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STUDIES ON THE NATURE, DISTRIBUTION, RELEASE, PHYSIOLOGY AND PATHOPHYSIOLOGY OF PANCREATIC POLYPEPTIDE.

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ABSTRACT

Pancreatic polypeptide (PP) is a straight chained hormonal peptide first discovered as a major impurity of insulin preparations. PP has been purified from several mammalian species and pharmacology of the bovine peptide in the dog revealed a broad spectrum of gastrointestinal actions.

A precise and specific radioimmunoassay was set up which was capable of detecting changes between adjacent samples of 4 pmol PP/l plasma. The assay was used to determine the distribution, release, physiology and pathophysiology of PP in man.

PP was localised almost entirely to the pancreas in both adult and foetal man. Chromatography revealed that the predominant molecular form in tissue was similar to pure PP and this was also the major form in plasma (greater than 95%).

PP was found to circulate in plasma and concentrations rose promptly following the ingestion of food and remained elevated for several hours. Protein and fat were the most potent releasers of PP but glucose and gastric distension also evoked release. Intravenous nutrients, however, did not alter PP levels. PP was absent from the circulation of pancreatectomised subjects indicating that the pancreas was the only source of the circulating peptide. It was clear that an indirect signal was responsible for the postprandial rise. A substantial rise occurred following insulin hypoglycaemia but this was abolished by vagotomy. Postprandial release in vagotomised subjects was quite normal, however, indicating that the vagus was not the only mechanism for PP release. Studies on the isolated perfused canine pancreas demonstrated that acetyl choline and several gastrointestinal hormones could release PP. Gut hormones also released PP in man but this could only be blocked by

atropine. It would appear that a degree of cholinergic tone is essential for PP release and that the "entero-PP-axis" involves both cholinergic nerves and circulating hormones.

Infusion studies in man, at doses which mimicked the postprandial response demonstrated an inhibition of pancreatic exocrine secretion and relaxation of the gall bladder. In contrast PP had no effect on gastric secretion or emptying, nor did it affect circulating metabolites.

Patients with steatorrhoea due to chronic pancreatitis or cystic fibrosis had a greatly reduced PP response to food. A large proportion of pancreatic endocrine tumours contained PP and circulating basal levels in these patients exceeded the normal peak postprandial values. Measurement in such cases may sometimes be of diagnostic use.

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CHAPTER 1. INTRODUCTION AND HISTORICAL BACKGROUND

The Endocrine Pancreas

The endocrine pancreas in adult man consists of about 1.5 grams of tissue and is composed of specialised cells, a network of thin nerve fibres and an abundance of small blood vessels. In the average healthy human this tiny heap of tissue exists sub-divided into about one million specks, the Islets of Langerhans. Each islet is a functioning multi-hormonal endocrine system, sensing and responding to chemical signals and neural impulses in an integrated and homeostatic fashion.

History of the Endocrine Pancreas

An understanding of the structure and function of the pancreatic endocrine system accompanied the study of the cause of diabetes mellitus. The syndrome had been recognised for many centuries but it was in 1889 that Von Mehring and Minkowski demonstrated that diabetes could be produced in dogs by pancreatectomy, thus associating the condition with this organ (Von Mehring and Minowski, 1890). This evoked a considerable interest in the pancreas and in particular the cells described earlier by Langerhans (Langerhans, 1869). The definitive experiment, that proved the endocrine function of the pancreas, was performed by Banting and Best in 1922. These workers produced diabetes by removing the pancreas from a dog and were able to reduce the resultant hyperglycaemia by the administration of pancreatic extract. They further demonstrated that these same preparations could be used in the treatment of patients suffering from diabetes. The name "insulin" was applied to the internal secretion present in their pancreatic extracts. Almost as soon as the first

insulin preparations began to be administered to diabetic patients, it was observed that these substances induced an unexpected though transient hyperglycaemia before lowering the blood sugar. Murlin and his colleagues suggested that the unknown hyperglycaemia factor was a second pancreatic hormone which they called glucagon (Murlin at al, 1922). However it was some 30 years later that glucagon was isolated and characterised (Staub et al, 1955). The elucidation of the function of the pancreatic D cells, which had remained a mystery for so long, followed the work of Alberti and colleagues who demonstrated that somatostatin could suppress insulin release in man (Alberti et al, 1973). It is now established that somatostatin is normally present in the D cells of the Islets of Langerhans of the mammalian pancreas. More recently Kimmel and Chance working quite independently discovered yet another peptide hormone as a contaminant of avian and bovine pancreas respectively (Kimmel et al, 1968, Chance et al, 1976). This peptide has been termed "pancreatic polypeptide" as its function in normal physiology is as yet unknown.

The Discovery of Pancreatic Polypeptide

The discovery of avian pancreatic polypeptide (APP) was an accidental event associated with purification studies of chicken insulin in the laboratory of Kimmel et al, 1968. The work involved the isolation of insulin from pancreas and APP was encountered as a major and almost the only contaminant of the insulin in the final stages of the purification.

The subsequent purification of APP was undertaken with the idea that this contaminant was avian glucagon which had not been characterised. Even after amino acid analysis this possibility still existed, but in the preliminary steps of the amino acid sequence determination it became apparent that the molecular had major sequence differences from porcine

glucagon. The finding of NH_2-terminal glycine was one significant difference since histidine is in the NH_2-terminal position of all known glucagons but the most important feature was the amidated tyrosine residue in the C terminal position, a finding quite unexpected for glucagon. An amidated C terminal amino acid is a fairly common feature of peptide hormones, particularly those found in the gastrointestinal tract such as cholecystokinin and secretin (Jorpes and Mutt, 1961, Mutt and Jorpes, 1971). Morphologists had described cells other than the A (glucagon producing) or B (insulin producing) cell types but the products of these small granuled cells were unknown. Kimmel and colleagues continued in their attempts to purify PP because of the likelihood that PP was a hormone secreted from one of these cell types. The complete amino acid sequence of APP is shown below. (Kimmel et al, 1975).

Gly - Pro - Ser - Gln - Pro - Thr - Tyr - Pro - Gly - Asp - Asp - Ala

Pro - Val - Glu - Asp - Leu - Ile - Arg - Phe - Tyr - Asp - Asn - Leu

Gln - Gln - Tyr - Leu - Asn - Val - Val - Thr - Arg - His - Arg - Tyr-NH_2

While Kimmel's work was in progress in Kansas City, quite independently, Chance and colleagues in Indianapolis isolated and sequenced similar peptides from several mammalian species. (Chance and Jones. 1974). The peptides isolated from human, bovine, porcine, canine and ovine pancreas were all very similar and each with 36 amino acids. There were only two or three differences in amino acid sequence between BPP and the other mammalian peptides. (Chance and Jones, 1974).

Purification of Pancreatic Polypeptide

A unique feature of the purification of pancreatic polypeptide is that no bioassay was used to monitor the isolation. Both groups of

Kimmel and Chance purified their respective peptides (APP & BPP) from side fractions of insulin purification. Biological activity was only assessed once pure material became available. This is in contrast to the purification of most other peptide hormones where a substance is isolated and purified using a bioassay to monitor each step.

Structure of Mammalian Pancreatic Polypeptide

The PP molecule is a 36 amino acid straight chain peptide from all species so far investigated. PP molecules from mammalian sources are all very similar as can be seen below (Chance et al, 1979).

Structure of HPP and Related Homologs

1	2	3	4	5	6	7	8	9
Ala -	Pro -	Leu -	Glu -	Pro -	Val -	Tyr -	Pro -	Gly
10	11	12	13	14	15	16	17	18
Asp -	Asn -	Ala -	Thr -	Pro -	Glu -	Gln -	Met -	Ala
19	20	21	22	23	24	25	26	27
Gln -	Tyr -	Ala -	Ala -	Asp -	Leu -	Arg -	Arg -	Tyr
28	29	30	31	32	33	34	35	36
Ile -	Asn -	Met -	Leu -	Thr -	Arg -	Pro -	Arg -	Tyr - NH_2

	Amino acid at position			
	2	6	11	23
Species differences compared to HPP				
Human (HPP)	Pro	Val	Asn	Asp
Bovine (BPP)		Glu		Glu
Ovine (OPP)	Ser	Glu		Glu
Porcine (PPP)			Asp	Glu
Canine (CPP)				

Avian PP differs considerably from mammalian PP, only 16 of the 36 amino acid residues being identical although C terminal hexapeptide sequence is similar. Four of the 6 residues are identical (see below).

C terminal hexapeptide of PP

APP Val - Thr - Arg - His - Arg - Tyr - NH_2

HPP Leu - Thr - Arg - Pro - Arg - Tyr - NH_2

This C terminal hexapeptide of bovine PP appears to exhibit full biological activity in the mammal (Lin and Chance, 1978).

Neither avian nor mammalian PP are related to any other known peptide. Rodent PP may well have significant differences in structure from PP in other species as antibodies raised to human or bovine PP do not in general cross-react to PP in rat or mouse pancreas. The C terminal tyrosinamide appears to be essential for biological activity in the dog at least on the suppression of pentagastrin stimulated acid secretion (Lin and Chance, 1978).

Cellular Localisation of Pancreatic Polypeptide

Distribution of PP cells

The cellular localisation of pancreatic polypeptide was first assigned to an endocrine cell type by Larrson et al, 1976. There appears to be considerable variation in the distribution of PP cells in different species. In the dog, rat and mouse they are more common in the juxta-duodenal part than in the tail of the pancreas. In most

species PP cells are found scattered in the exocrine parenchyma as well as in the periphery of the islets. In some species, particularly the dog, scattered PP cells can be found in the gastric and upper small intestine mucosa.

In man the most PP cells are found in the periphery of the Islets of Langerhans and are more concentrated in the head than in the tail of the pancreas. (Gingerich et al, 1978, Orci et al, 1978).

Ultrastructure of PP cells

There has been some controversy regarding the ultrastructural characteristics of the PP cell which is apparently due, at least in part, to species differences. In the dog and cat PP cells appear identical with the F cell described by Munger et al, 1965. (Larrson et al, 1976, Forssmann et al, 1977). However in some other species such as man cells with ultrastructural characteristics of the F cell do not occur. In man PP is secreted by cells with granules of much smaller size (less than 180 nm) and of a quite different appearance. Heitz et al, 1976, suggested that the human PP cells were identical to the D_1 cell of the revised Wiesbaden classification (Solcia et al, 1973) but this view has been disputed (Larrson et al, 1976, Sundler and Hakanson, 1976, Capella et al, 1977).

Human PP cells are rather small and show round or slightly ovoid granules of variable size, but generally small, and of moderate to high electron density. The granule core is sometimes separated from the limiting membrane by a very narrow clear space (Larrson et al, 1976).

At the international symposium on gut hormones held in Lausanne in 1977, eminent morphologists and other specialists gathered to discuss the classification of gastroenteropancreatic cells (Solcia et al, 1978). This group suggested the use of the functional label "PP cell" to describe the small granuled pancreatic endocrine cell type which secretes PP (Solcia et al, 1978).

Pharamcology of Pancreatic Polypeptide

The localisation of PP in the pancreas along with the established metabolic hormones, insulin and glucagon, initially suggested a metabolic role for this peptide. Indeed, early studies on circulating PP demonstrated large rises in plasma levels following the ingestion of food both in the chicken and the dog. This finding of a substantial and prolonged postprandial increase in plasma PP concentrations is strongly suggestive of either a metabolic or gastrointestinal action. Thus it was effects on the gastrointestinal tract, both secretor and motor, and on metabolism which have been studied in both the chicken and the dog.

Biological activity of APP in the chicken

As feeding is associated with absorption of nutrients and storage and transformation of carbohydrate, lipid and amino acids, Kimmel and co-workers in Kansas City undertook a programme to establish whether APP could have a metabolic role in the chicken; in addition they studied the effects of the peptide on the avian gastrointestinal tract.

Their initial studies demonstrated that APP could stimulate proventricular secretion in the adult chicken. Single intravenous bolus injections of APP (12.5, 25 or 50 μg/kg) in anaesthetised birds caused a substantial increase in proventricular flow for a period of 80 minutes. Injection of pentagastrin (1 μg/kg) caused a rapid elevation in flow rate of similar magnitude but this rapidly subsided to pre-injection levels within 30 minutes (Hazelwood et al, 1973). The total proventricular flow in APP injected birds was far greater than that seen following pentagastrin. Acid and pepsin concentrations increased in parallel with fluid flow. In the chicken APP had no effect on gall bladder tone, biliary flow, gizzard secretion and motility or pancreatic secretion (Hazelwood et al, 1973).

The potential metabolic actions of APP were first investigated by

measuring hepatic glycogen in unanaesthetised, non-fasting, 5-6 week old chicks following APP injection (100 ug/kg). Liver glycogen content rapidly decreased following either APP or glucagon injection. However glycogen content had nearly returned to normal within 50 minutes in the glucagon treated group, whereas in the APP treated birds glycogen was still significantly depressed. The glucagon treated birds exhibited the expected hyperglycaemia and hyperglycerolaemia, however, in the APP treated birds glucose levels did not rise and glycerol concentrations actually fell. These results suggest either an increase in tissue utilisation of glucose together with enhanced glycogenolysis following APP administration or an increased rate in the hepatic metabolism of glucose (Kimmel et al, 1978). Such activity would be expected to increase circulating concentrations of glucose metabolites. Following APP injection plasma glycerol concentrations were significantly reduced whilst plasma triglyceride concentrations rose by some 25% for a period of an hour. In fasted birds a fall in non-esterified fatty acids was also observed. A striking finding following these APP injections in the chicken was a decrease in concentration of all plasma amino acids. The effect on alanine was particularly marked with a fall to 50% of the basal level for at least an hour (Kimmel et al, 1978). A possible explanation for these findings is that APP stimulates hepatic lipogenesis by one or more of the following mechanisms; fatty acid synthesis from acetate, triglyceride formation or synthesis and mobilisation of lipoprotein. Glycogenolysis could therefore be secondary to the utilisation of acetate and glycerol-3-phosphate in which case hyperglycaemia would not be expected. In the chicken 90% of all lipid synthesis occurs in the liver and feeding is a potent stimulus for this process. A fall in plasma amino acid concentrations would accompany an increased rate of apolipoprotein synthesis in connection with lipid mobilisation.

It is clear that APP can produce changes in certain metabolic

parameters as well as gastrointestinal function although it is not known whether these actions are physiological.

Biological actions of PP in the dog.

Soon after the isolation and purification of bovine PP by Chance (in Indianpolis) this group undertook a systematic study of potential actions of the peptide in particular on the canine gastrointestinal tract.

Unlike insulin and glucagon BPP had no effect on blood glucose concentrations in the dog. In addition no change in plasma electrolytes or urine volume was observed following administration of PP. (Lin et al, 1977).

In fasting dogs acid secretion was stimulated gradually by an infusion of PP (40-100 $\mu g\ kg^{-1}\ hr^{-1}$). The maximal response of 10 mmol acid/hr occurred at the end of a 2 hour infusion (100 $\mu g\ kg^{-1}\ hr^{-1}$), was about one third of the response seen to pentagastrin infusion (4 $\mu g\ kg^{-1}\ hr^{-1}$) in these dogs. Thus BPP had a weak stimulatory effect on gastric parietal cells in the fasting state (Lin et al, 1977).

During sub-maximal steady state secretion induced by pentagastrin (0.5 $\mu g\ kg^{-1}\ hr^{-1}$) two separate doses of PP caused a significant inhibition of 32% (10 $\mu g\ kg^{-1}\ hr^{-1}$) and 72% (40 $\mu g\ kg^{-1}\ hr^{-1}$) respectively. (Lin et al, 1977).

In fasting dogs PP infusion (10 $\mu g\ kg^{-1}\ hr^{-1}$) reduced water and bicarbonate secretion by 80%. Steady secretion induced by cholecystokinin (CCK 0.5 IDU $kg^{-1}\ hr^{-1}$) and secretin (0.2 $\mu g\ kg^{-1}\ hr^{-1}$) was significantly inhibited by PP (1-5 $\mu g\ kg^{-1}\ hr^{-1}$). Maximal inhibitions in this stimulated state were 70% for protein secretion and 91% for volume and bicarbonate (Lin et al, 1977).

Endogenous CCK and secretin were released by continuous intraduodenal infusion of an iso-osmolar solution of NaCl, phenylalanine and tryptophan. The steady state secretion of water, bicarbonate and

protein was inhibited by BPP (1-5 μg kg^{-1} hr^{-1}) in a dose related manner (Lin and Chance, 1978).

Thus the inhibition of PP of pancreatic secretion induced by exogenous CCK-secretin was similar to that caused by endogenous CCK release.

In the fasting state intraluminal gall bladder pressure was reduced and resistence of the choledochal sphincter was increased during infusion of BPP (20 μg kg^{-1} hr^{-1}). Maximum decrease in gall bladder pressure was about 40% whilst choledochal sphincter pressure increased by 24% at the end of a two hour infusion. These results indicated that BPP had a relaxing effect on the gall bladder, a moderate stimulatory action on choledochal sphincter tone. BPP had no significant effect on bile flow from the liver (Lin and Chance, 1978).

Gut motility of conscious dogs was monitored by the use of implanted micro strain gauges, induction coils, unipolar electrodes and intraluminal catheters.

At high doses of BPP (40-50 μg/kg), administered as an intravenous bolus injection, violent contraction of the gut was observed which resulted in vomiting and defaecation (Lin and Chance, 1978).

BPP increased the motility of the antrum, duodenum, ileum and colon when infused at 2 to 25 μg kg^{-1} hr^{-1}. The frequency of action potentials recorded from the antrum and duodenum also increased significantly. This increased activity was followed by a long period of quiescence (1-2 hours) following termination of the infusions (Lin and Chance, 1978).

Effect of BPP on Gastric Emptying Rate and Intestinal Transit in Rodents

Gastric emptying in the rat was determined by the degree of retention of a phenol red containing pectin meal. At doses of between

1 and 100 µg/kg (intraperitoneal injection) gastric emptying was significantly enhanced. Average emptying of a 2 ml pectin meal was enhanced by 13% over a 15 minute period (Lin and Chance, 1978).

The intestinal transit of mice was studied using a charcoal-gum acacia meal. In BPP treated mice (10 µg/kg intraperitoneal injection) transit over a 15 minute period was significantly enhanced by 15% (Lin and Chance, 1978).

Possible Physiological Roles of Pancreatic Polypeptide

In mammals clues as to the physiological role of PP are found in its potent actions on the gastrointestinal tract. Inhibition of the exocrine pancreas, enhancement of gall bladder storage and stimulation of intestinal motility are all potential physiological effects.

In the chicken it would appear that the avian peptide counterpart of mammalian PP could have a metabolic role.

Lin and Chance concluded that the physiological role of PP could not be ascertained until comparative changes in plasma levels of PP physiologically released and pharmacologically induced are carefully determined (Lin and Chance, 1978).

CHAPTER 2. RADIOIMMUNOASSAY OF PANCREATIC POLYPEPTIDE

INTRODUCTION

History of Radioimmunoassay

During investigation of the metabolic fate of insulin in human subjects Berson and co-workers demonstrated that radioactively labelled insulin disappeared more slowly from the plasma of insulin treated diabetes than from normal subjects (Berson et al, 1956). Their results indicated that insulin binding antibodies had been produced in response to the administration of insulin (Berson et al, 1956). Using ^{131}I-labelled insulin they were able to show that the antigen-antibody complexes formed did not precipitate, that the binding of insulin to antibody was reversible and that at a constant antibody concentration the ratio of bound insulin to free insulin was an inverse function of the concentration of insulin.

As a direct result of these observations, Berson and Yalow developed the first radioimmunoassay system which enabled them to measure bovine, porcine, ovine and equine insulin concentrations by competitive inhibitions of binding at ^{131}I-insulin to human antisera (Berson and Yalow, 1958). In 1959 they reported an assay for the measurement of fasting human plasma insulin using antibodies raised in guinea pigs immunised with crystalline bovine insulin.

In the years that have followed this outstanding discovery, the radioimmunoassay system, with its overall simplicity and enormous potential for sensitivity and specificity has become the most important methodological advance in endocrinology. The techniques of Berson and Yalow have been applied to the measurement in tissue and plasma of most peptide hormones and this has given rise to an increased understanding

of both the physiology of such hormones and an insight into their role in disease.

General principles of radioimmunoassay

There are three basic requirements for the development of a radioimmunoassay. Antibodies which are specific for the hormone or other antigen to be measured, radioisotopically labelled hormone is required as a tracer and pure hormone (antigen) is needed to provide reference standards.

In principle the radioimmunoassay depends on the competition between labelled hormone and cold hormone (unknown sample or reference standard) for a limited number of binding sites on the antibody. The percentage of labelled hormone bound to the antibody will be inversely proportional to the amount of unlabelled hormone present. As a result of this competitive inhibition, the percentage of labelled hormone bound to antibody is reduced progressively as the concentration of unlabelled hormone in known standard solutions or in unknown samples is increased. The second phase of the assay system is separation of the "free" antigen from that which is antibody bound. After determining the proportion of radioactivity in each fraction the concentration of unknown antigen (i.e. endogenous hormone in a plasma sample) may be determined by comparison with the distribution of radioactivity in tubes from a standard curve.

Advantages of radioimmunoassay

When compared with bioassays the radioimmunoassay offers several advantages. Radioimmunoassay is more sensitive, often capable of detecting a few femtomoles of hormone which is well beyond the capabilities of most bioassays. Secondly, there is a potential for the radioimmunoassay to be extremely specific. Thirdly, the simplicity of the system permits

the simultaneous estimation of a large number of samples and the results are therefore in general more precise and reproducible than those of the bioassay. Finally, owing to their sensitivity radioimmunoassays allow the assay of small volumes.

Problems of radioimmunoassay

The radioimmunoassay is a structural assay, recognising a particular molecule configuration rather than biological activity of a peptide hormone. The antigenic determinant, or site on a peptide recognised by the binding site, of an antibody molecule usually comprises of only three or four amino acids although if the peptide has a folded structure these need not be adjacent residues. As the antibody recognises structure, in addition to hormone being measured, inactive precursors or fragments of the hormone, as well as unrelated proteins which happen to exhibit the amino acid sequence of the antigenic determinant, may be detected. It should be remembered therefore that this type of structural assay should not be equated with biological activity.

An additional problem is that any external agent other than the antigen which alters the binding of antigen to its specific antibody will cause interference in the assay and will give rise to error. Much effort has been made to eliminate or at least control assay interference but the variety of causes has made this task difficult and many remain unidentified. Factors in individual plasma samples which are known to cause interference include high blood urea, abnormal plasma proteins and haemoglobin released from haemolysed red cells. In some assays standards are incubated in buffer alone and thus differ from the unknown samples in that plasma is absent. Because the antigen-antibody reaction may be influenced by small changes in pH, ionic strength and protein concentration of the assay medium it is possible that the samples behave in a different way to the

standards. This problem may be overcome either by extracting the hormone from the unknown sample and assaying the extract in a buffer system or by the addition of hormone free plasma to the standard curve. Both of these methods have obvious disadvantages, the former in that the extraction method is tedious and may not recover all the endogenous hormone or fail to isolate the hormone free from all interfering material and the latter is that the method used to render the plasma hormone-free (e.g. adsorption to charcoal or resin) will remove other molecules as well. This last technique has been improved in recent years by the development of specific immunoadsorbents.

Adsorption of peptide to plastic or glass surfaces may be a problem but this can be minimised by the addition of inert protein to the assay buffer.

Stability of peptides is often poor and degradation may be reduced by the addition of proteolytic enzyme inhibitors and bactericidal agents to the buffer and by incubation of the assay at $4^{o}C$.

Above all it is important to know what the nature of the immunoreactive hormone is in an unknown sample. Antibodies should be fully characterised including, where possible, cross-reaction studies with synthetic hormone fragments. In addition the molecular forms of immunoreactive hormone can be measured by radioimmunoassay following chromatographic separation.

Production of antibodies

A radioimmunoassay is only useful if adequately sensitive and is specific for the hormone being measured. The production of a high affinity, specific antibody is therefore fundamental to the development of a radioimmunoassay. Antibodies are usually raised in laboratory animals (rabbits and guinea pigs) by giving repeated injections of hormone

in emulsified form with an adjuvant. Although large proteins make excellent immunogens small peptides are often not very antigenic. Small peptides may however be treated as haptens and when covalently linked to a large carrier molecule such as serum albumin, make excellent immunogens.

Immunogen dosage does not need to be large and antibodies may be successfully raised using primary doses 30-50 nmol with later boosting with half of that dose. The site of immunisation is probably not of great importance but multiple injections given intramuscularly, subcutaneously or into the lymph nodes are often employed.

Radioactive labelling of antigen

The most common isotope used for radiolabelling of peptides is ^{125}I partly because of the ease of iodination reactions and partly because this isotope has a relatively long half life (60 days) coupled with reasonable efficiency in counting the low energy gamma radiation emitted.

Ideally the labelled antigen should be of high specific activity, homogenous and possess an affinity for the specific antiserum similar to that of unlabelled antigen. In addition it should be stable so that frequent labelling is unnecessary.

The reaction involves oxidation of sodium ^{125}iodide (the chemical form in which the isotope is supplied) to free ^{125}Iodine which then undergoes direct substitution into the aromatic ring of a tyrosyl residue in the peptide molecule. Termination of the reaction is affected by addition of a reducing agent, usually sodium metabisulphite, which converts unreacted iodine back to the iodide form. The most commonly used oxidising agent is chloramine T as used by Hunter and Greenwood (1962), however some workers have employed the milder oxidation conditions offered by

enzymatic iodination using lactoperoxidase (Morrison et al, 1971, Thorell and Johansson, 1971).

Following iodination the tracer is purified to remove damaged or non-iodinated antigen, unreacted iodide and other reactants. Purification methods include chromatography and ion exchange chromatography. Gel filtration using "Sephadex" columns is one of the most widely adopted methods, simply separating ^{125} Iodide and the other reagents from the iodinated antigen on a molecular size and shape basis. Although rapid, much care is necessary in handling nanomole quantities of peptide on this system as heavy losses may occur due to adsorption to the glass column or to the gel itself. By selecting a grade of "Sephadex" higher than one which would be required for simple desalting it is sometimes possible to broaden to peptide peak so that some fractions show higher immunoreactivity than others. Ion exchange chromatography or electrophoresis on starch or polyacrylamide gel, provides a more discriminating separating system for the fractionation of iodated hormones. With ion exchange systems the rapid separation of pure monoiodinated peptides is possible and such high specific activity labels tend to be stable over several months

RADIOIMMUNOASSAY OF PANCREATIC POLYPEPTIDE

Source of Pancreatic Polypeptide (PP)

Both bovine pancreatic polypeptide (BPP)and human pancreatic polypeptide (HPP) were isolated from acid-ethanol extracts of pancreas using multiple gel filtration and ion-exchange chromatographic steps (Chance and Jones, 1974). The materials used on these studies were all generously donated by Dr.R.E.Chance.

Immunisation

Antibodies against BPP and HPP were raised in Dr.Chance's laboratory using New Zealnd white rabbits according to the following general schedule of injections. Each animal received a primary immunisation consisting of 1.0 mg of immunogen dissolved in 0.6 ml distilled water and emulsified with 0.6 ml complete Freund's adjuvant (Difco Laboratories), Detroit, Michigan) and injected at different sites (0.3 ml in each hind footpad, 0.2 ml subcutaneously, 0.2 ml intramuscularly). A booster immunisation was given one month later using 0.4 mg immunogen dissolved in 0.3 ml distilled water and emulsified with 0.3 ml incomplete Freund's adjuvant (Difco) and administered at multiple sites (0.1 ml each hind footpad, 0.2 ml subcutaneously and 0.2 ml intramuscularly). Similar booster immunisations were repeated at 6 to 12 month intervals.

The rabbits were bled at one or two week intervals following each booster immunisation.

Radioiodination of PP

Labelled ^{125}I HPP was prepared by a modification of the conventional chloramine T method (Hunter and Greenwood, 1962). 250 pmol (1 µg) HPP was iodinated with 0.5 nmol carrier free Na ^{125}I (IMS 30, Radiochemical Centre, Amersham, Bucks) using 70 nmol chloramine T as an oxidising agent, in a total volume of 200 µl 0.05 M phosphate buffer pH 7.4 for 15 seconds at room temperature. The reaction was stopped by the addition of 100 nmol sodium metabisulphite and 500 ul of column buffer containing 50 mmol sodium iodide.

Purification of Radio-labelled PP

The radioactively labelled PP was purified by gel permeation chromatography using Sephadex G50 superfine (Pharmacia Ltd). The label produced was eluted from a 0.9 x 60 cm column (K9 x 60, Pharmacia Ltd) with 0.1 M formic acid containing 150 µmol/l Human Serum Albumin (Lister Institute. The buffer was pumped to the column at a rate of 3 ml/hour and 0.5 ml fractions were collected. Fractions containing radioactivity were tested under normal assay conditions for adsorption to charcoal (blank) and for binding to antiserum at the normal assay concentrations and also in excess. Fractions demonstrating the highest antibody binding and the lowest charcoal blank were pooled, diluted in a special solution for freeze drying (see below, standards) lyophilised at -20°C in portions each sufficient for one assay of 500 tubes.

Preparation of Standards

Vials of standard hormone were prepared from the lyophilised material generously donated by Dr.R.E.Chance (Lilly Research Laboratories).

This was dissolved in a solution designed to inhibit losses by surface adsorption or oxidation and which was easily solubilised after lyophilisation. The constituents of this solution were 0.14 mol lactose/l, 0.04 mol BSA/l, 11 nmol citric acid/l, 6 nmol cysteine hydrochloride/l and 1600 KIU aprotinin (Trasylol, Bayer Ltd.) /ml in 0.1M formic acid. Vials containing 1 pmol PP were freeze dried to 10^{-2} torr, sealed in vacuo and stored at -20°C.

Assay Procedure

Samples were assayed in duplicate 2 ml polystyrene tubes (LP3, Luckham Ltd). The plasma sample (200 µl) was added to 600 µl 0.05 M sodium barbitone buffer pH 8.0, containing 1% HSA and 1.5 fmol labelled PP (approximately 100 counts/S). Antiserum was added to make a final dilution of 1 : 800 000. Tubes for the standard curve and other control tubes contained 200 µl human plasma pooled from healthy fasting subjects, from which the endogenous PP had been removed. The preparation of this "hormone free plasma" was effected by charcoal adsorption (Albano et al, 1972). PP was extracted by the addition of 50 g charcoal/l (Norit GSX, Hopkin and Williams Ltd), the slurry being constantly stirred for 20 minutes at 4°C. The suspension was then separated by centrifugation for one hour at 2000 g at 4°C. Final separation of the remaining traces of charcoal was effected by overnight centrifugation (16 h) at 2000 g at 4°C.

Standard curves were derived by the addition to this plasma of pure human PP in the range of 0-100 fmol/tube.

The assay constituents were incubated for 120 hours at 4°C before separation.

Separation of antibody bound from free PP was achieved using dextran-coated (clinical grade dextran, approximate molecular weight 60-90 000 : Sigma Co Ltd) activated charcoal (Norit GSX). This

as added to 500 µl slurry in 0.05 M barbitone buffer, pH 8.0 to each assay tube. The assay tubes were then mixed, incubated for 10 minutes and centrifuged at 200 g for 20 minutes at 4°C. The supernatant fraction (containing the antibody bound fraction) was aspirated and both this and the charcoal precipitate were counted sequentially in the same well of a multi detector gamma counter (NE 1600, Nuclear Enterprises Ltd). The dose response metameter used was percentage antibody bound.

Charcoal Separation

The optimum concentration of charcoal was determined for each new batch of charcoal by serial additions of buffer containing charcoal in decreasing amounts from 64 - 2 mg/tube, each dilution being made up with 100 g dextran/l and plasma added at 10% (volume). This was added to the tubes containing no antibody (blank), normal antibody concentration (zero), excess antibody and in addition to tubes containing normal antibody concentration and 10 fmol of standard HPP.

Antibody Specificity

Antiserum specificity was tested by the serial addition of 1 pmol/l to 10 nmol/l of pure glucagon, insulin, vasoactive intestinal peptide, gastrin inhibitory peptide, secretin, gastrin, motilin, neurotensin, cholecystokinin, somatostatin, substance P, bombesin and urogastrone. In addition standard curves of bovine and porcine PP were compared with those of human material.

Stability of PP in Plasma

Initial studies indicated that endogenous and exogenous PP was stable for at least 24 hours in both plasma and whole blood kept at room temperature.

The stability of PP in plasma was further tested by the addition of 25 pmol/l and 100 pmol/l of pure human PP to aliquot of a sample of plasma from freshly drawn, heparinised blood taken from five non-fasted healthy subjects. The three fractions (1) containing only endogenous PP, (2) containing endogenous PP + 25 pmol/l added PP, (3) containing endogenous PP + 100 pmol/l added PP.) were then subdivided into 5 aliquots for stability testing. One set of aliquots was frozen immediately, two sets kept at room temerature for 3 days and 10 days respectively and two further aliquots which were frozen and thawed 5 times and 20 times respectively. All samples were then stored at $20^{o}C$ and estimated in a single assay.

Assay Reproducibility

Assay reproducibility was evaluated by the inclusion in twenty consecutive assays of four quality control plasma samples. These were chosen from plasma containing high or low endogenous PP concentrations and were divided and lyophilised as 1 ml samples. Prior testing of recovery after lyophilisation of plasma with added PP had shown that losses due to this procedure of less than 2%. Within assay variation was calculated by including the same quality control samples in five batches of quadruplicates in a single assay.

The recovery of PP added to a nominal concentration of 100 pmol/l plasma from five patients who had undergone total surgical pancreatectomy was also tested.

Testing of Assay Incubation Conditions

Plasma effects on the antiserum were investigated by setting up two standard curves, one in buffer alone and the other in PP free plasma. Three sets of such "interlocked" curves were incubated for 3, 5 and 7 days respectively. This test was also used to establish optimum incubation times for buffer and plasma assays. In addition, samples of plasma from three fasting and three postprandial normal subjects and three fasting patients with pancreatic endocrine tumours secreting PP (PP omas) were serially diluted in PP free plasma and then assayed to determine parallelism.

To test the effect of pH and buffer type on the assay standard curves were set up in three different buffers both in the presence and absence of plasma. The buffers used were 0.05 M barbitone pH 8.0, 0.05 M phosphate pH 7.4 and 0.05 M acetate pH 6.5. These curves were interlocked and incubated for 5 days.

Analysis of Plasma Molecular Forms

To analyse the molecular forms of PP in the circulation plasma samples were taken from six healthy volunteers both after an overnight fast and 30 minutes after lunch. These were chromatographed on a 0.9 x 100 cm column containing Sephadex G-50 superfine gel (Pharmacia Ltd) and were eluted overnight with 0.1 M formic acid pumped at a rate of 5 ml/h. Dextran blue, cytochrome C and $Na^{125}I$ were added to each sample as internal markers of molecular size. Column calibration was carried out with 2 ml PP free plasma (charcoal adsorption) to which 1 pmol of pure human PP was added. This was performed before and after chromatography of a series of plasma samples. The column conditions

remained stable throughout. All fractions collected were stored at -20^{o}C before being assayed. Elutions coefficients of the molecular forms of PP detected by gel permeation chromatography were calculated as average elution coefficients by the method of Laurent and Killander (1964).

RESULTS

Immunisation and Selection of Antibodies

One of four rabbits immunised with BPP developed a particularly high titre antiserum six weeks after the first booster injection. This antiserum Lot No.615-R110-146-6 was found to be useful for radioimmunoassay at high dilution.

The development of an acceptable HPP antiserum took longer with only one of six animals responding with a good antiserum after either the second or third booster immunisation. For example, the working dilutions necessary to bind 60% of the ^{125}I-Hpp were as follows: (a) first booster (7-13 week bleedings) 1 : 3000 (b) second booster (week 4 bleeding) 1 : 80 000 (c) third booster (week 4 bleeding) 1 : 120 000 (d) fourth booster (week 2-4 bleedings) 1 : 70 000. The antiserum collected after the third booster (Lot No.615-1054B-249-19) is the main antiserum used for the HPP assay. The antisera was diluted in normal rabbit serum, lyophilised and stored at -20^{o}C.

In the standard radioimmunoassay system for plasma, this antibody bound 51% of 2 fmol ^{125}I-labelled PP at a final dilution of 1 in 800 000. The binding affinity of the antiserum in buffer according to the method of Scatchard (1949) was $6.6 \pm 0.4 \times 10^{11}$ 1/mol (mean $\pm$ SEM n = 6) and was similar to that seen with the addition of plasma ($6.8 \pm 0.4 \times 10^{11}$ 1/mol n = 6).

Radioiodination

Chromatography of the iodination products resulted in two major peaks of radioactivity, the first of which contained PP immunoreactivity

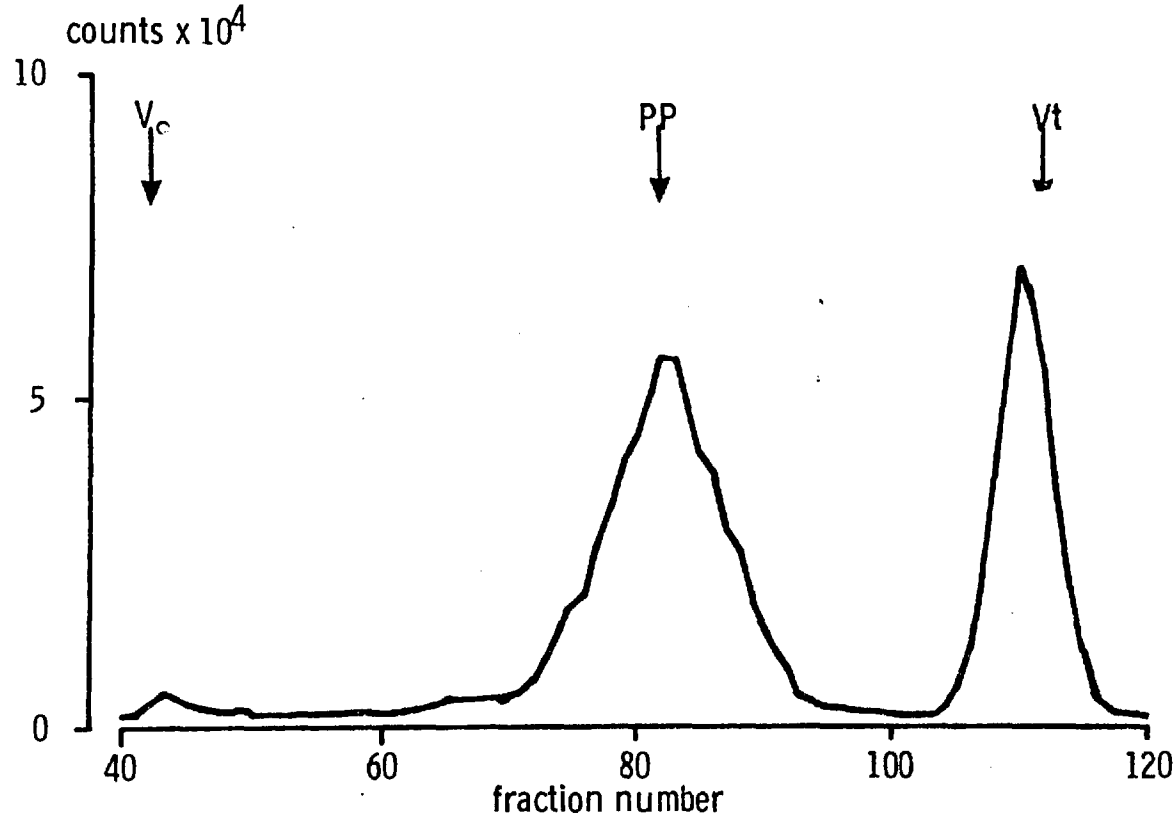


Figure 201:

The iodination profile of PP separated on Sephadex G-50 superfine. Two major peaks of radioactivity were eluted, the first is immunoreactive PP and the second is unreacted ^{125}Iodine. Fractions from the ascending side of the PP peak usually showed lowest non-specific binding and highest binding to antibody

(see Figure 201). Fractions from the ascending side of this peak demonstrated the highest binding of excess antibody and the lowest charcoal blank. The specific activity of this peptide calculated by self-displacement on the standard curve was 67 $\pm$ 9 Bq/fmol (n = 8) and was similar to that obtained by stoichiometric analysis of the iodination reaction. Preparations tested after a period of three months showed that the specific activity had dropped to 44 $\pm$ 5 Bq/fmol (n = 8).

Radioimmunoassay Procedure

The standard curve of the assay in plasma and in buffer is shown in Figure 202. Addition of 3 fmol PP caused displacement of binding of labelled antigen from 50% to 40%. The sensitivity of the assay defined as two SD of the zero standard was 0.6 fmol/tube (3 pmol/l plasma).

The optimum charcoal concentration for separation of the free from bound hormone was found to be 4 mg/tube, but half or double this amount affected the zero binding by only about 1%. See Figure 203. Non-specific binding at this concentration of charcoal was 6% zero binding 52% and excess antibody binding 95%. These conditions were found to be identical for buffer and plasma assays.

The non-specific binding (charcoal blank) of fresh label preparations was 6 $\pm$ 1% and after three months this had risen to 12 $\pm$ 2% (n = 10).

Standard curves with exogenous PP in buffer or plasma were parallel (Figure 202) and this was also the case for endogenous PP in plasma from subjects taken both fasting and postprandially.

Antibody Specificity

The antiserum showed no significant cross-reactivity with all the

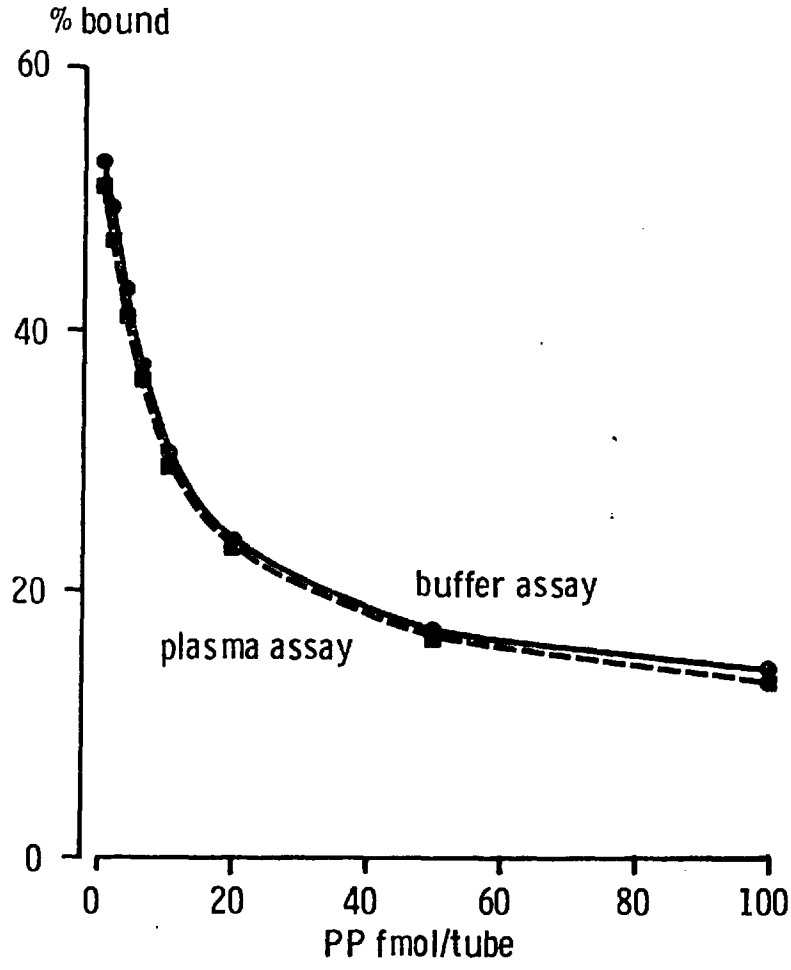


Figure 202:

Standard curves of PP in plasma and buffer. These curves were interlocked and incubated for 7 days at $4^{o}C$.

