STUDIES ON ASPECTS OF AUTONOMIC NEUROPATHY IN NON INSULIN DEPENDENT DIABETES

by

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For my mother and father and to the memories of my brother David and my grandmother.

ABSTRACT

Autonomic neuropathy (AN) is a well-recognized complication of diabetes mellitus. It has multiple clinical features and is often associated with debilitation and an increased mortality. However, there appears to be a paucity of knowledge with regard to its effects on hormone release and its biochemical sequelae. Recent evidence has accumulated suggesting a role for the autonomic nervous system in regulating release of a number of gastrointestinal hormones. It is therefore possible that autonomic dysfunction may be associated with altered gastrointestinal hormone release and indeed various studies have revealed abnormalities in the release of these peptides in diabetes mellitus.

The prime purpose of this thesis was to provide evidence which might contribute to an understanding of the interrelationship between non insulin dependent diabetes (NIDDM), gastrointestinal hormone release and the autonomic nervous system. Thus studies to evaluate basal and stimulated release of the gastrointestinal hormones glucagon (IRG), pancreatic polypeptide (PP), gastrin, gastric inhibitory polypeptide (GIP) and somatostatin (SRIF-LI) have been undertaken in NIDDM. Studies were also carried out to assess whether AN may be implicated in abnormal release of these five gut hormones in diabetes and to determine if one of the hormones, PP, might be used as a marker for AN. Finally the effect of dietary fibre on glucose tolerance was studied to assess whether its effect would be altered in the presence of AN in NIDDM.

All studies were undertaken in NIDDM with and without AN and matched controls whenever possible. The studies were designed to determine basal concentrations of the hormones on three separate occasions: after a 12 hr overnight fast; after a 12 hr insulin infusion to induce fasting normoglycaemia; and after overnight gastric aspiration to ensure complete emptying of the stomach. Concentrations of the five hormones were measured in response to insulin hypoglycaemia, with and without prior induction of fasting normoglycaemia and after ingestion of a mixed meal. The effects of dietary fibre supplementation on glucose tolerance were evaluated following the ingestion of two test meals, one with 5 g guar and 5 g pectin added and the other without.

The results of the studies on basal hormone release revealed that in the diabetics with AN, an elevated basal plasma IRG concentration was present, which could not be corrected by the prior establishment of fasting normoglycaemia or by complete gastric emptying. Basal serum PP levels were also elevated, but only after the establishment of fasting normoglycaemia. Abnormalities in

stimulated hormone release in the presence of AN were demonstrated as follows: impairment of the plasma IRG response to hypoglycaemia which could be corrected by the prior establishment of fasting normoglycaemia; a non-correctable uniform impairment of the serum PP response to hypoglycaemia; a delayed early serum PP peak following ingestion of the mixed meal and a delayed and impaired plasma GIP response to the meal. The addition of fibre to a test meal did not affect glucose or gut hormone responses to the meal in the diabetics with AN in contrast to those without AN who showed improved glucose tolerance.

When the diabetics were regarded as a single group abnormalities in basal and stimulated hormone release were present. These were: elevated basal plasma IRG concentrations, impaired serum PP responses to hypoglycaemia and impaired plasma GIP responses to ingestion of a mixed meal.

These findings suggest that AN in NIDDM may be associated with altered release of certain gastrointestinal hormones and indicate that AN may be relevant in the abnormal hormone release observed in NIDDM. Further the findings suggest that serum PP responses to insulin hypoglycaemia may have a role as a biochemical marker for AN and that dietary fibre is ineffective in improving glucose tolerance in NIDDM with AN, at least in the short term.

These studies, therefore, provide early evidence of an interrelationship between NIDDM, gastrointestinal hormone release and the autonomic nervous system and reveal the importance of determining the existence or absence of AN in groups of subjects in whom hormone release is to be studied. Furthermore the studies suggest that the serum PP response to insulin hypoglycaemia may indeed be useful as a marker for AN and that fibre supplementation of diets may have a real role in the treatment of diabetes which may however be limited in cases complicated by AN.

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Abstract

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ABBREVIATIONS

AN	autonomic neuropathy
APP	avian pancreatic polypeptide
BPP	bovine pancreatic polypeptide
CCK	cholecystokinin
^{o}C	degree celcius
cm	centimetre
cpm	counts per minute
ECG	electrocardiograph
EDTA	disodium ethylene-diamine-tetra-acetic acid
fig	figure
g	gram
g	gravity
GIP	gastric inhibitory polypeptide
GLI	glucagon-like immunoreactivity
hr	hour
^{125}I	^{125}Iodine
IDDM	insulin dependent diabetes
IRG	immunoreactive pancreatic-type glucagon
kg	kilogram
l	litre
M	molar (mole per litre)
mCi	millicurie
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mm H_2O	millimetres of water
mm Hg	millimetres of mercury
µg	microgram
µl	microlitre
µg/kg	microgram per kilogram
µU/ml	microunits per millilitre
NIDDM	non insulin dependent diabetes
NSB	non specific binding
pg	picogram
pg/ml	picograms per millilitre
pmol/l	picomoles per litre
PN	peripheral neuropathy
PP	pancreatic polypeptide

sec	second
SEM	standard error of the mean
SRIF	somatostatin
SRIF-LI	somatostatin-like immunoreactivity
Tc	total counts
tyr	tyrosine
U	units
U/hr	units per hour
U/ml	units per millilitre
U/kg	units per kilogram
VIP	vasoactive intestinal peptide
x	mean
yr	year

PREFACE

The work described in this thesis originated from a study on impotence in diabetes conducted by investigators of the University of Cape Town/Medical Research Council Endocrine and Diabetes Research Group. That study was directed towards an evaluation of the incidence and pathogenetic factors of impotence in male patients attending the Groote Schuur Hospital Diabetic Clinic.

On analysis of some of the early data it became apparent that differences existed in the release of certain gut hormones (which were measured coincidentally) in diabetics with autonomic neuropathy as compared to diabetics without autonomic neuropathy. This observation, together with the realization that previously described abnormalities in the release of various hormones had been found in patients in whom no reference to the existence of autonomic neuropathy had been made, led to the commencement of specific studies to evaluate gastrointestinal hormone release in a particular type of diabetic, namely non insulin dependent diabetics, in the presence and absence of autonomic neuropathy. In addition, the recent surge of interest shown in the effects of dietary fibre on glucose tolerance; the suggestions of its use as an adjunct to the therapy of diabetes and the observation of altered gastrointestinal hormone release in diabetics with autonomic neuropathy led to the question whether the effect of dietary fibre on glucose tolerance would be altered in the presence of autonomic neuropathy. These are the problems which were researched in this study.

While these studies were in progress three reports evaluating the effect of autonomic neuropathy on the release of the gastrointestinal hormones glucagon, gastrin, and pancreatic polypeptide in insulin dependent diabetics were published in the literature. The results in the first two reports contrasted with the findings in the present study, but the third study is in agreement. Furthermore the authors of the last study suggested that impairment of the serum PP response to hypoglycaemia might be used as a sign of autonomic neuropathy, a conclusion reached in this study. These reports are included in the literature review which extends as far as December 1979 on pages 39-41.

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CHAPTER I

REVIEW OF LITERATURE

"Although diabetic neuropathy is probably the most common complication of diabetes it remains the least understood, the least explored and the least appreciated."

Max Ellenberg (1976)

The description by Ellenberg of diabetic neuropathy or neuropathies as he preferred to call them, encompassed both autonomic and peripheral neuropathies. Whilst the clinical manifestations of both forms of neuropathy are well recognized and their pathogenesis, particularly that of peripheral neuropathy (PN) has received widespread attention, the hormonal effects and biochemical consequences of autonomic neuropathy (AN) remain largely unknown.

In this work the studies undertaken to investigate diabetic AN and its effect on the release of certain of the gastrointestinal hormones are described. In view of the paucity of literature pertaining to this relationship, the review of the literature has been designed to provide a comprehensive but not exhaustive background to the studies undertaken.

The literature review comprises five sections :-

(1) a brief resumé of the five gastrointestinal hormones studied namely glucagon (IRG), pancreatic polypeptide (PP), somatostatin (SRIF or SRIF-LI), gastric inhibitory polypeptide (GIP) and gastrin.

(2) autonomic regulation of the release of the five hormones.

(3) diabetes mellitus and the five hormones.

(4) diabetic autonomic neuropathy.

(5) dietary fibre.

A brief historical note prefaces each part of the review.

SECTION 1 : AN OVERVIEW OF THE GASTROINTESTINAL HORMONES

Introduction

In 1902 Bayliss and Starling demonstrated that brisk secretion of pancreatic juice was elicited by the instillation of hydrochloric acid into a

Table I.1 : Gastrointestinal Hormones

- insulin
- glucagon
- gastrin
- cholecystokinin
- secretin
- pancreatic polypeptide
- gastric inhibitory polypeptide
- enteroglucagon
- motilin
- somatostatin

Table I.2 : Peptides of the brain and gut

- Substance P
- Somatostatin
- Vasoactive intestinal peptide
- Enkephalin
- Bombesin
- Gastrin
- Cholecystokinin

denervated loop of small intestine. Realizing the implications of their findings, Bayliss and Starling suggested that an unidentified blood-borne "chemical messenger" was released from the acidified intestine to mediate the observed response. However, more than fifty years elapsed before the significance of the gastrointestinal hormones was realized. The most likely reasons for the time lapse were the difficulties encountered in isolating and identifying the hormones as well as the considerable controversy over the very existence of some of them. The situation started to change in the early sixties and now almost twenty years later the mucosa of the gastrointestinal tract is known to be the largest endocrine gland in the body.

The gastrointestinal hormones and related peptides are located in endocrine cells dispersed throughout the mucosa of the gastrointestinal tract. The widespread nature of this distribution has made it almost impossible to study the actions of the gastrointestinal hormones in the classical manner, i.e. surgical removal of the source of hormone release and examination of the effect of its absence. Only a few of the numerous peptides localized to the gastrointestinal tract however, have been found to fit the criteria for a gastrointestinal hormone as indicated in Table I.1 (Johnson, 1977; Bloom and Polak, 1978; Grossman, 1979; Pimstone, Sheppard, Shapiro, et al, 1979; Schusdziarra, Harris, Arimura and Unger, 1979).

The functions of the gastrointestinal hormones are numerous, but fall into a few major categories : stimulation or inhibition of water, electrolyte, enzyme and endocrine secretion from the gut, stimulation or inhibition of gut motility, trophic effects, regulation of metabolism and effects on intestinal absorption (Johnson, 1977).

A recent exciting development in the field of gastrointestinal endocrinology has been the finding of many peptides initially identified in the gut but subsequently found in the brain and conversely peptides of the brain which were later found in the gut. The peptides shown in Table I.2 have been localized to nervous tissue in the brain and in the gut as well as to specific endocrine cell types in the gut (Bloom and Polak, 1978; Fuxe, Anderson, Hökfelt, et al, 1979; Loonen and Soudijn, 1979; Polak and Bloom, 1979).

Originally gut hormones were thought to exert their actions by passing via the bloodstream to a distant site and eliciting a physiological response at their destination. However, there appear to be two further major ways in which gut hormones or peptides (since they do not all fulfill the criteria for hormones)

may act; as a peptidergic neurotransmitter and/or as a locally acting paracrine substance (Bloom and Polak, 1978). Thus nervous control, local control and control via the circulation may form a triple control system by which gastro-intestinal functions are regulated (Bloom and Polak, 1978).

Since only five gut hormones have been selected for study, not all the recognized gut hormones are discussed, but the review is limited to those chosen for study. They are IRG, PP, gastrin, GIP and SRIF. In this section an overview of the literature pertaining to the general characteristics of the five hormones is given.

I (i) GLUCAGON (IRG)

In 1921 Banting and Best noticed that a mild but reproducible hyper-glycaemia preceded the hypoglycaemic effect of injecting pancreatic extracts into depancreatized dogs. These workers thought that the hyperglycaemic phenomenon was due to liberation of epinephrine and did not pursue the subject. Shortly afterwards, Murlin, Clough, Gibbs and Stokes, in 1923 reported that aqueous pancreatic extracts caused a marked elevation of blood sugar levels in depancreatized dogs. They believed this effect was due to a glucogenic hormone which they named Glucagon or mobiliser of glucose.

Glucagon, which initially was considered to occur only in the islets of Langerhans in the pancreas, was subsequently also found in extrapancreatic tissue. Moreover glucagon was shown to have multiple rather than single form. The extrapancreatic glucagons form a heterogenous group of peptides which are for the most part, poorly characterized and have been localized primarily to the stomach, duodenum, ileum and colon depending on the species studied (Polak, Bloom, Coulling and Pearse, 1971; Larsson, Holst, Hakanson and Sundler, 1975; Grimelius, Capella, Buffa, et al, 1976).

The extrapancreatic glucagons are glucagon-like in that they may possess glucagon-like immunoreativity (GLI) but may or may not have glucagon-like biological activity (Holst, 1978). The availability of specific immunological techniques has enabled the differentiation of GLI from immunoreactive pancreatic-like glucagon (IRG). However, it has been well-established that one peptide which is indistinguishable from true pancreatic glucagon, may be found in extrapancreatic gastro-intestinal tissue in numerous species (Holst, 1978). A definite physiological role for these extrapancreatic glucagons has not been established as yet.

Further discussion of glucagon in this work applies solely to immunoreactive pancreatic-like glucagon (IRG) which is itself heterogenous. The predominent form of IRG is a straight chained 29 amino acid polypeptide with a molecular weight of 3500, but other molecular weight forms have also been found (Valverde, Villanueva, Lozano and Marco, 1974; Weir, Knowlton and Martin, 1975; Conlon, Ipp and Unger, 1978). By histochemical techniques IRG has been localized to the alpha cell, which is generally found on the periphery of the pancreatic islets (Munger, 1972) as well as in the gastric mucosa in certain species excluding man (Larsson, Holst, Hakanson and Sundler, 1975; Sundler, Alumets, Holst, Larsson and Håkanson, 1976).

IRG is a well recognized hormone but perhaps its most important physiological action is its role in the maintenance of glucose homeostatis. Its effects are opposed by those of insulin. IRG stimulates glucose output from the liver by activation of glycogenolysis with resultant inhibition of glycogen synthesis and stimulation of gluconeogenesis (Park and Exton, 1972). IRG also plays a role in lipid metabolism by promoting free fatty acid and glycerol mobilization from adipose tissue and the liver (Lefebvre, 1972). In addition IRG is an important regulator of amino acid metabolism (Marliss, Aoki and Cahill, 1972).

In summary, IRG is a well recognized metabolic hormone which is primarily localized to the pancreas and has its major role as one of the regulating hormones of carbohydrate homeostasis.

I (ii) PANCREATIC POLYPEPTIDE (PP)

PP, a 36 amino acid residue, was discovered independently by Kimmel, Pollock and Hazelwood (1968) and by Chance (1972). In the course of purifying chicken insulin Kimmel and co-workers extracted and purified a polypeptide which was named avian pancreatic polypeptide (APP). Chance isolated bovine pancreatic polypeptide (BPP) during the purification of bovine insulin and subsequently isolated and characterized a similar peptide from pig (PPP), sheep (OPP) and man (HPP) (Lin and Chance, 1974).

By immunohistochemistry PP has been localized to a population of endocrine cells in the islets of Langerhans, distinctive from the IRG (A), insulin (B) and SRIF-LI (D) cells. Further in most species PP cells also occur in the exocrine parenchyma of the pancreas (Larsson, Sundler and Hakanson, 1976; Heitz, Polak, Bloom and Pearse, 1976; Pelletier and Leclerc, 1977), and predominate in a specific lobe thereof (Larsson, Sundler and Hakanson, 1976; Orci, Malaisse-Lagae, Baetens and Perrelet, 1978). In addition small

quantities of PP have been found throughout the gastrointestinal tract (Adrian, Bloom, Bryant, et al, 1976).

At present the physiological function of PP is not known. However studies with APP in the chicken and with BPP in the dog have led to suggestions that it may have gastrointestinal and possibly metabolic functions. In the chicken infused APP induced reductions in liver glycogen, plasma glycerol, free fatty acids and amino acids, an increase in plasma triglyceride and a striking stimulation of H^+ and pepsin secretion. Although large doses of APP were used in the studies it has been assumed that the findings may have some meaning with regard to physiological activity (Kimmel, Pollock and Hayden, 1978). In the dog low pharmacological doses of BPP suppressed pancreatic secretion, enhanced gastric emptying and intestinal transit and initially stimulated but later inhibited gut motility (Lin and Chance, 1978; Taylor, Solomon, Walsh and Grossman, 1979). BPP has also been infused in man when suppression of plasma motilin levels, pancreatic exocrine secretion and bilirubin output were noted and the promotion of gallbladder storage (Floyd, 1979).

Thus PP has been localized primarily to endocrine cells and the exocrine parenchyma of the pancreas. While the physiological function of PP in man is not known it would appear that its biological actions are directed towards moderation of the rate or amount of pancreatic exocrine secretion and bile that reach the duodenum.

I (iii) GASTRIN

As early as 1905 Edkins reported that extracts of antral mucosa stimulated acid secretion when injected intravenously into anaesthetized cats (Edkins, 1905a) and named the active principal gastrin (Edkins, 1905b). His extracts however, contained histamine as well as gastrin and for many years there was controversy as to whether they contained an acid stimulant other than histamine. Finally in 1964, all doubts were dispelled when Gregory and Tracey isolated pure gastrin from the antral mucosa of the dog. The compounds isolated by Gregory and Tracey were two identical 17 amino-acid polypeptides named Gastrin I and II, differing only in the presence of a sulphate group on the tyrosine of Gastrin II. These gastrin heptadecapeptides have since been isolated from the antral mucosa of other species including man (Gregory, Tracey and Grossman, 1966). Subsequently further molecular forms of gastrin in tissues and serum have been isolated (Yalow and Berson, 1972; Rehfeld, Stadil, Malmstrøm and Miyata, 1975; Walsh and Grossman, 1975) but the heptadecapeptide appears to be the most potent form (Walsh and Grossman, 1975).

By immunohistochemistry gastrin containing cells (G cells) have been localized largely to the pyloric glands of the antrum, particularly to the mid-portion of these glands (McGuigan and Greider, 1971). An extensive quantitative study on the gastrin content of tissues in the gut has been carried out and in man it has been estimated that the duodenum, due to its greater bulk may contain as much gastrin as the antrum (Nilsson, Yalow and Berson, 1973). Controversy exists as to whether gastrin is found in the pancreas. Very small amounts of gastrin have been extracted from human pancreas by some investigators, and some workers have found G cells in the pancreas by immunohistochemistry, whereas other investigators have failed to confirm these observations using extraction and immunohistochemical techniques (Walsh and Grossman, 1975).

Gastrin in pharmacological doses has been found to have numerous and diverse actions on water, electrolyte and enzyme secretion, absorption of nutrients, bowel motility, release of hormones, gastric blood flow and stimulation of protein synthesis in gastrointestinal tissues (Walsh and Grossman, 1975). However, few of these actions have been demonstrated at physiological concentrations of gastrin. The accepted physiological actions of gastrin are limited to : stimulation of gastric acid and pepsin secretion, a stimulatory effect on the lower oesophageal sphincter and stimulation of DNA, RNA and protein synthesis in the stomach, duodenum, ileum and colon (Walsh and Grossman, 1975; Johnson, 1977).

In summary, gastrin is a gastrointestinal hormone localized primarily to the gastric antrum and the duodenum in man. Its major functions are promotion of gastric acid and pepsin secretion and a trophic action on certain gastro-intestinal tissues.

I (iv) GASTRIC INHIBITORY POLYPEPTIDE (GIP)

The possible existence of a polypeptide derived from porcine duodenum and jejunum with the biological actions of inhibiting acid and pepsin secretion and inhibiting motor activity was postulated by Brown and Pedersen (1970). Support for this hypothesis was provided by the isolation of a polypeptide during the purification of cholecystokinin-pancreozymin which inhibited acid and pepsin secretion from the canine stomach. The polypeptide named GIP was found to be a straight-chain, containing 43 amino acids and to have a molecular weight of 5105 (Brown, Mutt and Pedersen, 1970; Brown, 1971; Brown and Dryburgh, 1971). GIP has been found to circulate in two forms, one with a molecular weight of 5105 and the other as a larger molecular weight species (Brown, Dryburgh, Ross and Dupré, 1975). The N (amino) terminal amino acid

sequence of GIP appears to be similar to that of glucagon, secretin and vasoactive intestinal polypeptide (VIP) (Brown, Dryburgh, Ross and Dupré, 1975).

By immunohistochemistry GIP has been localized to cells situated predominantly in the midzone of the glands in the duodenum and to a lesser degree in the jejunum in man (Polak, Bloom, Kuzio, Brown and Pearse, 1973). This distribution of GIP containing cells has been confirmed following radioimmunoassay of tissue extracts (Thomas, Shook, O'Domsio, et al, 1977).

The known actions of GIP in man and animals are diverse. Effects on the gastrointestinal tract and on hormone secretion have been demonstrated. In the dog GIP causes inhibition of pentagastrin- and histamine- induced gastric acid secretion (Pederson, Brown, 1972). In man however, the role of GIP on gastric secretion is not as well documented. GIP infused at doses calculated to achieve supraphysiological concentrations inhibited pentagastrin-stimulated gastric acid secretion (Brown, Dryburgh, Frost, Otte and Pederson, 1978). Thus the relevance of this finding in physiological terms is uncertain. GIP is a well accepted insulinotropic hormone. In vitro, in isolated islets, and in vivo in man, GIP is a potent stimulator of insulin release (Dupré, Ross, Watson and Brown, 1973; Crockett, Cataland, Falko and Mazzaferri, 1976; Pederson and Brown, 1976). Indeed GIP was originally postulated to be the incretin ie. an enteric peptide released during glucose absorption and a stimulator of insulin secretion (McIntyre, Holdsworth and Turner, 1965), but it is now held that GIP may be only one of numerous incretins (Creutzfeldt, 1979). In addition GIP has been shown to induce IRG release in the isolated perfused rat and canine pancreas (Pederson, Dryburgh, Brown and Dupré, 1978; Adrian, Bloom, Hermansen and Iverson, 1978).

Thus GIP is a polypeptide hormone found in the duodenum and jejunum. It has numerous biological actions involving motility and secretion in the gastrointestinal tract and may have physiological relevance as an incretin.

I (v) SOMATOSTATIN (SRIF)

In 1973 whilst searching for growth hormone releasing factor, Brazeau, Vale, Burgus, et al, isolated and structurally identified a peptide capable of inhibiting growth hormone release from the ovine hypothalamus. The peptide was named somatotrophin release inhibiting factor or somatostatin and was shown to be a cyclic tetradecapeptide (Brazeau, Vale, Burgus, et al, 1973).

Following the original extraction of SRIF from the hypothalamus, it was subsequently found in other sites in the rest of the nervous system, ie. in

various areas of the brain, in the spinal cord and spinal ganglia (Hökfelt, Schultzberg, Johansson, et al, 1978; Luft, Efendic and Hökfelt, 1978) as well as in peripheral sympathetic ganglia (Hökfelt, Elfvin, Elde, et al, 1977). Outside the nervous system, SRIF has been found in various sites in the gastro-intestinal tract; in the antrum of the stomach where the largest number of SRIF cells are seen in man, in the D cells at the periphery of the islets in the pancreas, in the duodenum, jejunum and to a lesser extent in the colon (Arimura, Sato, Du Pont, Nichi and Schally, 1975; Dubois, 1975; Hökfelt, Efendic, Hellerström, et al, 1975; Orci, Baetens, Dubois and Rufener, 1975; Polak, Pearse, Grimelius, Bloom and Arimura, 1975).

The widespread distribution and actions of SRIF have led to suggestions that it may act as both a neuromodulator and a hormone (Luft, Efendic and Hökfelt, 1978; Pimstone and Berelowitz, 1978). While SRIF has multiple physiological actions, only its effects in the gastrointestinal tract will be referred to. SRIF has actions on endocrine and exocrine pancreatic and gastric secretion, gastrointestinal hormone secretion, gut motility and gut absorption (Konturek, 1976; Gomez-Pan and Hall, 1977; Gerich and Patton, 1978). Basal and stimulated insulin and IRG secretion is reduced by somatostatin as are plasma glucose levels (Gomez-Pan and Hall, 1977; Gerich and Patton, 1978). The hypoglycaemic effect of SRIF is transitory and is thought to be a consequence of IRG suppression (Sakurai, Dobbs and Unger, 1974). Unger and Orci (1977) have postulated that paracrine inter-relationships between insulin, IRG and SRIF in pancreatic islets may modify the resultant secretion of insulin and IRG and may thus account for some of the abnormalities seen in the secretion of these hormones in diabetes.

Somatostatin also impairs the release of a number of gastrointestinal peptides; GIP, CCK, secretin, motilin, GLI, IRG, VIP, PP and gastrin (Gomez-Pan and Hall, 1977; Gerich and Patton, 1978). Further, SRIF inhibits absorption of xylose, glucose and certain mono- and disaccharides (Wahren and Felig, 1976; Wagner, Hengst, Jansen and Gerlach, 1978) leading to the suggestion that SRIF may regulate nutrient homeostasis by modulating the absorption of nutrients from the intestine (Schusdziarra, Harris, Arimura and Unger, 1979). In addition SRIF reduces gut motility and inhibits gastric acid and pepsin secretion (Gomez-Pan and Hall, 1977; Gerich and Patton, 1978).

Thus SRIF is a peptide found primarily in central and peripheral nervous tissue and in endocrine cells of the gastrointestinal tract. This localization of SRIF together with its multiple actions have given rise to suggestions that

it may act as a hormone as well as a neuromodulator. In the gastrointestinal tract the major functions of SRIF are thought to be regulation of nutrient homeostasis and co-ordination of endocrine and exocrine secretion.

In this section a brief review of the literature was given to provide an overview of the 5 gastrointestinal hormones selected for study. Special emphasis was placed on the localization of the endocrine cells of origin of the hormones and the physiological functions of the hormones. In the next section the literature regarding regulation of the release of the 5 gastrointestinal hormones by the autonomic nervous system is reviewed.

SECTION 2 : AUTONOMIC NERVOUS CONTROL OF GASTROINTESTINAL HORMONE SECRETION

"I propose the term autonomic nervous system for the sympathetic system and applied nervous system of the cranial and sacral nerves and for the local nerves of the gut."

Langley, 1898

Introduction

Present knowledge has considerably extended the confines of the original description of the autonomic nervous system. Although sympathetic and parasympathetic pathways are probably the major components of the autonomic nervous system recent work has identified neuropeptides other than the classic neurotransmitters in nerves and ganglia of this system (Uvnäs-Wallensten, Efendic and Luft, 1978; Larson and Rehfeld, 1979; Loonen and Soudijn, 1979; Lundberg, Hökfelt, Kewenter, et al, 1979; Polak and Bloom, 1979). In addition immunocytochemical studies have revealed nerves in the gut containing neuropeptides in their entire length e.g. VIP, substance P (Loonen and Soudijn, 1979; Polak and Bloom, 1979). These observations together with others have led to the suggestion of peptidergic pathways as a component of the autonomic nervous system (Daniel, 1978; Polak and Bloom, 1979).

The autonomic nervous system provides a dual supply to the gastrointestinal tract, similar to other systems. The parasympathetic innervation of the oesophagus through to the pelvic colon derives from the subdiaphragmatic vagal trunks and the pelvic splanchnic nerves. The former supplying fibres to the gut as far as the splenic flexure of the colon and the latter fibres to the rest of the colon. The sympathetic innervation of the gut from the oesophagus through to the pelvic colon arises from the sympathetic trunks, the greater, lesser and lumbar splanchnic nerves. Fibres from the greater and lesser splanchnic nerves pass to the gut, from the oesophagus to the splenic flexure of the colon and fibres from the lumbar splanchnic nerves innervate the rest of the colon. Fibres from these nerves, particularly the sympathetic fibres, generally pass to ganglia such as the coeliac, superior and inferior mesenteric ganglia from which fibres extend to the various organs. However, vagal fibres also pass directly to the organs concerned and terminate in plexuses within their walls, e.g. myenteric and submucosal plexuses in the intestine (Pick, 1970; Barr, 1979; Chusid, 1979).

Thus it is apparent that there is a rich supply of nerves to the stomach, pancreas and small intestine, the primary sites of origin of the endocrine

cells of the 5 gut peptides. The parasympathetic fibres generally pass from plexuses within the walls of the organs and end shortly on smooth muscle and mucosal cells. However, sympathetic fibres generally follow the blood vessels to the organs, thereafter extending to the mucosal cells and smooth muscle (Lockhart, Hamilton and Fyfe, 1974). In addition to the fibres described above, which are all motor, sensory fibres also pass via the vagus and splanchnic nerves and subserve pain and distension in the gut (Lockhart, Hamilton and Fyfe, 1974; Barr, 1979).

Although there can be little doubt regarding the rich neural innervation of the gut, little information is available on direct neural innervation of gastro-intestinal endocrine cells. However there is some suggestive evidence with regard to the pancreas. Indeed a rich innervation of the pancreatic islets was recognized by Langerhans himself (1869). More recent work has revealed nerve fibres at the periphery of the islets and within the centre of the islets. Further nerve terminals of three types have been demonstrated in close association with islet endocrine cells (Woods and Porte, 1974) and microscopy has revealed nerve terminals applied to the surface of the alpha cell (Munger, 1972; Renold, 1972). In addition serotonin and dopamine, both amines, have been found within islet alpha (A) and beta (B) cells (Woods and Porte, 1974). These data indicate that the gut is plentifully supplied with neural fibres and that the pancreatic endocrine cells too are richly innervated. It is feasible that further studies would reveal a similar situation with regard to other endocrine cells of the gut. In addition the innervation of the endocrine cells of the gut by the peptidergic nerve fibres, which are likely to be in plentiful supply, remains to be elucidated.

Having reviewed the autonomic nervous innervation of the gut, at this stage it is pertinent to discuss the neurotransmitters and their agonists and antagonists, for without neurotransmitters nerve impulses could not reach their destination. Neurotransmitters are the chemical mediators of nerve impulse transmission at synapses and a number of criteria should be fulfilled before a substance may be classified as a transmitter (Fahrenkrug, 1979). Although it is perhaps appropriate to mention that few of the classical neuro-transmitters fulfil most of the criteria which are listed below:

(1) The substance must be present in neurons.
(2) The neurons must possess the necessary mechanisms to synthesize the transmitter.
(3) The presence of the various precursors and intermediaries in the synthetic pathway should be demonstrable.

(4) There should be systems for the inactivation of the transmitter.
(5) During stimulation the substance proposed as transmitter must be released from the neurons.
(6) The effect of the transmitter should be mimicked by exogenous application of the substance.
(7) Pharmacological agents which interact with the synaptically released transmitter should interact with the suspected transmitter in an identical manner.
(8) The demonstration of specific receptors for the transmitter substance on postsynaptic membranes.

The principle neurotransmitters at most sympathetic postganglionic endings are adrenaline and noradrenaline. Acetylcholine is the mediator at synapses between pre- and post-ganglionic fibres while dopamine and occasionally 5-hydroxytryptamine are mediators at some postganglionic endings in the sympathetic pathways. The ganglia, both pre- and postganglionic, of the parasympathetic pathways are mediated by acetylcholine (Johnson and Spalding, 1974; Ganong, 1979). In addition, an increasing number of neuropeptides are being found which comply with most of the stringent criteria for neuro-transmitters e.g. VIP and substance P (Fahrenkrug, 1979; Loonen and Soudijn, 1979). Thus the autonomic nervous system may in future be separated into cholinergic, adrenergic and also peptidergic divisions based on the chemical mediator released.

However, further subdivision in the adrenergic system has been recognized. These subdivisions were revealed by Ahlquist (1948) when he found that various catecholamines had two types of responses in physiological systems. He postulated the existence of two types of receptors; namely alpha-adrenergic and beta-adrenergic receptors and classified them according to the order of potency with which they were affected by sympathomimetic drugs. Subsequent developments have demonstrated that each of the types of receptors exist in more than one form (Lands, Arnold, McAuliff, Luduena and Brown, 1967; Wood, Arnett, Clarke, Tsai and Lefkowitz, 1979). The adrenergic receptors are still classified by their different sensitivities to certain drugs. Thus it is apparent that there are various subtypes of adrenergic receptors which mediate distinct physiological functions.

There are two further terms which require discussion. Various drugs may affect acetylcholine as well as adrenergic receptors. These drugs are known as agonists and antagonists. Agonist drugs are those capable of inducing the

response of the particular neurotransmitter while an antagonist reduces the effect of the neurotransmitter or its agonist. These terms have relevance to the discussion in this section.

The innervation of the gut by the autonomic nervous system has been described earlier. While disorders of the alimentary tract due to autonomic lesions may be suspected, few investigations are available which might confirm the existence of dysfunction of autonomic nervous innervation of the gut. However, one of these may prove to be of value in the investigation of the effect of AN on hormone secretion. The standard test of integrity of vagal innervation to the stomach is the gastric acid secretory response to insulin hypoglycaemia, as hypoglycaemia will only cause acid secretion if the vagal supply to the stomach is intact (Hollander, 1946; Johnson and Spalding, 1974). However, hypoglycaemia has subsequently been found to increase circulating catecholamine levels too (Vendsalu, 1960; Garber, Cryer, Santiago, et al, 1976). Thus it might appear that insulin hypoglycaemia activates both the sympathetic and parasympathetic nervous systems and in this way is known to stimulate the release of certain hormones e.g. PP (Adrian, Bloom, Besterman, et al, 1977; Schwartz, Holst, Fahrenkrug, et al, 1978).

Thus investigation of the effect of insulin hypoglycaemia on hormone secretion in AN may shed some light on the possible existence of altered hormone secretion in AN.

A further possible means of assessing dysfunction of the autonomic innervation of the gut relates to the fact that the autonomic nervous system regulates secretion of the gut. Indeed gastric acid secretion in response to a meal is induced by a number of mechanisms, one of which is neuronal (Soll and Grossman, 1978). Cephalic stimuli via the vagus nerves and gastric distension which also activates neural reflexes produce gastric acid secretion (Soll and Grossman, 1978) but also causes secretion of certain hormones e.g. gastrin (Soll and Grossman, 1978) and PP (Taylor, Feldman, Richardson and Walsh, 1978; Schwartz, Stenquist and Olbe, 1979). Ingestion of a meal is a known stimulus for the release of a number of gut hormones e.g. gastrin (Kormen, Soveny and Hansky, 1971), PP (Adrian, Bloom, Bryant, et al, 1976) and IRG (Gerich, Lorenzi, Karam, Schneider and Forsham, 1975). However, whether the release of these hormones relates to the meal per se or solely to activation of the autonomic nervous system, or possibly to a combination of these two factors, is not known. Thus it is conceivable that alterations in the release of certain hormones in the presence of AN may become apparent in their response to meal ingestion. Hence it would appear that both insulin hypoglycaemia and ingestion

of a mixed meal may provide valuable information regarding the effect of AN on gut hormone secretion.

The functions of the autonomic nervous system are numerous and diverse. In the gastrointestinal tract, the area of interest in this work the major actions are on motility and secretion. This action extends throughout the gastrointestinal tract and the actions of the parasympathetic and sympathetic divisions often promote opposite effects. The major functions of the parasympathetic innervation of the gut are the following : to increase peristalsis in the oesophagus, stomach, gall bladder, small and large intestines; to cause relaxation of sphincters in the oesophagus, stomach, small and large intestines; to increase exocrine secretion from the stomach, small and large intestine and possibly from the pancreas, and the conduction of pain impulses from the lower colon. The major functions of the sympathetic innervation of the gut are : diminution of motility in the oesophagus and small intestine; reduction of peristalsis in the stomach, gall bladder, bile ducts and large intestine; relaxation of sphincters in the oesophagus and small intestine; inhibition of exocrine secretion from the stomach, small and large intestines and possibly from the pancreas and conduction of pain impulses from multiple organs (Johnson and Spalding, 1974).

Although the functions of the peptidergic nerves remain unknown they too are likely to be involved in regulation of motility and secretion of the gut. Furthermore it is possible that a major function of peptidergic nerves in regulation of the release of gut hormones may come to light. Nevertheless it is known that sympathetic and parasympathetic pathways have an effect on gut hormone release. In this section literature regarding autonomic nervous regulation of the release of the five gut hormones is reviewed.

2 (i) GLUCAGON

In vivo and in vitro experiments in animals have provided evidence for a neuroregulatory role of IRG release. However, conflicting reports on studies conducted in man have led to uncertainty with regard to the importance of autonomic nervous regulation of IRG release.

Neuroregulatory effects on IRG release have been studied in animals, in vitro in the isolated perfused pancreas and in vivo and support a neuroregulatory role in IRG release. Perfusion of epinephrine and norepinephrine, providing combined alpha and beta adrenergic receptor stimulation, induced a significant increase in plasma IRG levels in the isolated perfused canine pancreas. Stimulation of beta adrenergic receptors by isoproterenol induced a marked increase in plasma

IRG levels which was completely abolished by propranolol, a beta adrenergic receptor antagonist. However, alpha adrenergic blockade did not alter IRG release (Iversen, 1973a). Acetylcholine in the isolated perfused pancreas also induced IRG release which could be abolished by atropine (Iversen, 1973b). These data indicate that adrenergic and cholinergic influences mediate IRG release in vitro.

Animals studied in vivo further support a neuroregulatory role in IRG release. Electrical stimulation of the mixed autonomic nerves innervating the pancreas in unanaesthetized dogs resulted in almost a doubling of the pancreaticouduodenal vein IRG output (Marliss, Girardier, Seydoux, et al, 1973). Electrical stimulation of the peripheral ends of the thoracic vagus nerve in adrenal-ectomized calves, in whom the splanchnic nerves had been sectioned, produced a significant rise in plasma IRG levels. In the normal calf atropine lowered basal plasma IRG levels and also delayed the rise in plasma IRG levels in response to insulin hypoglycaemia (Bloom, Edwards and Vaughan, 1974). In dogs sham feeding increased plasma IRG levels which could be markedly reduced by atropine (Nillson and Uvnäs-Wallensten, 1977). These observations tend to support a role for parasympathetic mediation of IRG release. Studies investigating the effects of the sympathetic nervous system on IRG release in animals have also been reported. Splanchnic nerve stimulation in the adrenalectomized calf, dog, cat and sheep induced a prompt increase in plasma IRG levels (Bloom, Edwards and Vaughan, 1973; Bloom and Edwards, 1975). Further studies in the calf showed that alpha or beta adrenoreceptor blockade did not suppress this elevation (Bloom and Edwards, 1978). In addition electrical stimulation of the ventromedial hypothalamus, the central sympathetic centre, induced an increase in IRG release in the rat (Frohman and Bernardis, 1968). These data thus support a role for neural mediation, both sympathetic and parasympathetic, of IRG release in animals.

The regulation of IRG release by the parasympathetic nervous system has been studied in man but conflicting reports have resulted in a lack of clarity on the subject. Atropine was originally reported to decrease basal plasma IRG levels and to reduce arginine-induced IRG release. Further truncal vagotomy was associated with a reduced rise in plasma IRG levels during insulin hypoglycaemia but did not alter basal plasma IRG levels (Bloom, Vaughan and Russell, 1974). However, a study by Palmer, Werner, Hollander and Ensinck (1979) contradicted these findings. In their study truncal vagotomy too did not alter basal plasma IRG levels. However neither bethanocol chloride, which mimics the effects of acetylcholine at cholinergic nerve terminals, nor edrophonium, a cholinesterase blocking agent, which results in accumulation of acetylcholine at cholinergic

nerve terminals, caused any change in basal plasma IRG concentrations. In addition the plasma IRG response to insulin hypoglycaemia was not influenced by atropine administration or by truncal vagotomy. The reasons for the contradictory observations in the reports by Bloom et al and Palmer et al are not clear as the criteria used in their vagotomized subjects were similar. Although a major point of difference in the two studies was the use of different IRG antisera, it is uncertain whether this factor could be responsible for the discrepancies reported. However, McLoughlin, Hayes, Buchanan and Kelly (1978), using two different glucagon antisera also found that atropine did not alter the IRG response to hypoglycaemia, tending to mitigate against antisera differences as the cause for the discrepant results reported. The effect of parasympathetic blockade on the hyperglucagonaemia induced by exercise was determined by the use of atropine, which caused no alteration in exercise-induced IRG release (Galbo, Christensen and Holst, 1977). While it might seem presumptuous to comment on the role that the parasympathetic nervous system may have on IRG release in man, it seems likely that its role would only be a minor one.

Adrenergic influences on IRG release have also been studied in man and have indeed been found to have an effect. Epinephrine infusion, producing combined alpha and beta receptor stimulation, was associated with a 50% rise in plasma IRG levels (Gerich, Karam and Forsham, 1973). Infusion of isoproterenol, a pure beta receptor agonist, induced a similar elevation of plasma IRG levels which was prevented by propranolol, a beta receptor antagonist, although propranolol itself had no effect on basal IRG release. In addition infusion of methoxamine, an alpha receptor agonist significantly reduced plasma IRG levels while phentolamine, an alpha receptor antagonist increased plasma IRG levels (Gerich, Langlois, Noracco, et al, 1974; Schneider and Forsham, 1974). However, adrenergic agents had no effect on stimulated plasma IRG concentrations induced by insulin hypoglycaemia or exercise. Propranolol and phentolamine did not alter insulin hypoglycaemia-induced IRG release (Walter, Dudl, Palmer and Ensinck, 1974; McLoughlin, Hayes, Buchanan and Kelly, 1978). Further, neither adrenalectomy (Ensinck, Walter, Palmer, Brodaws and Campbell, 1976) nor cervical cord transaction (Palmer, Henry, Benson, Johnson and Ensinck, 1976) reduced the plasma IRG response to hypoglycaemia. In addition IRG released during exercise was not altered by phentolamine administration (Galbo, Christensen and Holst, 1977). Thus while adrenergic mechanisms appear to have an effect on basal IRG release no associated effect was apparent on stimulated IRG release induced by exercise or insulin hypoglycaemia in man.

Dopaminergic agents have also been shown to effect IRG release (Rayfield, George, Eichner and Hsu, 1975; Leblanc, Lachelin, Abu-Fadil and Yen, 1977).

L-Dopa is a catecholamine precursor and could thus alter IRG release via catecholamine stimulation or may itself have a direct action on dopaminergic receptors. Dopamine in man increased plasma IRG levels and this increase was not antagonized by alpha or beta adrenergic receptor blocking agents (Lorenzi, Karam, Tsalikian, et al, 1979). Thus dopaminergic mechanisms too may play a part in the mediation of IRG release.

In animals there is fairly conclusive evidence of a neuroregulatory role via both the sympathetic and parasympathetic nervous systems of IRG release. However, in man the parasympathetic nervous system appears to play only a minor role, sympathetic influences appear to be involved only in basal IRG release and dopaminergic pathways may also play a part in mediation of IRG release.

2 (ii) PANCREATIC POLYPEPTIDE

Considerable evidence has accrued that the autonomic nervous system and in particular vagal cholinergic pathways play an important part in the regulation of PP release.

The following data derived from animal and human experiments provides evidence of a major role for the parasympathetic nervous system in the regulation of PP release. Electrical stimulation of the vagus nerves in anaesthetized pigs induced a ninefold increase in portal serum PP concentrations within 30 seconds. This response was inhibited by atropine and eliminated by hexamethonium, but unaltered by either alpha or beta adrenergic blockade (Schwartz, Holst, Fahrenkrug, et al, 1978). In addition acetylcholine stimulated the secretion of PP from the isolated perfused procine and canine pancreata and this secretion could be blocked by atropine (Schwartz, Holst, Fahrenkrug, et al, 1978; Adrian, Bloom, Hermansen and Iversen, 1978). In man the prominent rise in serum PP levels observed in response to insulin hypoglycaemia was totally abolished following vagotomy (Adrian, Bloom, Besterman, et al, 1977; Schwartz, Holst, Fahrenkrug, et al, 1978) and strongly inhibited by atropine (Schwartz, Holst, Fahrenkrug, et al, 1978).

Moreover there is considerable evidence that a part of the stimulus for PP release following food ingestion in animals and man is vagally mediated. In dogs after truncal vagotomy there was no early or late rise in serum PP levels after food ingestion (Taylor, Impicciatore and Walsh, 1977). In man Schwartz, Rehfeld, Stadil, et al (1976) found that there was a true biphasic serum PP response to food ingestion and that after truncal vagotomy the first phase was eliminated and the second significantly reduced. After truncal vagotomy and pyloroplasty patients were found to have greatly diminished early and late

serum PP responses to food (Hansky, Ho, Korman and Stern, 1978). Although data in two other reports showed a reduction in serum PP levels after food ingestion in patients with truncal vagotomy, the PP responses were adjudged to have been unaffected by vagotomy (Adrian, Bloom, Besterman, et al, 1977; Taylor, Feldman, Richardson and Walsh, 1978).

Further studies have attempted to evaluate more clearly the role of the vagus nerves in the first early phase of the PP response to food ingestion. Cephalic stimulation has been studied by adequate sham and modified sham feeding experiments. These experiments evoked a rapid rise in serum PP levels which was reduced by truncal vagotomy and abolished by atropine and benzilonium, an antimuscarinic agent with minimal cerebral effects (Taylor, Feldman, Richardson and Walsh, 1978; Schwartz, Stenguist and Olbe, 1979; Feldman, Richardson, Taylor and Walsh, 1979). The effect of a gastric stretch neural mechanism was evaluated by distension of the stomach by a rubber balloon, intragastric saline instillation and intragastric food. All induced an early small rise in serum PP levels which was reduced following vagotomy or probantheline, an anti-cholinergic agent (Schwartz and Rehfeld, 1977; Taylor, Feldman, Richardson and Walsh, 1978). Gastric distension and food stimuli activate local vagal reflexes (Grossman, 1962) to cause PP release. Ingestion of a mixed meal induced a much greater early rise in serum PP levels than either cephalic stimulation or gastric distension produced individually. It would indeed appear that the vagus nerve and in particular vagal cholinergic pathways play an important role in the mediation of PP release and specifically the early serum PP response to food and the serum PP response to insulin hypoglycaemia.

It is generally accepted that vagal integrity exerts a less prominent effect on the second late phase of PP release after a meal (Floyd, 1979). Although atropine administered intravenously at intervals up to 120 min after ingestion of a protein meal caused a rapid return of markedly elevated serum PP levels to near basal concentrations, it is likely that the delayed serum PP response to food is mediated by an interaction of chemical, humoral and vagal stimuli (Floyd, 1979).

The effects of the adrenergic nervous system on PP release are less prominent than those of the parasympathetic nervous system. Stimulation of splanchnic nerves in the anaesthetized pig failed to induce PP release (Schwartz, Holst, Fahrenkrug, et al, 1978). Adrenaline induced a small rise in serum PP levels in the isolated perfused dog pancreas (Adrian, Bloom, Hermansen and Iversen, 1978) as did isoproterenol, a beta adrenergic agonist. The latter stimulus could be

blocked by prior infusion of propranolol, a beta adrenergic antagonist (Samols, Weir, Patel, Loo and Gabbay, 1977). Adrenaline in man also produced a small rise in serum PP levels which was significantly increased with alpha adrenergic blockade. Further exercise-induced PP release was inhibited by beta adrenergic blockade but increased by alpha adrenergic blockade (Floyd, 1979). Hence it would appear that there is some adrenergic modulation of PP release.

From the above evidence there appears to be conclusive evidence that neural influences are important mediators of PP release. The serum PP response to insulin hypoglycaemia appears to be mediated by vagal mechanisms. The early serum PP response to food appears to be mediated by vagal cholinergic factors while vagal stimuli together with those of chemical and humoral factors probably mediate the delayed PP response to food. Furthermore there appears to be some degree of adrenergic modulation of PP release.

2 (iii) GASTRIN

Neural regulation of gastrin release has been the subject of much investigation in man and animals. For the most part the dog has been the animal studied although certain experiments have also been conducted in the cat.

The following data was derived from animal studies. Electrical stimulation of the vagus nerves induced a large increase of serum gastrin levels in anaesthetized cats (Uvnäs, Uvnäs-Wallensten and Nilsson, 1975) and in dogs (Smith, Kewenter, Connell, et al, 1975). Also in the dog, vagal stimulation by sham feeding and insulin hypoglycaemia induced gastrin release which was abolished by vagotomy and blocked by atropine (Nilsson, Simon, Yalow and Berson, 1972; Tepperman, Walsh and Preshaw, 1972; Csendes, Walsh and Grossman, 1972). Also in the dog, application of acetylcholine to the antral mucosa strongly stimulated gastrin release (Tepperman, Walsh and Preshaw, 1972), while distension of the antrum of the stomach induced gastrin release which could be blocked by vagal denervation (Debas, Konturek, Walsh and Grossman, 1974). Thus in the dog and to a lesser extent in the cat there is abundant evidence to support the importance of cholinergic vagal regulation of gastrin release.

In contrast to the dog, in man it would appear that there is a vagal cholinergic inhibitory mechanism of gastrin release. In man atropine has been shown variously to increase, to decrease and not to alter basal serum gastrin concentrations (Korman, Soveny and Hansky, 1971; Becker, Reeder and Thompson, 1974; Feldman, Richardson, Taylor and Walsh, 1979). However, all forms of vagotomy resulted in elevated basal serum gastrin concentrations (Stadil, 1974).

Vagotomy also resulted in greater serum gastrin responses to a meal (Korman, Hansky and Scott, 1972) and atropine further enhanced the serum gastrin response to a meal in vagotomized subjects (Hansky and King, 1977). In addition in healthy men atropine caused an augmented rise in serum gastrin levels after a meal (Becker, Reeder and Thompson, 1974) and potentiated gastrin release induced by sham feeding (Feldman, Richardson, Taylor and Walsh, 1979). Although a significant rise in serum gastrin levels in response to insulin hypoglycaemia in healthy men has not been found by all investigators, including McLoughlin, Hayes, Buchanan and Kelly (1978), these workers observed a significant increase in serum gastrin levels after prior atropinization. The investigators who have noted significant gastrin release with insulin hypoglycaemia reported that serum gastrin levels still rose during insulin hypoglycaemia in vagotomized subjects (Stadil, 1974). Thus it seems likely that there is a vagal-cholinergic pathway in man which normally acts to inhibit gastrin release.

The effect of dopaminergic stimulation on gastrin release has also been studied in animals. Using the acute antral pouch in the cat, Uvnäs-Wallensten, Efendic and Luft, (1978) demonstrated gastrin release in response to apomorphine, a dopamine receptor stimulant, which could not be inhibited by atropine. Although the apormorphine-induced effect on gastrin release might have been mediated by activation of dopaminergic receptors, mediation via the vagal nerves and via adrenergic pathways might also have been taken place. Further work is required before the proposed dopaminergic effect on gastrin release can be evaluated.

The effect of sympathetic nervous stimulation on gastrin release has also been studied and the data has revealed that adrenergic pathways too mediate gastrin secretion. Splanchnic nerve stimulation in anaesthetized cats after separation of acid and gastrin-secreting parts of the stomach resulted in a significant reduction in gastrin release (Blair, Grund, Reed, et al, 1975). The meaning of this finding is uncertain however, as the observed effects might have been due to altered blood flow as well as due to direct effects of nerve stimulation on gastrin release.

In man an important role has been suggested for the sympathetic nervous system in the mediation of gastrin release. Indeed elevated serum gastrin levels which returned to normal with surgical removal of the tumour, have been reported in two patients with phaeochromocytoma (Hayes, Ardill, Kennedy, Shanks and Buchanan, 1972). In addition epinephrine infusion in man induced a prompt

increase in serum gastrin levels (Stadil and Rehfeld, 1973; Christiansen and Stadil, 1976) which could be completely suppressed by prior administration of a beta adrenergic blocking agent (Stadil and Rehfeld, 1973). Infusion of isoproterenol, a beta receptor stimulant, resulted in a rise in serum gastrin levels which was prevented by prior beta adrenergic blockade (Brandsborg, Brandsborg and Christensen, 1976). These data indicate that there is beta adrenergic modulation of basal gastrin secretion.

The effect of beta adrenergic modulation of stimulated gastrin secretion has been investigated by evaluating the effect of beta adrenergic blockade on serum gastrin responses to stimuli. Oral or intravenous propranolol administered before ingestion of a meal did not alter the serum gastrin response to the meal (Kronberg, 1975). Infusion of a beta agonist produced a slight enhancement of the meal-induced gastrin release, this enhancement was prevented by prior infusion of a beta adrenergic antagonist (Brandsborg, Brandsborg and Christensen, 1976). Christiansen and Stadil (1976) assessed the effects of three beta adrenergic blocking agents on the serum gastrin response to hypoglycaemia. They found that propranolol eliminated, pindolol reduced and practolol caused no alteration in the serum gastrin response. In another study in which no significant rise in serum gastrin levels was observed in response to insulin hypoglycaemia, propranolol did not alter the serum gastrin response (McLoughlin, Hayes, Buchanan and Kelly, 1978). These data indicate that beta adrenergic pathways may be partly involved in the mediation of insulin hypoglycaemia-induced gastrin release but not in meal-induced gastrin release.

However, there is little data on alpha adrenergic modulation of gastrin secretion. In the two patients with phaeochromocytoma the elevated basal serum gastrin levels returned to normal with alpha adrenergic receptor blockade (Hayes, Ardill, Kennedy, Shanks and Buchanan, 1972). This data is suggestive but not conclusive of alpha adrenergic modulation of gastrin secretion.

The evidence presented above indicates that neural regulation plays an important part in mediation of gastrin release. There is considerable data to suggest that vagal cholinergic pathways normally provide an inhibitory effect on gastrin release, both basal and stimulated. As regards the adrenergic nervous system, it would appear that alpha and beta adrenergic pathways are involved in regulation of basal gastrin release. Further beta adrenergic receptor stimulation may play a part in the mediation of insulin hypoglycaemia-induced gastrin release, but not in meal-induced gastrin release. Further studies are required before an accurate assessment of the effect of dopaminergic stimulation on gastrin release can be made.

2 (iv) GASTRIC INHIBITORY POLYPEPTIDE

Little is known regarding possible neural regulation of GIP release. Basal plasma GIP levels and glucose-stimulated GIP release have been reported after vagotomy and vagotomy and plyoroplasty respectively. While the other reported studies involved the use of atropine.

A threefold elevation in basal plasma GIP levels has been reported in patients after truncal vagotomy, leading Ebert and Creutzfeldt (1978) to suggest that an intact autonomic innervation seemed to be important for the basal state of the GIP producing cells. Following truncal vagotomy and pyloroplasty, exaggerated plasma GIP responses to glucose ingestion were observed (Thomford, Sirinek, Crockett, Mazzaferri and Cataland, 1974). This observation might have been due to a direct effect of vagotomy or alterations in small intestinal transit time and altered gastric emptying. In view of the fact that GIP hypersecretion might be an intrinsic defect in patients with duodenal ulcers (Cataland, O'Dorisio, Brooks and Mekhijian, 1977) the effect of atropine administration on GIP release induced by intraduodenal perfusion of glucose was assessed in healthy subjects (Larrimer, Mazzaferri, Cataland and Mekhijian, 1978). Atropine was found to blunt the plasma GIP response to intraduodenal glucose, which could not be related to reduced gastrointestinal absoprtion of glucose and was thought to be due to an effect on the GIP cells. The effect of atropine on food-stimulated GIP release in the dog has also been studied (Baumert, Cataland, Tetirick, Pace and Mazzaferri, 1978). In this study the plasma GIP response to food ingestion was almost completely inhibited. Suggested mechanisms for this latter inhibitory effect of atropine on GIP release were a delay in gastric emptying of the meal or a dependence of GIP release on the vagus nerve.

Thus elevated basal plasma GIP levels have been observed in patients after truncal vagotomy and exaggerated plasma GIP responses were found in patients after truncal vagotomy and pyloroplasty. These observations may possibly be related to altered GIP release in duodenal ulcer. However atropine-induced inhibition of plasma GIP responses to intraduodenal glucose in man and to a meal in dogs might possibly suggest a cholinergic dependence of GIP release. Alternatively the atropine effect might have been related to other known actions of this drug such as altered gastric emptying.

2 (v) SOMATOSTATIN (SRIF-LI)

Few reports are available in which autonomic regulation of gastro-intestinal release of somatostatin-like immunoreactivity (SRIF-LI) has been investigated. However, the effect of cholinergic agonism and adrenergic

modulation of pancreatic SRIF-LI has been reported, while the effect of vagal activation and cholinergic control of gastric SRIF-LI release and the effect of electrical vagal stimulation of SRIF-LI release have also been studied.

Samols and Weir (1979) and Samols, Weir, Ramseur, Day and Patel (1978) found that there was adrenergic but little cholinergic modulation of pancreatic SRIF-LI secretion. Using the isolated perfused canine pancreas they infused various adrenergic agents to provide specific alpha and beta receptor stimulation and inhibition. They found that beta adrenergic stimulation induced a significant increase of SRIF-LI secretion which was abolished by beta adrenergic antagonism. Alpha adrenergic stimulation moderately decreased SRIF-LI secretion and this effect was abolished by infusion of alpha adrenergic antagonists. Thus SRIF-LI secretion from the D cell in the pancreas was inhibited by alpha adrenergic agonism and stimulated by beta adrenergic agonism (Samols and Weir, 1979). The effect of cholinergic agonism on the pancreatic D cell was also studied in the isolated perfused canine pancreas by Samols, Weir, Ramseur, Day and Patel (1978). Acetylcholine infused into the pancreas induced a mild suppression while atropine alone did not alter SRIF-LI release.

