STUDIES ON ASPECTS OF THE PHYSIOLOGY AND PATHOPHYSIOLOGY OF PANCREATIC POLYPEPTIDE

ALAN AVROM SIVE

A Thesis submitted for the degree of

Doctor of Medicine

University of Cape Town August 1982

ABSTRACT

Pancreatic polypeptide (PP) is a 36 amino-acid residue peptide which has recently been recognised in pancreatic endocrine cells. Although PP has a number of effects on gastrointestinal and pancreatic exocrine function, its physiological function has not been clarified. Studies were undertaken to elucidate four aspects of the physiology and pathophysiology of this candidate hormone. Firstly, factors affecting the release of human PP (hPP) were assessed in healthy subjects; secondly polyacrylamide gel electrophoresis was used to investigate molecular heterogeneity of the peptide; thirdly, the organ uptake and half-life time of endogenous PP was measured in pigs; and finally, basal and stimulated serum hPP concentrations were assayed in patients with acute pancreatitis, chronic pancreatitis and maturity-onset diabetes mellitus.

A sensitive, specific radioimmunoassay was established to measure hPP concentrations. The mean ($\pm$ S.E.) basal serum hPP in 50 healthy subjects was 34 $\pm$ 2,38pmol/l. In seven subjects after eating a mixed meal, serum hPP concentration rose to peak at 648 $\pm$ 166,5pmol/l. The levels then fell, but remained significantly elevated above the basal concentration for 3 hours. 1 ml/kg of oral lipid and 1ml/kg of lipid infused intravenously caused hPP concentrations to rise to 282 $\pm$ 154,6pmol/l and 160 $\pm$ 50,1pmol/l, respectively. 50g of oral glucose caused a doubling, whereas 25g of intravenous glucose was associated with a significant fall in basal hPP concentrations to 20 $\pm$ 2,6pmol/l. Intravenous arginine had no effect on hPP levels. 2CHRU/kg of 'Boots' secretin, 85 CHRU of 'Boots' cholecystokinin and 5ug/kg of synthetic pentagastrin caused serum hPP concentrations to rise to 489 $\pm$

104,3pmol/l, 67 $\pm$ 21,2pmol/l and 272 $\pm$ 97,1pmol/l respectively. The hPP response to 'Boots' secretin was dose-related, and did not differ in subjects who had undergone bilateral truncal vagotomy. Insulin-induced hypoglycaemia caused hPP levels to rise to 220 $\pm$ 54,6pmol/l. An infusion of 6ug/min of epinephrine caused a small hPP response. Adrenergic beta-receptor stimulation achieved by the concomitant infusion of epinephrine and 0.5mg/min of phentolamine caused a significant rise in the serum hPP concentration to 351 $\pm$ 60,5pmol/l. Adrenergic alpha-receptor stimulation during epinephrine and 0.1mg/min of propranolol infused simultaneously caused elevated serum hPP concentrations to fall. Somatostatin, given as a bolus injection of 200ug and followed by an infusion of 2ug/min, depressed basal serum hPP levels. An infusion of 2ug/min of somatostatin inhibited the hPP response to insulin-hypoglycaemia. Neither L-dopa, a dopamine agonist, metoclopramide, a dopamine antagonist, nor cimetidine a H_2-receptor blocking agent significantly modified hPP release.

These findings suggest that the regulation of hPP release is multifactorial involving nutrients, gastrointestinal factors and the autonomic nervous system. The relevance of each, and their interrelationship will require further study.

10% polyacrylamide disc gel electrophoresis of hPP in the peripheral circulation distinguished 3 immunoreactive molecular forms of hPP. The major peak of hPP migrated with hPP of molecular weight 4200. Peaks of immunoreactive hPP that migrated in positions more cathodal and more anodal than ^{125}I-hPP were identified. Chromatography did not distinguish between hPP released by insulin-hypolycaemia or 'Boots' secretin.

Following total pancreatectomy in pigs, PP concentrations in the portal circulation decreased rapidly and showed two phases of disappearance. The early phase had a $t_{\frac{1}{2}}$ of 6,3 $\pm$ 1,63 min and the second phase of $t_{\frac{1}{2}}$ of 37,4 $\pm$ 3,30 min. This suggests that PP has a short half life but that there is redistribution of PP in a large pool or that continued release of PP from an extrapancreatic source occurs. There was a PP gradient of 42% and 48% across the liver and kidney, respectively, suggesting PP uptake by these organs.

In patients with acute pancreatitis, the mean hPP concentration did not differ significantly from the values in healthy subjects and did not reflect the presence or extent of pancreatic damage. In patients with chronic pancreatic serum hPP responses to a mixed meal, oral and intravenous lipid, 'Boots' secretin and to insulin-hypoglycaemia were uniformly impaired and significantly lower than the responses in healthy subjects. These findings are compatible with a reduced PP cell mass in chronic pancreatitis and suggest that these stimuli may provide non-invasive techniques for detecting patients with chronic pancreatitis. In patients with maturity-onset diabetes mellitus who were screened to exclude autonomic neuropathy, basal hPP levels were not significantly higher than in age-matched controls. hPP responses to insulin-hypoglycaemia were similar to the levels in the controls. The findings suggest that glucose intolerance per se does not affect hPP levels or responses.

These studies suggest that hPP is a candidate hormone which responds to numerous stimuli. It circulates in multiple molecular forms and has a short half-life in keeping with other pancreatic peptide hormones. hPP responses in patients with chronic pancreatitis are clearly abnormal. A biological role for PP has not been identified.

CONTENTS

ACKNOWLEDGEMENTS

I wish to thank and acknowledge the help and support of:

Professor A.I. Vinik who kindled my interest in diabetes during my days as a student, and then during my internship stimulated me to undertake a research project. It has been a privilege to work under him both clinically and as a research fellow, and to experience the abundant enthusiasm and optimism which he generates. His constant source of ideas, invaluable guidance and confidence have indeed made this work possible.

Professor W.P.U. Jackson in whose laboratory this work was undertaken, for his support, encouragement and facilities.

The many persons who kindly volunteered for the numerous studies.

Dr S.V. van Tonder and Mrs A. Lund for invaluable assistance with the assay of pancreatic polypeptide.

The technical staff of the Endocrine/Diabetes Research Group - Mrs M. Paul, Mrs P. Child, Mrs M. Tyler, Mrs L. Schatz and Mr M. Davids for the estimations of glucose, insulin, free fatty acids, triglycerides, glucagon, and chromatography.

Mrs A. Mason for liaison with the volunteers and many additional tasks.

The gastrointestinal clinic of Groote Schuur Hospital for access to patients with chronic pancreatitis.

Drs. van Hoorn-Hickman and van Hoorn for their unstinting support and guidance, and performing the surgery on the pigs.

Dr G.O. Barbezat for the study of acute pancreatitis.

Dr T.W. Schwartz for assaying samples from patients with acute pancreatitis.

Dr R.E. Chance of Lilly Research Laboratories for the generous gifts of human and bovine pancreatic polypeptide as well as the antiserum to human pancreatic polypeptide.

Dr E Polakow of Ayerst Laboratories for a gift of synthetic cyclic somatostatin.

Drs J.L. Botha, L.J. Klaff and N.S. Levitt for their companionship and many helpful discussions.

Mrs Phillips, Mrs Garschagen and Mrs Heuer for painstakingly typing the final manuscript.

Mrs L. Coetzee for photography of the figures.

And lastly my wife, Rosemary, for her constant support, her ability to enjoy movie after movie alone, and typing and retyping drafts of the manuscript.

I also wish to acknowledge that this work forms part of the programme of the Endocrine/Diabetes Research Group, jointly sponsored by the

Medical Research Council of South Africa and the University of Cape Town. Grants from the Nellie Atkinson Bequest, the Mauerberger Trust Foundation and the Atomic Energy Board are gratefully acknowledged.

GLOSSARY OF ABBREVIATIONS AND TERMS

aPP	-	avian pancreatic polypeptide
APUD	-	amine precursor uptake and decarboxylation
BP	-	British Pharmacopoeia
bPP	-	bovine pancreatic polypeptide
^{o}C	-	degrees centigrade
CCK-PZ	-	cholecystokinin-pancreozymin
CHRU	-	Crick-Harper-Raper Units
CLIP	-	corticotropin-like intermediate peptide
cm	-	centimetres
C-terminus	-	carboxy terminus
DNA	-	deoxyribonucleic acid
Dopa	-	dihydroxyphenylalanine
FFA	-	free fatty acids
g	-	grams
g/kg	-	grams per kilogram
GIP	-	gastric inhibitory polypeptide
hPP	-	human pancreatic polypeptide
^{125}I	-	^{125}Iodine
IRG	-	immunoreactive glucagon
kg	-	kilograms
M	-	molar
M/l	-	moles per litre
MEA	-	multiple endocrine adenomatosis
mEq/l	-	milliequivalents per litre
mg	-	milligrams
mg/kg	-	milligrams per kilogram
mg/kg/hr	-	milligrams per kilogram per hour

mg/kg/min	-	milligrams per kilogram per minute
mg/min	-	milligrams per minute
mg/ml	-	milligrams per millilitre
min	-	minutes
ml	-	millilitres
ml/kg	-	millilitres per kilogram
ml/kg/min	-	millilitres per kilogram per minute
mmol/l	-	millimoles per litre
mU/l	-	milliunits per litre
ng/kg/hr	-	nanograms per kilograms per hour
ng/ml	-	nanograms per millilitre
nm	-	nanometres
nmol/kg	-	nanomoles per kilogram
oPP	-	ovine pancreatic polypeptide
PEG	-	polyethylene glycol
pg	-	picograms
pg/kg/hr	-	picograms per kilogram per hour
pg/ml	-	picograms per millilitre
pmol	-	picomoles
pmol/min	-	picomoles per minute
pmol/l	-	picomoles per litre
PP	-	pancreatic polypeptide
pPP	-	porcine pancreatic polypeptide
PTH	-	parathyroid hormone
R.F.	-	reference front values
rpm	-	revolutions per minute
S.D.	-	standard deviation
S.E.	-	standard error of the mean
somatostatin	-	growth hormone release inhibiting hormone
$t_{\frac{1}{2}}$	-	half-life time

T_4	-	thyroxine
U	-	units
U/kg	-	units per kilogram
µg	-	micrograms
µg/kg	-	micrograms per kilogram
µg/kg/hr	-	micrograms per kilogram per hour
µg/min	-	micrograms per minute
µg/ml	-	micrograms per millilitre
µl	-	microlitres
µmol/l	-	micromoles per litre
VIP	-	vasoactive intestinal polypeptide
WDHA	-	watery diarrhoea, hypokalaemia, achlorhydria syndrome
w/v	-	weight volume
$\bar{X}$	-	mean

The terms used in the description of the radioimmunoassay procedure are listed below.

Antiserum	rabbit anti-human pancreatic polypeptide serum
Assay buffer	0,04M phosphate buffer with 0,1 M sodium sodium chloride, 0,1% bovine serum albumin and 0,02% merthiolate, at pH 7,4.
Eluent buffer	0,5 M phosphate buffer with 0,1% bovine serum albumin at pH 7,5.
ID_{50}	the concentration of standard at which 50% of the binding of the tracer to the antiserum is inhibited.
Incorporation	the percentage of ^{125}Iodine incorporated into bPP by the iodination procedure.
N.S.B.	non-specific binding.
Standards	serial dilutions of the standard antigen.

Standard curve	dose-response curve
Tracer	^{125}I labelled bovine pancreatic polypeptide

CHAPTER I

AN INTRODUCTION

CHAPTER I.

AN INTRODUCTION

In many older textbooks of endocrinology, the pituitary gland is referred to as the "master gland" or the "leader of the endocrine orchestra". However, in the past decade or two, the rich endocrine potential of the gastrointestinal tract has been recognized and a multitude of new hormones or candidate hormones have been discovered in its mucosa (reviewed by Grossman, 1974; Pearse, Polak and Bloom, 1977). The pancreas too, like the mucosa of the gastrointestinal tract, has yielded numerous "new" peptides.

In addition to the established hormones of the pancreas - insulin and glucagon, several other peptides have been reported in the pancreas. Moreover, at least seven morphologically distinct endocrine-like cell types have been described in the pancreas (Capella, Solcia, Frigerio et al, 1977). In the pancreas of adult man, however, insulin, glucagon, somatostatin (Dubois, 1975; Polak, Grimelius, Pearse et al, 1975), vasoactive intestinal polypeptide (Buffa, Capella, Solcia et al, 1977) corticotropin-like intermediate peptide (C.L.I.P.) (Larsson, 1977) and pancreatic polypeptide (Larsson, Sundler and Hakanson, 1975) have, by immunohistochemical techniques, been localised to specific endocrine-like cell populations. One of these newly recognized pancreatic peptides, the candidate hormone pancreatic polypeptide is the subject of this thesis.

One of the interests of the Endocrine and Diabetes Research Group of

the University of Cape Town and Medical Research Council has been the study of the pancreatic and gastrointestinal endocrine secretion in genetic and more specifically, in acquired diabetes resulting from chronic pancreatitis. The discovery of a "new" pancreatic hormone was thus of obvious interest to the group. On joining the laboratory, I was given the opportunity of studying pancreatic polypeptide in chronic pancreatitis. This study was made possible by a generous gift of purified pancreatic polypeptide and its antiserum to Professor A.I. Vinik by Dr. R.E. Chance of Lilly Research Laboratories, U.S.A. This allowed the establishment of a radioimmunoassay capable of measuring physiological concentrations of pancreatic polypeptide in the circulation. Since very little was known about pancreatic polypeptide when I commenced this study in 1977, I first set out to investigate aspects of the normal physiology, before delving into its relationship to pancreatic disease or diabetes. This work was carried out over a 3 year period from 1977 to 1979. Much of the data has subsequently been published (Sive, Vinik, Van Tonder et al, 1978; Sive, Vinik and van Tonder, 1979; Sive, Vinik, Levitt et al, 1980; Glaser, Vinik, Sive et al, 1980; Levitt, Vinik, Sive et al, 1980; Sive, Vinik, Barbezat et al, 1980). The literature review has been brought up to date and aspects of the work have been collated to constitute this thesis. The former aspect will thus constitute the major part of this thesis.

Pancreatic polypeptide is a rather cumbersome name and it is generally known by its abbreviation, PP. For those who find this abbreviation somewhat indecent, I would relate a story told by Professor Roger Unger on a visit to Cape Town. Professor Unger tells that he met Drs. Kimmel and Chance, the independent co-discovers of PP, at a conference. They began discussing this new hormone, including what was, in Professor

Unger's opinion, its ridiculous name. He suggested that since PP was discovered by accident, serendipitously, so to speak, they should rename it "Serendipitin". Quite taken by the idea, Drs. Chance and Kimmel agreed to think it over. A day or so later, they again met Professor Unger and informed him that, after serious consideration, they had rejected his proposed name. According to the dictionary "serendipity" was defined as "discovered by chance alone", which would therefore be unfair to Dr. Kimmel. So this candidate hormone of the pancreas retains its short but possibly silly name. An account of its serendipitous discovery and subsequent history as published until 1981 are reviewed in the next chapter.

CHAPTER II

A REVIEW OF THE LITERATURE ON PANCREATIC POLYPEPTIDE

CHAPTER II A REVIEW OF THE LITERATURE ON PANCREATIC POLYPEPTIDE

CHAPTER II

A REVIEW OF THE LITERATURE ON PANCREATIC POLYPEPTIDE

2.1 The discovery and isolation of pancreatic polypeptide.

Pancreatic polypeptide (PP) has a relatively short history. It was discovered by accident when Kimmel, Pollock and Hazelwood (1968) at the University of Kansas Medical Centre were isolating and purifying chicken insulin. During anion exchange chromatography in the latter stages of the insulin purification procedure, an elution peak distinct from that for insulin was consistently found. Analysis of this peak revealed that it contained a peptide which differed from insulin and glucagon and was homologous on both paper chromatography and disc gel electrophoresis. As it was isolated from chicken pancreas, this contaminant of insulin, was called avian pancreatic polypeptide and abbreviated to aPP.

The discoverers of aPP found that this peptide was not confined to chickens. Purification of pancreatic extracts from turtles, also yielded a constant contaminant of insulin, which corresponded to aPP on displacement chromatography, thus suggesting that it was present in other species as well.

At about the same time as these discoveries in Kansas, Dr. R.E. Chance of Lilly Research Laboratories, Indiana, was purifying insulin and glucagon from bovine pancreata. He also isolated a contaminating peptide which was called bovine pancreatic polypeptide or bPP (Chance, 1972; Chance and Jones, 1974). Mammalian pancreatic polypeptide

FIGURE 2.1

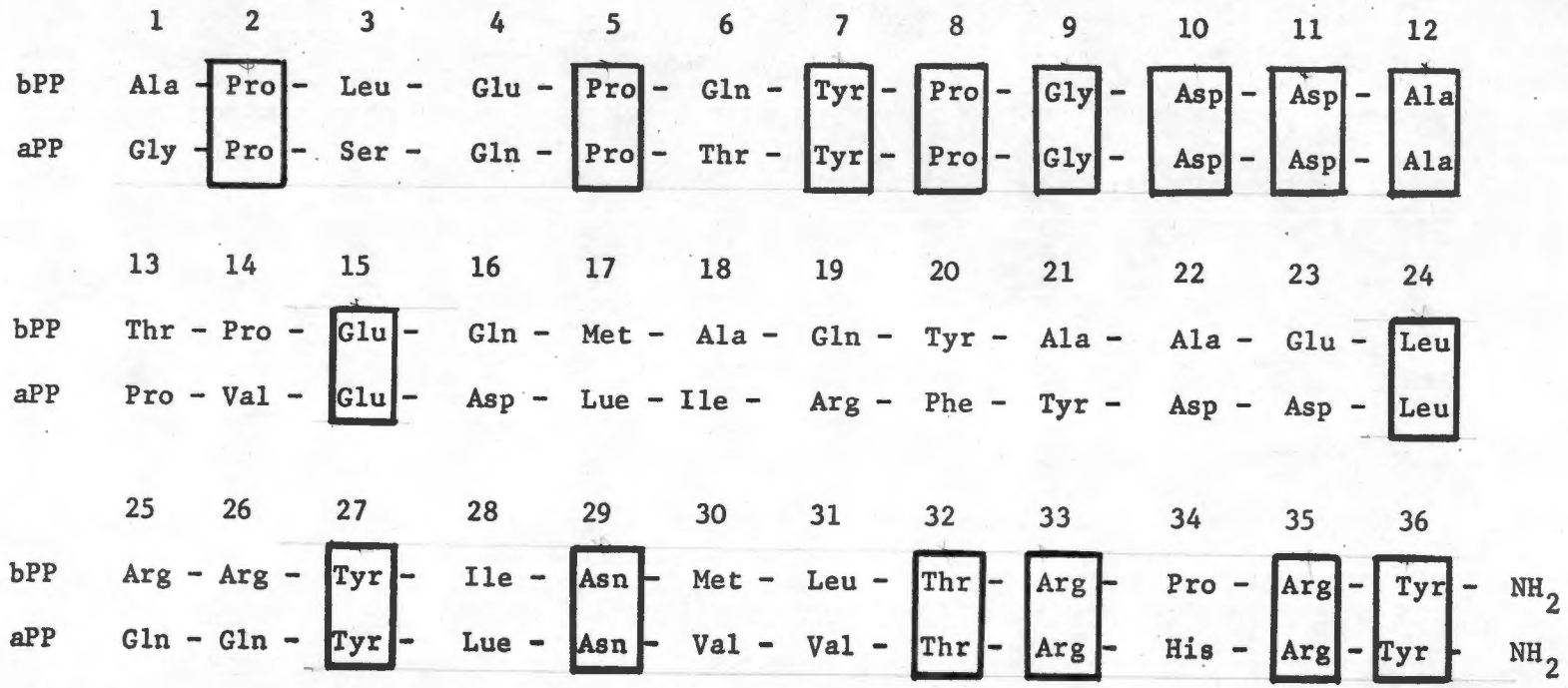


FIGURE 2.1 A comparison of the amino acid sequences of bovine pancreatic polypeptide (bPP) and avian pancreatic polypeptide (aPP). The amino acid residues which are common to both peptides are blocked in.

was subsequently isolated from the pancreas of humans (hPP), pigs (pPP) and sheep (oPP) (Lin and Chance, 1974; Chance and Jones, 1974). The mammalian varieties of PP are similar to each other but differ somewhat from the avian variety in amino-acid sequence.

2.2. Amino-acid sequence and molecular characteristics of pancreatic polypeptide from various species.

Figure 2.1 compares the amino-acid sequence of aPP and bPP. Sixteen amino-acid residues are common to the peptides. The shared amino-acids predominate in the amino-terminal segment where the positions of the proline residues are maintained, and in the carboxy-terminal portion which is basic (Kimmel, Hayden and Pollock, 1975). these authors also state that the number of mutations necessary to convert avian to bovine pancreatic polypeptide is less than would be expected by chance alone and thus suggests homology of the two peptides.

All the mammalian varieties of PP are similar (Floyd, Fajans, Pek and Chance, 1977). Human pancreatic polypeptide (hPP) differs from bPP in amino-acid composition only at positions 6, 10, 11 and 23, where valine, asparagine, asparagine and aspartic acid replace glycine, aspartic acid, aspartic acid and glutamine respectively. Ovine PP and porcine PP each differ from bPP by only one amino-acid residue.

There appears to be some homology between avian pancreatic polypeptide and chicken glucagon in that 8 amino-acid residues have identical positions (Kimmel, Hayden and Pollock, 1975). However, there is no evidence that any of the mammalian varieties of PP are homologs of

glucagon, secretin, gastric inhibitory polypeptide, gastrin, cholecystokinin or vasoactive intestinal polypeptide (Floyd, Fajans, Pek and Chance, 1977).

The molecular configuration of PP of the various species is not known but structural studies by Pitts, Jenkins, Tickle et al (1977) suggest that the central portion of the aPP molecules are helical. The terminal half of the molecule (amino-acid residues 17 to 34) show alternating regions of hydrophobic and hydrophilic residues which would imbue the helix with well defined hydrophilic and hydrophobic surfaces.

The molecular weight of APP is 4240 daltons and the isoelectric point has not been defined exactly but is between pH 6-7 (Kimmell, Hayden and Pollock, 1975). The average molecular weight of mammalian pancreatic polypeptide is 4200 daltons. bPP has an isoelectric point of pH 6,8, and at a wavelength of 276nm, the coefficient of absorption for hPP is 1,42 compared to 1,41 for bPP using a 1mg/ml solution and a 1cm pathlength (Chance and Jones 1974). Thus, in summary PP is a 36 amino-acid residue peptide with a molecular weight of about 4200. Avian and mammalian PP are homologous and the mammalian varieties of PP i.e. ovine, porcine, human and bovine, differ only slightly from each other.

2.3 Species distribution of pancreatic polypeptide

Avian PP has been found in chickens and turtles, and mammalian PP in cattle, sheep, pigs and man. It was distinctly possible that PP was present in other species as well, and Langslow, Kimmel and Pollock (1973) investigated this possibility. They successfully raised an antiserum in guinea pigs specific to aPP, and established a radio-

immunoassay which could detect aPP in extracts of chicken pancreas and in chicken plasma. They then prepared acid alcohol extracts of pancreas from:

1. Mammals - man, pig, cattle, dog and rabbit.
2. Birds - chicken, duck, goose, pigeon, guinea fowl, great horned owl, red-tailed hawk and roseate spoonbill.
3. Amphibians - marine toad and bullfrog.
4. Reptiles - rattlesnake, alligator and turtle.

Immunoreactive aPP was found in all the avian and in two of the reptile species - alligators and turtles. No immunoreactive aPP was found in any of the mammalian or the amphibian species nor in the rattlesnake. A pure preparation of bovine PP at concentrations of 2,4umol/l (10ug/ml) did not cross-react with aPP antiserum, explaining why no PP was detected in the mammalian species, in spite of probable homology between the two peptides.

This necessitated the production of an antiserum specific to mammalian PP. Chance (Chance, Moon and Johnson, 1979) successfully raised two such antisera in rabbits, one against hPP and the second against bPP.

Using these antisera, immunoreactive PP has been measured in the plasma of man (Floyd, Chance, Hayashi et al, 1975; Schwartz, Rehfeld, Stadil et al, 1976; Adrian, Bloom, Bryant et al, 1976; Taylor, Richardson, Feldman et al, 1977; Marco, Hedo and Villanueva, 1977), dogs (Taylor, Walsh, Wood et al, 1977) and pigs (Schwartz, Fahrenkrug, Holst et al, 1977), In addition PP has been found in tissue extracts from the baboon and the monkey (Adrian, Bloom, Bryant et al, 1976) and by immunohistochemistry in pancreatic cells of many species including the

mouse, rat, hampster, guinea pig, chinchilla, rabbit, opossum, cat, dog, sheep, cow, horse man (Larsson, Sundler, Hakanson, 1976) and the tree shrew (Forssman, Helmstaedter and Chance, 1977).

The presence of PP in a multitude of species from the lower to the highest orders, may suggest that retention of this peptide carries biological advantage and would then presuppose a biological function for PP. Conversely, PP may not have a biological function in the higher species, but has not yet been lost in the evolutionary process.

2.4. Localization of immunoreactive pancreatic polypeptide-containing cells by immunohistochemistry.

As alluded to above, immunoreactive techniques have localised PP to specific cells. These cells have the morphological features of peptide secreting endocrine cells (see 2.5). The exact localization of these endocrine cells varies from species to species but they are found in the islets of Langerhans of the pancreas, in the pancreatic acinar tissue and, in some species, in the mucosa of the gastrointestinal tract.

In man, PP containing cells (PP cells) are few in number (Larsson, Sundler and Hakanson, 1975). Generally, one to three cells are found at the periphery of the islets of Langerhans (Erlandsen, Hegre, Parsons et al, 1976; Pelletier and Leclerc, 1977). Furthermore, isolated PP cells are scattered in the exocrine parenchyma (Heitz, Polak, Bloom et al, 1976) and in the epithelium of small and medium sized ducts (Larsson, Sundler and Hakanson, 1975; Pelletier and Leclerc, 1977). Histological sections from the body and from the tail of the pancreas showed no

difference in the number or distribution of the PP cells (Larsson, Sundler and Hakanson, 1976). However, Orci, Malaise-Lagae, Baetens et al (1978) found most of the hPP in the uncinate process and little in the body and tail of the pancreas. This is similar to some species, especially rabbit, cat and dog in which PP cells are more numerous in the uncinate process (duodenal lobe) than in the tail of the pancreas (Larsson, Sundler and Hakanson, 1976; Gingerich, Greider, Chance et al, 1977). In the rat, PP cells are almost entirely situated in the islets of Langerhans, (Larsson, Sundler, Hakanson, 1976), whereas in chickens aPP cells are rarely found in the islets and are evenly scattered throughout the exocrine parenchyma (Larsson, Sundler and Hakanson et al, 1974).

Immunoreactive PP cells have been found in the oxyntic and antero-pyloric mucosa in the opossum and in the dog by Larsson, Sundler and Hakanson (1976). The presence of immunoreactive PP cells in the anteropyloric area of the gastric mucosa has been confirmed in the dog (Beatens, Rufener and Orci, 1976) and in addition the presence of ileal PP cells reported (Frigerio, Ravazola, Ito et al, 1977). Although extracts of baboon gastrointestinal tract yielded measurable amounts of PP (Adrian, Bloom, Bryant, et al, 1976) a cell of origin has not been identified and this may indicate uptake and not necessarily a site of production of PP. However, supporting the assertion that PP is found in the gastrointestinal tract in primates, Forssman, Helmstaedter and Chance (1977) reported the presence of PP containing cells in the mucosa of the gastric fundus and the upper duodenum of Tupaia belangeri, the tree shrew. Gastrointestinal immunoreactive PP cells are not found in all mammalian species. They are not found in adult rats (Sundler, Hakanson and Larsson, 1977); neither are immunoreactive aPP cells found

in the gastrointestinal tract of chickens (Larsson, Sundler, Hakanson et al 1974). Just as there is variability in the pancreatic localization of PP cells from species to species, so is there variability in the gastrointestinal presence of these cells in the various species.

More recently an aPP-like peptide has been demonstrated by immunocytochemical techniques in mammalian and avian central and peripheral nervous systems (Loren, Alumets, Hakanson et al, 1979); Hokfelt, Lundberg, Terenius et al, 1981). Neural bPP-like material could not be demonstrated by Loren et al, but was found in rat brain by Olschowka, O'Donohue and Jacobowitz (1981). bPP-like peptide has also been found and partially characterized in the brain of an insect, the blowfly, Calliphora vomitoria (Duve, Thorpe, Neville et al, 1981). These preliminary findings suggest that PP may play a role in the nervous system, possibly as a neurotransmitter or as a modulator.

2.5. Morphology of the immunoreactive pancreatic polypeptide-containing cells.

Numerous investigators concur that the PP cell in man contains small secretory granules of about 100 - 150 nm in diameter which have moderate to high electron density. By using the peroxidase anti-peroxidase staining technique of Sternberger (1974), PP has been localised to these granules (Larsson, Sundler and Hakanson, 1976; Bergstrom, Loo, Hirsch et al, 1977; Pelletier and Leclerc, 1977; Pelletier, 1977). These investigators also agree that the ultrastructure of the immunoreactive PP cell is typical of peptide hormone secreting cells.

What is not so clear, however, is to which cell the PP containing cell corresponds in previous morphological classifications of pancreatic endocrine cells. Deconinck, Potvliege and Gepts (1971) described 5 cell types in the islets of Langerhans of the adult pancreas. The A and B cells are the glucagon and insulin producing cells respectively; the other cells described are types III, VI and V. The type V cell occurs in the islets of Langerhans as well as in the acinar tissue of the pancreas. Larsson, Sundler and Hakanson (1976) are of the opinion that this cell is the same as the PP cell. Heitz, Polak, Bloom et al (1976a) stated that PP cells in endocrine tumours of the pancreas correspond to the type IV cells of Deconinck et al, but subsequently reported that the PP cells in normal human pancreata obtained at surgery, correspond to the type V cell (Heitz, Polak, Bloom et al, 1976). This type V cell also corresponds to the D1 cell in the revised Wiesbaden classification of gut endocrine cells (Solcia, Pearse, Grube et al, 1973) - the accepted nomenclature of endocrine cells of the gut and pancreas. In dogs, the PP cell is thought by Forssmann, Helmstaedter, Metz et al (1977) to correspond to the F cell described by Munger, Caramia and Lacy (1965).

Despite this inconsistency in nomenclature, what is certain, is that PP is found in specific pancreatic endocrine cells which differ from the insulin-, glucagon- and somatostatin-containing cells (Pelletier, 1977). Moreover, PP is found in secretory granules suggesting that it is produced and stored in these cells. In view of possible confusion regarding the name of the immunoreactive PP-containing cells, for convenience, they will simply be called the "PP cells" in this thesis.

2.6. Amine Precursor Uptake and Decarboxylation (APUD) characteristics of pancreatic polypeptide cells.

In 1938, a German pathologist, Friedrich Feyrter, published that a series of cells characterised by similar morphological and staining properties could be linked together as a diffuse endocrine system. Later, however, he believed that these cells were "paracrine" i.e. they acted directly on neighbouring cells. With the development of techniques to study the cytochemical and ultrastructural characteristics of cells, the concept of a series of endocrine cells which are not confined to specific anatomical glands had grown.

In 1966, Pearse reported that cytochemical and ultrastructural features common to peptide producing (endocrine) cells link them together. These cells share the amine handling properties described as Amine Precursor Uptake and Decarboxylation and the anagram denotes the name of this series of cells. Furthermore, the amine storage mechanism and the presence of cholinesterase in the APUD cells suggest that they are of neural crest origin (Pearse, 1969; Pearse and Polak, 1971) and to quote Pearse (1977), "the APUD cells wherever they occur, can properly be regarded as neuroendocrine".

The ultrastructural features of these PP cells suggest that they may belong to the APUD series. PP cells in opossum, exhibited formaldehyde-induced fluorescence, indicative of dopamine storage and the PP cells of the guinea-pig were able to take up exogenous amine precursors and convert them to their corresponding amines (Larsson, Sundler and Hakanson, 1976). PP cells in chickens were also able to take up and decarboxylate L-dopa (Larsson, Sundler, Hakanson et al,

1974) suggesting that they, too, are endocrine cells of the APUD series.

At present it is not known whether PP can make further claim to inclusion in the APUD series. Whether PP cells do, in fact, originate in the neural crest requires to be assessed.

2.7 Ontogeny of pancreatic polypeptide cells.

The development of the PP cells during foetal life has been incompletely studied in man. In abortuses of 18 to 20 weeks gestation, PP cells were abundant in the head of the pancreas, where they were situated at the periphery of the "mantle" or primitive islets, but were far less frequent in the tail of the pancreas (Larsson, Sundler and Hakanson, 1977).

In Wistar rats, where the ontogeny has been studied in detail, PP cells were not present during foetal life and first appeared during the perinatal period (Sundler, Hakanson and Larsson, 1977). At this stage the cells were found both in the islets of Langerhans and in the pancreatic acinar tissue. At five to seven days of neonatal life, the number of cells in the islets increased sharply and were scattered throughout the islets. By 10 days, however, the PP cells were found only at the periphery of the islets and were virtually absent from the pancreatic parenchyma. During these first 10 days of life, PP cells were also present in the antral mucosa of the stomach, whereas in adult rats, PP cells were not found in this site.

In chicks, aPP containing cells were very numerous at the time of hatching, but decreased in number over the following few days to the

proportions found in adult chickens (Larsson, Sundler, Hakanson et al, 1974). In very young chicks, aPP containing cells were also found in the duodenal mucosa, but subsequently disappeared. This transient appearance of a gastrointestinal peptide-hormone producing cell in a specific site only to disappear with maturation, has previously been reported for gastrin (Larsson, Rehfeld, Sundler et al, 1976), and appears to relate to PP as well.

In the species in which the development of the PP cell has been studied, they appear to be more numerous during late foetal and early neonatal life than in the adults. In addition in some species (chicken, rat), PP cells transiently appear in the gastrointestinal tract. Whether these phenomena relate to the function of the peptide, or are purely stages in the maturation of differentiation of the PP cell is conjectural.

It has been established that PP is a unique pancreatic peptide which originates from a specific endocrine-like cell. It has also been mentioned that PP has been measured in the plasma of man and some animals (2.3). As yet this does not imply that PP is a hormone and circulating pancreatic polypeptide is now examined in greater detail, in an attempt to elucidate this point.

2.8. The measurement of pancreatic polypeptide concentrations by radioimmunoassay.

To date, apart from Gates and Lazarus (1977), all investigators who have measured mammalian PP by radioimmunoassay have obtained the PP for iodination as tracer and for use as the standard reference preparation, as well as the PP antiserum, from Dr. R.E. Chance of Lilly Research

Laboratories, Indiana. Dr. Chance has successfully raised antisera to human pancreatic polypeptide (hPP) and to bovine pancreatic polypeptide (bPP) in rabbits. These two antisera show no difference in their reactions (Chance, Moon and Johnson, 1979). Furthermore, the antisera do not cross-react with gastric inhibitory polypeptide, vasoactive intestinal polypeptide, synthetic human heptadecapeptide gastrin I, cholecystokinin, secretin, glucagon, insulin, proinsulin, somatostatin (Floyd, Fajans, Pek and Chance, 1977) or motilin (Adrian, Bloom, Bryant et al, 1976). The use of the same antisera by all except one group of investigators, should have ensured uniformity of the peptide measured, if not of the results obtained. However, a direct comparison between the concentrations of PP measured by different investigators is not possible. This results from the fact that the assay methods differ considerably from investigator to investigator. These differences include the method of preparing the tracer, the assay buffers used, the duration of incubation of the assay, and the method of separating antibody-bound from free antigen. Furthermore, some investigators used a homologous assay, i.e. an assay in which the radiolabelled tracer and the standard antigen preparation were from the same species (hPP), whereas other investigators used a hybrid assay where bovine pancreatic polypeptide was radiolabelled to act as the tracer and human pancreatic polypeptide constituted the standard preparation.

In spite of such differences in the assay methodologies, PP concentrations have been measured in the plasma or serum of man and various animal species (see 2.3). In man, PP measurement in serial dilutions of sera as well as hPP added to sera have shown that the PP values obtained differed by less than 15% from those expected (Schwartz, Rehfeld, Stadil et al, 1976) suggesting that there is no significant

interference in the assay system and also that there is good recovery of PP by the assay. Thus basal or fasting plasma PP concentration as well as the stimulated concentrations have been measured in man and animals and will be discussed in section 2.10 and 2.13.

Although the specificity of the PP antisera as well as the assay systems are established, it is possible that the antiserum may not detect PP of a single molecular size. It is feasible that PP may be present in multiple molecular forms, all of which are immunoreactive, and which can all be recognized by a single antiserum, a phenomenon which is common to many endocrine peptides and their antisera.

2.9 Molecular forms of pancreatic polypeptide.

It has been recognized that peptide hormones may circulate in heterogeneous molecular forms which have different biological potencies but react equally with a specific antiserum (reviewed by Yalow, 1974). At present there is conflicting evidence whether PP occurs in more than one molecular form.

Adrian, Bloom, Bryant el al (1976) reported that tissue extracts of human as well as baboon pancreas, when column chromatographed with Sephadex G-50 superfine, yielded PP of a single molecular weight. However, PP in human plasma appears to be heterogenous. Villanueva, Hedo and Marco (1977) used Biogel P30 and Sephadex G100 to separate the molecular forms of hPP in plasma. Immunoreactive PP in plasma from fasting people eluted as 3 separate peaks of Biogel P30 and as 2 peaks on Sephadex G100 chromatography. With both columns, the first peak eluted in the void volume and the second peak coincided with the elution

position or radiolabelled PP. The 3rd peak (which was found only with the Biogel P30) eluted just before the free iodine. The authors suggest that the immunoreactive PP in this peak has a molecular weight of 1500 daltons. Plasma in which the PP concentration was elevated following endogenous PP release by insulin-hypoglycaemia, was then chromatographed on the Biogel P30 column. Only the component of PP which eluted in the position of the radiolabelled PP was increased. Boden, Master, Owen et al (1980) found 3 peaks when plasma was filtered through a Sephadex G-50 fine column. Their third peak coincided with the PP marker, while the second peak was intermediate between this and the peak that eluted in the void volume. This pattern was similar to that reported by Adrian, Bloom, Besterman el al (1978) in patients with pancreatic vipomas.

The existence of large and small molecular weight PPs have yet to be confirmed using different methods. Whether or not these could be precursor and degradation forms or molecules bound to plasma proteins also needs further investigation, as does the possibility that PP of different molecular charges in addition to molecular weights may exist.

Since the discovery of PP, several investigators have directed their attention to finding a physiological role for this peptide. The establishment of radioimmunoassays has permitted investigators to measure changes in the circulating PP concentrations in response to various manipulations, and thus determine factors controlling the release of this candidate hormone.

2.10 Factors affecting the release of pancreatic polypeptide.

2.10.1 Basal serum pancreatic polypeptide concentrations in healthy subjects.

PP concentrations have been measured in the plasma of fasting subjects by numerous investigators. Floyd, Fajans, Pek et al (1977) reported that the mean ($\pm$ S.D.) PP concentrations in 120 young subjects whose mean age was 25 years $\pm$ 3,4 (S.D.) was 51 $\pm$ 27 pg/ml (12 $\pm$ 6,4 pmol/l). The levels in older subjects were higher, the mean being approximately 200 pg/ml (47 pmol/l) in subjects in the 7th decade. Moreover, they reported a significant positive correlation between basal PP concentrations and age (Berger, Crowther, Floyd et al, 1978). The age dependent rise in basal PP concentrations has been confirmed by Track, Watters and Gauldie (1979). Obesity, too, appears to affect basal - stimulated PP concentrations, the levels being lower in obese subjects (Marco, Zulueta, Correas et al, 1980; Lassmann, Vague, Vialettes et al, 1980). Exercise is associated with a rise in the plasma hPP concentrations, but this elevation is markedly decreased following endurance training (Gingerich, Hickson, Hagberg et al, 1979). In pregnancy basal PP levels are lower than post-partum levels, as is the PP response to oral glucose (Hornnes and Kuhl, 1981).

In 21 healthy subjects who were 23 to 40 years old, Schwartz, Rehfeld, Stadil et al (1976) found that the mean ($\pm$ SD) basal serum PP concentration was 180 $\pm$ 76,9 pg/ml (43 $\pm$ 18,3 pmol/l) and the range was 84 to 370 pg/ml (20-88 pmol/l). These investigators used a hybrid assay (see 2.8) whereas Floyd et al used a homologous assay, which may explain the differences in the mean basal concentrations between the studies.

Adrian, Bloom, Bryant et al (1976), who also used a homologous assay, reported that the fasting plasma PP concentrations in 25 subjects (mean age 31 yrs) was 131 $\pm$ 130,2 pg/ml (31,2 $\pm$ 31 pmol/l).

These differences in basal concentrations may relate to the assay factors discussed or age differences and accord can only be reached by an interlaboratory exchange of samples and materials, thus enabling comparative values to be obtained in the various assays. Furthermore, strict age matching would appear to be essential in discussing comparative results.

2.10.2 The effects of nutrient ingestion on serum pancreatic polypeptide concentrations.

The initial observation that eating causes a rise in the circulating PP concentrations was made in chickens (Langslow, Kimmel and Pollock, 1973). Avian pancreatic polypeptide was not detected in plasma from fasted chickens, but rose to concentrations of greater than 4 ug/ml following feeding.

Mammalian PP, too, is released in response to a mixed meal (Floyd, Fajans and Pek, 1976; Schwartz, Rehfeld, Stadil et al, 1976; Adrian, Bloom, Bryant et al, 1976). The ingestion of a protein-containing meal caused a large release of PP into the circulation. Within a few minutes of eating, the serum PP concentration rose sharply to reach levels 5-8 times the basal concentration. Thereafter the concentrations declined but remained significantly elevated above the basal concentration for at least 6 hours (Schwartz, Reheld, Stadil et al, 1976; Adrian, Bloom, Besterman et al, 1977). Schwartz, Rehfeld and

Stadil et al (1976) suggested that this pattern of release was biphasic i.e. the initial sharp phase of release followed by a later phase of prolonged elevation constituted two separate phases to which Bloom and Adrian (1977) replied that in their hands the two different phases could not be confirmed statistically. Floyd, Fajans, Pek et al (1977) found a single phase of PP release after the ingestion of 500 g of cooked, ground beef. This meal caused a 10 fold elevation in the plasma PP concentrations ten minutes after eating, and the concentrations remained elevated in this region for 4 hours thereafter.

Thus, although it is agreed that a protein-containing meal is a potent stimulus for PP release, the pattern of release is not quite clear.

With regard to the individual nutrient fuels in a meal, the PP response to oral glucose and oral fat have been reported. After 1,75 g/kg of oral glucose an early small rise in the plasma PP concentration at 15 minutes was followed by an 8 fold rise in 4 hours (Floyd, Fajans and Pek, 1976). In the absence of glucose measurements, whether this relates to the concentrations of glucose attained is unknown, but it is unlikely that in normal subjects the glucose was still elevated at that time. Marco, Hedo and Villanueva (1977) also reported that oral glucose caused a rise in the plasma PP concentrations in gastrectomised subjects. These investigators subsequently showed that following the ingestion of 1.75 g/kg of glucose, there was a prompt but a small rise in the plasma PP concentration. This may have been an effect of the elevated plasma glucose per se, but may also have resulted directly from a gastro-pancreatic reflex. There was a far greater rise in plasma PP levels when glucose concentration fell below the basal level (Marco,

Hedo an Villanueva, 1978). To bypass a possible oral stimulus for PP release, glucose was instilled into the stomach via a nasogastric tube in healthy subjects. The intragastric instillation of glucose via a nasogastric tube was associated with a rise in the plasma PP concentration (Taylor, Feldman, Richardson et al, 1978); the same volume of saline instilled into the stomach also caused a rise in the PP concentration which was of a similar magnitude. Thus the effect of glucose per se on the release of PP is not clear and raises the possibility of "gastro-pancreatic" reflex causing the release of PP. The ingestion of 100ml of "Lipomul", a dietary supplement, which contains 70g of fat, caused a 3 fold elevation in PP concentration (Floyd, Fajans, Pek and Chance, 1977). In the absence of measurements of changes in the plasma triglyceride and free fatty acid concentrations, it is not known whether this relates to the fat per se, or possibly reflects PP released by a gastro-pancreatic reflex mechanism. In dogs with chronic gastric and pancreatic fistulas, intraduodenal instillation of phenylalanine was associated with a rise in plasma PP concentrations. This release was inhibited by atropine (Beglinger, Hacki, Gyr et al, 1981).

2.10.3 The effect of intravenously administered nutrients on pancreatic polypeptide release.

Glucose: The PP response to the intravenous administration of glucose is not clear. Adrian, Bloom, Besterman et al (1977) reported that 50g of glucose infused as a 50% solution over 30 minutes did not significantly change the basal PP concentration. In contrast, Floyd, Fajans, Pek and Chance (1977) reported that 30g of glucose of an unspecified concentration infused over 60 minutes caused a significant

fall in the PP concentration, but this could not be repeated in their subsequent studies. However, Marco, Hedo and Villanueva (1978), also found that intravenous glucose administration depressed the basal serum PP concentration and reduced the response to a protein rich mean (Marco, Hedo, Castillo-Olivares et al, 1980).

Fat: Neither Adrian, Bloom, Besterman et al (1977) who infused 8g of fat as "Intralipid" over 30 minutes nor Floyd, Fajans, Pek and Chance (1977) who infused 40g of fat also as "Intralipid" over 90 minutes, found a change in the basal plasma PP concentration. In contrast, Hedo Villaneuva and Marco (1979) reported that intravenous lipid and intravenous heparin, both of which significantly elevated the plasma free fatty acid concentrations, markedly depressed the basal and meal stimulated PP levels. Nicotinic acid, which lowered the free fatty acid concentrations to below basal, did not significantly affect plasma PP levels.

Amino-acids: The infusion of 0,41g/kg body weight of L-arginine over 30 minutes caused a late, modest rise in the plasma PP concentrations. Infusions of alanine and leucine also caused small late elevations of the plasma PP concentration (Floyd, Fajans, Pek and Chance, 1977). The same investigators found that a mixture of 10 essential amino acids infused over 30 minutes, caused a small, early rise in the PP concentrations which then fell to basal concentrations, but rose again to remain elevated from 40 through 150 minutes after the infusion. The infusion of 25g of "Aminosol", which also contains a mixture of amino acids, failed to change the circulating PP concentrations (Adrian, Bloom, Besterman et al, 1977) and thus as with glucose, a discrepancy in the PP response to intravenous amino acids was found by the various

investigators.

Thus, in summary, a protein-containing meal is a potent stimulus for the release of PP. Elevated concentrations are observed in the circulation for 6 hours after the meal, but whether the initial high concentrations are maintained is not clear. Both oral fat and oral glucose cause a rise in the circulating PP concentration but this elevation is far lower than that seen after a mixed meal. Whether the individual nutrient fuels directly stimulate PP release is not known, since in the case of glucose, PP concentrations are only markedly elevated a few hours after the glucose ingestion, and with oral fat, concomitant measurements of serum triglycerides and free fatty acids have not been reported. Furthermore, intravenous glucose and intravenous lipid may depress the plasma PP concentrations. Following intravenous administration of amino-acids, the circulating PP concentrations were only modestly elevated. The findings suggest that in addition to the individual nutrient fuels per se, other factors are implicated in PP release after the ingestion of a meal.

2.10.4 The effect of gastrointestinal hormones on pancreatic polypeptide release.

The massive PP response to a meal, the lesser responses to the individual nutrient fuels, and the lack of response to intravenous nutrients suggests the possibility that a gastrointestinal factor released by food may stimulate PP release. Such factors would accord with the recognized influence of gastrointestinal hormones on both pancreatic exocrine and endocrine release - often referred to as the gastrointestinal-pancreatic axis.

The intravenous administration of "Boots" secretin, a crude secretin preparation, containing 1% of secretin, caused a rapid ten-fold rise in the plasma PP concentration (Adrian, Bloom, Besterman et al, 1977). However, purified secretin (NIH secretin, Karolinska Institute, Stockholm) had no effect on the PP concentration (Schwartz and Rehfeld, 1977). Cholecystokinin (CCK - 99% pure) infused at doses of 40, 80 and 160 ug/hr did not change the plasma PP concentration (Taylor, Feldman, Richardson et al, 1978), nor did synthetic human heptadecapeptide gastrin (SHG-I) infused simultaneously with the CCK. In constrast, Lonovics, Guzman, Devitt et al (1980), reported a three fold rise in the PP level when they infused CCK (99% pure) at approximately the same rate into normal subjects. They also found that high doses of SHG-I stimulated PP release in dogs (Guzman, Lonovics, Chayvialle et al, 1980). 20% pure CCK infused at sequentially increasing doses, caused a dose-related rise in the PP concentration (Beglinger, Meyer, Hacki et al, 1981). The CCK-induced rise in PP concentrations was significantly lower after vagotomy in dogs (Guzman, Lonovics, Devitt et al, 1981). Caerulein, a peptide which is found in the skin of the frog Hyla Caerulia, and which is very similar in amino-acid sequence and action to the C-terminus of CCK in man (Anastasi, Erspamer and Endean, 1968), was infused at a rate of 100mg/kg/hr by Adrian, Bloom, Besterman et al (1977). At this dose a striking PP response was reported. Caerulein-stimulated PP release was blunted by prior treatment with atropine (Tsuda, Seino, Sakurai et al, 1980). Another peptide found in the skin of frogs, bombesin (Erspamer and Melchiorri, 1973) but also found in the human gastrointestinal tract (Polak, Bloom, Hobbs et al, 1976), caused a dose-related rise in the plasma PP concentration in dogs (Taylor, Walsh, Wood et al, 1977). This

release of PP by bombesin is dependent on cholinergic factors (Modlin, Lamers and Jaffe, 1980). Not surprisingly, PP release was inhibited by somatostatin. Macro, Hedo and Villanueva (1977) reported that an infusion of 9ug per minute of somatostatin in gastrectomised individuals induced a fall in basal, and abolished the oral glucose-stimulated rise in PP concentrations. Inhibition of basal and stimulated PP release by somatostatin has been confirmed by other workers (Adrian, Bloom, Besterman et al, 1978; Kayasseh, Hacki, Gyr et al, 1978; Floyd, Fajans and Pek, 1978; Hermansen and Schwartz, 1979).

In vitro, gastric inhibitory polypeptide (GIP) and vasoactive intestinal polypeptide (VIP), gastrin and caerulein caused release of PP from the perfused canine pancreas (Adrian, Bloom, Hermansen et al, 1978). Incubation of tissue slices from the uncinate process of the dog pancreas, with 2ug/ml of gastrin, 20ng/ml of caerulein and 5 clinical units/ml of secretin, each significantly elevated PP concentrations in the medium bathing the tissues by 31%, 51% and 79% respectively (Gingerich, 1977).

In addition to the influence of the recognised gastrointestinal hormones and candidate hormones on PP release, other hormones and chemical mediators affect its release. 15(R)-15 methyl prostaglandin E_2 (Petersen, Feldman, Taylor et al, 1979) and 16,16-dimethyl-prostaglandin E_2 (Materia, Modlin, Albert et al, 1981) blunted meal and bombesin-induced elevation of PP concentrations respectively in dogs and man. Morphine abolished PP released by a meal (Feldman, Walsh and Taylor, 1980). Glucagon and insulin did not affect PP release in the isolated perfused canine pancreas (Weir, Samols, Loo et al, 1979),

Glucocorticoids (Lantigua, Streck, Lockwood et al, 1980) and growth hormone (Zipf, Kelch, Floyd et al, 1981) were reported to suppress PP secretion. Although metoclopramide caused release of PP in man, this release was probably due to a cholinergic effect rather than its dopaminergic blocking properties (Spitz, Zylber, Jersky et al, 1979). Calcium had a modest effect on increasing PP release in an isolated perfused canine pancreatico-duodenal preparation (Hermansen and Schwartz, 1979) whereas lithium had an inhibitory effect on bombesin-stimulated PP release in dogs (Modlin, Ehrlich, Lamers et al, 1981).

In summary, in man a crude secretin preparation (Boots secretin), has been shown to stimulate the release of PP. In addition the anuran peptide caerulein, which resembles the C-terminus of CCK stimulates the release of PP. The infusion of bombesin into dogs causes the release of PP. Investigators differ as to whether CCK and gastrin release PP. In in-vitro studies, GIP, VIP, gastrin, secretin and caerulein caused a rise in PP concentration. Thus in the experimental situation, some gastrointestinal hormones and candidate hormones may mediate the release of PP. Whether they do so under physiological circumstances and what role the autonomic nervous system plays is not quite clear.

2.10.5 The effect of autonomic nervous factors on pancreatic polypeptide release

The autonomic nervous system can regulate both pancreatic endocrine and exocrine secretions and its effect on PP release will now be considered.

2.10.5.1 Cholinergic modulation.

There is little doubt that under certain circumstances the vagus nerve influences the release of PP. Patients with duodenal ulcertaion who had undergone a truncal vagotomy showed an impaired PP response to a meal after the operation (Schwartz, Rehfeld, Stadil et al, 1976). The early phase of release was impaired to a greater degree than the later phase, suggesting that the vagus exerts a greater influence on the former phase. In a similar study, Adrian, Bloom, Besterman et al (1977) reported that the PP response in subjects who had undergone bilateral truncal vagotomy, were not significantly different from the responses in control subjects. However, an examination of the figure depicting these results, shows that there is a delayed rise in the plasma PP concentrations, and that soon after eating the peak PP concentrations attained are markedly different. Possibly because these authors expressed the response to the meal in terms of the integrated release over 6 hours, differences in response between the groups could not be demonstrated. However, Taylor, Feldman, Richardson et al (1978) also reported that food-stimulated PP release was not affected by previous vagotomy but was markedly inhibited by propantheline. On the other hand, dogs following truncal vagotomy had significantly reduced PP release after a meal (Taylor, Impicciatore, Carter et al, 1978). With time, following vagotomy, there may be a return towards normal of the prolonged phase of PP release (Becker, Borger and Schafmayer, 1979; Taylor, 1980; Lovgren, Poulsen and Schwartz, 1981) but this has not been found by all investigators (Stern, Hansky, Korman et al, 1980). Atropine abolished PP release by a meal in dogs (Taylor, Impicciatore, Carter et al 1978; Modlin, Albert, Crochett et al, 1981) and by sham feeding in man (Feldman, Richardson, Taylor et al, 1979; Schwartz,

Stenquist and Olbe, 1979). Atropine also lowered basal PP concentration in man (Schwartz, Stenquist, Olbe et al, 1979). Adrian, Bloom, Besterman et al (1977) reported that insulin-hypoglycaemia induced release of PP, and that this release was abolished following vagotomy. It was also found that the insulin-hypoglycaemia-induced PP response was inhibited by atropine (Schwartz, Holst, Fahrenkrug et al, 1977). In addition to decreasing the PP released by a meal and by insulin-hypoglycaemia, vagotomy also abolished PP release following the distention of the gastric fundus by the inflation of a rubber balloon (Schwartz and Rehfeld, 1977).

Further animal studies have confirmed that the vagus has a role in regulating PP release. Direct electrical stimulation of the vagus in anaesthetised pigs, caused release of PP into the portal vein. This was inhibited by the cholinergic receptor blocking agent atropine and was abolished by the ganglion blocking agent, hexamethonium (Schwartz, Holst, Fahrenkrug et al, 1978). Perfusion of the isolated canine pancreas with acetylcholine (0,05 - 100 x 10^{-6} M/1) resulted in the release of PP (Iversen, Bloom, Adrian et al, 1977). In the isolated perfused porcine pancreas, 0,05 - 100 x 10^{-6} M/1 of acetylcholine induced dose-related PP release that could be blocked by atropine (Schwartz, Holst, Fahrenkrug et al, 1978).

Interestingly, even the effects of gastrointestinal hormones on PP release are influenced by vagal-cholinergic factors. Atropine was able to suppress the PP release in response to intravenous bombesin in dogs (Taylor, Walsh, Wood et al, 1978; Modlin, Lamers, Jaffe, 1980) and to caerulein in man (Tsuda, Seino, Sakurai et al, 1980). PP responses to

infusions of CCK and SHG-I in dogs were also blunted following vagotomy except when high doses of 99% pure CCK (1ug/kg/hr) or CCK octopeptide (0.25ug/kg/hr) were used (Guzman, Lonovics, Devitt et al, 1981). Vagotomy was, however, reported not to alter bombesin-induced PP release (Singer, Niebel, Lamers et al, 1981). These studies suggest that there is an interplay between gastrointestinal and vagal-cholinergic factors which mediate PP release after a meal.

2.10.5.2 Adrenergic modulation.

The parasympathetic nervous system does not appear to have the only modulating action on PP release. Although an infusion of 5ug/min of epinephrine did not alter the circulating PP concentration in 9 healthy subjects, adrenergic beta-receptor stimulation achieved by the concomitent infusions of epinephrine and the adrenergic alpha-receptor blocking agent phentolamine at a dose of 500ug/min, caused a significant rise in the plasma PP concentrations (Floyd, Pek, Knopf et al, 1977). On the other hand, adrenergic alpha-receptor stimulation using epinephrine and propranolol at a dose of 125ug/min, caused a small although insignificant fall in the plasma PP levels. Vigorous exercise which stimulates the sympathetic nervous system, was associated with PP release, enhanced by phentolamine administration (Berger, Floyd, Lampman et al, 1980), and inhibited by propranalol infusion (Berger, Floyd, Lampman et al, 1980; Feurle, Wirth, Diehm et al, 1980). Alpha-receptor blockade and beta-receptor adrenergic stimulation enhanced the PP response to the intraduodenal infusion of glucose whereas beta-receptor blockade with propranolol decreased the release (Flaten and Myren, 1981).

Not all investigators have found that adrenergic factors affect PP release. In adrenalectomized subjects, in whom insulin-induced hypoglycaemia did not cause the plasma adrenalin to rise, the PP response did not differ from that observed in control subjects (Jarhult, Farnebo, Hamberger et al, 1981). Furthermore, perfusion of the isolated perfused canine pancreas with 11nmol/l of adrenalin failed to elicit a PP response (Adrian, Bloom, Hermansen et al, 1978). Alpha and beta-receptor-adrenergic blockade failed to inhibit PP release caused by electrical stimulation of the vagus in pigs (Schwartz, Holst, Fahrenkrug et al, 1978).

In summary, numerous factors have been shown to mediate the release of PP. A mixed meal causes a large and prolonged release of PP into the circulation, ingestion of the individual nutrient fuels, glucose and fat, cause a lesser degree of release, and intravenous administration of these nutrients has been reported to inhibit the release of PP. In vivo, the administration of Boots secretin, bombesin, the anuran peptide caerulein and possibly CCK and gastrin, cause PP release and in vitro GIP, VIP and gastrin, too appear to stimulate the release of PP. Furthermore, the vagus nerve may have an important role in mediating PP release but adrenergic stimulation, specifically adrenergic beta-receptor stimulation may cause PP release as well. Inhibition of PP release has been reported during the infusion of pharmacological doses of somatostatin, and possibly by adrenergic alpha-receptor stimulation. Intravenous glucose and fat, too, may have an inhibitory effect on PP release.

These findings suggest that there is a complex interplay of mechanisms modulating PP release and it would appear that the PP released by a

mixed meal is not the result of a single stimulus. A great deal remains to be investigated both with regard to the control of PP release, and the implications thereof.

2.11 Metabolism and turnover kinetics of pancreatic polypeptide.

The circulating concentrations of PP under basal circumstances and following stimulation have been described in previous sections. It appears as though the PP cells are capable of releasing large amounts of PP into the circulation and it is presumed that the PP is released by the pancreas. In baboons and monkeys, by measuring the immunoreactive PP concentration of various tissue extracts, it has been reported that the highest concentration of PP is found in the pancreas (Adrian, Bloom, Bryant et al, 1976), and following pancreatectomy PP virtually disappears from the circulation.

The fate of the circulating endogenous peptide in normal man, has not been determined. Disease states have provided some insight into the possible metabolism of PP. Hallgren, Lundqvist and Chance (1977) found that plasma PP concentrations were raised in patients with chronic renal failure. Moreover, the degree of renal impairment correlated with the serum PP levels, suggesting renal clearance of PP. The PP concentration in renal arterial plasma was significantly higher than that in renal venous plasma in patients with cirrhosis of the liver and normal renal function (Boden, Master, Owen et al, 1980) Renal clearance of PP was calculated as 151 $\pm$ 47 ml/min. In infants with nesidioblastosis, a difference in PP concentration was found between the hepatic portal vein and a peripheral vein (Loo, Hirsch and Gabbay, 1977) suggesting hepatic extraction of PP. However, plasma PP

concentrations did not differ significantly from normal in patients with cirrhosis of the liver and in these patients, portal and hepatic vein PP concentrations were not significantly different. There was also no difference between brachial artery and hepatic vein PP concentrations (Boden, Master, Owen et al, 1980).

By infusing porcine PP into dogs to attain steady state circulating PP concentrations, the metabolic clearance rate, the distribution volume and disappearance half-time of exogenous PP were calculated (Taylor, Solomon, Walsh et al, 1977). The metabolic clearance rate was 26,6ml/kg/min, equal to approximately one third of the cardiac output; the distribution volume of 209ml/kg suggests that PP is distributed in the intravascular and almost the entire extravascular compartment; and the half life time of 5,5 $\pm$ 1 minute, indicates rapid turnover of the molecule. After infusing bPP into normal man the calculated mean disappearance half time was 6.9 minutes, the metabolic clearance rate 5.1ml/kg/min and the volume of distribution 51 ml/kg (Adrian, Greenberg, Besterman et al, 1978). In pigs the half life time was 4.5 to 6.9 minutes (Schwartz, Holst, Fahrenkrug et al, 1978). These findings of a molecule which has a relatively rapid turnover are in accord with the kinetics of other gastrointestinal peptide hormones. Further studies are required to determine if these findings are more generally applicable and whether in other species the liver has a role in PP extraction, as it does for other peptide hormones.

