fM to 0.1 nM BN was completely abolished by somatostatin treatment, whereas 81% inhibition was achieved with the highest concentration of BN tested, 10 nM (from 4.16 $\pm$ 0.34 %TCC to 2.40 $\pm$ 0.08 %TCC). Somatostatin alone did not significantly alter basal gastrin release (1.98 $\pm$ 0.11 %TCC without somatostatin vs. 1.54 $\pm$ 0.23 %TCC with somatostatin).

4. Effect of Somatostatin $\pm$ S10 Antibody on Basal Gastrin Release (Figure 51):

Somatostatin from 10 pM to 10 nM did not significantly affect basal gastrin secretion (Figure 51). On the other hand, inclusion of S10 antibody (10 μg/ml) modestly elevated gastrin levels and this increase was significant at 10 pM and 10 nM somatostatin (from 1.34 $\pm$ 0.14 %TCC to 2.21 $\pm$ 0.27 %TCC and from 1.27 $\pm$ 0.22 %TCC to 2.12 $\pm$ 0.42 %TCC, respectively, $P < 0.05$).

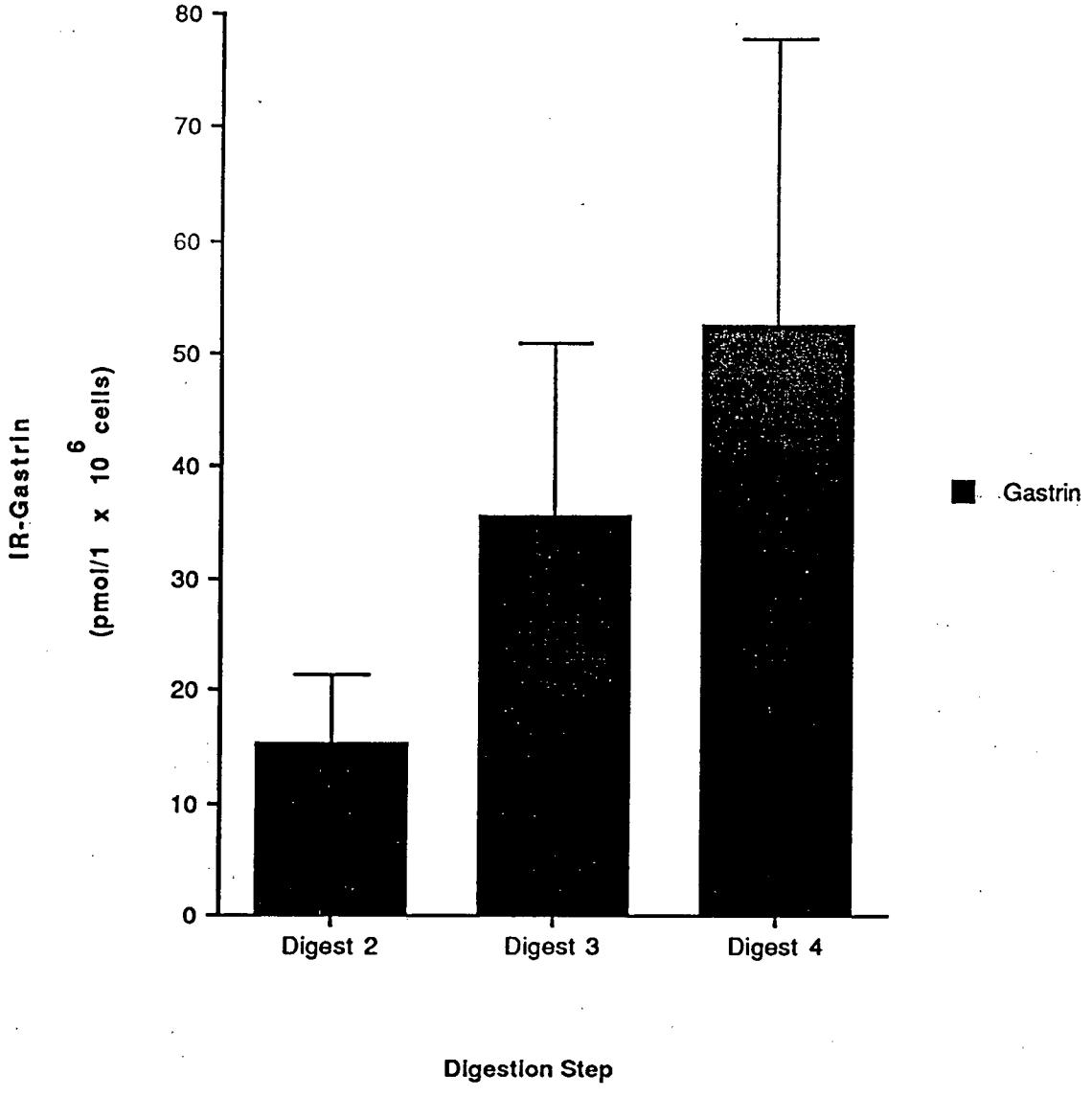


Figure 40 : Immunoreactive-gastrin content in cell extracts (n=4) after sequential collagenase and EDTA digestion. Levels are expressed as mean ± standard error of the mean in pmol/10^6 cells.

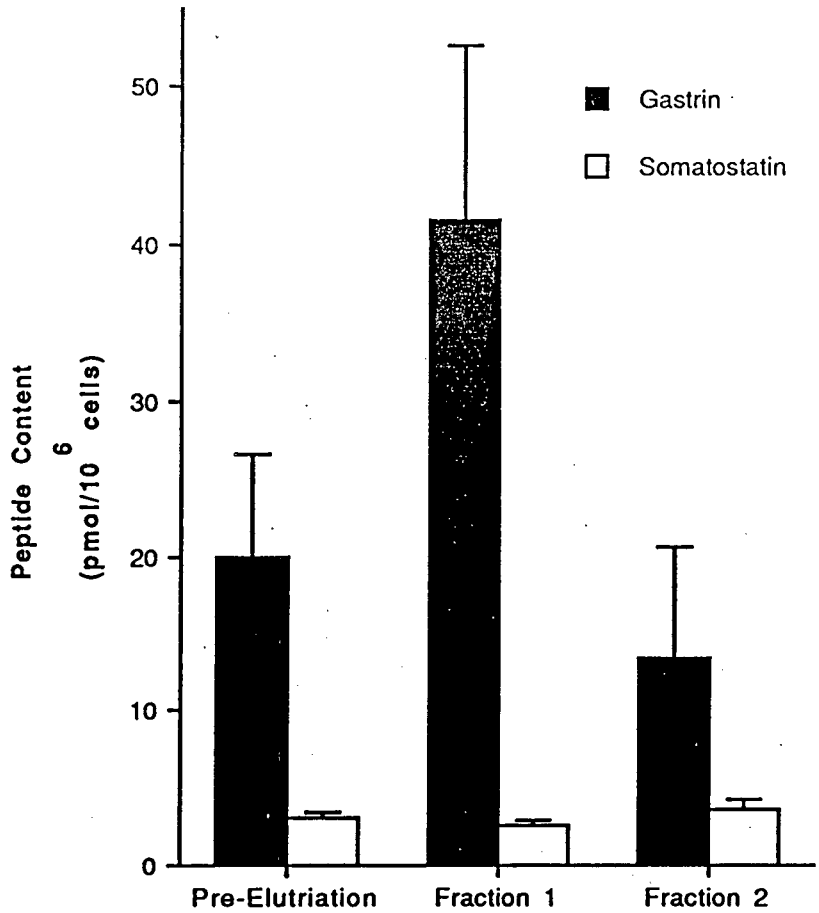


Figure 41 : Immunoreactive-gastrin (n=11) and somatostatin (n=5) content in cell extracts prior to and after centrifugal elutriation. Peptide levels are expressed as mean ± standard error of the mean in pmol/10^6 cells.

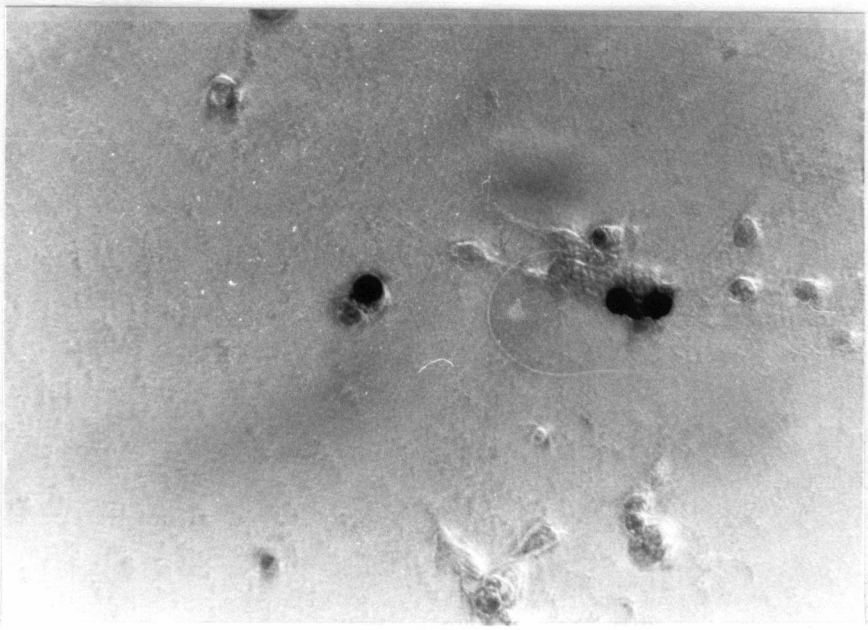


Figure 42a : Light micrograph of a representative group of immunoreactive-gastrin cells after a 2 day culture (n= 9). Magnification, x400 .

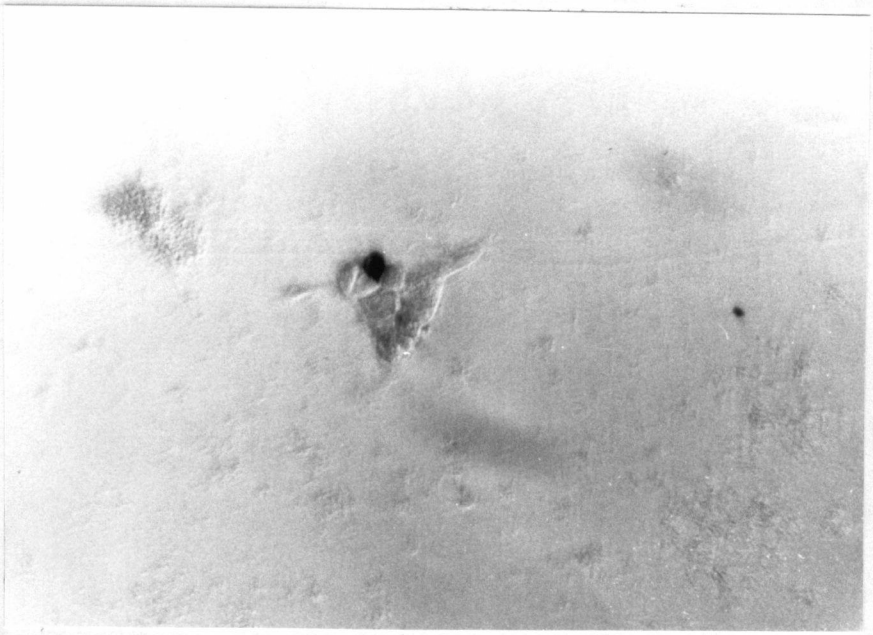


Figure 42b : Light micrograph of a representative group of cells demonstrating a single somatostatin-immunoreactive cell after a 2 day culture (n=9). Magnification, x400.

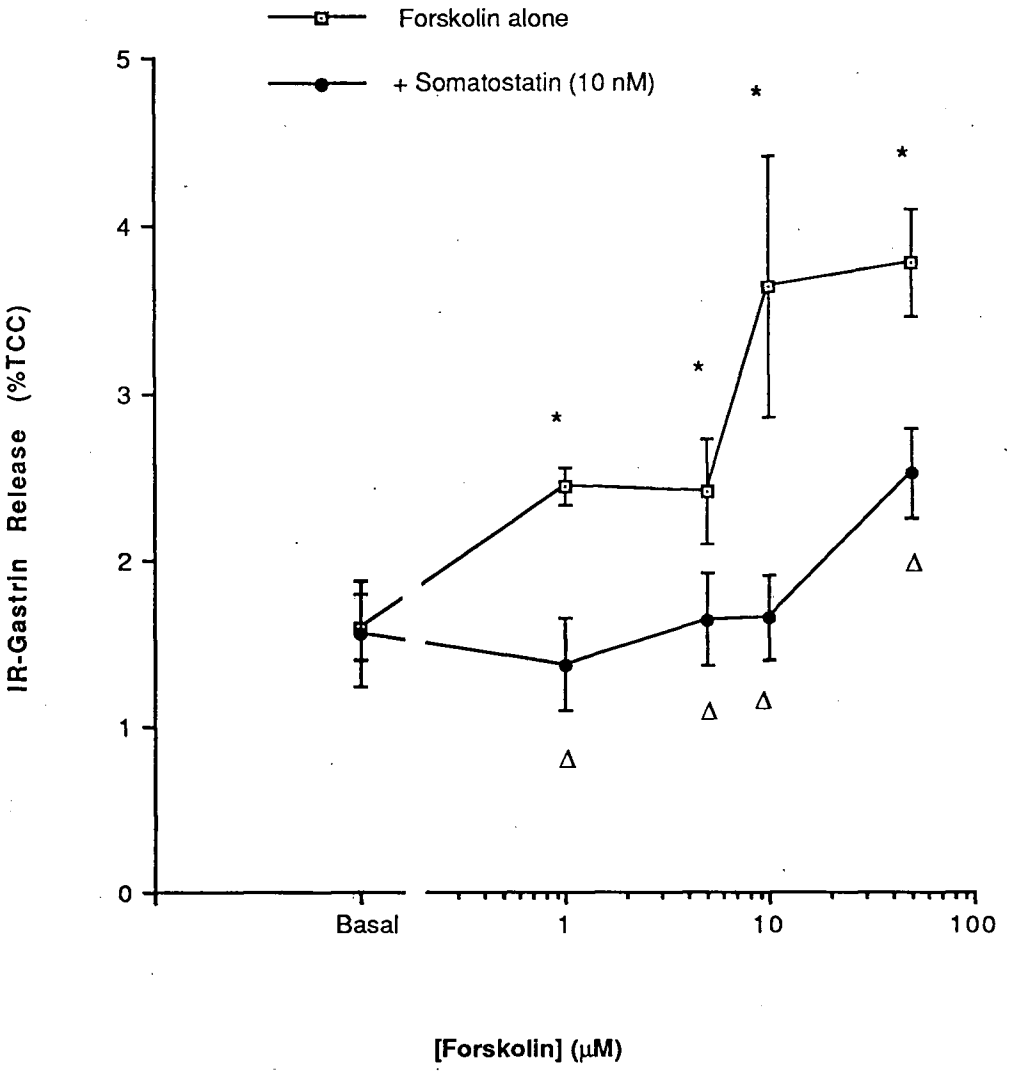


Figure 43 : The effect of forskolin (1 to 50 μM) on basal gastrin release in the absence and presence of 10 nM somatostatin (n=5). In this and subsequent figures, levels are expressed as mean ± standard error of the mean in % of total cell content (%TCC). Forskolin-stimulated gastrin release was compared to basal gastrin secretion and * denotes significance to at least $P < 0.05$. The gastrin responses to forskolin were compared in the presence and absence of somatostatin and Δ denotes significance to at least $P < 0.05$.

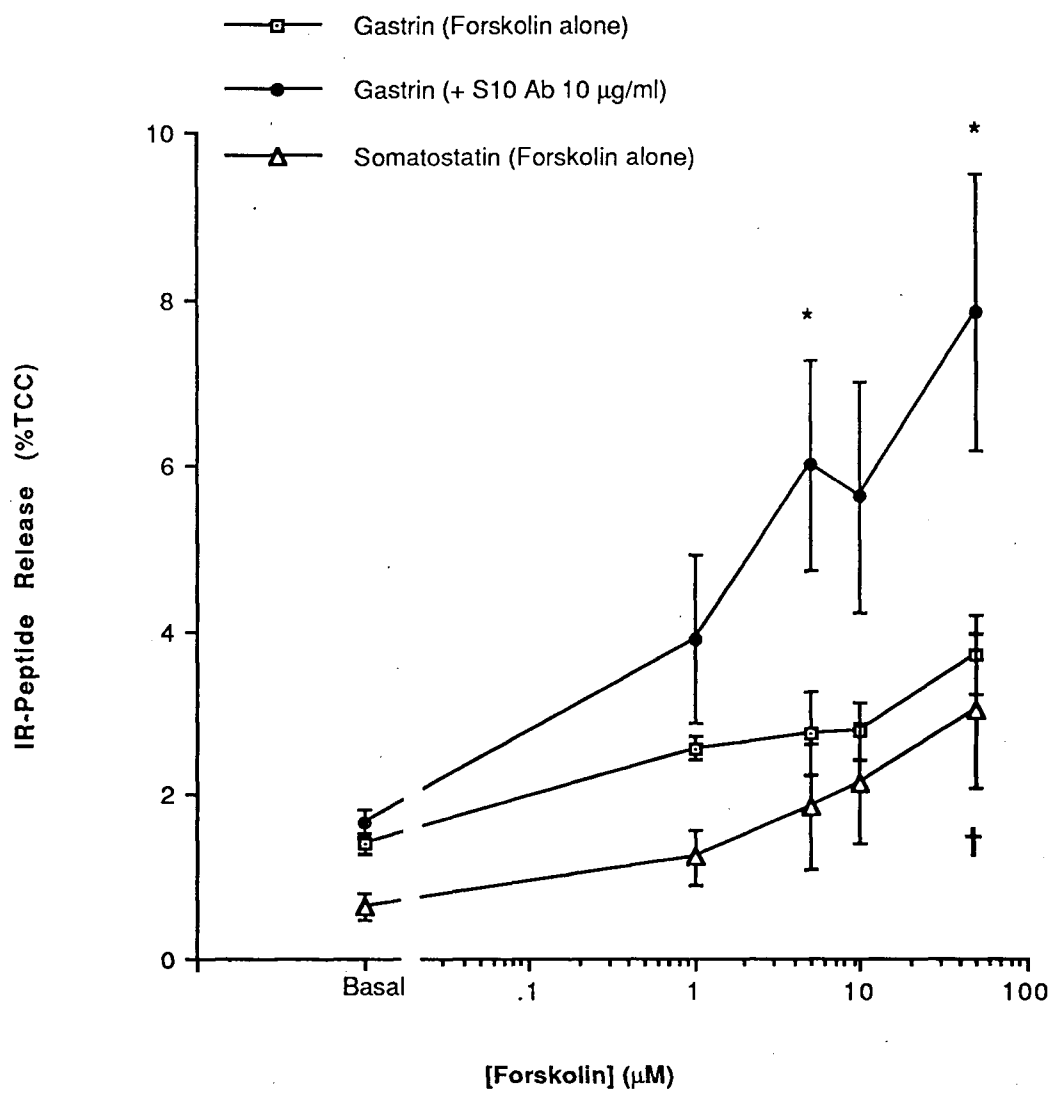


Figure 44 : The effect of S10 antibody (10 µg/ml), a monoclonal antibody directed against somatostatin, on forskolin-mediated (1 to 50 µM) gastrin (n=5) and somatostatin (n=3) secretion. The gastrin responses to forskolin were compared in the presence and absence of S10 antibody and * denotes significance to at least $P < 0.05$. Forskolin-stimulated somatostatin release was compared to basal somatostatin secretion and † denotes significance to at least $P < 0.05$.

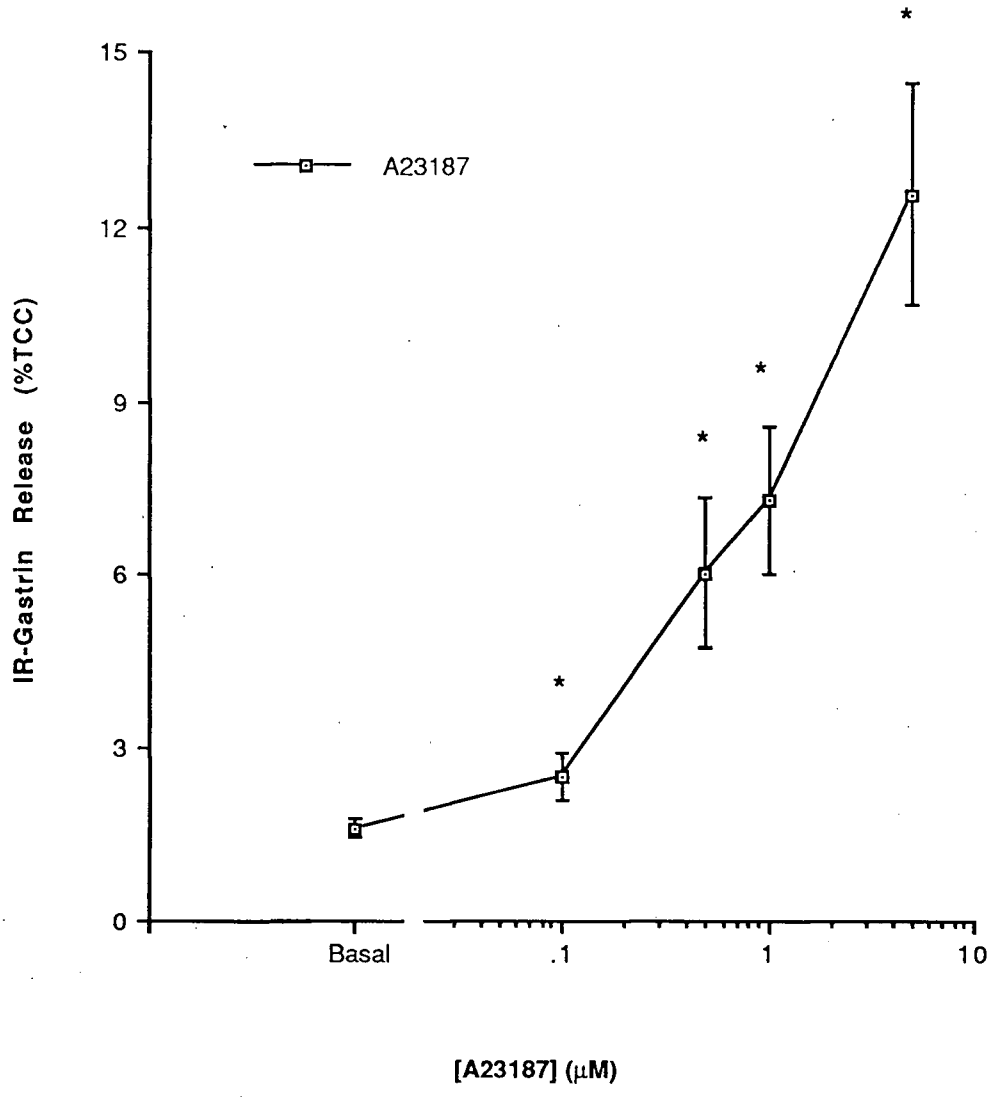


Figure 45 : The effect of the calcium ionophore, A23187 (0.1 to 5 μM), on basal gastrin release (n=6). * denotes statistically significant increases over basal gastrin secretion at $P < 0.05$.