The effect of vagal activation on SRIF-LI release in animals has also been studied. Vagal stimulation in cats has been shown to release SRIF-LI into the antral lumen (Uvnäs-Wallensten, Efendic and Luft, 1978). Electrical vagal stimulation in the dog produced a rise in portal plasma SRIF-LI levels, which peaked after the end of the stimulation period (Guzman, Chayvaille, Banks, Rayford and Thompson, 1979). In addition truncal vagotomy in the dog was associated with an increase in basal plasma SRIF-LI levels and like atropine, a reduced rise in plasma SRIF-LI levels in response to an intragastric meal (Schusdziarra, Rouiller, Harris and Unger, 1979). Furthermore, SRIF-LI released from the stomach in response to intraduodenal infusion of food in the dog was altered by vagotomy as well as by an atropine infusion (Schusdziarra, Rouiller, Pietri, et al, 1979). These data suggest that there is vagal and cholinergic mediation of gastrointestinal SRIF-LI release, although not necessarily by a direct effect. In addition dopamine receptor stimulation by apormorphine induced SRIF-LI release into the antral lumen. This SRIF-LI release was atropine-resistant (Uvnäs-Wallensten, Lundberg and Efendic, 1978). However, it is not known whether the apormorphine-induced SRIF-LI release was mediated via catecholamine, vagal or dopaminergic stimulation.

Thus it would appear from the literature available, that there is some degree of autonomic regulation of gastrointestinal SRIF-LI release. Vagal,

adrenergic and dopaminergic stimulation were associated with alterations in SRIF-LI secretion. However, the relevance of these mechanisms in the regulation of basal and stimulated SRIF-LI secretion remains unknown.

In this section literature pertaining to autonomic nervous regulation of the release of the five gastrointestinal hormones has been reviewed. It is apparent that only in the cases of PP is there conclusive evidence that neural factors and in particular vagal cholinergic influences are important mediators of its release. With regard to IRG, parasympathetic pathways appear to play only a minor role in its release in man, while sympathetic influences do appear to be involved in the regulation of basal IRG release. There is considerable data to support a continuous inhibitory effect of vagal cholinergic pathways on gastrin release and a regulatory role for adrenergic influences on the basal release of gastrin. There is data to suggest that vagal influences may partly mediate GIP release while early work has indicated that vagal, adrenergic and dopaminergic pathways effect SRIF-LI release.

Thus in this section the existence of at least a partial regulatory role for autonomic nervous pathways on the release of the five hormones has been determined. In addition the release of many hormones including these five gut hormones may also be affected in diabetes mellitus. Thus at this stage it is pertinent to review the literature of the effect of diabetes on the secretion of the five hormones.

SECTION 3 : DIABETES MELLITUS AND HORMONE RELEASE

The history of diabetes dates back many centuries. In the Ebers Papyrus in Egypt at about 1500 BC, reference was made to a condition which might have been diabetes. Later, in 400 BC "honey urine" was noted by Sushrutha in India. The first good clinical description of the disease came from Celsus and the name diabetes was introduced by Aretaeus, both Roman physicians living in the 1st century AD. However, it was not until the nineteenth century that major contributions to the understanding of the biochemistry, pathology and clinical features of diabetes were made. Indeed in this century considerable advances in the understanding of all aspects of diabetes have been made.

By definition, diabetes is "a chronic hereditary disease characterized by an abnormally high level of glucose in the blood and the excretion of that sugar in the urine. The basic defect is an absolute or relative lack of insulin which leads to abnormalities of metabolism, not only of carbohydrate but also of protein and fat" (Marble, White, Bradley and Krall, 1971). This definition is not completely satisfactory since some forms of diabetes are not hereditary, not all diabetics have glucosuria and insulin deficiency is not always the basic defect. Indeed it is unlikely that any definition of diabetes would receive universal acceptance.

A new classification of diabetes has been formulated by an international work group and was recently published by the National Diabetes Data Group (1979). This classification was based on contemporary knowledge of diabetes and included three clinical classes. Only two of these classes have relevance for the present studies, insulin dependent diabetes (IDDM) and non insulin dependent diabetes (NIDDM). IDDM is usually characterized clinically by a rapid onset of symptoms, insulinopenia, dependence on exogenous insulin to sustain life and proneness to ketosis. Classically this form of diabetes occurs in juveniles and was previously termed juvenile diabetes. However it may occur at any age. Thus diagnosis based on age at onset is inappropriate. Genetic and environmental factors may precipitate the disease and altered immune responses and autoimmunity are thought to play an etiological role.

NIDDM frequently present with minimal or no symptoms referable to the metabolic abnormalities of diabetes. Patients with NIDDM are not prone to ketosis and are not dependent on insulin for the prevention of ketonuria, but may require insulin for correction of symptomatic or persistent fasting hyperglycaemia that is resistant to dietary or oral hypoglycaemic management. Such patients may develop ketosis under special circumstances. In NIDDM insulin levels may be normal, above normal or low. NIDDM usually occurs in patients over 40 years of age and was

previously known as adult or maturity onset diabetes. However NIDDM may also occur in young people. NIDDM also has a genetic basis but environmental factors too are considered important in the onset of the disease. NIDDM may be separated into two sub-types, obese and non obese. Although each of the types of diabetes described above has a set of features that distinguish it from the other, on occasion it may be difficult to assign an individual to one class because insufficient information is available on that patient. However it should be possible to classify the patient once the information is obtained (National Diabetes Data Group, 1979).

Although there is general agreement that altered insulin secretion is basic to the aetiology of diabetes the role of other hormones, particularly pancreatic IRG and SRIF-LI, in the causation of diabetes has not been fully elucidated. At present there is tremendous controversy with regard to the role of pancreatic IRG (Unger, 1978; Lefebvre and Luyckx, 1979), while recent evidence has tended to suggest that SRIF-LI too may play a part in the development of diabetes (Unger and Orci, 1977). Setting the controversy regarding the relationship of gastrointestinal hormones and insulin secretion in the development of diabetes aside, diabetes per se has been shown to be associated with altered secretion of a number of hormones, both pituitary and gastrointestinal in origin. In this section literature referring to the effect of diabetes on the 5 gastrointestinal hormones chosen for study, will be reviewed.

3 (i) GLUCAGON

IRG secretion in diabetes has been the subject of many studies and there is evidence of disturbed secretion of the alpha cell in this disorder.

In the first place abnormalities in basal plasma IRG concentrations have been reported in various forms of diabetes in man. Significantly elevated basal plasma IRG concentrations have been demonstrated in IDDM (Gerich, Tsalikian, Lorenzi, et al, 1975), NIDDM (Pek, Fajans, Floyd, et al, 1972; Day and Anderson, 1973; Alford, Bloom and Nabarro, 1977) and in patients with diabetes secondary to chronic pancreatitis (Kalk, Vinik, Paul, Keller and Jackson, 1975). However in other reports basal plasma IRG concentrations have been similar in NIDDM, IDDM and healthy subjects (Aguilar-Parada, Eisentraut and Unger, 1969; Müller, Faloona, Aguilar-Parada and Unger, 1970; Unger, Aguilar-Parada, Muller and Eisentraut, 1970). The striking feature of these studies was that absolute elevation of basal plasma IRG levels as well as similar levels to those of healthy controls occurred, despite the presence of hyperglycaemia. In contrast, in healthy non-diabetic subjects in whom glucose was infused to produce hyper-glycaemia comparable to that observed in diabetics, a fall in basal plasma IRG

levels occurred (Unger, Aguila-Parada, Müller and Eisentraut, 1970). Thus the terms absolute and relative fasting hyperglucagonaemia have been used.

The mechanism of the fasting hyperglucagonaemia, both relative and absolute, is not known but there are suggestions of an insulin-related defect. Marked elevation in basal plasma IRG concentrations has been observed in diabetic ketoacidosis with reversion to normal levels after institution of treatment (Müller, Faloona and Unger, 1973). In addition supraphysiological doses of insulin reduced basal plasma IRG levels to within the normal range in NIDDM but not in IDDM (Raskin and Unger, 1978). However, restoration of normal basal plasma IRG levels has been reported following prolonged insulin infusion in IDDM (Gerich, Tsalikian, Lorenzi, et al, 1975; Raskin, Fujita and Unger, 1975). Furthermore, insulin treatment reduced the elevated basal plasma IRG levels to normal in newly diagnosed diabetics (Matsuyama, Hoffman, Dunbar, Foa and Foa, 1975). These observations seem to indicate that insulin lack per se, or metabolic derangements related to insulin lack induce the relative or absolute elevation of basal plasma IRG concentrations in NIDDM and IDDM. However, Unger and Orci (1977b) have suggested that altered intra-islet SRIF-LI secretion may also be involved in the development of fasting hyperglucagonaemia.

In diabetes in man numerous forms of stimulation have resulted in abnormalities in the release of IRG. Ingestion of a carbohydrate meal by healthy subjects was associated with a fall in plasma IRG levels. In contrast no reduction in plasma IRG levels was observed in IDDM and NIDDM after ingestion of this meal (Müller, Faloona, Aguilar-Parada and Unger, 1970). In the same report ingestion of a protein meal induced a similar rise in plasma IRG levels in healthy controls, NIDDM and IDDM despite hyperglycaemia in the diabetics. When comparable hyperglycaemia was induced in the healthy subjects, no rise in plasma IRG levels was observed in response to protein ingestion. Two to three-fold higher plasma IRG responses to ingestion of a mixed meal was also observed in IDDM (Gerich, Lorenzi, Karam, Schneider and Forsham, 1975). Furthermore an exaggerated rise in plasma IRG levels was noted in response to arginine and alanine stimulation in IDDM and NIDDM (Unger, Aguilar-Parada, Müller and Eisentraut, 1970; Wise, Hendler and Felig, 1973). Impaired plasma IRG responses to hypoglycaemia have been observed in IDDM (Gerich, Langlois, Noacco, Karam and Forsham, 1973), in unstable IDDM (Reynolds, Molnar, Horwitz, et al, 1977) and in IDDM with AN (Maher, Tanenberg, Greenberg, et al, 1977). The cause of the impaired IRG responses to hypoglycaemia is not clear, but may relate to the presence of AN and as such, will be discussed later. However, the data presented above clearly demonstrates abnormalities of stimulated IRG secretion

in diabetes in man.

The effects of insulin and SRIF infusions have been evaluated on some of the abnormalities in stimulated IRG release in diabetes. Insulin infusion during a carhohydrate meal reduced the abnormal plasma IRG response in both NIDDM and IDDM, decreasing it to the level in healthy subjects in the latter group alone, and then only when supraphysiological levels of insulin and high glucose levels were attained (Aydin, Raskin and Unger, 1977). Similarly supraphysiological quantities of insulin and prolonged insulin infusion respectively normalized the plasma IRG response to arginine in IDDM (Gerich, Tsalikian, Lorenzi, et al, 1975; Raskin, Aydin and Unger, 1976). However supraphysiological doses of insulin had no effect on the plasma IRG response to arginine in NIDDM (Raskin, Aydin and Unger, 1976). Nevertheless, insulin administration alone did not normalize the excessive plasma IRG response to ingestion of a mixed meal in IDDM, but SRIF infusion alone and when administered with insulin prevented a rise in plasma IRG levels (Gerich, Lorenzi, Karam, Schneider and Forsham, 1975). Aggressive insulin therapy providing improved control in NIDDM and IDDM, induced a significant reduction in mean daily plasma IRG levels in both groups, compared to a period of poor control, but in the latter group, the mean plasma IRG concentration was still significantly higher than in healthy subjects (Raskin and Unger, 1978). SRIF infused for five or 24 hours suppressed plasma IRG levels in NIDDM (Tamborlane, Sherwin, Hendler and Felig, 1977; Christensen, Hansen and Lundbaek, 1978), and caused the same effect in IDDM when infused for 24 hours (Christensen, Hansen, Weeke and Lundbaek, 1978). These data indicate that abnormalities in stimulated IRG secretion in IDDM and NIDDM may on occasion be corrected by insulin while SRIF may generally correct the abnormalities in both.

The mechanisms whereby hyperglucagonaemia occurs in diabetes are unclear as indicated previously, although there are several possibilities. Metabolic clearance rate and plasma acute disappearance times have been studied in IDDM (Alford, Bloom and Nabarro, 1976). Despite a similar metabolic clearance rate in healthy subjects and diabetics, the acute plasma disappearance time was prolonged in the latter. Thus it is possible that sluggish clearance of released IRG may be in part responsible for the basal and stimulated hyperglucagonaemia of IDDM, but it is not known if the same would apply to NIDDM. No studies are available to confirm this hypothesis. Other possible mechanisms involve abnormal release of IRG from the alpha cell, which may be an independent defect, may be secondary to insulin deficiency or loss of contact between A and B cells in the islets (Pek, 1977; Unger and Orci, 1977a). The inability of supraphysiological insulin infusions to totally correct the various forms of hyperglucagonaemia as described earlier, tends to mitigate insulin deficiency

per se being the only mechanism involved. A final possible mechanism postulated by Orci and Unger (1977b) suggests that SRIF-LI may alter IRG release from the pancreatic alpha cell by it's paracrine influence. This last postulate may indeed be responsible for at least part of the hyperglucagonaemia of diabetes but further studies are required to fully evaluate the role of somatostatin. Various mechanisms have been proposed to explain the impaired IRG responsiveness to hypoglycaemia observed in IDDM, including an alpha cell gluco-receptor defect (Gerich, Langlois, Noacco, Karam and Forsham, 1973) and AN (Maher, Tanenberg, Greenberg, et al, 1977). In view of the latter suggestion, further discussion will follow in the next section.

In summary, elevated basal plasma IRG concentrations relative and absolute, have been found in all forms of diabetes. Furthermore marked derangement in stimulated plasma IRG concentrations have also been demonstrated in diabetes thus a number of mechanisms have been suggested which might explain the abnormal IRG release of diabetes, but the precise mechanism remains unknown.

3 (ii) PANCREATIC POLYPEPTIDE

PP secretion in diabetes has received less attention than was the case with IRG.

One of the reasons for this lesser attention may be related to problems encountered in the measurement of serum PP levels in diabetics. First, serum PP levels increase with age in healthy subjects as well as diabetics (Berger, Crowther, Floyd, Pek and Fajans, 1978), thus unless groups are age-matched, the validity of the levels measured are questionable for comparison. Second, conventional insulin treatment is associated with the development of circulating antibodies to PP in up to 64 per cent of patients. Such circulating PP antibodies invalidate the levels of PP obtained when measured by a conventional radioimmunoassay for PP (Floyd, Fajans, Pek and Chance, 1977; Bloom, West, Polak, Barnes and Adrian, 1978).

Basal serum PP levels have been determined in diabetics and the levels observed appear to be related to the degree of hyperglycaemia. Floyd, Fajans, Pek and Chance (1977) found significantly higher basal serum PP levels in IDDM and insulin treated NIDDM compared to healthy subjects, but NIDDM treated with oral agents or diet had similar basal serum PP levels to controls. Amongst the four groups of diabetics studied by Floyd, et al, the mean basal serum PP level rose in relation to the mean fasting blood glucose level. Elevated basal serum PP levels which were twice that of age-matched healthy controls were reported by

Villanueva, Hedo, Castillo-Olivares and Marco (1978) in diabetics with marked fasting hyperglycaemia and presumably IDDM. In contrast a group of IDDM well stabilized on monocomponent insulin therapy had similar basal serum PP levels to those of age-matched controls (Adrian, Bloom, Besterman and Bryant, 1978). Introduction of insulin therapy in previously untreated NIDDM reduced their basal serum PP, with one exception, to levels which could not be considered elevated (Berger, Floyd, Pek and Fajans, 1978). These data suggests that diabetes is more often associated with elevated basal serum PP levels if the diabetes is uncontrolled.

The following data suggests that stimulated PP secretion is altered in diabetes. Villanueva, Hedo, Castillo-Olivares and Marco (1978) found that diabetics had exaggerated serum PP responses to ingestion of a protein meal compared to controls. Although Villanueva and associates did not specify the type of diabetic subject studied, they had marked fasting hyperglycaemia and were probably IDDM. In contrast Sive, Vinik, Van Tonder and Lund (1978) found impaired serum PP responses to insulin hypoglycaemia in patients with chronic pancreatitis and pancreatic diabetes. Impaired PP release after a meal was also found in patients with chronic pancreatitis and steatorrhoea of whom a small proportion were diabetic (Adrian, Besterman, Mallison, Garalotis and Bloom, 1979). The mechanism of the impaired serum PP responses is likely to be related to the reduced PP cell mass in the destroyed fibrotic pancreas, or alternatively to defective PP release resulting from gross distortion of the architecture of the pancreas due to the disease process of chronic pancreatitis. The mechanisms of the exaggerated serum PP responses in the diabetics reported by Villanueva et al are unclear, but may be related to altered intra-islet paracrine relationships and insulin deficiency.

Serum PP responses to insulin hypoglycaemia have also been studied in diabetic AN (Krarup, Schwartz, Hilsted, et al, 1979) and impaired responses have been found in IDDM with AN as compared with IDDM without AN. In the latter group however, impaired serum PP responses, although not to the same extent as in the former, were observed in the patients with a long duration of diabetes. In addition the impairment in the PP response in hypoglycaemia even on those without AN, was found to correlate inversely with the threshold of vibration sense. This suggests the existence of undiagnosed AN in the patients without AN and also reveals abnormal PP secretion in diabetic AN. This report is also discussed in the next section.

Thus it is apparent that abnormalities in basal and stimulated PP secretion in diabetes have been reported in the literature. The mechanisms whereby most of

these abnormalities occur are unknown, but suggestions have been made in each case. Nevertheless further work is required to fully elucidate the mechanisms underlying the various abnormalities reported.

3 (iii) GASTRIN

Gastrin secretion in diabetes mellitus has been the subject of one study. Feldman, Corbett, Ramsay, Walsh and Richardson (1979) studied gastrin secretion in IDDM; their patients were divided into two groups on the basis of the presence of the symptoms nausea and vomiting. Basal and food stimulated gastrin concentrations were found to be similar in both groups of diabetics, but were two- to threefold higher than in normal subjects. In addition features of PN and AN as well as delayed gastric emptying rates were present in both groups of diabetics. These data tend to suggest that the observed abnormal gastrin secretion was the consequence of AN and not diabetes per se. In view of this conclusion the data will be discussed in more detail in the next section.

3 (iv) GASTRIC INHIBITORY POLYPEPTIDE

GIP secretion, both basal and stimulated, has been studied in all forms of diabetes in man and there is some evidence to suggest abnormal secretion in this condition.

In the first instance fasting plasma GIP levels have been reported in various forms of diabetes. Normal basal plasma GIP levels have been found in non-obese NIDDM with fasting hypoglycaemia treated with dietary restriction alone (Ross, Brown and Dupré, 1977). Elevated basal plasma GIP levels were reported in obese subjects who had a pathological glucose tolerance test (Ebert and Creutzfeldt, 1978) while still higher basal levels were found in obese NIDDM (Crockett, Mazzaferri and Cataland, 1976; Ebert and Creutzfeldt, 1978). These data suggest that fasting plasma GIP concentrations are related to either obesity per se or to the hyperinsulinaemia which accompanies obesity. In addition elevated basal plasma GIP levels were found in subjects with mild acquired pancreatic diabetes (Botha, Vinik and Brown, 1976). In subjects with more severe diabetes secondary to chronic pancreatitis, insulin withdrawal induced a further rise in basal plasma GIP levels which returned to normal on reinstitution of insulin treatment, suggesting that insulin treatment lowers basal plasma GIP levels, possibly via a metabolic effect related to insulinopenia (Botha, Vinik and Child, 1978). In untreated ketotic IDDM, five-fold higher basal plasma GIP levels than those of controls have been observed, suggesting that insulin deficiency or elevated blood ketones may lead to higher basal plasma GIP levels (Ebert and Creutzfeldt, 1978).

Stimulated plasma GIP concentrations hae also been reported in diabetics and

various abnormalities have been found. However, neither in untreated ketotic IDDM nor in well controlled IDDM did the plasma GIP responses to ingestion of a test meal differ from those in healthy controls (Ebert, Frerichs and Creutzfeldt, 1976; Ebert and Creutzfeldt, 1978). Contrariwise in poorly controlled IDDM there was a significantly reduced plasma GIP response to oral glucose compared to controls (Reynolds, Tronsgard, Gibbons, Blix and Rubenstein, 1979). In contrast, obese and non-obese NIDDM with fasting hyperglycaemia had exaggerated plasma GIP responses to an oral glucose tolerance test and a mixed meal (Crockett, Mazzaferri and Cataland, 1976; Ebert, Frerichs and Creutzfeldt, 1976; Ross, Brown and Dupre, 1977). However no enhancement of plasma GIP responses to oral glucose or oral corn oil were observed in obese and non obese NIDDM who did not require oral hypoglycaemia therapy and who did not have fasting hyperglycaemic (May and Williams, 1978). Thus abnormalities in stimulated GIP secretion have been found in obese and non obese NIDDM with fasting hyperglycaemia and in certain IDDM but not in obese and non obese NIDDM with fasting normoglycaemia. These data in the NIDDM suggest that abnormal stimulated GIP secretion is related to the combination of fasting hyperglycaemia and NIDDM regardless of the presence of obesity. The discrepant results obtained in the IDDM are difficult to explain and do not appear to be related solely to insulin deficiency. Furthermore the precise mechanism of the altered stimulated GIP secretion observed in certain IDDM and NIDDM is unknown.

Hence abnormal GIP secretion occurs in the major forms of diabetes in man. In addition differences exist in the form of the altered GIP secretion in these various types of diabetes. These differences might be explained by some of the characteristics of the types of diabetes such as the presence of insulinopenia and obesity. It is apparent however that studies are required to elucidate the mechanisms underlying the observed alterations in GIP secretion in IDDM and NIDDM.

3 (v) SOMATOSTATIN

The effect of diabetes on SRIF-LI secretion in man is largely unknown due to the fact that the measurement of circulating SRIF-LI levels in this species is in it's infancy. Although considerable controversy has been raging over the estimation of circulating SRIF-LI levels in man, the recent description of a number of assay systems for this measurement should result in a flurry of reports including some on secretion in diabetes.

The reported studies on SRIF-LI secretion in diabetes have primarily involved evaluation of SRIF-LI secretion in the insulin-deficient dog and immunohisto-

chemistry and radioimmunoassay of pancreatic tissue extracts of various species. In the insulin-deficient alloxan diabetic dog, marked abnormalities in SRIF-LI secretion have been demonstrated. Significantly elevated basal plasma SRIF-LI levels have been observed in alloxan diabetic dogs compared to control dogs (Schusdziarra, Dobbs, Harris and Unger, 1977; Schusdziarra, Rouiller, Harris, Conlón and Unger, 1978). Despite insulin treatment lowering the elevated basal plasma SRIF-LI levels, the lowered concentrations were still significantly higher than those of control dogs (Schusdziarra, Dobbs, Harris and Unger, 1977). In addition in alloxan diabetic dogs plasma SRIF-LI responses to intragastric low and high fat protein meals were initially attenuated but ultimately exceeded those in control dogs. Further, insulin administration abolished the rise in plasma SRIF-LI levels in response to the intragastric meal in the diabetic dogs (Schusdziarra, Rouiller, Harris, Conlon and Unger, 1978). These data indicate the presence of altered SRIF-LI secretion in alloxan diabetic dogs and tend to suggest that insulin deficiency may play a part in this altered secretion.

In other forms of insulin-deficient diabetes, alterations in the SRIF-LI (D) cell mass and in SRIF-LI content of the pancreas have been reported. In IDDM in man and in streptozotocin-induced diabetes in rats, hypertrophy and hyperplasia of D cells and in the latter increased islet content of SRIF-LI, have been found (Orci, Baetens, Rufener, et al, 1976; Patel and Weir, 1976). However, in spontaneously diabetic mice, conflicting reports have appeared. In the insulinopenic spontaneously diabetic mouse, Baetens, Coleman and Orci (1976) found an increase in the number of D cells while in the same mice, not as yet insulinopenic, Patel, Orci, Bankier and Cameron (1976) found decreased pancreatic SRIF-LI content. The reason for the discrepancy in the latter two reports probably related to different ages of the animals studied, as in the former the mice were insulinopenic and were thus presumably older than those in the former study. Nevertheless it would appear that insulin deficient forms of diabetes are associated with altered SRIF-LI secretion and abnormalities in SRIF-LI content and D cell mass in the pancreas.

Little is known with regard to SRIF-LI secretion in non insulin-deficient forms of diabetes, although alterations in numbers of pancreatic D cells and in pancreatic SRIF-LI content have been reported in spontaneously diabetic animals. An increased pancreatic SRIF-LI content was observed in hyperinsulinaemic spontaneously diabetic mice (Dolais-Kitabchi, Marchand-Brustel and Freychet, 1979), but there was no associated increase in the number of D cells (Makino, Matsushima, Kanatsuka, et al, 1979). Conversely in the same hyperinsulinaemic spontaneously diabetic mice, a decreased pancreatic SRIF-LI content was noted (Patel, Orci, Bankier and Cameron, 1976) and further a reduced release of

SRIF-LI from the islets was observed (Petersson, Ludgvist and Andersson, 1979). Once again age differences in the animals studied may explain the discrepant results reported, although it is possible that varying laboratory techniques too may be responsible. Despite the conflicting reports, it is clear that alterations exist in pancreatic SRIF-LI content and release in hyperinsulinaemic forms of diabetes in animals. Whether the same applies to man remains to be assessed.

The sequelae of the aforementioned alterations in SRIF-LI secretion and pancreatic SRIF-LI content in the various forms of diabetes are unclear, but the importance of the reduced release of SRIF-LI from the pancreatic islets of hyperinsulinaemic mice as reported by Petersson and co-workers cannot be overlooked. Indeed it is feasible that altered islet SRIF-LI release in insulin deficient and non insulin-deficient forms of diabetes may produce changes in the normal intra-islet paracrine interrelationships and may in some way be related to the abnormalities in islet hormone release in diabetes (Unger and Orci, 1977).

In summary in this section literature regarding the effect of diabetes on the five gastrointestinal hormones has been reviewed. It is apparent that abnormalities in IRG, PP and GIP secretion are a common feature of diabetes. However little work is available on SRIF-LI secretion in diabetes and that which is available stems from animal work. Nonetheless the animal studies have provided evidence of abnormalities in SRIF-LI secretion which may also occur in diabetes in man. In addition it is possible that altered SRIF-LI secretion may in some way be related to the abnormalities in the islet hormones in diabetes. The finding of altered gastrin secretion in diabetes in the only report available was probably related to altered neural innervation of the G cell rather than to diabetes per se.

Thus diabetes may be associated with altered hormone secretion. In addition diabetes may be complicated by the presence of AN and by the same token hormone secretion in diabetes may be affected if AN is present. Hence in the next section literature relating to AN and its pathogenesis, clinical tests of its integrity and known hormonal effects, will be reviewed.

SECTION 4 : DIABETIC AUTONOMIC NEUROPATHY

Introduction

Prior to 1850 the high frequency of neurological abnormalities in diabetes mellitus led many workers to believe that diabetes was caused by a lesion in the nervous system. However, De Calvi in 1864 suggested that diabetes might be the cause, rather than the result of the neurological lesions. Almost without exception, early reports of neurological involvement in diabetes referred to peripheral neuropathy (PN), but in two reports involvement of autonomic fibres of peripheral nerves was described (Auché, 1890; Pryce, 1893). A detailed description of the features of AN did not appear until 1945 when Rundles, in a review of 125 cases with diabetic neuropathy, documented the presence of gastrointestinal and genito-urinary disturbances and abnormalities in the regulation of blood pressure, sweating and temperature of the skin in a proportion of the diabetics studied.

To date the numerous clinical manifestations of AN have been well documented. Moreover the fact that the symptoms of AN may be severe and incapacitating and that an increased mortality rate may be associated with AN (Ewing, Campbell and Clarke, 1976) are still well accepted. In this section literature pertaining to the pathogenesis and the clinical features of AN and the use of tests to confirm the presence of AN is covered. The known biochemical and hormonal sequelae of AN are discussed in more detail.

4 (i) PATHOGENESIS OF AUTONOMIC NEUROPATHY

Studies on the pathogenesis of diabetic neuropathy have focussed on PN and there is no comparable data on AN. In addition morphological studies of nerves in diabetic neuropathy have generally been limited to PN. However, there is evidence of similar morphological changes in peripheral and autonomic nerves in PN and AN, particularly with regard to segmental loss of myelin and axonal degeneration (Clarke, Ewing, Campbell, 1979). Thus it is possible that PN and AN may share a common pathogenesis. For this reason literature pertaining to the pathogenesis of PN will be reviewed briefly. The main hypothesis with regard to the pathogenesis of PN may be grouped as metabolic and vascular.

(a) Metabolic theories

Abnormalities in carbohydrate and lipid metabolism have been reported in peripheral nerves particularly within the myelin sheath and the Schwann cell, the metabolically active cell of peripheral nerves.

The Polyol Pathway

The first abnormality was described in the polyol pathway which is present in the Schwann cell and is the pathway in which free glucose is converted to fructose (Gabbay, Merola and Field, 1966). In diabetic peripheral nerves the pathway is very active leading to accumulation of large amounts of glucose, fructose and the intermediate product sorbitol. In studies by Ward (1972) and Gabbay (1973), the accumulation of sorbitol was related directly to the level of blood glucose and fell with improved control of blood sugar concentrations by insulin administration. Furthermore, increased sorbitol concentrations in peripheral nerves were associated with slowing of motor nerve conduction velocity, which improved when the blood sugar levels were lowered (Gabbay, 1973).

Myoinositol Metabolism

Abnormalities in myoinositol metabolism hae also been demonstrated in diabetes and several studies hae suggested that chronic depletion of myoinositol may occur in diabetes. In uncontrolled diabetes in man, excessive amounts of myoinositol were excreted in the urine but the amount diminished toward normal with improved control (Anderson, 1976). Myoinositol is a polyol that has an important role in lipid metabolism and is essential for normal structural integrity of peripheral nerves. Reduced myoinositol content of peripheral nerves in diabetic animals was observed in association with impaired motor nerve conduction velocity (Green, de Jesus and Winegrad, 1975). The addition of myoinositol to the diet of diabetic animals led to an improvement in the motor nerve conduction velocity and an increase in the myoinositol content of the nerves. Furthermore the decrease in peripheral nerve myoinositol content following induction of streptozotocin diabetes could be prevented by good control of the diabetes (Green, de Jesus and Winegrad, 1975). In addition in diabetics with neuropathy there was an improvement in motor conduction velocities of multiple nerves when high daily myoinositol intakes were instituted (Clements, Vourganti and Darnell, 1978).

Abnormal Glycosylation of Protein

A further possible metabolic mechanism is abnormal glycosylation of structural protein in nerves. Abnormalities in the metabolism of glycoproteins have been detected in diabetics and accumulation of excessive amounts of glycoprotein have been found on the glomerular basement membrane in this disease. Increased glycosylation might involve proteins occurring on basement and cell membranes and could conceivably contribute to nerve damage in diabetes (Clarke, Ewing and Campbell, 1979).

(b) Vascular theory

Changes in vascular supply to peripheral nerves have also been implicated in the pathogenesis of diabetic PN. Lundbaeck (1954) proposed a theory of wide-spread small vessel disease as the underlying factor in most diabetic com-

plications based on clinical observation of the kidney, eye and nerves. Histological studies of peripheral nerves have confirmed this theory (Timperley, Ward, Preston, Duckworth and O'Malley, 1976).

In this section metabolic and vascular factors which are thought to be involved in the pathogenesis of peripheral neuropathy, have been discussed. The relevance of these factors in terms of the pathogenesis of AN is not clear although it is possible that the two forms of neuropathy do share a common pathogenesis.

4 (ii) CLINICAL MANIFESTATIONS OF AUTONOMIC NEUROPATHY

The clinical features of AN are multiple and diverse, involving a number of organ systems. The symptoms are often unpleasant, may be life-threatenting and are usually associated with PN.

(a) Gastrointestinal Tract

Oesophagus: Symptoms of autonomic dysfunction affecting the oesophagus are rare. However, investigators have shown that disordered oesophageal motility may be detected by manometry or cineradiography in diabetics symptomatic of AN (Mandelstam, Siegel, Lieber and Siegel, 1969; Silber, 1969).

Stomach: Autonomic dysfunction of the stomach may induce alterations in gastric motility and acid secretion. Delayed gastric emptying has been reported in diabetic AN, which may result in gastric stasis (Kassander, 1958; Ellenberg, 1976; Hosking, Bennett and Hampton, 1978) and a variable degree of symptomatology may ensue. Nausea, vomiting, anorexia or a persistent feeling of fullness after meals are the usual symptoms. Alteration in gastric acid secretion has also been reported in diabetic AN (Hosking, Moody, Stewart and Atkinson, 1975).

Intestine: AN may result in diabetic enteropathy, which is characterized by intermittent diarrhoeal attacks with spontaneous exacerbations and remissions of varying length. Periods of alternating diarrhoea and constipation may occur with diarrhoea usually occurring at night or in the early morning. Nocturnal faecal incompetence may also occur (Ellenberg, 1964; Ellenberg, 1976).

Gall Bladder: A syndrome of neurogenic gall bladder in diabetics has been postulated by Gitelson, Schwartz, Fraenkel and Chowers (1963). They reported that diabetics with other evidence of autonomic dysfunction had large poorly contractile, but asymptomatic, gall bladders.

(b) Genitourinary System

Sexual dysfunction: Abnormality in sexual function is a frequent complaint in diabetes. Impotence has been found in 28-59% of diabetic males (Rundles, 1945; Rubin and Babbott, 1958; Schöffling, Federlin, Ditschuneit and Pfeiffer, 1963; Ellenberg, 1971; Kolodny, Kahn, Goldstein and Barnett, 1974). AN is thought to be an important pathogenetic factor (Keen, 1959; Ellenberg, 1971). Retrograde ejaculation as evidenced by infertility may also be a feature of AN (Ellenberg and Weber, 1966). Sexual dysfunction in female diabetics with AN might also be expected, although Kolodny (1971) was unable to detect any causal relationship.

The Bladder: Bladder dysfunction known as neurogenic vesical dysfunction has been reported in AN (Buck, Reed, Siddig, et al, 1976; Ellenberg, 1976). Clinically the onset is insiduous with gradual progression of bladder paralysis resulting in urinary retention. The symptoms initially are a reduced rate of micturition with straining followed by hesitation, dribbling, weakness of the stream and a sensation of incomplete bladder emptying. Later in the course infection intervenes and the symptoms alter (Ellenberg, 1976).

(c) Thermoregulation

Disturbances in sweating have been found in AN. Intolerance to heat accompanied by hyperhidrosis of the head, face, neck, axillae, chest and upper back with a lack of sweating below the waist has been reported (Goodman, 1966). An interesting feature of AN is extensive facial sweating induced by eating (Watkins, 1973; Bronshvag, 1978), a similar picture being found after cervical sympathectomy (Greenhalgh, Rosengarten and Martin, 1971), and in the auriculo-temporal or Frey syndrome following parotid surgery (Hemenway, 1960; Friedman and Pomarica, 1974). Gustatory sweating in the auriculo-temporal syndrome is thought to be due to damaged parasympathetic secretomotor fibres destined for the parotid gland being reanastomazed with sympathetic fibres supplying sweat glands (Hemenway, 1960).

Changes in vasomotor tone may also occur in diabetic AN, causing failure of circulatory adaption to alterations in environmental temperature. Persistent coldness of the feet may be a common symptom thereof (Martin, 1953; Keen, 1959; Ellenberg, 1976; Clarke, Ewing and Campbell, 1979).

(d) Cardiovascular System

A resting tachycardia has been observed in diabetics with AN (Page and Watkins, 1977), but diabetic subjects as a group have a faster mean heart rate than healthy subjects (Ewing, Irving, Kerr, Wildsmith and Clarke, 1974). Diabetics with AN were also found to have a fixed heart rate which was

unresponsive to manoeuvres that influence it reflexly (Ewing, 1978). In addition diminished cyclical variations in heart rate have been demonstrated in diabetics with AN (Wheeler and Watkins, 1973; Ewing, 1978).

Postural hypotension is the most prominent clinical expression of AN to affect the cardiovascular system. It tends to be a late feature, often associated with other clinical features of AN. Although postural hypotension may be asymptomatic, the main symptoms are dizziness, vertigo or syncope which occur on standing but occasionally may also be present when sitting. The symptoms are usually transient lasting a few minutes, but sometimes they are sustained and cause visual disturbances during which falls or loss of consciousness may result (Rundles, 1945; Keen, 1959; Ellenberg, 1976; Hosking, Bennett and Hampton, 1978; Clarke, Ewing and Campbell, 1979). Insulin administration may accentuate or produce postural hypotension in healthy subjects, but particularly in diabetics with AN (Miles and Hayter, 1968; Page and Watkins, 1976).

Painless myocardial infarction may also be a feature of diabetic AN (Faerman, Faccia, Milei, et al, 1977) although patients with severe AN may develop typical cardiac pain during myocardial infarction (Campbell, Ewing and Clarke, 1978). Furthermore cardiorespiratory arrests have been observed in relatively young diabetics with severe AN, and sudden death occurred in one case (Page and Watkins, 1978). In most cases interference of respiration with anaesthesia, drugs or pneumonia occurred, leading to suggestions that the arrests were caused by defective respiratory and not cardiovascular reflexes.

(e) Pupillary Changes

Reduced pupillary diameter particuarly in the dark and delayed or absent reflex to light and diminished hippus have been reported in AN (Rundles, 1945; Smith, Smith, Brown, Fox and Sönksen, 1978). However, no symptoms have been reported to ensue.

(f) Unawareness of Hypoglycaemia

This feature is common in diabetics with AN whereby loss of the normal premonitory signs and symptoms of impending hypoglycaemia occurs (Sussman, Crout and Marble, 1963) and may have a profound effect on the lifestyle of the patient.

4 (iii) AUTONOMIC NEUROPATHY AND HORMONE RELEASE

Abnormalities in the secretion of a number of hormones has been reported in diabetic AN, but the studies have been limited to IDDM. In IDDM with obvious clinical features of AN flat plasma IRG responses to insulin hypoglycaemia were observed. However, plasma cortisol and plasma growth hormone responses to insulin hypoglycaemia were not impaired in these diabetics (Maher, Tanenberg,

Greenberg, et al, 1977). In addition arginine stimulation induced a rise in plasma IRG levels in the diabetics with AN. These findings indicated that while the capacity to release IRG during hypoglycaemia was lost in AN, IRG responsiveness to arginine was maintained. Furthermore growth hormone and cortisol responsiveness to hypoglycaemia appeared to be independent of autonomic nervous integrity.

Serum gastrin concentrations were investigated in response to the intragastric instillation of food in IDDM. The patients were divided into two groups viz those with and those without nausea and vomiting, symptoms of delayed gastric emptying, and features of AN (Feldman, Corbett, Ramsey, Walsh and Richardson, 1979). The results revealed that basal and food-stimulated serum gastrin concentrations were two- to threefold higher in both groups of diabetics compared to normal subjects. In addition a similar degree of delayed gastric emptying was present in the two groups of diabetics. Thus it is possible that AN was present in both groups of diabetics; indeed evidence of PN was present in half of those subjects without nausea and vomiting and postural hypotension too was present in 20% of them. The study of Feldman, et al (1979) suggested that in long standing IDDM with either clinical evidence of AN or even PN, auto-vagotomy may be present which may in turn result in hypergastrinaemia. Although the hypergastrinaemia in their patients was not associated with hypersecretion of gastric acid.

Serum PP responses to insulin hypoglycaemia have also been studied in IDDM with AN, in whom markedly impaired responses were observed as compared with IDDM without AN (Krarup, Schwartz, Hilsted, et al, 1979). However certain of the patients without AN who had a long duration of diabetes, also had impaired serum PP responses, although not to the same extent as those with AN. Furthermore even in the diabetics without AN the impairment of the PP response to hypoglycaemia correlated inversely with the threshold of the sense of vibration. These data would tend to indicate the existence of undiagnosed AN in at least some of the patients in the group without AN.

Thus AN may be associated with alterations in the release of certain gastro-intestinal hormones. Moreover, Maher and associates (1977) have postulated that there is a relationship between unstable diabetes and AN. Their postulate was based on their observation that in patients with AN and unstable diabetes both separately (Maher et al, 1977; Reynolds, Molnar, Horwitz, et al, 1977) and in combination (Maher et al, 1977), there was an impaired IRG response to insulin hypoglycaemia.

Hence single reports on the effect of AN on IRG, PP and gastrin release have appeared in the literature. These reports have been limited to studies in IDDM. It is apparent that much work is required to evaluate fully the effect of AN on gastrointestinal hormone release in other forms of diabetes release and to elucidate neuropeptide release in diabetes of all kinds.

4 (iv) ASSESSMENT OF AUTONOMIC NERVE FUNCTION

In the last decade a number of tests which are simple, reproducible and non invasive have been developed to assess autonomic nerve function. These tests have enabled investigators to confirm the presence of AN although quantitation of the deficit is not possible at present. Most of the newer tests are based on cardiovascular reflexes and by inference they are assumed to reflect autonomic nerve change in other systems. The literature pertaining to the autonomic function tests which were described prior to the studies undertaken in this manuscript, is reviewed briefly.

(a) Tests based on blood pressure

With standing there is immediate pooling of blood in the legs with a fall in blood pressure, but provided there is normal baroreceptor function, this is rapidly corrected by peripheral vasoconstriction and tachycardia. Thus, Ewing, Campbell, Burt and Clarke (1973) first described the use of postural hypotension which was detected using a cuff sphygmomanometer and measuring the fall in systolic blood pressure as a measure of AN. These workers defined a fall of 30 mm Hg or more in systolic blood pressure as significant. This remains one of the simplest methods of detection of possible AN.

Another simple test which is based on blood pressure responses to sustained muscle exercise has been defined. Sustained muscle exercise produces an increase in systemic blood pressure and cardiac output which is dependent on heart rate but which does not alter peripheral vascular resistance. Ewing, Irving, Kerr, Wildsmith and Clarke (1974) devised a test based on this reflex in which a handgrip dynamomanometer, standardized at 30% of the maximum voluntary contraction was used together with measurement of blood pressure. They observed that there was an abnormally small rise in diastolic blood pressure in diabetics with AN. Thus a rise in diastolic blood pressure of 16 mm Hg or more was defined as normal, between 11 and 15 mm Hg as borderline and less than 10 mm Hg as abnormal (Ewing, Campbell, Burt and Clarke, 1973). This test although being effort-dependent is relatively simple and reproducible.

The third test based on measurement of blood pressure involved applying negative pressure to the lower body below the iliac crests which results in pooling of blood which in turn elicits cardiovascular reflexes to maintain system blood pressure (Brown, Goei, Greenfield and Plassaras, 1966). Diabetics with AN

showed a hypotensive response to this manoeuvre despite forearm vaseconstriction (Bennett, Hosking and Hampton, 1979). This test, unlike the first two, is time consuming, complex and requires special equipment.

(b) Tests based on heart rate

Heart rate changes during the Valsalva manoeuvre have been described as a test of autonomic function. Tachycardia and peripheral vasoconstriction during the valsalva manoeuvre is followed by an overshoot bradycardia and rise in blood pressure immediately after the manoeuvre and represent the baroreceptor response to the manoeuvre (Levin,1966). Heart rate changes during and after the manoeuvre are considered a reliable guide to the haemodyamic events and have been used to evaluate responses to the manoeuvre (Elsberg, 1963; Levin, 1966). The subject's response to the manoeuvre is expressed as the valsalva ratio i.e. the ratio of the longest R-R interval after the manoeuvre to the shortest R-R interval during the manoeuvre. In diabetics a ratio of 1.10 or less is considered abnormal, 1.11 - 1.20 as borderline and greater than 1.21 as normal (Ewing, Campbell, Burt and Clarke, 1973; Clarke, Ewing and Campbell, 1979). The test is simple but requires patient co-operation and a certain degree of effort.

Beat-to-beat variation in heart rate, which is dependent on parasympathetic innervation of the heart (Wheeler and Watkins, 1973) has been used in a test to evaluate autonomic function. In the test beat-to-beat variation in heart rate was analysed during deep breathing which produces maximum variation in heart rate (Wheeler and Watkins, 1973). The test is simple and does not require complex equipment.

Changing from lying to standing produces an integrated reflex response of the cardiovascular system which includes alterations in heart rates and blood pressure (Johnson and Spalding, 1974) and has been used as a test of autonomic function. The test involves measurement of the 15th and 30th RR intervals after standing on an ECG recording to give the 30:15 ratio. In normal subjects the ratio is greater than 1.03 while in diabetics with AN the ratio is 1.00 or less (Ewing, Campbell, Murray, Neilson and Clarke, 1978). The test is simple, reproducible and requires little patient co-operation.

(c) Gastric Acid Secretion

Reduced gastric acid secretion in response to insulin hypoglycaemia in the presence of a normal acid secretory response to pentagastrin suggests disturbance of vagal integrity (Langer, 1972; Hosking, Moody, Stewart and Atkinson, 1975). This may be used as a test for vagal integrity but requires special equipment, is invasive and may be uncomfortable for the patient.

(d) Impaired Sweating

Areas of reduced sweating due to sudomotor dysfunction may be elicited by dusting the patient with a starch and iodine compound or quinizarin and inducing an increase in core temperature by at least 1°C (Guttmann, 1946; Goodman, 1966). This has been used as a test of autonomic integrity but may be cumbersome and disagreeable.

(e) Testicular Pain Sensation

Reduced testicular pain sensation has been reported in patients with impotence and other features of AN (Campbell, Ewing, Clarke and Duncan, 1974) and has been suggested as a possible test of autonomic dysfunction. The test requires patient co-operation and may indeed prove to be unpleasant. Furthermore it is subjective and difficult to quantitate precisely.

(f) Drug Tests

Heart rate responses to various agents such as phenylephrine, atropine and propranolol which induce alterations in blood pressure and produce cardiac blockade have been evaluated in diabetics with AN (Lloyd-Mastyn and Watkins, 1975) and poor heart rate responses have been observed.

The tests are invasive and not as simple as some of those described earlier. Local instillation of various drugs with observation of pupil sizes may also be useful for assessing autonomic innervation of the pupil. Instillation of dilute solutions of hydroxyamphetamine, epinephrine, phenylephrine and cocaine may be used to detect the presence of sympathetic lesions. Instillation of cholinomimetic drugs such as pilocarpine and methacholine may elicit the presence of interrupted parasympathetic innervation of the pupil (Moskowitz, 1977).

Thus a large number of tests have been described whereby autonomic nerve function may be determined. Some of the tests evaluate parasympathetic innervation such as gastric acid secretory responses and beat-to-beat variation in heart rate. Integrity of sympathetic innervation may be determined by the blood pressure response to sustained handgrip and assessment of postural hypotension. In view of the patchy involvement of AN which may be manifest by intact sympathetic innervation and selective damage of parasympathetic pathways (Lloyd-Mostyn and Watkins, 1975), an evaluation of autonomic function therefore requires a variety of autonomic function tests.

In summary in this section literature pertaining to AN has been reviewed. While little has been reported with regard to the pathogenesis of AN, the numerous and diverse clinical features of AN have all been well documented. A number of tests, for the most part based on cardiovascular reflexes, have been described to assist in the confirmation of the presence of AN. Hormonal and biochemical effects of AN have been assessed in three reports revealing abnormalities in IRG, PP and gastrin release. In addition metabolic sequelae of AN based on altered IRG release have been postulated. The increased mortality rate and possible metabolic consequences of AN emphasize the necessity for greater understanding of this complication of diabetes.

It is apparent from the literature reviewed thus far that AN may effect gastrointestinal hormone secretion and may have relevance in the stability of control in these patients. A new exciting field has recently opened with the finding that dietary fibre improves glucose tolerance and with suggestions that fibre supplementation of diets may have a role to play in the therapy of diabetes. However the mechanisms whereby fibre exerts its effect on glucose tolerance are not fully understood, although altered gut hormone secretion amongst others have been proposed as possible mechanisms. Furthermore it is not known whether fibre would have an equally beneficial effect in the presence or absence of AN if the fibre-induced effect on glucose tolerance is somehow related to autonomic nervous integrity. In the next section existing knowledge of dietary fibre will be explored with particular reference to its proposed mechanisms of action and its effect in diabetes.

SECTION 5 : DIETARY FIBRE

Introduction

Dietary or plant fibres have been defined as those portions of edible plants that are not digested in or absorbed from the human small intestine (Anderson, Midgley and Wedman, 1979). Although a definite classification of the multitude of plant fibres cannot be made at present, from a biological viewpoint dietary fibres have been divided into:

(a) Structural fibres

(b) Gums and mucilages

(c) Storage polysaccharides

The structural fibres are components of plant cell walls and include cellulose, pectins, lignins and different hemicelluloses. The gums and mucilages serve specialized functions for the plant such as repair of injured areas. The storage polysaccharides are usually dispersed throughout the endosperm of the plant and are stored for later use as energy sources (Anderson and Chen, 1979).

Epidemiological studies have suggested that fibre depleted diets may play a role in the higher prevalence of cancer of the colon and rectum, diverticular disease of the colon, varicose veins, haemorrhoids and ischaemic heart disease noted in Western societies compared with rural African society (Trowell, 1973; Burkitt, Walker and Painter, 1974; Burkitt, 1978). In addition it was suggested that fibre depleted diets may play a causative role in the development of clinical diabetes (Trowell, 1973; Trowell, 1975; Trowell, 1978). These suggestions have precipitated tremendous interest in dietary fibres in recent years.

Various studies have revealed that dietary fibre has a number of effects. Indeed in semi and highly purified forms, and as part of high fibre foods dietary fibre has been shown to reduce serum cholesterol levels (Jenkins, Leeds, Newton and Cummings, 1975; Anderson and Chen, 1979), and serum triglyceride levels in patients with hypertriglyceridaemia (Anderson and Chen, 1979) and to improve glucose tolerance (Jenkins, Leeds, Gassull, Cochet and Alberti, 1977; Jenkins, Wolever, Leeds, et al, 1978; Monnier, Pham, Aguirre, Orsetti and Mirouze, 1978; Munoz, Sandstead, Jacobs, Johnson and Mako, 1979).

In this section literature pertaining to dietary fibre in diabetes and its postulated mechanisms of action in improving glucose tolerance is reviewed.

5 (i) MECHANISMS OF ACTION

Dietary fibre is thought to exert its effect on glucose tolerance through actions on the gastrointestinal tract. Four mechanisms have been postulated. First, a delay in gastric emptying may result in a lower absorption of carbohydrate. Soluble fibres such as guar and pectin slow gastric emptying (Holt, Heading, Carter, Prescott and Tothill, 1979). However whole bran appears to accelerate gastric emptying and has a less prominent effect on glucose tolerance than the soluble fibres (Anderson and Chen, 1979). Second, the gel forming properties of the soluble fibres may result in slower absorption of carbohydrate. Fibres such as pectin and guar form gelatinous masses in the gut which results in delayed gastric emptying and altered intestinal transit time (Anderson, Chen, 1979; Eastwood and Kay, 1979). Third, available carbohydrate that is packaged within fibre may be insulated from digestive enzymes and may not be digested or absorbed in the small intestine. Thus available carbohydrate may escape into the colon and be lost in the faeces or be digested by bacteria (Anderson and Chen, 1979). Fourth, altered gut hormone release i.e. GIP and GLI which has been demonstrated with fibre supplementation might be responsible for the reduced insulin secretion and in some way be related to the improved glucose tolerance (Anderson and Chen, 1979; Morgan Goulder, Tsiolakis, Marks and Alberti, 1979).

5 (ii) DIETARY FIBRE IN DIABETES

Two pieces of epidemiological evidence formed the basis of the theory that fibre depleted diets might play a part in the development of diabetes. First, rural societies in Africa have diets containing considerably more dietary fibre than Western societies and rarely suffer from diabetes (Trowell, 1973). Second, the increase in the wheat bran content of bread in Britain during world war II was found to be associated with a fall in mortality figures for diabetics (Trowell, 1973).

Numerous studies during recent years have revealed that fibre has a beneficial effect on control of diabetes and may have therapeutic value.

A number of studies have evaluated the acute effect of fibre on glucose tolerance and have revealed that fibres has a beneficial effect in the short term. After Jenkins, Wolever, Leeds, et al (1978) reported that of six different fibres guar produced the greatest flattening of the serum glucose response to oral glucose, and pectin too was effective, most further studies were confined to these two fibres. Addition of fibre to meals induced a significant reduction in plasma glucose concentrations after the meals in IDDM and NIDDM (Jenkins, Goff, Leeds,

et al, 1976; Morgan, Goulder, Tsiolakis, Marks and Alberti, 1979). Serum insulin responses to ingestion of a meal were also reduced in the NIDDM. In the latter study GIP responses were reduced as well leading to suggestions that fibre may be a useful adjunct in diabetic therapy, and that its effect on insulin secretion may be mediated through GIP.

Further studies have assessed the effect of long term fibre therapy on glucose tolerance. One week's therapy with guar resulted in a 10% reduction of mean urinary glucose excretion in IDDM and in one diabetic who was treated on dietary restriction alone (Jenkins, Wolever, Hockaday, et al, 1977). High fibre diets containing 20 g crude fibre maintained for 2 weeks resulted in a marked reduction in mean plasma glucose and IRG levels in IDDM, (Miranda and Horwitz, 1978). High fibre diets containing 15 g crude fibre for 2 weeks resulted in significant reduction in fasting plasma glucose levels, discontinuation of sulphonylurea therapy in NIDDM and a marked reduction in insulin requirements in IDDM on less than 30 units of insulin per day. However, in diabetics requiring 40-55 units of insulin, fibre induced no change in fasting plasma glucose levels or in insulin requirements (Kiehm, Anderson and Ward, 1976). High fibre, high carbohydrate diets for a period of 15 months produced a significant reduction in fasting plasma glucose levels and insulin or sulphonylurea requirements in IDDM and NIDDM. The reduction in insulin requirements was also noted in a single patient on 55 units of insulin per day (Anderson and Ward, 1978). This data suggests that fibre need not necessarily be of little value in patients on high daily doses of insulin.

Thus it is apparent that fibre, in either its purified or crude form has prominent effects on glucose tolerance in both the short and long term. Indeed fibre supplementation of diets may have a place in the therapy of diabetes. However, problems may be encountered in the implementation of these diets. The first problem concerns the question of palatability, flatulence and altered bowel habits. However, Anderson and Ward (1978) reported that despite increased flatulence, increased defaecation and initial problems with the large quantities of vegetables in the diet, patients tolerated and accepted the diets remarkably well. In addition, Jenkins, Wolever, Nineham, et al (1978) described the use of guar in crisp bread "the first palatable guar formulation therefore represents an important advance in food technology". Thus it would appear that high fibre diets are acceptable to patients. The second problem concerns the question of mineral depletion and vitamin deficiency. In short term studies of 20-38 days high fibre diets were associated with increased faecal losses of calcium, magnesium, phosphorus, zinc (Reinhold, Faradji, Abadi and Ismail-Beigi, 1976; Ismail-Beigi, Reinhold, Faraji and Abadi, 1977) and silicone (Kelsay, Behall and

Prather, 1979). In addition reduced plasma concentrations of calcium, phosphorus, zinc and iron were noted, but the reductions were sustained in the first two alone (Reinhold, Nasr, Lahimgarzadeh and Hedayati, 1973). In contrast in a long term study of 5-44 months no depletion of calcium, magnesium, iron or fat soluble vitamins were observed (Anderson, Sieling and Ferguson, 1979). These data would tend to indicate that fibre may have a deleterious effect on mineral balance in the short but not the long term. Further if there is adequate mineral intake, mineral balance may not suffer in the long term. However further studies are required to fully evaluate the effect of dietary fibre on mineral and vitamin balance in the long term.

A major point of interest with regard to fibre revolves around the mechanisms whereby fibre exerts its action on glucose tolerance, because AN in diabetes may result in delayed gastric emptying (Ellenberg, 1976; Hosking, Bennett and Hampton, 1978) and diabetic enteropathy (Ellenberg, 1964) as described earlier. Thus the question that arises is would fibre still exert its well recognized effect on glucose tolerance if there was no longer autonomic nervous integrity?