2.12 The actions of pancreatic polypeptide.

A number of biological actions have been attributed to PP, but whether these occur under physiological conditions or have physiological

relevance is not certain.

2.12.1 The effects of pancreatic polypeptides on nutrient metabolism.

The injection of 100ug/kg of avian PP into 5-6 week old chicks, did not affect the blood glucose concentration but did deplete the liver of glycogen and lower the plasma glycerol concentration (Hazelwood, Turner, Kimmel et al, 1973). In older chicks 10ug/kg of aPP caused a significant fall in plasma glycerol and plasma alanine concentrations and a rise in plasma triglyceride levels (Kimmel, Pollock and Hayden, 1978). The plasma aPP concentrations achieved, were, however, four times higher than those found after a meal. An intramuscular injection of 10ug/kg of bPP in dogs did not change the blood glucose concentration (Lin, Evans, Chance et al, 1977). In man, bPP infused intravenously at rates of between 10 and 150pmol/kg/hr (0.042 and 0.63ug/kg/hr) had no effect on blood levels of glucose, lactate, pyruvate, alanine, ketone bodies, free fatty acids, insulin or pancreatic glucogon (Adrian, Greenberg, Besterman et al, 1978). bPP incubated with isolated rat liver cells did not effect glucose or urea production by these cells (Schwartz, Corkey, Williamson et al, 1980).

In the pathological situation, the administration of either aPP or bPP to New Zealand obese mice, caused a diminution of the hyperinsulinaemia, hyperglycaemia and weight in these diabetic animals (Gates and Lazarus, 1977). Again the relevance of this finding in regard to a physiological role for PP is not certain but it has been reported that in mice PP decreased food intake and body mass (see 2.12.3), findings that may have influenced the glycaemia.

2.12.2 The effects of pancreatic polypeptide on the gastrointestinal tract.

To investigate the effects of pancreatic polypeptide on aspects of gastrointestinal exocrine secretion and on tone and motility and to investigate a possible tropic action for PP on the gastrointestinal tract, investigators have administered PP to animals and man and observed the reponse and changes caused by various doses of the peptide.

2.12.2.1 The effects of pancreatic polypeptide on gastrointestinal exocrine secretion.

To assess the effect of bPP on gastric acid secretion, Lin and co-workers infused bPP at rates of 40, 80 and 100ug/kg/hr into dogs. These doses of bPP stimulated acid secretion from both vagally innervated and denervated canine stomachs. However, when 10 and 40ug/kg/hr of bPP were infused during pentagastrin-stimulated acid secretion, the acid secretion was inhibited by 32% and 72% respectively (Lin, Evans, Chance et al, 1977). The circulating PP concentrations were not measured, but assuming a half-life time for PP of 5,5 min (Taylor, Solomon, Walsh et al, 1979) and the dogs to weigh 18 to 20 kg, the circulating PP levels would have been approximately 5 to 50 times higher than the concentrations following a meal. At these non-physiological concentrations, a physiological role in gastric acid secretion cannot be ascribed to PP. Confirming this, Parks, Gingerich, Jaffe et al (1979) reported that porcine PP infused at 1 and 2.25ug/kg/hr did not effect histamine or pentagastrin-stimulated gastric acid secretion; neither did bPP infused into man (Adrian, Greenberg, Besterman et al, 1978).

The effect of PP on pancreatic exocrine secretion appears more physiological. Lin, Evans, Chance et al (1977) reported inhibition of basal pancreatic water and bicarbonate output when they infused bPP into dogs at 10ug/kg/hr. At 1 to 5ug/kg/hr bPP significantly inhibited CCK- and secretin-stimulated pancreatic protein and bicarbonate secretion. When secretin was continuously infused, bPP initially increased the volume and bicarbonate output, and thereafter, decreased these to control values. Protein output was depressed throughout. Infusions of 50, 100, 200, 400 and 800pmol/kg/hr (0.21, 0.42, 0.84, 1.68, 3.36 ug/kg/hr) of porcine PP into dogs, raised the serum PP concentrations to levels which did not exceed those encountered after a meal. Even at the lowest infusion rate, basal pancreatic protein and bicarbonate secretion were inhibited. 100pmol/kg/hr (0.42ug) inhibited caerulein and secretin-stimulated bicarbonate secretion and 200pmol/kg/hr (0.84ug) inhibited stimulated protein output (Taylor, Solomon, Walsh et al, 1979). Endogenous stimulation of pancreatic exocrine secretion by duodenal instillation of phenylalanine and tryptophan, (Lin, Evans, Chance et al, 1977) and acid (Lonovics, Guzman, Devitt et al, 1981) was inhibited by exogenous PP administration. bPP infused into man at 200pmol/kg/hr (0.84ug/kg/hr), which achieved a plasma PP concentration of 711 $\pm$ 69pmol/l, caused marked inhibition of secretin-stimulated pancreatic protein and trypsin secretion but had little effect on the bicarbonate output. Inhibition of pancreatic secretion in dogs was achieved with the carboxy-terminal hexapeptide which therefore appears to be the biologically active part of the molecule (Chance, Cieszkowski, Joworek et al, 1981). bPP did not influence secretion in the jejunum, ileum or colon in rabbits (Camilleri, Cooper, Adrian et al, 1981).

2.12.2.2 The effects of pancreatic polypeptide on gastrointestinal motility.

By the implantation of induction coils in the wall of the gut, changes in tone and motility were measured in dogs following the administration of various doses of bPP. An infusion of 5 - 10ug/kg/hr of PP, relaxed the pyloric sphincter, duodenum, ileocaecal sphincter and descending colon (Lin and Chance, 1974). At higher doses of PP (50 - 100ug/kg/hr) gastrointestinal motility was stimulated as evidenced by vomiting and defaecation (Lin and Chance, 1974). In view of the high doses used, these latter phenomena may be toxic effects. Infusions of 10 - 250pmol/kg/hr (0.42 - 1.05ug/kg/hr of bPP in man did not cause diarrhoea (Adrian, Greenberg, Besterman et al, 1978) and 60 and 120pmol/kg/hr (0.25 and 0.5ug/kg/hr) did not alter the gastric emptying rate after a carbohydrate-rich breakfast (Adrian, Greenberg, Fitzpatrick et al, 1981)

In studies using small doses of 0,125 to 2ug/kg/hr, bPP relaxed the gall bladder, increased choledochal tone but did not change the bile flow (Lin and Chance, 1974), in dogs with chronic gall bladder and choledochal fistulae. In man, infusions of bPP at 150 - 214pmol/kg/hr (0.63 - 0.90ug/kg/hr) caused a significant decrease in bilirubin output (Greenberger, McCloy, Chadwick et al, 1979) which did not occur in patients who had undergone cholecystectomy (Bjornsson, Adrian, Dawson et al, 1979) suggesting that PP affects bile storage not hepatic bile production. In vitro, however, porcine PP did not cause relaxation of strips of rabbit or dog gallbladder, nor did it inhibit CCK-induced gallbladder contraction (Lonovics, Devitt, Rayford et al, 1979). In view of the smaller doses used in man which, by calculation, would have produced levels in the physiological range, these effects may reflect a

biological function of PP.

2.12.2.3 The gastrointestinal trophic effects of pancreatic polypeptide.

It has been shown that some gastrointestinal hormones may be important trophic factors for gastrointestinal tissue growth (Johnson, 1976). Greenberg, Mitznegg and Bloom (1977) have suggested that PP may have a trophic role in promoting pancreatic tissue growth. Following the injection of a single intraperitoneal dose of 12nmoles/kg (50ug/kg) of bPP in rats, the rate of DNA synthesis in pancreatic tissue was doubled as evidenced by the incorporation of ^{3}H-thymidine in DNA. The octapeptide of cholecystokinin-pancreozymin, injected at a similar dose, caused a similar increase in the rate of pancreatic DNA synthesis. Neither bPP nor cholecystokinin-pancreozymin promoted hepatic, duodenal or gastric increase in DNA synthesis; neither did bPP enhance the effects of cholecystokinin-pancreozymin, or vice versa.

In embryonic chicks, aPP may play a trophic role in the growth of the proventriculus (Laurentz and Hazelwood, 1979).

Thus bPP may have a trophic action on rat pancreatic tissue as evidenced by the increased rate of synthesis of DNA, but the concentration of PP attained was not measured and it is not known whether this would approximate the physiological concentrations in the pancreas under physiological conditions.

2.12.2.4 The effects of pancreatic polypeptide on gastrointestinal endocrine secretion.

In addition to its affects on the gastrointestinal tract as discussed above, it is possible that PP may have yet another action i.e. on the release of the gastrointestinal endocrine peptides. This would be in keeping with the effects of other candidate hormones of the gut e.g. vasoactive intestinal polypeptide, which has been shown to decrease gastric exocrine secretion (Said, 1974) and to release insulin and glucagon (Kaneto, Kaneko, Kajinuma et al, 1977).

In rats, synthetic human PP suppressed somatostatin levels in hepatic portal and in systemic plasma at a dose of 5ug/100g (Arimura, Meyers, Case et al, 1979). PP also inhibited gastric somatostatin release from the isolated rat stomach (Chiba, Taminato, Kadowaki et al, 1980). Hepatic portal plasma levels of insulin but not glucagon were decreased by synthetic hPP injected into rats (Murphy, Fries, Meyers et al, 1981). The suppression of insulin was achieved only by injection of the whole molecule, not the carboxy-terminal end which inhibits pancreatic secretion.

Although 60pmol/kg/hr (0,25ug/kg/hr) of bPP decreased basal plasma motilin concentration by 80% in fasted subjects (Adrian, Greenberg, Barnes et al, 1980), bPP infused at this dose did not affect plasma motilin, insulin, glucagon, gastrin, secretin, enteroglucagon, GIP, or neurotensin levels after a meal (Adrian, Greenberg, Fitzpatrick et al, 1981). PP infused at 0.8 to 2.1ug/kg/hr did not alter CCK, secretin (Lonovics, Guzman, Devitt et al, 1981) or gastrin (Chance, Cieszkowski, Jaworek et al, 1981) release in dogs.

2.12.3 The effects of pancreatic polypeptide on the regulation of food intake

Mice of the C57 BL/6J strain on which the ob/ob gene is superimposed, have a predisposition to hyperphagia, obesity, hyperglycaemia and hyperinsulinaemia. Intraperitoneal injections of 5 - 10ug/kg of bPP in these mice, was associated with a reduction in weight gain and in food intake compared with saline treated mice (Malaisse-Lagae, Carpentier, Patel et al, 1977). These effects and the fact that PP is released following feeding, suggested to these investigators that PP may be the "satiety factor" which is thought to be absent in these animals (Coleman, 1973). However, it is possible that in mice, like in dogs, certain doses of PP may cause vomiting and defaecation, which could have contributed to the reduced intake of food.

This review of the biological actions of PP indicates that a biological role for PP has not been defined. At present, the most likely biological actions relate to effects on pancreatic exorcrine secretion, and gall bladder and choledochal tone.

2.13 Pancreatic polypeptide concentrations in disease states.

Because PP has been localised to pancreatic endocrine-like cells, and its known actions relate to the gastrointestinal tract, the diseases in which PP concentrations have been measured involve pathology of these organs.

2.13.1 Diseases involving the pancreas.

Plasma PP concentrations have been measured in a number of diseases of the pancreas, both under basal and stimulated conditions. As discussed previously, the PP levels recorded vary from investigator to investigator which makes definitive assessment of the results difficult.

2.13.1.1. Plasma pancreatic polypeptide concentrations in pancreatectomised man.

Adrian, Bloom, Bryant et al (1976) reported that plasma PP concentrations were detectable in the plasma of pancreatectomised man, and that in these patients, the levels did not rise after the ingestion of a mixed meal, a potent stimulus for PP release. On the other hand, Floyd, Fajans, Pek et al (1977) recorded low but measurable circulating PP levels in patients who had undergone total pancreatectomy. The fasting levels were about one fifth of those in normal fasting individuals but the effect of feeding was not investigated in these patients.

These findings suggest that the pancreas is the major source of circulating PP, both in the basal and stimulated states, but the findings of Floyd et al do not rule out an extrapancreatic source for PP.

2.13.1.2. Pancreatic polypeptide concentrations in acute pancreatitis.

Acute pancreatic inflammation would lend itself to the investigation of the circulating pancreatic polypeptide concentrations, especially as the

PP cells are found both in the exocrine and endocrine parts of the pancreas (see 2.4). In view of the massive concentrations of amylase and glucagon found in the circulation in this condition (Waterworth, Barbezat, Vinik et al, 1977) it seems likely that the PP cells would be affected in this condition. Indeed, experimental acute pancreatitis induced in dogs, was associated with an immediate and sustained elevation of the serum PP concentration (Pappas, Yovos, Ellison et al, 1981).

2.13.1.3 Pancreatic polypeptide concentrations in patients with chronic pancreatitis

Basal and stimulated plasma PP concentrations were measured by Adrian, Besterman, Mallinson et al (1979) in patients with chronic pancreatitis and compared to the levels in healthy controls. The patients who had chronic pancreatitis were divided into two groups: one group had proven pancreatic insufficiency, and the second group, evidence of pancreatic calcification but had no evidence of loss of exocrine function. The investigators found that the basal PP concentrations were within the normal range, that patients with exocrine pancreatic insufficiency had impaired PP responses to a mixed meal, but that the patients with pancreatic calcification only, had a normal response. They then suggested that the PP responses to a meal may be used to evaluate pancreatic exocrine function. Investigators concur that PP release is impaired in chronic pancreatitis. They differ, however, as to its validity as a test for detecting this disease and determining its severity. Valenzuela, Taylor, Walsh et al (1979) and Anderson, Hagen, Klein et al (1980) found that the PP response to food was impaired but the correlation between PP response and pancreatic exocrine secretion

was poor. On the other hand, Yamamura, Mori, Tatsumi et al (1981) reported a significant correlation between PP release after food and total amylase output during CCK and secretin administration. Using 'Boots' secretin as a pancreatic exocrine and a PP secretogogue, Stern and Hansky (1981) found good correlation between basal to peak PP ratios and pancreatic bicarbonate output. There may, therefore, be a place for PP as a screening test for chronic pancreatitis.

2.13.1.4 Pancreatic polypeptide concentrations in patients with diabetes mellitus.

Patients with diabetes mellitus, irrespective of the treatment they were receiving, were reported to have basal PP concentrations which were significantly higher than those in healthy people (Floyd and Fajans, 1976). The levels in 10% of the diabetics were about 10 times higher than those in the other 90% of the patients. These high levels occurred only in insulin requiring diabetics but they were later found to be artifactual. They related to the presence of circulating interfering antibodies, which were directed at contaminating PP in the insulin preparations (Floyd, Fajans, Pek et al, 1977) and bound PP in the assay system, thus giving falsely high values.

Although it was found that plasma PP concentrations rise with age (Floyd, Fajans, Pek et al, 1977), it did not appear that the elevated PP concentrations in diabetics could be explained on the basis of age alone. When the age factor was taken into account and the levels corrected, diabetics still had elevated basal plasma PP levels.

The type of diabetes, and the mode of treatment appeared to have an effect on basal PP concentrations. The mean PP level in juvenile onset diabetics was 1,5 times higher than in maturity onset diabetics (170pg/ml and 113pg/ml respectively; i.e. 40,2 vs 26,9pmol/l). The basal level corrected for age in control subjects was 85pg/ml (20,2pmol/l). The maturity onset diabetics, who were treated with insulin, had PP levels that were higher than those in patients treated with diet alone, or diet plus oral hypoglycaemic agents (sulphonylureas), whereas the patients treated with sulphonylureas had PP levels higher than those treated with diet alone. These findings suggest that the basal PP concentrations may reflect the severity of the diabetes.

The mean blood glucose concentrations in the groups outlined above also related to the type of diabetes and the treatment the patients were receiving. Thus the juvenile onset diabetics had the highest glucose levels, the levels in insulin-requiring maturity-onset diabetics were lower, and in the diabetics on sulphonylureas and diabetics treated with diet only, the lowest levels were found. The trends of glucose concentrations and PP concentrations thus appeared to parallel each other, but the authors do not state whether there was a statistical correlation between blood glucose concentrations and the plasma PP levels observed.

Not only were basal PP levels elevated in diabetes, but the PP responses to oral glucose (Tsuda, Sakurai, Seino et al, 1980) and food (Berger, Floyd and Pek, 1981) were exaggerated. In patients with diabetic ketoacidosis, PP levels were grossly elevated and decreased with

treatment (Skare, Hanssen and Lundqvist, 1980). Normalization of the blood sugar in insulin-dependent diabetic subjects was associated with significant decrease in basal and stimulated PP release (Berger, Floyd and Pek, 1981).

Immunohistochemical studies on pancreata from patients with longstanding juvenile-onset diabetes showed that these patients had hyperplasia of the PP cells, which in some cases replaced the entire islet (Gepts, De Mey and Marichal-Pipeleers, 1977). These findings would be in accord with the elevated plasma PP concentrations in patients with juvenile-onset diabetes. There are however, no studies on the PP cell in patients with maturity-onset diabetes to explain the elevated PP concentrations in these patients.

In the light of limited knowledge of the biological actions of PP, speculation on the pathophysiological significance of these findings would appear to be premature.

2.13.1.5 Pancreatic polypeptide concentrations in plasma and in endocrine tumours of the pancreas.

Investigators have reported that a large proportion of patients with endocrine tumours of the pancreas have elevated concentrations of PP in the systemic circulation. In 15 patients with islet cell tumours, Floyd, Chance, Hayashi et al (1975) reported that plasma PP concentrations were greater than 3 times the range in healthy controls. According to Polak, Bloom, Adrian et al (1976), approximately 60% of patients with vipomas, insulinomas, glucagonomas and gastrinomas of the pancreas had plasma PP concentrations above 1000pg/ml (240pmol/l). It

was also found that basal PP levels were raised in some patients with multiple endocrine adenomatosis (MEA) type 1 (Floyd and Fajans, 1976). This condition in which pancreatic islet cell tumours may occur in conjunction with pituitary and parathyroid adenomas, and occasionally with adenomas of the adrenal cortex, renal cortex and thyroid and with carcinoid tumours, may in some cases be familial. It is of interest that elevated PP concentrations were found in some non-affected family members of patients with familial MEA Type 1 (Floyd, Fajans, Pek et al, 1977; Friesen, Kimmel and Tomita, 1980). A number of patients with proven pancreatic endocrine tumours had normal PP concentrations in plasma (Polak, Bloom, Adrian et al, 1976). Basal plasma PP levels were elevated (above 240pmol/l in only three of eight patients with the Zollinger-Ellison syndrome (Taylor, Walsh, Rotter et al, 1978). In patients with insulinomas, the elevated PP concentrations correlated negatively with the plasma glucose level, suggesting that PP elevation was secondary to hypoglycaemia (Nelson, Service, Ilstrup et al, 1980). These findings mitigate against the use of serum PP levels as a useful diagnostic criterion in these tumours.

That the elevated plasma PP concentrations in patients with pancreatic tumours derives from the tumours, was suggested by Polak, Bloom, Adrian et al (1976). They reported that the majority of the tumours contained immunoreactive PP cells and had higher concentrations of PP that found in normal pancreatic extracts. Lymphatic and hepatic secondaries from malignant tumour also contained PP cells (Polak, Bloom, Adrian et al, 1976; Heitz, Polak, Bloom et al, 1976). However, Larsson, Schwartz, Lundqvist (1976) have reported that although PP cells were present in pancreatic endocrine tumours, striking hyperplasia of the PP cells in the surrounding exocrine pancreas was apparent and that PP cells were no

more or less frequent than other cell types in mixed pancreatic endocrine tumours. The presence of hyperplastic PP cells in the non-tumorous exocrine pancreas may explain the failure of plasma PP concentrations to return to normal after removal of the neoplasm as reported by Floyd, Chance, Hayashi et al (1975). On the other hand, in two patients plasma PP levels did return to normal following excision of the tumours (Friesen, Kimmel and Tomita, 1980).

In infants with nesidioblastosis, immunohistochemical examination of the pancreas showed that PP cells were increased in number when compared with normal neonatal pancreata (Heitz, Kloppel, Hacki et al, 1977). However, the other endocrine cell types were also hyperplastic, resulting in endocrine rather than selective PP hyperplasia.

Tumours of the pancreas consisting only of PP cells - a PP-oma have been described (Larsson, 1976; Adrian, Bloom, Besterman et al, 1978; Friesen, Kimmel and Tomita, 1980). Larsson (1976) described a patient with the watery diarrhoea, hypokalaemic achlorhydria (WDHA) syndrome, who had normal plasma vasoactive intestinal polypeptide (VIP) levels, a thousand-fold increase in the basal plasma PP concentration and a pancreatic endocrine tumour consisting almost entirely of immunoreactive PP cells. Whether the PP was the cause of the diarrhoea though is not known.

It has been proposed that the WDHA or the Verner-Morrison syndrome, results from increased tumour production of vasoactive intestinal polypeptide (VIP) and that the plasma levels are always raised in this condition (Bloom and Polak, 1975). However, in some cases of this syndrome, elevated VIP levels in serum are not found, and this condition

has been called the pseudo-Verner-Morrison syndrome (Bloom and Polak, 1976). There are a number of candidates as the diarrhoeogenic agent in this situation, of which PP is one. Pancreatic polypeptide in large doses has, in dogs, been shown to cause diarrhoea, to inhibit pentagastrin-stimulated acid secretion and to cause relaxation of the gall bladder (see 1.12.1), a feature of prominence in the WDHA syndrome. PP does not, however, affect secretion in the small intestine of rats (Wu, O'Dorisio, Cataland et al, 1979) or rabbits (Camilleri, Cooper, Adrian et al, 1981). A role for prostaglandins has also been suggested in this condition (Jaffe, Kopen, De Schryver-Kecskmeti et al, 1977). Thus although PP remains a candidate as the agent causing diarrhoea in the patient described by Larsson, its role is by no means proven, especially since prostaglandin levels were not reported.

2.13.2 Diseases of the gastrointestinal tract.

The diseases of the gastrointestinal tract in which PP concentrations have been measured include the watery diarrhoea hypokalaemic achlorhydria syndrome which was discussed in the previous section (2.13.1.4 above) and peptic ulceration, which now merits consideration.

2.13.2.1 Plasma pancreatic polypeptide concentrations in patients with duodenal ulceration.

The ability of large doses of bPP to stimulate gastric acid secretion in dogs (Lin and Chance, 1974) suggested to Schwartz, Rehfeld, Stadil et al (1976), that PP hypersecretion may be a factor in the pathogenesis of duodenal ulceration. They reported that basal PP concentrations were significantly raised in 14 patients with proven duodenal ulcers to 2,5

times the concentration in healthy controls. However, it is not clear how the ages compared in the two groups. Adrian, Bloom, Besterman et al (1977), disputed the fact that the plasma PP concentrations were higher than normal in patients with duodenal ulcers, after they found that the levels were identical in 11 patients with duodenal ulceration and 10 healthy controls whose mean ages were 39 and 41 years respectively. The PP response to a protein-rich meal was similar in duodenal ulcer patients and control subjects, but patients with gastric ulcers had significantly lower levels (Stern and Hansky, 1981). Thus, whether the circulating PP concentrations in patients with peptic ulcers differ significantly from normal is still unresolved.

It has also been reported by Lin and Chance (1974) that infusions of bPP in dogs inhibited pentagastrin-stimulated acid secretion. It is thus feasible that in an attempt to counteract the action of the gastrin hypersecretion in the Zollinger-Ellison (gastrinoma) syndrome, PP, too would be released in excess and that the plasma concentrations would be raised. Polak, Bloom, Adrian et al (1976) reported that of 8 patients with the Zollinger-Ellison syndrome, the plasma PP concentrations were elevated to above 1000 pg/ml (240pmol/l) in five. However, they also found that plasma PP concentrations were elevated in a large proportion of all their patients with endocrine tumours of the pancreas, mitigating against elevated PP concentrations being a specific marker for the Zollinger-Ellison syndrome. Taylor, Walsh, Rotter et al (1978) concur with this finding.

2.14 Summary

In this chapter, the history of pancreatic polypeptide (PP) from its discovery in 1968 to its status in December 1981 has been reviewed. What emerges is the story of a newly recognized pancreatic peptide which has been isolated from the pancreas of chickens and a number of mammalian species including man. The amino-acid composition and sequence of PP from avian and mammalian species has been determined and in each instance revealed a 36 amino-acid peptide of molecular weight 4200 daltons. The mammalian varieties differ slightly from each other in amino-acid composition, and avian PP shares 16 amino-acid residues with the bovine molecule, showing species homology.

Immunohistochemical techniques have localised PP to specific endocrine-like cells, which, in most species, are found both in pancreatic islets of Langerhans as well as the exocrine parenchyma. Furthermore, in some species, but not in man, PP cells have been demonstrated in the gastrointestinal tract and preliminary studies have suggested that the PP cells may belong to the APUD series.

Radioimmunoassays have been established which can quantitatively assay PP both in plasma or serum and in tissue extracts. However, variations in assay methodologies have made it difficult to compare the results from different laboratories.

Although facets of the character of PP have been elucidated, there are many hiatuses in the understanding of its behaviour. PP is measurable in plasma and serum of normal fasting man, but the levels rise with age, and due to variation in assays, the levels differ from laboratory to

laboratory, thus making it difficult to determine the normal range. Circulating PP levels rise strikingly after the ingestion of a mixed meal which is a potent stimulus for PP release. It has been suggested but it is not uniformly accepted, that the meal causes a biphasic pattern of release which may indicate that a number of factors are responsible for the release of PP. In this regard, the nutrients per se, gastrointestinal factors and control by the vagus nerve have been investigated. Although ingestion of glucose and fat caused the release of PP, the plasma concentrations observed were not of the same magnitude as those seen following the ingestion of a meal. Intravenous amino-acid infusion was reported to cause a modest rate in plasma PP concentrations; intravenous fat infused over a prolonged period had no effect or decreased plasma PP levels and intravenous glucose caused a fall in the plasma PP concentrations. In man, infusion of a crude secretin preparation (Boots secretin) was associated with marked release on PP. In dogs, bombesin caused a rise in plasma PP levels and gastrin and CCK are also likely PP secretogogues. Somatostatin inhibited PP release. Gastric inhibitory polypeptide, vasoactive intestinal polypeptide, gastrin, secretin and the anuran peptide, caerulein, caused release of PP in in-vitro studies with isolated perfused pancreata or pancreatic tissue slices. It has also been shown that electrical stimulation of the vagus nerve caused the release of PP, and that in man, bilateral truncal vagotomy abolished the PP response to insulin-induced hypoglycaemia, and decreased the PP response after a mixed meal. It is possible that the adrenergic nervous system may play a role in mediating PP release, as it was shown that plasma PP concentrations rose with adrenergic beta-receptor stimulation and exercise.

Thus numerous stimuli may cause PP to rise in the circulation but due to differences in investigative procedures, consensus about their effects has not been reached. Moreover, which of these stimuli act under physiological conditions and what their relevance is, is not clear.

Examination of circulating PP by molecular sieve chromatography with regard to its molecular forms has suggested that immunoreactive PP of at least two molecular weights may be present in the circulation. However, extracts of pancreatic tissue yielded PP of a single molecular weight on column chromatography. It is thus possible that PP exists in more than one immunoreactive form and confirmation thereof is required.

The fate of circulating PP is not known. Studies in pathological conditions in man have suggested that PP may be cleared by the liver and by the kidney, but whether this is the situation in health is not known. In dogs exogenously infused porcine PP had a half-life of approximately five minutes which suggests that PP may be a molecule of fairly rapid turnover, and the turnover kinetics of endogenous PP is required to confirm this finding.

Although PP is released into the circulation in amounts comparable to that of other gastrointestinal endocrine-like peptides, its actual biological role is not known. Studies in dogs using doses which may have achieved supraphysiological concentrations suggest that PP affects many aspects of gastrointestinal function including stimulation of gastric acid secretion, inhibition of pentagastrin-stimulated acid secretion and increased gastrointestinal motility. At more physiological concentrations PP inhibited pancreatic protein and bicarbonate secretion and relaxed the gall bladder and choledochus. No

effect on metabolism has been shown for PP in mammals but in chicks large doses (100ug/kg) of avian PP depleted the liver of glycogen and caused the plasma glycerol levels to fall. Thus the physiological role of PP is not clear and further investigations in this respect are required.

Finally, the concentrations of PP in disease states was reviewed. It was found that plasma PP concentrations in pancreatectomised man were low or undetectable and did not respond to stimulation. The plasma PP levels in patients with acute pancreatitis have not been measured but in patients with chronic pancreatitis, depending on the severity of the disease, the PP responses to a meal were impaired. The fasting plasma PP concentrations in patients with diabetes mellitus were higher than in control subjects and tended to be higher in insulin- than in non-insulin-requiring diabetics. Plasma PP concentrations were also elevated in some patients with endocrine tumours of the pancreas but this was not a universal finding. Furthermore, extracts of many of these tumours yielded high concentrations of PP, and many of the tumours in addition to the functioning cells, contained immunoreactive PP cells as well. It was also found that the surrounding pancreas showed PP cell hyperplasia, which may suggest an additional source for the elevated circulating PP concentrations found in some of the patients.

The PP story has thus unfolded to reveal a gastrointestinal peptide that has been isolated, purified, sequenced and given a cellular home. However, numerous gaps in our understanding of the regulation of its release, how it circulates, how it is metabolised, its pathophysio-

logical significance and what it does, are present. These problems have been alluded to in this chapter and provide scope for specific investigations.

CHAPTER III

AIMS AND OBJECTIVES

CHAPTER III AIMS AND OBJECTIVES

3.0 Introduction

3.1 The regulation of pancreatic polypeptide release

3.1.1 The effects of nutrients on serum pancreatic polypeptide concentrations

3.1.2 The effect of gastrointestinal hormones on serum pancreatic polypeptide concentrations

3.1.3 Neural regulation of pancreatic polypeptide release

3.1.3.1 Adrenergic modulation of pancreatic polypeptide release

3.1.3.2 Cholinergic modulation of pancreatic polypeptide release

3.1.3.3 Dopaminergic control of pancreatic polypeptide release

3.1.4 The effects of substances of profound systemic influence on pancreatic polypeptide release

3.1.4.1 The effect of somatostatin on pancreatic polypeptide release

3.1.4.2 The effect of histamine on pancreatic polypeptide release

3.2 Investigation of the molecular forms of circulating immunoreactive pancreatic polypeptide

3.3 Investigation of the metabolism and turnover kinetics of pancreatic polypeptide

3.3.1 Organ uptake of pancreatic polypeptide

CHAPTER III

AIMS AND OBJECTIVES

3.0 Introduction

At the time that this study was designed, much of the data about pancreatic polypeptide reviewed in the previous chapter was unpublished. Thus the aims and objectives described here, pertain to factors that were relevant at that stage, but also reflect issues that remain unresolved and important.

For a peptide that has been purified, characterised, sequenced and has a cell of origin, the obvious and principal question is "What does it do"? One of the ways of finding the answer is to administer the purified peptide to experimental animals or to man. From the historical review it is apparent that by using this approach, although numerous actions have been demonstrated, a physiological role for PP has not been defined. It has been shown that gastrointestinal hormones, in addition to their primary actions, may in supraphysiological concentrations have additional actions. Thus gastrin, in addition to its primary action on gastric acid secretion, increases gastrointestinal motor activity, releases insulin and calcitonin, inhibits absorption of water, electrolytes and glucose by the small intestine and increases blood flow in stomach, small intestine and pancreas (reviewed by Walsh and Grossman, 1975). It is thus important when infusing a peptide in an attempt to determine its primary action, to mimic the physiological circulating concentrations. To achieve this, it is of the essence, firstly to know its physiological concentrations under various circumstances; secondly, since many peptides circulate in multiple

molecular forms which may have different biological potencies, to know if these are present for the specific peptide; and thirdly, to know how the peptide is cleared and its rate of turnover.

When planning these studies, PP was not available for infusion, which necessitated an alternate approach to investigate its function. This chapter deals with factors which motivate the formulation of specific questions whose answers aim to give insight into the PP concentrations under various circumstances and may provide a better understanding of its behaviour and characteristics. Moreover, they may provide a basis for assessing how PP can be infused to mimic physiological levels under various circumstances and may also in a more indirect way help to answer the question "What does PP do"?

This chapter and the ensuing studies are considered in four sections, each of which deals with separate aspects of the study. The sections are:

1. The regulation of pancreatic polypeptide release.
2. Molecular forms of circulating pancreatic polypeptide.
3. Metabolism and turnover kinetics of pancreatic polypeptide.
4. Pancreatic polypeptide concentrations in disease states.

3.1 The regulation of pancreatic polypeptide release.

One of the classical approaches to investigate a candidate hormone is to determine factors regulating its release. For a hormone to have a homeostatic effect a number of factors must be present. There must be a signal to the cell of origin for its release; the cell must then effect release; the product must be transported to the site(s) of action to exert that action; the hormone must then be cleared and a feedback message sent to the cell of origin to inhibit further release. The complexity of the control varies from short, single, negative feedback loops, to multiple short and long loops which cause regulation at various levels. In the former instance, the glucose-insulin relationship may be cited as an example, whereas the control of thyroxine release may serve as a case in point for the latter.

Thus, although a hormone regulates a physiological reaction, the substrates and products of that reaction in turn may regulate its secretion. Studying the patterns of release of a candidate hormone may provide an indication of its control and field of actions. Numerous factors which may regulate PP release, possibly in terms of the more simple insulin-glucose type relationship, will be considered and are discussed below.

3.1.1 The effects of nutrients on serum pancreatic polypeptide concentrations.

Because PP is found in cells situated both in the endocrine and exocrine parts of the pancreas, it is possible that PP may be released together with the pancreatic exocrine secretions into the lumen of the gut to

exert an action on nutrient absorption or metabolism. However, in this study only the circulating PP concentrations will be assessed. The reports on the release of PP following a mixed meal differ as to the pattern of release found (see 2.10.2) and this needs to be assessed. Meal induced PP release may occur with all or only with some of the nutrient components and thus, the effect of a mixed meal and of individual nutrient fuels on PP release are to be investigated.

3.1.2 The effect of gastrointestinal hormones on serum pancreatic polypeptide concentrations.

It is well recognized that gastrointestnal hormones influence the release of the pancreatic hormones, insulin and glucagon. For example, secretin (Dupré, Rojas, White et al, 1966), cholecystokinin-pancreozymin (CCK-PZ) (Unger, Ketterer, Dupré et al, 1967) and gastric inhibitory polypeptide (GIP) (Dupré, Ross, Watson et al, 1973) have all been shown to influence insulin release, and GIP (Rabinovitch and Dupré, 1974) and CCK-PZ (Unger, Ketterer, Dupré et al, 1967) to release glucagon. In many instances, the release of insulin or glucagon is not a primary action of these hormones and does not occur at physiological concentrations. As PP is released in response to a meal, the gastrointestinal hormones released by the food may act as PP secretogogues and play a part in mediating the PP response. Thus, although gastrointestinal hormones are capable of causing the release of pancreatic hormones including PP, there are discrepancies in the literature with regard to which hormones release PP.

3.1.3 Neural regulation of pancreatic polypeptide release.

The nerve supply of the pancreas consists of autonomic and visceral afferent fibres. The parasympathetic nervous fibres come from the vagal trunks and the sympathetic from the greater and middle splanchnic nerves. At an ultrastructural level, two types of neural elements have been identified in the islets of Langerhans. These are, firstly, small agranular electron-lucent vesicles and secondly, small vesicles with dense granular cores (Kobayashi and Fujita, 1969). The former may well be cholinergic and the latter, adrenergic nerve endings (Richardson, 1964). The nerve fibres penetrate the islets of Langerhans and the terminals lie in close proximity to the endocrine cells (Kobayashi and Fujita, 1969). These morphological findings suggest that the autonomic nervous system plays a role in mediating pancreatic endocrine secretion.

By using catecholamines, their agonists and antagonists, as well as cholinergic agents and their agonists and antagonists, the separate influences of adrenergic and cholinergic innervation of the endocrine pancrease have been further defined.

3.1.3.1 Adrenergic modulation of pancreatic polypeptide release.

Pharmacological studies have clearly shown that stimulation of the adrenergic alpha-receptors inhibits, whereas stimulation of the beta-receptors stimulates the release of insulin in man (Porte, Graber, Kuzuyaet et al, 1966; Porte and Williams, 1966; Porte 1967; Karam, Grasso, Wegienka et al, 1966; Buse, Johnson, Kuperminc et al, 1970) and in animals (Kansal and Buse, 1967; Hertelendy, Machlin and Kipnis,

1969). Glucagon release, too, is modulated by adrenergic mechanisms (Gerich, Karam and Forsham, 1972; Bloom, Edwards and Vaughan, 1973). The role of the adrenergic nervous system in the control of PP release is not clear and therefore of interest.

Insulin-induced hypoglycaemia, which is a potent stimulus for PP release in normal people, activates both the sympathetic (Vendsalu, 1960) and the parasympathetic (Johnson and Spalding, 1974) nervous systems. This suggests that both may play a role in the PP response to insulin-hypoglycaemia. Patients who had undergone bilateral truncal vagotomy, however, failed to release PP during insulin-hypoglycaemia (Adrian, Bloom, Besterman et al, 1977) suggesting that the response is mediated only by the parasympathetic nervous system, but this does not necessarily exclude a role for the adrenergic nervous system in PP release. Like insulin, it is possible that adrenergic alpha-receptor stimulation inhibits and that beta-receptor stimulation excites PP release, thus causing no nett change in the circulating PP concentration with combined alpha- and beta-receptor stimulation. The predominance of either alpha- or beta-receptor stimulation may, however, cause the PP concentration to change.

To assess these possibilities, an investigation into the effects of adrenergic stimulation on PP release will be carried out.

3.1.3.2 Cholinergic modulation of pancreatic polypeptide release.

The cholinergic regulation of pancreatic polypeptide has been discussed in detail (see 2.10.4.1) and it is apparent that cholinergic nervous impulses play an important role in the control of PP release. It was

found that vagotomy abolished the rise in PP during insulin-induced hypoglycaemia (Adrian, Bloom, Besterman, 1977), indicating the key role of the vagus in this response.

To establish the range and the responses in control subjects in this laboratory by using the techniques and materials available, and to act as control data for subsequent studies, the effect of insulin-induced hypoglycaemia on PP release will be assessed.

3.1.3.3 Dopaminergic control of pancreatic polypeptide release.

It has been recognized for a fairly long time that dopamine is a neurotransmitter in the central nervous system (Fuxe, 1963) but only recently has it become apparent that dopamine is also a neurotransmitter of the autonomic nervous system (Thorner, 1975). Indeed, dopaminergic receptors have been described in postganglionic cells of sympathetic ganglia and on presynaptic terminals of adrenergic nerve endings (reviewed by Reid, 1977). In the pancreas, dopamine has been shown to alter insulin (Quickel, Feldman and Lebovitz, 1971) and glucagon (George and Rayfield, 1974) release. Indeed, Lebovitz and Feldman (1973) have proposed that the biogenic amines play an important integrative role in the control of insulin release. Although a dopamine antagonist, metoclopramide, may affect PP release (Spitz, Zylber, Jersky et al, 1979), the role of dopaminergic agents on PP secretion have not been fully delineated and require further investigation.

In addition to the effects of nutrients, gastrointestinal hormones, and the autonomic nervous system, other substances must be considered which may regulate the release of PP. In many cases the exact modes of action

of these substances have not been clearly defined, thus making classification difficult. In the next section they will, for convenience, be grouped together as substances which may have profound systemic effects and may in addition regulate the release of PP.

3.1.4 The effects of substances of profound systemic influence on pancreatic polypeptide release.

In this miscellaneous category, the effect of somatostatin and histamine will be considered.

3.1.4.1 The effects of somatostatin on pancreatic polypeptide release.

A number of peptides which are found both in the gastrointestinal tract and in the brain (reviewed by Pearse, 1977), have gained prominence as substances which may function as peptide neurotransmitters. These include neurotension, substance P, vasoactive intestinal polypeptide, and somatostatin. The stringent criteria which must be fulfilled before these substances can be classified as neurotransmitters, precludes the classification of somatostatin as a true neuropeptidergic substance but it is possible that it acts in this way. However, somatostatin is also considered a hormone (Luft, Efendic and Hokfelt, 1978) and its action could then be considered under the section on the effects of gastrointestinal hormones on PP release. There is a third way in which somatostatin may act. Feyrter (1938), published the concept of the diffuse endocrine system (see 2.6), but believed that these cells may in fact be "paracrine" i.e. that their products act directly on neighbouring cells. The theory of paracrine control of the

hormone-secreting cells of the islets of Langerhans of the pancreas, was proposed by Orci and Unger (1975) and suggests that somatostatin may directly affect the release of insulin and glucagon by its proximity to A and B cells. Since the PP cells are also found in the islets, it is possible that somatostatin may affect PP release in a paracrine fashion.

Somatostatin inhibits the release of many peptide hormones (reviewed by Primstone, Berelowitz, Kronheim, 1976) and in pharmacological doses, inhibits PP release as well (Marco, Hedo, Villanueva, 1977). Its effect on PP release will be investigated.

3.1.4.2 The effects of histamine on pancreatic polypeptide release.

The second substance which is considered in this miscellaneous category is histamine. It is clear that this ubiquitous amine exerts its action on at least two receptors, the H_1 and H_2 receptors - the H_2 receptor being principally involved in gastric acid secretion (reviewed by Code, 1977). The action of histamine on the H_2-receptor is antagonised by cimetidine (Brimblecombe, Duncan, Durant et al, 1975) but H_2-receptor blockade does not appear to have a direct effect on gastrin release (Bank, Barbezat, Vinik et al, 1977) suggesting that histamine may not affect peptide hormone release. However, recently it was suggested that the H_2-receptor blocking agent, cimetidine, may in fact have an effect on peptide hormone release. Della Fara, Tamburrano, Magistris et al (1977) reported that the serum prolactin levels were elevated in patients treated with cimetidine.

Whether cimetidine can affect the circulating concentrations of other peptides particularly gastrointestinal peptides is not known, but in

view of a possible effect of H_2-receptor blockade on the release of one peptide hormone, and the proposed action of PP relating to acid secretion (see 2.12.2.1) the effect of cimetidine on PP release will be investigated.

In summary, the first section of this chapter deals with the release of pancreatic polypeptide and aims to examine the effects of a mixed meal, of individual nutrient fuels and gastrointestinal hormones on changes in the circulating PP concentrations. Furthermore, it aims to establish the normal range for insulin-hypoglycaemia induced PP release in this laboratory, and whether the sympathetic nervous system modulates PP release as well. The possible effects of dopaminergic neurotransmission, of somatostatin, and of histamine H_2- receptor blockade will also be assessed.

Although many of these factors may release PP, it is possible that like many gastrointestinal peptides, multiple molecular forms of immunoreactive PP may be found in the circulation, and this possibility will be considered in the next section.

3.2 Investigation of the molecular forms of circulating pancreatic polypeptide.

Many peptide hormones have been isolated in more than one chemical form. These heterogeneous molecular forms may represent pro-hormones, polymerised forms, protein-bound hormones, degradation fragments or cross-reacting immunoreactive material (Yalow, 1974). Gastrin, for example, demonstrates some of these phenomena. Rehfeld, Stadil and Vikelsoe (1974) reported on the heterogeneity of gastrin in serum. They found that gastrin immunoreactivity was distributed into four components: Component I was gastrin of approximately the same molecular weight as proinsulin, and was converted to 'little' gastrin i.e. heptadecapeptide (G17) gastrin by tryptic digestion; Component II eluted as a double or biphasic component which corresponded to 'big' or G34 gastrin; Component III was also biphasic, and corresponded to 'little' (G17) gastrin; and the biphasic Component IV corresponded to 'minigastrin' i.e. G14 gastrin. The biphasic elution patterns were indicative that G34, G17 and G14 each occurred in sulphated and non-sulphated forms. An additional, larger form of immunoreactive gastrin (Big Big gastrin) was reported by Yalow and Berson (1972) but Rehfeld, Schwartz and Stadil (1977) reported that this form of gastrin was an artefact in normal serum, but may have been real in some gastrinomas.

Certain antisera may react with all these various forms of gastrin (Rehfeld, Stadil and Vikelsoe, 1974) or they may be distinguished by antisera specific to a particular gastrin molecule (Dockray and Taylor, 1976). In the absence of such specific antisera, as is the case with PP, separation of the various forms of the peptide by

criteria of a molecular weight and/or charge is required to establish heterogeneity of the peptide.

Heterogeneity of peptide hormones is not merely a molecular curiosity but is important in understanding the biological potency of the peptide. Frequently the smaller (hormonal) peptide has greater biological potency than the larger form. Thus, to use gastrin as an example once more, it has been shown that with similar serum concentrations, heptadecapeptide (G17) gastrin is six times more potent than G34 gastrin (Dockray and Taylor, 1976; Walsh, 1977). As a further example, proinsulin, the insulin precursor, has less than 20% of the biological activity of insulin (Sherman, Gorden, Roth et al, 1971) even though it largely shares immunoreactivity with insulin in many radioimmunoassays (Rubenstein, Cho and Steiner, 1968).

As yet, there is little conformity about the molecular forms of PP and this aspect requires clarification. Furthermore, since there are a number of different stimuli for PP release, the nature of the PP released by diverse methods and the immunoreactive forms of circulating PP will be investigated.

The next step in this investigation of aspects of the physiology of pancreatic polypeptide, is to ascertain what happens to the peptide after it has been released into the circulation.

3.3. Investigation of the metabolism and turnover kinetics of pancreatic polypeptide

"Kinetics" is defined by Stedmans medical dictionary (1966) as "the study of motion and acceleration under the influence of forces". A study of PP embracing all aspects of this comprehensive definition would elucidate many unanswered questions but such a task is beyond the scope of this thesis. In this section "kinetics" has been limited to asking some questions about the site(s) of release and uptake, the organs of clearance and the half-life time ($t_{\frac{1}{2}}$) of PP. The answers to these questions in themselves cannot indicate a role for PP, but they may contribute to an understanding of its physiology.

3.3.1 Organ uptake of pancreatic polypeptide.

Because PP is released by pancreatic and possibly by gastrointestinal cells, it must traverse the liver before it appears in the systemic circulation. Whether or not the liver removes PP during this passage is therefore of importance, since it may indicate a site of action and/or degradation of PP. Similarly, the uptake of PP by other organs e.g. the kidney is likely to indicate a site of metabolism, excretion or action of PP. Although it has been reported that plasma PP concentrations are elevated in patients with renal failure (Hällgren, Lundqvist and Chance, 1977) this may not necessarily relate to failure of renal clearance of PP, but could also indicate enhanced release and thus the renal clearance of PP in non-pathological situations needs to be established.

3.3.2. The half-life time of disappearance of pancreatic polypeptide.

Serum PP concentrations are elevated for at least 6 hours after a meal (Adrian, Bloom, Besterman et al, 1977). The prolonged elevation may reflect a long half-life time ($t_{\frac{1}{2}}$) and a slow rate of turnover of the peptide, or, on the other hand, a rapid turnover with continued release. In general, the rate of turnover of biologically active substances reflects the duration of its action. Thus substances with a short $t_{\frac{1}{2}}$ e.g. the catecholamines, which have a calculated half-life of less than 20 seconds in the systemic circulation (Ferreira and Vane, 1967), have a short-lived, dynamic action. These are substances of acute need. Conversely substances with a long $t_{\frac{1}{2}}$ have a slower onset and a more prolonged duration of action. For example, in adult human beings, the biological half-life of thyroxine (T4) in serum is 6-11 days. After a single intravenous dose of thyroxine, the maximum oxygen consumption is observed after one half-life time and the effect is still discernable 4 to 5 half-lives after the injection has been given (van Middlesworth, 1974) thus suggesting that this is not a hormone of acute need. Thus calculation of the $t_{\frac{1}{2}}$ of PP may add to an understanding of the mode and duration of its action. To date, studies to determine the half-life time of PP have involved the infusion of exogenous PP into man or animals. The aim of this study is to determine the half-life of endogenous PP and see if it compares to that found by other investigators.

The aims of the preceding three sections involved an investigation of the behaviour of pancreatic polypeptide in non-pathological situations to gain insight into its physiology. Thus, the regulation of PP

release, the molecular forms of circulating PP and the metabolism and turnover kinetics of PP will be examined in healthy subjects or in animals. In the next section, circulating PP concentrations in various disease states will be investigated which may add to an understanding of the biological relevance of this candidate hormone.

3.4 Serum pancreatic polypeptide concentrations in patient with diseases of the pancreas.

The aim of measuring serum PP concentrations in patients with certain diseases is three fold. Firstly, a comparison of the PP concentrations in these patients with those found in matched healthy subjects, can contribute to an understanding of a biological action of PP when related to facets of the disease; secondly, abnormal PP concentrations in these patients may serve a useful diagnostic and possibly prognostic purpose; and thirdly, in the long run, when the biological function of PP is more clear, these abnormal levels may elucidate unexplained features of the disease process.

The pancreatic origin of this peptide suggests that diseases of the pancreas should receive priority investigation.

3.4.1 Serum pancreatic polypeptide concentrations in patients with acute pancreatitis.

Apart from the measurements of serum amylase, there are no adequate non-invasive indices for the diagnosis of acute pancreatitis, nor is there a measure of the extent of pancreatic damage. Glucagon concentrations are raised in patients with acute pancreatitis (Waterworth, Barbezat, Vinik et al, 1977; Drew, Joffe, Vinik et al, 1978; Drew, Joffe, Vinik et al, 1978a) but these do not reflect the severity of the destruction. It is possible that the serum PP concentrations in acute pancreatitis may reflect acute pancreatic damage and also the degree of damage sustained.

3.4.2 Serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

Insulin levels in chronic pancreatitis are relatively normal until the disease is well advanced and only on intensive beta-cell stimulation can a diminished reserve be demonstrated (Vinik, Kalk, Botha et al, 1976). abnormalities of glucagon secretion occur in less severe disease, and include hyperglucagonaemia and an exaggerated response to arginine (Kalk, Vinik, Bank et al, 1974) and tolbutamide (Kalk, Vinik, Paul et al, 1975).

Whether the basal PP concentrations in chronic pancreatitis are relatively normal, like insulin, or, like glucagon, become abnormal early in the disease warrants investigation.

Once the basal serum PP concentrations are established, it would be of interest to compare the response in these patients with those observed in healthy subjects. The PP responses may indeed be abnormal. Since patients with chronic pancreatitis have normal insulin responses until the disease is well advanced and they have exaggerated gastric inhibitory polypeptide (GIP) concentrations and responses early in the disease process (Botha, Vinik and Brown, 1976), it has been suggested that the maintained insulin response is a result of enhanced GIP stimulation. In view of the finding of Iversen, Bloom, Adrian et al (1977) that GIP may be a PP secretogogue, it is possible that elevated GIP levels in patients with chronic pancreatitis may contribute to abnormal PP concentrations and responses in these patients. However, distortion of the pancreatic architecture by fibrosis (Comfort, Gambill and Baggenstoss, 1946) may limit the ingress and diffusion of circu-

lating hormones to the pancreatic cells and the fibrosis may also distort and destroy the autonomic nerve fibres discussed earlier (see 3.1.4), thus causing further abnormalities in release. It would therefore be of particular interest to observe the effects of nutrients, of intravenously administered stimulating and inhibiting hormones and of vagal stimulation on PP responses in patients with chronic pancreatitis.

3.4.3 Serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

Floyd and Fajans (1976) reported that PP levels were elevated in patients with diabetes mellitus, regardless of whether they were insulin dependent or independent. Gates and Lazarus (1977), however, reported that the PP levels in diabetic New Zealand obese mice were low, and that administration of pancreatic polypeptide to these mice, caused the elevated blood sugar to fall. Although the differences between the PP concentrations in diabetic man and diabetic mouse may relate to species and to disease specificity, it is possible that the PP concentrations in patients with diabetes mellitus are elevated in an attempt to lower the blood sugar. Alternatively, it is possible that although basal PP concentrations are slightly elevated in patients with diabetes mellitus, the ability to release PP in response to stimuli is impaired and this may contribute to the pathology. Therefore, the basal PP concentrations and the ability of patients with diabetes mellitus to release PP appropriately, merits investigation.

3.5 Summary

To elucidate aspects of the physiology and pathophysiology of pancreatic polypeptide secretion this study aims to examine factors affecting the regulation of PP release, the molecular forms of circulating PP, the organ clearance and turnover kinetics of PP and the serum PP concentrations in some disease states which involve the pancreas. With regard to factors effecting the release of PP, the PP responses to a mixed meal, to individual nutrient fuels and to gastrointestinal hormones are to be investigated. The effects of insulin-induced hypoglycaemia, of manipulation of the sympathetic nervous system and of dopaminergic neurotransmission will be assessed, as will the effects of somatostatin and histamine H_2-receptor blockade.

To determine whether the PP released by the cells circulates as a single or as multiple molecular forms, the molecular homogeneity of circulating PP will be assessed. The organ uptake and half-life time of PP will then be investigated to indicate possible sites of action or degradation of PP and possibly its duration of action. Finally, serum PP concentrations will be assessed in patients with acute pancreatitis, with chronic pancreatitis and with diabetes mellitus to determine whether the PP concentrations may have diagnostic or prognostic indications in these conditions. Furthermore, the release of PP in response to stimulation will be assessed, which may help to elucidate features of the disease process.

These studies outlined above can only be accomplished if serum PP concentrations can be accurately measured. A specific, sensitive radioimmunoassay would provide such a measurement and forms the basis of all studies undertaken.

CHAPTER IV

THE EXPERIMENTAL DESIGN

CHAPTER IV THE EXPERIMENTAL DESIGN

4.0 The measurement of pancreatic polypeptide by radioimmunoassay

4.0.1 Establishment of a radioimmunoassay for pancreatic polypeptide

4.0.2. Validation of the radioimmunoassay

4.1 The regulation of pancreatic polypeptide release

4.1.1 The effects of nutrients on pancreatic polypeptide release

4.1.1.1 The effects of a mixed meal on serum pancreatic polypeptide concentrations

4.1.1.2 The effects of individual nutrients on serum pancreatic polypeptide concentrations

4.1.1.2.1 The effects of amino-acids on serum pancreatic polypeptic concentrations

4.1.2.2 2 The effects of glucose on serum pancreatic polypeptide concentrations

4.1.1.2.3 The effects of lipid on serum pancreatic polypeptide concentrations

4.1.1.2.4 The effects of water and electrolytes on serum pancreatic polypeptide concentrations

4.1.2 The effect of gastrointestinal hormones on serum pancreatic polypeptide concentrations

4.1.2.1 The effects of secretin on serum pancreatic polypeptide concentrations

4.1.2.2 The effects of cholecystokinin-pancreozymin on serum pancreatic polypeptide concentrations

4.1.2.3 The effects of gastrin on serum pancreatic polypeptide concentrations

4.1.3 The neural regulation of pancreatic polypeptide release

4.1.3.1 Adrenergic modulation of pancreatic polypeptide release

4.1.3.2 The effects of cholinergic stimulation on serum pancreatic polypeptide concentrations

4.1.3.3 Dopaminergic effects on pancreatic polypeptide release

4.1.4 The effects of miscellaneous substances on serum pancreatic polypeptide concentrations

4.1.4.1 The effects of somatostatin on serum pancreatic polypeptide concentrations

4.1.4.2 The effects of histamine H_2-receptor blockade on serum pancreatic polypeptide concentrations

4.2 Investigation of the molecular forms of circulating immunoreacitve pancreatic polypeptide by 10% polyacrylamide disc gel electrophoresis

4.3. Investigation of the metabolism and turnover kinetics of pancreatic polypeptide

4.3.1 Organ uptake of pancreatic polypeptide

4.3.2 The half-life time of disappearance of pancreatic polypeptide

CHAPTER IV

THE EXPERIMENTAL DESIGN

This chapter established the experimental design to answer the questions posed previously. Of cardinal importance and in common with all the studies is the accurate, reproducible and reliable measurement of serum PP concentrations. A radioimmunoassay was established to fulfil these criteria.

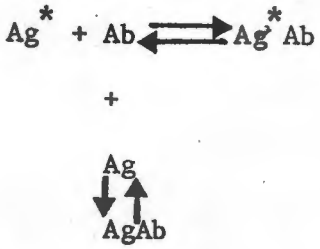
4.0 The measurement of pancreatic polypeptide by radioimmunoassay.

Since the establishment of the first radioimmunoassay for insulin by Yalow and Berson in 1959, this technique has become widely accepted and is possibly the most commonly used tool in endocrine research.

The principles of radioimmunoassay will be briefly discussed, but it must be constantly borne in mind that a radioimmunoassay measures the IMMUNOLOGICAL activity of a specific peptide in terms of its ability to bind to a specific antibody and that this immunological activity does not necessarity correlate with its BIOLOGICAL activity. This is especially true of PP, for which a physiological action has not been determined with any degree of certainty.

In brief, the basis of the radioimmunoassay is the competitive inhibition by a non-radioisotope-labelled hormone of the binding of a radio-isotope labelled hormone to a specific antibody.

Expressed diagrammatically,



Where Ag = Free hormone

Ag^* = Free radioisotope-labelled hormone

Ab = Specific antibody

Ag^*Ab = Labelled hormone - antibody complex

AgAb = Unlabelled hormone - antibody complex

it can be seen that as the concentration of the unlabelled hormone (Ag) is increased, the antibody (Ab) available for binding the labelled hormone (Ag^*) decreases because of the formation of Ag-Ab complexes. Thus by reacting unlabelled hormone of increasing and known concentrations (standards) with a fixed amount of Ag^* and Ab, one is able to produce increasing displacement of the Ag^* from the Ab. If the Ag^*Ab and AgAb complexes are separated from the Ag^* and Ag, a plot can be constructed of either Ag^* or the Ag^*Ab complex as a function of the unlabelled hormone concentrations (standards) added. This plot of the dose-response is known as the "standard curve". The concentration of a hormone in a plasma sample is determined by comparing the displacement of labelled hormone it causes, with the displacement caused by the known standard preparations.

4.0.1 Establishment of a radioimmunoassay for pancreatic polypeptide.

Thus in order to establish a radioimmunoassay, a purified hormone, radioisotope labelled hormone and an antiserum containing an antibody specific to the hormone are prerequisite . In the case of the PP radioimmunoassay, the following reagents were used:

(a) PURE hPP, for use as the standard reference preparation was a gift from Dr. R.E. Chance (See acknowledgements).

(b) hPP ANTISERUM, too, was a gift from Dr. Chance. It was raised in rabbits by initially injecting lmg of highly purified hPP homogenized in complete Freud's adjuvant, both subcutaneously and intramuscularly. Subsequent injections were given as 0,5mg hPP homogenized in complete Freud's adjuvant.

(c) RADIOISOTOPE - LABELLED bPP (the "tracer") was prepared by reacting radioactive iodine ^{125}I, with bPP which was obtained from Dr. Chance. A modification of the method described by Hunter and Greenwood (1962) for preparing radiolabelled growth hormone of high specific activity was used.

In preparing the tracer, the following factors were taken into account:

(i) the "specific activity" i.e. the degree to which the hormone was labelled.

(ii) the purity and stability of the tracer after the labelling procedure and in the assay system.

(d) Polythylene glycol (PEG) of molecular weight 6000 for affecting SEPARATION of the antibody-bound from the free hormones.

The details of the methodology to establish the assay and the results obtained are described in Appendix I.

4.0.2 Validity of the radioimmunoassay.

Once a "standard curve" was obtained, i.e. there was dose dependent inhibition by the hPP standards by the binding of the ^{125}I bPP to the antibody, the validity of the assay for measuring experimental serum samples was established.

(a) The effects of plasma and serum on the assay and the recovery of immunoreactive PP in serum samples was established.

(b) The cross-reaction and interreaction of the assay system with other hormones, biological substances and infusates was determined.

(c) The sensitivity, precision and reproducibility of the assay was assessed.

The methodologies and results of the validation procedures are reported in the Appendix.

Once the assay for measuring PP was validated and proven reliable, studies were designed to investigate the factors reviewed in Chapter III.

4.1 The regulation of pancreatic polypeptide release.

General Principles.

As stated previously, it is generally believed that the release of hormones and putative hormones is governed by feedback mechanisms. These feedback systems may vary from hormone to hormone. An elementary example may involve the effect of glucose on glucagon release. When there is a fall in the blood glucose concentration, glucagon is released to effect an increase in the glucose concentration and this in turn, "switches off" glucagon secretion (Foa, Santamaria, Berger et al, 1952; Unger, Eisentraut, McCall et al, 1962; Ohneda, Aguilar-Parada, Eisentraut et al, 1969). It is possible that such a simple feedback mechanism is involved in PP release. Using provocative stimulation to upset the homeostasis and open the feedback loops, may thus cause changes in the circulating PP concentration. This would then indicate factors effecting the release of PP and may suggest processes in which PP plays a part.

At the outset it must be stated that the stimuli chosen may not reflect the way in which stresses operate under the usual living conditions. The ingestion of pure lipid or pure glucose is not how these nutrients are normally eaten, since they are generally components of a mixed meal. However, the stress imposed by individual nutrients of a meal may help to elucidate the effects of a mixed meal. Furthermore the ingestion of a single nutrient may have additional uses, thus the oral glucose tolerance test certainly has value in the diagnosis of diabetes.

4.1.1 The effects of nutrients on pancreatic polypeptide release.

In assessing the effects of nutrients on serum pancreatic polypeptide concentrations, the effects of a mixed meal and of individual nutrients of a mixed meal will be investigated.