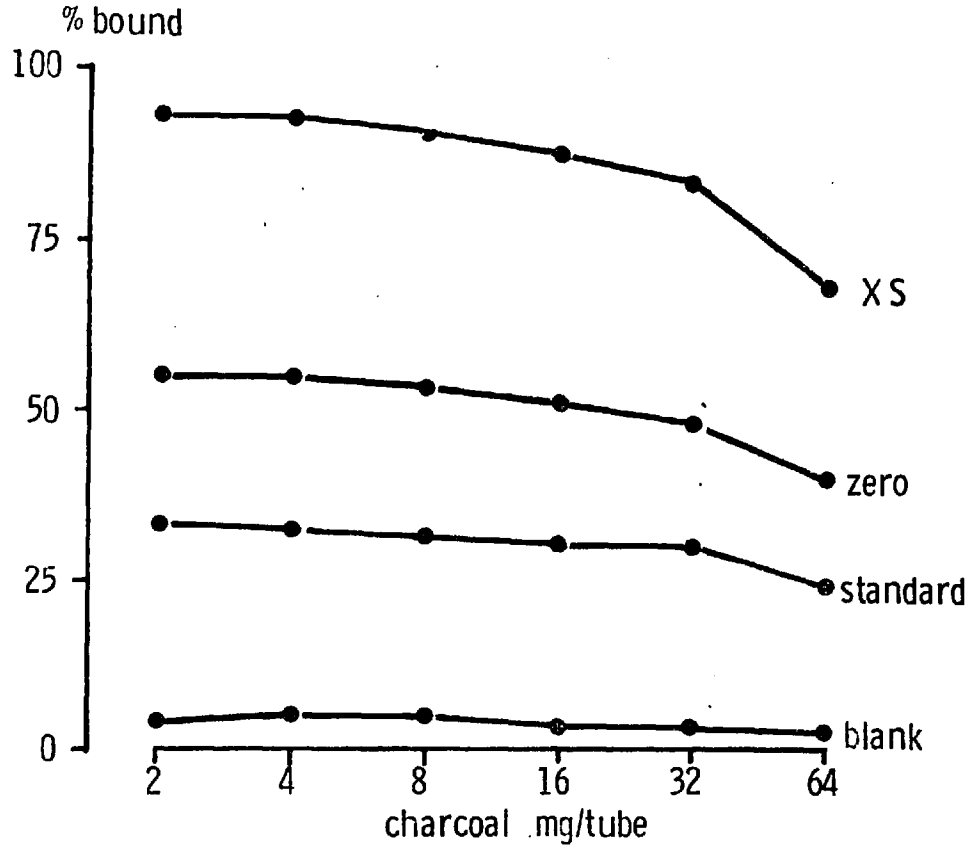


Figure 203

The effect of increasing charcoal concentration on binding of labelled PP in the absence of antibody (blank), presence of antibody (zero), antibody and standard (10 fmol/tube) and excess antibody. Charcoal effects were identical in buffer and plasma assay systems.

other peptides tested with the exception of human and porcine PP which gave essentially superimposable standard curves compared with the human material.

Stability in Plasma

Both exogenous and endogenous immunoreactive PP remained completely stable under all the conditions tested and losses were less than 5% (Table 201). Thus PP is stable in whole blood for at least 24 hours, in sterile plasma for at least 10 days and in addition is unaffected by repeated freezing and thawing up to 20 times. Unlike several other gut hormones the addition of a proteolytic enzyme inhibitor is not required.

Assay Reproducibility

The coefficient of variation between assays for the four plasma samples was 8.0%. 8.4%, 6.1% and 6.5% respectively. The within assay variation for the same samples was 5.4%, 5.6%, 4.0% and 3.6% respectively. Between assay variation for these same samples can be seen in Figure 204.

Incubation Conditions

As can be seen from Table 202 there was a small increase in assay sensitivity from 3 days to 5 days incubation in both buffer and plasma assays (10% fall in binding 5 fmol/tube at 3 days, 3 fmol/tube at 5 days) and plasma assays (10% fall in binding 25 pmol/l at 3 days, 15 pmol/l at 5 days). There was no further increase in sensitivity from 5 days to 7 days in either system.

THE STABILITY OF PP IN PLASMA

	ENDOGENOUS PP	EXOGENOUS (25 pmol/1) AND ENDOGENOUS PP	EXOGENOUS (100 pmol/1) AND ENDOGENOUS PP
PLASMA SEPARATED AND FROZEN IMMEDIATELY	19.8 ± 4.5	45.5 ± 5.6	119.2 ± 7.6
PLASMA AT ROOM TEMP. 3 DAYS THEN FROZEN	20.8 ± 4.3	46.0 ± 5.0	118.1 ± 8.0
PLASMA AT ROOM TEMP 10 DAYS THEN FROZEN	18.5 ± 4.3	44.3 ± 5.0	116.8 ± 7.8
PLASMA FROZEN AND THAWED 5 TIMES	18.3 ± 4.1	44.5 ± 5.1	121.1 ± 6.9
PLASMA FROZEN AND THAWED 20 TIMES	19.8 ± 4.6	44.5 ± 5.0	118 ± 7.3

TABLE 201: Plasma PP concentrations (pmol/1) after different conditions of storage. Samples were taken from five healthy subjects and exogenous pure human PP was added to portions of the sample.

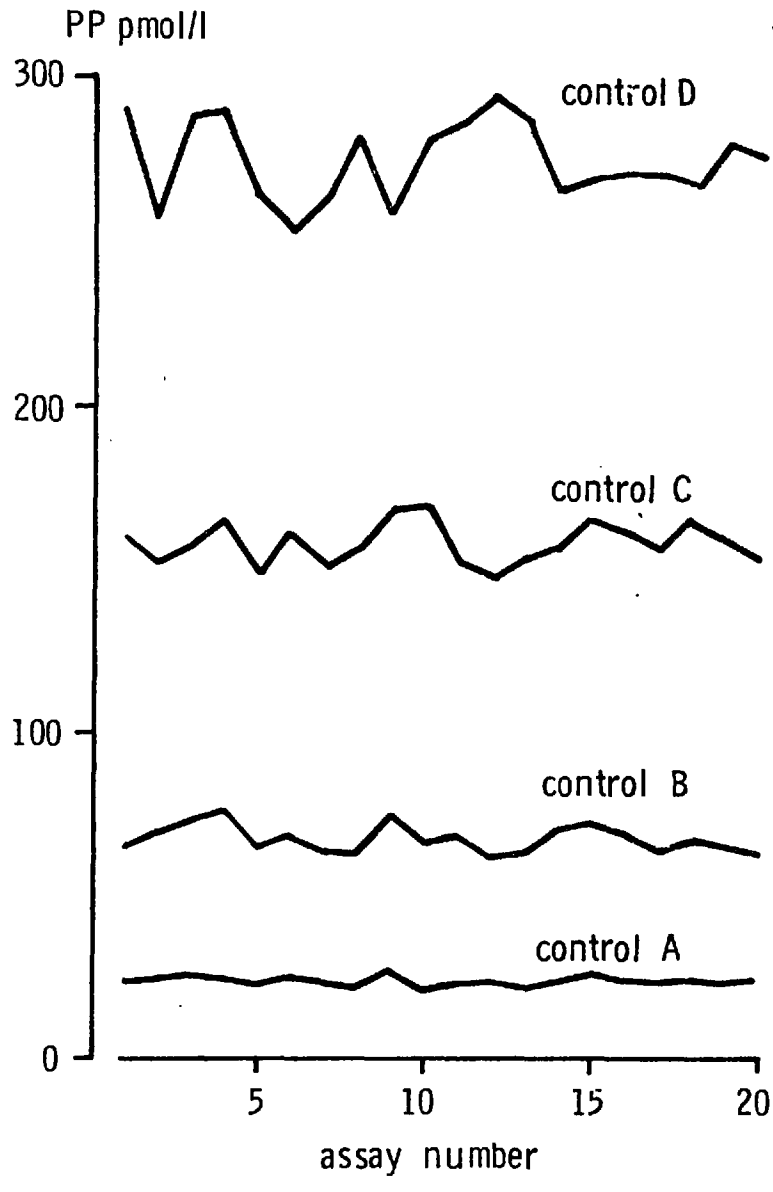


Figure 204:

The concentrations of four freeze dried quality control plasma samples run in each of 20 consecutive assays.

TEST FOR OPTIMUM INCUBATION TIME OF PP ASSAY

	BUFFER ASSAY			PLASMA ASSAY		
DAYS	3	5	7	3	5	7
BLANK	5	5	6	5	5	5
ZERO	46	50	53	44	49	50
10 fmol STANDARD	29	29	30	28	30	30
EXCESS ANTIBODY	93	92	94	94	94	94

TABLE 202: The effect of incubation time on % binding in the absence of antibody (blank), in the presence of antibody (zero), antibody and standard (10 fmol/tube) and in the presence of antibody in excess.

Table 203 shows the effect of different buffers on the assay of PP both in buffer and plasma assay systems. Barbitone and phosphate buffers (pH 8.0 and 7.4 respectively) give superimposable standard curves with identical sensitivity. The acetate buffer also gave similar sensitivity to the other buffers but antibody binding in the excess situation was slightly reduced as was zero binding in the buffer assay system.

Analysis of Normal Circulating Molecular Forms

In plasma from healthy fasting subjects only one major peak of PP like immunoreactivity was detectable after gel permeation chromatography. This peak eluted in the position of the pure human standard. The average elution coefficient (Kav) of this peak was 0.45 ± 0.02 ($n = 6$). The size of the peak increased substantially in all six subjects following the ingestion of food. The individual elution profiles can be seen in Figure 205.

TEST OF BUFFER TYPE FOR PP ASSAY

	BUFFER ASSAY			PLASMA ASSAY		
BUFFER TYPE	BARBITONE pH 8.0	PHOSPHATE pH 7.4	ACETATE pH 6.5	BARBITONE pH 8.0	PHOSPHATE pH 7.4	ACETATE pH 6.5
BLANK	6	4	4	5	5	5
ZERO	53	53	49	52	53	54
10 fmol STANDARD	33	33	28	30	31	32
EXCESS ANTIBODY	93	93	89	94	94	92

TABLE 203: The effect of buffer type on % binding in the absence of antibody (blank), presence of antibody (zero), antibody and standard (10 fmol/tube) and in the presence of excess antibody.

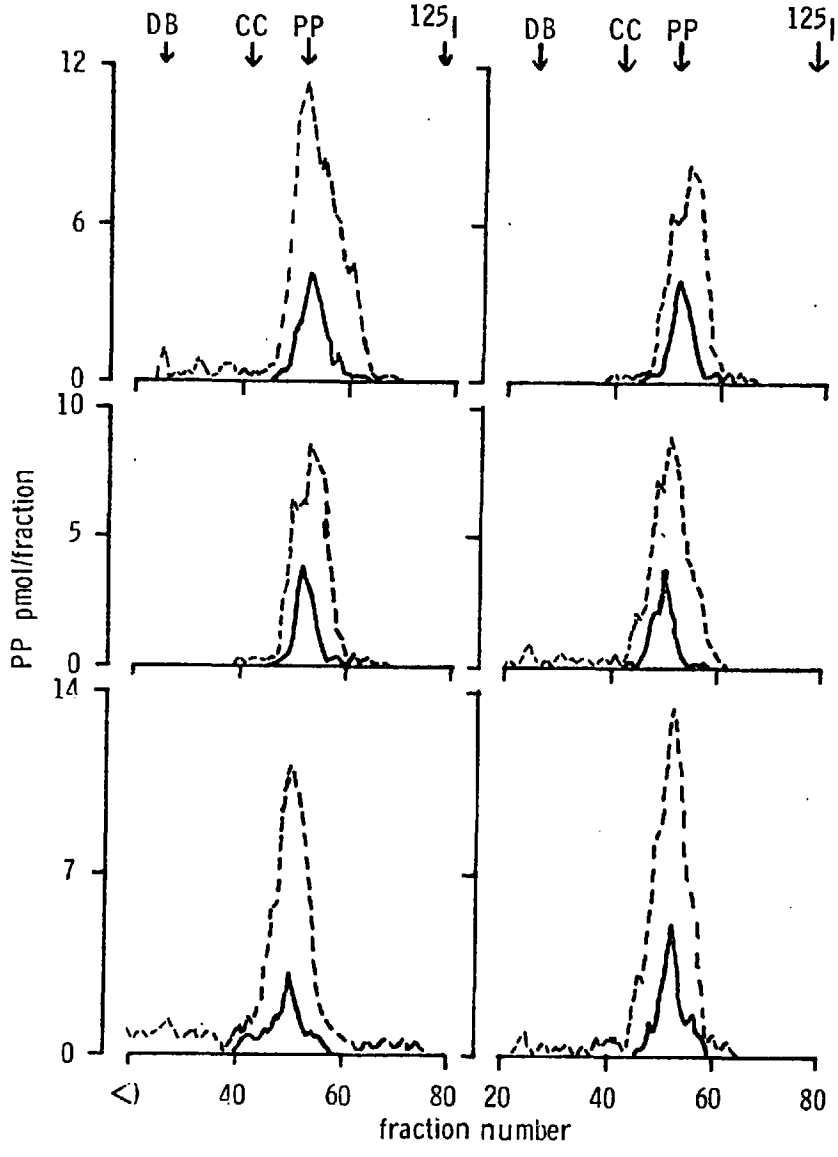


Figure 205:

Plasma immunoreactive PP column profiles from 6 healthy subjects taken before and after lunch (Sephadex G50 superfine) Dextran blue, cytochrome C and ^{125}I were added to each sample as molecular weight markers

DISCUSSION

Radioimmunoassay of Pancreatic Polypeptide

The radioimmunoassay of pancreatic polypeptide in human plasma has presented no major difficulties. A unique situation has arisen in that all the reported radioimmunoassays for this peptide use the same antibody and pure peptide preparations which have been made available to all interested research groups by Dr.R.E.Chance (Chance et al, 1978). This similarity between methodologies in different centres had led to an unusual high degree of agreement between different laboratories on both circulating and tissue levels of PP.

The sensitivities of all the assays described in the literature are similar and although sensitivity is readily improved still further, this is not usually necessary as circulating concentrations of the peptide are relatively high. Sensitivity can be improved by preincubation of the sample with antibody followed by a second incubation period with labelled PP. In addition the use of ion exchange chromatography or polyacrilamide gel electrophoresis for the purification of labelled PP can produce a homogenous product of high specific activity and consequent improvement in assay sensitivity (Marco et al, 1978).

The similarity of the K values of the antiserum in buffer and plasma would suggest that it is not unduly sensitive to protein interference thereby implying little benefit from plasma extraction procedures as such.

The full characterisation of this antibody must await the availability of synthetic fragments of PP in order to establish to which sequence or part of the molecule the antibody is directed.

Endogenous PP in plasma and exogenously added pure material are extremely stable and therefore the addition of proteolytic enzyme inhibitors to sample for PP measurement is not essential.

Assay conditions for PP do not appear to be critical and several different buffers have been used with success including phosphate, barbitone and acetate buffers in pH ranges from 6 to 8.5 (Chance et al, 1979). Similarly several separation methods appear to be successful in the PP assay including double antibody, charcoal adsorption and alcohol precipitation (Chance et al, 1979).

Compared with the radioimmunoassay of several other peptide hormones the assay of PP is relatively straightforward and the published results suggest a high degree of reproducibility and agreement between different groups of workers.

CHAPTER 3. DISTRIBUTION OF PANCREATIC POLYPEPTIDE

INTRODUCTION

Distribution of Pancreatic Polypeptide

As both avian and mammalian PP were isolated from pancreatic extracts they were clearly produced in that organ. The distribution throughout the rest of the gastrointestinal tract however was unknown. The distribution of PP was investigated in sub-human primates and in adult and foetal man. In addition, the PP content of pancreatic endocrine tumours was investigated.

It has become apparent in recent years that most polypeptide hormones exist in several molecular forms. Some, like proinsulin, are precursor molecules but for most of the gastrointestinal hormones the role of different molecular forms is uncertain.

Gel chromatographic studies were utilised to establish whether one or several molecular forms of PP-like immunoreactivity could be detected in both normal and neoplastic tissue.

METHODS

Distribution of PP in the Primate Gastrointestinal Tract

The complete intestinal tract was removed after brief anaesthesia from three adult baboons and two monkeys (Macaca Iris) all of whom had been fed on a human diet for several years. In addition, heart and liver were taken as control tissues. After cleansing the bowel with cold running water and rapid dissection into anatomical regions, all tissues

were plunged into freezing acid ethanol (75% ethanol containing 100 mmol/l hydrochlorine acid at -40°C) to prevent enzymic degradation of PP. The tissues were subsequently stored separately in acid ethanol at -20°C until the time of extraction when they were weighed while still frozen, minced, and then homogenised at 10,000 rpm for 10-15 minutes in 5 ml acid ethanol/g of tissue wet weight. In order to minimise losses of peptide material which may have been released during initial freezing and storage, the tissues were homogenised in the acid ethanol in which they had been stored. The resulting homogenates were allowed to stand at 40°C with constant stirring for one hour in order to solubilise the peptides and then centrifuged at 2500 g for 30 minutes. The clear supernatants were decanted and saved and the tissue pellets re-extracted another three times with 4 ml, 3 ml and finally 2 ml of acid ethanol/g of tissue. After final centrifugation, the tissue debris was discarded and the supernatants from each region of the gut pooled. The extracts were neutralised and the resulting precipitate removed by further centrifugation. The precipitate was dried, redissolved in 100 mmol/l formic acid and assayed to determine whether any loss of hormonal immunoreactivity occurred in this precipitate. Aliquots of the supernatant peptide liquors were dried down under nitrogen and reconstituted in buffer before assay. The bulk of the peptide fraction was stored at -20°C for subsequent column gel chromatographic studies of hormone immunoreactivity. For PP radioimmunoassay samples of the extracts were added in duplicate 10 fold serial dilutions from 100 µl to 0.001 µl of extract per assay tube.

In order to assess the ability of PP to withstand the extraction procedure, a series of separate pilot studies was performed. The recovery of endogenous PP and radioactive PP (using ^{125}I - PP) was monitored at each step of the process outlined previously. The label was introduced into the system by addition to the acid ethanol used for the first homogenisation. In an attempt to determine the degree of damage caused during

extraction an evaluation was made of the ability of iodinated PP recovered at each step to bind to anti PP antiserum. These studies allow assessment of PP recovery once the hormone is solubilised but does not assess the efficiency of the extraction itself.

Distribution of PP in Adult Man

Pieces of tissue, removed at operation and subsequently assessed as normal by histological examination were used to study the distribution of PP in man. In addition human brain removed at autopsy 8 hours after death was obtained. All tissues were kept deep frozen until the time of extraction. Each region was boiled for 10 minutes in water (5 ml/g wet tissue), centrifuged 2500g for 30 mins) and the sediment homogenised in water (5 ml/g wet tissue) and allowed to extract for 30 minutes after each homogenisation. The centrifugation and extraction was repeated once in water and twice in 0.1 M formic acid. The water and formic acid extracts for each tissue sample were pooled independently. Recovery and damage was quantified by adding ^{125}I-PP to some tissue samples after the first homogenisation. The ability of the iodinated PP to bind to antibody was assessed at each step in the extraction procedure. Recovery of added exogenous PP was also assessed in tissue samples.

For PP radioimmunoassay samples of both water and formic acid extracts were added in duplicate 10 fold serial dilutions from 100 µl to 0.001 µl of extract per assay tube.

Distribution of PP in the Human Foetus

Foetal tissue was obtained from therapeutic abortions (n = 9) and from stillbirths (n = 11) and in addition was obtained at autopsy from 5 infants with sudden infant death (cot death). The tissues used in the study were assessed histologically and when found to be normal were used

to assess the distribution of PP. All tissues were kept deep frozen (below 20°C) until the time of extraction. Each region was boiled for 10 minutes in water (5 ml/g wet tissue), centrifuged (2500g for 30 mins) and the sediment homogenised in water (5 ml/g wet tissue) and allowed to extract for 30 minutes after each homogenisation. The centrifugation and extraction was repeated once in water and twice in 0.1 M formic acid. The water and formic acid extracts were pooled independently.

For PP radioimmunoassay samples of both the water and formic acid extracts were added in duplicate 10 fold serial dilutions from 100 µl to 0.001 µl of extract per assay tube.

PP Content of Pancreatic Endocrine Tumours

Surgical samples of endocrine tumours (apudomas) were derived from a number of patients with established syndromes due to insulinoma, vipoma (Bloom 1978) gastrinoma, glucagonoma (Mallinson et al, 1974) and from patients with the carcinoid syndrome. The tissues were kept deep frozen (below -20°C) until the time of extraction. Each sample was boiled for 10 minutes in water (5 ml/g wet tissue), centrifuged (2500g for 30 mins) and the sediment homogenised in water (5 ml/g wet tissue) and allowed to extract for 30 minutes after each homogenisation. The centrifugation and extraction was repeated once in water and twice in 0.1 M formic acid. The water and formic acid extracts were pooled independently.

For PP radioimmunoassay samples of both water and formic acid extracts were added in duplicate 10 fold serial dilutions from 100 µl to 0.001 µl of extract.

Chromatographic Analysis of the Molecular Forms of PP in Normal and Neoplastic Tissue.

The molecular forms of PP in normal pancreas (n = 6) and in pancreatic

endocrine tumours (n = 8) and carcinoid tumours (n = 2) were investigated using gel permeation chromatography.

Chromatographic analysis was carried out using a 60 x 0.9 cm column of Sephadex 6.50 superfine eluted with 0.06 M phosphate buffer pH 7.4 containing 0.2 M Na Cl, 0.01 M EDTA (ethylene diamine tetra acetic acid) and 70 uM H.S.A.(human serum albumin). The column buffer was pumped at the rate of 3 ml/1 and fraction size was 0.5 ml. The column was precalibrated with pure HPP standard and dextran blue (void volume), cytochrome C, and Na ^{125}I (total column volume) were mixed with each sample and run as column markers. The total sample volume in each case was 0.5 ml.

RESULTS

Distribution of PP in the Primate Intestine

PP from both baboon and monkey pancreas diluted out to give a curve parallel to the pure human PP standard curve. Extracts of heart and liver, taken as control tissues, showed no inhibition of binding of labelled hormone to antibody. The protein content of these extracts (determined by 280 nm absorption) was greater than that of the gut tissue extracts, thus confirming previous observations that interference by non-specific protein effects within the PP assay are negligible at the dilutions used.

From the pilot studies it was found that less than 5% damage was caused to PP during the extraction procedure and the recovery of PP in the extracts was 94 $\pm$ 4% (mean $\pm$ SEM).

The distribution of PP in the primate intestine can be seen in Figure 301. High concentrations of PP (123 $\pm$ 16 pmol/g mean $\pm$ SEM) were found in the pancreas and a small but detectable amount was found in the fundus

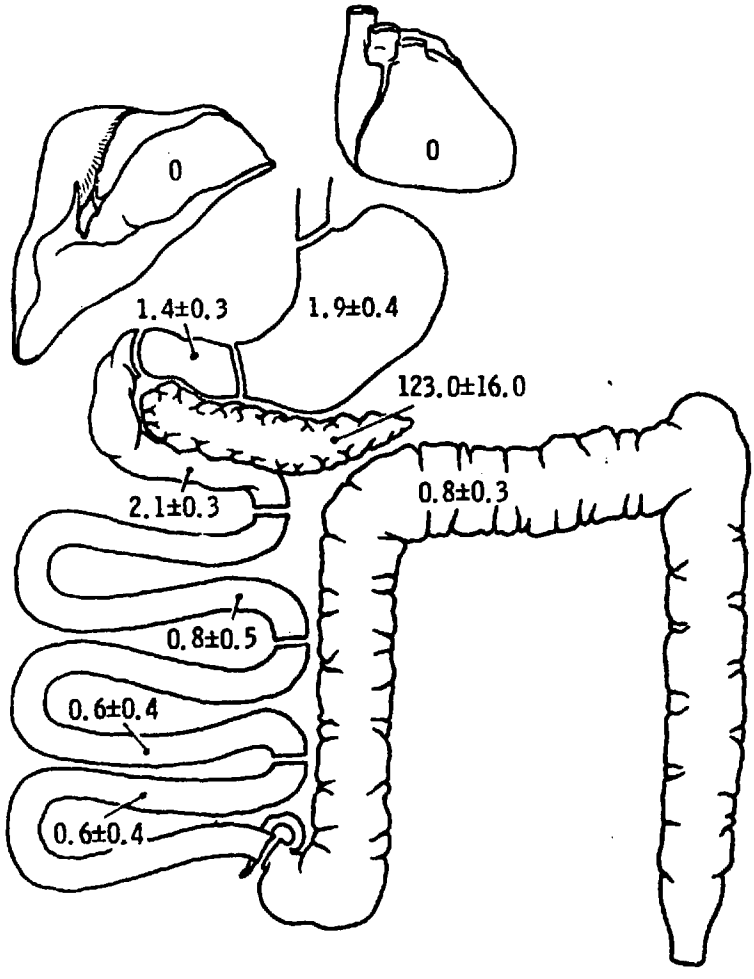


Figure 301:

Distribution of pancreatic polypeptide (PP) in the primate gastrointestinal tract. Concentrations are given in pmol/g wet tissue.

(1.9 ± 0.3 pmol/g, $p < 0.005$) and antrum of the stomach (1.4 ± 0.3 pmol/g, $p < 0.02$) and in the duodenum (2.1 ± 0.3 pmol/g, $p < 0.005$). Statistically significant amounts of PP were not detectable in oesophagus. jejunum, ileum, colon, heart or liver (p 0.1).

Distribution of PP in Adult Man

The experimental detection limit was found to be 200 fmol PP/g wet tissue. PP in all extracts diluted out to give curves parallel to the pure human PP standard. Extracts of control tissue (liver, lung,etc) showed no inhibition of binding of labelled hormone to antibody.

From the pilot studies it was found that less than 5% damage was caused to PP during the extraction procedure and the recovery of PP in the extracts was 97 ± 6% (mean ± SEM).

The distribution of PP in the human adult intestine can be seen in Figure 302. Highest concentration of 907 ± 284 pmol/g wet weight (mean ± SEM, n = 8) were found in the pancreas and a small but significant amount was also found in the gastric fundus and pyloric antrum and also in the duodenum and rectum. No immunoreactive PP was detected in oesophagus, jejunum, ileum, colon, salivary gland or brain. It is notable that although a small amount of immunoreactive PP can be detected in the stomach, duodenum and rectum the concentration of this material is only 0.1% - 0.4% of the concentration found in the pancreas.

Within the pancreas the highest PP concentration was in the head of the organ (2013 ± 621 pmol/g) which was significantly higher than that found in the body and tail of the gland (270 ± 69 and 283 ± 50 pmol/g respectively, $p < 0.01$.

Distribution of PP in the Human Foetus

The PP concentration in the pancreas of all the foetuses studied was 1093 ± 207 pmol/g wet tissue which is similar to the concentration in

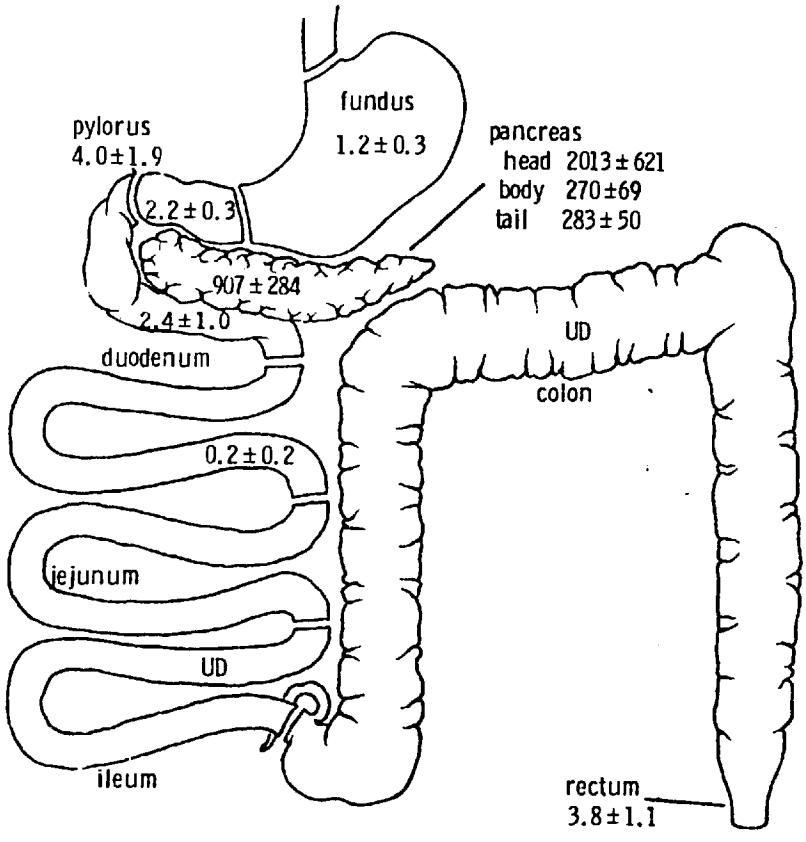


Figure 302:

Distribution of PP in the gastrointestinal tract of adult man. Concentrations are quoted in pmol/g wet tissue.

tissue from adult man. From the third month of gestation there was no significant change in pancreatic PP content of the foetus. In foetuses of less than 20 weeks gestation the pancreatic PP content was 1212 $\pm$ 225 pmol/g (n = 5), from 20-30 weeks it was 890 $\pm$ 163 pmol/g (n = 8) and from 30 weeks to term 983 $\pm$ 111 pmol/g (n = 7).

In the gastrointestinal tract of the foetus a small amount of PP was detectable, notably in the oesophagus, stomach and duodenum although the concentration was less than 1% of that in the pancreas (Figure 303). It is of interest that early in the gestational period (10-20 weeks) there is a moderate amount of PP in the caecum (6.9 $\pm$ 1.6 pmol/g) and colon (10.6 $\pm$ 2.0 pmol/g) although concentrations fall significantly towards term (caecum 1.1 $\pm$ 0.2, colon 2.6 $\pm$ 0.8, $p < 0.005$). By term, significant amounts of PP-like immunoreactivity are seen in the oesophagus, gastric fundus, antrum and duodenum, a similar distribution to that found in the adult.

PP Content of Pancreatic Endocrine Tumours

The PP content of the individual pancreatic tumour extracts is shown in Figure 304. It is clear that although PP is detectable in extracts of most pancreatic exocrine tumours the concentration in the majority is below that of normal pancreas. Of 48 primary pancreatic tumours extracted only 8 had PP concentrations outside the range for normal adult pancreas whereas 3 out of 7 liver metastases extracted had PP concentrations above those for normal pancreas.

Chromatographic Analysis of Molecular Forms of PP

Column chromatograms revealed that the major form of PP-like immunoreactivity in normal pancreas eluted in a position identical to that of the pure PP standard (Figure 305). Some tissue samples show very small

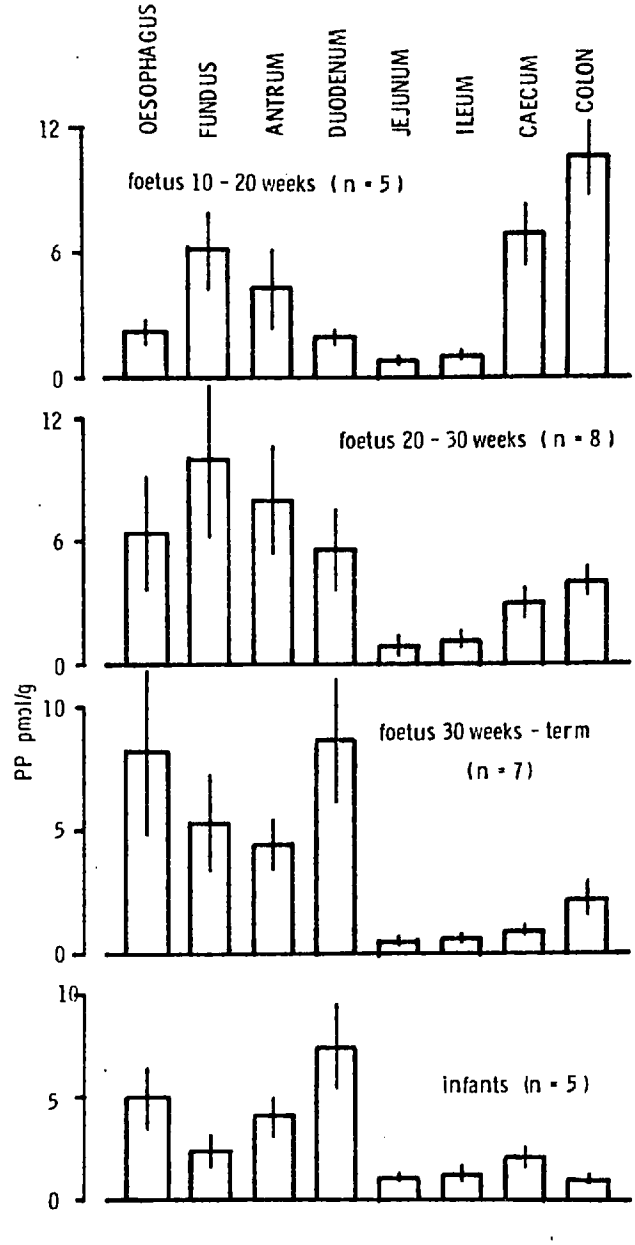


Figure 303:

Distribution of PP in the gastrointestinal tract of the human foetus. Concentrations are quoted in pmol/g wet tissue. For comparison the concentration of PP in the pancreas was 1212 $\pm$ 225 pmol/g (less than 20 weeks gestation, n = 5), 890 $\pm$ 163 pmol/g (20-30 weeks, n = 8) and 983 $\pm$ 111 pmol/g (greater than 30 weeks, n = 7).

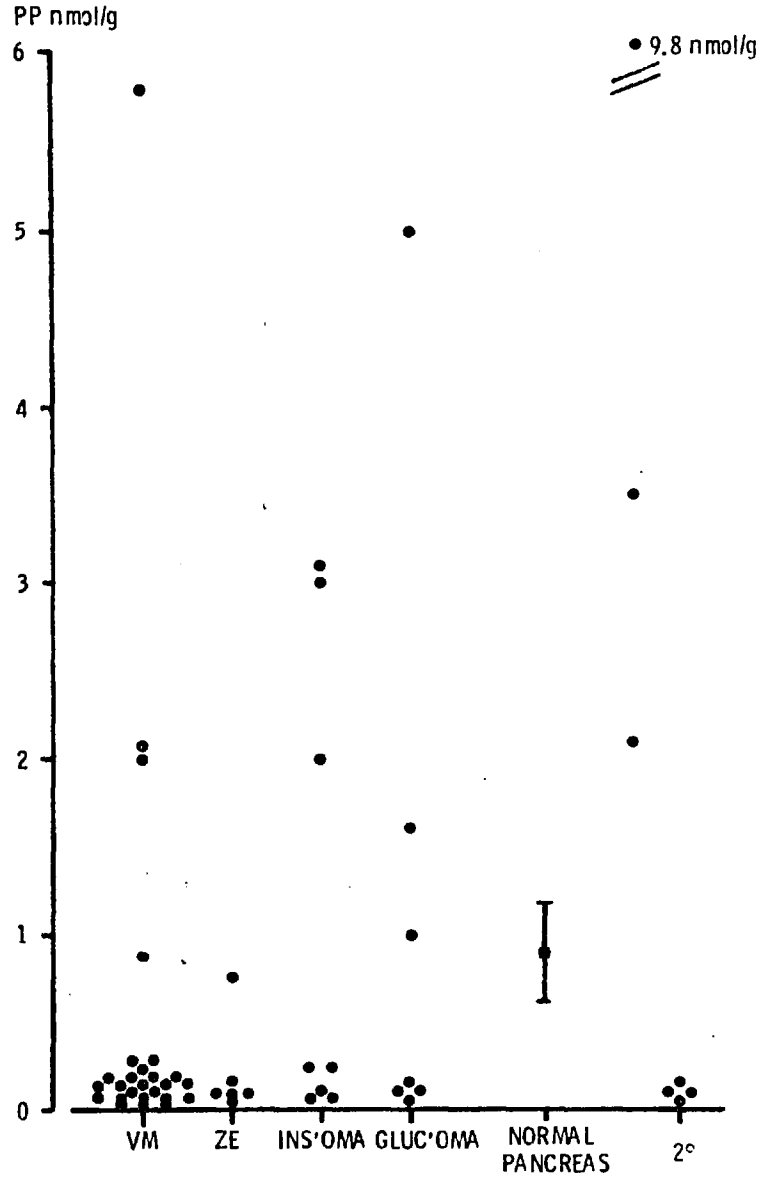


Figure 304:

PP content of pancreatic endocrine tumours. The PP concentration of normal pancreas (mean $\pm$ SEM, n = 8) was included for comparison.

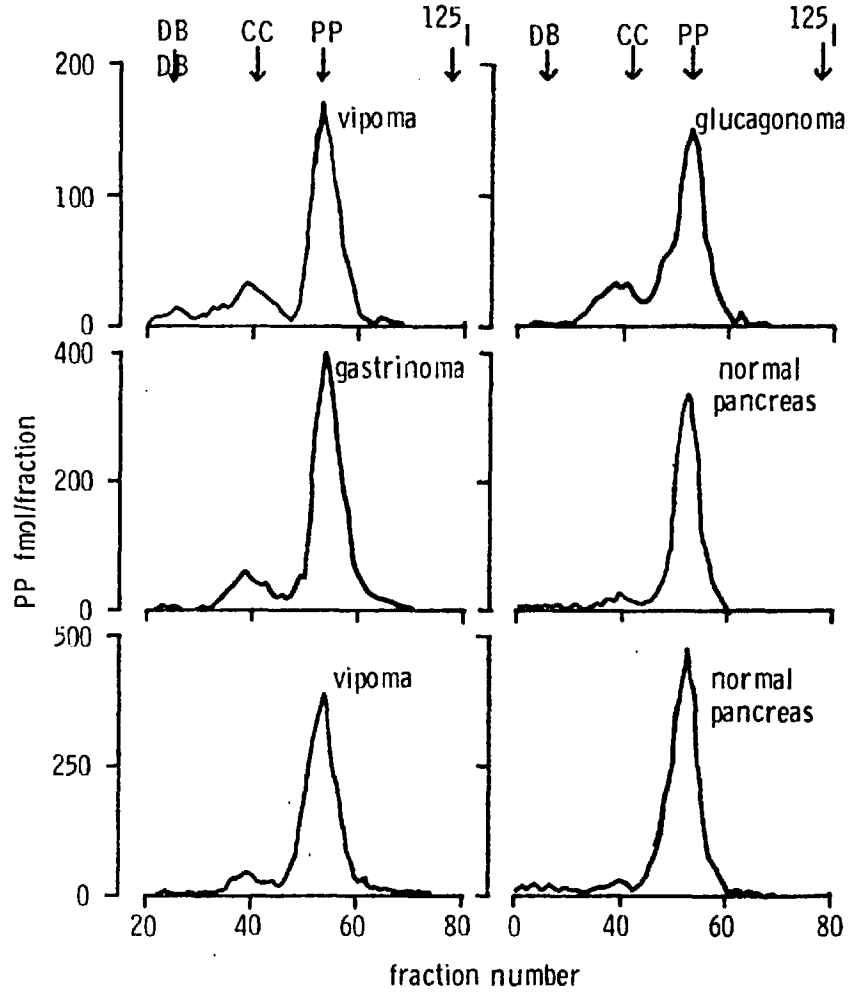


Figure 305:

Gel chromatograms (Sephadex G50 superfine) of pancreatic tumour extracts showing PP-like immunoreactivity. Dextran blue, cytochrome C and ^{125}I were added to each sample as molecular weight markers.

amounts of immunoreactive PP eluting in a similar position to cytochrome C suggesting material of a higher molecular weight. In no case did this peak of apparently higher molecular weight PP exceed 5% of the total PP immunoreactivity.

Tumour extract chromatograms were broadly similar to those of normal pancreas, most with a small but detectable amount of PP immunoreactivity eluting in a similar position to cytochrome C. However, the peak eluting in the same position as pure PP was always the predominant form often accounting for more than 90% of the PP-like immunoreactivity (Figure 305). Chromatograms of two carcinoid tumours revealed only one immunoreactive PP peak which eluted in a similar position to pure PP.

DISCUSSION

Distribution of Pancreatic Polypeptide

Pancreatic polypeptide (PP) has been isolated from several mammalian species and was first discovered as a major contaminant during insulin purification (Chance et al, 1976). As expected therefore the concentration of PP in sub-human primate and human pancreas was high. A small amount of PP-like immunoreactivity was present in the stomach and duodenum of adult and foetal man although in concentration this only represented approximately 0.2% or 1% of the pancreatic content respectively. Small numbers of PP cells have been reported to be present in the gastrointestinal tract of several mammalian species (Larsson et al, 1976). The concentration of PP in the foetal pancreas was found to be similar to that in adult man. This is in keeping with immunocytochemical evidence (Helmstaedter et al, 1978).

In the human pancreas there was a significant difference in the

intrapancreatic distribution of PP with concentrations in the pancreatic head being approximately 10-fold higher than in the body and tail. This finding is in keeping with the observation that in the dog pancreas the uncinate process or head contains considerably more PP than the body and tail (Gingerich et al, 1978). A similar distribution, with greater numbers of PP cells in the pancreatic head, has been described using immunocytochemical analysis of human adult pancreas removed at autopsy (Orci et al, 1978).