In this section available literature of dietary fibre has been discussed with special reference to its known actions in diabetics and its proposed mechanisms of action in altering carbohydrate absorption and metabolism. Fibre in different forms has been shown to improve glucose tolerance in the acute situation as well as in the long term. In addition fibre supplementation of the diet for 2 weeks to 15 months has been responsible for reducing or obviating the need for oral hypoglycaemic or insulin therapy in two groups of diabetics. However, in one study fibre had no effect on glucose tolerance or insulin requirements in patients on more than 40 units of insulin per day, while in another study, fibre had a beneficial effect on glucose tolerance insulin requirements in a patient on 55 units of insulin per day. Altered intestinal transit time secondary to numerous factors and altered gut hormone release have been postulated as the mechanisms whereby fibre exerts its action on glucose tolerance. While fibre supplementation may seem an ideal adjunct to the existing forms of therapy in diabetics, problems such as lack of effect in patients on high daily doses of insulin, possible mineral and vitamin depletion, and the question of its efficiency in the presence of AN need to be investigated.

SUMMARY

This review reveals gaps in present knowledge not only of AN and release of the five gastrointestinal hormones, but also in the pathogenesis of AN, in autonomic nervous regulation of the release of some of the hormones and in the effect of diabetes on them. It is apparent that little is known about the pathogenesis of AN. Studies have revealed abnormalities in carbohydrate and lipid metabolism in peripheral nerves of patients with PN leading to suggestions that metabolic factors may be causative in PN. In addition, vascular factors have been postulated to be involved in the pathogenesis of PN. Similarities in histological studies of peripheral and autonomic nerves in PN and AN respectively lend credence to the possibility that PN and AN may share a common pathogenesis. Although beyond the scope of this manuscript, further studies are required to evaluate the causation of AN and to assess whether PN and AN indeed have a common pathogenesis.

In spite of work having been done concerning autonomic nervous regulation of gastrointestinal hormone release, it is evident that hiatuses exist in present understanding. It is fairly definite that PP release is regulated, at least in part, by vagal cholinergic pathways, but the role of the sympathetic nervous system needs to be elucidated. As regards gastrin there seems little doubt that vagal cholinergic pathways provide a constant inhibitory effect on its release, while it would appear that adrenergic pathways are involved in regulation of basal, but not stimulated gastrin release. A great deal of contradictory data has been reported on autonomic, and in particular, parasympathetic regulation of IRG release. It would appear however that adrenergic pathways modulate basal IRG secretion but the true effect of the parasympathetic nervous system on IRG secretion needs to be fully evaluated. Early work has revealed that the vagus may be involved in mediation of GIP release, but studies are required to fully assess parasympathetic and sympathetic modulation of GIP release. Animal studies have suggested that SRIF-LI too may be regulated at least in part, by autonomic pathways but in this instance too, further studies are required before an adequate assessment of the importance of autonomic regulation of SRIF-LI release can be made.

In the reports on the effect of AN on hormone release gastrin, IRG and PP were the hormones studied and abnormalities in the secretion of all were found. The three studies were limited to IDDM and one stimulation test for each hormone was used. The early evidence of autonomic modulation of GIP and SRIF-LI release might also indicate that abnormalities in their release would occur in the presence of AN.

With regard to dietary fibre, considerable evidence has accumulated which demonstrates that fibre has a beneficial effect on glucose tolerance in IDDM and NIDDM in the short and long term. Indeed fibre supplementation has even resulted in a reduction of insulin requirements in patients on moderate daily doses of insulin and cessation of oral hypoglycaemic therapy. It is not clear however, that fibre would be effective in patients in whom loss of autonomic nervous integrity was present.

Finally, gaps have been revealed in our present knowledge, particularly of the pathogenesis of AN, the effect of AN on hormone release, autonomic control of hormone release and the efficacy of dietary fibre in the presence of AN. In this thesis the effect of AN on gastrointestinal hormone release and the relative value of dietary fibre supplementation in the presence of AN are the subjects studied.

CHAPTER II

OBJECTIVES OF THE STUDY

CHAPTER II

OBJECTIVES OF THE STUDY

From the literature review it is apparent that gaps in knowledge exist in almost every field of study alluded to. Hiatuses were revealed particularly with regard to : the pathogenesis of AN, the effect of AN on gastrointestinal hormone release, autonomic nervous regulation of the release of certain gastrointestinal hormones, the effect of diabetes on gastrointestinal hormone release and the efficacy of dietary fibre in the presence of AN. To attempt to fill all these gaps is a massive undertaking and well beyond the scope of a single project.

The primary aim of this work is to provide evidence which may contribute to an understanding of the interrelationship between NIDDM, gastrointestinal hormone release and the autonomic nervous system. Thus, in an attempt to interrelate these aspects, the objectives of this thesis which have been limited to the study of five gastrointestinal hormones were as follows:-

First, to compare the basal levels of IRG, PP, gastrin, GIP and SRIF-LI in NIDDM with and without AN.

As has been indicated basal release of the five gastrointestinal hormones may be regulated, at least in part, by the autonomic nervous system. If this is indeed the case, differences in the basal concentrations of these hormones may be apparent in NIDDM with AN as opposed to NIDDM without AN. Also, diabetes per se appears to have an effect on the basal release of most of the five gastrointestinal hormones. In such a situation the basal concentrations of the hormones should be similar in NIDDM with AN and NIDDM without AN but may differ from those in healthy subjects. However, should the basal hormone concentrations differ in the NIDDM with AN and those without AN, this may have relevance to the altered basal hormone release found in NIDDM per se. Thus the second objective is to investigate whether abnormalities in basal hormone release found in NIDDM could relate to the presence of AN.

The third objective is to compare stimulated levels of IRG, PP, gastrin, GIP and SRIF-LI in NIDDM with and without AN. As stated earlier, not only basal but also stimulated release of the five hormones may be regulated, at least in part, by the autonomic nervous system. Thus in this situation too, differences

in the stimulated concentrations of the five hormones may occur in the NIDDM with and without AN. However, altered stimulated secretion of the majority of these hormones may also be found in diabetes per se. Thus the stimulated concentrations of the hormones may be similar in the NIDDM with and without AN but may differ from those in normal people. In the event of a relationship between AN and altered stimulated hormone release, and diabetes per se and altered stimulated hormone release, however, this may indicate that AN is involved in the disturbed stimulated release of the hormones in NIDDM per se. Hence the fourth objective is to assess whether AN may contribute to the abnormalities found in stimulated release of the five hormones in NIDDM.

The fifth objective is to determine the existence of a biochemical marker for AN. There is little doubt that AN may have unpleasant and even dangerous consequences for the patient, e.g. postural hypotension, unawareness of hypoglycaemia and sudden cardiorespiratory arrest. At present various clinical tests are available to detect AN. The majority of these tests are based on cardiovascular reflexes, and testing of gastrointestinal nervous integrity relies on the gastric acid secretory response to insulin hypoglycaemia. If there is indeed autonomic nervous control of gastrointestinal hormone release and if this could be elicited easily and reproducibly, then the response of such a hormone may be an accurate marker for gastrointestinal autonomic integrity and as such may add a useful test to the armoury of those used for investigation of autonomic nervous integrity.

The final objective is to evaluate whether the beneficial effect of dietary fibre on glucose tolerance could be altered by the presence of AN in NIDDM. Fibre supplemented diets have been shown to improve glucose tolerance and have been suggested to have a role in the therapy of diabetes. Also suggested is the possible dependence for this action of dietary fibre on mechanisms which may be related to autonomic nervous integrity such as delayed gastric emptying and altered gastrointestinal hormone secretion. If the effect of dietary fibre on glucose tolerance is mediated by a factor related to autonomic nervous integrity, differences may be apparent in NIDDM with and without AN. However the converse may also apply.

To attain these objectives controlled studies have been designed and undertaken.

CHAPTER III

EXPERIMENTAL DESIGN

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CHAPTER III

EXPERIMENTAL DESIGN

SECTION I : THE SUBJECTS

Previous reports of hormone concentrations in diabetics have sometimes been the result of studies in poorly defined groups, containing perhaps unknown numbers of patients with AN. Thus an attempt is made in these studies to control strictly the type of diabetic studied, the presence and absence of AN and the matching of patients.

I (i) THE TYPE OF DIABETES

NIDDM formed the patient group for study. They were selected because of the high proportion of NIDDM attending the Groote Schuur Hospital Diabetes Clinic and because they were not treated with insulin and were therefore not prone to the formation of antibodies e.g. to PP, which may result from treatment with ordinary commercial insulins (Bloom, West, Polak, Barnes and Adrian, 1978; Klaff, Vinik, Berelowitz and Jackson, 1978).

I (ii) MATCHING OF SUBJECTS

In all instances groups of subjects who were compared, were either age-matched or within a similar age range. This was necessary because the concentrations of certain hormones are known to rise with increasing age e.g. PP, IRG (Berger, Crowther, Floyd, Pek and Fajans, 1978). In addition only males were studied in order to exclude sex differences between patients as a possible cause for differences in hormone concentrations. Similarly only non-obese subjects were studied in order to exclude obesity as a possible factor in differences in hormone concentrations. Wherever possible care was taken to study patients with a similar duration of diabetes to exclude varying durations of diabetes as a possible factor in differences in hormone concentrations.

I (iii) THE PRESENCE OF AUTONOMIC NEUROPATHY

As the field of study was the effect of AN on gastrointestinal hormone release, it would have been ideal to localize the AN to the gut. However only one test has been described which evaluates autonomic function of the gut viz gastric acid secretory responses to insulin hypoglycaemia and pentagastrin (Hosking, Moody, Stewart and Atkinson, 1975). A battery of 7 other tests were therefore added, namely postural hypotension (Ewing, Burt, Williams, Campbell and Clarke, 1976), beat-to-beat variation on deep breathing (Wheeler and

Watkins, 1973), the Valsalva Manoeuvre (Ewing, Campbell, Burt and Clarke, 1973), sustained handgrip (Ewing, Campbell, Kerr, Wildsmith and Clarke, 1974), sweating in response to insulin hypoglycaemia (Guttmann, 1940; Editorial, Lancet, 1976). testicular sensation (Campbell, Ewing, Clarke and Duncan, 1974) and immediate heart rate responses to standing (Ewing, Campbell, Murray, Neilson and Clarke, 1978).

The majority of these tests were based on cardiovascular reflexes and had the advantages of simplicity, non-invasiveness and reproducibility. Disadvantages of some of these tests were their effort dependence e.g. Valsalva and in the case of beat-to-beat variation with deep breathing, the fact that this variation diminishes over the age of 50 in healthy subjects. The chief disadvantage of the tests not based on cardiovascular reflexes was the unpleasantness which may be associated with hypoglycaemia. A further disadvantage of the tests was that they are not quantitative and cannot precisely localize a lesion of the autonomic nervous system. However their prime purpose in this study was to provide an overall assessment of the presence of AN and not to localize the lesion.

There are no specific criteria for the diagnosis of AN. Abnormal responses to three or more of the autonomic function tests together with the presence of symptoms of AN were defined as the criteria for the diagnosis of AN in this study. The criteria for the absence of AN were the lack of symptoms of AN and normal responses to all eight autonomic function tests.

SECTION 2 : THE SCOPE OF THE STUDIES

Using the patients as outlined above, measurement of their gastro-intestinal hormone status was undertaken under basal and stimulated conditions.

2 (i) THE HORMONES

The hormones were selected primarily on the basis of availability of antisera and reliable radioimmunassays. Further reasons for their selection relate to their altered release in diabetes per se and/or their at least partial dependence on autonomic nervous regulation. The hormones measured initially were IRG, PP, gastrin and GIP. SRIF-LI was added to the four hormones only after the initial insulin hyperglycaemia test had been carried out.

2 (ii) THE TESTS OF HORMONE RELEASE

In these tests whenever possible normal healthy subjects acted as controls for the studies. The diabetics were initially considered as a single group regardless of the presence or absence of AN, in order to document the concentrations of the hormones in the group of NIDDM selected for study.

Thereafter an assessment was made as to whether any differences might exist in the concentrations of the hormones in the diabetics with and without AN.

(a) Tests of Basal Hormone Concentrations:

Studies were designed to evaluate the effect of AN on basal hormone concentrations. In the first study, circulating hormone concentrations were determined after a 12 hour overnight fast. The second study took cognizance of the chronic hyperglycaemia of diabetes and was therefore designed to assess whether basal hormone concentrations were related to the level of fasting glycaemia. The effects of the acute establishment of fasting normoglycaemia were therefore assessed on basal hormone concentrations. The normalization of blood glucose levels was achieved by a continuous overnight insulin infusion, using a rapidly acting monocomponent insulin. An infusion rate of 0.5-0.8 U/hr was considered adequate to produce fasting normoglycaemia in moderately hyperglycaemia NIDDM. However in order to ensure a satisfactory fall in blood glucose levels over the 12 hr infusion period, blood glucose estimations were carried out hourly and the infusion rate altered accordingly.

Motor dysfunction of the stomach with consequent dilatation and delayed gastric emptying, although often asymptomatic, is a feature of diabetic AN (Campbell, Heading, Tothill, et al, 1977). This manifestation may contribute to poor diabetic control by the irregular and delayed entry of food into the intestine (Kassander, 1958). Therefore the third study was designed in order to exclude the possibility that gastric retention may result in continuous stimulation of hormone release due to a slow 'leak' of food through the pylorus. The study was designed to provide 'slow' drainage of the stomach by overnight gastric aspiration to ensure that all subjects had complete emptying of the stomach before the estimation of basal concentrations was carried out.

(b) Provocative Stimulation Tests:

The ensuing studies involved measurement of the concentrations of the five hormones after provocative stimulation. In the first study, hormone concentrations were measured in response to the stimulus of insulin hypoglycaemia. Insulin hypoglycaemia is a known stimulus for the release of IRG (Gerich, Schneider, Dippe, et al, 1974) and PP (Adrian, Bloom, Besterman, et al, 1977; Marco, Hedo and Villaneuva, 1978) while there is conflicting evidence with regard to its effectiveness as a stimulus for gastrin release (Stadil, 1974; McLoughlin, Hayes, Buchanan and Kelly, 1978), as also for GIP (Service, Nelson, Rubenstein and Go, 1978; Reynolds, Tronsgard, Gibbons, Blix and Rubenstein, 1979).

The effect of insulin hypoglycaemia on SRIF-LI release was not known. Hollander in 1946 originally described the insulin test to detect the presence of intact vagal fibres after vagal operations for peptic ulcer disease. In his description of this test, Hollander suggested that the insulin injection induced hypoglycaemia, which in turn activated the vagus centre in the medulla resulting in stimulation of gastric acid secretion. More recent work has however indicated that insulin hypoglycaemia increases circulating catecholamine levels, in addition to activation of the vagus nerves (Vendsalu, 1960; Garber, Cryer, Santiago, et al, 1979). Indeed the release of certain of the hormones e.g. PP (Adrian, Bloom, Besterman, et al, 1977; Schwartz, Holst, Fahrenkrug, et al, 1979) and gastrin (Stadil, 1974), has been shown to be altered in response to hypoglycaemia after vagotomy. However, conflicting evidence has been presented with regard to the effect of vagotomy on IRG secretion (Bloom, Vaughan and Russell, 1974; Palmer, Werner, Hollander and Ensinck, 1979). The effect of vagotomy on plasma GIP and plasma SRIF-LI responses to hypoglycaemia was not known. Nevertheless by using the stimulus of insulin hypoglycaemia, an assessment could be made of the effect of autonomic nervous dysfunction on hormone release. The diabetics received a larger dose of insulin than the healthy controls because of the higher level of fasting glycaemia in the diabetics.

As the level of fasting hyperglycaemia may influence either the rate of change, or the absolute changes in circulating hormone levels in response to a given stimulus, a modification to this second study was made and a study was designed to evaluate whether the hormone responses to insulin hypoglycaemia were dependent on the degree of fasting glycaemia. Thus in the third study the effect of fasting normoglycaemia on the hormone concentrations during hypoglycaemia in the diabetic patients was assessed. Fasting normoglycaemia was induced acutely by a continuous insulin infusion.

In the next study hormone concentrations were measured in response to ingestion of a mixed meal. This stimulus was chosen because it is known that IRG (Gerich, Lorenzi, Karam, Schneider and Forsham, 1975), GIP (Kuzio, Dryburgh, Malloy and Brown, 1974), PP (Adrian, Bloom, Bryant, et al, 1976) and gastrin (Korman, Soveny and Hansky, 1971) are all released in response to ingestion of a mixed meal. While instillation of intragastric food in the dog is associated with SRIF-LI release (Schusdziarra, Rouillier, Harris, Conlon and Unger, 1975), it was not known whether ingestion of a mixed meal would induce SRIF-LI release in man. Ingestion of a mixed meal containing protein, carbohydrate, fat, minerals and fluid, constituents of a normal balanced diet should constitute a good physiological stimulus for the release of most gastrointestinal hormones. The

second reason for the selection of a mixed meal was related to the fact that neural, and in particular vagal, pathways have been implicated in the cephalic and gastric phases of PP (Schwartz and Rehfeld, 1977; Taylor, Feldman, Richardson and Walsh, 1978; Schwartz, Stenquist and Olbe, 1979), gastrin (Grossman, 1967; Soll and Grossman, 1978) and gastric acid (Grossman, 1967; Soll and Grossman, 1978) responses to food. Thus ingestion of a mixed meal should provide not only a good physiological stimulus for hormone release, but also an assessment of the integrity of vagal innervation of certain of the hormones.

2 (iii) FIBRE SUPPLEMENTATION

The final study was designed to assess whether the effectiveness of dietary fibre supplementation on glucose tolerance would be altered by the presence of AN in NIDDM. Dietary fibre has been shown to have a beneficial effect on glucose tolerance in healthy man as well as diabetics (Jenkins, Wolever, Leeds, et al, 1978; Monnier, Pham, Aquirre, Orsetti and Mirouze, 1978; Monuz, Sandstead, Jacob, Johnson and Mako, 1979). In addition the use of high fibre, high carbohydrate diets has led to a reduction in insulin requirements in some IDDM and discontinuation of sulphonylurea therapy in NIDDM (Kiehm, Anderson and Ward, 1976; Anderson and Ward, 1978). However it was uncertain whether fibre would hae an equally beneficial effect on glucose tolerance in diabetics with and without AN.

Two possible means of assessing the effectiveness of dietary fibre on glucose tolerance were considered, namely, addition to glucose in the form of an oral glucose tolerance test or by its addition to a mixed meal. The latter was chosen as it was felt that a meal would represent the more physiological stimulus.

The next issue which arose was the selection of the particular fibre or fibres to be used. Indeed a large number of dietary fibres are available, but in reported studies guar and pectin, particularly the former, were the most favoured. One major factor in favour of the choice of guar was the report in which out of six different fibres it was shown to have the most marked effect on postprandial glucose responses in healthy volunteers (Jenkins, Wolever, Leeds, et al, 1978). Pectin was also reported to be effective on postprandial glucose concentrations (Jenkins, Leeds, Gassull, et al, 1977; Jenkins, Wolever, Leeds, et al, 1978). Guar is a galactomannan, a storage polysaccharide, derived from the cluster bean Cyamopsis tetragonotoba. Pectin is a constituent of plant and cell walls, and thus a structural fibre.

The dosage of guar and pectin was the next problem. In most previous reports 10 to 16 g of guar and 10-15 g of pectin were used (Jenkins, Goff, Leeds, et al,

1976; Jenkins, Leeds, Gassull, et al, 1977; Jenkins, Wolever, Leeds, et al, 1978; Morgan, Goulder, Tsiolakis, Marks and Alberti, 1979). In the present study 15 g guar and 10 g pectin were tried. The guar was added to 20 g cooked oats and the pectin to 20 g honey. However, neither the honey nor the oats were found to be palatable after the two fibres were added. The addition of 5 g of guar to the cooked oats and 5 g of pectin to the honey of the previous standard test meal was found to be quite palatable however and was used in this study. Hence the effect of fibre supplementation could be assessed on post-prandial glucose and hormone concentrations, using the standard test meal mentioned earlier as the control.

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CHAPTER IV

METHODOLOGY

SECTION I : THE PATIENTS AND THE DIAGNOSIS OF AUTONOMIC NEUROPATHY

The male NIDDM who attended the Groote Schuur Hospital Diabetes Clinic were specifically questioned for the symptoms of autonomic dysfunction, such as impotence, diarrhoea, gastric fullness and dizziness on standing. Impotence was found to be the most common symptom. Of those questioned, fifteen whose only symptom of autonomic dysfunction was impotence, agreed to undergo testing in order to confirm the presence of AN. A further 20 male diabetics, asymptomatic of AN agreed to undergo the same tests. The patients underwent a series of eight autonomic function tests and the diagnosis of AN was made if the patients had three or more abnormal responses to the eight tests. Only patients who had normal responses to all the tests carried out were included in the group without AN. The tests were as follows:

i) Postural Hypotention: With the subject lying in the supine position, a standard sphygmomanometer cuff was attached to the patient's upper arm. The systolic blood pressure was recorded and was repeated within 30 seconds of the subject assuming the standing position. The procedure was done three times at 5 minute intervals. Postural hypotension was judged as being present if a fall in systolic blood pressure of 30mm Hg or more was present immediately on assuming the standing from the supine position, on all occasions (Ewing, Burt, Williams, Campbell and Clarke, 1976).

ii) Beat-to-beat variation on deep breathing: The subject lay in the supine position for the duration of the test. A standard electrocardiograph machine (Cardiopan 531, Philips) was used. Lead II strips were utilized for measurement of the heart rate. With the electrocardiograph (ECG) recording the rate, the patient breathed in and out deeply three times during a 20 second period. The beginning of each inspiration and each expiration was recorded on the ECG tracing by a marker. The minimum R-R interval for each inspiration and the maximum R-R interval for each expiration was measured and the beats per minute calculated in each instance. The procedure was repeated twice at 5 minute intervals. A mean beat-to-beat variation of less than 10 beats per minute in the 3 procedures was defined as abnormal (Wheeler and Watkins, 1973).

iii) Valsalva Manoeuvre: The subject was studied in the semi-recumbent position and was trained to perform the Valsalva manoeuvre by blowing through a mouthpiece attached to a sphygmomanometer and maintaining a pressure of 40 mm Hg for

15 sec. Heart rate changes as measured by the R-R interval variation on a continuously recording ECG were used as the index of response. Once the subject had mastered the technique, the test was performed with an ECG continuously recording the heart rate changes during the manoeuvre and for 15 sec after the pressure had been abruptly released. The manoeuvre was performed three times at 5 minute intervals. The results were expressed as the Valsalva ratio which was calculated from the ratio of the longest R-R interval after the manoeuvre to the shortest R-R interval during the manoeuvre. A mean valsalva ratio was calculated from the ratios of the 3 manoeuvres. A valsalva ratio of 1.10 or less was defined as an abnormal response (Ewing, Campbell, Burt and Clarke, 1973).

iv) Sustained Handgrip: After instruction in the use of the handgrip dynamo-manometer (Stoelting Company), the subject remained seated throughout with their dominant arm gripped maximally for a few seconds and this was repeated twice. In this way the maximum voluntary contraction (MVC) was obtained by taking the highest value of the three contractions. The patient then maintained the hand-grip at 30% of MVC for 5 minutes, or alternatively for as long as possible, to a maximum of 5 minutes. Blood pressure was measured with a sphymomanometer on the non-dominant arm. Three basal diastolic blood pressure recordings were taken at 5 minute intervals. Further recordings were taken at 1 minute intervals during contraction. A rise in diastolic blood pressure of 10 mm Hg or less was defined as abnormal (Ewing, Irving, Kerr, Wildsmith and Clarke, 1974).

v) Sweating in response to Insulin Hypoglycaemia: The subject was rendered hypoglycaemic by a 0.20 U/kg intravenous insulin injection (Actrapid monocomponent insulin, Novo Laboratories Industries Ltd) after an overnight fast. Quinizarin powder was dusted onto sellotape which was in turn applied to the subject's forehead, palms of the hands and soles of the feet. In the presence of sweating in response to hypoglycaemia, the powder altered in colour from orange-red to purple. Absent or reduced sweat on the lower limbs in comparison with that apparent on the forehead was defined as abnormal (Guttmann, 1940: Editorial, Lancet, 1976).

vi) Testicular Sensation: Testicular sensation was tested only in those diabetics complaining of impotence. The subject's scrotum was held in one hand and each testicle was firmly squeezed in turn between the thumb and two fingers of the other hand. Pressure was increased until pain was felt. A normal response was considered as a peculiar 'sickening' pain on moderate pressure. The response was considered diminished if considerable pressure elicited only an unpleasant but tolerable discomfort and absent if full pressure evoked no response. Diminished or absent testicular sensation in impotent patients was defined as abnormal (Campbell, Ewing, Clarke and Duncan, 1974).

Table IV.1 CLINICAL DETAILS AND INDIVIDUAL RESPONSES TO THE AUTONOMIC FUNCTION TESTS IN THE PATIENTS STUDIED

No.	Name	Age (yr)	Weight (kg)	Height (cm)	Duration of diabetes (yr)	Therapeutic agent used	Postural Hypotension	Beat-to-Beat Variation	Valsalva	Sustained Handrip	Sweating	Testicular Sensation	Heart rate response	Gastric Acids
Diabetics with AN														
1	SH	51	75	167	16	chlorpropamide + phenformin	N	A	A	N	A	N	A	A
2	RB	52	63	164	2	diet	N	A	A	N	A	N	A	N
3	CT	45	56	161	6	glibenclamide + metformin	N	A	A	A	N	N	A	N
4	WR	45	91	179	6	chlorpropamide + phenformin	N	A	N	N	A	A	A	A
5	CL	63	84	180	4.5	tolbutamide	N	N	N	N	N	A	A	A
6	TW	41	74	180	6	glibenclamide + metformin	N	A	N	N	N	N	A	A
7	JB	47	77	168	8	tolbutamide + metformin	N	N	A	N	N	A	A	N
8	MA	52	63	173	10	chlorpropamide + metformin	N	A	A	N	A	N	A	N
9	DC	58	94	169	6	chlorpropamide	N	N	A	A	A	N	A	N
10.	LP	50	64	168	8	glibenclamide	A	A	A	A	-	A	A	-
Diabetics without AN														
A	JL	46	63	172	5	chlorpropamide	N	N	N	N	N	-	N	N
B	DG	35	65	165	5	chlorpropamide	N	N	N	N	N	-	N	N
C	PR	58	63	164	2	glibenclamide	N	N	N	N	N	-	N	N
D	WN	54	70	171	10	chlorpropamide + metformin	N	N	N	N	N	-	N	N
E	WG	49	69	167	3	glibenclamide + metformin	N	N	N	N	N	-	N	N
F	PH	45	86	183	7	chlorpropamide + metformin	N	N	N	N	N	-	N	N
G	EG	59	76	170	8	chlorpropamide	N	N	N	N	N	-	N	N
H	PL	45	93	180	3	glibenclamide	N	N	N	N	N	-	N	N
I	HK	61	73	178	10	chlorpropamide + phenformin	N	N	N	N	N	-	N	N
J	DG	70	76	176	20	chlorpropamide	N	N	N	N	N	-	N	N
K	GA	41	81	182	5	tolbutamide	N	N	N	N	-	-	N	-
L	MW	66	80	187	6	chlorpropamide + metformin	N	N	N	N	-	-	N	-
M	AD	52	74	181	7	glibenclamide	N	N	N	N	-	-	N	-
N	PB	54	68	163	4	metformin	N	N	N	N	-	-	N	-
O	VE	60	70	179	5	glibenclamide + metformin	N	N	N	N	-	-	N	-
P	GF	38	67	168	8	glibenclamide	N	N	N	N	-	-	N	-
Q	SF	41	84	179	2	chlorpropamide	N	N	N	N	-	-	N	-
R	CD	51	60	161	5	chlorpropamide	N	N	N	N	-	-	N	-
S	VV	41	60	168	3	chlorpropamide + metformin	N	N	N	N	-	-	N	-

vii) Immediate heart rate response to standing: The subject lay quietly for three minutes, then stood up within 5 seconds and remained motionless for 2 minutes. The manoeuvre was performed with an ECG continuously recording the heart rate and a marker was used to identify the time of standing. The ECG was recorded from 30 seconds before standing to 1 minute after standing. The R-R intervals at beats 15 and 30 after standing were measured. The results were expressed as the 30 : 15 ratio, ie. the length of the R-R interval at beat 30 divided by the length of the R-R interval at beat 15. A ratio of 1.00 or less was considered abnormal (Ewing, Campbell, Murray, Neilson and Clarke, 1978).

viii) Gastric Acids: The subject was tested in the morning on two separate occasions, each time after a 12 hour overnight fast. With the subject in a semi-recumbent position, on each occasion a radio-opaque nasogastric tube (Rusch, West Germany) was passed into the stomach and the correct positioning confirmed by radiological screening. Then using constant suction at a pressure of 3 mm H_2O, the stomach was emptied of any resting gastric juice. Basal secretions were collected over the ensuing hour. Thereafter the appropriate stimulation was given. On the one occasion an intramuscular injection of 6 μg/kg pentagastrin (Peptavlon, Imperial Chemical Industries Ltd., Macclesfield, Great Britain) was given and gastric juice collected over the next hour. On the other occasion 0.2 U/kg insulin was given. Gastric juice was collected for the next two hours. An abnormal gastric acid response to insulin hypoglycaemia as assessed by Hollander's criteria (1946), in the presence of a gastric acid secretory response to pentagastrin stimulation was defined abnormal (Hosking, Moody, Stewart and Atkinson, 1975).

It is apparent in Table IV.1 that not all patients underwent the full battery of eight autonomic function tests. Patient 10 had such a wide range of abnormal responses to six of the tests that the more cumbersome tests were considered unnecessary. Some of the diabetics without AN, who were only utilized to increase the numbers for the study on basal plasma IRG secretion had only six of the eight autonomic function tests. Again the more complex and cumbersome tests were excluded. All the other patients underwent the full battery of tests. On the basis of their responses to the eight autonomic function tests, AN was diagnosed in ten of the fifteen diabetics who complained of impotence but not in the remaining five. These five patients had abnormal reponses to less than three of the tests and were excluded from the study. In addition nineteen of the twenty diabetics who had no symptoms of AN were indeed found to be free of AN, while in the one remaining diabetic AN was diagnosed.

The clinical details and individual responses to the autonomic function tests of the ten diabetics with AN and the nineteen diabetics without AN, who were included in the ensuing studies, are given in Table IV.1. None of the diabetics was obese and the age ranges in the two groups were alike. The normal subjects,

also referred to as controls, healthy controls or healthy subjects, who acted as controls in the studies were drawn from the laboratory and cleaning staff at the University of Cape Town Medical School. They were all healthy non-obese, non-diabetic males with no history of any endocrine or other medical disorders and with no family history of diabetes mellitus.

All the studies were approved by the Ethical Review Committee of the University of Cape Town. Prior to each test written informed consent was obtained from each person after a careful explanation of the nature of the test.

In the various studies intravenous cannulae were used to enable the withdrawal of multiple blood samples. The cannulae were all 19 gauge Wings infusion sets (AHSC South Africa (Pty) Ltd) which were inserted into an antecubital vein and kept patent by filling the needle with heparinized saline.

The studies on hormone secretion were divided into three sections : basal hormone concentrations, stimulated hormone concentrations, and the effect of fibre supplementation on glucose and hormone concentrations.

SECTION 2 : BASAL HORMONE SECRETION

Basal plasma IRG, serum PP, serum gastrin, plasma GIP and plasma SRIF-LI concentrations were estimated in diabetics and healthy controls of similar ages, divisible into those with AN and those without AN. The effects of the acute establishment of fasting normoglycaemia and of complete gastric emptying on the basal levels of the five aforementioned hormones were also assessed in the diabetics after separation into the two groups. In this and the following sections the diabetics included in the studies on the various hormones are referred to by the number or letter ascribed to them in Table IV.1.

Basal plasma IRG concentrations were estimated in twentysix diabetics, eight with AN (1,2,3,4,5,8,9 and 10) and eighteen without AN (A,B,C,D,E,F,H,I,J,K,L, M,N,O,P,Q,R and S). However the detailed studies on the effect of prior normalization of fasting blood glucose levels and complete gastric emptying on basal plasma IRG concentrations were conducted in ten diabetics, five with AN (1,2,3,4 and 5) and five without AN (A,B,C,D and E). Basal serum PP concentrations were studied in 12 diabetics : 6 with AN (2,3,4,5,6 and 7) and 6 without AN (A,B,C,E,F and G), basal plasma GIP concentrations in 12 diabetics : 6 with AN (1,3,4,5,6 and 7) and 6 without AN (A,B,C,D,E and F), basal serum gastrin concentrations in 12 diabetics : 6 with AN (1,2,3,4,5 and 6) and 6 without AN (A,B,C,D,F and G), and basal plasma SRIF-LI levels in 12 diabetics : 6 with AN (1,3,4,5,6 and 7) and 6 without AN (A,B,C,D,E and F).

Basal plasma IRG, serum PP, plasma SRIF-LI and serum gastrin concentrations were estimated in 17 healthy subjects aged 35-62 yr, weighing 61-97 kg and 166-190 cm in height. Basal plasma GIP concentrations were estimated in 12 healthy controls aged 35-55 years, 166-190 cm tall and weighing 61-97 kg. The detailed studies were not done in the healthy subjects.

Basal secretion of the hormones was studied under the following conditions :

A. After a 12 hr overnight fast:

The diabetics and controls fasted overnight for 12 hr. In the morning at 08.00 hr an indwelling cannula was inserted and after reclining comfortably for half-an-hour a basal blood sample was collected. The next two protocols were conducted with the diabetics admitted to a hospital ward.

B. After a 12 hr overnight insulin infusion:

Once in the hospital ward, at 20.00 hr, three hr after the evening meal, an indwelling catheter was inserted into each arm. The one catheter was used for blood sampling and the other to administer a continuous insulin infusion. 2.5 units of Actrapid insulin was added to 50 ml haemaccel (Behring Institute, West Germany). The haemaccel was used in order to prevent the insulin from adhering to the syringe walls. The infusion was maintained by a B. Brown infusion pump (Apparatebau, Melsungen). The infusion was commenced at 20.00 hr and a rate of 0.5 - 0.8 U/hr was maintained. With a view to obtaining normalization of fasting blood glucose concentrations, blood samples were withdrawn at hourly intervals throughout the night, blood glucose estimations were done using dextrostix (Ames Company, Miles Laboratories Ltd) and the Ames Eyetone Reflectometer, and the infusion rate adjusted accordingly. In the morning at 08.00 hr, the infusion was stopped and basal blood samples collected over thirty minutes.

C. After overnight gastric aspiration:

In the evening on the second day of hospital admission, 5 hours after eating his evening meal, a radio-opaque naso-gastric tube was passed in each diabetic subject. The correct positioning was confirmed radiologically. Two hourly gastric aspiration was carried out during the night. In the morning at 08.00 hr the nasogastric tubes were removed, an indwelling cannula inserted and basal blood samples withdrawn.

SECTION 3 : PROVOCATIVE STIMULATION

Hormone secretion was examined following provocative stimulation in NIDDM who could be sub-divided into the two groups, and in the healthy controls. The stimulated secretion of IRG, PP, GIP, Gastrin and SRIF-LI was studied according to the following protocols:

A. Insulin Hypoglycaemia without prior induction of fasting normoglycaemia

Plasma IRG, plasma GIP, serum gastrin and serum PP concentrations were estimated during insulin hypoglycaemia without prior induction of fasting normoglycaemia. However, plasma SRIF-LI responses were not evaluated in this study.

The fifteen diabetics who took part in this study on IRG concentrations consisted of the five diabetics with AN included in the previous study, with an additional two diabetics with AN (8 and 9). The diabetics without AN comprised four subjects from the previous study (A,C,D and E) plus an additional four subjects (F,H,I and J). Serum PP concentrations were estimated in 14 patients, two (5 and I) being added to the twelve participants in the previous study. Serum gastrin concentrations were estimated in the same twelve diabetics participating previously; while plasma GIP levels were estimated in ten diabetics, five with AN (1,2,3,4 and 5) and five without AN (A,C,E,F and I).

Twelve healthy people aged 35-59 yr, weighing 70-88 kg and 164-182 cm in height acted as controls for all the hormones except GIP, for which there were seven controls, aged 38-50 yr, 170-186 cm tall and weighing 70-89 kg.

The insulin hypoglycaemia test was done on an outpatient basis.

Following an overnight fast, an indwelling catheter was inserted. After half an hour at rest in a recumbent position, a basal blood sample was withdrawn. Then an intravenous bolus injection of 0.2 U/kg Actrapid insulin was given in the diabetics, while 0.1 U/kg insulin was given in the controls. Blood samples were withdrawn 20, 40, 60 and 90 minutes after the insulin injection.

B. Insulin Hypoglycaemia after Induction of fasting normoglycaemia

The effect of prior normalization of fasting blood glucose concentrations was assessed on plasma IRG, serum PP and serum gastrin concentrations during insulin hypoglycaemia in the diabetics. On this occasion plasma was also collected for SRIF-LI estimations. In view of the lack of effect of hypoglycaemia on plasma GIP levels in the preceding study, samples were not collected for GIP estimations in this study. Plasma IRG responses were assessed in 5 diabetics with AN (1,2,3,4 and 5) and 5 diabetics without AN (A,C,D,E and F). Serum PP responses were evaluated in 5 diabetics with AN (2,3,5,6 and 7) and 5 diabetics without AN (A,B,C,F and G). Serum gastrin responses were assessed in the same 12 patients who took part in the preceding protocol while plasma SRIF-LI responses were determined in 5 diabetics with AN (1,3,4,5 and 6) and 5 diabetics without AN (A,B,D,E and F). Ten healthy people aged 35-55 yr, 166-180 cm tall and weighing 73-92 kg acted as controls for the study on SRIF-LI. They underwent the insulin hypoglycaemia test described in the preceding protocol.

The diabetics were admitted to a hospital ward for the study. In the

Table IV.2 CONSTITUENTS OF THE MIXED MEAL

	Weight (g)	CHO (g)	Proetin (g)	Fat (g)	Calories
2 boiled eggs	108	1.0	14.0	12.4	166
250 ml cow's milk	250	12.0	8.5	9.0	165
30 g skim milk powder	30	15.5	10.7	0.1	107.5
2 slices wholewheat bread	60	28.2	4.9	2.1	136.8
20 g honey	20	15.3	0.1	-	61.2
20 g butter	20	-	0.1	16.4	148
	488	72	38.3	40	784.5

C.I. Church and H.N. Church : Food values of Portions commonly used
12th ed Rev.
J.B. Lippincott, 1975
Philadelphia.

evening, at 20.00 hr, three hours after the evening meal, a 12 hr continuous insulin infusion was started in each diabetic with a view to establishing fasting normoglycaemia. The details of the infusion are described in section 1.3. The infusion completed, basal blood samples were collected over the following thirty min and the insulin hypoglycaemia test described in the preceding protocol repeated.

C. Mixed Meal

Concentrations of all of the abovementioned hormones were estimated after ingestion of a mixed meal. The study on IRG secretion was conducted in the same 10 diabetics, who took part in the detailed studies on basal plasma IRG secretion. The studies on PP, gastrin and GIP secretion were carried out in the same patients participating in each individual hormone study in the first section. SRIF-LI secretion in response to ingestion of a meal was evaluated in the same diabetics studied for SRIF-LI in the preceding protocol. Twelve healthy subjects aged 33-62 yr, weighing 62-97 kg and 170-186 cm tall acted as controls for the study on all the hormones besides gastrin. Six healthy subjects aged 35-62 yr, 170-179 cm tall and weighing 61-97 kg acted as controls for the study on serum gastrin concentrations.

The diabetics and controls ate a mixed meal the morning after an overnight fast. Simultaneously with the overnight fast, the diabetics had their stomachs slowly emptied to ensure complete gastric emptying.

In the evening, on the second day of admission, five hr after the evening meal, a nasogastric tube was passed in each patient and the overnight gastric aspiration as described in detail in Section I begun. At 08.00 hr, the nasogastric tubes were removed, an indwelling catheter inserted and basal blood samples collected over the ensuing 15 minutes. In the healthy subjects the study also commenced at 08.00 hrs, with the pacing of an intravenous catheter in an antecubital vein and the collection of basal blood samples over fifteen minutes. In all the subjects, after the basal samples were taken, the mixed meal, the constituents and caloric contents of which are given in Table IV.2, was eaten in ten minutes. One blood sample was taken half-way during the meal and further samples were collected 5, 15, 30, 45, 60, 90, 120, 180 and 240 minutes after completion of the meal.

SECTION 4 : FIBRE SUPPLEMENTATION

The effects of fibre supplementation on serum glucose, serum insulin, plasma IRG and plasma GIP responses to ingestion of a meal were assessed in six diabetics with AN, six age-matched diabetics without AN and in twelve healthy controls in the same age range. Patients 1,3,4,5,6 and 7 comprised the diabetics with AN and patients A,B,C,D,E and F the diabetics without AN. The twelve healthy subjects ranged in age from 35-62 yr, were 170-187 cm in height and

weighed 60-97 kg. The subjects ingested a test meal on two separate occasions, 1-2 weeks apart, in each instance after an overnight fast. The test meal was the same as that described in Section 3 C and detailed in Table IV.2. In the fibre-supplemented meal, 5 g guar was added to the cooked oats which was substituted for one slice of bread and 5 g of pectin was added to the honey. Otherwise the constituents of the meal were the same as in the test meal. The carbohydrate and caloric contents of the fibre-supplemented meal were 28 g and 794.5 g respectively. On each occasion in indwelling cannula was inserted, basal blood samples withdrawn over 15 min and the meal ingested in 10 minutes. One blood sample was collected during the meal and further samples were withdrawn 5,15,30,45,60,90,120,180 and 240 minutes after completion of the meal.

SECTION 5 : COLLECTION OF SAMPLES

In all the aforementioned studies, blood samples for plasma GIP, plasma IRG and plasma SRIF-LI estimations were collected in heparinized test tubes containing 1,000 U/ml of Trasylol (Aprotinin, Bayer). The plasma was immediately separated by centrifugation and the aliquots were stored at -20°C. Blood samples for serum glucose, serum PP, serum insulin and serum gastrin estimations were collected in plain glass tubes and allowed to clot. Thereafter the serum was separated by centrifugation and the aliquots stored at -20°C for later assay.

SECTION 6 : LABORATORY METHODS

Serum glucose concentrations were estimated by means of the ferricyanide method (Hoffman, 1937; Varley, 1967) using the Technicon Auto Analyser I (Technicon Instrument Corporation, Tarrytown, New York).

Concentrations of the various hormones in blood were measured by specific radioimmunoassays.

Plasma glucagon immunoreactivity (IRG) was assayed by the method described by Faloona and Unger (1974).

Serum PP immunoreactivity was measured by radioimmunoassay by the method described by Sive, Vinik, Van Tonder and Lund (1978).

Plasma GIP immunoreactivity was estimated by radioimmunoassay using the method described by Kuzio, Dryburgh, Malloy and Brown (1974).

Serum gastrin immunoreactivity was measured by radioimmunoassay using the SORIN gastrin radioimmunoassay kit (International CIS).

Plasma somatostatin immunoreactivity (SRIF-LI) was assayed by the method described by Kronheim, Berelowitz and Pimstone (1978).

Serum insulin immunoreactivity was measured by radioimmunoassay using the SORIN insulin radioimmunoassay kit (International CIS).

<u>Promblems associated with the SRIF-LI assay</u>:

The SRIF-LI assay using unextracted plasma developed by Kronheim, Berelowitz and Pimstone (1978) was utilized in this study. Kronheim and associates hae gone to considerable lengths to authenticate this assay and have demonstrated that : the integrity of the labelled SRIF can be maintained in unextracted plasma and the SRIF-LI measured in this system shared immunological identity with synthetic SRIF (Kronheim, Berelowitz and Pimstone, 1978; Kronheim and Pimstone, 1980). However, doubt still remains concerning the authenticity of SRIF-LI measured by this means. In particular Arimura, Lundqvist, Rothman, et al (1978), and Penman, Wass, Lund, et al (1979), have found that plasma SRIF-LI levels measured in extracted plasma are much lower than those estimated in unextracted plasma using the present assay. These differences may be due to other SRIF-LI like substances or to the measurement of different forms of SRIF-LI or non-specific effects in unextracted plasma. In particular the antiserum used in the present assay appears to be specific for the middle portion of SRIF and may thus measure other forms of SRIF such as those recently described. Recently biologically active higher molecular weight forms of SRIF have been isolated and structurally identified in porcine intestine (Pradayrol, Jornvall, Mutta and Ribet, 1980) and porcine and ovine hypothalami (Schally, Huang, Chang, et al, 1980; Brazeau, Ling, Esch, et al, 1980) and have been demonstrated to be released from ovine stalk median eminence synaptosomes (Dr R.P. Miller, University of Cape Town, personal communication). Thus the quantitation of SRIF-LI in plasma will depend on the relative crossreactivity of the antisera used with these N terminally extended forms of SRIF. Whatever the explanation for the differences it is clear that the material measured in unextracted plasma has been at least partially specified as SRIF-LI. However, the controversy will remain until such time as unequivocal specification of the material measured in unextracted plasma as SRIF-LI is demonstrated by its actions in a bioassay. Accepting these reservations the SRIF-LI assay using unextracted plasma as mentioned earlier, and which appears to measure a substance that is immunologically related to synthetic SRIF and is altered in response to physiological stimuli, was used in the present studies.

The details of the laboratory techniques are described in the appendix.

SECTION 7 : STATISTICAL ANALYSIS

Statistical analysis of the data was carried out according to the methods described by Snedcor and Cochrane (1967). The data on plasma IRG, plasma SRIF-LI, plasma GIP, serum PP, serum gastrin, serum insulin and serum glucose concentrations were all analysed by means of non-parametric statistical tests. The Mann-Whitney U test was used for unpaired data and the Wilcoxon Signed Rank test for paired data. The detailed methods of the statistical tests are described in the appendix.

CHAPTER V

RESULTS

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CHAPTER V

RESULTS

In this chapter the results of the studies carried out are given, following the same sequence as the preceding chapter. The results are expressed as the mean ± the standard error of the mean (SEM). Wherever appropriate, diagrams appear on the page opposite the relevant results. On occasion the results are given in table form.

In the sections : (a) on basal hormone secretion,

and

(b) hormone secretion in response to ingestion of a mixed meal,

and

(c) insulin hypoglycaemia,

the results are initially given for the diabetics considered as a single group. Results after subdivision into diabetics with and without AN follow.

The studies on the various hormones utilized combinations of the patient group. However the differences in the composition of the study groups made no difference to the glucose and insulin results which were obtained. In order to avoid repetition therefore, the glucose and where relevant, the insulin results in each section, will be described first.

SECTION I : BASAL HORMONE SECRETION : EFFECTS OF OVERNIGHT INSULIN INFUSION AND OVERNIGHT GASTRIC ASPIRATION

I (i) GLUCOSE AND INSULIN

Diabetics vs Controls

The 26 diabetics had a significantly higher mean basal serum glucose concentration than the 17 healthy subjects (8.2 ± 0.6 vs 4.9 ± 0.2 mmol/l) ($p < 0.01$). However the mean basal serum insulin concentrations were similar in the diabetics (16.2 ± 1.5 μU/ml) and the controls (13.8 ± 1.5 μU/ml).

Diabetics without AN vs Diabetics with AN

The mean basal serum glucose concentrations were similar in the 18 diabetics without AN (8.2 ± 0.7 mmol/l) and in the 8 diabetics with AN (8.2 ± 0.9 mmol/l) but were elevated relative to the controls. The basal serum insulin concentrations too were similar in the diabetics without AN (16 ± 1.3 μU/ml) and those

Table V.1

Basal serum insulin and serum glucose concentrations and the effects thereon of overnight insulin infusion and overnight gastric aspiration in 5 diabetics with and 5 diabetics without AN

	Serum Insulin μU/ml			Serum Glucose mmol/l		
	Basal (After Overnight Fast)	After Overnight Insulin Infusion	After Overnight Gastric Aspiration	Basal (After Overnight Fast)	After Overnight Insulin Infusion	After Overnight Gastric Aspiration
Diabetics Without AN	13.8 ± 1.8	24.8 ± 5 *	12.6 ± 1.6	8 ± 1.9	5 ± 0.4*	8.1 ± 0.6
Diabetics With AN	17.2 ± 3.2	29 ± 8.5*	15.2 ± 2.5	7.9 ± 1.5	5.5 ± 0.4*	10.1 ± 1.7

* $p < 0.01$ significantly different after 12 hr insulin infusion

with AN (16.5 ± 2 μU/ml) and were not different from the controls.

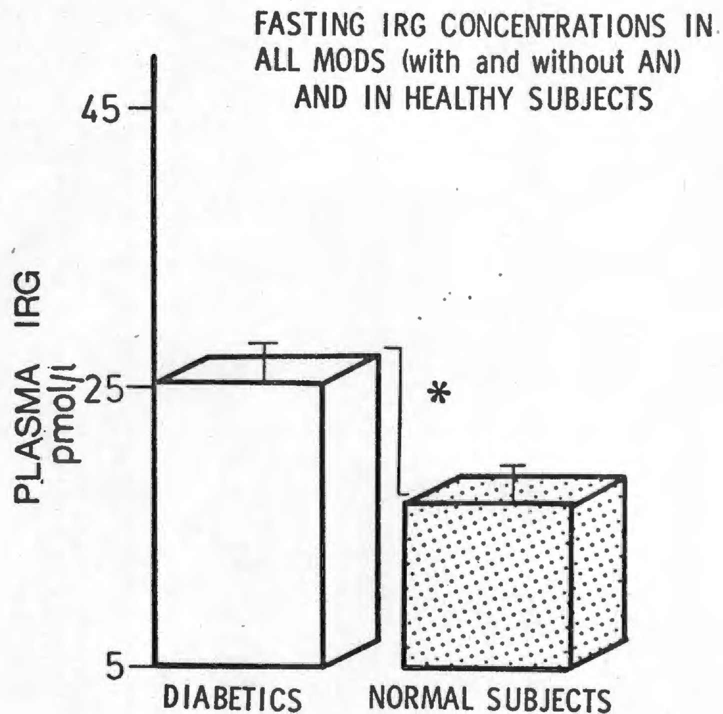
In the smaller group of patients selected for detailed studies (Table V.1) the mean basal serum glucose and insulin concentrations remained similar in the two groups of diabetics.

The overnight insulin infusion reduced the mean basal serum glucose concentrations to within the normal fasting range and raised the mean basal serum insulin concentrations in both the diabetics with AN and those without AN. There were no significant differences between the two groups.

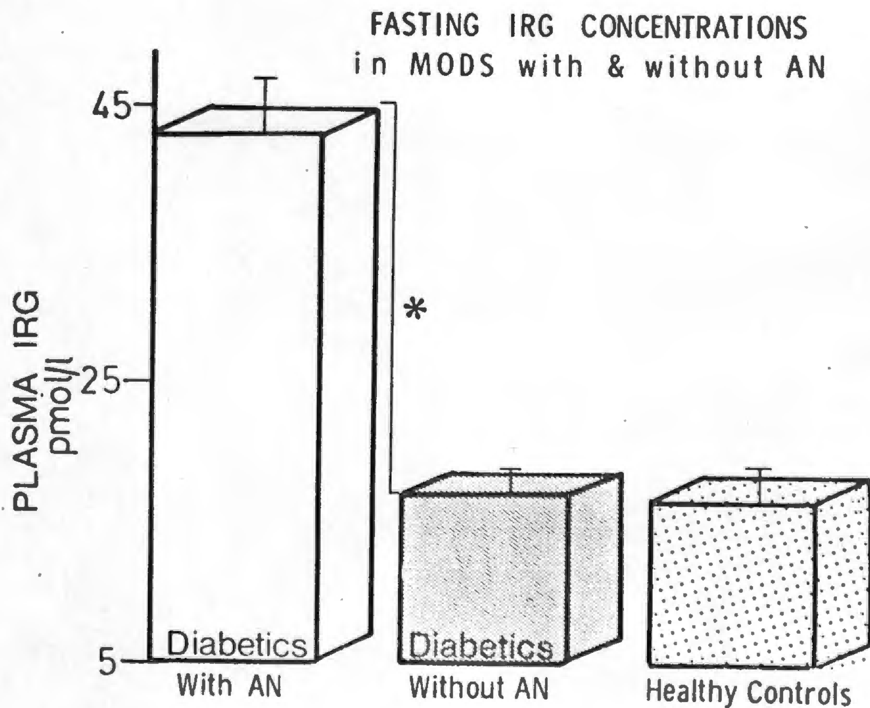
The overnight gastric aspiration did not alter the mean basal serum insulin or glucose concentrations in either group of diabetics. Once again no significant differences were apparent between the two groups.

Comment:

The diabetics had significant fasting hyperglycaemia but similar basal serum insulin concentrations to those of the controls. The diabetics with and without AN had similar basal serum insulin and glucose concentrations. The overnight gastric aspiration did not alter these concentrations in either group of diabetics. The overnight insulin infusion induced fasting normoglycaemia and raised the basal serum insulin levels to a similar degree both in the diabetics with and without AN.



(Fig. V.1) Basal plasma IRG concentrations in 17 healthy subjects and 26 diabetics. The vertical bars represent the SEM in this and all following figures. The asterisk indicates $p < 0.05$ difference between the two groups.



(Fig. V.2) Basal plasma IRG concentrations in 8 diabetics with AN, 18 diabetics without AN and 17 healthy subjects. Asterisk indicates $p < 0.01$ difference between the diabetics with AN and the other two groups.

Table V.2

Basal plasma IRG concentrations (pmol/l) and the effects thereon of overnight insulin infusion and overnight gastric aspiration in 5 diabetics with AN and 5 diabetics without AN.

	Basal (After Overnight Fast)	After Overnight Insulin Infusion	After Overnight Gastric Aspiration
Diabetics without AN	16.2 $\pm$ 2.1	18.8 $\pm$ 2.9	30.4 $\pm$ 2.8
Diabetics with AN	49.4 $\pm$ 5.9*	48.6 $\pm$ 6.4*	52.1 $\pm$ 6.4*

* $p < 0.01$ difference between the two groups.

I (ii) GLUCAGON

Diabetics vs Controls (Fig. V.1)

The mean basal plasma was 25.3 ± 2.8 pmol/l in the 26 diabetics and 16.8 ± 2.5 pmol/l in 17 healthy controls. The difference was statistically significant ($p < 0.05$).

Diabetics with AN vs Diabetics without AN

When the diabetics were divided into those with and without AN, prominent differences in their basal plasma IRG concentrations became apparent (Fig. V.2). In the 18 diabetics without AN, the mean basal plasma IRG level was 17.4 ± 1.3 pmol/l, similar to that observed in the controls. In contrast the diabetics with AN had a mean basal plasma IRG level of 43 ± 4.2 pmol/l, significantly higher ($p < 0.01$) than in the diabetics without AN and in the controls.

In 5 diabetics in each group selected for the detailed studies, the mean basal plasma IRG concentration was significantly higher ($p < 0.01$) in the diabetics with AN than in the diabetics without AN (Table V.2).

The overnight insulin infusion which induced fasting normoglycaemia did not alter the mean basal plasma IRG concentrations in either group of diabetics.

The overnight gastric aspiration which was done to ensure complete emptying of the stomach, also did not alter the mean basal plasma IRG concentrations in either group of diabetics.

On both occasions the mean basal plasma IRG levels were significantly higher ($p < 0.01$) in the diabetics with AN than in the diabetics without AN.

Comment:

The diabetics had a significantly higher mean basal plasma IRG concentration than the healthy controls. However, the mean basal plasma IRG concentration in the diabetics without AN was similar to that in the controls, but were significantly elevated in the diabetics with AN. Further, neither the overnight insulin infusion which induced fasting normoglycaemia, nor the overnight gastric aspiration which ensured complete emptying of the stomach, induced any change in the basal plasma IRG concentrations in the diabetics with AN and those without AN.

Table V.3

Basal serum PP concentrations (pmol/l) with and without prior overnight insulin infusion and overnight gastric aspiration in 6 diabetics with AN and 6 diabetics without AN.

	Basal (After Overnight Fast)	After Overnight Insulin Infusion	After Overnight Gastric Aspiration
Diabetics without AN	35 ± 11.5	35 ± 6	48.6 ± 8.8
Diabetics with AN	47 ± 10.8	86 ± 16.6*	52.6 ± 12.1

* $p < 0.05$ difference between the two groups.

I (iii) PANCREATIC POLYPEPTIDE

Diabetics vs Controls

The mean basal serum PP concentrations were similar in the 12 diabetics (40.6 ± 7.7 pmol/l) and the 17 healthy subjects (42.5 ± 6.3 pmol/l).

Diabetics without AN vs Diabetics with AN (Table V.3)

The mean basal serum PP concentrations were found to be similar in the two groups of diabetics and were not different from the contols.

The overnight insulin infusion did not alter the mean basal serum PP concentration in the diabetics without AN. In the diabetics with AN, however, there was an increase in the mean basal serum PP level. This increase was not significant despite the insulin infusion resulting in an elevation of fasting serum PP levels in five of the six diabetics with AN. On this occasion the mean basal serum PP concentration was significantly higher in the diabetics with AN than in those without AN and also to the controls ($p < 0.05$).

The overnight gastric aspiration induced an insignificant increase in the mean basal serum PP levels in both groups of diabetics.

The differences between the two groups of diabetics were not significant nor were the levels different from the mean basal levels in the controls.