4.1.1.1 The effects of a mixed meal on serum pancreatic polypeptide concentrations.

A balanced diet requires the intake of carbohydrates, proteins, fat, vitamins, minerals and fluid. All these elements contained in a mixed meal would thus constitute a physiological stimulus to PP release. The effect of a mixed meal will thus be assessed.

4.1.1.2 The effects of individual nutrients on serum pancreatic polypeptide concentrations.

It is possible that only one or two constituents of the meal may affect PP release and thus the effects of some of the individual nutrients will be assessed. Furthermore, it is necessary to separate out the effects of the nutrients per se from the effects of the nutrients plus gastrointestinal factors (the "gastro-entero-pancreatic axis") on PP release. Therefore the nutrients will be administered both orally and intravenously and the effects compared.

4.1.1.2.1 The effects of amino acids on serum pancreatic polypeptide concentrations.

The amino acid arginine stimulates the release of the peptide hormones

insulin (Fajans, Floyd, Knopf et al, 1967), glucagon (Unger, Aguilar-Parada, Müller et al, 1970), and growth hormone (Knopf, Conn, Fajans et al, 1965) when administered intravenously. Arginine will be administered in a dose similar to that shown to stimulate the release of these hormones. It will be used in preference to a mixture of amino acids but other single amino acids e.g. alanine could be used as well. However, arginine is readily available in an acceptable form for injection and since studies in this laboratory using arginine infusion have been reported (Kalk, Vinik, Bank et al, 1974; Kalk, Vinik, Bank et al, 1974a), the technique has been validated.

4.1.1.2.2. The effects of glucose on serum pancreatic polypeptide concentrations.

The effects of oral and intravenous glucose will be compared in non-obese subjects, all of whom are to have normal glucose tolerance. No attempt will be made to attain similar blood glucose concentrations, but blood will be sampled earlier and more frequently following the administration of intravenous glucose. Oral and intravenous glucose tolerance tests using 50g and 25g of glucose as a 50% solution respectively are routine tests of glucose tolerance and will therefore be used in this study.

4.1.1.2.3. The effects of lipid on serum pancreatic polypeptide concentrations.

(a) Oral and intravenous lipid administration.

Because the effects of oral and intravenous lipid on PP release are to be compared, a commercially available lipid preparation suitable

for intravenous infusion will be used for both tests. "Intralipid" will be administered in the same way as the glucose i.e. by a rapid intravenous infusion, and by ingestion in non-obese subjects on separate occasions.

"Intralipid" is a fat emulsion which contains 100g of fractionated soybean oil, 12g of fractionated egg lecithin and 22,5g of glycerine BP per litre of distilled water. Since triglycerides are funnelled through a free fatty acid pool which is a dynamic, highly turning over pool, it is possible that a response to "Intralipid" may not indicate an effect of triglycerides per se, but rather be due to changes in the free fatty acid concentration. Thus it has been demonstrated that plasma glucagon for example, responds to changes in free fatty acid concentrations (Luyckx and Lefebvre, 1970) and the effect of free fatty acids on PP release will therefore be investigated.

4.1.1.2.4. The effects of water and electrolytes on serum pancreatic polypeptide concentrations.

The effect of water, in a similar volume to the lipid and glucose ingested, will be assessed. The PP response to a slow infusion of normal saline will also be investigated. Water is a nutrient in its own right but in addition, also acts as a control experiment for gastric distension, which may initiate a gastro-pancreatic neural reflex to cause PP release.

4.1.2 The effects of gastrointestinal hormones on serum pancreatic polypeptide concentrations.

Many hormones and candidate hormones may affect PP release. Because of their vast number, the hormones to be studied will be limited to the accepted and recognized gastrointestinal hormones - gastrin, secretin and cholecystokinin-pancreozymin. Although gastric inhibitory polypeptide has recently been added to the list of acknowledged gastrointestinal hormones, it was not available for infusion, and will therefore be excluded from the studies.

4.1.2.1. The effects of secretin on serum pancreatic polypeptide concentrations.

(a) Dose dependent release of pancreatic polypeptide.

"Boots" secretin is the only secretin preparation available in South Africa for use in humans. It is a crude secretin preparation and probably contains contaminating gastrointestinal peptides. Therefore, in addition to the secretin, the impurities in the preparation may cause PP release. Although under physiological circumstances the meal-stimulated release of gastrointestinal hormones is prolonged, in these experiments, the hormones will be administered as boluses, and the results will therefore be purely an "all or none" effect and will not relate to a physiological situation. However, it will be investigated whether secretin could have a dose-dependent action on PP release. In this laboratory, it will not be possible to measure the circulating secretin concentrations attained to compare with the concentrations observed after a meal, nor to relate the concentrations to the PP release.

(b) The effect of secretin on serum pancreatic polypeptide concentrations in vagotomised individuals.

Flushing and nausea are reported to commonly occur during the secretin infusions and may indicate stimulation of the autonomic nervous system. Since PP release depends on the integrity of the vagus (Schwartz, Rehfeld, Stadil et al, 1976), the secretin may affect PP release via vagal stimulation. The effect of secretin will therefore be assessed in patients who have undergone bilateral truncal vagotomy, and who have no evidence of insulin-hypoglycaemia induced gastric acid secretion six to twelve months after the procedure. The responses will be compared to those obtained in healthy subjects using the same doses of secretin.

4.1.2.2. The effects of cholecystokinin-pancreozymin on serum pancreatic polypeptide concentrations.

The "Boots" cholecystokinin-pancreozymin (CCK-PZ) preparation, like the secretin preparation, is not pure. It is feasible that shared impurities in the two "Boots" preparations may cause PP release. Like secretin, the Boots CCK-PZ will be administered as bolus doses.

4.1.2.3. The effects of gastrin on serum pancreatic polypeptide concentrations.

The carboxy tetrapeptide of gastrin is the biologically active portion of the molecule for gastric acid secretion. Pentagastrin will thus be administered intravenously as a bolus, to assess its affect on PP release. Although the amino-acid sequence of this portion of the

molecule is the same as the C-terminal portion of cholecystokinin-pancreozymin, the dose, the molecular size and the differences in the half-life time of the two peptides may cause differences in actions.

4.1.3 The neural regulation of pancreatic polypeptide release.

Since it is intended to study PP regulation in man, pharmacological manipulation of the autonomic nervous system will be used.

4.1.3.1. Adrenergic modulation of pancreatic polypeptide release.

The study will investigate the effects of combined adrenergic alpha- and beta-receptor stimulation and selective alpha- and beta-receptor agonism on PP release in normal people. Combined adrenergic stimulation will be achieved by infusing epinephrine alone, and the simultaneous infusion of either an alpha- or a beta- receptor blocking agent, will be used to cause independent stimulation of the unblocked receptor. Propronalol will be used as the adrenergic beta-receptor blocking agent and phentolamine to block alpha-receptors. In one group of subjects, combined adrenergic stimulation is to be followed by a period of alpha-receptor stimulation, followed by combined stimulation again, then by selective adrenergic beta-receptor stimulation. In another group of subjects, the periods of alpha- and beta-receptor stimulation will be reversed. As epinephrine is readily inactivated by oxidation, a reducing agent (ascorbic acid) will be added to all the epinephrine infusates to preserve their potency in these subjects. In an additional study, the effects of epinephrine which has not been preserved with ascorbic acid will be assessed.

An alternate method to cause independent alpha- and beta-receptor agonism, is to infuse their agonists, methoxamine and isoproterenol, respectively. These are synthetic pharmacological agents, whereas the method chosen allows selective stimulation by the naturally occurring catecholamine, epinephrine, and appears to be widely accepted in endocrine investigation.

4.1.3.2 The effects of cholinergic stimulation on serum pancreatic polypeptide concentrations.

The fact that in normal, healthy people, gastric acid secretion can be induced by insulin-hypoglycaemia, whereas subjects who have undergone a surgical vagotomy are unable to secrete acid during hypoglycaemia, indicates that hypoglycaemia causes stimulation of the vagus nerve. Thus, Hollander's test (1946) to assess the effectiveness of the vagotomy procedure, relies on the fact that insulin-induced hypoglycaemia is a stimulus of vagal, cholinergic activity.

Insulin-hypoglycaemia will thus be induced in healthy subjects to confirm that this procedure causes PP release, and to ascertain the levels of response in this laboratory. However, the reduction in blood sugar per se, may cause PP release, but as Adrian, Bloom, Besterman et al (1977) have shown that PP is not released by hypoglycaemia in vagotomised subjects, a positive response to hypoglycaemia in the subjects to be tested would indicate vagal stimulation of PP release.

4.1.3.3 Dopaminergic effects on pancreatic polypeptide release.

(a) Dopamine agonism: to achieve dopaminergic stimulation L-dopa will be administered orally to volunteers. L-dopa has a prolonged action (up to 8 hours) and the test will therefore be divided into two parts. Firstly, the effects of L-dopa alone, on serum PP concentrations will be assessed; and secondly, after a 90 minute interval, the subjects will eat the standard test meal (see 4.1.2.1) to determine whether an altered PP response is observed.

L-dopa will be used in preference to apomorphine as the dopaminergic agent for the following reasons: it is administered orally, is readily available and it may act directly on pancreatic dopaminergic receptors as opposed to apomorphine which may only have a central dopaminergic action (Lorenzi, Tsalikian, Bohannon et al, 1977). The ingestion of L-dopa with water, may by causing gastric distension, trigger PP release via a gastro-pancreatic neural reflex. A period of 90 minutes will be allowed in which to assess the PP response before the subjects are given the meal to eat.

As a marker for the effectiveness of the L-dopa, serum growth hormone concentrations will be measured as the release of growth hormone after L-dopa administration has been repeatedly documented (Boyd, Lebovitz and Pfeiffer, 1970; Eddy, Jones, Chakmakjian et al, 1971; La Rossa, Agrin and Melby, 1977).

(b) Dopamine antagonism: in addition to dopamine agonism, a dopamine antagonist will be administered to normal volunteers. Metoclopramide is a centrally acting dopaminergic-receptor blocking agent and has been

shown to cause prolactin release possibly by antagonizing dopamine-mediated secretion of prolactin inhibitory factor (McCullum, Sowers, Hershman et al, 1975). In addition to its anti-dopaminergic effect, metoclopramide also has cholinergic effects which could influence PP release. However, whether metoclopramide only has a central dopaminergic effect or has a peripheral action as well, is not known. Metoclopramide will be administered intravenously in a similar dose as that which has been used to assess the prolactin response (Minuto, Marugo, Giusti et al, 1977).

4.1.4 The effects of miscellaneous substances on serum pancreatic polypeptide concentrations.

In this category, studies to investigate the effects of somatostatin and of histamine H_2-receptor blockade will be assessed.

4.1.4.1 The effects of somatostatin on serum pancreatic polypeptide concentrations.

The inhibitory effect of somatostatin on insulin and growth hormone release appears to be dependent on the dose infused (Giustina, Peracchi, Reschini et al, 1975; Christensen, Nerup, Hansen et al, 1976). Furthermore, the doses of somatostatin required to suppress basal and stimulated hormone release may differ and thus, in assessing the effect of somatostatin on PP release, various doses will be infused.

The metabolic clearance rate and the half-life time of somatostatin in normal man have been calculated as 26,0 $\pm$ 3,9 ml/min-kg and 2,14 $\pm$ 0,20 min respectively (Sheppard, Shapiro, Berelowitz et al,

1977). Using these values, infusions of somatostatin, ranging from 2 to 10μg/min, can be calculated to raise the steady state systemic plasma concentrations of somatostatin from a basal of 274 ± 9pg/ml (Pimstone, Berelowitz, Kronheim et al, 1977) to approximately 500 - 2500pg/ml, which represents a 2 to 10 fold increase above the fasting concentrations. These increases in plasma somatostatin concentration far exceed the increase following nutrient stimulation in man (Pimstone, Berelowitz, Kronheim et al, 1977). However, as hepatic extraction of somatostatin occurs in rats (Pimstone, Berelowitz, Kronheim et al, 1977) and may occur in man, the somatostatin concentration in the portal vein may be far higher than in the systemic circulation and the levels at which a physiological effect of somatostatin occurs is therefore unknown.

In these studies, the effect of somatostatin will be assessed in the following ways:

(a) A "low-dose" of somatostatin will be infused to assess its effect on basal PP concentrations.

(b) The effect of this low dose of somatostatin on stimulated PP responses will be examined.

(c) The infusion of the low-dose of somatostatin will be preceded by a large priming bolus injection and the effect on basal PP concentrations will then be measured.

The dose of somatostatin to be used (2μg/min) can be calculated to cause a 2 fold rise (to approximately 570pg/ml) in the basal somatostatin concentration. After an initial priming dose of 200μg, the somatostatin concentration should then reach 6×10^4 pg/ml

initially, and still be approximately 10 times the basal concentration at 10 minutes. This latter level is unlikely to be in the physiological range, and it is conjectural to speculate that it approaches the concentrations at the pancreatic cells.

4.1.4.2 The effects of histamine H_2-receptor blockade on serum pancreatic polypeptide concentrations.

The effect of acute administration of cimetidine on PP release is to be assessed. As discussed earlier (see 4.1.1.2.4) the oral ingestion of water may have an effect on PP release, by initiating neural release of PP. Therefore, a volume of 150ml of water will be ingested half an hour before the cimetidine, and the cimetidine then given in an equal volume of water. The PP response will be measured basally, after water ingested alone and after cimetidine plus water.

Following these studies to assess the effects of nutrients, gastrointestinal hormones, manipulation of the sympathetic and parasympathetic nervous systems, dopaminergic substances, somatostatin and cimetidine on serum PP concentrations, the molecular forms of PP released into the circulation will be investigated.

4.2 Investigation of molecular forms of circulating immunoreactive pancreatic polypeptide by 10% polyacrylamide disc gel electrophoresis.

As there is doubt about the molecular homogeneity of PP, it was proposed to investigate for the presence of multiple immunoreactive molecular forms of PP in human serum Heterogeneous forms of the peptide may exist during basal conditions or only after provocative stimulation. The fact that PP may be released by the diverse effects of the vagus nerve and gastrointestinal hormones, suggests that provocation of PP release by these stimuli, may emphasize differences in molecular forms, should they be present.

Thus, in healthy subjects, PP release will be induced by the administration of Boots secretin and by activating the vagus by insulin-induced hypoglycaemia. The serum samples will then be fractionated to determine if more than one form of immunoreactive PP is present in the circulation.

Although differences in plasma PP have been found by criteria of molecular weight (Marco, Hedo and Villaneuva, 1977), it is possible that further separation of the molecules can be induced, using criteria of molecular charge as well as weight. A technique which combines both these attributes is polyacrylamide disc gel electrophoresis. This procedure has the further advantage of separating very small amounts of peptide, which can be relatively easily recovered for measurement by radioimmunoassay. To facilitate the separation procedure , and reduce the volume to be electrophoresed, the peptides in serum are concentrated after 95% ethanol extraction, a procedure which precipitates large molecular weight proteins. This

means that a peptide bound to a carrier protein and which could therefore be interpretted as being of a large molecular weight, would not appear as a distinct migration peak when separation is accomplished by polyacrylamide disc gel electrophoresis after alcohol extraction of the sample.

The density of the gel and the pH at which the electrophoresis is carried out, are variables that determine the rate and extent of migration. These factors can be adjusted to achieve maximum migration and separation. However, the physical properties of the gels, often determine the density at which they are used. Moreover, the pH at which the gel is prepared affects its handling properties. However, the techniques of polyacrylamide disc gel electrophoresis have previously been applied in this laboratory (Vinik and Jackson, 1975; Botha, Vinik, Child et al, 1977), thus allowing assessment of the reliability and validity of the procedure in the proposed studies.

To assess the relative migration of hPP during electrophoresis, known peptide "markers" will be co-electrophoresed with the unknown samples. The markers chosen will be:

1. Monocomponent insulin
2. Proinsulin
3. Glucagon

These are peptides of known purity and migration, and will therefore reflect not only the relative migration of the unknown sample, but also the uniformity and quality of the procedure. The migration of these peptides using the technique in this laboratory have previously been published (Botha, Vinik, Child et al, 1977).

4. ^{125}Iodine labelled hPP
5. ^{125}Iodine labelled bPP.

Both human and bovine PP are used in the radioimmunoassay (see Appendix). They differ from each other by two amino-acid residues and this may cause differences in their electrophoretic mobility.

In addition to the radiolabelled PP markers, non-radioactive or "cold" markers of PP will be used. hPP will be added to:

6. Phosphate (assay) buffer
7. Charcoal stripped (hormone-free) plasma
8. Freshly drawn heparinised blood, prior to separation of the plasma by centrifugation.

These markers will compare the electrophoretic properties of exogenous, highly purified hPP with endogenously released hPP, and furthermore will permit the calculation of the recovery of hPP after electrophoresis.

Thus in these studies it is hoped to gain insight into whether more than one immunoreactive component of PP is detected by the PP antiserum, and whether a number of such fractions are present in the circulation. Furthermore, an idea of the molecular weights or charges of various molecular forms of PP may be gained. It is then intended to investigate what happens to PP released into the circulation, in order to further understand the physiology of PP.

4.3 Investigation of the metabolism and turnover kinetics of pancreatic polypeptide.

Numerous techniques are available whereby the kinetics and metabolism of hormones in the body are assessed. They all have their advantages, disadvantages and their limitations. In general, these methods involve the injection of exogenous biological material into an experimental animal or subject, or the stimulation or inhibition of the endogenous biological substance. The kinetic behaviour of the substance is then followed by making serial measurements of its concentration. Exogenous material is injected as a bolus or as an infusion aimed at achieving steady state conditions. Furthermore, the material may be radiolabelled or may be in the native (cold) form. These methods presuppose the availability of such material for administration and that they behave like endogenous material, although in the case of gastrointestinal hormones, they are generally administered into a peripheral vein, whereas biologically they are released into the hepatic portal venous system. Other problems include deiodination of radiolabelled material, the purity of the material injected, the presence of molecular heterogeneity, and monitoring the circulating levels attained.

The lack of PP for injection, either as "hot" or "cold" material, coupled to the ethical considerations of injecting a "newly recognized substance" into man, dictated that the kinetic behaviour of endogenously released PP be studied.

4.3.1 The organ uptake of pancreatic polypeptide.

The technique of sampling blood simultaneously from multiple sites, thereby allowing measurement of PP concentrations across various organs, precludes the use of human volunteers in these studies. Pigs will therefore be used as experimental animals because they are routinely used as large experimental animals in this department, and are therefore readily available. This animal model has previously been used in this department for studies on the kinetics of gastrin (Vinik, Hickman and Grant, 1978) and glucagon (van Hoorn, Vinik and van Hoorn-Hickman, 1978). Simultaneous fasting blood samples from the hepatic portal vein, the hepatic vein, the carotid artery, the renal vein and the femoral vein, will provide samples to determine the PP concentration across the liver, heart-lungs, kidney and hind limb. However, calculation of the changes in the amount of peptide across these organs requires measurements of their blood flow. These data are available in animals of this type and weight (Vinik, Hickman and Grant, 1978) and will be used to estimate organ uptake of PP.

The multiple catheter technique is best undertaken in starved animals during anaesthesia. The effect of anaesthesia may well influence the results, which cannot therefore be described as basal, but may simulate fasting conditions.

4.3.2 The half-life time of disappearance of pancreatic polypeptide.

The half-life time ($t_{\frac{1}{2}}$) of endogenously released PP will be studied by inhibiting its release. It has been reported that 93% of PP is found in the pancreas in monkeys and baboons (Adrian, Bloom, Bryant

et al, 1976) but whether this applies to pigs is not known. The surgical removal of the pancreas may abolish the source of secretion if the situation in pigs is similar to monkeys. The procedure of total pancreatectomy in pigs has been successfully established by Drs. Van Hoorn and van Hoorn-Hickman (Van Hoorn, Vinik and Van Hoorn-Hickman, 1978) who kindly agreed to provide serial samples of blood following pancreatectomy in pigs for the determination of serum PP concentrations. The blood samples will be taken pre-operatively, intra-operatively, and post-operatively from the portal vein and the rate of disappearance of PP in the portal circulation will then be determined. The assessment of $t_{\frac{1}{2}}$ using this procedure, presumes that the pancreas is the sole source of PP and could thus bias the results in favour of a prolonged $t_{\frac{1}{2}}$. The pharmacological inhibition of PP release by somatostatin, would cause similar difficulties if total suppression of secretion were not achieved. Thus briefly, these studies are designed to investigate how endogenous PP is removed from the circulating pool. In an animal model, the pig, the organ uptake of PP as well as the rate of disappearance after total pancreatectomy will be investigated.

4.4 Serum pancreatic polypeptide concentrations in patients with diseases of the pancreas.

In diseases of the pancreas, altered circulating PP concentrations may be observed for numerous reasons. There may be a change in the rate or quantity of synthesis; the rate of secretion may be affected; a change in the sensitivity to release may occur; or there may be a change in the rate of removal or deactivation of the peptide. Thus examination of the serum PP concentration may be a product of these various phenomena and may not elucidate a specific reason for increased or decreased serum PP concentrations or responses in a specific disease. However, this approach is intended as the first step in defining abnormalities in the conditions to be discussed.

4.4.1 Serum pancreatic polypeptide concentrations in patients with acute pancreatitis.

In this study, serial changes in PP concentration will be measured in patients with acute pancreatitis. Initially the patients will all be fasted but with recovery will begin to eat. As a special precaution the administration of anticholinergic drugs will be withheld during the period of the study. The serum PP concentrations will be compared to levels in healthy subjects who will fast but not undergo any stress.

4.4.2 Serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

Patients who fulfil the diagnostic criteria of Bank, Marks, Moshal et al (1963) will be accepted as having chronic pancreatitis.

These criteria are:

1. The presence of one of:

a. a pancreatic effluent of less than 100 ml in 80 minutes

b. a mean duodenal bicarbonate concentration of less than 60mEq/l

c. a mean duodenal amylase concentration of less than 5,0 units/ml

or 2. The presence of any combination of two of:

a. a pancreatic effluent of 100 - 140 ml in 80 minutes

b. a mean duodenal bicarbonate concentration of 60-70mEq/l

c. a mean duodenal amylase concentration of 5,0-6,0 units/ml

after sequential pancreatic stimulation with "Boots" secretin and cholecystokinin.

The basal and stimulated PP concentrations will be measured in these patients.

4.4.2.1 Basal serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

The serum PP concentrations will be measured after an overnight fast. Diabetes will not be a contra-indication to inclusion in the study. A retrospective correlation of the PP concentration with the degree of pancreatic insufficiency will not be attempted in patients who had previously been diagnosed, as such a correlation may be invalidated by ongoing disease.

4.4.2.2 Stimulation of pancreatic polypeptide release in patients with chronic pancreatitis.

Examination of PP release in the patients with chronic pancreatitis will follow the guide lines discussed for normal subjects.

4.4.2.2.1 The effects of nutrients on serum pancreatic polypeptide concentrations.

A mixed meal, intravenous arginine and oral and intravenous lipid will be administered to patients with chronic pancreatitis to compare the effects with those in normal matched subjects. With lipid, the chronic pancreatitis patients will be divided into two groups viz those with and those without symptomatic steatorrhoea. The patients with steatorrhoea may have poorer lipid absorption than the patients without steatorrhoea, which may be reflected in the responses to PP.

4.4.2.2.2 The effects of gastrointestinal hormones on serum pancreatic polypeptide concentrations.

In this study, only secretin will be used to assess its effects on PP release in patients with chronic pancreatitis. The responses will be compared to those found in healthy subjects.

4.4.2.2.3 The effects of insulin-hypoglycaemia on serum pancreatic polypeptide concentrations.

The effect of insulin-induced hypoglycaemia on PP release will be compared in chronic pancreatitis patients and normal controls.

However, as a consequence of diabetes, patients with chronic pancreatitis may have vagal neuropathy and thus exhibit imparied release of PP during hypoglycaemia. Therefore, only patients without signs and symptoms of overt autonomic neuropathy will be tested.

4.4.2.3 Inhibition of pancreatic polypeptide release in patients with chronic pancreatitis.

It is possible that somatostatin exerts its effects directly on the cell to inhibit release of its products. If diffusion of somatostatin from the systemic or the portal circulations to the cells of the pancreas were inhibited by pancreatic fibrosis and distortion, somatostatin may fail to exert its action. Thus the effects of an infusion of somatostatin on insulin-hypoglycaemia induced PP release will be investigated in patients with chronic pancreatitis.

4.4.3 Serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

As in the patients with chronic pancreatitis, basal and stimulated concentrations of PP will be measured. Many insulin-treated diabetics have a factor in their blood which interferes with the PP radioimmunoassay. These patients are reported to have circulating antibodies to PP (Klaff, Vinik, Berelowitz et al, 1978) and will be excluded from the studies. All measurements of PP will therefore be made in maturity-onset diabetics who are not insulin-requiring and are treated with oral hypoglycaemic agents.

4.4.3.1 Basal serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

Fasting PP concentrations will be measured in maturity-onset diabetics between the ages of 35 and 65 years.

4.4.3.2 Stimulated serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

Insulin-hypoglycaemia, a reproducible provocative stimulus of PP release in normal subjects, will be used in diabetics. As in the chronic pancreatitis patients, autonomic neuropathy will be excluded by numerous tests of autonomic function. The results will then be compared with those obtained in matched subjects.

4.5 Summary.

These studies have been designed to examine the release of PP under various conditions, to investigate the molecular forms of PP released into the circulation, to assess the organ uptake and rate of disappearance of circulating PP and to determine how the serum PP concentrations differ from normal in patients with acute pancreatitis, chronic pancreatitis and diabetes mellitus.

Thus, in healthy subjects the serum PP response to a mixed meal, to intravenous arginine, to oral and intravenous glucose, to oral and intravenous lipid and to the endogenous release of free fatty acids will be assessed. The effect of secretin, cholecystokinin-pancreozymin and pentagastrin will be studied, and it will further be determined whether secretin causes dose-dependent release of PP and whether this release is dependent on vagal integrity. The influence of combined and independent adrenergic α- and β-receptor stimulation, of insulin-induced hypoglycaemia and of dopamine agonism and antagonism will be determined; the effect of histamine H_2-receptor blockade by cimetidine on serum PP concentrations will be investigated and finally, the effect of somatostatin on both basal and stimulated release of PP will be assessed.

By using polyacrylamide disc gel electrophoresis, the molecular forms of immunoreactive PP in the circulation will be investigated, after stimulation of release has been achieved by the administration of secretin and activation of the vagus by insulin-induced hypoglycaemia. The organ uptake and rate of disappearance of PP in pigs will then be determined, by simultaneously taking multiple blood samples from different sites to determine PP gradients across various organs and by following

the disappearance of PP from the circulation after total pancreatectomy in these animals.

The final section deals with serum PP concentrations in patients who have various diseases involving the pancreas - both exocrine and endocrine. The serum PP levels in these patients will be compared to the levels and responses in healthy subjects. The PP levels in patients with acute pancreatitis during the acute illness and with recovery will be examined. The basal PP levels in patients with chronic pancreatitis, as well as the responses to a meal, to lipid, to secretin, to insulin-induced hypoglycaemia and to somatostatin will be compared to the levels in healthy subjects. Finally, the serum PP concentrations in non-insulin treated diabetics and the responses to insulin-induced hypoglycaemia will be assessed.

Thus the studies outlined above are predominantly applied clinical studies in healthy volunteers and in patients with diseases involving the pancreas. In addition, an animal model, the pig, will be used to study aspects of the metabolism and turnover of endogenous PP, since it will not be feasible to carry out these studies in man.

A detailed account of the subjects, the materials and the experimental procedures employed, is described in the next chapter.

CHAPTER V

MATERIALS AND METHODS

CHAPTER V MATERIALS AND METHODS

CHAPTER V

MATERIALS AND METHODS

5.0 General protocol.

To prevent repetition, the general protocol describes factors which are common to all studies carried out in man.

Each person who volunteered for a study, signed a written, informed consent after a careful explanation of the tests. These studies, where indicated, had been approved by the Ethical Review Committee of the University of Cape Town Medical School. All the tests were started between 8 and 9 a.m., after the volunteers had fasted for at least 12 hours. The tests were carried out with the subjects at rest, either comfortably seated or reclining on couches.

In the ensuing text, "normal" or "healthy" people, or "controls" refers to persons who had no personal or immediate family history of endocrine abnormalities, were not taking any medication and had no clinical or biochemical evidence of diabetes, metabolic disease or chronic illness. They had no recent history of trauma and none had been hospitalised in the preceding 2 years. Blood was sampled and intravenous test substances were administered via a 19 gauge infusion set (Wings, AHCS, SA (Pty) Ltd.) inserted into an antecubital vein and kept patent by a slow infusion of normal saline. At least two basal blood samples were taken 15 minutes apart before the administration of test substances. Blood samples were centrifuged to separate the plasma or serum and the samples were then stored as multiple 1ml aliquots in polystyrene cuvettes (LKB-Producter, Sweden) at -20^{o}C until specific laboratory measurements

were made.

Serum or plasma glucose concentrations were measured on the Technicon Auto Analyser II (Technicon Instrument Corporation, Tarrytown, New York) using either the ferricyanide method of Hoffman (1937) or the glucose oxidase method (Trinder, 1969). Serum triglycerides were also measured on the Technicon Auto Analyser II using the method described in the Technicon Auto Analyser II publication - Clinical Methods No. SE 4 - 0023 FF3. Free fatty acid (FFA) concentrations were measured by the colorimetric method of Duncombe (1964) as modified by Itaya and Ui (1965). Immunoreactive insulin and human growth hormone were assayed using kits purchased from Sorin, Italy. These assays have been used and validated in this laboratory for numerous previous publications (Vinik, Kalk, Keller et al, 1973; Vinik, Kalk, Botha et al, 1976; Botha, Vinik, Le Roith et al, 1977).

Serum PP concentrations were measured by a specific radioimmunoassay which is described in detail in the Appendix.

5.0.1 Materials.

For convenience, the details of the materials and drugs used and their sources of supply are listed below. The details are not again included under the description of the specific clinical studies.

1. Neutral monocomponent porcine insulin (Actrapid insulin) was obtained from Novo Industries, Copenhagen, Denmark.

2. Sterile, lyophilised secretin and cholecystokinin-pancreozymin (CCK-PZ) were obtained from the Boots Company, Ltd., Nottingham, England. Each vial of secretin was reconstituted with normal saline to a concentration of 10 Crick-Harper-Raper units per ml immediately before use. The CCK-PZ was reconstituted with 20 ml of normal saline.

3. Sterile L-arginine monochloride (Merck Laboratories) buffered to pH 7,6 in saline was obtained from the Groote Schuur Hospital dispensary.

4. Fifty grams of dextrose monohydrate BP, was obtained locally and dissolved in 200 ml of water before oral ingestion.

5. Sterile 50ml ampoules of Dextrose BP 25g as a 50% (w/v) solution, were obtained from Saphar Laboratories.

6. "Intralipid", a 10% solution of emulsified fat, containing 100g of fractionated soybean oil, 12g of fractionated egg lecithin and 22,5g of glycerin BP per litre was manufactured by Vitrum, Sweden and purchased locally.

7. Peptavlon - synthetic pentagastrin, was obtained from ICI, Macclesfield, Great Britain.

8. Epinephrine (Petersens Ltd.), ascorbic acid injection, Propranolol (Inderal, ICI, Macclesfield, Great Britain), and Phentolamine (Regitine, Ciba, Switzerland) were purchased locally.

9. Lyophilised synthetic cyclic somatostatin was kindly donated by Dr. E. Polakow of Ayerst Laboratories. It was dissolved in normal saline to the concentration required before administration.

10. L-Dopa (Larodopa, Roche) as 500 mg tablets were purchased locally.

11. Metoclopramide (Primperan) for injection was a gift from Berk Pharmaceuticals.

12. Cimetidine (Tagamet) was purchased locally from Smith, Kline and French Laboratories. Two 200mg tablets were dissolved in 150 ml of water before ingestion by the test subjects.

5.0.2 Statistical Analysis.

Statistical analysis of the data was carried out on a Hewlett-Packard 9830A desk-top calculator and a 9866A printer (Hewlett-Packard, Calculator Products Division, Colorado, USA) according to the methods described by Snedecor and Cochrane (1967). All the PP results were analysed for significance using non-parametric statistics by the Wilcoxon signed rank test for paired data and the Mann-Whitney U test for unpaired data. Other parameters were analysed using the Student t-test, the t-test, and analysis of variance. In all studies, statistical significance was accepted at the 5% level i.e. $p < 0,05$. The Institute of Biostatistics of the Medical Research Council of South Africa was consulted for statistical advice.

TABLE 5.1 THE TEST MEAL

	Protein (g)	Fat (g)	Carbohydrate (g)	KJoules	(Cals)
2 boiled eggs	14	14	-	756	181
250 ml milk	8	8	12	714	171
30g skim milk powder	10	-	15	420	100
2 slices bread	4,4	1	30	605	145
20g butter	-	20	-	756	181
20g honey	-	-	15	252	60
TOTAL	36,4	43	72	3503	838

TABLE 5.1 Protein, Fat and Carbohydrate content and energy value of the test meal.

5.1 The regulation of pancreatic polypeptide release.

The details of the tests as they were carried out are described. They follow the order of the previous chapters.

5.1.1 The effects of nutrients on pancreatic polypeptide release.

5.1.1.1 The effects of a mixed meal on serum pancreatic polypeptide concentrations.

The PP responses to a mixed meal were measured in 7 normal subjects. They were 32 to 54 years old, weighed 51 to 82 kg and were 156 to 183 cm tall. All were within the desirable weight for height range according to tables of the Metropolitan Life Insurance Company as quoted in Documenta Geigy.

The test meal comprised two boiled eggs, two slices of bread, 20g of butter, 20g of honey and 250ml of milk to which 30g of skim milk powder had been added. The meal provided 36,4g of protein, 43g of fat and 72g of carbohydrate. A comprehensive analysis of the meal is shown in Table 5.1.

Three basal blood samples were taken at 15 minute intervals, after which the meal was eaten. Five minutes after starting to eat, a blood sample was taken and further blood samples were then drawn 5, 15, 30, 45, 60, 90, 120 and 180 minutes after its completion.

5.1.1.2 The effects of individual nutrients on serum pancreatic polypeptide concentrations.

5.1.1.2.1 The effects of amino-acids on serum pancreatic polypeptide concentrations.

Five healthy male volunteers who were 50-59 years old, weighed 59 - 78kg and were 169 - 179cm tall were tested. After two basal blood samples had been taken, arginine was infused at a rate of 10mg/kg/min via an indwelling canula in one arm for 30 minutes using a constant infusion pump Type 71100 (B. Braun, Germany). Blood was sampled from an indwelling canula in the opposite arm at 10, 20, 30, 40, 60 and 90 minutes. The serum glucose and PP concentrations were measured.

5.1.1.2.2 The effects of glucose on serum pancreatic polypeptide concentrations.

Nine persons consented to undergo both oral and intravenous glucose tolerance tests on separate occasions. 50g of oral glucose and 25g of intravenous glucose, injected over 3 minutes, were given in random order. Blood samples for glucose and PP measurements were taken 30, 60 and 90 minutes after oral and 3, 6, 10, 20, 30 and 60 minutes after the intravenous glucose administration.

5.1.1.2.3 The effects of lipid on serum pancreatic polypeptide concentrations.

a. <u>Oral and intravenous lipid administration.</u>

Seven persons of whom 6 were men, were each tested on two occasions.

They were 30 to 60 years old, weighed 55 to 72 kg and were 165 to 173 cm tall. Each subject was given 1ml/kg of "Intralipid" orally on one occasion, and on the other, the same volume of "Intralipid" was infused intravenously over 3 minutes. The order of the tests was randomised. Blood samples were taken 5, 10, 20, 30, 45, 60, 90 and 120 minutes after the lipid administration. Serum triglycerides, glucose, free fatty acids and PP concentrations were measured for each subject.

b. Endogenous free fatty acid release.

Eight normal subjects who were 28 to 68 years old, weighed 64 to 88kg and were 164 to 190cm tall, each received 3 intravenous bolus doses of heparin of 3000U, 15 minutes apart. Blood was sampled 10, 20, 30, 40, 60 and 90 minutes after the first heparin injection. Serum free fatty acid, glucose and PP concentrations were measured.

5.1.1.2.4 The effects of water and electrolytes on serum pancreatic polypeptide concentrations.

150ml of water was given to 6 normal people (ages 28 to 41 years, weights 51 to 83 kg, and height 156 to 184 cm) to drink. Blood was sampled 5, 15 and 30 minutes later. A further six people (age 33 to 51 years, weights 59 to 74 kg, and height 169 to 177 cm) received a slow intravenous infusion of normal saline over 30 minutes and blood samples were taken at 5, 10, 20 and 30 minutes. The serum PP concentrations were measured.

5.1.2 The effects of gastrointestinal hormones on serum pancreatic polypeptide concentrations.

"Boots" secretin, "Boots" cholecystokinin-pancreozymin and synthetic pentagastrin, were administered to healthy volunteers on separate occasions as rapid infusions each over two minutes, to assess the effects on serum PP concentrations.

5.1.2.1 The effects of secretin on serum pancreatic polypeptide concentrations.

a. The dose-dependent effects of secretin.

Five normal subjects aged 29 to 49 years, who weighed 46 to 80kg and were 164 to 180 cm tall were tested. Secretin doses of 0,5, 1,0, 1,5 and 2,0 Crick-Harper-Raper Units (CHRU) per kg were sequentially infused, each over 2 minutes, at 30 minute intervals. Blood was sampled 1, 3, 5, 10, 15 and 30 minutes after each secretin infusion.

b. The effect of secretin administration in vagotomised individuals.

The completeness of a surgical truncal vagotomy in seven people was confirmed by the absence of a gastric acid response to insulin-hypoglycaemia (Hollanders test; Hollander, 1946). These tests were carried out 6 to 12 months after the surgical procedure. They were 29 - 51 years old, weighed 47 - 94 kg and were 168 to 188cm tall. Each was given an intravenous infusion of 2 CHRU/kg of secretin over 2 minutes. Blood was sampled 1, 3, 5, 15, 30, 45 and 60 minutes later.

c. The effect of secretin administration in healthy controls.

The seven control subjects were 29 to 48 years old, weighed 54 to 85kg

and were 166 to 190cm tall. They received intravenous secretin as described in 5.1.2.1b above, i.e. 2 CHRU/kg of secretin was administered over 2 minutes and blood was sampled 1, 3, 5, 15, 30, 45 and 60 minutes later.

5.1.2.2 The effects of cholecystokinin-pancreozymin on serum pancreatic polypeptide concentrations.

Seven normal people aged 30 to 42 years, who weighed 50 to 82kg and were 156 to 193cm tall, each received 85 CHRU of cholecystokinin-pancreozymin (CCK-PZ) intravenously over two minutes. Blood was sampled 1, 3, 5, 15, 30, 45 and 60 minutes later.

5.1.2.3 The effects of gastrin on serum pancreatic polypeptide concentrations.

An intravenous infusion of 5µg/kg of pentagastrin was infused into seven healthy people over 2 minutes. They were 28 to 39 years old, weighed 56 to 77kg and were 161 to 184cm tall. Blood was also sampled 1, 3, 5, 15, 30, 45 and 60 minutes after the pentagastrin had been given.

In the preceding experiments when secretin, CCK-PZ and pentagastrin were infused, i.e. 5.1.2.1, 5.1.2.2 and 5.1.2.3 serum PP concentrations were measured.

5.1.3 The neural regulation of pancreatic polypeptide release.

5.1.3.1 The effects of adrenergic modulation on pancreatic polypeptide release.

Eighteen healthy subjects between the ages of 22 and 58 years, were divided into 3 aged-matched groups. Each group contained five males and a female. They weighed 55 to 100kg and were 160 to 185cm tall. An intravenous cannula was inserted into an antecubital vein of each arm, one for the administration of the test substances and the other, for blood sampling.

The studies were then carried out in 3 separate parts, a separate group of subjects being used for each part. In the first protocol, epinephrine was infused alone and no attempt was made to prevent inactivation of the epinephrine by oxidation. In the second and third parts of the study, epinephrine which was preserved with ascorbic acid, was first infused alone, and then infused together with either the adrenergic α-receptor antagonist, phentolamine, or with the adrenergic β-receptor antagonist, propranolol. In the second and third protocols, the epinephrine was diluted in normal saline containing 20mg/100ml of ascorbic acid to prevent inactivation of the epinephrine by oxidation. As an additional step to ensure potency of the epinephrine for the duration of the study, freshly prepared infusates were started every 30 minutes. In all the studies epinephrine was infused at a rate of 6μg/min by using a constant infusion pump, Type 71100 (B. Braun, Germany).

In the first part of the study, i.e. in protocol I, after 3 basal samples had been taken at 15 minute intervals, an infusion of normal saline was given for one hour and blood was sampled at 15 minute intervals. Then epinephrine at a rate of 6μg/min was infused simul-

taneously with the saline for a further 2 hours and blood was sampled at 5, 10, 20, 30, 60, 75, 90 and 120 minutes. A single epinephrine infusate was given throughout the two hour period, and ascorbic acid was not added to the infusion.

In the second group of subjects, after a 30 minute basal period, a 6µg/min infusion of epinephrine was started to run for the duration of the study. During the first 30 minutes, blood samples were taken at 15 minute intervals. A 10mg bolus of propranolol was then given over two minutes and propranolol then infused at a rate of 0,1mg/min to run concurrently with the epinephrine infusion. Blood was sampled 3, 5, 15, 30, 45 and 60 minutes later. At 60 minutes the propranolol infusion was stopped and epinephrine infused alone for a further 60 minutes. Blood was sampled at 75, 90 and 120 minutes. A 5mg bolus of phentolamine was then injected over two minutes and followed by an infusion of 0,5mg/min, again to run concurrently with the epinephrine until termination of the study 60 minutes later. Blood was sampled at 123, 125, 135, 150, 165 and 180 minutes.

In the third protocol, the initial epinephrine infusion was prolonged to 60 minutes during which time blood was sampled at 15 minute intervals. The protocol was then repeated except that the periods of propranolol and phentolamine infusions were reversed, thus allowing the adrenergic β-receptor stimulation to precede the period of α-receptor stimulation. Blood samples were taken at the times described above.

In all the subjects serum glucose, free fatty acids, insulin and PP concentrations were estimated.

5.1.3.2 The effects of cholinergic stimulation on serum pancreatic polypeptide concentrations.

Five normal males who were 50 to 59 years old, weighed 59 to 78kg . and were 169 to 179cm tall, were made hypoglycaemic by an intravenous injection of 0,1 U/kg of porcine monocomponent insulin. Blood was sampled 10, 20, 30, 45, 60, 70, 80 and 90 minutes after the insulin injection for measurement of blood glucose and serum PP concentrations.

5.1.3.3 Dopaminergic effects on pancreatic polypeptide release.

To examine the effects of dopamine agonism and antagonism on serum PP concentrations, L-dopa and metoclopramide were administered to different individuals on separate occasions.

a. Dopamine agonism: six people aged 27 to 59 years, who weighed 54 to 70 kg and were 155 to 184 cm tall, were tested. The subjects each ingested one 500mg L-dopa tablet with 100ml of water. Blood was sampled at intervals of 15 minutes for 90 minutes. The test meal as described in 5.1.2.1 was then eaten and blood was sampled as previously, during the meal and 15, 30, 45, 60, 90, 120 and 180 minutes after its completion.

b. Dopamine antagonism: one ampoule of metoclopramide (10mg) was given as an intravenous injection over two minutes to six healthy people. They were 28 to 40 years old, weighed 51 to 82 kg, and were 156 to 186 cm tall. Blood for PP assay was sampled 1, 3, 5, 10, 20, 40, 60 and 90 minutes later.

5.1.4 The effects of miscellaneous substances on serum pancreatic polypeptide concentrations.

5.1.4.1 The effects of somatostatin on serum pancreatic polypeptide concentrations.

a) "Low" doses of somatostatin.

The subjects in whom the effects of insulin-hypoglycaemia had been assessed (see 4.1.3.2) were tested again. Somatostatin in normal saline was infused at 2µg/min using a constant infusion pump while blood samples were taken at intervals for an hour. Then PP release was provoked by the injection of 0,1U/kg of monocomponent insulin and further blood samples were taken 10, 20, 30, 45, 60, 70, 80 and 90 minutes later. The blood glucose and serum PP concentrations were measured.

b) A preceding "priming" dose of somatostatin.

In 6 males aged 29 to 51 years, who weighed 59 to 74kg and were 169 to 177cm tall, a 200µg bolus of somatostatin was injected and this was followed by a constant infusion of 2µg/min of somatostatin for 30 minutes. Blood was sampled at 5, 10, 20, 30 and 45 minutes to assess the effect on basal PP concentrations.

5.1.4.2 The effects of histamine H_2-receptor blockade on serum pancreatic polypeptide concentrations.

The effects of cimetidine on serum PP concentrations were assessed in six subjects. They were 28 to 41 years old, weighed 51 to 83 kg and were 156 to 184 cm tall. They were given 150ml of tap water to drink and blood was sampled 5, 15 and 30 minutes after drinking the water. At 30 minutes, they were again given 150ml of water

to drink, this time containing 400mg of cimetidine in solution. Blood was sampled at 35, 40, 45, 60, 90, 120 and 150 minutes for assessing serum PP concentration.

5.2 Investigation of the molecular forms of circulating immunoreactive pancreatic polypeptide by 10% polyacrylamide disc gel electrophoresis.

In order to assess whether PP circulates in multiple molecular forms, the release of PP was provoked in six healthy, young persons who were 19 to 22 years old. Three were made hypoglycaemic with insulin according to the procedure described in 5.1.3.2 while three each received an infusion of 2CHRU/kg of secretin as described in 5.1.3.1c above. Aliquots of serum from these tests were assayed to determine their PP concentrations and 1 ml of the serum samples containing the peak PP concentrations from each person were then prepared for electrophoresis.

The following additional samples were also prepared:

1. 2ml of blood was taken from a healthy fasting volunteer and injected into a test tube containing 50U of heparin. 100 000pg of hPP in 100µl of 0,25M phosphate buffer at pH 7,4 was immediately added to the sample which was then agitated and centrifuged, after which 1ml of plasma was decanted and stored at -20°C until fractionated.

2. Charcoal stripped plasma was prepared by adding 20mg of washed activated charcoal (Sigma Chemical Company) to each millilitre of old, pooled human plasma. The mixture was agitated and then centrifuged to precipitate the charcoal. The procedure was repeated twice on the supernatant. Before electrophoresis 100 000pg of hPP in 100µl of 0,25M phosphate buffer at pH 7,4 was added to 0,9ml of the charcoal treated plasma.

3. A solution of 20 000pg/ml of hPP in 0,25M phosphate buffer at pH 7,4 was prepared. 100µl was electrophoresed.

4. Solutionsof 1mg/ml of "monocomponent" insulin, proinsulin and glucagon were prepared. They were kindly donated by Dr. Lise Heding of Novo Institute, Denmark. 2,5µg of each was fractionated.

5. Lastly, radiolabelled hPP and bPP were both prepared as described in the Appendix. 50µl of each was electrophoresed.

The serum samples from the test persons and the marker plasmas (1 and 2 above) were then extracted in ethanol and concentrated. To 1 ml os sample, 1,8ml of dehydrated alcohol BP was added. The tubes were thoroughly mixed and centrifuged at 2000rpm at 4°C for 30 minutes. The supernatants were then decanted and evaporated to dryness in a stream of nitrogen. Prior to fractionation the samples were redissolved in 200µl of distilled water and 100µl of the resultant sample was then fractionated by electrophoresis as described below.

Electrophoresis was carried out using 10% polyacrylamide gels in Tris-glycine buffer at pH 8,5 and at 4°C. A Shandon SAE 2731 apparatus was used for the electrophoresis, which was carried out according to the method of Cain and Pitney (1968) as modified by Lazarus, Gutman and Recant (1971). The method is fully described in the Appendix.

After completion of the electrophoretic procedure, the gels containing the three markers, insulin, proinsulin and glucagon were stained with 0,5% amido black 10B (Merck Laboratories), then destained with a 7% acetic acid solution to show up the peptide band in each. The other gels were sliced into segments each of which were 3mm in length.

The radioiodine containing gel segments were counted on a Packard Auto-gamma scintillation spectrometer Model 5260, whereas the segments from the remaining gels were incubated overnight in 1,5ml of 0,1M ammonium hydroxide at 4°C. They were vigorously mixed, lyophilised, and finally they were reconstituted in 500µl of the PP assay buffer, prior to the assay being carried out. The PP content of the gels was determined for each 3mm segment. The migration of each of the markers and of the unknown samples was expressed in relation to the extent of migration of bromphenol blue, the reference material added to each gel which migrated the most anodally to indicate the gel front. The distance each substance migrated was assigned a value, known as the R.F. (reference front) value which was derived from the distance it migrated over the distance the bromphenol blue migrated from the point of origin. The extent of migration of the substances in gels of unequal length could thus be compared by plotting the PP concentrations against the R.F. values.

5.3 Investigation of the metabolism and turnover kinetics of pancreatic polypeptide.

The procedure performed in healthy pigs to determine the organ uptake and the half-life time of endogenous PP are described below.

5.3.1. Organ uptake of pancreatic polypeptide.

Six young pigs (Landrace X Large Whites) of either sex, weighing 18 to 22kg were anaesthetised after 24 hours of starvation. Anaesthesia was induced with 2 - 3mg/kg of thiopentone sodium injected into an ear vein and was maintained with oxygen and nitrous oxide, administered via a cuffed endotracheal tube. Catheters were then inserted into:

1. The right carotoid artery
2. The right internal jugular vein for slow normal saline administration
3. A hepatic vein via the right external jugular vein
4. The proximal part of the hepatic portal vein
5. The left renal vein via the left femoral vein
6. The right femoral vein.

The portal vein catheters were introduced intra-abdominally via a branch of the splenic vein and the hepatic vein catheter was manipulated into position by intra-abdominal palpation.

The catheter in the carotid artery was used to monitor arterial pressure as well as for arterial blood sampling.

After completion of the operative procedure a 30 minute period was allowed for stabilization of the animals during which time the blood pressure was carefully monitored. Blood samples were then simultaneously taken from the arterial, the hepatic, the portal, the renal and femoral venous catheters. In three pigs, samples were also taken from the pancreatico-duodenal vein by needle puncture. Serum was separated by centrifugation and the samples stored at -20°C for later assay of PP concentrations.

To determine the hepatic and renal uptake of PP, the total hepatic and portal hepatic as well as the renal blood flow measurements were required. The hepatic blood flow (Hickman, Saunders and Terblanche, 1974; Vinik, Hickman and Grant, 1978) and the renal blood flow (Dr. Rosemary van Hoorn-Hickman, personal communication) have been determined in pigs of similar size and weight. These figures were used to estimate the amount of PP taken up by the liver and kidney.

The mass of PP in pmols retained by the liver per minute was calculated by the equation:

$$\text{Hepatic uptake (pmol/min)} = \left[(PPf \times PPc) + (Af \times Ac) \right] - HfxHc$$

where PPf = proximal portal blood flow in ml/min

PPc = proximal portal concentration of pancreatic polypeptide in pmol/ml

Af = hepatic arterial blood flow in ml/min

Ac = concentration of pancreatic polypeptide in the arterial circulation in pmol/ml

Hf = hepatic vein blood flow in ml/min

Hc = concentration of pancreatic polypeptide in hepatic vein in pmol/ml.

The percentage of pancreatic polypeptide which was retained by the liver in relation to the total mass presented to it was therefore:

$$\% \text{ PP retained by the liver per minute} = \frac{\left[(PPf \times PPc) + (Af \times Ac) - (Hf \times Hc)\right] \times 100}{(PPf \times PPc) + (Af \times Ac)}$$

The renal uptake of PP was similarly calculated, but in this case it was assumed that the total renal inflow of blood equalled the outflow.

Thus the amount of PP in pmols taken up by the left kidney per minute was calculated by the equation

$$\text{Renal uptake (pmol/min)} = (Ac \times Rf) - (Rc \times Rf)$$

where Ac = concentration of pancreatic polypeptide in the arterial circulation in pmol/ml

Rf = renal vein blood flow in ml/min

Rc = concentration of pancreatic polypeptide in the renal vein in pmol/l

The percentage (%) of PP retained by the kidney was calculated by the equation:

$$\% \text{ PP retained by the kidney per minute} = \frac{(Ac - Rc) \times 100}{Ac}$$

The renal and hepatic uptake of PP were individually calculated for each pig.

5.3.2 The half-life time of disappearance of pancreatic polypeptide.

Total pancreatectomies were kindly performed in 4 large White Landrace X pigs, weighing 20 to 25 kg by Drs. Van Hoorn and van Hoorn-Hickman as has been previously described (Van Hoorn, Vinik, van Hoorn-Hickman, 1978). A catheter in the internal jugular vein was used to infuse normal saline and a catheter in the hepatic portal vein to sample blood. The pigs were starved for 24 hours before the operative procedure. Portal vein blood samples were taken before the pancreatectomy was started, during the pancreatectomy, and 1, 3, 5, 10, 20 and 30 minutes after the last pancreatic vein permitting pancreatic venous effluent to enter the portal vein was ligated.

The PP concentrations in these latter samples were used to calculate the half-life time ($t_{\frac{1}{2}}$) of PP in the portal circulation, according to the formula $t_{\frac{1}{2}} = 0{,}693/K$ (Conard, Farnckson, Basterie et al, 1953) where K is derived from the linear regression of the $\log_e$ of PP concentration against time using a Hewlett Packard 9830A calculator and 9862A printer.

5.4 Serum pancreatic polypeptide concentrations in patients with diseases of the pancreas.

In most instances, the experimental procedures in this section are identical to those carried out in the healthy subjects and to avoid repetition, the reader is referred to the appropriate section. However, this is not the case for patients with acute pancreatitis.

5.4.1 Serum pancreatic polypeptide concentrations in patients with acute pancreatitis.

Seventeen patients aged 30 - 50 years with a typical history of acute pancreatitis and an initial serum amylase concentration of greater than 600 Pimstone units per 10ml (normal range 40-140; Pimstone, 1964) had basal blood samples taken on admission. The patients stated that they had not eaten or taken any medication for 4 to 6 hours before admission. Alcohol was an aetiological factor in pancreatitis in the majority of cases.

Nine of the 17 patients then had further blood samples taken 3 hourly for 12 hours, 6 hourly for the next 36 hours and then daily until they were discharged from the hospital. They were all fasted for at least 24 hours, received an intravenous infusion of 0,25% saline with 5% dextrose, had nasogastric decompression and analgesics. Anticholinergic drugs were withheld.

Blood samples for glucagon and PP radioimmunoassays were collected in heparinised tubes with added aprotinin (Trasylol, Bayer Leverkusen, Germany). 0,5ml of the plasma was lyophilised and sent to Dr. T.W.

TABLE 5.2 THE CLINICAL AND PANCREATIC STATUS OF THE PATIENTS WITH CHRONIC PANCREATITIS

PANCREATIC FUNCTION STUDIES

PANCREATIC EFLUENT

Patient	Age years	Weight kg	Height cm	OGTT[1]	Volume ml	Bicarbonate mEq/l	Amylase units	Additional evidence
1	43	75	172	N - D[2]	98	39	10,10	calcification[4]
2	65	54	174	D[3]	110	18	0,39	"
3	51	55	182	D	164	25	1,78	laparotomy
4	20	56		D	128	36	0,11	"
5	68	75	185	D	128	16	0,13	
6	55	57		D	97	22	0,79	calcification
7	53	60	160	D	2,34	26	1,30	"
8	39	55	160	D	63	15	0,82	"
9	54	57		N - D	154	66	2,35	"
10	51	60	167	N - D	80	60	1,13	"
11	62	65		D	187	21	0,18	"
12	40	83		D	132	45	0,72	
13	28	44	162	N - D	122	39	1,25	calcification
14	65	54	160	D	100	30	1,82	laparotomy
15	28			N - D	laparatomy confirms calcification			
16	48	61		D	37	32	1,47	
17	36	51	177	N - D				calcification
18	45	55		N - D	84	25	0,62	"
19	41	51	170	D	laparotomy confirms calcification			
20	43	60	181	N - D	119	28	1,50	
21	58	65	164	D	292	44	0,68	calcification
22	36	55	179	N - D	278	57	1,38	
23	52	61	167	N - D	172	20	0,06	calcification
24	41	45	170	N - D	39	32	2,05	
25	58	65	160	N - D	129	46	2,82	calcification
26	39	74	180	N - D	138	78	12,80	"
27	58	44	160	D	72	46	8,70	"
28	53	50	174	N - D	83	74	0,51	"
29	34	61	180	N - D	469	84	6,14	"

OGTT - oral glucose tolerance test

N - D - not diabetic

D - diabetic Criteria of Jackson and Vinik, 1973

GTT criteria

Criteria for abnormal pancreatic function (Bank, Marks, Moshal et al, 1963):

1. Presence of one of the following:
   a) 80-min volume of < 100ml
   b) mean duodenal bicarbonate concentration of < 60 mEq/litre
   c) mean duodenal amylase concentration of < 5,0 U/ml
2. Presence of any combination of two of the following:
   a) 80-min volume of 100-140 ml
   b) mean duodenal bicarbonate concentration of 60-70 mEq/litre
   c) mean duodenal amylase concentration of 5,0 - 6,0 U/ml

[1] Fifty gram oral glucose tolerance test

[2] Not diabetic

[3] Diabetic by criteria of Jackson and Vinik (1970)

[4] Pancreatic calcification on an X-ray of the abdomen

Schwartz at the Institute of Medical Biochemistry, University of Aarhus, Denmark, who kindly assayed these samples for PP according to his method which has been published (Schwartz, Rehfeld, Stadil et al, 1976). At this time, the radioimmunoassay for PP was being validated in this laboratory, and Dr. Schwartz kindly agreed to assay these samples. Dr. Schwartz further confirmed that lyophilisation and reconstitution of plasma samples did not adversely affect PP measurements in the assay. The PP measurements in these patients are thus not comparable to PP measurements obtained in the assay described in this thesis, but can be compared with measurements in healthy subjects as reported by Schwartz, Rehfeld, Stadil et al (1976) in his assay system.

Immunoreactive glucagon (IRG) was measured by radioimmunoassay using Ungers 30K antiserum (Dr. R. Unger, Dallas) according to the method described by Vinik and Hardcastle (1974), and blood glucose by Auto Analyser (Technicon, Tarrytown, New York) using the automated neocuproine method (Bittner and Manning, 1966).

5.4.2 Serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

Table 5.2 lists the ages, weights and heights as well as the pancreatic functions and glucose tolerance of the patients with chronic pancreatitis who were tested in the subsequent studies. In these patients measurements of basal as well as stimulated serum PP concentrations were carried out.

5.4.2.1 Basal serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

Each patient listed in table 5.2 had blood taken on at least two occasions for assay of fasting PP concentrations. The basal results are presented as the average of these observations for each patient. For comparison, blood was taken from fifty healthy subjects in the same age range. Again, at least two samples were taken from each subject.

5.4.2.2 Stimulation of pancreatic polypeptide release in patients with chronic pancreatitis.

As was the case in the healthy subjects, numerous stimuli were administered to the patients with chronic pancreatitis to assess their effects on the serum PP concentrations. The tests followed the format described for the healthy subjects who were thus used as controls for the subsequent studies. Thus in the ensuing sections, the details of the tests are not repeated, but reference is made to the appropriate sections where the protocols are given.

5.4.2.2.1 The effects of nutrients on serum pancreatic polypeptide concentrations.

a. The test meal as described in 5.1.1.1 was carried out in seven patients with chronic pancreatitis.

b. Intravenous arginine as described in 5.1.1.2.1 wad administered to 5 patients with chronic pancreatitis.

c. Oral and intravenous lipid (see 5.1.1.2.3a) was administered to 13 patients with chronic pancreatitis.Seven of the patients (23-29, table 5.2) had symptomatic steatorrhoea and were treated with pancreatic enzyme supplements; the other 6 were asymptomatic. On the day of the test procedure, pancreatic enzyme supplements were not given. A further 8 patients, two of whom had steatorrhoea, were given intravenous heparin (see 5.1.2.2.3b) to cause elevation of the endogenous free fatty acid concentrations.

5.4.2.2.2 The effects of gastrointestinal hormones on serum pancreatic polypeptide concentrations.

Seven patients were given an intravenous secretin infusion of 2CHRU/kg as described in 5.1.2.1c.

5.4.2.2.3 The effects of insulin-hypoglycaemia on serum pancreatic polypeptide concentrations.

Hypoglycaemia was induced in 5 patients by an intravenous insulin injection of 0,1U/kg according to the protocol described in 5.1.3.2.

5.4.2.3 Inhibition of pancreatic polypeptide release in patients with chronic pancreatitis.

In the 5 patients who had undergone insulin tolerance tests (5.4.2.2.3) the test was repeated during a concommitant infusion of 2µg/min of somatostatin as described in 5.1.4.1a.

In all the above studies, blood was sampled at the same intervals as in the healthy subjects.

5.4.3 Serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

Patients with maturity onset diabetes mellitus, who were not insulin requiring and who attended Groote Schuur Hospital diabetes clinic, were tested. In all, the presence of autonomic neuropathy was excluded by a series of tests as outlined below.

In none of the patients was there:

a. A fall in the systolic blood pressure of more than 20mmHg on assuming the standing from the supine position (Campbell, Ewing, Clarke et al, 1974).

b. A resting heart rate of more than 90 beats per minute.

c. A valsalva ratio of less than 1,2 (Levin, 1966). This ratio was determined by measuring the shortest R-R interval on an electrocardiogram tracing while the patients exhaled for 15 seconds against a pressure of 40mmHg on a pressure gauge and the longest R-R interval within 15 seconds of stopping to forcibly exhale.

d. A variation in heart rate of less than 15 beats per minute with deep respiration (Campbell, Ewing, Clarke et al, 1974). An electrocardiogram tracing of the heart rate was made while the patients breathed in and out deeply. From the shortest R-R interval during inspiration, the heart rate per minute was computed; this was similarly done for the longest R-R interval during expiration.