It is of interest that immunoreactive PP is present in the caecum and colon early in gestation but this is lost before parturition. Similar apparently anomalous findings can be seen with other gastrointestinal hormones, for example, gastrin, which is found in the foetal pancreas but is absent from the adult organ. It is tempting to speculate that PP was present in the gastrointestinal tract of more primitive animals and that it has evolved to a predominantly pancreatic localisation. Presence of PP in the gastrointestinal tract of the foetus and to a lesser extent the adult could explain the occurrence of PP cells in carcinoid tumours of both fore and hind gut.

It is apparent that PP is a frequent component of pancreatic endocrine tumours (Polak et al, 1976), although the incidence is greater in VIPomas and glucagonomas than it is in gastrinomas and insulinomas. Since high concentrations are found in hepatic and lymph node metastases, it can be assumed that PP is a primary product of these tumours. Further, many patients with pancreatic endocrine tumours have high circulating PP levels which is further evidence of the abnormal PP production by these tumours (Polak et al, 1976, Adrian et al, 1978).

It is apparent that the PP content of pancreatic apudomas is often below that of normal pancreas although the plasma levels of PP are grossly elevated. This finding would suggest that the tumour produced PP is frequently secreted in an autonomous manner rather than being stored in the

tissue. Synthesis must occur at a phenomenal rate in some tumours however. One patient studied with a malignant pancreatic tumour secreting VIP and PP suffered from torrential diarrhoea. The PP content of the primary tumour and its liver metastases was very high (5800 and 9700 pmol/g respectively) and plasma levels varied between 20,000 and 40,000 pmol/l which is 1000 times higher than fasting levels seen in healthy subjects.

Although it would appear from column chromatographic studies that the major tissue form of PP is the 36 amino acid peptide isolated by Chance (Chance, 1976) there is evidence from these studies of a different molecular form of high molecular weight. An additional peak is usually seen eluting in a similar positon to cytochrome C (M wt 12 000). In normal tissue this minor form of PP-like immunoreactivity accounts for less than 5% of the total. Some pancreatic tumours have considerable amounts of the higher molecular weight peak (eluting with cytochrome C). It is tempting to speculate that this larger molecular weight form is a biosynthetic precursor of PP.

CHAPTER 4. RELEASE OF PANCREATIC POLYPEPTIDE IN ADULT MAN

INTRODUCTION

Release of Pancreatic Polypeptide in Adult Man

Soon after the discovery of pancreatic polypeptide it became evident that this substance was potentially a third circulating peptide hormone secreted from this gland. Studies in the dog had suggested that PP circulated in plasma and that levels rose in response to food (Lin and Chance, 1974). Studies in the chicken with avian pancreatic polypeptide (APP) confirmed these findings (Kimmel et al, 1975). It was therefore of importance to establish the factors responsible for the release of this peptide in man.

As PP was evidently released from the pancreas following the ingestion of food it was important to investigate the nature of the signal involved in its release. The release of insulin and glucagon from the pancreas is complex, involving circulating nutrients, hormones and neural factors. It was clear that any of these mechanisms could be involved in the release of PP. Indeed the extremely rapid elevation of plasma PP concentrations which was seen within a few minutes of food led to the suggestion that the mechanism may be entirely neural (Schwartz et al, 1976). Experiments were therefore designed to investigate the influence of oral and intravenous nutrients, the role of the autonomic nervous system, in particular the vagus, and also the influence of the gastrointestinal hormonal peptides on the release of PP in man.

Patients who had undergone total surgical pancreatectomy were studied after a meal to investigate the possibility that some circulating PP was derived from an extrapancreatic source.

The effect of gastric distension on circulating PP levels was studied using both balloon distension and oral water ingestion.

Vagal influence was studied using both a test meal and insulin induced hypoglycaemia in normal volunteers and patients who had undergone truncal vagotomy.

SUBJECTS AND METHODS

The Release of PP by Oral Nutrients

Six healthy fasting subjects four male and two female, mean age 27 (range 22-35) (103 $\pm$ 3% ideal body weight) received the following isocaloric meals in random order on different days.

a) mixed meal consisting of two medium sized boiled eggs, 60g bread as toast, 10g butter, 35g marmalade and 150 ml unsweetened orange juice. The meal contained 18g protein, 22g fat and 66g carbohydrate (530 calories).

b). 680g steamed cod with a water drink to a total volume of 500 ml (530 calories).

c). 115g double cream with a water drink to a total volume of 500 ml (530 calories).

d). 138g glucose with a water drink to a total volume of 500 ml (530 calories).

The standard test breakfast used above was also consumed by eight

patients who had previously undergone total surgical pancreatectomy. Mean age 42 (range 32-67).

Release of PP by an Oral Water Load

The following protocols were carried out on separate days on different groups of six healthy overnight fasted subjects, aged 25-35 years, weight 70-85 kg.

a) 7.5 ml/kg of distilled water, temperature 20°C, was given orally.

b) 15 ml/kg distilled water as above.

c) 15 ml/kg experiment repeated with water given 2 minutes after intravenous atropine (600 mg).

The mean ingestion time for water in all groups was 2 minutes.

PP Release Following Antral Distension

The effect of graded distension of the antral part of the stomach was studied in seven healthy volunteers, mean age 23 years (range 22-24). A thin walled balloon attached to a nasogastric tube was placed in the antrum. After a basal period of 60 minutes the balloon was inflated with contrast medium to a volume of 100 ml for a further period of 60 minutes. The position of the balloon was checked frequently.

Effect of Intravenous Nutrients on PP Release

Four fasting male subjects mean age 25 (range 22-30) (103 $\pm$ 2% of ideal body weight) received the following 30 minute isocaloric intravenous

infusions in random order on separate days.

a) glucose, 20g in 40 ml of "50% Dextrose" (Baxter Ltd).

b) amino acids, 25g as 250 ml of "10% Aminosol" (Kabi Vitrum Ltd).

c) fat, 8g as 40 ml of 20% intralipid (Kabi Vitrum Ltd).

Twenty healthy fasting subjects, mean age 42 (range 24-67) (105 $\pm$ 6% ideal body weight) received an intravenous arginine infusion (0.5 kg^{-1} hr^{-1}) for 30 minutes.

Nine healthy fasting subjects, mean age 32 (range 24-40) (98 $\pm$ 3% ideal body weight) received an intravenous bolus injection of glucose (20g in 200 ml water).

The Influence of the Vagus on PP Release - Insulin Induced Hypoglycaemia

An intravenous bolus injection of insulin (0.2u/kg) was administered to the following groups of fasting subjects.

a) eight healthy volunteers aged 26-54 (mean 36).

b) nineteen duodenal ulcer patients aged 27-63 (mean 38).

c) seventeen duodenal ulcer patients who had undergone a successful truncal vagotomy (Hollander negative) at least 6 months before the study, aged 32-68 (mean 41).

d) eight patients who had undergone selective vagotomy (proximal (gastric) vagotomy) at least 6 months before the study, aged 30-65 (mean 39).

e) eight patients who had undergone highly selective vagotomy (partial cell vagotomy) at least 6 months before the study, aged 28-66 (mean 38).

PP Release by Food in Vagotomised Subjects

A standard hospital lunch (79g carbohydrate, 44g fat and 28g protein was eaten by the following groups of overnight fasted subjects.

a) 10 normal volunteers, mean age 41 (range 27-64).

b) 11 patients with duodenal ulcer, mean age 39 (range 29-63).

c) 6 patients who had undergone a successful truncal vagotomy, mean age 42 (range 34-68).

Effect of Gastrointestinal Hormonal Peptides on PP Release

Several gastrointestinal peptides were infused in healthy subjects following an overnight fast. The peptide used, infusion dose, number of subjects and mean age is shown in Table 401.

The plasma levels of secretin, GIP, motilin and neurotensin were within or near to those seen following physiological stimuli. The doses of pentagastrin and somatostatin used were pharmacological and were included as part of other studies. The doses of caerulein used were just sufficient to cause maximal pancreatic enzyme secretion (70 pmol kg^{-1} hr^{-1}) and one tenth of this dose (7 pmol kg^{-1} hr^{-1}). The dose of cholecystokinin produces sub-maximal enzyme secretion.

Effect of Atropine on Release of PP by Humoral Agents.

Five healthy fasting subjects mean age 29, received an intravenous

RELEASE OF PP BY GASTROINTESTINAL PEPTIDES

PEPTIDE	SOURCE	DOSE pmol kg^{-1} hr^{-1}	n	SUBJECTS + − Age or mean age
PENTAGASTRIN	PEPTAVLON SYNTHETIC (ICI)	900	8	35
CHOLECYSTOKININ	PURE NATURAL (GIH Laboratories)	40	4	27
CAERULEIN	CAERULETIDE SYNTHETIC (Farmitalia)	70	5	27
	AS ABOVE BUT LOW DOSE	7	7	37
SECRETIN	PURE NATURAL (GIH Laboratories)	12	5	40
GASTRIC INHIBITORY PEPTIDE	PURE NATURAL (Prof. J.C.Brown)	120	7	29
MOTILIN	PURE NATURAL (Prof. J.C.Brown)	120	10	32
NEUROTENSIN	SYNTHETIC BOVINE (UCB)	140	5	30
BOMBESIN	SYNTHETIC (Farmitalia)	15	5	33
SOMATOSTATIN	SYNTHETIC (Salk Institute)	700	6	28

TABLE 401

infusion over one minute of 2 Crick Harper Raper units/kg of Boots' "secretin" (Boots Ltd), a crude extract of porcine intestine. The above infusion protocol was repeated on a separate day 30 minutes after intramuscular injection of atropine (1.2 mg).

In addition these five subjects received caerulein (70 pmol $kg^{-1}hr^{-1}$ Caeruletide, Farmitalia Ltd), in the fasting state (see Table 402) both with and without atropine 30 minutes before commencing the infusion.

RESULTS

Release of PP by Oral Nutrients

The effect of a normal mixed breakfast on circulating PP concentration in healthy young subjects can be seen in Figure 401. Plasma levels rose promptly from the low fasting concentrations of 22 $\pm$ 3 pmol/l to a peak at 12 minutes of 198 $\pm$ 38 pmol/l. Levels then remained elevated throughout the study. Figure 402 shows the effect of the isocaloric oral nutrients in the same subjects as above. Protein and fat caused the greatest rise in PP levels whereas the effect of glucose was relatively small. The peak following protein or fat was lower than that seen following the mixed meal in spite of these stimuli being isocaloric.

PP was undetectable in both the fasting and postprandial plasma of all eight subjects who were previously pancreatectomised.

Release of PP by an Oral Water Load

Plasma PP following the 15 ml/kg oral water load with and without atropine is shown in Figure 403. The 7.5 ml/kg water load caused PP levels to rise from a fasting level of 20 $\pm$ 5 pmol/l (mean $\pm$ SEM) to a peak at 15 minutes of 63 $\pm$ 9 pmol/l ($p < 0.05$). Levels then fell returning

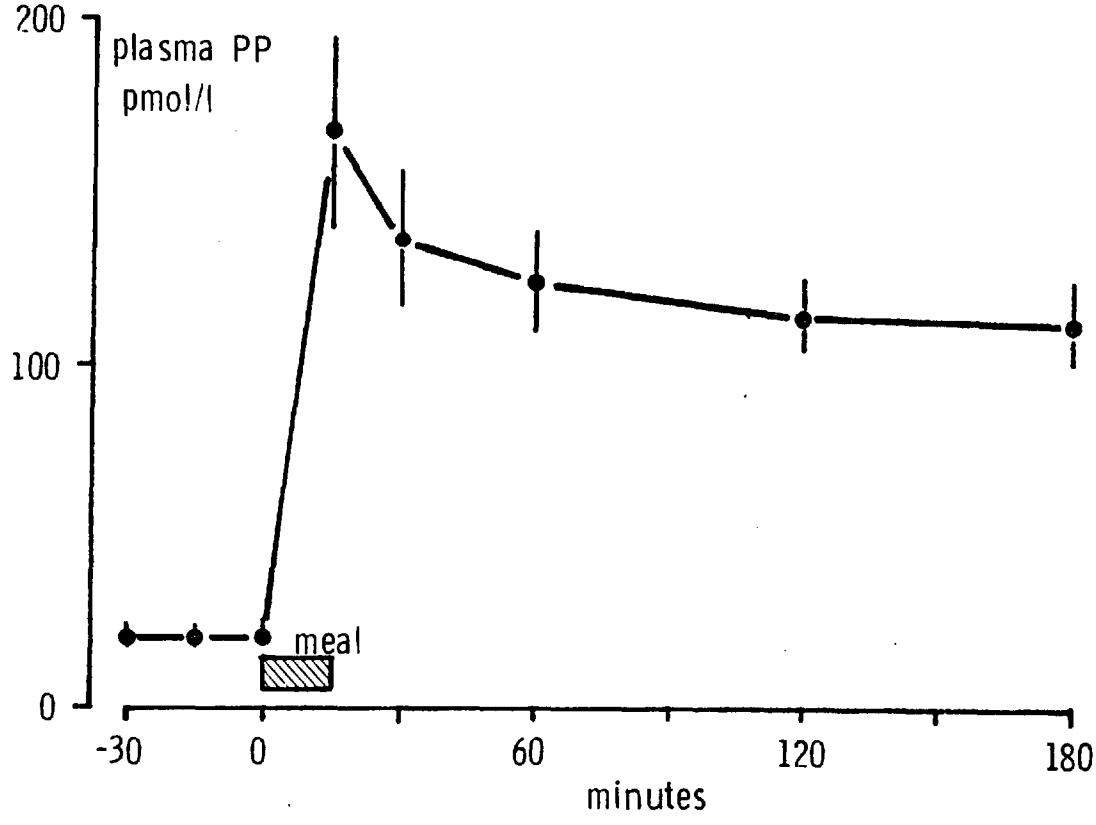


Figure 401: Effect of a small mixed breakfast on plasma PP concentrations in six healthy fasting subjects.

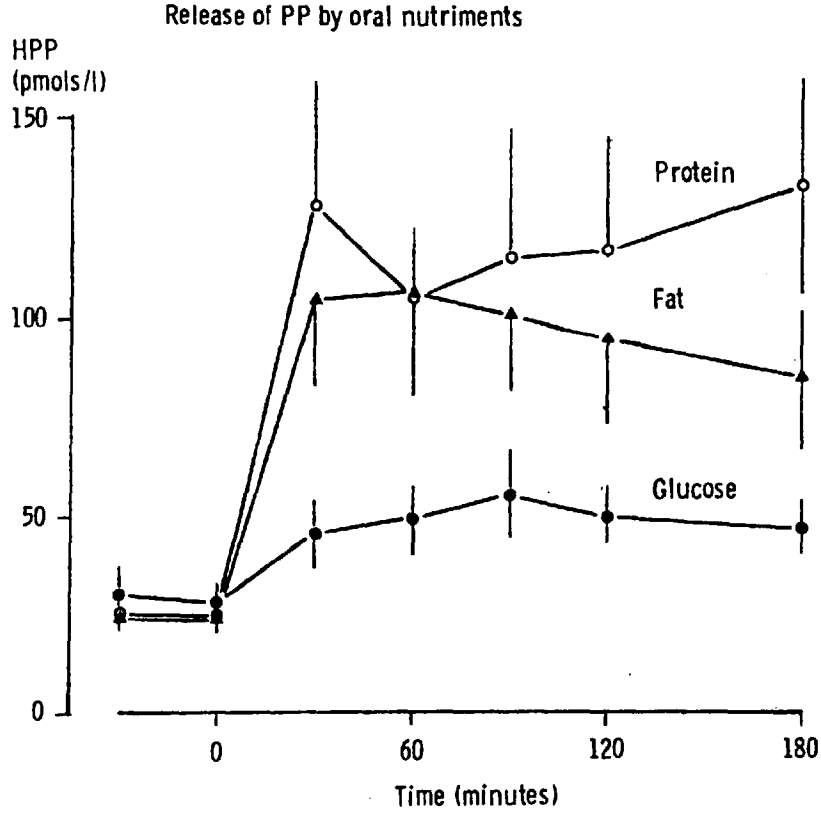


Figure 402: Effect of isocaloric oral nutrients on plasma PP levels in six healthy fasting subjects.

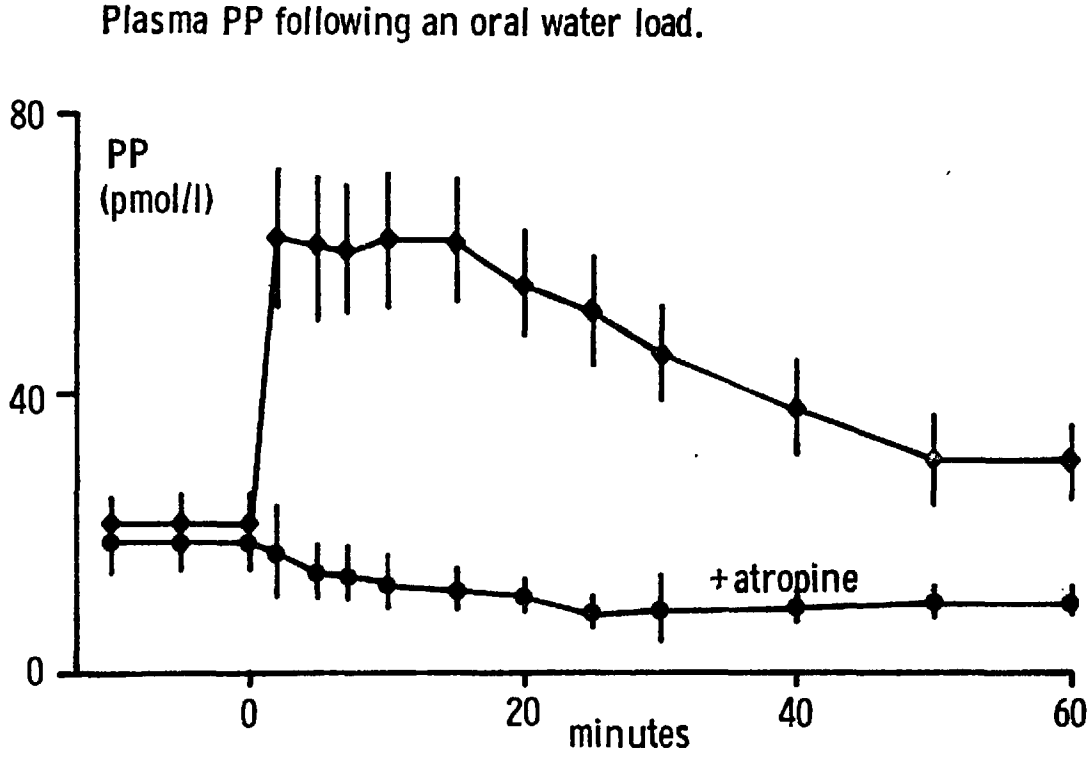


Figure 403: Plasma PP concentrations following a 15 ml/kg oral water with and without intravenous atropine (600 mg) in six healthy fasting subjects.

to a value not significantly different from basal at 40 minutes. Following ingestion of the 15 ml/kg water load plasma PP levels rose from a fasting level of 21 $\pm$ 4 pmol/l to an early peak value at 10 minutes of 63 $\pm$ 12 pmol/l (p 0.05) and fell slowly to basal at 50 minutes. In the atropinised subjects PP levels fell from a zero value of 19 $\pm$ 4 pmol/l to a median nadir value at 30 minutes of 9 $\pm$ 5 pmol/l ($p < 0.05$). Levels remained significantly below basal until the end of the experiment at 60 minutes.

PP Release following Antral Distension

Plasma PP concentrations rose following balloon distension from a basal of 15 $\pm$ 4 pmol/l to a peak at 10 minutes of 42 $\pm$ 10 pmol/l ($p < 0.02$) and slowly declined towards basal at the end of the distension hour (Figure 404). PP levels had returned to basal within 30 minutes of deflating the balloon.

Effect of Intravenous Nutrients on PP Release

The effect of intravenous infusion of glucose, amino acids and fat on circulating PP concentrations can be seen in Figure 405. In the period during the 30 minute infusions and in the 30 minutes following the infusion there was no significant change in fasting PP concentrations from those seen in the fasting state. The pharmacological nature of this study is underlined by the fact that the intravenous glucose load caused an elevation in blood glucose from 4.6 $\pm$ 0.2 mmol/l to 10.4 $\pm$ 0.3 mmol/l. A rise which greatly exceeds the response to a normal meal.

Arginine infusion had no significant effect on plasma PP concentrations which were 26 $\pm$ 5 pmol/l fasting, 28 $\pm$ 6 pmol/l at the end of infusion and 26 $\pm$ 4 pmol/l 30 minutes after the end of the infusion.

Fasting PP concentrations of 25 $\pm$ 7 pmol/l were not significantly

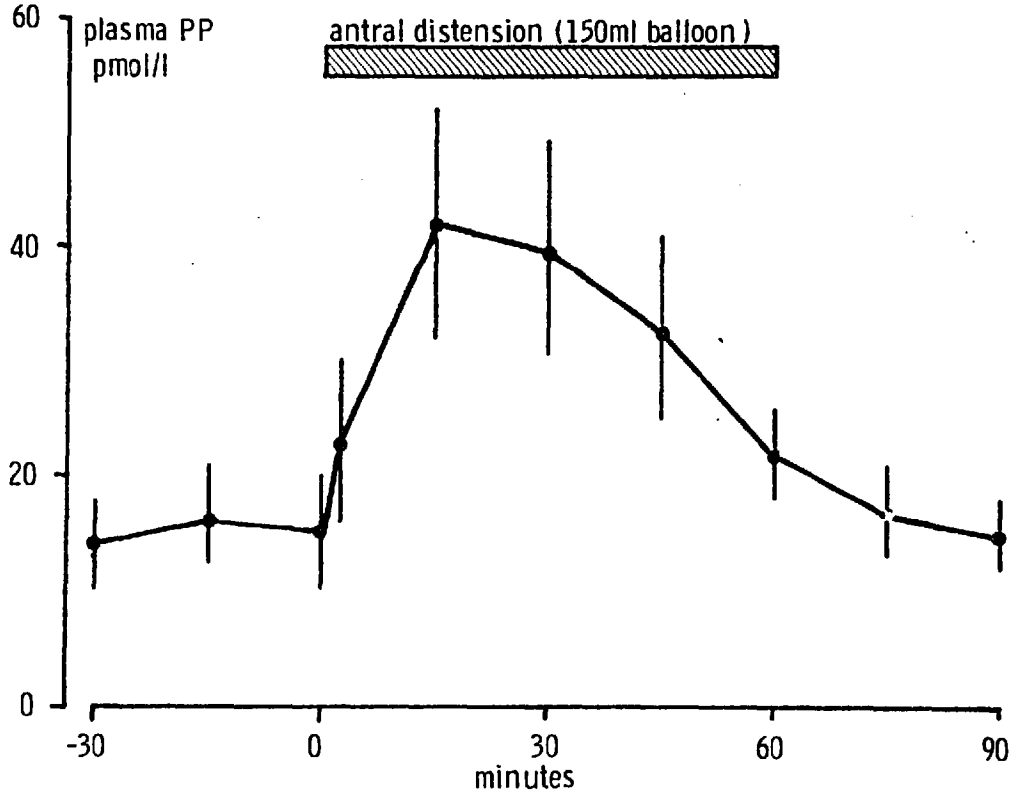


Figure 404: Plasma PP concentrations following balloon distension (150 ml) of the gastric antrum in seven healthy volunteers.

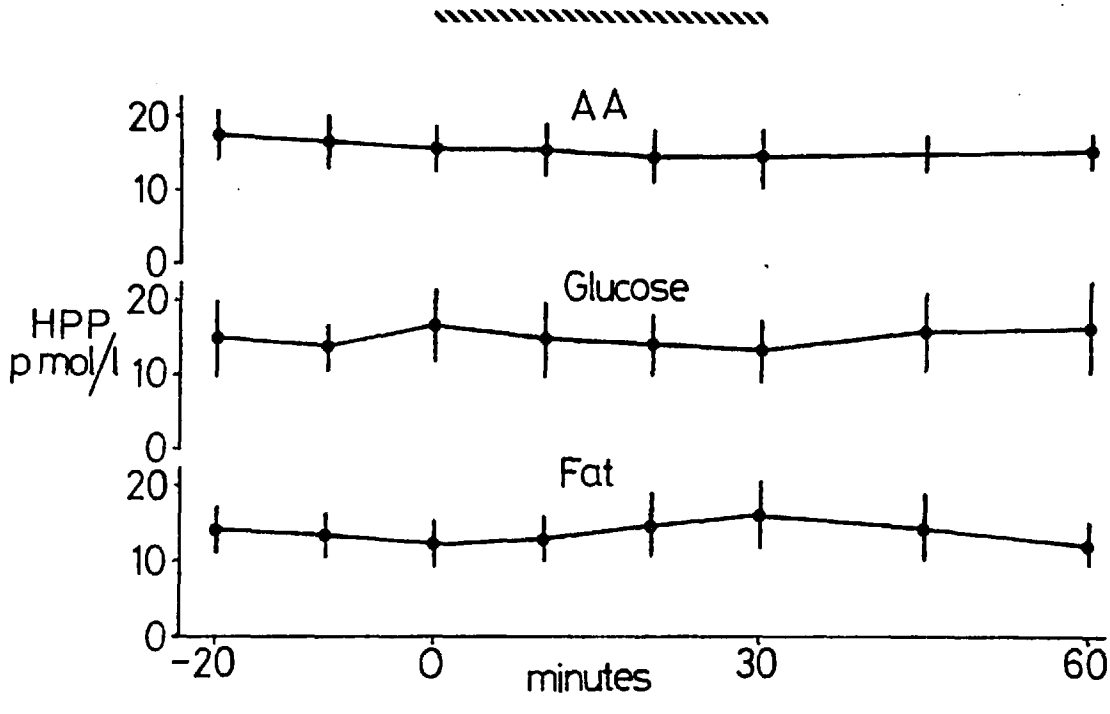


Figure 405: Plasma PP concentrations during isocaloric intravenous infusions of glucose, amino acids and fat in four healthy fasting subjects.

changed following an intravenous bolus injection of glucose. The level at 15 minutes was 25 $\pm$ 6 pmol/l, at 30 minutes was 28 $\pm$ 6 pmol/l and at 60 minutes 18 $\pm$ 7 pmol/l.

The Influence of the Vagus on PP Release - Insulin Induced Hypoglycaemia

The nineteen patients with duodenal ulcer all had a substantial rise in plasma PP concentrations from a basal of 23 $\pm$ 3 pmol/l to a mean 50 minute peak of 235 $\pm$ 68 pmol/l, coinciding with the lowest blood glucose (2.1 $\pm$ 0.1 mmol/l) and this was similar to that in the eight normal subjects (basal PP 26 $\pm$ 1,peak 219 $\pm$ 85, glucose nadir 2.1 $\pm$ 0.1) Figure 406. The rise was absent in the seventeen patients who had undergone a successful truncal vagotomy (basal PP 20 $\pm$ 3, 50 minutes 28 $\pm$ 7) in spite of a greater fall in blood glucose (50 minutes nadir 1.8 $\pm$ 0.3 mmol/l). In the patients who had undergone selective vagotomy the PP response was reduced (basal 26 $\pm$ 3, peak 73 $\pm$ 19) as it was in the patients who had the highly selective procedure (basal 28 $\pm$ 3, peak 114 $\pm$ 21). Figure 407 shows the effect of insulin induced hypoglycaemia in the duodenal ulcer patients, all of the patients after selective vagotomy (selective and highly selectives) and the patients after truncal vagotomy.

PP Release by Food in Vagotomised Subjects

Fasting and postprandial PP concentrations were similar in the normal subjects (basal 28 $\pm$ 4 pmol/l, peak 355 $\pm$ 52 at 30 minutes) and the duodenal ulcer patients (basal 33 $\pm$ 5, peak 332 $\pm$ 46 at 30 minutes). There was no signiciant difference in the PP response to the meal in the patients who had undergone truncal vagotomy when compared with either of the above groups (Figure 408). The initial sharp peak of PP was blunted in the truncal vagotomy group but the difference did not attain statistical significance at any time point.

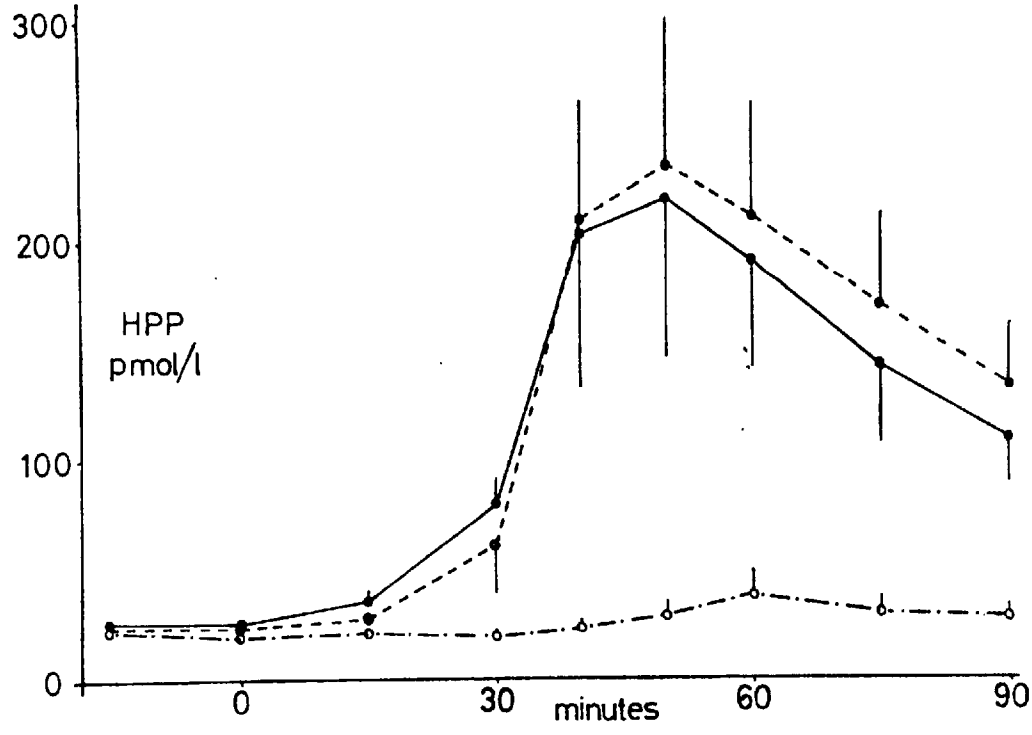


Figure 406: Effect of insulin induced hypoglycaemia on plasma PP concentrations in 8 healthy subjects ●——●, 19 patients with duodenal ulcer ●- - -●, 17 patients studied at least 6 months after a successful truncal vagotomy o-·-·o.

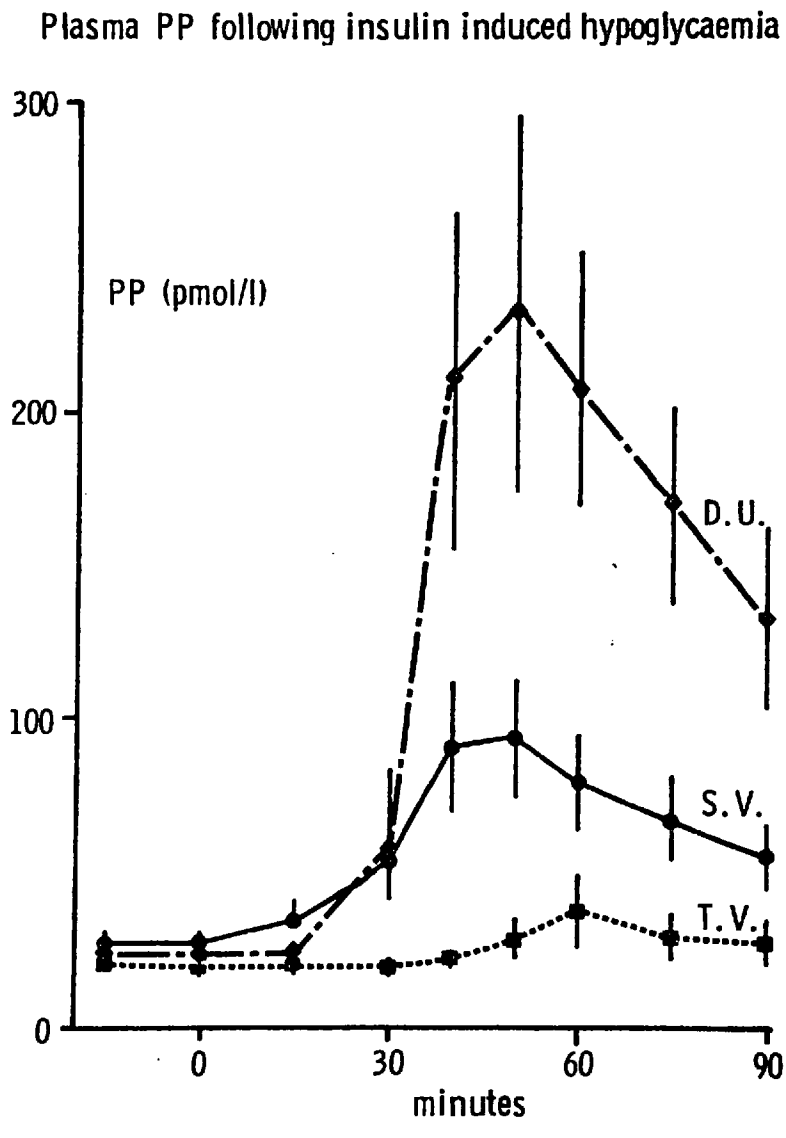


Figure 407: Plasma PP concentrations following insulin induced hypoglycaemia in 19 duodenal ulcer patients (DU), 18 patients studied at least six months after selective vagotomy (SV) and 17 patients studied at least six months after truncal vagotomy (TV)

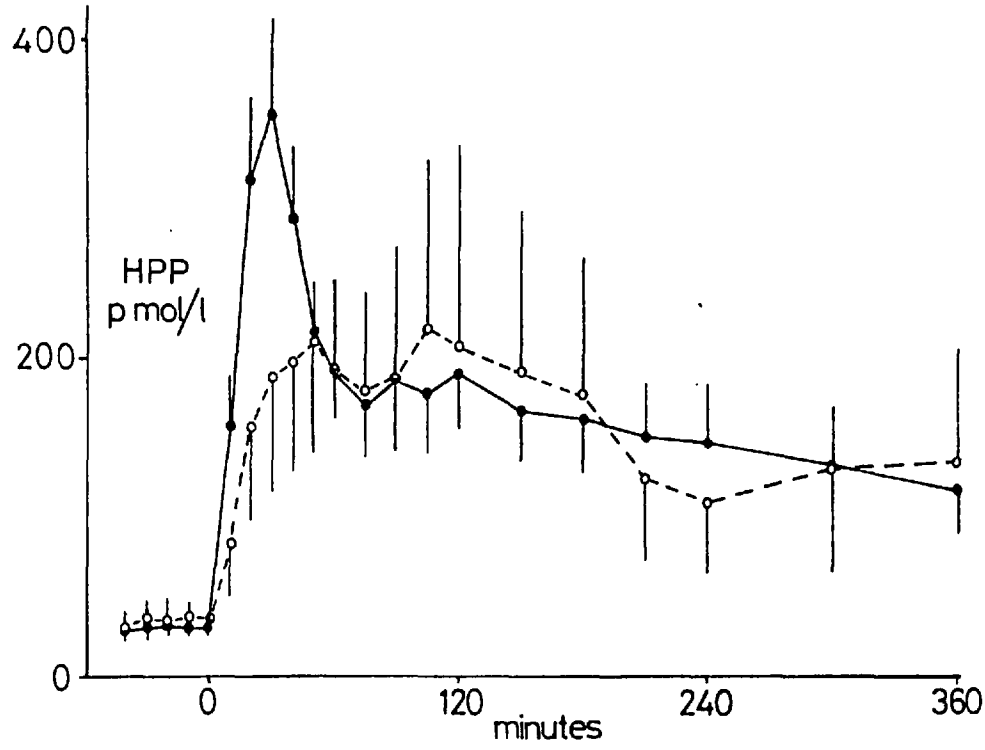


Figure 408: Plasma PP concentrations following a standard hospital lunch in 10 healthy subjects and 6 patients studied at least six months after a successful truncal vagotomy.

Effect of Gastrointestinal Hormones on PP Release

The effect of gastrointestinal hormones on PP release in the fasting state is summarised in Table 402.

No significant effect at the doses infused was observed with pentagastrin or pure secretin. There was a significant rise in plasma PP concentrations with cholecystokinin, caerulein, both at high (Figure 409) and low doses (Figure 410), gastric inhibiting peptide, motilin, neurotensin (Figure 411) and bombesin.

Somatostatin caused a significant reduction in fasting PP levels (Figure 412).

Effect of Atropine on the Release of PP by Humoral Agents

With Boots' secretin injection there was a rapid rise in plasma PP concentrations from a basal of 19 $\pm$ 3 pmol/l to a peak of 165 $\pm$ 22 pmol/l at 6 minutes. When the test was preceded by atropine the PP response to Boots' secretin was completely abolished (basal 21 $\pm$ 4 pmol/l, 6 minutes 14 $\pm$ 2 pmol/l) (Figure 413). Indeed basal PP concentrations fell significantly from 21 $\pm$ 4 pmol/l to 8 $\pm$ 2 pmol/l following atropinisation (p 0.01).

Atropine administration also greatly obtunded the PP response to caerulein (Figure 414). Following caerulein infusion, PP concentrations were 19 $\pm$ 6 pmol/l at 30 minutes in the post-atropine experiments and 85 $\pm$ 14 pmol/l following caerulein alone.

PEPTIDE	DOSE pmol kg^{-1} h^{-1}	FASTING PP CONCENTRATION pmol/l	PEAK PP CONCENTRATION pmol/l	STATISTICAL SIGNIFICANCE (Paired t test)
PENTAGASTRIN	900	24 ± 7	23 ± 7	NS
CHOLECYSTOKININ	40	21 ± 5	94 ± 11	$p < 0.001$
CAERULEIN	70	18 ± 3	85 ± 14	$p < 0.001$
CAERULEIN	7	28 ± 6	59 ± 14	$p < 0.02$
SECRETIN	12	23 ± 4	24 ± 3	NS
GASTRIC INHIBITORY PEPTIDE	120	16 ± 4	51 ± 12	$p < 0.02$
MOTILIN	120	21 ± 5	46 ± 11	$p < 0.05$
NEUROTENSIN	140	14 ± 3	185 ± 62	$p < 0.001$
BOMBESIN	15	23 ± 6	87 ± 28	$p < 0.05$

TABLE 402: Effect of gastrointestinal hormones of PP release in man.

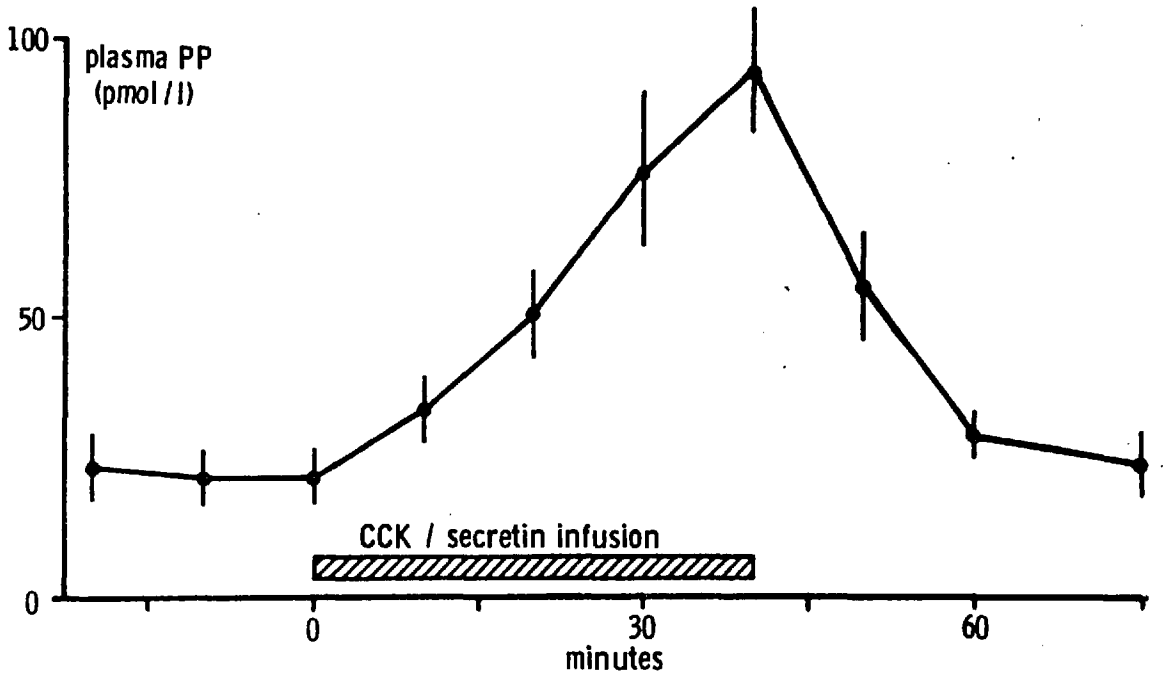


Figure 409: Plasma PP concentrations following an intravenous infusion of cholecystokinin (40 pmol kg^{-1} l^{-1}) and secretin (12 pmol kg^{-1} l^{-1}) in four healthy subjects.