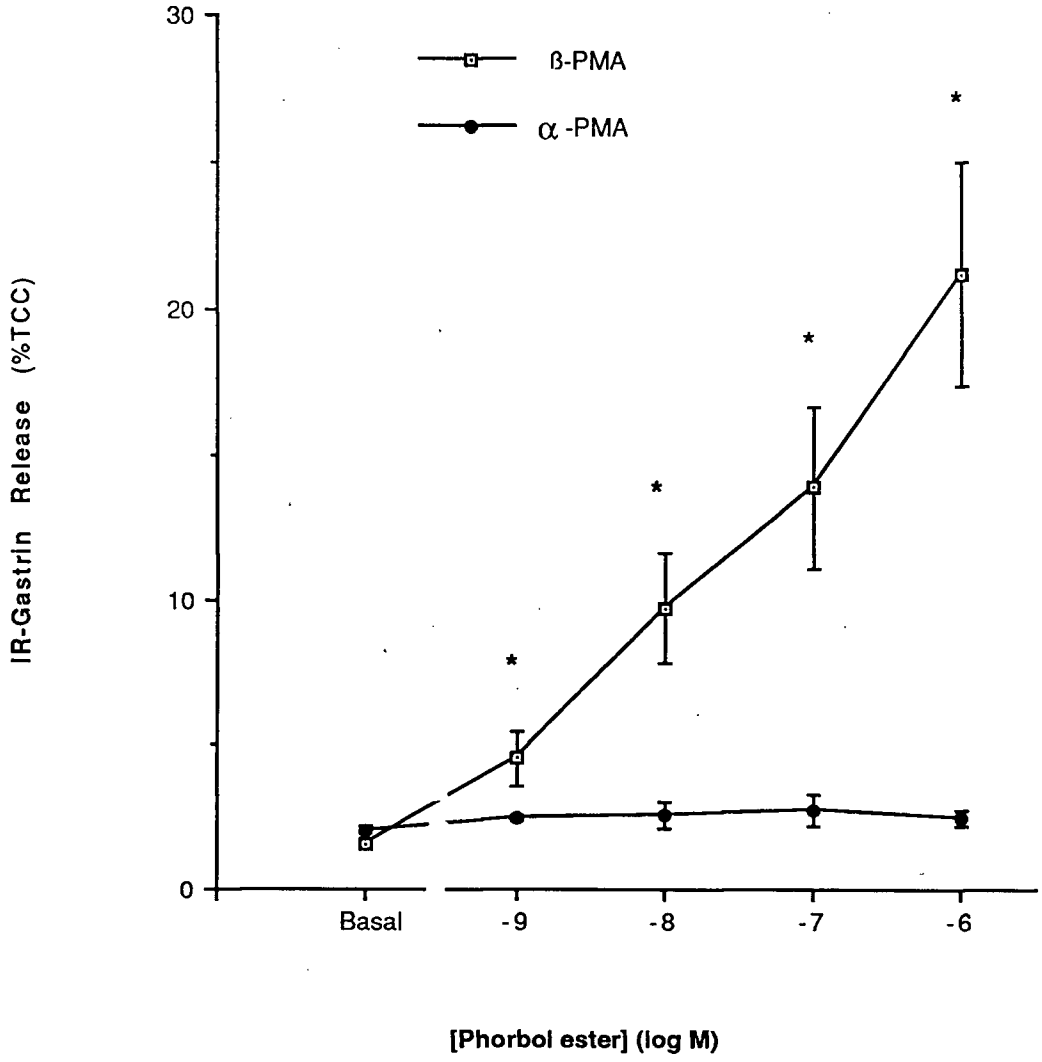


Figure 46 : The effect of the phorbol esters, α- and ß-PMA (1 nM to 1 μM), on basal gastrin release (n=4 and n=7,respectively). * denotes statistically significant increases over basal gastrin secretion at $P < 0.05$.

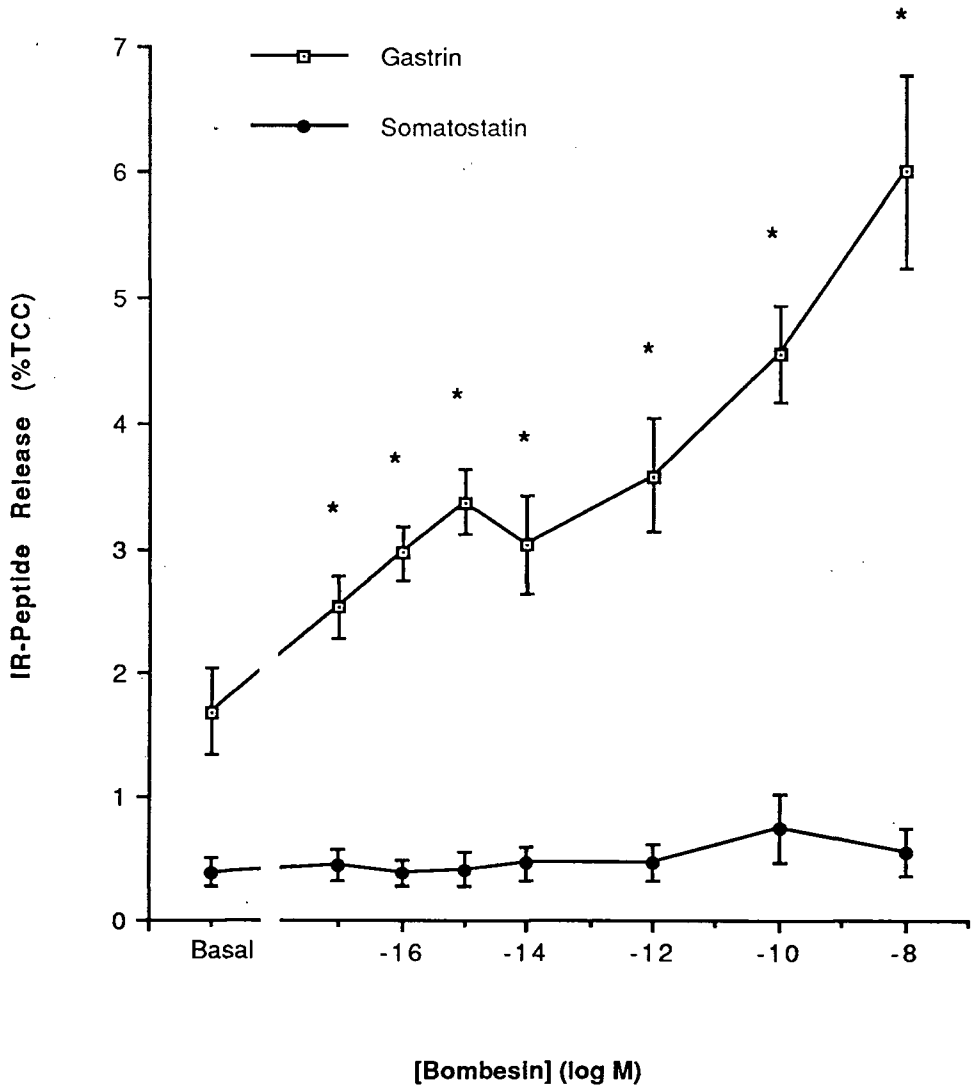


Figure 47 : The effect of bombesin (0.01 fM to 10 nM) on basal gastrin (n=5) and somatostatin (n=5) release. * denotes statistically significant increases over basal peptide secretion at $P < 0.05$.

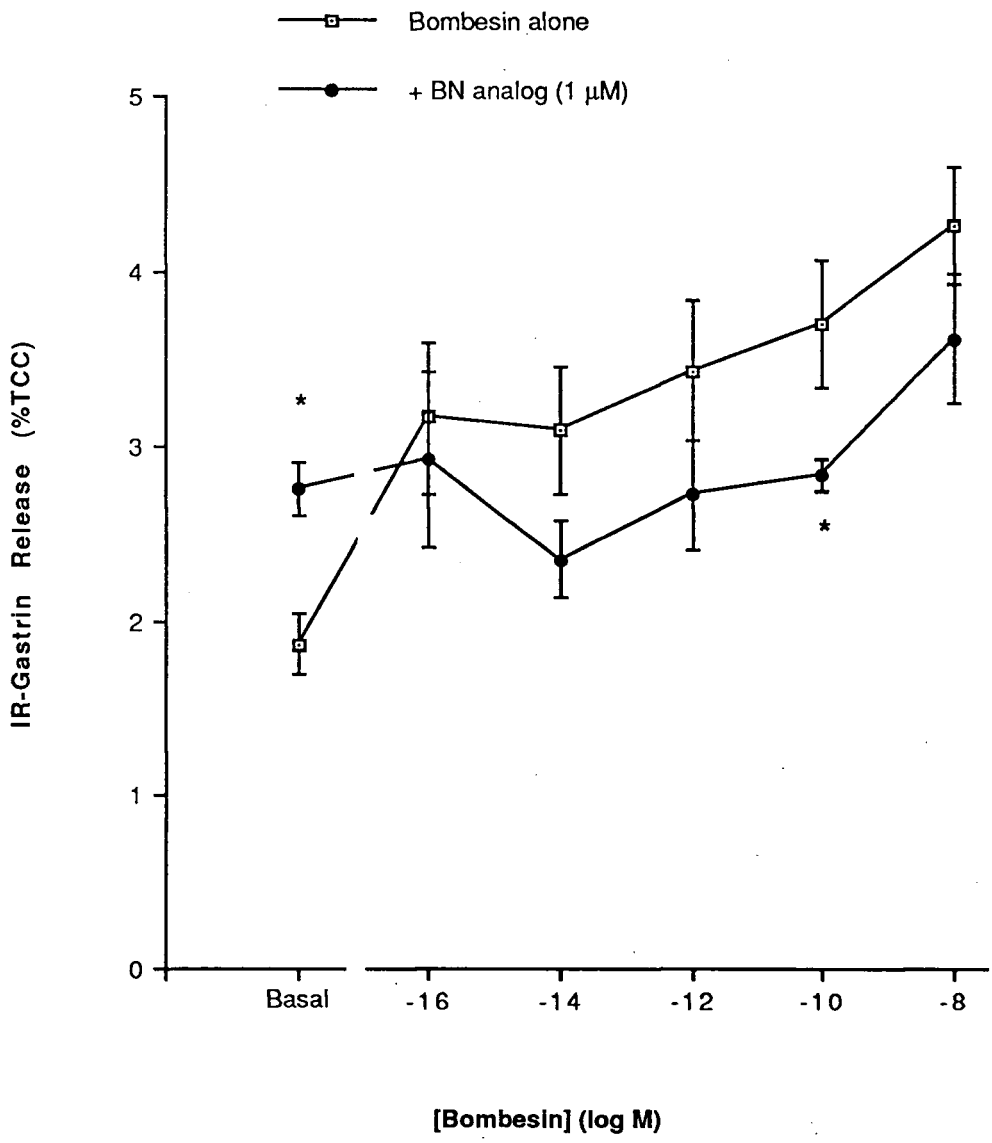


Figure 48 : The effect of [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin (1 μM) on bombesin-stimulated (0.1 fM to 10 nM) gastrin release (n=5). The gastrin responses to bombesin were compared in the presence and absence of [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin and * denotes significance to at least $P < 0.05$.

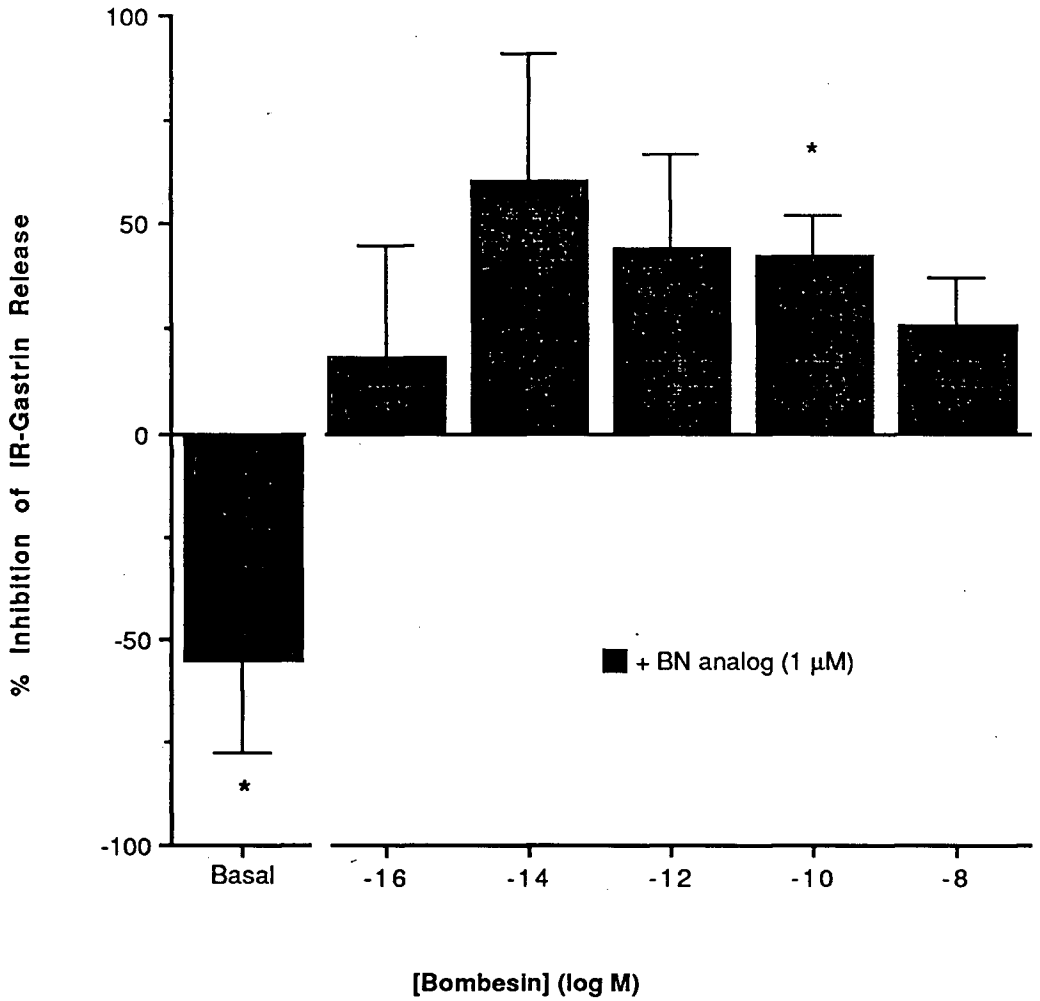


Figure 49 : Degree of inhibition of basal and bombesin-stimulated gastrin release by [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin (1 μM, n=5). * denotes statistically significant differences at $P < 0.05$.

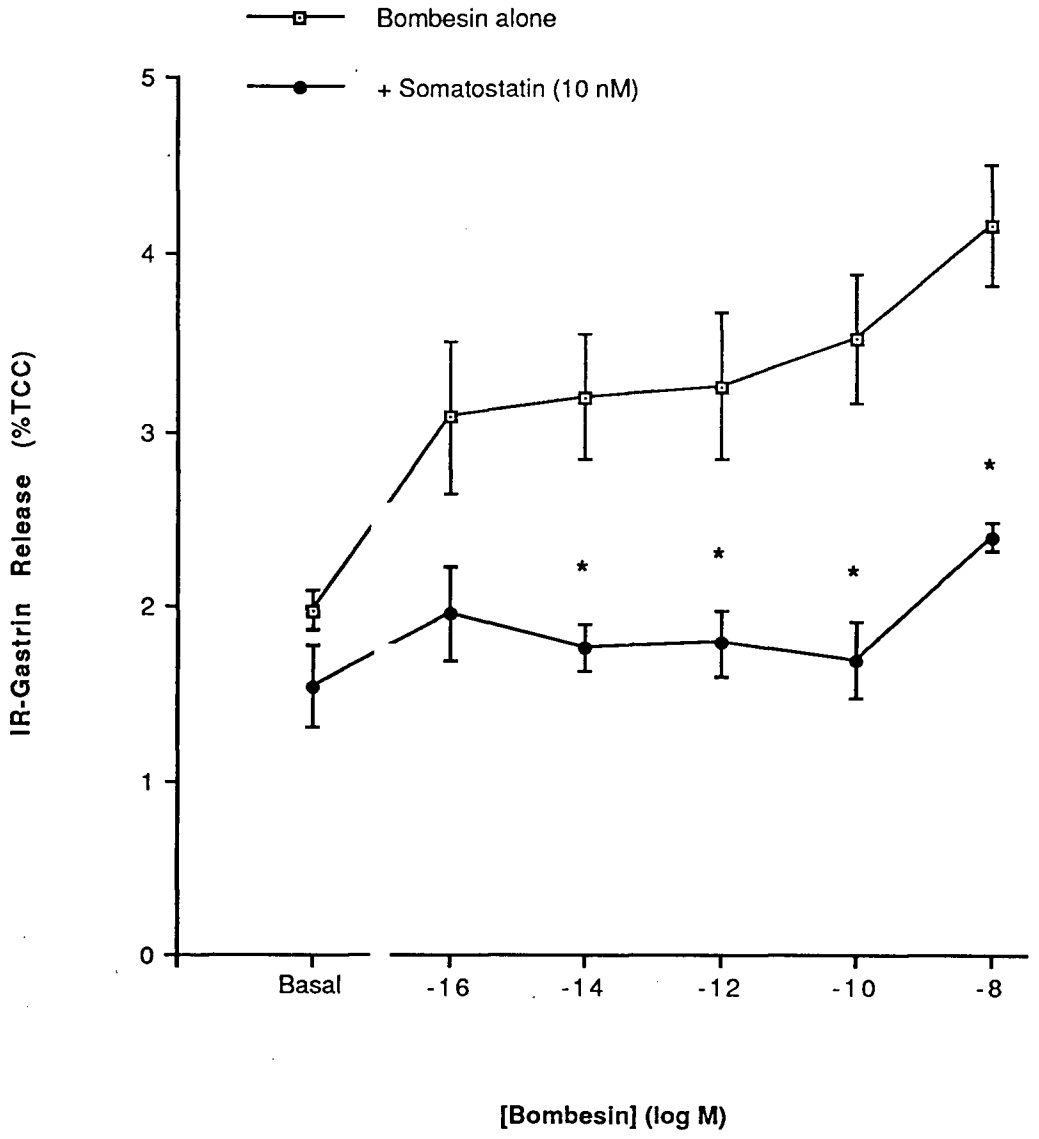


Figure 50 : The effect of somatostatin (10 nM) on bombesin-stimulated (0.1 fM to 10 nM) gastrin release (n=5). The gastrin responses to bombesin were compared in the presence and absence of somatostatin and * denotes statistical significance to at least $P < 0.05$.

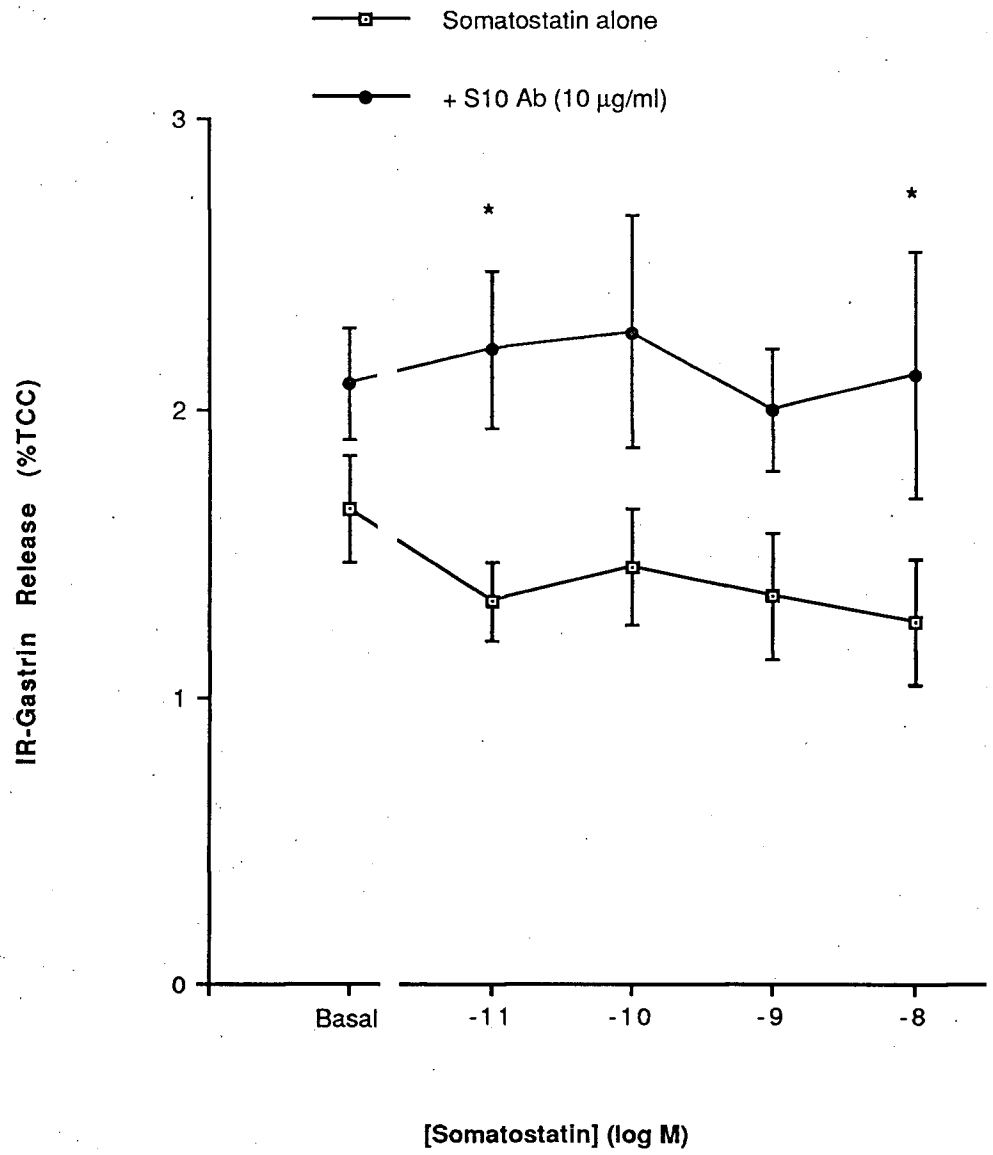


Figure 51 : The effect of somatostatin (10 pM to 10 nM) on basal gastrin release in the absence and presence of 10 µg/ml S10 antibody (n=5). The gastrin responses to somatostatin were compared to basal gastrin secretion and in the presence and absence of S10 antibody. * denotes statistical significance to at least $P < 0.05$.

DISCUSSION

The primary goal of this thesis was to investigate the mechanisms that regulate gastrin secretion in normal and pathophysiological states. *In vivo* and *in vitro* experimental preparations were used to this end. An *in vivo* model of obesity, the Zucker "fatty" rat, along with two *in vitro* preparations, the isolated vascularly perfused stomach preparation and the isolated cultured G-cell preparation, were employed in studies reported here. This discussion is divided in two parts beginning with an analysis of gastrin release in lean and obese Zucker rats, with emphasis on the possible underlying basis for the hypergastrinemia of the obese phenotype, and concluding with a preliminary inquiry into the control of gastrin secretion in the dog and human at the cellular level.

Evidence exists that adequate food intake is related to normal turnover of gastrin cells and circulating gastrin levels. The relationship of gastrin cell morphology and secretion to hyperphagia and obesity is less clear. The Zucker "fatty" (fa/fa) rat provided a model of obesity with hyperphagia present as one of many neuroendocrine abnormalities. The present study has shown that fasting circulating gastrin levels of obese Zucker pups are normal compared to their lean littermates 3 weeks after birth, but obese animals become hypergastrinemic by 3 months, a condition which increases in severity with age (Figure 11). In addition, the administration of oral peptone induced markedly higher peak gastrin concentrations in the plasma of obese animals, although the relative response was similar in both groups (Figure 12 a & b). Such elevated levels of circulating gastrin during fasted and stimulated conditions have also been reported in other animal models of genetic obesity (Lichtenberger and Ramaswamy, 1979; Morton et al, 1985).

Basal gastrin hypersecretion was also observed from the isolated perfused stomach of obese animals (Figure 13). The degree of separation between the secretory rates of lean and obese rats (3-fold) is consistent with the difference in their fasting plasma gastrin

concentrations *in vivo*. As the *in vitro* stomach preparation is a single pass system, it is unlikely that the hypergastrinemia of the obese rat is due to a reduction in the clearance rate of gastrin from the circulation.

The possibility that a hyperactive post-ganglionic cholinergic component is involved in the gastrin hypersecretion of the obese Zucker rat was investigated by infusing 10 μM atropine during basal conditions (Figure 14). Atropine treatment did not modify basal gastrin release from either lean or obese stomachs suggesting that the cholinergic inputs to the antral G-cell population of obese rats are normal. This conclusion differs from that found in rats made hypergastrinemic by vagotomy (Pederson et al, 1984). Post-vagotomy hypergastrinemia was blocked by the addition of hexamethonium or atropine indicating the involvement of a cholinergic drive at the ganglionic and G-cell levels (Pederson et al, 1984). It is unlikely that the lack of an effect with atropine treatment reported here is concentration-related since identical amounts of atropine were used to produce quantitative inhibition of vagotomy-induced hypergastrinemia.