The difference in these rates indicated the heart rate variation with deep respiration.

5.4.3.1 Basal serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

Twenty patients with diabetes mellitus, had blood taken on at least two occasions for assay of fasting PP concentrations. The patients ranged in age from 35 to 72 years, whereas the ages in the healthy subjects ranged from 21 to 65 years and in the patients with chronic pancreatitis from 28 to 65 years. As previously, the basal PP level for each patient was taken as the average value of the observations.

5.4.3.2 Stimulated serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

Insulin tolerance tests were performed in 6 patients aged 42 to 72 years. In addition, the procedure was also carried out in 5 age matched (45 to 68 years) controls. After the basal blood samples had been taken, the diabetics were given 0,2U/kg of monocomponent porcine insulin as an intravenous bolus, whereas the controls were given the standard dose of 0,1U/kg. Blood was sampled 20, 40, 60 and 90 minutes thereafter for measurement of the serum glucose and PP concentrations.

5.5 Summary.

This chapter described the details of the subjects, materials and the experimental procedures used in these studies. The laboratory procedures are not described in this chapter but are to be found in the Appendix. The first section (5.1) described the applied clinical studies in healthy subjects, the majority of whom were 30 to 60 years old, who were not obese and none of whom had any history of acute or chronic illness. All the studies were carried out after the volunteers had fasted overnight. In groups of between 5 and 10 subjects, the serum PP concentrations were measured in response to: a standardised mixed meal; an intravenous infusion of arginine at 10mg/kg/min; 50g of oral glucose and 25g of intravenous glucose on separate occasions; 1ml/kg of oral and the same volume of intravenous "Intralipid" on separate days; intravenous heparin, administered as 3 bolus doses each of 3000U, to raise endogenous circulating free fatty acid concentrations; oral water and intravenous saline; the gastrointestinal hormones - "Boots" secretin, cholecystokinin-pancreozymin and gastrin administered intravenously; intravenous epinephrine, epinephrine plus propranolol (adrenergic α-receptor stimulation) and epinephrine plus phentolamine (adrenergic β-receptor stimulation); insulin-induced hypoglycaemia; 500mg of oral L-dopa and a dopamine antagonist, metoclopramide given as a 10mg intravenous injection; somatostatin infused at a rate of 2μg/min; and 400μg of the histamine H_2-receptor blocking agent, cimetidine, which was administered orally.

The second section of the chapter (5.2) described the method employed to determine whether PP in the serum circulates in more than one immunoreactive form. Thus in 6 healthy subjects, endogenous PP release was

provoked by insulin-induced hypoglycaemia or by an intravenous dose of "Boots" secretin, and serum samples then fractionated on 10% polyacrylamide disc gel electrophoresis. The migration of endogenous PP was compared to ^{125}I-bPP, ^{125}I-hPP, and pure hPP added to the assay buffer, to whole human blood and to human plasma.

The hepatic, renal and peripheral uptake of endogenous PP was then determined in 6 pigs (5.3) by assessing concentration gradients across the liver, kidney and the hind limb. Blood was simultaneously sampled from multiple sites in starved, anaesthetised pigs, and since the blood flow to the liver and kidney was known in animals of this type and weight, the molar uptake of PP by these organs could be determined. Furthermore, to establish the half-life time of endogenous PP in pigs, the disappearance of PP from the portal vein was measured following total pancreatectomy in 4 animals.

In the last section of the chapter (5.4), the serum PP concentrations were assessed in patients with acute pancreatitis, in patients with chronic pancreatitis and in patients with diabetes mellitus. In 17 patients with diagnosed acute pancreatitis, the serum PP concentrations were measured when the patients were admitted to hospital. Changes in the serum PP concentrations were then followed in 9 of these patients at regular intervals during their recovery. To examine the effects of chronic pancreatitis on serum PP concentrations, fasting measurements were made in 29 patients with established pancreatic exocrine insufficiency. The serum PP responses to the standardised mixed meal, intravenous arginine, oral and intravenous "Intralipid", intravenous heparin, intravenous "Boots" secretin, insulin-induced hypoglycaemia, and intravenous somatostatin, were compared to the responses observed

in healthy subjects under the same experimental conditions.

Finally, in 20 patients with non-insulin requiring maturity-onset diabetes mellitus, the fasting PP concentrations were measured and in 6 of these patients, the PP response to insulin-induced hypoglycaemia was compared to the response in healthy, matched control subjects.

In all these studies statistical analysis of the PP results was done using non-parametric statistics on a Hewlett Packard 9830A calculator and significance was accepted at the 5% level.

The next chapter will describe the results of these studies.

CHAPTER VI

RESULTS

CHAPTER VI RESULTS

CHAPTER VI

RESULTS

6.0 Introduction

A description of the salient characteristics of the results obtained are described in this chapter. The results are presented as the mean ± standard errors (S.E.) but for detailed reference the PP results are tabulated in the Appendix. In all the studies where significant changes were observed, the results are graphically illustrated on the page preceding the text. The order of the previous chapter is repeated.

Although the basal or unstimulated serum PP concentrations varied from group to group, it must be stated at the outset that no statistical significance was found in this variation. The responses, however, differed markedly.

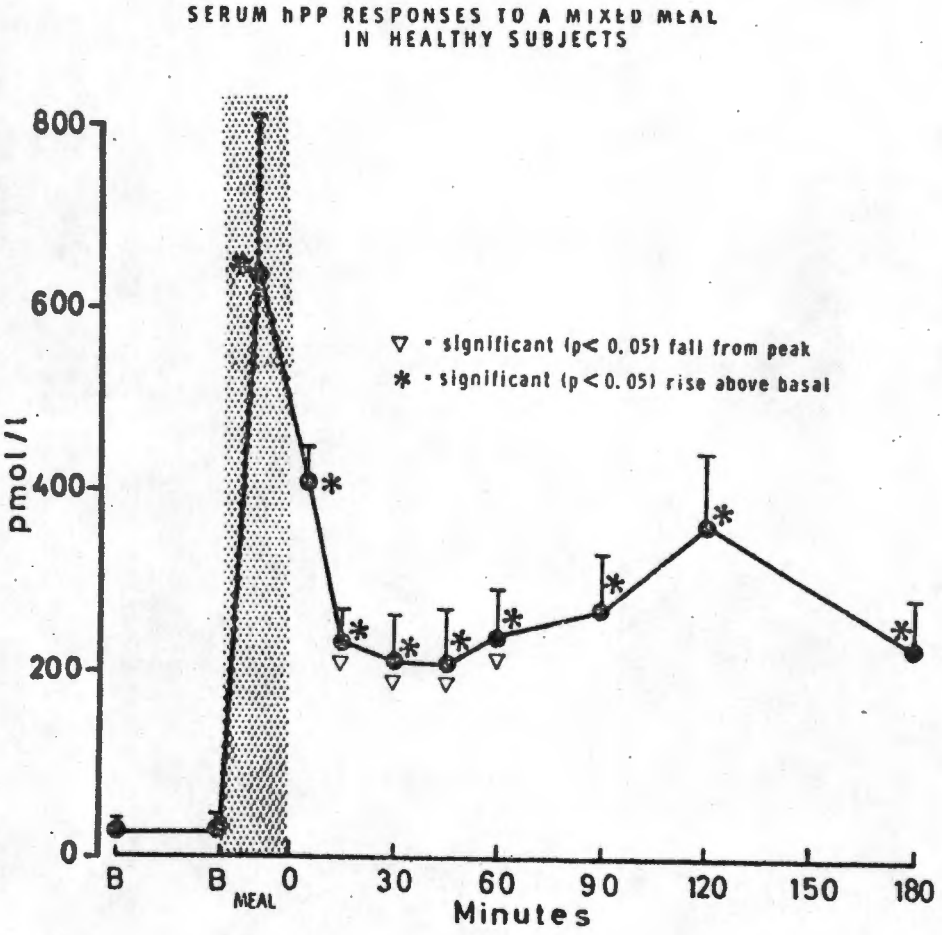


Figure 6.1

Mean $\pm$ S.E. serum hPP responses to a mixed meal in 7 healthy subjects. The cross-hatched area indicates the duration of the meal; the asterisks indicate a significant ($p < 0.05$) rise above the basal concentration and the triangles a significant ($p < 0,05$) fall from the peak concentration.

6.1 The regulation of pancreatic polypeptide release

6.1.1 The effects of nutrients on pancreatic polypeptide release.

6.1.1.1 The effects of a mixed meal on serum pancreatic polypeptide concentrations.

In the 7 healthy subjects, there was a significant rise in the serum hPP concentration during the meal (Fig. 6.1). The serum hPP concentrations rose from a basal level of 17 ± 2,8pmol/l to a peak of 648 ± 166,5 pmol/l 5 minutes after starting to eat. The serum hPP concentrations fell significantly after completion of the meal, to a trough concentration of 215 ± 61,4pmol/l at 45 minutes, which was still significantly elevated above the basal concentrations. A second small rise was then observed, but although significantly greater than the basal concentration, was not significantly higher than the trough concentration. This second peak was not significantly lower than the first peak concentration at 5 minutes. During the entire period over which blood samples were taken, the hPP concentration remained significantly elevated above the basal concentrations.

6.1.1.2 The effects of individual nutrients on serum pancreatic polypeptide concentrations.

6.1.1.2.1 The effects of amino-acids on serum pancreatic polypeptide concentrations.

The intravenous infusion of 10mg/kg/min of arginine was not associated with a change from basal in the serum hPP concentrations; nor was

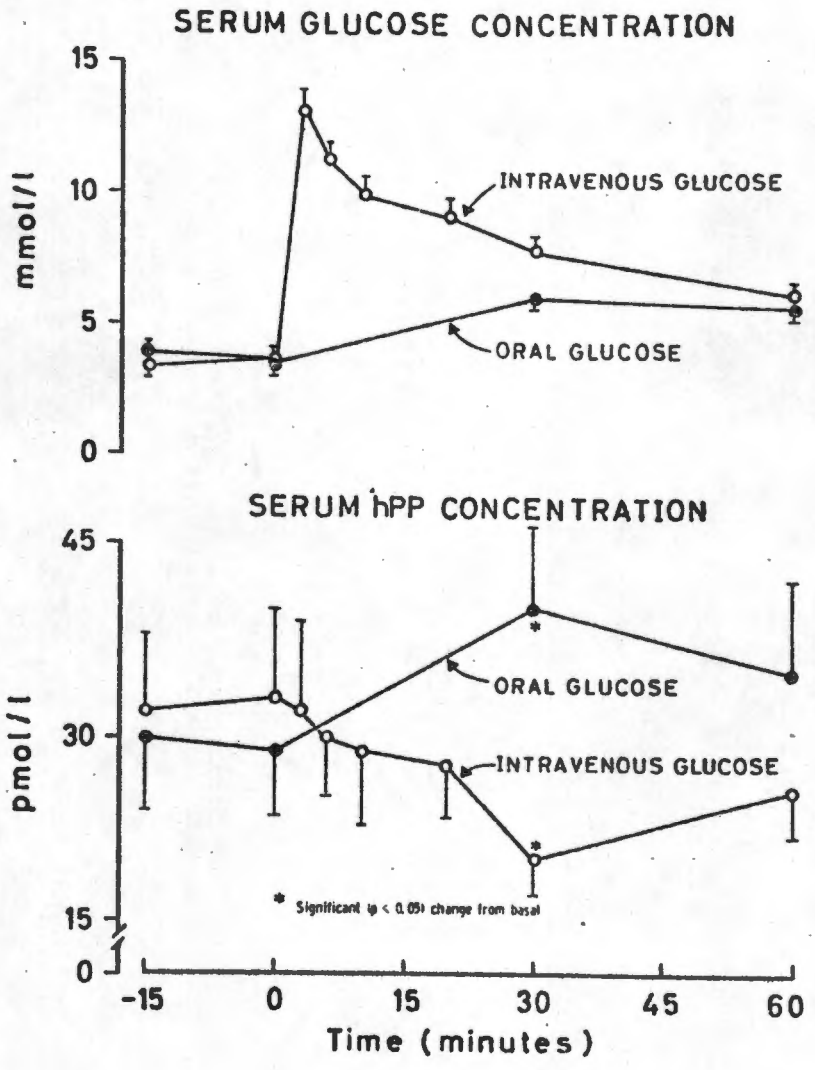


Figure 6.2

Mean $\pm$ S.E. serum glucose and hPP responses to 50g of oral, and 25g of intravenous glucose infused over 3 minutes in 9 healthy subjects. Each subject underwent both tests in random order.

there a change in the blood glucose levels during this time.

6.1.1.2.2 The effects of glucose on serum pancreatic polypeptide concentrations.

The serum hPP responses to oral and to intravenous glucose were very different. Oral glucose caused a rise, and intravenous glucose a fall, in the serum hPP concentrations (Fig. 6.2).

Glucose tolerance was normal in all the subjects by the criteria of Jackson and Vinik (1970). After oral glucose, serum glucose rose from 3,6 $\pm$ 0,13 mmol/l to 6,4 $\pm$ 0,60 mmol/l at 60 minutes and the serum hPP concentration rose significantly from 28 $\pm$ 4,5 pmol/l to 40 $\pm$ 6,2 pmol/l at 30 minutes. After the intravenous glucose bolus, the peak glucose concentration of 13,2 $\pm$ 0,76 mmol/l was observed at 3 minutes. The serum hPP concentration, however, showed a slow steady fall from 32 $\pm$ 5,9 pmol/l to a nadir of 20 $\pm$ 2,6 pmol/l which occurred at 30 minutes ($p < 0,04$). The fall in serum hPP concentrations thus lagged behind the peak glucose concentration but may have been associated with the falling glucose concentration.

Neither after oral nor after intravenous glucose was rebound hypoglycaemia observed. Serum hPP concentrations also did not show a rebound phenomenon in any of the studies and was returning towards the basal concentrations at the end of the studies.

After oral glucose ingestion there was a significant correlation between the changes in the mean glucose concentration and the mean hPP concentration ($r = 0,9473$, $p < 0,01$) but a correlation was not found between

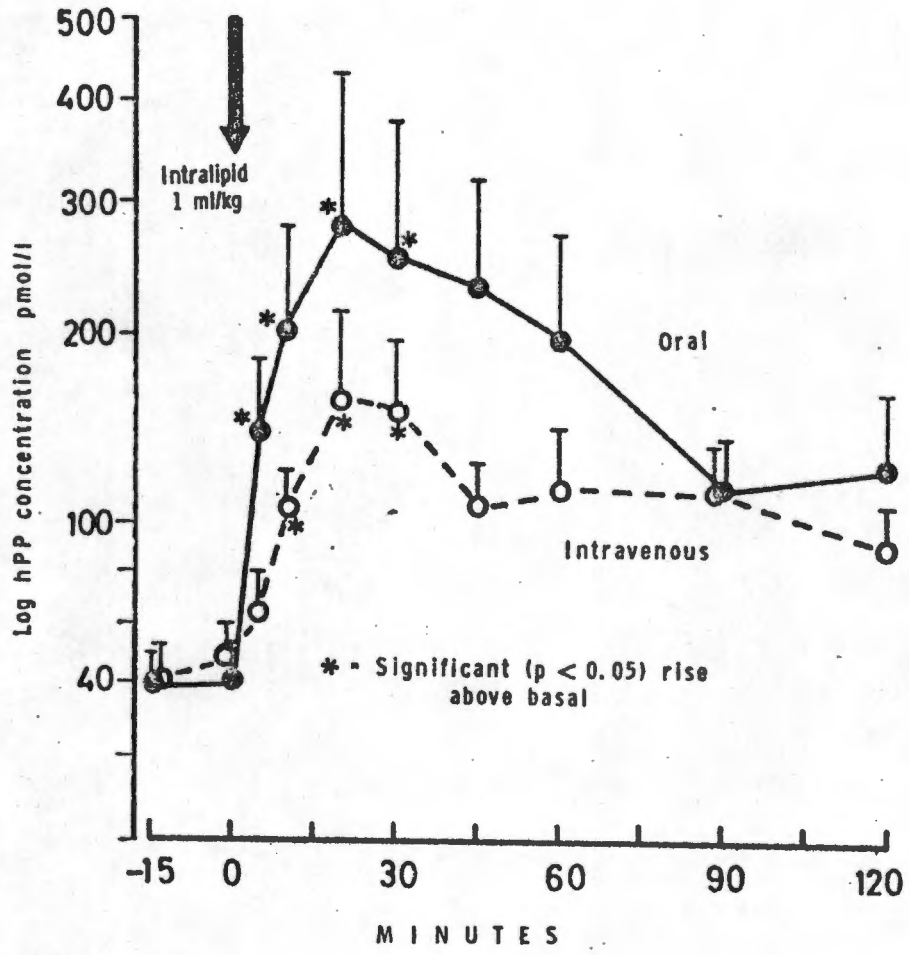


Figure 6.3

Mean $\pm$ S.E. serum hPP responses to oral and intravenous lipid in 7 healthy subjects. Each subject received 1ml/kg of 'Intralipid' orally and intravenously over 3 minutes on separate occasions.

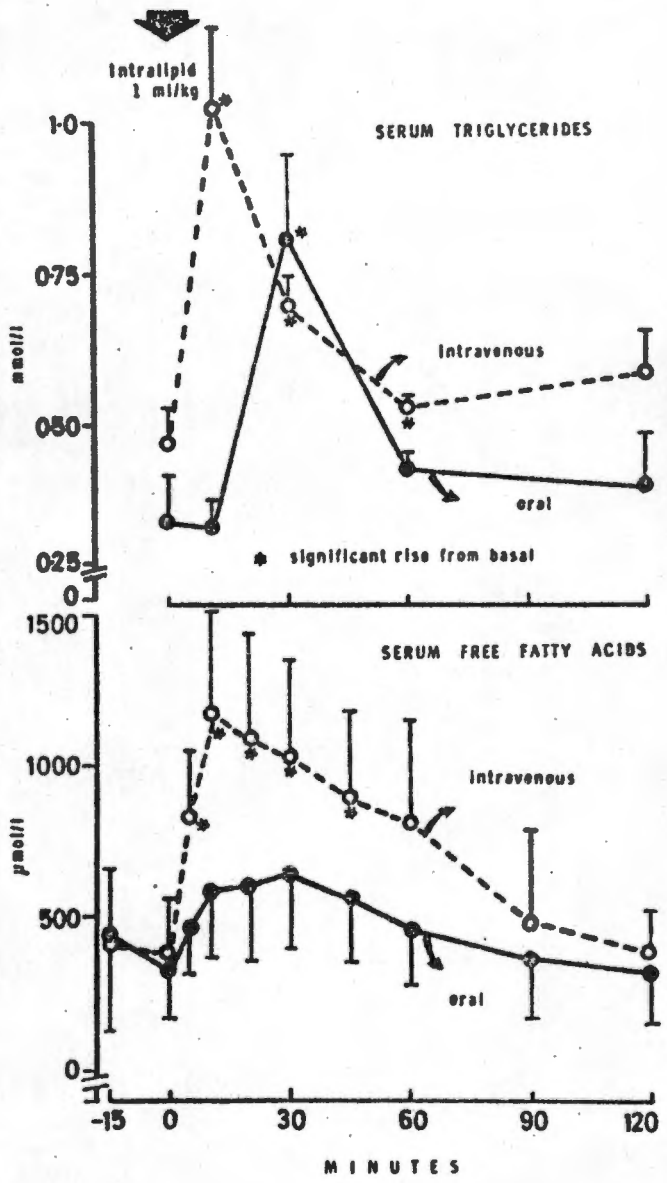


Figure 6.4

Mean $\pm$ S.E. serum triglyceride and free fatty acid responses to oral and intravenous lipid in 7 healthy subjects. Each subject received 1ml/kg of 'Intralipid' orally and intravenously over 3 minutes on separate occasions.

the serum glucose and serum hPP concentrations after intravenous glucose administration.

6.1.1.2.3 The effects of lipid on serum pancreatic polypeptide concentrations.

a. Oral and intravenous lipid administration.

Both oral and intravenous "Intralipid" caused a significant rise in the serum hPP concentration, this despite a marked interpatient variation in the basal levels and responses. To reduce this variability for purposes of presentation, the mean hPP concentrations are illustrated on a semi-logarithmic plot (Fig. 6.3). Although oral lipid caused a rise in the mean serum hPP concentration to 282 ± 154,5 pmol/l at 20 minutes and intravenous lipid to only 160 ± 50,1 pmol/l, which also occurred at 20 minutes, the differences in the peak responses were not statistically significant.

Both oral and intravenous "Intralipid" caused the serum triglyceride levels to rise significantly. After oral "Intralipid", the triglyceride concentrations rose from 0,39 ± 0,10 to 0,93 ± 0,16 mmol/l (triolein equivalents) at 30 minutes, whereas after the intravenous infusion, the peak concentration of 1,17 ± 0,18 mmol/l was observed within 10 minutes (Fig. 6.4). There was no significant rise in the serum free fatty acid concentrations after oral "Intralipid", but with intravenous "Intralipid" a significant increase from 902µmol/l (oleic acid equivalents) to 1670µmol/l was observed at 10 minutes (Fig. 6.4).

b. Endogenous free fatty acid release.

Intravenous heparin induced a four-fold rise in the serum FFA concentration from 528 ± 96,4 µmol/l to 2333 ± 239,9 µmol/l (Fig. 6.5).

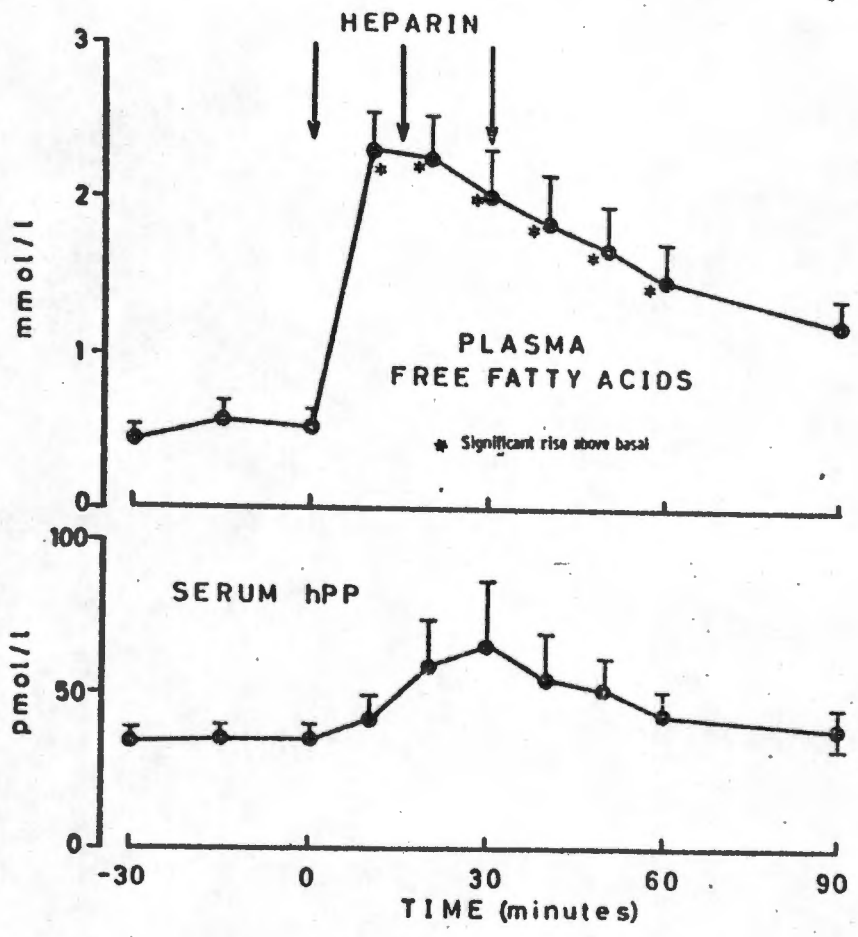


Figure 6.5

Mean $\pm$ S.E. plasma free fatty acid and serum hPP responses to intravenous heparin in 8 healthy subjects. Bolus infections, each of 3000u of heparin, were given at 15 minute intervals.

Although a small rise in the serum hPP concentration was associated with the elevation in FFA concentrations, the hPP elevation was not statistically significant.

6.1.1.2.4 The effects of water and electrolytes on serum pancreatic polypeptide concentrations.

Neither a drink of 150 ml of water, nor a saline infusion caused significant change in the serum hPP concentrations.

Summary of the effects of nutrients on serum hPP concentrations.

The pattern of the serum hPP responses to these nutrients was very variable. The mixed meal caused prolonged release of hPP, which was seen as a sharp, early rise in the serum hPP concentration and was followed by prolonged elevation at a lower level. Oral glucose, oral "Intralipid" and intravenous "Intralipid" administration also caused the serum hPP concentrations to rise, but to lower levels than those observed after the meal, and which returned to basal much earlier. Intravenous glucose was associated with a fall in the serum hPP concentrations, and intravenous arginine, endogenous free fatty acid release, intravenous saline and a drink of water had no significant effect on basal serum hPP concentrations.

6.1.2 The effects of gastrointestinal hormones on serum pancreatic polypeptide concentrations.

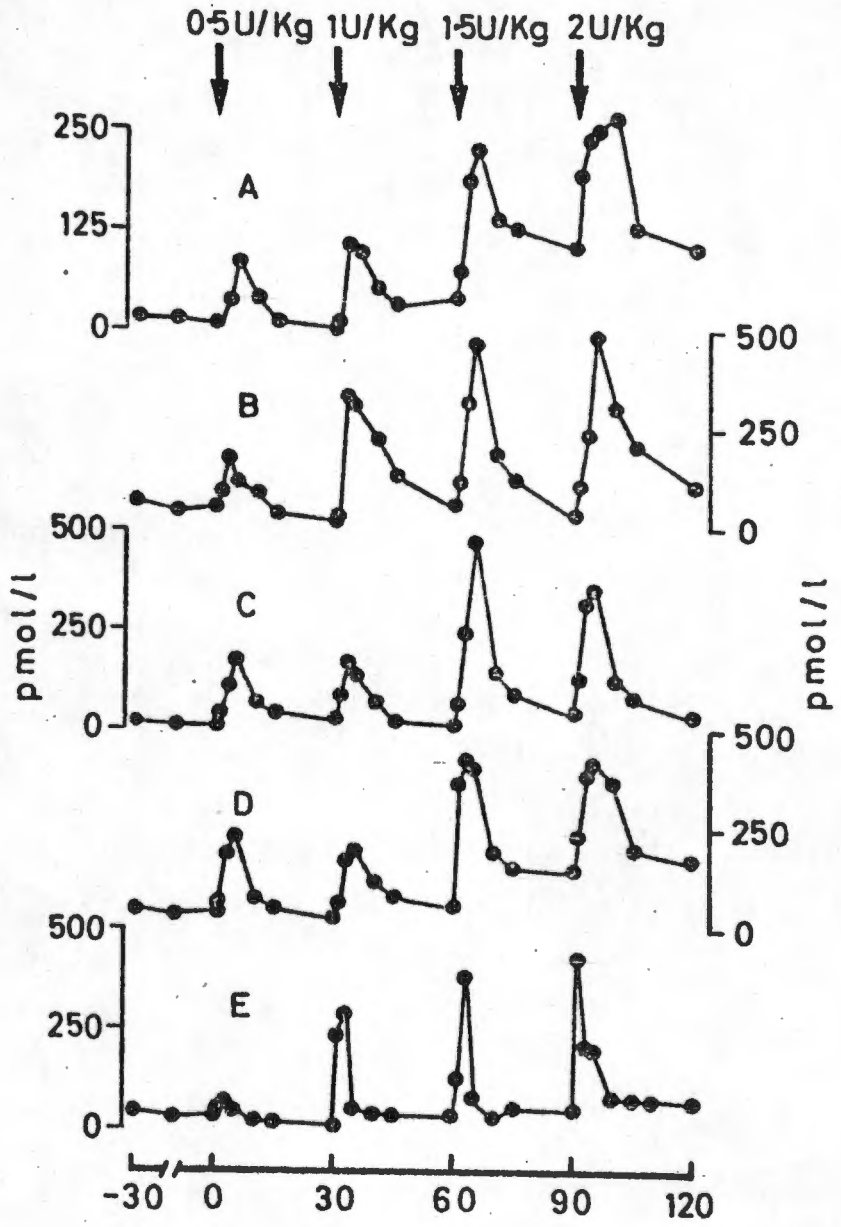


Figure 6.6

Serum hPP respones to 'Boots' secretin given by bolus injection in sequentially increasing doses at 30 minute intervals in 5 healthy subjects.

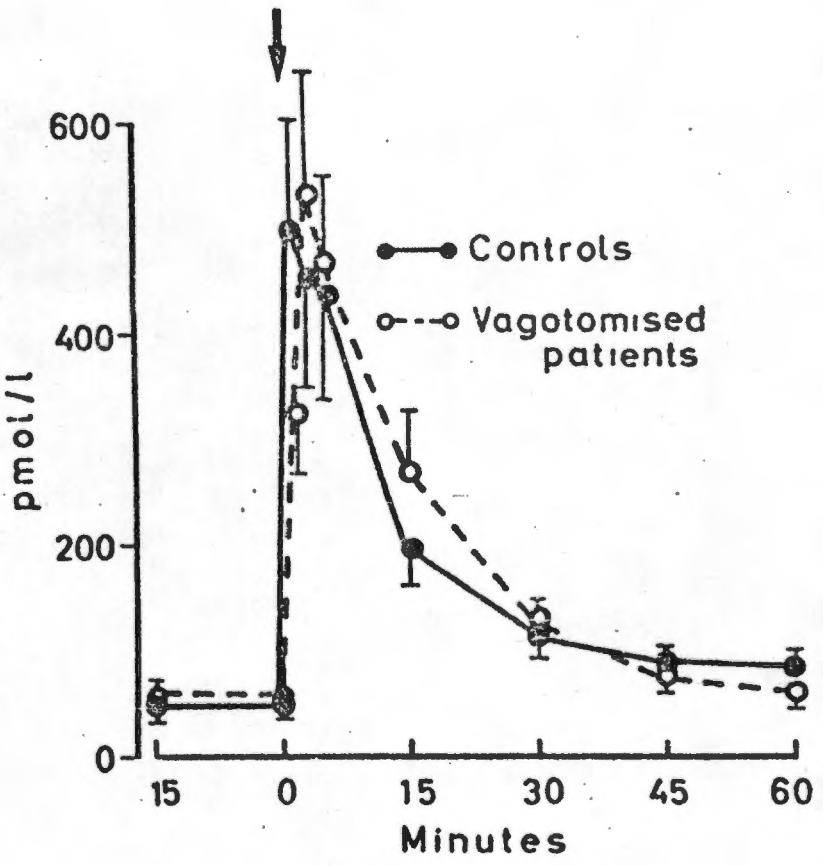


Figure 6.7

Mean ± S.E. serum hPP responses to 2 CHRU/kg 'Boots' secretin injected over 2 minutes in 7 healthy subjects and in 7 truncally vagotomised patients. The arrow indicates the time of the secretin injection.

6.1.2.1 The effects of secretin on serum pancreatic polypeptide concentrations.

a. Dose dependent release of pancreatic polypeptide.

The individual serum hPP responses to the sequentially administered increasing doses of "Boots" secretin are shown in Fig. 6.6. A dose dependent increase in the serum hPP concentration was seen which reached a maximum concentration with 1,5CHRU/kg of secretin.

b and c. The effects of secretin on serum pancreatic polypeptide concentrations in vagotomised individuals and in normal controls.

In the control subjects, the "Boots" secretin infusion of 2CHRU/kg caused a rapid rise in the mean serum hPP concentration from 51 $\pm$ 10,1 pmol/l to a peak concentration of 498 $\pm$ 104,3 pmol/l one minute after terminating the injection of secretin. By 45 minutes, the hPP concentration were no longer significantly elevated and had returned to basal levels by 60 minutes.

In the vagotomised subjects the basal serum hPP concentration was 61 $\pm$ 9,2 pmol/l and secretin caused the levels to rise to a peak concentration of 534 $\pm$ 114,7 pmol/l which, however, occurred three minutes after terminating the injection of secretin (Fig. 6.7).

6.1.2.2 The effects of cholecystokinin-pancreozymin on serum pancreatic polypeptide concentrations.

After 85CHRU of "Boots" CCK-PZ administered intravenously, the serum hPP concentration rose from a basal level of 36 $\pm$ 9,1 pmol/l to a peak concentration of 67 $\pm$ 21,2 pmol/l three minutes after the infusion.

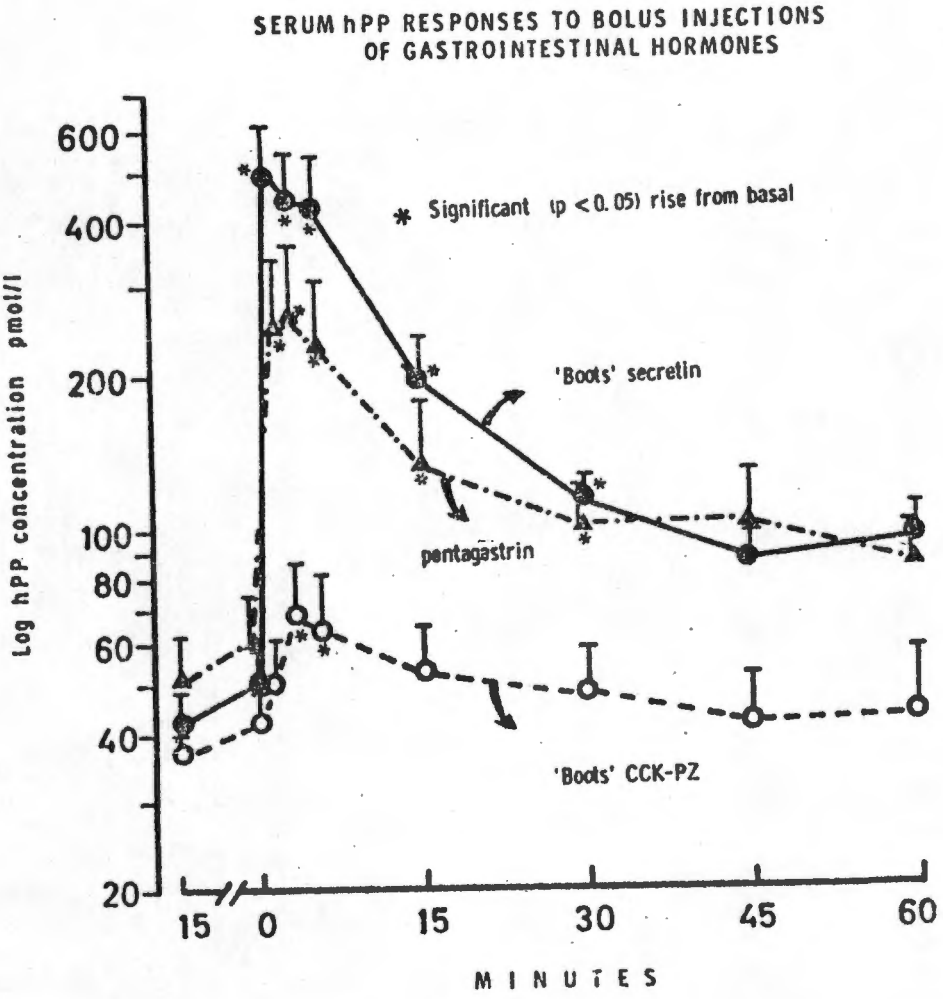


Figure 6.8

Mean $\pm$ S.E. serum hPP responses to 2 CHRU/kg 'Boots' secretin, 85 CHRU 'Boots' CCK-PZ and 5ug/kg synthetic pentagastrin each injected over 2 minutes in 7 healthy subjects.

This small, but statistically significant rise, was far lower than the response to 2CHRU/kg of "Boots" secretin (Fig. 6.8).

6.1.2.3 The effects of gastrin on serum pancreatic polypeptide concentrations.

The pentagastrin infusion of 5µg/kg caused a significant rise in the serum hPP concentrations from 55 ± 13,6 pmol/l to 272 ± 97,1 pmol/l which occurred three minutes after the pentagastrin had been infused. The rise in the serum hPP concentration induced by pentagastrin was intermediate between that seen with CCK-PZ and secretin (Fig. 6.8).

Summary of the effects of gastrointestinal hormones on serum hPP concentrations.

All three gastrointestinal hormones, at the doses infused, were associated with a significant rise in the serum hPP concentrations. Secretin caused hPP release in a dose related manner which was not dependent on vagal integrity; cholecystokinin-pancreozymin caused a small, but significant rise in the hPP concentration and 5µg/kg of pentagastrin a response which was intermediate between the two.

TABLE 6.1 SERUM PANCREATIC POLYPEPTIDE RESPONSES TO COMBINED ADRENERGIC STIMULATION IN 6 HEALTHY PEOPLE

	BASAL			SALINE				EPINEPHRINE							
Time (min)	-90	-75	-60	-45	-30	-15	0	5	15	30	45	60	75	90	120
Mean	52	55	52	52	57	60	65	58	59	60	52	56	58	55	54
± S.E.	6,9	10,1	8,7	8,6	10,2	14,3	12,2	7,3	8,4	9,1	7,1	5,3	7,1	6,1	8,1

No significant changes in hPP concentration were found.

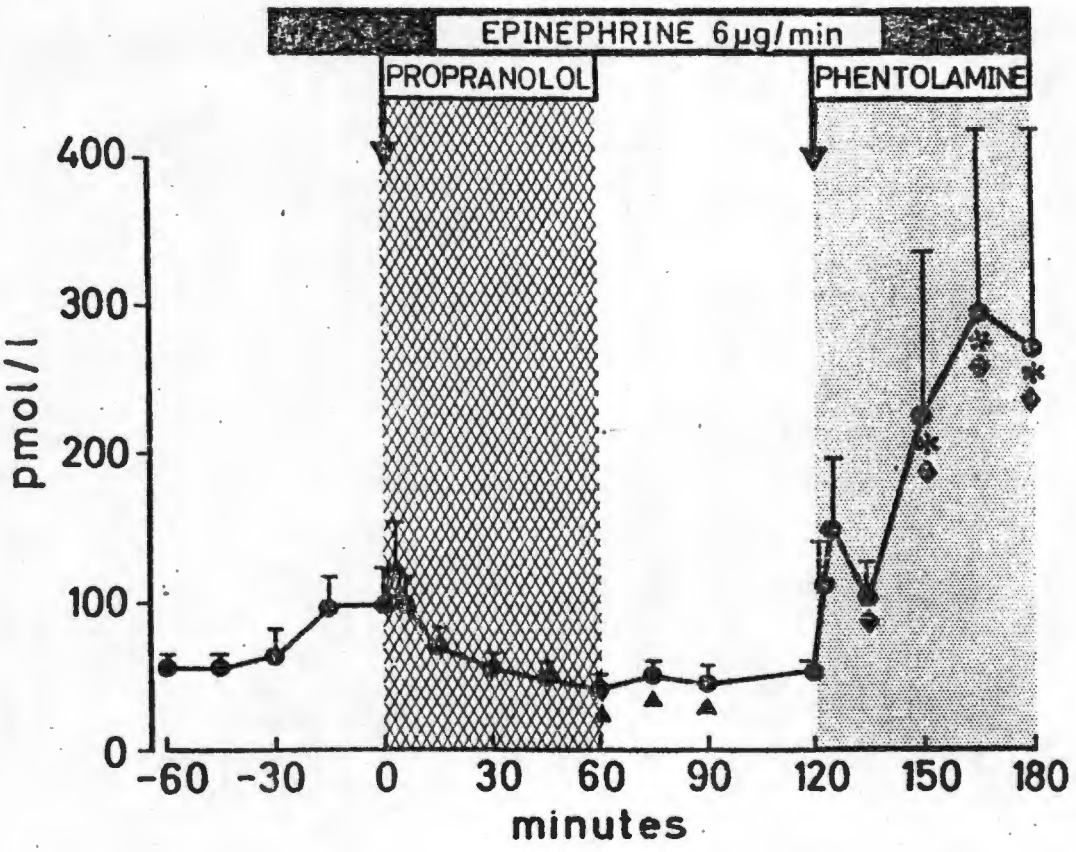


Figure 6.9

Mean $\pm$ S.E. serum hPP responses to adrenergic modulation in 6 healthy subjects. The propanolol infusion of 0,1mg/min and the phentolamine infusion of 0,5mg/min were preceded by a bolus dose of 10mg and 5mg, respectively, as indicated by the arrows. The asterisks indicate a significant ($p < 0.05$) change from the basal concentration, the triangles a significant ($p < 0.05$) fall from the concentration during the initial epinephrine infusion, and the diamonds a significant ($p < 0.05$) rise above the concentrations during the second phase of mixed adrenergic stimulation.

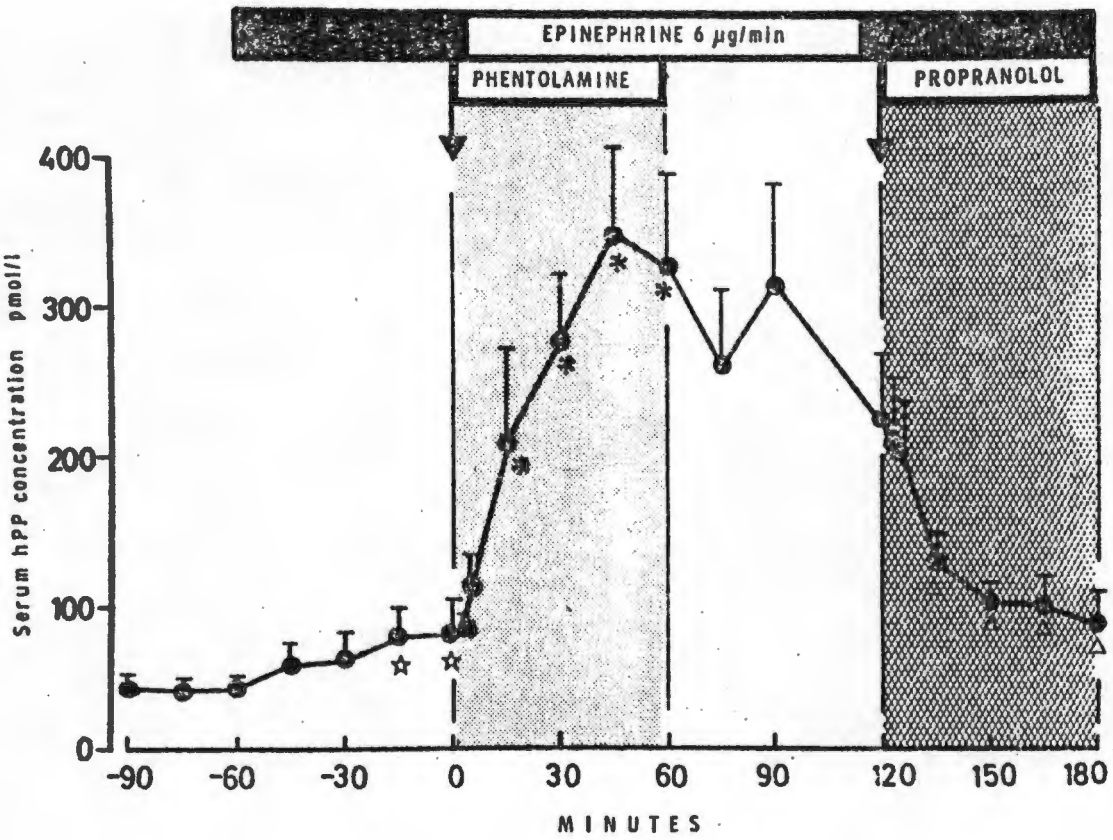


Figure 6.10

Mean $\pm$ S.E. serum hPP responses to adrenergic modulation in 6 healthy subjects. The phentolamine infusion of 0,5mg/min and the propranolol infusion of 0,1mg/min were preceded by a bolus dose of 5mg and 10mg, respectively, as indicated by the arrows. The stars indicate a significant ($p < 0.05$) rise above the basal concentration, the asterisks a significant ($p < 0.05$) rise above the concentration during the first phase of mixed adrenergic stimulation and the triangles a significant ($p < 0.05$) fall from the concentrations during the second phase of mixed adrenergic stimulation.

6.1.3 The neural regulation of pancreatic polypeptide release.

6.1.3.1 The effects of adrenergic modulation on pancreatic polypeptide release.

In the 18 patients who were studied using three different protocols, the serum hPP responses depended on the nature of the adrenergic stimulus and also on the state of preservation of the epinephrine.

Serum hPP concentrations:

In the first protocol in which unpreserved epinephrine at the rate of 6µg/min was infused after a saline infusion, neither saline, nor epinephrine caused a change in the serum hPP concentrations. The mean basal serum hPP concentration was 52 ± 6,9 - 55 ± 10,1 pmol/l and during the subsequent saline and epinephrine infusions, the concentrations varied from 52 ± 8,6 - 65 ± 12,2 pmol/l, but these changes were not significant (Table 6.1). When the epinephrine was preserved with ascorbic acid and infused at the rate of 6µg/min, in the second group of subjects, after 30 minutes of infusion, a small but insignificant rise in the serum hPP concentration was observed (Fig. 6.9). However, when the initial epinephrine infusion was prolonged to 60 minutes in the third group of subjects (Fig 6.10), the rise in the serum hPP concentrations reached statistical significance after 45 minutes at a level of 82 ± 23,1 pmol/l.

When the second protocol was followed, i.e. propranolol was administered immediately after the epinephrine infusion in 6 subjects (Fig. 6.9), there was a small rise, then a fall in the serum hPP concentration to a nadir of 44 ± 7,8 pmol/l, 60 minutes after starting the propranolol. The nadir concentration was significantly lower than

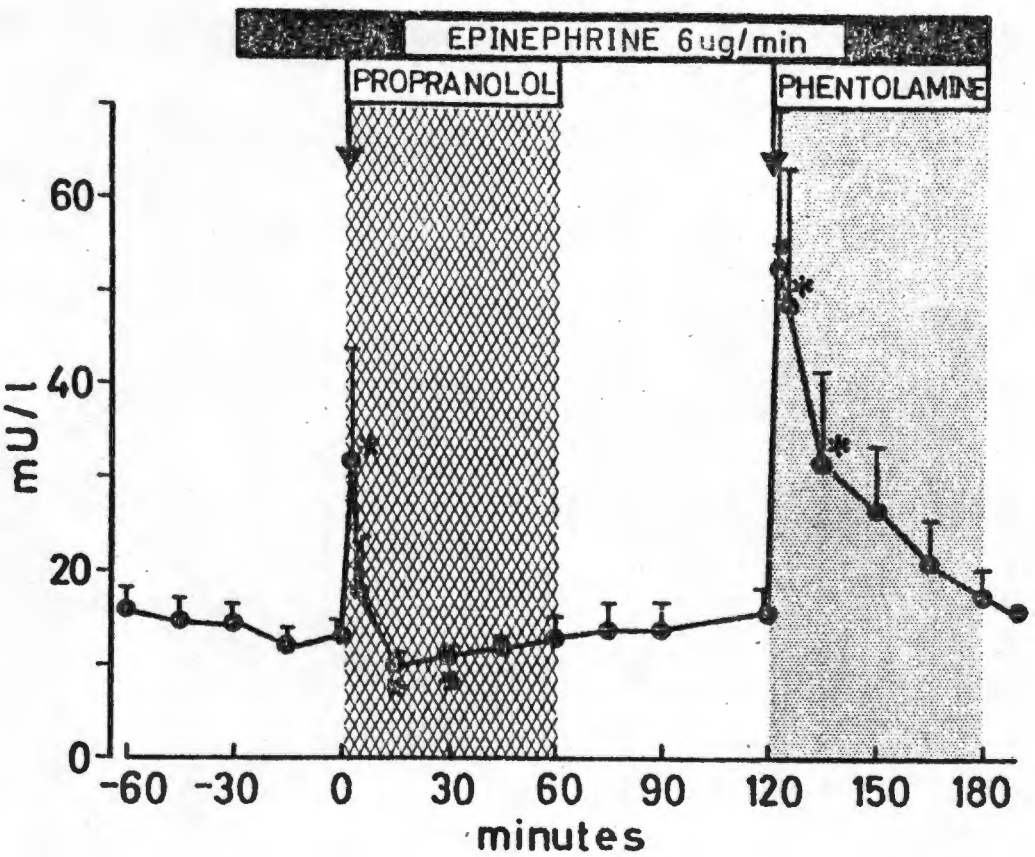


Figure 6.11

Mean $\pm$ S.E. serum insulin responses to adrenergic modulation in 6 healthy subjects. The propranolol infusion of 0,1mg/min and the phentolamine infusion of 0,5mg/min were preceded by a bolus dose of 10mg and 5mg, respectively, as indicated by the arrows. The asterisks indicate a significant ($p < 0.05$) change from the basal concentration.

the pre-propranolol hPP concentration of 99 ± 25,2 pmol/l but was not significantly lower than the basal concentration. The low serum hPP levels persisted during the second period of epinephrine infused alone. Following the addition of the phentolamine i.e. with adrenergic β-receptor stimulation, serum hPP concentrations rose steadily to a peak of 297 ± 125,3 pmol/l 45 minutes after starting the infusion of phentolamine.

In the third group of 6 subjects (Fig. 6.10) in whom the initial epinephrine infusion was succeeded by the period of adrenergic β-receptor stimulation, the epinephrine-induced rise in serum hPP concentration was immediately followed by a slow and steady but pronounced rise in serum hPP concentration which reached a peak of 351 ± 60,4 pmol/l 45 minutes after starting the phentolamine. Stopping the phentolamine infusion was associated with a fall in the hPP concentration, which continued during the subsequent period of adrenergic α-receptor stimulation i.e. when propranolol was added to the infusion.

Serum insulin concentrations:

In the first protocol in which a saline infusion was followed by the infusion of unpreserved epinephrine, neither saline alone, nor saline and epinephrine significantly affected the plasma insulin concentrations.

In the second group of subjects (Fig. 6.11) in whom the initial epinephrine infusion was followed by the propranolol infusion, the basal serum insulin concentration was 15 ± 2,7 mU/l. Epinephrine infused alone, caused a small decline from basal in the serum hPP levels.

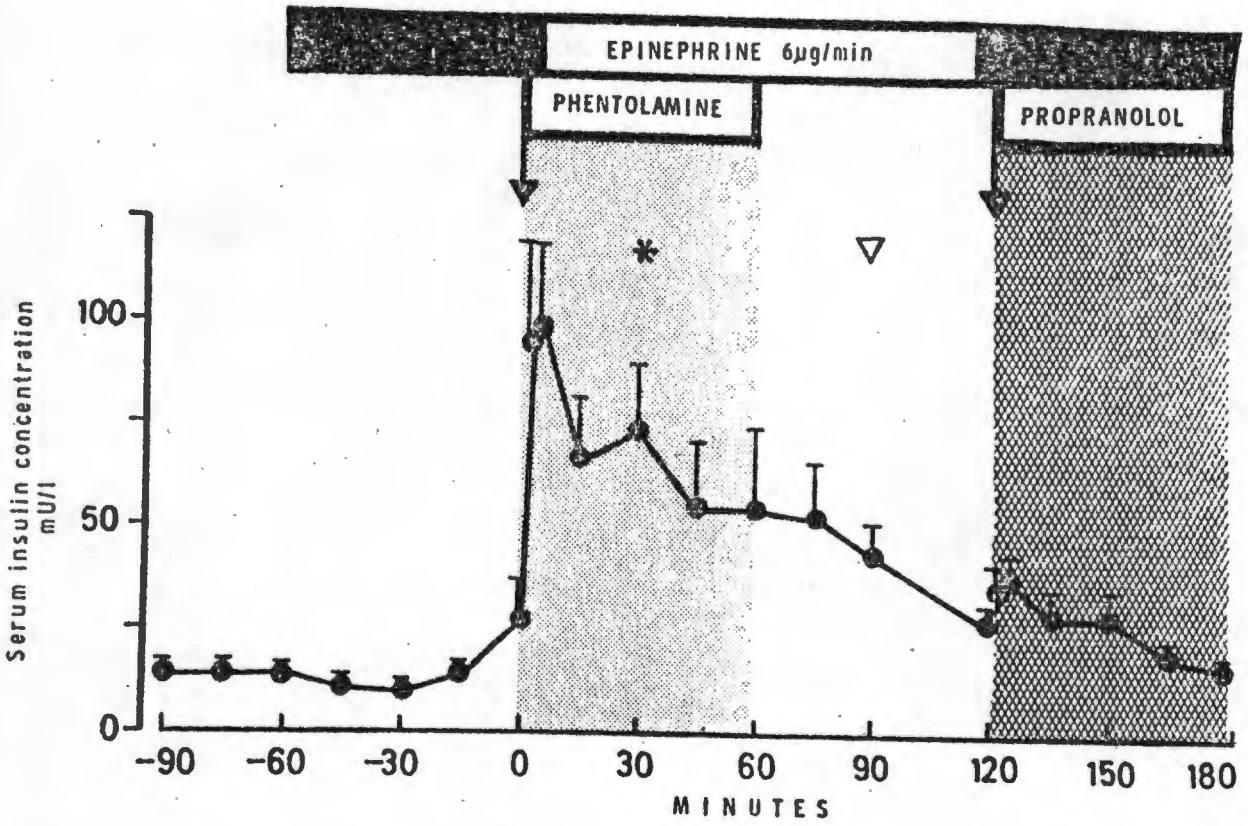


Figure 6.12

Mean $\pm$ S.E. serum insulin responses to adrenergic modulation in 6 healthy subjects. The phentolamine infusion of 0,5mg/min and the propranolol infusion of 0,1mg/min were preceded by a bolus dose of 5mg and 10mg, respectively, as indicated by the arrows. The asterisk indicates that each concentration during the phentolamine infusion was significantly ($p < 0.05$) elevated above the basal concentration. The triangle indicates that each concentration during the second period of mixed adrenergic stimulation was significantly ($p < 0.05$) lower than the peak concentration.

The addition of propranolol to the epinephrine infusion, caused a significant rise, then a fall in insulin concentration to a level which was significantly below the basal serum insulin concentration. During the subsequent period of combined adrenergic α- and β-receptor stimulation, the insulin concentration rose slowly towards basal levels. Selective adrenergic β-receptor stimulation caused a rapid rise, followed by a fall in the serum insulin concentration, but basal insulin levels had not been reached by the end of the experiment 60 minutes later.

When the order of the stimulation was reversed (Fig. 6.12) again there was a small decline in the serum insulin concentration followed by a small rise during the initial epinephrine infusion. The addition of the phentolamine infusion caused a significant, sustained elevation of serum insulin levels. Thereafter, during the subsequent period of combined α- and β-receptor stimulation, and selective α-receptor stimulation, a slow steady fall in serum insulin concentrations was observed but the levels did not at any time fall to below the basal serum insulin concentration. As was previously seen, the initial propranolol bolus injection was associated with a small and transient rise in the mean insulin concentration.

Serum glucose concentrations:

The mean fasting serum glucose concentration was 4,5 ± 0,28 mmol/l in the subjects who received only saline plus epinephrine. Epinephrine raised the serum glucose concentration to 7,2 ± 0,43 mmol/l, which was significantly above the basal concentration (Fig. 6.13).

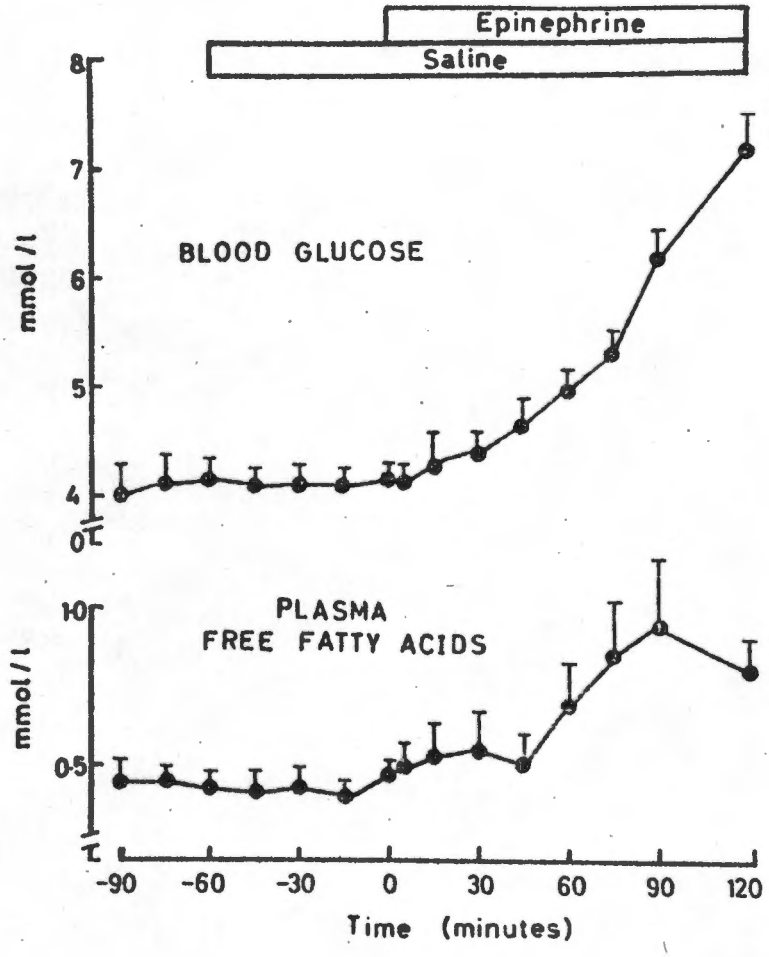


Figure 6.13

Mean $\pm$ S.E. serum glucose and free fatty acid responses to adrenergic stimulation. Epinephrine was infused at 6ug/min. The asterisks indicate a significant ($p < 0.05$) rise above the basal concentration.

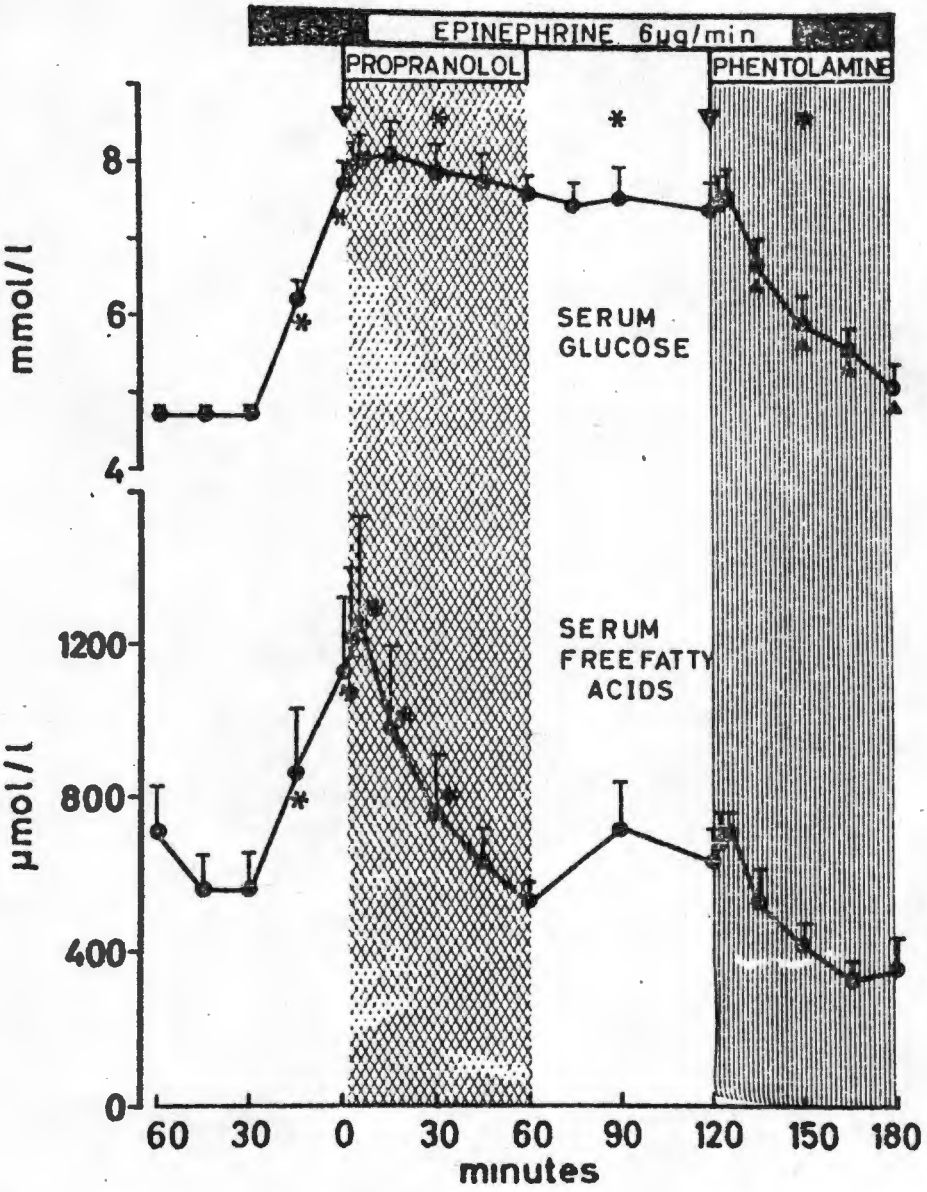


Figure 6.14

Mean $\pm$ S.E. serum glucose and free fatty acid responses to adrenergic modulation. The propranolol infusion of 0,1mg/min and the phentolamine infusion of 0,5mg/min were preceded by a bolus dose of 10mg and 5mg, respectively, as indicated by the arrows. The asterisks indicate a significant ($p < 0.05$) rise above the basal concentration and the triangles indicate a significant ($p < 0.05$) fall from the concentration during the second phase of mixed adrenergic stimulation.