Comment:

The 12 diabetics and the 17 healthy subjects had similar mean basal serum PP concentrations. Diabetics with and without AN had similar mean basal serum PP concentrations which were not different from those of the healthy controls. Overnight gastric aspiration induced an insignificant rise in mean serum PP levels in both groups of diabetics but in neither group was the level significantly different from the normal mean basal level. Overnight insulin infusion induced an elevation in the mean basal serum PP level only in the diabetics with AN. On this occasion the mean basal serum PP concentration was significantly higher in the diabetics with AN than in those without AN and the controls.

Table V.4

Basal serum gastrin concentrations (pmol/l) with and without prior overnight insulin infusion and overnight gastric aspiration in 6 diabetics with AN and 6 diabetics without AN.

	Basal (After Overnight Fast)	After Overnight Insulin Infusion	After Overnight Gastric Aspiration
Diabetics without AN	27.4 ± 5.1	31.8 ± 5.1	26.1 ± 4.8
Diabetics with AN	34 ± 4	30.5 ± 1.7	26 ± 1.3

I (iv) GASTRIN

Diabetics vs Controls

The mean basal serum gastrin concentration was 24.6 ± 1.9 pmol/l in the 17 healthy controls and 30.7 ± 3.2 pmol/l in the 12 diabetics. The difference was not statistically significant.

Diabetics without AN vs Diabetics with AN (Table V.4)

The mean basal serum gastrin concentrations were similar in both groups of diabetics, and were not significantly different compared to the controls.

The overnight insulin infusion induced a slight rise in the mean basal serum gastrin level in the diabetics without AN and a minor fall in the diabetics with AN.

Overnight gastric aspiration did not alter the mean basal serum gastrin level in the diabetics without AN, but caused a small fall in the diabetics with AN. On all occasions tested, the mean basal serum gastrin concentrations were similar in both groups of diabetics and were not different from the levels in the healthy subjects.

Comment:

The diabetics as a single group or divided into two groups, ie. those with and without AN, all had normal mean basal serum gastrin concentrations. Furthermore neither the insulin infusion nor the overnight gastric aspiration resulted in a deviation of the mean basal serum gastrin levels from the normal range in either group of diabetics.

Table V.5

Basal plasma GIP concentrations and the effects thereon of overnight insulin infusion and overnight gastric aspiration in 6 diabetics with AN and 6 diabetics without AN.

	Plasma GIP pmol/l		
	Basal (After Overnight Fast)	After Overnight Insulin Infusion	After Overnight Gastric Aspiration
Diabetics without AN	58.6 ± 10.9	65.2 ± 12.1	73.1 ± 13.2
Diabetics with AN	58 ± 9	77 ± 11.1	56.2 ± 10.3

I (v) GASTRIC INHIBITORY POLYPEPTIDE

Diabetics vs Controls

The mean basal plasma GIP concentrations were similar in the 12 diabetics (58.3 $\pm$ 6.8 pmol/l) and in the 12 controls (59.4 $\pm$ 5.0 pmol/l).

Diabetics without AN vs Diabetics with AN (Table V.5)

The mean basal plasma GIP concentrations were similar in the diabetics without AN and those with AN.

The overnight insulin infusion did not significantly alter the mean basal plasma GIP levels in either group of diabetics.

The overnight gastric aspiration too did not significantly alter the mean basal plasma GIP levels in the two groups of diabetics. In the diabetics without AN, there was a small rise in mean basal plasma GIP levels after gastric aspiration but not in the diabetics with AN. On all occasions the mean basal plasma GIP levels in the two groups of diabetics did not differ from those in the healthy subjects.

Comment:

The mean basal plasma GIP concentrations were similar in the diabetics and controls. Both the diabetics with and without AN had similar mean basal plasma GIP concentrations, which were not different to that of the normals. Furthermore, gastric aspiration and insulin infusion did not notably alter the mean basal plasma GIP concentrations in either group of diabetics.

Table V.6

Basal plasma SRIF-LI concentrations (pg/ml) with and without prior overnight insulin infusion and overnight gastric aspiration in 6 diabetics with AN and 6 diabetics without AN.

	Basal (After Overnight Fast)	After Overnight Insulin Infusion	After Overnight Gastric Aspiration
Diabetics without AN	215 ± 60.4	162 ± 30.2	177 ± 40.8
Diabetics with AN	236 ± 44.8	203 ± 60.9	230 ± 47

I (vi) SOMATOSTATIN

Diabetics vs Controls

The mean basal plasma SRIF-LI concentration was 225 ± 36 pg/ml in the 12 diabetics and 192.2 ± 16.1 pg/ml in the 17 healthy controls. The difference was not statistically significant.

Diabetics without AN vs Diabetics with AN (Table V.6)

The mean basal plasma SRIF-LI levels were similar in the two groups of diabetics, and neither were significantly different from that in the controls.

The overnight insulin infusion did not induce any significant alteration in the mean basal plasma SRIF-LI concentrations in either of the diabetic groups. However there was a slight reduction in the mean basal plasma SRIF-LI concentration in both groups.

Similarly, the overnight gastric aspiration induced negligible alterations in the mean basal plasma SRIF-LI levels in both groups of diabetics. On all occasions, the basal plasma SRIF-LI concentrations in the two groups of diabetics remained within the normal fasting range.

Comment:

In the diabetics, whether considered as one group or sub-divided into those with and without AN, the mean basal plasma SRIF-LI concentrations were within the normal range. In addition, neither the overnight insulin infusion nor the overnight gastric aspiration induced the basal plasma SRIF-LI levels to fall outside the normal basal range in the diabetics with AN or in those without AN.

Table V.7

Summary of the data on basal secretion of the five gastrointestinal hormones in the diabetics as a single group, the diabetics without AN and the diabetics with AN relative to the normal subjects.

	IRG	PP	GASTRIN	GIP	SRIF-LI
Diabetics	↑↑	N	N	N	N
Diabetics without AN	N	N	N	N	N
Diabetics with AN	↑↑	N	N	N	N

↑↑ - elevated

N - normal

SUMMARY: (Table V.7)

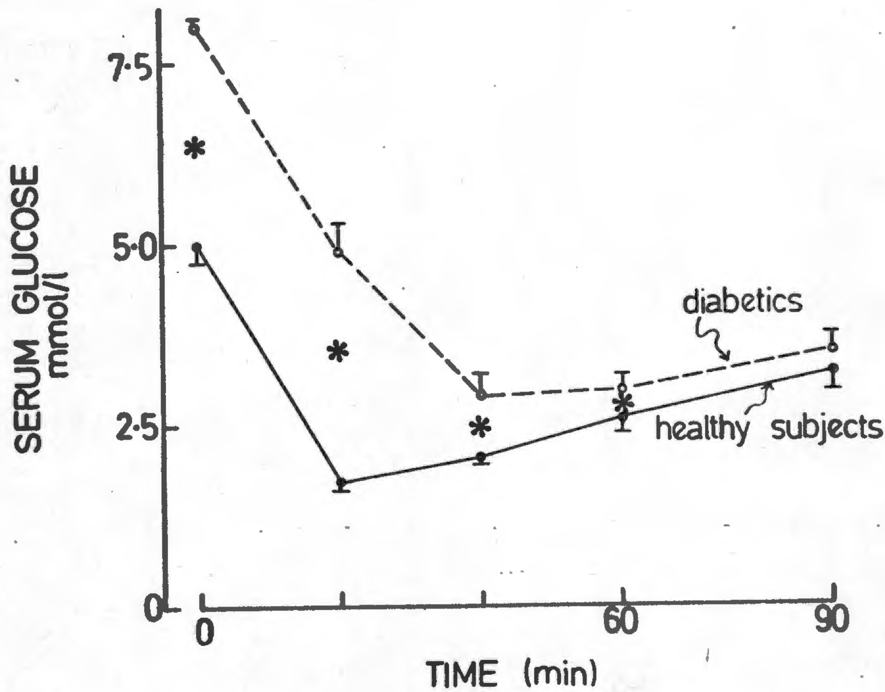
Although the diabetics had fasting hyperglycaemia, their mean basal plasma GIP, plasma SRIF-LI, serum PP and serum gastrin concentrations were similar to those in the healthy controls. However the mean basal plasma IRG concentrations were significantly higher in the diabetics than the controls.

The mean basal plasma GIP, plasma SRIF-LI, serum PP and serum gastrin concentrations were similar in the diabetics with AN and in those without AN, and were not different from the mean concentrations in the healthy subjects. The basal plasma IRG concentrations presented a different picture from the one observed with the aforementioned hormones. There was a significant difference in the basal hormone concentrations in the two groups of diabetics. The diabetics without AN and the healthy controls had similar mean basal plasma IRG concentrations, despite the presence of fasting hyperglycaemia in the former group. In contrast the diabetics with AN had a two-fold higher mean basal plasma IRG concentration than the diabetics without AN and also the controls.

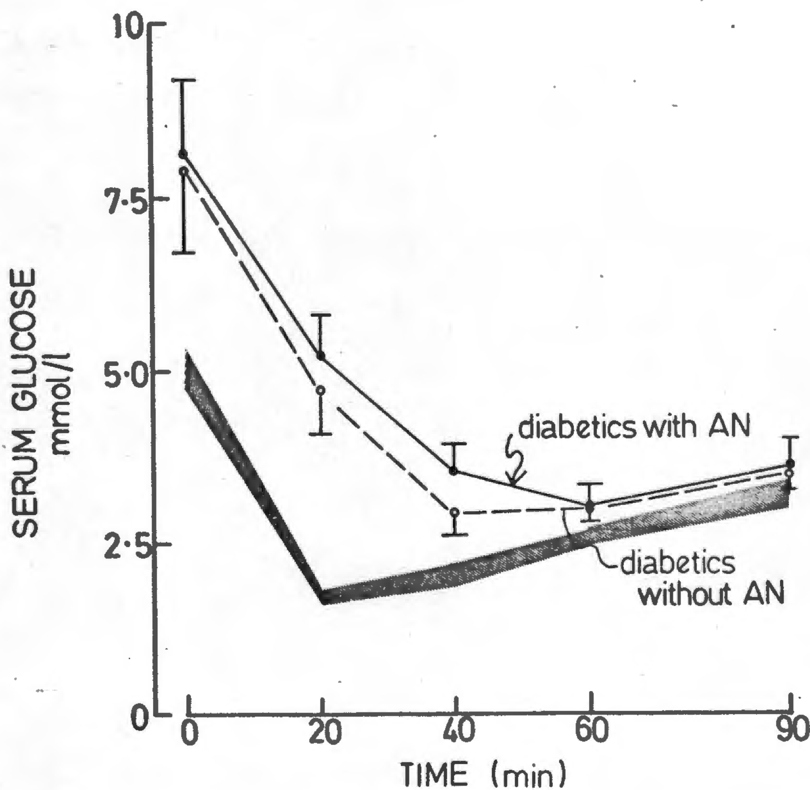
The overnight insulin infusion which induced fasting normoglycaemia did not alter the mean basal plasma SRIF-LI, plasma GIP or serum gastrin concentrations in either group of diabetics and all remained similar to the levels observed in the controls. The overnight insulin infusion also did not alter the mean basal plasma IRG concentrations in either of the groups of diabetics. Thus the mean basal plasma IRG concentration remained twofold higher in the diabetics with AN than in those without AN. After the overnight insulin infusion, the mean serum PP level rose only in the diabetics with AN and was significantly higher than that in the diabetics without AN.

The overnight gastric aspiration, which ensured complete emptying of the stomach, did not induce any deviation from the normal fasting range of the mean basal plasma GIP, plasma SRIF-LI, serum PP or serum gastrin levels in the two groups of diabetics. The plasma IRG levels too were unaffected by the gastric aspiration. Thus once again the mean basal plasma IRG level was twofold higher in the diabetics with AN than in those without AN.

Thus abnormalities in basal IRG secretion were apparent in the diabetics as a group and in the diabetics with AN, but not in those without AN. The basal secretion of the other hormones remained unaltered either in the diabetics per se or in the presence of AN.



(Fig. V.3) Serum glucose responses to insulin hypoglycaemia in 12 healthy subjects and 15 diabetics. Asterisks indicate p 0.01 difference between groups.



(Fig. V.4) Serum glucose responses to insulin hypoglycaemia in 8 diabetics without AN and 7 diabetics with AN. Hatched area in this and ensuing figures represents mean ± SEM responses in healthy subjects.

SECTION 2 : PROVOCATIVE STIMULATION

A INSULIN HYPOGLYCAEMIA WITHOUT PRIOR INDUCTION OF FASTING NORMOGLYCAEMIA

2 A (i) GLUCOSE

Diabetics vs Controls (Fig. V.3)

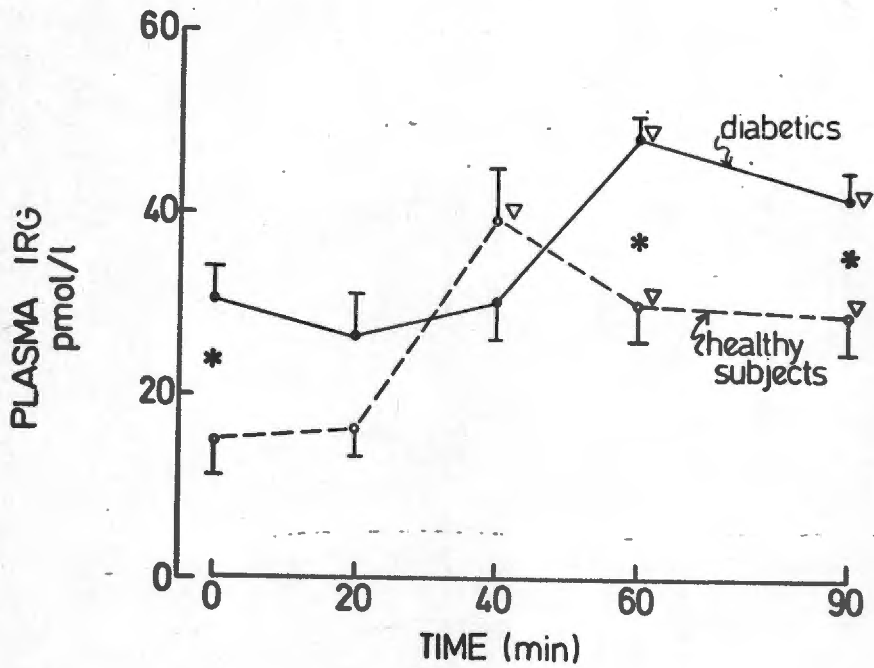
In the 12 controls serum glucose levels fell rapidly after the insulin bolus, from the mean basal level of 5 ± 0.3 mmol/l to 1.7 ± 1.1 mmol/l at 20 min, rising thereafter. In the 15 diabetics the mean basal serum glucose level was 8 ± 0.8 mmol/l, significantly higher than in the controls ($p < 0.01$). Furthermore, the serum glucose levels fell more slowly and not as far as in the controls, with a mean level of 2.9 ± 0.3 mmol/l occurring at 40 min.

Diabetics without AN vs Diabetics with AN (Fig. V.4)

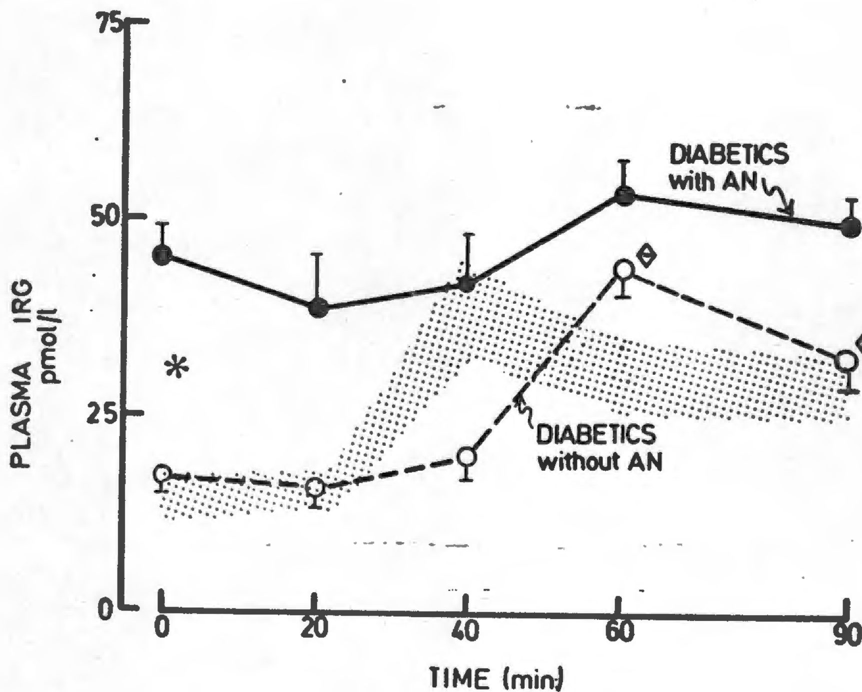
The mean basal serum glucose concentrations as well as the serum glucose responses to the insulin injection were similar in both groups of diabetics and were equally different from those of the controls. In the 8 diabetics without AN serum glucose fell from the mean basal level of 7.9 ± 1.2 mmol/l to 2.9 ± 0.3 mmol/l at 40 min. In the 7 diabetics with AN the mean basal glucose level was 8.1 ± 1.1 mmol/l and fell to 3 ± 0.3 mmol/l at 60 min.

Comment:

There was significant fasting hyperglycaemia, a delayed fall and higher absolute serum glucose levels in the diabetics than the controls. The diabetics with and without AN had similar degrees of fasting hyperglycaemia and serum glucose responses.



(Fig. V.5) Plasma IRG responses to insulin hypoglycaemia in 12 healthy subjects and 15 diabetics. Asterisks indicate $p < 0.01$ difference between groups. Triangles indicate $p < 0.01$ rise above mean basal concentrations.



(Fig. V.6) Plasma IRG responses to insulin hypoglycaemia in 7 diabetics with and 8 diabetics without AN. Asterisk indicates $p < 0.01$ difference between groups and triangles $p < 0.01$ rise above the mean basal concentrations.

2 A (ii) GLUCAGON

Diabetics vs Controls (Fig. V.5)

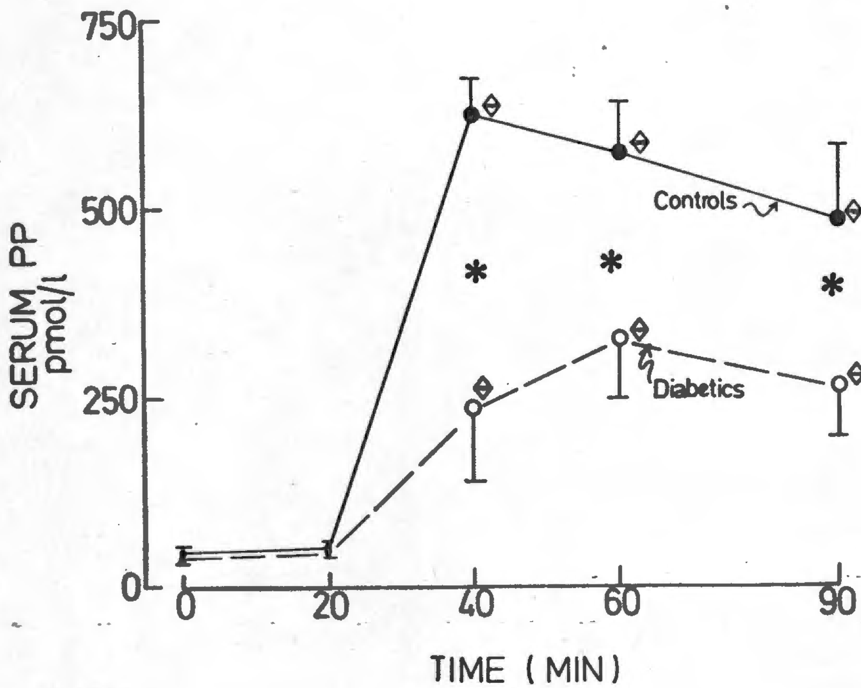
Insulin hypoglycaemia induced a significant rise in plasma IRG levels in the 15 diabetics and the 12 controls. The mean basal plasma IRG concentration was 30.5 ± 4.2 pmol/l in the 15 diabetics and 14.9 ± 2.8 pmol/l in the 12 healthy controls. The difference was statistically significant ($p < 0.01$). In the healthy controls, plasma IRG concentrations rose significantly above basal at 40, 60 and 90 min after the insulin bolus ($p < 0.01$) and a mean peak concentration of 41.5 ± 5.4 pmol/l was attained. In the diabetics plasma IRG concentrations only rose significantly above the mean basal concentration at 60 and 90 min ($p < 0.01$) but the maximum incremental rise was similar in the diabetics (20.5 ± 4.1 pmol/l) and in the controls (26.5 ± 3.6 pmol/l). The mean peak plasma IRG concentration was 51 ± 2.6 pmol/l in the diabetics. Significant differences between the two groups were apparent at 60 and 90 min ($p < 0.01$).

Diabetics without AN vs Diabetics with AN (Fig. V.6)

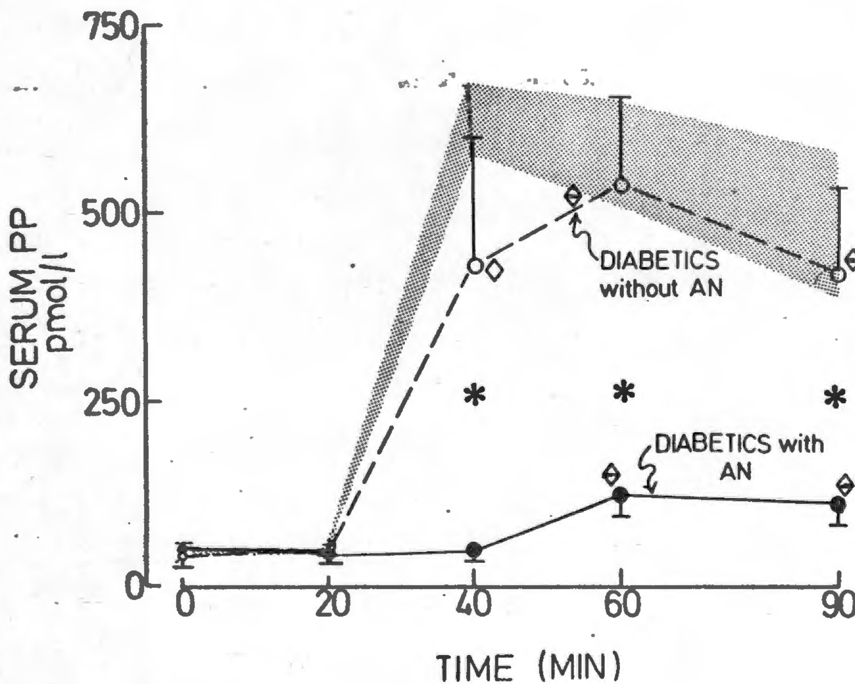
In the diabetics without AN plasma IRG concentrations were significantly elevated above the mean basal concentration of 18.1 ± 1.9 pmol/l at 60 and 90 min (Fig. V.20). The mean peak plasma IRG concentration of 45.7 ± 3.5 pmol/l (maximum increment 27.5 ± 4.6 pmol/l) was observed in the diabetics without AN. Thus both the mean basal and mean peak plasma IRG levels were similar in the diabetics without AN and the controls. The mean basal plasma IRG concentration of 44.7 ± 4.4 pmol/l in the diabetics with AN was significantly higher than in the other two groups ($p < 0.01$). Furthermore, there was no significant rise in plasma IRG concentrations in response to the insulin bolus in the diabetics with AN. In these patients the mean peak plasma IRG concentration was 57.1 ± 2.5 pmol/l and the mean maximum increment 12.5 ± 5.9 pmol/l.

<u>Comment</u>:

The magnitude of the plasma IRG response to the insulin injection was similar in the diabetics as a whole and the controls. Diabetics without AN were found to have a similar twofold rise in plasma IRG concentrations to that seen in the controls. In contrast, the diabetics with AN, who started with a twofold higher mean basal plasma IRG concentration than the diabetics without AN and the healthy subjects, had a flattened and insignificant rise in plasma IRG levels in response to the insulin injection.



(Fig. V.7) Serum PP responses to insulin hypoglycaemia in 12 healthy subjects and 14 diabetics. Triangles indicate $p < 0.01$ rise above the mean basal concentrations. Asterisks indicate $p < 0.05$ difference between groups.



(Fig. V.8) Serum PP responses to insulin hypoglycaemia in 7 diabetics with AN and 7 diabetics without AN. Asterisks indiate $p < 0.01$ difference between groups and triangles $p < 0.01$ rise above the mean basal concentrations.

2 A (iii) PANCREATIC POLYPEPTIDE

Diabetics vs Controls (Fig. V.7)

The mean basal serum PP levels were similar in the healthy subjects and the diabetics, but the rise in serum PP levels in response to insulin hypoglycaemia was significantly greater in the former. In the healthy subjects serum PP concentrations rose from the mean basal level of 44.9 $\pm$ 6.5 pmol/l to reach significant elevation above basal from 40 min onwards ($p < 0.01$), although there was a small fall in PP levels after 40 min. The mean peak serum PP level of 752.4 $\pm$ 60.1 pmol/l, a seventeenfold rise, was attained in the healthy controls. In the diabetics serum PP levels rose from 42.4 $\pm$ 6.4 pmol/l, reaching significant elevation above basal from 40 to 90 min ($p < 0.01$). The mean serum PP levels attained were significantly lower than those of the controls ($p < 0.05$). The mean peak serum PP level attained in the diabetics was 409.7 $\pm$ 99.9 pmol/l, a tenfold rise and significantly lower than that of the controls ($p < 0.05$).

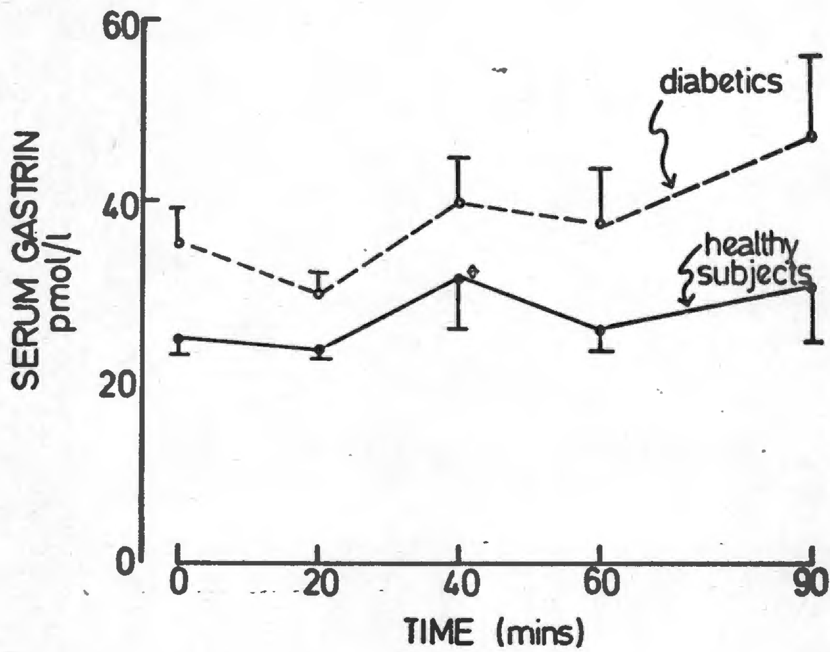
Diabetics without AN vs Diabetics with AN (Fig. V.8)

In the diabetics without AN there was a significant rise in serum PP concentrations from the mean basal level of 37.6 $\pm$ 9.3 pmol/l, apparent from 40 to 90 min ($p < 0.01$) and the mean peak concentration of 676.9 $\pm$ 134.9 pmol/l (an eighteenfold rise) was attained. At no time was the PP response in the diabetics without AN and the controls different.

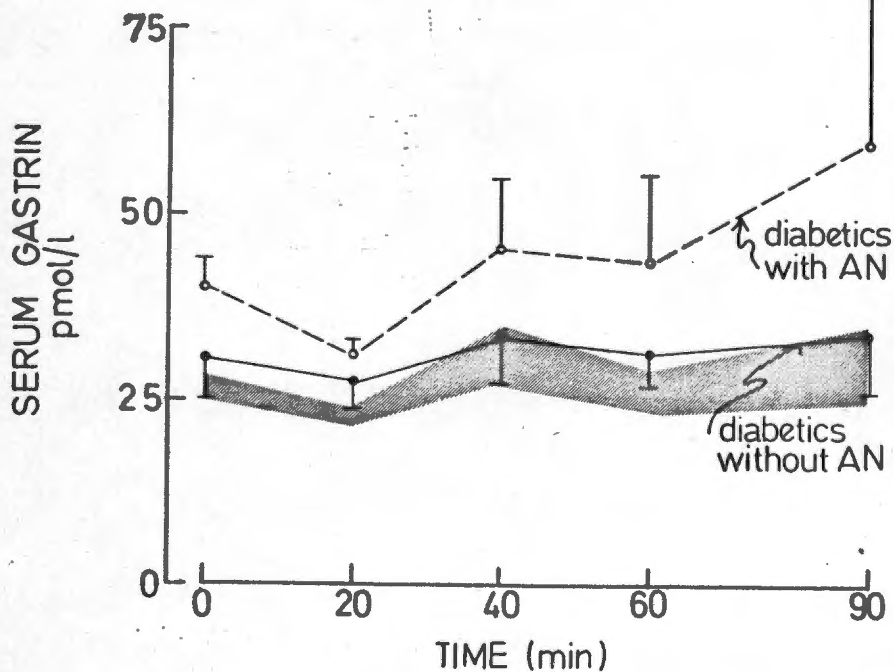
In the diabetics with AN serum PP levels rose from 47.1 $\pm$ 9.1 pmol/l, reaching significant elevation above basal from 60 to 90 min ($p < 0.01$) and attaining the mean peak level of 142.5 $\pm$ 35.9 pmol/l (a threefold rise). There was significant impairment of the serum PP response to hypoglycaemia at 40, 60 and 90 min as well as the mean peak level in the diabetics with AN relative to the controls and the diabetics without AN ($p < 0.01$).

Comment:

The diabetics as a whole had a significantly impaired rise in serum PP levels. The diabetics without AN had a similar rise in serum PP levels to that of the controls, but in the diabetics with AN there was a severely truncated PP rise.



(Fig. V.9) Serum gastrin responses to insulin hypoglycaemia in 12 healthy controls and 12 diabetics. Triangles indicates $p < 0.01$ rise above the mean basal concentration.



(Fig. V.10) Serum gastrin responses to insulin hypoglycaemia in 6 diabetics with and 6 diabetics without AN.

2 A (iv) GASTRIN

Diabetics vs Controls (Fig. V.9)

The mean basal serum gastrin concentrations were 24.9 ± 2.4 pmol/l in the 12 controls and 35.6 ± 3.5 pmol/l in the 12 diabetics. The difference was not statistically significant. In the healthy subjects serum gastrin levels rose in response to insulin hypoglycaemia, reaching significance at 40 min ($p < 0.05$). The mean peak concentration of 36.4 ± 5.3 pmol/l was noted in the controls and was also significantly elevated above the mean basal level ($p < 0.01$). Although there was a rise in serum gastrin levels after the insulin injection in the diabetics too, only the mean peak concentration (49.6 ± 8.8 pmol/l) was significantly elevated above basal ($p < 0.05$). However the mean maximum incremental rises were similar in the diabetics (14 ± 6.9 pmol/l) and the controls (11.5 ± 3.7 pmol/l).

Diabetics without AN vs Diabetics with AN (Fig. V.10)

There was only a small and insignificant rise in serum gastrin levels in the diabetics without AN, and a larger but still insignificant rise in the diabetics with AN in response to insulin hypoglycaemia. In the diabetics without AN serum gastrin levels rose from a mean basal concentration of 30.8 ± 5.6 pmol/l to a mean peak concentration of 38.3 ± 6.1 pmol/l ($p > 0.05$). In the diabetics with AN the mean basal level was 40.4 ± 3.8 pmol/l and the mean peak level 61 ± 15.9 pmol/l ($p > 0.05$). In contrast, the mean maximum incremental rise in gastrin levels was slightly higher in the diabetics with AN (20.7 ± 13.2 pmol/l) than the similar rises of 7.4 ± 4.3 pmol/l in the diabetics without AN and 11.5 ± 3.7 pmol/l in the controls.

Comment:

There was a significant serum gastrin response to insulin hypoglycaemia in the controls. Although the response in the diabetics and the controls were not notably different only the mean peak serum concentration was significantly elevated above basal in the former. There was an insignificant rise in serum gastrin levels in response to the insulin injection in the diabetics with and without AN. The maximum incremental serum gastrin increases were not different in either group of diabetics relative to the controls.

Table V.8

Plasma GIP concentrations (pmol/l) before and during insulin hypoglycaemia in 10 diabetics and 7 healthy subjects.

Time after insulin bolus (min)	0	20	40	60	90
Healthy subjects	60.6 ± 7.8	54.1 ± 10.8	59.2 ± 6	64.5 ± 12.9	59 ± 10.6
Diabetics	76.2 ± 6.8	73.3 ± 5.9	63.7 ± 4.3	77.4 ± 7	82.8 ± 6.7

Table V.9

Plasga GIP concentrations (pmol/l) before and during insulin hypoglycaemia in 5 diabetics without AN and 5 diabetics with AN.

Time after insulin bolus (min)	0	20	40	60	90
Diabetics without AN	75.5 ± 5.8	77.7 ± 10.2	63.9 ± 4.4	75.3 ± 11.8	80.4 ± 5.9
Diabetics with AN	77.1 ±12.2	68.8 ± 6.5	63.5 ± 8.1	78.2 ± 8.9	85.1 ± 12.9

2 A (v) GASTRIC INHIBITORY POLYPEPTIDE

Diabetics vs Controls (Table V.8)

There was no significant alteration in plasma GIP levels after the insulin injection in either the diabetics or the controls.

Diabetics without AN vs Diabetics with AN (Table V.9)

Insulin hypoglycaemia induced no significant alteration in GIP levels in either group of diabetics.

Comment:

Insulin hypoglycaemia failed to induce any significant alteration in plasma GIP concentrations in the healthy controls, the diabetics as a whole, or after subdivision into those with AN and those without AN.

Table V.10

Summary of the responses of the four gastrointestinal hormones to insulin hypoglycaemia in the healthy subjects, the diabetics as a single group, and after subdivision into those with AN and those without AN, relative to responses in the healthy controls.

	IRG	PP	GIP	GASTRIN
Healthy subjects	↑	↑	-	↑
Diabetics	N	↓	→	± ↑
Diabetics without AN	N	N	→	→
Diabetics with AN	↓	↓	→	→

↑ - significant response

→ - no significant response

N - normal

↓ - impaired

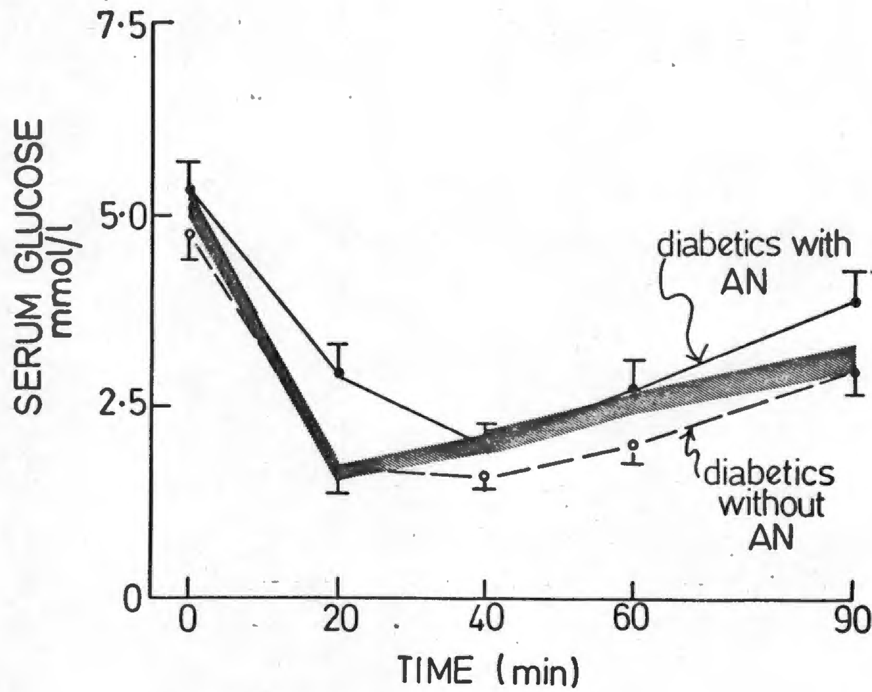
↑± - significant response only in mean peak concentration

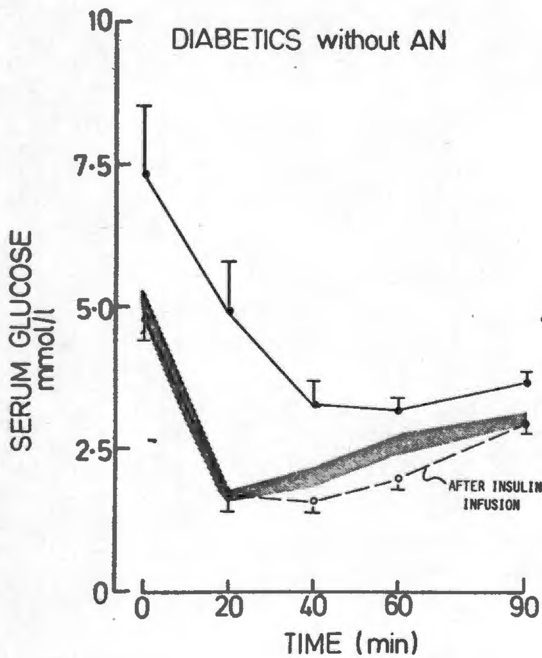
SUMMARY (Table V.10)

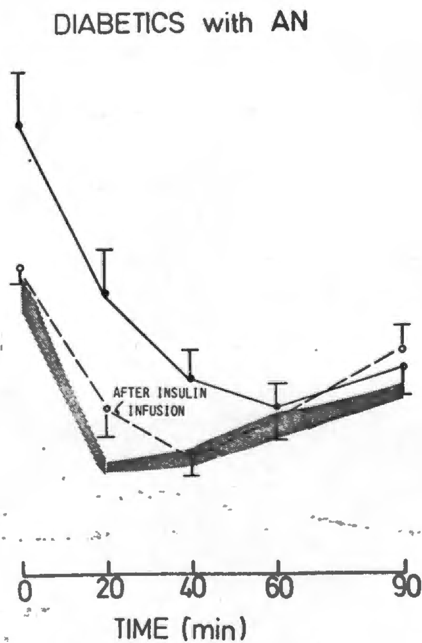
The diabetics as a group had fasting hyperglycaemia before PP, a delayed fall and higher absolute serum glucose levels during insulin hypoglycaemia than the healthy subjects. In the latter, insulin hypoglycaemia induced significant increases in the concentrations of circulating IRG, PP and gastrin but not GIP, while in the former significant increases in serum PP, plasma IRG concentrations and the mean peak serum gastrin were noted. However only in the case of PP was the response in the diabetics significantly impaired relative to the controls.

On subdivision of the diabetics, differences in the response to hypoglycaemia of certain of the hormones became apparent, despite a similar degree of fasting hyperglycaemia and similar rates and extents of decline in serum glucose levels in the two groups of diabetics. Insulin hypoglycaemia induced significant increases in plasma IRG, and serum PP but not serum gastrin or plasma GIP concentrations in the diabetics without AN. In all cases the responses were not different from those in the controls. In the diabetics with AN only serum PP levels rose notably in response to hypoglycaemia. However, significantly impaired serum PP and plasma IRG responses were evident in the diabetics with AN.

Thus insulin hypoglycaemia was not a stimulus for GIP release in any group. Abnormalities in PP secretion alone were detected in the diabetics as a whole. However the diabetics with AN had abnormal serum PP and plasma IRG responses to hypoglycaemia. The latter findings could not be accounted for by differences in fasting hyperglycaemia or glucose responses to hypoglycaemia in the diabetics with and without AN.







(Fig.V.11) The effect of prior induction of fasting normoglycaemia by an overnight insulin infusion on serum glucose responses to insulin hypoglycaemia in 5 diabetics without AN (left) and 5 diabetics with AN (right). Serum glucose responses to insulin hypoglycaemia after prior induction of fasting normoglycaemia in the abovementioned diabetics (top).

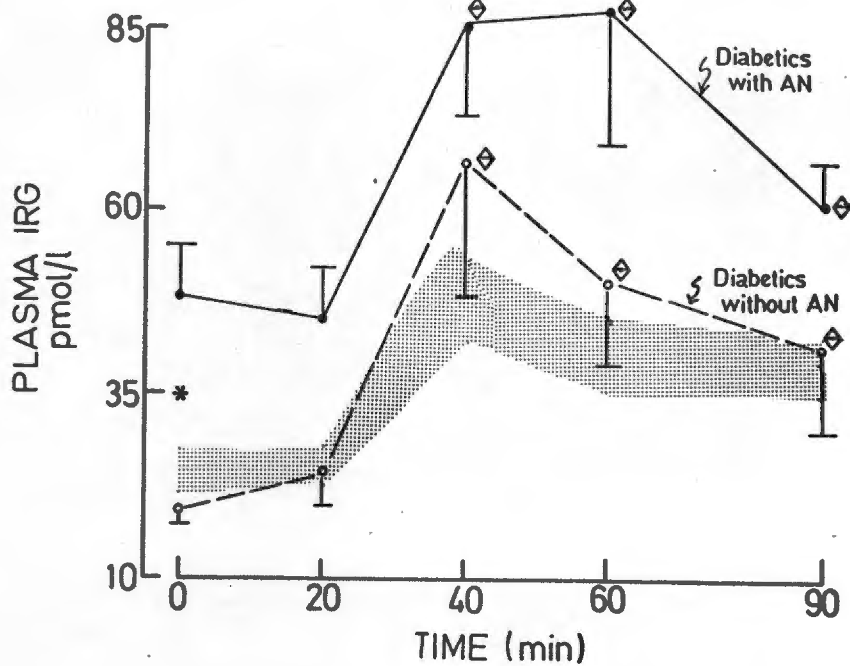
2 B INSULIN HYPOGLYCAEMIA AFTER INDUCTION OF FASTING NORMOGLYCAEMIA IN DIABETICS WITH AND WITHOUT AN

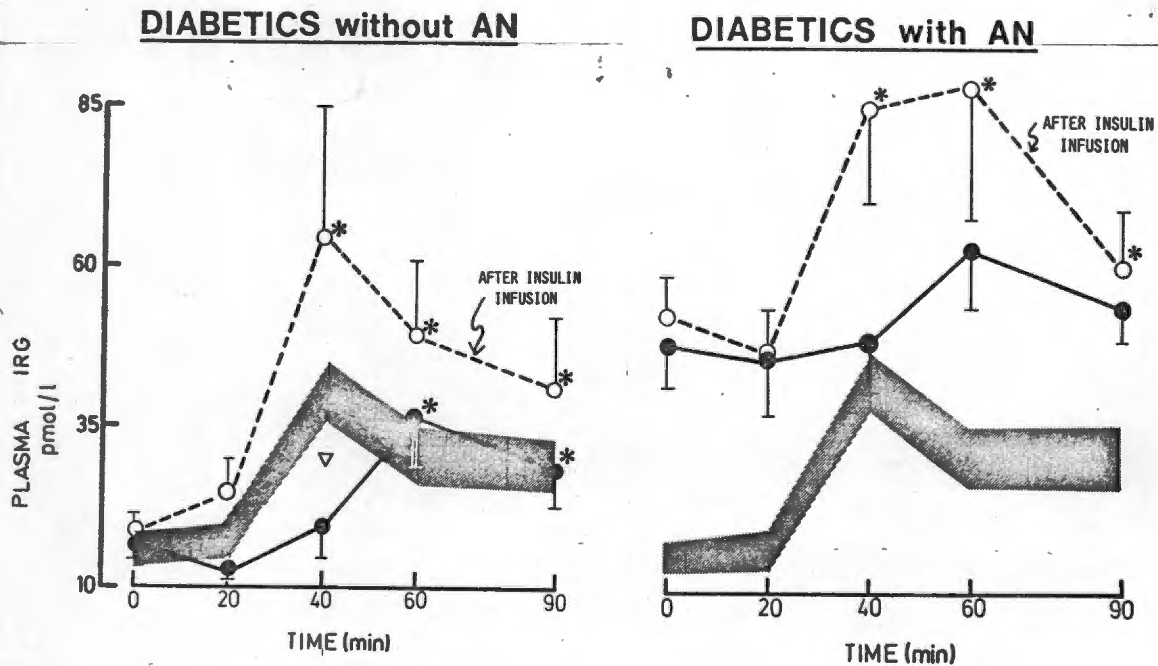
2 B (i) GLUCOSE (Fig. V.11)

The overnight insulin infusion resulted in a marked reduction in the mean basal serum glucose levels as well as a more rapid fall and a greater degree of hypoglycaemia in response to the insulin injection in both groups of diabetics. On this occasion the mean basal serum glucose level was 4.8 $\pm$ 0.4 mmol/l falling to 1.6 $\pm$ 0.3 mmol/l at 40 min in the diabetics without AN. In the diabetics with AN the mean basal serum glucose concentration was 5.4 $\pm$ 0.3 mmol/l falling to 2 $\pm$ 0.3 mmol/l also at 40 min. The mean basal serum glucose levels and the glucose responses on this occasion were not different in the two groups of diabetics and further approximated the corresponding levels in the healthy controls.

Comment:

The overnight insulin infusion induced fasting normoglycaemia and a more rapid and greater hypoglycaemia response to the insulin injection in the diabetics with and without AN. The alterations induced were comparable in the two groups of diabetics and their responses now corresponded with that of the healthy controls.





(Fig. V.12) The effect of prior induction of fasting normyglycaemia by an overnight insulin infusion on plasma IRG responses to hypoglycaemia in 5 diabetics without AN (left) and 5 diabetics with AN (right). Asterisks indicate $p < 0.01$ rise above the mean basal concentrations. Triangle indicates $p \leq 0.01$ difference between the two tests. Plasma IRG responses to insulin hypoglycaemia after prior induction of fasting normoglycaemia in the abovementioned diabetics. Asterisks indicates $p \leq 0.01$ difference between the two groups. Triangles indicate $p < 0.01$ rise above the mean basal concentrations.

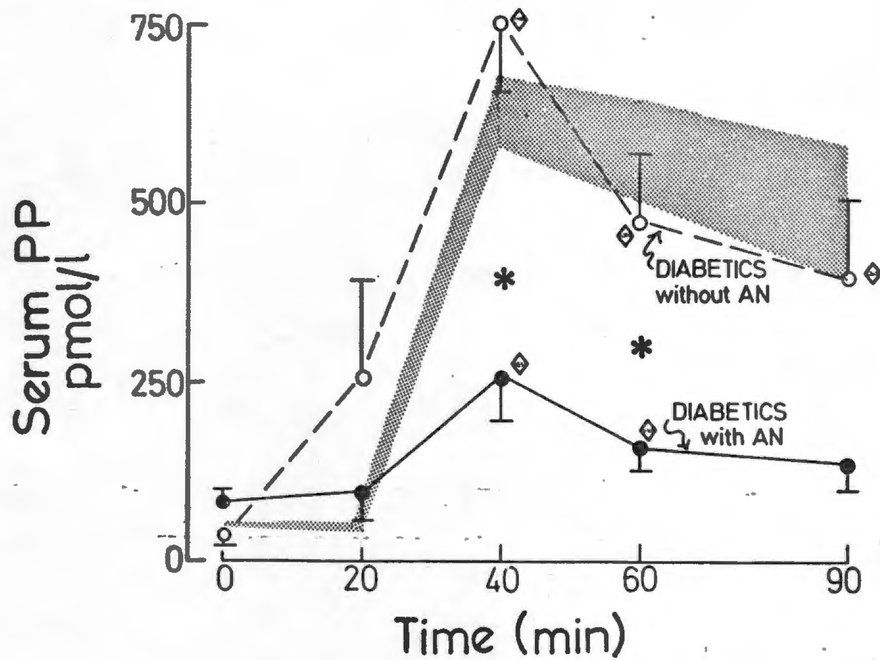
2 B (ii) GLUCAGON (Fig. V.12)

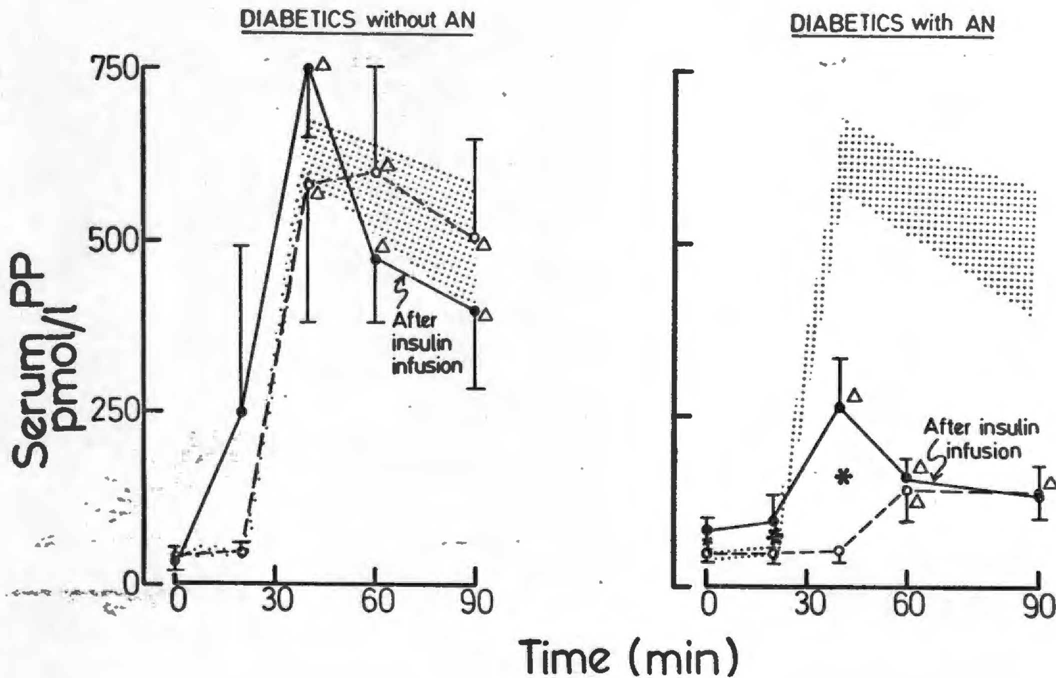
The overnight insulin infusion which ensured fasting normoglycaemia, induced a marked improvement in the plasma IRG response to insulin hypoglycaemia in both groups of diabetics. In the diabetics without AN plasma IRG levels rose from 19.4 $\pm$ 2.3 pmol/l to reach a mean peak concentration of 66.4 $\pm$ 18.6 pmol/l; significant elevation ($p < 0.01$) above the basal level was noted at 40, 60 and 90 min. In contrast, in the initial test in the diabetics without AN, plasma IRG levels rose from 19.1 $\pm$ 1.7 pmol/l to a peak of 44.3 $\pm$ 4.1 pmol/l but significant elevation ($p < 0.01$) above basal occurred later at 60 and 90 min.

In the diabetics with AN in the second insulin test, plasma IRG levels rose from the mean basal concentration of 48.6 $\pm$ 6.3 pmol/l to reach a mean peak concentration of 92.4 $\pm$ 16.6 pmol/l, with significant elevation ($p < 0.01$) above basal being noted at 40, 60 and 90 min. In the initial insulin test in contrast, there was no significant elevation in plasma IRG levels above the mean basal concentration of 45.4 $\pm$ 5.9 pmol/l, the mean peak concentration being 60.2 $\pm$ 3.0 pmol/l ($p < 0.05$). Thus there was no longer an impaired rise in plasma IRG levels in response to hypoglycaemia in the diabetics with AN.

Comment:

Thus the overnight insulin infusion, while not altering the basal plasma IRG levels, resulted in a marked improvement in the plasma IRG responses to insulin hypoglycaemia in both groups of diabetics. Furthermore in the diabetics with AN the rise in IRG levels in response to insulin hypoglycaemia was no longer impaired.





(Fig.V.13) The effect of the prior establishment of fasting normoglycaemia by an overnight insulin infusion on serum PP responses to insulin hypoglycaemia in 5 diabetics without AN (left) and 5 diabetics with AN (right). Asterisks indicate $p < 0.01$ difference between the two tests and triangles $p < 0.01$ rise above the mean basal concentrations.Serum PP responses to insulin hypoglycaemia after induction of fasting normoglycaemia in the abovementioned diabetics. Asterisks indicate $p < 0.01$ difference between the two groups and triangles $p < 0.01$ rise above the mean basal concentrations (top).

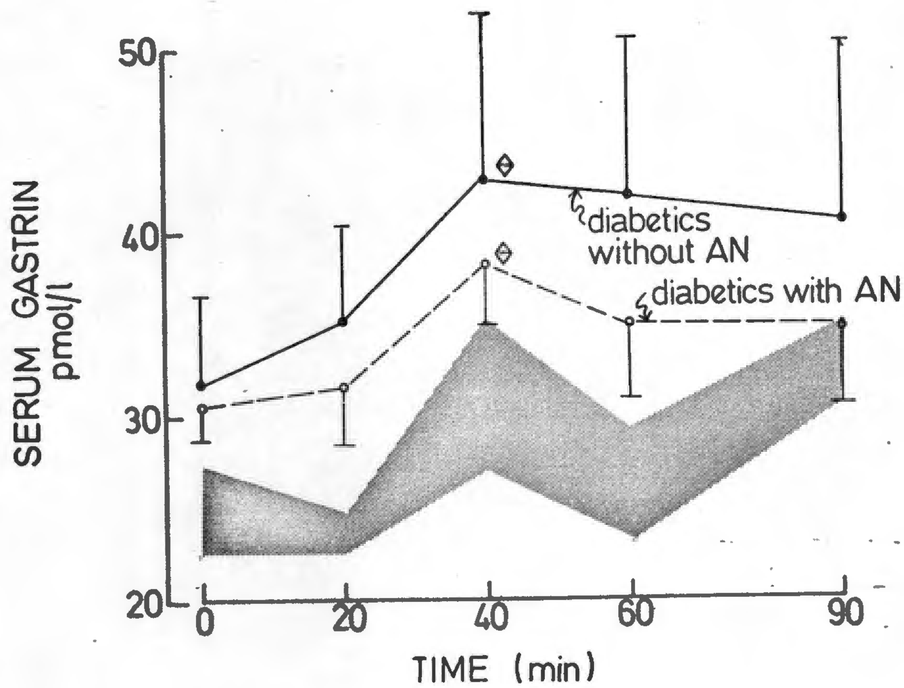
2 B (iii) PANCREATIC POLYPEPTIDE (Fig. V.13)

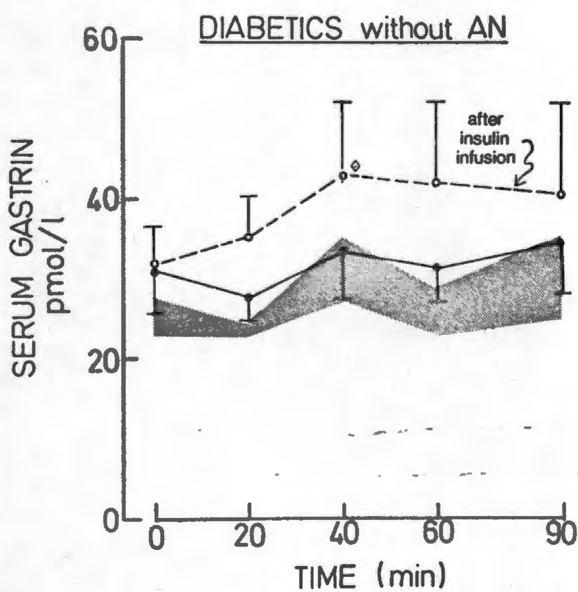
The overnight insulin infusion altered the serum PP response to insulin hypoglycaemia in the diabetics with AN but not those without AN. In the diabetics without AN serum PP levels rose from 39.7 $\pm$ 12.6 pmol/l to the mean peak concentration of 800 $\pm$ 154.2 pmol/l in the initial test and in the second from 33.4 $\pm$ 7 to 756.7 $\pm$ 109.6 pmol/l. The only difference was the more rapid rise in the second test when significant elevation above the basal level was noted from 20 min and PP levels stated falling from 40 in place of 60 min.