The existence of a normal cholinergic tone acting on the G-cell population of obese animals was further supported by the gastrin response of obese stomachs to electrical stimulation of the vagus nerve (Figure 15). Vagally-evoked gastrin release was elevated to the same degree in lean and obese rats and this effect was completely suppressed by atropine in both groups. Related studies investigating the mechanisms responsible for the hyperinsulinemia of the obese Zucker rat demonstrated attenuation of the augmented insulin response of obese animals to a glucose load *in vivo* by pre-treatment with atropine, indicating the presence of an increased cholinergic influence (Rohner-Jeanrenaud et al, 1983; Fletcher and McKenzie, 1988). However, it has also been shown that hyperinsulinemia in obese animals is unaffected by vagotomy (Opsahl and Powley, 1974) and that insulin secretion from the isolated perfused pancreas in response to acetylcholine is not different in lean and obese Zucker pups (Chan et al, 1985). These discrepancies may reflect the non-specific or indirect effects of atropine treatment *in vivo* and suggest that

abnormal secretion of gastrin and insulin in obese animals may be governed by different mechanisms.

The inhibitory effect of somatostatin on G-cell activity is well known (Bloom et al, 1974; Raptis et al et al, 1975). Furthermore, a functional linkage between somatostatin and gastrin has been proposed based on reciprocal changes in their secretory rates from the isolated perfused rat stomach in response to cholinomimetics (Saffouri et al, 1980; Martindale et al, 1982), vagal activation (McIntosh et al, 1981), transmural stimulation (Schubert et al, 1982) and infusions of various agents (Chiba et al, 1980; Saffouri et al, 1980). Indeed, the existence of a somatostatinergic tone on basal gastrin secretion was revealed in immunoneutralization experiments wherein IR-G levels increased dramatically upon inclusion of somatostatin antibodies into the perfusion medium (Saffouri et al, 1979; Short et al, 1985; Koop et al, 1988; Tang et al, 1988). Basal somatostatin release from the stomachs of obese rats was found to be similar to that of lean littermates (Figure 16). In addition, the density of the antral D-cell population of lean and obese phenotypes did not differ either at 3 weeks or 6 months of age (Figures 18 & 19). These results strongly indicate that basal hypergastrinemia in the obese animal is independent of somatostatin. Evidence exists to support the uncoupling of gastrin and somatostatin secretion under certain conditions. For example, bombesin stimulated the release of both peptides while the reverse was observed with methionine-enkephalin (DuVal et al, 1981; Martindale et al, 1982; Chiba et al, 1979). Subdiaphragmatic vagotomy resulted in gastrin hypersecretion in rats without alterations in SLI levels (Pederson et al, 1981) whereas isoproterenol (Koop et al, 1982; Koop et al, 1983) and GIP (Pederson et al, 1981) produced the opposite results.

Antral G-cell hyperplasia as a possible cause of hypergastrinemia was investigated by immunocytochemical localization and quantification (Figures 17 & 19). It had been previously demonstrated that islet ß-cell hyperplasia preceeded the appearance of increased insulin levels in the obese Zucker rat (Chan et al, 1985). An enlarged ß-cell population was evident in obese pups as early as 7 days after birth, whereas glucose-stimulated

hyperinsulinemia did not appear until 21 days of age and fasting insulin levels were not elevated until 35 days of age. In this study, no difference was observed in the density of the antral G-cell population of lean and obese animals at 3 weeks of age. However, a doubling of the G-cell number of obese rats compared to lean controls became evident by 6 months of age, revealing a strong correlation between the number of gastrin-containing cells and circulating plasma gastrin concentrations. The reduction in G-cell density found in the antra of adult lean rats compared to 3 week-old animals agreed with the observations of Lichtenberger and Johnson (1974). These investigators showed that in rats, antral gastrin concentration is greatest at 21 days of age whereafter it decreases by about 25%.

Several conclusions were drawn from these intial studies. Hypergastrinemia is a progressive condition in obese Zucker rats that manifests itself between 3 weeks and 3 months of age. Elevated gastrin levels may be related to the developing hyperphagia of obese weanlings since G-cell number has been shown to decrease during fasting in rats and to revert to normal upon refeeding (Bertrand and Willems, 1980). The hyperphagic behavior of obese animals is not present during the suckling period, but appears subsequent to weaning (Godbole et al, 1981). Secondly, hypergastrinemia does not appear to be mediated by an increased postganglionic cholinergic drive as the gastrin responses of the perfused stomachs of obese rats to electrical stimulation of the vagal trunks and to muscarinic inhibition by atropine were similar in degree compared to those observed in lean animals. Increased G-cell numbers in obese antra following weaning suggests G-cell hyperplasia as a cause of gastrin hypersecretion. However, the doubling of the G-cell population in adult obese rats cannot completely account for the markedly elevated levels of gastrin (3-fold) *in vivo* and *in vitro* during basal conditions. It is unlikely that the G-cell population of obese animals is hypersensitive to stimulation as the relative increase in gastrin output (2-fold) was similar in both lean and obese rats in response to oral peptone and vagal stimulation.

The most readily apparent mechanism that could account for G-cell hyperplasia and gastrin hypersecretion in obese Zucker rats was that of abnormal feeding behaviour. Prolonged fasting (Lichtenberger et al, 1975; Bertrand and Willems, 1980; Koop et al, 1982; Schwarting et al, 1986) as well as intravenous feeding (Johnson et al; 1975a; Johnson et al, 1975b; Track, 1980; Campos et al, 1987) resulted in a significant depression of serum and antral gastrin content (Johnson et al; 1975a; Johnson et al, 1975b; Track, 1980; Campos et al, 1987) and G-cell hypoplasia was cited as the cause of gastrin deficiency in starved (Lichtenberger et al, 1975; Bertrand and Willems, 1980; Schwarting et al, 1986) and parenterally-fed animals (Campos et al, 1987). Furthermore, normalization of gastrin levels after food deprivation (Lichtenberger et al, 1975) or total parenteral nutrition (Wheeler, 1988) was achieved upon re-feeding with solid rat chow. Conversely, increased food intake has been associated with elevated gastrin concentrations (Lichtenberger and Johnson, 1974; Lichtenberger et al, 1976). Because of the association between oral intake of food and gastrin levels, hyperphagia as the source of excess gastrin secretion was examined in studies where the food allocations of obese Zucker pups were matched with the daily food intake of lean animals between 5-8 weeks of age.

At the start of the pair-feeding regimen (5 weeks of age), fasting plasma gastrin levels and mean body weights of lean and obese animals were not significantly different (Table III & Figure 21). The mean body weights of the obese pair-fed and obese *ad libitum* groups began to deviate 5 days after the initiation of food restriction and persisted until the end of the test period. However, diet-controlled animals remained phenotypically obese. Similar observations have been reported in obese Zucker rats given 70% of their normal daily consumption from 5-10 weeks of age (Muller and Cleary, 1988). Preferential conversion of nutrients into fat in obese animals is unlikely to be due to increased glucose metabolism in adipocytes (Muller and Cleary, 1988), but may be related to augmented fatty acid production due to elevated liproprotein lipase activity in adipose tissue (Dugail et al, 1988). It should be noted that the mean body weight of lean animals was significantly

greater than that of pair-fed obese rats from 45-56 days of age (Table III & Figure 21). This observation is most likely due to the fact that food allocations for the pair-fed animals were determined according to the mean daily intake of the lean group from the previous day. Although the mean body weight of lean animals was not significantly different from freely-feeding obese rats until 47 days after birth, the trend of increased weight gain in obese rats was evident by 30 days of age.

Dietary restriction failed to reduce the fasting basal hypergastrinemia of 8 week-old obese animals (Figure 21) to levels found in age-matched lean controls. In addition, gastrin hypersecretion persisted from the isolated perfused stomachs of pair-fed obese rats (Figure 22). Although fasting plasma gastrin concentrations and perfusate gastrin levels from the *in vitro* stomach preparation of the diet-restricted obese rat were lower compared to its freely-feeding obese counterpart, these reductions were not statistically significant (Figure 21 & 22b). Thus, increased food consumption does not appear to play a major role in the hypergastrinemia of the obese Zucker rat. Similar findings have been obtained in pair feeding studies involving other animal models of obesity. Elevated antral and serum gastrin concentrations persisted in 10 week-old genetically obese (ob/ob) mice that had ingested an equivalent amount of food as lean mice since weaning (Morton et al, 1985). This phenomenon was attributed to a reduction in the gastric acid secretory capacity thereby providing an attenuated inhibitory signal on the G cell. Likewise, tissue and serum gastrin concentrations remained elevated in obese, genetically diabetic (db/db) mice after an 18-day restricted diet (Lichtenberger and Ramaswamy, 1979), although the exact cause could not be determined. In pair-fed VMH- lesioned rats, basal plasma gastrin levels were similar to sham-operated controls, but the gastrin response of obese food-restricted animals to insulin was markedly greater than that of the control group (Chikamori et al, 1983).

Therefore, hypergastrinemia appears to be independent of increased food ingestion in the obese rat. Another pathological characteristic of this animal, hyperinsulinemia, has also been shown to be resistant to diet restriction. The glucose, insulin and GIP responses

to an oral glucose challenge of obese rats fasted to 80% of their original body weight were unchanged compared to overnight-fasted obese animals (Chan, 1985). Furthermore, insulin secretion from the isolated perfused pancreas of food-deprived obese animals stimulated by glucose and GIP was similar to that observed from obese controls.

In comparison, pair-feeding resulted in normalization of the antral G-cell number of obese animals compared to lean littermates (Figure 23). On the other hand, a significantly greater number of gastrin-containing cells was observed in the antra of the obese ad libitum group compared to the lean and the diet-restricted obese animals. These results parallelled the changes in the antral G-cell population of obese diabetic mice induced by dietary restrictions (Lichtenberger and Ramaswamy, 1979). Thus, abnormal feeding behavior in the obese Zucker rat appears to be causally related to the developing G-cell hyperplasia, but its role in hypergastrinemia is much less pronounced. In addition, these results indicate that G-cell hyperplasia does not play a significant role in the hypergastrinemia of the 2 month-old obese rat, but probably contributes to the increasing severity of this condition in older adult obese animals.

Another possible explanation for augmented gastrin secretion was an increase in the degree of stimulation received by the G-cell from vagal, non-muscarinic inputs during basal conditions. Vagally-induced gastrin release that is not mediated by muscarinic receptors has been clearly established (Smith et al, 1975; Uvnas et al, 1975; Dockray and Tracy, 1980; Nishi et al, 1985; Holst et al, 1987). Circumstantial evidence implicating bombesin-like peptides as the stimulatory postganglionic neurotransmitter has been obtained from studies showing copious secretion of gastrin evoked by bombesin/gastrin releasing peptide (BN/GRP) that is atropine-resistant (Bertaccini et al, 1974; McDonald et al, 1978; DuVal et al, 1981; Martindale et al, 1982; Richelsen et al, 1983). In addition, two known stimulants of gastrin release, electrical activation of the vagus (Holst et al, 1983; Knuhtsen et al, 1984; Nishi et al, 1985) and acetylcholine (Schusdziarra et al, 1983), have also proven to be secretagogues for GRP. Recently, direct and sensitive stimulation of gastrin release by BN

(Giraud et al, 1987; Sugano et al, 1987) as well as the presence of high affinity BN/GRP receptors (Vigna et al, 1988) were demonstrated on isolated antral G cells of the dog. Immunoneutralization of endogeous BN/GRP with antiserum raised against BN elicited a 60% reduction in the gastrin responses to field stimulation and nicotinic-receptor agonism (Schubert et al, 1982). These results, taken together with the identification of BN/GRP-like immunoreactive fibers in the antral mucosa (Polak et al, 1978; Dockray et al, 1979; Yanaihara et al, 1981; Moghimzadeh et al, 1983), provide convincing evidence for the involvement of BN/GRP in the postganglionic vagal activation of G-cell function. Thus, the final series of experiments investigating the cause of hypergastrinemia in the obese Zucker rat was devoted to examining the possibility that an endogenous BN/GRP drive was responsible for this condition.

Radioimmunoassay of gastric tissue extracts revealed a 3.5-fold greater content of GRP in the antral tissue of obese animals compared to lean littermates (Table III). This provided the basis for the possible increased activation of G-cells in the obese rat due to exposure to abnormally high levels of GRP.

The presence of a bombesinergic drive was further investigated by infusing exogenous bombesin into the isolated perfused stomach preparation on the premise that if enhanced tonic BN/GRP release was responsible for hypergastrinemia, the relative gastrin response of obese stomachs to BN stimulation would be expected to be less than that observed from lean controls. During a 0-10 nM BN gradient, the rate of gastrin secretion from lean stomachs rose immediately (6 min) and dramatically to a peak value 337% greater than the initial secretion rate at 1.7 nM BN (Figure 24), whereas a comparable maximal response from obese stomachs (324% above the initial secretion rate) was observed much later into the gradient (34 min) at 9.4 nM BN. The maximal gastrin output observed from lean animals is somewhat greater than that reported by DuVal et al (1981) who demonstrated a 200% increase over basal at 10 nM BN in Sprague-Dawley rats, which may represent an inter-strain variation. Nevertheless, the attenuated response of obese stomachs

to BN infusion compared to lean stomachs suggested the presence of a tonic BN/GRP drive in the "fatty" rat.

In addition to a difference in sensitivity to exogenous BN, the gastrin profiles of lean and obese stomachs during the BN gradient were distinctive (Figure 24). IR-G levels in obese animals rose progressively with increasing BN concentration. In contrast, the gastrin response of lean rats was biphasic: the first phase displayed a peak response 4.5 times greater than the initial secretion rate 6 min into the gradient corresponding to 1.7 nM BN, whereas the 2nd phase consisted of a constant secretion rate approximately 3-fold higher than that in control animals. The drop in gastrin levels observed from lean stomachs to increasing concentrations of BN during the 2nd phase may be indicative of desensitization in this preparation. The phenomenon of desensitization to BN/GRP has also been reported in the dog (Larson et al, 1983) and in the pig (Holst et al, 1987). Holst et al (1987) showed that pre-perfusion of the isolated perfused pig antrum with 10 nM GRP abolished the gastrin responses to subsequent vagal stimulation or 0.1nM GRP for up to 30 min. Desensitization to BN has also been observed with enzyme secretion from dispersed pancreatic acini of the guinea pig (Lee et al, 1980; Pandol et al, 1982). Furthermore, desensitization in pancreatic acini appears to be receptor specific as stimulation of enzyme release was observed with other secretagogues, such as cholecystokinin, carbachol, vasoactive intestinal peptide or the calcium ionophore, A23187, after BN treatment (Gardner and Jensen, 1986).

Parallel determinations of SLI levels were performed on samples collected during the 0-10 nM BN gradient in order to ascertain if BN-stimulated gastrin release may be mediated through inhibition of somatostatin secretion. Figure 25 shows no difference in the somatostatin secretion rate from the perfused stomachs of BN-treated lean or obese animals compared to their correseponding control groups. These results contradicted previous reports of potent stimulation of somatostatin release, up to 200% over basal, with 10 nM BN using the same organ preparation (DuVal et al, 1981; Martindale et al, 1982).

This discrepancy is not easily explained, but may be related to potential desensitization of somatostatin cells to activation by BN similar to that seen with gastrin secretion in lean animals (Figure 24). In order to circumvent difficulties in interpreting results associated with studies of BN-stimulated hormone release during a continuous gradient, experiments utilizing single concentrations of BN were performed to construct concentration-hormone release response curves.

Bombesin, from 0.1 nM to 100 nM, stimulated gastrin secretion in a concentration-dependent fashion from the perfused stomachs of both lean and obese rats (Figure 26). Maximal gastrin ouput (270% of basal) in obese animals was observed at 10 nM BN, although comparable stimulation (273% of basal) was observed at 100 nM BN. This agrees with earlier experiments employing a BN gradient (Figure 24) wherein peak IR-G levels (approximately 300% of basal) were achieved at 9.8 nM BN. In contrast, the gastrin response from the perfused stomachs of lean animals was markedly greater than that observed in obese rats at all concentrations of BN tested (Figure 26). Furthermore, a true maximal gastrin output in lean rats could not be determined as the peak value (499% of basal) was obtained at the highest concentration of BN used, 100 nM. In comparison, somatostatin levels were unaffected by the lower doses of BN in both phenotypes (Figure 27), but were modestly elevated with 10 and 100 nM BN in lean animals (149% and 154% of basal, respectively) and 100 nM BN in obese animals (126% of basal). More importantly, somatostatin outputs from lean and obese stomachs did not differ during infusion with BN. Thus, it is unlikely that the augmented responses of lean stomachs to BN stimulation compared to obese rats are due to differential release of somatostatin. Rather, these results are indicative of an endogenous bombesinergic tone that attenuates the gastrin secretory capacity of obese rats to exogenous BN. Support for this conclusion comes from studies examining the etiology of post-vagotomy hypergastrinemia in Wistar rats (Pederson et al, 1984). These investigators reported gastrin responses from vagotomized animals that were 4-6 fold lower than those seen in sham-operated controls

during stimulation with methacholine. Since post-vagotomy hypergastrinemia was inhibited by atropine and hexamethonium, these authors concluded that attenuated gastrin responses to methacholine were suggestive of the presence of an enhanced cholinergic tone in denervated animals.

In this study, weak enhancement of somatostatin release by BN disagrees with the findings of DuVal et al (1981) and Martindale et al (1982). In addition, stimulation of somatostatin release from the perfused rat stomach by the nicotinic agonist, DMPP, has been shown to be completely abolished by the inclusion of BN antibodies in the perfusion medium (Schubert et al, 1985). Potent secretion of somatostatin was also observed from the perfused porcine antrum during the infusion of 1 and 10 nM GRP (Holst et al, 1987). However, other investigators were unable to confirm these findings. Nishi and his colleagues (1985) demonstrated GRP-induced inhibition of somatostatin levels from the perfused stomachs of Wistar rats. Furthermore, 100 nM BN did not elicit any increase in somatostatin release from perifused antropyloric glands of Wistar rats (Richelsen et al, 1983). The discrepancy in results from these experiments in the bombesinergic control of somatostatin secretion may be attributable to differences in methods, experimental models and species. However, it should be noted that all studies exhibiting enhancement of SLI levels by BN in the rat were observed using the Sprague-Dawley strain; this effect was not evident in the Wistar rat. Therefore, the regulation of somatostatin secretion by BN/GRP may vary between and within species. Further studies utilizing a specific BN/GRP antagonist are required to clarify the role of this peptide in the control of somatostatin release in each animal and experimental model.

Results from radioimmunoassay determinations of antral GRP content and from the perfusion experiments with BN presented in this thesis provide circumstantial evidence for the involvement of BN/GRP in the hypergastrinemia of the obese Zucker rat. However, it is fundamentally important to be able to demonstrate inhibition of action at the receptor level before a specific functional role can be assigned to any chemical messenger. With this in

mind, two putative BN/GRP antagonists, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P and [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin, were tested on basal and BN-stimulated gastrin secretion in lean and obese rats.

A preliminary examination of the effect of substance P (SP) on gastrin secretion was required since a substance P analog was to be employed as a BN antagonist. Figure 28 shows that 10 nM SP did not alter basal gastrin levels from the perfused stomachs of obese rats. In lean animals, very modest inhibition of gastrin secretion was observed during the 2nd 5-min collection period of a 10-min SP infusion. Similar findings have been reported with up to 1 μM SP (Kwok et al, 1988). It was concluded that any reduction in gastrin secretion that might be elicited by the SP analog in lean or obese animals would most likely be indicative of antagonism at BN and not SP receptor sites.

Neither the SP analog nor the BN analog altered basal gastrin release from the *in vitro* stomachs of lean and obese rats (Figures 29 & 30). Furthermore, both analogs were unable to evoke significant inhibition of the gastrin responses of lean and obese stomachs to 1 nM BN (Figures 31-34). These results are in contrast to the findings of Holst et al (1987) who demonstrated the abolition of the gastrin response from the isolated perfused pig antrum to 100 pM GRP with the infusion of 0.66 μM SP analog. In addition, the SP analog has been shown to reverse BN-stimulated amylase release from dispersed pancreatic acini (Jensen et al, 1984; Jensen et al, 1988) as well as attenuate BN-mediated mitogenesis in Swiss 3T3 fibroblasts (Layton et al, 1988; Woll and Rozengurt, 1988). Similar observations regarding the inhibitory capacity of the BN analog have been reported (Coy et al, 1988; Woll et al, 1988). The inability of both analogs to antagonize gastrin release in response to exogenous BN suggests that the BN-receptor type present on the antral G cells of Zucker rats are of a distinct class from those on pancreatic acini and Swiss 3T3 cells. Thus, a definitive role for BN/GRP in the hypergastrinemia of the obese rat cannot be ascertained from these studies using BN antagonists.

A number of conclusions can be drawn from these studies into the regulation of gastrin secretion in lean and obese Zucker rats. Gastrin hypersecretion in the "fatty" rat is independent of a hyperactive post-ganglionic cholinergic tone and is not a consequence of reduced somatostatin secretion or cell numbers. Antral G-cell hyperplasia in the obese rat is secondary to increased food consumption. Although G-cell hyperplasia correlated well with the hypergastrinemia of the obese animal and probably contributes to the increasing severity of this condition, hyperplasia does not appear to be the primary causal defect as pair-feeding did not normalize gastrin secretion in the obese rat *in vivo* and from the *in vitro* stomach preparation. Elevated antral GRP content and attenuated gastrin responses to exogenous BN provide some indirect evidence for the existence of an endogenous bombesinergic drive in obese rats.

Further studies are required to determine the mechanism(s) underlying gastrin excess in the "fatty" rat. Clearly, the precise involvement of BN/GRP in the hypergastrinemia of the obese Zucker rat is worth pursuing. However, the success of this endeavour awaits the development and testing of novel and highly specific receptor antagonists. The hypergastrinemic condition is unlikely to be due to reduced feedback inhibition of acid on G-cell activity since normal gastric acid secretion has been shown in the obese rat (Opsahl and Powley, 1974). It is conceivable, however, that intrinsic defects in the transmission and/or reception of this signal may result in defective feedback inhibition at the level of the G cell. A similar argument may be made for somatostatin in that hypergastrinemia may be a product of hyposensitivity to somatostatin inhibition.

Despite the existence of a voluminous amount of data describing the stimulation and inhibition of gastrin release from a variety of animal and experimental models, conclusions concerning its regulation at the cellular level have for the most part remained speculative because of the presence of multiple factors that could affect G-cell function. In systems ranging from preparations of partially digested antral mucosa to the intact conscious animal,

the potential for interaction among stimuli is enhanced as a result of increasing structural integrity. Consequently, the goal for scientists investigating the local regulation of gastrin secretion has been to work towards an isolated, single cell preparation that is sufficiently enriched for G cells in order to carry out secretory and mechanistic studies. Fortunately, compared to other gastrointestinal endocrine cells, gastrin-containing cells are distributed within a relatively small region of the gut, the pyloric antrum and duodenum. Recent developments in cell isolation and culture techniques have permitted the study of the local determinants of gastrin secretion using canine antral G cells in primary culture (Giraud et al, 1987; Sugano et al, 1987). Removal of the G cells from the region of the antrum where they are susceptible to modulation by paracrine, neural, endocrine and luminal signals allows for selective study of G-cell function by measurement of the cell's responsiveness to agents added singly or in combination. The remainder of this discussion will be concerned with studies performed on canine and human antral G cells in primary culture.

In the first series of experiments, the effect of two putative BN antagonists, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] SP and [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN on bombesin-stimulated gastrin release from isolated canine G cells was examined. These studies were felt to be necessary to support conclusions regarding the functional role of BN/GRP in the control of gastrin secretion. Furthermore, earlier studies reported here, demonstrated the inability of these analogs to inhibit BN-induced gastrin secretion from the perfused stomachs of lean and obese Zucker rats (Figures 31-34). The existence of distinct BN/GRP receptors among species was proposed to account for this observation and the canine model provided an opportunity to test this hypothesis.

Preferential enrichment of gastric endocrine cells by centrifugal elutriation and short-term tissue culture resulted in antral mucosal cultures in which G cells and D cells accounted for 8.5% and 1%, respectively, of the total population of viable cells that adhered to the culture wells. Studies by others have shown that the numbers of somatostatin cells reported here are not sufficient to influence gastrin release. Giraud et al

(1987) observed that the gastrin response to BN-stimulation is unaltered in isolated cell preparations containing 1.5% somatostatin cells even in the presence of a monoclonal antibody to somatostatin, S8. Therefore, inhibition of basal and BN-mediated gastrin release by somatostatin from D cells in culture plates in this preparation is probably not present.