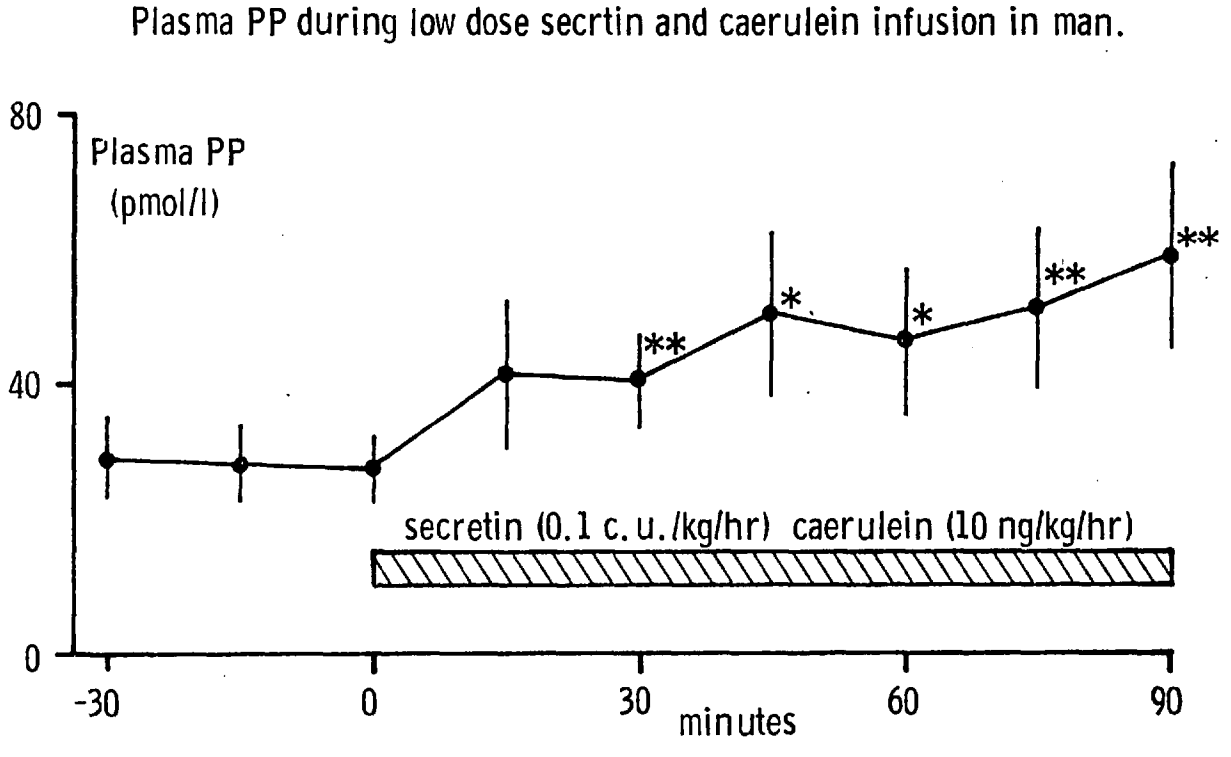


Figure 410: Plasma PP concentrations following an intravenous infusion of caerulin (7 pmol kg^{-1} l^{-1}) and secretin (12 pmol kg^{-1} l^{-1}) in seven healthy subjects.

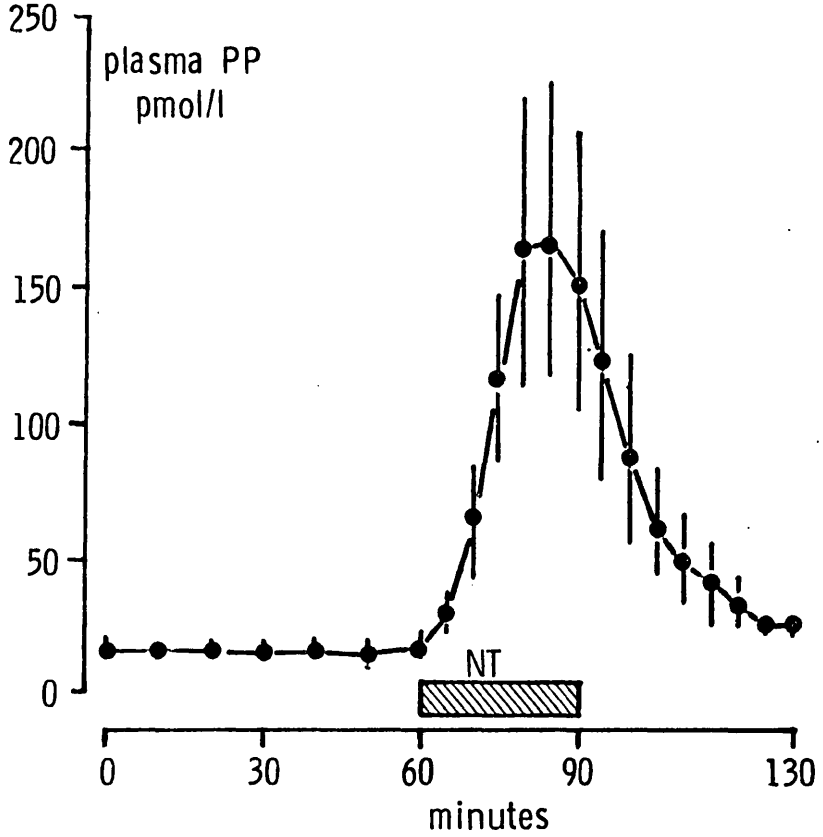


Figure 411: Plasma PP concentrations during intravenous infusion of neurotensin (140 pmol kg^{-1} h^{-1}) in 5 healthy fasting subjects.

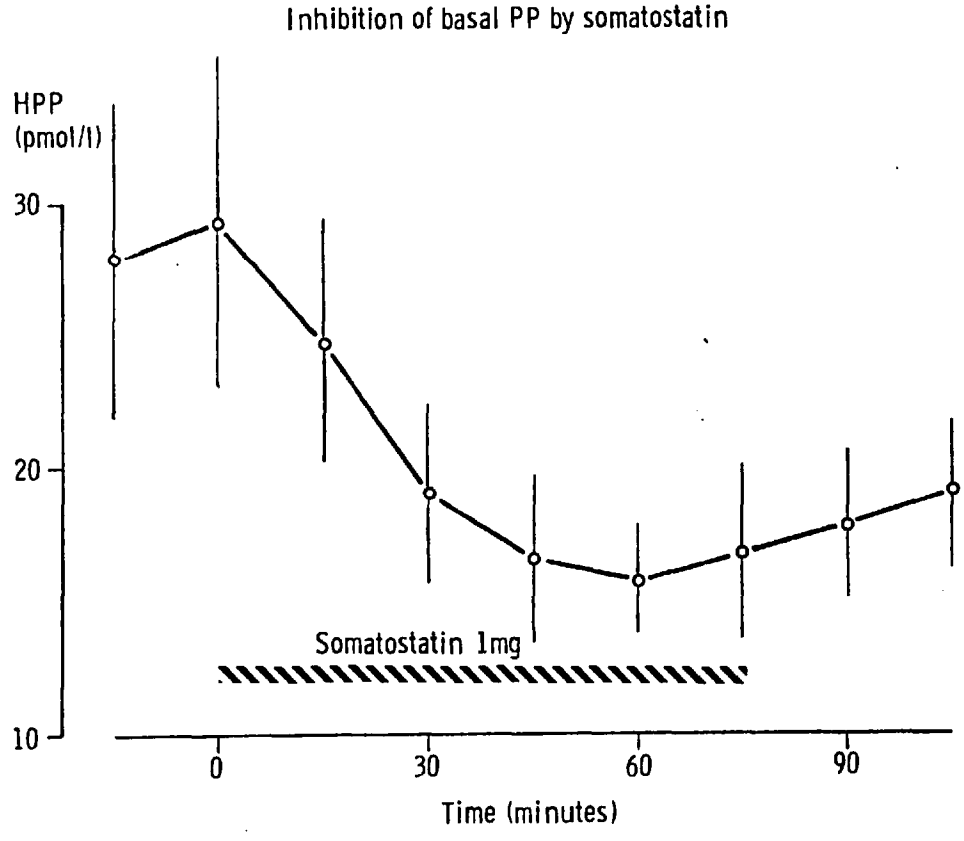


Figure 412: Plasma PP concentrations during intravenous infusion of somatostatin (700 pmol kg^{-1} h^{-1}) in 6 healthy fasting subjects.

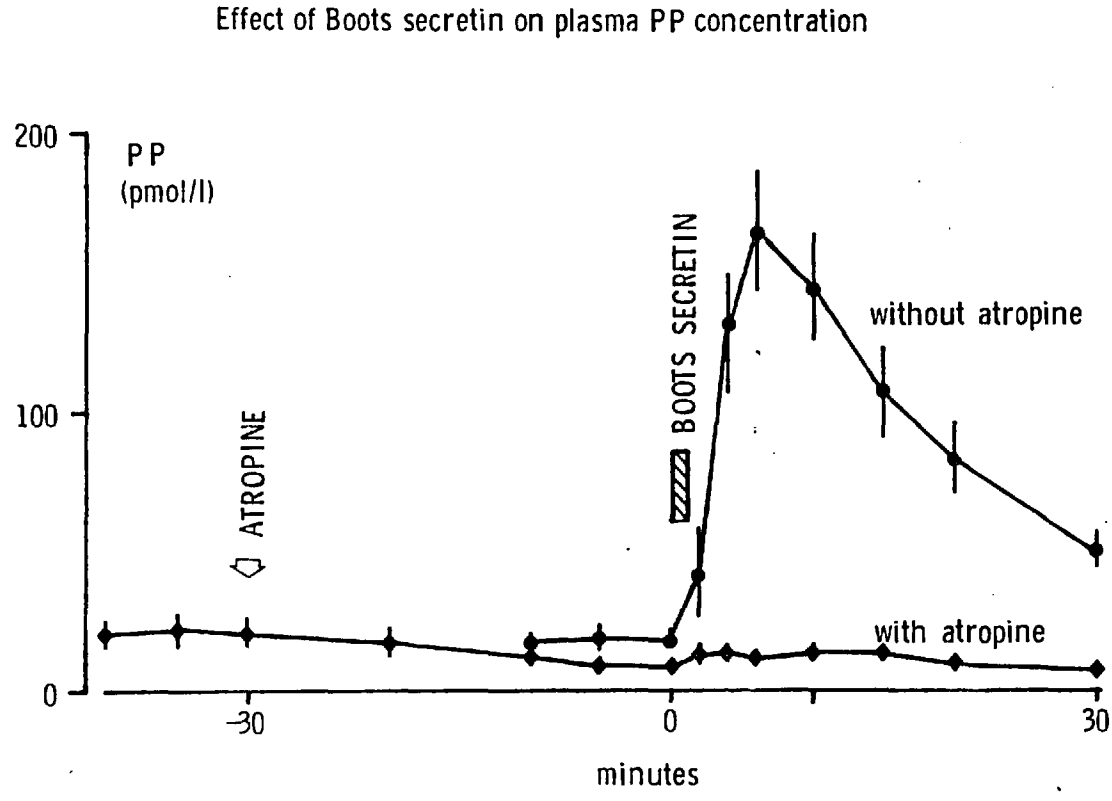


Figure 413: Effect of intravenous Boots' secretin on fasting plasma PP concentrations in six healthy subjects both with and without prior atropinisation.

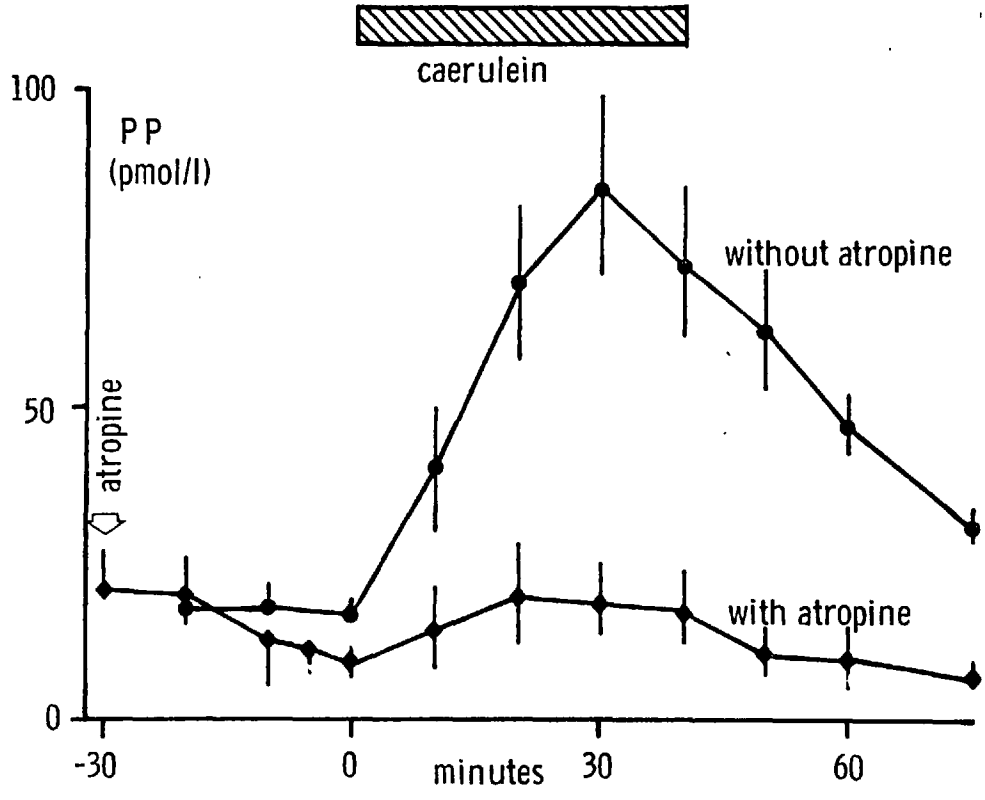


Figure 414: Plasma PP concentrations during infusion of caerulein (100 ng kg^{-1} l^{-1}) in six healthy subjects both with and without prior administration of atropine.

DISCUSSION

Release of PP by Oral Nutrients

Plasma pancreatic polypeptide (PP) circulates in plasma and it is clear from these studies that plasma levels rise in response to food. This postprandial elevation has been noted by several groups (Adrian et al, 1978; Floyd et al, 1976; Schwartz et al, 1976; Taylor et al, 1978a). Protein appears to be the most potent oral stimulus for PP release and this has been noted by several groups (Adrian et al, 1978; Floyd et al, 1977; Schwartz et al, 1976). It would appear from these studies that fat is also a potent stimulus for PP release. Floyd et al (1977) reported that Lipomul (a dietary fat supplement) was relatively ineffective at releasing PP compared with beef. The most likely reason for this discrepancy is that the dose used (70g fat) was compared with 500g cooked ground beef and was therefore only about one third of the caloric load. Glucose is clearly less effective than either protein or fat in causing a release of PP; this finding has been confirmed by other groups (Adrian et al, 1978; Floyd et al, 1977; Marco et al, 1978).

It is clear that the pancreas is the only source of PP in the plasma from the absence of detectable circulating PP in patients who have undergone total surgical pancreatectomy.

Effect of Gastric Distension on PP Release

Distension of the stomach either following the ingestion of water or by the use of an air filled balloon results in a moderate increase in plasma PP levels. This increase is blocked by prior atropinisation indicating the cholinergic dependence of the mechanism involved. These studies have been confirmed by Schwartz et al (1978) who have also demonstrated that highly selective vagotomy inhibits the PP response to

fundic distension.

Effect of Intravenous Nutrients on PP Release

The studies of isocaloric amounts of intravenous nutrients suggested that the PP cell was not influenced by circulating nutrients. In addition no significant change was observed during arginine infusion. Other groups have investigated the effect of intravenous nutrients on the release of PP. Arginine, leucine or alanine infusions are reported to have no effect on PP levels (Floyd et al, 1976). However, these workers reported a very small (12 pmol/l rise) transient increase of PP levels following the infusion of the essential amino acids (Floyd et al, 1977). In addition a small rise may be observed after amino acid infusions are terminated depending on the protocol used. This late increase is associated with decreasing blood glucose levels (Floyd et al, 1976; Floyd et al, 1977). Intravenous infusions of fat are reported not to affect plasma PP concentrations (Adrian et al, 1977; Floyd et al, 1977). Several groups have investigated the effect of intravenouse glucose on circulating PP levels. Some reports suggest a modest suppression of basal PP levels by glucose (Floyd et al, 1976; Marco et al, 1978; Sive et al, 1978), but this has not been demonstrable in all groups of experiments (Adrian et al, 1977; Floyd et al, 1977), even when mean blood glucose reached 16 mmol/l (Floyd et al, 1977). Additional evidence for an absence of significant effect of glucose or arginine on the PP all comes from in vitro studies on canine pancreas where PP secretion does not appear to be affected by changes in perfusate glucose or arginine concentrations (Adrian et al, 1978; Gingerich et al, 1978).

Following the termination of glucose infusions, and as is sometimes seen after amino acid infusions are terminated, plasma levels of glucose may fall below basal levels. When this happens PP levels may increase

(Floyd et al, 1976; Marco et al, 1978). Recent work by Marco and colleagues suggest that substantial hyperglycaemia can reduce the postprandial response of PP (Marco et al, 1978).

The Influence of the Vagus on PP Release

There is a substantial response to insulin induced hypoglycaemia in normal subjects and duodenal ulcer patients. In patients who have undergone truncal vagotomy this response is abolished suggesting that it is mediated via a central vagal signal. The reduced PP response to hypoglycaemia in patients who have had selective or highly selective vagotomy would suggest that either some of this response is mediated through local vagal fibres to the pancreas from the stomach or, alternatively, that some of the fibres to the pancreas are damaged by selective or even parietal cell vagotomy procedures.

The release of PP during insulin induced hypoglycaemia has been reported by several groups (Adrian et al, 1977; Floyd et al, 1977; Schwartz et al, 1978) and the cholinergic mechanism of this response has been confirmed by Schwartz et al (1978) who showed it to be atropine sensitive.

The influence of the vagus on potential PP secretion has been a controversial issue. It has been reported that PP release after food was greatly reduced following vagotomy (Schwartz et al, 1976; Taylor et al, 1977; Hansky et al, 1978). Other reports suggested that postprandial PP release was not significantly affected by truncal vagotomy (Adrian et al, 1977; Taylor et al, 1978). It is now apparent that the time of study after vagotomy is of importance and that normal PP release returns within a few months of vagotomy although the gland remains unresponsive to a central vagal stimulus (insulin induced hypoglycaemia) indicating the completeness of vagotomy.

Pancreatic exocrine function is also greatly reduced immediately

after vagotomy and this too returns after a few months (Tiscornia,1977; Thambugala and Baron, 1971).

Schwartz has demonstrated that the PP response to fundic distension is abolished by proximal gastric vagotomy. These results suggest that the PP response to gastric distension is regulated via the afferent part of the vagal pathway (Schwartz et al, 1979). It is likely that part of the early PP response to food is via a gastropancreatic reflex through a long vagovagal pathway present in man.

It is clear that the vagus may be an important factor in the phsyiological control of PP release. However it is evident, from the substantial postprandial PP increase in truncal vagotomy patients, that the vagus is not the only mechanism for the release of PP. A vagal stimulus is possibly an important factor in the early release of PP (first 30 minutes) as the response to food appears to be reduced in this early period.

Effect of Gastrointestinal Hormones on PP Release

It is clear that several gastrointestinal hormones can evoke a significant response of pancreatic polypeptide (PP) when infused in the fasting state. Cholecystokinin and caerulein, its analogue from frog skin, appear to be quite potent in this respect and neurotensin is an extremely powerful agent. Several other gut hormones, gastric inhibiting peptide, neurotensin, bombesin and motilin all cause a significant elevation in plasma PP concentrations. Gastrin and pure secretin do not appear to influence PP secretion in man. The latter finding suggests that the release evoked by Boots' secretin is due to some contaminant in this impure preparation of porcine intestine rather than the secretin itself.

The absence of PP response to caerulein and Boots' secretin in atropinised subjects together with a reduction in basal PP levels following atropine, suggests that both basal and gut hormone-stimulated PP release

is dependent on cholinergic tone.

It is difficult to assess if any of these hormones are involved in the postprandial release of PP. Cholecystokinin is an obvious candidate, being released by protein and fat, the very stimuli which enhance PP secretion. Gastric inhibitory peptide (GIP) is unlikely to be of great importance because of its potent release by oral glucose, an agent which is poor at effecting an elevation of PP levels. Motilin is at best only transiently elevated following the ingestion of food (Christofides et al, 1979) and is thus unlikely to be an important factor in the postprandial release of PP. Vasoactive intestinal peptide (VIP) and bombesin are neuropeptides and are not thus expected to act normally via the circulation. However, the pancreas contains fairly high concentrations of VIP which may have a local influence on PP secretion. Little is known of the release or physiology of the recently discovered neurotensin but this peptide may well be another circulating gut hormone.

In common with the other circulating pancreatic hormones, glucagon and insulin, and also the other gastrointestinal hormones so far investigated PP levels in plasma are substantially reduced during somatostatin infusion.

The Entero-PP Axis

It is clear from these studies that PP in plasma is derived entirely from the pancreas. In addition levels of this circulating PP rise rapidly following the ingestion of food. However, dramatic increases in circulating nutrient concentrations, resulting from intravenous infusion, have no significant influence on PP secretion from the pancreas. It is apparent that an indirect signal is involved in the postprandial release of this peptide. We postulate that an entero-PP axis exists (Adrian et al, 1977) which is comparable to the well-established enteroinsular axis (McIntyre et al, 1964).

This entero-PP axis appears to have two components, the vagus and gastrointestinal hormones. The vagus appears to be responsible in part for the early release of PP, the first phase of the biphasic response. The mechanism of vagal control may be in part a vago-vagal signal from the gastric fundus in response to distension of that organ.

The vagus is not the only mechanism for the release of PP however as is suggested by the long time course of PP release, the selective potent effect of protein and fat and most important of all the normal PP response seen in patients studied several months after complete vagotomy. Several gastrointestinal hormonal peptides can potently release PP in man and it is probable that one or more of these peptides is involved in the physiological PP response to food. Control of the PP cell is complex however and the maintainance of fasting levels and also the response to gastrointestinal hormones appears to be dependent on cholinergic tone. This is analogous to the release of enzymes from the pancreatic acinar cell where both neural and hormonal stimuli appear to be required for an effective enzyme release (Solomon and Grossman, 1979).

The gastric parietal cell also is dependent on acetyl choline, histamine and gastrin for the release of its product and the inhibition of any one of these factors effectively blocks gastric acid secretion (Soll, 1978).

The implications of these findings to endocrinology are profound as they suggest that the control of hormone secretion may be far more complex than is generally realised. This seems to be of particular relevance to the endocrine pancreas where the release of insulin and glucagon secretion is controlled by hormonal and nervous factors as well as by circulating metabolites.

CHAPTER 5. CIRCULATING PANCREATIC POLYPEPTIDE IN THE HUMAN NEONATE

INTRODUCTION

The presence of PP in the foetal pancreas and the observation that circulating PP levels in the adult rise with age (Floyd et al, 1977), suggested that study of the developmental physiology of PP would be interesting.

Basal plasma PP concentrations and the response to milk was investigated in both term and preterm neonates and in addition a group of preterm infants who had never received oral nutrition were studied.

PATIENTS AND METHODS

Forty-three normal term infants (mean birth weight 3290 $\pm$ 50 (mean $\pm$ SEM) g and gestation 39.5 $\pm$ 0.11 weeks and 128 healthy preterm infants (1970 $\pm$ 30 g and 33.5 $\pm$ 0.1 weeks), were studied. Each infant contributed only one venous blood sample taken at the same time as blood was required for routine clinical purposes.

The term infants were studied at 6 days and were breast-fed 4-hourly over 25-35 minutes with a mean feed volume 20 ml/kg. Blood samples were taken either before a feed or at 55, 90 or 150 minutes after the beginning of the feed.

Preterm infants were either 6 (range 5-7), 13 (11-17) or 24 (18-42) days old and were fed 3-hourly using pooled human milk given by naso-gastric tube over 5 minutes (mean feed volume of 22 ml/kg).

Blood was taken either before a feed or at 30, 60 or 120 minutes after the feed. These infants were nursed in incubators at environmental

temperatures appropriate to their weight and gestation. None had any clinical problems other than prematurity.

A further group of 10 preterm infants were studied (mean age 6 ± 0.5 days, birth weight 1875 ± 243 g, gestation 33 ± 1 weeks), who had received only intravenous dextrose since birth on account of inability to tolerate enteral feeds because of hyaline membrane disease. These unfed infants required ventilatory assistance and enriched inspired oxygen concentrations, but none was hypoxic Pa O_2, maintained between 90-60 torr or acidotic (pH above 7.25) at the time of the study.

In addition, umbilical venous blood was taken at vaginal delivery for 20 term and 6 preterm healthy infants, and control blood taken from 12 healthy fasting adults (age range 22-34 years).

RESULTS

Plasma PP in the Human Neonate

Mean umbilical venous plasma PP levels were similar in term and preterm infants at 13 ± 3 and 17 ± 3 pmol/l (mean $\pm$ SEM). These levels were significantly lower than the young healthy fasting adults' values of 28 ± 1 ($n = 12$, $p < 0.01$).

In term infants the mean basal level on the sixth day was 28 ± 6, significantly higher than the cord value ($p < 0.05$), and similar to the adult value (Figure 501). There was no change in PP level following a feed (see Table 501).

In preterm infants the basal PP cincentrations rose from the cord level to 43 ± 9 on the sixth day ($p < 0.02$) and to 65 ± 11 on the thirteenth day ($p < 0.005$), which was significantly higher than the adult level ($p < 0.005$), but subsequently it fell to 34 ± 5 at 24 days ($p < 0.05$)

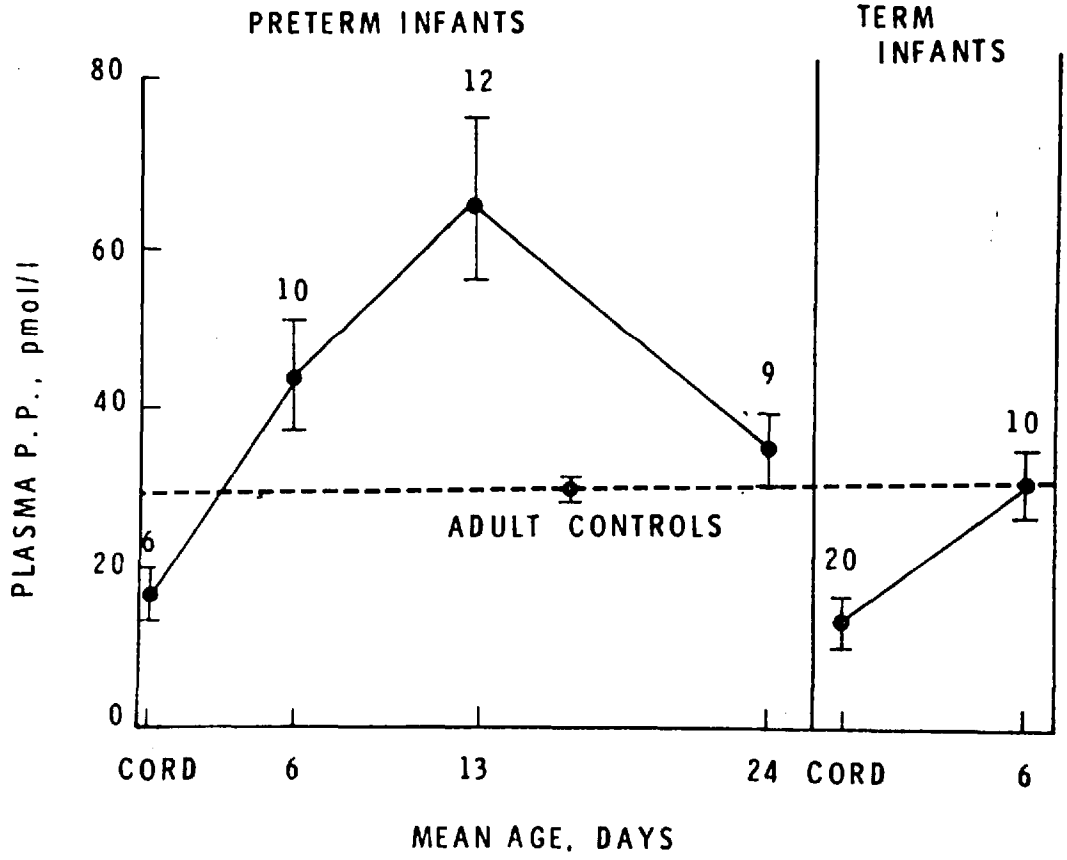


Figure 501:

Basal plasma pancreatic polypeptide (PP) (pmol/l $\pm$ SEM) in preterm (left) and term (right) infants, in venous cord blood and post-natally. Plasma PP concentration in healthy young fasting adults

TABLE 501

PLASMA PANCREATIC POLYPEPTIDE (pmol/l ± SEM)

RESPONSE TO FEED ON SIXTH POSTNATAL DAY.

TERM INFANTS				
Time, minutes	0	55	90	150
	28 ± 6	30 ± 5	21 ± 3	32 ± 6
	n = 10	n = 12	n = 12	n = 9

PRETERM INFANTS				
Time, minutes	0	30	60	120
	43 ± 9	30 ± 9	43 ± 14	30 ± 5
	n = 10	n = 11	n = 12	n = 13

(Figure 501). There were no significant postprandial changes in PP at 6 days, but at 13 days there was a significant fall at 30 minutes ($p < 0.02$) (Figure 502a) and at 24 days there was a small and non-significant elevation (Figure 502b).

The group of preterm infants with hyaline membrane disease who had not been fed enterally from birth had a mean plasma PP concentration of 41 ± 8 pmol/l.

DISCUSSION

Basal Plasma PP and the Response to Feeding in the Human Neonate

At birth, plasma concentrations of plasma pancreatic polypeptide were found to be low compared with the fasting adult level. This observation is consistent with the demonstration by Floyd et al, of increasing plasma PP levels in adults with advancing age (1976). However, after birth in both term and preterm infants there was a sharp rise in basal plasma PP and in 13 day-old preterm infants, there was a peak basal concentration which significantly exceeded the level in young adults. The results of this study show that the increase in basal PP levels by the sixth day of life in both preterm and term infants, was also seen in preterm infants who had not received enteral feeds. It seems therefore that this elevation of PP may not be due to enteral feeding itself.

Greenberg et al, have shown a stimulatory effect of pharmacological doses of PP on pancreatic DNA synthesis, and it is therefore possible that the postnatal elevation of basal PP may be important in the adaptation to extra-uterine life, by stimulating pancreatic growth. (Greenberg, 1977).

In adults the PP response to feeding is impressive. Fat and protein exert the greatest stimulatory effects (Adrian et al, 1978) and these potentiate each other. Following a mixed meal, given to fasting healthy

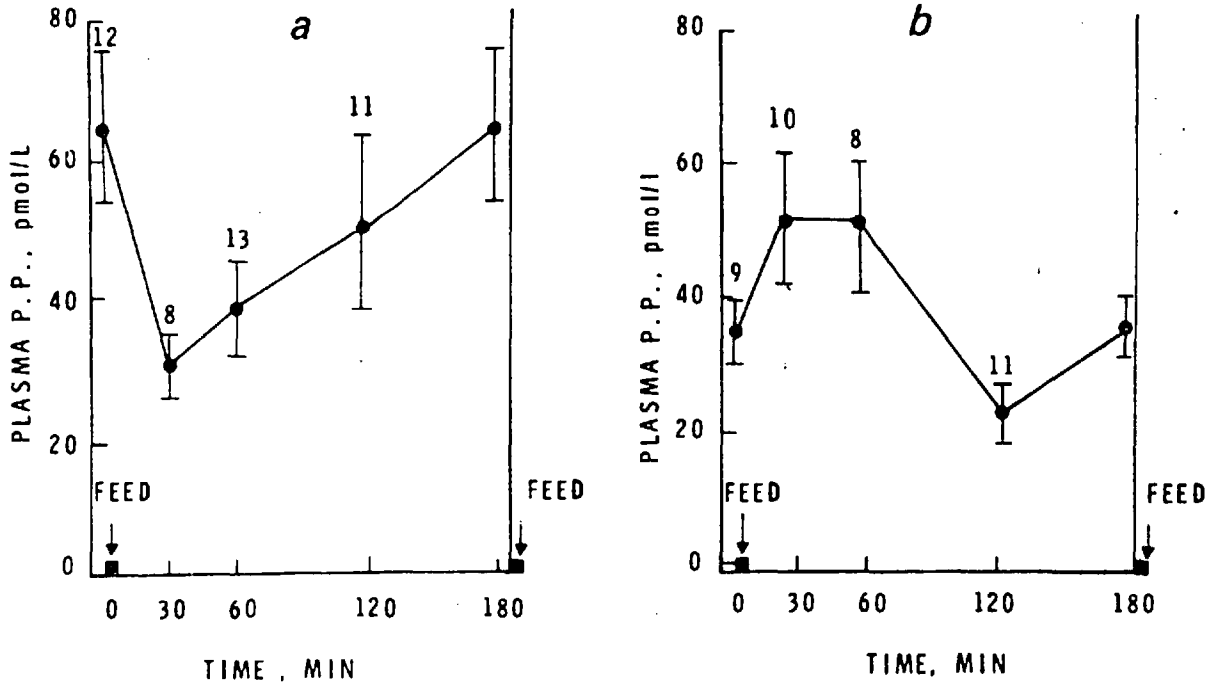


Figure 502:

Change in plasma pancreatic polypeptide (pmol $\pm$ SEM) after a nasogastric feed of human milk in preterm infants at (a) 13 days and (b) 24 days after birth. n values are shown above the mean and standard error range bars. The "zero" observations are shown also at 180 min prior to the next feed.

adults, there is a prompt tenfold elevation in plasma PP, which is maintained for over 6 hours. It is thought that this elevation is brought about by both hormonal and vagal influences (Adrian et al, 1977; Schwartz et al, 1976). In these studies there was no apparent rise in PP after a milk feed in breast-fed term infants on the sixth day or in preterm infants up to 24 days of age. In fact on the thirteenth day, when basal levels were highest, preterm infants showed an actual fall in PP 30 minutes after the feed, suggesting that PP output may be suppressed by feeding at this age. At no point in the neonatal period did PP levels after feeding reach even half the adult postprandial levels reported. It is possible, however, that there had been a transient PP elevation before the first postprandial sampling time at 30 minutes.

A possible explanation for the absence of postprandial plasma PP elevation is that the short feed-to-feed interval in neonates (3-4 hours) prevents a true basal level of PP being achieved. In adults the post-prandial plasma PP concentrations remain elevated above basal, though declining for more than 6 hours. PP levels were not lower in a group of term infants who were fed 6-hourly than those who were fed 4-hourly. Alternatively, the lack of PP elevation following a feed in neonates compared with adults, could be due to relative insensitivity of PP release mechanisms or be secondary to absence of appropriate stimuli. Since PP is an inhibitor of exocrine pancreatic and biliary secretion (Greenberg et al, 1978), it is possible that the lack of PP response to feeding is "adaptive", allowing maximum stimulation of exocrine secretions in the neonatal period. Thus it is probable that the entero-PP-axis does not operate fully in the early weeks of life.

CHAPTER 6. RELEASE OF PP FROM THE ISOLATED PERFUSED CANINE PANCREAS

INTRODUCTION

It is now clear that the pancreatic islets form a complex endocrine unit. They contain three types of cell, A, B and PP cell, which undoubtedly secrete their product into the circulation and other less numerous cell types producing, for example, somatostatin which is thought to act mainly locally. In addition there is a complex innervation which includes the three neural elements, sympathetic, parasympathetic and peptidergic (e.g. VIPergic). The release of insulin and glucagon from the islets has been extensively studied using the isolated perfused canine pancreas (Iversen 1971, 1973a, 1973b, 1974, Iversen and Miles, 1971). This model was considered to be an extremely valuable tool to investigate the relative potency of several PP releasing agents at the organ level.

Gastrointestinal hormones have been shown to be potent releasers of PP in man and dog and probably play a major role in the rise in plasma levels of this hormone after a meal (Adrian et al, 1977; Floyd et al, 1977; Taylor et al, 1978). The isolated perfused canine pancreas was used in an attempt to establish whether the observed effect of gut hormones on the PP cell was direct or indirect, and also to compare the potencies of the various gut hormones in the release of PP with that of acetyl choline and adrenaline.

METHODS

The Isolated Perfused Canine Pancreas

Male mongrel dogs weighing 20-30 kg and fed ad libitum with tripe, Purina chow and water, served as pancreas donors. The animals were fasted overnight, anaesthetised with thiopentone, intubated and ventilated with positive pressure respiration, using oxygen and nitrous oxide in a ratio of 1:1:3. The operative procedure was carried out and the perfusion system established as described in detail by Iversen (1971). The preparation consisted of the pancreas and the proximal 10 cm of attached duodenum. The coeliac artery, the splenic artery and the portal vein were catheterised. The pancreas was perfused, without recirculation, with an oxygenated synthetic medium which consisted of Krebs Ringer buffer pH 7.4 adjusted to the electrolyte concentrations of dog plasma with 40 g/l dextran, bovine serum albulin at 0.35 mmol/l and glutamate, fumarate and pyruvate each at concentrations of 0.5 mmol/l. The perfused flow rate was 18-20 ml/min (approaximately 0.25 ml/min/g of pancreas wet weight) and the perfusion pressure was 30-40 mm Hg. Both parameters were constant during the perfusion experiment. The oxygen uptake of the isolated pancreas was 0.005 ml/min/g (Iversen, 1974). Each perfusion experiment was carried out with hourly alterations using glucose concentrations of 1.4 and 8.3 mmol/l respectively.

Hormone Perfusion

The pure peptide hormones, human gastrin (synthetic human gastrin 1. ICI Ltd), caerulein - an analogue of cholecystokinin (synthetic "caeruletide", Farmilatia Ltd), porcine pancreatic glucagon (Novo Ltd), bovine pancreatic polypeptide (BPP, Lilly Research Laboratories), porcine gastric inhibiting peptide (GIP) (Professor J.C.Brown), porcine vasoactive intestinal peptide (VIP) (Professor S.I.Said) and bombesin (synthetic

Farmilatia Ltd), were added to the perfusate in ramdom order at a concentration of 1 nmol/l. Acetyl choline was perfused at a concentration of 1 μmol/l and adrenaline at 11 nmol/l. When investigating the effect of inhibitors on the release of the pancreatic hormones, synthetic cyclic ovine somatostatin was added to the perfusate at 60 nmol/l and atropine at 25 μmol/l. The pancreas preparations were perfused with each substance for 10 minutes with a recovery period of at least 20 minutes before the next test substance. Samples were taken every minute from the influx and the efflux perfusate. Even though there is no evidence that proteolytic enzymes escape into the venous drainage from the perfused pancreas (Iversen, 1973a) a proteolytic enzyme inhibitor, approtinin (Trasylol, Bayer Ltd) was added to the tubes in which the efflux samples were collected to give a final concentration of 400 K I units/ml. PP concentrations in the efflux perfusate were measured by the radioimmunoassay described in Chapter 2 using the buffer system.

RESULTS

Release of PP from the Isolated Perfused Canine Pancreas

The release of PP by the gut peptide hormones can be seen in Figure 601 and the mean zero peak of each stimulus is summarised in Table 601. The basal and stimulated PP concentrations were not altered by the different glucose concentrations used : for example, at 8.3 mmol/l glucose concentrations basal PP was 23 $\pm$ 6 pmol/l (mean $\pm$ SEM) and the peak after caerulein 66 $\pm$ 2 pmol/l while at 1.4 mmol/l glucose concentrations basal PP was 23 $\pm$ 6 pmol/l and caerulein peak 62 $\pm$ 4 pmol/l (both measured in the same four preparations). The results in Table 601 therefore represent the concentrations of PP after the first application of the stimulus

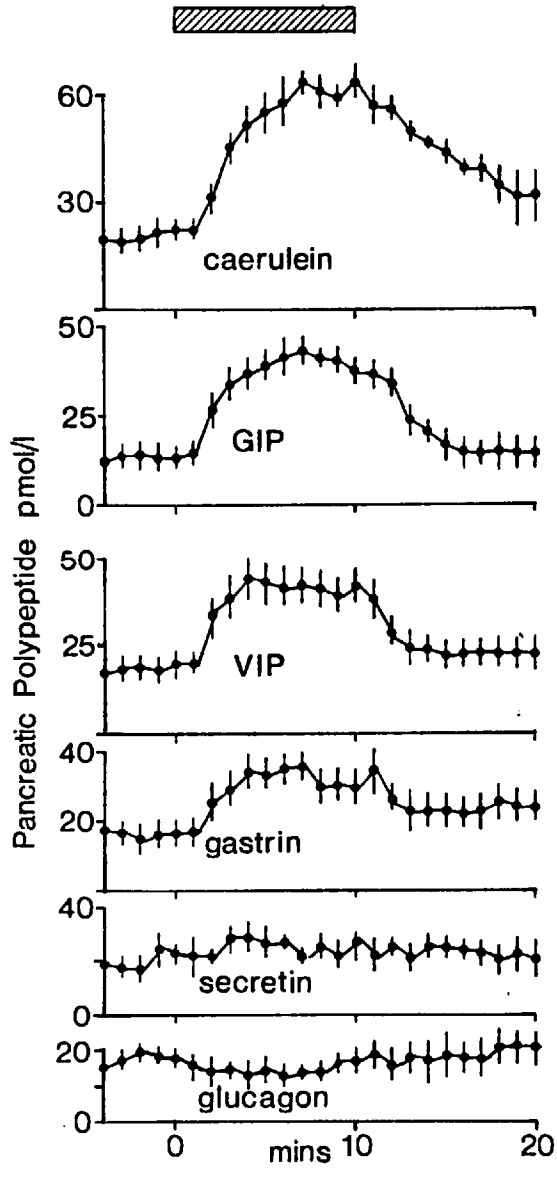


Figure 601: The effect of 10 minute perfusion of gut peptide hormones (1 nmol/l) on efflux pancreatic polypeptide concentrations from the isolated perfused canine pancreas. The number of experiments for each hormone is given in Table 601.

TABLE 601:

PERFUSED SUBSTANCE	NUMBER OF PREPARATIONS	MEAN PP CONCENTRATION pmol/l Zero	Peak	P <
Caerulein	5	22.8 ± 3.9	64.4 ± 2.7	0.001
Gastrin	6	17.7 ± 4.4	35.5 ± 4.3	0.05
Glucagon	4	18.3 ± 2.1	18.3 ± 1.3	NS
Secretin	5	16.2 ± 2.2	23.0 ± 4.0	NS
GIP	6	13.3 ± 4.1	43.5 ± 4.4	0.005
VIP	6	20.5 ± 4.1	44.8 ± 7.4	0.05
Bombesin	2	10.0	12.0	-
Acetyl Choline	6	18.5 ± 4.6	223 ± 11	0.001
Adrenaline	6	20.8 ± 4.4	27.7 ± 5.2	NS

The mean basal and peak PP concentrations in pmol/l in the efflux perfusate of the isolated dog pancreas during perfusion with hormones and neurotransmitters.

in each pancreas preparation regardingless of the glucose concentrations. Caerulein and GIP caused a highly significant release of PP from all preparations and the release elicited by gastrin and VIP were also significant. Secretin and glucagon, however, did not release PP at the dosed used in this in vitro preparation. Acetyl choline is extremely potent in its capacity to release PP (Figure 602). The data shown represent experiments carried out with a dose of 1 μmol/l acetyl choline. However, in a single experiment a dose of 10 pmol/l gave a response which was 50% maximum. A small rise in perfusate PP concentrations was observed during adrenaline perfusion but this did not reach statistical significance (Figure 602). Bombesin was perfused in only two preparations and no effect on PP was observed (Figure 602). Atropine completely abolished the release of PP elicited by acetyl choline by 97.3 ± 1.7 ($n = 4$) inhibition, but had no significant effect on caerulein induced PP release in two preparations. Somatostatin inhibited the release of PP evoked by acetyl choline by $61.3 \pm 9.8\%$ ($n = 4$) and completely blocked the release of PP induced by caerulein in two preparations.