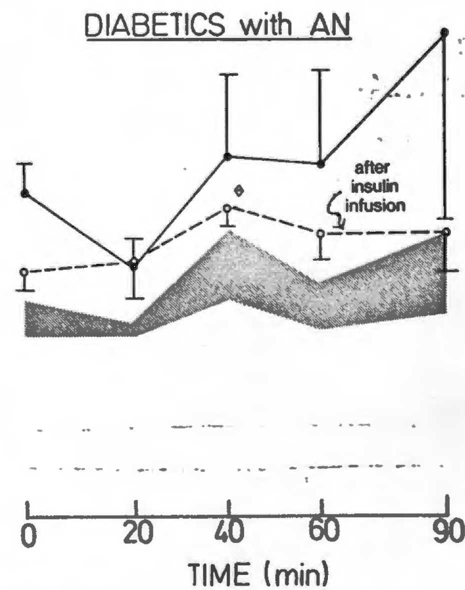
In contrast there was a significant increase in the mean basal serum PP level as well as in the PP response to hypoglycaemia in the diabetics with AN during the test after the overnight insulin infusion. The mean basal serum PP levels were 49.5 $\pm$ 12.7 and 81.7 $\pm$ 19.6 pmol/l in the first and second tests respectively. Significantly higher serum PP levels were noted from 40 to 90 min in the second test compared to the first. In addition there was a more rapid response and the mean peak level rose from 167.6 $\pm$ 46.3 to 275.7 $\pm$ 67.3 pmol/l ($p < 0.01$). However the diabetics with AN still had a significantly impaired serum PP response relative to those without AN ($p < 0.01$).

Comment:

Although the basal serum PP levels as well as the PP response to hypoglycaemia were significantly greater in the diabetics with AN, when the hypoglycaemia was preceded by the overnight insulin infusion, they still had significantly impaired PP responses to hypoglycaemia relative to the diabetics without AN (threefold vs twentytwofold rise). In both groups of diabetics there was a more rapid rise in serum PP levels when insulin hypoglycaemia was preceded by the overnight insulin infusion.







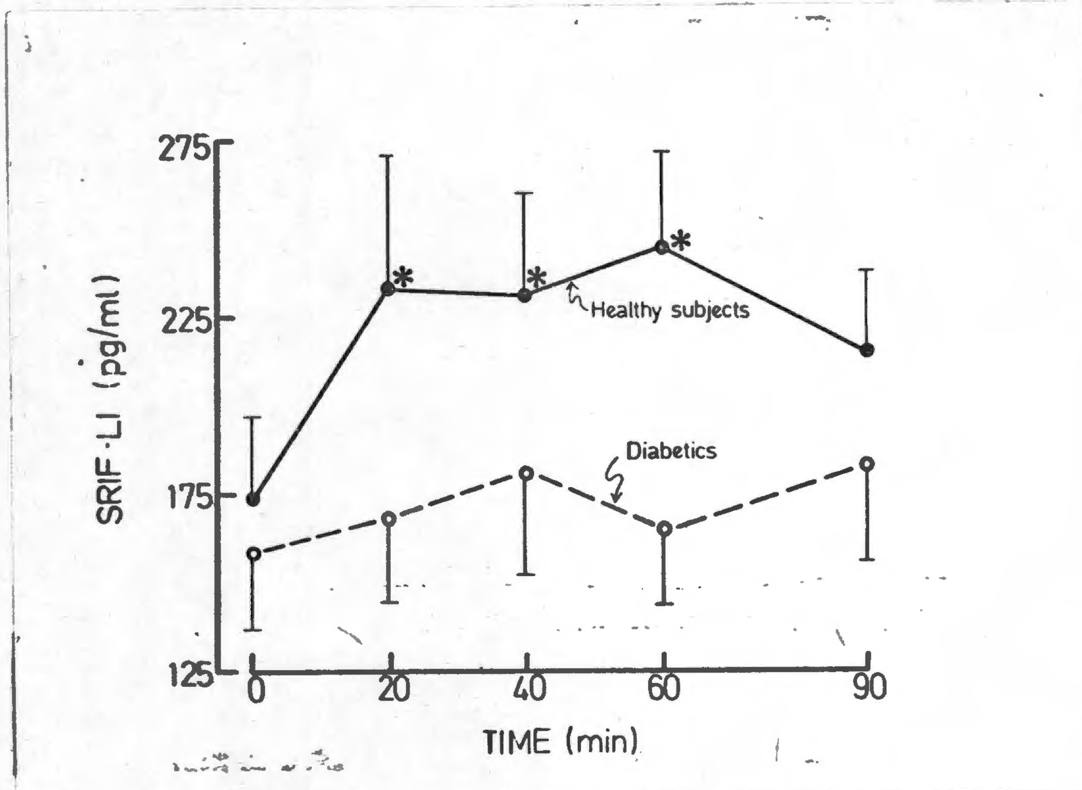
(Fig.V.14) The effect of the prior induction of fasting normoglycaemia by an overnight insulin infusion on serum gastrin responses to insulin hypoglycaemia in 6 diabetics without AN (left) and 6 diabetics with AN (right). Serum gastrin responses to insulin hypoglycaemia after the establishment of fasting normoglycaemia in the abovementioned diabetics (top). Triangles indicate $p < 0.01$ rise above the mean basal concentrations.

2 B (iv) GASTRIN (Fig. V.14)

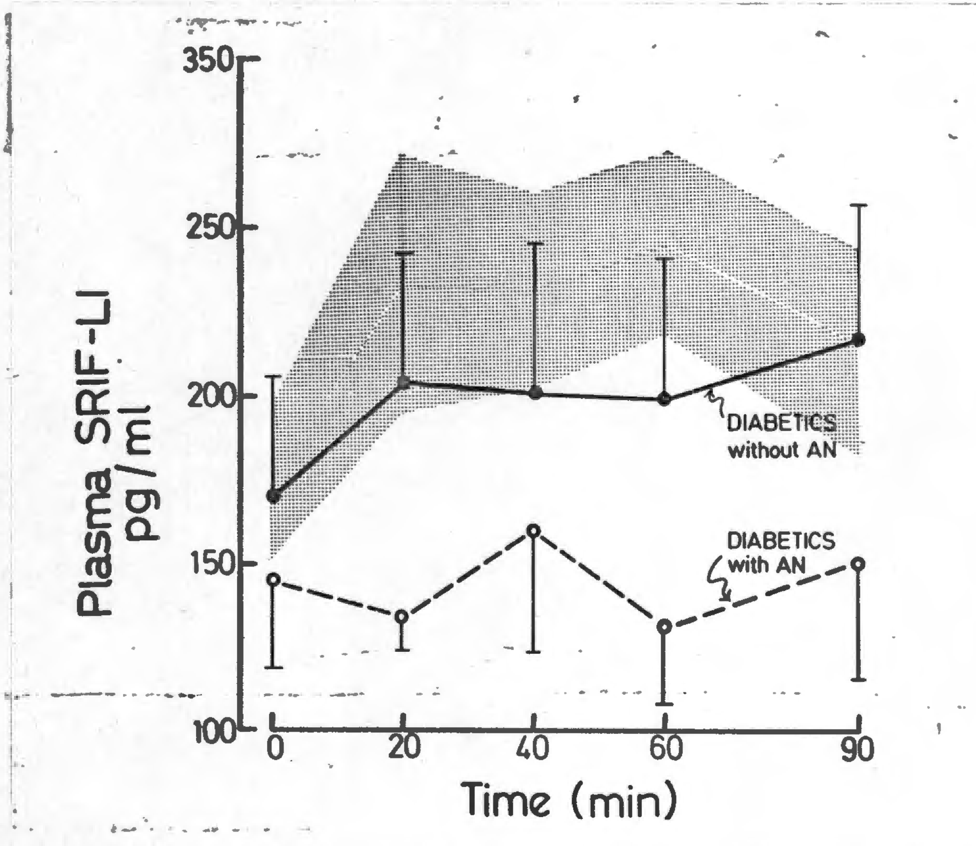
After the overnight insulin infusion there was a more prominent gastrin response to insulin hypoglycaemia in both groups of diabetics. In the diabetics without AN serum gastrin levels rose significantly from the mean basal level of 31.8 $\pm$ 5.1 pmol/l to a mean peak concentration of 47.6 $\pm$ 11.7 pmol/l ($p < 0.01$). In the initial insulin hypoglycaemia test, there was no significant elevation above the basal serum gastrin level in the diabetics without AN. Although the diabetics with AN had a slightly lesser gastrin response in the second compared to the initial test, the rise in serum gastrin levels was significant, contrasting with the insignificant rise in the first test. In the diabetics with AN the mean basal serum gastrin level was 30.5 $\pm$ 1.7 pmol/l and rose significantly ($p < 0.01$) to the mean peak level of (44.9 $\pm$ 2.5 pmol/l). The rise in serum gastrin levels on this occasion was similar in the two groups of diabetics.

Comment:

When the overnight insulin infusion, with its resultant normalization of fasting blood glucose levels, preceded the insulin hypoglycaemia test, serum gastrin levels rose significantly in both groups of diabetics. This finding contrasted with the insignificant responses when basal hyperglycaemia had been present.



(Fig. V.15) Plasma SRIF-LI responses to insulin hypoglycaemia in 10 healthy subjects and 10 diabetics. The insulin hypoglycaemia was preceded by the induction of fasting normoglycaemia in the diabetics. Asterisks indicate $p < 0.05$ rise above the mean basal concentrations.



(Fig. V.16) Plasma SRIF-LI responses to insulin hypoglycaemia preceded by the induction of fasting normoglycaemia in 5 diabetics with AN and 5 diabetics without AN.

2 B (v) SOMATOSTATIN

In view of the fact that plasma SRIF-LI concentrations were not estimated in the initial insulin hypoglycaemia test, a comparison of plasma SRIF-LI response to the two insulin hypoglycaemia tests could not be carried out. Therefore the plasma SRIF-LI responses to the second test will be given in the diabetics and controls as well as in the diabetics with and without AN.

Diabetics vs Controls (Fig. V.15)

Insulin hypoglycaemia induced a significant rise in plasma SRIF-LI concentrations in the controls but not in the diabetics. In the controls plasma SRIF-LI levels rose rapidly from the mean basal level of 174 $\pm$ 23.5 pg/ml to reach significance at 20 min, remaining thus until 60 min ($p < 0.05$), but by 90 min basal levels had almost been regained. In the diabetics there was no notable rise in plasma SRIF-LI concentrations above the mean basal level of 158 $\pm$ 21.4 pg/ml-

Diabetics without AN vs Diabetics with AN (Fig. V.16)

Neither the diabetics without AN nor those with AN had a significant rise in plasma SRIF-LI levels in response to insulin hypoglycaemia. In the diabetics without AN the mean basal plasma SRIF-LI levels was 170 $\pm$ 35.6 pg/ml and there was only a minor rise in plasma SRIF-LI levels thereafter. In the diabetics with AN the mean basal plasma SRIF-LI concentration was 146 $\pm$ 26.7 pg/ml and once again no notable rise was observed in response to hypoglycaemia. However the plasma SRIF-LI response in the diabetics without AN tended to be similar to that of the controls whilst the diabetics with AN tended to have a flatter response.

Comment:

In the controls, but not in either group of diabetics, a significant increase in plasma SRIF-LI levels was observed in response to insulin-induced hypoglycaemia. However the diabetics with AN tended to have a slightly flatter plasma SRIF-LI response.

Table V.11

Summary of the data on the responses of the four gastrointestinal hormones to insulin hypoglycaemia preceded by an overnight insulin infusion in the diabetics with and without AN, relative to their responses and those of the healthy subjects in the initial insulin test.

	IRG	PP	GASTRIN	SRIF-LI
Diabetics without AN	I/N	N	I/N	→
Diabetics with AN	I/N	↓	I/N	→
(healthy subjects)	↑	↑	↑	↑

↑ - significant response

I - significantly improved response

N - normal response

↓ - impaired response

→ - no significant response

SUMMARY (Table V.11)

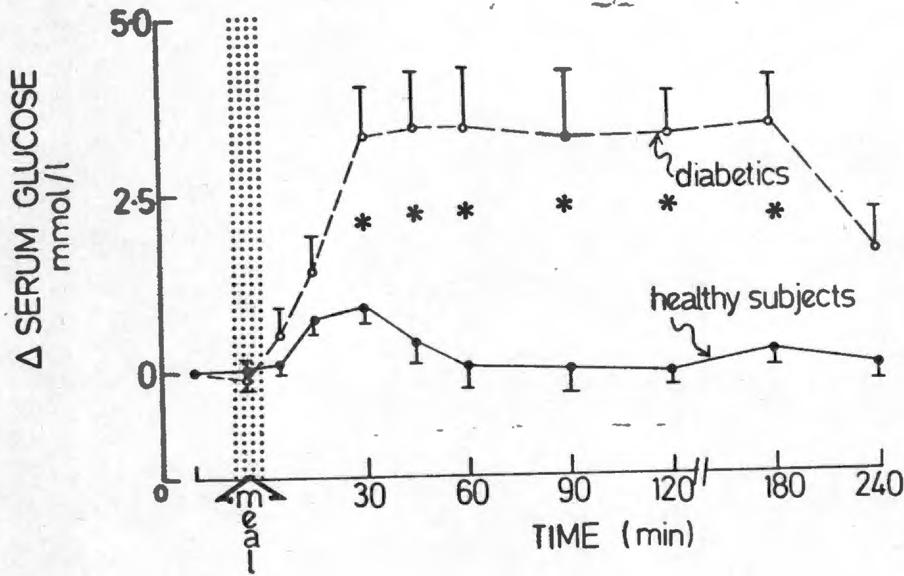
The overnight insulin infusion resulted in the establishment of fasting normoglycaemia and a more rapid and greater degree of hypoglycaemia in response to the insulin bolus in both groups of diabetics, compared to the first test.

In the diabetics without AN the plasma IRG and serum gastrin, but not serum responses to insulin hypoglycaemia, were significantly increased when hypoglycaemia was preceded by the overnight insulin infusion. Furthermore on this occasion no notable differences were apparent in the responses of any of the hormones in the diabetics without AN compared to the controls.

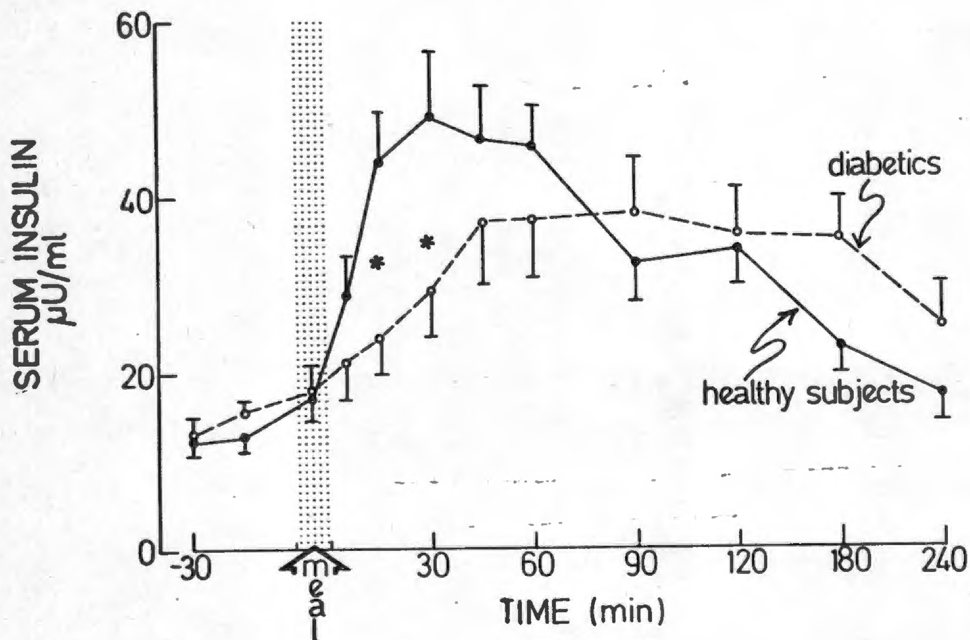
In the diabetics with AN the overnight insulin infusion resulted in significant plasma IRG and serum gastrin responses to hypoglycaemia compared with their insignificant responses in the initial test. Furthermore normalization of the previously impaired plasma IRG response ensued. However serum PP responses remained significantly impaired relative to the diabetics without AN and the controls.

Plasma SRIF-LI concentrations were not estimated in the initial insulin hypoglycaemia test thus preventing an evaluation of the effect of the prior overnight insulin infusion or its responses to hypoglycaemia. Thus only the plasma SRIF-LI responses to the test after prior overnight insulin infusion can be commented on.

Although insulin hypoglycaemia induced a significant rise in plasma SRIF-LI levels in the controls, this was not the case in the diabetics as a whole or in those with and without AN. Thus insulin hypoglycaemia preceded by an overnight insulin infusion was associated with correction of the impaired plasma IRG response to hypoglycaemia in the diabetics with AN. However, their impaired serum PP response remained uncorrected.



(Fig.V.17) Serum glucose responses to ingestion of a mixed meal in 12 healthy subjects and 10 diabetics. Asterisks indicate $p < 0.01$ difference between groups.



(Fig.V.18) Serum insulin responses to ingestion of a mixed meal in 12 healthy subjects and 10 diabetics. Asterisks indicate $p < 0.05$ difference between groups.

2.C INGESTION OF A MIXED MEAL

2.C (i) GLUCOSE AND INSULIN

Diabetics vs Controls

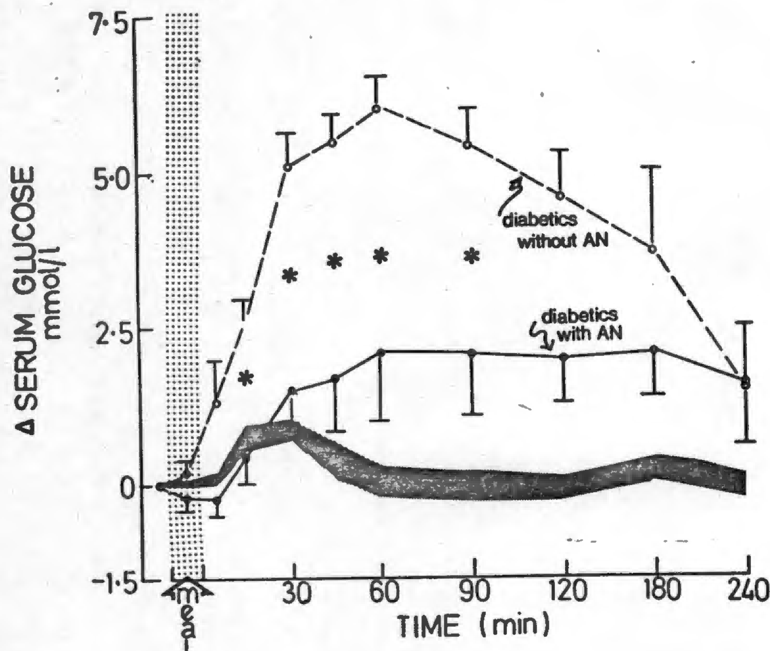
The mean basal serum glucose concentration was significantly higher in the diabetics (9.1 $\pm$ 0.9 mmol/l) than in the controls (5.1 $\pm$ 0.1 mmol/l) ($p < 0.01$). Furthermore (Fig. V.17) the diabetics had a significantly greater rise in serum glucose levels after the meal than in the controls. The peak serum glucose concentration was 5.9 $\pm$ 0.2 mmol/l in the healthy subjects compared with 12.5 $\pm$ 1.1 mmol/l in the diabetics ($p < 0.01$).

The mean basal serum insulin levels were similar in the diabetics and the controls. In the controls serum insulin levels rose from a mean basal level of 12.8 $\pm$ 1.6 μU/ml to a peak of 49.6 $\pm$ 6.9 μU/ml. In the diabetics, serum insulin levels rose from a mean basal concentration of 14.3 $\pm$ 1.9 μU/ml to apeak of 38.2 $\pm$ 6.4 μU/ml. The diabetics had significantly lower serum insulin levels at 15 and 30 min after completion of the meal relative to the controls ($p < 0.05$). (Fig. V.18)

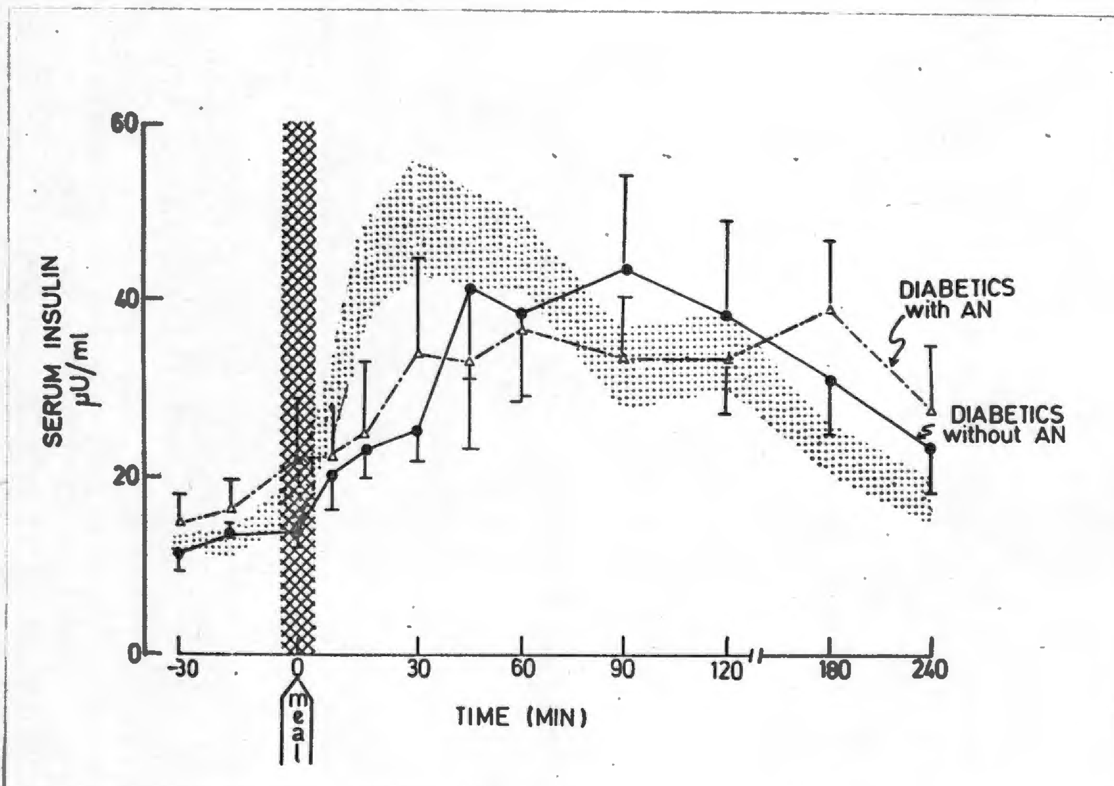
Diabetics without AN vs Diabetics with AN

The mean basal serum glucose concentrations were not significantly different in the diabetics with AN (10.1 $\pm$ 1.7 mmol/l) and in those without AN (8 $\pm$ 0.7 mmol/l). There was also a similar degree of fasting hyperglycaemia in the two groups of diabetics relative to the controls. In addition the rise in serum glucose concentrations after the meal was significantly greater in both groups of diabetics than in the controls. However, the diabetics with AN had a lesser rise in serum glucose levels than the diabetics without AN. The maximum incremental rise in serum glucose levels was 1.5 $\pm$ 0.1 mmol/l in the diabetics with AN, significantly lower than that of 5.3 $\pm$ 0.4 mmol/l in the diabetics without AN ($p < 0.01$). (Fig. V.19)

The mean basal serum insulin levels were similar in the two groups of diabetics and were not different from the controls. In the diabetics without AN serum insulin levels rose from 12.7 $\pm$ 1.3 μU/ml to a peak of 43.3 $\pm$ 10.8 μU/ml at 90 min, decreasing gradually thereafter (Fig. V.20). Compared to the normal subjects the diabetics without AN had significantly lower serum insulin concentrations at 15 and 30 min ($p < 0.01$). In the diabetics with AN serum insulin levels rose from 15.8 $\pm$ 3.7 μU/ml to a peak of 39 $\pm$ 7.6 μU/ml at 180 min; their response was similar to that in the diabetics without AN and was not different from the insulin response in the controls.



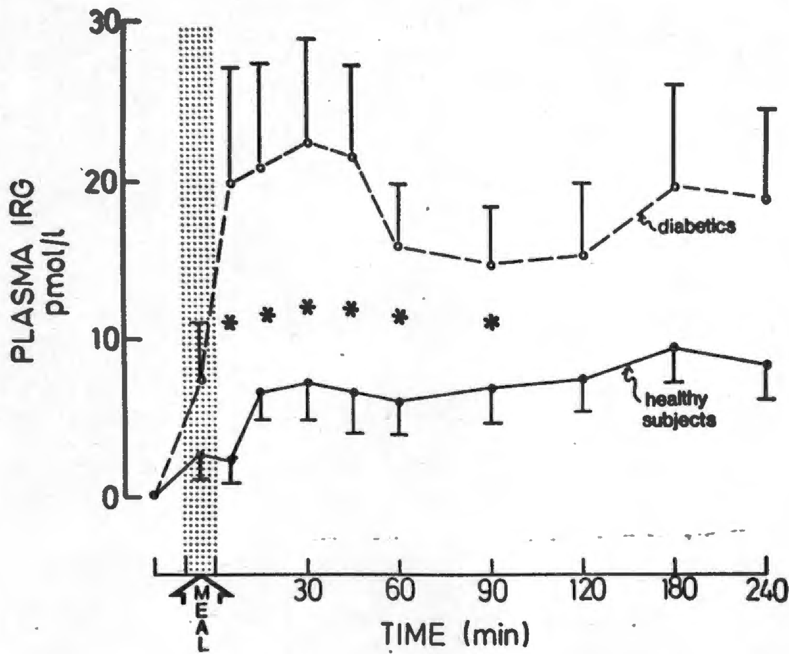
(Fig.V.19)Serum glucose responses to ingestion of a mixed meal in 5 diabetics with and 5 diabetics without AN. Asterisks indicate $p < 0.05$ difference between groups.



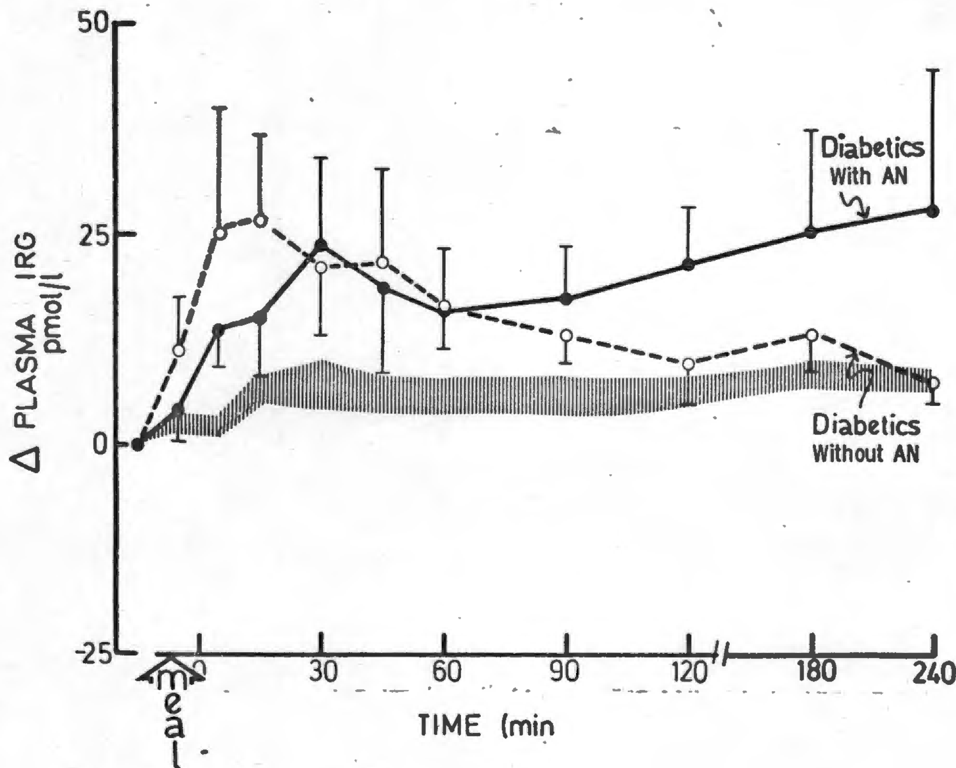
(Fig.V.20) Serum insulin responses to ingestion of a mixed meal in 5 diabetics with and 5 diabetics without AN.

Comment:

The diabetics had fasting hyperglycaemia and a greater hyperglycaemic response to the meal than the controls. The diabetics and controls had similar basal serum insulin levels, but the diabetics had an impaired insulin response to the meal. The diabetics with AN had a slightly greater degree of fasting hyperglycaemia than those without AN, but the former had a significantly lesser serum glucose response to the meal. The serum insulin responses were similar in those with and without AN, but the latter had an impaired early response compared to the controls.



(Fig.V.21) Plasma IRG responses to ingestion of a mixed meal in 12 healthy subjects and 10 diabetics. Asterisks indicate p 0.05 difference between groups.



(Fig.V.22) Plasma IRG responses to ingestion of a mixed meal in 5 diabetics with and 5 diabetics without AN.

2 C (ii) GLUCAGON

Diabetics vs Controls (Fig. V.21)

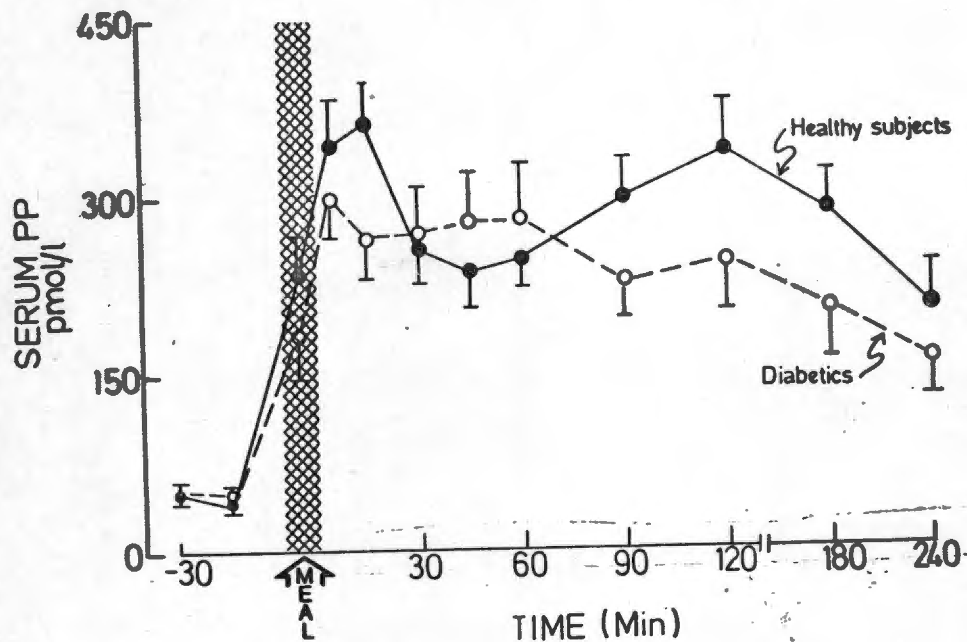
The mean basal plasma IRG concentration was 36.2 ± 6.2 pmol/l in the 10 diabetics and 16.8 ± 2.9 pmol/l in the 12 healthy subjects. The difference was statistically significant ($p < 0.01$). In addition the diabetics had an exaggerated plasma IRG response to the meal. In the diabetics plasma IRG levels rose significantly ($p < 0.05$) 5 min after completion of the meal and remained so for 4 hr. The mean peak concentration of 58.2 ± 10.4 pmol/l (maximum increment 22.4 ± 6.3 pmol/l) occurred at 30 min. In the healthy controls plasma IRG levels rose significantly ($p < 0.05$) 15 min after the meal; the peak concentration of 24.6 ± 4.3 pmol/l (maximum increment 9.4 ± 1.9 pmol/l) was noted at 3 hr but levels remained elevated for 4 hr.

Diabetics without AN vs Diabetics with AN (Fig V.22)

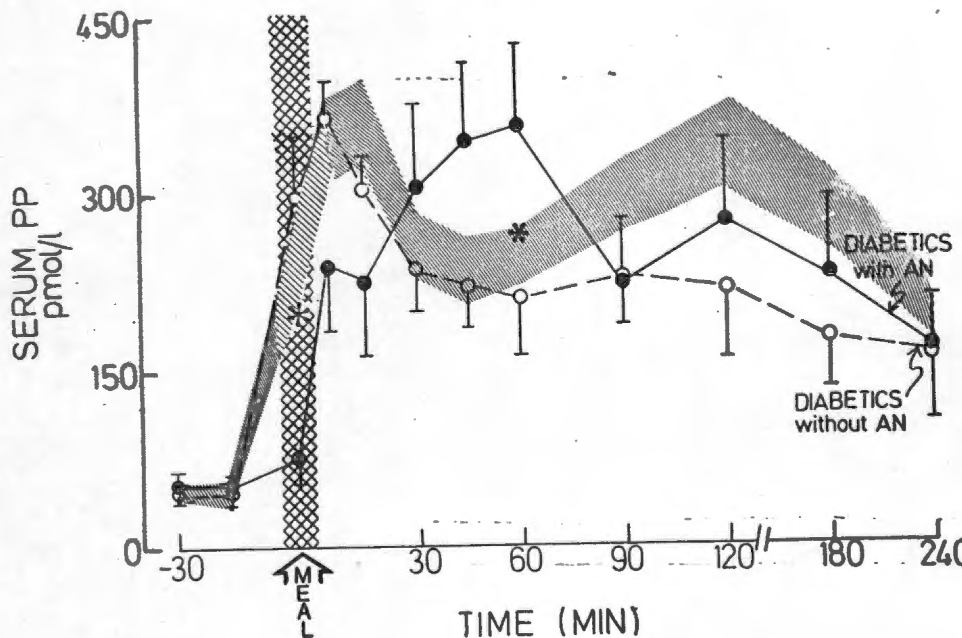
The mean basal plasma IRG concentration was 20.4 ± 2.8 pmol/l in the diabetics without AN, similar to that in the healthy controls, but significantly lower than that of 52.1 ± 6.4 pmol/l in the diabetics with AN ($p < 0.01$). In the diabetics without AN significant elevation ($p < 0.01$) in mean plasma IRG levels was first observed during the meal and was maintained for 4 hr. The peak IRG concentration of 46.2 ± 11.8 pmol/l (maximum increment 26.8 ± 10.4 pmol/l) was noted at 15 min. Despite starting at a significantly higher mean basal level, the diabetics with AN had an IRG response similar to that seen in the diabetics without AN. However, in the diabetics with AN the peak concentration of 79.9 ± 20.3 pmol/l (maximum increment 28 ± 16.6 pmol/l) occurred later, after 4 hr. The plasma IRG response to the meal were similarly exaggerated in the two groups of diabetics relative to the healthy controls.

Comment:

The diabetics considered as a single group had a significantly greater rise in plasma IRG levels after the meal than the healthy subjects. The diabetics with and those without AN had a similarly exaggerated plasma IRG response to the meal despite the fasting hyperglugonaemia in the former.



(Fig.V.23) Serum PP responses to ingestion of a mixed meal in 12 healthy subjects and 12 diabetics.



(Fig.V.24) Serum PP responses to ingestion of a mixed meal in 6 diabetics with and 6 diabetics without AN. Asterisks indicates $p < 0.01$ difference between groups.

2 C (iii) PANCREATIC POLYPEPTIDE

Diabetics vs Controls (Fig. V.23)

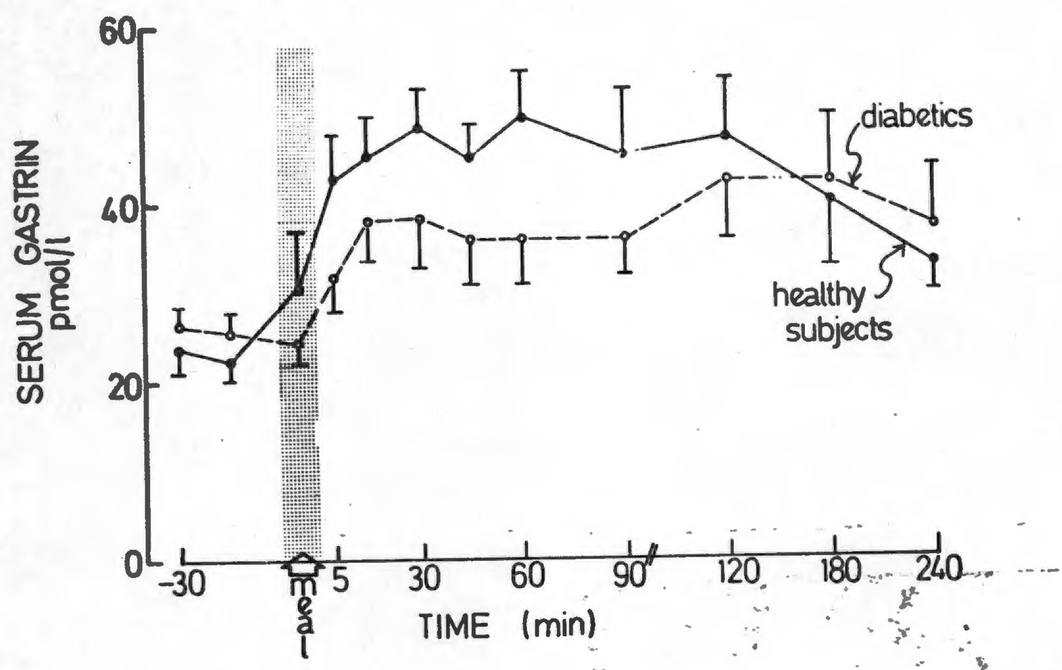
As illustrated in Fig. V.23 ingestion of the mixed meal induced a prominent and comparable rise in serum PP concentrations in the 12 diabetics and the healthy controls. Serum PP concentrations rose rapidly and significantly in the healthy subjects from the mean basal level of 46.5 $\pm$ 7.9 pmol/l to 232.2 $\pm$ 36.4 pmol/l during the meal, increasing further to a peak of 363.5 $\pm$ 36.2 pmol/l, 15 min after completion of the meal. Thereafter serum PP levels fell to the level of 235.3 $\pm$ 29.5 pmol/l at 45 min, which was significantly lower than the peak concentration ($p < 0.01$). Serum PP concentrations again rose significantly ($p < 0.01$) to 336.8 $\pm$ 49.4 pmol/l at 120 min, and remained significantly elevated above the basal concentration for the duration of the study ($p < 0.01$). In the 12 diabetics serum PP levels also rose rapidly and significantly ($p < 0.01$) from the mean basal concentration of 50.6 $\pm$ 7.2 pmol/l to 185.3 $\pm$ 43.1 pmol/l during the meal, increasing to reach 300.6 $\pm$ 35.6 pmol/l at 5 min. The serum PP levels fluctuated thereafter, falling gradually but at 240 min were still significantly elevated above basal ($p < 0.01$). However there was no true biphasic PP response in the diabetics unlike in the controls, while the time taken to reach the mean peak concentration was not significantly different in the diabetics (20.8 $\pm$ 6.2 min) and the controls (11.3 $\pm$ 2.2 min).

Diabetics without AN vs Diabetics with AN (Fig. V.24)

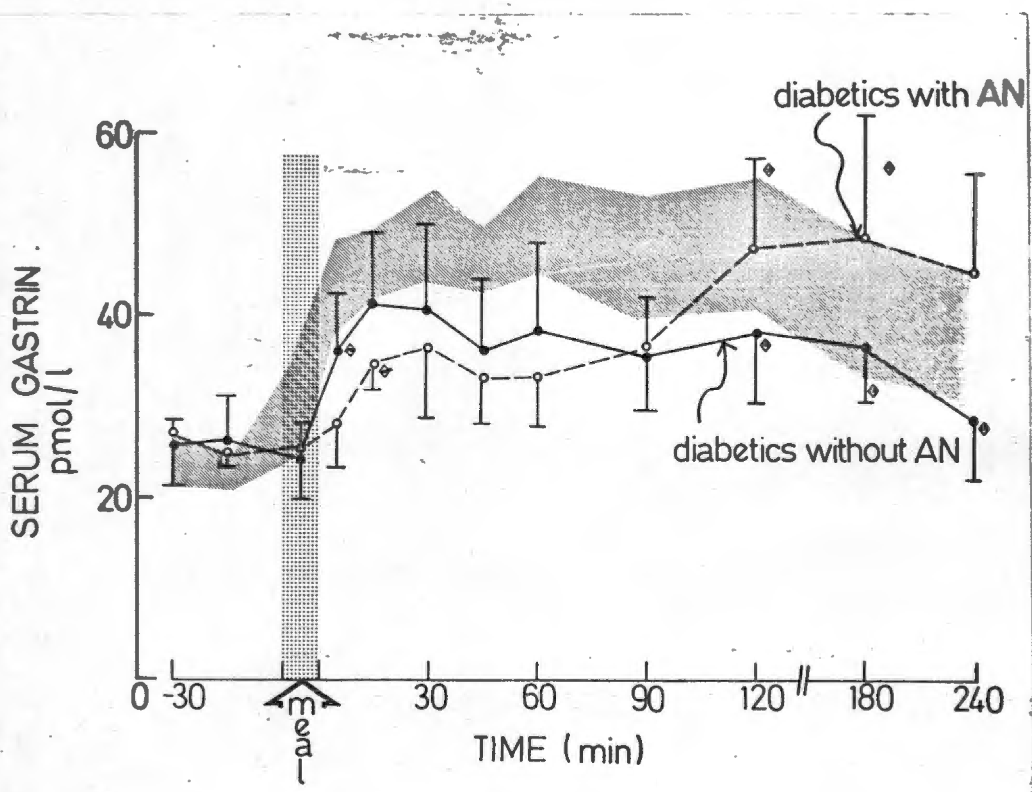
In the diabetics without AN there was a prompt and significant increase in serum PP levels, which became manifest during the meal ($p < 0.01$), similar to the controls. Serum PP levels rose from 37.1 $\pm$ 8.8 pmol/l to 363.1 $\pm$ 32.5 pmol/l at 5 min, thereafter remaining significantly elevated until 240 min ($p < 0.01$). There was no true biphasic PP response to the meal in the diabetics without AN, in whom the time taken to reach the mean peak concentration was 8.3 $\pm$ 4.4 min, similar to that in the controls. In the diabetics with AN there was a delayed early rise in serum PP levels compared to the controls and the diabetics without AN. Serum PP levels rose from the mean basal level of 52.6 $\pm$ 12.1 pmol/l, reaching significance 5 min after completion of the meal ($p < 0.01$). The serum PP levels increased gradually to 352 $\pm$ 74.9 pmol/l and remained significantly elevated above basal until 240 min. As in the diabetics without AN, there was no true biphasic PP response to the meal in the diabetics with AN. However, the time taken to reach the mean peak concentration was 23.4 $\pm$ 4.1 min in the diabetics with AN, significantly delayed relative to the diabetics without AN ($p < 0.05$) and the controls ($p < 0.01$).

Comment:

Ingestion of the mixed meal resulted in a comparable rapid, and marked increase in serum PP levels in the controls and the diabetics when considered as a single group. However only in the controls was the postprandial rise in serum PP levels biphasic. The diabetics without AN had a similar rise in serum PP levels to that seen in the controls. In contrast, in the diabetics with AN the early rise in serum PP levels was significantly delayed, but the magnitude of the rise was not impaired.



(Fig.V.25)Serum gastrin responses to ingestion of a mixed meal in 6 controls and 12 diabetics.



(Fig.V.26) Serum gastrin responses to ingestion of a mixed meal in 6 diabetics with and 6 diabetics without AN. Triangles indicate $p < 0.01$ rise above the mean basal concentrations.

2 C (iv) GASTRIN

Diabetics vs Controls (Fig. V.25)

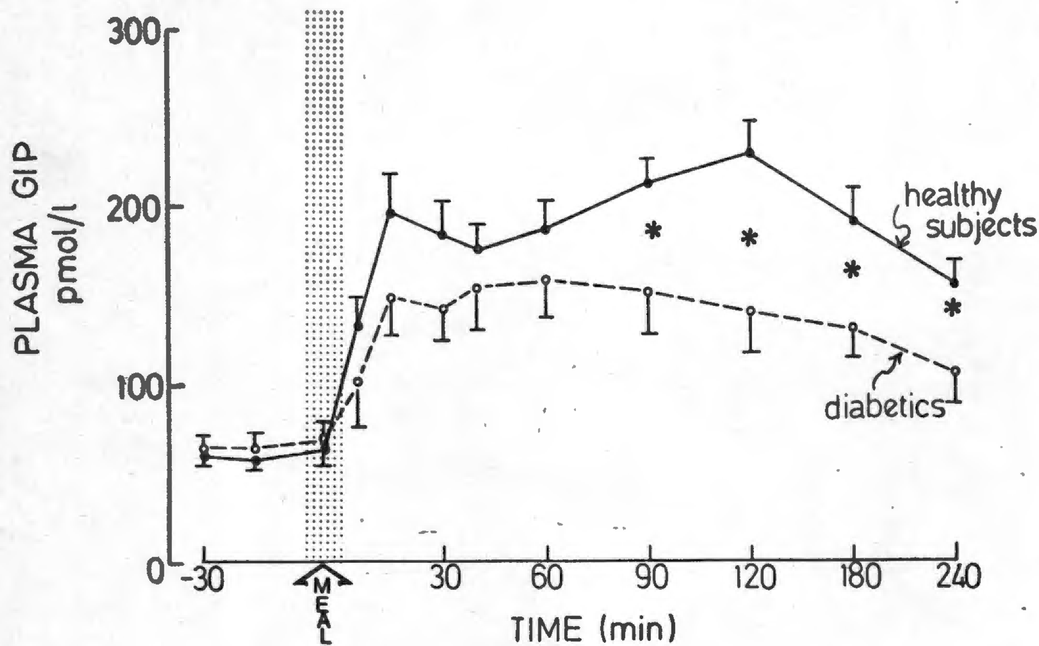
The mean basal serum gastrin concentrations were similar in the 12 diabetics (26.1 ± 2.4 pmol/l) and in the healthy controls (23.4 ± 2.4 pmol/l). There was a rapid rise in serum gastrin levels in the controls, reaching significance ($p < 0.01$) within 5 min of completion of the meal and remaining thus for 4 hr. In the diabetics, significant elevation above the mean basal serum gastrin level was first noted at 15 min and also remained elevated for 4 hr. The magnitude of the rise in serum gastrin concentrations was similar in the diabetics and the controls; the peak concentration was 42.7 ± 6.5 pmol/l in the former and 49.8 ± 5.3 pmol/l in the latter group.

Diabetics without AN vs Diabetics with AN (Fig. V.26)

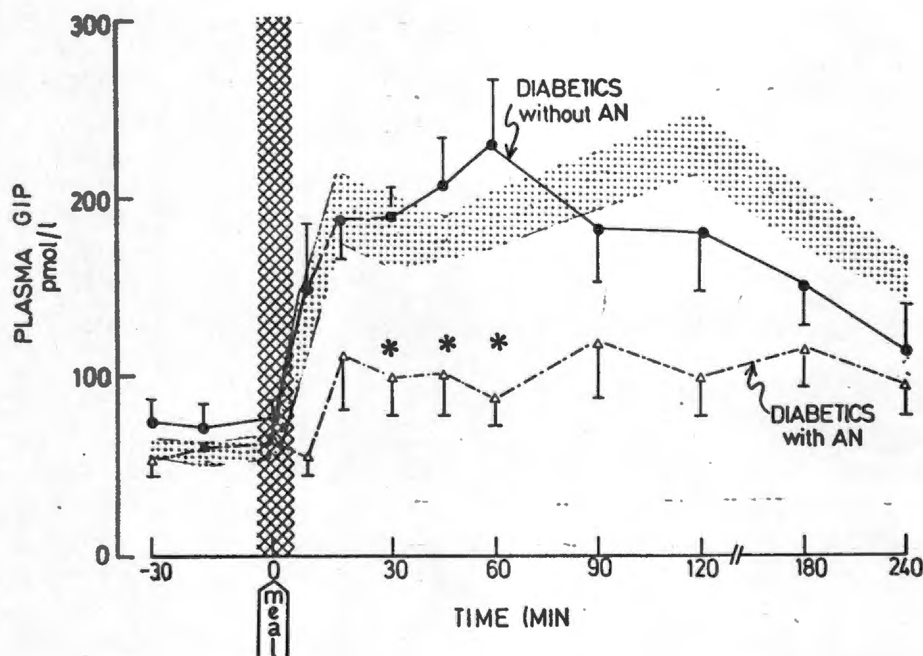
No prominent differences in the serum gastrin levels were found when the diabetics were divided into those with and without AN. The mean basal serum gastrin concentration was 26.1 ± 4.8 pmol/l in the diabetics without AN and 26 ± 1.3 pmol/l in the diabetics with AN, similar to each other as well as to the healthy controls. Furthermore the rise in serum gastrin levels after the meal was similar in both groups of diabetics as well as in the controls. In the diabetics without AN serum gastrin concentrations rose within 5 min of completion of the meal, thereafter plateaued and by 4 hr fasting levels had almost been regained. In these subjects though, because of the large standard errors, significant elevation ($p < 0.01$) above basal levels was noted only at 5, 120 and 180 min. The peak serum gastrin concentration of 41.4 ± 8.6 pmol/l was observed at 15 min. There was a slightly delayed rise in serum gastrin levels in the diabetics with AN, but a significant rise ($p < 0.01$) was observed 15 min after completion of the meal. Thereafter serum gastrin levels gradually increased to reach a peak of 48.6 ± 14.4 pmol/l at 3 hr.

Comment:

The magnitude of the rise in serum gastrin levels after the mixed meal was similar in the diabetics and controls. The diabetics with AN and without AN had similar serum gastrin responses to the meal, which were not significantly different from that of the controls.



(Fig.V.27) Plasma GIP responses to ingestion of a mixed meal in 12 healthy subjects and 12 diabetics. Asterisks indicate p ≤ 0.05 difference between groups.



(Fig.V.28)

Plasma GIP responses to ingestion of a mixed meal in 6 diabetics with and 6 diabetics without AN. Asterisks indicate p ≤ 0.05 difference between the two groups.

2 C (v) GASTRIC INHIBITORY POLYPEPTIDE

Diabetics vs Controls (Fig. V.27)

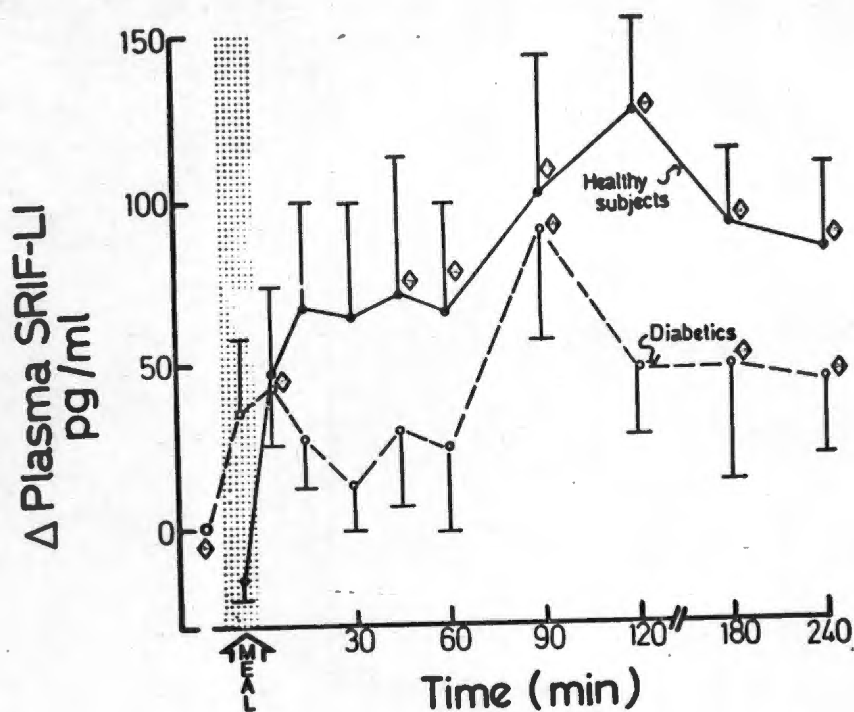
Mean basal plasma GIP concentrations were similar in the 12 diabetics and the healthy controls. In the healthy subjects the mean basal plasma GIP concentration was 59.4 ± 5 pmol/l; after ingestion of the meal plasma GIP levels rose significantly and rapidly, a rise was exhibited at 5 min and was sustained for 4 hrs ($p < 0.01$). The peak plasma GIP concentration of 229.6 ± 19.9 pmol/l occurred after 2 hrs in the controls. There was also a rapid rise in plasma GIP levels after the meal in the diabetics; by 5 min plasma GIP levels were significantly elevated above the mean basal concentration of 64.7 ± 8.4 pmol/l and the rise was maintained for 4 hrs ($p < 0.01$). However the magnitude of the plasma GIP response was lower in the diabetics, significantly so for the last 2½ hrs of sampling ($p < 0.05$). The peak GIP concentration was 158.2 ± 20.9 pmol/l in the diabetics.

Diabetics without AN vs Diabetics with AN (Fig. V.28)

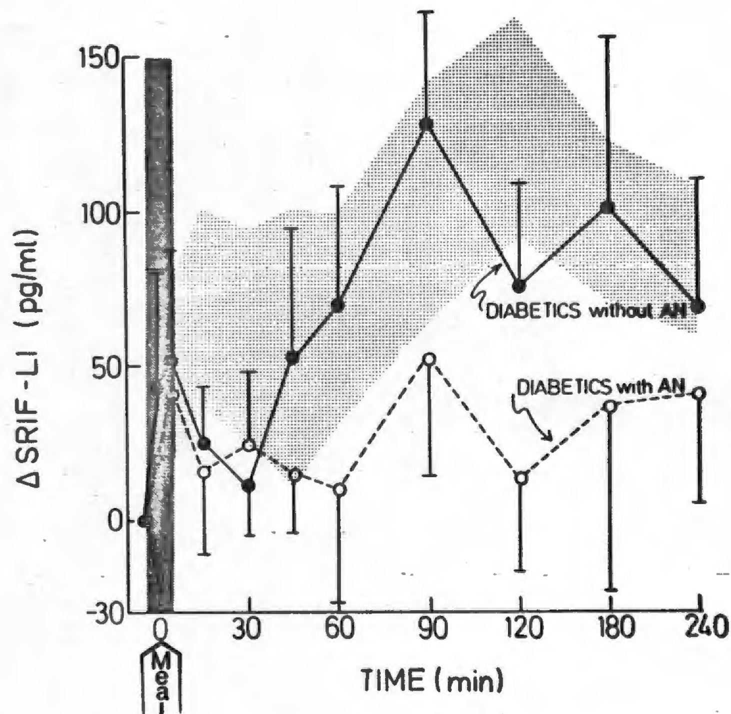
Differences in the plasma GIP response to the mixed meal were also noted when the diabetics were separated into those with and without AN. However the mean basal plasma GIP concentrations were similar in the two groups of diabetics as well as in the controls. In the diabetics without AN there was a rapid rise in plasma GIP levels from the mean basal concentration of 73.1 ± 13.2 pmol/l, to reach significance at 5 min, remaining significantly elevated until 2 hr ($p < 0.01$). The peak level of 229 ± 37.7 pmol/l occurred at 1 hr. The plasma GIP response to the meal was similar in the diabetics without AN and the controls. However, in the diabetics with AN there was an initially delayed and significantly impaired ($p < 0.05$) plasma GIP response after completion of the meal, compared to the healthy controls and the diabetics without AN. In the diabetics with AN, plasma GIP levels rose from the basal level of 56.2 ± 10.3 pmol/l to the peak concentration of 119.6 ± 30.5 pmol/l at 90 min which was significantly impaired ($p < 0.05$) relative to the peak concentrations noted in the diabetics without AN and the healthy subjects.

Comment:

Plasma GIP levels rose significantly after ingestion of the meal in the diabetics and the healthy subjects but the rise in the diabetics was impaired. The latter diabetics without AN had a similar postprandial GIP response to the controls. The diabetics with AN however, had an initially delayed and impaired GIP response relative to the diabetics without AN and the controls.



(Fig.V.29) Plasma SRIF-LI responses to ingestion of a mixed meal in 12 healthy subjects and 10 diabetics. Triangles indicate $p < 0.05$ rise above the mean basal concentrations.



(Fig.V.30) Plasma SRIF-LI responses to ingestion of a mixed meal in 5 diabetics with and 5 diabetics without AN.

2 C (vi) SOMATOSTATIN

Diabetics vs Controls (Fig. V.29)

The mean basal plasma SRIF-LI concentrations were similar in the healthy controls (191 ± 21.7 pg/ml) and in the 10 diabetics (185 ± 27.7 pg/ml). The plasma SRIF-LI levels fell significantly during the meal and rose gradually after completion of the meal in the controls, reaching significant elevation above basal initially at 5 min and then again at 45 min, then again remaining elevated for 4 hrs ($p < 0.05$). The peak concentration of 318 ± 53.6 pg/ml (maximum increment 126.3 ± 36.9 pg/ml) occurred at 120 min. In the diabetics, plasma SRIF-LI levels rose more rapidly after the meal reaching significance at 5 min, fluctuating thereafter. The peak concentration of 276 ± 53 pg/ml (maximum increment 90 ± 34.5 pg/ml) was noted at 90 min and was significantly elevated above basal. At all times after 5 min plasma SRIF-LI levels were lower in the diabetics than the controls.