Results from this study (Figure 36) were consistent with previous reports attributing a direct stimulatory role to BN/GRP in the control of gastrin secretion in the dog (Giraud et al, 1987; Sugano et al, 1987). Indeed, immunocytochemical studies have demonstrated dense innervation of the canine antral mucosa by GRP/BN-positive nerves (Furness et al, 1987). Furthermore, the gastrin responses to both vagal stimulation (Smith et al, 1975; Dockray et al, 1980) and exogenous GRP (McDonald et al, 1978) in the dog have been shown to be atropine resistant. Using direct radioligand-receptor binding techniques, the presence of high-affinity sites for BN on antral mucosa sections (Vigna et al, 1987) and isolated antral G cells (Vigna et al, 1988) of the dog have been revealed. The dramatic increase in gastrin levels by the addition 0.01 pM BN (Figure 36), when taken together with the above findings provide evidence for a physiological role of BN/GRP in the vagal control of G-cell function in the dog.

The high potency of BN/GRP in the stimulation of gastrin secretion has also been observed in the intact dog (Bunnett et al, 1985). These investigators reported half-maximal stimulation of gastrin release with the infusion of two GRP fragments (23 and 27 amino acids) to circulating concentrations that were only 20 pM over basal values. In addition, marked increases in gastrin secretion rates in response to 10 pM peptide histidine isoleucine have been shown from perfused rat stomachs (Schusdziarra et al, 1986) further indicating the presence of high affinity binding sites for regulatory peptides on the G cell. The absence of diffusion barriers, peptidases and other modulatory substances in the isolated cell preparation used here undoubtedly contributes to the extremely sensitive response of canine G cells to BN stimulation.

Receptors for BN/GRP have recently been found on canine antral G cells (Vigna et al, 1988). These binding sites are of a single class and display a much greater affinity for BN than those located on guinea pig pancreatic acini (Pandol et al, 1982), rat brain membranes (Moody et al, 1978), a rat pituitary cell line (Westendorf and Schonbrunn, 1983), human small lung cancer cells (Moody et al, 1985) and Swiss 3T3 fibroblasts (Zachary and Rozengurt, 1985). Further characterization of these receptors is predicated on the development of specific antagonists. A number of approaches have been adopted in this endeavor. The first approach was based on the fact that bombesin-like peptides are structurally related to the tachykinins of which SP is a member (Erspamer, 1983). Thus, molecular analogs of SP have emerged not only as antagonists of SP action but that of BN as well (Jensen et al. 1984; Jensen et al, 1988). Two other strategies used in the development of BN receptor antagonists have been founded upon the observation that the biological activity of BN resides in the C-terminal region (Coy et al, 1988). The first of these included [D-Phe^{12}] BN analogs (Heinz-Erian et al, 1987), whereas the second class of BN analogs featured the replacement of natural peptide bonds with a methylamide (CH_2NH) group (Coy et al, 1988).

Competitive antagonism of BN-stimulated amylase release from dispersed pancreatic acini (Jensen et al, 1984; Jensen et al, 1988) and of BN-mediated proliferation of Swiss 3T3 fibroblasts (Layton et al, 1988; Woll and Rozengurt, 1988) has been demonstrated with the SP analog, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] SP. Furthermore, no agonistic effect was observed when the analog was given alone. In the present study, however, addition of 1 μM of the SP analog elicited only modest inhibition of BN-stimulated gastrin secretion (Figures 37 & 39) and in fact stimulated gastrin release by itself. These results support earlier findings in lean and obese Zucker rats (Figures 31 & 32) wherein the SP analog was ineffective in suppressing BN-evoked gastrin secretion from the isolated perfused rat stomach preparation. The relatively low potency of the SP analog for attenuating BN-stimulated amylase release (half-maximal inhibition at 7.2 μM)

and its four-fold greater affinity for the SP receptor than for the BN receptor (Jensen et al, 1984; Jensen et al, 1988) may account for the present observations. On the other hand, Holst et al (1987) demonstrated complete inhibition of GRP-stimulated gastrin release from the perfused pig antrum with a concentration of the SP analog similar to that used here. In such a preparation, the preservation of luminal and neural influences raises the possibility that GRP activation of gastrin secretion may be indirect. Alternatively, these findings may reflect the existence of species-specific differences in gastric BN/GRP receptors.

In the present study, the BN analog, [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN, displayed potent competitive antagonism of BN action on cultured canine G cells (Figures 38 & 39). This antagonist was shown to completely suppress the gastrin response to 0.01-1 pM BN and to yield greater than 50% inhibition at 10 and 100 pM BN. This coupled with its lack of stimulation of the gastrin cell when given alone makes it a potentially powerful tool for BN-receptor characterization studies and an investigation of the involvement of bombesin-like peptides in cell function. It is not clear why this analog did not induce significant suppression of BN-stimulated gastrin secretion in lean and obese Zucker rats (Figures 33 & 34). Restrictions in molecular conformation conferred upon the BN analog by the reduced peptide bond (CH_2NH group) may have dramatically reduced its affinity for BN/GRP receptors in this species. Also, these findings strengthen the case for the existence of BN/GRP receptor sub-types among mammals. The results of this investigation are supported by studies of BN-stimulated amylase release from dispersed guinea pig pancreatic acini (Coy et al, 1988) and BN-induced growth of Swiss 3T3 cells (Coy et al,1988; Woll et al, 1988) wherein half-maximal inhibition was achieved at 35 nM and 18 nM, respectively. In addition, the BN analog did not exhibit any affinity for substance P receptors and successfully inhibited the binding of [^{125}I-Tyr^4] BN to pancreatic acini (Coy et al, 1988). The binding characteristics of this analog represents a 100-fold enhancement over other available BN antagonists. Therefore, it is possible that the BN analog could be an effective inhibitor of BN-stimulated gastrin secretion at concentrations lower than the 1

μM used in this study. Furthermore, the extremely high binding affinity of the BN analog makes it the agent of choice for studies requiring a continuous infusion of a BN antagonist. Indeed, bombesin-like peptides have been shown to act as autocrine growth factors in human small-cell lung cancer (Cuttitta et al, 1985). Thus, development of specific and potent BN antagonists may be clinically relevant in the management of this disease. Perhaps the most significant contribution of the BN analog lies in its structure. The substitution of peptide bonds in the biologically active sites of regulatory peptides with a CH_2NH moiety represents a novel approach in peptide modification and predicts the development of families of peptide antagonists with greater binding affinity and reduced agonistic properties.

In summary, the isolated, cultured antral G cell preparation was employed to demonstrate direct stimulation of gastrin release by BN in the dog. Effective inhibition of BN function was achieved with the BN analog, [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN, but not with the SP analog, [D-Arg^1, D-Pro^2, D-$Trp^{7,9}$, Leu^{11}] SP. These results further underscore the necessity for the development of suitable antagonists of regulatory peptides in order to elucidate their functional roles in normal and pathophysiological states.

Scientific investigation of gastrin release in humans has been problematic for a variety of reasons. Foremost of these is the potential for dangerous side-effects brought about by chemical manipulations used to expose mechanisms of action. In addition, much of the data available has been obtained from human subjects displaying symptoms of gastrointestinal and/or endocrine disease. Conclusions from such investigations can be relevant, but must be treated with caution when applied to normal G-cell function. The complexity of gastrin regulation *in vivo* is another significant consideration. The availability of gastric tissue from healthy organ donor transplant patients provided the unique opportunity to circumvent some of these difficulties. Modifications of the techniques used in the isolated canine G-cell model allowed the study of local regulation of

gastrin secretion and the mechanisms underlying stimulus-secretion coupling in enriched cultures of human antral G cells.

Dispersion of human antral tissue by sequential incubation with collagenase and EDTA (Tabel II) progressively increased the gastrin content of the resulting cell suspensions (Figure 40). Centrifugal elutriation resulted in the greater proportion of G cells eluting first followed by D cells (Figure 41). This elution order was opposite to that seen with canine gastric endocrine cells. After a 40-48 h culture period, gastrin-containing cells and somatostatin-containing cells accounted for 15% and 5%, respectively, of the total adhered cell population (Figure 42). The degree of enrichment of G cells cannot be determined with certainty from these studies as G-cell content was not measured in crude pre-elutriation cell suspensions due to the poor adherence of acutely dispersed cells to glass slides. Assuming that G cells before elutriation accounted for approximately 1% of the total cell population, as had been shown for the dog (Giraud et al, 1987), then the final cell culture preparations obtained here represent a 15-fold enrichment in G-cell content. The integrity of the cultured G cells was evidenced by the fact that basal gastrin secretion over a 2 h incubation period was < 2% of the total cell content and that these cells responded to stimulation in a concentration-related manner to various secretagogues. This system was therefore considered to be suitable for studying the local regulation of gastrin release from human antral G cells.

The responsiveness of cultured human G cells was initially tested using pharmacological activators of the cAMP and phosphatidylinositide second messenger systems. Forskolin, a plant-derived peptide which directly activates adenylate cyclase thereby increasing intracellular cAMP concentrations (Seamon et al, 1981), stimulated gastrin secretion from human antral G cells in a dose-dependent fashion (Figure 43). This agrees with previous reports showing enhancement of gastrin release from partially digested rat antral mucosa (DeSchryver-Kecskemeti et al, 1977) and antral and duodenal mucosal strips of the guinea pig (Schebalin et al, 1977) by the combined addition of

dibutyryl cAMP (dbcAMP) and theophylline, an agent which inhibits phosphodiesterase-mediated inactivation of cAMP. Furthermore, arginine-stimulated gastrin release from isolated pieces of rat gastric antrum was augmented by dbcAMP or theophylline (Hayes and Williams, 1975). Recently, enhanced secretion of gastrin from isolated canine G cells was demonstrated with dbcAMP (Sugano et al, 1987) and forskolin (Giraud et al, 1987). Studies reported here, when taken with reports of others, support the contention that cAMP-dependent pathways can mediate gastrin release from human as well as animal G cells.

In addition, Figure 43 shows potent suppression of forskolin-stimulated gastrin secretion by somatostatin. Studies in pituitary cells (Bilezikjian and Vale, 1983; Cronin et al, 1983; Reisine et al, 1983) and canine enteric endocrine cell cultures enriched for neurotensin and enteroglucagon cells (Barber et al, 1987) revealed that somatostatin treatment results in a marked reduction of the cytosolic cAMP content. Somatostatin inhibition of peptide release was not associated with an increase in phosphodiesterase activity (Reisine et al, 1983). Rather, it appeared to involve a dual mode of action including adenylate cyclase-dependent and independent mechanisms (Barber et al, 1987).

Concomitant determination of SLI levels in the release medium after forskolin treatment revealed the potent secretagogue function of forskolin on human antral D cells (Figure 44). Somatostatin secretion in response to dbcAMP and forskolin has been demonstrated in isolated canine fundic D cells (Soll et al, 1984; Yamada et al, 1984) and in human cell cultures enriched for antral D cells (Buchan et al, 1989), respectively. These findings indicate that, as with gastrin, somatostatin secretion can be modulated by cAMP-dependent mechanisms. Forskolin-mediated gastrin release was markedly enhanced by the addition of a monoclonal antibody specific for somatostatin, S10 (Buchan et al, 1985; Figure 44), suggesting that endogenously-released somatostatin is biologically active. Preliminary studies indicate that the predominant molecular form of somatostatin that is released into the medium corresponds to somatostatin 14 (Buchan et al, 1989), similar to

that seen with canine fundic D cells (Soll et al, 1984). Thus, it appears that forskolin directly activates G-cell function, but indirectly modulates this response via its secretory action on D cells.

The hydrolysis of phosphatidyl inositol, a common phospholipid located in plasma membranes, into inositol triphosphate (IP_3) and diacylglycerol (DAG) has been shown to result in the initiation of an least two distinct intracellular events: the mobilization of Ca^{2+} and the activation of a membrane-bound kinase (protein kinase C), leading to a complex cascade of protein phosphorylation and the eventual cellular response (reviewed in Berridge, 1987). In this study, pharmacological agents that mimic the actions of IP_3 and DAG were employed to examine the role of the phosphatidyl inositide transduction pathway in gastrin secretion from human antral G cells. The calcium ionophore, A23187, which increases cytosolic calcium concentrations by facilitating the entry of Ca^{2+} into the cell, evoked potent secretion of gastrin into the medium in a concentration-dependent manner (Figure 45). A similar response to A23187 has been reported in partially dispersed rat antral mucosa (DeSchryver-Kecskemeti et al, 1981) and in cultured canine G cells (Giraud et al, 1987). Indeed, the successful activation of G-cell function by bombesin has recently been demonstrated to be dependent on the influx of extracellular calcium in the isolated perfused rat stomach preparation (Guo et al, 1988) and in isolated canine G cells in primary culture (Sugano et al, 1987). In addition, ß-PMA, a phorbol ester that directly activates protein kinase C, also enhanced basal gastrin levels in a concentration-dependent fashion, reaching maximal values 1250% over basal (Figure 46). In contrast, the inactive isomer of this phorbol ester, α-PMA had no effect on gastrin secretion suggesting that stimulation of gastrin cell function by ß-PMA is a specific effect mediated through its action on protein kinase C and is not a non-specific response to phorbol esters. These results were consistent with the observations of Giraud et al (1987) investigating gastrin secretion from cultured G cells of the dog. Furthermore, they provide convincing support for the

involvement of the bifurcating signals of the phosphatidyl inositide transduction pathway in the secretion of gastrin from human G cells.

A precedent for stimulation of peptide hormone secretion via the adenylate cyclase and phosphatidyl inositide second messenger systems exists in the pancreatic ß cell (reviewed in Malaisse, 1988). Nutrient-induced insulin secretion is believed to involve increases in cytosolic Ca^{2+} concentrations first, followed by hydrolysis of inositol phospholipids and cAMP accumulation. In comparison, insulin release by cholinergic agonists is primarily dependent upon the activation of protein kinase C and the mobilization of intracellular calcium. These findings suggest that the adenylate cyclase and phosphatidyl inositide transduction pathways may be ubiquitous in peptide hormone-containing cells. In addition, the presence of interactions between these second messenger systems in the ß cell predicts similar occurrences in the gastrin cell.

In the final series of experiments, peptidergic regulation of gastrin release was investigated. Bombesin produced potent stimulation of gastrin levels but did not alter basal somatostatin release over the concentration range of 0.01 fM to 10 nM (Figure 47). The extremely high sensitivity of human G cells to BN activation parallels earlier findings using the canine counterpart of this preparation (Figure 36; Giraud et al, 1987). The removal of diffusion barriers, peptidases and interfering signals have been proposed to contribute to the particularly high potency of BN in this system. The lack of stimulation of somatostatin release by BN supported previous observations in lean and obese Zucker rats (Figures 25 & 27) and in the dog (P. 104), but was contrary to studies performed in the Sprague-Dawley rat (DuVal et al, 1981; Martindale et al, 1982; Schubert et al, 1985) and in the pig (Holst et al, 1987). Intra- and inter-species variation in the receptor composition of the D cell may be responsible for the discrepant results. Furthermore, these observations indicate that the stimulatory action of BN on the human G cell is direct and not mediated via inhibition of D-cell function.

Bombesinergic control of gastrin secretion was further investigated using the BN analog, [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN. As discussed earlier, this analog has been shown to be a potent antagonist of BN action in dispersed pancreatic acini (Coy et al, 1988) and in Swiss 3T3 cells (Coy et al,1988; Woll et al, 1988) with half-maximal inhibition achieved in the nM range. In the present study, however, [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN elevated immunoreactive-gastrin levels by > 50% during basal conditions and produced only modest inhibition of BN-stimulated gastrin secretion (Figures 48 & 49). In the presence of the BN analog, BN-evoked gastrin release was not significantly lower than basal gastrin secretion at all concentrations of BN tested. Partial agonist activity of [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN has also been recently reported in frog esophageal peptic cells (Dickinson et al, 1988) and in the rat central nervous system (Cowan et al, 1988). Collectively, these studies provide support for previous observations of ineffective inhibition of BN-stimulated gastrin secretion from the perfused stomachs of lean and obese Zucker rats (Figures 33 & 34) and suggest the existence of heterogeneous binding sites for BN/GRP.

In comparison, the addition of 10 nM somatostatin completely suppressed BN-induced increases in gastrin secretion from cultured human G cells up to 0.1 nM BN (Figure 50). Concentration-dependent inhibition of BN-mediated gastrin release by somatostatin has also been demonstrated in cultured canine G cells (Giraud et al, 1987; Sugano et al, 1987). These studies mimic potential interactions that may occur at the cellular level and suggest that somatostatin has a direct inhibitory role in gastrin secretion via specific and sensitive receptors. Furthermore, modulation of BN-stimulated G-cell function by somatostatin presumably occurs at a site distal to the early events of signal transduction since the modes of action of BN and somatostatin are believed to involve Ca^{2+}- (Sugano et al, 1987; Guo et al, 1988) and cAMP-dependent (Barber et al; 1987) mechanisms, respectively.

Finally, basal gastrin release was not significantly enhanced by the addition of the somatostatin-specific antibody, S10, (Figure 51), indicating that endogenous somatostatin

levels did not influence basal G-cell activity in this preparation. Exogenous somatostatin did not significantly reduce basal gastrin levels, but may in fact produce mild inhibition of basal gastrin release as evidenced by the modest increases in gastrin levels observed in S10 antibody-treated cells.

Several conclusions can be drawn from these studies. Firstly, a method for the isolation and enrichment of antral G cells originally described for the dog has been modified and applied to successfully yield enriched cultures of isolated human antral G cells that possess functional integrity. In addition, pharmacological examination of the possible signal transduction systems involved in stimulus-secretion coupling in human G cells revealed the participation of cAMP- and well as Ca^{2+}-protein kinase C-dependent pathways. Lastly, results from these experiments provide support for direct stimulation and inhibition of G-cell function by bombesin and somatostatin, respectively, via specific and high-affinity receptors. Inhibition experiments employing the BN analog, [Leu^{13}-ψ-CH_2NH-Leu^{14}] BN, suggested that the human antral G cell receptor for BN/GRP is biochemically distinct from its counterpart in canine antral G cells, but may share some functional properties with the BN/GRP receptor in the Zucker rat antrum. These results are the first of their kind to be reported using enriched cultures of isolated human antral G cells. The isolated human antral G-cell model holds much promise for the study of the local regulation of gastrin secretion and will undoubtedly facilitate the elucidation of the complex control of gastrin release *in vivo*.

SUMMARY AND CONCLUSIONS

Studies presented in this thesis were designed to investigate the complex regulation of gastrin secretion in health and disease. To this end, *in vivo* and *in vitro* techniques were employed to determine the etiology of the hypergastrinemic condition of obese Zucker rats, whereas isolated cultured antral G cells of the dog and human were used to probe local events associated with normal gastrin release.

Hypergastrinemia in the obese rat became evident at a point after weaning but sometime before 2 months of age. This condition was exacerbated by aging. Gastrin hypersecretion persisted from the isolated perfused stomach preparation suggesting that varying clearance rates of gastrin from the circulation was not responsible for abnormal gastrin levels. Cholinergic blockade with atropine did not affect basal gastrin release in either lean or obese rats and the relative responses of perfused stomachs of both phenotypes to vagal stimulation alone or superimposed upon atropine infusion were similar. These results indicated that a hyperactive post-ganglionic cholinergic drive was not involved in the hypergastrinemia of the obese animal. Furthermore, differential tonic inhibition of G-cell function by somatostatin is an unlikely candidate as the source of gastrin hypersecretion since somatostatin secretion and cell numbers were not different in the two groups of animals. In contrast, the doubling of the antral G-cell population of obese rats compared to lean animals suggested a causal relationship between G-cell hyperplasia and hypergastrinemia. It was proposed that post-weaning hyperphagia initiated the proliferation of G cells in obese rats leading to augmented gastrin secretion. Pair-feeding experiments were undertaken to test this hypothesis. Indeed, obese animals whose daily food consumption was restricted to match that of lean controls from 5-8 weeks of age did not exhibit G-cell hyperplasia, supporting the view that abnormal feeding behaviour in obese rats is the signal for G-cell proliferation. However, fasting gastrin concentrations

and basal gastrin release in the diet-controlled obese rat were not significantly different from its freely-feeding obese counterpart suggesting that: a) mechanism(s) independent of food intake mediated gastrin hypersecretion and b) G-cell hyperplasia is not the primary cause of hypergastrinemia but probably contributes to its severity in older animals. Because BN/GRP has been shown to be a potent secretagogue for gastrin and neurons containing BN/GRP-like immunoreactivity have been localized in the intrinsic innervation of the antral mucosa, studies were performed to examine the role of this peptide in the hypersecretion of gastrin. Elevated GRP levels in antral tissue extracts of obese rats and the attenuated gastrin response of perfused obese stomachs to exogenous BN provided indirect support for an increased endogenous BN/GRP drive as the cause of hypergastrinemia. However, additional support could not be obtained from studies employing BN/GRP blocking agents since the two putative antagonists used, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P and [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin, were ineffective in inhibiting basal and BN-stimulated gastrin release in both lean and obese rats. These findings suggested the involvement of bombesin-like peptides in the hypergastrinemia of obese animals, but corroborating evidence demonstrating inhibition of this proposed bombesinergic tone awaits the development of more specific and potent antagonists.

Using isolated canine antral G cells in primary culture, direct activation of gastrin release by BN was confirmed by studies reported in this thesis. This response was shown to be concentration-dependent and sensitive to very low amounts of BN (10 fM) supporting the occurrence of high-affinity BN/GRP receptors on the G cell. Complete suppression of BN-induced gastrin secretion from canine G cells was achieved with [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin. In comparison, [D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P displayed partial agonism in this preparation and consequently, produced only modest inhibition of BN-stimulated gastrin release. The ability of antagonists used to block BN-mediated gastrin release in this system but not from the isolated perfused stomachs of

Zucker rats implied that a heterogeneous population of gastric BN/GRP receptors exists across species. It was concluded that peptide bond replacement with an ethylamide group may represent a breakthrough in the development of effective competitive antagonists for BN/GRP as well as other regulatory peptides.

Finally, successful application of cell culture methods resulted in the production of human antral mucosal cultures preferentially enriched for G cells. Gastrin-containing cells accounted for 15% of the total adhered cell population and it was estimated that this represented a 15-fold enrichment compared to freshly dispersed cell suspensions. Concentration-dependent increases in gastrin levels were observed in cultures challenged with pharmacological activators of the adenylate cyclase and phosphatidyl inositide second messenger systems over a 2 h period. It was concluded that gastrin release from human G cells could be mediated by the accumulation of cAMP, mobilization of extracellular Ca^{2+} or stimulation of protein kinase C. Furthermore, BN also augmented gastrin secretion in a concentration-dependent fashion supporting a direct stimulatory role for BN-like peptides in this system. As in the canine model, the presence of high-affinity BN/GRP receptors on human antral G cells was inferred since significant enhancement of gastrin levels was observed with 0.01 fM BN. However, unlike findings in the canine preparation, [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin exhibited partial agonism which contributed to its weak attenuation of BN-stimulated gastrin release. This provided further evidence that BN/GRP receptors in the Zucker rat, dog and human possess distinct biochemical and functional properties. Exogenous somatostatin elicited potent inhibition of the gastrin responses to forskolin and BN, but had no significant effect on basal gastrin release. Several conclusions concerning the control of gastrin secretion at the level of the G cell were drawn from these studies: 1) BN and somatostatin exert their effects directly on the G cell via specific and sensitive stimulatory and inhibitory receptors; 2) BN/GRP-receptor subtypes exist among species and tissues thereby complicating the development of reliable and potent BN antagonists and 3) Stimulus-secretion coupling in human antral G cells can be

modulated by the adenylate cyclase and phosphatidyl inositide transduction systems. The success of this preliminary inquiry into the local regulation of gastrin secretion from human G cells predicts the usefuleness of this model for future investigations.

A diagrammatic representation of the findings contained in this thesis is summarized in Figures 52 & 53. It is clear from these studies that gastrin secretion is the product of a complex number of interactions between inhibitory and excitatory stimuli acting directly or indirectly on the G cell. Further elucidation of these mechanisms is dependent on advances in experimental methods and materials, and demands the integration of information obtained from a spectrum of biological models.