An interesting result was obtained in a single preparation in which a VIP perfusion followed a short time after acetyl choline (Figure 603). The PP release by VIP was very much greater than that observed with other experiments. It was thus excluded from the group for statistical analysis.

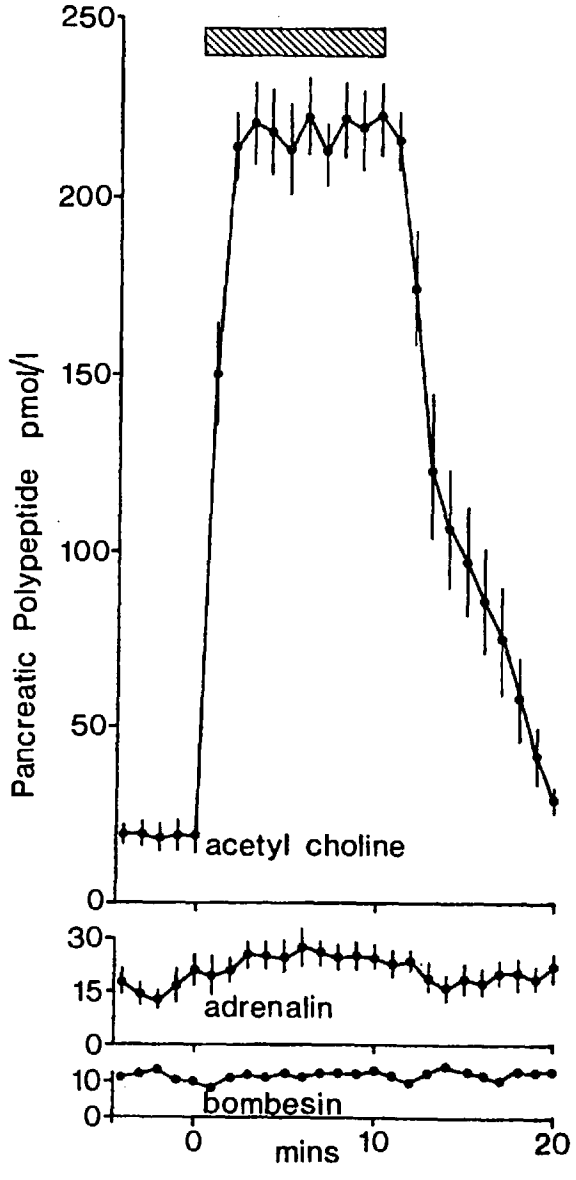


Figure 602:

The effect of 10 minute perfusion of acetyl choline (1 μmol/l), adrenaline (11 nmol/l) and bombesin (1 nmol/l) on efflux pancreatic polypeptide concentrations from the isolated perfused canine pancreas. The number of experiments from each stimulus is given in Table 1.

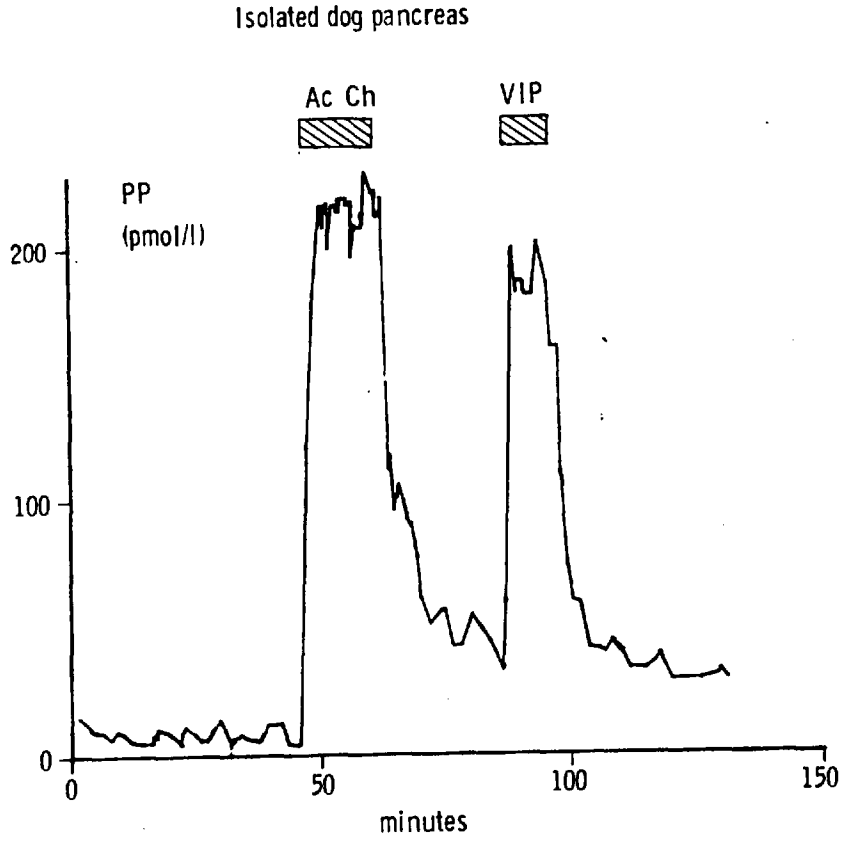


Figure 603:

The enhanced release of PP evoked by 1 nmol/l VIP perfusion when following closely after a perfusion of 1 µmol/l acetyl choline in the isolated perfused canine pancreas.

DISCUSSION

Release of PP from the Isolated Perfused Canine Pancreas

It is now well established that PP circulates in plasma and there is a rapid and substantial rise after a meal (Floyd et al, 1976; Adrian et al, 1976; Schwartz et al, 1976). As PP is found almost exclusively in the pancreas, it has been proposed that an entero-PP axis (Adrian et al, 1977) analogous to the well established entero-insular axis, is responsible for the release of this hormone following intestinal stimulation by food. The entero-PP axis appears to be mediated partly through the vagal innervation and partly through circulating gut hormones. The absence of any glucose effect on PP release from the isolated pancreas is consistent with the lack of effect of circulating nutrients noted in man. The experiments reported here have demonstrated at the tissue level that circulating hormones and acetyl choline can release PP directly and thus are possible physiological components of the entero-PP axis. The release by acetyl choline is in agreement with the findings reported by other workers (Schwartz et al, 1978), as is the minor effect of catecholamines on PP release (Samols et al, 1977). As expected, the PP release by acetyl choline is totally blocked by atropine. Somatostatin reduced the PP response to the high dose of acetyl choline used in the experiments and effectively blocked the PP release induced by caerulein; this is expected from previous work in man (Adrian et al, 1977; Marco et al, 1977). The apparent synergism observed between acetyl choline and VIP is of great interest and may be presumed to reflect the in vivo situation where some degree of continuous vagal tone is probable.

In spite of the anatomical grouping of the A, B and PP cells in the Islets of Langerhans the release of PP does not appear to be integrated with the release of insulin and glucagon. This is perhaps not surprising as in some species, e.g. dog, many of the PP cells lie outside the islets,

e.g. between the acinar cells and in duct walls (Larsson et al, 1976). PP is mainly influenced by circulating hormones, acetyl choline and peptidergic neurotransmitters whilst insulin and glucagon release is in addition, powerfully affected by metabolites and catecholamines. These experiments demonstrate directly how the observed influence of the gut on the hormones of the endocrine pancreas can be accounted for by the combined integrated influence of metabolites, hormonal and nervous control.

It has recently been established that in addition to the well recognised adrenergic and cholinergic nerve fibres a third component of the autonomic nervous system exists (Bloom and Polak, 1978). This is the peptidergic system, which may be anatomically identical, in part, with the previously described system to which the term "purinergic" has been applied. Thus the islets of Langerhans are richly innervated with, for example, VIP-containing nerve fibres (Bryant et al, 1976). It is impossible at present to evaluate the possible concentration of peptide neurotransmitter which may be present at the target cell. Our finding of the effectiveness of low concentrations of VIP in releasing PP as well as glucagon and insulin, however, adds considerable emphasis to its possible role in the physiological control mechanisms of the endocrine pancreas.

CHAPTER 7. PHYSIOLOGY OF PANCREATIC POLYPEPTIDE-INFUSION STUDIES IN MAN

INTRPDUCTION

Physiology of Pancreatic Polypeptide-Infusion Studies

When infused into the dog bovine pancreatic polypeptide had a broad spectrum of biological actions on the gastrointestinal tract including stimulation of basal gastric acid secretion, inhibition of pentagastrin stimulated acid, inhibition of pancreatic enzyme secretion and relaxation of the gall bladder (Lin et al, 1977). Although BPP had no apparent metabolic effects in the dog APP caused hepatic glycogenolysis in the chicken (Kimmel et al, 1978). In rodents PP has been shown to enhance the rate of gastric emptying of solid food (Lin and Chance, 1978).

In an attempt to assess which, if any, of these observed biological actions in animals could be considered to be physiological in man we undertook infusion studies in a total of 55 healthy volunteers (mean age 39, range 22-64). Of these 55 subjects 26 received infusions of BPP in the basal state at a dose which achieved plasma PP levels of equal to that seen after a normal test breakfast (approximately 200 pmol/l, n = 6) and at doses giving 3 (n = 9) and 5 (n = 11) times this level. Full informed consent was obtained from each subject and all experiments had prior approval from the Royal Postgraduate Medical School Ethical Committee. Temperature, blood pressure, ECG and routine haematological and biochemical (including hepatic and renal) measurements were monitored. The infusions did not produce any subjective side effects or change in pulse rate, respiration or blood pressure. There was no change in red cell or platelet counts. Similarly biochemical parameters of renal and hepatic function and plasma electrolytes including calcium did not change.

Pharmacokinetic studies were carried out on 23 of these subjects.

The effect of PP on basal gastric acid and pepsin secretion was assessed in 8 subjects at the high dose and the effect on basal biliary and pancreatic exocrine function in 7 subjects at the high dose. The effects of PP in circulating metabolite concentrations and on gastrointestinal and pancreatic hormones was assessed in 7 subjects at the low dose and 10 at the high dose.

In experiments to assess the effect of PP on stimulated gastric secretion PP was infused during submaximal stimulation by pentagastrin in 8 subjects at the middle dose level mentioned above. The middle dose was also used to assess the effect of PP on secretin stimulated pancreatic exocrine function in 5 subjects. The effect of the low dose of PP on pancreatic secretion was assessed in 7 normal subjects and 4 cholecystectomised patients during stimulation with a low dose of caerulein and secretin. In order to study the effect of PP on the rate of gastric emptying, PP was infused at 1 and 2 pmol kg^{-1} min^{-1} in 5 subjects before and during the ingestion of a small carbohydrate rich breakfast. The effect of PP on postprandial concentrations of circulating metabolites and pancreatic and gastrointestinal hormones was also assessed during these studies.

METHODS

Infusion Procedure

In all infusion studies subjects were fasted overnight and an antecubital vein was catheterised at 0.15 M saline with human serum albumin (200 µmol HSA/l) was infused from a syringe ram pump for 30 minutes. BPP was added to the infusate at nominal doses of 1, 3 and 5 pmol kg^{-1} min^{-1}. At the end of each infusion a sample of infusate

was collected from the venous end of the catheter for PP measurement by radioimmunoassay. Blood samples were collected from a second in-dwelling catheter inserted in the opposite arm.

Pharmacokinetics of PP

Twenty-three healthy volunteers (mean age 37, range 22-48) were included in this study. BPP infusions were carried out as described above at 1 (n = 6), 3 (n = 9) and 5 (n = 8) pmol kg^{-1} min^{-1} for 1 hour. Blood samples were collected at 10 minute intervals before and during the PP infusion and at 5 minutes before and 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 minutes after termination of the infusion for half-life studies on the infused PP. Calculation of pharmacokinetic data were based on the plateau principle described by Goldstein et al (1974). To determine the half time of decay ($t\frac{1}{2}$) on PP each subject's mean basal level was substracted from all subsequent values. The post-infusion values were normalised by expressing them as a percentage of the previous steady state level. These values were then converted to their natural logarithms and were plotted against time. The t $\frac{1}{2}$ value for each infusion was derived from the resulting straight line plot. In addition, the mean of the normalised data for all 23 infusions for each time period was calculated. These values were converted to their natural logarithms and linear regression analysis was performed. The metabolic clearance rate (MCR) was calculated by dividing the infusion rate (in pmol kg^{-1} min^{-1}) by the steady state increment in plasma PP (in pmol/ml). The apparent space of distribution (DS) was calculated from the formula ; DS (ml/kg) = MCR x $t\frac{1}{2}$ x 1.44 (Goldstein et al, 1974).

Effect of PP on Basal Gastric Secretion

Following an overnight fast, a modified Andersen tube (ANIO. H.W. Andersen Inc., New York), was positioned under fluoroscopic control in

the body of the stomach. Throughout the experiment a non-absorbable marker, phenol red (0.15% was infused intragastrically at 12 ml/hour via a separate polyvinyl catheter (internal diameter 1 mm) attached to the gastric tube. Gastric juice was aspired for 3 hours by continuous suction (Air Shields Inc., Hatboro, PA), and pooled in 10 minute collections. PP was infused at 5 pmol kg^{-1} min^{-1} for 1 hour in 8 subjects as described above, during the middle hour of a three hour measurement of basal acid and pepsin production. Gastric aspirates were analysed for volume acidity (electrometric autotitration against 0.1 M sodium hydroxide to end point pH 7.0) and pepsin (antoanalyser haemoglobin substrate method, Vatier et al, 1978). Phenol red concentration was estimated colorimetrically and used to correct gastric juice volume for pyloric loss (Hobsley and Silen, 1969).

Effect of PP on Pentagastrin-Stimulated Gastric Secretion

Intubation and gastric juice collection was carried out as described above (Effect of PP on basal gastric acid and pepsin output). After a 30 minute period of basal gastric juice collection, pentagastrin was infused intravenously for 150 minutes at a dose (0.7 μg kg^{-1} hr^{-1}) which achieved about 50% of maximal acid output. After 60 minutes of pentagastrin alone BPP was infused for 50 minutes at 3 pmol kg^{-1} min^{-1}. A final 30 minute basal collection followed the pentagastrin infusion. Laboratory analysis of gastric juice was carried out as described above (Effect of PP on basal gastric acid and pepsin output).

Effect of PP on Basal Pancreatic and Biliary Secretion

Following an overnight fast, a modified Andersen tube (ANIO. H.W. Andersen Inc., New York) was positioned under fluoroscopic control with its tip in the fourth part of the duodenum. Isotonic saline containing a non-absorbable marker polyethlene glycol(4,000, 5 g/l) was infused

into the second part of the duodenum at a constant rate of 5 ml/min via a separate polyvinyl catheter (internal diameter 1 mm) attached to the duodenal tube. Duodenal contents were aspirated 30 cm distally by continuous suction (Air Shields, Hatboro, PA) and pooled over ice in 10 minute collections. A separate gastric tube (ANIO. H.W.Andersen Inc., New York) was positioned in the body of the stomach for continuous suction of gastric contents. Absence of contamination of duodenal aspirates by gastric juice was confirmed by a duodenal pH greater than 6.5. The effect of PP at a dose of 5 pmol kg^{-1} min^{-1} on a three hour basal pancreatic (bicarbonate and trypsin) and biliary (bilirubin) outputs was studied by infusion PP during the middle hour in the manner described above. Duodenal juice bicarbonate concentration was measured by electrometric autotitration, by the addition of 0.1 M hydrochloric acid and back titration with 0.1 M sodium hydroxide. Trypsin was measured by the method of Haverback et al (1963), bilirubin by the method of Malloy and Evelyn (1937) and polyethlene glycol (PEG) by the method of Hyden (1955). Aspirated volumes were corrected from the recovery fraction (RF) or PEG:

$$RF = \frac{Vd \times PEG\ d}{Vi \times PEG\ i}$$

Vd	=	volume of duodenal aspirate
PEG d	=	concentration of PEG in duodenal sample
Vi	=	volume of marker infused
PEG i	=	concentration of PEG in infusate
$\frac{Vd}{RF}$	=	corrected volume

The secretion volumes were calculated from the corrected volumes by substraction of the infused volume. The outputs of bilirubin, bicarbonate and trypsin were calculated from the concentrations and secretion volumes.

Effect of PP on Secretin Stimulated Pancreatic Secretion

Following an overnight fast the subjects were intubated under fluoroscopic control : a double lumen tube was positioned in the second part of the duodenum and the nasogastric tube in the antrum. Gastric and duodenal juice was aspirated continuously and the latter kept on ice and divided into 10 minute samples. After the resting duodenal juice had been removed, two 10 minute basal samples were collected. Secretin (GIH 0.15 cu kg^{-1} hr^{-1}) was infused for 3 hours into an antecubital vein using a syringe ram pump via an in-dwelling intravenous catheter. During the middle hour PP was added to the infusate at 3 pmol kg^{-1} min^{-1}. Duodenal juice bicarbonate concentrations and tryptic activity were measured as described above (Effect of PP on basal pancreatic and biliary secretion). Total protein concentration was measured by the method of Lowry et al (1951).

Effect of a Low Dose of PP on Stimulated Pancreatic and Biliary Secretion.

Seven healthy subjects mean age 37 years and 4 patients who had had cholecystectomy (mean age 45 years) without common bile duct exploration or sphincteroplasty, 6 months to 3 years earlier, took part in this study. All were healthy and had no current gastrointestinal symptoms.

Intubation and duodenal juice collection was carried out as described above (Effect of PP on basal pancreatic and biliary secretion). The effect of PP on pancreatic (bicarbonate and trypsin) and bilary (bilirubin) outputs was studied by intravenous infusions of secretin (GIH, 0.1 cu kg^{-1} hr^{-1}) and caerulein (7.5 pmol kg^{-1} hr^{-1}) for 220 minutes, to mimic normal postprandial pancreatico-biliary secretions. After 90 minutes PP was infused at 1 pmol kg^{-1} min^{-1} for 60 minutes. Duodenal juice bicarbonate and bilirubin concentrations and trypsin

activity were measured and corrected as described above (Effect of PP on basal pancreatic and biliary secretion).

Effect of PP on the Rate of Gastric Emptying During Breakfast.

Five healthy subjects aged 26-45 were studied. The rate of gastric emptying of a solid marker mixed with a test breakfast in each subject was measured on 3 separate days. On one day 0.15 M saline alone was infused and on the other two days PP was infused at 1 and 3 pmol kg^{-1} min^{-1} respectively. The infusions were given in random order and care was taken to ensure that the subject was unaware of which infusion was being given either before or after the study. The gastric emptying method used was that of Cowley et al, (1973) which was modified by the use of ^{99m}Tc-tin colloid in the place of ^{129}Cs absorbed onto a zirconium phosphate resin, as a marker for gastric emptying. There were several reasons for this modification. Radioactive technetium gives a lower radiation dose than radioactive caesium and the half life is shorter (6 hours for ^{99m} and 32 hours for ^{129Cs}). ^{99m}TC emits gamma rays with an energy of 140 KeV which are better for imaging than ^{129}Cs which emits several gamma rays, the main ones being at 370 and 410 KeV, The ^{99mm}Tc-tin colloid could be prepared as required from a commercial kit (Radiochemical Centre N112) whereas ^{129}Cs was available only one day per week which would have limited studies to that particular day. The ^{99mm}Tc-tin colloid was prepared in the standard way by adding 1 mCi ^{99m}TC O_4 in 8 ml 0.15 M saline to the kit. A dose of 100 μCi was dispensed for the study together with a standard activity for comparison with the plasma samples. After positioning the subject under the gamma camera in a sitting position and a basal period of 20 minutes, a two hour infusion of either PP or saline was started. A standard test breakfast was taken 20 minutes after

the start of the infusion, the duration of the meal was between 5 and 8 minutes. The breakfast consisted of 25.5g porridge oats, 15g unskimmed milk, 20g melba toast, 5g margarine, 19.5g marmalade and 200 ml unsweetened orange juice and contained 8g protein, 4g fat, 70g carbohydrate (370 Cals). This carbohydrate rich breakfast was chosen as it elicited only a small release of endogenous PP. The 100 μCi dose of ^{99m}Tc-tin colloid was mixed with the porridge oats. Collection of data from the gamma camera commenced immediately following complete ingestion of the meal and recording was continued for 90 minutes. Data was stored in digital form on a magnetic tape and the rate of decrease of counts in the stomach was computed against time. The selection of the stomach area was based on the distribution of activity in the first four minutes of acquisition of data, but the position of the stomach was checked throughout the study to confirm that it remained wholly within the area of count analysis. The assessment of the gastric area was performed blind by an independent medical physicist.

Effect of PP on Circulating Metabolites

The effect of PP infusion on fasting plasma concentrations of glucose, lactate, 3 hydroxybutyrate, alanine, glycerol (Lloyd et al, 1978) and non-esterified fatty acids (Ho Meng, 1968), was measured during infusion rates of 1 pmol kg^{-1} min^{-1} (n = 7) and 4 pmol kg^{-1} min^{-1} (n = 10). In addition these circulating metabolites were measured during the posprandial infusions described above. (Effect of PP on the rate of gastric emptying during breakfast). These studies on post-prandial metabolite concentrations were again carried out at two dose levels 1 pmol kg^{-1} min^{-1} and 2 pmol kg^{-1} min^{-1}.

Effect of PP on Pancreatic and Gastrointestinal Hormones

Plasma concentrations of insulin (Albano et al, 1972), glucagon

(Alford et al, 1977) gastrin (Russell et al, 1976), secretin (Hacki et al, 1977), gastric inhibitory peptide (GIP) (Sarson et al, 1980), motilin (Bloom et al, 1976), neurotensin (Blackburn and Bloom, 1979), enteroglucagon (Thompson and Bloom, 1976), were all measured during infusion of PP at doses of 1 pmol kg^{-1} min^{-1} (n = 7) and 4 pmol kg^{-1} min^{-1} (n = 10). In addition plasma levels of these circulating pancreatic and gastrointestinal hormones were monitored in the gastric emptying experiments described above (Effect of PP on the rate of gastric emptying during breakfast). The studies on postprandial release of gastrointestinal hormones, glucagon and insulin were again carried out at two dose levels of PP pmol kg^{-1} min^{-1} and 2 pmol kg^{-1} min^{-1}.

RESULTS

The measured infusion dose and the mean basal and plateau plasma PP concentrations in all studies are shown in Table 701.

Pharmacokinetics of PP

The mean PP dose measured by radioimmunoassay and the half life, metabolic clearance rate, and distribution space calculated for each of the three infusion doses can be seen in Table 702. For all the 23 infusions the mean half life was 5.9 $\pm$ 0.3 (mean $\pm$ SEM) minutes, MCR 5.1 $\pm$ 0.2 ml/kg/min, and apparent space of distribution 51 $\pm$ 3 ml.

The mean plasma levels of PP in nine subjects during infusion of the middle PP infusion dose (2.84 $\pm$ 0.18 pmol/kg/min) is shown in Figure 701. The steady state increments of plasma PP reflect the grading of the three infusion doses (1:2.8:4.6) as would be expected if endogenous PP secretion were not suppressed during the administration of exogenous PP. This finding validates the substraction of baseline levels when

	NUMBER OF SUBJECTS	INFUSION DOSE pmol kg^{-1} min^{-1}		PLASMA PP pmol/l	
		NOMINAL	MEASURED mean ± SEM	BASAL	PLATEAU mean ± SEM
PHARMACOKINETICS	6	1	0.98 ± 0.13	20 ± 5	203 ± 34
PHARMACOKINETICS	9	3	2.84 ± 0.18	18 ± 4	575 ± 73
PHARMACOKINETICS	8	5	4.88 ± 0.34	22 ± 3	930 ± 48
BASAL GASTRIC SECRETION	8	5	4.50 ± 0.55	24 ± 7	907 ± 52
BASAL PANCREATIC SECRETION	7	5	5.33 ± 0.47	23 ± 5	934 ± 56
STIMULATED ACID SECRETION	8	3	2.88 ± 0.42	24 ± 5	687 ± 76
SECRETIN STIMULATED PANCREATIC SECRETION	5	3	3.36 ± 0.22	23 ± 4	711 ± 69
CAERULEIN & SEC.STIM. PANCREATIC SECRETION	7	1	1.08 ± 0.08	28 ± 6	215 ± 20
CAERULEIN & SEC.STIM. PANCREATIC SECRETION IN CHOLECYSTECTOMISED SUBJECTS	4	1	1.10 ± 0.12	31 ± 4	218 ± 27
GASTRIC EMPTYING	5	1	1.04 ± 0.07	25 ± 4	218 ± 20
GASTRIC EMPTYING	5	2	2.11 ± 0.13	25 ± 4	410 ± 27

TABLE 701: The number of subjects, measured PP infusion dose, basal and plateau plasma PP concentrations in the subjects receiving PP infusions.

PP doses (pmol/kg/min)	0.98 ± 0.13 (n = 6)	2.84 ± 0.18 (n = 9)	4.58 ± 0.34 (n = 8)
Basal plasma PP Concn (pmol/l)	20 ± 5	18 ± 4	22 ± 3
Plateau plasma PP Concn (pmol/l)	203 ± 34	575 ± 73	930 ± 48
$t_{\frac{1}{2}}$	6.9 ± 0.5	6.9 ± 0.6	7.0 ± 0.6
MCR (ml/kg/min)	5.2 ± 0.4	5.0 ± 0.4	5.1 ± 0.4
DS (ml)	50 ± 2	50 ± 6	53 ± 5

TABLE 702: Basal and plateau plasma PP concentrations, half-time for decay ($t_{\frac{1}{2}}$), metabolic clearance rate (MCR), and apparent distribution space (DS) for BPP infusions in 23 subjects at three dose levels.

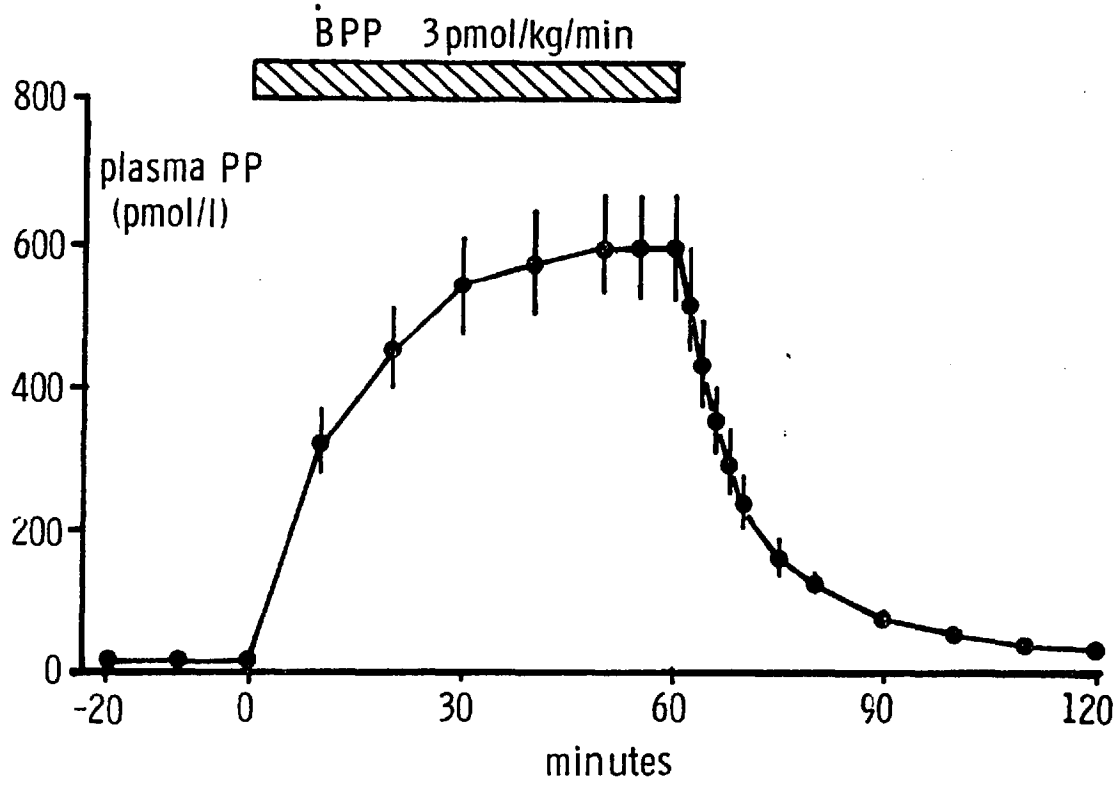


Figure 701: Plasma PP concentrations during a 3 pmol kg^{-1} min^{-1} pure bovine pancreatic polypeptide infusion over 60 minutes in nine healthy subjects (SEM marked with bar).

calculating the pharmacokinetic data.

Figure 702 shows the normalised data of the post-infusion period plotted semi-logarithmically versus time. A good fit to the calculated linear regression line was noted with a correlation coefficient of 0.999.

Effect of PP on Basal Gastric Secretion

Basal volume, acid and pepsin output remained unchanged during the infusion of PP and in the subsequent hour (Table 703).

Effect of PP on Pentagastrin Stimulated Gastric Secretion

Acid output increased significantly from a fasting level of 6 ± 2.4 mmol/hr to a plateau of 25.2 ± 4.4 mmol/hr during infusion of pentagastrin ($p < 0.005$). However, PP had no effect on this pentagastrin stimulated acid or pepsin output at the dose used in this study (Table 704).

Effect of PP on Basal Pancreatic and Biliary Secretion

Changes in duodenal measurements during each hour of the study are summarised in Table 705 while the temporal relationship with rising plasma concentrations of bovine PP is shown in Figure 703.

The effect of PP on basal net fluid accummulation in the duodenum is shown in Figure 703. A significant ($p < 0.02$) four-fold reduction in volume was observed throughout the PP infusion, followed by recovery in the post-PP hour. A progressive inhibition of trypsin output during PP was also observed (Figure 703) with outputs at 120 and 140 minutes being significantly ($p < 0.02$) lower than in the pre-infusion hour. After the PP, trypsin output then returned to pre-PP values. The most profound effect of PP was observed on bilirubin output (Figure 703). After 20 minutes of infusion, PP inhibited bilirubin output by 98% of pre-infusion levels ($p < 0.02$). Although there was a return of bilirubin to the post-infusion hour, the magnitude of rise was less than that observed for volume and

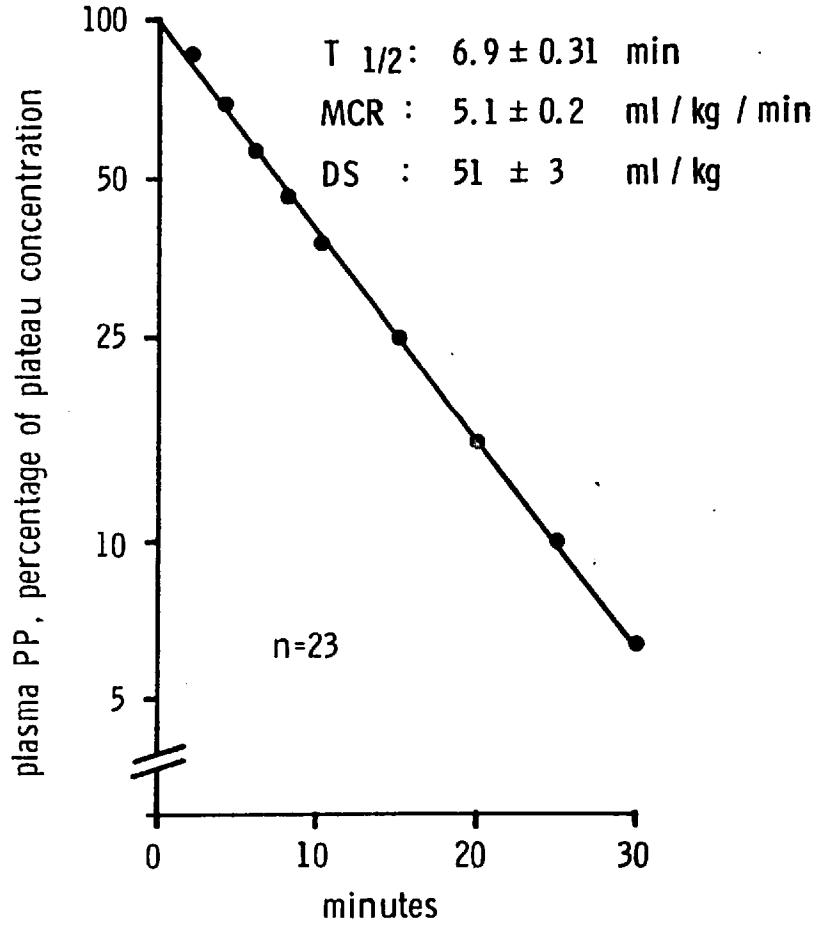


Figure 702: Plasma PP decay after discontinuing intravenous infusion plotted as mean percentage of steady state infusion increment. Linear decay suggests first order kinetics (n = 23).

	BASAL	PP	BASAL	P
VOLUME (ml/30 min)	60 (3-73)	67 (18-108)	53 (4-114)	ns
ACID (mmol/30 min)	2.7 (0.2-4.5)	1.8 (0.1-2.4)	2.3 (0.3-3.7)	ns
PEPSIN (mg/30 min)	(12-63)	(10-43)	(10-49)	ns

The effect of PP on basal volume, acid and pepsin output. (Results are expressed as median and range for last 30 minutes of each study hour).

ns = not significant (Wilcoxon's signed rank test).

TABLE 703

	BASAL	PENTAGASTRIN	PENTAGASTRIN + PP	PENTAGASTRIN	BASAL
ACID (mmol/30/min)	3 ± 1.2	12.6 ± 2.2	13.2 ± 2.1	12.5 ± 1.8	8.4 ± 1.3
PEPSIN (mg/30/min)	21 ± 9	41 ± 9	39 ± 10	36 ± 8	20 ± 6

TABLE 704: The effect of PP on pentagastrin stimulated gastric acid and pepsin secretion. (Results mean $\pm$ SEM of output for the last 30 minutes of each study period).

PARAMETERS (n7)	BASAL	DURING BPP		FROM BPP	
Volume (ml/h)	55 (13 to 189)	-27 (-7 to -115)	a	+57 (+4 to +166)	a
Trypsin output (IU/h)	142 (40 to 1632)	-92 (-40 to -1212)	a	+106 (+18 to +1230)	a
Bilirubin output (mg/h)	2.9 (0.3 to 10.6)	-2.9 (-0.3 to -10.5)	a	+1.9 (0 to 5.1)	a
Bicarbonate output (mmol/h)	0.3 (0 to 2.0)	+0.1 (-1.1 to +1.5)	b	0 (-2.2 to +1.5)	b

TABLE 705: Effect of Bovine Pancreatic Polypeptide on pancreatic and biliary functions in the fasting state.
(a. $p < 0.02$ b. non-significant)

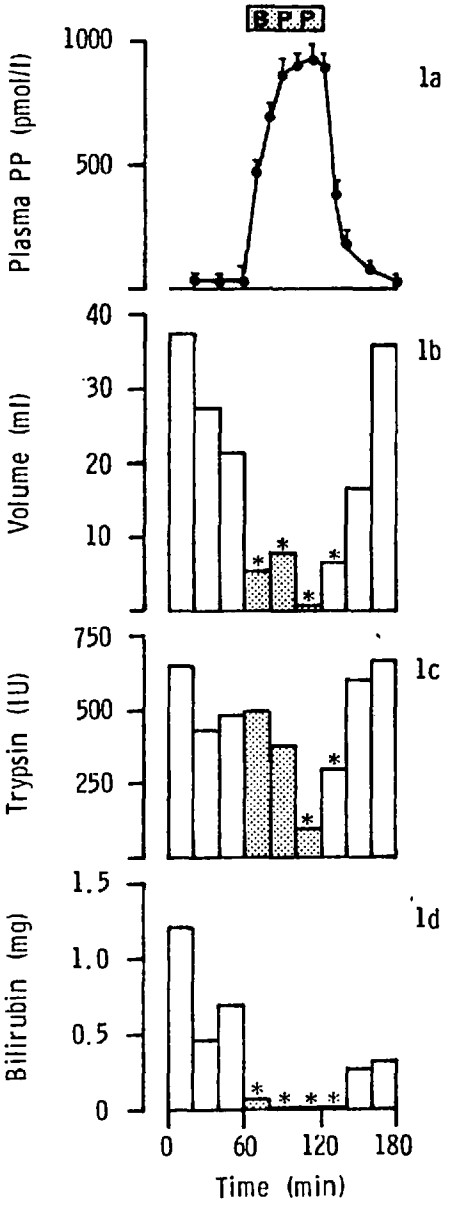


Figure 703: The effect of PP on fasting pancreatic and biliary secretion. Temporal relationship between plasma PP concentrations (mean $\pm$ SEM) and duodenal volume, trypsin and bilirubin outputs in seven healthy subjects (median) $p < 0.02$).

trypsin. Basal bicarbonate output was low, and during PP was not significantly different from basal (Table 705).

Effect of PP on Secretin Stimulated Pancreatic Secretion

The mean volume and bicarbonate output of the recovered duodenal juice is shown in Figure 704 and the mean bilirubin, protein, and trypsin output are shown in Figure 705. Because of the skewed nature of this data it was normalised by expressing the half hourly output of each parameter as a percentage of the 30 minute period immediately before the PP period for each individual. Statistical analysis was then carried out using paired t tests; the results are summarised in Table 706. The bicarbonate concentration of duodenal juice reached a plateau by the fourth 10 minute period of the secretin infusion and remained steady at approximately 140 mmol/l thereafter. The change in bicarbonate output during the PP infusion therefore reflects the change in the volume of duodenal juice. Enzyme output, measured both by specific tryptic activity and also by total protein concentration, was significantly reduced during the PP infusion and did not return to the pre-PP levels when the PP was stopped. Bilirubin output was reduced markedly and juice collected during the second half of the PP infusion was always colourless. In three subjects pigment did not return until 40-60 minutes after the end of the PP period and in the other two subjects pigment was seen in the post PP hour. The response to the infusion of Boots' pancreozymin and secretin at the end of the experiment was substantial for this stimulus indicating a normally functioning pancreas. In all subjects the second 10 minute period mean volume had risen to 46 ± 5 ml, bicarbonate output to 5.73 ± 0.86 mmol/10 min, protein to 266 ± 116 mg/10 min, trypsin to 358 ± 122 units/10 min, and bilirubin to 4.2 ± 1.3 pmol/10 min.

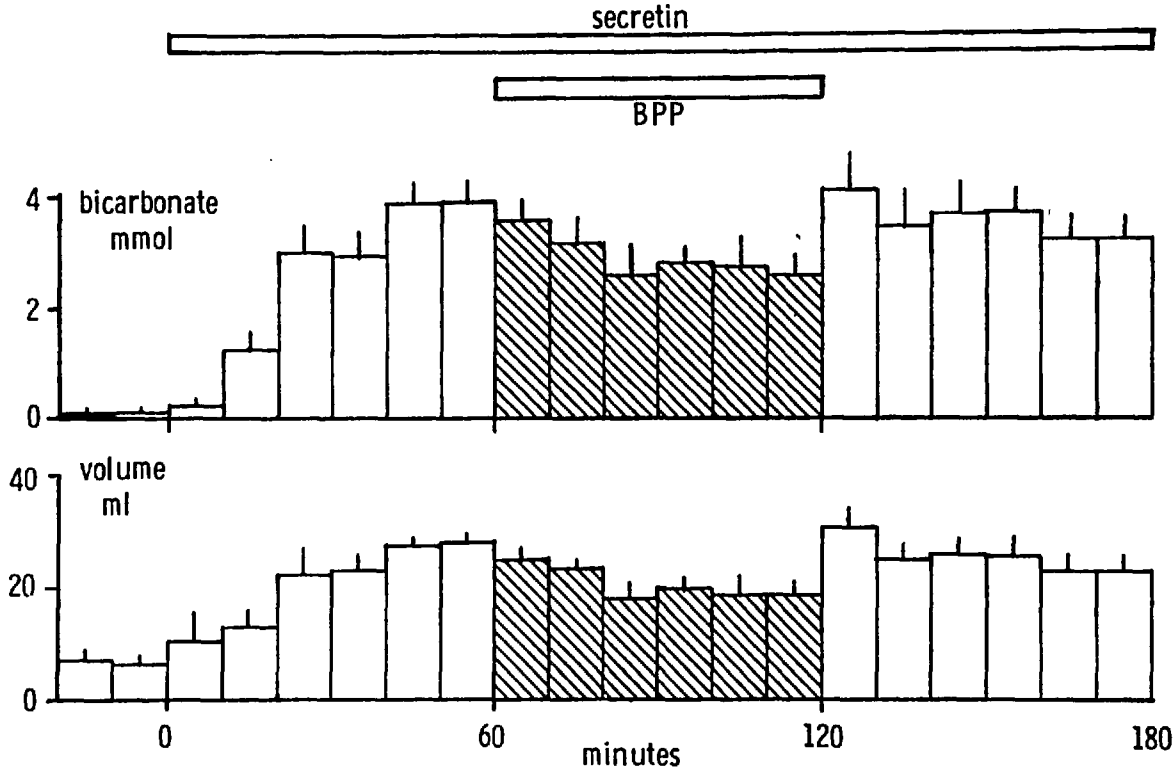


Figure 704: The effect of PP on secretin stimulated pancreatic secretion. Water and bicarbonate output fell significantly ($p < 0.05$) during PP infusion in the middle hour of a three hour secretin infusion in five healthy subjects.