Diabetics without AN vs Diabetics with AN (Fig. V.30)

No prominent differences in the postprandial SRIF-LI concentrations became apparent when the diabetics were subdivided. The mean basal plasma SRIF-LI concentrations were similar in the diabetics without AN (180 ± 49.8 pg/ml) and the diabetics with AN (191 ± 31.6 pg/ml), and were not different from the value in the controls. In the diabetics without AN there was a small early rise and a larger late rise in plasma SRIF-LI levels, however significant elevation above the basal concentration was only noted at 120 and 180 min ($p < 0.01$). The peak concentration of 308 ± 90.4 pg/ml (maximum increment 128 ± 56.5 pg/ml). occurred at 90 min in the diabetics without AN. There were no significant differences in plasma SRIF-LI levels after the meal between the two groups of diabetics, or in either group compared to the controls. However there was no notable rise in plasma SRIF-LI levels after the meal in the diabetics with AN. In these patients the peak concentration of 243 ± 63 pg/ml which constituted a maximum incremental rise of 52 ± 38.2 pg/ml also occurred at 90 min.

Comment:

Ingestion of the mixed meal induced a significant increase in plasma SRIF-LI levels in the controls and the diabetics. Only in the diabetics without AN was the rise in plasma SRIF-LI levels significantly different from basal. Nevertheless no significant differences were apparent in the magnitude of the rise in plasma SRIF-LI concentrations between the two groups of diabetics.

Table V.12

Summary of the Responses of the five gastrointestinal hormones to ingestion of the mixed test meal in the healthy subjects, the diabetics as a single group, the diabetics without AN and the diabetics with AN.

	IRG	PP	GASTRIN	GIP	SRIF-L
Healthy subjects	↑	↑	↑	↑	↑
Diabetics	↑↑	N	N	↓	N
Diabetics without AN	↑↑	N	N	N	N
Diabetics with AN	↑↑	D	N	đ	N

↑ - significant response

↑↑ - exaggerated response

N - normal response

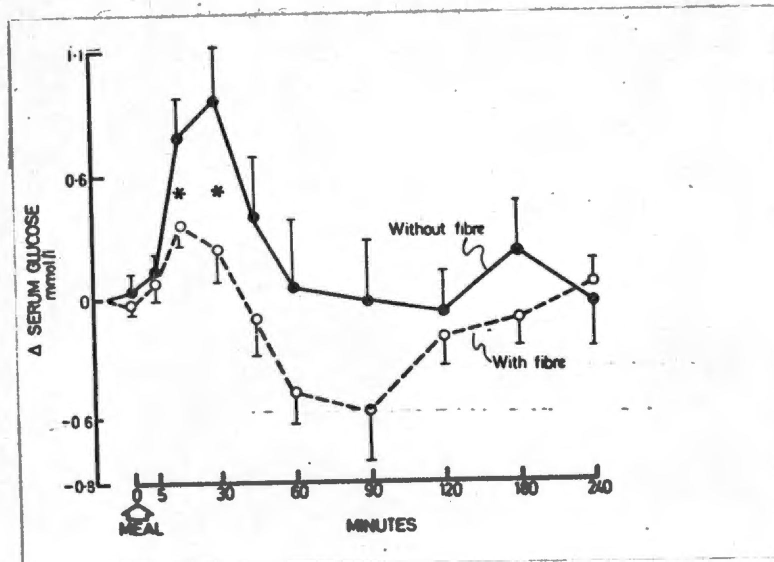
đ - impaired response

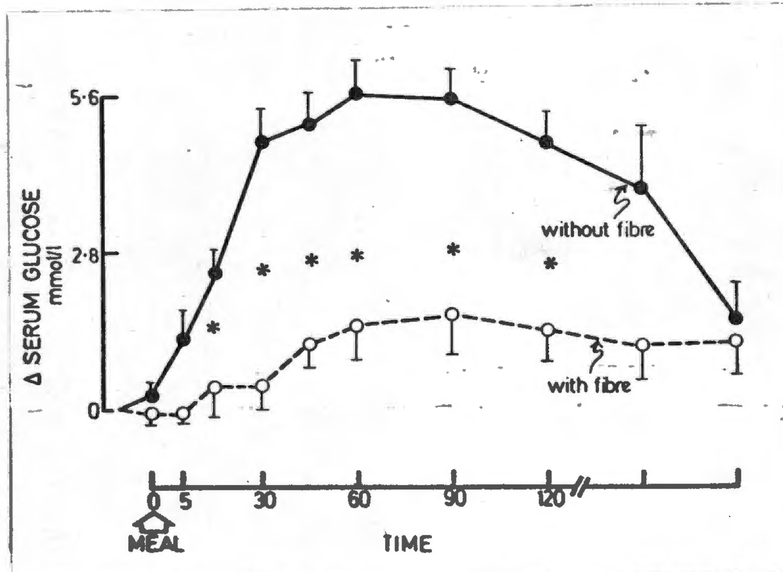
D - delayed response

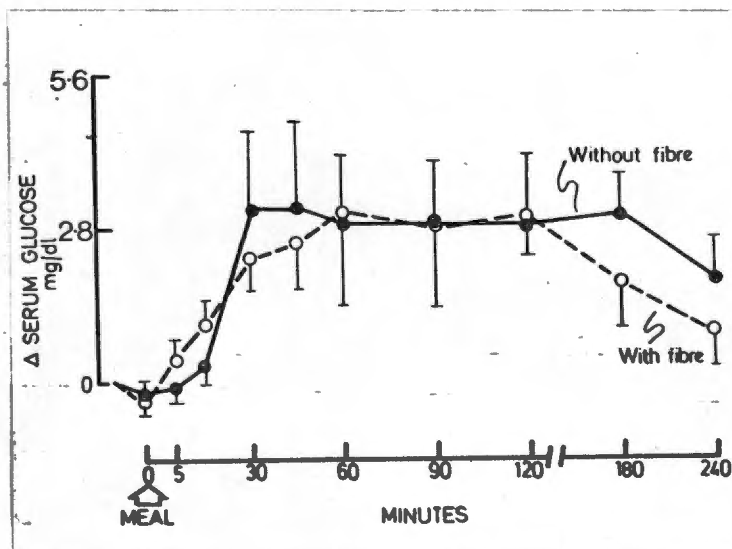
SUMMARY (Table V.12)

The diabetics as a group had fasting hyperglycaemia and greater hyperglycaemic responses after ingestion of the mixed meal than the controls. The plasma IRG concentrations followed a similar pattern. In the diabetics there was fasting hyperglucagonaemia and an exaggerated rise in plasma levels after the meal compared to the controls. In contrast the serum gastrin and plasma SRIF-LI levels before and after the meal were similar in the diabetics as a group and the controls. However in the diabetics, the rise in plasma GIP levels after the meal was impaired, as was the rise in serum insulin levels, relative to the controls.

Several differences in the glucose and hormone responses to the mixed meal became apparent when the diabetics were divided into those with AN and those without AN. The level of fasting hyperglycaemia was generally slightly higher in the diabetics with AN relative to the diabetics without AN. However the diabetics without AN had a greater rise in glucose levels after the meal than the diabetics with AN. The two groups of diabetics had similarly exaggerated plasma IRG responses compared to the controls. The serum gastrin and plasma SRIF-LI responses to the meal were similar in both groups of diabetics and were not different from the controls. The rise in serum insulin levels was also similar in the two groups of diabetics, but there was an early impaired rise in the diabetics without AN compared to the controls. In contrast there was an initially delayed and a significantly impaired increase in plasma GIP levels after the meal in the diabetics with AN relative to the diabetics without AN and the controls, in whom the GIP response was similar. In addition there was a significantly delayed early rise in serum PP levels after the meal in the diabetics with AN compared to the controls and those without AN.







(Fig. V.31) Serum glucose responses to ingestion of a test meal with and without added fibre in 12 healthy subjects (top), 6 diabetics without AN (middle) and 6 diabetics with AN (bottom). Asterisks indicate $p < 0.01$ differences between the two meals.

SECTION 3 : FIBRE SUPPLEMENTATION

3 (i) GLUCOSE (Fig. V.31)

Controls

In the healthy subjects, the mean basal serum glucose concentration was 5 $\pm$ 0.1 mmol/l before the fibre supplemented meal and 5.1 $\pm$ 0.1 mmol/l before the test meal. When fibre was added to the meal there was a smaller rise in serum glucose levels and a prolonged depression below the basal level for over 2 hrs. The mean area under the serum glucose curve was 24 $\pm$ 21.5 mmol/l 240 min after the fibre supplemented meal, significantly lower than that of 135.7 $\pm$ 54.6 mmol/l 240 min observed after the test meal (111.7% change $p < 0.05$).

Diabetics without AN

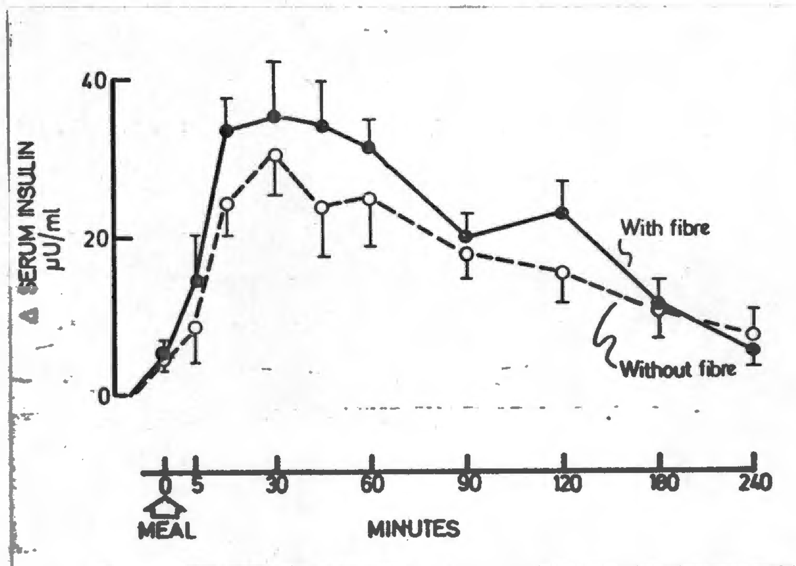
Fibre supplementation also had a profound effect on the rise in postprandial glucose concentrations in the diabetics without AN. In these patients, the mean basal serum glucose concentration was 8.2 $\pm$ 0.6 mmol/l before the test meal and 9.2 $\pm$ 2.2 mmol/l before the fibre-supplemented meal. The difference was not statistically significant. With the addition of fibre to the meal, there was a marked diminution in the serum glucose concentrations throughout the 4 hr of sampling. In addition there was a 61% reduction ($p < 0.01$) in the mean area under the glucose curve after the fibre supplemented meal (372.9 $\pm$ 97.1 mmol/l 240 min) compared to the test meal (972.1 $\pm$ 124.4 mmol/l 240 min).

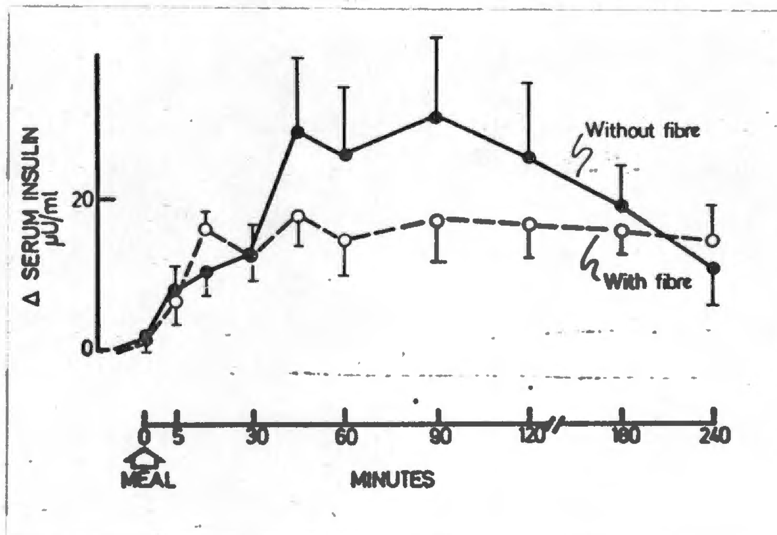
Diabetics with AN

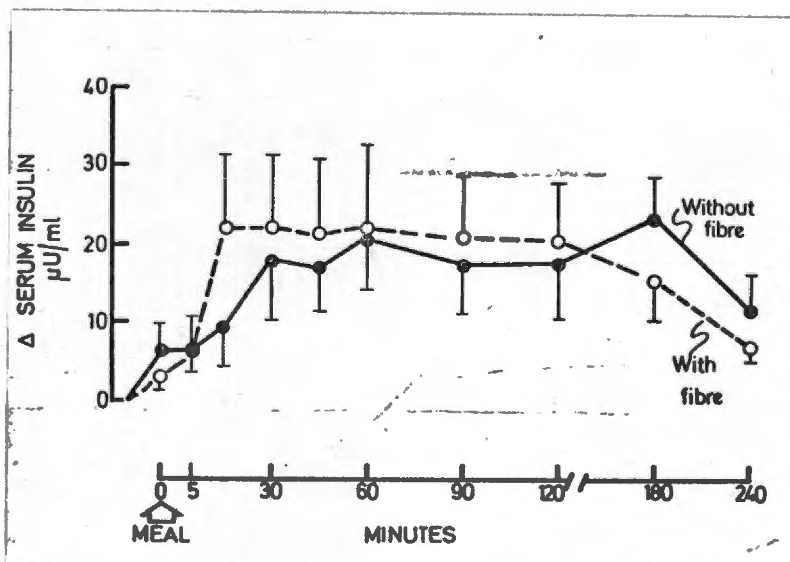
In the diabetics with AN, fibre supplementation had little effect on the rise in serum glucose levels after ingestion of the meal. The mean basal serum glucose concentrations in these patients were similar before the fibre-supplemented meal (12.1 $\pm$ 2.0 mmol/l) and before the test meal (11.5 $\pm$ 1.2 mmol/l). There were no significant differences in the rise in the serum glucose levels after the fibre supplemented meal compared to the test meal. The mean area under the glucose curve was 673 $\pm$ 119.4 mmol/l 240 min after the test meal and 573 $\pm$ 193.6 mmol/l 240 min after the meal with fibre, only a 15% fall ($p > 0.05$).

Comment:

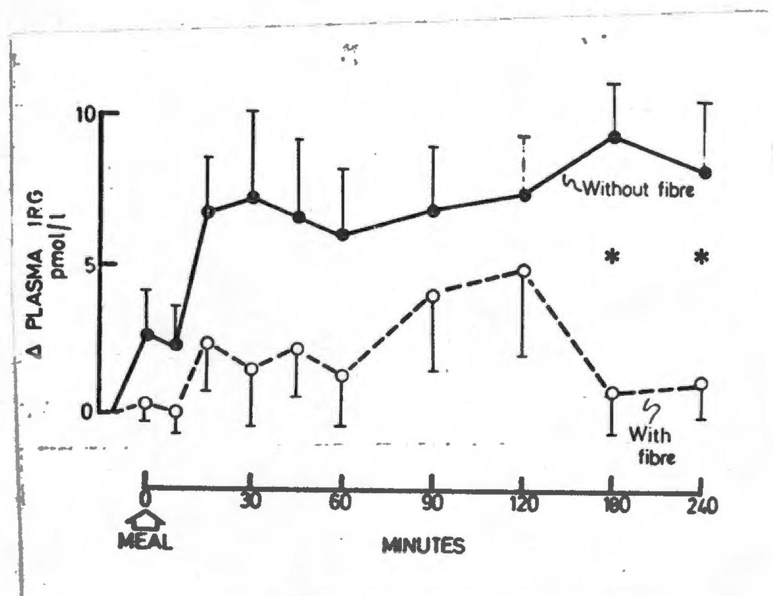
Fibre supplementation of the meal was associated with a significant reduction in the serum glucose concentrations in the diabetics without AN and the controls, but no notable alteration was observed with fibre supplementation in the diabetics with AN.

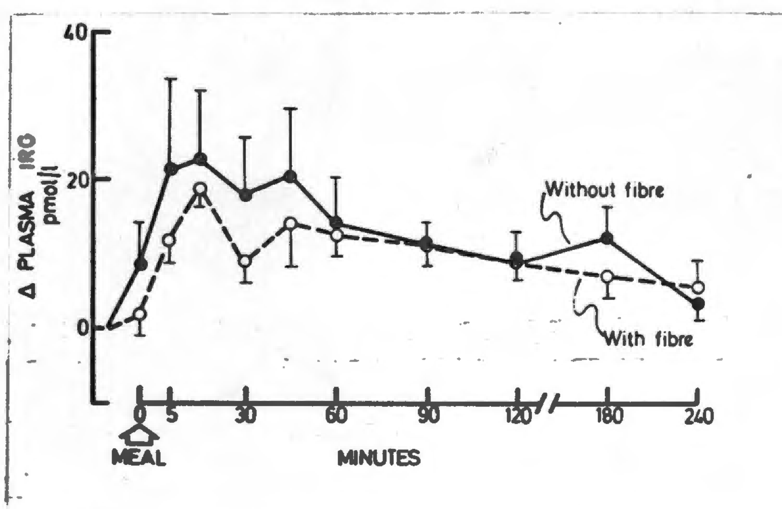


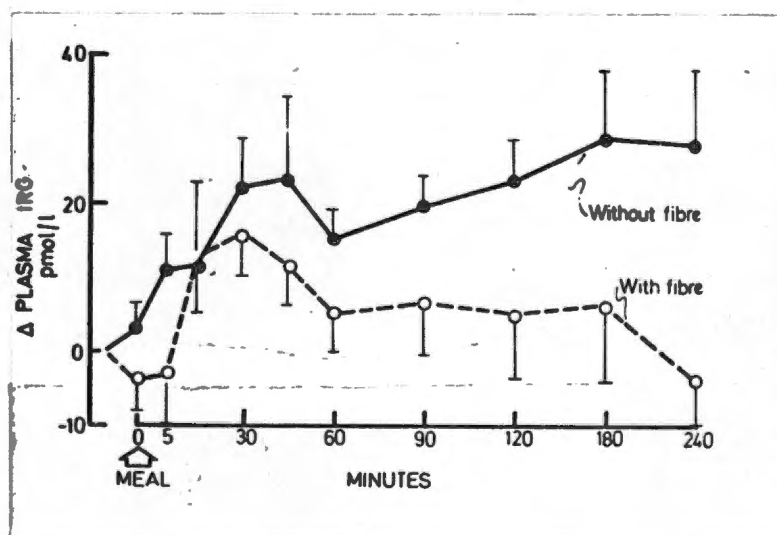




(Fig. V.32) Serum insulin responses to ingestion of a test meal with and without added fibre in 12 healthy subjects (top), 6 diabetics without AN (middle) and 6 diabetics with AN (bottom).







(Fig. V.33) Plasma IRG responses to ingestion of a test meal with and without added fibre in 12 healthy subjects (top), 6 diabetics without AN (middle) and 6 diabetics with AN (bottom). Asterisks indicate $p < 0.05$ differences between the two meals.

3 (ii) INSULIN (Fig. V.32)

In the healthy subjects the mean basal serum insulin concentrations were similar before the test meal with added fibre (14.8 ± 1.3 μU/ml) and before the test meal alone (13.7 ± 1.7 μU/ml). The serum insulin response was marginally lower after the fibre supplemented meal, compared to the test meal, but no significant differences were noted at any of the sampling times. The mean area under the insulin curve was 4662.8 ± 431.7 μU/ml 240 min after the test meal and 4389.5 ± 749.5 μU/ml 240 min after the meal with added fibre, a 6% change which was not statistically significant.

Diabetics without AN

In the diabetics without AN too, the mean basal serum insulin concentrations were similar before the fibre-supplemented meal (13.7 ± 1.7 μU/ml) and before the test meal (12.7 ± 1.3 μU/ml). The addition of fibre to the meal did not alter the early insulin response but lowered, albeit insignificantly, the mean serum insulin levels after 30 min. Fibre supplementation reduced the mean area under the insulin curve by 26.2% (3622 ± 1003.2 μU/ml 240 min vs 4908 ± 1299.5 μU/ml 240 min) ($p > 0.05$).

Diabetics with AN

The mean basal serum insulin concentrations in the diabetics with AN were also similar before the fibre supplemented (18 ± 3.3 μU/ml) and the test meal (15.8 ± 3.7 μU/ml). The addition of fibre to the meal did not alter the serum insulin response in these patients. The mean area under the insulin curve was 4344 ± 1002.8 μU/ml 240 min after the test meal and 3954 ± 1183.2 μU/ml 240 min after the meal with added fibre, a 9% diminution ($p > 0.05$).

Comment:

Addition of fibre to the test meal had a minimal effect on the postprandial insulin concentrations in the controls and the diabetics with AN. In spite of a 26% reduction in the mean area under the serum insulin curve in the fibre supplemented meal in the diabetics without AN, the reduction was not significant.

3 (iii) GLUCAGON (Fig. V.33)

Fibre supplementation had different effects on the plasma IRG responses to the mixed meal in the normal subjects and the diabetics.

Controls

The mean basal plasma IRG concentration in the healthy subjects was similar before the fibre supplemented meal (19.4 ± 2.8 pmol/l) and the test meal (16.8 ± 2.9 pmol/l). With the addition of fibre to the meal, there was a smaller rise in plasma IRG levels throughout the sampling time, although the differences were significant only at 180 and 240 min. The mean area under the IRG curve was 1917 ± 442.7 pmol/l 240 min after the test meal and 767 ± 420.11 pmol/l 240 min after the addition of the fibre to the meal, a 60% and significant diminution ($p < 0.05$).

Diabetics without AN

In the diabetics without AN, the addition of fibre did not effectively alter the plasma IRG response to the meal. In these patients the mean basal plasma IRG concentration was 18.4 ± 2.5 pmol/l before the fibre supplemented meal and 21.1 ± 2.4 pmol/l before the test meal. The difference was not significant. Although the initial rise in plasma IRG levels was lower after the fibre supplemented meal, the differences were not significant and indeed the levels for the last 3 hrs of the test were similar on both occasions. The mean area under the IRG curve was 3340 ± 1072.7 pmol/l 240 min after the test meal and 2349.1 ± 381.8 pmol/l 240 min after the meal with added fibre, and constituted a 30% reduction. However the reduction was not statistically significant.

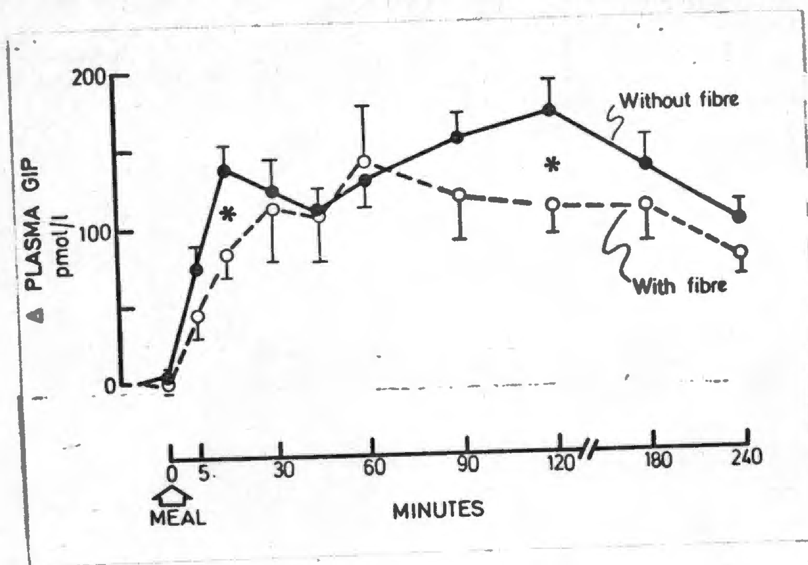
Diabetics with AN

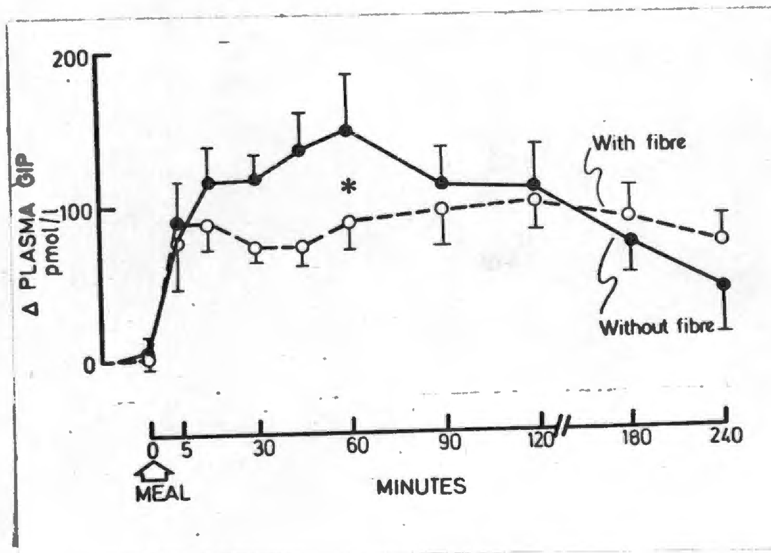
In the diabetics with AN, the mean basal plasma IRG concentrations were similar before the fibre supplemented (57.1 ± 10.1 pmol/l) and the test meals (48.5 ± 6.2 pmol/l). With the addition of fibre to the meal, the rise in plasma IRG levels was lower throughout almost the entire sampling time. However, at no point were the differences in mean plasma IRG levels after the test and fibre supplemented meals significantly different. Addition of fibre to the meal reduced the mean area under the plasma IRG curve from 5744.3 ± 1728.8 to 1557.7 - 1566.7 pmol/l 240 min i.e. a change of 72.9%, which was not statistically different.

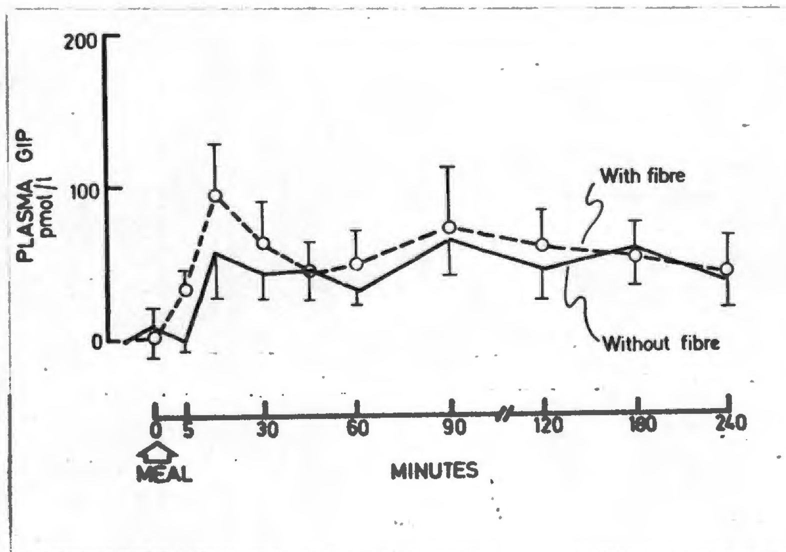
Comment:

Fibre supplementation of the meal induced a significant reduction in the rise in

plasma IRG levels in the controls but not in the diabetics without AN. Although plasma IRG levels were lower after the fibre supplemented meal in the diabetics with AN, the reduction was not statistically significant.







(Fig. V.34) Plasma GIP responses to ingestion of a test meal with and without added fibre in 12 healthy subjects (top), 6 diabetics without AN (middle) and 6 diabetics with AN (bottom). Asterisks indicate $p < 0.05$ difference between the two meals.

3 (iv) GASTRIC INHIBITORY POLYPEPTIDE (Fig. V.34)

Controls

The mean basal plasma GIP concentrations in the normal subjects were similar before the fibre supplemented meal (68.3 ± 5.9 pmol/l) and the test meal (59.4 ± 5 pmol/l). There was a lower early and late rise in plasma GIP levels after completion of the fibre supplemented meal. Although there was a 22% reduction in the mean area under the GIP curve, with the addition of fibre to the meal, the reduction was not significant (33945 ± 2633.4 vs 26397.4 ± 3754.7 pmol/l 240 min).

Diabetics without AN

In the diabetics without AN too, the plasma GIP concentrations were similar before the meal with added fibre (74.3 ± 12.5 pmol/l) and the test meal (73.1 ± 13.2 pmol/l). In these patients although the initial rise in plasma GIP levels was similar after ingestion of the test meal with and without added fibre, from 15 to 60 min the GIP levels were lower after the fibre supplemented meal. According to the mean area under the GIP curve, the addition of fibre to the meal reduced the GIP response by 10.5% (23208.2 ± 4792.2 vs 20782.9 ± 3224.8 pmol/l 240 min) ($p > 0.05$).

Diabetics with AN

The mean basal plasma GIP concentration was 62.9 ± 3.5 pmol/l before the meal with added fibre and 56.2 ± 10.3 pmol/l before the test meal in the diabetics with AN. In these patients the plasma GIP response was similar after ingestion of the two meals although marginally higher after the meal with added fibre. Indeed, the mean area under the GIP curve was 14245.3 ± 5522.9 pmol/l 240 min after the fibre supplemented meal and 11806.9 ± 3364.5 pmol/l 240 min after the test meal, a 20.7% rise ($p > 0.05$).

Comment:

Fibre supplementation led to a reduction in the plasma GIP concentration after the test meal in the controls and diabetics without AN, but in neither group was the reduction in the mean area under the GIP curve significant. In the diabetics with AN there was a small increase in GIP levels after the fibre supplemented meal.

Table V.13

Summary of the data on the effect of fibre supplementation on glucose and hormone responses to ingestion of a mixed meal in 12 healthy subjects, 6 diabetics without AN and 6 diabetics with AN.

	Glucose	Insulin	IRG	GIP
Healthy controls	♁♁	→	↓	→
Diabetics without AN	♁♁	→	→	→
Diabetics with AN	→	→	→	→

♁♁ - significantly reduced
→ - no significant change
↓ - impaired response

<u>SUMMARY</u> (Table V.13)

In the healthy subjects fibre supplementation of the meal induced a significant reduction in the serum glucose and plasma IRG responses to meal ingestion. However, there were only minor associated reductions in the serum insulin and plasma GIP responses.

In the diabetics without AN the serum glucose response to the mixed meal was significantly reduced. No significant alterations in the plasma IRG, serum insulin or plasma GIP responses were found in diabetics without AN.

In sharp contrast to the controls and the diabetics without AN, addition of fibre to the meal in the diabetics with AN did not alter the serum glucose response to food ingestion. Furthermore, fibre supplementation induced only minor insignificant changes in the serum insulin, plasma GIP and plasma IRG concentrations after the meal.

SUMMARY OF CHAPTER V:

The first section deals with basal hormone secretion. The only abnormality detectable in the diabetics as a whole was a significantly elevated basal plasma IRG concentration compared to the controls. After division of the diabetics significant fasting hyperglucagonaemia was also the other abnormality detected in the diabetics with AN. Neither the overnight insulin infusion nor the overnight gastric aspiration significantly altered the mean basal concentrations of any of the hormones in either group of diabetics.

The second section deals with the effects of insulin hypoglycaemia on hormone release. Insulin hypoglycaemia was not a stimulus for GIP secretion. The diabetics as a group had a normal plasma IRG, an impaired serum PP and a non significant serum gastrin response to hypoglycaemia. When the diabetics were subdivided, no abnormalities were detected in hormone secretion in response to hypoglycaemia in the diabetics without AN. In contrast impaired plasma IRG and serum PP responses were observed in the diabetics with AN despite similar basal glucose levels and similar serum glucose responses in the diabetics with and without AN.

In the third section results are given of the effect of prior establishment of fasting normoglycaemia on hormone secretion during insulin hypoglycaemia. This manoeuvre ensured correction of the plasma IRG but not the serum PP response to hypoglycaemia in the diabetics with AN. In addition there was improvement in the serum gastrin and plasma IRG responses in the diabetics without AN and the former also improved in the diabetics with AN. Further, both groups of diabetics as well as the diabetics as a whole had an insignificant rise in plasma SRIF-LI levels in response to hypoglycaemia, in comparison with the significant rise in the controls.

In the fourth section results of the glucose and hormone response to ingestion of a mixed meal are given. In the diabetics considered as a single group, exaggerated plasma IRG responses, normal serum PP, serum gastrin and plasma SRIF-LI responses occurred, while impaired plasma GIP responses were observed. The diabetics with AN had impaired plasma GIP, delayed serum PP and smaller serum glucose responses to food ingestion relative to the diabetics without AN.

In the final section the results of the study on fibre supplementation of the mixed meal are given. Fibre was found to have no effect on the postprandial rise in serum glucose concentrations in the diabetics with AN, in sharp

contrast to the diabetics without AN and the controls, where a prominent reduction was apparent. In addition serum insulin and plasma GIP response to the meal were unchanged by fibre in either of the three groups, but fibre significantly reduced the plasma IRG response in the healthy controls.

Thus it is apparent that abnormalities in basal and stimulated hormone secretion occur in diabetics per se and particularly in diabetics with AN. In addition it is apparent that fibre benefits glucose tolerance only in the absence of AN.

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CHAPTER VI

DISCUSSION

In this chapter we will discuss our findings on the effect of AN on basal and stimulated hormone release in NIDDM; the possibility of the existence of a hormonal marker for AN, and whether fibre supplementation of a meal would have a similar effect on glucose tolerance and hormone secretion in the presence or absence of AN. In order to avoid repetition, literature which has been discussed in earlier chapters will be referred to by page number and will not be discussed in detail unless immediately relevant.

SECTION 1 : THE DIAGNOSIS OF AUTONOMIC NEUROPATHY

The clinical features of AN are numerous and diverse and include sweating abnormalities, intermittent nocturnal diarrhoea, constipation, bladder problems, impotence and postural hypotension (pages 37-39). However, many of these features are non-specific. In these studies, the only symptom of autonomic dysfunction elicited from the diabetics was that of impotence and its non-specificity as a feature of AN was confirmed by the finding that only 10 of the 15 diabetics complaining of impotence, were found on testing to have AN.

Previously the non specific nature of many of the symptoms of AN, together with the complex nature of the tests of autonomic nerve function and the difficulty in their interpretation due to the lack of control data, often led to the diagnosis of AN being made by exclusion. However, the description in the last decade of a new generation of simple, reproducible and generally non-invasive tests has greatly facilitated the diagnosis of AN. In addition it has become apparent that abnormal responses to autonomic function tests may occur in the absence of symptoms of AN (Murray, Ewing, Campbell, Neilson and Clarke, 1975; Gunderson and Neubauer, 1977), as was observed in patient No. 2 in this study. Further, these newly described tests have confirmed the patchy involvement of AN where patients have been found to have normal responses to some tests, yet abnormal responses to others (Bennett, Hosking and Hampton, 1975). Intact sympathetic innervation in the presence of severe parasympathetic damage has been reported previously (page 43) and was observed in patient No. 5 in these studies. Therefore a battery of tests assessing both sympathetic and parasympathetic involvement should be used in any evaluation of autonomic function.

In these studies AN was said to exist if abnormal responses to three or more of the eight autonomic function tests were present. This was an arbitrary decision as a case could be made for the diagnosis of AN if a single abnormal response to autonomic function testing was detected, for some degree of impairment of neural function is likely to have been present in such a situation. Although at present there is no real means of quantifying the degree of AN, it might be assumed that the more abnormal responses found on testing, the more severe the autonomic dysfunction. Indeed, the grounds for the selection of three or more abnormal responses rested primarily on the desire to evaluate the effect of fairly severe AN on hormone secretion in the NIDDM.

SECTION 2 : BASAL HORMONE SECRETION

2 (i) GLUCOSE AND INSULIN

Fasting hyperglycaemia with normal basal serum insulin levels, as observed in the diabetics as a whole in this study, are well recognized features of NIDDM. However, the similarity in basal serum insulin and glucose concentrations in the diabetics with and without AN has not been reported previously in NIDDM.

The significant increase in basal serum insulin levels after the overnight infusion in the two groups of diabetics, together with the fasting normoglycaemia achieved in both groups, suggests a lack of insulin resistance in these patients. The effect of overnight gastric aspiration on serum insulin and glucose concentrations has not been reported previously. However, the lack of effect noted in this study may indicate that either delayed gastric emptying was not present in those with AN or alternatively that the delay was not great enough to alter either basal serum glucose or insulin concentrations.

2 (ii) GLUCAGON

The finding of an elevated basal plasma IRG level in the NIDDM as a whole, relative to the healthy subjects is in agreement with reports by numerous workers (page 26). However, basal plasma IRG levels similar to those in healthy subjects have also been reported in NIDDM (page 26).

The striking feature of this study was that the diabetics with AN had significantly elevated basal plasma IRG levels compared to the diabetics without AN and the control subjects, in whom similar levels occurred. This has not been reported previously. As all diabetics were male, of similar ages, had similar degrees of obesity, similar durations of diabetes and similar fasting glucose levels, these factors must be discounted as causes of the differences in basal plasma IRG levels compared in the two groups of diabetics. Further overnight gastric aspiration failed to alter the basal plasma IRG levels compared in either group. This latter observation would tend to exclude delayed gastric emptying (page 37), as a cause for the elevated basal plasma IRG levels in the diabetics with AN. In addition the 12 hr insulin infusion failed to reduce the fasting plasma IRG levels in either group of diabetics. Thus the difference in basal plasma IRG levels in the two groups of diabetics might best be explained by a loss or impairment of the neural innervation of the alpha cell in the diabetics with AN, there being no other differences between the two groups which might account for their differing basal plasma IRG levels.

The mechanism of the elevated basal plasma IRG levels in the diabetics with AN is uncertain. Recent evidence has led to the belief that the parasympathetic nervous system exerts little effect on basal IRG release (pages 15-16). However, the diabetics with AN had mixed sympathetic and parasympathetic neuropathy and sympathetic pathways appear to influence basal IRG release (page 16). Thus interruption of the sympathetic regulation of basal IRG release and in particular loss of the inhibitory alpha adrenic tone might be the underlying cause for the elevated basal plasma IRG levels in the diabetics with AN.

Although there can be little doubt that fasting hyperglucagonaemia, either relative (ie. elevated in relation to the prevailing glucose levels) or absolute (ie. significantly elevated as compared to healthy controls) may be a feature of NIDDM, the mechanism thereof is poorly understood. Theories which have been advanced include insulin deficiency, impairment of the action of endogenous insulin or an abnormality of intracellular carbohydrate metabolism (Day and Anderson, 1973), abnormal secretion of IRG and/or GLI (Pek, 1977) or abnormal intra-islet paracrine relationships (Unger and Orci, 1976). To these AN should be added, as in the present study, the marked fasting hyperglucagonaemia in the diabetics with AN, in the face of the relative fasting hyperglucagonaemia in those without AN, conferred absolute fasting hyperglucagonaemia on the whole group of diabetics. Thus it is possible that undiagnosed AN might have been an underlying pathogenetic factor of the observed fasting hyperglucagonaemia which has previously been reported in NIDDM. The mechanism of relative fasting hyperglucagonaemia as observed in the diabetics without AN remains conjectural.

2 (iii) PANCREATIC POLYPEPTIDE

The observation of normal basal serum PP levels in the diabetics considered as a single group in this study is in accord with a previous report in NIDDM (page 29). In addition the similarity in basal serum PP levels in the diabetics with and without AN is in agreement with the finding in IDDM with and without AN (Krarup, Schwartz, Hilsted, et al, 1979).

The latter observations may suggest that autonomic dysfunction does not have a prominent effect on basal PP release. However, this conflicts with available evidence suggestive of an effect of neural influences on basal PP release. Schwartz, Strenquist, Olbe and Stadil (1979) argued that the spontaneous secretion of PP is regulated mainly by tonic vagal activity. Their argument was based on the findings that atropine and benzilonium both suppressed basal PP release. Further, that truncal vagotomy normalized basal serum PP concentrations in duodenal ulcer patients with the highest pre-operative levels

(Schwartz, Rehfeld, Stadil, et al, 1976). However, the lack of effect of vagotomy on normal pre-operative basal serum PP levels (Adrian, Bloom, Besterman, et al, 1976; Schwartz, Rehfeld, Stadil, et al, 1976; Taylor, Feldman, Richardson and Walsh, 1978) may indicate that vagal activity is not the sole regulator of basal PP release. Indeed adrenergic influences too alter basal PP release (page 19). Thus the normal basal serum PP levels in the diabetics with AN may have been due to combined defective sympathetic and parasympathetic innervation of basal PP release. Alternatively, there may have been residual neural function in these patients or it may be that neural influences are not of prime importance in regulation of basal PP release.

In the present study a 12 hr overnight insulin infusion did not significantly alter the mean basal serum PP level in the 6 diabetics without AN (PP levels rose in 3 and fell in 3 patients). In contrast there was a rise in basal serum PP levels in 5 of the 6 diabetics with AN, leading to a mean concentration significantly above that of the healthy controls and the diabetics without AN. This latter observation tends to contradict numerous reports for which there is no apparent explanation (pages 29-30). The mean basal serum glucose levels were only insignificantly higher in the diabetics with AN than in those without, both initially and after the insulin infusion, providing no explanation of the increase in basal serum PP levels in the former. However, it is possible that the acute fluctuation in blood glucose levels produced an acute alteration in the glucose sensitive metabolic pathways in certain autonomic nerves, resulting in improved conduction of the vagus nerve and unrestrained defective alpha adrenergic activity, causing the observed increased basal serum PP levels.

In the present study there was an insignificant rise in serum PP levels after overnight gastric aspiration in both groups of diabetics. Thus the possible existence of delayed gastric emptying did not have a prominent effect on basal serum PP concentrations in the diabetics with AN. The small rise in mean serum PP levels after aspiration in both groups of diabetics might have been due to the mere presence of the nasogastric tube in the stomach, which interacted with receptors in the gastric wall.

2 (iv) GASTRIN

There appears to be a paucity of existing knowledge concerning gastrin release in NIDDM and the normal basal levels observed in the diabetics as a whole in this study have not been reported previously. However, twice to three-fold elevated basal serum gastrin concentrations relative to healthy subjects

have been reported in long-standing IDDM who were thought to have AN (Feldman, Corbett, Ramsay, Walsh and Richardson, 1979).

In this study, by contrast, the diabetics with and without AN had similar and normal basal serum gastrin levels, which remained unaltered by the prior establishment of fasting normoglycaemia and the overnight gastric aspiration. Thus the presence of fasting hyperglycaemia and the possible existence of a slow leak of food through the pylorus played no part in the normal basal serum gastrin concentrations in the patients with AN.

In view of the evidence of neural regulation, both sympathetic and vagal cholinergic (pages 19-21) on basal gastrin release, the reason for the normal basal serum gastrin concentrations in the diabetics with AN is unclear. However, combined sympathetic and vagal cholinergic dysfunction might result in normal basal serum gastrin concentrations. Alternatively residual neural function in these patients would produce the results observed. Indeed the difference in the effect of AN on basal serum gastrin concentrations in the NIDDM in this study and the IDDM in Feldman, et al's study, might have been due to selective vagal impairment in their patients.

2 (v) GASTRIC INHIBITORY POLYPEPTIDE

The finding of normal basal plasma GIP levels in the diabetics considered as a single group in the present study, none of whom were obese, is in accord with previous findings in non-obese NIDDM (page 31). However, comparable and normal basal plasma GIP levels in the diabetics with and without AN noted in this study, have not been reported previously. Similarly the lack of effect of an overnight insulin infusion and overnight gastric aspiration on basal plasma GIP levels observed in the diabetics with and without AN have not been reported. These findings indicate that fasting hyperglycaemia did not alter basal GIP release in either group and that the possible existence of delayed gastric emptying did not alter basal GIP release in those with AN.

The present findings indicate that basal GIP secretion is unaltered in the presence of AN in NIDDM. However, an explanation of this is hindered by a lack of knowledge of the effect of the autonomic nervous system on basal GIP release. In the only report available a threefold elevation in fasting plasma GIP levels was described in patients after truncal vagotomy (page 22), leading the author of the report to suggest that intact autonomic innervation seemed to be important for the basal control of GIP cells. The present findings, taken in conjunction with the patients' responses to the autonomic function tests and

the prominent effect AN had on stimulated PP levels, tends to mitigate against their suggestion.

2 (vi) SOMATOSTATIN

Controversy surrounds the measurement of SRIF-LI in unextracted plasma, particularly as the levels measured are higher than those measured in extracted plasma. Thus the basal plasma SRIF-LI concentrations found in the present study were similar to those previously reported in unextracted plasma in man (Kronheim, Berelowitz and Pimstone, 1978), but were much higher than those found in extracted plasma in man (Penman, Wass, Lund, et al, 1979). However, similar forms of stimulation resulted in a significant increase in plasma SRIF-LI levels in both extracted and unextracted plasma, which will be discussed later. Thus while there may be some doubt with regard to the measurement of SRIF-LI in unextracted plasma, the fact that the SRIF-LI measured by this method shares immunological identity with synthetic SRIF (Kronheim, Berelowitz and Pimstone, 1978) and is found to increase after stimulation, as is that measured in extracted plasma, suggests that some form of SRIF-LI is being measured in our system. Furthermore, measurement of different somatostatin moeities, antisera differences and losses incurred during extraction may be responsible for the different levels found in the two methods.

In the present study the diabetics considered as a single group and the healthy subjects had similar basal plasma SRIF-LI levels. Furthermore, the diabetics with and without AN had comparable basal plasma SRIF-LI levels, which did not differ from those of the healthy controls. The former observation contrasts with the significantly elevated basal plasma SRIF-LI levels reported in alloxan diabetic dogs compared to normal dogs, (page 33). This disparity is likely to be due to differing SRIF-LI secretion in insulin-deficient and non insulin-deficient forms of diabetes.

In the present study the overnight insulin infusion did not alter basal plasma SRIF-LI levels in the diabetics with AN and those without AN. In contrast it was reported that reinstitution of insulin therapy in insulin deprived alloxan diabetic dogs reduced their elevated basal plasma SRIF-LI levels, but to levels still significantly higher than those of control dogs (page 33). These observations tend to reaffirm the differences in SRIF-LI secretion in insulin deficient and non-insulin deficient diabetes, and the similarity in basal SRIF-LI release in the diabetics regardless of the presence of AN. Overnight gastric aspiration too did not alter basal plasma SRIF-LI levels in either the diabetics with AN or those without AN, suggesting that the possible existence of delayed gastric emptying had no effect on basal SRIF-LI in the former.

The observation of similar and normal basal plasma SRIF-LI concentrations in the diabetics with and without AN tends to suggest that autonomic dysfunction does not have a major effect on basal SRIF-LI release. This is supported by the findings of a lack of effect of atropine on basal plasma SRIF-LI levels in the dog (Schusdziarra, Rouiller, Harris and Unger, 1979), and on SRIF-LI release from the isolated canine pancreas (page 23). The suggestion is contradicted by the finding that vagotomy in the dog resulted in significant elevation of basal plasma SRIF-LI levels (page 23). In addition in the isolated perfused canine pancreas beta adrenergic blockade abolished SRIF-LI release stimulated by beta adrenergic agonism (page 23).

In view of the data indicating neural mediation of basal SRIF-LI release, the lack of effect of AN on this release is surprising. However, it may well be that neural influences are not of prime importance in regulation of basal SRIF-LI release in man. Alternatively the neuropathy may not have been severe enough to cause a noticeable effect on basal SRIF-LI release.

SECTION 3 : PROVOCATIVE STIMULATION

A INSULIN HYPOGLYCAEMIA

3 A (i) GLUCOSE

Although the absolute nadir serum glucose concentration in the initial insulin hypoglycaemia test was lower in the healthy controls than the diabetics as a whole, or after division into those with and without AN, the actual fall in serum glucose concentrations was greater in the diabetics. This indicated sensitivity to insulin administration in the diabetics. However the dosage of insulin given to the diabetics was twice that given to the controls. This larger dose was administered in order to produce an adequate degree of hypoglycaemia in the diabetics. Indeed although the absolute nadir serum glucose concentrations were higher in the diabetics, the vast majority experienced symptoms of hypoglycaemia, comparable to those of the controls. This observation suggests that the features associated with hypoglycaemia may be induced by factors other that a nadir serum glucose concentration of less than 2.8 mmol/l.

The serum glucose responses to insulin administration in the diabetics with and without AN were similar. Had the release of the counter-regulatory hormones such as growth hormone, the catecholamines and IRG been impaired in the diabetics with AN, a delayed recovery from hypoglycaemia might have ensued. Although IRG release was impaired in the diabetics with AN in the initial insulin hypoglycaemia test, but not when hypoglycaemia was preceded by the overnight insulin infusion, there was no impairment in the recovery from hypoglycaemia on either occasion. This observation may suggest that the release of the other counter-regulatory hormones was unaltered in the diabetics with AN in this study. This would in turn account for the unimpaired recovery from hypoglycaemia.

The third major point of the study was the fact that the overnight insulin infusion normalized the glucose responses to the insulin bolus in both groups of diabetics. This indicates an equivalent degree of sensitivity to short term insulin therapy regardless of the presence of AN in the diabetics studied.

3 A (ii) GLUCAGON

The prominent plasma IRG response to insulin hypoglycaemia observed in the healthy subjects in the present study confirmed present beliefs (page 55). Previous reports of plasma IRG responses to insulin hypoglycaemia in diabetics have been limited to IDDM. Impaired responses have been reported in IDDM, unstable IDDM and IDDM with AN (page 27).

In contrast to these reports in IDDM, the NIDDM considered as a single group in this study did not have an impaired plasma IRG response to insulin hypoglycaemia, nor indeed did those without AN. This finding suggests that impaired plasma IRG responses to insulin hypoglycaemia are not a feature of NIDDM. Indeed it would appear that the existence of fasting hyperglycaemia and a higher nadir serum glucose level did not induce an abnormal plasma IRG response to hypoglycaemia in the NIDDM compared with the controls.

When in this study the diabetics with AN were found to have an impaired plasma IRG response to the initial insulin hypoglycaemia test, various factors which may possibly have been implicated in this impaired response were evaluated. There were no differences in age, degrees of obesity, degrees of fasting hyperglycaemia, the level of hypoglycaemia attained, expressed either as absolute values or as percentage change from basal, or in the rate of fall in serum glucose levels to account for the different plasma IRG responses in the two groups of diabetics. After exclusion of the abovementioned factors it seemed likely that the impaired IRG response in the diabetics with AN might be explained on the basis of defective neural regulation of IRG release. In agreement with this suggestion was the observation that impaired IRG responses to hypoglycaemia were present in IDDM with AN (Maher, Tannenberg, Greenberg, et al, 1977).

However, when insulin hypoglycaemia was repeated after prior establishment of fasting normoglycaemia, the plasma IRG response improved in both groups of diabetics. Furthermore the plasma IRG response was no longer impaired in the diabetics with AN. Thus it would appear that factors other than the autonomic innervation of the alpha cell play the major role in the regulation of the IRG response to insulin hypoglycaemia. This is supported by recent evidence indicating that neural mechanisms are probably unimportant in mediation of the IRG response to hypoglycaemia (pages 15-16).

The exact mechanism whereby IRG release is stimulated by insulin hypoglycaemia remains unknown, but the rate of fall of blood glucose levels or hypoglycaemia per se may be the initiator of IRG release. It may well be that hypoglycaemia is such a potent alpha cell stimulus that it overrides neural control of the alpha cell. If that is the case, the impaired plasma IRG responses to hypoglycaemia observed in IDDM with AN (Maher, Tannenberg, Greenberg, et al, 1977) might have been related to the presence of unstable diabetes. This suggestion is based on the following. Unstable IDDM were reported to have impaired IRG responses to insulin hypoglycaemia (Reynolds, Nolnar, Horwitz, et al, 1977) and most of the IDDM with AN had unstable diabetes, while in none of the IDDM

without AN was their diabetes unstable. Therefore there would appear to be different situations with regard to IRG responsiveness to hypoglycaemia in IDDM and NIDDM. Further, the improvement in the plasma IRG responses to hypoglycaemia in both groups of diabetics after the overnight insulin infusion in the present study may have been due to the greater degree of absolute hypoglycaemia attained.

3 A (iii) PANCREATIC POLYPEPTIDE

The stimulatory effect of insulin hypoglycaemia on PP release in man is well recognized (page 55) and was confirmed in the healthy subjects in this study. However, PP responses to insulin hypoglycaemia have not previously been reported in NIDDM in whom, when considered as a single group, impaired responses were noted.

In IDDM, impaired serum PP responses to hypoglycaemia have been found in patients without AN and greatly impaired responses in patients with AN (Krarup, Schwartz, Hilsted, et al, 1979). In the IDDM without AN impairment of the PP response to hypoglycaemia was related to duration of diabetes, as well as to increased threshold of vibratory sensation. This probably indicates the existence of undiagnosed AN in the patients with a longer duration of diabetes, as their patients with a short duration of diabetes had a normal serum PP response to hypoglycaemia. Similarly the truncated serum PP responses to hypoglycaemia observed in the NIDDM as a whole in this study appeared to be due to the overriding effect of the markedly impaired responses in the diabetics with AN, particularly as the magnitude of the rise in serum PP levels was similar in the diabetics without AN and the control subjects.

In the present studies the diabetics with AN had a significantly impaired rise in serum PP levels during hypoglycaemia relative to the diabetics without AN. This impairment occurred regardless of whether hypoglycaemia was preceded by the establishment of fasting normoglycaemia. Indeed there appeared to be an improvement in the magnitude of the rise in serum PP levels in the diabetics with AN when hypoglycaemia was preceded by the establishment of fasting normoglycaemia. However their basal serum PP levels were higher on this occasion and they still had only a threefold rise in serum PP levels, which remained significantly impaired relative to the diabetics without AN. The uniformly impaired rise in serum PP levels in the diabetics with AN compared to those without AN could not be explained by any differences in the two groups, in whom chronic pancreatitis, which may also induce impaired serum PP responses to insulin hypoglycaemia (page 30) had been excluded, other than the presence of autonomic dysfunction in those with AN. This suggestion is in agreement with

the study in IDDM by Krarup et al, and in keeping with the vagal cholinergic mediation of this aspect of PP release (page 17). Furthermore the likely mechanism of the impaired serum PP responses to insulin hypoglycaemia in the diabetics with AN is defective vagal cholinergic activity. Once again this suggestion is in line with the conclusions reached by Krarup and associates.

In order to determine the possible value of the PP response to insulin hypoglycaemia as a marker for AN, an assessment was made of correlations between the patients' PP responses to hypoglycaemia and their responses to the individual criteria used to determine the presence or absence of AN. However, the degree of impairment of the PP responses did not correlate with the individual absolute criteria of autonomic dysfunction in the patients. The PP response to both the initial hypoglycaemia test and the test preceded by the establishment of fasting normoglycaemia was uniformly impaired in all patients who had AN, and normal in those without AN. Thus it is possible that the PP responses to insulin hypoglycaemia might have been an additional marker of AN, in the small number of patients studied. The inability to correlate the PP response to hypoglycaemia with the other indices of AN in this study might possibly have been due to the qualitative nature of the latter. These data suggest that the impairment of the PP response to hypoglycaemia may prove to be a quantitative marker of AN.

3 A (iv) GASTRIN

Insulin hypoglycaemia has not been reported as an unequivocal stimulus for gastrin release in healthy man (page 55), but proved to be a stimulus in the control subjects in this study. The effect of insulin hypoglycaemia on gastrin release has not previously been reported in diabetics and their response when considered as a single group in this study was similar to that of the controls, but only the mean peak serum gastrin concentration was significantly elevated above basal. The reason for this is unclear but may relate to greater variability in the serum gastrin responses in the diabetics.

Recently it has been shown that vagal intactness is not a prerequisite for the gastrin response to hypoglycaemia (page 20). Whereas, infusion studies have suggested that the rise in serum gastrin levels in response to hypoglycaemia may at least in part be mediated via beta adrenergic pathways (page 21). Thus it is apparent that in diabetes complicated by autonomic dysfunction an altered gastrin response could occur. However, in the present study the diabetics with and without AN had similar gastrin responses to insulin hypoglycaemia whether preceded by the overnight insulin infusion or not, although when the test was preceded by the insulin infusion, the serum gastrin response to hypoglycaemia

became significant in both groups. This improvement was probably related to the greater degree of hypoglycaemia attained in the second test.

The mechanism of the normal gastrin response to hypoglycaemia in the diabetics with AN is unclear, but there are a number of possibilities which are similar to those discussed earlier with regard to the normal basal gastrin release in these patients.