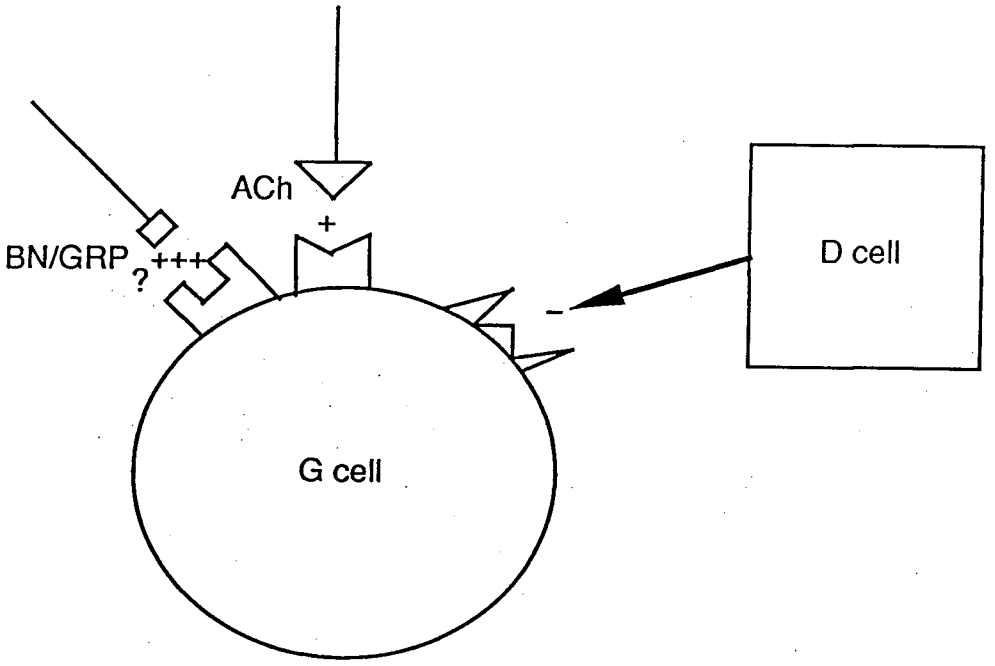


Figure 52: Proposed model for the control of the hypergastrinemia of the obese Zucker rat. Post-ganglionic cholinergic inputs to the G cell of the obese animal appear to be normal compared to the its lean littermate. Gastrin hypersecretion in the obese rat does not involve a reduction in somatostatin secretion or cell numbers. There is some indirect evidence to suggest that hypergastrinemia may be due to increased stimulation by BN/GRP intramural neurons. G-cell hyperplasia could not account for gastrin excess, but probably contributes to the severity of this condition in older animals.

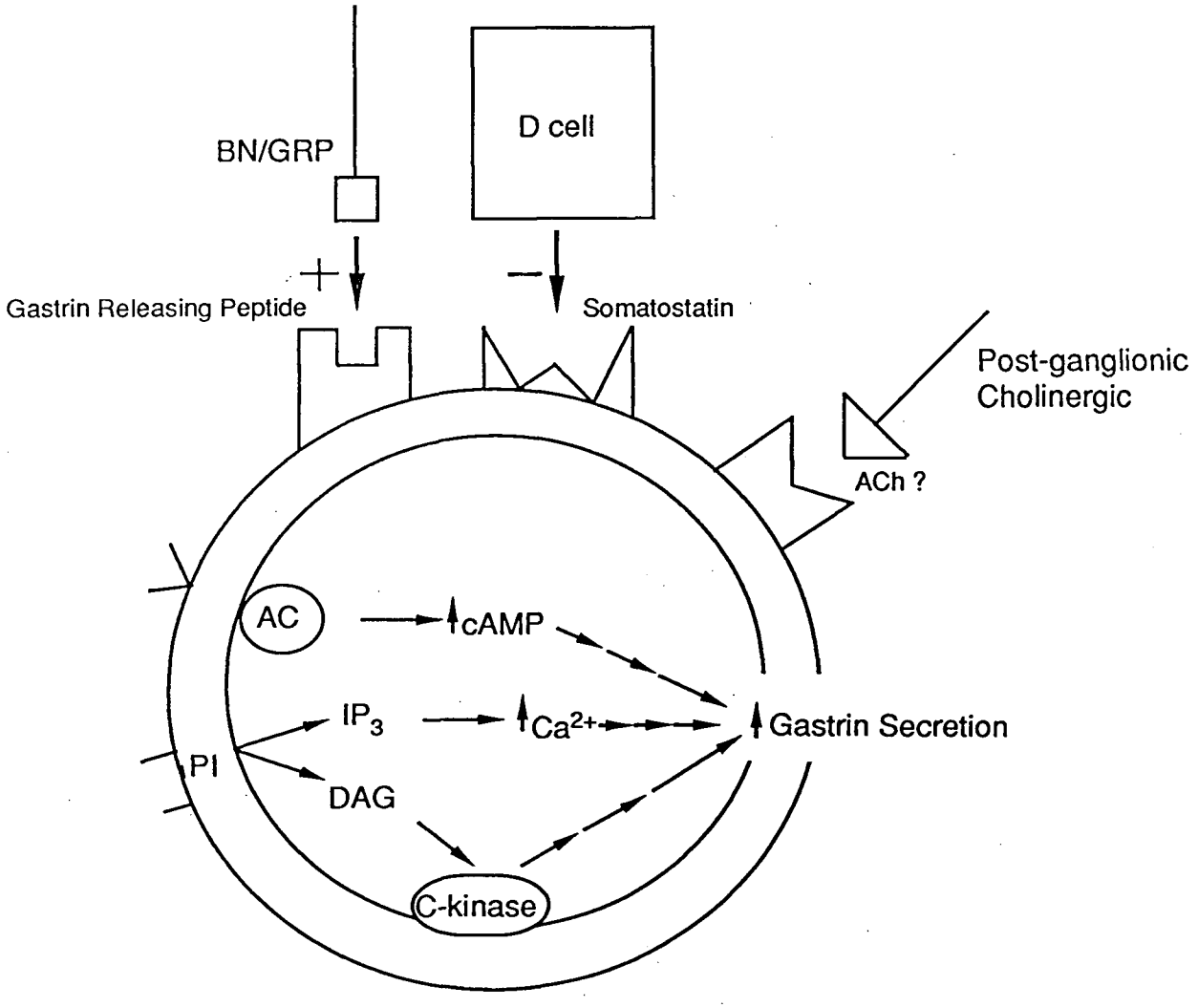


Figure 53: Proposed model for the control of gastrin secretion from human antral G cells. Somatostatin inhibits and BN/GRP stimulates G-cell function directly via specific and sensitive receptors. The gastric BN/GRP receptor of the human G cell possesses functional properties distinct from the canine and Zucker rat form of this receptor. The exact role of post-ganglionic cholinergic neurons in the regulation of gastrin secretion from human antral G cells has yet to be determined. Stimulus-secretion coupling in the human G cell is mediated by events associated with the adenylate cyclase and phosphatidyl inositide transduction pathways.

REFERENCES

Agarwal , K.L, Beacham, J., Bentley, P.H., Gregory, R.A., Kenner, G.W., Sheppard, R.C. and Tracy, H.J. (1968): Isolation, structure and synthesis of ovine and bovine gastrin. Nature 219:614-615.

Agarwal, K.L., Kenner, G.W. and Sheppard, R.C. (1969a): Structure and synthesis of canine gastrin. Experientia 25:346-348.

Agarwal, K.L., Kenner, G.W. and Sheppard, R.C. (1969b): Feline gastrin. An example of peptide sequence analysis by mass spectrometry. J. Amer. Chem. Soc. 91:3096-3097.

Alumets, J., Ekelund, M., El Munshid, H.A., Hakanson, R., Loren, I. and Sundler, F. (1979): Topography of somatostatin cells in the stomach of the rat: Possible functional significance. Cell. Tiss. Res. 202:177-188.

Anastasi, A., Erspamer, V. and Bucci, M. (1971): Isolation and structure of bombesin and alytesin, two analogous active peptides from the skin of the European amphibians Bombina and Alytes. Experientia 27:166-167.

Andersen, B., Abramovich, D., Brand, S., Petersen, B.and Rehfeld, J.F. (1985): Complete sulfation of jejunal gastrin in the human fetus. Reg. Peptides 10:329-338.

Anderson, B. (1984): Measurement of occurrence of sulfated gastrins. Scand. J. Clin. Lab. Invest. Suppl. 168:5-24.

Anderson, J.C., Barton, , M.A., Gregory, R.A., Hardy, P.M., Kenner, G.W., MacLeod, J.K., Preston, J., Sheppard, R.C. and Morley, J.S. (1964): The antral hormone gastrin. Synthesis of gastrin. Nature 204:933-934.

Atkinson, R.L., Dahms, W.T., Bray, G.A., Lemmi, C. and Schwartz, A.A. (1979): Gastrin secretion after weight loss by dieting and intestinal bypass surgery. Gastroenterology 77:696-699.

Aures, D., Johnson, L.R. and Way, L.W. (1970): Gastrin: Obligatory intermediate for activation of gastric histidine decarboxylase. Am. J. Physiol. 219:214-216.

Barber, D.L., Gregor, M. and Soll, A.H. (1987): Somatostatin and muscarinic inhibition of canine enteric endocrine cells: cellular mechanisms. Am. J. Physiol. 253:G684-G689.

Barry, W.S. and Bray, G.A. (1965): Plasma triglycerides in genetically obese rats. Metabolism 18:833-839.

Basso, N., Lezoche, E., Ciri, S., Percoco, M. and Speranza, V. (1977): Acid and gastrin levels after bombesin and calcium infusion in patients with incomplete antrectomy. Am. J. Dig. Dis. 22:125-128.

Bayliss, W.M. and Starling, E.H. (1902): The mechanism of pancreatic secretion. J. Physiol. 28:325-353.

Becker, H.D., Reeder, D.D., Scurry, M.T. and Thompson, J.C. (1974): Inhibition of gastrin release and gastric secretion by calcitonin in patients with peptic ulcer. Am. J. Surg. 127:71-75.

Becker, H.D., Reeder, D.D. and Thompson, J.C. (1973): Effect of glucagon on circulating gastrin. Gastroenterology 65:28-35.

Becker, H.D., Reeder, D.D. and Thompson, J.C. (1974a): Effect of atropine on basal and food-stimulated serum gastrin levels in man. Surgery 75:701-704.

Becker, H.D., Reeder, D.D. and Thompson, J.C. (1974b): Gastrin release by ethanol in man and in dogs. Ann. Surg. 179:906-909.

Bedi, B.W., Debas, H.T., Gillespie, G. and Gillespie, I.E. (1971): Effect of bile salt on antral gastrin release. Gastroenterology 60:256-262.

Bentley, P.H., Kenner, G.W. and Sheppard, R.C. (1966): Structures of human gastrins I and II. Nature 209:583-585.

Berridge, M.J. (1987): Inositol triphosphate and diacylglycerol: two interacting second messengers. Ann. Rev. Biochem. 56:159-193.

Berson, S.A., Walsh, J.H. and Yalow, R.S. (1973): Radioimmunoassay of gastrin in human plasma and regulation of gastrin secretion. In: Frontiers in Gastrointestinal Hormone Research, S. Andersson, ed., Almqvist & Wiksell, Stockholm, pp. 57-68.

Berson, S.A. and Yalow, R.S. (1971): Nature of immunoreactive gastrin extracted from tissues of gastrointestinal tract. Gastroenterology 60:215-222.

Berson, S.A. and Yalow, R.S. (1972): Radioimmunoassay in gastroenterology. Gastroenterology 62:1061-1084.

Bertaccini, G., Erspamer, V., Melchiorri, P. and Sopranzi, N. (1974): Gastrin release by bombesin in the dog. Br. J. Pharmacol. 52:219-225.

Bertrand, P. and Willems, G. (1980): Induction of antral gastrin cell proliferation by refeeding of rats after fasting. Gastroenterology 78:918-924.

Besterman, H.S., Sarson, D.L., Blackburn, A.M., Cleary, J., Pilkington, T.R.E. and Bloom, S.R. (1978): The gut hormone profile in morbid obesity and following jejuno-ileal bypass. Scand. J. Gastroenterol. Suppl. 13(49):15 (Abstract).

Bilezikjian, L.M. and Vale, W.W. (1983): Stimulation of adenosine 3',5'-monophosphate production by growth hormone-releasing factor and inhibition by somatostatin in anterior pituitary cells *in vitro*. Endocrinology 113:1726-1731.

Bloom, S.R., Ghatei, M.A., Christofides, N.D., Blackburn, A.M., Adrian, T.E., Lezoche, E., Basso, N., Carlei, F. and Speranzo, F. (1979): Bombesin infusion in man, pharmacokinetics and effect on gastro-intestinal and pancreatic hormonal peptides. J. Endocrinol. 83:51P (Abstract).

Bloom, S.R., Mortimer, C.H., Thorner, M.O., Besser, G.M., Hall, R., Gomez-Pan, A., Roy, V.M., Russell, R.C.G., Coy, D.H., Kastin, A.J. and Schally, A.V. (1974): Inhibition of gastrin and gastric-acid secretion by growth-hormone release-inhibiting hormone. Lancet ii:1106-1109.

Brand, S., Andersen, B. and Rehfeld, J.F. (1984): Complete tyrosine-O-sulphation of gastrin in neonatal rat pancreas. Nature 309:456-458.

Bray, G.A. (1969): Studies on the composition of adipose tissue from the genetically obese rats. Proc. Soc. Exp. Biol. Med. 131:1111-1114.

Bray, G.A. (1970): Metabolic and regulatory obesity in rats and man. Horm. Metab. Res. Suppl. 2:175-180.

Bray, G.A., Luong, D. and York, D.A. (1974): Regulation of adipose tissue mass in genetically obese rodents. In: The Regulation of the Adipose Tissue Mass, J. Vague and J. Boyer, eds., Excerpta Medica Foundation, Amsterdam, pp. 111-121.

Bray, G.A. and York, D.A. (1971): Genetically transmitted obesity in rodents. Physiol. Rev. 51:598-646.

Bremond, J., Orosco, M., Bonte, D., Pernes, J.F., Nurit, Y. and Jacquot, C. (1982): Relationship between experimental obesity and plasma lipid fractions. Inter. J. Obesity 6:191-196.

Brown, T.R., Bagchi, N., Mack, R.E., Booth, E. and Jones, D.P. (1976): Isolation of monoiodinated gastrin using DEAE-Sephadex and its characteristics in gastrin radioimmunoassays. Clin. Chim. Acta 67:321-323.

Bryce, G.F., Johnson, P.R., Sullivan, A.C. and Stern, J.S. (1977): Insulin and glucagon: plasma levels and pancreatic release in the genetically obese Zucker rat. Horm. Metab. Res. 9:366-370.

Buchan, A.M.J., Curtis, S.B. and Meloche, R.M. (1989): Release of somatostatin-immunoreactivity from human antral D cells in culture. Submitted for publication to Gastroenterology.

Buchan, A.M.J., Polak, J.M., Solcia, E. and Pearse, A.G.E. (1979): Localisation of intestinal gastrin in a distinct endocrine cell type. Nature 277:138-140.

Buchan, A.M.J., Sikora, L.K.J., Levy, J.G., McIntosh, C.H.S., Dyck, I. and Brown, J.C. (1985): An immunocytochemical investigation with monoclonal antibodies to somatostatin. Histochemistry 83:175-180.

Buffa, R., Solovieva, I., Fiocca, R., Giorgino, S., Rindi, G., Solcia, E., Mochizuchi, T., Yanaihara, C. and Yanaihara, N. (1982): Localization of bombesin and GRP (gastrin releasing peptide) sequences in gut nerves of endocrine cells. Histochemistry 76:457-467.

Bunnett, N.W., Clark, B., Debas, H.T., del Milton, R.C., Kovacs, T.O.G., Orloff, M.S., Pappas, T.N., Reeve, J.R., Rivier, J.E. and Walsh, J.H. (1985): Canine bombesin-like gastrin-releasing peptides stimulate gasrin release and acid secretion in the dog. J. Physiol. 365:121-130.

Bunnett, N.W., Debas, H.T., Turner, A.J., Kobayashi, R. and Walsh, J.H. (1988): Metabolism of gastrin and cholecystokinin by endopeptidase 24.11 from the pig stomach. Am. J. Physiol. 255:G676-G684.

Campos. R.V., Wheeler, M.B., Pederson, R.A., Buchan, A.M.J. and Brown, J.C. (1987): The effect of total parenteral nutrition (TPN) on gastrin release in the rat. Reg. Peptides 19:281-289.

Capella, C. and Solcia, E. (1972): The endocrine cells of the pig gastrointestinal mucosa and pancreas. Arch. Histol. Jpn. 35:1-29.

Carter, D., Taylor, I., Elashoff, J. and Grossman, M.I. (1979): Reappraisal of the secretory potency and disappearance rate of pure human minigastrin. Gut 20:705-708.

Chan, C.B. (1985): On the role of the enteroinsular axis in obesity. Ph. D. Thesis. University of British Columbia, Vancouver.

Chan, C.B., Pederson, R.A., Buchan, A.M.J., Tubesing, K.B. and Brown, J.C. (1985): Gastric inhibitory polypeptide and hyperinsulinemia in the Zucker (fa/fa) rat: a developmental study. Inter. J. Obesity 9:137-146.

Chew, C. and Hersey, S. (1982): Gastrin stimualtion of isolated gastric glands. Am. J. Physiol. 242:G504-G512.

Chiba, T., Kadowaki, S.,Taminato, T., Chihara, K., Seino, Y., Matsukura, S. and Fujita, T. (1981): Effect of antisomatostatin g-globulin on gastrin release in rats. Gastroenterology 81:321-326.

Chiba, T., Taminato, T., Kadowaki, S., Abe, H., Chihara, K., Seino, Y., Matsukura, S. and Fujita, T. (1980): Effects of glucagon, secretin, and vasoactive intestinal polypeptide on gastric somatostatin and gastrin release from isolated perfused rat stomach. Gastroenterology 79:67-71.

Chiba, T., Taminato, T., Kadowaki, S., Fujita, T., Seino, Y., Imura, H. and Yanaihara, N. (1979): Somatostatin and gastrin release from isolated perfused rat stomach. In: Gut Peptides, A. Miyoshi, ed., Kodansha Ltd., Tokyo, pp. 233-236.

Chikamori, K., Fukushima, Y., Yamashita, H., Sato, K., Nishimura, N. and Mori, H. (1983): Alterations of gastrin secretion in obese rats with ventromedial hypothalamic lesions. Inter. J. Obesity 7:563-567.

Chikamori, K., Masuda, K., Izumi, H., Isaka, K. and Tezuka, U. (1977): Effect of vagotomy on hyperinsulinemia in obese rats with hypothalamic lesions. Endocrinol. Jpn. 24:251-258.

Christensen, K. (1984): Specific beta-adrenergic mechanisms in the hypoglycaemic activation of gastrin and gastric acid secretion. Scand. J. Gastroenterol. 19:339-342.

Clendinnen, B.G., Reeder, D.D., Brandt Jr., E.N. and Thompson, J.C. (1973): Effect of nephrectomy on the rate and pattern of the disappearance of exogenous gastrin in dogs. Gut 14:462-467.

Cohen, S. and Lipshutz, W. (1971): Hormonal regulation of human lower esophageal sphincter competence: interaction of gastrin and secretin. J. Clin. Invest. 50:449-454.

Costa, M., Furness, J.B., Yanaihara, N., Yanaihara, C. and Moody, T.W. (1984): Distribution and projections of neurons with immunoreactivity for both gastrin-releasing peptide and bombesin in the guinea pig small intestine. Cell Tissue Res. 235:285-293.

Cowan, A., Wheeler, H. and Coy, D.H. (1988): Studies *in vivo* with Leu^{13}-ψ-[CH_2NH]-Leu^{14}-bombesin, a proposed competitive antagonist of bombesin. FASEB J. 2:A784 (Abstract).

Coy, D.H., Heinz-Erian, P., Jiang, N., Sasaki, Y., Taylor, J., Moreau, J., Wolfrey, W.T., Gardner, J.D. and Jensen, R.T. (1988): Probing peptide backbone function in bombesin. J. Biol. Chem. 263:5056-5060.

Crean, G.P., Marshall, M.W. and Rumsey, R.D.E. (1969): Parietal cell hyperplasia induced by the administration of pentagastrin to rats. Gastroenterology 57:147-155.

Creutzfeldt, W., Arnold, R., Creutzfeldt, C., Feurle, G. and Ketterer, H. (1971): Gastrin and G-cells in the antral mucosa of patients with pernicious anemia, acromegaly, and hyperparathyroidism and in a Zollinger-Ellison tumour of the pancreas. Eur. J. Clin. Invest. 1:461-479.

Cronin, M.J., Rogol, A.D., Myers, G.A. and Hewlett, E.L. (1983): Pertussis toxin blocks the somatostatin-induced inhibition of growth hormone release and adenosine 3',5'-monophosphate accumulation. Endocrinology 113:209-215.

Csendes, A., Walsh, J.H. and Grossman, M.I. (1972): Effects of atropine and of antral acidification on gastrin release and acid secretion in response to insulin and feeding in dogs. Gastroenterology 63:257-263.

Cuttitta, F., Carney, D.N., Mulshine, J., Moody, T.W., Fedorko, J., Fischler, A. and Minna, J.D. (1985): Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. Nature 316:823-826.

Dale, H.H. and Laidlaw, P.P. (1910): The physiological action of ß-imidazolylethylamine. J. Physiol. 41:318-344.

Davenport, H.W. and Kauffman, G.L. (1975): Plasma shedding by the canine oxyntic and pyloric glandular mucosa induced by topical action of acetylcholine. Gastroenterology 69:190-197.

Debas, H.T., Csendes, A., Walsh, J.H. and Grossman, M.I. (1974): Release of antral gastrin. In: Endocrinology of the Gut, W.Y. Chey and S.P. Brooks, eds., Charles B. Slack, Thorofare, New Jersey, pp. 222-232.

Debas, H.T., Hollinshead, J., Seal, A., Soon-Shiong, P. and Walsh, J.H. (1984): Vagal control of gastrin release in the dog: pathways for stimulation and inhibition. Surgery 95:34-37.

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

Debas, H.T., Walsh, J.H. and Grossman, M.I. (1974): Pure human minigastrin: secretory potency and disappearance rate. Gut 15:686-689.

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

Debas, H.T., Walsh, J.H. and Grossman, M.I. (1976): After vagotomy atropine suppresses gastrin release by food. Gastroenterology 70:1082-1084.

Deschodt-Lanckman, M., Najdovski, T., Dimaline, R., Dockray, G.J. and Pauwels, S. (1987): Degradation of unsulphated and sulphated gastrin heptadecapeptides by endopeptidase 24.11 in vitro, and identification of the products in vivo. In: Gastrin and Cholecystokinin: Chemistry, Physiology and Pharmacology, J.-P. Bali and J. Martinez, eds., Excerpta Medica, Elsevier, Amsterdam, pp. 41-44.

Deschodt-Lanckman, M., Pauwels, S., Najdovski, T., Dimaline, R. and Dockray, G.J. (1988): In Vitro and in vivo degradation of human gastrin by endopeptidase 24.11. Gastroenterology 94:712-721.

DeSchryver-Kecskemeti, K., Greider, M.H., Rieders, E.R., Komyati, S.E. and McGuigan, J.E. (1981): *In vitro* gastrin secretion by rat antrum: effects of neurotransmitter agonists, antagonists, and modulators of secretion. Lab. Invest. 44:158-163.

DeSchryver-Kecskemeti, K., Greider, M.H., Saks, M.K., Rieders, E.R. and McGuigan, J.E. (1977): The gastrin-producing cells in tissue cultures of the rat pyloric antrum. Lab. Invest. 37:406-410.

Desmond, H., Dockray, G.J. and Spurdens, M. (1985): Identification by specific radioimmunoassay of two novel peptides derived from the C-terminus of porcine preprogastrin. Reg. Peptides 11:133-142.

Dickinson, K.E.J., Uemura, N., Sekar, M.C., McDaniel, H.B., Anderson, W., Coy, D.H. and Hirschowitz, B.I. (1988): Partial agonist activity of the bombesin-receptor antagonist [Leu^{14}-ψ-CH_2-NH-Leu^{13}]-bombesin in frog peptic cells. Biochem. Biophys. Res. Comm. 157:1154-1158.

Dockray, G.J. (1980): Immunochemical studies of big gastrin using NH2-terminal specific antisera. Reg. Peptides 1:169-186.

Dockray, G.J., Best, L. and Taylor, I.L. (1977): Immunochemical characterization of gastrin in the pancreatic islets of normal and genetically obese mice. J. Endocrinol. 72:143-151.

Dockray, G.J., Gregory, R.A., Tracy, H.J. and Zhu, W. (1981): The transport of cholecystokinin-octapeptide-like immunoreactivity toward the gut in afferent vagal fibres in cat and dog. J. Physiol. 314:501-511.

Dockray, G.J. and Tracy, H.J. (1980): Atropine does not abolish cephalic vagal stimulation of gastrin release in dogs. J. Physiol. 306:473-480.

Dockray, G.J., Vaillant, C. and Hopkins, C. (1978): Biosynthetic relationships of big and little gastrins. Nature 273:770-772.