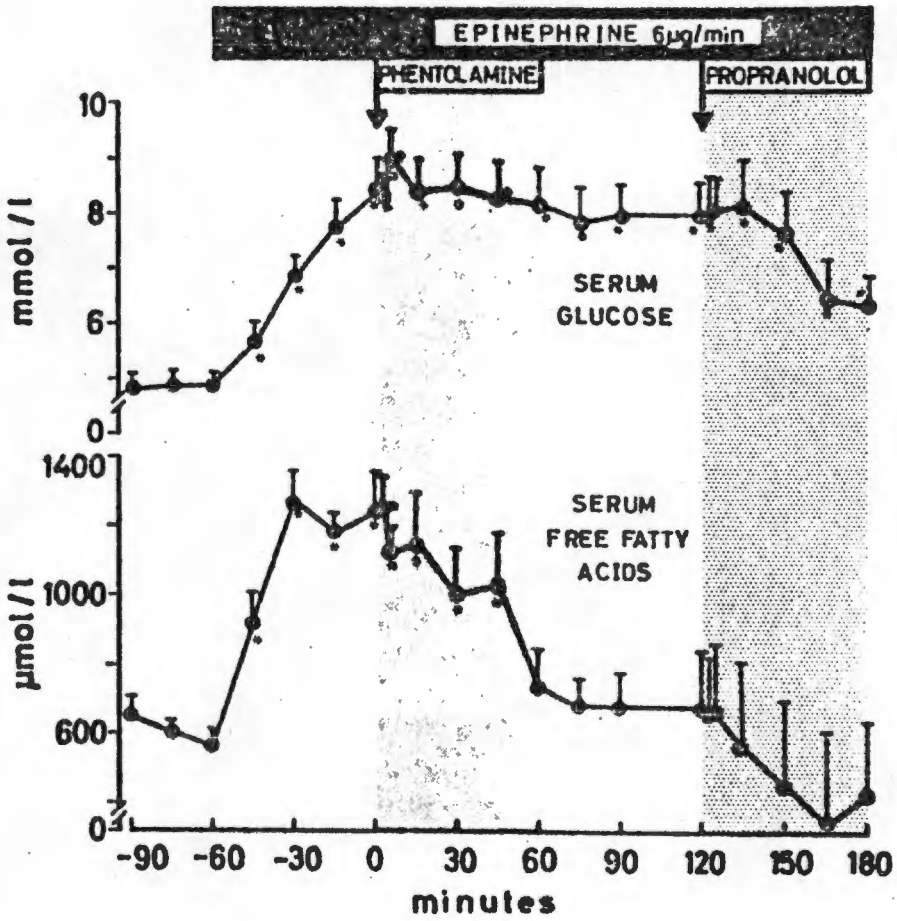


Figure 6.15

Mean $\pm$ S.E. serum glucose and free fatty acid responses to adrenergic modulation. The phentolamine infusion of 0,5mg/min and the propranolol infusion of 0,1mg/min were preceded by a bolus dose of 5mg and 10mg, respectively, as indicated by the arrows. The asterisks indicate a significant ($p < 0.05$) rise above the basal concentrations.

During the second protocol (epinephrine - propranolol - epinephrine - phentolamine), the mean basal glucose concentration was 4,7 ± 0,10 mmol/l (Fig. 6.14). A significant rise in the glucose concentration to 7,8 ± 0,30 mmol/l was observed during the initial epinephrine infusion. The serum glucose concentration remained elevated (8,2 ± 0,39 - 7,4 ± 0,38 mmol/l) during the subsequent infusions of propranolol plus epinephrine and epinephrine alone. With the introduction of adrenergic β-receptor stimulation, the glucose concentrations fell towards basal levels.

The mean basal glucose concentration of 4,9 ± 0,26 mmol/l was similar in the third group of subjects in whom the order of the selective stimulation was reversed (Fig. 6.15). Epinephrine infused alone, caused the serum glucose concentration to rise to 8,4 ± 0,61 mmol/l and this elevation was sustained during the subsequent phentolamine plus epinephrine and epinephrine infusions. A small decline in the glucose levels was observed during the period of adrenergic α-receptor stimulation.

Serum free fatty acid concentrations:

In the first group of subjects who received only combined adrenergic α- and β-receptor stimulation together with saline, the serum FFA concentrations rose from a basal level of 515 ± 121,6 µmol/l to 945 ± 198,4 µmol/l (Fig. 6.13).

In the second group of 6 subjects in whom the initial epinephrine infusion was followed by the period of selective adrenergic α-receptor stimulation (epinephrine plus propranolol infusions), the serum free fatty acid concentration rose from a basal concentration of

563 ± 96,9 µmol/l to 1131 ± 224,8 µmol/l during the initial epinephrine infusion (Fig. 6.14). When propranolol was infused simultaneously with the epinephrine, the FFA concentration declined to 525 ± 50,5 µmol/l after 60 minutes of selective adrenergic α-receptor stimulation. With combined adrenergic stimulation the levels stabilized but the subsequent period of selective β-receptor stimulation was associated with a further fall in the serum FFA concentrations to 367 ± 78,2 µmol/l.

In the third group of subjects in whom the order of selective stimulation was reversed i.e. selective adrenergic β-receptor stimulation was followed by the α-receptor stimulation, epinephrine alone induced a rise in the serum FFA concentrations from 563 ± 68,6 µmol/l to 1239 ± 120,9 µmol/l (Fig. 6.15). The subsequent epinephrine plus phentolamine infusion was associated with FFA concentrations which remained elevated for 30 minutes, but then declined to 722 ± 108,0 µmol/l over the subsequent 30 minutes. The FFA concentrations stabilized at 689 ± 85,7 - 685 ± 88,1 µmol/l during the subsequent period of combined adrenergic α- and β-receptor stimulation but with the addition of propranolol, a further fall in FFA concentration to 342 ± 72,6 µmol/l was observed.

In these studies when the data were analysed separately for each protocol or as pooled data for all the protocols there was no statistical correlation between the serum glucose, serum FFA or serum insulin concentrations and the serum hPP concentrations.

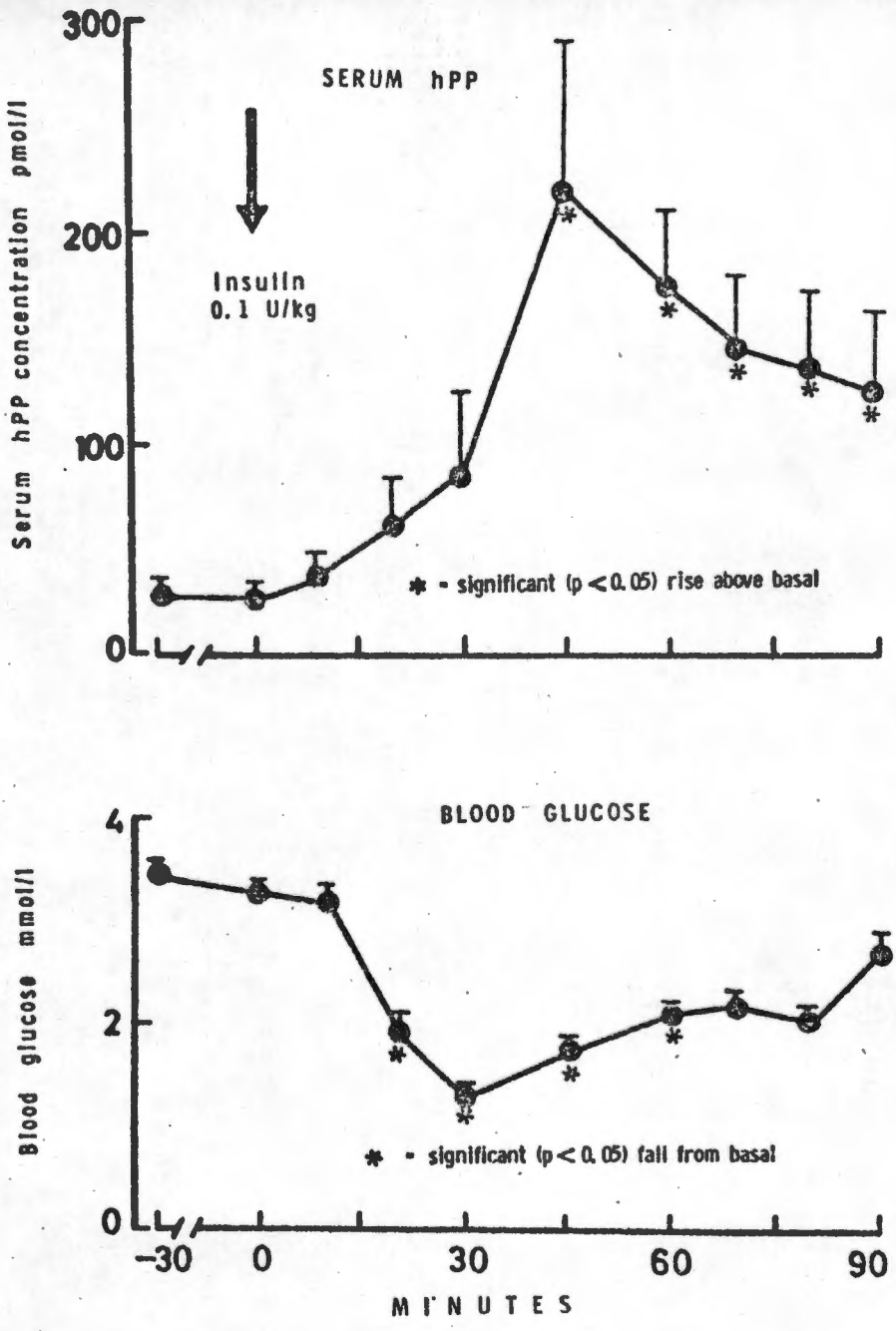


Figure 6.16

Mean $\pm$ S.E. serum hPP and blood glucose reponses to insulin-induced hypoglycaemia in 5 healthy subjects. 0,1U/kg of monocomponent insulin was injected at time zero.

TABLE 6.2 SERUM HUMAN GROWTH HORMONE RESPONSES IN ng/ml TO 500mg L-DOPA FOLLOWED BY THE INGESTION OF THE TEST MEAL IN 6 HEALTHY SUBJECTS

	BASAL			L-DOPA				POST MEAL							
TIME minutes	-120	-105	-90	-60	-30	0	IM*	5	15	30	45	60	90	120	180
PATIENT															
1	0,5	0,65	0,85	10	16,5	5,3	4,45	4,15	2,15	1,8	1,1	1,0	0,85	1,2	0,55
2	0,62	0,65	1,15	1,78	2,35	1,6	1,5	1,25	1,18	0,9	0,85	0,5	0,6	0,55	0,45
3	13,0	13,5	12,25	9	20	16,5	15	10	15	4,45	2,5	1,78	1,2	0,75	1,95
4	1,1	1,95	1,6	0,8	3,35	13,5	13,5	9,5	7,5	3,9	2,45	1,7	0,8	1,35	1,35
5	0,7	0,6	0,5	0,5	1,1	0,62	0,65	0,65	0,55	0,65	0,75	0,4	0,4	0,38	0,5
6	0,55	0,5	0,55	3,5	6,75	1,9	1,45	1,1	0,8	0,57	0,6	0,55	0,49	0,95	0,5
mean	2,7	2,8	2,8	4,3	8,34	6,6	4,6	4,4	4,5	2,0	1,4	0,9	0,7	0,8	0,9
S.E.	2,05	2,15	1,89	1,7	3,2	2,7	1,73	1,75	2,34	2,93	0,35	0,25	0,12	0,95	0,25

*intra meal sample

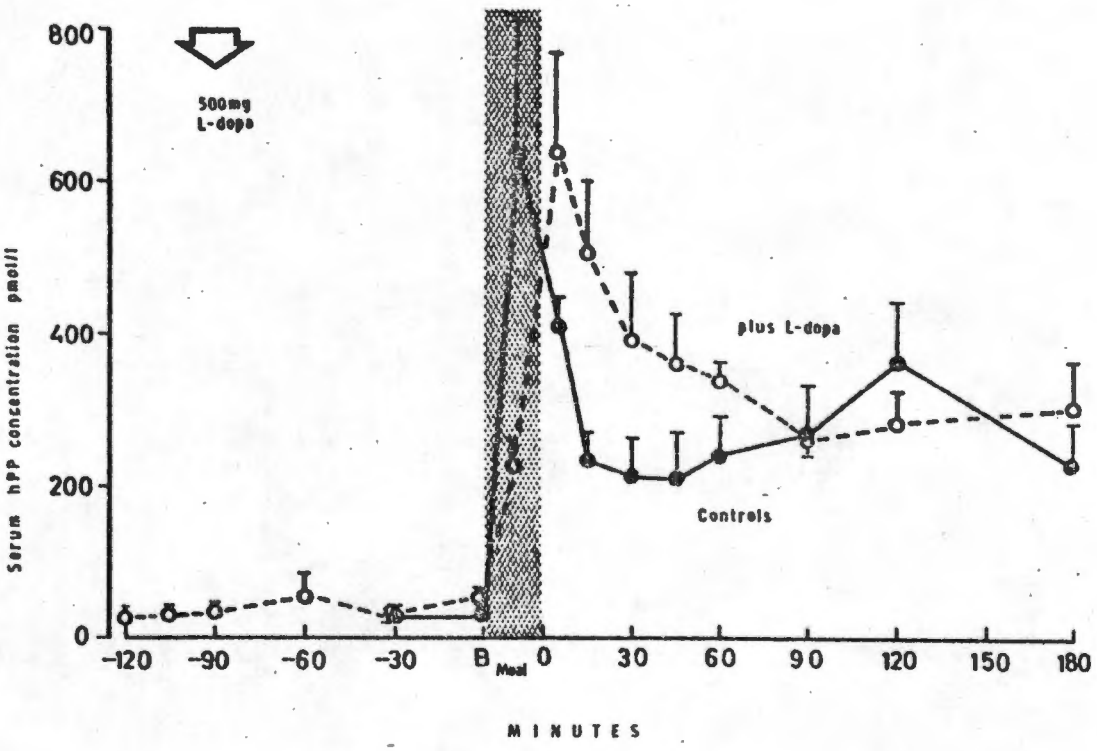


Figure 6.17

Mean $\pm$ S.E. serum hPP concentrations after the ingestion of 500mg of L-Dopa followed by a meal in 6 subjects, or a meal only, in 7 subjects. hPP concentrations did not differ significantly between the groups. The meal was started at time B and completed at time zero.

6.1.3.2 The effects of cholinergic stimulation on serum pancreatic polypeptide concentrations.

In the 5 healthy subjects who received an intravenous bolus dose of 0,1 U/kg of monocomponent insulin, the blood glucose concentration fell from 3,5 ± 0,12 mmol/l to a nadir concentration of 1,5 ± 0,06 mmol/l 30 minutes later. In contrast, the mean serum hPP concentration rose from a basal level of 28 ± 4,3 pmol/l to a peak concentration of 220 ± 54,6 pmol/l which occurred 45 minutes after the insulin had been given (Fig. 6.16).

6.1.3.3 Dopaminergic effects on pancreatic polypeptide release.

a) Dopamine agonism: Ingestion of 500mg of L-Dopa with water did not itself significantly alter the mean basal hPP concentration, nor was the response to a mixed meal significantly changed when compared with the serum hPP response seen to a meal without the ingestion of L-Dopa (Fig. 6.17). The serum growth hormone concentrations rose significantly (Table 6.2) after ingestion of the L-dopa.

b) Dopamine antagonism: In the study to assess the effect of a dopamine antagonist on the serum hPP response, the intravenous administration of 10 mg of metoclopramide did not cause a significant change in the serum hPP concentrations. However, in this study the serum growth hormone levels were also not significantly changed.

Summary of the effects of neural regulation of PP release.

The pharmacological manipulation of the autonomic nervous system caused striking changes in the serum hPP concentrations. Although combined adrenergic α- and β- receptor stimulation caused only a modest rise in the serum hPP levels, selective β-receptor adrenergic stimulation caused a 6 - 7 fold rise in the hPP levels to a peak concentration of 297 ± 125,3 pmol/l in one study and to 351 ± 60,4 pmol/l in another. On the other hand, adrenergic α-receptor stimulation was associated with a decline in serum hPP concentrations. The changes in the serum hPP concentrations were independent of the changes in serum glucose, insulin and FFA concentrations induced by the adrenergic stimulation.

During insulin-induced hypoglycaemia, the mean serum hPP concentration rose from a basal level of 28 ± 4,3 pmol/l to 220 ± 54,6 pmol/l, an 8 fold increase. The peak hPP concentration was observed 15 minutes after the nadir glucose concentration of 1,5 ± 0,06 mmol/l.

Neither the dopamine agonist, L-dopa, nor the dopamine antagonist, metoclopramide, significantly affected the serum hPP concentrations. L-dopa caused a significant rise in the serum growth hormone concentrations, whereas the levels did not change with metoclopramide.

6.1.4 The effects of miscellaneous substances on serum pancreatic polypeptide concentrations.

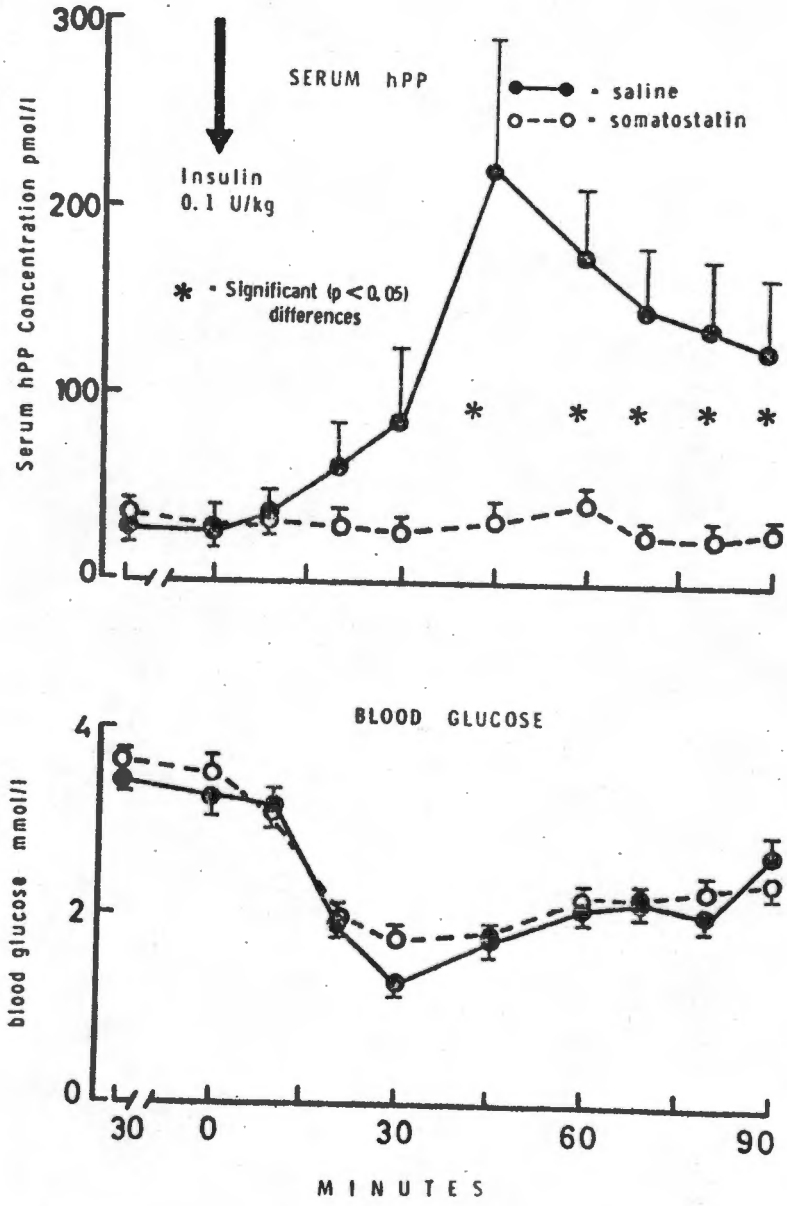


Figure 6.18

Mean $\pm$ S.E. serum hPP and blood glucose responses in 5 healthy subjects to insulin-hypoglycaemia plus a somatostatin or saline infusion. 2ug/min of somatostatin was infused throughout the period of study and 0,1U/kg of monocomponent insulin was injected at time zero. The asterisks indicate significant ($p < 0.05$) differences between the saline and somatostatin infusions.

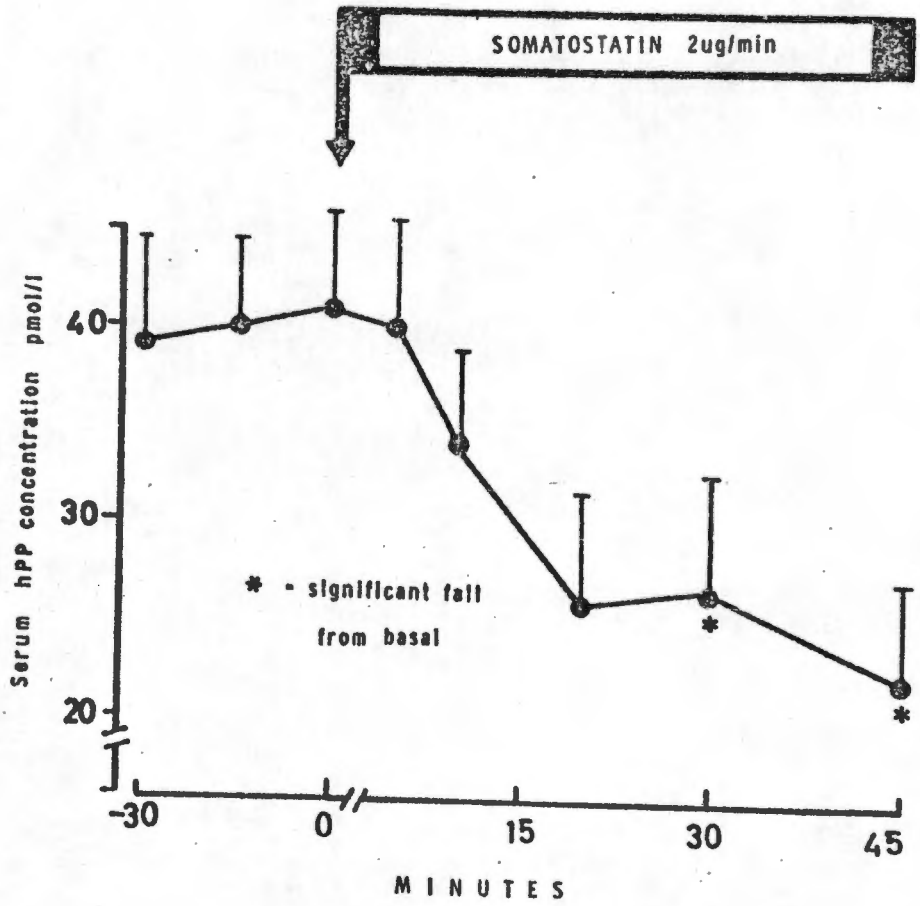


Figure 6.19

Mean $\pm$ S.E. serum hPP concentrations during a somatostatin infusion in 6 healthy subjects. The somatostatin infusion was preceded by a bolus injection of 200ug as indicated by the arrow. The asterisks indicate a significant ($p < 0.02$) fall from the basal concentration.

6.1.4.1 The effects of somatostatin on serum pancreatic polypeptide concentrations.

a) "Low" doses of somatostatin.

An infusion of somatostatin at a rate of 2µg/min did not significantly affect the basal hPP concentration of 34 ± 7,6 pmol/l. However, the rise in serum hPP concentrations to 220 ± 54,6 pmol/l induced by insulin-hypoglycaemia (see 6.1.3.2) was abolished by this dose of somatostatin (Fig. 6.18). There were no significant differences in the blood glucose concentrations in the two tests (Fig. 6.18), which fell to a nadir of 1,7 ± 0,14 mmol/l when somatostatin was infused and to 1,5 ± 0,06 mmol/l when insulin was administered without the somatostatin infusion.

b) A preceding "priming" dose of somatostatin.

Following the administration of a 200µg bolus of somatostatin preceding the somatostatin infusion of 2µg/min, a significant fall in the serum hPP concentration from a basal level of 46,6 pmol/l to 26,4 pmol/l ($p < 0,02$) at 30 minutes was observed (Fig. 6.19).

6.1.4.2 The effects of histamine H_2-receptor blockade on serum pancreatic polypeptide concentrations.

Neither an initial drink of 150 ml of water, nor 400 mg of cimetidine in water taken 30 minutes later, caused the serum hPP concentration to change over a period of two and a half hours.

The results described in this section have shown that numerous factors

cause changes in the serum hPP concentration. A mixed meal, adrenergic β-receptor stimulation, insulin-induced hypoglycaemia and "Boots" secretin all caused the serum hPP concentration to rise to levels 6 - 10 times above the basal concentration. In the next section, the molecular heterogeneity of circulating immunoreactive PP, which was released by insulin-hypoglycaemia and "Boots" secretin, will be described.

6.2 Investigation of the molecular forms of circulating immunoreactive pancreatic polypeptide by 10% polyacrylamide disc gel electrophoresis

The administration of 2 CHRU/kg of "Boots" secretin and inducing hypoglycaemia with an intravenous injection of 0,1 U/kg of insulin caused the serum hPP concentration to rise in each subject. The hPP levels attained are shown in Table 6.3.

The peak serum samples were then electrophoresed using 10% polyacrylamide gels, and the migration of the immunoreactive hPP was compared with that of radiolabelled hPP (^{125}I-hPP), radiolabelled bPP (^{125}I-bPP) and cold hPP added to the PP assay buffer, to whole blood and to charcoal-treated plasma.

Fig. 6.20 shows the electrophoretic patterns of the radiolabelled markers. ^{125}I-hPP migrated as a single peak in a position more cathodal than ^{125}I-bPP which also migrated as a single peak. By calculation, approximately 93% of the ^{125}I-bPP and 55% of the ^{125}I-hPP constituted their respective peaks of migration.

In contrast, the cold hPP markers did not migrate as homogenous immunoreactive substances (Fig. 6.21) and more than one peak was observed in each. Thus cold hPP added to the assay buffer, migrated predominantly to the position of ^{125}I-hPP but two additional, more poorly defined peaks were present. One migrated in a position more cathodal than the ^{125}I-hPP marker and the other to a position more anodal than this marker. hPP added to whole blood, migrated as two fractions of unequal size. The smaller peak was seen at the position

TABLE 6.3 PEAK SERUM hPP CONCENTRATIONS (pmol/1) IN 6 SUBJECTS AFTER STIMULATION OF PP RELEASE WITH 0,1 U/kg PER MONOCOMPONENT PORCINE INSULIN OR 2 CHRU/kg OF "BOOTS" SECRETIN.

SUBJECT	AGENT	PEAK SERUM hPP CONCENTRATION pmol/1
A	Insulin	350
D	Insulin	145
E	Insulin	58
B	Secretin	129
C	Secretin	337
F	Secretin	200

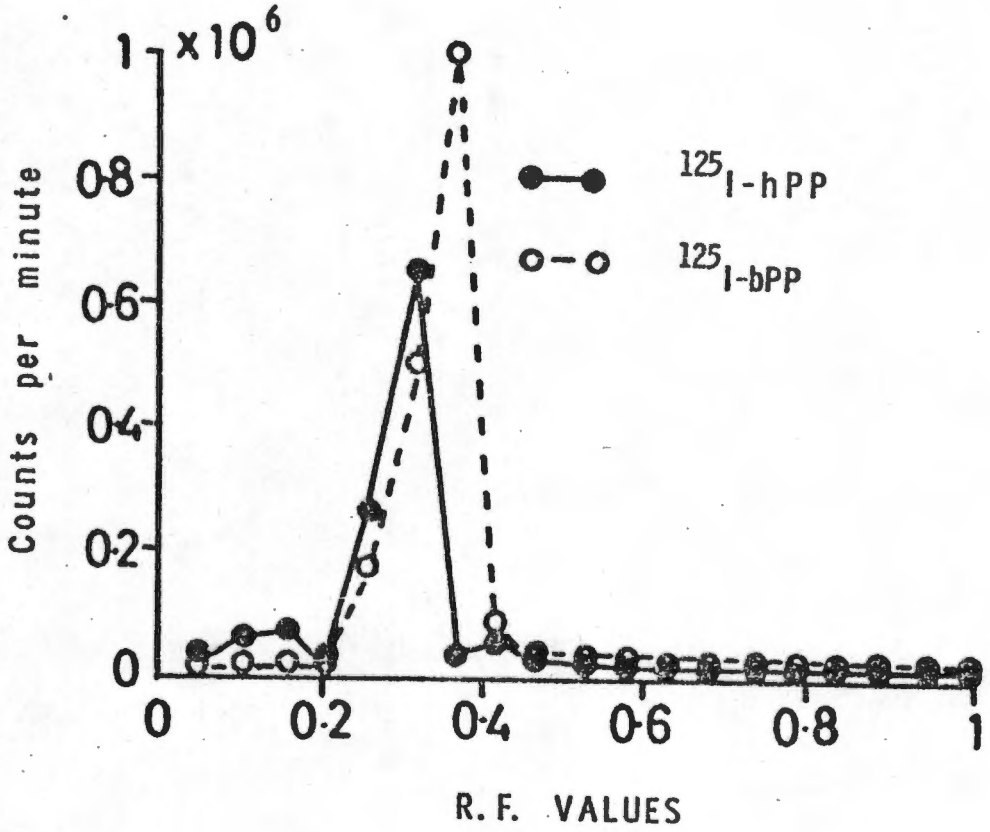


Figure 6.20

10% polyacrylamide disc gel electrophoresis of ^{125}I-labelled hPP and bPP.

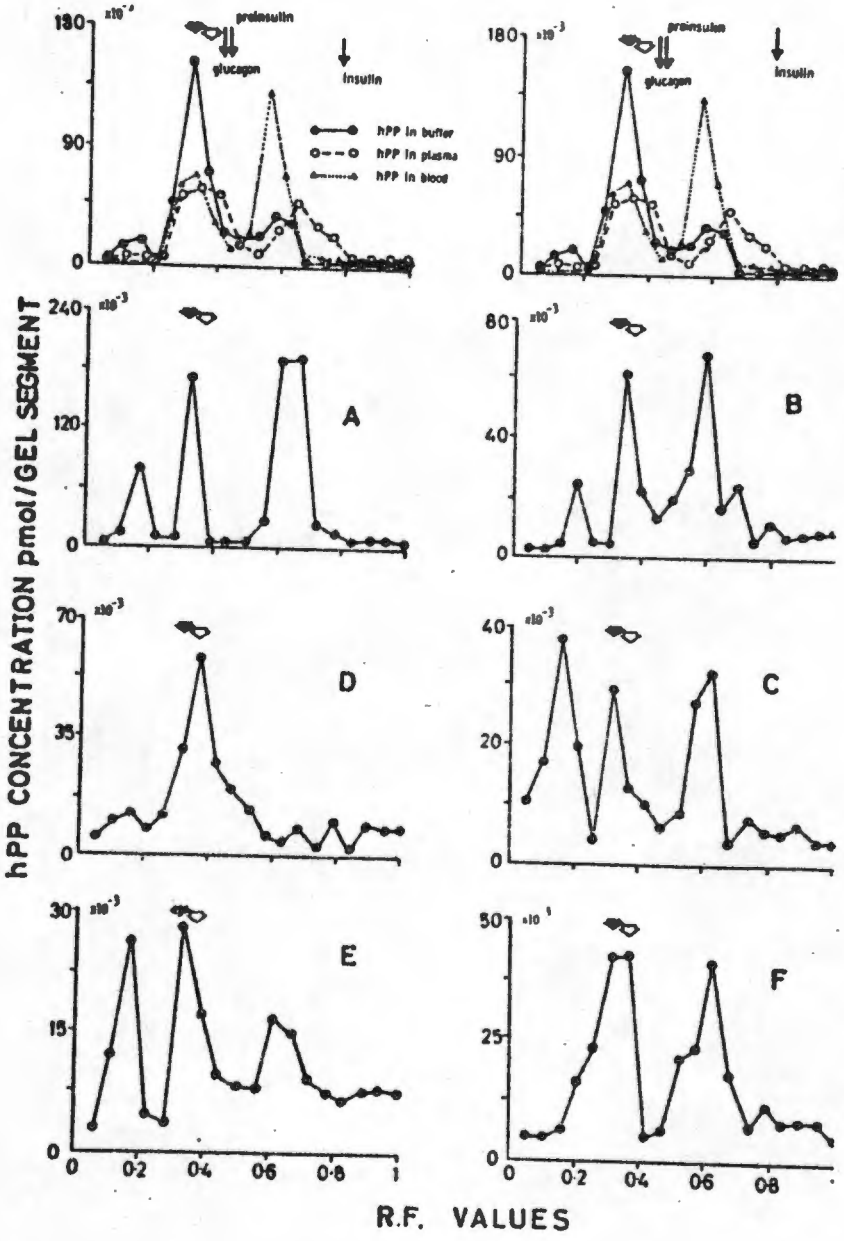


Figure 6.21

10% polyacrylamide disc gel electrophoresis of hPP. The upper panels show the electrophoretic pattern of hPP added to buffer, whole blood and charcoal-treated plasma. The migration of the markers, ^{125}I-hPP (solid arrows), ^{125}I-bPP (open arrows), glucagon, proinsulin and insulin are shown. Figures on the left (A,D,E) show the migration of hPP released by insulin-hypoglycaemia and the figures on the right (B,C,F) hPP released by 'Boots' secretin.

of ^{125}I-hPP, whereas the second, larger fraction was found in a position anodal to this marker. Similarly, the cold hPP added to charcoal stripped human plasma, migrated as two components which were not clearly defined. The cathodal peak was present in the area of the ^{125}I-hPP marker and the anodal fraction in the vicinity of the anodal fraction of cold hPP added to buffer and of cold hPP added to whole blood. The peaks which coincided with the position of the ^{125}I-hPP marker, were more cathodal than the positions of glucagon, proinsulin and insulin, whereas the anodal hPP fractions migrated to a position between the proinsulin and insulin markers.

The electrophoretic patterns of migration of hPP in the unknown serum samples are shown in Fig. 6.21. For purposes of reference, the migrations of the markers are also included in the diagram, which in the column on the left, shows the migration of hPP in the serum samples in which hPP was released by insulin-induced hypoglycaemia, and on the right, the sera in which elevated hPP levels were provoked by intravenous "Boots" secretin administration. Individual variability in the patterns of migration were present regardless of the method by which elevation of the hPP concentrations were achieved. In the majority of the samples, three distinct peaks of migration were present, and in each of the sera, a peak was present which migrated either in the position of the ^{125}I-hPP marker or slightly more anodal, towards the position of the ^{125}I-bPP marker. The additional peaks migrated to the positions of the cathodal peak of the cold hPP in buffer, and/or to the position of the anodal peaks of hPP added to blood, assay buffer or charcoal-treated plasma. The serum sample from subject D, however, showed only a single peak at the position of the ^{125}I-bPP marker,

TABLE 6.4 THE RECOVERY OF ^{125}I-bPP AFTER CONCENTRATION OF SERUM SAMPLES BY ETHANOL EXTRACTION AND EVAPORATION TO DRYNESS

Sample No.	Counts per minute of ^{125}I-bPP added	Counts per minute of ^{125}I-bPP recovered	% Recovery
1	13985	8049	57,5
2	14032	8856	63,1
3	11077	7422	67,0
4	10192	6959	68,2
5	10798	7456	69,0
6	11140	7434	66,7
Mean ± S.E.			65,3 ± 1,75%

TABLE 6.5 THE CALCULATED RECOVERY OF hPP FROM SERUM SAMPLES AFTER FRACTIONATION BY 10% POLYACRYLAMIDE DISC GEL ELECTROPHORESIS

Sample No.	Initial serum hPP concentration (pmol/l)	Calculated amount of hPP fractionated (pmol)	hPP recovered (pmol)	% recovery
A	350	0,110	0,082	74
D	145	0,046	0,027	60
E	58	0,018	0,021	114
B	129	0,041	0,045	104
C	337	0,106	0,025	24
F	200	0,063	0,037	59
Mean ± S.E.				72 ± 13,4%

whereas in the serum from subject F, only two anodal peaks were discernable.

The recovery of hPP from the gels.

Because the serum samples and the blood and charcoal-treated plasma markers were concentrated by ethanol extraction before they were electrophoresed, the recovery of PP after the initial extraction procedure was determined. The extraction procedure was repeated on serum samples to which known amounts of radiolabelled ^{125}I-bPP were added. The radioactive ^{125}I-bPP content of the samples in counts per minute was determined before and after the ethanol extraction procedure and is shown in Table 6.4. In 6 samples, between 57,5 and 69% of the radiolabelled hPP was recovered, the mean of 65,3% being used for further calculations.

Although the concentrated, dried samples were reconstituted in 200µl of distilled water, only 100µl of the resultant solution was electrophoresed. Thus the calculated amount of hPP added to the gels was used to assess the recovery of hPP after the electrophoresis procedure. The mean recovery of hPP was 72 $\pm$ 13,4% but ranged from 24 to 114% (Table 6.5). Sixty percent of the added hPP was recovered from whole blood, 53% from charcoal treated plasma and 87% from the assay buffer. However, since the whole blood sample was not free from endogenous PP, the figure of 66% recovery may be higher than the actual recovery.

Thus, more than one component of immunoreactive PP was present in all except one of the serum samples. In all these samples, a component of hPP was present in the position of migration of ^{125}I-hPP, and in addition fractions of immunoreactive hPP migrated to positions that were more anodal and cathodal than the ^{125}I-hPP.

6.3 Investigation of the metabolism and turnover kinetics of pancreatic polypeptide.

Studies to determine the organ uptake and the $t_{\frac{1}{2}}$ of PP were carried out in pigs. The kinetic behaviour of endogenous PP was examined by assessing the serum PP concentration in numerous sites at the same time, and by following the serum concentrations of PP after total pancreatectomy in these animals.

6.3.1 Organ uptake of pancreatic polypeptide.

The multiple site simultaneous blood sampling in the pigs yielded concentrations of PP which decreased in the following order: pancreatico-duodenal vein, hepatic portal vein, hepatic vein, carotid artery, renal vein and femoral vein. The actual concentrations in these vessels are shown in Table 6.6. In only three animals was the PP concentration measured in the pancreatico-duodenal vein, but in all instances the highest concentrations were observed in these samples. The PP concentrations in the pancreatico-duodenal vessels were 3 - 6 times higher than those in the portal vein.

6.3.1.1 The hepatic uptake of pancreatic polypeptide.

Previous studies from this laboratory have shown that hepatic blood flow is relatively constant in pigs of the same breed, age and weight. Thus, in Landrace X pigs of similar ages and weights to those used in this study, Vinik, Hickman and Grant (1978) determined that the total hepatic blood flow was 580 $\pm$ 24,0 ml/min of which 432 $\pm$ 35,6 ml/min or 75% derived from the hepatic portal vein and 147 $\pm$ 34,7 ml/min from the hepatic artery. Using these data for the mean hepatic blood

TABLE 6.6 PP CONCENTRATIONS IN pmol/l IN SERA SAMPLED SIMULTANEOUSLY FROM MULTIPLE SITES IN ANAESTHETISED PIGS

Pig	Pancreatico-duodenal vein	Hepatic portal vein	Hepatic vein	Arterial	Renal vein	Femoral vein
1		202	87	85	64	29
2		262	226	140	43	119
3		345	114	102	45	83
4	1416	250	145	117	40	81
5	1009	476	310	83	62	83
6	2333	702	250	250	140	143
mean		372	188	129	66	90
S.E.		76,7	35,4	25,6	15,4	15,9

TABLE 6.7 THE HEPATIC UPTAKE OF PP IN ANAESTHETISED PIGS

Pig	Mass of PP 'entering' the liver pmol/min	Mass of PP 'leaving' the liver pmol/min	% retention of PP
1	99,9	50,4	49,5
2	138,8	97,7	27,0
3	164,2	66,2	57,9
4	125,2	84,2	32,7
5	218	179,5	17,7
6	365	145,0	60,4
mean	184	104	42
S.E.	39,7	20,1	7,3

TABLE 6.8 THE RENAL UPTAKE OF PP IN ANAESTHETISED PIGS

Pig	Arterial PP concentration pmol/l	Renal venous PP concentration pmol/l	% retention of PP
1	85	64	24,7
2	140	43	69,3
3	102	45	55,9
4	117	40	65,8
5	83	62	25,3
6	250	140	44,0
mean			48
S.E.			7,9

flow estimations, the mass of PP entering and leaving the liver was calculated for each pig (Table 6.7). The mean calculated mass of PP extracted by the liver was 81 ± 29,5 pmol/min, or expressed differently, 42 ± 7,3% of the mass of PP presented to the liver per minute was retained.

6.3.1.2 The renal uptake of pancreatic polypeptide.

In calculating the renal extraction of PP, it was assumed that the afferent and efferent renal blood flow were equal; furthermore, that the PP concentration in the renal artery approximated the concentration in the carotid artery. Thus, to calculate the retention of PP as a percentage of the amount presented to the left kidney per unit time, a measurement of the renal blood flow was not needed. The calculated mean renal uptake was 48 ± 7,9%. The values for the individual animals are shown in Table 6.8.

The renal blood flow in pigs of this type and size varies according to the weight of the kidney, but is between 40 and 50 ml per kidney (personal communication from Dr. Rosemary van Hoorn-Hickman). By using an average value of 45 ml/min to calculate the approximate mass of PP taken up by the kidney, it was estimated that 29 ± 0,69 pmol/min of PP was retained by the left kidney. Thus the renal uptake of PP was far lower than the amount taken up by the liver.

6.3.1.3 The peripheral uptake of pancreatic polypeptide.

In all except one of the animals the PP concentration in the femoral vein was lower than the arterial level. The mean arterial PP concen-

TABLE 6.9 PORTAL VEIN PP CONCENTRATIONS IN pmol/l BEFORE, DURING AND AFTER PANCREATECTOMY IN STARVED, ANAESTHETISED PIGS

			MINUTES - POST-OP						
Pig	Pre-op	Intra-op	0	1	3	5	10	20	30
1	385	600	410	320	290	270	260	200	190
2	470	560	380	310	210	190	142	123	100
3	785	850	740	470	300	165	135	105	100
4	468	500	435	348	330	277	240	200	180
mean	527	627	491	362	282	225	194	157	142
S.E	88,2	76,9	83,6	36,8	26,6	29,2	32,4	25,7	24,6

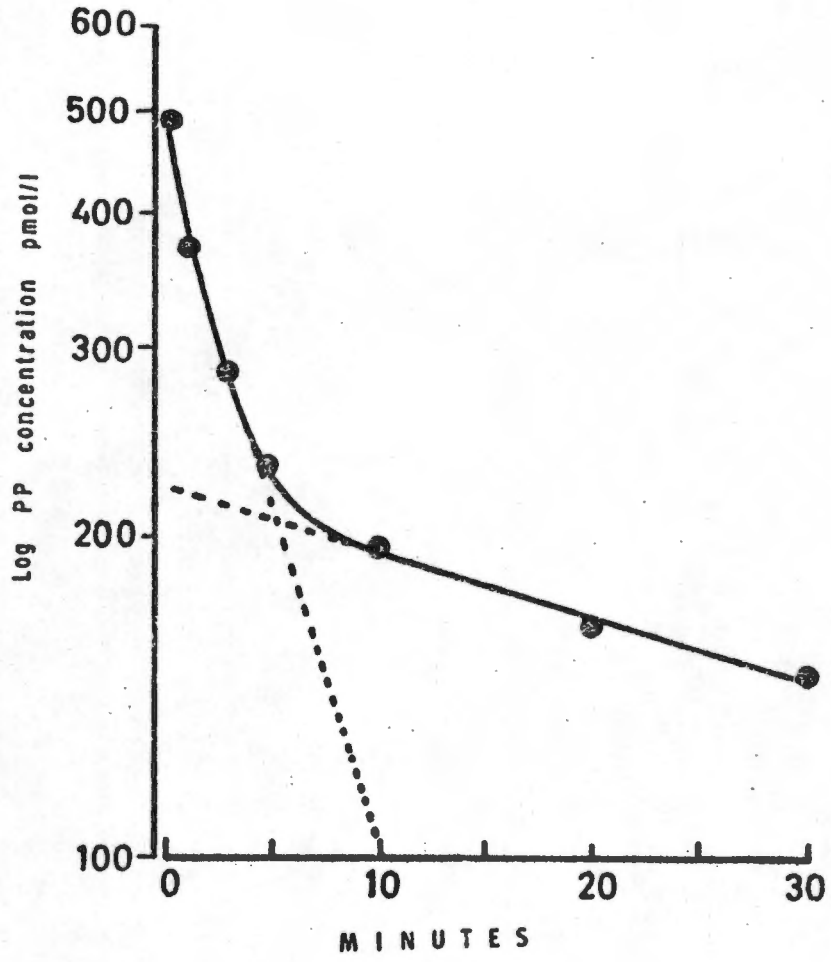


Figure 6.22

Mean PP concentrations in hepatic portal vein serum after total pancreatectomy in 4 pigs. At time zero the last pancreatic vein draining into the portal vein was ligated.

tration was 129 ± 25,6 pmol/l, whereas the mean femoral concentration was 90 ± 15,9 pmol/l and the mean reduction in concentration was 29 ± 9,5%. As before, the calculations assume that under steady state conditions, the afferent and efferent blood flow across the hind limb were equal. However, since the rate of flow was not known, the molar uptake of PP could not be estimated.

6.3.2 The half-life time of disappearance of pancreatic polypeptide.

The rate of disappearance of PP in the hepatic portal vein was determined following total pancreatectomy in anaesthetised pigs.

During the operative procedure there was a rise in the portal vein PP concentration from a pre-operative level of 527 ± 88,2 to 627 ± 76,9 pmol/l. The levels then declined again to 491 ± 83,6 pmol/l at the time of cessation of pancreatic venous drainage into the portal vein. Thereafter there was a steady fall in PP concentrations in all the pigs (Table 6.9).

The disappearance of PP after pancreatectomy is shown in Fig. 6.22. The mean (± S.E.) half-life time was 22,0 ± 4,42 minutes. This long half life appeared to be due to a decrease or flattening in the disappearance curve after 5 minutes which seemed to indicate that two phases of disappearance were present. The disappearance rate was then treated as if two phases were present - a first phase of 5 minutes and a second phase from 5 to 30 minutes, and the $t_{\frac{1}{2}}$ was then calculated for each phase. The calculated $t_{\frac{1}{2}}$ for these phases were 6,3 ± 1,63 and 37,4 ± 3,30 minutes respectively.

Summary

Thus, it was shown that in starved anaesthetised pigs, the PP concentrations in serum, from samples taken from multiple sites, decreased in the following order: pancreatico-duodenal vein, hepatic portal vein, hepatic vein, carotid artery, renal vein and femoral vein. From the serum PP concentrations in these vessels and knowledge of blood flow to the liver and kidney, it was calculated that the liver retained 42 ± 7,3% of the mass of PP entering it, and the kidney 48 ± 7,9% of the mass of PP presented to it. On a molar basis, however, the liver retained 81 ± 29,5 pmol of PP per minute and both kidneys approximately 58 pmol per minute.

Following total pancreatectomy in pigs, serial measurement of PP concentration in the hepatic portal vein were made and the half-life time of disappearance of PP was calculated. The disappearance of PP appeared to occur in two phases and the $t_{\frac{1}{2}}$ was calculated for each. The $t_{\frac{1}{2}}$'s for these phases of disappearance were 6,3 ± 1,63 min and 37,4 ± 3,30 min respectively, whereas the overall $t_{\frac{1}{2}}$ was 22,0 ± 4,42 min.

The preceding sections have detailed the results regarding aspects of the physiology of PP in man and in pigs, and the next section will describe serum PP concentrations in patients with pathological conditions which affect the pancreas.

6.4 Serum pancreatic polypeptide concentrations in patients with diseases of the pancreas.

In the first section of this chapter (6.1), the results of factors affecting serum hPP concentrations in healthy subjects were presented. In similar studies, measurements of basal and stimulated hPP levels were made in patients with chronic pancreatitis and diabetes mellitus. The results of these studies will be presented and compared with those in the healthy subjects. However, firstly the serum hPP concentrations in patients with acute pancreatitis will be described.

6.4.1 Serum pancreatic polypeptide concentrations in patients with acute pancreatitis.

Seventeen patients with a history and clinical findings that were typical of acute pancreatitis, as well as a serum amylase concentrations of greater than 600 Pimstone units per 10 ml were studied. Blood samples from these patients were taken when they were admitted to hospital, and the mean hPP concentration of these initial samples was 33 $\pm$ 6,9 pmol/l (range 11 - 92 pmol/l) as shown in Fig. 6.23. The mean value and range is similar to the hPP concentrations in healthy people of a similar age, (mean 43 $\pm$ 4 pmol/l; range 20 - 88 pmol/l) reported by Schwartz et al (1976) who kindly measured the hPP levels in the plasma samples from these patients. No correlation was found between the initial plasma hPP concentration and the serum amylase concentration.

The serum hPP concentrations were monitored in 9 of these patients at regular intervals to observe changes in their hPP concentrations with recovery (Fig. 6.24). There was a marked fluctuation in the mean

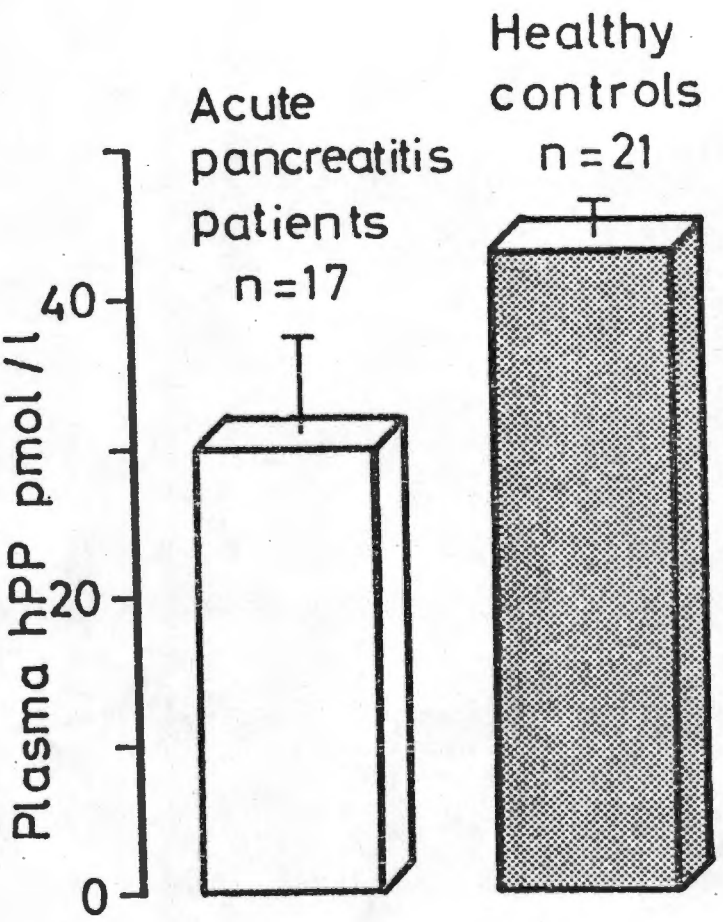


Figure 6.23

Mean ± S.E. plasma hPP concentrations in 17 patients with acute pancreatitis taken on admission to hospital and basal hPP concentrations in 21 healthy subjects after an overnight fast.

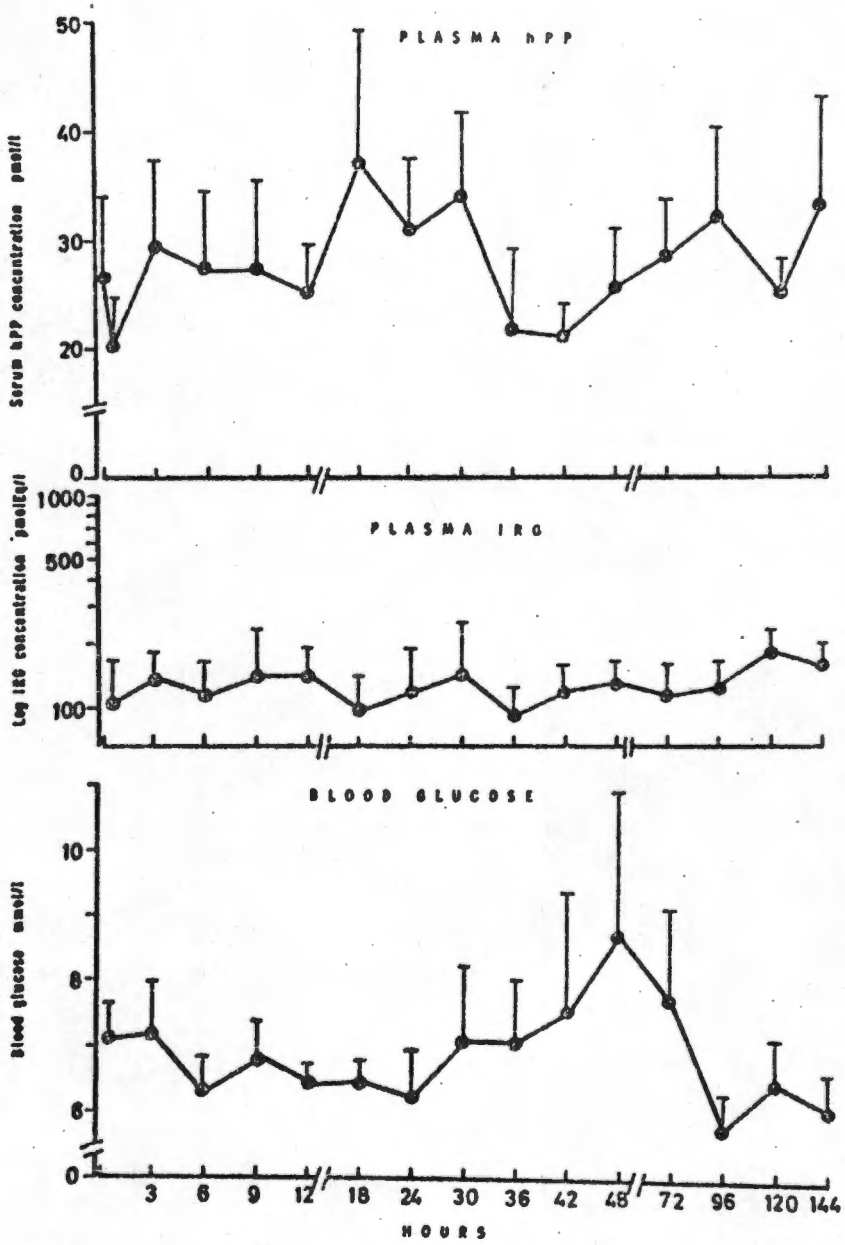


Figure 6.24

Mean $\pm$ S.E. plasma hPP, immunoreactive glucagon and blood glucose concentrations in 9 patients with acute pancreatitis on admission to hospital and during the subsequent course of the illness.

serum hPP concentrations during this period, but in only one patient were very high levels of hPP observed. This patient, however, developed hypocalcaemia, renal failure and the adult respiratory distress syndrome and died 17 days after admission to hospital. On the day of his death, the serum hPP concentration was 214 pmol/l.

Contrasting with the relatively normal serum hPP concentrations in these patients, the initial plasma immunoreactive glucagon (IRG) concentrations were 5 to 10 times the normal value of 11,4 $\pm$ 2,85 pmolEq/l (normal fasting range for this laboratory in fifty, healthy subjects). These elevated plasma IRG levels persisted throughout the patients stay in hospital (Fig. 6.24). The mean blood glucose concentration on admission to the hospital ward was 7,2 $\pm$ 0,46 mmol/l. The concentration fell over the next 24 hours, but thereafter fluctuations in the glucose concentrations were observed.

The mean plasma hPP, plasma IRG and blood glucose concentrations are shown in Fig. 6.24.

6.4.2 Serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

As opposed to the patients with acute pancreatitis, in whom relatively normal hPP concentrations were found, patients with chronic pancreatitis had serum hPP concentrations which were lower than the levels in normal controls, although the differences were not always statistically significant.

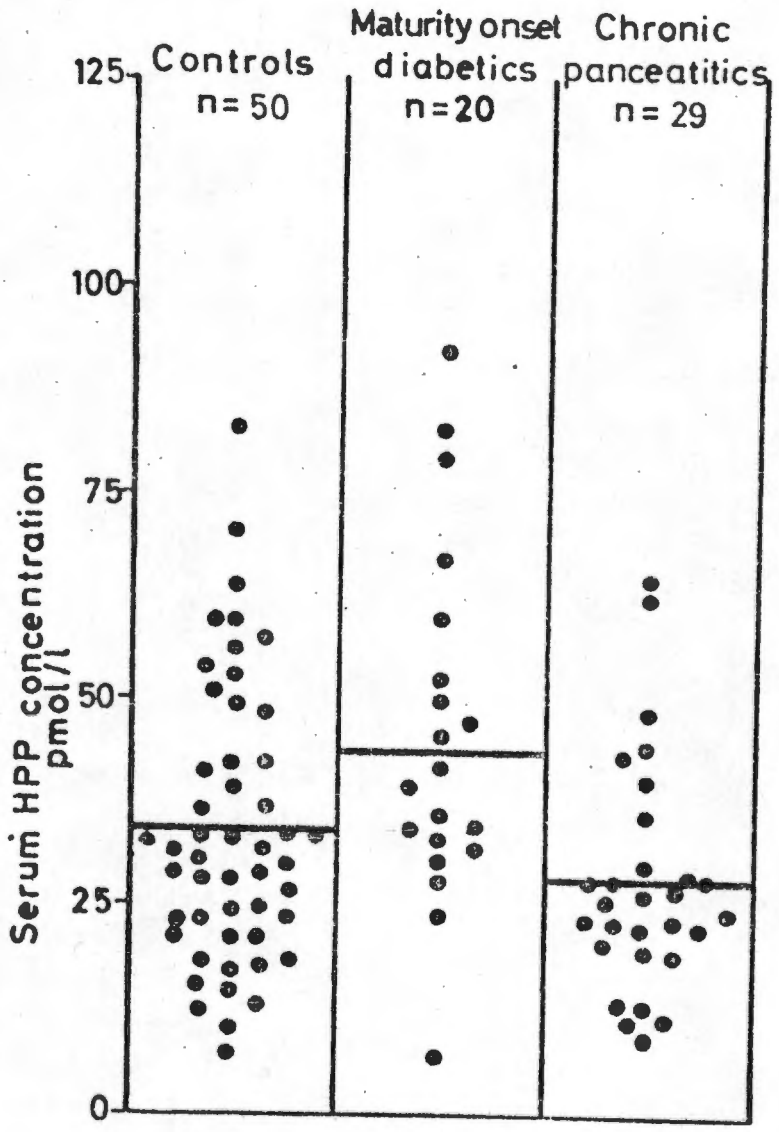


Figure 6.25

Basal serum hPP concentrations in healthy subjects, patients with maturity-onset diabetes and patients with chronic pancreatitis. The bars indicate the mean concentration for each group.

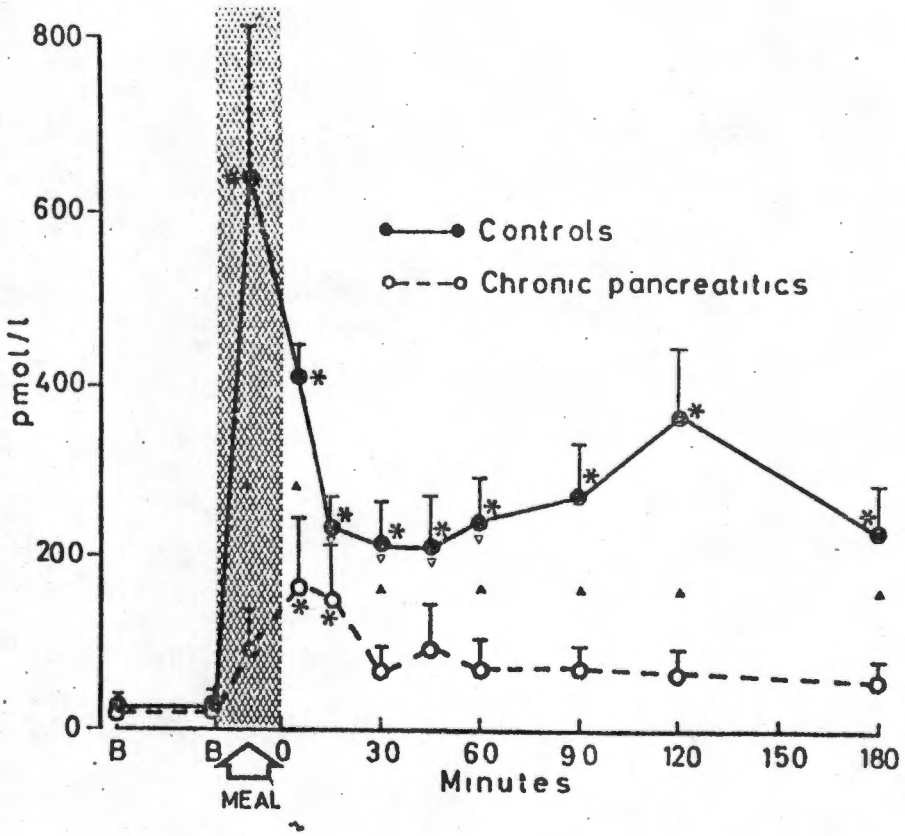


Figure 6.26

Mean $\pm$ S.E. serum hPP responses to a mixed meal in 7 healthy subjects and in 7 patients with chronic pancreatitis. The cross-hatched area indicates the duration of the meal. The asterisks indicate significant ($p < 0.05$) differences between the groups.