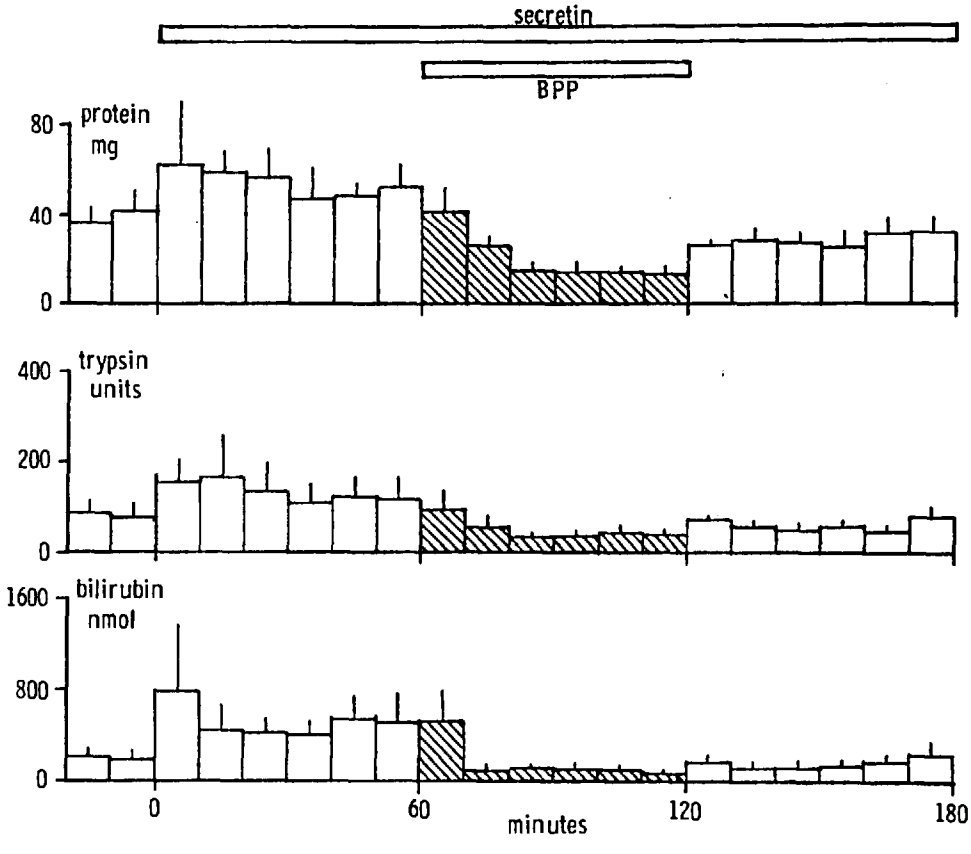


Figure 705: The effect of PP on secretin stimulated pancreatic secretion. Protein, trypsin and bilirubin output fell significantly ($p < 0.001$) during PP infusion in the middle hour of a three hour secretin infusion in five healthy subjects.

	VOLUME	BICARBONATE	PROTEIN	TRYPSIN	BILIRUBIN
Depression of output during first half hour of PP infusion.	86 ± 7	86 ± 8	58 ± 6	59 ± 7	57 ± 13
p <	ns	ns	.005	0.005	0.05
Depression of output during second half hour of PP infusion.	75 ± 8	76 ± 8	32 ± 7	42 ± 6	33 ± 12
p <	0.05	0.05	0.001	0.001	0.01
Recovery % of pre-PP period. First half hour.	105 ± 10	105 ± 13	61 ± 8	68 ± 13	41 ± 14
p <	ns	ns	0.01	ns	0.02
Recovery % of pre-PP period. Second half hour.	92 ± 10	95 ± 11	59 ± 8	66 ± 13	52 ± 17
p <	ns	ns	0.01	ns	0.05

TABLE 706: Effect of PP on secretin stimulated pancreatic secretion. The change in output during and after PP infusion expressed as a percent of the pre-PP infusion 30 minute period. (mean ± SEM ns - not significant).

Effect of a low dose of PP on Stimulated Pancreatic and Biliary Secretion

The mean fasting PP was 28 $\pm$ 6 pmol/l and rose significantly ($p < 0.02$) after caerulein and secretin to 59 $\pm$ 14 pmol/l (Figure 706) and 20 minutes after the bovine PP infusion plasma concentrations levelled off after a mean incremental rise of 187 $\pm$ 20 pmol/l. During the secretin infusion plasma secretin concentrations rose by 6 $\pm$ 1 pmol/l.

Duodenal net fluid accummulation significantly decreased ($p < 0.01$) after 20 minutes of the PP infusion and then returned to control levels 10 minutes after the PP infusion had ended (Figure 707, upper graph). A small but significant ($p < 0.05$) decrease in bicarbonate output was observed only in the last 10 minutes of the PP infusion, reflecting the change in volume (Figure 707, lower graph). In the cholecystectomised patients changes in volume and bicarbonate during the PP infusion were similar to those in control subjects (Table 707).

Trypsin output fell four-fold ($p < 0.001$) in control subjects 20 minutes after PP while bilirubin output was almost totally inhibited (Figure 708). Both then gradually returned to control levels in the post-PP hour. The decrease in trypsin and bilirubin output was a product of reductions both in volume and in concentration. By contrast, in cholecystectomised patients, PP inhibited trypsin output as in control subjects (Table 707) but bilirubin output was not significantly changed.

Effect of PP on the rate of Gastric Emptying During Breakfast

The mean PP doses as measured in the infusate were 1.04 $\pm$ 0.07 pmol kg^{-1} min^{-1} and 2.11 $\pm$ 0.13 pmol kg^{-1} min^{-1} (mean $\pm$ SEM) respectively. Plasma PP concentrations during the breakfast with and without PP infusion are shown in Figure 709.

The half emptying time for saline and both infusion doses of PP is shown in Figure 710. The mean percentage of the radioactive meal marker

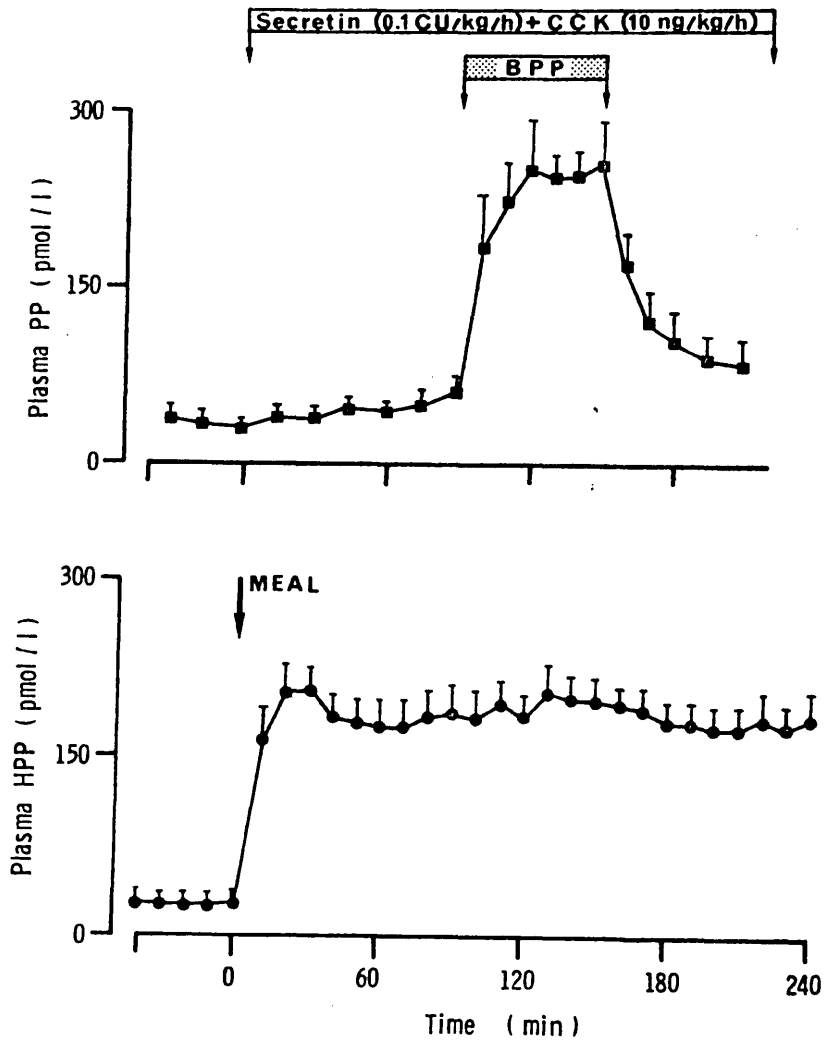


Figure 706:

Top: Plasma PP concentrations during a low dose infusion of secretin and caerulein and during PP infusion in seven healthy subjects.

Bottom: Plasma PP concentrations during a mixed breakfast in these seven subjects.

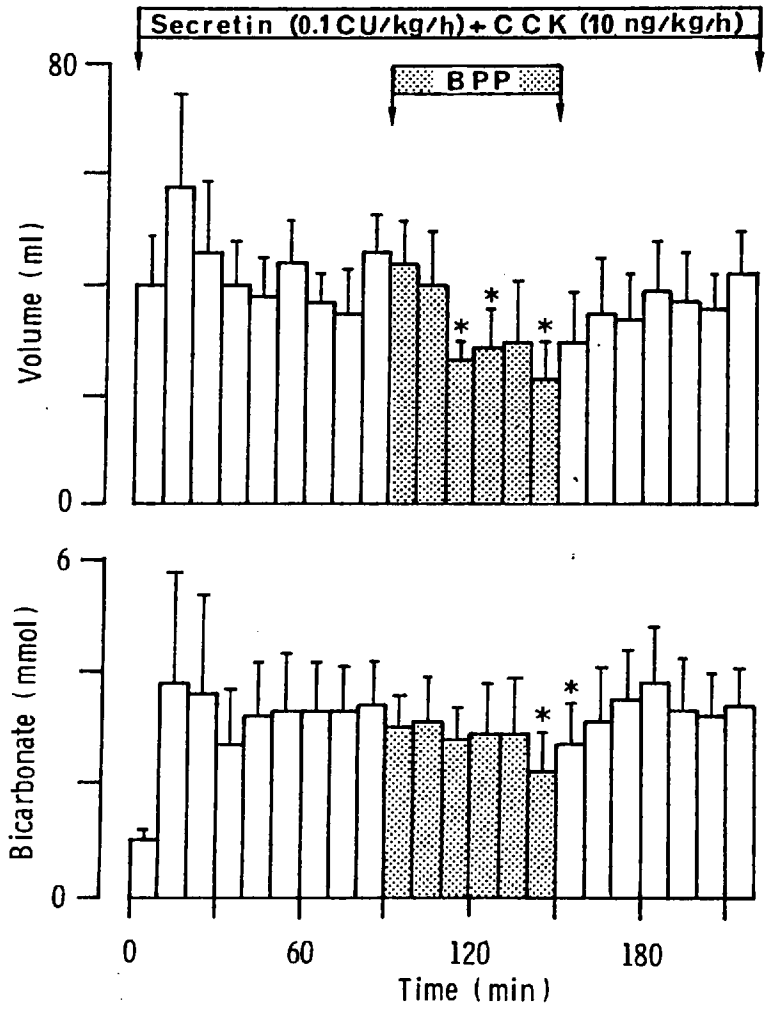


Figure 707: The effect of PP on volume and bicarbonate output during pancreatic stimulation with a low dose of secretin and caerulein in seven healthy subjects ($p < 0.05$; † $p < 0.01$).

	Control (n=7)		Cholecystectomy (n=4)	
	Basal	PP	Basal	PP
Volume (ml/h)	246 (36)	154 (45) ($p < 0.01$)	251 (70)	181 (69)
Trypsin output (IU/h)	20400 (2700)	8500 (2300) ($p < 0.001$)	24000 (3600)	10900 (3200) ($p < 0.001$)
Bilirubin output (mg/h)	8.9 (1.5)	1.2 (0.6) ($p < 0.01$)	5.0 (1.5)	4.3 (1.5)
Bicarbonate output (mmol/h)	20 (4.8)	16 (5.1)	19 (4.3)	14 (1.9)

TABLE 707: Effect of PP infusion on pancreatic and biliary functions during stimulation with a low dose of caerulein and secretin. Values are mean SEM.

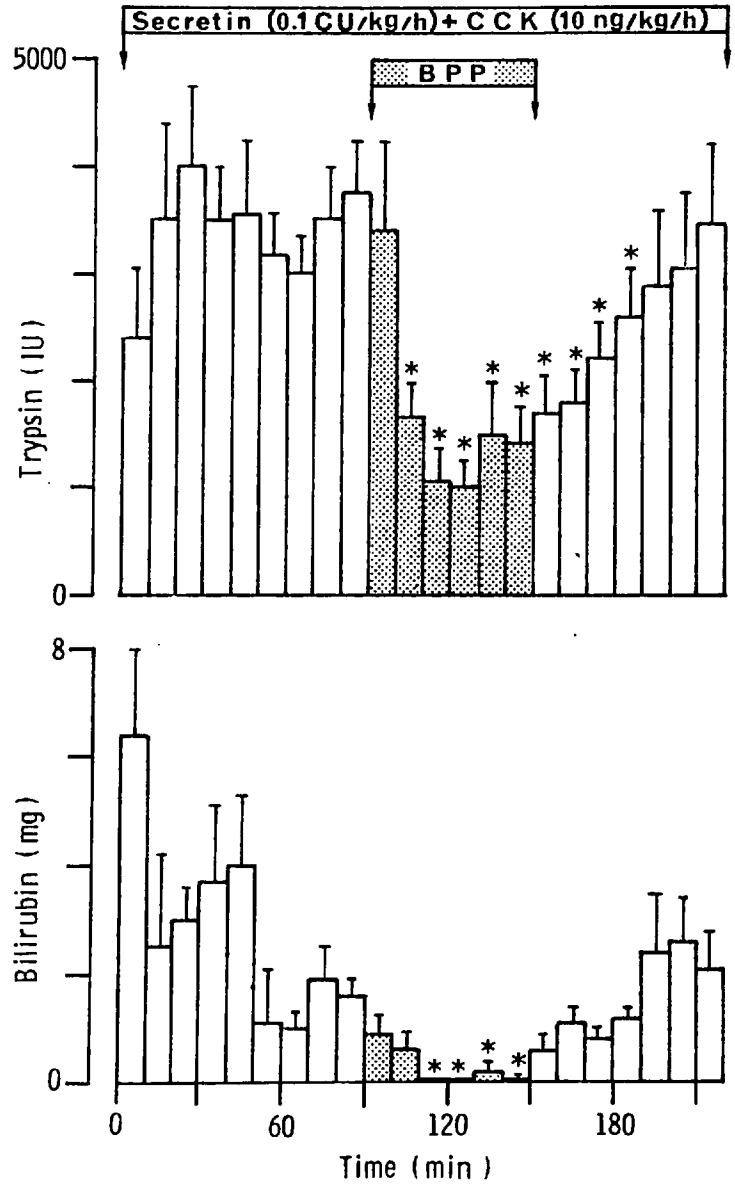


Figure 708: The effect of PP on trypsin and bilirubin output during pancreatic stimulation with a low dose of secretin and caerulein in seven healthy subjects. (*$p < 0.05$; † $p < 0.01$; †† $p < 0.001$).

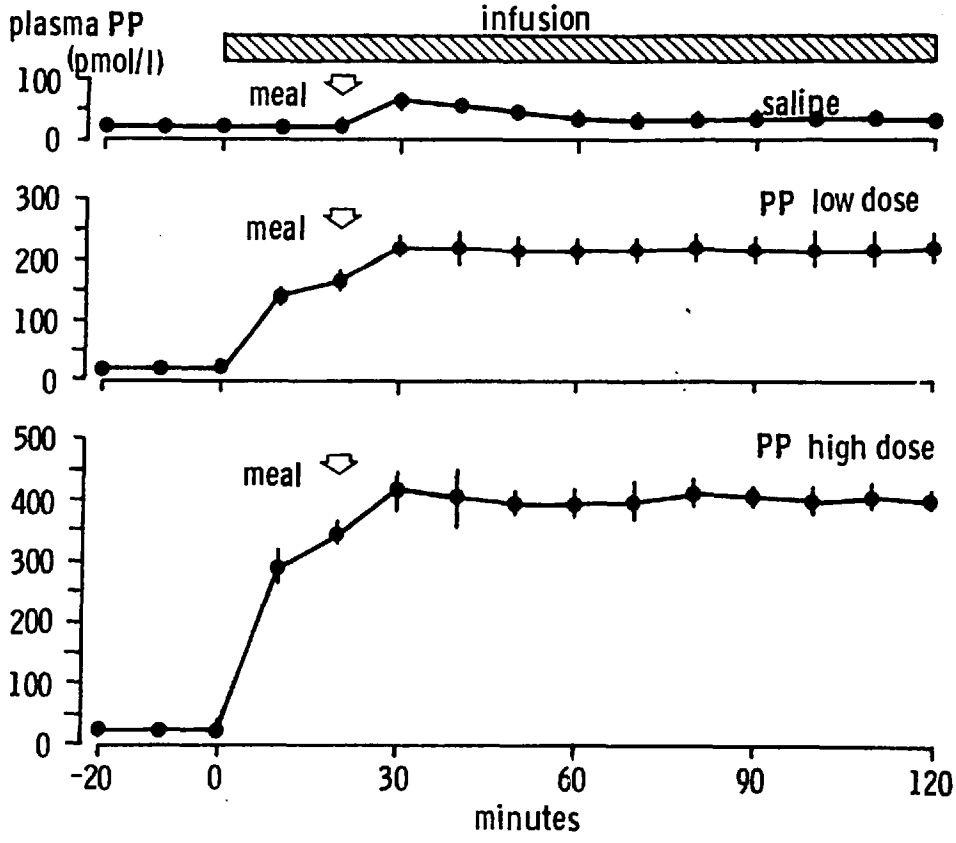


Figure 709: Plasma PP concentrations following a standard carbohydrate-rich breakfast during a saline infusion and PP infusions at doses of 1 pmol kg^{-1} min^{-1} and 2 pmol kg^{-1} min^{-1} respectively in five healthy subjects. Points represent mean $\pm$ SEM.

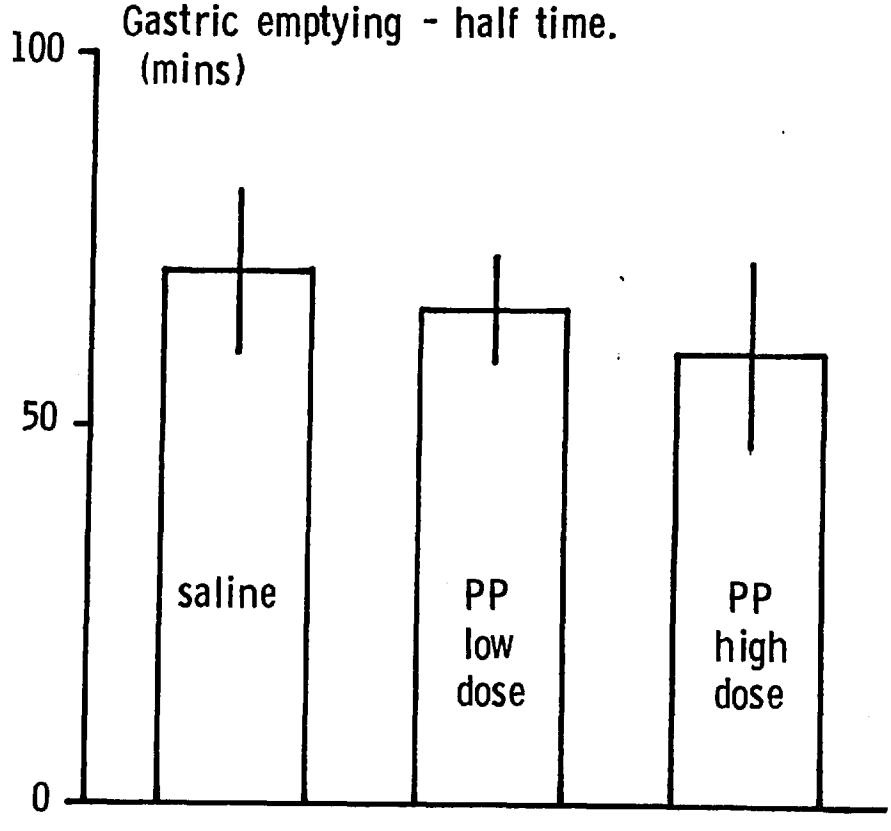


Figure 710: Half time for gastric emptying of a standard breakfast during a saline infusion and PP infusions at doses of 1 pmol kg^{-1} min^{-1} and 2 pmol kg^{-1} min^{-1} in five healthy subjects. Bars represent mean $\pm$ SEM.

remaining in the stomach area at a given time was not significantly different between the day with saline when compared to either study with PP. $T\frac{1}{2}$ for saline was 71 $\pm$ 12 min and was 66 $\pm$ 7 min and 60 $\pm$ 12 min for the low and high doses of PP respectively.

Effect of PP on Circulation Metabolites

Plasma PP concentrations during BPP infusion in the fasting state and during breakfast are shown in Figures 711 and 709 respectively.

PP had no significant effect on any of the circulating metabolite concentrations during the fasting PP studies, as shown in Table 708. Although a small rise in 4-hydroxybutyrate and a small fall in alanine levels was observed these changes were consistent with continued fasting, as they were also seen in the group receiving saline alone.

Following the ingestion of the carbohydrate-rich meal blood glucose, lactate and alanine concentrations rose and 3-hydroxybutyrate, glycerol and free fatty acid levels fell as expected. The postprandial concentrations of these metabolites were not significantly influenced by either dose of PP when compared with the control saline infusion (Table 709).

Effect of PP on Circulating Pancreatic and Gastrointestinal Hormones

Plasma PP concentration during BPP infusion in the fasting state and during breakfast are shown in Figures 711 and 709 respectively.

The effect of PP on fasting concentrations of pancreatic and gastrointestinal hormones are shown in Table 710. Infusion of PP caused no significant changes in plasma insulin, glucagon, gastrin, secretin, enteroglucagon gastric inhibitory peptide (GIP) or neurotensin concentrations. However in contrast PP caused a marked reduction in plasma motilin concentrations from 60 $\pm$ 10 pmol/l to 13 $\pm$ .3 pmol/l (mean $\pm$ SEM, $p<0.001$) (Figure 712). The concentrations of pancreatic and gastrointestinal hormones before and after the ingestion of the breakfast are

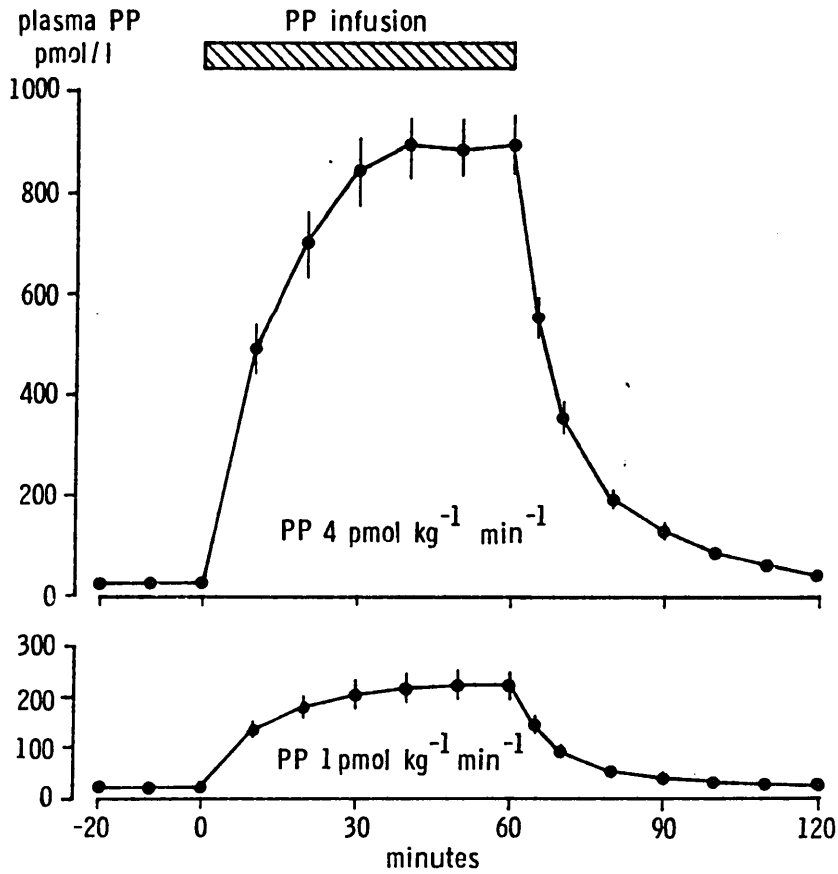


Figure 711: Plasma PP concentrations during PP infusions of 1 pmol kg^{-1} min^{-1} (n = 7) and 4 pmol kg^{-1} min^{-1} (n = 10) respectively. (mean $\pm$ SEM).

MINUTES	SALINE 0'	30'	60'	90'	120'	PP 1 pmol kg^{-1} min^{-1} 0'	30'	60'	90'	120'	PP 4 pmol kg^{-1} min^{-1} 0'	30'	60'	90'	120'
GLUCOSE															
Mean	4.66	4.63	4.60	4.61	4.59	4.83	4.87	4.87	4.87	4.85	4.89	4.85	4.87	4.83	4.83
± SEM	0.25	0.24	0.26	0.23	0.26	0.06	0.11	0.07	0.08	0.09	0.18	0.14	0.16	0.13	0.14
LACTATE															
Mean	0.64	0.70	0.69	0.65	0.7o	0.71	0.74	0.67	0.64	0.69	0.78	0.77	0.80	0.78	0.79
± SEM	0.03	0.04	0.04	0.04	0.07	0.07	0.09	0.08	0.08	0.08	0.08	0.07	0.08	0.07	0.08
3-HYDROXYBUTYRATE															
Mean	0.12	0.13	0.14	0.14	0.14	0.10	0.11	0.13	0.14	0.14	0.10	0.14	0.15	0.16	0.16
± SEM	0.03	0.04	0.04	0.06	0.06	0.04	0.06	0.08	0.08	0.07	0.05	0.05	0.06	0.07	0.08
GLYCEROL															
Mean	0.07	0.08	0.08	0.08	0.08	0.07	0.07	0.08	0.08	0.09	0.09	0.09	0.09	0.09	0.09
± SEM	0.01	0.02	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
ALANINE															
Mean	0.23	0.22	0.22	0.22	0.22	0.28	0.26	0.24	0.23	0.24	0.29	0.27	0.26	0.24	0.25
± SEM	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.01	0.02
NON-ESTERIFIED FATTY ACIDS															
Mean	0.63	0.66	0.65	0.72	0.69	0.65	0.70	0.75	0.76	0.74	0.75	0.81	0.79	0.79	0.79
± SEM	0.91	0.10	0.09	0.07	0.08	0.09	0.08	0.06	0.08	0.09	0.07	0.08	0.07	0.06	0.07

TABLE 708: Blood metabolite concentrations (mmol/l, mean ± SEM) during infusion of PP at doses of 1 pmol kg^{-1} min^{-1} (n = 7) and 4 pmol kg^{-1} min^{-1} (n = 10) respectively and during saline infusion (n = 6) in healthy fasting subjects.

MINUTES	SALINE 0'	30'	60'	90'	120'	PP 1 pmol kg^{-1} min^{-1} 0'	30'	60'	90'	120'	PP 2 pmol kg^{-1} min^{-1} 0'	30'	60'	90'	120'
GLUCOSE															
Mean	4.68	4.86	6.98	5.53	4.78	4.80	5.23	7.36	6.01	5.46	4.77	5.59	7.71	6.76	5.47
± SEM	0.15	0.24	0.53	0.72	0.55	0.17	0.30	0.14	0.45	0.57	0.16	0.34	0.47	0.71	0.60
LACTATE															
Mean	0.77	0.79	0.40	1.43	1.43	0.79	0.88	1.49	1.46	1.08	0.74	0.82	1.46	1.55	1.28
± SEM	0.10	0.09	0.23	0.13	0.08	0.07	0.08	0.13	0.16	0.08	0.13	0.10	0.07	0.18	0.09
3-HYDROXYBUTYRATE															
Mean	0.09	0.09	0.05	0.02	0.02	0.09	0.09	0.04	0.02	0.02	0.08	0.08	0.04	0.02	0.01
± SEM	0.05	0.04	0.01	0.01	0.01	0.05	0.04	0.01	0.01	0.00	0.00	0.03	0.01	0.01	0.00
GLYCEROL															
Mean	0.07	0.08	0.07	0.05	0.04	0.06	0.06	0.05	0.04	0.04	0.06	0.06	0.05	0.04	0.03
± SEM	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01
ALANINE															
Mean	0.27	0.27	0.35	0.35	0.37	0.28	0.30	0.37	0.37	0.37	0.27	0.27	0.37	0.39	0.38
± SEM	0.02	0.03	0.04	0.03	0.03	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02
NON-ESTERIFIED FATTY ACIDS															
Mean	0.73	0.77	0.60	0.43	0.35	0.68	0.70	0.57	0.46	0.33	0.67	0.70	0.59	0.41	0.28
± SEM	0.08	0.09	0.06	0.04	0.03	0.07	0.09	0.07	0.07	0.04	0.04	0.08	0.06	0.08	0.04

TABLE 709: Blood metabolite concentrations (mmol/l, mean ± SEM) before and after a standard carbohydrate-rich breakfast given 20 minutes after the start of a PP infusion at two dose levels with a control saline infusion in 5 healthy fasting subjects.

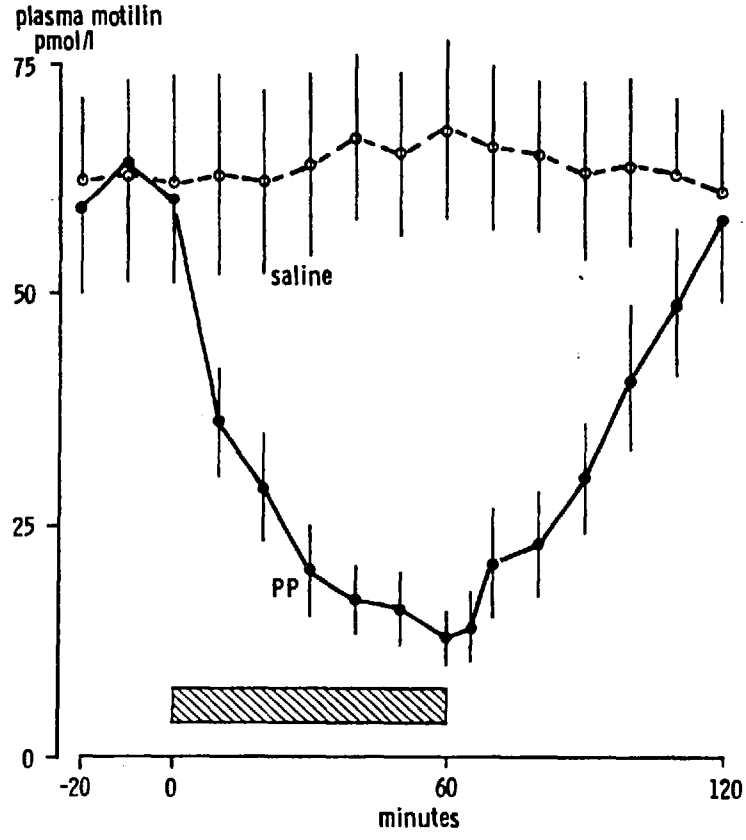


Figure 712: Plasma motilin concentrations during infusion of saline (n = 6) and PP at a dose of 1 pmol kg^{-1} min^{-1} (n = 7) in healthy fasting subjects. (mean $\pm$ SEM).

MINUTES	SALINE 0'	SALINE 30'	SALINE 60'	SALINE 120'	PP 1 pmol kg^{-1} min^{-1} 0'	PP 1 pmol kg^{-1} min^{-1} 30'	PP 1 pmol kg^{-1} min^{-1} 60'	PP 1 pmol kg^{-1} min^{-1} 120'	PP 4 pmol kg^{-1} min^{-1} 0'	PP 4 pmol kg^{-1} min^{-1} 30'	PP 4 pmol kg^{-1} min^{-1} 60'	PP 4 pmol kg^{-1} min^{-1} 120'
INSULIN	12 ± 4	13 ± 3	12 ± 3	11 ± 2	13 ± 3	14 ± 3	12 ± 3	13 ± 3	11 ± 4	10 ± 4	12 ± 3	11 ± 3
GLUCAGON	12 ± 2	12 ± 3	11 ± 2	11 ± 2	12 ± 3	14 ± 2	13 ± 3	12 ± 3	11 ± 3	10 ± 3	12 ± 4	10 ± 3
GASTRIN	5 ± 2	5 ± 2	5 ± 1	6 ± 1	6 ± 2	6 ± 1	7 ± 2	5 ± 1	7 ± 4	6 ± 3	7 ± 3	6 ± 3
SECRETIN	0.7±0.1	0.8±0.1	0.8±0.1	0.6±0.1	0.6±0.2	0.6±0.2	0.8±0.2	0.7±0.1	0.7±0.2	0.6±0.2	0.6±0.2	0.8±0.2
ENTEROGLUCAGOB	23 ± 7	24 ± 9	21 ± 8	23 ± 8	26 ± 5	23 ± 6	24 ± 7	25 ± 9	25 ± 8	23 ± 9	21 ± 7	24 ± 6
GIP	14 ± 3	14 ± 3	15 ± 2	13 ± 4	15 ± 4	14 ± 3	16 ± 3	15 ± 3	13 ± 4	12 ± 3	13 ± 4	15 ± 4
MOTILIN	61 ± 15	72 ± 15	68 ± 12	63 ± 15	60 ± 10	20 ± 5	13 ± 3	58 ± 10	53 ± 11	26 ± 6	18 ± 4	46 ± 13
NEUROTENSIN	17 ± 3	16 ± 2	15 ± 3	16 ± 3	16 ± 3	16 ± 2	16 ± 2	15 ± 2	15 ± 2	16 ± 2	16 ± 2	14 ± 1

TABLE 710: Plasma concentrations of pancreatic and gastrointestinal hormones (pmol/l, mean ± SEM) during PP infusion at 1 pmol kg^{-1} min^{-1} (n = 7) and 4 pmol kg^{-1} min^{-1} (n = 10) and during saline infusion (n = 6) in healthy fasting subjects.

	SALINE				PP 1 pmol kg^{-1} min^{-1}				PP 2 pmol kg^{-1} min^{-1}			
MINUTES	Zero	30'	60'	120'	Zero	30'	60'	120'	Zero	30'	60'	120'
INSULIN	13 ± 3	20 ± 6	313 ± 108	149 ± 22	11 ± 3	14 ± 3	228 ± 83	143 ± 46	10 ± 3	14 ± 4	237 ± 79	167 ± 32
GLUCAGON	14 ± 1	14 ± 3	8 ± 3	11 ± 3	12 ± 3	13 ± 3	9 ± 2	6 ± 1	14 ± 4	13 ± 3	8 ± 1	6 ± 3
GASTRIN	6 ± 2	6 ± 2	7 ± 1	8 ± 2	6 ± 2	6 ± 2	10 ± 3	9 ± 4	7 ± 3	10 ± 3	10 ± 3	10 ± 3
SECRETIN	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	1.0 ± 0.3	0.8 ± 0.1	1.0 ± 0.3	0.7 ± 0.2	0.7 ± 0.1	0.9 ± 0.2	0.7 ± 0.1
ENTEROGLUCAGON	25 ± 9	26 ± 6	21 ± 6	29 ± 6	26 ± 8	27 ± 7	23 ± 8	26 ± 8	26 ± 6	30 ± 7	27 ± 6	28 ± 7
GIP	15 ± 5	17 ± 6	26 ± 6	32 ± 7	16 ± 4	19 ± 6	30 ± 7	34 ± 4	16 ± 5	24 ± 5	28 ± 6	32 ± 4
MOTILIN	39 ± 6	42 ± 9	25 ± 9	20 ± 8	30 ± 8	29 ± 12	21 ± 13	15 ± 7	36 ± 7	21 ± 7	12 ± 5	8 ± 3
NEUROTENSIN	24 ± 5	28 ± 5	31 ± 6	30 ± 3	24 ± 2	26 ± 2	29 ± 3	33 ± 3	26 ± 2	28 ± 2	33 ± 4	35 ± 4

TABLE 711: Plasma concentrations of pancreatic and gastrointestinal hormones (pmol/l mean ± SEM) before and after a standard carbohydrate-rich breakfast given 20 minutes after the start of a PP infusion at two dose levels with a control saline infusion in 5 healthy fasting subjects.

shown in Table 711. The postprandial insulin concentrations were lower during the PP infusions than on the control day in spite of the blood glucose concentrations being higher although neither change reached statistical significance. Postprandial plasma levels of glucagon, gastrin, secretin, enteroglucagon, GIP and neurotensin were similar during the PP infusions to those of the saline control day. In contrast to the marked effect of PP on basal motilin concentrations, there was no significant difference in postprandial plasma motilin concentrations during PP infusion when compared with saline infusion; however, there was a substantial postprandial fall in motilin concentrations following the breakfast in all three groups.

DISCUSSION

Pharmacokinetics of PP

These studies show that exogenously administered BPP is rapidly cleared from the circulation in man. The course of disappearance follows first order kinetics with a half life of seven minutes.

The apparent distribution space suggests that PP was distributed mainly in the plasma volume. A similar small volume of distribution would be expected for a substance bound to a carrier molecule in plasma. This is unlikely,however, as results of gel chromatograms of plasma from normal subjects show only one peak of PP immunoractivity eluting in the same position as pure PP.

The amino acid sequence of BPP is very similar to that of the human peptide, the only difference being in position 6 in which glycine replaces valine and in position 23 where glutamic acid replaces aspartic acid (Chance and Jones, 1974). Because of this similarlity in structure,

differences in pharmacokinetics between BPP and HPP are not anticipated, however, this can be determined only when sufficient pure human peptide is available. Certainly the decay rate of endogenous PP following somatostatin suppression gives a similar half life (approxmately eight minutes) to that observed here. Plasma PP levels rise substantially on the ingestion of food (Adrian et al, 1976; Floyd et al, 1976; Schwartz et al, 1976), particularly protein and fat (Adrian et al,1978), and levels of approximately 200 pmol/l are seen after the ingestion of a small mixed breakfast in young adults (Adrian et al, 1976). After a larger meal PP levels rise to a peak of approximately 350 pmol/l in healthy young subjects (Adrian et al, 1977). Thus the plasma levels achieved with our PP infusions at the lowest dose are within the physiological range and the higher doses reflect rises two to three times greater than those observed after a normal lunch.

Effect of PP on Gastric Acid and Pepsin Secretion

Bovine pancreatic polypeptide, when infused into man at levels comparable to those observed postprandially does not affect either basal or pentagastrin-stimulated acid or pepsin output. From these experiments it would appear unlikely, therefore, that PP plays an important physiological role in the modulation of these secretions in man (Greenberg et al, 1978).

In contrast to these results in man, BPP in the dog has powerful actions on acid secretion. It strongly stimulates basal acid, but inhibits pentagastrin-stimulated acid production. One possible explanation for this discrepancy is the species difference variation in the structure of PP. However, the full biological activity of PP is found in the C-terminal hexapeptide and this active site is identical in bovine and human PP. (Lin and Chance, 1978). A more likely reason is that in the dog the minimum dosage of PP which affected acid secretion, was more than ten times the maximum dose used in this study.

Effect of PP on Basal Pancreatic and Biliary Secretion

The results demonstrate that at this dose PP substantially diminished trypsin output and even more markedly inhibited bilirubin output in the fasting state.

Because the decline in bilirubin and trypsin output was attributable to a decrease in both volume and concentration, there are at least three possible explanations which, alone or together, might account for our observations. Firstly, PP could have a direct and independent inhibitory effect on basal hepatic bile flow and on pancreatic trypsin production. Alternatively, a diminished output of these secretions could be secondary to alterations in motor tone of the gall bladder and the sphincter of Oddi as a consequence of the PP. Finally, because other hormones, notably secretin and cholecystokinin, directly affect small bowel sodium and water absorption (Moritz et al, 1973), the dminished net duodenal fluid accummulation could have been due, in part, to a stimulatory action by PP on these mechanisms. However, the design of this study does not allow for a more precise definition of the site of action of PP.

Effect of PP on Secretin Stimulated Pancreatic Secretion

The mean plasma PP concentration achieved during BPP infusions was approximately twice the mean concentration seen half an hour after lunch in healthy young adult subjects and may therefore be considered to be just within the physiological range.

BPP has a marked inhibitory action on pancreatic secretion reducing volume, enzyme activity and total protein concentration. On the other hand, PP appeared to have no effect on bicarbonate concentration. In the dog, total water and bicarbonate responses to BPP were reported to parallel each other, as in this study in man, but occasionally a biphasic response, with augmentation followed by inhibition, was observed (Lin et al, 1977). This may reflect either a dose or a species difference.

The slow recovery of protein secretion after inhibition of PP was also observed in the dog studies. Similarly the protein concentration did not return to pre-PP levels until 45 or 60 minutes after PP infusion ceased (Lin et al, 1977). Since in our experiments the infusion was not continued after three hours, we were unable to ascertain when a full return to pre-PP levels occurred. During the PP infusions reported here the duodenal juice became colourless and the bilirubin content was dramatically reduced, perhaps reflecting increased gall bladder storage of bile, an effect also observed in the dog (Lin et al, 1977). However, a change in biliary secretion rate cannot ne excluded by this study.

Effect of a Low Dose of PP on Stimulated Pancreatic and Biliary Secretion

Pancreatic and biliary secretion was measured during stimulation with the minimum dose of caerulein required for gall bladder concentration and the increment in plasma secretin (6 pmol/l) produced was within the range observed after food (Schaffalitzky de Muckadell and Fahrenkrug, 1978). Our results demonstrate that under these conditions PP at plasma levels equivalent to those observed after a meal substantially inhibits the output of trypsin and, even more substantially, bilirubin.

The most likely explanation for our observations is that PP had independent effects on pancreatic trypsin production and on biliary secretion. However, alterations in motor tone of the gall bladder and sphincter of Oddi could also account for the decrease in bilirubin output. The demonstration that in patients without gall bladders the same magnitude of inhibition in trypsin concentration and output occurred without a significant change in bilirubin,supports both a direct motor effect by PP on the biliary tree and an independent inhibition of trypsin secretion. This is in agreement with its reported pharmacological action in dogs

(Lin et al, 1977), where a major relaxation of gall bladder tone and a small increase in choledochal sphincter pressures were seen with no change in bile flow. Bicarbonate output during PP paralleled changes in volume and, as in dogs (Lin et al, 1977), bicarbonate concentration was unaltered. However, PP also occasionally first stimulated bicarbonate output then inhibited it (Lin et al, 1977). We found in man only a small inhibition in bicarbonate output at the end of the PP infusion. This discrepancy may reflect species or dose differences.

Effect of PP on the Rate of Gastric Emptying

This study was designed to assess the effect of PP at physiological concentrations on the rate of gastric emptying of a solid meal in man. As PP is released promptly and substantially after the ingestion of food (Adrian et al, 1976; Floyd et al, 1976; Schwartz et al, 1976) a normal meal was chosen which provoked a minimum increment in plasma PP concentrations, thereby minimising the effect of endogenously released PP. These results demonstrate that PP, at plasma levels both equal to and twice those seen after a normal mixed breakfast (Greenberg et al, 1978), has no significant effect on the rate of gastric emptying. (Adrian et al, 1980).

In the rat BPP caused a very small but significant enhancement (13%) of the rate of gastric emptying of a 2 ml pectin meal, however, the doses used in rats were rather higher than in this present study (5-500 fold) and were administered as an intraperitoneal bolus (Lin and Chance, 1978). Therefore the route of administration, the dose or a species difference are all possible reasons for this discrepancy.