Dockray, G.J., Vaillant, C. and Walsh, J.H. (1979): The neuronal origin of bombesin-like immunoreactivity in the rat gastrointestinal tract. Neuroscience 4:1561-1568.

Dockray, G.J. and Walsh, J.H. (1975): Amino terminal gastrin fragment in serum of Zollinger-Ellison syndrome patients. Gastroenterology 68:220-230.

Dockray, G.J., Walsh, J.H. and Passaro Jr., E. (1975): Relative abundance of big and little gastrin in the tumours and blood of patients with the Zollinger-Ellison syndrome. Gut 16:353-358.

Dugail, I., Quignard-Boulange, A., Brigant, L., Etienne, J., Noe, L. and Lavau, M. (1988): Increased lipoprotein lipase content in the adipose tissue of suckling and weaning obese Zucker rats. Biochem. J. 249:45-49.

DuVal, J.W., Saffouri, B., Weir, G.C., Walsh, J.H., Arimura, A. and Makhlouf, G.M. (1981): Stimulation of gastrin and somatostatin secretion from the isolated rat stomach by bombesin. Am. J. Physiol. 241:G242-G247.

Edkins, J.S. (1898): Mechanism of secretion of gastric, pancreatic, and intestinal juices. In: Textbook of Physiology, E.A. Schafer, ed., Pentland, Edinburgh, pp. 531-558.

Edkins, J.S. (1905): On the chemical mechanism of gastric secretion. Proc. R. Soc. (Lond.) 76:376.

Edkins, J.S. (1906): The chemical mechanism of gastric secretion. J. Physiol. 34:133-144.

Ekblad, E., Ekman, R., Hakanson, R.and Sundler, F. (1984): GRP neurons in the rat small intestine issue long anal projections. Reg. Peptides 9:279-287.

Elwin, C.E. (1969): Stimulation of gastric acid secretion by irrigation of the antrum with some aliphatic alcohols. Acta Physiol. Scand. 75:1-11.

Elwin, C.E. (1974): Gastric acid responses to antral application of some amino acids, peptides, and isolated fractions of a protein hydrolysate. Scand. J. Gastroenterol. 9:239-247.

Emas, S. and Grossman, M.I. (1967): Effect of truncal vagotomy on acid and pepsin responses to histamine and gastrin in dogs. Am. J. Physiol. 34:133-144.

Erspamer, V. (1983): Amphibian skin peptides in mammals -- looking ahead. Trends Neurosci. 6:200-201.

Erspamer, V., Falconieri-Erspamer, G. and Inselvini, M. (1970): Some pharmacological actions of alytesin and bombesin. J. Pharm. Pharmac. 22:875-876.

Erspamer, V. and Melchiorri, P. (1975): Actions of bombesin on secretions and motility of the gastrointestinal tract. In: Gastrointestinal Hormones, J.C. Thompson, ed., University of Texas Press, Austin, pp.575-589.

Eysselein, V., Maxwell, V., Reedy, T., Wunsch, E. and Walsh, J.H. (1984): Similar acid stimulatory potencies of synthetic human big and little gastrins in man. J. Clin. Invest. 73:1284-1290.

Farooq, O. and Walsh, J.H. (1975): Atropine enhances serum gastrin response to insulin in man. Gastroenterology 68:662-666.

Feldman, M., Isenberg, J. and Grossman, M.I. (1981): Gastric acid and gastrin response to decaffeinated coffee and a peptone meal. JAMA 246:248-250.

Feldman, M., Richardson, C.T., Taylor, I.L. and Walsh, J.H. (1979): Effect of atropine on vagal release of gastrin and pancreatic polypeptide. J. Clin. Invest. 63:294-298.

Feldman, M. and Walsh, J.H. (1980): Acid inhibition of sham feeding-stimulated gastrin release and gastric acid secretion: effect of atropine. Gastroenterology 78:772-776.

Fletcher, D.R., Shulkes, A., Bladin, P.H.D. and Hardy, K.J. (1983): The effect of atropine on bombesin- and gastrin releasing peptide-stimulated gastrin, pancreatic polypeptide and neurotensin release in man. Reg. Peptides 7:31-40.

Fletcher, J.M. and McKenzie, N. (1988): The parasympathetic nervous system and glucocorticoid-mediated hyperinsulinemia in the genetically obese (fa/fa) Zucker rat. J. Endocrinol. 118:87-92.

Forrest, J.A.H., Fettes, M., McLoughlin, G. and Heading, R.C. (1978): The effect of long-term cimetidine on gastric acid secretion, serum gastrin and gastric emptying. In: Cimetidine, C. Wastell and P. Lance, eds., Churchill Livingstone, Edinburgh, pp.57-65.

Forssman, W.G., Feurle, G., Yanaihara, N., Helmstaedter, V., Glotzer, J., Buchler, M., Greenberg, J., Tischbirek, K., Lehy, T., Ito, S. and Lichtenberger, L. (1980): Ultrastructure and immunocytochemistry of gastrin cells (G cells). Biomed. Res. Suppl. 1:54-66.

Forssman, W.G., Pictet, R., Renold, A.E. and Rouiller, C. (1969): The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. J. Cell Biol. 40:692-715.

Frank, S.A., Walker, C.O. and Fordtran, J.S. (1973): The effect of continuous pentagastrin (PG) infusion on lower esophageal sphincter pressure (LES). Gastroenterology 64:728 (Abstract).

Furness, J.B., Miller, A.S. and Costa, M. (1987): Bombesin-like peptides in the enteric plexuses. Reg. Peptides 19:109 (Abstract).

Fyro, B. (1967): Reduction of antral and duodenal gastrin activity by electrical vagal stimulation. Acta Physiol. Scand. 71:334-340.

Gardner, J.D. and Jensen, R.T. (1986): Receptors mediating the actions of secretagogues on pancreatic acinar cells. In: The Exocrine Pancreas: Biology, Pathobiology, and Diseases, V.L.W. Go, J.D. Gardner, F.P. Brooks, E. Lebenthal, E.P. DiMagno and G.A. Scheele, eds., Raven, New York, pp. 109-122.

Gedde-Dahl, D. (1974): Radioimmunoassay of gastrin. Fasting serum levels in humans with normal and high gastric acid secretion. Scand. J. Gastroenterol. 9:41-47.

Giraud, A.S., Soll, A.H., Cuttitta, F. and Walsh, J.H. (1987): Bombesin stimulation of gastrin release from canine gastrin cells in primary culture. Am. J. Physiol. 252:G413-G420.

Glaser, B., Vinik, A.I., Valtysson, G. and Zoghlin, G. (1981): Truncal vagotomy abolishes the somatostatin response to insulin-induced hypoglycemia in man. J. Clin. Endocrinol. Metab. 52:823-828.

Godbole, V.Y., Grundleger, M.L., Pasquine, T.A. and Thenen, S.A. (1981): Composition of milk from day 5 to 20 of lactation and milk intake of lean and preobese Zucker pups. J. Nutr. 111:480-487.

Godbole, V.Y. and York, D.A. (1978): Lipogenesis *in situ* in the genetically obese Zucker fatty rat (fa/fa): role of hyperphagia and hyperinsulinemia. Diabetologia 14:191-197.

Goldstein, R.M., Hebiguchi, T., Luk, G.D., Taqi, F., Guilarte, T.R., Franklin Jr., F.A., Niemiec, P.W. and Dudgeon, D.L. (1985): The effects of total parenteral nutrition on gastrointestinal growth and development. J. Pediatr. Surg. 20:785-791.

Goodman, J.M. (1964): Duodenal submucosal islet-cell adenoma and chronic gastroduodenitis. Am. J. Surg. 10:726-729.

Greenberg, G., Chan, B., McDonald, T.J. and Alleyne, J. (1985): The role of vagal integrity in gastrin releasing peptide stimulated gastroenteropancreatic hormone release and gastric acid secretion. Reg. Peptides 10:179-187.

Greenwood, M.R.C., Cleary, M., Steingrimsdottir, L. and Vasselli, J.R. (1983): Adipose tissue metabolism and genetic obesity: the LPL hypothesis. In: Recent Advances in Obesity Research III, P. Bjorntorp, M. Cairella and A.N. Howard, eds., John Libbey, London, pp. 75-79.

Gregory, R.A. (1974): The gastrointestinal hormones: a review of recent advances. J. Physiol. 241:1-32.

Gregory, R.A. (1988): Gastrin. In: Advances in Metabolic Disorders Volume 11: Gastrointestinal Hormones, V. Mutt, ed., Academic Press, California, pp. 163-198.

Gregory, R.A., Dockray, G.J., Reeve, J.R., Shiveley, J.E. and Miller, C. (1983): Isolation from porcine antral mucosa of a hexapeptide corresponding to the C-terminal sequence of gastrin. Peptides 4:319-323.

Gregory, H., Hardy, P.M., Jones, D.S., Kenner, G.W. and Sheppard, R.C. (1964): The antral hormone gastrin. Structure of gastrin. Nature 204:931-933.

Gregory, R.A. and Tracy, H.J. (1961): The preparation and properties of gastrin. J. Physiol. 156:523-543.

Gregory, R.A. and Tracy, H.J. (1964): The constitution and properties of two gastrins extracted from hog antral mucosa. I. The isolation of two gastrins from hog antral mucosa. II. The properties of two gastrins isolated from hog antral mucosa. Gut 5:103-117.

Gregory, R.A. and Tracy, H.J. (1972): Isolation of two "big gastrins" from Zollinger-Ellison tumour tissue. Lancet 2:797-799.

Gregory, R.A. and Tracy, H.J. (1974): Isolation of two minigastrins from Zollinger-Ellison tumour tissue. Gut 15:683-685.

Gregory, R.A. and Tracy, H.J. (1975): The chemistry of the gastrins: Some recent advances. In: Gastrointestinal Hormones, J.C. Thompson, ed., University of Texas Press, Austin, pp.13-24.

Gregory, R.A., Tracy, H.J., Agarwal, K.L. and Grossman, M.I. (1969): Amino acid constitution of two gastrins isolated from Zollinger-Ellison tumour tissue. Gut 10:603-608.

Greider, M.H. and McGuigan, J.E. (1971): Cellular localization of gastrin in the human pancreas. Diabetes 20:389-396.

Greider, M.H., Steinberg, V. and McGuigan, J.E. (1972): Electron microscopic identification of the gastrin cell of the human antral mucosa by means of immunocytochemistry. Gastroenterology 63:572-583.

Grossman, M.I. (1970): Gastrin, cholecystokinin, and secretin act on one receptor. Lancet 1:1088-1089.

Grossman, M.I. (1979): Vagal stimulation and inhibition of acid secretion and gastrin release: which aspects are cholinergic? In: Gastrins and the Vagus, J.F. Rehfeld and E. Amdrup, eds., Academic Press, London, pp. 105-113.

Guo, Y., Thompson, J.C. and Singh, P. (1988): Role of Ca^{2+} in bombesin-stimulated release of gastrin and somatostatin from isolated perfused rat stomach. Am. J. Physiol. 255:G627-G632.

Gustavsson, S. and Lundquist, G. (1978): Participation of antral somatostatin in the local regulation of gastrin release. Acta Endocrinol. 88:339-346.

Guzman, S., Chayvialle, J.-A., Banks, W.A., Rayford, P.L. and Thompson, J.C. (1979): Surgery 86:329-336.

Hakanson, R., Blom, H., Carlsson, E., Larsson, H., Ryberg, B. and Sundler, F. (1986): Hypergastrinemia produces trophic effects in stomach but not in pancreas and intestines. Reg. Peptides 13:225-233.

Hammer, R. and Giachetti, A. (1982): Muscarinic receptor subtypes: M1 and M2 -- biochemical and functional characterization. Life Sci. 31:2991-2998.

Harty, R.F., Maico, D.G. and McGuigan, J.E. (1988): Comparison of adrenergic and cholinergic receptor-mediated stimulation of gastrin release from rat antral fragments. Peptides 9:463-468.

Hayes, J.R. and Williams, R.H. (1975): The effect on gastrin secretion of agents which increase the intracellular concentration of 3',5'-adenosine monophosphate. Endocrinology 97:1210-1214.

Heidenhain, R. (1879): Ueber die absonderung der fundusdrusen des magens. Pfluger's Archiv fur die gesamte Physiologie 19:148-166.

Heinz-Erian, P., Coy, D.H., Tamura, M., Jones, S.W., Gardner, J.D. and Jensen, R.T. (1987): [D-Phe^{12}] bombesin analogues: a new class of bombesin receptor antagonists. Am. J. Physiol. 252:G439-G442.

Heldsinger, A. and Vinik, A. (1984): Action of gastrin in guinea pig oxyntic cells: studies using quantitative cytochemistry. J. Clin. Invest. 74:124-132.

Higgs, R.H., Smyth, R.D. and Castell, D.O. (1974): Gastric alkalinization: effect on lower-esophageal-sphincter pressure and serum gastrin. N. Engl. J. Med. 291:486-490.

Hirschowitz, B.I. and Gibson, R.G. (1978): Cholinergic stimulation and suppression of gastrin release in gastric fistula dogs. Am. J. Physiol. 235:E720-E725.

Hirschowitz, B.I. and Gibson, R.G. (1979): Augmented vagal release of antral gastrin by 2-deoxyglucose after fundic vagotomy in dogs. Am. J. Physiol. 236:E173-E179.

Hokfelt, T., Efendic, S., Hellerstrom, C., Johansson, O., Luft, R. and Arimura, A. (1975): Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A1-cells of the pancreatic islets and to the hypothalamus. Acta. Endocrinol. Suppl. 80(200):1-41.

Hollinshead, J., Debas, H.T., Yamada, T., Elashoff, J., Osadchey, B. and Walsh, J.H. (1985): Hypergastrinemia develops within 24 hours of truncal vagotomy in dogs. Gastroenterology 88:35-40.

Holst, J.J., Jensen, S.L., Knuhtsen, S., Nielsen, O.V. and Rehfeld, J.F. (1983): Effect of vagus, gastric inhibitory polypeptide, and HCl on gastrin and somatostatin release from perfused pig antrum. Am. J. Physiol. 244:G515-G522.

Holst, J.J., Knuhtsen, S., Orskov, C., Skak-Nielsen, T., Poulsen, S.S., Jensen, S.L. and Nielsen, O.V. (1987): GRP nerves in pig antrum: role of GRP in vagal control of gastrin secretion. Am. J. Physiol. 253:G643-G649.

Holst, J.J., Knuhtsen, S., Orskov, C., Skak-Nielsen, T., Poulsen, S.S. and Nielsen, O.V. (1987): GRP-producing nerves control antral somatostatin and gastrin secretion in pigs. Am. J. Physiol. 253:G767-G774.

Hsu, S.M., Raine, L., Fanger, H. (1981): The use of antiavidin antibody and avidin-biotin-peroxidase complex in immunoperoxidase techniques. Am. J. Clin. Pathol. 75:816- .

Hsu, W.H. and Cooper, C.W. (1977): Serum gastrin in the rat: cholinergic and adrenergic effects. Proc. Soc. Exp. Biol. Med. 154:401-406.

Impicciatore, M., Debas, H., Walsh, J.H., Grossman, M.I. and Bertaccini, G. (1974): Release of gastrin and stimulation of acid secretion by bombesin in dog. Rendicoti di Gastroenterologia 6:99-101.

Impicciatore, M., Walsh, J.H. and Grossman, M.I. (1977): Low doses of atropine enhance serum gastrin response to food in dogs. Gastroenterology 72:995-996.

Inoue, S. and Bray, G.A. (1977): The effects of subdiaphragmatic vagotomy in rats with ventromedial hypothalamic obesity. Endocrinology 100:108-114.

Isenberg, J.I., Grossman, M.I., Maxwell, V. and Walsh, J.H. (1975): Increased sensitivity to stimulation of acid secretion by pentagastrin in duodenal ulcer. J. Clin. Invest. 55:330-337.

Isenberg, J.I. and Maxwell, V. (1978): Intravenous infusion of amino acids stimulates gastric acid secretion in man. N. Engl. J. Med. 298:27-29.

Iwanaga, T. (1983): Gastrin releasing peptide (GRP)/bombesin-like immunoreactivity in the neurons and paraneurons of the gut and lung. Biomed. Res. 4:93-104.

Jacobson, E.D., Linford, R.H. and Grossman, M.I. (1966): Gastric secretion in relation to mucosal blood flow studied by a clearance technique. J. Clin. Invest. 45:1-13.

Jaffe, B.M., Glendinneu, B.G., Clark, R.J. and Williams, J.A. (1974): The effect of selective and proximal gastric vagotomy on serum gastrin. Gastroenterology 66:944-953.

Jaffe, B.M. and Walsh, J.H. (1978): Gastrin and related peptides. In: Methods of Hormone Radioimmunoassay, B.M. Jaffe and H.R. Behrman, eds., Academic Press, New York, New York, pp. 455-477.

Jensen, D.M., McCallum, R. and Walsh, J.H. (1978): Failure of atropine to inhibit gastrin-17 stimulation of the lower esophageal sphincter in man. Gastroenterology 75:825-827.

Jensen, R.T., Jones, S.W., Folkers, K. and Gardner, J.D. (1984): A synthetic peptide that is a bombesin receptor antagonist. Nature 309:61-63.

Jensen, R.T., Heinz-Erian, P., Mantey, S., Jones, S.W. and Gardner, J.D. (1988): Characterization of ability of various substance P antagonists to inhibit action of bombesin. Am. J. Physiol. 254:G883-G890.

Johnson, L.R. (1973): Effect of gastric mucosal acidification on the action of pepsigogues. Am. J. Physiol. 225:1411-1415.

Johnson, L.R. (1974): Gut hormones on growth of gastrointestinal tract mucosa. In: Endocrinology of the Gut, W.Y. Chey and F.P. Brooks, eds., Charles B. Slack, Inc., New Jersey, pp. 163-177.

Johnson, L.R. (1976): The trophic action of gastrointestinal hormones. Gastroenterology 70:278-288.

Johnson, L.R. (1977): New aspects of the trophic action of gastrointestinal hormones. Gastroenterology 72:788-792.

Johnson, L.R., Castro, G.A., Lichtenberger, L.M., Copeland, E.M. and Dudrick, S.J. (1974): The significance of the trophic action of gastrin. Gastroenterology 66:718.

Johnson, L.R., Copeland, E.M., Dudrick, S.J., Lichtenberger, L.M. and Castro, G.A. (1975b): Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. Gastroenterology 68:1177-1183.

Johnson, L.R., Lichtenberger, L.M., Copeland, E.,M., Dudrick, S.J. and Castro, G.A. 1975a): Action of gastrin on gastrointestinal structure and function. Gastroenterology 68:1184-1192.

Johnson, P.R., Zucker, L.M., Cruce, J.A.F. and Hirsch, J. (1971): Cellularity of adipose depots in the genetically obese Zucker rat. J. Lipid Res. 12:706-714.

Kahlson, G., Rosengren, E., Svahn, D. and Thunberg, R. (1964): Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. J. Physiol. 174:400-416.

Kanatsuka, A., Osegawa, M., An, T., Suzuki, T., Hashimoto, N. and Makino, H. (1984): Augmented gastrin responses in diabetic patients with vagal neuropathy. Diabetologia 26:449-452.

Keast, J.R., Furness, J.B. and Costa, M. (1984): Somatostatin in human enteric nerves. Distribution and characterization. Cell. Tiss. Res. 237:299-308.

Knuhtsen, S., Holst, J.J., Knigge, U., Olesen, M. and Nielsen, O.V. (1984): Radioimmunoassay, pharmacokinetics, and neuronal release of gastrin-releasing peptide in anaesthetized pigs. Gastroenterology 87:372-378.

Knutson, U. and Olbe, L. (1974): The effect of exogenous gastrin on the acid sham feeding response in antrum-bulb-resected duodenal ulcer patients. Scand. J. Gastroenterol. 9:231-238.

Komarov, S.A. (1938): Gastrin. Proc. Soc. Exp. Biol. Med. 38:514-516.

Koop, H., Behrens, I., Bothe, E., Koschwitz, H., McIntosh, C.H.S., Pederson, R.A., Arnold, R. and Creutzfeldt, W. (1983): Adrenergic control of rat gastric somatostatin and gastrin release. Scand. J. Gastroenterol. 18:65-71.

Koop, H., Behrens, I., Bothe, E., McIntosh, C.H.S., Pederson, R.A., Arnold, R. and Creutzfeldt, W. (1982): Adrenergic and cholinergic interactions in rat gastric somatostatin and gastrin release. Digestion 25:96-102.

Koop, H., Bothe, E., Eissele, R., Dionysius, J. and Arnold, R. (1988): Somatostatin-gastrin interactions in the rat stomach. Res. Exp. Med. 188:115-121.

Koop, H., Schwab, E., Arnold, R. and Creutzfeldt, W. (1982): Effect of food deprivation on rat gastric somatostatin and gastrin release. Gastroenterology 82:871-876.

Korman, M.G., Brough, B.J., and Hansky, J. (1972): Gastrin and acid studies in the pouch dog. II. Effect of truncal vagotomy on response to food and insulin hypoglycemia. Scand. J. Gastroenterol. 7:525-529.

Korman, M.G., Hansky, J. and Scott, P.R. (1972): Serum gastrin in duodenal ulcer. III. Influence of vagotomy and pylorectomy. Gut 13:39-42.

Korman, M.G., Layer, M.C. and Hansky, J. (1972): Hypergastrinemia in chronic renal failure. Br. Med. J. 1:209-210.

Korman, M.G., Soveny, C. and Hansky, J. (1971): Serum gastrin in duodenal ulcer. Basal levels and effect of food and atropine. Gut 12:899-902.

Koop, H., Behrens, I., Bothe, E., McIntosh, C.H.S., Pederson, R.A.. Arnold, R. and Creutzfeldt, W. (1982): Adrenergic and cholinergic interactions in rat gastric somatostatin and gastrin release. Digestion 25:96-102.

Krejs, G.J., Sauberli, H.A., Largiader, F., Walsh, J.H. and Blum, A.L. (1977): Acta Hepato-Gastroenterol. 24:106-109.

Kronborg, O., Stadil, F., Rehfeld, J.F. and Christiansen, P.M. (1973): Relationship between serum gastrin concentrations and gastric acid secretion in duodenal ulcer patients before and after selective and highly selective vagotomy. Scand. J. Gastroenterol. 8:491-496.

Kwok, Y.N., McIntosh, C.H.S., Sy, H. and Brown, J.C. (1988): Inhibitory actions of tachykinins and neurokinins on release of somatostatin-like immunoreactivity from the isolated perfused rat stomach. J. Pharmacol. Exp. Ther. 246:726-731.

Lam, S.K., Chan, P.K.W., Wong, J. and Ong, G.B. (1978): Fasting and postprandial serum gastrin levels before and after highly selective gastric vagotomy, truncal vagotomy with pyloroplasty and truncal vagotomy with antrectomy: is there a cholinergic antral gastrin inhibitory and releasing mechanism. Br. J. Surg. 65:797-800.

Lam, S.K., Isenberg, J.I., Grossman, M.I., Lane, W.H. and Walsh, J.H. (1980): Gastric acid secretion is abnormally sensitive to endogenous gastrin released after peptone test meals in duodenal ulcer patients. J. Clin. Invest. 65:555-562.

Lambert, J.R., Hansky, J., Soveny, C. and Hunt, P. (1984): Comparative effects of bombesin and porcine gastrin-releasing peptide in the dog. Dig. Dis. Sci. 29:1036-1040.

Lamers, C.B.H.W. (1981): Clinical usefulness of the secretin provocation test. J. Clin. Gastroenterology 3:255-259.

Lamers, C.B.H.W. (1988): The significance of gastrin in the pathogenesis and therapy of peptic ulcer disease. Drugs Suppl. 35(3):10-16.

Lamers, C.B., Harrison, A., Ippoliti, A. and Walsh, J.H. (1979): Molecular forms of circulating gastrin in normal subjects and duodenal ulcer patients. Gastroenterology 76:1179 (Absrtact).

Lamers, C.B., Ruland, C.M., Joosten, H.J.M., Verkooyen, H.C.M., Van Tongeren, J.H.M. and Rehfeld, J.F. (1978): Hypergastrinemia of antral origin in duodenal ulcer. Am. J. Dig. Dis. 23:998-1002.

Lamers, C.B., Walsh, J.H., Jansen, J., Harrison, A., Ippoliti, A. and Van Tongeren, J. (1982): Evidence that gastrin 34 is preferentially released from the human duodenum. Gastroenterology 83:233-239.