6.4.2.1 Basal serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

In the 29 patients with chronic pancreatitis studied, the mean basal serum hPP concentration of 28 ± 13,9 pmol/l (mean ± S.D.) was lower than the mean level of 34 ± 16,8 pmol/l (mean ± S.D.) in fifty healthy subjects. The difference in the mean values was not statistically significant and Fig. 6.25 shows the overlap in the two groups.

6.4.2.2 Stimulation of pancreatic polypeptide release in patients with chronic pancreatitis.

6.4.2.2.1 The effects of nutrients on serum pancreatic polypeptide concentrations.

a) The serum hPP responses to the test meal.

Figure 6.26 compares the serum hPP responses after the standard mixed meal in the patients with chronic pancreatitis and in healthy subjects. In the patients with chronic pancreatitis, the peak hPP concentration of 166 ± 83,0 pmol/l was observed five minutes after completion of the meal, whereas in the healthy subjects, the peak concentration of 648 ± 166,5 pmol/l was observed five minutes after starting to eat. Furthermore, in the former, the hPP levels were no longer significantly elevated 30 minutes after completing the meal, whereas in the healthy subjects, the serum hPP concentrations were still significantly elevated at 180 minutes. At all times sampled, the post-prandial hPP concentrations were significantly lower in the patients with chronic pancreatitis than in the healthy control subjects.

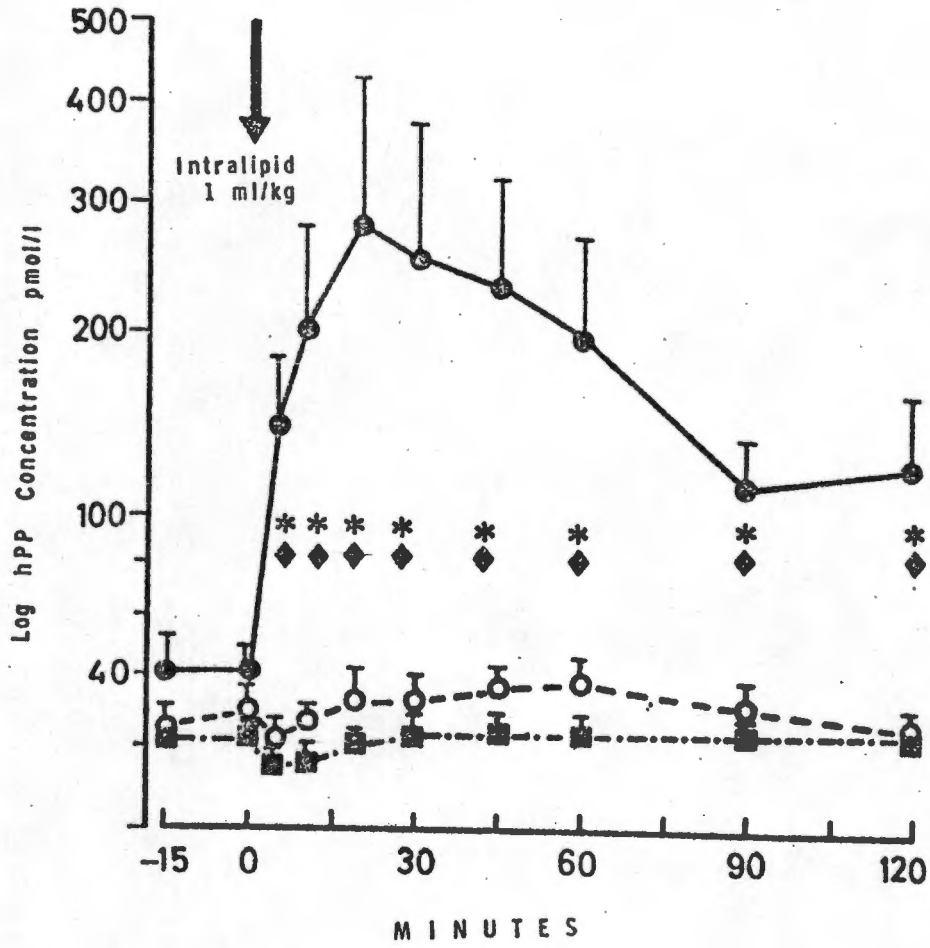


Figure 6.27

Mean $\pm$ S.E. serum hPP responses to oral lipid in 7 health control subjects (closed circles), 6 chronic pancreatitis patients who did not have steatorrhoea (open circles) and 7 chronic pancreatitis patients who had steatorrhoea (squares). The asterisks indicate significant ($p < 0.05$) differences between the normal subjects and patients without steatorrhoea, and the diamonds between controls and patients with steatorrhoea.

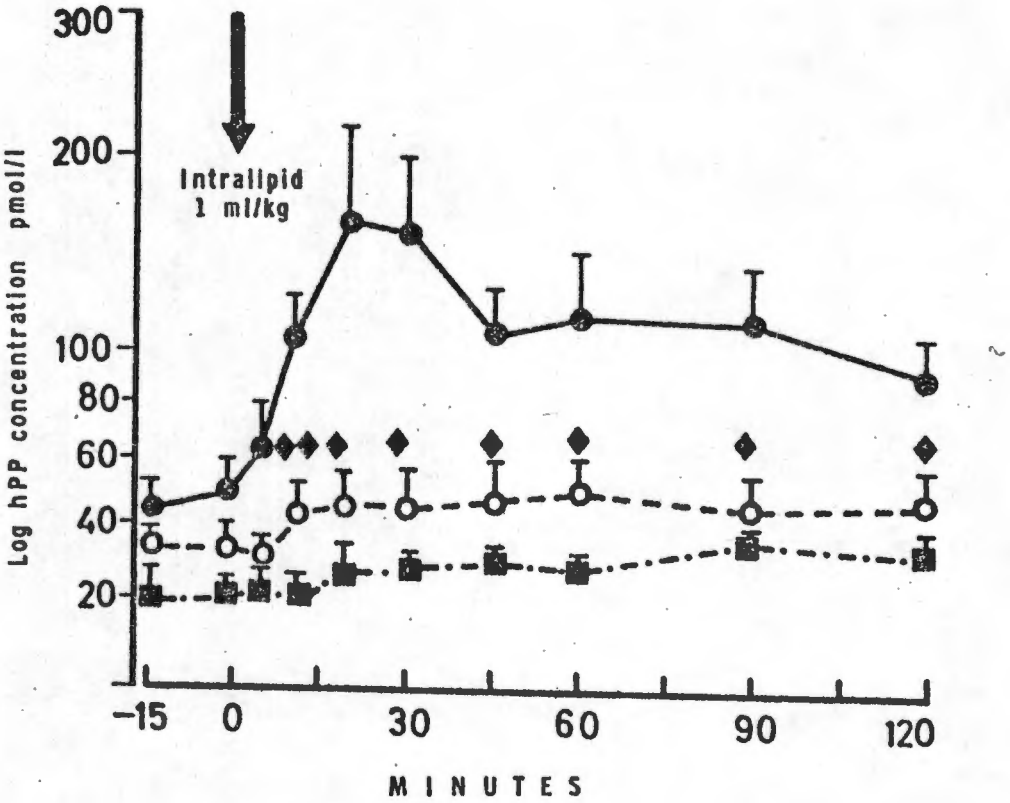


Figure 6.28

Mean $\pm$ S.E. serum hPP responses to intravenous lipid in 7 healthy control subjects (closed circles), 6 chronic pancreatitis patients who did not have steatorrhoea (open circles) and 7 chronic pancreatitis patients who had steatorrhoea (squares). The diamonds indicate significant ($p < 0.05$) differences between the control subjects and patients with steatorrhoea.

b) The effect of intravenous arginine on serum hPP concentrations.
The intravenous arginine infusion of 10 mg/kg/min did not change the serum hPP concentrations in five patients with chronic pancreatitis. This finding was in accord with the results in healthy subjects in whom arginine also had no effect on the serum hPP levels.

c) The effect of lipid on serum hPP concentrations.
Neither oral nor intravenous "Intralipid" administration was associated with significant changes in the serum hPP concentrations in patients with chronic pancreatitis, regardless of whether or not they had evidence of steatorrhoea. This contrasted with the results in healthy subjects in whom oral "Intralipid" caused the serum hPP to rise from a mean basal concentration of 42 $\pm$ 9,4 pmol/l to a mean peak concentration of 282 $\pm$ 154,5 pmol/l. In these healthy subjects, intravenous "Intralipid" administered as a bolus injection, caused the serum hPP concentration to rise from 49 $\pm$ 10,1 pmol/l to 160 $\pm$ 50,1 pmol/l.

After an initial small fall in the mean serum hPP concentration in the patients with chronic pancreatitis, oral "Intralipid" ingestion was associated with a modest increase in the hPP concentration from a basal level of 30 $\pm$ 9,2 to 38 $\pm$ 9,6 pmol/l in the patients without steatorrhoea and with a return to the basal concentration of 23 $\pm$ 4,4 pmol/l in the patients who had steatorrhoea (Fig. 6.27). These changes were not statistically significant. Although a slightly greater response to intravenous "Intralipid" administration was seen in the patients without steatorrhoea, the rise in the mean serum hPP concentration to 49 $\pm$ 11,5 pmol/l was also not significant (Fig. 6.28).

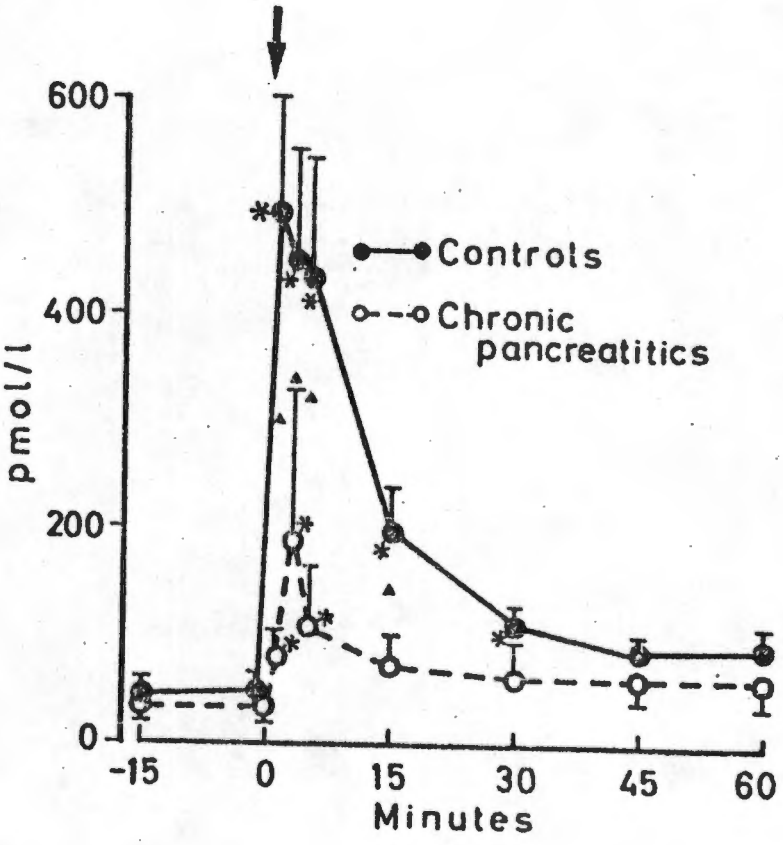


Figure 6.29

Mean $\pm$ S.E. serum hPP responses to 2 CHRU/kg of 'Boots' secretin in 7 healthy subjects and 7 patients with chronic pancreatitis. The secretin was injected intravenously over 3 minutes at time zero as indicated by the arrow. The asterisks indicate a significant ($p < 0.05$) rise above the basal concentrations, and the triangles indicate significant ($p < 0.05$) differences between the groups.

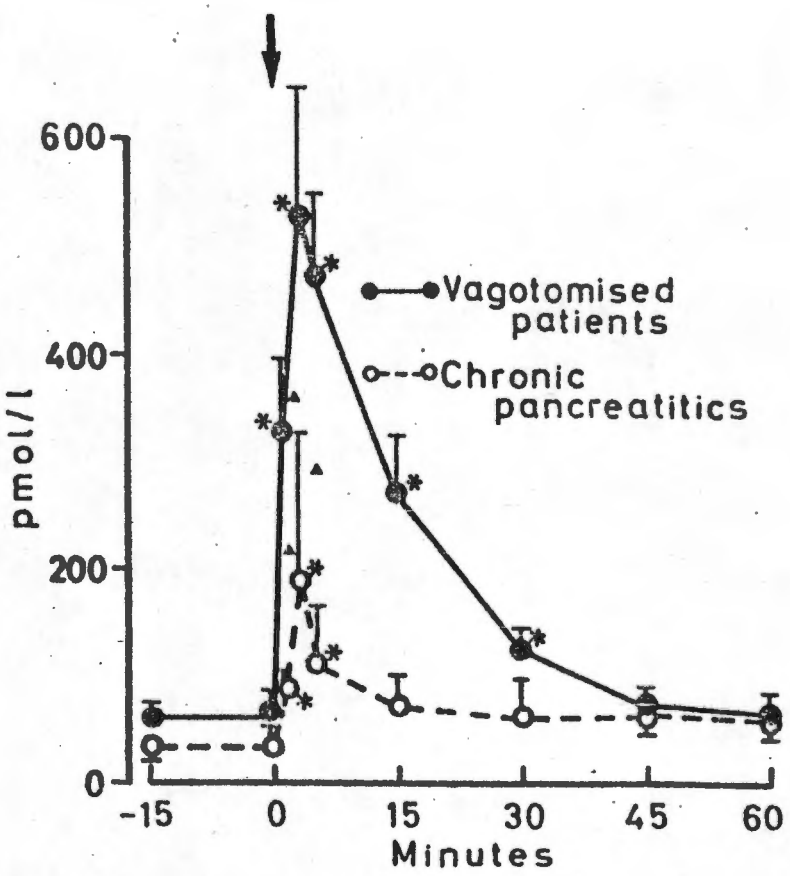


Figure 6.30

Mean ± S.E. serum hPP responses to 2 CHRU/kg of 'Boots' secretin in 7 patients with chronic pancreatitis and 7 patients who had undergone truncal vagotomy for duodenal ulceration. The secretin was injected intravenously over 3 minutes at time zero as indicated by the arrow. The asterisks indicate significant ($p < 0.05$) differences between the groups.

As in the healthy subjects, elevation of endogenous free fatty acids with heparin was not associated with a significant change in the serum hPP concentrations.

6.4.2.2.2 The effects of gastrointestinal hormones on serum pancreatic polypeptide concentrations.

In healthy subjects, "Boots" secretin infused intravenously was a potent stimulus for PP release (6.1.2.1), and 2CHRU/kg of the secretin preparation caused the serum hPP concentration to rise from a basal level of 50 ± 10,1 pmol/l to 498 ± 104,3 pmol/l. In contrast, in the patients with chronic pancreatitis this dose of secretin caused the serum hPP concentrations to rise to a significantly lower mean level of 188 ± 143,0 pmol/l (Fig. 6.29). The serum hPP response to this dose of secretin was also significantly lower in the patients with chronic pancreatitis than in the subjects who had undergone bilateral truncal vagotomy (Fig. 6.30).

6.4.2.2.3 The effects of insulin-hypoglycaemia on serum pancreatic polypeptide concentrations.

The administration of 0,1 U/kg of monocomponent insulin to five patients with chronic pancreatitis caused the serum hPP levels to rise from a mean basal concentration of 30 ± 7,1 pmol/l to a peak concentration of 53 ± 14,7 pmol/l, significantly lower than the peak response of 220 ± 54,6 pmol/l in healthy subjects (Fig. 6.31). The patients with chronic pancreatitis had a significantly higher mean fasting blood glucose concentration (4,6 ± 0,5 mmol/l) than the control subjects (3,5 ± 0,12 mmol/l). However, insulin injection caused the mean blood glucose concentration to fall to a nadir of 1,7 ±0,10 mmol/l

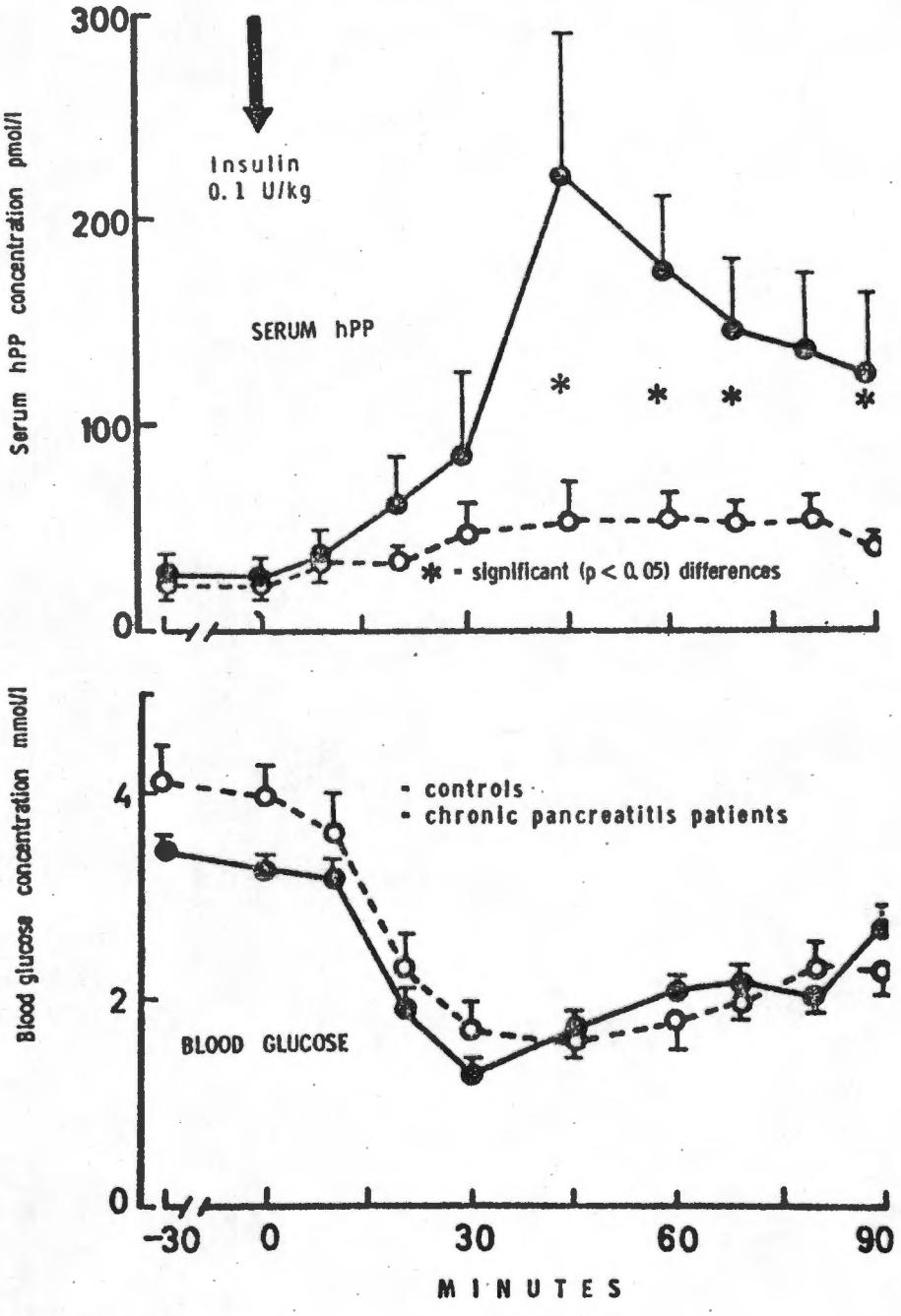


Figure 6.31

Mean $\pm$ S.E. serum hPP and blood glucose reponses to insulin-induced hypoglycaemia in 5 healthy subjects and in 5 patients with chronic pancreatitis. 0,1U/kg of monocomponent insulin was injected at time zero.

in the patients with pancreatitis and to 1,5 ± 0,06 mmol/l in the healthy subjects, values that were not significantly different. Thus despite a relatively greater fall in the mean blood glucose concentration in the patients with chronic pancreatitis, the hPP responses to insulin-induced hypoglycaemia was significantly impaired.

6.4.2.3 Inhibition of pancreatic polypeptide release in patients with chronic pancreatitis.

The infusion of synthetic cyclic somatostatin at a rate of 2µg/min in the patients with chronic pancreatitis, abolished the attenuated hPP response to insulin-induced hypoglycaemia. The failure of serum hPP concentrations to rise during the hypoglycaemia in the presence of an infusion of somatostatin, was in accord with the findings in healthy subjects.

Summary of serum hPP concentrations in patients with chronic pancreatitis.

In patients with chronic pancreatitis, although the basal hPP levels were not significantly lower than the concentrations in healthy subjects, the hPP responses to provocative stimulation were uniformly impaired. Thus the diverse stimuli of a mixed meal, "Boots" secretin and insulin-induced hypoglycaemia elicited serum hPP responses which were significantly smaller than the responses in healthy subjects. The hPP responses to oral and intravenous "Intralipid" administration, too, were impaired and appeared to be poorer in patients with, as opposed to patients without, evidence of steatorrhoea. However, as in healthy

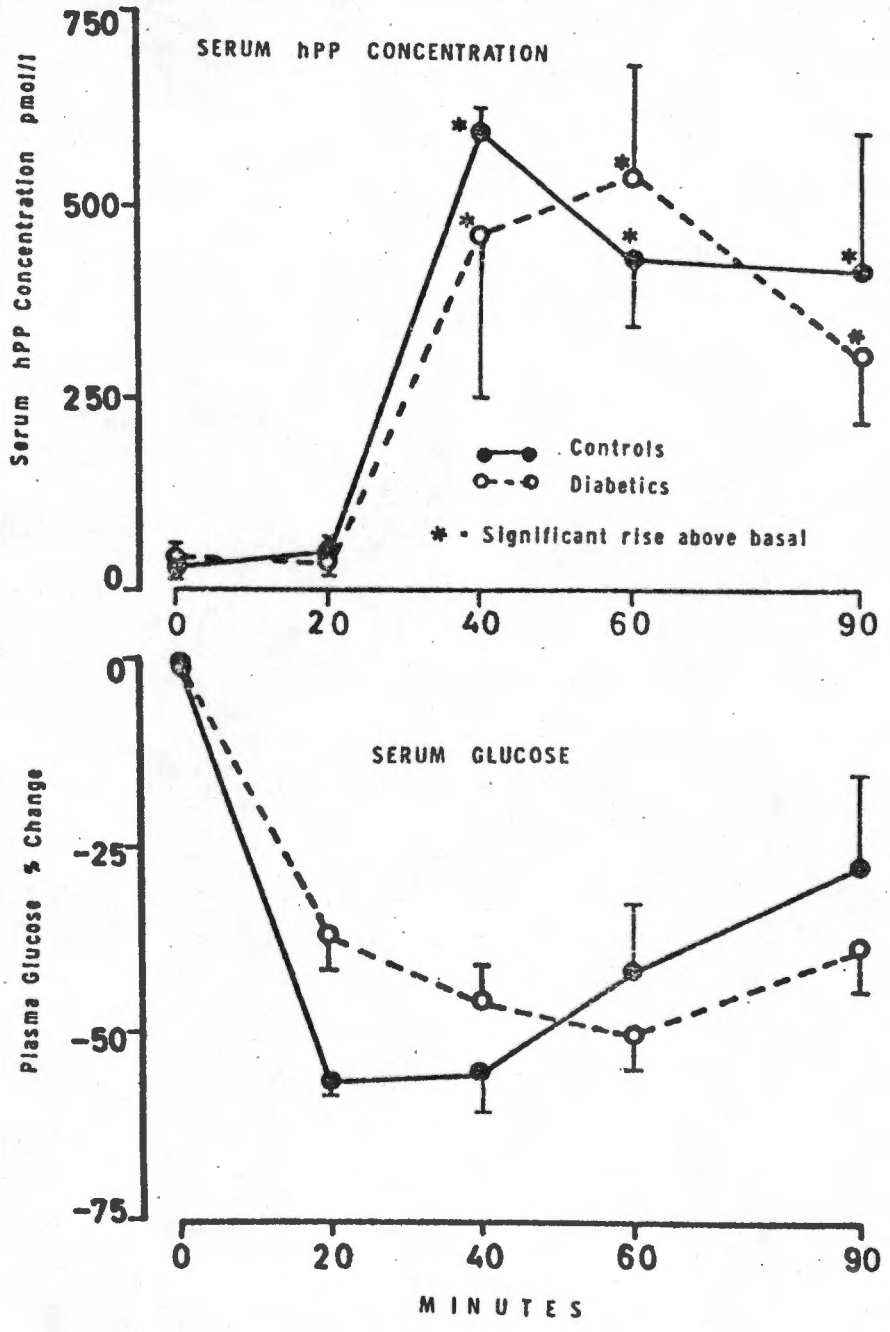


Figure 6.32

Mean $\pm$ S.E. serum hPP and glucose responses to insulin-induced hypoglycaemia in 6 patients with maturity-onset diabetes and in 5 healthy subjects. The diabetic subjects receivied 0,2U/kg and the healthy subjects 0,1U/kg of monocomponent insulin intravenously at time zero. The asterisks indicate a significant ($p < 0.05$) rise above the basal concentrations.

control subjects, somatostatin suppressed the hPP response to insulin-induced hypoglycaemia.

6.4.3 Serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

6.4.3.1 Basal serum pancreatic polypeptide concentration in patients with diabetes mellitus.

In twenty patients with maturity-onset diabetes mellitus, who were 35 to 72 years old, the mean (± S.D.) basal serum hPP concentration was 44 ± 20,9 pmol/l. This was higher, but not significantly so, than the mean (± S.D.) concentration of 34 ± 16,8 pmol/l in fifty non-age matched healthy subjects. The fasting hPP concentrations in the healthy subjects, in patients with chronic pancreatitis and in the patients with diabetes mellitus are compared in Fig. 6.25.

6.4.3.2 Stimulated serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

Insulin-induced hypoglycaemia caused the hPP concentrations to rise from a mean basal concentration of 30 ± 11,3 pmol/l to 539 ± 139,4 pmol/l in 6 patients with diabetes mellitus. This response was not significantly different from the response observed in age-matched healthy subjects (Fig. 6.32). In the control subjects used for this study, the serum hPP concentrations rose from 33 ± 9,9 pmol/l to 597 ± 28,8 pmol/l, the peak concentration occurring 40 minutes after the insulin injection, whereas in the diabetics, the peak concentration was observed at 60 minutes.

In the subjects with diabetes mellitus, the mean basal serum glucose concentration was 7,9 ± 1,79 mmol/l, and after insulin administration fell to a nadir concentration of 4,1 ± 0,97 mmol/l. The mean basal glucose concentration in the controls was 4,6 ± 0,52 mmol/l and the nadir concentration was 1,7 ± 0,24 mmol/l (Fig. 6.32). The fall in serum glucose concentrations represented a decrease of 48% in the diabetics and of 58% in the controls. The nadir glucose concentration occurred at 20 minutes in the controls, and at 40 minutes in the diabetics.

Thus, in summary, although the mean basal serum hPP concentration was higher in patients with diabetes mellitus than in non-age matched controls, the difference was not statistically significant. Serum hPP responses to insulin-induced hypoglycaemia were similar in diabetics and in matched controls, although the peak serum hPP concentration occurred 20 minutes later in the diabetic subjects. However, in these subjects, the nadir glucose concentration was also reached 20 minutes later.

6.5 A summary of the results.

This chapter described the observations which were made when the regulation of PP release in healthy subjects, the molecular forms of circulating immunoreactive hPP, the organ uptake and disappearance rate of PP, and the serum hPP concentrations in patients with acute and chronic pancreatitis and diabetes mellitus were studied.

To summarise the salient features of these results, it was found that in 50 healthy subjects, the mean (± S.D.) basal serum hPP concentration was 34 ± 16,8 pmol/l and ranged from 7 to 83 pmol/l. Ingestion of a mixed meal, caused a sharp rise in the serum hPP concentrations to 648 ± 166,5 pmol/l and although the levels then fell, they remained significantly elevated above the basal concentration for 3 hours. The ingestion of 50g of glucose, of 1 ml/kg of "Intralipid" also caused serum hPP concentrations to rise, but to concentrations far lower than those observed after a mixed meal. Intravenous lipid administration caused a 50% rise from basal in the serum hPP concentrations but intravenous glucose administered as a bolus, caused the hPP concentrations to fall by 40%. An intravenous arginine infusion had no effect on the serum hPP concentrations. The intravenous administration of 2CHRU/kg of "Boots" secretin was associated with a 9 fold rise in the serum hPP concentrations both in healthy subjects and in subjects who had undergone bilateral truncal vagotomy. "Boots" cholecystokinin-pancreozymin and pentagastrin administered as rapid intravenous infusions, also caused significant elevations in the serum hPP concentration.

Serum hPP concentrations were modulated by perturbations of the sympathetic and parasympathetic nervous systems. Selective adrenergic

β-receptor stimulation caused an 8-fold rise in the serum hPP concentrations, but adrenergic α-receptor stimulation was associated with a fall in the hPP concentrations. Insulin-induced hypoglycaemia also caused the serum hPP concentration to rise to 8 times above the basal concentration. The hypoglycaemia-induced rise in the serum hPP concentrations was abolished when somatostatin was infused at a rate of 2μg/min. The dopamine agonist, L-dopa, had no effect either on basal or on meal stimulated hPP responses and the dopamine antagonist, metoclopramide, too had no effect on basal serum hPP concentrations.

Fractionation of serum hPP by using 10% polyacrylamide disc gel electrophoresis, revealed that the circulating hPP consisted of more than one immunoreactive component. In sera from 6 subjects, a component of hPP was present which migrated in the position of ^{125}I-hPP, but in addition, in the majority of the sera, components which migrated in more anodal and in more cathodal positions were also detected.

PP released into the portal circulation in pigs was taken up by the liver and by the kidneys, to the extent of 81 ± 29,5 pmol/min by the liver and approximately 58 pmol/min by the kidneys. Following pancreatectomy in 4 pigs, the disappearance of PP from the hepatic portal vein appeared to occur in two phases. The calculated $t_{\frac{1}{2}}$ for the first phase of disappearance was 6,3 ± 1,63 min and that for the second phase 37,4 ± 3,30 min.

Finally, observations in the patients with diseases involving the pancreas, revealed that in patients with acute pancreatitis plasma hPP concentrations were similar to the concentrations in healthy fasting

subjects, but that in 29 patients with chronic pancreatitis, although the mean (± S.D.) basal serum hPP concentration of 28 ± 13,9 pmol/l (range 7 to 65 pmol/l) was not significantly different from that for 50 healthy subjects (34 ± 16,8 pmol/l), the serum hPP response to insulin-induced hypoglycaemia, to a mixed meal, and to "Boots" secretin administration were impaired. After the stimuli, the serum hPP levels only rose to concentrations that were 3 - 4 times lower than the concentrations in healthy subjects. On the other hand, in patients with diabetes mellitus, the serum hPP response to insulin-induced hypoglycaemia was of the same magnitude as in matched control subjects. In 20 patients with diabetes mellitus, the mean (± S.D.) basal serum hPP concentration of 44 ± 20,9 pmol/l (range 24 to 93 pmol/l) was not significantly higher than the mean concentration of 34 ± 16,8 pmol/l in the non-aged matched healthy subjects.

Following this description of the results of these studies their significance in relation to the findings of other investigators and in relation to the physiology and pathophysiology of other gastro-intestinal hormones and candidate hormones will be discussed.

CHAPTER VII

DISCUSSION

CHAPTER VII DISCUSSION

- Serum pancreatic polypeptide concentrations in patients with chronic pancreatitis
- Serum pancreatic polypeptide concentrations in patients with diabetes mellitus

CHAPTER VII

DISCUSSION

7.0. Introduction

In 1968, Kimmel, Pollack and Hazelwood isolated from pancreata of chickens a peptide, which up to that stage had not been known to exist. Homologous peptides were also isolated from pancreata of man, cows, sheep and pigs by Chance (1972). These peptides were sequenced and were each found to consist of 36 amino-acid residues and to have a molecular weight of 4200 daltons. Later, this pancreatic peptide which was called pancreatic polypeptide (PP), was localised to specific pancreatic endocrine-like cells and was found to circulate in the blood in concentrations similar to those of many gastrointestinal hormones. It was suggested that this newly-recognized peptide was in fact a new pancreatic hormone. However, a physiological role for PP was not defined.

Although it was found that PP was released into the circulation during feeding and had actions on many aspects of gastrointestinal function, these findings were not adequate to label PP a hormone. Additional data on factors affecting the release of PP became known. Thus it was reported that not only nutrients but also gastrointestinal hormones and the autonomic nervous system, especially the vagus nerve, were important factors in controlling the release of PP. However, with the growth in knowledge of PP, so too came discrepancies in the findings of different investigators. For instance, Floyd, Fajans, Pek et al (1977) reported that in healthy subjects intravenous glucose administration caused the plasma hPP concentration

to fall whereas Adrian, Bloom, Besterman et al (1977) found that plasma PP concentrations were not significantly changed by intravenous glucose.

Similarities in the characteristics of PP and other endocrine-like peptides were sought. Thus Villanueva, Hedo and Marco (1977) reported that PP, like many peptide hormones, circulated in the blood in more than one immunoreactive form. However, PP in extracts of pancreata was present in only one molecular form (Adrian, Bloom, Bryant et al, 1976). It was further reported that like other gastrointestinal peptides, PP has a relatively short $t_{\frac{1}{2}}$ of 5,5 $\pm$ 1 min (Taylor, Solomon, Walsh et al, 1977), but this $t_{\frac{1}{2}}$ was calculated for porcine PP infused into dogs.

With regard to the pathophysiology of PP, it was reported that fasting PP levels were elevated in patients with diabetes mellitus and in some patients with endocrine tumours of the pancreas, and that the serum hPP responses to a meal were impaired in patients with calcific chronic pancreatitis. However, an analysis of PP responses and levels were not known in other situations. Thus, for example, the serum PP levels in patients with acute pancreatitis had not been measured, nor had the responses to provocative stimulation been determined in patients with diabetes mellitus.

Although this brief historical description does not encompass the total body of knowledge of PP at the time of undertaking the studies, this resumé highlights areas where it was proposed to further investigate aspects of PP physiology. In an attempt to fill hiatuses in our knowledge of the regulation PP release, to clarify discrepancies in our knowledge of PP, to define factors involved in the kinetics of PP release and uptake, and to assess serum PP concentrations and

responses in patients with certain diseases of the pancreas, a radioimmunoassay was established which enabled the measurement of PP in serum.

Studies in healthy people were then designed and carried out, which examined the serum PP responses to oral and intravenously administered nutrients, intravenous gastrointestinal hormone administration, manipulation of the sympathetic, parasympathetic and dopaminergic nervous systems, and the administration of the histamine H_2-receptor antagonist, cimetidine, and to somatostatin. Further studies in normal people examined the molecular forms of circulating immunoreactive hPP, after separation of these forms by criteria of molecular weight and charge. In order to examine the kinetics of endogenous PP, organ uptake and $t_{\frac{1}{2}}$ of PP were studied in pigs. Attention then reverted to man, but in this instance to compare the basal and stimulated serum PP levels in patients with acute and chronic pancreatitis, and diabetes mellitus, with the levels in healthy subjects.

During the time that the studies outlined above were in progress, additional knowledge of the characteristics and behaviour of PP became available in the literature. Thus, in the discussion which follows, the results of these studies will be assessed in the light of the present knowledge and understanding of PP. In an attempt to present a cohesive interpretation of the results, the format of the discussion will not strictly follow the order of the preceding chapters.

7.1 The regulation of pancreatic polypeptide release.

One of the most striking observations in the studies which were carried out in healthy subjects was the serum PP response to a standardised mixed meal. The meal was a potent stimulus for PP release, and caused the serum hPP concentrations to rise to a mean level of 648 $\pm$ 166,5pmol/l five minutes after starting to eat. Although the hPP concentrations then fell significantly, they remained significantly elevated above the basal concentration for 3 hours which was the duration of the study. This pattern of response was similar to that reported by Schwartz, Rehfeld, Stadil et al (1976), who described the response that they observed as "biphasic". In the study carried out in this laboratory, two separate and distinct peaks in the serum hPP concentration were not observed, which may indicate that a true biphasic phenomenon was not present. However, there appeared to be a clear distinction between the early, sharp response and the more prolonged elevation at a lower level. Thus there may indeed be early and later phases of PP release which could suggest that several factors may play a role in regulating the meal-induced release of PP. It may further suggest that in trying to assess the factors controlling the release of PP, the early release during eating and the post-prandial elevation at a lower level, should be dealt with separately.

The early release of hPP after ingestion of a meal.

Within five minutes of starting to eat, a rapid rise in the serum hPP concentration was observed. This very early rise is unlikely to be a result of the absorption of nutrients, because the hPP concentration

then fell, only to rise again 90 minutes later. The fact that this early release can be abolished by truncal vagotomy (Schwartz, Rehfeld, Stadil et al, 1976) suggests that it is mediated by a neural mechanism. Furthermore, distension of the gastric fundus by a thin walled rubber balloon inflated with 150ml of saline, was reported to cause an immediate rise in the serum PP concentration, which was also abolished by truncal vagotomy (Schwartz and Rehfeld, 1977). This supports the contention that a gastro-pancreatic neural reflex is implicated in the early release of PP within a short time, in this instance, 5 minutes, of starting to eat.

The degree of gastric distension required to trigger the reflex is not known, but a drink of 150ml of water did not raise the serum hPP concentration in this study, whereas Schwartz and Rehfeld (1977) reported that inflation of a rubber balloon in the gastric fundus with 150ml of saline did cause the release of PP. Further inflation of the balloon did not enhance the PP response (Schwartz, Grotzinger, Schöön et al, 1979). It is possible that a rubber balloon inflated with 150ml of saline, because of the enclosed space, exerted a greater pressure on the stomach than 150ml of water which was free and able to find its own level over a greater area. Thus there may be a critical pressure which could trigger the reflex. Taylor, Feldman, Richardson, et al (1978) reported that 600ml of saline instilled into the stomach via a nasogastric tube, caused a significant rise in the hPP levels in healthy volunteers. This release, however, was significantly smaller than that caused by the instillation of 600ml of a liquidised mixed meal. It would thus appear that a "stretch" mechanism may trigger a gastro-pancreatic reflex to cause the release of hPP, but another mechanism must be invoked to explain the different hPP response to the same volumes of saline and a

mixed meal. An explanation is also required why in this study, in similar volumes, oral glucose caused only a modest rise in the hPP concentration, oral lipid a far greater response, and water no response at all, although, admittedly, blood was sampled at different intervals in these experiments. It is possible that an osmotic effect exerted by the food may have an effect on PP release, but as yet there is no evidence for this suggestion. Deutch and Wang (1977) have proposed that there are nutrient receptors in the stomach which may differentiate between the primary nutrients and communicate this information to a cephalic centre. A gastro-cephalic- pancreatic reflex could thus transmit a signal for the release of PP and may explain the different hPP reponses to equal volumes of food and saline in Taylor et al's study and to fat, glucose and water in these studies.

A further cephalic factor, however, may be operative. Taylor, Feldman, Richardson et al (1978) also reported that "sham-feeding" - chewing the food without swallowing it - in human volunteers, was associated with a small hPP response, and that sham-feeding enhanced the hPP response to gastric instillation of food. Whether this relates to an oral stimulus for hPP release or is a conditioned response similar to the salivary response in Pavlov's dogs (Pavlov, 1927) is not known. It is feasible that a cephalic centre may influence pancreatic hormone release. Goldfine, Abriara, Grunewald et al (1970) have shown that insulin was released by hypnotic suggestion which reflects that higher centres may contribute to pancreatic hormone release. It has been realised since the classic work of Claude Bernard (1849) that alterations in the functioning of the brain can lead to changes in the blood sugar concentration. Electrical stimulation of the ventrolateral hypothalamic nuclei were reported to cause an increase in the plasma insulin

concentrations whereas stimulation of ventromedial hypothalamic nuclei were reported to decrease plasma insulin and increase plasma glucagon concentrations (Frohman and Bernardis, 1971). Thus stimulation of these nuclei of the hypothalamus can affect the release of pancreatic hormones, but whether they are excited during sham-feeding or during hypnosis is not known. Moreover, whether stimulation of these nuclei could affect the release of PP is also not known.

Factors in addition to those already discussed may contribute to the early release of hPP. It is unlikely that the PP response is regulated by changes in gastric or duodenal pH, as both neutralization of gastric acidity with antacids (Taylor, Walsh, Hogan et al, 1978) and duodenal acidification by the instillation of hydrochloric acid (Floyd, Fajans, Pek et al, 1977) had no significant effect on PP responses. Furthermore, these investigators reported that the hPP response to a mixed meal did not differ from normal in patients with achlorhydria and pernicious anaemia. On the other hand, it is possible that massive gastric acid secretion may enhance PP release. Thus Polak, Bloom, Adrian et al (1976) reported that a number of patients with the Zollinger-Ellison (gastrinoma) syndrome had basal plasma PP concentrations that were elevated above 240pmol/l. However, as only a proportion of these patients had PP hypersecretion and as PP cell hyperplasia has been found in these tumours (Heitz, Polak, Bloom et al, 1976) and in the surrounding non-tumorous pancreas (Larsson, Schwartz, Lundqvist et al, 1976) it is unlikely that the acid per se is the cause of the increased PP release in this condition.

It would thus seem that neural factors are of importance in regulating the early release of PP in response to a meal. It is possible that PP

is released by a gastro-pancreatic reflex which is triggered by gastric distension of an unknown magnitude, and by nutrient sensors. In addition, higher cephalic centres may be implicated in the PP response shortly after eating. However, investigation is still required to elucidate whether different primary nutrients would cause varying release of PP, and whether this may relate to the nutrient per se, the osmotic concentration, or the physical pressure it may exert.

In discussing the early release of PP shortly after eating, the possibility was raised that the nature of the nutrients even before absorption may to some extent have determined PP release. Indeed, it is possible that nutrient receptors in the lumen of the gut may register the type of nutrient, and thus constitute an initial stage in providing a hormonal response. Moreover, the active process of absorption, the absorption of the nutrient into the circulation and the actual concentrations of the nutrients in the blood may be factors which could also determine the release of PP.

The prolonged release of hPP after the meal.

After completion of the meal, the PP concentrations fell to a level of $215 \pm 61{,}4$pmol/l which was significantly lower than the peak concentration of $648 \pm 166{,}5$pmol/l observed 5 minutes after starting to eat. This lower level was still significantly higher than the basal concentration and remained significantly elevated for 3 hours after the meal. In fact, elevated hPP levels have been measured for 6 hours after eating (Adrian, Bloom, Besterman et al, 1977). The short half-life of PP in pigs (see 6.3.2.) and in dogs (Taylor, Solomon, Walsh et al, 1978) of 6,3 and 5,5 min respectively, suggests that there is sustained

release of hPP during this time, and that the elevated concentrations are not due to a slow rate of removal of PP.

In the studies of Schwartz, Rehfeld, Stadil et al (1976), truncal vagotomy diminished the hPP concentration during the period of prolonged release after a meal but did not abolish it. In contrast, Adrian, Bloom, Besterman et al (1977) reported that truncal vagotomy did not cause a decrease in the integrated plasma hPP response to a meal which suggests that additional factors are responsible for the release of PP. Some of these are considered below.

The effects of nutrients on hPP release

Oral nutrients may exert their effects in a number of ways to cause PP release. Clearly a neural effect may be implicated; secondly, after absorption, the nutrients per se may have a direct effect on the PP cell; and thirdly, they may cause release of a gut factor which acts as a PP secretogogue. The effects of the individual nutrients that were tested and the possible ways in which they may have acted to cause changes in the serum PP concentrations will be considered below.

The effects of amino-acids on hPP release.

The intravenous administration of arginine had no effect on the hPP concentrations. This is in accord with a report from Floyd, Fajans, Pek et al (1977) although they found that 180 minutes after the arginine infusion, there was a small rise in the hPP concentration. Whether, at this late stage, the release relates to the arginine per se is doubtful especially as they found the same pattern of release with an infusion

of normal saline. It is however possible, that the period of reactive hypoglycaemia which occurred after stopping the arginine infusion may have caused the rise in the plasma hPP levels. However, after the saline infusion hPP concentrations still rose, yet there was no decline in the blood glucose concentrations. Infusions of alanine and leucine also only caused late release of hPP in their hands. However, a mixture of 10 essential amino-acids caused sustained release of hPP. This finding is contrary to a report from Adrian, Bloom, Besterman et al (1977) that an undefined mixture of amino-acids had no effect on the plasma hPP concentration. It is possible that the differences in the hPP responses in the two studies may depend on the volume infused, the pH of the infusate, the concentrations of the amino-acids or differences in the amino-acid compositions. Intraduodenal instillation of an amino-acid mixture containing 8 essential and 7 non-essential amino-acids in dogs, caused PP release of a similar magnitude to that seen after a meal (Wilson, Boden and Owen, 1978). Whether the release of hPP observed by Floyd et al and Wilson et al is a direct effect of specific amino-acids is not known. It has been shown that certain amino-acids viz. a mixture of methionine, phenylalanine, tryptophan and valine caused the release of cholecystokinin when instilled into the duodenum, whereas a mixture of arginine, histidine, isoleucine, leucine, lysine and threonine caused release of gastric inhibitory polypeptide without affecting the release of CCK (Thomas, Sinar, Mazzaferri et al, 1978). Thus the various amino-acids appear to release different gut factors when instilled into the duodenum, but whether this would apply to the intravenous administration of these amino-acids and more specifically, whether only some would release PP when administered intravenously is not known. Clearly, however, arginine per se does not cause hPP release and further investigation is required to ascertain

which amino-acids are PP secretogogues.

The effects of glucose on hPP release.

In contrast to arginine, both the oral ingestion and the intravenous administration of glucose caused perturbations in the serum hPP concentrations. Oral glucose caused a rise, whereas with intravenous glucose a fall in the serum hPP concentration was observed. The rise with oral glucose confirms the finding of Floyd, Fajans, Pek et al (1977) but may relate to the gastro-pancreatic reflex previously discussed and not be an effect of glucose per se. Alternatively, an indirect mechanism, via the release of a gastrointestinal factor may have been responsible for the release of hPP. Gastric inhibitory polypeptide (GIP) and vasoactive intestinal polypeptide (VIP) in-vitro (Iversen, Bloom, Adrian et al, 1977), and secretin in-vivo (Adrian, Bloom, Besterman et al, 1977) have been reported to cause hPP release. Oral glucose has been shown to cause GIP release (Pedersen, Schubert and Brown, 1975; Cataland, Crockett, Brown et al, 1974), but glucose by intraduodenal instillation had no effect on VIP concentrations (Schaffalitzky de Muckadell, Fahrenkrug, Holst et al, 1977). The plasma secretin response to oral glucose is uncertain. Some investigators have reported a rise (Chisholm, Kraegen, Young et al, 1971) whereas others (Fahrenkrug and Schaffalitzky de Muckadell, 1977) were unable to confirm this response. Thus of these three gastrointestinal factors, GIP is the most likely candidate as the gastrointestinal mediator of PP release following glucose ingestion and indeed in the isolated perfused canine pancrease GIP did release PP (Adrian, Bloom, Hermansen et al, 1978). However, this stimulus to the release of hPP may not occur in-vivo under physiological conditions.

The fall in the serum hPP concentration after the intravenous glucose bolus of 25g, is in accord with the findings of Marco, Hedo and Villanueva (1978) who reported that in healthy subjects, the plasma hPP concentrations fell by approximately 50% during glucose infused at a rate of 0,6g/min, and by 40% after a bolus injection of 0,33g/kg.

Glucose was reported not to cause the isolated perfused canine pancreas to release PP (Iversen, Bloom, Adrian et al, 1977), but it has been reported to cause a transient increase in pancreatic blood flow, which preceded the release of insulin (Fisher, Hommel and Schmid, 1975). It is unlikely that glucose could change the rate of inactivation or disappearance of PP. Intravenous glucose does, however, cause a rise in the somatostatin concentration in the hepatic portal vein in rats and causes a small but insignificant rise in the peripheral somatostatin concentration in man (Pimstone, Berelowitz, Kronheim et al, 1977). However, it is feasible that after intravenous glucose, the hepatic portal vein concentration of somatostatin may be elevated in man as well, which may then cause inhibition of hPP release.

The effects of lipid on hPP concentration.

As with oral glucose it is possible that the rise in the serum hPP concentration to 282 $\pm$ 154,5pmol/l after the ingestion of 1ml/kg of "Intralipid" was partly mediated by a neural mechanism. However, the possibility that oral lipid in its own right may stimulate hPP release is supported by the observation that 1ml/kg of intravenous "Intralipid" also caused a significant rise in the hPP concentration to 160 $\pm$ 50,1pmol/l. The peak triglyceride concentration attained with intravenous "Intralipid" preceded and exceeded that after oral lipid,

yet the hPP response to oral lipid was greater. The greater response after oral lipid ingestion may result from the combined stimuli of a gastro-pancreatic reflex plus lipid absorption, and may indicate that a gastro-pancreatic stretch and/or chemical reflex is the dominant stimulus.

The hPP response to intravenous lipid administration is at variance with the findings of Hedo, Villanueva and Marco (1979). They reported that intravenous "Intralipid" depressed the plasma hPP levels to values below the basal level. In that study, "Intralipid" was administered as a constant infusion at 4ml/min over 90 minutes. Differences in the experimental design may have contributed to the contradictory findings. In this study, free fatty acid release induced by 3 bolus doses of heparin, each of 3000u, did not significantly alter the serum hPP concentration despite a 4-fold rise in the FFA concentration. Again, this differs from the findings of Hedo et al who found that 3 bolus doses of heparin, each of 5000u, lowered serum hPP levels. They suggest that free fatty acids may have a direct depressant effect on the PP cell but that hypothesis is not compatible with the findings in this study. Further work would be required to explain the discrepant results.

Since fat is a potent stimulus to CCK release, the PP response to oral "Intralipid" may have been mediated by this hormone. Infusions of 99% pure CCK in physiological doses caused serum hPP to rise to levels similar to those seen after a meal (Lonovics, Guzman, Devitt et al, 1980). This suggests that CCK is an important mediator of PP release under physiological circumstances. It further suggests that fat may be important in the prolonged phase of PP release after a mixed meal.

In summary, although glucose and lipid caused perturbations in the serum hPP concentration, there is no concrete evidence that these nutrient fuels exerted a direct effect on the PP cells. The ingestion of glucose was associated with a 50% rise in the serum hPP concentrations but intravenous glucose administration was associated with a significant fall in the hPP concentrations, thus indicating that glycaemia per se does not have a constant effect on the PP cells. Both oral and intravenous lipid administration caused a rise in the serum hPP concentration, but a greater response was observed after oral than after the intravenous lipid. The difference in responses suggests that although lipid may itself have had an effect on the PP cells, additional stimuli may have contributed to the PP response. The ingestion of glucose and lipid may have stimulated a gastro-pancreatic reflex, which possibly was triggered by pressure or by chemical receptors in the gastrointestinal tract. In addition the ingested glucose and lipid may have acted via a gastrointestinal humoral factor, to cause PP release. CCK has received attention as this factor.

The effects of gastrointestinal hormones on hPP release.

Clearly, the hPP responses to feeding are not entirely due to an effect of the nutrients per se. The possibility that gastrointestinal hormones may mediate the PP response to feeding therefore arises.

The effects of "Boots" secretin, "Boots" CCK-PZ and synthetic pentagastrin on hPP release were investigated. All three of these substances when injected intravenously caused the serum hPP concentrations to rise significantly. "Boots" secretin stimulated hPP release in a dose-dependent manner and in vagotomised subjects the hPP

response was shown to be unimpaired and therefore independent of the vagus. The rise in serum hPP concentration after an infusion of 2 CHRU/kg of "Boots" secretin confirms the finding of Adrian, Bloom, Besterman et al (1977). Although "Boots" secretin was found to contain 2,42pmol of immunoreactive PP per 1 CHRU, the injected PP is unlikely to account for the rise in the serum hPP concentration observed. The calculated hPP concentration (of approximately 60pmol/l) which would have been attained by the injection of 2 CHRU/kg of "Boots" secretin, is only 12% of the serum hPP concentration observed in the healthy, normal subjects. Furthermore, the maximum hPP response was observed at a secretin dose of 1,5 CHRU/kg and increasing the dose to 2 CHRU/kg did not enhance the hPP response. Also mitigating against the possibility that exogenous PP was responsible for the rise observed, were the significantly lower serum hPP concentrations in the patients with chronic pancreatitis, despite the administration of a supramaximal dose of secretin.

Although the serum hPP response to secretin confirms the findings of Adrian, Bloom, Besterman et al (1977), it is contrary to the experience of other investigators (Schwartz and Rehfeld, 1977). The conflicting observations may be explained by difference in the secretin preparations infused. Purified porcine secretin (GIH secretin, Karolinska Institute, Sweden) was infused by Schwartz and Rehfeld, whereas in this study and the study of Adrian et al, "Boots" secretin, a semipure commercial preparation (Crick, Haper and Raper, 1950) was used. It is feasible that a contaminant in the "Boots" preparation is a PP secretogogue, either enhancing or causing the PP release. However, further studies on the effect of secretin on hPP release have shown that GIH secretin was equipotent with "Boots" secretin in releasing hPP (Glaser, Vinik, Sive

et al, 1980). When GIH secretin was injected as a bolus and when serum hPP levels were corrected for the estimated increase in serum hPP concentrations due to PP contained in the "Boots" preparation, the hPP responses were similar for both preparations. Secretin may adhere to plastic surfaces (Bitar, Zfass and Makhlouf, 1978) which could reduce the actual amount of secretin infused and account for the failure of prolonged infusions to release hPP (Schwartz and Rehfeld, 1977; Adrian, Bloom, Besterman et al, 1978).

Boden, Diniso and Owen (1974) reported that GIH secretin is 5000 times as immunoreactive as "Boots" secretin and that 1 clinical unit of GIH secretin is equipotent with 4 CHRU of "Boots" secretin. From these data the calculated plasma secretin concentration which would have been achieved by 2 CHRU/kg of secretin was approximately 1000pmol/l. Even at the lowest dose of secretin infused (0,5 CHRU/kg), the initial circulating secretin concentration was probably 100-fold higher than the post-prandial plasma secretin concentration (Fahrenkrug and Schaffalitzky de Muckadell, 1977), suggesting that these pharmacological doses of secretin did not reflect physiological release of PP. Moreover, the intraduodenal instillation of HCl, a potent stimulus to endogenous secretin secretion (Fahrenkrug and Schaffalitzky de Muckadell, 1977) had no effect on hPP release (Floyd, Fajans, Pek et al, 1977). Thus, although secretin has strong PP-releasing properties in the experimental situation, its role as a physiological PP secretogogue appears limited.

Despite the strong emphasis of vagal control on PP release (Schwartz, Holst, Fahrenkrug et al, 1978) the response to 2 CHRU/kg of secretin did not differ significantly in the normal controls and the patients who had

undergone truncal vagotomy. This suggests that the response to "Boots" secretin is independent of vagal control. In a subsequent study however, it was shown that during a concommitant infusion of atropine, secretin failed to release hPP (Glaser, Vinik, Sive et al, 1980). This may indicate that there is an extravagal cholinergic mechanism mediating the secretin-induced hPP release. It may also suggest that like the prolonged phase of PP release following a meal (Becker, Borger and Schafmayer, 1979), the hPP response to secretin becomes normal with time following the vagotomy.

Although a small rise in serum hPP concentrations was observed following the cholecystokinin-pancreazymin injection, this preparation had significantly less effect on hPP release than did the secretin preparation. Taylor, Feldman, Richardson et al (1978) and Regan, Go and DiMagno (1980) reported that CCK infused in man did not cause hPP release whereas Lonovics, Guzman, Devitt et al (1980) found that 99% pure CCK did release PP in man and dogs (Guzman, Lonovics, Devitt et al, 1981). They suggest that CCK may lose its biologic potency during sterilization (Lonovics, Suddith, Guzman et al, 1979), a problem they overcame by using a specially coated silver filter. Caerulein, the decapeptide isolated from the skin of the frog, Hyla caerulia, and which has a similar carboxy terminus to CCK (Anastasi, Ersparmer and Endean, 1968) infused at a rate of 100ng/kg/hr for 40 minutes, caused plasma hPP to rise to concentrations higher than those observed with "Boots" CCK-PZ in these subjects (Adrian, Bloom, Besterman et al, 1977). The differences in the PP response in this study compared with other studies may relate to differences in the assays and to differences in the purity and the type of CCK used. It has been shown that the whole molecule of CCK-PZ is biologically less potent than C-terminus by Mutt and Jorpes

(1968) and Bertoccini, De Caro and Melchiorri (1970). The doses of caerulein and CCK-PZ used and the different methods of administration may further explain differences in the results.

Although circulating immunoreactive plasma CCK levels have been measured in man (Harvey, Dowsett, Hartog et al, 1973; Schlegel, Raptis, Harvey el al, 1977) and shown to respond to the ingestion of lipid, it is not known how the circulating levels of CCK attained after a meal or lipid ingestion would compare with the levels after the intravenous administration of 85 CHRU of "Boots" CCK-PZ. The difficulty in trying to compare the levels, stems from the heterogeneous nature of the CCK measured by the assays and from inadequate knowledge of the purity of the "Boots" preparation which was infused. Commercial preparations of CCK-PZ also contain GIP (Kuzio, Dryburgh, Malloy et al, 1974) which may have had an affect on hPP release. However, since fat is a potent releaser of CCK, it is feasible that "Intralipid" ingestion stimulated the release of CCK-PZ which may have effected the hPP response following lipid ingestion.

The intravenous injection of 5ug/kg of pentagastrin caused a significant 4-fold rise in the serum hPP concentrations. The hPP response to pentagastrin is in accord with the observations of Floyd, Fajans, Pek et al (1977) that a bolus injection of pentagastrin caused a 3-fold rise in the plasma hPP concentrations in 5 healthy subjects and with Parks, Gingerich, Jaffe et al (1979) that an infusion of pentagastrin elevated PP levels in dogs. In view of the similarities between the amino-acid sequence of pentagastrin and the C-terminal part of cholecystokinin, it is not surprising that at these pharmacological doses pentagastrin caused hPP release. However, as with secretin, it is probable that this

dose of pentagastrin caused the serum gastrin-like activity to rise to levels which would exceed those found under physiological circumstances.

In contrast to the hPP-releasing properties of the gastrointestinal hormones secretin, CCK and gastrin, somatostatin inhibited the release of PP. As alluded to previously, although somatostatin may be a neuropeptidergic or a paracrine substance, its inhibitory action on PP release will be discussed in this section on gastrointestinal hormones as it is possible that it may act in this way as well (Luft, Efendic and Hökfelt, 1978). The physiological function of somatostatin has not yet been established, although its inhibitory action on multiple secretory processes is well recognized (reviewed by Pimstone, Berelowitz and Kronheim, 1976).

Somatostatin infused at a rate of 2ug/min which was calculated to cause a 2-fold rise in the basal somatostatin concentrations, did not inhibit the basal serum hPP concentration. However, when a bolus of 200ug of somatostatin was followed by a constant infusion of 2ug/min the basal serum hPP levels fell significantly to 56% of the original value. It is probable that higher circulating somatostatin concentrations were achieved with the latter regime and that as has been shown for insulin, glucagon and growth hormone release (Leblanc, Rigg and Yen, 1975), the inhibitory action of somatostatin on PP may be dose-related. Marco, Hedo and Villanueva (1977) reported that 9ug/min of somatostatin induced a fall in the basal plasma hPP concentration, but this was carried out in gastrectomised individuals with four times the concentrations used in these studies.

In this study, somatostatin abolished the hPP response to insulin-

hypoglycaemia. As vagotomy has the same effect (Adrian, Bloom, Besterman et al, 1977) it is feasible that somatostatin in this instance exerts its effect via the vagus. However, the inhibitory action of somatostatin is still present in the denervated, isolated perfused canine pancreas (Adrian, Bloom, Besterman et al, 1978) which suggests that somatostatin exerts its effects directly on the PP cells.
In discussing the fall in serum hPP concentration after intravenous glucose administration, the possibility that release of somatostatin mediated the hPP response was mentioned. Orci and Unger (1975) proposed that in the islets of Langerhans, the beta, alpha and D cells form a functional unit in which their respective products can modify the secretory activity of at least one of the adjoining cells thus giving rise to a paracrine system of secretory regulation. PP, being an additional pancreatic peptide, may enter into this scheme. However, since a proportion of the PP cells are situated in the pancreatic parenchyma as well as in the islets of Langerhans (Heitz, Polak, Bloom et al, 1976), it is unlikely that the all PP cells are situated in close proximity to the D cells. Thus the anatomical distribution of the cell types suggests that although somatostatin may have a paracrine effect on PP release, it is possible that it may act in an additional way e.g. as a hormone to inhibit PP release.

Thus to summarise the effects of the gastrointestinal hormones on PP release, "Boots" secretin, "Boots" CCK-PZ and pentagastrin each caused a significant rise in the serum hPP concentrations. It is probable that the circulating hormone levels achieved by the infusions exceeded their levels or activities under physiological circumstances. Thus whether the release of hPP by secretin, CCK or gastrin is a physiological action of these hormones requires further investigation, although studies by

Lonovics, Guzman, Devitt et al (1980) suggest that CCK may be a physiological PP secretogogue.