3 A (v) GASTRIC INHIBITORY POLYPEPTIDE

The major observation of this study was the notable failure of insulin hypoglycaemia to induce an alteration in plasma GIP levels in any of the subjects studied. Prior to the commencement of these studies the effect of insulin hypoglycaemia on GIP release had not been determined, but recently two contradictory reports on this subject have appeared (page 55). In the first Service, Nelson, Rubenstein and Go (1978) described a significant rise, ie. 78 $\pm$ 40 pg/ml in plasma GIP levels in healthy subjects in response to insulin hypoglycaemia. In the second report hypoglycaemia induced by the same dose of insulin as used in the first report, failed to significantly alter GIP levels in healthy subjects and IDDM (Reynolds, Tronsgard, Gibbons, Blix and Rubenstein, 1979). There is no ready explanation for the contradictory reports. However the findings in this study support those of Reynolds et al. In addition it would appear that vagal activation and catecholamine release induced by insulin hypoglycaemia have little effect on GIP release in man.

3 A (vi) SOMATOSTATIN

In this study insulin hypoglycaemia induced a significant rise in plasma SRIF-LI levels in the healthy subjects, but not the diabetics as a whole. Although insulin hypoglycaemia in the latter was preceded by the induction of fasting normoglycaemia. The reason for the differential effect in the controls and the diabetics is not clear, although it may not be important in view of the fact that the response in the latter was not significantly different from that of the former. In a recent report the stimulatory effect of insulin hypoglycaemia on SRIF-LI secretion in healthy subjects has been confirmed by Wass, Penman, Medbak, et al (1980). However plasma SRIF-LI responses to hypoglycaemia have not been reported previously in diabetics.

In the diabetics with and without AN the rise in plasma SRIF-LI levels during hypoglycaemia was insignificant in both cases, but the response seemed to be flatter in those with AN. The significance of this finding is uncertain in view of the small groups studied and the lack of knowledge with regard to the

mechanisms whereby insulin hypoglycaemia induce SRIF-LI release. It is possible that significant differences in the SRIF-LI responses in the diabetics with and without AN might be apparent if larger groups were studied. Such a finding would tend to support a neural role in mediation of the SRIF-LI response to hypoglycaemia.

B THE MIXED MEAL

3 B (i) GLUCOSE AND INSULIN

The exaggerated rise in serum glucose concentrations and impaired serum insulin responses following ingestion of the meal in the diabetics considered as a single group in this study, are a well recognized feature of NIDDM.

The major point of interest in this study was the delayed and significantly lower serum glucose response in the diabetics with AN compared to those without AN, while their postprandial insulin responses were similar. There are a number of possible explanations for the lower serum glucose response in the diabetics with AN. They include a delay in gastric emptying, which has previously been reported in diabetic AN (page 37). This was not investigated in the present study. Second, defective innervation of the small intestine which may result in defective absorption and/or altered gut hormone release. There is no evidence for defective innervation of the small intestine in these patients, but altered gut hormone release e.g. GIP or SRIF-LI, might have been involved.

In view of the fact that the insulin response to food ingestion may be partially neurally mediated (Woods and Porte, 1974; Young and Landsberg, 1977), it was interesting to note the similar serum insulin responses to the meal in the diabetics with and without AN. This observation may indicate that neural factors are not of prime importance in mediation of the insulin response to food ingestion. Alternatively, that the neuropathy was not severe enough to induce any alteration in the insulin response in these patients.

3 B (ii) GLUCAGON

The small, but significant, rise in plasma IRG levels noted after ingestion of the mixed meal in the healthy subjects in the present study is in agreement with a previous report (page 56). Further the exaggerated rise in plasma IRG levels observed in the ten diabetics in this study has also been found after a mixed meal in IDDM (page 27). However, the similarly exaggerated plasma IRG responses to the mixed meal in the diabetics with and without AN observed in this study have not previously been described.

Although the groups studied were small, the latter findings suggest that autonomic dysfunction does not alter the plasma IRG response to food. Furthermore the findings tend to suggest that factors other than the integrity of the autonomic nervous system might be primarily responsible for the food-induced rise in plasma IRG levels. The latter suggestion is supported by the fact that only a cephalic phase of IRG release in response to food has been identified, and then only in the dog (page 15) and the calf (Edwards and Vaughan, 1978). Whereas other factors are known to play a prominent role in meal-induced IRG release, such as protein or fat which cause release of IRG (Unger and Orci, 1979), gastrointestinal hormones such as GIP, which are released after ingestion of a meal and have IRG-stimulating activity, may be the enteric signal to the alpha cell (Unger and Orci, 1979).

3.B (iii) PANCREATIC POLYPEPTIDE

The biphasic release of PP following ingestion of a mixed meal observed in the healthy subjects in this study is in agreement with a previous report (page 17). However, the similarity in the serum PP responses to ingestion of the meal in the diabetics as a whole and the controls in this study contrasts with the exaggerated serum PP responses to a protein meal in diabetics previously reported (page 30). However the previous report did not define the type of diabetic studied. Furthermore their patients had a greater degree of fasting hyperglycaemia than those of the present study.

Of major interest in this study was the delayed early PP peak in the diabetics with AN compared to the diabetics without AN and the controls, particularly in view of the evidence of a dependence of this phase on vagal cholinergic pathways (pages 17-18). All six diabetics with AN had vagal neuropathy as evidenced by their impaired PP responses to hypoglycaemia and abnormal responses to the autonomic function tests which assessed vagal integrity. Thus it seems likely that the delayed early rise in serum PP levels noted in these patients was due to the presence of vagal neuropathy. Although the fact that the magnitude of the early rise in serum PP levels was not impaired in these patients suggests residual vagal function and that the neuropathy was not severe enough to effect this phase of PP release in its entirety.

It is generally believed that an interaction of humoral and chemical stimuli and vagal activity mediate the prolonged second phase of PP release after food and it has been suggested that mechanisms other than neural are dominant in controlling this phase (page 18). In the diabetics with and without AN, there was no significant difference in their second phase of PP release, which

supports the aforementioned suggestion.

3 B (iv) GASTRIN

Gastrin responses to meal ingestion have not previously been reported in NIDDM, but the diabetics as a whole and the healthy subjects had insignificantly different serum gastrin responses in this study.

The diabetics with and without AN also had similar serum gastrin responses to the meal, and their responses did not differ from those of the controls. This latter observation contrasts with a report in IDDM. Feldman, Corbett, Ramsey, Walsh and Richardson (1979) studied gastrin responses to the intragastric installation of homogenized food in two groups of long-standing IDDM. Their two groups of patients were supposed to comprise one group with AN and one group without AN. However, both groups of patients were found to have vagal neuropathy and in addition had twice to threefold higher food-stimulated serum gastrin concentrations than the normal controls.

In the light of the report in the IDDM with AN and the evidence of vagal but not adrenergic (pages 20-21) mediation of gastrin release in response to food, the mechanism of the normal serum gastrin responses in the diabetics with AN in this study is unclear. However, the likely explanation is the presence of residual vagal function in these patients.

3 B (v) GASTRIC INHIBITORY POLYPEPTIDE

The observation of an impaired plasma GIP response to the mixed meal in the diabetics as a whole in this study differs from the findings of other workers. Besterman, Sarson, Turner and Bloom (1978) observed normal plasma GIP responses to a test meal in NIDDM, similar to the diabetics without AN in this study, whilst Ebert and Creutzfeldt (1978) reported exaggerated GIP responses after a test meal in NIDDM. The diabetics in the present study comprised an equal number of subjects with and without AN and only the former subgroup was characterized by an impaired plasma GIP response to the meal. Thus it appears likely that the diabetics with AN conferred an impaired plasma GIP response on the entire group. The normal plasma GIP responses reported by Besterman et al, and noted in the diabetics without AN on the one hand, and the exaggerated plasma GIP responses observed by Ebert et al on the other, might be explained by the presence of obesity in the patients studied by Ebert et al. Alternatively, antisera differences may be the explanation for the differing results.

The most interesting feature of this study was the impaired plasma GIP response to the meal in the diabetics with AN. This finding suggests that autonomic

dysfunction may have altered the sensitivity of the GIP cell to the meal. However, studies evaluating neural regulation of food-stimulated GIP release have reported findings comparable with either the effects of atropine per se or a neural dependence of GIP release (page 22). Thus the impaired plasma GIP responses in the diabetics with AN might have been due to other factors. Ebert, Creutzfeldt, Brown, Frerichs and Arnold (1976) suggested that the GIP response to a meal was dependent on at least two factors: the rate of absorption of nutrients and the capacity of the beta cell for insulin secretion. Insulin secretion was similar in the diabetics with and without AN, thus excluding altered insulin secretion as the cause for the impaired GIP response in the former. Although having slightly higher basal serum glucose levels, the diabetics with AN had lower postprandial glucose responses than the diabetics without AN. Thus the impaired plasma GIP response to food ingestion might, in fact, have been related to altered absorption of nutrients, although glucose was the only nutrient measured. In support of this suggestion was the report by Creutzfeld, Ebert, Arnold, Frerichs and Brown (1976) in which significantly impaired plasma GIP responses to a mixed meal were noted in patients with malabsorption due to coeliac disease. Their patients had significantly lower glucose and insulin responses compared with controls. Patients with chronic pancreatitis and fat malabsorption in contrast, had exaggerated GIP responses to a mixed meal, which was postulated to be due to endocrine (insulin) insufficiency (Ebert, Creutzfeld, Brown, Frerichs and Arnold, 1976). Thus it would appear that impaired plasma GIP responses to food ingestion may be related solely to poor absorption of glucose. Indeed it is attractive to suggest that there is defective absorption of nutrients, in particular glucose, in diabetics with AN in order to explain their impaired plasma GIP responses to food ingestion. Had changes in fat levels been measured the problem might have been nearer resolution. The mechanism of the defective absorption of nutrients in turn may have been related to delayed gastric emptying, as alluded to earlier.

3 B (vi) SOMATOSTATIN

The stimulatory effect of meal ingestion on SRIF release noted in the healthy subjects in this study has recently been confirmed by Wass, Penman, Dryburgh, et al (1980). However SRIF-LI release following a meal has not previously been reported in diabetics. In the present study plasma SRIF-LI responses to meal ingestion were similar in the diabetics considered as a single group and the healthy controls. This finding contrasts with the report of a delayed but ultimately higher rise in plasma SRIF-LI levels in alloxan diabetic dogs after protein-fat meals (page 33). The discrepancy in the findings of the two studies, like that for the basal SRIF-LI concentrations, is likely to be related to the different forms of diabetes studied.

The major point of interest in the present study was that the plasma SRIF-LI responses to the meal were not significantly different in the diabetics with and without AN although the responses in the former group tended to be flatter, particularly compared to the controls.

The precise mechanism whereby ingestion of a mixed meal induced stimulation of SRIF-LI release is unknown. Cephalic factors have not been identified as yet and would tend to be excluded by the present findings. Animal experiments have revealed a gastric phase for SRIF-LI release (page 23). In addition the existence of an intestinal phase of the SRIF-LI response to food, mediated by gut hormones, nutrients and neural pathways has been suggested by Schusdziarra, Rouiller, Pietri, et al (1979). Thus two phases have been identified in the SRIF-LI response to ingestion. However the lack of significant differences in the response in the two groups of diabetics may suggest that in man neural factors are probably not of prime importance in mediation of the SRIF-LI response to food.

SECTION 4 : FIBRE SUPPLEMENTATION

These studies confirm the observation that fibre added to meals lowers the postprandial rise in serum glucose concentrations in normal subjects and NIDDM and further confirm that fibre improves glucose tolerance (pages 46-47). Although the effect of fibre on glucose tolerance in diabetics with AN has not previously been reported, our findings indicate that improvement in glucose tolerance is observed only in the absence of AN in NIDDM.

The reason for the lack of effect of fibre supplementation on the serum glucose responses to food ingestion in the diabetics with AN is not clear. However, it is conceivable that one or another of the mechanisms which have been postulated to explain the effects of fibre on glucose tolerance (page 46) may not be fully operative in the presence of AN. Delayed gastric emptying is a well recognized feature of diabetic AN (page 37), although not assessed in the present study as stated earlier. Nevertheless it is possible that the diabetics with AN already had asymptomatic delayed gastric emptying which could not be further delayed by fibre. This suggestion has subsequently been confirmed (Dr M. Rosman of the University of Cape Town, personal communication).

Significantly smaller rises in serum glucose levels following ingestion of a mixed meal were documented earlier in this work in diabetics with AN compared to those without AN. This observation may indicate the presence of altered absorption of nutrients in the diabetics with AN, which may be secondary to delayed gastric emptying or defective innervation of the small intestine. At this stage it is uncertain whether altered nutrient absorption may occur in diabetic AN per se and may thus negate the postulated fibre-induced delayed nutrient absorption.

Intestinal transit time may be reduced in diabetics with AN affecting the bowel (Scarpello and Sladen, 1978). In addition delayed gastric emptying per se may result in prolonged transit times in these patients. Hence it is possible that the lack of effect of fibre in the diabetics with AN may be related to intestinal transit time, which was already slowed by the presence of AN.

It is apparent from our studies that diabetics with AN may have altered gut hormone release. Altered IRG (Maranda and Horwitz, 1978; Munoz, Sandstead, Jacob, Johnson and Mako, 1979), GIP (page 47) and GLI release (Morgan, Goulder, Tsiolakis, Marks and Alberti, 1979) have been reported with fibre supplementation. However in this study, only IRG secretion was significantly altered by fibre and then only in the healthy subjects, although reduced plasma GIP

concentrations, albeit statistically insignificant, were noted in the healthy subjects and both groups of diabetics. While it is possible that altered gut hormone release may play a part in mediation of the fibre-induced effects on glucose tolerance, the lack of significant changes in their release in the diabetics without AN, despite the observed improvement in glucose tolerance, makes this unlikely. In addition it seems unlikely that the lack of effect of fibre in the diabetics with AN was related to the existence of altered gut hormone secretion.

After due consideration of the various mechanisms proposed to explain the effect of fibre on glucose tolerance, the presence of existing delayed gastric emptying in the diabetics with AN appears to be the most likely cause for the lack of effect of fibre in this group of subjects.

A further point of interest in the present study was that in contrast to previous reports in NIDDM (Jenkins, Goff, Leeds, et al, 1976) and healthy subjects (Jenkins, Leeds, Gassull, Cochet and Alberti, 1977; Morgan and associates, 1979) there was no associated reduction in serum insulin responses with fibre supplementation of the test meal. However, Munoz and co-workers (1979) also found that in healthy subjects, fibre supplemented diets did not alter serum insulin responses to meal ingestion. The reason for the disparity in fibre-induced insulin secretion in the various studies is not clear, but there are a number of possible explanations. The first relates to the type of fibre used. Guar and pectin administered either separately or together were used in the studies by Jenkins, Goff, Leeds, et al; Jenkins, Leeds, Gassull, et al; Morgan et al and in present study, while Munoz and co-workers used various sources of dietary fibre. The second explanation relates to the report of significantly reduced GIP secretion in association with significantly diminished insulin secretion with fibre supplemented meals by Morgan et al. They suggest that GIP may be involved in the fibre-induced effects on insulin secretion. Thus the lack of effect of fibre on insulin secretion in the present study might have been due to the lack of significant alteration in GIP secretion. The third possible explanation relates to the different dosages of fibre used. In the present study 5 g each of guar and pectin were used. Morgan and co-workers gave 10 g guar only, Jenkins and co-workers used 14.5 g guar and 10 g pectin and 16 g guar and 10 g pectin in different studies and Munoz and co-workers administered 26 g each of various fibres. Thus it is apparent that real differences exist in the dosages of fibre administered in the various studies. It might therefore be of value to determine the effects of increasing doses of fibre, since it is probable that the effect of fibre on insulin secretion and glucose tolerance is

dose-dependent. Although cognisance was taken of reports in the literature in which guar and pectin were administered, the relatively low dosages of 5 g of each were selected for this study because of their palatability. The doses of the two fibres in the meal were gradually reduced from 15 g each until the meal became palatable. However, in spite of the relatively low quantities of fibre used, marked reduction in serum glucose levels were observed in the absence of a prominent effect on insulin secretion. This suggests that fibre might exert its effect on glucose tolerance independent of altered insulin secretion.

There seems to be little doubt that dietary fibre has a beneficial effect on glucose tolerance in both healthy subjects and diabetics. A reduction in insulin requirements in IDDM and obviation of the need for oral hypoglycaemic therapy in NIDDM have been reported with fibre-supplemented diets (page 47). However, high fibre diets were not universally successful in IDDM on relatively high doses of insulin (page 47) and fibre was not effective in the present study in NIDDM with AN. Thus although further studies are required to fully elucidate the mechanisms whereby fibre improves glucose tolerance and to assess the reason for its lack of effect in diabetics with AN, dietary supplementation with fibre may have a real role in the management of diabetes, even in the presence of AN.

COLLATION OF DATA ON INDIVIDUAL HORMONES

The prime purpose of these studies was to provide evidence which might contribute to an understanding of the inter-relationship between NIDDM, gastrointestinal hormone release and the autonomic nervous system. The various studies carried out however, revealed some interesting features of the individual hormones, a short summary of which follows :

(i) GLUCAGON

The data revealed that IRG release stimulated by insulin hypoglycaemia and a mixed meal was unaltered by the presence of AN in NIDDM, but that basal IRG release was affected. This data suggested selective involvement of the basal unstimulated alpha cell by AN. The lesion in the autonomic innervation of the alpha cell which was most compatible with the abnormal basal IRG release in these patients, was loss of the inhibitory alpha adrenergic influences on basal IRG release.

The possible consequences of the significant fasting hyperglucagonaemia in the diabetics with AN depends on two main factors. First, the importance of IRG in the induction of metabolic abnormalities observed in diabetics. Second, the relative importance of abnormal basal only as opposed to abnormal basal and stimulated IRG secretion in the induction of metabolic abnormalities.

(ii) PANCREATIC POLYPEPTIDE

The PP data revealed normal basal PP release except after the establishment of fasting normoglycaemia, an impaired serum PP response to hypoglycaemia and a delayed early serum PP response to the mixed meal in the diabetics with AN. These findings indicate that stimulated and to a lesser extent basal PP release were affected by autonomic nervous dysfunction in the patients studied.

These findings provide confirmatory evidence for a prominent neuroregulatory role in the mediation of PP release. However, the data also suggests that the various facets of PP release are governed by neural pathways, but to varying degrees. The lesion in the autonomic innervation of PP release most compatible with the abnormalities in it's stimulated release was loss or impairment of vagal cholinergic integrity. In contrast defective alpha adrenergic activity might have been involved in the single instance of abnormal basal PP release.

The consequences of the abnormal PP release in the diabetics with AN are

uncertain and will largely depend on the physiological functions uncovered for this newly described hormone.

(iii) GASTRIN

The data revealed that AN did not induce any major alterations in basal or stimulated gastrin release. This might have been due to the presence of residual neural function in the patients and the possibility that a greater degree of neural impairment may be required before an effect on gastrin release is observed.

(iv) GASTRIC INHIBITORY POLYPEPTIDE

The data revealed that basal GIP release was unaffected by AN, while food-induced GIP release was affected in these patients. These data might have suggested selective involvement of stimulated GIP release by autonomic dysfunction. However, the impaired GIP response to food ingestion in the diabetics with AN was probably secondary to defective glucose absorption and not due to defective neural innervation of GIP release. As yet the consequences of the impaired stimulated GIP release in the diabetics with AN are unknown.

(v) SOMATOSTATIN

The data revealed a lack of effect of AN on all facets of SRIF-LI release studied. However the fact that flatter, albeit insignificantly so, SRIF-LI responses to the mixed meal and insulin hypoglycaemia were observed in the diabetics with AN than in those without AN, may indicate that significant differences would appear if larger groups were studied. Furthermore whether SRIF-LI release from nerves is altered in the presence of AN and the consequences thereof remain to be determined.

CHAPTER VII

CONCLUSIONS

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The major purpose of this thesis was to provide evidence which might contribute to an understanding of the interrelationship between NIDDM, gastrointestinal hormone release and the autonomic nervous system.

Thus it was attempted to :

a) evaluate the effect of AN on the release of certain gastrointestinal hormones in NIDDM. The hormones studied were IRG, PP, gastrin, GIP and SRIF-LI.

b) assess whether any of the abnormalities in the release of these gastrointestinal hormones described in NIDDM could be accounted for by undiagnosed AN.

c) evaluate whether the PP response to hypoglycaemia could be used as a biochemical marker for AN, and finally,

d) assess whether the action of dietary fibre on glucose tolerance would be altered in the presence of AN.

To these ends studies were designed, formulated and conducted. The results revealed abnormalities in the release of certain of the hormones studied. The major findings were in the NIDDM with AN.

i) significantly elevated basal plasma IRG concentrations which were not correctable by the prior establishment of fasting normoglycaemia or by complete gastric emptying.

ii) significantly elevated basal serum PP concentrations only after the establishment of fasting normoglycaemia.

iii) an impaired plasma IRG response to hypoglycaemia which was corrected by the prior induction of fasting normoglycaemia by an overnight insulin infusion.

iv) an impaired serum PP response to hypoglycaemia which was not corrected

by the prior establishment of fasting normoglycaemia.

v) a delayed early serum PP peak after ingestion of a mixed meal.

vi) an impaired plasma GIP response to ingestion of the mixed meal.

vii) a lack of effect of fibre supplementation on serum glucose concentrations and hormone responses following ingestion of a mixed meal.

These were all new findings.

In all the diabetics studied, ie in the combined group of patients with and without AN it was found that :

i) basal plasma IRG concentrations were significantly elevated.

ii) the serum PP response to insulin hypoglycaemia was impaired.

iii) the plasma GIP response to ingestion of the mixed meal was impaired.

The findings revealed that an interrelationship does exist between NIDDM, gastrointestinal hormone release and the autonomic nervous system. Further that AN does have an effect on the release of certain of the hormones studied, although many of the precise mechanisms are not fully understood as yet. Certain abnormalities in the release of the gut hormones in NIDDM may be related to the presence of undiagnosed AN. In addition serum PP responses to hypoglycaemia may be a biochemical marker for AN in NIDDM. Finally in NIDDM the effect of dietary fibre on glucose tolerance is altered in the presence of AN.

Therefore these findings suggest that the discrepancies in hormone release reported in diabetes in the literature may be related to variable numbers of patients with undiagnosed AN in the study groups. Further studies may shed more light on this suggestion and may reveal involvement of AN in various of the other as yet unexplained biochemical abnormalities found in patients with diabetes.

CHAPTER VIII

SUGGESTED DIRECTIONS FOR FUTURE RESEARCH

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The present findings have reinforced the belief that many facets of diabetes mellitus are poorly understood. Furthermore the meaning of these findings in terms of biochemical sequelae and patient care remain uncertain. Further studies are needed to provide a better understanding of these factors.

Suggested directions for future research would include the following :

i) To provide a better understanding of the precise underlying mechanisms for the abnormalities in gastrointestinal hormone release revealed in the presence of AN. Disturbed release of certain gut hormones has now been demonstrated in IDDM and NIDDM with AN. A more complete understanding of the autonomic nervous regulation of the gut hormones may shed some light on the precise mechanisms involved. Studies in patients with autonomic nervous dysfunction not secondary to diabetes, such as familial dysautonomia may also be of value in this regard.

ii) To provide a more complete understanding for the lack of effect of dietary fibre on glucose tolerance in the presence of AN in NIDDM and to evaluate whether the long term effects of fibre may also be altered in the presence of AN. If the precise mechanism for the lack of effect of fibre noted in the NIDDM with AN could be identified and countered, fibre supplementation may prove to be beneficial even in the presence of AN. This together with a study of the long term effects of fibre supplementation in diabetics with AN might have relevance in the therapy of diabetes.

iii) To evaluate PP as a quantitative marker for AN. Although numerous clinical tests are available to detect the presence of AN, there appears to be no ready means of quantifying the degree of AN. A quantitative marker of AN would enable identification of patients at risk from the increased mortality as a result of AN. Improved control of diabetes and close medical attention may result in an improved outlook for these patients.

iv) To extend the studies of the five hormones to include aspects of their release, as well as to other gut hormones, with regard to the effect of AN thereon and the possible effect of undiagnosed AN on abnormalities in their release found in diabetes per se. Studies on the 24 hr profile of the various gut hormones may provide valuable evidence of the effect of AN on the daily secretory pattern of the hormones.

v) To determine whether AN has any important metabolic sequalae in NIDDM. An association between instability of diabetic control and AN has been suggested for IDDM but whether the same holds for NIDDM is uncertain. Studies on 24 hr profiles of blood glucose, free fatty acids, triglyrides and amino acids in the presence and absence of AN may shed some light on the issue.

vi) To evaluate a possible effect of AN on neuropeptide release, which may have relevance to certain of the abnormalities demonstrated in gut hormone release in AN. An effect of AN on neuropeptide release may have greater relevance in terms of the overall functioning of the gastrointestinal tract, particularly with regard to motility, absorption, endocrine and exocrine secretion on a nutrient homeostasis.

APPENDICES

APPENDIX A

BIOCHEMICAL METHODS

1. Measurement of serum glucose
2. Radioimmunoassays
   - (O) Principles
   - (i) Radioimmunoassay of Glucagon
   - (ii) Radioimmunoassay of Pancreatic Polypeptide
   - (iii) Radioimmunoassay of Gastrin
   - (iv) Radioimmunoassay of Gastric Inhibitory Polypeptide
   - (v) Radioimmunoassay of Somatostatin
   - (vi) Radioimmunoassay of Insulin

APPENDIX B

STATISTICAL METHODS

1. Mean
2. Standard error of mean
3. Wilcoxan Signed-Rank test
4. Mann Whitney U test

APPENDIX A

BIOCHEMICAL METHODS

1. MEASUREMENT OF SERUM GLUCOSE (Hoffman, 1937; Varley, 1967)

Principle: Potassium ferricyanide is reduced to ferrocyanide by glucose in alkaline solution and measured by an autoanalyser (Technicon Autoanalyser I)

Reagents: (a) Cyanide-saline diluent, 0.5% sodium cyanide in 0.9% sodium chloride with 0.5 ml Brij 35/1.

(b) Alkaline ferricyanide: 0.03g potassium ferricyanide, 20 g anhydrous sodium carbonate, 9 g sodium chloride /1 with 0.5 ml Brij 35/1.

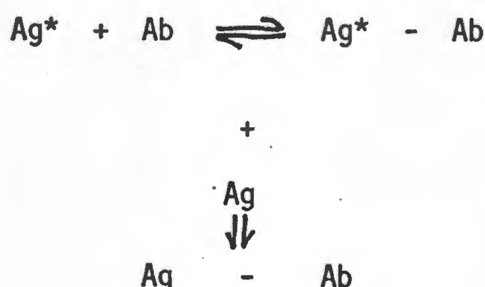
Standards: Standards were prepared to cover the ranges 25 - 400 mg glucose/ 100 ml and 50 - 800 mg glucose/100 ml.

Procedure: The sensitivity of the method was increased by the inclusion of sodium cyanide in the saline diluent. The standard Auto analyser technique utilizing dialysis to remove non-reducing substances was employed. Absorption was recorded at 420 mu and the results calculated from the recorded peak heights.

2. RADIOIMMUNOASSAYS

2 (0) Principles of Radioimmunoassay (Yalow and Berson, 1971)

The general principles of radioimmunoassay may be illustrated by the following reactions:



where Ag* = radioactively labelled antigen
Ab = specific antibody
Ag* - Ab = labelled antigen - antibody complex
Ag = unlabelled antigen
Ag - Ab = unlabelled antigen - antibody complex

The principle of radioimmunoassay is based on the competition of radio labelled antigen with unlabelled antigen for a limited amount of antibody.

Separation of the bound from free labelled antigen may be accomplished by a

number of methods. For example, charcoal is used in the IRG, SRIF-LI and gastrin assays. The charcoal adsorbs the free unbound labelled antigen which is then counted in an automatic gamma spectrometer. Separation methods which precipitate the antibody-bound label are also made use of. These include polyethylene glycol (PEG) which is used in the PP assay and microfiltration, used in the insulin assay.

CALCULATIONS

All samples and standards are assayed in duplicate. In addition duplicate tubes containing the total radioactivity added are counted (TC) and duplicated tubes are set up to determine the non specific binding (NSB).

The maximum binding (BO) in the absence of unlabelled Ag is calculated thus:

$$\frac{BO}{TO} = \frac{BO - NSB}{TC - NSB} \times 100$$

and chosen to be between 40 - 60%.

The percentage binding of standards and unknowns is calculated as below:

$$\%\ \text{binding} = \frac{S - NSB}{TC - NSB}$$

where S = radioactivity of standards and unknowns.

A standard curve is constructed by plotting percentage binding of the reference standards. The percentage binding of the unknown samples are calculated and the hormone concentration read off the graph.

The basic requirements for a radioimmunoassay are therefore:

1. unlabelled antigen
2. labelled antigen
3. antibody
4. a method for separation of bound from free antigen.

2 (i) Radioimmunoassay of Glucagon (IRG) (Faloona and Unger, 1974)

The assay used was modified from that described by Faloona and Unger (1974). The modifications were as follows:

a) alteration of the quantities of reagents placed in assay tubes and a final volume of 1 ml as opposed to 1.2 ml.

b) incubation was for 3 as opposed to 4 days.

c) horse not sheep serum was used in the separation.

d) incubation with charcoal was reduced from 45 to 20 min.

Reagents

(a) Antiserum: The antiserum (30k) was raised in rabbits against beef-pork pancreatic glucagon and purchased from Dr R. Unger. The cross-reactivity with GLI was $< 4\%$ and with GIP, VIP, cholecystokin octapeptide, substance P, Leu Enkephalin and SRIF $< 1\%$. The final dilution of the antiserum in the radio-immunoassay was 1:40,000.

(b) Standards: The reference standards used were pure porcine pancreatic IRG obtained from Eli Lilly Laboratories, Indianappolis, U.S.A. The unlabelled antigen was reconstituted on the day of assay and diluted to give standards of 5, 10, 20, 50, 100, 200, 500 pg IRG.

(c) ^{125}I IRG: The above pure porcine pancreatic IRG was labelled with ^{125}I by the technique of Greenwood, Hunter and Glover (1936). The label was purified on a 30 x 0.7 cm Sephadex QAE A25 anion exchange column in 0.08 M TRIS HCl - sodium chloride buffer, pH 8.6.

(d) Assay buffer: The buffer used was 0.2M glycine in 0.1M sodium chloride, pH 8.8, containing 0.25% human serum albumen, 1% normal rabbit serum and 5% trasylol.

Procedure

600 μl assay diluent, 200 μl standard or unknown sample, 100 μl antiserum and 100 μl ^{125}I IRG (7000 counts per minute; cpm) were incubated in plastic disposable tubes for 72 hr at 4^oC. Separation of antibody-bound from free ^{125}I IRG was accomplished using 500 μl 0.5% charcoal (Sigma Chemical, USA) and 0.25% dextran T70, mixing and incubating for 20 min at 4^oC. The tubes were then centrifuged at 1.2×10^3 g, the supernatant decanted and the pellet with free ^{125}I IRG was counted in a Packard automatic gamma spectrometer for either 10 min or 10,000 counts.

Results

The sensitivity, taken as the lowest amount of IRG that caused a significant displacement of ^{125}I IRG ($p < 0.01$) (Feldman and Rodbard, 1971), was 6 pmol/l.
The inter-assay coefficient of variation was 15.9 ($n = 9$).
The intra-assay coefficient of variation was 14.1% ($n = 10$).

2 (ii) Radioimmunoassay of Pancreatic polypeptide (PP) (Sive, Vinik, Van Tonder and Lund, 1978.

Reagents

(a) Antiserum: The antiserum which was raised in a rabbit against purified hPP (Lot 615-10548-248-19), was a generous gift from Dr R.E. Chance of Eli Lilly Laboratories, USA. There was no cross-reactivity of the antiserum with monocomponent insulin, glucagon, GIP or human gastrin 1. The antiserum was used in a final dilution of 1:100,000.

(b) Standard: Highly purified hPP (Dr R.E. Chance) was used as standard.

(c) ^{125}I bPP: bPP was iodinated using the Chloramine-T method (Greenwood, Hunter and Glover, 1963) and purified on a 0.9 x 50 cm. Sephadex G-50 superfine column.

(d) Assay Buffer: The buffer was a 0.04M sodium phosphate buffer, pH 7.4, containing 1 g human albumen, 1% normal rabbit serum and 0.2 g merthiolate and 6 g sodium chloride.

Procedure

The assay tubes containing 700 μl buffer, 100 μl antiserum and 100 μl standard or sample were incubated in duplicate at 4°C for 48 hr. ^{125}I bPP (5000 cpm) in 100 μl buffer was added and the tubes incubated for a further 24 hr. Separation of antibody bound from free ^{125}I bPP was carried out by addition of 1 ml of 25% PEG to the tubes.

Results

The sensitivity of the assay was 4 pmol/l.
The inter-assay coefficient of variation was 14% at 50% of the maximum binding (n = 10).
The intra-assay coefficient of variation was 9%.

2 (iii) Radioimmunoassay of Gastrin

The Sorin gastrin kit (Sorin Biomedica, Gruppo Radiochimica, Italy) was used for all radioimmunoassays.

Reagents

All reagents were supplied in the Sorin gastrin kit.

(a) Antiserum: The antiserum was raised in rabbit against human gastrin conjugated to bovine serum albumen. The cross-reactivity of the antiserum with cholecystokinin octapeptide was 10%, with caerulein $<$ 5% and with bombesim, insulin, IRG and VIP $\leq$ 1%.

(b) Standards: The reference standard was synthetic human gastrin - I (1 - 17).

(c) ^{125}I Gastrin: The above synthetic human gastrin I was used for labelling.

(d) Assay Buffer: The buffer was a 0.02M veronal buffer pH 8.2 - 8.6

Procedure

700 μl buffer, 100 μl sample or standard, 100 μl ^{125}I Gastrin (10,000 cpm) and 100 μl antiserum were added to assay tubes in duplicate. The tubes were mixed and incubated at 2 - 6°C for 24 hr. Before separation 100 μl inactivated horse serum was added to all standard tubes. Separation of antibody bound from free ^{125}I Gastrin was achieved by the addition of charcoal and centrifugation at 1.2 x 10^{3}g for 15 min. The radioactivity in the charcoal pellet was counted in a gamma spectrometer for either 10 min or 10,000 counts.

Results

The sensitivity of the assay was 8 pmol/l.
The inter-assay coefficient of variation was 17.2% (n = 10).
The intra-assay coefficient of variation was 6.8% (n = 10).

2 (iv) Radioimmunoassay of Gastric Inhibitory Polypeptide (GIP) (Kuzio, Dryburgh, Malloy and Brown, 1974)

The assay used was modified from that described by Kuzio, et al (1974). The modifications were as follows:

(a) Sephadex QAE - A25 column was used in place of a Sephadex G - 15 column in the purification of ^{125}I GIP.

(b) The incubation volume was increased to 1 ml in place of 0.5 ml and the preincubation period increased to 72 hr.

(c) The separation of bound from free ^{125}I GIP was accomplished using 25% PEG instead of charcoal.

Although the materials used in the assay were the same as those used in the assay reported by Kuzio, et al, difficulties encountered in establishing a stable, reproducible assay resulted in major modifications being made.

Reagents

(a) Antiserum: Antiserum (GP01) for the assay was obtained from Dr J.C. Brown and was used in a final dilution of 1:35,000.

(b) Standards: The reference standard used was guinea pig GIP (EG 111) received from Dr J.C. Brown.

(c) ^{125}I GIP: The above guinea pig GIP (EG 111) was used for labelling. 5 μg GIP (in 50 μl 0.5 M phosphate buffer, pH 7.5) was reacted with 1.0 mCl Sodium ^{125}I (Radiochemical Centre, Amersham) using 10 μg chloramine T. The reaction was terminated after 10 sec by the addition of 20 μl of 0.24% sodium metabisulphite. 50 μl 1% Potassium Iodide and 10 μl 1M TRIS were added before applying to the 30 x 0.7 cm Sephadex QAE - A25 column and eluted with 0.03 M TRIS/Sodium chloride buffer pH 8.6 at 10 ml/hr. 1 ml fractions were collected and the fractions showing the greatest immunoreactivity and least damage were used in the assay.

(d) Buffer: The assay buffer was 0.04M phosphate, pH 7.4 with added 1% normal rabbit serum, 0.25% human serum albumen and 5% trasylol.

Procedure

700 μl assay buffer, 100 μl sample or standard and 100 μl antiserum were placed in a tube and preincubated at 4°C for 72 hr. After 72 hr 100 μl ^{125}I GIP (5,000 cpm) was added and the mixture incubated at 4°C for a further 24 hr. Separation of antibody bound from free ^{125}I GIP was accomplished using 1 ml of 25% PEG. Plasma content was equalized by the addition of aged charcoal treated human plasma to standards and water to sample tubes. The tubes were centrifuged at 1.8 x 10^3g for 45 min, the supernatant decanted and the PEG precipitate counted in a spectrometer for a minimum of 10 min or 10,000 counts.

Results

The sensitivity of the assay was 29.4 pmol/l.
The inter-assay coefficient of variation was 9% (n = 8).
The intra-assay coefficient of variation was 8% (n = 9).

2 (v) Radioimmunoassay of Somatostatin (SRIF-LI) (Kronheim, Berelowitz and Pimstone, 1978

Reagents

(a) Antiserum: Antibodies to somatostatin were produced in rabbit in response to immunization with cyclic somatostatin conjugated by carbodiimide condensation to Haliotis sp. haemocyanin (Goodfriend, Levine and Fasman, 1964). The antiserum showed no cross-reactivity with a wide range of hypothalamic peptides, posterior pituitory neurohormones, pituitary and gut hormones and neurotransmitters. The antiserum ($S_1$6) was used in the assay at a final dilution of 1:125,000.

(b) Standard: The reference standard used was synthetic cyclic somatostatin (AY24910) obtained from Dr E. Polakow, Ayerst Laboratories.

(c) ^{125}I SRIF: N - tyrosylated - somastostatin obtained from Dr D.H. Coy, was labelled with ^{125}I by the Greenwood-Hunter-Glover technique (1963). The label was purified on a 5 x 1 cm Whatman CM52 cellulose column.

(d) Buffer: The assay buffer was 0.5% bovine serum albumen 0.01M sodium phosphate in 0.15M sodium chloride, pH 7.8 with 0.05M disodium ethylene-diamine-tetra-acetic acid (EDTA). The final buffer pH of the buffer was 5.6.

Procedure

200 μl buffer, 100 μl antiserum, 100 μl ^{125}I-tyr'- somatostatin (4,000 to 6,000 cpm) and 100 μl reference standard in serial dilutions or sample were incubated in tubes at 4°C for 20 hr. Separation of antibody-bound from free label was achieved using 500 μl 0.1% charcoal (NORIT "A") coated with 0.01% dextran in phosphate buffer saline in the presence of 100 μl inactivated horse serum (Wellcome), incubated for 40 min at 4°C. The tubes were centrifuged at 2.5 x 10^{3} g for 20 min at 4°C and the radioactivity in the charcoal pellet counted in a gamma spectrometer for a minimum of 10 min or 10,000 counts.

Results

(a) The sensitivity of the assay was approximately 10 pg/tube.
(b) The intra-assay coefficient of variation was 17.4% at 400 pg/ml (n = 23).
(c) The inter-assay coefficient of variation was 19.2% at 480 pg/ml (n = 8).

2 (vi) Radioimmunoassay for Insulin (IRI) (Sorin Biomedica, Gruppo Radiochimica, Italy)

The Sorin insulin radioimmunoassay kit was used in the present studies.

Reagents

These were all contained in the Sorin Insulin Radioimmunoassay kit.

(a) Antiserum: Insulin antibodies were obtained by pre-reacting human insulin antiserum raised in guinea pigs with an anti-serum pig gamma globulin serum raised in rabbits. The cross-reactivity of the antiserum with pro-insulin was 7% and with glucagon and C-peptide $<$ 1%.

(b) Standards: Human insulin was used as the reference standard.

(c) ^{125}I Insulin: Labelled insulin was provided.

(d) Buffer: Lyohilized buffer of sodium phosphate, EDTA sodium salt and bovine albumen was dissolved in distilled water to obtain a solution of pH 7.4.

Procedure

100 µl sample or standard and 100 µl antiserum were incubated in assay tubes at 2 - 8°C for 6 hr. Thereafter 100 µl ^{125}I Insulin was added and the tubes were incubated for a further 18 hr at 2 - 8°C. Separation was carried out by microfiltration using a phosphate buffer, the filter disc was transferred to a counting tube and the radioactivity measured in a gamma spectrometer.

Results

The sensitivity of the assay was 2 µU/ml.

The inter-assay coefficient of variation was 12.8% (n = 10).

The intra-assay coefficient of variation was 8.6% (n = 10).

APPENDIX B

STATISTICAL METHODS

All statistical analysis of data was performed using the appropriate programme for a Hewlett-Packard Computer, model 9830A according to the method described by Snedcor and Cochran (1967).

1. The Mean: $\bar{x} = \frac{1}{n} \Sigma x$

   where x = the observations

   Σ = the sum of

   n = the number of observations.

2. Standard Error of the Mean (SEM)

   for $n < 30$ $\quad SEM = \dfrac{\sqrt{\Sigma \dfrac{(x - \bar{x})^2}{n - 1}}}{\sqrt{n}}$

3. Wilcoxan Signed-Ranked Test (paired data) :
   As the data did not fit a normal distribution curve, a non parametric method of analysis was applied to all results obtained -

   i) for each matched pair, determine the signed ($\pm$) difference (d) between the two values.

   ii) Rank the d's without respect to sign. With tied d's, assign the average of the tied ranks (ie. first 3 tied) rank $(1 + 1 + 3)/_2 = 2$; next rank $= 4$.

   iii) Affix to each rank the sign ($\pm$) of the d which it represents.

   iv) Determine T = the smaller of the sums of the like-signed ranks.

   v) n = total no. of d's with a sign (excludes d = 0).

   vi) for $n < 25$, critical T values are read from the appropriate tables for n degrees of freedom and significance.

4. The Mann Whitney U test (for unpaired samples).

   i) All observations in the groups for comparison are put in a single array.

   ii) Ranks are then assigned to the combined array. With tied ranks the average of the ranks is given.

   iii) The rank numbers in the group that has the smaller sum are added.

   iv) The smaller sum of ranks, T is referred to the appropriate table to determine significance.
   When the samples are of unequal size (n_1, n_2), an extra step is required:

      i) Find the total T_1 of the ranks of the sample that has the smaller size, say n_1

      ii) Compute $T_2 = n_1 (n_1 + n_2 + 1) - T_1$

      iii) Then T = the smaller of T and T_2 and is read off the appropriate table.

5. Levels of Significance:

   Values of p were determined from the appropriate tables for the Mann Whitney U test and the Wilcoxan Signed-Rank test.

   Two tailed tests were used in the determination of p, and where p was greater than 0.05 ($p > 0.05$) the differences were not regarded as significant.

BIBLIOGRAPHY

ADRIAN T.E., BESTERMAN H.S., MALLINSON C.N., GARALOTIS C. and BLOOM S.R. (1979)
Impaired pancreatic polypeptide release in chronic pancreatitis with steatorrhoea.
(Gut 20 : 98-101)

ADRIAN T.E., BLOOM S.R., BESTERMAN H.S., BARNES A.J., COOKE T.J.C., RUSSELL R.C.G. and FABER R.G. (1977)
Mechanism of pancreatic polypeptide release in man.
(Lancet 1 : 161-163)

ADRIAN T.E., BLOOM S.R., BESTERMAN H.S. and BRYANT M.G. (1978)
PP - Physiology and Pathology. In: Gut Hormones.
pp 254-260, Ed. Bloom, S.R.
(Churchill Livingstone : Edinburgh, London and New York)

ADRIAN T.E., BLOOM S.R., BRYANT M.G., POLAK J.M., HEITZ P.H. and BARNES A.J. (1976)
Distribution and release of human pancreatic polypeptide.
(Gut 17 : 940-944)

ADRIAN T.E., BLOOM S.R., HERMANSEN K. and IVERSEN J. (1978)
Pancreatic polypeptide, glucagon and insulin secretion from the isolated perfused canine pancreas.
(Diabetologia 14 : 413-417)

AGUILAR-PARADA E., EISENTRAUT A.M. and UNGER R.H. (1969)
Pancreatic glucagon secretion in normal and diabetic subjects.
(American Journal of the Medical Sciences 257 : 415-419)

AHLQUIST R.P. (1948)
A study of the adrenotropic receptors.
(American Journal of Physiology 153 : 586-600)

ALFORD F.P., BLOOM S.R. and NABARRO J.D.N. (1976)
Glucagon metabolism in man. Studies on the metabolic clearance rate and the plasma acute disappearance time of glucagon in normal and diabetic subjects.
(Journal of Clinical Endocrinology and Metabolism 42 : 830-836)

ALFORD F.P., BLOOM S.R. and NABARRO J.D.N. (1977)
Glucagon levels in normal and diabetic subjects : use of a specific immunoabsorbent for glucagon radioimmunoassay.
(Diabetologia 13 : 1-6)

ANDERSON J.W. (1976)
Metabolic abnormalities contributing to diabetic complications. 11. Peripheral nerves.
(American Journal of Clinical Nutrition 29 : 402-408)

ANDERSON J.W. and CHEN W.J.L. (1979)
Plant Fibre : carbohydrate and lipid metabolism.
(The American Journal of Clinical Nutrition 32 : 346-363)

ANDERSON J.W., MIDGLEY W.R. and WEDMAN B. (1979)
Fibre and diabetes.
(Diabetes Care 2 : 369-379)

ANDERSON J.W., SIELING B. and FERGUSON S. (1979)
Long-term effects of high-fibre diets on mineral and fat soluble vitamin status in persons with diabetes.
(Diabetes 28 : 384)

ANDERSON J.W. and WARD K. (1978)
Long-term effects of high-carbohydrate, high-fibre diets on glucose and lipid metabolism : a preliminary report on patients with diabetes.
(Diabetes Care 1 : 77-82)

ARIMURA A., LUNDQVIST G., ROTHMAN J., CHANG R., FERNANDEZ-DURANGO R., ELDE R., COY D.H., MEYERS C. and SCHALLY Q.V. (1978)
Radioimmunoassay of somatostatin.
(Metabolism 27 (Suppl. 1) : 1139-1144)

ARIMURA, SATO, DU PONT, NICHI and SCHALLY (1975)
Somatostatin : abundance of immunoreactive hormone in rat stomach and pancreas.
(Science 189 : 1007-1009)

AYDIN J., RASKIN P. and UNGER R.H. (1977)
The effect of short-term intravenous insulin administration on the glucagon response to a carbohydrate meal in adult onset and juvenile type diabetes.
(Diabetologia 13 : 629-636)

BAETENS D., COLEMAN D.L. and ORCI L. (1976)
Islet cell population in ob/ob and db/db mice.
(Diabetes 25 (Suppl. 1) : 344)

BANTING F.G. and BEST C.H. (1922)
The internal secretion of the pancreas.
(Journal of Laboratory and Clinical Medicine 7 : 251-266)

BARR M.L. (1979)
The human nervous system : an anatomical viewpoint.
(Harper and Row : Maryland)

BAUMERT J.E. (Jr), CATALAND S., TETERICK C.E. (Jr), PACE W.G. and MAZZAFERRI E.L. (1978)
Effect of atropine on meal-stimulated gastrin and gastric inhibitory polypeptide (GIP) release.
(Journal of Clinical Endocrinology and Metabolism 46 : 473-476)

BAYLISS W.M. and STARLING E.H. (1902)
The mechanism of pancreatic secretion.
(Journal of Physiology 28 : 325-353)

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

BENNETT T., HOSKING D.J. and HAMPTON J.R. (1975)
Cardiovascular control in diabetes mellitus.
(British Medical Journal 2 : 585-587)

BENNETT T., HOSKING D.J. and HAMPTON J.R. (1979)
Cardiovascular responses to lower body negative pressure in normal subjects and in patients with diabetes mellitus.
(Cardiovascular Research 13 : 31-38)

BERGER D., CROWTHER R.C., FLOYD J.C. (Jr), PEK S. and FAJANS S.S. (1978)
Effect of age on fasting plasma levels of pancreatic hormones in man.
(Journal of Clinical Endocrinology and Metabolism 47 : 1183-1189)

BERGER D., FLOYD J.C. (Jr), PEK S. and FAJANS S.S. (1978)
Effect of insulin treatment on pancreatic polypeptide levels in diabetes mellitus.
(Clinical Research 26 : 677A)

BESTERMAN H.S., SARSON D.L., TURNER R.C. and BLOOM S.R. (1978)
GIP and insulin release in diabetes mellitus and alimentary disease.
(Scandinavian Journal of Gastroenerology 13 (Suppl.49) : 17)

BLAIR E.L., GRUND E.R., REED J.D., SANDERS D.J., SANGER G. and SHAW B. (1973)
The effect of sympathetic nerve stimulation on serum gastrin, gastric acid secretion and mucosal blood flow responses to meat extract stimulation in anaesthetized cats.
(Journal of Physiology 253 : 493-504)

BLOOM S.R. and EDWARDS A.V. (1975)
The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation.
(Journal of Physiology 253 : 157-173)

BLOOM S.R. and EDWARDS A.V. (1978)
Certain pharmacological characteristics of the release of pancreatic glucagon in response to stimulation of the splanchnic nerves.
(Journal of Physiology 280 : 25-35)

BLOOM S.R., EDWARDS A.V. and VAUGHAN N.J.A. (1973)
The role of the sympathetic innervation in the control of plasma glucagon concentration in the calf.
(Journal of Physiology 233 : 457-466)

BLOOM S.R., EDWARDS A.V. and VAUGHAN N.J.A. (1974)
The role of the autonomic innervation in the control of glucagon release during hypoglycaemia in the calf.
(Journal of Physiology 236 : 611-623)

BLOOM S.R. and POLAK J.M. (1978)
Gut hormone overview. In: Gut Hormones.
pp 3-17, Ed. Bloom S.R.
(Churchill Livingstone : Edinburgh, London and New York)

BLOOM S.R., VAUGHAN N.J.A., RUSSELL R.C.G. (1974)
Vagal control of glucagon release in man.
(Lancet 2 : 546-549)

BLOOM S.R., WEST S.A.M., POLAK J.M., BARNES A.J. and ADRIAN T.G. (1978)
Hormonal contaminents of insulin. In: Gut Hormones.
pp 318-322, Ed. Bloom S.R.
(Churchill Livingstone : Edinburgh, London and New York)

BOTHA J.L., VINIK A.I. and BROWN J.C. (1976)
Gastric inhibitory polypeptide (GIP) in chronic pancreatitis.
(Journal of Clinical Endocrinology and Metabolism 42 : 791-797)

BOTHA J.L., VINIK A.I. and CHILD P.T. (1978)
Gastric inhibitory polypeptide in acquired pancreatic diabetes : effects of insulin treatment.
(Journal of Clinical Endocrinology and Metabolism 47 : 543-549)

BRANDSBORG O., BRANDSBORG M. and CHRISTENSEN N.J. (1976)
The role of the beta-adrenergic receptor in the secretion of gastrin : studies in normal subjects and in patients with duodenal ulcers.
(European Journal of Clinical Investigation 6 : 395-401)

BRAZEAU P., LING N., ESCH F., BOHLEN P., BENOIT R. and GUILLEMIN R. (1980)
Haute activité biologique des répliques synthétique de somatostatine - 28 et somatostatine - 21 hypothalamiques.
(Compte Rendue Academie Science de Paris 290 : 1369-1371)

BRAZEAU P., VALE W., BURGUS R., LING N., BUTCHER M., RIVIER J. and GUILLEMIN R. (1973)
Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone.
(Science 179 : 77-79)

BRONSHVAG M.M. (1978)
Spectrum of gustatory sweating, with especial reference to its presence in diabetics with autonomic neuropathy.
(American Journal of Clinical Nutrition 31 : 307-309)

BROWN J.C. (1971)
A gastric inhibitory polypeptide I : the amino acid composition and the tryptic peptides.
(Canadian Journal of Biochemistry 49 : 255-261)

BROWN J.C. and DRYBURGH J.R. (1971)
A gastric inhibitory polypeptide II : the complete amino acid sequence.
(Canadian Journal of Biochemistry 49 : 867-872)

BROWN J.C., DRYBURGH J.R., FROST J.L., OTTE S.C. and PEDERSON R.A. (1978)
Properties and actions of GIP. In: Gut Hormones.
pp 277-282, Ed. Bloom S.R.
(Churchill Livingstone : Edinburgh, London and New York)

BROWN J.C., DRYBURGH J.R., ROSS S.A. and DUPRE J. (1975)
Identification and actions of gastric inhibitory polypeptide.
(Recent Progress in Hormone Research 31 : 487-532)

BROWN E., GOEI J.S., GREENFIELD A.D.M. and PLASSARAS V. (1966)
Circulatory responses to simulated gravitational shifts of blood in man induced by exposure of the body below the iliac crests to sub-atmospheric pressure.
(Journal of Physiology 183 : 607-627)

BROWN J.C., MUTT V. and PEDERSON R.A. (1970)
Further purification of a polypeptide demonstrating enterogastrone activity.
(Journal of Physiology 209 : 57-64)

BROWN J.C. and PEDERSON R.A. (1970)
A multiparameter study on the action of preparations containing cholecystokinin-pancreozymin.
(Scandinavian Journal of Gastroenterology 5 : 537-541)

BUCK A.C., REED P.I., SIDDIA Y.K. CHISHOLM G.D. and FRASER T.R. (1976)
Bladder dysfunction and neuropathy in diabetes.
(Diabetologia 12 : 251-258)

BURKITT D.P. (1978)
Colonic-rectal cancer : fibre and other dietary factors.
(American Journal of Clinical Nutrition 31 : 558-564)

BURKITT D.P., WALKER A.R.P. and PAINTER N.S. (1974)
Dietary fibre and disease.
(Journal of the American Medical Association 299 : 1068-1074)

CAMPBELL I.W., EWING D.J. and CLARKE B.F. (1978)
Painful myocardial infarction in severe diabetic autonomic neuropathy.
(Acta Diabetologica Latina 15 : 201-204)

CAMPBELL I.W., EWING D.J., CLARKE B.F. and DUNCAN L.J.P. (1974)
Testicular pain sensation in diabetic autonomic neuropathy.
(British Medical Journal 2 : 638-639)

CAMPBELL I.W., HEADING R.C., TOTHILL P., BUIST T.A.S., EWING D.J. and CLARKE B.F. (1977)
Gastric emptying in diabetic autonomic neuropathy.
(Gut 18 : 462-467)

CATALAND S., O'DORISIO T.M., BROOKS R. and MEKHJIAN H.S. (1977)
Stimulation of gastric inhibitory polypeptide in normal and duodenal ulcer patients.
(Gastroenterology 73 : 19-22)

CHANCE R.E. (1972)
Discussions of presentations.
(Diabetes 21 (Suppl. 2) : 536)

CHRISTENSEN S.E., HANSEN P. and LUNDBAEK K. (1978)
Somatostatin in maturity-onset diabetes.
(Diabetes 27 : 1013-1019)

CHRISTENSEN S.E., HANSEN P., WEEKE J. and LUNDBAEK K. (1978)
24-Hour studies of the effects of somatostatin on the levels of plasma growth hormone, glucagon and glucose in normal subjects and juvenile diabetics.
(Diabetes 27 : 300-306)

CHRISTENSEN K.C. and STADIL F. (1976)
On the beta-adrenergic contribution to the gastric acid and gastrin responses to hypoglycaemia in man.
(Scandinavian Journal of Gastroenterology 11 (Suppl. 37) : 81.86)

CHRISTENSEN K.C. and STADIL F. (1976)
Effect of epinephrine and norepinephrine on gastrin release and gastric secretion of acid in man.
(Scandinavian Journal of Gastroenterology 11 (Suppl. 37) : 87-92)

CHUSIO J.G. (1979)
Correlative neuroanatomy and functional neurology.
(Lange : Los Altos, California)

CLARKE B.F., EWING D.J. and CAMPBELL I.W. (1979)
Diabetic autonomic neuropathy.
(Diabetologia 17 : 195-212)

CLEMENTS R., VOURGANTI B., and DARNELL B. (1978)
Effect of low and high dietary myoinositol (MI) content upon nerve conduction velocities (NCVS) in neuropathic diabetics.
(Diabetes 27 (Suppl. 2) : 436)

CONLON J.M. IPP E. and UNGER R.H. (1978)
The molecular forms of immunoreactive glucagon secreted by the isolated, perfused dog pancreas.
(Life Sciences 23 : 1655-1658)

CREUTZFELDT W. (1979)
The incretin concept today.
(Diabetologia 16 : 75-85)

CREUTZFELDT W., EBERT R., ARNOLD R., FRERICHS H. and BROWN J.C. (1976)
Gastric inhibitory polypeptide (GIP), gastrin and insulin : response to test meal in coeliac disease and after duodeno-pancreatectomy.
(Diabetologia 12 : 279-286)

CROCKETT S.E., CATALAND S., FALKOJ.M. and MAZZAFERRI E.L. (1976)
The insulinotropic effect of endogenous gastric inhibitory polypeptide in normal subjects.
(Journal of Clinical Endocrinology and Metabolism 42 : 1098-1103)