Larson, T., Sanchez, J. and Taylor, I.L. (1983): Bombesin-induced tachyphylaxis markedly enhances gastrin response to a meal. Am. J. Physiol. 244:G652-G655.

Larsson, L.-I. (1984): Evidence for anterograde transport of secretory granules in processes of gastric paracrine (somatostatin) cells. Histochemistry 80:323-326.

Larsson, L.-I., Goltermann, N., DeMagistris, L., Rehfeld, J.F. and Schwartz, T.W. (1979): Somatostatin cell processes as pathway for paracrine secretion. Science 205:1393-1395.

Larsson, L.-I., Ljungberg, O., Sundler, F., Hakanson, R., Borg, I., Rehfeld, J.F. and Holst, J.J. (1973): Antro-pyloric gastrinoma associated with pancreatic nesidioblastosis and proliferation of islets. Virchows Arch. Pathol. Anat. 360:305-314.

Larsson, L.-I. and Rehfeld, J.F. (1977): Characterization of antral gastrin cells with region-specific antisera. J. Histochem. Cytochem. 25:1317-1321.

Larsson, L. -I., Rehfeld, J.F. and Goltermann, N. (1977): Gastrin in the human fetus. Distribution and molecular forms of gastrin in the antro-pyloric gland area, duodenum and pancreas. Scand. J. Gastroenterol. 12:869-872.

Layton, J.E., Scanlon, D.B., Soveny, C. and Morstyn, G. (1988): Effects of bombesin antagonists on the growth of small cell lung cancer cells *in vitro*. Cancer Res. 48:4783-4789.

Leander, S., Ekman, R., Uddman, R., Sundler, F. and Hakanson, R. (1984): Neuronal cholecystokinin, gastrin-releasing peptide, neurotensin, and ß-endorphin in the intestine of the guinea pig. Cell Tissue Res. 235:521-531.

Lee, P.C., Jensen, R.T. and Gardner, J.D. (1980): Bombesin-induced desensitization of enzyme secretion in dispersed acini from guinea pig pancreas. Am. J. Physiol. 238:G213-G218.

Lefebvre, P.J. and Luyckx, A.S. (1977): Factors controlling gastric glucagon release. J. Clin. Invest. 59:716-722.

Lenz, H., Ferrari-Taylor, J. and Isenberg, J.I. (1983): Wine and five percent ethanol are potent stimulants of gastric acid secretion in humans. Gastroenterology 85:1082-1087.

Levant, J.A., Walsh, J.H. and Isenberg, J.I. (1973): Stimulation of gastric secretion and gastrin release by single oral doses of calcium carbonate in man. N. Engl. J. Med. 289:555-558.

Lewin, K. Yang, K., Ulich, T., Elashoff, J. and Walsh, J.H. (1984): Primary gastrin cell hyperplasia: report of five cases and a review of the literature. Am. J. Surg. Pathol. 8:821-832.

Lichtenberger, L.M., Delansorne, R. and Graziani, L. (1982): Importance of amino acid uptake and decarboxylation in gastrin release from isolated G cells. Nature 295:698-700.

Lichtenberger, L.M. and Johnson, L.R. (1974): Gastrin in the ontogenic development of the small intestine. Am. J. Physiol. 227:390-395.

Lichtenberger, L.M., Lechago, J. and Johnson, L.R. (1975): Depression of antral and serum gastrin concentration by food deprivation in the rat. Gastroenterology 68:1473-1479.

Lichtenberger, L.M., Nance, D.M. and Gorsk, R.A. (1976): Sex related differences in antral and serum gastrin levels in the rat. Proc. Soc. Exp. Biol. Med. 151:785-788.

Lichtenberger, L.M. and Ramaswamy, K. (1979): Alterations in serum and antral gastrin levels in genetically diabetic mice. Gastroenterology 77:1276-1282.

Lin, T.M., Southard, G.L. and Spray, G.F. (1976): Stimulation of gastric acid secretion in the dog by the C-terminal penta, tetra, and tripeptides of gastrin and their O-methyl esters. Gastroenterology 70:733-736.

Loly, J., Depresseu, J., Brassinne, A. and Nizet, A. (1982): Renal control of the peripheral uptake of exogenous gastrin in the dog. Eur. J. Physiol. 395:171-174.

Longhi, E.H., Greenlee, H.B., Bravo, J.L., Guerrero, J.D. and Dragstedt, L.R. (1957): Question of an inhibitory hormone from the gastric antrum. Am. J. Physiol. 191:64-70.

Lucey, M.R., Wass, J.A.H., Fairclough, P., Webb, J., Webb, S., Medback, S. and Rees, L.H. (1985): Autonomic regulation of postprandial plasma somatostatin, gastrin, and insulin. Gut 26:683-688.

Lundell, L., Svensson, S.E. and Nilsson, G. (1976): Further studies of the mode of action of isoprenaline on gastric secretion in conscious rat. Br. J. Pharmacol. 58:17-25.

Malaisse, W.J. (1988): Stimulus-secretion coupling in the pancreatic B cell. In: Current Topics in Neuroendocrinology, Volume 9, D. Ganten and D. Pfaff, eds., Springer-Verlag, Berlin, pp. 231-251.

Martindale, R., Kauffman, G.L., Levin, S., Walsh, J.H. and Yamada, T. (1982): Differential regulation of gastrin and somatostatin secretion from isolated perfused rat stomachs. Gastroenterology 83:240-244.

Maxwell, J.G., Moore, J.G., Dixon, J. and Stevens, L.E. (1971): Gastrin levels in anephric patients. Surg. Forum 22:305-306.

McArthur, K.E., Isenberg, J., Hogan, D. and Dreier, S. (1983): Intravenous infusion of L-isomers of phenylalanine and tryptophan stimulates gastric acid secretion at physiologic plasma concentrations in normal subjects and after parietal cell vagotomy. J. Clin. Invest. 71:1254-1261.

McCallum, R.W. and Walsh, J.H. (1979): Relationship between lower esophageal sphincter pressure and serum gastrin concentration in Zollinger-Ellison syndrome and other clinical settings. Gastroenterology 76:76-81.

McDonald, T.J. (1988): The gastrin-releasing polypeptide (GRP). In: Advances in Metabolic Disorders Volume 11: Gastrointestinal Hormones, V. Mutt, ed., Academic Press, California, pp. 199-250.

McDonald, T.J. and Fox, J.E.T. (1984): Effects of porcine gastrin-releasing peptide (GRP) on canine motility and gastrin release in vivo. Life Sci. 35:1415-1422.

McDonald, T.J., Ghatei, M.A., Bloom, S.R., Adrian, T.E., Mochizuki, T., Yanaihara, C. and Yanaihara, N. (1983): Dose-response comparisons of canine plasma gastroenteropancreatic hormone responses to bombesin and the porcine gastrin-releasing peptide (GRP). Reg. Peptides 5:125-137.

McDonald, T.J., Ghatei, M.A., Bloom, S.R., Track, N.S., Radziuk, J., Dupre, J. and Mutt, V. (1981): A qualitative comparison of canine plasma gastroenteropancreatic hormone responses to bombesin and the porcine gastrin-releasing peptide. Reg. Peptides 2:293-304.

McDonald, T.J., Jornvall, H., Nilsson, G., Vagne, M., Ghatei, M.A., Bloom, S.R. and Mutt, V. (1979): Characterization of gatrin releasing peptide from porcine non-antral gastric tissue. Biochem. Biophys. Res. Commun. 90:227-233.

McDonald, T.J., Jornvall, H., Tatemoto, K. and Mutt, V. (1983): Identification and characterization of variant forms of the gastrin-releasing peptide. FEBS Lett. 156:349-356.

McDonald, T.J., Nilsson, G., Vagne, M., Ghatei, M.A., Bloom, S.R. and Mutt, V. (1978): A gastrin-releasing peptide from the porcine non-antral gastric tissue. Gut 19:767-774.

McGuigan, J.E. (1968): Gastrin mucosal intracellular localization of gastrin by immunoflourescence. Gastroenterology 55:315-327.

McGuigan, J.E., Jaffe, B. and Newton, W.T. (1970): Immunochemical measurements of endogenous gastrin release. Gastroenterology 59:499-504.

McGuigan, J.E. and Trudeau, W.L. (1968): Immunochemical measurement of elevated levels of gastrin in the serum of patients with pancreatic tumors of the Zollinger-Ellison variety. N. Engl. J. Med. 278:1308-1313.

McGuigan, J.E. and Trudeau, W.L. (1972): Serum gastrin levels before and after vagotomy and pyloroplasty or vagotomy and antrectomy. N. Engl. J. Med. 296:184-188.

McIntosh, C.H.S., Arnold, R., Bothe, E., Becker, H., Koberling, J. and Creutzfeldt, W. (1978): Gastrointestinal somatostatin: extraction and radioimmunoassay in different species. Gut 19:655-663.

McIntosh, C.H.S., Kwok, Y.N., Tang, C. and Brown, J.C. (1987): The use of monoclonal antibodies in RIA for gastrointestinal hormones. J. Clin. Immunoassay. 10:79-84.

McIntosh, C.H.S., Pederson, R.A., Muller, M. and Brown, J.C. (1981): Autonomic nervous control of gastric somatostatin secretion from the perfused rat stomach. Life Sci. 29:1477-1483.

Merali, Z., Merchant, C.A., Crawley, J.N., Coy, D.H., Heinz-Erian, P., Jensen, R.T. and Moody, T.W. (1988): [D-Phe^{12}] bombesin and substance P analogues function as central bombesin receptor antagonists. Synapse 2:282-287.

Moghimzadeh, E., Ekman, R., Hakanson, R., Yanaihara, N and Sundler, F. (1983): Neuronal gastrin-releasing peptide in the mammalian gut and pancreas. Neuroscience 10:553-563.

Moody, T.W., Carney, D.N., Cuttitta, F., Quattrochi, K., Gadzar, A.F. and Minna, J.D. (1985): High affinity receptors for bombesin/GRP-like peptides on human small cell lung cancer. Life Sci. 37:105-113.

Moody, T.W., Pert, C.B., Rivier, J. and Brown, M.R. (1978): Bombesin-specific binding to rat brain membranes. Proc. Natl. Acad. Sci. 75:5272-5276.

Morley, J.S. (1968): Structure-function relationships in gastrin-like peptides. Proc. R. Soc. (Lond.) Biol. 170:97-111.

Morley, J.S., Tracy, H.J. and Gregory, R.A. (1965): Structure-function relationships in the active C-terminal tetrapeptide sequence of gastrin. Nature 207:1356-1359.

Morton, A.P., Hanson, P.J. and Dockray, G.J. (1985): Elevated serum and antral gastrin in obese (ob/ob) mice. Gastroenterology 88:945-950.

Muller, S. and Cleary, M.P. (1988): Glucose metabolism in isolated adipocytes from *ad Libitum*- and restricted-fed lean and obese Zucker rats at two different ages. Proc. Soc. Exp. Biol. Med. 187:398-407.

Neuburger, P.H., Lewin, M. and Bonfils, S. (1972): Parietal and chief cell populations in four cases of the Zollinger-Ellison syndrome. Gastroenterology 63:937-942.

Nilsson, G. (1976): Regulationsmechanismen der magensauresekretion. In: Vagotomie, H. Burge et al, eds., George Thieme Verlag, Stuttgart, pp. 14-25.

Nilsson, G. and Brodin, K. (1977): Increase in gastrin concentration in the duodenal mucosa of dogs following resection of the gastric antrum. Acta Physiol. Scand. 98:510-512.

Nilsson, G., Simon, J., Yalow, R.S. and Berson, S.A. (1972): Plasma gastrin and gastric acid responses to sham-feeding and feeding in dogs. Gastroenterology 63:51-59.

Nilsson, G., Yalow, R.S. and Berson, S.A. (1973): Distribution of gastrin in the gastrointestinal tract in human, dog, cat and hog. In: Frontiers in Gastrointestinal Hormone Research, S. Andersson, ed., Almqvist and Wiksell, Stockholm, pp. 95-101.

Nishi, S., Seino, Y., Takemura, J., Ishida, H., Seno, M., Chiba, T., Yanaihara, C., Yanaihara, N. and Imura, H. (1985): Vagal regulation of GRP, gastric somatostatin, and gastrin secretion in vitro. Am. J. Physiol. 248:E425-E431.

Oberhelman Jr., H.A. and Nelson, T.S. (1964): Surgical consideration of the management of ulcerogenic tumours of the pancreas and duodenum. Am. J. Surg. 108:132-140.

Oberhelman Jr., H.A., Rigler, S.P. and Dragstedt, L.R. (1957): Significance of innervation in the function of the gastric antrum. Am. J. Physiol. 190:391-395.

Oberhelman Jr., H.A., Woodward, E.R., Zubiran, J.M. and Dragstedt, L.R. (1952): Physiology of the gastric antrum. Am. J. Physiol. 169:738-748.

Olbe, L. (1964): Potentiation of sham-feeding response in Pavlov pouch dogs by subthreshold amounts of gastrin with and without acidification of denervated antrum. Acta Physiol. Scand. 61:244-254.

Olbe, L. (1974): Differences between human and animal gastric acid secretion. In: Syllabus for American Gastroenterological Association Postgraduate Course on Peptic Ulcer Disease, American Gastroenterological Association, San Francisco, pp. 6-10.

Olbe, L., Ridley, P.T. and Uvnas, B. (1968): Effects of gastrin and histamine on vagally induced acid and pepsin secretion in antrectomized dogs. Acta Physiol. Scand. 72:492-497.

Olesen, M., Holst, J.J., Sottimano, C. and Nielsen, O.V. (1987): Autonomic nervous control of fundic secretion of somatostatin and antral secretion of gastrin and somatostatin in pigs. Digestion 36:24-35.

Opsahl, C.A. and Powley T.L. (1974): Failure of vagotomy to reverse obesity in the genetically obese Zucker rat. Am. J. Physiol. 226:34-38.

Pandol, S.J., Jensen, R.T. and Gardner, J.D. (1982): Mechanism of [Tyr4] bombesin-induced desenstitization in dispersed pancreatic acini from guinea pig pancreas. J. Biol. Chem. 257:12024-12029.

Pavlov, I.P. (1883): The centrifugal nerves of the heart. Thesis. St. Petersburg. (Russian).

Pavlov, I.P. (1897): Lectures on the work of the principal digestive glands. St. Petersburg. (Russian)

Pederson, R.A., Chan, C.B. and Buchan, A.M.J. (1985): Gastrin release in obese Zucker rats. Gastroenterology 88:1534 (Abstract).

Pederson, R.A., Kwok, Y.N., Buchan, A.M.J., McIntosh, C.H.S. and Brown, J.C. (1984): Gastrin release from isolated perfused rat stomach after vagotomy. Am. J. Physiol. 247:G248-G252.

Pederson, R.A., McIntosh, C.H.S., Mueller, M.K. and Brown, J.C. (1981): Absence of a relationship between immunoreactive-gastrin and somatostatin-like immunoreactivity secretion in the perfused rat stomach. Reg. Peptides 2:53-60.

Pederson, R.A., O'Dorisio, T., Howe, B., McIntosh, C.H.S., Mueller, M., Brown, J.C. and Cataland, S. (1981): Vagal release of IR-VIP and IR-Gastrin from the isolated perfused rat stomach. Mol. Cell. Endocrinol. 23:225-231.

Peters, M.N., Feldman, M., Walsh, J.H. and Richardson, C.T. (1983): Effect of gastric alkalinization on serum gastrin concentrations in humans. Gastroenterology 85:35-39.

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

Pe Thein, M. and Schofield, B. (1959): Release of gastrin from the pyloric antrum following vagal stimulation by sham feeding in dogs. J. Physiol. 148:291-305.

Pointner, H. and Flegel, U. (1976): Acta Hepato-Gastroenterol. 23:201-209.

Polak, J.M., Ghatei, M.A., Wharton, J., Bishop, A.E., Bloom, S.R., Solcia, E., Brown, M.R. and Pearse, A.G.E. (1978): Bombesin-like immunoreactivity in the gastrointestinal tract, lung and central nervous system. Scand. J. Gastroenterol. Suppl. 13(49):148 (Abstract).

Polak, J.M., Pearse, A.G.E., Grimelius, L., Bloom, S.R. and Arimura, A. (1975): Growth-hormone release-inhibiting hormone in gastrointestinal and pancreatic D cells. Lancet i:1220-1222.

Popielski, L. (1902): Ueber das peripherische reflectorische centrum der magendrusen. Centralblatt fur Physiologie 16:121-123.

Popielski, L. and Panek, K. (1909): Chemische untersuchungen uber das vasodilatin, den wirksamen korper der extrakte aus samtlichen teilen des verdauungskanals, den gehirn, pankreas und pepton witte. Pfluger's Archiv fur die gesamte Physiologie 128:222-225.

Popielski, L. (1920): ß-imidazolylathylamin und die organextrakte; ß-imidazolylathylamin als machtiger erreger der magendrusen. Pfluger's Archiv fur die gesamte Physiologie 178:214-236.

Powell, C., Ney, C., Aran, P. and Agarwal, K.L. (1985): A gastrin gene is expressed in both porcine pituitary and antral mucosal tissues. Nucl. Acids Res. 13:7299-7305.

Power, D.M., Bunnett, N.W., Turner, A.J. and Dimaline, R. (1987): Degradation of endogenous heptadecapeptide gastrin by endopeptidase 24.11 in the pig. Am. J. Physiol. 253:G33-G39.

Powley, T.L. and Opsahl, C.A. (1974): Ventromedial hypothalamic obesity abolished by subdiaphragmatic vagotomy. Am. J. Physiol. 226:25-33.

Raptis, S., Dollinger, H.C., von Berger, L., Schlegel, W., Schroder, K.E. and Pfeiffer, E.F. (1975): Effects of somatostatin on gastric secretion and gastrin release in man. Digestion 13:15-26.

Rayford, P.L., Villar, H.V., Reeder, D.D. and Thompson, J.C. (1974): Effect of GIP and VIP on gastrin release and gastric secretion. Physiologist 17:319.

Redfern, J., Thirlby, R., Feldman, M. and Richardson, C. (1985): Effect of pentagastrin on gastric mucosal histamine in dogs. Am. J. Physiol. 248:G369-G375.

Redford, M. and Schofield, B. (1965): The effect of local anesthesia of the pyloric antral mucosa on acid inhibition of gastrin-mediated acid secretion. J. Physiol. 180:304-320.

Reeder, D.D., Becker, H.D. and Thompson, J.C. (1974): Effect of intravenously administered calcium on serum gastrin and gastric secretion in man. Surg. Gynecol. Obstet. 138:847-851.

Reeve Jr., J.R., Dimaline, R., Shiveley, J.E., Hawke, D., Chew, P. and Walsh, J.H. (1981): Unique amino terminal structure of rat little gastrin. Peptides 2:453-458.

Rehfeld, J.F. (1974): What is gastrin? A progress report on the heterogeneity of gastrin and tissue. Digestion 11:397-405.

Rehfeld, J.F. (1978a): Immunochemical studies on cholecystokinin II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. J. Biol. Chem. 252:4022-4030.

Rehfeld, J.F. (1978b): Localization of gastrins to neuro- and adenohypophysis. Nature 271:771-773.

Rehfeld, J.F., Hansen, H.F., Larsson, L.-I., Stengaard-Petersen, K. and Thorn, N.A. (1984): Gastrin and cholecystokinin in pituitary neurons. Proc. Natl. Acad. Sci. U.S.A. 81:1902-1905.

Rehfeld, J.F. and Iversen, J. (1973): Secretion of immunoreactive gastrin from the isolated, perfused canine pancreas. VIII Congress of the International Diabetes Federation, Brussels, Excerpta Medica, Elsevier, Amsterdam, p. 119 (Abstract).

Rehfeld, J.F. and Larsson, L.-I. (1979): The predominating antral gastrin and intestinal cholecystokinin is the common COOH-terminal tetrapeptide amide. In: Gastrins and the Vagus, J.F. Rehfeld and E. Amdrup, eds., Academic Press, London, pp. 85-94.

Rehfeld, J.F. and Larsson, L.-I. (1981): Pituitary gastrins: different processing in corticotrophs and melanotrophs. J. Biol. Chem. 256:10426-10429.

Rehfeld, J.F., Schwartz, T.W. and Stadil, F. (1977): Immunochemical studies on macromolecular gastrins. Evidence that "big big" in blood and mucosa are artifacts -- but truly present in some large gastrinomas. Gastroenterology 73:469-477.

Rehfeld, J.F. and Stadil, F. (1973): Gel filtration studies on immunoreactive gastrin in serum from Zollinger-Ellison patients. Gut 14:369-373.

Rehfeld, J.F., Stadil, F. and Vikelsoe, J. (1974): Immunoreactive gastrin components in human serum. Gut 15:102-111.

Reisine, T.D., Zhang, Y. and Sekura, R.D. (1983): Pertussis toxin blocks somatostatin's inhibition of stimulated cyclic AMP accumulation in anterior pituitary tumor cells. Biochem. Biophys. Res. Comm. 115:794-799.

Richardson, C.T., Walsh, J.H., Cooper, K.A., Feldman, M. and Fordtran, J.S. (1977): Studies on the role of cephalic-vagal stimulation in the acid secretory response to eating in normal human subjects. J. Clin. Invest. 60:435-441.

Richardson, C.T., Walsh, J.H., Hicks, M. and Fordtran, J. (1976): Studies on the mechanisms of food-stimulated gastric acid secretion in normal human subjects. J. Clin. Invest. 58:623-631.

Richelsen, B., Rehfeld, J.F. and Larsson, L.-I. (1983): Antral gland cell column: a method for studying release of gastric hormones. Am. J. Physiol. 245:G463-G469.

Rohner-Jeanrenaud, F., Hochstrasser, A.-C. and Jeanrenaud, B. (1983): Hyperinsulinemia of preobese and obese fa/fa rats is partly vagus nerve mediated. Am. J. Physiol. 244:E317-E322.

Royston, C.M.S., Brew, D.S.J., Garnham, J.R., Stagg, B.H. and Polak, J.M. (1972): The Zollinger-Ellison syndrome due to an infiltrating tumour of the stomach. Gut 13:638-642.

Rubin, W. (1972): Endocrine cells in the normal human stomach. Gastroenterology 62:784-800.

Rudick, J., Werther, J.L., Chapman, M.L., Janowitz, H.D. and Kark, A.E. (1972): Effects of physiologic and supramaximal doses of pentagastrin on mucosal blood flow in antral and fundic pouches. Surgery 71:405-411.

Rufener, C., Dubois, M.P., Malaisse-Lagae, F. and Orci, L. (1975): Immuno-fluorescent reactivity to anti-somatostatin in the gastro-intestinal mucosa of the dog. Diabetologia 11:321-324.

Sacks, J., Ivy, A.C., Burgess, J.P. and Vandolah, J.E. (1932): Histamine as the hormone for gastric secretion. Am. J. Physiol. 101:331-338.

Saffouri, B., Weir, G.C., Bitar, K.N. and Makhlouf, G.M. (1979): Stimulation of gastrin secretion from the perfused rat stomach by somatostatin antiserum. Life Sci. 25:1749-1754.

Saffouri, B., Weir, G.C., Bitar, K.N. and Makhlouf, G.M. (1980): Gastrin and somatostatin secretion by perfused rat stomach: functional linkage of antral peptides. Am. J. Physiol. 238:G495-G501.

Sasaki, H., Nagulesparan, M., Dubois, A., Straus, E., Samloff, I.M., Lawrence, W.H., Johnson, G.C., Sievers, M.L. and Unger, R.H. (1983): Hypergastrinemia in obese noninsulin-dependent diabetes: a possible reflection of high prevalence of vagal dysfunction. J. Clin. Endocrinol. Metab. 56:744-750.

Sawitsch, V. and Zeliony, G. (1913): Zur physiologie des pylorus. Pfluger's Archiv fur die gesamte Physiologie 150:128-138.

Schafmayer, A., Teichmann, R., Swierczek, J., Rayford, P. and Thompson, J.C. (1978): Influence of vagus on mechanisms for stimulation and inhibition of gastrin release. Surgery 84:711-715.

Schebalin, M., Zfass, A.M. and Makhlouf, G.M. (1977): Mediation of guinea pig gastrin secretion *in vitro* by cyclic nucleotides. Gastroenterology 73:79-83.

Schiller, L.R., Walsh, J.H. and Feldman, M. (1980): Distention-induced gastrin release: effects of luminal acidification and intravenous atropine. Gastroenterology 78:912-917.