"Boots" secretin caused the serum hPP concentration to rise in a dose related manner, but a maximum hPP concentration was observed at a dose of 1,5 CHRU/kg, and the administration of 2 CHRU/kg did not enhance the response. As the release of hPP after an intravenous injection of 2 CHRU/kg was not significantly different in patients who had undergone bilateral truncal vagotomy compared with healthy subjects, the secretin-induced release of hPP did not appear to depend on the integrity of the vagus but may depend on extravagal cholinergic factors.

In these studies somatostatin infused at a rate of 2ug/min abolished the serum hPP rise to insulin-induced hypoglycaemia whereas a 200ug bolus of somatostatin which preceded an infusion of 2ug/min lowered the basal hPP concentration. It is assumed that somatostatin acted directly on the PP cells to cause inhibition of hPP release, but whether it affected inhibition as a paracrine substance, a hormone or a neurotransmitter is not known. Moreover, the physiological relevance of the inhibitory effect of somatostatin on PP rlease is also not clear.

The neural regulation of hPP release.

In the literature reviewed and preceding sections of this discussion, the role of neural modulation, specifically the effects of the vagus nerve were mentioned. It was pointed out that in patients who had undergone truncal vagotomy, the hPP response to a meal, especially early release which occurs shortly after eating may be impaired (Schwartz, Rehfeld, Stadil et al, 1976); that the PP response to insulin-induced

hypoglycaemia was abolished (Adrian, Bloom, Besterman et al, 1977); and that the PP release after gastric distension was absent (Schwartz and Rehfeld, 1977). Moreover, electrical stimulation of the vagus in the pig (Schwartz, Holst, Fahrenkrug et al, 1978) and perfusion of the isolated canine (Iversen, Bloom, Adrian et al, 1977) and porcine (Schwartz, Holst, Fahrenkrug et al, 1978) pancreas with acetylcholine released PP which was inhibited by atropine.

These experimental data have made attractive the hypothesis that the vagus plays an important role in mediating PP release and Schwartz, Rehfeld, Stadil et al (1976) were of the opinion that "hPP is a digestive hormone under vagal control". However, since vagotomy did not abolish the release of PP in all studies (Adrian, Bloom, Besterman et al, 1977; Taylor, Feldman, Richardson et al, 1978; Glaser, Vinik, Sive et al, 1980) it seems that other mechanisms also regulate the release of PP. It is well recognised that the secretory processes of the endocrine pancreas may be altered by a number of neurotransmitters including epinephrine, norepinephrine, acetylcholine, serotonin and dopamine (reviewed by Woods and Porte, 1974). Insulin-hypoglycaemia, although it activates the vagus, also causes the release of catecholamines (Vendsalu, 1960). It seems that acetylcholine mediates PP release but what of the catecholamines?

In this study adrenergic beta-receptor stimulation by the concomitant infusion of epinephrine and phentolamine caused a rise in the hPP concentration of a similar magnitude to that observed following insulin-hypoglycaemia. However, since phentolamine also has histamine-like properties which may be affected by atropine (Nickerson and Collier, 1975) it is possible that phentolamine mediated hPP release

via this mechanism. Indeed, in complementary studies (Sive, Vinik and Levitt, 1980) it was shown that phentolamine alone caused a doubling of the hPP concentration that could be blocked by atropine. It is possible that phentolamine may directly stimulate a cholinergic receptor on the PP cell. However, an infusion of the pure beta-receptor agonist, isoproterenol, caused a four-fold rise and phentolamine plus epinephrine a seven-fold rise in serum hPP levels, suggesting that there is a separate adrenergic beta-receptor effect on hPP release. Moreover, Lantigua, Lilavivathana, Campbell et al (1980) found that phentolamine infused at the same dose as used here, augmented, whereas propranolol suppressed the hPP response to insulin-induced hypoglycaemia. These studies strengthen the argument that the vagus is not the only neural mediator of PP release.

Although Floyd, Pek, Knopf et al (1977) reported a rise in hPP concentration with beta-adrenergic stimulation using a similar technique to that which was used in this study, the rise in hPP concentration was smaller than that observed here. The difference may relate to the assays or the smaller doses of epinephrine and phentolamine used by Floyd et al. Furthermore, these studies demonstrated that the state in which the epinephrine is preserved to prevent its oxidation, could have an effect on its potency.

Combined alpha- and beta-receptor stimulation by epinephrine infused alone caused a small rise in hPP concentration. The rise was considerably less than that which was observed with pure beta-receptor stimulation suggesting that adrenergic alpha-receptor stimulation partially inhibited the response to epinephrine. The fall in the hPP concentration during the propranolol infusions also suggests that

adrenergic alpha-receptor stimulation may inhibit hPP release. However, the small rise in the hPP concentrations immediately after the propranolol (beta-blocker) injection (see Fig. 6.9) which has also been observed by Lantigua, Lilavivathana, Campbell et al (1980) may reflect the minor intrinsic sympathomimetic activity of the beta-blocker (Black, Crowther, Shanks et al, 1964). This beta-receptor agonism may also explain the initial rise in insulin concentrations observed immediately after administration of the propranolol (Fig. 6.11). After stopping the propranolol infusion in the second protocol (Fig. 6.9) elevation of hPP concentrations to pre-propranolol infusion levels did not occur and the failure of hPP to rise during the second period of epinephrine was possibly due to a carry-over effect of propranolol (Shand, Nuckolls and Oates, 1970). Floyd, Pek, Knopf et al (1977) too found a small fall in hPP concentration with adrenergic alpha-receptor stimulation, but in their study statistical significance was not reached.

When the periods of selective stimulation were reversed (Fig. 6.10), the hPP response to the periods of alpha- and beta-receptor stimulation were similar i.e. adrenergic beta-receptor stimulation causing a rise and alpha-receptor stimulation being associated with a fall in serum hPP concentrations. These findings suggest that the antagonistic effects of adrenergic alpha-receptor inhibition and beta-receptor stimulation may play a role in maintaining hPP homeostasis.

Although the half life time ($t_{\frac{1}{2}}$) of hPP is not known, it is feasible that adrenergic modulation may change the rate of disposal of hPP by altering blood flow, thus apparently causing increased or decreased release. In the pig, PP is removed from the circulation predominantly by the liver and the kidney (see 6.3.1.1. and 6.3.1.2). Adrenergic

beta-receptor stimulation increases, whereas alpha-receptor stimulation decreases blood flow to these organs (Neil, 1975). Increased delivery to the site of inactivation should shorten the $t_{\frac{1}{2}}$ and cause a fall in the circulating level, contrary to what was observed. This suggests that adrenergic stimulation may exert its effect directly by stimulating or inhibiting hPP release.

Serum PP concentrations mirrored the insulin responses which may suggest an insulin effect on hPP. However, during epinephrine infused alone, the hPP concentrations rose slightly whereas the serum insulin concentrations declined and the insulin responses were rapid while those of hPP were gradual, suggesting rather than the pancreatic beta cell and the PP cell have independent responses to adrenergic stimulation. The changes in serum hPP concentration were independent of the serum glucose and FFA concentrations. The marked rise in glucose and FFA concentrations with epinephrine were associated only with a small rise in the serum hPP concentration. Moreover, a rise in blood glucose concentration induced by intravenous glucose administration caused a fall (see 6.1.1.2.2; Marco, Hedo, Villanueva, 1978; Floyd, Fajans, Pek et al, 1977) and a rise in FFA induced by heparin injection, an insignificant rise (see 6.1.1.2.3) in hPP concentrations. The possibility that prolonged elevation of glucose concentration induced by epinephrine was the cause of the fall in hPP concentration and not the propranolol infusion cannot be excluded. However, the infusion of epinephrine and saline for two hours did not cause a late fall in hPP concentration despite elevation of the glucose levels.

Thus in these studies hPP release was modulated by adrenergic manipulation. Whether this indicates that PP may be released or

inhibited during stress is not clear. From these studies it would appear that either adrenergic beta-receptor or adrenergic alpha-receptor activity would have to predominate to cause a rise or fall in the serum hPP concentration during stress. Until changes in the hPP concentrations have been demonstrated in such situations, the possibility of PP responding like a stress hormone remains ill-defined even though these studies and the discussion of the results would support the contention that PP release may be mediated by both the sympathetic and parasympathetic nervous systems. Thus acetylcholine and epinephrine would appear to modulate hPP release. But what of dopamine, which is also recognized as a neurotransmitter of the autonomic nervous system (Thorner, 1975). Indeed dopaminergic substances have been shown to affect both insulin (Quickel, Feldman and Lebovitz, 1971) and glucagon (George and Rayfield, 1974) release.

Dopaminergic substances may modulate hormone release in a number of ways: by an anticholinergic effect (Thorner, 1975); by a direct action on peripheral dopamine receptors (Thorner, 1975); by being metabolised to norephinephrine and/or epinephrine (Ericson, Ekholm, Lundqvist, 1971); or by acting centrally on the nervous system (Iversen, 1975). In these studies the dopaminergic agonist, L-dopa, did not significantly alter either basal or stimulated hPP concentrations. The central dopaminergic effect of L-dopa was evidenced by a significant rise in the serum growth hormone concentration as has been reported by Boyd, Lebovitz and Pfeiffer (1970).

Although Spitz, Zylber, Le Roith et al (1978) reported that 5 mg of metoclopramide given intravenously caused significant elevation of the circulating hPP concentrations both in healthy subjects and in patients

with diabetes mellitus this elevation could be blocked by prior treatment with atropine (Spitz, Zylber, Jersky et al, 1979). In this study, 10mg of metoclopramide injected as an intravenous bolus did not cause a significant rise in the serum hPP concentration. The reasbn for this disparity is not apparent, even though in both studies blood samples were taken for one hour. Furthermore, in this study, there was no significant change in the serum growth hormone concentrations as an index of the effectiveness of the infusate. However, each subject in the study, complained of agitation and motor restlessness which were possibly manifestations of the extrapyramidal side effects of metoclopramide as reported by Robinson (1973). In addition to its anti-dopaminergic effect (Peringer, Jenner and Marsden, 1975), metoclopramide also has anti-atropine like actions on the gastro-intestinal tract. Because of the multiplicity of its actions, the failure of metoclopramide to affect hPP release in this study, does not rule out the possibility that pure dopamine antagonism may in fact modulate PP release. Furthermore, since the effects of biogenic amines on pancreatic hormone release are relatively species specific (Mahoney and Feldman, 1977), these findings do not exclude the possibility that other monoamines may affect PP release in man or that the biogenic amines may modulate PP release in other species.

A summary of factors regulating the release of PP.

One of the cardinal observations in these studies was the serum hPP response to the ingestion of a mixed meal. During eating and within five minutes of completing the meal, a massive rise in the serum hPP concentration was observed, which then fell significantly, but remained significantly elevated above the fasting PP concentrations for 3 hours.

It thus appears that the release of PP could be viewed in two parts, which relate to the concentrations of hPP attained and the time at which they occurred. It has been suggested that the early release of PP is neurally mediated, possibly by the vagus, but the stimuli activating the nervous system have not been adequately defined. It is possible that gastric distension, chemical receptors for nutrients in the gastrointestinal tract, and higher cephalic centres may play a role in the activation of the neural reflex.

However, it appears that in addition to neural mechanisms other factors probably contribute to the regulation of PP release. The individual nutrients fuels lipid, glucose and the amino-acid, arginine, were therefore tested. In these studies it was found that lipid and oral glucose caused the serum hPP concentration to rise, that intravenous glucose administration was associated with a 35% fall in the hPP concentrations and that intravenous arginine did not affect the hPP concentration. None of the nutrients were associated with changes in the PP concentration that were equivalent in magnitude to the changes observed after a mixed meal. Although these nutrients may have had a direct effect on the PP cell, the possibilities that they acted indirectly, e.g. via a neural mechanism or gastrointestinal hormone release cannot be excluded.

The gastrointestinal hormones gastrin, secretin and cholecystokinin-pancreozymin all caused the serum hPP concentrations to rise when administered intravenously. The impurity of the secretin and cholecystokinin preparations used in this study and the circulating levels that would have been attained with the doses infused, precludes the conclusion that these hormones are PP secretogogues under physiological

circumstances. However, secretin was shown to cause the serum hPP concentration to rise in a dose-related manner and to cause similar hPP responses in healthy subjects and subjects who had undergone bilateral truncal vagotomy, suggesting that all stimuli for PP release are not mediated via the vagus. Somatostatin infused at a high dose (200ug bolus followed by an infusion of 2ug/min) caused a fall in the basal hPP concentrations and lower doses of somatostatin (an infusion of 2ug/min)abolished hypoglycaemia-induced hPP release but did not cause the basal hPP concentration to fall. Whether somatostatin acts as a paracrine substance or as a hormone to inhibit PP release, and whether under physiological conditions PP homeostasis is maintained by somatostatin is not clear.

That the vagus, stimulated electrically or by pharmacological means, can cause PP release seems certain. These studies have also suggested that the sympathetic nervous system may play a role in modulating PP release. Thus combined adrenergic alpha- and beta-receptor stimulation caused a small rise in the serum hPP concentrations, selective adrenergic beta-receptors stimulation caused a far greater response, and alpha-receptor stimulation was associated with a fall in hPP concentrations. It is possible that the antagonistic effects of adrenergic alpha-receptor inhibition and beta-receptor stimulation may play a role in maintaining hPP homeostasis. Whether the adrenergic modulation of hPP means that PP may respond in times of stress is not clear and requires further investigation. Manipulation of dopaminergic activity in man by the ingestion of L-dopa and metoclopramide, had no effect on altering serum hPP concentrations. However, as the biogenic amines are relatively species specific, the possibility cannot be excluded that other substances with amine-like properties may affect the

release of PP in man, and that in other species dopamine would have an effect on PP release.

It thus appears that the release of hPP is not mediated by a single stimulus and one regulating mechanism. There may be an interplay of numerous factors including nutrients, gastrointestinal hormones and the autonomic nervous system which all contribute to the regulation of PP release. As yet, however, their interactions and relative importance have not been defined.

These studies to elucidate aspects regarding the regulation of PP release have all been based on measuirng the circulating immunoreactive hPP concentration. They did not attempt to investigate the regulation of PP in relation to various immunoreactive components that may exist in the circulation. In the section that follows, the results of the studies to identify hPP heterogeneity will be discussed.

7.2. Investigation of the molecular forms of circulating immunoreactive pancreatic polypeptide by 10% polyacrylamide disc gel electrophoresis.

Before the era of radioimmunoassays, the measurement of hormones depended on measuring their biological potency in bioassay systems. Thus substances that may have been closely related in structure to an active hormone e.g. precursor or degradation fragments, because they had low biological activity were not detectable. The advent of radioimmunoassays caused the pendulum to swing in the opposite direction so that substances that shared immunoreactivity with active hormones could be detected and possibly be interpreted as having biological activity. Thus, athough the phenomenon of shared immunoreactivity between related but non-identical substances may have caused difficulty in relating the circulating concentrations to the biological activity, the detection of various immunoreactive forms was, nevertheless, an initial step in gaining insight into the biosynthesis and metabolism of some hormones.

The radioimmunoassay of parathyroid hormone (PTH) first described by Berson, Yalow, Aurbach et al (1973) may serve as an example. In 1968 Berson and Yalow reported that when using 3 different PTH antisera, one of the antisera measured PTH concentrations that were lower than the concentrations measured by the other two. Moreover, following parathyroidectomy, the disappearance of PTH from the circulation was more rapid with the antiserum that initially meausred the lower PTH concentrations. They recognized that more than one form of PTH was present in the circulation and later confirmed this chromatographically (reviewed by Yalow, 1974). Studies by other investigators revealed that

the amino-terminus of the PTH molecule is the biologically active fragment and that it has a rapid rate of turnover, whereas the carboxy-terminal fragment is slowly degraded and has a longer half-life time (Potts, Murray, Peacock et al, 1971). Thus PTH assays which measure the whole PTH molecule plus the carboxy-terminus give higher PTH values than do amino-terminal-specific assays. However, amino-terminal assays which measure the whole molecule of PTH and the amino-terminal fragment demonstrate rapid changes in PTH concentration and reflect more accurately the biological activity of PTH (Arnaud, Goldsmith, Bordier et al, 1974).

This example of the heterogeneity of PTH suggests that using antisera specific to regions of a molecule is one method of establishing molecular heterogeneity. However, if only one antiserum is available an alternate method must be used to assess if more than one immunoreactive form of a hormone is present in the circulation.

Thus in these studies, fractionation of serum hPP was carried out on 10% polyacrylamide disc gel electrophoresis to separate hPP by criteria of molecular weight and molecular charge. The migration of endogenous hPP, which had been released either by insulin-induced hypoglycaemia or by an intravenous injection of "Boots" secretin, was compared to the migration of radiolabelled hPP and bPP as well as to purified hPP which was added to a buffer (the assay buffer), to whole blood and to charcole-treated plasma.

The radiolabelled PP markers migrated predominantly as single fractions, and ^{125}I-hPP migrated more cathodally than ^{125}I-bPP. The more cathodal migration of ^{125}I-hPP is in keeping with the findings of Chance et al

that hPP is one charge more basic than bPP (Chance, Moon and Johnson, 1979). Both radiolabelled hPP and bPP migrated in positions more cathodal than insulin, proinsulin or glucagon. The hPP which was added to buffer has a molecular weight of 4200 and as it migrated in the same position as ^{125}I-hPP, it suggests that hPP has a more basic chemical nature than insulin or proinsulin which both have higher molecular weights than hPP.

The radiolabelled hPP, although it migrated predominantly as a single fraction, showed a small component which migrated in a more cathodal position than the major peak. This smaller component may have resulted from fractions of hPP labelled to different degrees in the iodination procedure thus causing changes in its charge, and not be due to differences in the molecular weight or charge of the hPP extracted from the pancreas. However, cold hPP added to the assay buffer showed a similar small component, but this component was absent when cold hPP was added to whole blood or to charcoal-treated plasma. In all three of these cold hPP markers, a component of hPP was present that migrated in a position more anodal than ^{125}I-bPP, between the positions of insulin and proinsulin. No such component of hPP was detected in the radiolabelled hPP but was found in 5 of the 6 serum samples that contained endogenous hPP. These sera all had the component of hPP that migrated in the position of ^{125}I-hPP, which is equivalent to hPP of molecular weight 4200, but in addition in 4 of the sera, a component of hPP was detected which migrated more cathodally than 4200 hPP. The patterns of migration of hPP did not appear to relate to the method in which hPP release was stimulated, suggesting that the different stimuli did not cause hPP of different molecular sizes or charges to be released.

Previous studies to assess the molecular forms of immunoreactive hPP have fractionated PP by criteria of molecular size only. Thus Adrian, Bloom, Bryant et al (1976) found PP of a single molecular weight in extracts of human pancreata. On the other hand, molecular sieve column chromatography of plasma yielded 3 components of immunoreactive hPP (Villanueva, Hedo and Marco, 1977). The first component eluted in the void volume suggesting that it was hPP of a large molecular weight or alternatively, hPP bound to a plasma protein; the second component eluted in the position of hPP isolated from the pancreas (molecular weight 4200); and the third component appeared to have a molecular weight of approximately 1500 daltons. Although Boden, Masters and Owen (1980) also found 3 hPP peaks with Sephadex G50 chromatography, they described a peak which migrated between the largest molecule weight fraction and PP of molecular weight 4200. In studies to examine the biosynthesis of pancreatic polypeptide using extracts of endocrine cells purified from canine pancreatic tissue, Schwartz, Gingerich and Tager (1980) found that gel chromatography yielded immunoreactive PP-like material with molecular weights of 4300 and 9000. The peptide with a molecular weight of 4300 appeared compatible with PP. They further described that in pulse-chase experiments, the larger molecule yielded two peptides, one compatible with 4200mw PP and another which did not react with PP antiserum. This second peptide is thus not compatible with the smaller molecular weight immunoreactive PP found in this study and the study of Villanueva et al. These studies would support the contention that hPP exists in more than one form.

Although hPP in the circulation was present in 3 molecular forms in the studies using molecular sieve chromatography as in this study, it is not possible to relate the various molecular forms to each other. Because

PP used as the marker was purified and donated by Dr Chance for both this study and that of Villanueva et al, it is likely that the hPP that migrated in the position of ^{125}I-hPP in this study corresponds to the elution of the second component of hPP in the study of Villanueva et al. However, it is not known whether the hPP components that migrated in a position more cathodal than ^{125}I-hPP in this study reflects hPP that has a higher molecular weight or hPP that is chemically more basic than the marker 4200 molecular weight PP; nor is it known whether the component of hPP that migrated in the position more anodal than the ^{125}I-hPP is of a lower molecular weight or less basic in nature. To assess this situation, an estimate of molecular size would have to be carried out using a detergent to eliminate the charge effect of the polyacrylamide gels.

Although hPP of different molecular weights or charges were found in these studies, the relevance of these observations in terms of the physiology of hPP is not clear. The biological activity of PP with respect to its action on pancreatic exocrine inhibition, resides in the carboxy - terminal end (Chance, Joworck et al, 1981). How this relates to the multiple molecular circulating forms of PP is not known. This and whether some of these components of hPP could possibly be implicated in the process of degradation of hPP provides scope for further studies.

It thus appears that the antiserum used in the PP assay reacts with more than one molecular form of human pancreatic polypeptide. Whether this applies to other species as well, or more specifically whether or not porcine PP also circulates in more than one immunoreactive form is not known. Thus in the studies where the metabolism and kinetics of PP were examined in pigs, it is possible that PP of different molecular forms

could have different rates of organ uptake or half-life time. Thus in the discussion which follows on the release, organ uptake and rate of disappearance of endogenous PP in pigs, the results reflect total immunoreactive PP, but may be markedly influenced by only one of the components of immunoreactive PP in the circulation.

7.3. Investigation of the metabolism and turnover kinetics of pancreatic polypeptide.

This section of the discussion differs from the preceding and succeeding sections, in that it deals with PP concentrations in pigs and not in man. Heterogeneity of circulating pancreatic polypeptide was demonstrated in man, but whether this applies to porcine PP is not known and thus, care must be taken extrapolating these data to the situation in man.

The investigations which were carried out, aimed to characterise what happens to PP after it has been released into the circulation. There are numerous ways to approach such an investigation. PP may be infused into animals or man and the kinetics of exogenous PP then determined. Altenatively, as was the case in these studies, the kinetic behaviour of endogenous PP was studied, one of the reasons being that PP was not available for infusion. Although the investigations were designed to examine the fate of PP in the unstimulated situation, the effects of anaesthesia on PP release or uptake are now known, and could influence the results. Thus the data presented reflect the situation in starved anaesthetised pigs and do not truly reflect the basal situation.

In these studies the serum PP concentration was measured in samples taken from various sites at the same time. The serum PP concentrations were determined in the pancreatico-duodenal, hepatic portal, hepatic, renal and femoral veins as well as in the arterial circulation in six animals. From the concentrations and the rate of blood flow, the hepatic and renal uptake of PP could be determined.

Organ uptake of pancratic polypeptide

The serum PP concentrations at the various sites not only give an indication of the disappearance or organ uptake of PP, they may, in addition, give some insight into the sites and the extent of PP release in pigs. Thus in these animals the highest concentrations of PP were found in samples taken from the pancreatico-duodenal vein and the hepatic portal veins and the implications of this observation will be discussed here.

The release of pancreatic polypeptide in pigs.

The serum pancreatic polypeptide concentrations in the pancreatico-duodenal vein of 3 pigs ranged from 2333 to 1009pmol/l. These concentrations were 3 to 6 times higher than the mean concentration of $372 \pm 76,7$pmol/l in the hepatic portal vein samples in 6 pigs, and were far higher than the PP concentrations in the hepatic portal veins in the 3 corresponding samples. In the absence of blood flow measurements in the pancreatico-duodenal vein, the mass of PP in this vessel cannot be compared with the mass of PP in the portal vein and it is thus speculative to suggest on these grounds that the pancreatico-duodenal vein drains the primary site of PP release. However, Adrian, Bloom and Bryant (1976) reported that in monkeys and baboons 93% of the PP in the gastrointestinal tract culd be extracted from the pancreas, suggesting that the pancreas is indeed the primary source of PP and it is likely this applies to other species like the pig. If it is assumed that this situation does in fact apply to pigs and that the PP content of 123pmol per gram of pancreas (wet weight) in monkeys (Adrian, Bloom, Bryant el

al, 1976) is similar to that in pigs, and if it is further assumed that all the PP released by the pancreas drains into the portal vein, then an idea can be obtained of the capacity of the pancreas to release PP. Thus since the mean PP concentration in the portal vein was 372pmol/l and the mean portal vein blood flow in pigs of this type and weight was 432ml/min (Vinik, Hickman and Grant, 1978), approximately 161pmol of PP passed through the portal vein per minute. In pigs of this type and weight the mean pancreatic mass (wet weight) was 45g (personal communication from Dr Rosemary van Hoorn-Hickman). Thus if the pancreas is assumed to contain approximately 123pmol/gm, then the total PP content would be approximately 5535pmol. Thus to maintain an output of 161pmol of PP per minute the pancreas would release only 2,9% of its PP content. However, since the half-life time of disappearance of PP in the portal circulation was calculated to be 6,3 $\pm$ 1,63 min, the pancreas would only have to release 0,5% of its total content to maintain steady state PP concentrations.

It is thus possible that there is a large pancreatic reserve of PP, which can be released rapidly on stimulation. It is also possible that because of this reserve, the basal hPP concentration in patients with chronic pancreatitis were not significantly lower than the basal concentrations in healthy subjects (see 6.4.2.1). However, following stimulation of PP release in the patients with chronic pancreatitis it is possible that a diminished reserve did not permit the serum PP levels to rise to the concentration observed in healthy controls. On the other hand it is possible that the pancreas is not the sole source of pancreatic polypeptide. Indeed, Adrian, Bloom and Bryant et al (1976) reported that in the higher primates, monkeys and baboons, 7% of gastrointestinal PP was not found in the pancreas. It is thus possible

that if this 7% of PP is synthesised in the rest of the gastrointestinal tract, and if this source of PP is rapidly turning over, the gastro-intestinal tract could contribute to basal hPP concentrations. Indeed, Floyd, Fajans, Pek et al (1977) detected low levels of circulating hPP in patients who had undergone total pancreatectomy. However, Adrian, Bloom, Besterman et al (1977) did not detect PP in pancreatectomised man in the basal state, nor after the ingestion of a meal. Thus although it is feasible that the extrapancreatic release of PP may contribute to the basal PP concentrations, it is unlikely that this extra-pancreatic PP contributes significantly to PP release after a meal.

Since the vast majority of PP is released by the pancreas, it must travel via the portal circulation and traverse the liver to enter the systemic circulation. In these studies a PP concentration gradient was found across the liver, suggesting hepatic uptake of porcine PP.

The hepatic uptake of pancreatic polypeptide in pigs.

In these studies, the hepatic uptake of PP was calculated after measuring the serum PP concentration in the hepatic portal vein, the hepatic vein and the arterial circulation. It was found that $42 \pm 7{,}3\%$ of the mass of PP which entered the liver per minute was retained. A similar degree of hepatic uptake of PP was found in children with nesidioblastosis who had plasma hPP concentrations that were elevated above the levels in healthy infants (Hirsh, Loo and Gabbay, 1977) On the other hand, in patients requiring cardiac catheterization and in patients with hepatic cirrhosis, Boden, Master, Owen et al (1980) found that there was not a significant transhepatic hPP gradient. The study of the hepatic uptake of pancreatic polypeptide in children represents a

pathological situation, whereas these data in pigs, the basal situation is anaesthetised animals, and neither are readily extrapolated to the post-prandial state in normal man.

The fate of the PP cleared by the liver in this study is unknown but the liver has been shown to metabolise peptide hormones. Forty to fifty percent of insulin in the portal circulation is extracted during one passage through the liver (Madison and Kaplan, 1958; Samols and Ryder, 1961). Glucagon (Assan, 1972) and secretin (Bridgewater, Kurozanegi and Chiles, 1962; Skillman, Silen and Harper, 1962; Way, Johnson and Grossman, 1969) too, are taken up by the liver. Some investigators have reported hepatic extraction of gastrin (Thompson, Reeder, Davidson et al, 1969; McGuigan, Jaffe and Newton, 1969) but the weight of evidence now suggests that the mass of gastrin entering and leaving the liver is unchanged (Reeder, Brandt, Watson et al, 1972; Sacks, Grant and Vinik, 1978). However, it has been reported that although the mass of gastrin entering and leaving the liver is unchanged, a qualitative change in gastrin may occur during hepatic transit (Vinik, Hickman and Grant, 1978), resulting in a larger molecular weight form, which may have an altered biological potency. As far as PP is concerned, it is not known whether the hepatic extraction of immunoreactive PP is accompanied by qualitative changes in the hepatic effluent, nor whether the hepatic extraction of PP implies degradation, or a site of biological action.

It is of interest that Adrian, Bloom, Bryant et al (1976) found that immunoreactive PP was not detectable in hepatic extracts from monkeys and baboons. Whether this reflects differences in hepatic uptake of PP in these primates compared with pigs, or indicates a hepatic effect on PP so that it is no longer immunoreactive, is not known. Studies are

thus required to assess the hepatic uptake of PP in other species, to determine whether PP may have a specific action in the liver, whether there is hepatic degradation of PP, and whether there is a change in the immunoreactive characteristics of PP in the liver.

The renal uptake of pancreatic polypeptide in pigs.

Like the liver, the kidney has a role in the metabolism of certain peptide hormones. Insulin (Katz and Rubenstein, 1973) glucagon (Lefebvre, Luyckx and Niezet, 1974; Assan, 1972), gastrin (Booth, Rheeder, Hjelmquist et al, 1973; Grace, Davidson and State, 1974) and CCK-PZ (Thompson, Fender, Rasmus et al, 1975) are known to be cleared by the kidney. In certain instances e.g. insulin (Rubenstein and Spitz, 1968) and somatostatin (Kronheim, Berelowitz and Pimstone, 1977) a proportion of the filtered peptide appears in the urine, whereas in other cases e.g. glucagon (Assan, 1972) only catabolites are found in the urine. Thus, renal filtration and excretion must be differentiated from intra-renal catabolism.

A fall in the PP concentration was uniformly found across the renal bed in all the pigs studies. There was a 48 $\pm$ 7,9% fall in the PP concentration across the left kidney and the renal uptake of PP by both kidneys was calculated to be 58pmol/min. Whether the renal uptake of PP is due to filtration of intra-renal metabolism of PP is not known, and studies are required to determine the renal clearance of PP, the urinary concentrations of PP and whether metabolites of PP occur in the urine in pigs. Supporting the assertion that the kidney may remove circulating PP are the findings of Hällgren, Lundqvist and Chance (1977) and Boden, Master, Owen et al (1980) in patients with chronic renal failure. In

these patients the fasting plasma hPP concentrations were elevated and the extent of the elevation correlated with the degree of renal impairment. It is likely that this elevation reflects impaired renal clearance of PP, and not enhanced release. Further studies are required to investigate the turnover of PP in renal failure and to ascertain the effects of the tubular and glomerular elements of the kidney on the handling of PP in healthy subjects.

Peripheral uptake of pancreatic polypeptide in pigs.

A gradient in the PP concentration between the aorta and the femoral vein was found in 5 of the 6 pigs. The mean arterial serum PP concentration was 129 $\pm$ 25,6pmol/l and the mean PP concentration in the femoral veins was 90 $\pm$ 15,9pmol/l. Although there was a 30% fall in the serum PP concentration between the cartoid artery and the femoral vein, this decline was not statistically significant and in the absence of measurements of blood flow in these and the other vessels supplying the limb, the hepatic extraction of PP by peripheral tissues cannot be calculated.

The half-life time of disappearance of pancreatic polypeptide in pigs

The half-life time ($t_{\frac{1}{2}}$) of PP was calculated from the disappearance of PP from the hepatic portal vein following total pancreatectomy in 4 pigs. The curve of disappearance was exponential and revealed an early phase of rapid disappearance followed by a prolonged second place of disappearance. In the absence of evidence that PP is released only from the pancreas and not from additional sites as well, some doubt as to the

calculated rates of disappearance of PP must exist. However, the calculated $t_{\frac{1}{2}}$ of 6,3 $\pm$ 1,63 mins for the first phase of disappearance is in reasonable agreement with the $t_{\frac{1}{2}}$ of 5,5 $\pm$ 1 min obtained by infusing porcine PP in to dogs (Taylor, Solomon, Walsh et al, 1979) and 6,9 $\pm$ 0.3 min with bPP infused in man (Adrian, Greenberg, Besterman et al, 1978). The prolonged second phase of disappearance with a $t_{\frac{1}{2}}$ of 37,4 $\pm$ 3,30 min may be explained by the redistribution of PP in a larger (extravascular) compartment calculated to be 209 $\pm$ 42ml/kg in dogs (Taylor, Solomon, Walsh et al, 1979) or by the continued release of PP from an extra-pancreatic source. Floyd, Fajans, Pek et al (1977) reported that in 3 patients who had undergone total pancreatectomies, low but detectable hPP concentrations were found in the basal state, suggesting that PP does not entirely disappear from the circulation after total pancreatectomy. However, Adrian, Bloom, Besterman et al (1977) could not detect hPP in pancreatectomized man but have reported that in higher primates a proportion of PP (7%) is found in extrapancreatic sites (Adrian, Bloom, Bryant et al, 1976). Thus it is possible that immunoreactive PP may be present in low concentrations in pancreatectomized pigs and measurements of serum PP concentrations at longer intervals after total pancreatectomy are required to assess whether there is continued release of PP from the extrapancreatic source.

The short $t_{\frac{1}{2}}$ found during the first phase of disappearance is in keeping with a short $t_{\frac{1}{2}}$'s of the first phases of disappearance of glucagon and gastrin in pigs of this type and weight. Thus van Hoorn, Vinik and van Hoorn-Hickman (1978) reported that the $t_{\frac{1}{2}}$ of exogenous glucagon infused into pancreatectomised pigs was 3,4 min and Vinik, Hickman and Grant (1978) found that the $t_{\frac{1}{2}}$ of gastrin was 3-4 min in healthy pigs. These

TABLE 7.1 THE HALF-LIFE TIMES OF SEVERAL PANCREATIC AND GASTROINTESTINAL HORMONES

Hormone	Species	Half-life min	Investigators
Insulin	rat	13,8	Rubenstein, Pottenger, Mako et al, 1972
Insulin	man	15-25	Berson and Yalow, 1966
Glucagon	man	± 10	Samols, Marri and Marks, 1966
Secretin	man	4,06	Kolts and McGuigan, 1977
VIP	dog	3.1	Strunz, Walsh, Bloom et al, 1977
Motilin	man	4,56	Mitznegg, Bloom, Domschke, 1977

data are also similar to the $t_{\frac{1}{2}}$'s of glucagon, gastrin, insulin, motilin, secretin and vasoactive intestinal polypeptide (VIP) measured in other species (Table 7.1).

The relatively short $t_{\frac{1}{2}}$ of PP may suggest that PP is required for rapid regulation of a biological function and that its concentration may rise and fall as its action is required.

A summary of the metabolism and turnover kinetics of pancreatic polypeptide.

These data suggest that in starved anaesthetised pigs, PP is released into the portal circulation predominantly from the pancreas via the pancreatico-duodenal vein. There appears to be a large pancreatic reserve of PP and only a small proportion is released to maintain steady state circulating concentrations. Following the release of PP, the liver retained 42 $\pm$ 7,3% of the PP entering it (18 $\pm$ 29,5pmol/min) but whether the hepatic retention implies that the liver is a site of action, activation or deactivation of PP is not known. The kidney also retained PP. The calculated renal uptake of both kidneys was of the order of 58pmol/min, which was approximately 30% lower than the hepatic uptake of PP. How the kidney handles PP is not known and whether there is renal filtration or intrarenal catabolism of PP requires investigation.

Following total pancreatectomy in four pigs, the serum PP concentration declined steadily in the hepatic portal vein. The disappearance of PP appeared to occur in two phases, the first having a $t_{\frac{1}{2}}$ of 6,3 $\pm$ 1,63 min and the second at $t_{\frac{1}{2}}$ of 37,4 $\pm$ 3,30 min. The $t_{\frac{1}{2}}$ of the first phase of

disappearance is in accord with that found in dogs following an infusion of porcine PP, whereas the second, prolonged phase of disappearance may be explained by redistribution of PP in a larger compartment or by the continued release of PP from an extrapancreatic source.

This, and the preceding sections of this chapter have discussed aspects of the physiology of PP regarding the regulation of its release, the circulating molecular forms, and the concentration gradients across and uptake by various organs and its rate of disappearance after release. All these facets of PP may be altered in patients with diseases involving the pancreas who may exhibit enhanced or impaired release, differences in the molecular forms, or changes in the rate of release or removal from the circulation. Thus in the next section, studies that were carried out in patients with acute pancreatitis, chronic pancreatitis and diabetes mellitus to assess the serum hPP concentrations in these disorders, will be discussed in relation to the specific diseases and to the findings in healthy subjects.

7.4. Serum pancreatic polypeptide concentrations in patients with diseases of the pancreas.

In these studies serum hPP concentrations were measured in the basal state and after provocative stimuli in patients with diseases involving the pancreas. As discussed in the previous section, an elevated or a reduced serum PP concentration may reflect increased or decreased release, or alternatively, an increase or decrease in the rate of clearance of circulating PP. However, in these patients the pancreas was primarily affected and this fact coupled to the $t_{\frac{1}{2}}$ of PP, makes it seem more likely that altered hPP concentrations may reflect predominantly a primary change in the release of PP rather than a change in the $t_{\frac{1}{2}}$.

The first study to be discussed examined the PP concentrations in patients with acute pancreatitis and in these patients provocative stimulation of PP release was not attempted. The study aimed rather to document the serum hPP concentrations during this acute pancreatic illness.

Serum pancreatic polypeptide concentrations in patients with acute pancreatitis

To document the plasma hPP concentrations in patients with acute pancreatitis, plasma hPP concentrations were measured in 17 patients with acute pancreatitis when they were admitted to a hospital ward, and serial hPP concentrations were then measured in 8 of these patients during a period when they were fasted for at least 24 hours and received only intravenous fluids and during their subsequent stay in hospital

until they were discharged.

The patients studied had the typical clinical features of acute pancreatitis associated with raised circulating amylase and glucagon concentrations. Presumably, the damaged pancreas "leaks" these substances into the circulation. It was therefore surprising to find that plasma hPP concentrations were not elevated, especially since a large proportion of the PP cells are situated in the pancreatic parenchyma (Heitz, Polak, Bloom et al, 1976). Experimental acute pancreatitis in dogs, was associated with a rapid rise in serum hPP levels, suggesting that PP may be released in this condition (Pappas, Yovos, Ellison et al, 1981). However, previous attacks of pancreatitis may have influenced the admission concentrations in some patients because in patients with chronic pancreatitis, although the basal levels were within the normal range, an impaired response to stimulation was found (see 6.4.2.2.). In contrast to the hPP concentrations that were in the normal fastng range, the plasma glucagon (IRG) concentrations were 5 - 10 times the normal level on admission. Whether endogenous glucagon could have had an effect on maintaining the normal hPP concentrations in this situation is not known.

Only one of the patients had very high hPP concentrations which varied from 123 to 214pmol/l but he also had renal failure. In this situation gross pancreatic destruction may have caused marked hPP release, but the renal failure may have impaired the removal of hPP (Hallgren, Lundqvist and Chance, 1977) and accounted for the very high circulating levels.

In summary, finding hPP concentrations in the normal range in patients

with acute pancreatitis, suggests that the measurement of the hPP concentrations in this condition may be of little diagnostic or prognostic value. In contrast, the hPP responses in chronic pancreatitis may be of clinical significance.

Serum pancreatic polypeptide concentrations in patients with chronic pancreatitis.

The mean fasting hPP concentrations in the patients with chronic pancreatitis was lower than that in 50 healthy subjects. However, there was considerable overlap in the hPP concentrations in these groups, which suggests that the measurement of the fasting hPP concentration is of little value in diagnosing or assessing the severity of this disorder. This could be in keeping with the observation in pigs that there is a large pancreatic reserve of PP.

The situation following stimulation was different. The test meal, insulin-induced hypoglycaemia and the "Boots" secretin infusion, all caused significantly lower hPP responses in the patients with chronic pancreatitis compared with the responses in healthy subjects.

Secretory defects of the A and B cells of the pancreatic islets have been described in patients with chronic pancreatitis (Kalk, Vinik, Bank et al, 1974; Vinik, Kalk, Botha et al, 1976) and it appears that hPP release, too, is abnormal in this condition. The poor hPP responses contrast with glucagon hypersecretion in this condition (Kalk, Vinik, Bank et al, 1974; Kalk, Vinik, Paul et al, 1975). It was also found that in patients with chronic pancreatitis the more severe the insulinopaenia, the higher was the glucagon concentration (Kalk, Vinik,

Bank et al, 1974). In this study, although the majority of the patients had impaired glucose tolerance, the insulin levels were not measured and whether they could have influenced the hPP concentrations is not certain even though in the isolated perfused canine pancreas insulin did not affect PP secretion (Weir, Samols, Loo et al, 1979). Glucose intolerance per se would not appear to influence the hPP response to insulin-induced hypoglycaemia, because in the maturity onset diabetics, hPP responses were not impaired (see 6.4.3.2.).

The significantly lower serum hPP responses to the intravenous administration of "Boots" secretin in the patients with chronic pancreatitis compared with the responses in the healthy subjects, is unlikely to be a result of vagal denervation. 2 CHRU/kg of "Boots" secretin caused similar serum hPP responses in control subjects and in patients who had undergone bilateral truncal vagotomy. The hPP response to insulin-induced hypoglycaemia, on the other hand, may have been impaired in the patients with chronic pancreatitis because they had autonomic (vagal) neuropathy. However, clinical tests showed that none of these patients with chronic pancreatitis had autonomic neuropathy as evidenced by the normal responses on autonomic function tests. It is possible that the impaired hPP responses following a meal may have been due to autonomic neuropathy or to reduced release of a gastrointestinal PP secretogogue. It has been reported that gastric inhibitory polypeptide (GIP) and gut glucagon-like immunoreactivity (GLI) are excessive in patients with chronic pancreatitis (Botha, Vinik and Brown, 1976; Both, Vinik, Child et al, 1977), suggesting that an inadequate release of GIP was not responsible for the impaired release of hPP in these patients.

TABLE 7.2 THE LIVER FUNCTIONS IN THE PATIENTS WITH CHRONIC PANCREATITIS WHO ATE THE TEST MEAL AND WERE GIVEN 2 CHRU/kg OF INTRAVENOUS "BOOTS" SECRETIN

PATIENT	AGE YRS	SEX	IDEAL BODY WEIGHT (%) *	SERUM PROTEIN g/l	SERUM ALBUMIN g/l	SERUM BILIRUBIN µmol/l	SERUM AAT units/ml	PROTHROMBIN INDEX, %
1	58	M	90	86	39	7	130	91
2	58	M	120	90	43	4	37	95
3	52	M	108	68	44	9	41	96
4	41	M	80	79	33	10	34	100
5	32	F	105	81	35	5	13	99
6	36	M	80	82	35	5	31	80
7	41	M	85	79	42	7	40	96
8+	47	M	85	65	41	4	23	100
9	47	F	105	77	40	36	175	90
10+	36	M	87	75	35	5	47	91
11	43	M	95	74	47	2	7	97
Normal range				60-80	35-50	2-17	10-50	80-100

+Steatorrhoea
*Documents Geigy, scientific tables

Could the significantly lower hPP concentrations in patients with chronic pancreatitis be explained by an increased rate of removal of the peptide? Although liver disease may occur in patients with chronic pancreatitis, the frequency of cirrhosis plus concomitant chronic pancreatitis is low in this population (Uys, Bank and Marks, 1973). Moreover, impaired liver function would tend to result in impaired removal of hPP thus causing higher circulating levels of hPP. Furthermore, the liver functions in the patients who received secretin and the meal were within the normal range (table 7.2) Therefore it is unlikely that liver disease was responsible for the significantly lower hPP concentrations observed in the patients with chronic pancreatitis. It is possible that distortion of the pancreatic architecture by fibrosis (Comfort, Gambill and Baggenstoss, 1946) may have prevented PP secretogogues from reaching the PP cells, either by altering blood flow or decreased diffusion across fibrotic tissue. An infusion of 2ug/min of somatostatin was effective in inhibiting hPP release during insulin-induced hypoglycaemia, so detracting from the possibility that fibrosis prevented humorally-borne test substances from reaching the PP cells. However, the fibrosis may have disrupted the anatomical arrangement of the autonomic fibres in the pancreas and thus have impaired the autonomic control of hPP release.

When the hPP responses to oral and intravenous lipid administration in the chronic pancreatitis patients who had steatorrhoea were compared with the responses in those patients who did not have steatorrhoea, no significant differences in the responses were found. However, both of these groups of patients had hPP responses that were far lower than the responses in the healthy subjects. Although neither oral, nor intravenous "Intralipid" administration caused a significant rise in

the hPP concentratons in the patients with chronic pancreatitis, in both instances the responses were lower in the patients with steatorrhoea. This may suggest that the degree of pancreatic destruction as evidenced by the presence of steatorrhoea may influence the hPP responses. It is of interest that unlike the healthy subjects in whom the hPP responses were greater after oral "Intralipid" ingestion, the patients with chronic pancreatitis had higher hPP levels after intravenous "Intralipid" although the differences were not statistically significant. It is thus possible that a small PP cell mass may not have been the only factor responsible for the attenuated hPP rise after oral lipid, but that impaired absorption too may have contributed to the lower levels. Unfortunately the serum triglyceride levels were not measured in this instance due to technical difficulties.

From these studies it is feasible that in patients with chronic pancreatitis the attenuated hPP responses to a mixed meal, oral and intravenous lipid, "Boots" secretin and insulin-induced hypoglycaemia may be explained by a reduced PP cell mass and immunohistochemical studies are required to determine the PP cell number in this condition. Finally it is possible that these stimuli to hPP release may constitute simple non-invasive techniques for identifying patients with chronic pancreatitis. Adrian, Besterman, Mallinson et al (1979), Valenzuela, Taylor and Walsh (1979) and Anderson, Hagen, Klein et al (1980) found that although PP responses to a meal were significantly lower in patients with chronic pancreatitis, poor correlation between hPP responses and exocrine function in individual patients was obtained. On the other hand, Stern and Hansky (1981) found that if, after a

"Boots" secretin injection, the basal to peak hPP ratio was less than 5, 90% of patients with chronic pancreatitis could be detected. A 10% false positive rate was obtained. Thus PP estimation may acquire a role as a non-invasive method for screening for patients with chronic pancreatitis.

Serum pancreatic polypeptide concentrations in patients with diabetes mellitus.

The mean basal hPP concentration was slightly higher in the diabetic patients than in the 50 healthy controls. However, the overlap in the ranges in this small group of patients prevents any meaningful assessment of these values. Floyd, Fajans, Pek et al (1977) have, however, reported that fasting serum hPP concentrations, when corrected for age, were elevated in maturity onset diabetics. Floyd, Fajans, Pek et al (1977) further reported that the type of diabetes and the severity of the disease may influence the basal hPP concentrations. Thus they found that basal hPP concentrations were highest in insulin-requiring juvenile-onset diabetics, were lower in maturity-onset diabetics who were treated with oral hypoglycaemic agents and were near normal in maturity-onset diabetics controlled on diet alone. Thus it is possible that the severity of the diabetes or the degree of hyperglycaemia may have influenced the basal hPP concentrations in these patients. In addition, obesity which lowers (Marco, Zulueta, Correas et al, 1980; Lassmann, Vague, Vialettes et al, 1980), and diabetic ketoacidosis which elevates (Skare, Hanssen and Lundqvist, 1980) hPP concentrations could have affected the hPP levels in this study. However, none of the patients studied here were obese or ketotic.

The elevated hPP concentrations in juvenile-onset diabetics may be explained by PP cell hyperplasia which has been reported in this condition (Gepts, De Mey and Marichal-Pipeleers, 1977), but whether hyperplasia of the PP cells occurs in patients with maturity-onset diabetes as well, is not known.

When the hPP responses to insulin-induced hypoglycaemia were compared in the diabetics and age-matched controls, no difference either in the basal or stimulated levels were apparent, although the mean response in the diabetics was higher than in non-age matched subjects. These diabetics were carefully screened to exclude the presence of autonomic neuropathy. The importance of vagal integrity in the hPP response to insulin-hypoglycaemia suggests that diabetics with autonomic neuropathy may exhibit impaired hPP responses and, indeed, it seems that diabetics with autonomic neuropathy do have impaired release of hPP following insulin-induced hypoglycaemia (Levitt, Vinik, Sive et al, 1980).

The relevance of the normal hPP response to insulin-induced hypoglycaemia in the diabetic is not clear, especially as Gates and Lazarus (1977) reported that avian and bovine PP injected into genetically diabetic mice restored normoglycaemia. The modestly increased release of hPP in the diabetics may be an attempt to restore normoglycaemia but PP was shown not to have an effect on blood glucose concentration in dogs (Lin and Chance, 1974) or man (Adrian, Greenberg, Besterman et al, 1978). As opposed to the elevated basal hPP levels reported in human diabetics, the New Zealand obese (NZO) mice, however, had low circulating PP concentrations when compared with other strains. Whether these findings relate to differences in the type of diabetes or pertain to differences in the species, or may relate to the

secretion of an immunologically competent but biologically incompetent form of PP in diabetes in conjectural.

It thus appears that these investigations of hPP concentrations and responses to insulin-induced hypoglycaemia in patients with diabetes to elucidate aspects of hPP release in this disease, have barely scratched the surface of the subject.

CHAPTER VIII

SUMMARY AND CONCLUSION

CHAPTER 8

SUMMARY AND CONCLUSIONS

Aspects of the physiology and pathophysiology of a newly recognised pancreatic peptide, pancreatic polypeptide (PP), have been investigated. Studies were undertaken to determine: 1) factors regulating the release of human PP (hPP) in healthy people; 2) the molecular forms of PP in the peripheral circulation of healthy people; 3) the metabolism and turnover kinetics of endogenous PP in pigs; 4) the basal and stimulated serum hPP concentrations in patients with acute pancreatitis, chronic pancreatitis and maturity-onset diabetes mellitus.

A sensitive specific radioimmunoassay was established to measure circulating PP. In applied clinical studies in healthy volunteers, changes in the hPP concentration were measured in response to manipulation of the physiological environment. The effects of nutrients, some gastrointestinal hormones and neurohumoral factors were assessed.

Human pancreatic polypeptide concentrations rose strikingly after eating a mixed meal. An early sharp rise in the serum hPP concentration was followed by a period of prolonged elevation which was significantly lower than the peak concentration. 1ml/kg of lipid administered either orally or intravenously and 50g of glucose taken orally caused significant uniphasic elevations of the serum hPP concentration. 25g of intravenous glucose caused a significant fall in hPP levels. Intravenous arginine had no effect on the serum hPP concentration.

2CHRU/kg of 'Boots' secretin, 85 CHRU/kg of 'Boots' cholecystokinin and 5ug/kg of synthetic pentagastrin administered intravenously, each caused significant elevation of the serum hPP concentration. The response to 'Boots' secretin was dose-dependent and was unaltered in vagotomised individuals, suggesting independence from vagal control.

These findings suggest that there is more than one factor influencing hPP release in response to a meal. The nutrients and gastrointestinal factors may play a part. Other workers have shown that the vagus is important for the early release of hPP after a meal and that CCK may play a role in mediating the prolonged phase of release. Additional investigations using gastrointestinal factors in a highly purified form at physiological concentrations will be required to define their effects as physiological hPP secretogogues.

Adrenergic manipulation caused significant changes in the serum hPP concentrations. Combined adrenergic alpha- and beta-receptor stimulation achieved by infusing epinephrine at 6ug/min caused a small rise in hPP concentrations. Unopposed beta-receptor stimulation during an infusion of epinephrine plus the alpha-adrenergic receptor antagonist, phentolamine, caused a striking rise in the serum hPP concentration. Unopposed alpha-receptor stimulation during an infusion of epinephrine plus propranolol, was associated with a fall in elevated hPP concentrations. The findings suggest that the antagonistic effects of adrenergic alpha-receptor inhibition and beta-receptor stimulation may play a role in maintaining PP homeostasis. Whether hPP is released during stress or could be a hormone of stress is not known.

Insulin-induced hypoglycaemia caused marked elevation of the serum hPP concentration. Other investigators have shown this release to be mediated by the vagus, which also plays a role in the hPP response to a meal. The insulin-hypoglycaemia induced rise in hPP concentration was abolished by somatostatin infused intravenously at 2ug/min. Somatostatin infused at 2ug/min but preceded by a 200ug bolus dose, was associated with a fall in serum hPP concentration to levels that were significantly lower than the basal concentration. A dopaminergic agonist, L-dopa and an antagonist, metoclopramide, did not significantly modify the release of hPP, nor did histamine H_2-receptor blockade with cimetidine.

The regulation of hPP release would appear to be multifactorial involving nutrients, gastrointestinal factors and the autonomic nervous system. Their relative importance and the conditions under which each exerts its maximum influence is not known and requires to be further investigated.

hPP released by insulin-induced hypoglycaemia (vagal release) and by the intravenous administration of 'Boots' secretin, were present in the peripheral circulation in three molecular forms when separated by 10% polyacrylamide disc gel electrophoresis. Chromatography did not differentiate between hPP released by insulin-hypoglycaemia or by 'Boots' secretin. These studies suggested that hPP circulates in more than one immunoreactive form. The major component of hPP migrated in the position of hPP (MW 4200 daltons) which is isolated from the pancreas. It was not apparent from these studies whether the two additional forms of hPP detected, had different molecular weights or charges from 4200 hPP.

Studies to determine the turnover kinetics of PP in the pig, showed that the highest concentration of PP was found in the pancreatico-duodenal vein and that PP drains into the portal circulation. The liver and the kidney retained 42% and 48% respectively of the PP presented to these organs. Following total pancreatectomy in pigs, the disappearance of endogenous PP in the portal circulation showed two phases of disappearance - an early rapid phase which had a half-life time of 6,3 $\pm$ 1,63 mins and a prolonged second phase of 37,4 $\pm$ 3,30 mins, suggesting that there is redistribution of PP in a larger second pool, or that continued release of PP from an extrapancreatic source occurs.

The release of hPP in disease states which involved the pancreas was not uniform. In patients with acute pancreatitis, the hPP concentrations were within the normal range and did not reflect the presence or extent of pancreatic disease. The mean fasting hPP concentration in maturity-onset diabetes without autonomic neuropathy was marginally elevated but was within the normal range, as was the response to insulin-induced hypoglycaemia. In these conditions measurement of the serum hPP concentration does not appear to be of diagnostic or prognostic consequence.

In chronic pancreatitis, although the basal serum hPP concentrations were also within the normal range, the release of hPP was impaired in response to eating a mixed meal, to the administration of "Intralipid" both orally and intravenously, to the intravenous administration of 'Boots' secretin, and to insulin-induced hypoglycaemia. These findings are consistent with a reduced functioning PP cell mass in chronic pancreatitis, and these stimuli may prove useful non-invasive techniques for identifying patients with chronic pancreatitis.

It is apparent that although insight has been gained into aspects of the behaviour of this candidate hormone, it still remains just that - an incumbent of the title of "hormone". Although PP may have roles in carbohydrate and lipid metabolism, or in the digestion process of nutrients, this work has not ascribed a biological function to PP. Clearly, there is much to be done to elucidate a biological role for PP. However, insight has been gained into factors that may regulate the release of PP in health and disease.

CHAPTER IX

THE FUTURE

CHAPTER 9

THE FUTURE

These studies on pancreatic polypeptide are in themselves neither conclusive nor definitive and invite speculation concerning its biological activity and the direction future research should take. The known biological actions of mammalian PP relate to pancreatic exocrine secretion and gall bladder and choledochal tone i.e. they are "secretomotor" actions. The striking release of PP after a meal, on a molar basis, is of the same order as insulin, a hormone with largely "metabolic" functions but which in addition may have trophic actions. Teleologically, if PP is purely a hormone of "secretion", the postprandial concentrations contrast markedly with the low post-prandial levels of the "secretory" hormones gastrin, cholecystokinin-pancreozymin and secretin. Although this may indicate that higher concentrations of PP are required for it to exert an action, it may indicate that there is a "metabolic" action of PP in addition. Indeed, in terms of evolution there is some support for the notion. The more primitive form of PP found in reptiles and birds, avian PP, has been shown to be metabolically active but at concentrations that would be higher than those observed after eating. With evolution, the PP molecule has been somewhat changed in its amino-acid sequence, but is far from discarded and still circulates in appreciable concentrations. Thus mammalian PP too, may have a "metabolic" role. Although PP infusions in man and dogs have failed to demonstrate an effect on metabolism, the reported studies have used heterologous PP i.e. PP from other species. There is a possibility that PP is species-specific and that effects with homologous PP may occur. In view of the hepatic uptake of PP described

in this thesis, and the known effects of the liver on nutrient metabolism, an investigation of the effects of PP on hepatic nutrient metabolism, possibly in an isolated perfused liver, may suggest a further role for PP. The binding of PP to liver cell membrane preparations may be of interest and if successful could constitute a radio-receptor assay for PP.

The hepatic uptake of PP raises another question of interest. It has been suggested by Greenberg, Mitznegg and Bloom (1977) that PP is trophic to the exocrine pancreas. In the light of the large hepatic uptake of PP and its proposed trophism, the search for a gastrointestinal or pancreatic factor having hepatotrophic properties, should include an investigation of pancreatic polypeptide. On the other hand, is it possible that PP is an example of biological waste? It seems strange that an "endocrine" peptide should be found in the systemic circulation in such large quantities when it acts adjacent to its site of release. In view of the diffuse scatter of PP cells in the exocrine parenchyma of the pancreas, one could visualise that PP acts in a "paracrine" fashion and this too, merits further investigation.

The most likely physiological action of PP appears to be its ability to inhibit pancreatic exocrine and bile secretion. It appears contradictory to expectations that the concentrations of a hormone with this biological action should be low during fasting and be released in response to a meal. Since the initial phase of PP release in response to food appears to be vagally mediated, it is possible that the same stimulus could provoke unnecessary pancreatic and bile secretion before food has reached the duodenum. The early PP release may act to suppress this secretion. During the phase of prolonged secretion, when nutrients in

the small bowel stimulate the release of many gastrointestinal factors, PP may act to modulate the effects of the recognized pancreatic and biliary secretogogues. Studies to examine the interaction of PP and these secretogogues may contribute to an understanding of pancreatic physiology. The use of analogues to block the action of PP may be an additional approach to delineate its function.

Finally, because I entered into this study of PP through an interest in diabetes mellitus, I would like to conclude on a speculative note about PP and diabetes. There are many ill-understood facets and phenomena of diabetes which possibly PP or the other newly recognized pancreatic peptides, somatostatin or vasoactive intestinal polypeptide may someday explain. When Banting and Best reported that replacement of pancreatic extracts reversed the hyperglycaemia in pancreatectomised dogs, the most significant breakthrough in the treatment of diabetes had been achieved. However, they realised that this was not the whole answer and could not explain all the phenomena they observed, as Best wrote to Dr Pierre Foá and was quoted by Dr R Unger in his Banting Memorial Lecture (1975).

"I have a very clear recollection of the immediate rise in blood sugar of about 5 - 10mg%. This lasted about half an hour. As you know, we thought this might have been due to epinephrine liberation and, for this reason, we failed to investigate it thoroughly".

It is now known that glucagon was responsible for this phenomenon, but there are other poorly understood phenomena following total pancreatectomy. These include the brittleness of diabetes, the insulin sensitivity, the easily provoked hypoglycaemia, the virtual absence of ketosis and complications, and an increased predisposition to gastric

hypersecretion and gastric, duodenal and even jejunal ulceration. It is possible that PP may yet fill a role in understanding some of these features.

APPENDIX I

RADIOIMMUNOASSAY OF PANCREATIC POLYPEPTIDE

APPENDIX I

THE RADIOIMMUNOASSAY OF PANCREATIC POLYPEPTIDE

Immunoreactive PP was measured by a specific radioimmunoassay using reagents kindly donated by Dr R.E. Chance of Eli Lilly, Indianapolis, USA.

A. THE BUFFERS

1. The assay buffer

   The assay buffer was a 0.04 M phosphate pH 7.4 buffer to which 1% Bovine Albumin Fraction V (Miles Laboratories), 0.005% Merthiolate and 0.1M Na Cl was added.

2. The eluting buffer

   0.05M phosphate buffer to which 0.1M Na Cl and 1% Bovine Albumin Fraction V were added.

3. Antiserum diluting buffer

   0.04M phosphate buffer pH 7.4, containing 0.1% Bovine Albumin Fraction V, 0.02% merthiolate and 1% normal rabbit serum.

B. THE STANDARD REFERENCE PREPARATION - the 'standards'

Human pancreatic polypeptide (hPP) Lot 615-1054B-200 was received in lyophilised form. It was reconstituted in the assay buffer to a concentration of 1 ug/ml and stored in 50 ul aliquots, at -20°C

Prior to use 20 ul of the stored concentrate was diluted in 2 ml of the assay buffer. The resulting concentration of 10 000 pg/ml was the highest concentration of standard used. Serial dilutions to 5 000, 2 500, 1 000, 500, 250, 125, 62.5 and 31.25 pg/ml were then made to constitute the concentrations for the dose-response ("standard") curve.

C. THE TRACER

Bovine pancreatic polypeptide (bPP) Lot 615-D63-188-9 was iodinated as tracer. The bPP was received and stored in a lyophilised form. At intervals, 10-20 ug of bPP was weighed out on a Mettler ME 30 balance and made up to a concentration of 0.1 ug/ul in 0.25 M phosphate buffer at pH 7.5. 50 ul aliquots were stored at -20°C. Freshly prepared bPP was iodinated on each occasion.