Effect of PP in Circulating Metabolites

In the chicken administration of APP resulted in a substantial fall in plasma levels of glycerol and amino acids, alanine in particular

(Kimmel et al, 1978). These observations suggest that PP may influence either tissue glucose utilisation or hepatic lipogenesis in the bird (Kimmel et al, 1978). A rise in plasma PP concentrations with prolonged fasting (Floyd et al, 1977) and during strenuous exercise has also led to the suggestion that PP may influence metabolism in man. In this study, at plasma concentrations similar to and above those seen after a normal mixed breakfast in man (Greenberg et al, 1978), PP has no significant influence on circulating concentrations of glucose, lactate, 3-hypoxy-butyrate, alanine, glycerol or non-esterified fatty acids.

A species difference may account for the apparent discrepancy between the metabolic influence of PP in the bird and man. In addition, however, the doses used in the chicken were somewhat larger (2.3 - 23 nmol/kg) which produced plasma PP levels of approximately 20 nmol/l. Circulating metabolite concentrations are a relatively crude measure of metabolic activity but the complete absence of any significant change in the concentration of a range of metabolites make it extremely unlikely that PP caused any major change in metabolic flux.

It is of interest that PP infusion did not affect circulating metabolite levels in man as plasma PP concentrations are elevated in patients with diabetes mellitus when compared with age matched controls (Floyd et al, 1977). These high PP concentrations were reduced following insulin therapy, suggesting that high PP levels may be related to the metabolic derangement in diabetes (Berger et al, 1978). These results would suggest that PP does not contribute to the abnormal metabolism in untreated diabetes.

Effect of PP on Circulating Pancreatic and Gastrointestinal Hormones

PP had no significant effect on basal or postprandial concentrations of insulin, glucagon, gastrin, secretin, enteroglucagon, gastric inhibitory peptide or neurotensin and it is unlikely that endogenous PP modifies

the secretion of these peptides. However, a profound reduction in basal motilin levels was observed during infusions at even the lowest dose. Plasma motilin levels rise only transiently in response to a mixed meal and then fall and this fall is substantial when carbohydrate is ingested (Christofides et al, 1979a). It remains to be established whether fluctuations in endogenous PP concentrations can influence motilin release in man. However, motilin, a peptide from the upper small intestine potently stimulates gastrointestinal smooth muscle and a physiological role in initiating the interdigestive myoelectric complex (Van Trappen et al, 1978), and controlling the rate of gastric emptying (Christofides et al, 1979b), in man has been suggested. It is therefore possible that PP influences motor actions in the gastrointestinal tract through inhibition of motilin release. Indeed, the observed actions of PP on the pancreatic and biliary secretion can readily be explained by motor actions of PP on the sphincter of Oddi and the gall bladder.

CONCLUSIONS

PP Infusion in Man

These studies demonstrate that PP is cleared rapidly from the circulation and therefore must be secreted continually for several hours following a meal (Adrian et al, 1978).

Pancreatic and biliary secretion are inhibited by PP in both the fasting state (Greenberg et al, 1979) and infusion of secretin (Adrian et al, 1979). This inhibition of exocrine pancreas and gall bladder is also observed at PP concentrations similar to those seen postprandially (Greenberg et al, 1978) and may therfore be considered as physiological. The observation that in cholecystectomised subjects PP inhibits enzyme

secretion but has no effect on bile flow suggests that PP has two separate sites of action; firstly the exocrine pancreas, where it is particularly potent at inhibiting enzyme secretion, and secondly causing relaxation of the gall bladder.

These actions of PP on the exocrine pancreas and gall bladder are directly opposing those of cholecystokinin. It may be considered strange, that a peptide released postprandially should inhibit enzyme and bile output, two functions which aid digestion. However this should be considered in the light of the knowledge that PP levels remain elevated for at least six hours postprandially, long after the levels of other gastrointestinal hormones have returned to their basal concentrations. PP may thus be of importance in the late interprandial period to conserve pancreatic enzymes and promote the storage of bile in anticipation of the next meal.

At the doses used on these studies PP had no significant effect on gastric secretion or on the rate of gastric emptying. In addition there was no significant effect on circulating metabolite or pancreatic hormone (insulin and glucagon) concentrations. The infusion doses used in these produced plasma PP concentrations similar to and four and five fold higher than those seen postprandially. This would suggest that PP is unlikely to have a marked effect on metabolism or on gastric function in man.

The inhibition of motilin release by PP is most interesting as PP had marked pharmacological motor effects on the canine gastrointestinal tract and its action on bile output may be due to relaxation of the gall bladder.

It should be remembered that BPP has differences in three amino acid residues from HPP and such a species difference could give rise to altered biological effects. This would seem unlikely however as the biologically active C-terminal hexapeptide is identical in the two molecules. Such

species differences can only be ruled out however when human PP is available in sufficent quantities to undertake infusion studies in man.

CHAPTER 8. PATHOPHYSIOLOGY OF PANCREATIC POLYPEPTIDE

INTRODUCTION

The Pathophysiology of Pancreatic Polypeptide

As early studies suggested that pancreatic polypeptide may be a circulating peptide hormone it was clearly of interest to see if the release of this peptide was associated with pancreatic disease. PP cells are found both in the periphery of the islets and in the exocrine parenchyma of the pancreas. In view of this location it was apparent that PP could be affected by endocrine or exocrine pancreatic disease.

Both maturity onset diabetes and juvenile type diabetes were studied to establish whether PP secretion was influenced by abnormal islet function. As physiological studies suggested that PP could reduce pancreatic exocrine secretion (Greenberg et al, 1978; Taylor et al, 1979), the release of PP was investigated in patients with chronic inflammatory pancreatitis with steatorrhoea and also children with pancreatic insufficiency due to fibrocystic disease. Because of the link between alcohol abuse and pancreatic disease PP release was assessed in chronic alcoholics. A further possible physiological role of PP was control of gall bladder function. As PP relaxed the canine gall bladder (Lin and Chance, 1978), PP release was therefore investigated in patients with cholelithiasis.

PP release was also investigated in several other gastrointestinal diseases. Pancreatic function is known to be reduced in conditions giving rise to mucosal atrophy of the small intestine. This is thought to be due to the failure of release of peptide hormones from the upper small intestine which stimulate exocrine secretion (e.g. secretin and cholecystokinin) and insulin release (glucose-dependent insulinotropic peptide, GIP). As an

enteric signal is important in the postprandial release of PP we studied the meal response of PP in patients with coeliac disease and acute tropical malabsorption (tropical sprue). Similarly to investigate the effect of potential removal of a PP releasing agent we studied patients who had undergone small and large intestinal resections and in addition patients who had undergone jejuno-ileal bypass for massive obesity.

When infused into the dog PP stimulated basal gastric acid secretion (Lin et al, 1977), and it was therefore of interest to study PP release in patients with peptic ulcer disease in whom acid secretion is enhanced.

Because of the stimulatory effects of PP on gastrointestinal motor activity observed in the dog (Lin and Chance, 1978), PP release was investigated in several conditions associated with altered bowel motility, including infective diarrhoea, diverticular disease and the irritable bowel syndrome. Two other conditions studied, Crohn's disease (regional enteritis) and ulcertative colitis are inflammatory conditions characteristically presenting with pain and diarrhoea.

Little is known about the regulation of appetite or the mediation of the sensation of satiety. The control of eating has been extensively studied in genetically obese mice. The obese hyperglycaemic mouse (ob/ob) is the most intensively studied animal model of human late onset diabetes mellitus, a condition associated with obesity. Parabiotic experiments have led to the conclusion that the hyperphagia and resulting weight gain is due to the lack of a circulating satiety factor from the gut which in normal animals controls food intake (Coleman, 1978). It has been reported that the administration of PP reverses the hyperphagia and resulting weight gain in these mice which subsequently become normoglycaemic (Malaisse-Lagae et al, 1977). Similar studies in the New Zealand obese mouse have also led to the suggestion that PP is the circulating satiety factor proposed by Coleman (Gates and Lazarus, 1977). In order to establish

whether PP could be involved in the regulation of appetite in man, patients with morbid obesity were studied and in addition patients with two disease states associated with altered food intake, thyrotoxicosis and anorexia nervosa.

An early report suggested that PP cells were present in more than half of the endocrine tumours of the pancreas and their metastases. In addition circulating levels of PP in these patients were grossly elevated (Polak et al, 1976). Circulating PP levels were measured in a large number of patients with pancreatic endocrine tumours and in addition patients with adenocarcinoma of the pancreas and other sites. The possibility that PP cells may be present in other endocrine tumours such as carcinoid tumours, phaechromocytomas, pituitary tumours and medullary carcinomas of the thyroid, was also investigated.

METHODS

Standard Test Breakfast

As a stimulus for PP release, a test breakfast was used consisting of two medium sized boiled eggs, 60g bread as toast, 10g butter, 35g marmalade and 150ml unsweetened orange juice. The breakfast which contained 18g protein, 22g fat and 66g carbohydrate (530 calories), was eaten by all subjects following an overnight fast.

Milk Test

In our experience the test meal stimulus is not readily tolerated by children whereas a milk drink is acceptable and is invariably drunk within a few minutes. The milk drink which consisted of 10ml pasteurised cows milk per Kg body weight was used as a stimulus for PP release in the

children with cystic fibrosis and the normal children who formed the control group.

Secretin Test

In addition to the test breakfast some patients with steatorrhoea due to chronic pancreatitis and a group of normal controls were given a bolus injection of 2 Crick Harper Raper units (CHRU) of secretin per Kg (Boots Ltd, Nottingham). This secretin stimulus stimulates the release of PP into the circulation but by-passes the normal enteric signal which is responsible for postprandial PP secretion. Pure secretin does not release PP in man and it is presumably some other substance in this partially purified extract of porcine intestine which is responsible for evoking a PP response.

SUBJECTS

Age Matching of Control Subjects

Fasting plasma PP concentrations increase with age in normal subjects (Floyd et al, 1977; Taylor et al, 1978). We have observed that the post-prandial PP response is also greater in older healthy subjects (Figure 801) (Adrian 1980). It is therefore of great importance when comparing groups of patients with normal subjects that the two groups are age matched.

The normal control groups (healthy subjects) were age and sex matched with each disease state group. The samples of test subject and control were then alternated throughout the assay to eliminate the effect of any minor assay drift.

Patients Studied Following a Test Breakfast

Brief clinical details of all patients studied in the test meal series

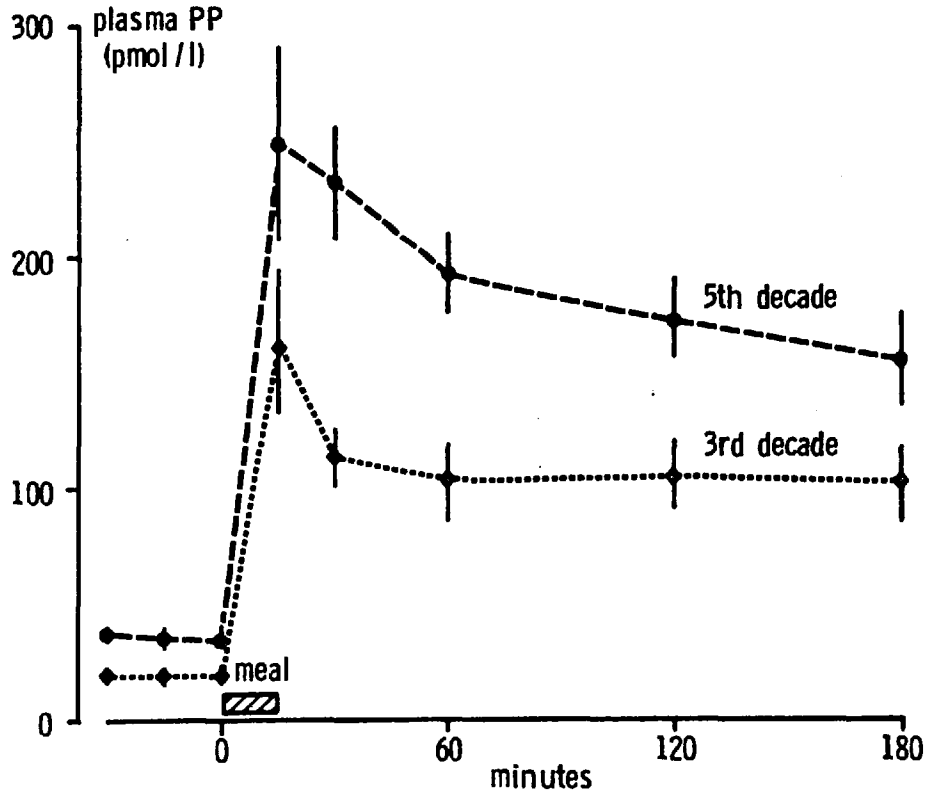


Figure 801: Plasma PP concentrations after the ingestion of a standard test breakfast in fasting normal volunteers in their 3rd. decade (n = 9) and 5th. decade (n = 7). The postprandial response was significantly different at 30 min. ($p < 0.001$), 60 min. ($p < 0.005$), 120 min. ($p < 0.02$) and 180 min. ($p < 0.05$).

	NUMBER OF SUBJECTS		AGE	WEIGHT kg
	M	F	mean (range)	(mean ± SEM)
DIABETES MELLITUS	10	8	48 (36-63)	68 ± 4
CHRONIC PANCREATITIS WITHOUT STEATORRHOEA	17	6	47 (23-80)	63 ± 2
CHRONIC PANCREATITIS WITH EXOCRINE DEFICIENCY	12	4	52 (27-85)	57 ± 3
CHRONIC ALCOHOLISM	9	0	41 (32-60)	68 ± 4
CHRONIC CHOLELITHIASIS	2	7	54 (35-72)	65 ± 5
PANCREATECTOMY	5	3	45 (35-65)	62 ± 6
DUODENAL ULCER	7	5	42 (26-53)	65 ± 4
COELIAC DISEASE ACTIVE	4	7	54 (29-74)	52 ± 3
COELIAC DISEASE TREATED	4	9	44 (23-69)	58 ± 3
TROPICAL SPRUE	8	4	29 (23-49)	58 ± 3
INFECTIVE DIARRHOEA	7	4	37 (19-67	64 ± 3
CROHN'S DISEASE	7	7	35 (13-70)	49 ± 4
ULCERATIVE COLITIS	10	14	38 (14-74)	59 ± 3
DIVERTICULAR DISEASE	3	9	63 (43-78	60 ± 4
IRRITABLE BOWEL DISEASE WITH DIARRHOEA	3	16	42 (20-77)	71 ± 4
IRRITABLE BOWEL DISEASE WITH CONSTIPATION	3	8	47 (21-87)	70 ± 3
SMALL INTESTINAL RESECTION	10	8	48 (20-72)	54 ± 3
LARGE INTESTINAL RESECTION	4	5	34 (21-62)	53 ± 4
MORBID OBESITY	2	17	35 (24-52)	129 ± 4
ILEO-JEJUNAL BYPASS	2	17	36 (25-54)	106 ± 4
THYROTOXICOSIS	0	6	29 (16-44)	62 ± 3
ANOREXIA NERVOSA	0	6	32 (23-50)	38 ± 3

TABLE 801: The number of each sex, age and weight of the patients investigated in the test meal study. Each group was matched for age and sex with healthy controls for assay.

including age, sex and weight are shown in Table 801.

Diabetes Mellitus

Fasting plasma samples were taken from the following groups of patients:-

1). 448 insulin-dependent diabetes, mean age 49 years (range 11-88) who had not received the new highly purified or monocomponent insulins.

2). 22 insulin-dependent diabetes, mean age 31 years (range 17-55) who had received only monocomponent insulin.

3). 180 maturity onset diabetes, mean age 58 years (range 20-86) who had never received insulin and were treated by diet or sulphonylureas.

In addition 18 patients with maturity onset diabetes received the standard test breakfast.

Chronic Pancreatitis

Twenty-three patients with chronic pancreatitis were studied, 16 having severe chronic steatorrhoea, mean age 53 years (range 27-85) and weight 57 $\pm$ 3 kg (mean $\pm$ SEM) and 23 having clinical evidence of pancreatic disease but no overt exocrine deficiency, mean age 46 years (range 23-80) and weight 63 $\pm$ 2 kg. Patients with PP antibodies resulting from insulin therapy for diabetes had to be excluded (Bloom et al, 1978).

The diagnosis of chronic pancreatitis was made on a typical history and one or more of the following: the finding of x-ray evidence of

pancreatic calcification (n = 20), gross ERCP abnormalities (n = 22) or by histological examination of biopsies obtained at laparotomy (n = 8). Patients categorised as having pancreatic insufficiency in addition had marked steatorrhoea, all subjects having faecal excretion above 50 mmol/day (mean 116 $\pm$ 24 mmol/day) and a resulting substantial weight loss of greater than 7 kg from their previous normal weight (mean 10.8 $\pm$ 1.8 kg). In those who underwent pancreatic secretion studies (n = 10) a trypsin excretion to less than 10% of normal was found (DiMagno et al, 1973). Of the 16 patients with steatorrhoea 5 were diabetic, 9 had pancreatic calcification and 8 had a history of alcohol abuse. Of the 23 patients with chronic pancreatitis but no steatorrhoea, 5 were diabetic, 11 had calcification and 14 a history of alcohol abuse.

As an additional test, 8 patients with chronic pancreatitis with overt exocrine deficiency and 8 normal controls were fasted overnight and given an injection over one minute at 2 Crick Harper Raper units (CHRU) secretin per kg (Boots Ltd, Nottingham).

Cystic Fibrosis

Ten patients with cystic fibrosis were studied. These children had a mean age of 10 years (range 5-15) and a weight of 28.6 $\pm$ 2.5 kg (mean $\pm$ SEM) 5 were male and 5 female. These patients all had a typical clinical history of cystic fibrosis and the diagnosis was confirmed by quantitative pilocarpine iontophoresis sweat test. All had sweat sodium concentrations above 70 mmol/l. All the fibrocystic children had pancreatic insufficiency on clinical grounds and all were on pancreatic enzyme replacement therapy. None were diabetic and none were ill at the time of study.

Six healthy children who had recovered from minor illnesses were studied as controls. (These had a mean age of 8 years (range 5-12) and a weight of 32.2 $\pm$ 4.4 kg, 3 were male and 3 female. None of these subjects

had a history or evidence of gastrointestinal or pancreatic disease. The study was performed at the time of a clinical indicated venepucture.

All studies were carried out with parenteral consent and approval for the study was obtained from the Local Ethical Committee. As a stimulus for gut hormone release pasteurised cow's milk (10 ml/kg) was consumed by each subject following an overnight 12 hour fast.

Chronic Alcoholism

Nine chronic alcohol subjects were studied on the second day of admission to a rehabilitation unit. These patients were studied again two weeks later having abstained totally from alcohol during that period. These patients had a long history of alcohol abuse but because of social status they were all well nourished.

Cholelithiasis

Ten patients with chronic cholelithiasis were studied prior to surgery. All patients presented with a typical history of biliary colic and in each case gall stones were diagnosed by cholecystography.

Coeliac Disease

Eleven patients with active coeliac disease were studied together with 13 patients who had been successfully treated with a gluten-free diet. All patients presented with steatorrhoea and a jejunal biopsy showed total or partial villous atrophy (Trier, 1973). Subsequent to this study the patients with active coeliac disease have all improved on a gluten-free diet.

Tropical Sprue

Twelve patients with post-infective tropical malabsorption (acute

tropical sprue) were previously healthy but had developed steatorrhoea following a trip to the tropics. A jejunal biopsy showed partial villous atrophy in all patients (Mathan, 1973).

Peptic Ulcer Disease

All 12 patients in this group presented with a typical history of abdominal pain and the diagnosis of duodenal ulcer was made on endoscopic examination.

Infective Diarrhoea

These 12 patients who had previously enjoyed good health were admitted to hospital for an acute episode of acute diarrhoea. All patients required intravenous fluid and electrolyte replacement and all recovered completely before discharge. Salmonella were isolated from the stool of 3 patients (Agona, Saint Paul and Tennessee), entamoeba histolitica from a fourth and no pathogens, ova, cysts or parasites were seen in the remaining 8. The test breakfast was ingested at an early stage of recovery when diarrhoea was still present.

Crohn's Disease

Fourteen patients with regional enteritis (Crohn's disease) were studied. The patients typically presented with pain and diarrhoea and the diagnosis was made on radiological and histological evidence.

Ulcerative Colitis

Twenty-four patients with ulcerative colitis were studied. These patients presented with diarrhoea and the diagnosis was made sigmoidoscopically together with histological evidence from colonic biopsy.

Diverticular Disease

Twelve patients with diverticular disease of the colon were studied. The patients presented with a history of pain and diarrhoea and the diagnosis was confirmed radiologically with a barium enema.

Irritable Bowel Syndrome

Thirty patients with the irritable bowel syndrome were studied. Of these 19 patients complained primarily of pain and diarrhoea and in 11 patients the disease was predominantly associated with constipation.

Short Bowel Syndrome

Eighteen patients with partial ileal resection were studied together with nine patients who had undergone partial colonic resection. Gut resection had been undertaken for inflammaroty bowel disease, neoplasia, radiation fibrosis or trauma, a minimum of one year prior to the study.

Morbid Obesity and Jejuno-ileal Bypass

Nineteen patients with morbid obesity ($225 \pm 7\%$ ideal body weight) and 21 patients who had undergone jejuno-ileal bypass were studied. The post-operative patients were studied between two and twelve months after surgery and their weight had fallen moderately ($181 \pm 8\%$ ideal body weight, mean weight loss 28 ± 2 kg).

Thyrotoxicosis

Six thyrotoxic patients were studied, one of the presenting symptoms in each case was a greatly increased appetite and food consumption. All had further symptoms and signs suggestive of thyrotoxicosis and the diagnosis was confirmed by elevated serum thyroxine and triiodothyronine concentrations and a corresponding low concentration of thyroid stimulating hormone.

Anorexia Nervosa

Six patients with anorexia nervosa were studied. The diagnosis was made by impartial clinicians and included a full psychiatric assessment. In each case organic disease had been excluded. All patients demonstrated a greatly reduced appetite and a resultant substantial weight loss of around 20 kg.

Pancreatic Endocrine Tumours

Fasting plasma samples were taken from 164 patients with pancreatic endocrine tumours in the following groups:

Vipoma: Forty-nine patients with the Verner Morrison syndrome (Verner and Morrison, 1958). All patients had pancreatic tumours associated with watery diarrhoea, hypokalaemia and wasting. In all cases plasma VIP concentrations were elevated (greater than 70 pmol/l).

Insulinoma: Eighteen patients with insulinoma (Wilder et al, 1927)

Gastrinoma: Seventy-five patients with the Zollinger-Ellison syndrome (Zollinger and Ellison, 1955). All patients had pancreatic tumours associated with multiple or recurrent peptic ulcers and elevated plasma gastrin concentrations (greater than 50 pmol/l).

Glucagonoma: Seventeen patients with the glucagonoma syndrome (Mallinson et al, 1974). All patients suffered from a characteristic skin rash, diabetes, weight loss, anaemia, glossitis and stomatitis. Plasma glucagon concentrations were elevated (greater than 50 pmol/l)

PP'oma: Two patients with PP'omas. These patients both presented with obstructive jaundice and a tumour was found at laparotomy. The finding of PP cells in the tumour was an incidental finding following conventional histology. Pre-operative fasting plasma samples were available on both patients.

Somatostatinoma: Three patients with somatostatinomas (Ganda et al, 1977). These patients presented with diabetes melitus (3) steatorrhoea (3) and cholelithiasis (2). Gastric acid output was low in all three patients.

Molecular Forms in Pancreatic Apudomas

The molecular forms of PP in plasma from 13 patients with pancreatic endocrine tumours were investigated using gel filtration chromatography using Sephadex G50 superfine as described in Chapters 3 and 4. Seven patients with VIPomas and six patients with glucagonomas were investigated.

Other Endocrine Neoplasms

Fasting plasma samples were taken from the following groups of patients.

Ganglioneuroblastoma: Nine children with neuroblastomas with watery diarrhoea. All of these samples had elevated VIP concentrations (greater than 70 pmol/l).

Phaeochromacytoma: Seven patients were studied in this group, all had elevated urinary HMMA concentrations.

Medullary carcinoma of the thyroid: Eleven patients with medullary carcinoma associated with diarrhoea were studied.

Cushing's syndrome: Twenty patients with ectopic ACTH tumours and remitting Cushing's syndrome. In most the site of the primary tumour was uncertain.

Carcinoid syndrome: Twenty-four patients with the carcinoid syndrome were studied. All patients had frequent flushing attacks and 20 of these also suffered from diarrhoea.

Prolactinoma: Six patients with pituitary tumours secreting prolactin were studied.

Acromegaly: Ten patients with growth hormone secreting pituitary tumours were investigated.

Adenocarcinoma

Fasting plasma samples were obtained from a total of 75 patients with adenocarcinoma. The primary site of the tumour was the pancreas (17), stomach (15), colon (5), rectum (8), bronchus (24) and breast (6).

RESULTS

Diabetes Mellitus

When results from the insulin treated diabetics were analysed it became apparent that the majority of these patients had circulating antibodies which were capable of binding the labelled PP in the absence of added antibody. PP measurement could not therefore be made by conventional methods in such patients. The fasting plasma PP concentrations in the remaining 168 insulin treated diabetics were 40 ± 5 pmol/l which

was significantly higher than that found in 125 healthy control subjects of similar age 22 $\pm$ 2 pmol/l ($p < 0.01$). In contrast the 22 patients who were treated only with highly purified or mono-component insulin preparations had fasting plasma PP concentrations of 24 $\pm$ 3 pmol/l which was similar to that seen in the controls. Fasting plasma PP concentrations in the 180 maturity onset diabetics treated with a diet of sulphonylureas was 32 $\pm$ 3 pmol/l which was significantly higher than in the control group ($p < 0.02$).

Endogenous PP Antibodies in Insulin Treated Diabetics

Of the 448 insulin dependent diabetics studied 280 were found to have PP antibodies. These were detected by an increase in percent binding of blank tubes containing patients' sample and radioactively labelled PP but no exogenous antibody. The "cut off" point taken was an increase in percent binding of at least three standard deviations above the mean binding for healthy subjects. In the majority of cases binding was much higher than this in positive cases even when the plasma sample was diluted 1 in 10 or 1 in 100 prior to assay. Further analysis revealed that the mean duration of insulin treatment in the antibody-positive patients was 12.6 $\pm$ 0.7 years (mean $\pm$ SEM) and dose was 65 $\pm$ 4 units insulin/day whereas in negative patients it was 9.8 $\pm$ 1.0 years and the dose 50 $\pm$ 3 units insulin/day. There was a strong inverse correlation of antibody titre with age (Figure 802). Mean age was 52 $\pm$ 2 years in the PP antibody-free patients (n = 168), 51 $\pm$ 3 years in patients with moderate titres (1/10) (n = 117), 41 $\pm$ 3 years in patients with titres of 1/10 to 1/100 (n = 84), and 34 $\pm$ 3 years in patients with very high titres (1/100) (n = 79). Patients under 25 (n = 76) had an 80% frequency of PP antibodies, whereas patients over 50 (n = 202) had a 45% frequency, although insulin duration and dose

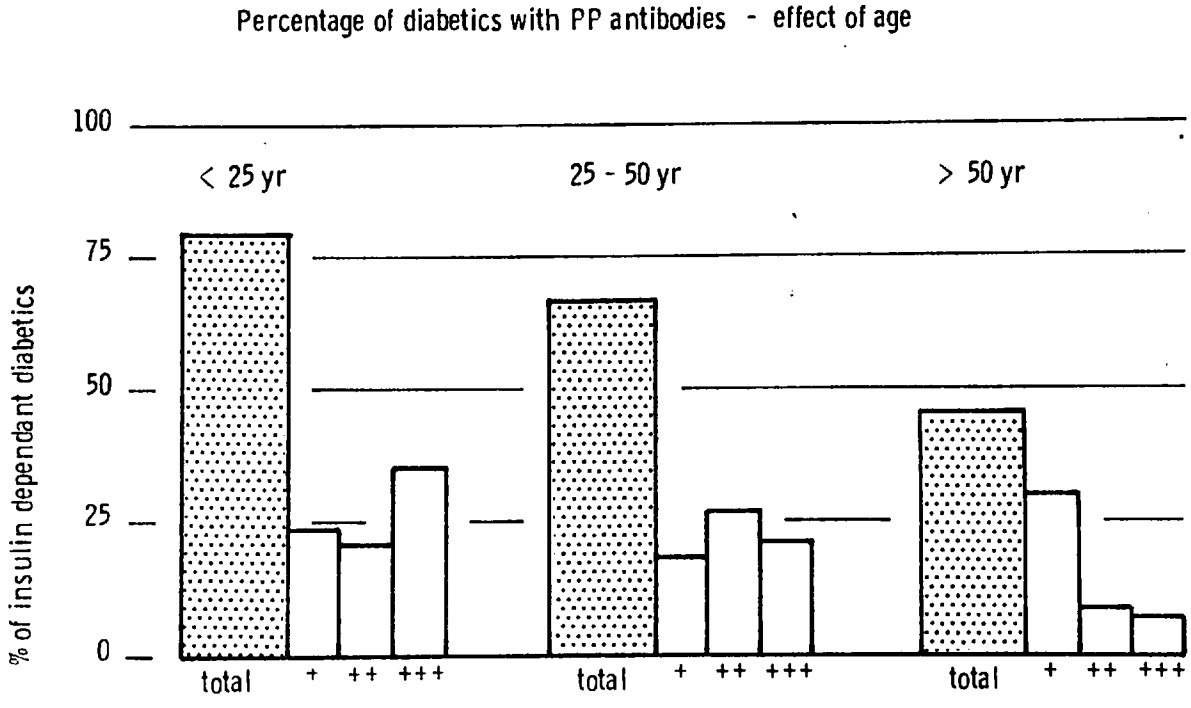


Figure 802: Influence of age on the incidence and titre of anti-PP antibodies in the plasma of insulin treated diabetics.

were very similar. Scathard analysis (see Chapter 2) showed that these antibodies were of high avidity and the affinity constants of six randomly chosen antibodies ranged from 2.2×10^{10} to 2×10^{11} litres/mole (mean s x 10^{10} 1/mol).

Cystic Fibrosis

Fasting plasma concentrations of PP were significantly lower in the cystic fibrosis patients ($p < 0.01$) and no rise was seen following the ingestion of milk, whereas the control subjects exhibited a substantial rise following the milk drink (Figure 803). The difference in post-stimulus PP levels between the two groups was highly significant (all $p < 0.005$ and at 15 minutes $p < 0.001$).

TEST MEAL RESULTS

The plasma PP concentrations during breakfast in the various groups of patients with pancreatic disease, chronic alcoholism and cholelithiasis are shown in Table 802, intestinal disorders in Tables 803 and 804, obesity, jejuno-ileal bypass, small and large bowel resection and pancreatectomy in Table 805 and anorexia nervosa and thyrotoxicosis are shown in Table 806. In each table the plasma PP concentrations in the respective groups of age and sex matched controls are also given.

Diabetes Mellitus

Although both basal and postprandial levels were slightly higher in the diabetes than controls this did not reach statistical significance at any time point (Table 802).

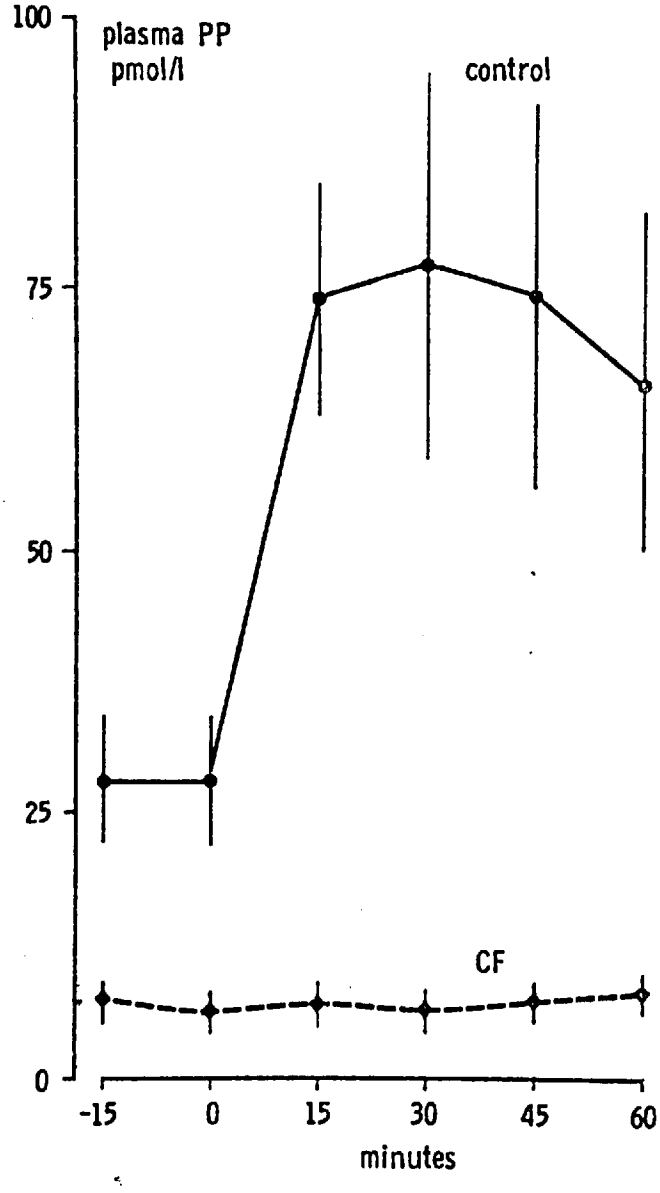


Figure 803: Plasma PP concentrations following a milk drink (10 ml/kg) in ten children with cystic fibrosis and six age matched healthy controls.

	-30	-15	0	15	30	60	120	180
DIABETES MELLITUS	33 ± 3	32 ± 2	32 ± 3	264 ± 41	275 ± 38	249 ± 40	190 ± 37	182 ± 36
CONTROLS	26 ± 3	23 ± 4	25 ± 3	186 ± 55	180 ± 51	144 ± 31	162 ± 45	120 ± 22
CHRONIC PANCREATITIS WITHOUT STEATORRHOEA	61 ± 17	50 ± 15	54 ± 15	207 ± 32	226 ± 36	208 ± 31	179 ± 32	142 ± 29
CONTROLS	31 ± 5	30 ± 6	31 ± 5	222 ± 39	218 ± 32	182 ± 32	210 ± 31	148 ± 26
CHRONIC PANCREATITIS WITH EXOCRINE DEFICIENCY	11 ± 3	9 ± 3	10 ± 3	36 ± 11	38 ± 11	44 ± 14	35 ± 13	29 ± 11
CONTROLS	31 ± 5	30 ± 6	31 ± 5	222 ± 39	218 ± 36	182 ± 32	210 ± 31	148 ± 26
CHRONIC ALCOHOLISM	88 ± 16	89 ± 17	91 ± 17	356 ± 65	351 ± 67	357 ± 61	304 ± 48	203 ± 44
CONTROLS	28 ± 5	28 ± 5	29 ± 5	143 ± 26	153 ± 28	118 ± 27	107 ± 16	98 ± 15
CHRONIC CHOLELITHIASIS	31 ± 7	32 ± 8	22 ± 8	184 ± 24	211 ± 27	184 ± 21	144 ± 20	141 ± 19
CONTROLS	23 ± 3	22 ± 4	22 ± 4	176 ± 29	170 ± 43	165 ± 30	147 ± 26	117 ± 18

TABLE 802: Plasma PP concentrations (pmol/l, mean ± SEM) during a standard test meal in patients with diabetes mellitus, pancreatitis, alcoholism and cholelithiasis. Each group was matched with controls of similar age.

	-30	-15	0	15	30	60	120	180
DUODENAL ULCER	28 ± 6	28 ± 6	23 ± 5	140 ± 17	125 ± 10	100 ± 11	125 ± 22	123 ± 23
CONTROLS	21 ± 6	22 ± 6	23 ± 8	175 ± 34	173 ± 36	142 ± 20	150 ± 27	116 ± 19
COELIAC DISEASE ACTIVE	29 ± 10	29 ± 10	31 ± 11	267 ± 41	232 ± 36	147 ± 37	130 ± 38	121 ± 42
CONTROLS	30 ± 7	33 ± 7	29 ± 7	239 ± 43	212 ± 44	135 ± 42	193 ± 41	154 ± 31
COELIAC DISEASE TREATED	29 ± 5	29 ± 6	27 ± 6	212 ± 24	221 ± 27	184 ± 31	154 ± 26	124 ± 23
CONTROLS	30 ± 7	33 ± 7	29 ± 7	239 ± 43	212 ± 44	185 ± 42	193 ± 41	154 ± 31
TROPICAL SPRUE	35 ± 7	39 ± 9	41 ± 9	230 ± 42	168 ± 33	121 ± 27	106 ± 19	84 ± 15
CONTROLS	30 ± 8	28 ± 8	29 ± 9	271 ± 69	216 ± 68	195 ± 59	179 ± 49	143 ± 36
INFECTIVE DIARRHOEA	43 ± 7	41 ± 8	54 ± 12	284 ± 50	253 ± 49	154 ± 17	120 ± 16	106 ± 19
CONTROLS	23 ± 4	20 ± 4	24 ± 6	173 ± 33	149 ± 32	105 ± 15	133 ± 18	119 ± 19

TABLE 803: Plasma PP concentrations (pmol/l, mean ± SEM) during a standard test meal in patients with peptic ulcer, coeliac disease, tropical malabsorption and infective diarrhoea. Each group was matched with controls of similar age

	-30	-15	0	15	30	60	120	180
CROHN'S DISEASE	29 ± 6	31 ± 8	26 ± 6	240 ± 48	277 ± 27	202 ± 46	199 ± 56	180 ± 52
CONTROLS	26 ± 6	23 ± 6	23 ± 6	211 ± 48	202 ± 54	197 ± 57	208 ± 63	119 ± 24
ULCERATIVE COLITIS	42 ± 8	37 ± 7	47 ± 12	226 ± 37	267 ± 41	199 ± 34	184 ± 30	155 ± 24
CONTROLS	26 ± 6	23 ± 6	23 ± 7	211 ± 48	205 ± 54	197 ± 57	208 ± 63	119 ± 24
DIVERTICULAR DISEASE	42 ± 11	42 ± 9	44 ± 12	234 ± 28	275 ± 42	232 ± 33	207 ± 38	185 ± 36
CONTROLS	21 ± 6	22 ± 6	25 ± 7	175 ± 34	173 ± 36	142 ± 20	150 ± 27	116 ± 19
IRRITABLE BOWEL SYNDROME WITH DIARRHOEA	42 ± 8	43 ± 9	42 ± 9	283 ± 33	304 ± 41	237 ± 41	208 ± 37	179 ± 29
CONTROLS	22 ± 3	22 ± 4	20 ± 3	222 ± 41	176 ± 35	146 ± 30	134 ± 28	123 ± 30
IRRITABLE BOWEL SYNDROME WITH CONSTIPATION	45 ± 11	45 ± 12	50 ± 14	247 ± 42	286 ± 42	208 ± 37	194 ± 22	186 ± 28
CONTROLS	22 ± 3	22 ± 4	20 ± 3	222 ± 41	176 ± 35	146 ± 30	134 ± 28	123 ± 30

TABLE 804: Plasma PP concentrations (pmol/l, mean ± SEM) during a standard test meal in patient with Crohn's disease, ulcerative colitis, diverticular disease and the irritable bowel syndrome. Each group was matched with controls of similar age.

	-30	-15	0	15	30	60	120	180
PANCREATECTOMY	-2 ± 1	-2 ± 1	-1 ± 1	-1 ± 1	-1 ± 1	-1 ± 1	-1 ± 1	-1 ± 1
CONTROLS	22 ± 5	22 ± 5	22 ± 5	171 ± 30	155 ± 25	126 ± 19	135 ± 25	110 ± 21
SMALL INTESTINAL RESECTION	85 ± 29	94 ± 36	90 ± 31	327 ± 60	431 ± 63	396 ± 64	372 ± 64	323 ± 66
CONTROLS	22 ± 5	22 ± 5	22 ± £	171 ± 30	155 ± 25	126 ± 19	135 ± 25	110 ± 21
LARGE INTESTINAL RESECTION	59 ± 13	59 ± 13	59 ± 13	386 ± 84	443 ± 87	368 ± 83	332 ± 93	264 ± 61
CONTROLS	22 ± 5	22 ± 5	22 ± 5	171 ± 30	155 ± 25	126 ± 19	135 ± 25	110 ± 21
MORBID OBESITY	26 ± 6	25 ± 4	25 ± 4	191 ± 27	325 ±118	151 ± 25	162 ± 30	134 ± 24
CONTROLS	20 ± 4	20 ± 4	20 ± 3	179 ± 33	144 ± 29	127 ± 27	128 ± 28	98 ± 16
ILEO-JEJUNAL BYPASS	34 ± 5	35 ± 5	36 ± 6	233 ± 26	211 ± 28	161 ± 26	145 ± 24	164 ± 28
CONTROLS	20 ± 4	20 ± 4	20 ± 3	179 ± 33	144 ± 29	127 ± 27	128 ± 28	98 ± 16

TABLE 805: Plasma PP concnetrations (pmol/l, mean ± SEM) following a standard test meal in patients after total pancreatectomy, intestinal resection and in patients with morbid obesity before and after ileo-jejunal bypass. Each group was matched with controls of similar age.

	-30	-15	0	15	30	60	120	180
THYROTOXICOSIS	28 ± 6	30 ± 7	28 ± 8	199 ± 41	158 ± 42	119 ± 31	113 ± 9	85 ± 7
CONTROLS	23 ± 4	20 ± 4	24 ± 6	173 ± 33	149 ± 32	104 ± 15	133 ± 18	119 ± 19
ANOREXIA NERVOSA	23 ± 5	23 ± 6	25 ± 7	253 ± 76	260 ± 80	227 ± 87	242 ±109	196 ± 70
CONTROLS	23 ± 4	20 ± 4	24 ± 6	173 ± 33	149 ± 15	104 ± 15	133 ± 18	119 ± 19

TABLE 806: Plasma PP concentrations (pmol/l, mean ± SEM) following a standard test meal in patients with thyrotoxicosis and anorexia nervosa. Both groups were matched with controls of similar age.

Chronic Pancreatitis

Patients with chronic pancreatitis without exocrine deficiency had basal and stimulated plasma PP concentrations which were not significantly different from normal. Those patients with chronic pancreatitis and exocrine deficiency (P1) however had significantly reduced fasting levels (P1 10 ± 2 pmol/l, controls 30 ± 6 pmol/l, $p < 0.005$) and the response to the breakfast was substantially reduced (Figure 804). The peak rise in circulating PP levels in the steatorrhoea group was 34 ± 12 pmol/l compared with 208 ± 32 pmol/l in the controls ($p < 0.001$) and the total integrated hormonal response was 5.6 ± 1.8 pmol l^{-1} min^{-1} compared with 30.8 ± 4.8 in controls.