Schiller, L.R., Walsh, J.H. and Feldman, M. (1982): Effect of atropine on gastrin release stimulated by an amino acid meal in humans. Gastroenterology 83:267-272.

Schonfeld, G., Felski, C. and Howald, M.A. (1974): Characterization of the plasma lipoproteins of the genetically obese hyperlipoproteinemic Zucker fatty rat. J. Lipid Res. 15:457-464.

Schrumpf, E., Giercksky, K.E., Nygaard, K. and Fausa, O. (1981): Gastrin secretion before and after gastric bypass surgery. Scand. J. Gastroenterol. 16:721-725.

Schrumpf, E. and Stradaas, J. (1974): Effect of gastric distension on motility and plasma gastrin concentration before and after secretin administration. Scand. J. Gastroenterol. 9:119-122.

Schubert, M.L., Bitar, K.N. and Makhlouf, G.M. (1982): Regulation of gastrin and somatostatin secretion by cholinergic and noncholinergic intramural neurons. Am. J. Physiol. 243:G442-G447.

Schubert, M.L. and Makhlouf, G.M. (1982): Regulation of gastrin and somatostatin secretion by intramural neurons: effect of nicotinic receptor stimulation with dimethylphenylpiperazinium. Gastroenterology 83:626-632.

Schubert, M.L., Saffouri, B., Walsh, J.H. and Makhlouf, G.M. (1985): Inhibition of neurally mediated gastrin secretion by bombesin antiserum. Am. J. Physiol. 248:G456-G462.

Schusdziarra, V. (1983): Somatostatin -- Physiological and pathophysiological aspects. Scand. J. Gastroenterol. Suppl. 82:69-84.

Schusdziarra, V., Bender, H. and Pfeiffer, E.F. (1983): Release of bombesin-like immunoreactivity from the isolated perfused rat stomach. Reg. Peptides 7:21-29.

Schusdziarra, V., Lawecki, J., Ditschuneit, H.H., Lukas, B., Maier, V. and Pfeiffer, E.F. (1985): Effect of low-dose somatostatin infusion on pancreatic and gastric endocrine function in lean and obese nondiabetic human subjects. Diabetes 34:595-601.

Schusdziarra, V., Schmid, R., Berder, H., Schusdziarra, M., Rivier, J., Vale, W. and Classen, M. (1986): Effect of vasoactive intestinal peptide, peptide isoleucine and growth hormone-releasing factor-40 on bombesin-like immunoreactivity, somatostatin and gastrin release from the perfused rat stomach. Peptides 7:127-133.

Schwarting, H., Koop, H., Gellert, G. and Arnold, R. (1986): Effect of starvation on endocrine cells in the rat stomach. Reg. Peptides 14:33-39.

Seamon, K.B., Padgett, W. and Daly, J.W. (1981): Forskolin: unique diterpene activator of adenylate cyclase in membranes and intact cells. Proc. Natl. Acad. Sci. 78:3363-3367.

Short, G.M., Doyle, J.W. and Wolfe, M.M. (1985): Effect of antibodies to somatostatin on acid secretion and gastrin release by the isolated perfused rat stomach. Gastroenterology 88:984-988.

Short, G.M., Reel, G.M., Doyle, J.W. and Wolfe, M.M. (1985): Effect of GRP on ß-adrenergic-stimulated gastrin and somatostatin release in the isolated rat stomach. Am. J. Physiol. 249:G197-G202.

Sjodin, L. and Nilsson, G. (1973): Gastric acid secretion and plasma gastrin levels during urecholine infusion in the dog. Acta Physiol Scand. Suppl. 396:63 (Abstract).

Sjodin, L. and Nilsson, G. (1974): Plasma gastrin levels and gastric acid secretion in dogs during administration of urecholine. Scand. J. Gastroenterol. 9:747-750.

Smith, C.L., Kewenter, A.M., Connell, J., Ardill, R., Hayes, R. and Buchanan, K. (1975): Control factors in the release of gastrin by direct electrical stimulation of the vagus. J. Digest. Dis. 20:13-22.

Soares, E.C., Zaterka, S. and Walsh, J.H. (1977): Acid secretion and serum gastrin at graded intragastric pressures in man. Gastroenterology 72:676-679.

Solcia, E., Vassalo, G. and Capella, C. (1969): Studies on the G-cells of the pyloric mucosa, the probable site of gastrin secretion. Gut 10:379-388.

Soll, A.H. (1978a): The actions of secretagogues on oxygen uptake by isolated mammal parietal cells. J. Clin. Invest. 61:370-380.

Soll, A.H. (1978b): The interaction of histamine with gastrin and carbamylcholine on oxygen uptake by isolated mammal parietal cells. J. Clin. Invest. 61:381-389.

Soll, A.H. (1982): Potentiating interactions of gastric stimulants on [14C] aminopyrine accumulation by isolated canine paritetal cells. Gastroenterology 83:216-233.

Soll, A.H., Amirian, D.A., Thomas, L.P., Reedy, T.J. and Elashoff, J.D. (1984): Gastrin receptors on isolated canine parietal cells. J. Clin. Invest. 73:1434-1447.

Soll, A.H., Yamada, T., Park, J. and Thomas, L.P. (1984): Release of somatostatinlike immunoreactivity from canine fundic mucosal cells in primary culture. Am. J. Physiol. 247:G558-G566.

Stadil, F. and Rehfeld, J.F. (1972): Preparation of 125I-labelled synthetic human gastrin I for radioimmunoanalysis. Scand. J. Clin. Lab. Invest. 30:361-368.

Stadil, F. and Rehfeld, J.F. (1973): Release of gastrin by epinephrine in man. Gastroenterology 65:210-215.

Stadil, F. and Rehfeld, J.F. (1974): Gastrin response to insulin after selective, highly selective, and truncal vagotomy. Gastroenterology 66:7-15.

Starling, E.H. (1905): The Croonian lectures on the chemical correlation of the functions of the body. Lancet 2:339-341.

Stein, B.A., Buchan, A.M.J., Morris, J. and Polak, J.M. (1983): The ontogeny of regulatory peptide-containing cells in the human fetal stomach. J. Histochem. Cytochem. 31:1117-1125.

Stern, D.H. and Walsh, J.H. (1973): Gastrin release in postoperative ulcer patients: evidence for release of duodenal gastrin. Gastroenterology 64:363-369.

Stock-Damge, C., Lhoste, E., Aprahamian, M. and Pousse, A. (1985): Gastrin modulation of pancreatic growth. Scand. J. Gastroenterol. Suppl. 112:68-74.

Strunz, U.T., Thompson, M.R., Elashoff, J. and Grossman, M.I. (1978): Hepatic inactivation of gastrins of various chain lengths in dogs. Gastroenterology 74:550-553.

Strunz, U.T., Walsh, J.H. and Grossman M.I. (1978a): Removal of gastrin by various organs in dogs. Gastroenterology 74:32-33.

Strunz, U.T., Walsh, J.H. and Grossman M.I. (1978b): Stimulation of gastric acid secretion and gastrin release in dogs by individual amino acids. Proc. Soc. Exp. Biol. Med. 157:440-441.

Sue, R., Toomey, M.L., Todisco, A., Soll, A.H. and Yamada, T. (1985): Pirenzepine-sensitive muscarinic receptors regulate gastric somatostatin and gastrin. Am. J. Physiol. 248:G184-G187.

Sugano, K., Park, J., Soll, A.H. and Yamada, T. (1987): Stimulation of gastrin release by bombesin and canine gastrin-releasing peptides. J. Clin. Invest. 79:935-942.

Svensson, S.O., Emas, S., Dorner, M. and Kaess, H. (1976): Cholinergic release of gastrin by feeding in cats. Gastroenterology 70:742-746.

Tang, C.L. (1988): The effects of a somatostatin monoclonal antibody on gastrin and insulin release. M.Sc. Thesis, University of British Columbia, Vancouver.

Taylor, I.L., Byrne, W., Christie, D., Ament, M. and Walsh, J.H. (1982): Effect of individual L-amino acids on gastric acid secretion and serum gastrin and pancretic polypeptide release in humnas. Gastroenterology 83:273-278.

Taylor, I.L., Dockray, G.J., Calam, J. and Walker, R. (1979): Big and little gastrin responses to food in normal and ulcer subjects. Gut 20:957-962.

Taylor, I.L., Walsh, J.H., Carter, D.C., Wood, J. and Grossman, M.I. (1979): Effects of atropine and bethanechol on bombesin-stimulated release of pancreatic polypeptide and gastrin in dog. Gastroenterology 77:714-718.

Tepperman, B.L., Walsh, J.H. and Preshaw, R.M. (1972): Effect of antral denervation on gastrin release by sham-feeding and insulin hypoglycemia in dogs. Gastroenterology 63:973-980.

Thompson, J.C., Hirose, F.M., Lemmu, C.A.E. and Davidson, W.D. (1968): Zollinger-Ellison syndrome in a patient with multiple carcinoid-islet cell tumours of the duodenum. Am. J. Surg. 115:177-184.

Thompson, J.C., Reeder, D.D., Buchman, H.H., Becker, H.D. and Brandt Jr., E.R.S. (1972): Effect of secretin on circulating gastrin. Ann. Surg. 176:384-393.

Thompson, J.C., Reeder, D.D., Davidson, W.D., Charters, A.C., Bruckner, W.L., Lemmi, C.A.E. and Miller, J.H. (1969): Effect of hepatic transit of gastrin, pentagastrin, and histamine measured by gastric secretion and by assay of hepatic vein blood. Ann. Surg. 170:493-503.

Track, N.S. (1980): Gastrointestinal hormones during total parenteral nutrition. Front. Horm. Res. 7:258-264.

Track, N., Creutzfeldt, C., Litzenberger, J., Neuhoff, C., Arnold, R. and Creutzfeldt, W. (1979): Appearance of gastrin and somatostatin in the human fetal stomach, duodenum and pancreas. Digestion 19:292-306.

Tracy, H.J. and Gregory, R.A. (1964): Physiological properties of a series of synthetic peptides structurally related to gastrin I. Nature 204:935-938.

Tranberg, K.-G., Hagander, P. and Von Schenck, H. (1982): Hepatic uptake of synthetic human gastrin I (1-17) in humans. Surgery 93:747-751.

Trudeau, W.L. and McGuigan, J.E. (1971): Relations between serum gastrin levels and rates of gastric hydrochloric acid secretion. N. Engl. J. Med. 284:408-412.

Uvnas, B. (1942): The part played by the pyloric region in the cephalic phase of gastric secretion. Acta Physiol. Scand. Suppl. 4(13):1-86.

Uvnas, B. (1945a): Further attempts to isolate a gastric secretory excitant from the pyloric mucosa of pigs. Acta Physiol. Scand. 9:296-305.

Uvnas, B. (1945b): The presence of a gastric secretory excitant in the human gastric and duodenal mucosa. Acta Physiol. Scand. 10:97-101.

Uvnas, B., Uvnas-Wallensten, K. and Nilsson, G. (1975): Release of gastrin on vagal stimulation in the cat. Acta Physiol. Scand. 94:167-176.

Uvnas-Wallensten, K. and Andersson, H. (1977): Effect of atropine and methiamide on vagally induced gastric acid secretion and gastrin release in anaesthetized cats. Acta Physiol. Scand. 99:496-502.

Uvnas-Wallensten, K., Efendic, S., Roovete, A. and Johansson, C. (1980): Decreased release of somatostatin into the portal vein following electrical vagal stimulation in the cat. Acta Physiol. Scand. 109:393-398.

Uvnas-Wallensten, K., Rehfeld, J.F., Larsson, L.-I. and Uvnas, B. (1977): Heptadecapeptide gastrin in the vagal nerve. Proc. Natl. Acad. Sci. U.S.A. 74:5707-5710.

Varner, A.A., Modlin, I.M. and Walsh, J.H. (1981): High potency of bombesin for stimulation of human gastrin release and gastric acid secretion. Reg. Peptides 1:289-296.

Vatn, M.H., Schrumpf, E., Hanssen, K.F. and Myren, J. (1977): The effect of somatostatin on pentagastrin-stimulated gastric secretion and on plasma gastrin in man. Scand. J. Gastroenterol. 12:833-839.

Vatn, M.H., Schrumpf, E. and Myren, J. (1975): The effect of carbachol and pentagastrin on the gastric secretion of acid, pepsin and intrinsic factor in man. Scand. J. Gastroenterol. 10:55-58.

Vigna, S.R., Giraud, A.S., Soll, A.H., Walsh, J.H. and Mantyh, P.W. (1988): Bombesin receptors on gastrin cells. In: Bombesin-like Peptides in Health and Disease. Annals of the New York Academy of Sciences Volume 547, Y. Tache, P. Melchiorri and L. Negri, eds., New York Academy of Sciences, New York, pp. 131-137.

Vigna, S.R., Mantyh, C.R., Giraud, A.S., Soll, A.H., Walsh, J.H. and Mantyh, P.W. (1987): Localization of specific binding sites for bombesin in the canine gastrointestinal tract. Gastroenterology 93:1287-1295.

Walsh, J.H. (1978): Pathogenetic role of the gastrins. In: Gastrins and the Vagus, J.F. Rehfeld and E. Amdrup, eds., Academic Press, London, pp. 181-188.

Walsh, J.H., Debas, H.T. and Grossman, M.I. (1974): Pure human big gastrin. Immunochemical properties, disappearance half time, and acid-stimulating action in dogs. J. Clin. Invest. 54:477-485.

Walsh, J.H. and Grossman, M.I. (1975): Medical progress: Gastrin. New Engl. J. Med. 292:1324-1332.

Walsh, J.H., Isenberg, J., Ansfield, J. and Maxwell, V. (1976): Clearance and acid-stimulating action of human big and little gastrins in duodenal ulcer subjects. J. Clin. Invest. 57:1125-1131.

Walsh, J.H. and Lam, S.K. (1980): Physiology and pathology of gastrin. In: Clinics in Gastroenterology Volume 9 Number 3, W. Creutzfeldt, ed., W.B. Saunders, London, pp. 567-591.

Walsh, J.H., Lamers, C.B. and Valenzuela, J.E. (1982): Cholecystokinin-octapeptidelike immunoreactivity. Gastroenterology 82:438-444.

Walsh, J.H. and Laster, L. (1973): Enzymatic deamidation of the C-terminal tetrapeptideamide of gastrin by mammalian tissues. Biochem. Med. 8:432-449.

Walsh, J.H., Richardson, C.T. and Fordtran, J.S. (1975): pH dependence of acid secretion and gastrin release in normal and ulcer subjects. J. Clin. Invest. 55:462-468.

Walsh, J.H. and Wong, J.H. (1979): Bombesin-like peptides. In: Methods of Hormone Radioimmunoassay, B.M. Jaffe and H.R. Berman, eds., Academic Press, New York, pp. 581-593.

Walsh, J.H., Yalow, R.S. and Berson, S.A. (1971): The effect of atropine on plasma gastrin response to feeding. Gastroenterology 60:16-21.

Wass, J.A.H., Penman, E., Medback, S., Dawson, A.M., Tsiolakis, D., Marks, V., Besser, G.M. and Rees, L.H. (1980): Immunoreactive somatostatin changes during insulin-hypoglycemia and operative stress in man. Clin. Endocrinology 12:269-275.

Westendorf, J.M. and Schonbrunn, A. (1983): Characterization of bombesin receptors in a rat anterior pituitary cell line. J. Biol. Chem. 258:7527-7535.

Wheeler, M.B. (1988): The effect of total parenteral nutrition on pancreatic and gastric endocrine secretion. Ph.D. Thesis. University of British Columbia, Vancouver.

Willems, G., Vansteenkiste, Y. and Limbosch, J.M. (1972): Stimulating effect of gastrin on cell proliferation kinetics in canine fundic mucosa. Gastroenterology 62:583-589.

Wolfe, M.M., Jain, D.K., Reel, G.M. and McGuigan, J.E. (1984): Effects of carbachol on gastrin and somatostatin release in rat antral tissue culture. Gastroenterology 87:86-93.

Woll, P.J., Coy, D.H. and Rozengurt, E. (1988): [Leu^{13}-ψ-CH_2NH-Leu^{14}] bombesin is a specific bombesin receptor antagonist in Swiss 3T3 cells. Biochem. Biophys. Res. Comm. 155:359-365.

Woll, P.J. and Rozengurt, E. (1988): Bombesin and bombesin antagonists: studies in Swiss 3T3 cells and human small cell lung cancer. Br. J. Cancer 57: 579-586.

Wood, S.M., Jung, R.T., Webster, J.D., Ghatei, M.A., Adrian, T.E., Yanaihara, N., Yanaihara, C. and Bloom, S.R. (1983): The effect of the mammalian neuropeptide, gastrin releasing peptide (GRP), on gastrointestinal and pancreatic hormone secretion in man. Clin. Sci. 65:365-371.

Wormsley, K.G. and Grossman, M.I. (1965): Maximal histalog test in control subjects and patients with peptic ulcer. Gut 6:427-435.

Wyllie, J.H., Stagg, B.H. and Temperley, J.M. (1974): Inactivation of pentagastrin by the liver. Br. J. Surg. 61:22-26.

Yalow, R.S. and Berson, S.A. (1960): Immunoassay of endogenous plasma insulin in man. J. Clin. Invest. 39:1157-1175.

Yalow, R.S. and Berson, S.A. (1970a): Size and charge distinctions between endogenous human plasma gastrin in peripheral blood and heptadecapeptide gastrins. Gastroenterology 58:609-615.

Yalow, R.S. and Berson, S.A. (1970b): Radioimmunoassay of gastrin. Gastroenterology 58:1-14.

Yalow, R.S. and Berson, S.A. (1971): Further studies on the nature of immunoreactive gastrin in human plasma. Gastroenterology 60:203-214.

Yalow, R.S. and Berson, S.A. (1972): And now, "big big" gastrin. Biochem. Biophys. Res. Commun. 48:391-395.

Yalow, R.S. and Wu, N. (1973): Additional studies on the nature of big big gastrin. Gastroenterology 65:19-22.

Yamada, T., Soll, A.H., Park, J. and Elashoff, J. (1984): Autonomic regulation of somatostatin release: studies with primary cultures of canine fundic mucosal cells. Am. J. Physiol. 247:G567-G573.

Yanaihara, N., Yanaihara, C., Mochizuki, T., Iwahara, K., Fujita, T. and Iwanaga, T. (1981): Immunoreactive GRP. Peptides Suppl. 2(2):185-191.

Zachary, I. and Rozengurt, E. (1985): High-affinity receptors for peptides of the bombesin family in Swiss 3T3 cells. Proc. Natl. Acad. Sci. 82:7616-7620.

Zollinger, R.M. and Ellison, E.M. (1955): Primary peptic ulceration of the jejunum associated with islet cell tumours of the pancreas. Ann. Surg. 142:709-728.

Zucker, L.M. (1965): Hereditary obesity in the rat associated with hyperlipemia. Ann. N.Y. Acad. Sci. 131:447-458.

Zucker, L.M. and Antoniades, H.N. (1972): Insulin and obesity in the Zucker genetically obese rat "fatty". Endocrinology 90:1320-1330.

Zucker, L.M. and Zucker, T.F. (1961): Fatty, a new mutation in the rat. J. Hered. 52:275-278.

Zucker, T.F. and Zucker, L.M. (1962): Hereditary obesity in the rat associated with high serum fat and cholesterol. Proc. Soc. Exp. Biol. Med. 110:165-171.

APPENDIX I
CHEMICAL SOURCES

CHEMICAL	SOURCE
Acetic acid (glacial)	BDH
Acetone	BDH
Ammonium acetate	Baker
Ammonium chloride	Fisher
Amphotericin B (Fungizone®)	Gibco
Aprotinin (Trasylol®)	Miles Pharmaceuticals
Bacto-peptone	Difco
Basal medium Eagle (powder)	Gibco
Bombesin	Institut Armand Frappier
Bovine serum albumin (fraction V)	Sigma
Bovine serum albumin (RIA grade)	Sigma & Miles
Calcium chloride	Fisher
Calcium ionophore (A23187)	Sigma
Carbon decolourizing neutral (activated charcoal)	Fisher
Chloramine T	Eastman Kodak
Collagenase (Type I & Type XI)	Sigma
Cytosine ß-D-arabinofuranoside	Gibco
[D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11}] substance P	Institut Armand Frappier
Deoxyribonuclease	Sigma
Dextran (clinical grade)	Sigma
Dextran T-70	Pharmacia
D-glucose (powder)	Fisher
Diaminobenzidine	BDH
Dimethylsulfoxide (DMSO)	Sigma
Disodium ethylenediaminetetraacetate (EDTA)	BDH
Dithiothreitol	Sigma
Dulbecco's modified Eagle's medium	Gibco & Sigma
Ethanol	Commercial Alcohol
Fetal calf serum	Gibco
Formaldehyde (histological grade)	Fisher
Forskolin	Sigma
Gastrin I (synthetic human)	Research Plus
Gentamycin sulphate	Sigma
Glucose (50% commercial solution)	Abbott
Glucose oxidase	Sigma
Glutamine	Sigma
Hank's balanced salt solution (powder)	Gibco
Hematoxylin	Fisher
Heparin	Fisher
HEPES	BDH
Hydrochloric acid	Fisher
Hydrocortisone	Sigma
Hydrogen peroxide (histological grade)	Fisher
Imidazole	Sigma

CHEMICAL	SOURCE
Insulin	Sigma
Lithium carbonate	Fisher
Magnesium sulphate	Fisher
Nerve growth factor	Collaborative Research
Paraffin (paraplast)	Monoject
Permamount	Fisher
Petroleum ether	Fisher
Phenol red	Sigma
Phorbol esters (α- & ß-PMA)	Sigma
Picric acid	BDH
Potassium chloride	Fisher
Potassium phosphate (monobasic)	Fisher
QUSO microfine silica, G-32	Philadelphia Quartz
Rat chow (pelleted and powder forms)	Purina
Sephadex CM-52	Pharmacia
Sephadex DEAE A-25	Pharmacia
Sodium acetate	Fisher
Sodium azide	Baker
Sodium barbital	Fisher
Sodium bicarbonate	Fisher
Sodium chloride	Fisher
Sodium hydroxide	Fisher
Sodium ^{125}iodide	Amersham
Sodium merthiolate	Eastman Kodak
Sodium metabisulphite	Fisher
Sodium pentobarbital	MTC Pharmaceuticals
Sodium phosphate (monobasic & dibasic)	Fisher
Sodium pyruvate	Gibco
Somatostatin (synthetic cyclic and Tyr^1)	Peninsula
Tris-HCl	Sigma
Triton X-100	Fisher
Xylene (histological grade)	Fisher

PUBLICATIONS

PAPERS:

1. Campos RV, Wheeler MB, Pederson RA, Buchan AMJ, and Brown JC. The effect of total parenteral nutrition (TPN) on gastrin release in the rat. Regulatory Peptides 19: 281-289, 1987.

2. Pederson RA, Campos RV, Wheeler MB, Chan CB, Buchan AMJ and Brown JC. Gastrin release in obese Zucker rats. Regulatory Peptides 24: 131-142, 1989.

3. Campos RV, Buchan AMJ, Coy DH, Pederson RA and McIntosh CHS. Inhibition of bombesin-stimulated gastrin release from isolated canine G cells by bombesin antagonists. Canadian Journal of Physiology and Pharmacology, In press.

ABSTRACTS:

1. Campos RV, Buchan AMJ, Meloche RM, Pederson RA. Regulation of gastrin secretion from isolated human antral G cells maintained in short term culture. Gastroenterology 96(5):A680, Abstracts from the 90th Annual Meeting of the American Gastroenterological Association, Washington, D.C., 1989.

2. Campos RV, Coy DH and Pederson RA. Inhibition of bombesin-stimulated gastrin release from isolated canine G cells by bombesin antagonists. Canadian Journal of Physiology and Pharmacology, 67:Av, 1989.

3. Campos RV and Pederson RA. The role of gastrin releasing peptide/bombesin in the hypergastrinemia of the obese Zucker rat. Canadian Journal of Physiology and Pharmacology 66: Aix, 1988.

4. Wheeler MB, Campos RV and Pederson RA. The effect of total parenteral nutrition (TPN) on insulin release in the rat. Canadian Journal of Physiology and Pharmacology 66: Axl, 1988.

5. Campos RV, Wheeler MB, Pederson RA, Buchan AMJ and Brown JC. The effect of total parenteral nutrition (TPN) on gastrin release in the rat. Physiology Canada 17: 180, 1986.

6. Wheeler MB, Campos RV and Pederson RA. The effect of glucose and arginine on insulin secretion after short term total parenteral nutrition. Physiology Canada 17: 252, 1986.