CROCKETT S.E., MAZZAFERRI E.L. and CATALAND S. (1976)
Gastric inhibitory polypeptide (GIP) in maturity onset diabetes mellitus.
(Diabetes 25 : 931-935)

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)

DANIEL E.E. (1978)
Editorial : Peptidergic nerves in the gut.
(Gastroenterology 75 : 142-144)

DAY J.L. and ANDERSON J. (1973)
Abnormalities of glucagon metabolism in diabetes mellitus.
(Clinical Endocrinology 2 : 211-217)

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)

DE CALVI M. (1864)
Recherches sur les accidénts diabétique et essai d'une théorie générale du diabète.
(Paris : P. Asselin)

DOLAIS-KITABGI J., LE MARCHAND-BRUSLEL Y. and FREYCHET P. (1979)
Somatostatin in the pancreas and hypothalamus of obese mice.
(Diabetologia 17 : 257-261)

DUBOIS M.P. (1975)
Immunoreactive somatostatin is present in discrete cells of the endocrine pancreas.
(Proceedings of the National Academy of Sciences of the USA 72 : 1340-1343)

DUPRE J., ROSS S.A., WATSON D. and BROWN J.C. (1973)
Stimulation of insulin secretion by gastric inhibitory polypeptide in man.
(Journal of Clinical Endocrinology and Metabolism 37 : 826-828)

EASTWOOD M.A. and KAY R.M. (1979)
A hypothesis for the action of dietery fibre along the gastrointestinal tract.
(American Journal of Clinical Nutrition 32 : 364-367)

EBERT R. and CREUTZFELDT W. (1978)
Aspects of GIP pathology. In: Gut Hormones.
pp 294-300, Ed. Bloom S.R.
(Churchill Livingstone : Edinburgh, London, New York)

EBERT R., CREUTZFELDT W., BROWN J.R., FRERICHS H. and ARNOLD, R. (1976)
Response of gastric inhibitory polypeptide (GIP) to test meal in chronic pancreatitis-relationship to endocrine and exocrine insufficiency.
(Diabetologia 12 : 609-612)

EBERT R., FRERICHS H. and CREUTZFELDT W. (1976)
Serum gastric inhibitory polypeptide (GIP) response in patients with maturity onset diabetes and in juvenile diabetics.
(Diabetologia 12 : 388)

EDITORIAL (1976)
The diagnosis of autonomic dysfunction.
(Lancet 1 :1115-1116)

EDKINS J.S. (1905a)
The Chemical Mechanism of gastric secretion.
(Journal of Physiology (London) 34 : 133-144)

EDKINS J.S. (1905b)
On the chemical mechanism of gastric secretion.
(Proceedings of the Royal Society of London B76 : 376)

EDWARDS A.V. and BLOOM S.R. (1978)
Nervous control of pancreatic hormones. In: Gut Hormones.
pp 394-405, Ed. Bloom, S.R.
(Churchill Livingstone : Edinburgh, London and New York)

ELISBERG E.I. (1963)
Heart rate response to the Valsalva manoeuvre as a test of circulatory integrity.
(Journal of the American Medical Association 186 : 200-205)

ELLENBERG M. (1964)
Diabetic diarrhoea.
(Texas State Journal of Medicine 60: 829-834)

ELLENBERG M. (1971)
Impotence in diabetes : the neurologic factor.
(Annals of Internal Medicine 75 : 213-219)

ELLENBERG M. (1976)
Diabetic neuropathy : clinical aspects.
(Metabolism 25 : 1627-1655)

ELLENBERG M. and WEBER H. (1966)
Retrograde ejaculation in diabetic neuropathy.
(Annals of Internal Medicine 65 : 1237-1246)

ENSINCK J.W., WALTER R.M., PALMER J.P., BRODOWS R.G. and CAMPBELL R.G. (1976)
Glucagon responses to hypoglycaemia in adrenalectomized man
(Metabolism 25 : 227-232)

EWING D.J. (1978)
Cardiovascular reflexes and autonomic neuropathy.
(Clinical Science and Molecular Medicine 55 : 321-327)

EWING D.J., BURT A.A., WILLIAMS I.R., CAMPBELL I.W. and CLARKE B.F. (1976)
Peripheral motor nerve function in diabetic autonomic neuropathy.
(Journal of Neurology, Neurosurgery and Psychiatry 39 : 453-460)

EWING D.J., CAMPBELL I.W., BURT A.D. and CLARKE B.F. (1973)
Vascular reflexes in diabetic autonomic neuropathy.
(Lancet 2 : 1354-1356)

EWING D.J., CAMPBELL I.W. and CLARKE B.F. (1976)
Mortality in diabetic autonomic neuropathy.
(Lancet 1 : 601-603)

EWING D.J., CAMPBELL I.W., MURRAY A., NEILSON J.M.M. and CLARKE B.F. (1978)
Immediate heart-rate response to standing : simple test for autonomic neuropathy in diabetes.
(British Medical Journal 1 : 145-147)

EWING D.J., IRVING J.B., KERR F., WILDSMITH J.A.W. and CLARKE B.F. (1974)
Cardiovascular responses to sustained handgrip in normal subjects and in patients with diabetes mellitus : a test of autonomic function.
(Clinical Science and Molecular Medicine 46 : 295-306)

FAERMAN I., FACCIO E., MILEI J., NUNEZ R., JADZINSKY M., FOX D. and RAPAPORT M. (1977)
Autonomic neuropathy and painless myocardial infarction in diabetic patients : histological evidence of their relationship.
(Diabetes 26 : 1147-1158)

FAHRENKRUG J. (1979)
Vasoactive intestinal polypeptide : measurement, distribution and putative neruotransmitter function.
(Digestion 19 : 149-169)

FALOONA G.R. and UNGER R.H. (1974)
Glucagon. In: Methods of hormone radioimmunoassay.
pp 317-330, Ed. Jaffe B.M. and Behrman H.R.
(Academic Press: New York and London)

FELDMAN M., CORBETT D.B., RAMSEY E.J., WALSH J.H. and RICHARDSON C.T. (1979)
Abnormal gastric function in longstanding insulin-dependent diabetic patients.
(Gastroenterology 77: 12-17)

FELDMAN M., RICHARDSON C.T., TAYLOR I.L. and WALSH J.H. (1979)
Effect of atropine on vagal release of gastrin and pancreatic polypeptide.
(Journal of Clinical Investigation 63 : 294-298)

FELDMAN H. and RODBARD D. (1971)
Mathematical theory of radioimmunoassay. In: Principles of competitive protein-binding assays.
pp 158-203, Eds. Odell W.D. and Daughaday W.H.
(J.P. Lippincott Company : Philadelphia)

FLOYD J.C. (Jr) (1979)
Human pancreatic polypeptide.
(Clinics in Endocrinology and Metabolism 8 : 379-399)

FLOYD J.C. (Jr), FAJANS S.S., PEK S. and CHANCE R.E. (1977)
A newly recognized pancreatic polypeptide : plasma levels in health and disease.
(Recent Progress in Hormone Research 33 : 519-570)

FRIEDMAN W.H., and POMARICO J.M. (1974)
The intratympanic correction of Frey Syndrome.
(Archives of Surgery 108 : 366-368)

FROHMAN L.A. and BERNARDIS L.L. (1971)
Effect of hypothalamic stimulation on plasma glucose, insulin and glucagon levels.
(American Journal of Physiology 221 : 1596-1603)

FUXE K., ANDERSSON K., HÖKFELT T., MUTT V., FERLAND V.L., AGNATI L.F. GANTEN D., SAID S., ENEROTH P. and GUSTAFSSON J.A. (1979)
Localization and possible functions of peptidergic neurones and their interactions with central catecholamine neurones, and the central actions of gut hormones.
(Federation Proceedings 38 : 2333-2340)

GABBAY K.H. (1973)
Role of sorbitol pathway in neuropathy.
(Advances in Metabolic Disorders (Suppl. 2) : 417-424)

GABBAY K.H., MEROLA L.O. and FIELD R.A. (1966)
Sorbitol pathway : presence in nerve and cord with substrate accumulation in diabetes.
(Science 151 : 209-210)

GALBO H., CHRISTENSEN N.J. and HOLST J.J. (1977)
Catecholamines and pancreatic hormones during autonomic blockade in exercising man.
(Acta Physiologica Scandinavica 101 : 428-437)

GANONG W.F. (1979)
Review of medical physiology.
(Lange : Los Altos, California)

GARBER A.J., CRYER P.E., SANTIAGO J.V., HAYMOND M.W., PAGLIARA A.S. and KIPNIS D.M. (1976)
The role of adrenergic mechanisms in the substrate and hormonal response to insulin-induced hypoglycaemia in man.
(Journal of Clinical Investigation 58 : 7-15)

GERICH J.E., KARAM J.H. and FORSHAM P.N. (1973)
Stimulation of glucagon secretion by epinephrine in man.
(Journal of Clinical Endocrinology and Metabolism 37 : 479-481)

GERICH J.E., LANGLOIS M., NOACCO C., KARAM J.H. and FORSHAM P.H. (1973)
Lack of glucagon response to hypoglycaemia in diabetes : evidence for an intrinsic pancreatic alpha cell defect.
(Science 182 : 171-173)

GERICH J.E., LANGLOIS M., NOACCO C., SCHNEIDER V. and FORSHAM P.H. (1974)
Adrenergic modulation of pancreatic glucagon secretion in man.
(Journal of Clinical Investigation 53 : 1441-1446)

GERICH J.E., LORENZI M., KARAM J.H., SCHNEIDER V. and FORSHAM P.H. (1975)
Abnormal pancreatic glucagon secretion and postprandial hyperglycaemia in diabetes mellitus.
(Journal of the American Medical Association 234 : 159-165)

GERICH J.E. and PATTON G.S. (1978)
Somatostatin : physiology and clinical applications.
(Medical Clinics of North America 62 : 375-397

GERICH J.E., SCHNEIDER V., DIPPE S.E., LANGLOIS M., NOACCO C., KARAM J.H., FORSHAM P.H. (1974)
Characterization of the glucagon response to hypoglycaemia in man.
(Journal of Clinical Endocrinology and Metabolism 38 : 77-82)

GERICH J.E., TSALIKIAN E., LORENZI M., SCHNEIDER V., BOHANNON N.V., GUSTAFSON G. and KARAM J.H. (1975)
Normalization of fasting hyperglucagonaemia and excessive glucagon responses to intravenous arginine in human diabetes mellitus by prolonged infusion of insulin.
(Journal of Clinical Endocrinology and Metabolism 41 : 1178-1180)

GITELSON S., SCHWARTZ A., FRAENKEL M. and CHOWERS I. (1963)
Gall-bladder dysfunction in diabetes mellitus : the diabetic neurogenic gall bladder.
(Diabetes 12 : 308-312)

GOMEZ-PAN A. and HALL R. (1977)
Somatostatin (Growth hormone-release inhibiting hormone).
(Clinics in Endocrinology and Metabolism 6 : 181-200)

GOODFRIEND T.L., LEVINE L. and FASMAN G.D. (1964)
Antibodies to bradykenin and augioteusin : a use of carbodiimides in immunology.
(Science 144 : 1344-1346)

GOODMAN J.I. (1966)
Diabetic Anhidrosis.
(American Journal of Medicine 41 : 831-835)

GREENE D.A., DE JESUS P.V. (Jr) AND WINEGRAD A.I. (1975)
Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes.
(Journal of Clinical Investigation 55 : 1326-1336)

GREENHALGH R.M., ROSENGARTEN D.S. and MARTIN P. (1971)
Role of sympathectomy for hyperhidrosis.
(British Medical Journal 1 : 332-334)

GREENWOOD F.C., HUNTER W.M. and GLOVER J.S. (1963)
The preparation of ^{131}I-labelled growth hormone of high specific radioactivity.
(Biochemical Journal 89 : 114-123)

GREGORY R.A. and TRACY H.J. (1964)
The constitution and properties of two gastrins extracted from hog antral mucosa : Part 1. The isolation of two gastrins from hog antral mucosa.
Part 11. The properties of two gastrins isolated from hog antral mucosa.
(Gut 5 : 103-117)

GREGORY R.A., TRACY H.J. and GROSSMAN M. (1966)
Human gastrin : isolation, structure and synthesis : Isolation of two gastrins from human antral mucosa.
(Nature 209 : 583)

GRIMELIUS L., CAPELLA C., BUFFA R., POLAK J.M., PEARSE A.G.E. and SOLCIA E. (1976)
Cytochemical and ultrastructural differentiation of enteroglucagon and pancreatic-type glucagon cells of the gastrointestinal tract.
(Virchows Archiv B Cell Pathology 20 : 217-228)

GROSSMAN M.I. (1962)
Secretion of acid and pepsin in response to distension of vagally innervated fundic gland area in dogs.
(Gasgroenterology 42 : 718-721)

GROSSMAN M.I. (1967)
Neural and hormonal stimulation of gastric secretion of acid.
In: Handbook of Physiology, Sect. 6, vol.2.
pp 835-863, Ed. Code C.F.
(American Physiological Society : Washington D.C.)

GROSSMAN M.I. (1979)
Chemical Messengers : a view from the gut.
(Federation Proceedings 38 : 2341-2343)

GUNDERSEN H.J.G. and NEUBAUER B. (1977)
A Long-term diabetic autonomic nervous abnormality : reduced variations in testing heart rate measured by a simple and sensitive method.
(Diabetologia 13 : 137-140)

GUTTMAN L. (1940)
Topographic studies of disturbances of sweat secretion after complete lesions of peripheral nerves.
(Journal of Neurology and Psychiatry 3 : 197-210)

GUZMAN S., CHAYVIALLE J.A., BANKS W.A., RAYFORD P.L. and THOMPSON J.C. (1979)
Effect of vagal stimulation on pancreatic secretion and on blood levels of gastrin, cholecystokinin, secretin, vasoactive intestinal peptide and somatostatin.
(Surgery 2 : 329-336)

HANSKY J., HO P., KORMAN M.G. and STERN A.I. (1978)
Pancreatic polypeptide release in man. Studies in ulcer disease and after gastric surgery.
(Scandinavian Journal of Gastroenterology 13 (Suppl. 49) : 78)

HANSKY J. and KING R.W.F. (1977)
Effect of atropine on food-stimulated gastrin release after truncal vagotomy in man.
(Gastroenterology 73 : 205-206)

HAYES J.R., ARDILL J., KENNEDY T.L.,SHANKS R.G. and BUCHANAN K.D. (1972)
Stimulation of gastrin release by catecholamines.
(Lancet 1 : 819-821)

HEITZ P.H., POLAK J.M., BLOOM S.R. AND PEARSE A.G.E. (1976)
Identification of the D_1-cell as the source of human pancreatic polypeptide.
(Gut 17 : 755-758)

HEMENWAY W.G. (1960)
Gustatory sweating and flushing.
(Laryngoscope 70 : 84-90)

HOFFMAN W.S. (1937)
A rapid photo-electric method for the determination of glucose in blood and urine.
(Journal of Biological Chemistry 120 : 51-55)

HÖKFELT T., EFENDIC S., HELLERSTROM C., JOHANSSON O., LUFT R. and ARIMURA A. (1975)
Cellular localization of somatostatin in endocrine cells and neurons of the rat with special reference to the A_1-cells of the pancreatic islets and to the hypothalamus.
(Acta Endocrinologica 80 (Suppl. 200) : 5-41)

HÖKFELT T., ELFVIN L.G. ELDE R., SCHULTZBERG M., GOLDSTEIN M. and LUFT R. (1977)
Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons.
(Proceedings of the National Academy of Sciences 74 : 3587-3591)

HÖKFELT T., SCHULTZBERG M., JOHANSSON O., LJUNGDAHL A., ELFVIN L., ELDE R., TERENIUS L., NILSSON G., SAID S. and GOLDSTEIN M. (1978)
Central and peripheral peptide producing neurons. In: Gut Hormones. pp 423-433, Ed. Bloom, S.R.
(Churchill Livingstone : Edinburgh, London and New York)

HOLLANDER F. (1946)
The insulin test for the presence of intact nerve fibres after vagal operations for peptic ulcer.
(Gastroenterology 7 : 607-614)

HOLST J.J. (1978)
Extrapancreatic glucagons.
(Digestion 17 : 168-190)

HOLT S., HEADING R.C., CARTER D.C., PRESCOTT L.F. and TOTHILL P. (1979)
Effect of gel fibre on gastric emptying and absorption of glucose and paracetamol.
(Lancet 1 : 634-639)

HOSKING D.J. BENNETT T. and HAMPTON J.R. (1978)
Diabetic autonomic neuropathy.
(Diabetes 27 : 1043-1054)

HOSKING D.J. MOODY F., STEWART I.M. and ATKINSON M. (1975)
Vagal impairment of gastric secretion in diabetic autonomic neuropathy.
(British Medical Journal 2 : 588-590)

ISMAIL-BEGI F., REINHOLD J.G., FARAJI B. and ABADI P. (1977)
Effects of cellulose added to diets of low and high fibre content upon the metabolism of calcium, magnesium, zinc and phosphorus by man.
(Journal of Nutrition 107 : 510-518)

IVERSEN J. (1973a)
Adrenergic receptors and the secretion of glucagon and insulin from the isolated, perfused canine pancreas.
(Journal of Clinical Investigation 52 : 2102-2116)

IVERSEN J. (1973b)
Effect of acelylcholine on the secretion of glucagon and insulin from the isolated perfused canine pancreas.
(Diabetes 22 : 381-387)

JENKINS D.J. GOFF D.V., LEEDS A.R., ALBERTI K.G.M.M., WOLEVER T.M.S. GASSULL M.A. and HOCKADAY T.D.R. (1976)
Unabsorbable carbohydrates and diabetes : decreased post-prandial hyperglycaemia.
(Lancet 2 : 172-174)

JENKINS D.J.A., LEEDS A.R., GASSULL M.A., COCHET B. and ALBERTI K.G.M.M. (1977)
Decrease in postprandial insulin and glucose concentrations by guar and pectin.
(Annals of Internal Medicine 86 : 20-23)

JENKINS D.J.A., LEEDS A.R., NEWTON C. and CUMMINGS J.H. (1975)
Effect of pectin, guar gum and wheat fibre on serum cholesterol.
(Lancet 1 : 1116-1117)

JENKINS D.J.A., WOLEVER T.M.S., HOCKADAY T.D.R., LEEDS A.R., HOWARTH R., BACON S., APLING E.C. and DILAWARI J. (1977)
Treatment of diabetes with guar gum : decrease of urinary glucose loss in diabetics.
(Lancet 2 : 779-780)

JENKINS D.J.A., WOLEVER T.M.S., LEEDS A.R., GASSULL M.A. HAISMAN P., DILAWARI J., GOFF D.V., METZ G.L. and ALBERTI K.G.M.M. (1978)
Dietary fibres, fibre analogues, and glucose tolerance : importance of viscosity.
(British Medical Journal 1 : 1392-1394)

JENKINS D.J.A., WOLEVER T.M.S., NINEHAM R., TAYLOR R., METZ G.L., BACON S. and HOCKADAY T.D.R. (1978)
Guar crispbread in the diabetic diet.
(British Medical Journal 2 : 1744-1746)

JOHNSON L.R. (1977)
Gastrointestinal hormones and their functions.
(Annual Review of Physiology 39 : 135-158)

JOHNSON R.H. and SPALDING J.M.K. (1974)
Disorders of the autonomic nervous system.
(Blackwell Scientific Publications : Oxford, London, Edinburgh, Melbourne)

KALK W.J., VINIK A.I., PAUL M., KELLER P. and JACKSON W.P.U. (1975)
Immunoreactive glucagon responses to intravenous tolbutamide in chronic pancreatitis.
(Diabetes 24 : 851-855)

KASSANDER P. (1958)
Asymptomatic gastric retention in diabetics (gastroparesis diabeticorum).
(Annals of Internal Medicine 48 : 797-812)

KEEN H. (1959)
Autonomic neuropathy in diabetes mellitus.
(Postgraduate Medical Journal 35 : 272-281)

KELSAY J.L., BEHALL K.M. and PRATHER E.S. (1979)
Effect of fibre from fruits and vegetables on metabolic responses of human subjects : calcium, magnesium, iron and silicone balances.
(American Journal of Clinical Nutrition 32 : 1876-1880)

KIEHM T.G., ANDERSON J.W. and WARD K. (1976)
Beneficial effects of a high carbohydrate, high fibre diet on hyperglycaemic diabetic men.
(The American Journal of Clinical Nutrition 29 : 895-899)

KIMMEL J.R., POLLOCK H.G. and HAYDEN L.J. (1978)
Biological activity of avian PP in the chicken. In: Gut Hormones. pp 234-241, Ed. Bloom S.R.
(Churchill Livingstone : Edinburgh, London and New York)

KIMMEL J.R., POLLOCK H.G. and HAZELWOOD R.L. (1968)
Isolation and characterization of chicken insulin.
(Endocrinology 83 : 1323-1330)

KLAFF L.J., VINIK A.I., BERELOWITZ M. and JACKSON, W.P.U. (1978)
Circulating antibodies in diabetics treated with conventional and purified insulins.
(South African Medical Journal 54 : 149-153)

KOLODNY R.C. (1971)
Sexual dysfunction in diabetic females.
(Diabetes 20 : 557-559)

KOLODNY R.C., KAHN C.B., GOLDSTEIN H. and BARNETT D.M. (1974)
Sexual dysfunction in diabetic men.
(Diabetes 23 : 306-309)

KONTUREK S.J. (1976)
Somatostatin and the gastrointestinal secretions.
(Scandinavian Journal of Gastroenterology 11 : 1-4)

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., SOVENY P and HANSKY J. (1971)
Effect of food on serum gastrin evaluated by radioimmunoassay.
(Gut 12 : 619-624)

KRARUP T., SCHWARTZ T.W., HILSTED J., MADSBAD S., VERLAGGE O. and SESTOFT L. (1979)
Impaired response of pancreatic polypeptide to hypoglycaemia : an early sign of autonomic neuropathy in diabetics.
(British Medical Journal 2 : 1544-1546)

KRONBERG O. (1975)
The effect of beta-adrenergic blockade upon basal and pentagastrin-stimulated gastric acid secretion and upon gastrin responses to food.
(Scandinavian Journal of Gastroenterology 10 : 757-762)

KRONHEIM S., BERELOWITZ M. and PIMSTONE B.L. (1978)
The characterization of somatostatin-like immunoreactivity in human serum.
(Diabetes 27 : 523-529)

KRONHEIM S. and PIMSTONE B.L. (1980)
Conditions for the measurement of somatostatin-like immunoreactivity in unextracted human plasma. In: Neuropeptides : Biochemistry and Physiology. Ed. Millar, R.P.
(Churchill Livingstone: Edinburgh in press)

KUZIO M., DRYBURGH J.R., MALLOY K.M. and BROWN J.C. (1974)
Radioimmunoassay for gastric inhibitory polypeptide.
(Gastroenterology 66 : 357-364)

LANDS A.M., ARNOLD A., MCAULIFF J.P., LUDUENA F.P. and BROWN T.G.(Jr) (1967)
Differentiation of receptor systems activated by sympathomumetic aminos.
(Nature 214 : 597-598)

LANGER L. (1972)
Pentagastrin- and insulin-induced secretion in diabetes mellitus.
(Acta Medica Scandinavica 191 : 471-475)

LANGERHANS P. (1937)
Contributions to the microscopic anatomy of the pancreas (M.D. Thesis). Berlin 1869 (Translated by H. Morrison, Baltimore : The John Hopkins Hospital Press)

LARRIMER J.N., MAZZAFERRI E.L., CATALAND S. and MEKHJIAN H.S. (1978)
Effect of atropine on glucose-stimulated gastric inhibitory polypeptide.
(Diabetes 27 : 638-642)

LARSSON L-I., HOLST J., HAKANSON R. and SUNDLER F. (1975)
Distribution and properties of glucagon immunoreactivity in the digestive tract of various mammals : An immunohistochemical and immunochemical study.
(Histochemistry 44 : 281-290)

LARSSON L-I. and REHFELD J.F. (1979)
Peptidergic and adrenergic innervation of pancreatic ganglia.
(Scandinavian Journal of Gastroenterology 14 : 433-437)

LARSSON L-I., SUNDLER F. and HAKANSON R. (1976)
Pancreatic polypeptide - a postulated new hormone : identification of its cellular storage site by light and electron microscopic immunocytochemistry.
(Diabetologia 12 : 211-226)

LEBLANC H., LACHELIN G.C.L. ABU-FADIL S. and YEN S.S.C. (1977)
The effect of dopamine infusion on insulin and glucagon secretion in man.
(Journal of Clinical Endocrinology and Metabolism 44 : 196-202)

LEFEBVRE P. (1972)
Glucagon and lipid metabolism. In: Glucagon.
pp 109-121, Ed. Lefebvre P.J. and Unger R.H.
(Pergamon Press : Oxford)

LEVIN A.B. (1966)
A simple test of cardiac function based upon the heart rate changes induced by the Valsalva manoeuvre.
(American Journal of Cardiology 18 : 90-99)

LIN T-M. and CHANCE R.E. (1974)
Gastrointestinal actions of a new bovine pancreatic polypeptide (BPP).
In: Endocrinology of the gut.
pp 143-145, Ed: Chey and Brooks.
(C.R. Slack : Thorofare, New Jersey)

LIN T-M. and CHANCE R.E. (1978)
Spectrum of Gastrointestinal actions of bovine PP. In: Gut Hormones.
pp 242-246, Ed: Bloom S.R.
(Churchill Livingstone : Edinburgh, London and New York)

LLOYD-MOSTYN R.H. and WATKINS P.J. (1975)
Defective innervation of heart in diabetic autonomic neuropathy.
(British Medical Journal 3 : 15-17)

LOCKHART R.D., HAMILTON G.F. and FYFE (1974)
Anatomy of the human body.
(Faber and Faber : London)

LOONEN A.J.M. and SOUDIJN W. (1979)
Peptides with a dual function : central neuroregulators and gut hormones.
(Journal of Physiology, Paris, 75 : 831-850)

LORENZI M., KARAM J.H., ISALIKIAN E., BOHANNON N.V., GERICH J.E. and FORSHAM P.H. (1979)
Dopamine during α - or β - adrenergic blockade in man : hormonal, metabolic and cardiovascular effects.
(Journal of Clinical Investigation 63 : 310-317)

LUFT R., EFENDIC S. and HÖKFELT T. (1978)
Somatostatin - both hormone and neurotransmitter?
(Diabetologia 14 : 1-13)

LUNDBAEK K. (1954)
Diabetic augiopathy : a specific vascular disease.
(Lancet 1 : 377-379)

LUNDBERG J.M., HÖKFELT T., KEWENTER J., PETERSSON G., AHLMAN H., EDIN R., DAHLSTRÖM A., NILSSON G., TERENIUS L., UVNÄS-WALLENSTEN and SAID S. (1979)
Substance P-, VIP- and enkephalin-like immunoreactivity in the human vagus nerve.
(Gastroenterology 77 : 468-471)

MAHER T.D., TANENBERG R.J., GREENBERG B.Z., HOFFMAN J.E., DOE R.P. and GOETZ F.C. (1977)
Lack of glucagon response to hypoglycaemia in diabetic autonomic neuropathy.
(Diabetes 26 : 196-200)

MAKINO H., MATSUSHIMA Y., KANATSUKA A., YAMAMOTO M., KUMAGAI, A. and NISHIMURA M. (1979)
Changes in pancreatic somatostatin content in spontaneously diabetic mice, as determined by radioimmunoassay and immunohistochemical methods.
(Endocrinology 104 : 243-247)

MANDELSTAM P., SIEGEL C.I., LIEBER A. and SIEGEL M. (1969)
The swallowing disorder in patients with diabetic neuropathy - gastroenteropathy.
(Gastroenterology 56 : 1-12)

MARBLE A., WHITE P., BRADLEY R.F. and KRALL L.P. (1973)
Joslin's Diabetes Mellitus.
(Lea and Febiger : Philadelphia)

MARCO J., HEDO J.A. and VILLANUEVA M.L. (1978)
Control of pancreatic polypeptide secretion by glucose in man.
(Journal of Clinical Endocrinology and Metabolism 46 : 140-145)

MARLISS E.B., AOKI T.T. and CAHILL G.F. (Jr) (1972)
Glucagon and amino acid metabolism. In: Glucagon.
pp 123-150, Ed. Lefebvre P.J. and Unger P.M.
(Pergamon Press : Oxford)

MARLISS E.B., GIRARDIER L., SEYDOUX J., WOLLHEIM C.B., KANAZAWA Y., ORCI L., RENOLD A.E. and PORTE D. (Jr) (1973)
Glucagon release induced by pancreatic nerve stimulation in the dog.
(Journal of Clinical Investigation 52 : 1246-1259)

MARTIN M.M. (1953)
Involvement of autonomic nerve-fibres in diabetic neuropathy.
(Lancet 1 : 560-565)

MATSUYAMA T., HOFFMAN W.H., DUNBAR J.C. FOÀ N.L. and FOÀ P.P. (1975)
Glucose, insulin, pancreatic glucagon and glucagon-like immunoreactive materials in the plasma of normal and diabetic children. Effect of the initial insulin treatment.
(Hormone and Metabolic Research 7 : 452-456)

MAY J.M. and WILLIAMS R.H. (1978)
The effect of endogenous gastric inhibitory polypeptide on glucose-induced insulin secretion in mild diabetes.
(Diabetes 27 : 849-855)

MCGUIGAN J.E. and GREIDER M.H. (1971)
Correlative immunochemical and light microscopic studies of the gastrin cell of the antral mucosa.
(Gastroenterology 60 : 223-236)

MCINTYRE N., HOLDSWORTH C.D. and TURNER D.S. (1965)
Intestinal factors in the control of insulin secretion.
(Journal of Clinical Endocrinology and Metabolism 25 : 1317-1324)

MCLOUGHLIN J.C., HAYES J.R., BUCHANAN K.D. and KELLY J.G. (1978)
Role of neural influences in the release of gastrin, glucagon and secretin during hypoglycaemia in man.
(Gut 19 : 632-639)

MILES D.W. and HAYTER C.J. (1968)
The effect of intravenous insulin on the circulatory responses to tilting in normal and diabetic subjects with special reference to baroceptor reflex block and atypical hypoglycaemic reactions.
(Clinical Science 34 : 419-430)

MIRANDA P.M. and HORWITZ D.L. (1978)
High-fibre diets in the treatment of diabetes mellitus.
(Annals of Internal Medicine 88 : 482-486)

MONNIER L., PHAM T.C., AGUIRRE L., ORSOTTI A. and MIROUZE J. (1978)
Influence of indigestible fibres on glucose tolerance.
(Diabetes Care 1 : 83-88)

MORGAN L.M., GOULDER T.J., TSIOLAKIS D., MARKS V. and ALBERTI K.G.M.M. (1979)
The effect of unabsorbable carbohydrate on gut hormones : modification of postprandial GIP secretion by guar.
(Diabetologia 17 : 85-89)

MOSKOWITZ M.A. (1977)
Diseases of the autonomic nervous system.
(Clinics in Endecrinology and Metabolism 6 : 745-768)

MÜLLER W.A., FALOONA G.R., AGUILAR-PARADA E. and UNGER R.H. (1970)
Abnormal alpha-cell function in diabetes : response to carbohydrate and protein ingestion.
(New England Journal of Medicine 283 : 109-115)

MÜLLER W.A., FALOONA G.R. and UNGER R.H. (1973)
Hyperglucagonaemia in diabetic ketoacedosis : Its prevalance and significance.
(American Journal of Medicine 54 : 52-57)

MUNGER B.L. (1972)
The histology, cytochemistry and ultrastructure of pancreatic islet A-cells.
In: Glucagon.
pp 7-25, Ed: Lefebvre and Unger.
(Pergamon Press, Oxford)

MUNOZ J.M., SANDSTEAD H.H., JACOB R.A., JOHNSON L. and MAKO M.E. (1979)
Effects of dietary fibre on glucose tolerance of normal men.
(Diabetes 28 : 496-502)

MURLIN J.R., CLOUGH H.D., GIBBS C.B.F. and STOKES A.M. (1923)
Aqueous extracts of pancreas : 1. Influence on the carbohydrate metabolism of depancreatized animals.
(Journal of Biological Chemistry 56 : 253-296)

MURRAY A., EWING D.J., CAMPBELL I.W., NEILSON J.M.M. and CLARKE B.F. (1975)
RR interval variations in young male diabetics.
(British Heart Journal 37 : 882-885)

NATIONAL DIABETES DATA GROUP (1979)
Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance.
(Diabetes 28 : 1039-1057)

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., and UVNÄS-WALLENSTEN K. (1977)
Effect of teasing and sham-feeding on plasma glucagon concentration in dogs.
(Acta Physiologica Scandinavica 100 : 298-302)

NILSSON G., YALOW R.S. and BERSON S.A. (1973)
Distribution of gastrin in the gastrointestinal tract of human, dog, cat and hog. In: Frontiers of Gastrointestinal hormone Research.
pp 95-101, Ed: Almqvist and Wiksell
(Stockholm)

ORCI L., BAETENS D., DUBOIS M.P. and RUFENDER C. (1975)
Evidence for the D-cell of the pancreas secreting somatostatin.
(Hormone and Metabolic Research 7 : 400-402)

ORCI L., BAETENS D., RUFENER C., AMHERDT M., RAVAZZOLA M., STUDER P., MALAISSE-LAGAE F. and UNGER R.H. (1976)
Hypertrophy and hyperplasia of somatostatin containing D-cells in diabetes.
(Proceedings of the National Academy of Sciences of the USA 73 : 1338-1342)

ORCI L., MALAISSE-LAGAE F., BAETENS B. and PERRELET A. (1978)
Pancreatic-polypeptide-rich regions in human pancreas.
(Lancet 2 : 1200-1201)

PAGE M.MCB. and WATKINS P.J. (1976)
Provocation of postural hypotension by insulin in diabetic autonomic neuropathy.
(Diabetes 25 : 90-95)

PAGE M.MCB. and WATKINS P.J. (1977)
The heart in diabetes : autonomic neuropathy and cardiomyopathy.
(Clinics in Endocrinology and Metabolism 6 : 377-388)

PAGE M.MCB. and WATKINS P.J. (1978)
Cardiorespiratory arrest and diabetic autonomic-neuropathy.
(Lancet i : 14-16)

PALMER J.P., HENRY D.P., BENSON J.W., JOHNSON D.G. and ENSINCK J.W. (1976)
Glucagon response to hypoglycaemia in sympathectomized man.
(Journal of Clinical Investigation 57 : 522-525)

PALMER J.P. WERNER P.L., HOLLANDER P. and ENSINCK J.W. (1979)
Evaluation of the control of glucagon secretion by the parasympathetic nervous system in man.
(Metabolism 28 : 549-552)

PARK C.R. and EXTON J.H. (1972)
Glucagon and the metabolism of glucose. In: Glucagon.
pp 77-108, Ed: Lefebvre P.J. and Unger R.H.
(Pergamon Press, Oxford)

PATEL Y.C., ORCI L., BANKIER A. and CAMERON D.R. (1976)
Decreased pancreatic somatostatin (SRIF) concentration in spontaneously diabetic mice.
(Endocrinology 99 : 1415-1418)

PATEL Y.C. and WEIR G.C. (1976)
Increased somatostatin content of islets from streptozotocin-diabetic rats.
(Clinical Endocrinology 5 : 191-194)

PEDERSON R.A. and BROWN J.C. (1972)
Inhibition of histomine-pentagastrin-and insulin-stimulated canine gastric secretion by pure "gastric inhibitory polypeptide".
(Gastroenterology 62 : 393-400)

PEDERSON R.A. and BROWN J.C. (1976)
The insulinotropic action of gastric inhibitory polypeptide in the perfused isolated rat pancreas.
(Endocrinology 99 : 780-785)

PEDERSON R.A., DRYBURGH J.R., BROWN J.C. and DUPREE A.H. (1978)
GIP effects on the isolated pancreas. In: Gut Hormones.
pp 283-287, Ed: Bloom S.R.
(Churchill Livingstone: Edinburgh, London and New York)

PEK S. (1977)
Glucagon and diabetes.
(Clinics in Endocrinology and Metabolism 6 : 333-344)

PEK S., FAJANS S.S., FLOYD J.C. (Jr), KNOPF R.F., WEISSMAN P.N. and CONN J.W. (1972)
Plasma levels of glucagon in patients with diabetes mellitus.
(Diabetes 21 (Suppl. 1) : 324)

PELLETIER G. and LECLERC R. (1977)
Immunohistochemical localization of human pancreatic polypeptide (HPP) in the human endocrine pancreas.
(Gastroenterology 72 : 569-571)

PENMAN E., WASS J.A.H., LUND A., LOWRY P.J., STEWART J., DAWSON A.M., BESSER G.M. and REES L.H. (1979)
Development and validation of a specific radioimmunoassay for somatostatin in human plasma.
(Annals of Clinical Biochemists 16 : 15-25)

PETERSSON B., LUNDQVIST G. and ANDERSSON A. (1979)
Somatostatin content and release of isolated pancreatic islets from obese-hyperglycaemic mice.
(Experientia 35:127-128)

PICK J. (1970)
The Autonomic Nervous System : morphological, comparative, clinical and surgical aspects.
(J.B. Lippincott: Philadelphia, Toronto)

PIMSTONE B.L. and BERELOWITZ M. (1978)
Somatostatin-paracrine and neuromodulator peptide in gut and nervous system.
(South African Journal of Medicine 53 : 7-9)

PIMSTONE B.L., SHEPPARD M., SHAPIRO B., KRONHEIM S., HUDSON A., HENDRICKS S. and WALIGORA K. (1979)
Localization in and release of somatostatin from brain and gut.
(Federation Proceedings 38 : 2330-2332)

POLAK J.M. and BLOOM S.R. (1979)
Neuropeptides of the gut : a newly discovered major control system.
(World Journal of Surgery 3 : 393-406)

POLAK J.M. BLOOM S., COULLING I. and PEARSE A.G.E. (1971)
Immunofluorescent localization of enteroglucagon cells in the gastrointestinal tract of the dog.
(Gut 12 : 311-318)

POLAK J.M. BLOOM S.R., KUZIO M., BROWN J.C. and PEARSE A.G.E. (1973)
Cellular localization of gastric inhibitory polypeptide in the duodenum and jejunum.
(Gut 14 : 284-288)

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 1 : 1220-1222)

PRADAYROL L., JÖRNVALL H., MUTT V. and RIBET A. (1980)
N-terminally extended somatostatin : the primary structure of somatostatin - 28.
(Federation of European Biochemical Societies 109 : 55-58)

PRYCE T.D. (1893)
On diabetic neuritis, with a clinical and pathological description of three cases of diabetic pseudo-takes.
(Brain 16 : 416-424)

RASKIN P., AYDIN I. and UNGER R.H. (1976)
Effect of insulin on the exaggerated glucagon response to arginine stimulation in diabetes mellitus.
(Diabetes 25 : 227-229)

RASKIN P., FUJITA Y. and UNGER R.H. (1975)
Effect of insulin-glucose infusions on plasma glucagon levels in fasting diabetics and non-diabetics.
(Journal of Clinical Investigation 56 : 1132-1138)

RASKIN P. and UNGER R.H. (1978)
Effect of insulin therapy on the profiles of plasma immunoreactive glucagon in juvenile-type and adult-type diabetics.
(Diabetes 27 : 411-419)

RAYFIELD E.J., GEORGE D.T., EICHNER H.L. and HSU T.H. (1975)
L-dopa stimulation of glucagon secretion in man.
(New England Journal of Medicine 293 : 589-591)

REHFELD J.F., STADIL F., MALMSTRØM J. and MIYATA M. (1975)
Gastrin heterogeneity in serum and tissue. A progress report.
In: Gastrointestinal Hormones. A symposium.
pp 43-58, Ed. Thompson J.C.
(University of Texas Press : Austin and London)

REINHOLD J.G., FARADJI B., ABADI P. and ISMAIL-BEIGI F. (1976)
Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fibre and phosphorus consumption as wheat bread.
(Journal of Nutrition 106 : 493-503)

REINHOLD J.G., NAST K., LAHIMGATZADEH A. and HEDAYATI H. (1973)
Effects of purified phytate and phytate-rich bread upon metabolism of zinc, calcium, phosphorus and nitrogen in man.
(Lancet 1 : 283-288)

RENOLD A.E. (1972)
The beta cell and its responses : summarizing remarks and some contributions from Geneva.
(Diabetes 21 : 619-631)

REYNOLDS C., MOLNAR G.D., HORWITZ D.L. RUBENSTEIN A.H., TAYLOR W.F. and JIANG N-S. (1977)
Abnormalities of endogenous glucagon and insulin in unstable diabetes.
(Diabetes 26 : 36-45)

REYNOLDS C., TRONSGARD N., GIBBONS E., BLIX P.M. and RUBENSTEIN A.H. (1979)
Gastric inhibitory polypeptide response to hyper- and hypoglycaemia in insulin-dependent diabetics.
(Journal of Clinical Endocrinology and Metabolism 49 : 255-261)

ROSS S.A., BROWN J.C. and DUPRÉ J. (1977)
Hypersecretion of gastric inhibitory polypeptide following oral glucose in diabetes mellitus.
(Diabetes 26 : 525-529)

RUBIN A. and BABBOTT D. (1958)
Impotence and diabetes mellitus.
(Journal of the American Medical Association 68 : 498-500)

RUNDLES R.W. (1945)
Diabetic Neuropathy : general review with report of 125 cases.
(Medicine 24 : 111-160)

SAKURAI H., DOBBS R. and UNGER R.H. (1974)
Somatostatin-induced changes in insulin and glucagon secretion in normal and diabetic dogs.
(Journal of Clinical Investigation 54 : 1395-1402)

SAMOLS E. and WEIR G.C. (1979)
Adrenergic modulation of pancreatic A, B and D cells : alpha adrenergic suppression and beta adrenergic stimulation of somatostatin secretion, alpha adrenergic stimulation of glucagon secretion in the perfused dog pancreas.
(Journal of Clinical Investigation 63 : 230-238)

SAMOLS E., WEIR G.C., PATEL Y.C., LOO S.W. and GABBAY K.H. (1977)
Autonomic control of somatostatin and pancreatic polypeptide by the isolated perfused canine pancreas.
(Clinical Research 25 : 499A)

SAMOLS E., WEIR G.C., RAMSEUR R., DAY J.A. and PATEL Y.C. (1978)
Modulation of pancreatic somatostatin by adrenergic and cholinergic agonism and by hyper- and hypoglycaemic sulphonamides.
(Metabolism 27 (Suppl. 1) : 1219-1221)

SCARPELLO J.H.B. and SLADEN G.E. (1978)
Diabetes and the gut.
(Gut 19 : 1153-1162)

SCHALLY A.V., HUANG W-Y., CHANG R.C.C., ARIMURA A., REDDING T.W., MILLAR R.P., HUNKAPILLER M.W. and HOOD L.E. (1980)
Isolation and structure of pro-somatostatin : a putative somatostatin precursor from pig hypothalamus.
(Proceedings of the National Acadmey of Sciences of the USA 77 : in press)

SCHOFFLING K., FEDERLIN K., DITSCHUNEIT H. and PFEIFFER E.F. (1963)
Disorders of sexual function in male diabetic.
(Diabetes 12 : 519-527)

SCHUSDZIARRA V., DOBBS R.E., HARRIS V. and UNGER R.N. (1977)
Immunoreactive somatostatin levels in plasma of normal and alloxan diabetic dogs.
(Federation of European Biochemical Societies 81 : 69-72)

SCHUSDZIARRA V., HARRIS V., ARIMURA A. and UNGER R.H. (1979)
Evidence for a role of splanchnic somatostatin in the homeostasis of ingested nutrients.
(Endocrinology 104 :1705-1708)

SCHUSDZIARRA V., ROUILLER D., HARRIS V. and UNGER R.H. (1979)
Gastric and pancreatic release of somatostatin-like immunoreactivity during the gastric phase of a meal : effects of truncal vagotomy and atropine in the anaesthetized dog.
(Diabetes 28 : 658-663)

SCHUSDZIARRA V., ROUILLER D., HARRIS V., CONLON J.M. and UNGER R.H. (1978)
The response of plasma somatostatin-like immunoreactivity to nutrients in normal and alloxan diabetic dogs.
(Endocrinology 103 : 2264-2273)

SCHUSDZIARRA V., ROUILLER D., PIETRI A., HARRIS V., ZYZNAR E., CONLON J.M. and UNGER R.H. (1979)
Pancreatic and gastric release of somatostatinlike immunoreactivity during intestinal phase of a meal.
(American Journal of Physiology 237 : 555-560)

SCHWARTZ T.W., HOLST J.J., FAHRENKRUG J., JENSEN S.L., NIELSEN O.V., REHFELD J.F., SCHAFFALITZKY D.E., MUCKADELL O.B. and STADIL F. (1978)
Vagal, Cholinergic regulation of pancreatic polypeptide secretion.
(Journal of Clinical Investigation 61 : 781-789)

SCHWARTZ T.W. and REHFELD J.J. (1977)
Mechanism of pancreatic-polypeptide release.
(Lancet 1 : 697-698)

SCHWARTZ T.W., REHFELD J.F., STADIL F., LARSON L-I., CHANCE R.E. and MOON N. (1976)
Pancreatic-polypeptide response to food in duodenal-ulcer patients before and after vagotomy.
(Lancet 1 : 1102-1105)

SCHWARTZ T.W., STENQUIST B. and OLBE L. (1979)
Cephalic phase of pancreatic-polypeptide secretion studied by sham feeding in man.
(Scandinavian Journal of Gastroenterology 14 : 313-320)

SCHWARTZ T.W., STENQUIST B., OLBE L. and STADIL F. (1979)
Synchronous oscillations in the basal secretion of pancreatic polypeptide and gastric acid : depression by cholinergic blockade of pancreatic polypeptide concentrations in plasma.
(Gastroenterology 76 : 14-19)

SERVICE F.J., NELSON R.L., RUBENSTEIN A.H. and GO V.L.W. (1979)
Direct effect of insulin on secretion of insulin, glucagon, gastric inhibitory polypeptide and gastrin during maintainance of normoglycaemia.
(Journal of Clinical Endocrinology and Metabolism 47 : 488-493)

SILBERT W. (1969)
Diabetes and oesophageal dysfunction.
(British Medical Journal 3 : 688-690)

SIVE A., VINIK A.I., VAN TONDER S. and LUND A. (1978)
Impaired pancreatic polypeptide secretion in chronic pancreatitis.
(Journal of Clinical Endocrinology and Metabolism 47 : 556-559)

SMITH C.L., KEWENTER J., CONNELL A.M., ARDILL J., HAYES R. and BUCHANAN K. (1975)
Control factors in the release of gastrin by direct electrical stimulation of the vagus.
(Digestive Diseases 20 : 13-22)

SMITH S.E., SMITH S.A., BROWN P.M., FOX C. and SÖNKSEN P.H. (1978)
Pupillary signs in diabetic autonomic neuropathy.
(British Medical Journal 2 : 924-927)

SNEDECOR G.W. and COCHRAN (1967)
Statistical methods, 6th edition.
(The Iowa State University Press : Iowa)

SOLL A.H. and GROSSMAN M.I. (1978)
Cellular mechanisms in acid secretion.
(Annual Review of Medicine 29 : 495-507)

STADIL F. (1974)
Gastrin and insulin hypoglycaemia : a review of studies on gastrin determination and hypoglycaemic release of gastrin in man.
(Scandinavian Journal of Gastroenterology 9 (Suppl. 23) : 1-45)

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

SUNDLER F., ALUMETS J., HOLST J., LARSSON L.I. and HAKANSON R. (1976)
Ultrastructural identification of cells storing pancreatic-type glucagon in dog stomach.
(Histochemistry 50 : 33-37)

SUSSMAN K.E., CROUT J.R., MARBLE A. (1963)
Failure of warning in insulin-induced hypoglycaemic reactions.
(Diabetes 12 : 38-45)

TAMBORLANE W.V., SHERWIN R.S., HENDLER R. and FELIG P. (1977)
Metabolic effects of somatostatin in maturity-onset diabetes.
(New England Journal of Medicine 297 : 181-183)

TAYLOR I.L., FELDMAN M., RICHARDSON C.T. and WALSH J.H. (1978)
Gastric and cephalic stimulation of human pancreatic polypeptide release.
(Gastroenterology 75 : 432-437)

TAYLOR I.L., IMPICCIATORE M. and WALSH J.H. (1977)
Effect of atropine and vagotomy on the pancreatic polypeptide response to a meal.
(Gastroenterology 72 : 1139)

TAYLOR I.L., SOLOMON T.E., WALSH J.H. and GROSSMAN M.I. (1979)
Pancreatic polypeptide : metabolism and effect on pancreatic secretion in dogs.
(Gastroenterology 76 : 524-528)

TEPPERMAN B.L., WALSH J.H. and PRESHAW R.M. (1972)
Effect of antral denervation on gastrin release by sham feeding and insulin hypoglycaemia in dogs.
(Gastroenterology 63 : 973-980)

TIMPERLEY W.R., WARD J.D., PRESTON F.E., DUCKWORTH T. and O'MALLEY B.C. (1976)
Clinical and histological studies in diabetic neuropathy : a reassessment of vascular factors in relation to intravascular coagulation.
(Diabetologia 12 : 237-243)

THOMAS F.B., SHOOK D.F., O'DORISIO T.M., CATALAND S., MEKHJIAN H.S., CALDWELL J.H. and MAZZAFERRI E.L. (1977)
Localization of gastric inhibitory polypeptide release by intestinal glucose perfusion in man.
(Gastroenterology 72 : 49-54)

THOMFORD N.R., SIRINEK K.R., CROCKETT S.E., MAZZAFERRI E.L. and CATALAND S. (1974)
Gastric inhibitory polypeptide : response to oral glucose after vagotomy and pylotoplasty.
(Archives of Surgery 109 : 177-182)

TROWELL H. (1973)
Dietary fibre, ischaemic heart disease and diabetes mellitus.
(Proceedings of the Nutrition Society 32 : 151-157)

TROWELL H.C. (1975)
Dietary-fibre hypothesis of the etiology of diabetes mellitus.
(Diabetes 24 : 762-765)

TROWELL H. (1978)
Diabetes mellitus and dietary fibre of starchy foods.
(The American Journal of Clinical Nutrition 31 : 553-557)

UNGER R.H., AGUILAR-PARADA E., MÜLLER W.A., EISENTRAUT A.M. (1970)
Studies of pancreatic alpha cell function in normal and diabetic subjects.
(Journal of Clinical Investigation 49 : 837-848)

UNGER R.H. and ORCI L. (1977) (a)
Role of glucagon in diabetes.
(Archives of Internal Medicine 137 : 482-491)

UNGER R.H. and ORCI L. (1977) (b)
Possible roles of the pancreatic D-cell in the normal and diabetic states.
(Diabetes 26 : 241-244)

UNGER R.H. and ORCI L.
Glucagon : secretion, transport, metabolism, physiologic regulation of secretion and derangements in diabetes. In: Endocrinology vol.2.
pp 959-981, Ed. De Groot, L.J.
(Grune and Stratton : New York)

UVNÄS B., UVNÄS-WALLENSTEN K. and NILSSON G. (1975)
Release of gastrin on vagal stimulation in the cat.
(Acta Physiologica Scandinavica 94 : 167-176)

UVNÄS-WALLENSTEN K., EFENDIC S. and LUFT R. (1977)
Vagal release of somatostatin into the antral lumen of cats.
(Acta Physiologica Scandinavica 99 : 126-128)

UVNÄS-WALLENSTEN K., LUNDBERG J.M. and EFENDIC S. (1978)
Dopaminergic control of antral gastrin and somatostatin release.
(Acta Physiologica Scandinavica 103 : 343-345)

VALVERDE I., VALLANUEVA M.L., LOZANO I. and MARCO J. (1974)
Presence of glucagon immunoreactivity in the globulin fraction of human plasma "Big plasma glucagon".
(Journal of Clinical Endocrinology and Metabolism 39 : 1090-1098)

VARLEY H. (1967)
Practical Chemical Biochemistry.
pp 80-96, 4th edition.
(William Heinemann Medical Books Ltd : London)

VENDSALU A.A. (1960)
Studies on adrenaline and noradrenaline in human plasma.
(Acta Physiologica Scandinavica 49 (Suppl. 173) : 33-35)

VILLANUEVA M., HEDO J., CASTILLO-OLIVARES J. and MARCO J. (1978)
Effect of exogenous and endogenous hyperglycaemia on human pancreatic polypeptide (hPP) secretion.
(Diabetologia 15 : 366)

WAGNER H., HENGST K., JANSEN H. and GERLACH U. (1978)
Effect of somatostatin on absorption of mono- and disaccharides in small intestine in vivo and in vitro in man and rats.
(Metabolism 9 (Suppl. 1) : 1329-1332)

WAHREN J. and FELIG P. (1976)
Influence of somatostatin on carbohydrate disposal and absorption in diabetes mellitus.
(Lancet 2 : 1213-1216)

WALSH J.H. and GROSSMAN M.I. (1975)
Gastrin (First of Two Parts). Chemistry and Physiology.
(New England Journal of Medicine 292 : 1324-1334)

WALTER R.M., DUDL R.J., PALMER J.P. and ENSINCK J.W. (1974)
The effect of adrenergic blockade on the glucagon responses to starvation and hypoglycaemia in man.
(Journal of Clinical Investigation 54 : 1214-1220)

WARD J.D. (1972)
Diabetic neuropathy.
(Clinics in Endoctrinology and Metabolism 1 : 809-827)

WASS J.A.H., PENMAN E., DRYBURGH J.R., TSIOLAKIS D., GOLDBERG P.L., DAWSON A.M., BESSER G.M. and REES L.H. (1980)
Circulating somatostatin after food and glucose in man.
(Clinical Endocrinology 12 : 569-574)

WASS J.A.H., PENMAN E., MEDBAK S., DAWSON A.M., TSIOLAKIS D., MARKS V., BESSER G.M. and REES L.H. (1980)
Immunoreactive somatostatin changes during insulin-induced hypoglycaemia and operative stress in man.
(Clinical Endocrinology 12 : 269-275)

WATKINS P.J. (1973)
Facial sweating after food : a new sign of diabetic autonomic neuropathy.
(British Medical Journal 1 : 583-587)

WEIR G.C., KNOWLTON S.D. and MARTIN D.B. (1975)
High molecular weight glucagon-like immunoreactivity in plasma.
(Journal of Clinical Endocrinology and Metabolism 40 : 296-302)

WHEELER T. and WATKINS P.J. (1973)
Cardiac denervation in diabetes.
(British Medical Journal 4 : 584-586)

WISE J.K., HENDLER R. and FELIG P. (1973)
Evaluation of alpha-cell function by infusion of alanine in normal, diabetic and obese subjects.
(New England Journal of Medicine 288 : 487-490)

WOOD C.L., ARNETT C.D., CLARKE W.R., TSAI B.S. and LEFKOWITZ R.J. (1979)
Subclassification of alpha-adrenergic receptors by direct binding studies.
(Biochemical Pharmacology 28 : 1277-1282)

WOODS S.C. and PORTE D. (Jr) (1974)
Neural control of the endocrine pancreas.
(Physiological Reviews 54 : 596-619)

YALOW R.S. and BERSON S.A. (1971)
Introduction and general considerations. In: Principles of Competitive Protein Binding Assays.
pp 1-21, Eds: Odell W.D. and Daughaday W.H.
(J.P. Lippincott Company : Philadelphia)

YALOW R.S. and BERSON S.A. (1972)
And now, "Big, Big" Gastrin.
(Biochemical and Biophysical Research Communications 48 : 391-395)

YOUNG J.B. and LANDSBERG L. (1977)
Catecholamines and the regulation of hormone secretion.
(Clinics in Endocrinology and Metabolism 6 : 657-695)

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The Author's Published Papers arising from this Project

LEVITT N.S., VINIK A.I., SIVE A.A., CHILD P.T. and JACKSON W.P.U. (1979)
Studies on plasma glucagon concentration in maturity onset diabetics with autonomic neuropathy.
(Diabetes 28 : 1015-1021)

LEVITT N.S., VINIK A.I., SIVE A.A., VAN TONDER S. and LUND A. (1980)
Impaired pancreatic polypeptides responses to insulin-induced hypoglycaemia in diabetic autonomic neuropathy.
(Journal of Clinical Endocrinology and Metabolism 50 : 445-449)

LEVITT N.S., VINIK A.I., SIVE A.A., CHILD P.T. and JACKSON W.P.U. (1980)
The effect of dietary fibre on glucose and hormone responses to a mixed meal in normal subjects and diabetics with and without autonomic neuropathy.
(Diabetes Care. In press)

LEVITT N.S., VINIK A.I., PIMSTONE B.L., PAUL M. and KRONHEIM S. (1980)
Somatostatin secretion in diabetic autonomic neuropathy.
In Neuropeptides : Biochemistry and Physiology.
Ed: R.P. Millar.
(Churchill Livingstone : Edinburgh, in press)

LEVITT N.S., VINIK A.I., CHILD P.T. and JACKSON W.P.U. (1980)
GIP secretion in diabetic autonomic neuropathy.
(Journal of Clinical Endocrinology and Metabolism. In press)

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