Three patients with insulin treated diabetes mellitus had circulating antibodies which bound PP and PP could therefore not be measured in these samples. The patients with chronic pancreatitis were also sub-grouped into those with diabetes mellitus and those without. The results in these sub-groups are shown in Table 807. Statistical analysis was not possible due to the small number of patients with diabetes but no PP antibodies (3 patients with chronic pancreatitis but no steatorrhoea and 3 patients with exocrine deficiency). In each diabetic group however the highest value (top of range) for basal, peak rise and total integrated response of PP were all lower than the corresponding mean levels in the patient groups without diabetes mellitus.

The PP response to intravenous injection of Boots' secretin was also substantially reduced in the patients with chronic pancreatitis and exocrine deficiency (Figure 805). The plasma PP concentration in normal subjects rose rapidly from the basal level of 16 ± 2 pmol/l to a peak of 122 ± 21 pmol/l at six minutes and then fell rapidly to basal levels within 30 minutes. The group with chronic pancreatitis with pancreatic insufficiency, however, showed a significantly reduced

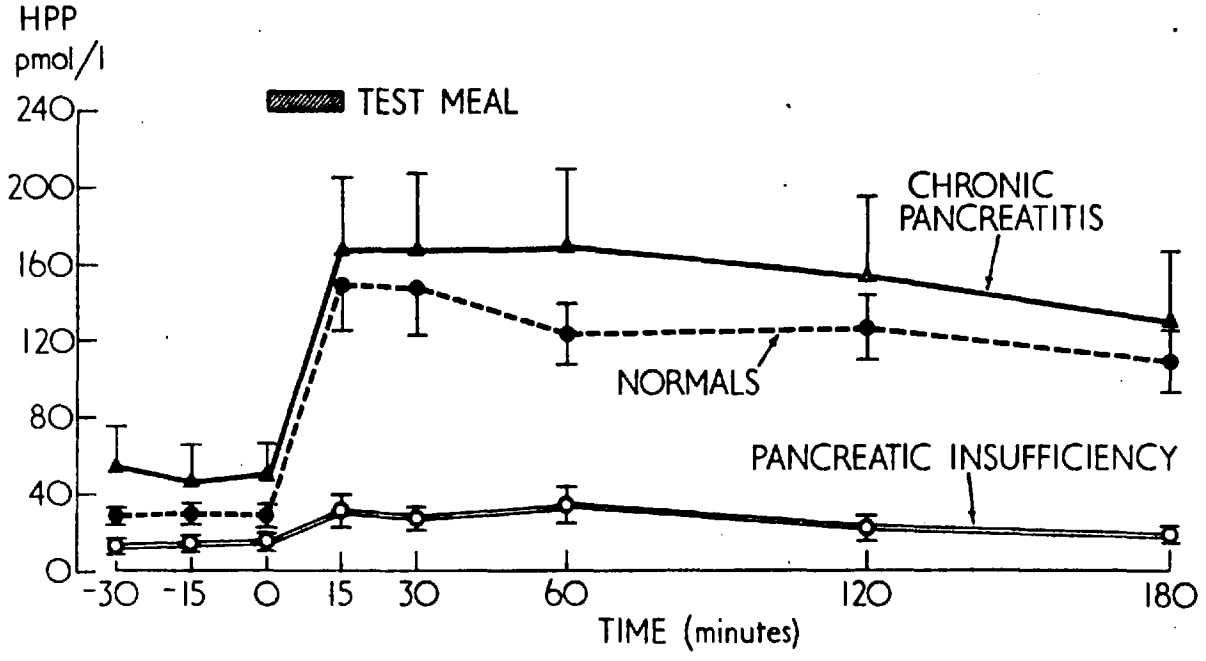


Figure 804: Plasma PP concentrations during a standard test breakfast in 22 patients with chronic pancreatitis with no steatorrhoea, 16 patients with chronic pancreatitis and exocrine insufficiency and 15 healthy age matched controls.

	NORMAL SUBJECTS	CHRONIC PANCREATITIS WITHOUT STEATORRHOEA		CHRONIC PANCREATITIS WITH EXOCRINE DEFICIENCY	
	n = 15	No Diabetes n = 19	With Diabetes n = 3	No Diabetes n = 11	With diabetes n = 3
BASAL	30 ± 6	47 ± 9	16 (range 3-22)	12 ± 3	5 (range 2-9)
PEAK RISE	247 ± 31	224 ± 36	122 (range 42-211)	42 ± 16	15 (range 3-33)
TOTAL INTEGRATED RESPONSE	30.8 ± 4.8	33.5 ± 5.5	16.2 (range 3.0-25.6)	7.3 ± 2.8	2.0 (range 0.3-3.6)

TABLE 807: Plasma PP concentrations in chronic pancreatitis both with and without diabetes mellitus and healthy controls. Basal and peak rise are in pmol/l and total integrated response are in pmol l^{-1} min^{-1}.

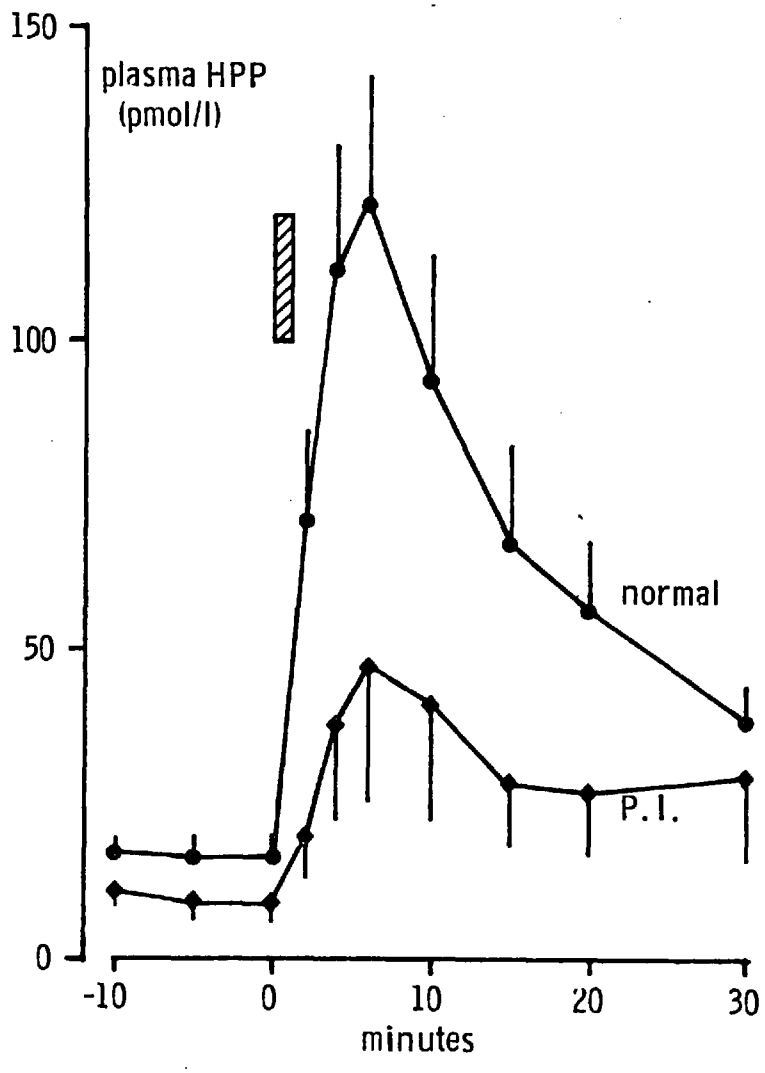


Figure 805: Plasma PP concentrations following a one minute infusion of Boots' secretin (2 Crick Harper Raper units/kg) in 8 patients with chronic pancreatitis and exocrine insufficiency (PI) and 8 healthy controls.

response to this exogenous stimulus (basal 9 $\pm$ 3 pmol/l to 47 $\pm$ 22 pmol/l at 6 minutes ($p < 0.025$).

Chronic Alcoholism

Both basal and postprandial PP concentrations were significantly elevated throughout the test on the day of admission in all the alcoholic patients ($p < 0.001$) (Figure 806). PP levels were still significantly elevated after two weeks of abstinence from alcohol.

Cholelithiasis

Basal and postprandial PP concentrations in patients with chronic cholelithiasis were not significantly different from age matched controls.

Gastrointestinal Disease

The plasma PP response to the standard test breakfast in the patients with active and treated coeliac disease, tropical sprue, duodenal ulcer, infective diarrhoea, Crohn's disease, ulcerative colitis and diverticular disease was not significantly different from that seen in age matched controls (Tables 803 and 804).

The patients with the irritable bowel syndrome however had basal and postprandial plasma PP concentrations which were significantly higher than controls (Table 804, $p < 0.05$). This moderate elevation of PP levels was seen in patients with constipation as well as in those with diarrhoea.

Short Bowel Syndrome

Both groups of patients with gut resection had significantly elevated fasting and postprandial PP concentrations compared with control subjects (Figure 807, Table 805). Basal PP concentrations in the small intestinal resection group were 90 $\pm$ 31 pmol/l and in the large bowel resection group 58 $\pm$ 13 pmol/l. These were significantly higher than

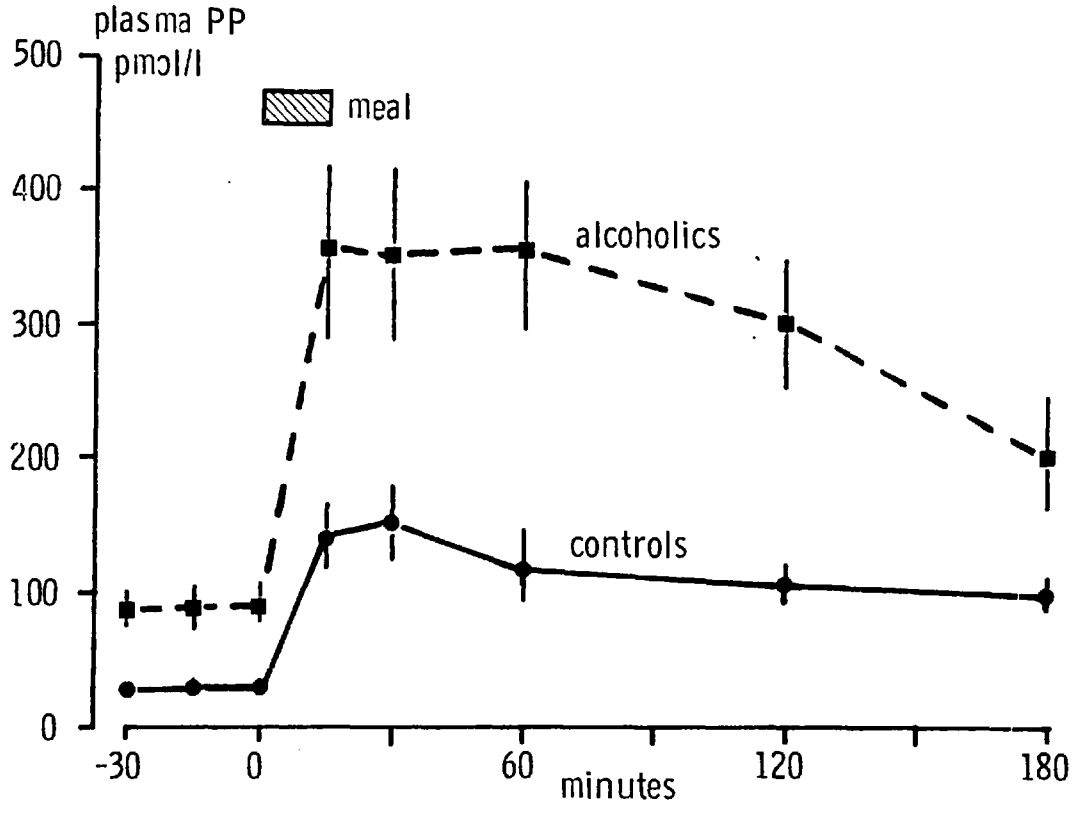


Figure 806: Plasma PP concentrations during a standard test breakfast in 9 chronic alcoholics and 9 healthy age matched controls.

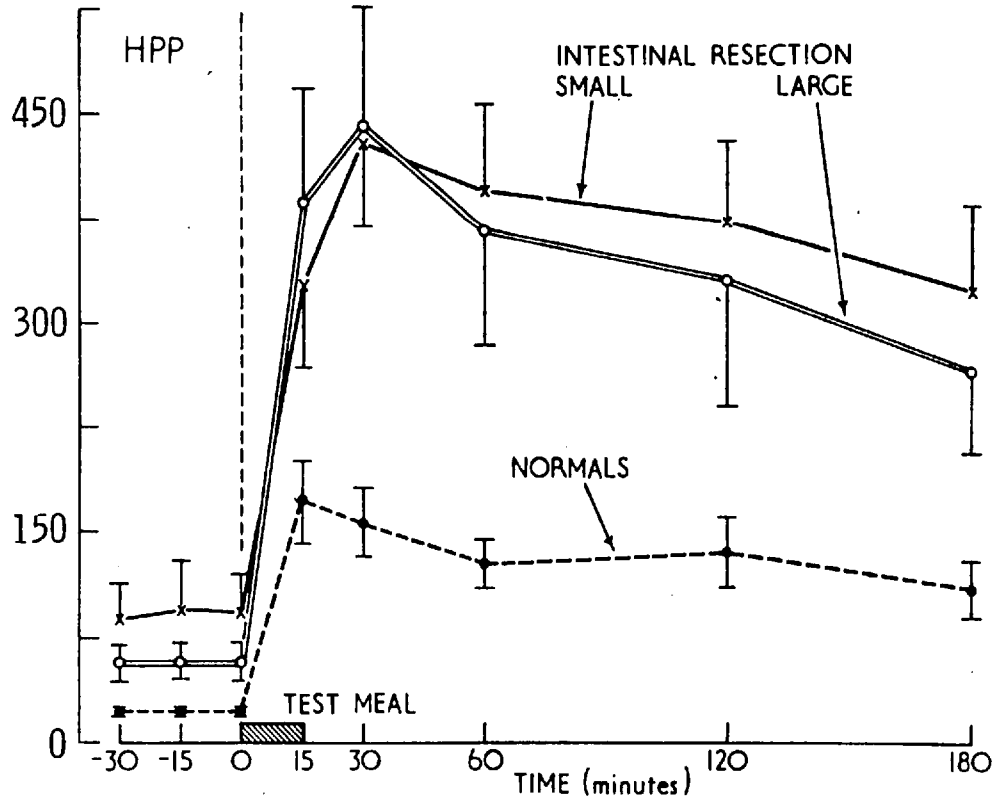


Figure 807: Plasma PP concentrations during a standard test breakfast in 18 patients who had undergone small intestinal resection, 9 patients after large bowel resection and 15 healthy age matched controls.

control values of 22 $\pm$ 4 pmol/l ($p < 0.05$ and $p < 0.02$ respectively). The peak rise in PP levels in the small bowel resection group was 407 $\pm$ 48 pmol/l and in the large bowel resection was 426 $\pm$ 79 pmol/l which were significantly higher than control values 174 $\pm$ 28 pmol/l ($p < 0.001$ and $p < 0.01$ respectively). The total integrated hormonal response in normal controls was 23.3 $\pm$ 3.9 pmol l^{-1} min^{-1} and was significantly higher in both the small bowel (64.4 $\pm$ 10.9 $p < 0.005$) and large bowel (59.3 $\pm$ 13.3, $p < 0.02$) resection groups respectively.

Morbid Obesity and Jejuno-Ileal Bypass

Postprandial PP levels tended to be higher in obese patients than controls but this did not reach statistical significance at any time point (Table 805). Similarly the PP response in patients who had undergone jejuno-ileal bypass was not significantly different from that of controls (Table 805).

Anorexia Nervosa and Thyrotoxicosis

The patients with anorexia nervosa and thyrotoxicosis had basal and postprandial PP levels which were similar to those seen in age matched healthy controls (Table 805).

Pancreatic Endocrine Tumours

Of a total of 164 patients with pancreatic endocrine tumours studied 66 (40%) had grossly elevated fasting plasma PP concentrations. The individual plasma PP concentrations are shown in Figure 808. Mean fasting PP concentrations in 125 healthy subjects were 22 $\pm$ 2 pmol/l and ranged from 1 to 100 pmol/l. For tumour cases however an arbitrary "cut off" point of 300 pmol/l was chosen to denote "elevated" levels as concentrations of up to 200 pmol/l are sometimes seen in very ill patients suffering from

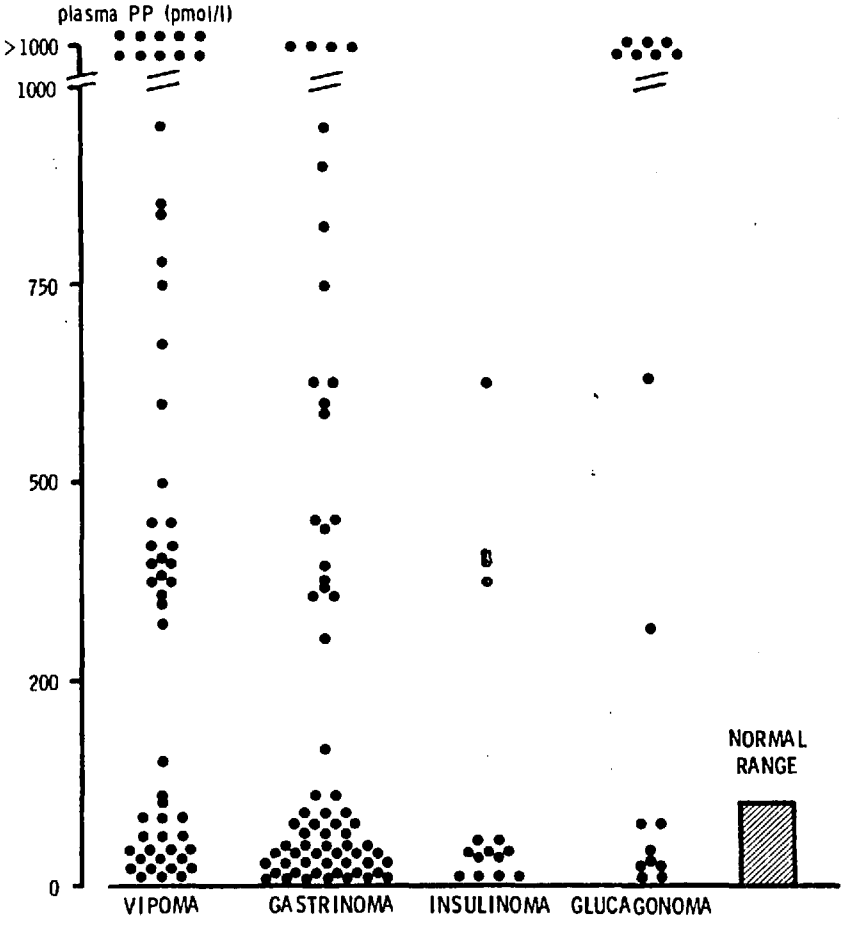


Figure 808: Fasting plasma PP concentrations in patients with pancreatic endocrine tumours; Vipoma (n = 49), gastrinoma (n = 75), insulinoma)n = 18) and glucagonoma (n = 17). The normal range for healthy fasting adults is also included (mean $\pm$ SEM).

a wide range of diseases. Of the 49 patients with pancreatic VIPoma 31 (63%) had elevated PP levels. Of 75 patients with gastrinoma 20 (27%) had elevated PP levels. Of 17 patients with glucagonoma 9 (53%) had elevated PP levels. Of 18 patients with insulinoma 4 had elevated PP levels. In contrast none of the 3 patients with pancreatic somatostatinoma had elevated PP levels. Three patients in whom an endocrine cell tumour was diagnosed histologically and subsequently shown to be comprised of PP cells by immunofluorescent studies, also had elevated levels of PP in fasting plasma.

The molecular form of PP in plasma of patients with pancreatic endocrine tumours is shown in Figure 809. In 12 out of 13 samples two distinct peaks of PP-like immunoreactivity were demonstrated. The major form (90%) corresponded to the pure PP marker (M wt 4300) whereas a second peak eluted close to cytochrome C (M wt 12 000). Both of these immunoreactive peaks were suppressed during somatostatin infusion (8 patients) indicating a rapid turnover for both molecular forms.

Carcinoid Syndrome

Using the arbitrary limit of 300 pmol/l to describe abnormal fasting PP levels, elevated concentrations were seen in 11 out of 26 (42%) patients with the carcinoid syndrome. The individual plasma PP levels are shown in Figure 810. The incidence of flushing and diarrhoea were similar between the groups with high and normal PP levels and, in addition, urinary 5H 1AA excretion was similar between the two groups. There was no correlation between PP levels and the site of the primary tumour.

Other Endocrine Neoplasms

Fasting plasma PP concentrations were not significantly different from normal in patients with ganglioneuroblastoma 40 $\pm$ 8 pmol/l,

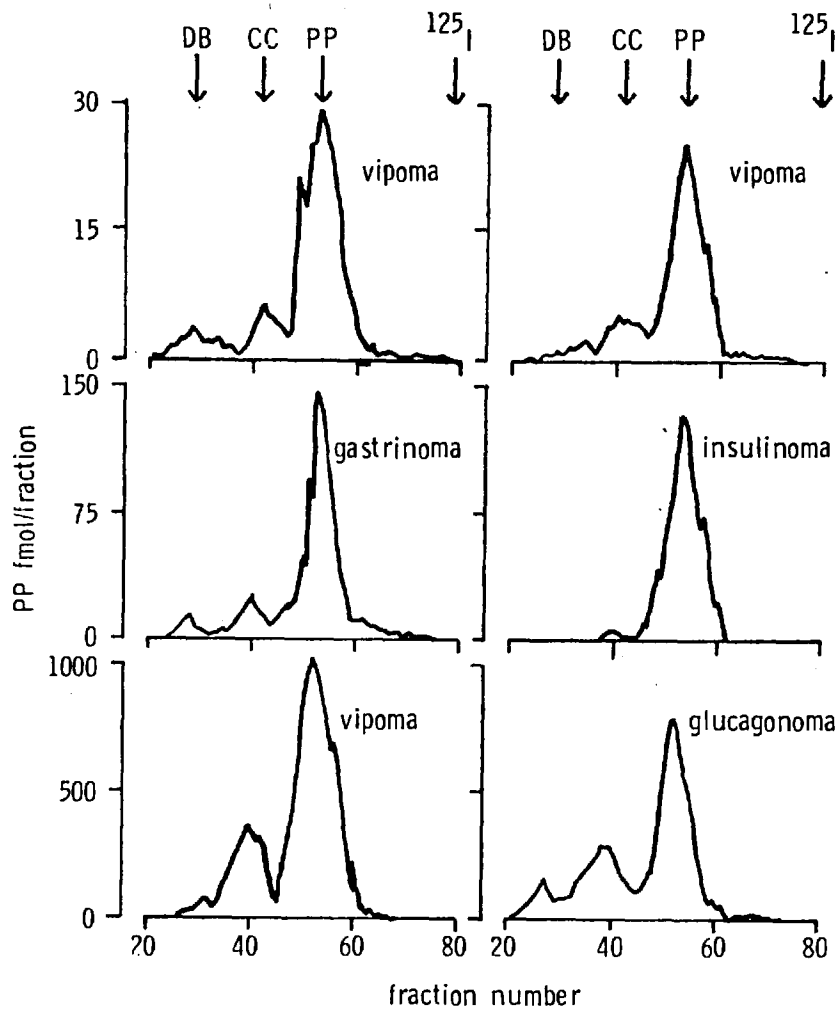


Figure 809: Gel chromatography of PP immunoreactivity in fasting plasma from three patients with pancreatic VIPomas and one patient with a glucagonoma. The positions of column calibration markers is indicated by the arrows, DB = dextran blue, CC = cytochrome C, PP = pure HPP standard and I = ^{125}I.

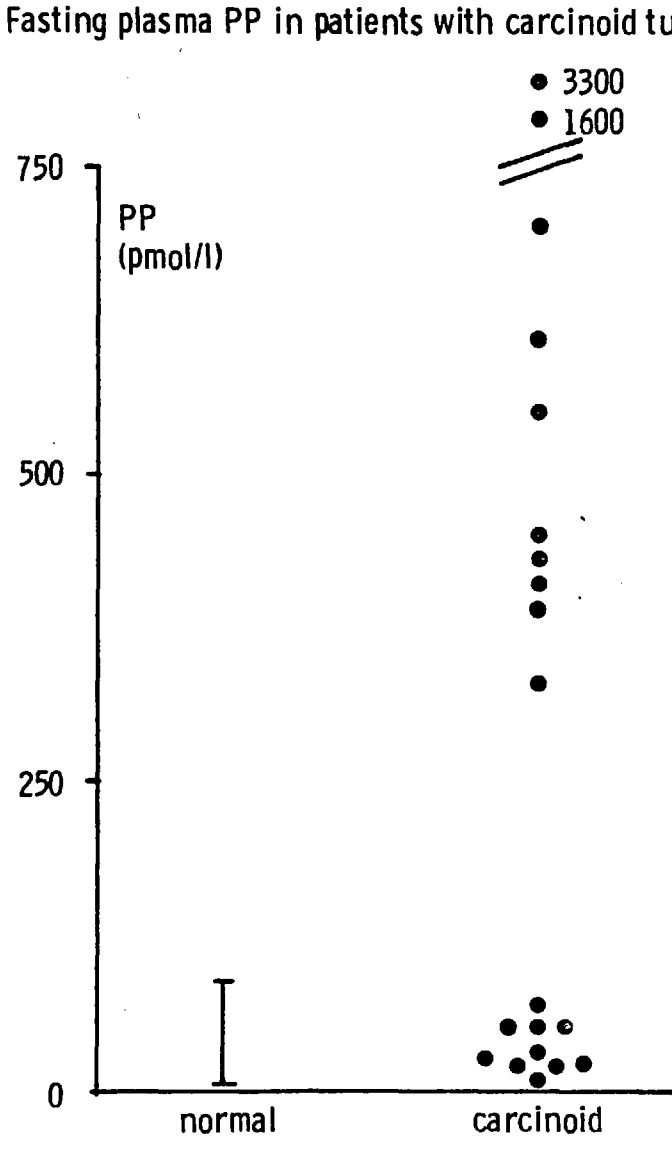


Figure 810: Fasting plasma PP concentrations in 20 patients with the carcinoid syndrome.

phaeochromacytoma 29 ± 8 pmol/l, medullary carcinoma of the thyroid 23 ± 4 pmol/l, ectopic ACTH secreting tumours 31 ± 4 pmol/l, prolactinomas 24 ± 8 pmol/l or acromegaly 17 ± 3 pmol/l. Basal levels in normal controls were 25 ± 2 pmol/l.

Adenocarcinoma

In 17 patients with adenocarcinoma of the pancreas fasting PP concentrations were 37 ± 10 pmol/l which was not significantly different from patients with a tumour in the stomach (36 ± 7, n = 15), colon (34 ± 2, n = 5), rectum (45 ± 6, n = 8), bronchus (42 ± 6, n = 24) or breast (50 ± 8, n = 6). They were also similar to values obtained from healthy controls of similar age.

DISCUSSION

The Pathophysiology of Pancreatic Polypeptide

The study of abnormal hormone secretion in disease has often shed light on the role of a hormone in normal physiology; as biological effects are usually more pronounced when hormone levels are very high.

PP in Pancreatic Disease

Because of the localisation of PP in the Islets of Langerhans as well as between the acinar cells and along the ducts of the exocrine tissue, it was clear that PP secretion could have been influenced by endocrine and exocrine pancreatic disease. Moderate elevations of fasting plasma PP were seen in patients with diabetes mellitus however these were not seen in patients carefully controlled with highly purified insulin and may well have been secondary to the metabolic derangement

(Adrian, 1980). Such elevations in diabetes mellitus have been reported previously (Floyd et al, 1977). Perhaps of more interest is the finding that two thirds of insulin dependent diabetics have endogenous antibodies to PP in their circulation as a result of the contamination of commercial insulin preparations with porcine and bovine PP (Bloom et al, 1978). These antibodies were particularly present in young diabetics (80%). In addition these antibodies have shown pronounced immunocytochemical staining with fluorescein-labelled or peroxidase-labelled antihuman gammaglobulin of the PP cells in normal pancreas sections. It is also noteworthy that the affinity of these antibodies is high rivalling that of antisera for radioimmunoassay (Bloom et al, 1978). There is as yet no data on the consequence of this induced autoimmunity.

The grossly impaired PP release following a test breakfast in patients with pancreatic exocrine deficiency due to chronic pancreatitis and cystic fibrosis presumably reflects the presence of PP cells in the exocrine parenchyma as well as in the Islets of Langerhans (Adrian et al, 1978; Adrian et al, 1979; Adrian et al, 1980). The finding that the response to the exogenous stimulus of intravenous Boots' secretin is also impaired in patients with pancreatic steatorrhoea provides further evidence in favour of a loss of functioning PP cells (Adrian et al, 1979). Indeed Sive and co-workers have demonstrated that the PP response to insulin induced hypoglycaemia is similarly impaired in chronic pancreatitis (Sive et al, 1978). The normal release of PP in chronic pancreatitis without clinical steatorrhoea would suggest that effective PP cell function is maintained in spite of extensive loss of acinar cell function as steatorrhoea does not develop until exocrine secretion is reduced by 90% or more (DiMagno et al, 1973). The greatly obtunded PP response in cystic fibrosis (Adrian et al, 1980) would perhaps be expected as the

steatorrhoea in these children is accompanied by glucose intolerance and sometimes frank diabetes mellitus (Handweger et al, 1969). In addition impaired insulin and glucagon responses have been described (Kjellman and Larsson, 1975; Redmond et al, 1977).

It is of interest that PP levels are grossly elevated in chronic alcoholics as this suggests a sub-clinical involvement of the endocrine pancreas in this condition. The link between pancreatic disease and alcohol abuse is well established although the role of PP in alcoholic disease is as yet unknown.

PP in Gastrointestinal Disease

During early experiments on the biological actions of BPP in the dog, Lin showed that low doses of PP could relax the gall bladder (Lin et al, 1978). Infusion studies in man have led to the suggestion that PP may be involved in the postprandial control of gall bladder function (Greenberg et al, 1978; Bjornsson et al, 1979; Adrian et al, 1979). The plasma PP response to food was however quite normal. This finding suggests that PP is unlikely to play a role in the development of gallstones.

The normal release of PP after a meal in patients with coeliac disease and tropical sprue suggests that steatorrhoea and malabsorption per se do not affect PP release. It is indeed of interest that PP release is normal in coeliac disease as pancreatic exocrine secretion after food is reported to be impaired in this condition. (Worning et al, 1967; DiMagno et al, 1969). The pancreas however responds quite normally to exogenous secretin and cholecystokinin and it is presumed that secretin and cholecystokinin are not released normally from the diseased small intestine mucosa in response to acid chyme. For example, a failure of secretin release has been demonstrated (Besterman et al, 1980). It is thus likely that the humoral signal responsible for the release of PP

after a meal may not be released solely from this part of the small bowel.

Pharmacological studies in the dog demonstrated that PP could stimulate basal gastric acid secretion although the doses at which this occurred were one or two orders of magnitude above those which influenced the exocrine pancreas. It was clearly of interest to investigate the normal release of PP in patients with peptic ulcer disease as postprandial acid secretion is high in these patients. PP secretion appeared to be normal in duodenal ulcer patients (Adrian et al, 1977). Schwartz et al, reported however that basal PP levels were moderately elevated in two out of nine patients with duodenal ulcers (Schwartz et al, 1976). The elevated PP levels in these subjects was suppressed by atropine and was therefore considered to be evidence of increased vagal tone in such patients which might also induce excessive acid secretion (Schwartz, 1978).

As PP was shown to be a powerful stimulant of intestinal motor activity in the dog (Lin et al, 1978) PP release was investigated in several gastrointestinal disorders associated with altered bowel motility. PP levels in patients with infective diarrhoea, Crohn's disease, ulcerative colitis and diverticular disease were not significantly different from normal (Besterman et al, 1978; Adrian et al, 1980). Patients with the irritable bowel syndrome had moderately elevated PP concentrations. However, these elevated basal and postprandial PP levels were seen in patients with constipation as well as those with diarrhoea. PP levels in patients with pancreatic endocrine tumours such as glucagonomas, can be up to 1000 times the normal fasting level without the development of altered bowel habit. This suggests that the moderately elevated levels seen in the irritable bowel syndrome are unlikely to cause symptoms of diarrhoea or constipation.

Patients with both small and large bowel resections had significantly elevated plasma PP concentrations (Besterman et al, 1978). The reason for this is obscure. BPP has been shown to increase DNA synthesis in the rat pancreas and hence may be trophic to the pancreas (Greenberg et al, 1977). No such effect was found in the stomach, duodenum or liver however, and effects in the ileum and colon were not investigated. Recent experimental work in rats has shown that PP can stimulate colonic water absorption (Mitchenere et al, 1980). The association between elevated PP levels and a loss of effective absorptive gut area may provide further clues into possible intestinal actions of PP.

PP in Obesity

The observation that in genetically obese mice PP could reduce hyperphagia and result in a loss of weight led to the suggestion that PP may be a satiety factor. If PP was involved in the regulation of appetite abnormalities may accompany morbid obesity in man, in addition, conditions of altered food intake such as anorexia nervosa and thyrotoxicosis may be accompanied by abnormal PP secretion. Basal and postprandial PP concentrations were completely normal in these three groups of patients (Besterman et al, 1978) and in addition PP secretion was not impaired following ileo-jejunal bypass operation for morbid obesity. Subsequent work has shown that plasma PP concentrations and the pancreatic tissue content of the obese hyperglycaemia mice were not significantly different from lean litter mates (Adrian et al, 1979). Additional evidence against PP being a circulating satiety factor is the observation that during more than 50 PP infusions in man, some at doses giving plasma levels of four or five times the normal postprandial rise, no subjective effect on appetite was noted (Adrian wt al, 1980).

PP in Neoplastic Disease

PP is a frequent component of pancreatic endocrine tumours and their metastases (Polak et al, 1976). Patients with such tumours often have fasting plasma levels which exceed even the postprandial levels seen in healthy subjects. Plasma concentrations may be as high as 40 nmol/l which exceeds the mean normal fasting level by 1000 times. Most of the tumours produced PP eluted from a gel permeation column in an idential position to pure PP suggesting that it is similar or identical to the normally produced hormone (Bryant et al, 1978). The small amount of higher molecular weight material seen might represent some precursor form which is not normally detectable in normal plasma because of low concentration.

Several groups have confirmed that plasma PP concentrations are often elevated in patients with pancreatic apudomas. Taylor et al reported that 4 out of 41 patients with the ZE syndrome had levels greater than 300 pmol/l and that a further seven patients had PP levels greater than the upper limit of normal (Taylor et al, 1978; Lamers et al, 1978). This latter group found a similar percentage of patients with elevated plasma PP (17%) to the present study (27%). Floyd et al reported that patients with insulinoma have plasma PP levels which are higher than normal subjects. (Floyd et al, 1977).

The high concentrations of PP found in hepatic and lymph-node metastases can be construed as further evidence that PP is a primary product of these tumours. PP producing cells have also been demonstrated by immunocytochemistry in both primary and metastatic tumour tissue (Polak et al, 1976). The tumours which appeared to produce PP alone presented with symptoms of biliary obstruction in all three cases, however no symptoms were apparent which could be attributed to hypersecretion of PP. It is therefore unlikely that PP contributes to the symptoms seen

in the other endocrine tumours such as diarrhoea and duodenal ulceration. The suggestion has been made that PP could be responsible for the watery diarrhoea seen in some patients with pancreatic endocrine tumours (Larsson et al, 1976). However the observation that in some patients PP levels of 40 000 pmol/l are not accompanied with diarrhoea make this suggestion inherently unlikely (Adrian et al, 1980). It is possible however that PP is responsible for the large flaccid gall bladders often seen in patients with pancreatic apudomas (Schwartz, 1980).

Nearly half of the patients with the carcinoid syndrome also had elevated PP levels although in general these were not as high as was seen in patients with pancreatic endocrine tumours (Adrian et al, 1978, 1980). PP was detectable in carcinoid tumour tissue, both primary and metastatic, and column chromatography revealed that this PP-like immunoreactivity eluted in the position of pure HPP. In the carcinoid syndrome PP levels did not appear to be related to the incidence of flushing or severity of the diarrhoea seen, neither did it correlate with the site of the primary tumour (Adrian et al 1978, 1980).

Patients with other endocrine tumours such as ganglioneuroblastoma, phaeochromacytoma, medullary carcinoma of the thyroid, ectopic ACTH tumours (Cushing's syndrome), prolactinomas and growth hormone secreting tumours (acromegaly) are associated with normal fasting PP levels. In addition patients with adenocarcinoma of the pancreas have similar fasting plasma PP concentrations to normal subjects and patients with adenocarcinoma of other sites.

It is clear that the measurement of PP in plasma may be a useful marker for pancreatic endocrine neoplasms. Being relatively stable in plasma and easy to measure, elevated levels of PP can give confirmatory evidence of a pancreatic tumour and may, in particular, be useful in the monitoring of the effectiveness of chemotherapy in these patients. PP

measurements may also be used to localise pancreatic tumours by selective venous sampling following percutaneous transhepatic portal venous catheterisation (Burcharth, 1979). One particular use of the PP assay is in distinguishing pancreatic VIPoma, where most patients have elevated PP levels, from VIP secreting tumours of the sympathetic chain, associated with normal PP levels.

It has become apparent in more recent years that pancreatic "islet cell tumours" frequently secrete more than one peptide and it may therefore be pertinent to measure several peptides to screen for such tumours including PP, glucagon, gastrin, VIP and somatostatin.

CHAPTER 9. CONCLUSIONS

Although the discovery of pancreatic polypeptide (PP) is a relatively recent event (Kimmel et al, 1968; Chance and Jones, 1974) considerable progress has been made in establishing PP as a third circulating pancreatic hormone. PP is localised in a distinct small granuled endocrine cell which is found in both the exocrine parenchyma and in the Islets of Langerhans of the pancreas (Larsson et al, 1976). PP circulates in plasma and levels rise in response to various stimuli, food in particular. PP has a broad spectrum of biological actions some of which are seen at plasma concentrations similar to those seen after food (Greenberg et al, 1978; Taylor et al, 1979). In addition circulating PP concentrations appear to be influenced by pancreatic and gastrointestinal diseases. There are, however, several questions still unresolved.

The discovery of PP was somewhat unusual in that the peptide was purified before its biological activity was investigated. However, the availability of pure PP has enabled several groups to measure the release of this peptide into blood and, in addition, infusion studies have been undertaken to investigate the physiological role of PP.

The radioimmunoassay of PP has proven to be relatively simple compared with that of other gastrointestinal hormones. The reliability of the assay is undoubtedly due, in part at least, to the remarkable stability of the molecule. Plasma concentrations of PP are similar in magnitude to insulin and therefore assay sensitivity does not constitute a problem in the measurement of PP.

As PP is released from the pancreas following the ingestion of food

but not by intravenous nutrients it is clear that an indirect signal is responsible for this release. This "entero-PP-axis" would appear to be complex. Vagal stimulation, for example during insulin induced hypoglycaemia, can potently release PP and may therefore contribute to the "axis". Although postprandial PP release is somewhat obtunded soon after truncal vagotomy this function returns to normal within a few months of operation. It is clear therefore that the vagus is not the only mechanism for PP release. Several gastrointestinal peptide hormones or hormonal candidates can release PP such as CCK, bombesin, GIP and neurotensin. Of these neurotensin and the CCK group appear to be the most potent. It is probable that one or several gut hormones is responsible in part for the release of PP seen following the ingestion of food. The release of PP by gastrointestinal hormones appears to be dependent on cholinergic tone however as it is atropine sensitive. Although it is clear that an indirect axis is involved in the normal release of PP after food the exact nature of this signal is still unresolved.

When infused into man or dog at concentrations which mimic the postprandial response, PP can inhibit pancreatic enzyme secretion. In addition, in man PP reduced bile flow into the duodenum. Studies in cholecystectomised subjects have suggested that PP acts at two independent sites in man, firstly the exocrine pancreas where it inhibits enzyme secretion and secondly the gall bladder which is relaxed by the peptide. Clearly PP could be involved in the physiological modulation of post-prandial pancreatic and biliary secretion, two actions which oppose those of cholecystokinin. It is also possible that PP has other physiological actions. PP potently inhibits the release of motilin, a peptide from the upper small intestine which stimulates gastric and small bowel motility. This inhibition of circulating motilin would suggest that PP may itself

influence motor activity, although infusion did not significantly affect the rate of gastric emptying of a mixed breakfast. Motilin can stimulate the interdigestive myolelectric motor complexes which sweep down the intestine in the interprandial period to keep it free of debris and undigested food. It is tempting to speculate that PP may have a role in "switching off" these complexes when food is eaten. PP has motor effects on both the small and large bowel when infused at high doses in the dog. The possibility exists therefore that PP may directly influence normal bowel motility, however this remains to be investigated. Although PP has no obvious metabolic effect the possibility exists that it may influence water or nutrient absorption from the small bowel and this is therefore another area in which investigation could prove fruitful.

It is of interest that PP release is impaired in conditions in which the function of the exocrine pancreas is impaired such as chronic pancreatitis and cystic fibrosis. PP measurement is however probably of little diagnostic use as these conditions are clinically obvious once steatorrhoea has developed. Study of other gut functions in these patients may however shed light on the normal physiology of PP. Elevated PP responses in patients with intestinal resection are also of interest but the cause and effects of these elevated PP levels as yet remains a mystery.

The increase in basal and postprandial PP concentrations in chronic alcoholics is evidence of sub-clinical pancreatic involvement in these patients. This is of particular interest because of the known associations between alcohol abuse and pancreatic disease.

There is a high incidence of endogenous PP antibodies in diabetic patients who have been treated with conventional insulin. Diabetics frequently complain of gastrointestinal symptoms and therefore a thorough investigation into the relationship between the existence of PP auto-immunity and the complications of diabetes is warranted.

The secretion of PP by pancreatic endocrine tumours has led to the use of this assay in the detection of such neoplasms and also in monitoring of the effectiveness of surgery and chemotherapy. These rare tumours are usually slow growing and patients frequently perish from the symptoms produced by their secretory products. Their early detection, coupled with possible resection, is therefore highly desirable. It has become apparent in recent years that these tumours frequently secrete more than one peptide and, although seemingly clinically silent, PP is perhaps the most common peptide product of these tumours. As well as early detection because of the grossly elevated PP levels which are often seen, the assay has also proven useful in the localisation of these tumours by selective venous sampling following transhepatic portal venous catheterisation.

Future studies will no doubt unravel the complex release mechanisms of PP and perhaps also bring to light as yet unknown physiological actions. It seems only reasonable to expect that a peptide (PP) which circulates in similar concentrations to insulin must have an important role to play in normal physiology.

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