1. The iodination procedure

Iodination of bPP was carried out at room temperature using the following reagents:

a) 5 ug of bPP in 50 ul of 0.25 M phosphate buffer at pH 7.5.

b) 10 ul (1 000 uCi) ^{125}I (Amersham, England)

c) 20 ul of Chloramine T at a concentration of 4 mg/ml

d) 20 ul of sodium metabisulphite at a concentration of 2.4 mg/ml.

e) 50 ul of potassium iodide at a concentration of 10 mg/ml-

f) 10 ul of charcoal stripped plasma

g) 250 ul of the eluting buffer.

Reagents b, c and d were dissolved in 0.05 M phosphate buffer pH 7.5.

The reagents were added sequentially. Before adding the sodium metabisulphite, the iodination mixture was agitated for 15 seconds.

2. The incorporation of ^{125}I into bPP

To assess the incorporation of ^{125}I into bPP, 10 ul of the final iodination mixture was added to 5 ul of bromophenol blue containing 2% human serum albumin. 10 ul of the resulting mixture was then electrophoresed using paper strips (Whatman, Ltd). The radioactivity remaining at the 'origin' i.e. ^{125}I incorporated into intact tracer was compared to the total radioactivity and the percentage incorporation calculated.

$$\% \text{ incorporation} = {}^{O}/_{T} \times 100$$

where O = counts per unit time at origin

T = total counts

The incorporation of ^{125}I in uCi per ug of bPP was also calculated.

Since 200 uCi ^{125}I was added per ug of bPP, the maximum incorporation would be 200 uCi/ug and the

$$\text{actual incorporation} = \frac{200 \times X}{100} \text{ uCi/ug}$$

where X = % incorporation.

Increasing the amount of chloramine T added to the iodination reaction, increased the percentage incorporation of ^{125}I.

Amount of Chloramine T	% Incorporation
10 ug	27
15 ug	37
20 ug	55

In 10 consecutive assays, using the iodination procedure described, the mean ($\pm$ S.D.) incorporation was $55 \pm 4.2\%$ or 110 ± 8.4 uCi/ug.

3. Purification of the tracer:

The tracer was purified on a 0.9 x 50 cm Sephadex G-50 superfine column (Pharmacia, Sweden). The sephadex was soaked overnight at room temperature in the eluting buffer. After pouring the column, it was equilibrated with the eluting buffer to run at 10-12 ml/hr using a peristaltic pump.

Following the iodination procedure, the iodination mixtue was placed on the column, washed, and eluted with the eluting buffer. Fractions of the eluent were collected at 5 minute intervals using an LKB 2070 Ultrarac fraction collector. 10 ul aliquots were counted for radioactivity.

4. Assessment of the integrity of the tracer

From the fractions in the elution peak, 10 ul aliquots were removed and electrophoresed on paper strips as described above. Intact tracer remains at the origin whereas fragments migrate with the human serum albumin and bromophenol blue marker.

The integrity of the tracer was then calculated.

$$\% \text{ Intact} = {}^{0}/_{0 + M} \times 100$$

where 0 = counts at the origin per unit time

M = counts at the area of migration per unit time.

The integrity of the peak fractions in a typical elution profile were:

Fraction No	% Intact
30	94
31	94
32	95
33	93
34	88

In 10 consecutive iodinations the mean ($\pm$ S.D.) integrity of the fractions used as the tracer was 95 $\pm$ 1.0% intact.

5. Storage and use of the tracer

The fraction used as the tracer was stored in 100 ul aliquots at -20°C. Prior to use, the tracer was diluted in the assay buffer to 6 000 to 7 000 counts per 100 ul per minute. Stored in this way, the tracer maintained its integrity for three weeks.

D. THE ANTISERUM

Rabbit anti-hPP serum Lot 615-1054B-248-19 was received in lyophilised form.

1. Diluting of the antiserum

The antiserum was diluted to the desired concentration in 0.4 M phosphate buffer pH 7.4, containing 0.1% bovine serum albumin, 0.02% merthiolate and 1% normal rabbit serum.

Binding of the tracer to the antiserum was dependent on the concentration of antiserum used. In the absence of cold hPP, binding of tracer to various concentrations of the antiserum is shown below.

Initial antiserum concentration	% binding
1 : 10000	67.5
1 : 20000	63.9
1 : 40000	59.2
1 : 80000	44.4
1 : 100000	34.4
1 : 150000	22.1

The antiserum was used at our initial dilution of 1 : 100 000. At 50% of the maximum binding (Id 50), the antiserum detected 468 pg/ml (111.5 pmol/l) of PP.

The calculated lowest detection limit of the assay was 1.7pg (4×10^{-4} pmol) per assay tube or 17pg/ml (4pmol/l).

2. Specificity of the antiserum

The addition of synthetic non-sulphated heptadecapeptide gastrin (Sorin), monocomponent insulin, highly purified glucagon (Eli Lilly and Co.), gastric inhibitory polypeptide (Dr J Brown, Vancouver), cholecystokinin (Karolinska Institute, Stockholm), and caerulein (Dr Erspamer, Farmitalia, Milan) in one thousand fold excess of the highest hPP concentration, did not displace the tracer. 'Intralipid', cimetidine, metoclopramide and L-dopa which were given to the subjects in the studies carried out, did not affect the binding of the tracer in the assay system.

"Boots" secretin contained 10200 pg (2.42pmol) of PP per unit of secretin.

Standard insulin preparations contained PP as shown below.

Type of Insulin		PP concentration in umol per mol of insulin
Wellcome Soluble	40 U/ml	391
	80 U/ml	196
Protamine zinc	40 U/ml	0.6
Boots Protamine zinc	40 U/ml	345
	80 U/ml	415
N.P.H.	40 U/ml	164
	80 U/ml	223
Lente	40 U/ml	1615
	80 U/ml	595

Commercial monocomponent insulins were free of PP.

E. THE ASSAY PROCEDURE

The standard reference preparations, tracer and antiserum were prepared as described above. The assay tubes containing 700 ul of the assay buffer, 100 ul of standard or serum sample and 100 ul of antiserum. The tubes were incubated for 48 hours at 4°C.

Incubation of the ^{125}I - labelled bPP at 4°C in the assay buffer caused it to degrade necessitating delayed addition of the tracer. At 48 hours, 100 ul of tracer was added and the incubation allowed to proceed for a further 24 hours.

```
 10  DIM N$(20), D$(10), T$(20), P$(20)
 20  REM... PEG ASSAY
 30  DISP "NAME OF ASSAY";
 40  INPUT N$
 50  DISP "DATE";
 60  INPUT D$
 70  DISP "TECHNICIAN";
 80  INPUT T$
 90  PRINT N$
100  PRINT
110  PRINT D$, "TECHNICIAN", T$
120  PRINT
130  DISP "HOW MANY TOTALS";
140  INPUT T
150  X=0
160  FOR J=1 TO T
170  DISP "ENTER TIME TOTAL" J;
180  INPUT T1
190  T2=10000/T1
200  X=X+T2
210  NEXT J
220  T3=X/T
230  PRINT "MEAN TOTAL BINDING = "T3" CPM"
240  PRINT
250  PRINT
260  DISP "PATIENT'S NAME";
270  INPUT P$
280  PRINT P$
290  DISP "HOW MANY NSB-TUBES";
300  INPUT N
310  DISP "TIME(0) OR COUNTS/5 MIN(1)";
320  INPUT C
330  Z=0
340  FOR K=1 TO N
350  IF C THEN 400
360  DISP "ENTER NSB TIME"K;
370  INPUT N1
380  N2=10000/N1
390  GOTO 430
```

```
400  DISP "ENTER NSB COUNTS"K;
410  INPUT N1
420  N2=N1/5
430  Z=Z+N2
440  NEXT K
450  N3=Z/N
460  PRINT
470  PRINT "MEAN NSB ="N3"CPM /"N3*100/T3"%"
480  PRINT
490  DISP "HOW MANY UNKNOWNS":
500  INPUT U
510  DISP "HOW MANY REPLICATES";
520  INPUT R
530  FOR I=1 TO U
540  A=0
550  FOR J=1 TO R
560  IF C THEN 610
570  DISP "ENTER UNKNOWN"I",REP"J"TIME";
580  INPUT R1
590  U1=10000/R1
600  GOTO 640
610  DISP "ENTER UNKNOWN"I",REP"J"COUNTS";
620  INPUT R1
630  U1=R1/5
640  U2=(U1-N3)*100/(T3-N3)
650  PRINT I"REP"J;U1;U2"%"
660  A=A+U2
670  NEXT J
680  PRINT TAB40"MEAN ="A/R"%"
690  PRINT
700  NEXT I
710  DISP "ANOTHER PATIENT YES=1,NO=0;
720  INPUT O
730  IF O THEN 240
740  END
```

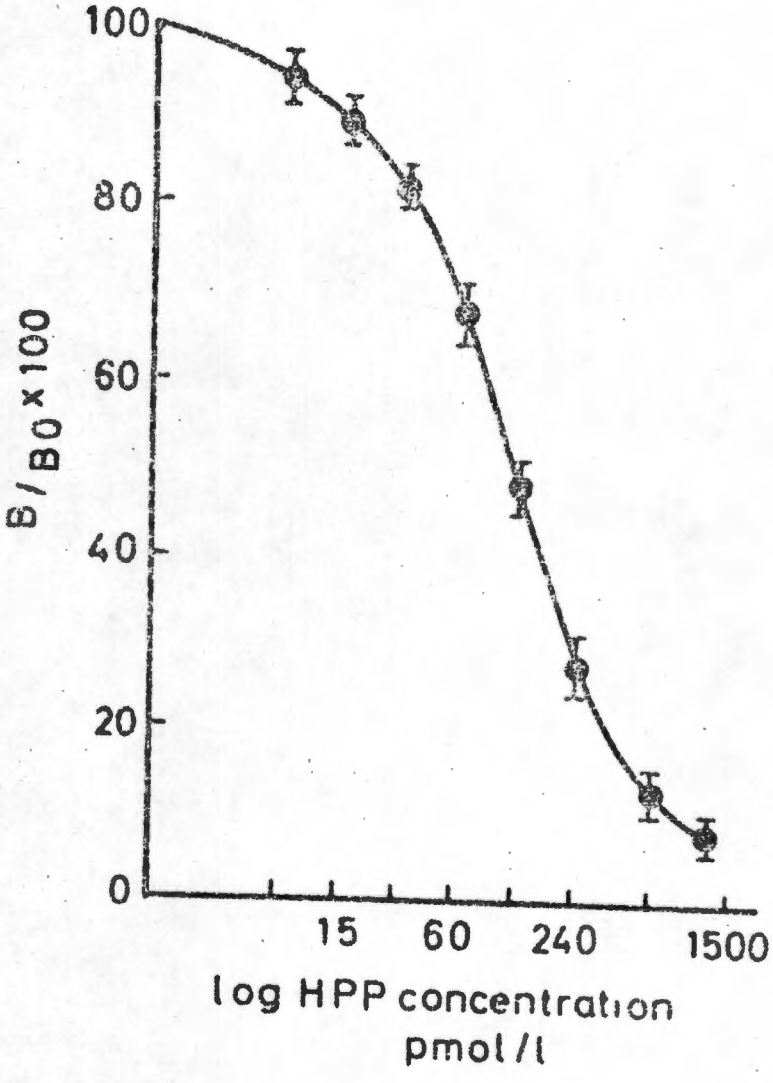


Figure A.1

A composite pancreatic polypeptide dose-response curve (standard curve) for 10 consecutive assays showing the mean ($\pm$ S.D.) of each point.

Separation of antibody-bound from free ^{125}I - labelled hPP was carried out by the addition of 1 ml of 25% (w/v) polyethylene glycol (m.w. 6 000) which was made up in 0.04 M phosphate buffer, pH 7.4. The tubes were mixed and centrifuged at 2 000 r.p.m. for 45 minutes at 4°C. The supernatant was then decanted and the precipitate counted using a Packard Antogamma Scintillation spectrometer, model S260.

For each assay, tubes containing buffer and tracer only were prepared to serve as the "total counts" for the subsequent calculation.

Non-specific binding (NSB) was measured for the assay and for each set of serum samples. These tubes did not contain hPP antiserum and were made to the same volume with assay buffer.

Each assay was carried out in duplicate. Control tubes with known hPP concentration were assayed at the same time to assess the interassay variation.

Binding of the tracer to the antibody was calculated on a Hewlett Packard 9830A desk top calculator and 9866A printer according to the program designed for this purpose and included opposite. The dose-response curve ('standard curve') was then manually plotted on semi-logarithm paper, and the unknown samples read from this curve. The mean of each unknown sample was recorded. The values in pg/ml were converted to S.I. units (pmol/l) for presentation.

A composite standard curve for 10 consecutive assays with the mean and standard deviation of each point is shown in Fig A.1. The mean ($\pm$ S.D.) non-specific binding in these assays was 13.5 $\pm$ 1.68% and the maximum binding (Bo), 36.5 $\pm$ 1.98%.

F. REPRODUCTIVITY AND VARIABILITY OF THE ASSAY

1. Assay of PP in serum

Adding known amounts of hPP to serum, resulted in 70% recovery of hPP. Dilutions of serum containing high hPP concentrations caused a predictable change in the serum hPP concentration suggesting parallel displacement of tracer from the antiserum compared with hPP standards.

Sample No	PP concentration in serum (pg/ml)	PP concentration 1:10 dilution of serum (pg/ml)	Corrected hPP concentration (pg/ml)
1	5300	560	5600
2	3100	340	3400
3	3500	310	3100
4	1050	90	900
5	2600	220	2200

2. Assay variation

Inter- and intra-assay coefficients of variation (C.V.) were determined by

$$C.V. = {}^{S.D.}/_{mean} \times 100$$

when SD = standard deviation of the mean.

a) hPP at the concentrations shown below was added to serum. Six measurements, each in duplicate was then made of these concentrations. The intra-assay coefficient of variation is tabulated.

pg/ml of hPP	Coefficient of variation
125	15%
250	11%
500	9%
1000	10%

b) In the ten 10 consecutive assays presented in Fig A.1 the coefficient of variation at 50% of the maximum binding ($I.D._{50}$) was 14%.

APPENDIX II

POLYACRYLAMIDE GEL ELECTROPHORESIS OF

PANCREATIC POLYPEPTIDE

APPENDIX II

10% POLYACRYLAMIDE DISC GEL ELECTROPHORESIS OF PANCREATIC POLYPEPTIDE

10% polyacrylamide disc gel electrophoresis was carried out by the method of Cain and Pitney (1968) as modified by Lazarus, Gutman and Recant (1971). The following were prepared:

A. THE RUNNING GEL

Reagent A:

3 M Tris-hydrochloride, pH 8.5 containing 0.23 ml Tetramethylethylenediamine (Temed, Merck) per 100 ml

Reagent C:

40 g acrylamide (Merck), 0.4 ml of ethylene diacrylate (Merck) 0.04 ml and Tween 80 (Merck) were made up to 100 ml in distilled water.

Reagent G:

0.006 M Ammonium persulphate was freshly prepared on the day of its use.

2 ml of Reagent A, 4 ml of reagent C, 8 ml of Reagent G and 2 ml of distilled water were mixed to constitute the running gel. The mixture was debubbled using vacuum suction.

B. THE STACKING GEL

Reagent B

0.5 M Tris-hydrochloride, pH 6.7 containing 0.4 ml of Temed per 100 ml.

Reagent D

20 g of acrylamide, 5 g and bisacrylamide (Merck) and 0.32 ml of ethylene diacrylamide were up to 100 ml in distilled water.

Reagent F

0.015 M Ammonium persulphate was freshly prepared on the day of its use.

2 ml of Reagent B, 4 ml of reagent D, 2 ml of reagent F and 8 ml of distilled water were mixed to constitute the stacking gel. The mixture was debubbled using vacuum extraction.

C. THE RUNNING BUFFER

An 0.01 M Tris buffer containing 0.077 M glycine, pH 8.3 was prepared.

D. THE APPARATUS

A Shandon apparatus for gel electrophoresis and standard Shandon 75 mm glass columns were used. The columns were siliconized before use. A Shandon SAE 2761 power pack regulated the amperage and maintained a constant current.

E. THE PROCEDURE

The gels were prepared on the day prior to the electrophoresis. 1 ml of the running gel was pipetted into each column and a few drops of water layered on top to attain an even surface. When the gels had set, the water was removed with tissue paper. 200 ul of the stacking gel was then layered on top of the running gel and water again used to achieve a smooth, level surface.

When set, the gel columns were fitted into the electrophoresis apparatus and the running buffer was then poured into the lower chamber to cover the electrode.

50 ul of sample or markers were mixed with an equal volume of 40% sucrose and applied to the gel. The upper chamber of the apparatus was then filled with buffer to which a few drops of a marker substance, 1% bromophenol blue had been added. This marker indicates the point of maximal migration.

Electrophoresis was carried out at 4°C. Until the buffer front had migrated through the stacking gel, 2 m amps per column were used. Thereafter the electrophoresis was carried out using 5 m amps per column. Columns were removed when the marker had migrated to 0.5 cm of the column end. The current was then appropriately reduced by 5 m amp per column removed.

The gels were removed from the glass column by gentle hydrostatic pressure and cut into 3 mm segments. Segments containing radiolabelled peptides were counted in the Packard Autogamma scintillation counter. The other segments were soaked overnight in 1 ml of the assay buffer at 4°C to elute the peptides prior to assay. The fractions were stored at -20°C until assayed.

APPENDIX III

TABULATION OF PANCREATIC POLYPEPTIDE RESULTS

THE EFFECT OF A STANDARDISED MEAL ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes										
	-15	0	1.M*	5	15	30	45	60	90	120	180
1	14	14	66	258	84	58	84	172	48	180	320
2	16	22	1286	572	358	404	452	392	358	476	358
3	14	14	1190	392	200	108	114	228	322	262	128
4	14	14	649	452	338	158	134	220	548	620	456
5	14	14	404	462	180	380	438	462	238	666	64
6	14	14	514	358	258	180	80	90	228	220	148
7	34	30	431	375	236	231	201	154	163	133	146
$\bar{X}$	17	17	648	410	236	217	215	245	272	365	231
S.E.	2.8	2.4	166.5	37.2	35.6	49.7	61.4	50.3	60.0	82.7	55.1

* 1.M denotes the intra-meal hPP concentration five minutes after starting to eat at time '0' and the times 5-180 minutes, the period after completing the meal.

THE EFFECT OF ARGININE INFUSED AT A RATE OF 10 mg/kg/min ON SERUM hPP CONCENTRATIONS IN pmol/l

Subjects	Minutes							
	Arginine 10 mg/kg/min							
	-15	0	10	20	30	45	60	90
1	47	40	36	37	32	29	23	26
2	16	11	29	21	33	21	25	19
3	27	26	19	19	17	27	22	12
4	26	24	21	30	18	20	24	28
5	42	54	83	45	47	41	45	41
$\bar{X}$	32	31	37	30	29	28	28	25
S.E.	5.6	7.4	11.7	4.9	5.5	3.8	4.3	4.8

THE EFFECTS OF ORAL (50g) AND INTRAVENOUS (25g) GLUCOSE ON SERUM hPP CONCENTRATIONS IN pmol/l.

ORAL GLUCOSE

Subjects	Minutes					
	-15	0*	30	60	90	120
1	36	36	44	38	12	39
2	32	18	49	53	42	29
3	13	20	32	15	26	23
4	15	22	42	26	33	30
5	19	22	18	30	30	31
6	18	22	24	20	21	7
7	54	47	73	83	51	57
8	44	44	54	30	33	41
9	33	30	53	53	112	94
$\bar{X}$	28	27	40	36	36	36
S.E.	4.5	3.9	6.2	7.0	9.3	7.9

INTRAVENOUS GLUCOSE

Subjects	Minutes							
	-15	0*	3	6	10	20	30	60
1	34	26	32	34	27	30	8	19
2	26	35	41	33	28	26	25	46
3	24	25	38	28	23	21	21	22
4	24	30	24	32	17	21	23	25
5	17	18	18	16	18	36	22	28
6	47	38	19	16	25	30	20	20
7	35	26	29	35	45	35	23	21
8	78	95	90	60	73	55	36	35
9	27	26	20	34	28	21	18	40
$\bar{X}$	32	33	32	30	29	28	20	26
S.E.	5.9	7.3	7.1	4.6	5.7	4.0	2.6	3.6

* The glucose was administered after the final basal sample at time '0'.

THE EFFECTS OF ORAL AND INTRAVENOUS 'INTRALIPID' (1 ml/kg) ON SERUM hPP CONCENTRATIONS.

ORAL 'INTRALIPID'

Subjects	Minutes									
	-15	0*	5	10	20	30	45	60	90	120
1	23	29	155	252	1190	1000	417	351	157	64
2	60	60	64	110	140	69	51	119	176	304
3	57	58	369	714	286	321	702	607	176	202
4	15	15	175	57	20	95	89	92	152	135
5	31	27	85	68	100	167	252	146	53	40
6	83	82	86	131	162	79	93	69	76	119
7	19	22	87	86	77	54	36	11	16	17
$\bar{X}$	41	42	145	202	282	255	234	199	115	126
S.E.	9.7	9.4	40.3	88.7	154.5	128.9	93.4	79.0	24.8	38.0

INTRAVENOUS 'INTRALIPID'

Subjects	Minutes									
	-15	0*	5	10	20	30	45	60	90	120
1	65	88	82	102	101	219	86	85	80	76
2	64	74	131	181	327	188	186	260	238	92
3	62	61	43	106	345	375	101	55	86	87
4	23	36	36	160	205	135	165	195	193	95
5	20	12	19	36	42	33	81	56	21	36
6	45	45	100	115	51	52	33	36	133	155
7	29	27	34	36	50	74	110	119	103	119
$\bar{X}$	44	49	64	105	160	154	109	115	122	94
S.E.	7.8	10.1	15.6	20.9	50.1	45.3	19.7	31.5	27.7	13.9

* The 'Intralipid' was administered as a bolus dose after the final basal sample at time '0'.

THE EFFECT OF HEPARIN ADMINISTERED AS 3 INTRAVENOUS DOSES OF 3 000 U EACH ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes									
	-30	-15	0*	10	* 20	30*	40	50	60	90
1	33	31	49	45	40	33	29	48	51	54
2	38	39	45	68	138	212	117	105	65	57
3	12	14	15	22	11	10	15	15	13	7
4	35	33	32	24	22	23	34	31	27	25
5	45	48	62	24	31	62	54	58	33	22
6	64	69	37	43	102	131	152	107	88	74
7	31	35	35	95	126	45	33	45	60	62
8	15	12	16	15	12	17	14	14	15	16
$\bar{X}$	34	35	36	42	60	67	56	53	44	40
S.E.	5.8	6.5	5.6	9.7	18.7	24.8	18.0	12.8	9.3	8.8

*The Heparin injections were given at time '0', 15 and 30 minutes.

THE EFFECT OF A DRINK OF 150 ml OF WATER ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes				
	-15	0*	5	15	30
1	24	24	21	16	42
2	18	14	26	7	10
3	14	14	7	7	7
4	33	34	24	28	43
5	37	49	43	31	20
6	41	35	38	49	35
$\bar{X}$	28	28	27	23	26
S.E.	4.4	5.6	5.2	6.6	6.5

*The drink of water was given after the final basal sample at time '0'.

THE EFFECT OF AN INTRAVENOUS SALINE INFUSION ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes						
				0,9 saline infusion			
	-15	0	5	10	15	20	30
1	32	39	41	42	38	38	41
2	34	42	41	47	47	54	54
3	33	20	28	32	32	33	30
4	22	23	21	22	21	21	21
5	22	26	22	23	22	12	21
6	17	18	17	26	21	30	35
$\bar{X}$	27	28	28	32	30	31	33
S.E.	2.9	4.1	4.3	4.3	4.4	5.9	5.2

THE EFFECTS OF INCREASING SEQUENTIAL DOSES OF "BOOTS" SECRETIN ON SERUM hPP CONCENTRATIONS IN pmol/l

Subjects	-30	-15	0*	1	3	5	10	15	30*	31	33	35	40	45	60*	61	63	65	70	75	90*	91	93	95	100	105	120 min.
1	47	30	46	44	63	51	21	18	11	228	286	51	38	37	33	128	380	81	31	57	54	428	190	190	79	81	74
2	18	13	17	33	104	171	64	42	23	84	167	136	68	19	8	66	238	476	142	90	43	131	310	345	123	79	35
3	52	33	45	62	190	228	75	48	23	54	167	192	116	76	53	357	428	416	192	152	147	228	381	416	369	200	173
4	14	15	7	8	40	88	43	13	7	10	109	100	35	57	45	76	190	231	142	131	109	195	238	250	262	131	107
5	73	43	59	88	178	119	90	40	18	62	333	321	232	138	59	119	321	476	187	124	33	109	238	488	208	309	109
$\bar{X}$	41	27	35	47	115	131	59	32	16	88	212	160	98	65	40	149	311	336	139	111	77	218	271	338	208	160	100
S.E	11,0	5,7	9,8	13,5	30,0	31,1	12,1	7,0	3,2	37,1	41,7	46,4	36,6	20,5	4,0	53,3	43,9	78,0	29,0	16,7	21,8	56,6	33,4	54,0	51,3	43,3	22,8

*0,5, 1,0, 1,5 and 2,0 CHRU/kg of secretin was administered intravenously at 0, 30, 60 and 90 minutes respectively.

THE EFFECT OF 2 CHRU/kg OF 'BOOTS' SECRETIN ADMINISTERED INTRAVENOUSLY ON SERUM hPP CONCENTRATIONS (pmol/l) IN NORMAL SUBJECTS.

Subjects	Minutes								
	-15	0*	1	3	5	15	30	45	60
1	40	40	343	310	267	79	90	117	110
2	28	28	286	383	333	310	99	105	122
3	67	90	667	332	476	205	121	58	66
4	30	75	467	467	467	202	136	90	108
5	53	48	390	429	314	124	112	102	119
6	14	14	286	186	157	96	50	36	12
7	67	66	1048	1048	1048	371	186	104	137
$\bar{X}$	42	51	498	450	438	198	113	87	96
S.E.	7.7	10.1	104.3	105.3	109.7	41.5	15.9	11.1	16.3

* The secretin was administered after the final basal sample at time '0'.

THE EFFECT OF 2 CHRU/kg OF 'BOOTS' SECRETIN ADMINISTERED INTRAVENOUSLY ON SERUM hPP CONCENTRATIONS (pmol/l) IN SUBJECTS WHO HAD UNDERGONE BILATERAL TRUNCAL VAGOTOMY.

Subjects	Minutes								
	-15	0*	1	3	5	15	30	45	60
1	57	79	148	714	810	500	148	77	57
2	50	31	476	524	548	429	200	124	93
3	55	58	381	357	393	210	112	90	82
4	124	106	145	429	381	114	67	31	38
5	52	60	500	1071	548	250	129	67	65
6	54	52	524	548	500	286	167	100	57
7	30	45	119	98	105	100	40	21	27
$\bar{X}$	60	61	327	534	469	270	123	73	60
S.E.	11.1	9.2	69.3	114.7	81.0	56.8	21.1	13.9	8.7

* The secretin was administered after the final basal sample at time '0'.

THE EFFECTS OF 85 U/kg OF CHOLECYSTOKININ - PANCREOZYMIN ADMINISTERED INTRAVENOUSLY ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes								
	-15	0*	1	3	5	15	30	45	60
1	86	81	110	190	176	119	86	95	143
2	34	57	67	68	57	43	44	38	24
3	28	28	30	38	42	30	31	30	27
4	17	17	24	25	34	25	20	21	21
5	16	14	28	61	26	28	10	11	16
6	28	48	47	41	47	52	79	62	38
7	44	52	45	44	62	72	68	40	36
$\bar{X}$	36	42	50	67	63	53	48	42	43
S.E.	9.1	9.1	11.4	21.2	19.3	12.7	11.2	10.6	16.8

* The cholecystokinin-pancreozymin was administered after the final basal sample at time '0'.

THE EFFECT OF 5 µg/kg OF INTRAVENOUS PENTAGASTRIN ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes									
	-30	-15	0*	1	3	5	15	30	45	60
1	29	18	24	89	89	99	33	50	40	26
2	17	15	20	155	107	71	23	60	48	86
3	43	81	62	298	238	190	86	51	46	46
4	31	54	31	129	179	106	51	81	152	126
5	119	109	83	714	833	690	298	162	130	100
6	78	56	145	274	286	333	174	174	129	119
7	71	112	60	166	171	129	286	148	205	105
$\bar{X}$	55	64	61	261	272	231	136	104	107	87
S.E.	13.6	15.0	16.5	80.7	97.1	83.4	44.5	20.9	24.1	14.2

* The pentagastrin was injected after the final basal sample at time '0'.

THE EFFECT OF EPINEPHRINE MODULATION ON SERUM hPP RESPONSES IN pmol/l.

Subjects				epinephrine 6 µg/min		epinephrine 6 µg/min + propranolol 0.1 mg/min						epinephrine 6 µg/min			epinephrine 6 µg/min + phentolamine 0.5 mg/min					
	-60	-45	-30	15	0	3*	5	15	30	45	60	75**	90	120	123	125	135	150	165	180 Minutes
7	33	79	50	126	142	238	157	100	101	32	55	77	35	35	228	327	92	164	380	140
8	57	39	30	89	92	97	108	63	38	55	50	63	70	69	188	278	219	785	880	1023
9	92	78	57	78	66	91	88	63	30	26	9	10	11	30	51	58	42	84	216	235
10	42	47	41	46	42	29	35	41	48	52	39	36	36	54	53	48	78	102	72	61
11	47	57	71	57	52	57	57	52	42	47	47	52	46	46	61	80	71	123	90	76
12	71	51	47	192	202	207	165	110	88	92	66	63	88	85	98	108	115	107	142	88
$\bar{X}$	57	58	51	98	99	120	102	72	58	51	44	50	48	53	113	150	103	225	297	271
S.E.	8.8	6.7	6.4	21.9	25.2	34.2	21.4	11.2	12.0	9.5	8.0	9.8	11.2	8.5	31.2	49.4	25.2	112.4	125.3	152.7

* The propranolol infusion was preceded by a propranolol bolus of 10 mg.

** The phentolamine infusion was preceded by a phentolamine bolus of 5 mg.

THE EFFECT OF ADRENERGIC MODULATION ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Basal			epinephrine 6 µg/min					epinephrine 6 µg/min + phentolamine 0.5 mg/min					epinephrine 6 µg/min				epinephrine 6 µg/min + propranolol 0.1 mg/min				
	-90	-75	-60	-45	-30	-15	0	3*	5	15	30	45	60	75	90	120	123**	125	135	150	165	180 Minutes
1	39	30	35	51	56	88	71	78	88	520	321	369	423	297	357	333	254	204	183	121		
2	20	24	24	24	20	36	39	76	130	119	133	181	205	204	142	135	82	102	62	81	80	78
3	20	20	17	28	26	24	20	38	72	88	247	219	228	188	152	114	116	106	79	80	54	33
4	42	46	51	90	71	64	73	58	64	85	452	523	476	333	440	202	273	261	160	67		
5	64	65	66	108	138	171	111	142	152	134	204	290	119	112	242	191	146	161	171	161	161	197
6	83	83	83	76	82	103	178	101	184	303	321	523	511	452	559	392	380	380	130	111	145	95
$\bar{X}$	45	45	46	63	66	81	82	82	115	208	280	351	327	264	315	228	209	202	131	104	110	101
S.E.	10.2	10.2	10.4	13.9	17.6	21.8	23.1	14.7	19.6	70.5	45.2	60.4	66.6	49.6	68.0	45.3	46.2	43.2	20.5	14.2	25.6	34.6

* The phentolamine infusion was preceded by a phentolamine bolus of 5 mg.

** The propranolol infusion was preceded by a propranolol bolus of 10 mg.

THE EFFECT OF INSULIN-HYPOGLYCAEMIA ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes									
	-30	0*	10	20	30	45	60	70	80	90
1	23	25	36	31	23	100	224	162	143	117
2	25	19	21	28	22	357	214	233	242	250
3	22	12	70	152	150	148	114	79	75	60
4	24	29	22	27	22	148	65	60	43	44
5	45	41	-	76	214	347	267	204	188	168
$\bar{X}$	28	25	37	63	86	220	177	148	138	128
S.E.	4.3	4.9	11.4	24.1	40.4	54.6	37.5	34.3	36.3	37.6

* 0.1 U/kg of monocomponent porcine insulin was injected intravenously after the final basal sample at time '0'.

THE EFFECT OF THE INGESTION OF 500 mg OF L-DOPA ON BASAL AND MEAL STIMULATED SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes															
	-120	-105	-90*	-60	-30	0**	1.M***	5	15	30	45	60	90	120	180
1	26	55	74	85	33	112	440	1048	702	690	231	333	300	271	226
2	7	57	31	30	29	60	405	452	571	262	214	229	229	429	571
3	23	40	25	7	10	62	483	619	548	264	302	300	368	236	219
4	45	40	83	188	47	31	483	1048	774	643	638	531	440	386	417
5	15	13	17	35	15	17	310	371	176	329	300	352	271	161	154
6	15	8	10	12	12	13	252	324	298	190	252	305	129	219	207
$\bar{X}$	22	35	39	59	24	49	277	643	511	396	365	341	289	283	298
S.E.	5.4	8.4	12.6	28.1	5.9	15.5	38.9	134.1	94.6	87.5	64.6	41.6	44.3	42.1	65.6

* 500 mg of L-dopa ingested after the final basal sample

** a standard mixed meal ingested

*** 1.M denotes intra-meal sample, and the times 5 to 180 minutes, the period after completing the meal.

THE EFFECT OF AN INTRAVENOUS BOLUS OF 10 mg OF METOCLOPRAMIDE ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects	Minutes									
	-15	0*	1	3	5	10	20	40	60	90
1	54	74	86	121	167	110	128	110	69	83
2	24	11	25	7	12	38	27	12	7	20
3	60	55	60	57	74	93	58	93	114	44
4	25	40	37	44	55	38	42	43	35	18
5	52	66	52	38	33	21	27	40	67	100
6	40	43	57	50	37	38	45	58		18
$\bar{X}$	42.5	48.2	52.8	52.8	63.0	56.3	54.5	59.3	58.4	47
S.E.	6.28	9.14	8.6	15.4	22.50	14.69	15.46	14.8	17.98	14.7

* The metoclopramide was injected after the final basal sample at time '0'.

THE EFFECT OF A CONSTANT INFUSION OF 2 µg/min OF SOMATOSTATIN ON BASAL SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects			2 µg/min		
	-15	0	15	30	45
1	32	34	38	38	23
2	26	26	27	24	42
3	27	33	29	33	36
4	22	27	21	14	13
5	64	-	57	51	33
$\bar{X}$	34	30	34	32	29
S.E.	7.6	2.0	6.3	6.3	5.1

THE EFFECT OF A CONSTANT INFUSION OF 2 µg/min OF SOMATOSTATIN ON INSULIN-HYPOGLYCAEMIA INDUCED hPP RELEASE IN pmol/l.

Subjects					2 µg/min					
	-30	0*	10	20	30	45	60	70	80	90
1	24	18	21	21	17	20	25	19	20	21
2	40	30	23	17	20	29	38	13	8	17
3	48	71	80	64	61	71	85	57	59	52
4	18	15	19	13	13	14	21	10	16	25
5	30	22	28	-	25	30	36	23	23	27
$\bar{X}$	32	31	34.2	29	27	33	41	24	25	28
S.E.	5.4	10.2	11.5	11.9	8.7	10.0	11.4	8.5	8.8	6.1

* 0.1 U/kg of monocomponent porcine insulin was injected intravenously at time '0'.

THE EFFECT OF SOMATOSTATIN ON SERUM hPP CONCENTRATIONS IN pmol/l.

Subjects			2 µg/min					
	-15	0*	5	10	15	20	30	45
1	3	52	50	38	33	22	30	27
2	45	39	57	54	45	23	36	36
3	54	50	46	57	52	55	50	37
4	48	45	36	32	31	24	17	10
5	29	38	45	45	33	20	26	13
6	15	19	18	15	13	11	7	7
$\overline{X}$	39	40	42	40	34	26	28	22
S.E.	5.9	4.9	5.6	6.3	5.5	6.2	6.1	5.4

* A 200 µg bolus dose of somatostatin was injected at time '0' and followed by a constant infusion of somatostatin at a rate of 2 µg/min.

THE EFFECT OF 400 mg OF ORAL CIMETIDINE ON SERUM hPP CONCENTRATIONS in pmol/l.

Subjects	Minutes											
	-15	0*	5	15	30**	35	40	45	60	90	120	150
1	24	24	21	10	42	51	43	34	30	40	33	10
2	18	14	26	7	10	7	7	19	30	20	7	19
3	14	14	7	7	7	7	7	7	7	17	21	21
4	33	34	24	28	43	38	64	52	62	55	62	69
5	52	60	61	44	28	33	33	23	31	27	30	25
6	83	69	77	98	69	90	88	83	71	64	83	64
$\bar{X}$	37	36	36	32	33	38	40	36	39	37	39	35
S.E.	10.6	9.6	10.9	14.4	9.5	12.7	13.1	11.2	9.7	7.9	11.4	10.3

* 150 ml of water was ingested at time '0'

** 400 mg of cimetidine in 150 ml of water was ingested at time '30'.

THE EFFECT OF A STANDARDISED MEAL ON SERUM hPP CONCENTRATIONS IN pmol/l IN PATIENTS WITH CHRONIC PANCREATITIS.

Subjects	Minutes										
	-30	-15	1.M*	5	15	30	45	60	90	120	180
1	7	7	26	90	83	67	62	62	95	69	60
2	15	19	7	7	21	7	14	7	7	7	7
3	13	33	333	329	286	183	381	267	161	167	155
4	7	7	76	90	15	7	7	7	12	15	27
5	34	39	179	595	595	155	160	110	169	167	129
6	7	7	16	27	34	19	26	29	40	55	29
7	7	10	12	24	11	10	15	7	19	7	7
$\bar{X}$	13	17	93	166	149	64	95	70	72	70	59
S.E.	3.7	5.1	46.2	83.0	82.9	28.4	51.8	35.9	26.5	26.7	22.6

* 1.M denotes the intra-meal hPP concentration five minutes after starting to eat at time '0' and the times 5-180 minutes, the period after completing the meal.

THE EFFECT OF ARGININE INFUSED AT A RATE OF 10 mg/kg/min ON SERUM hPP CONCENTRATIONS IN pmol/l IN PATIENTS WITH CHRONIC PANCREATITIS.

Subjects	Minutes							
	Arginine 10 mg/kg/min							
	-15	0	10	20	30	45	60	90
1	27	29	25	24	29	28	36	28
2	31	13	21	20	38	38	37	22
3	15	18	12	8	16	13	11	13
4	40	52	54	40	21	33	50	56
5	27	20	26	23	30	27	19	30
$\bar{X}$	28	26	28	23	27	28	30	30
S.E.	4.0	6.9	7.0	5.1	3.8	4.2	6.9	7.2

THE EFFECT OF ORAL 'INTRALIPID' (1 ml/kg) ON SERUM hPP CONCENTRATIONS IN pmol/l IN CHRONIC PANCREATITIS PATIENTS WITH AND WITHOUT STEATORRHOEA

WITHOUT STEATORRHOEA

Subjects	Minutes									
	-15	0*	5	10	20	30	45	60	90	120
1	8	12	14	7	10	15	21	40	34	28
2	42	52	42	45	44	51	69	76	62	42
3	49	60	43	43	79	64	50	44	44	39
4	21	38	17	15	10	19	27	38	27	27
5	7	7	8	23	48	29	35	17	8	7
6	17	15	7	24	9	13	12	9	18	11
$\bar{X}$	24	30	21	26	33	32	36	38	32	26
S.E.	7.2	9.2	6.7	6.2	11.7	8.6	8.5	9.6	7.8	5.8

WITH STEATORRHOEA

Subjects	Minutes									
	-15	0*	5	10	20	30	45	60	90	120
1	7	8	7	7	17	19	10	8	7	7
2	20	15	14	7	8	20	33	43	51	16
3	29	30	12	22	21	31	37	10	15	20
4	23	15	8	7	8	10	13	10	11	17
5	40	40	42	42	49	45	27	44	30	43
6	10	6	7	7	6	10	7	7	8	7
7	30	31	19	23	29	32	54	42	32	55
$\bar{X}$	23	21	15.6	16.4	20	24	24	23	22	24
S.E.	4.4	4.9	4.7	5.1	5.8	4.8	6.2	6.9	6.1	6.9

* The 'Intralipid' was administered after the final basal sample at time '0'.

THE EFFECT OF INTRAVENOUS 'INTRALIPID' (1 ml/kg) ON SERUM hPP CONCENTRATIONS IN pmol/l IN CHRONIC PANCREATITIS PATIENTS WITH AND WITHOUT STEATORRHOEA.

WITHOUT STEATORRHOEA

Subjects	Minutes									
	-15	0*	5	10	20	30	45	60	90	120
1	16	14	7	8	12	14	20	14	21	16
2	38	45	37	45	51	37	39	27	42	51
3	11	12	18	37	37	38	44	38	32	55
4	37	27	33	27	23	27	19	65	33	20
5	36	38	39	73	62	64	53	56	67	73
6	64	64	48	76	86	88	106	92	81	76
$\bar{X}$	34	33	30	44	45	45	47	49	46	49
S.E.	7.7	8.0	6.2	10.8	11.0	11.0	13.0	11.5	9.4	10.4

WITH STEATORRHOEA

Subjects	Minutes									
	-15	0*	5	10	20	30	45	60	90	120
1	11	7	7	8	9	8	7	10	7	7
2	7	7	7	7	7	7	7	7	7	7
3	21	29	22	27	17	34	18	21	30	38
4	19	27	10	7	7	9	21	9	15	12
5	30	19	23	23	19	19	21	27	36	12
6	40	40	53	64	96	111	71	60	120	119
7	15	14	19	12	18	11	49	30	21	12
$\bar{X}$	20	20	20	21	25	28	28	23	34	30
S.E.	4.3	4.6	6.1	7.7	12.0	14.2	9.0	7.0	14.9	15.4

* The 'Intralipid' was administered as a bolus dose after the final basal sample at time '0'.

THE EFFECT OF HEPARIN ADMINISTERED AS 3 INTRAVENOUS DOSES OF 3 000 U EACH ON SERUM hPP CONCENTRATIONS IN pmol/l IN PATIENTS WITH CHRONIC PANCREATITIS

Subjects	Minutes									
	-30	-15	0*	10 *	20	30	40	50	60	90
1	41	46	49	50	23	26	38	44	52	30
2	22	29	20	20	19	28	35	18	20	19
3	25	20	22	16	23	19	16	21	20	19
4	24	23	25	40	11	19	17	35	22	33
5	26	24	20	20	43	40	35	33	19	12
6	52	43	30	48	42	52	38	50	61	42
7	18	18	20	20	18	15	33	26	30	35
8	29	27	23	21	23	25	15	22	30	26
$\bar{X}$	30	29	26	29	25	28	28	31	32	27
S.E.	4.0	3.7	3.5	5.0	4.0	4.4	3.7	4.1	5.7	3.5

* The Heparin injections were given at time '0', 15 and 30 minutes.

THE EFFECT OF 2 CHRU/kg OF 'BOOTS' SECRETIN ADMINISTERED INTRAVENOUSLY ON SERUM hPP CONCENTRATIONS IN pmol/l IN PATIENTS WITH CHRONIC PANCREATITIS.

Subjects	Minutes								
	-15	0*	1	3	5	15	30	45	60
1	64	64	181	905	381	219	238	133	162
2	20	14	114	48	41	31	17	22	18
3	36	35	90	76	77	51	52	77	81
4	33	49	49	57	48	29	19	67	53
5	17	20	33	33	62	57	25	33	31
6	14	14	14	14	51	40	20	52	33
$\bar{X}$	31	32	80	188	110	71	61	64	63
S.E.	7.6	8.3	25.1	143.4	54.4	29.9	35.6	16.1	21.7

* The secretin was administered after the final basal sample at time '0'.

THE EFFECT OF INSULIN-HYPOGLYCAEMIA ON SERUM hPP CONCENTRATIONS IN pmol/l IN PATIENTS WITH CHRONIC PANCREATITIS.

Subjects	Minutes									
	-30	0*	10	20	30	45	60	70	80	90
1	36	28	43	31	105	143	93	77	77	57
2	37	23	31	28	25	21	25	23	21	22
3	11	13	7	11	40	36	83	73	62	37
4	50	56	56	62	49	36	40	50	86	52
5	20	30	27	19	16	26	23	39	25	30
$\bar{X}$	31	30	33	30	47	52	53	52	54	40
S.E.	6.8	7.1	8.2	8.7	15.6	22.8	14.7	10.2	13.3	6.6

* 0.1 U/kg of monocomponent porcine insuline was injected intravenously after the final basal sample at time '0'.

SERUM hPP RESPONSES IN pmol/l TO INSULIN-INDUCED HYPOGLYCAEMIA IN MATURITY ONSET DIABETICS AND IN AGE MATCHED CONTROLS.

Diabetics	Minutes				
	0*	20	40	60	90
1	27	31	129	250	219
2	21	12	93	619	357
3	85	57	1167	1119	679
4	20	40	21	274	68
5	9	10	10	286	129
6	16	58	536	690	381
$\bar{X}$	30	35	326	539	305
S.E.	11,3	8,6	185,9	139,4	90,0

Controls	Minutes				
	0*	20	40	60	90
1	57	62	488	714	1095
2	55	119	619	381	333
3	7	7	643	262	233
4	26	48	643	560	321
5	20	14	595	240	82
$\bar{X}$	33	50	597	431	413
S.E.	9,9	20,1	28,8	90,6	176,3

*The diabetics received 0,2 U/kg and the control subjects 0,1 U/kg of intravenous monocomponent insulin after the basal sample.

REFERENCES

REFERENCES

A

Adrian, T.E., H.S. Besterman, C.N. Mallinson, C. Garolotis, S.R. Bloom. Impaired pancreatic polypeptide release in chronic pancreatitis with steatorrhoea. Gut 20: 78-101, 1979.

Adrian, T.E., H.S. Besterman, C.N. Mallinson, G.R. Greenberg, S.R. Bloom. Inhibition of secretin stimulated pancreatic secretion by pancreatic polypeptide. Gut 20: 37-40, 1978.

Adrian, T.E., S.R. Bloom, H.S., Besterman, A.J., Barnes, T.J.C. Cooke, R.C.G. Russell, R.G. Faber. Mechanism of pancreatic polypeptide release in man. Lancet i: 161-163, 1973.

Adrian, T.E., S.R. Bloom, M.G. Bryant, J.M. Polak, Ph. Heitz, A.J. Barnes. Distribution and release of human pancreatic polypeptide. Gut 17: 940-944, 1976.

Adrian, T.E., S.R. Bloom, H.S. Besterman, M.G. Bryant. PP - physiology and pathology. In: Gut Hormones. P254-260. Ed. S.R. Bloom. Churchill Livingstone, 1978.

Adrian, T.E., S.R. Bloom, K. Hermansen, J. Iversen. Pancreatic polypeptide, glucagon and insulin secretion from the isolated perfused canine pancreas. Diabetologia 14: 413-417, 1978.

Adrian, T.E., G.R. Greenberg, A.J. Barnes, N.D. Christofides, K.G. Alberti, S.R. Bloom. Effects of pancreatic polypeptide on motilin and circulating metabolites in man. Eur. J. Clin. Invest. 10: 235-240, 1980.

Adrian, T.E., G.R. Greenberg, H.S. Besterman, S.R. Bloom. Pharmacokintics of pancreatic polypeptide in man. Gut 19: 907-909, 1978.

Adrian, T.E., G.R. Greenberg, H.S. Besterman, R.F. McCloy, V.S. Chadwick, A.J. Barnes, C.N. Mallinson, J.H. Baron, K.G.G.M. Alberti, S.R. Bloom. PP infusion in man - summary of initial investigations. In: Gut Hormones. P.265-267. Ed. S.R. Bloom. Churchill Livingstone, 1978.

Adrian, T.E., G.R. Greenberg, M.L. Fitzpatrick, S.R. Bloom. Lack of effect of pancreatic polypeptide in the rate of gastric emptying and gut hormone release during breakfast. Digestion 21: 214-218, 1981

Anastasi, A., V. Erspamer, R. Endean. Isolation and amino acid sequence of caerulein, the active decapeptide of the skin of Hyla caerulea. Arch. Biochem. Biophys. 125: 57-68, 1968.

Andersen, B.N., C. Hagen, H.C. Klein, F. Stadil, H. Worning. Correlation between exocrine pancreatic secretion and serum concentration of human pancreatic polypeptide in chronic pancreatitis. Scand. J. Gastroenterol. 15: 699-704, 1980.

Arimura, A., C.A. Meyers, W.L. Case, W.A. Murphy, A.V. Schally. Suppression of somatostatin levels in the hepatic portal and systemic plasma of the rat by synthetic human pancreatic polypeptide. Biochem. Biophys. Res. Commun. 89: 913-918, 1979.

Arnaud, C.D., R.S. Goldsmith, P.J. Bordier, G.W. Sizemore. Influence of immunoheterogeneity of circulating parathyroid hormone on results of radioimmunoassay of serum in man. Am. J. Med. 56: 785-793, 1974.

Assan, R. In vivo metabolism of glucagon. In: Glucagon. Molecular Physiology, Clinical and Therapeutic Implications P47-59. Eds. P.J. Lefebvre and R.H. Unger. Oxford Pergamon, 1972.

B

Baetens, D., C. Rufener, L. Orci. Bovine pancreatic polypeptide (BPP) in the pancreas and in the gastrointestinal tract of the dog. Experientia 32: 8, 1976.

Bank, S., G.O. Barbezat, A.I. Vinik, F. Halter, C.A. Helman. Cimetidine and serum gastrin levels in man. In: Cimetidine: Proceedings of the second international symposium on histamine H_2-receptor antagonists. Eds. W.L. Burland and M.A. Simkins. Amsterdam-Oxford, Excerpta Medica, 1977.

Bank, S., I.N. Marks, M.G. Moshal, G. Efron, R. Silber. Pancreatic function test, method and normal values. S. Afr. Med. J. 37: 1061-1066, 1963.

Becker, H.D., H.W. Börger, A. Schafmayer. Effect of vagotomy on gastrointestinal hormones. World. J. Surg. 3: 615-622, 1979.

Beglinger, C., W. Häcki, K. Gyr, G.A. Stalder. The release of pancreatic polypeptide by intraduodenal l-phenylalanine in the dog. Hepatogastroenterology 28: 116-117, 1981.

Beglinger, C., F. Meyer, W. Häcki, K. Gyr. The release of pancreatic polypeptide by exogenous CCK in man and dog. Digestion 22: 225-228, 1981.

Berger, D., R.C. Crowther, J.C. Floyd, Jr., S. Pek, S.S. Fajans. Effect of age on fasting plasma levels of pancreatic hormones in man. J. Clin. Endocrinol. Metab. 47: 1183-1189, 1978.

Berger, D., J.C. Floyd, Jr., R.M. Lampman, S.S. Fajans. The effect of adrenergic receptor blockade on the exercise-induced rise in pancreatic polypeptide in man. J. Clin. Endocrinol. Metab. 50: 33-39, 1980.

Berger, D., J.C. Floyd, Jr., S.B. Pek. The effect of treatment of type 2 (insulin independent) diabetes mellitus on plasma concentrations of pancreatic polypeptide and glucagon. Diabetologia 21: 120-125, 1981.

Bernard, C. Chiens rendus diabetiques. Compt. Rend. Soc. Biol. 1: 60, 1849.

Berson, S.A., R.S. Yalow. Insulin in blood and insulin antibodies. Am. J. Med. 40: 676-690, 1966.

Berson, S.A., R.S. Yalow. Immunocytochemical heterogeneity of parathyroid hormone in plasma. J. Clin. Endocrinol. Metab. 28: 1037-1047, 1968.

Berson, S.A., R.S. Yalow, G.D. Aurbach, J.T. Potts, Jr. Immunoassay of bovine and human parathyroid hormone. Proc. Natl. Acad. Sci. USA 49: 613-617, 1963.

Bertoccini, G., G. De Caro, P. Melchiorri. The effects of caerulein on insulin secretion in anaesthetised dogs. Br. J. Pharmac. 40: 78-85, 1970.

Bitar, K.N., A.M. Zfass, G.M. Makhlouf. Binding of secretin to plastic surfaces. Gastroenterology 75: 1080-1082, 1978.

Bittner, D.L., J. Manning. Automated neocuprine glucose method: critical factors and normal values. In: Automation in Analytical Chemistry, Technicon Symposia P 33-36, 1966.

Björnsson, O.G., T.E. Adrian, J. Dawson, R.F. McCloy, G.R. Greenberg, S.R. Bloom, V.S. Chadwick. Effects of gastrointestinal hormones on fasting gallbladder storage patterns in man. Eur. J. Clin. Invest. 9: 293-300, 1979.

Black, J.W., A.F. Crowther, R.G. Shanks, L.H. Smith, A.C. Dornhorst. A new adrenergic beta-receptor antagonist. Lancet i: 1080-1081, 1964.

Bloom, S.R., T.E. Adrian. Mechanisms of pancreatic-polypeptide release. Lancet i: 698, 1977.

Bloom, S.R., P.M. Daniel, D.I. Johnston, O. Ogawa, O.E. Pratt. Release of glucagon induced by stress. Quart. J. Exp. Physiol. 58: 99-108, 1973.

Bloom, S.R., A.V. Edwards, N.J.A. Vaughan. The role of the sympathetic innervation in the control of plasma glucagon concentration in the calf. J. Physiol. 233: 457-466, 1973.

Bloom, S.R., J.M. Polak. The role of VIP in pancreatic cholera. In: Gastrointestinal hormones. P 635-642. Ed. J.C. Thompson, Austin, University of Texas Press, 1975.

Bloom, S.R., J M. Polak. VIP measurements in distinguishing Verner-Morrison syndrome and pseudo Verner-Morrison syndrome. Clinical Endocrinol. 5: 223s-228s, 1976.

Boden, G., V.P. Diniso, O.E. Owen. Immunological potency and stability of native and synthetic secretins. Gastroenterology 67: 1119-1125, 1974.

Boden, G., R.W. Master, O.E. Owen, M.R. Rudnick. Human pancreatic polypeptide in chronic renal failure and cirrhosis of the liver: role of kidneys and liver in pancreatic polypeptide metabolism. J. Clin. Endocrinol. Metab. 51: 573-578, 1980.

Booth, R.A.D., D.D. Reeder, U.B. Helmquist, E.N. Brandt, J.C. Thompson. Renal inactivation of endogenous gastrin in dogs. Arch. Surg. 106: 851-854, 1973.

Botha, J.L., A.I. Vinik, J.C. Brown. Gastric inhibitory polypeptide (GIP) in chronic pancreatitis. J. Clin. Endocrinol. Metab. 42: 791-797, 1976.

Botha, J.L., A.I. Vinik, P.T. Child, W.P.U. Jackson. Inhibition of exaggerated gastrointestinal glucagon responses in chronic pancreatitis by somatostatin. J. Clin. Endocrinol. Metab. 45: 1265-1270, 1977.

Botha, J.L., A.I. Vinik, P.T. Child, M. Paul, W.P.U. Jackson. Pancreatic glucagon-like immunoreactivity in a pancreatectomized patient. Horm. Metab. Res. 9: 199-205, 1977.

Botha, J.L., A.I. Vinik, D. Le Roith, P.T. Child, W.P.U. Jackson. The effects of somatostatin on hormonal and metabolic responses in chronic pancreatitis. S. Afr. Med. J. 51: 872-875, 1977.

Boyd, A.E. III, H.E. Lebovitz, J.B. Pfeiffer. Stimulation of growth hormone secretion by L-Dopa. New Eng. J. Med. 283: 1425-1429, 1970.

Bridgewater, A.B., Y. Kurozanegi, Th. Chiles, H. Necheles. Secretin inactivating enzymes in liver. Proc. Soc. Exp. Biol. Med. 110: 852-855, 1962.

Brimblecombe, R.W., W.A.M. Duncan, G.J. Durrant, J.C. Emmett, C.R. Ganellin, M.E. Parsons. Cimetidine - a non-thiourea H_2-receptor antagonist. J. Int. Med. Res. 3: 86-92, 1975.

Brown, J.C., J.R. Dryburgh, S.A. Ross, J. Dupré. Identification and actions of gastric inhibitory polypeptide. Rec. Prog. Horm. Res. 31: 487-532, 1975.

Buse, M.G., A.H. Johnson, D. Kuperminc, J. Buse. The effect of alpha-adrenergic blockade on insulin secretin in man. Metabolism 19: 219-225, 1970.

C

Cain, D.F., R.E. Pitney. A method for the determination of the relative specific radioactivity of (^{3}H) proteins in acrylamide gel. Analytical Biochem. 22: 11-20, 1968.

Camilleri, M., B.T. Cooper, T.E. Adrian, S.R. Bloom, V.S. Chadwick. Effects of vasoactive intestinal peptide and pancreatic polypeptide in rabbit intestine. Gut 22: 14-18, 1981.

Campbell, I.W., D.J. Ewing, B.F. Clarke, L.J.P. Duncan. Testicular pain sensation in diabetic autonomic neuropathy. British Med. J. 2: 638-639, 1974.

Capella, C., E. Solcia, B. Frigerio, R. Buffa, L. Usellini, P. Fontana. The endocrine cells of the pancreas and related tumours. Ultrastructural study and classification. Virchows Arch. A. Path. Anat. and Histol. 373: 327-352, 1977.

Cataland, S., S.E. Crockett, J.C. Brown, E.L. Mazzaferri. Gastric inhibitory polypeptide (GIP) stimulation by oral glucose in man. J. Clin. Endocrinol. Metab. 39: 223-228, 1974.

Chance, R.E. Diabetes 21: Suppl. 2. 536, 1972.

Chance, R.E., M. Cieszkowski, J. Jaworek, S.J. Konturek, J. Swierczek, J. Tasler. Effect of pancreatic polypeptide and its C-terminal hexapeptide on meal and secretin induced pancreatic secretion in dogs. J. Physiol 314: 1-9, 1981.

Chance, R.E., W.E. Jones. Polypeptides from bovine, ovine, human and porcine pancreas. United States Patent Office 3,842,063, 1974.

Chance, R.E., N.E. Moon, M.G. Johnson. Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP). In: Methods of Hormone Radioimmunoassay P.657-672. Eds. B.M. Jaffe, H.R. Behrman. Academic Press, 1979.

Chiba, T., T. Taminato, S. Kadowaki, H. Abe, K. Chihara, S. Matsukura, Y. Goto, Y. Seino, J. Fujita. Effects of insulin and pancreatic polypeptide on gastric somatostatin release. Diabetes 29: 292-295, 1980.

Chisholm, D.J., E.W. Kraegen, J.D. Young, L. Lazarus. Comparison of secretin reponse to oral intraduodenal or intravenous glucose administration. Horm. Metab. Res. 3: 180-183, 1971.

Christensen, S.E., J. Nerup, A.P. Hansen, K. Lundbaek. Effects of somatostatin on basal levels of plasma growth hormone and insulin in acromegalics: dose-response studies and attempted total growth hormone suppression. J. Clin. Endocrinol. Metab. 42: 839-845, 1976.

Code, C.F. Reflections on histamine, gastric secretion and the H_2 receptor. New Eng. J. Med. 296: 1459-1462, 1977.

Coleman, D.L. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia 9: 294-298, 1973.

Comfort, M.W., E.E. Gambill, A.H. Baggenstoss. Chronic relapsing pancreatitis. Gastroenterology 6: 239-285, and 376-408, 1946.

Conard, V., J.R.M. Franckson, P.A. Bastenie, J. Kestens, L. Kovacs. Etude critique du triangle d'hyperglycemie intraveneux chez l'homme normal et determination d'un "coefficient d'assimilation glucidique". Arch. Int. Pharmacodyn Ther. 93: 132, 1953.

Crick, J., A.A. Harper, H.S. Raper. On the preparation of secretin and pancreozymin. J. Physiol 110: 367-376, 1950.

D

Deconink, J.F., P.R. Potvliege, W. Gepts. The ultrastructure of the human pancreatic islets. I. The islets of adults. Diabetologia 7: 266-282, 1971.

Della Fava, G.F., G. Tamburrano, L. de Magistris, C. Matoli, M.L. Santoro, R. Carratu, A. Torsoli. Gynaecomastia with cimetidine. Lancet i: 1319, 1977.

Deutsch, J.A., M-L. Wang. The stomach as a site for rapid nutrient reinforcement sensors. Science 195: 89-90, 1977.

Dockray, G.J., I.L. Taylor. Heptadecapeptide gastrin: measurement in blood by specific radio-immunoassay. Gastroenterology 71: 971-977, 1976.

Drew, S.I., B. Joffe, A. Vinik, H. Seftel, F. Singer. The first 24 hours of acute pancreatitis. Changes in biochemical and endocrine homeostasis in patients with pancreatitis compared with those in control subjects undergoing stress for reasons other than pancreatitis. Am. J. Med. 64: 795-803, 1978.

Drew S.I., B.I. Joffe, A.I. Vinik, H. Seftel, F. Singer. First 24 hours of acute pancreatitis. A biochemical and endocrine evaluation of initial versus repeated attacks. Am. J. Gastroenterology 70: 66-72, 1978a.

E

Eddy, R.L., A.L. Jones, Z.H. Chakmakjian, M.C. Silverthorne. Effect of levodopa (L-Dopa) on human hypophyseal trophic hormone release. J. Clin. Endocrinol. Metab. 33: 709-712, 1971.

Ericson, L.E., R. Ekholm, I. Lundqvist. Metabolism in L-dopa and 5-HTP in the pancreatic islets of the mouse studied with electron microscopic autoradiography. Acta Endocrinol. 67: Suppl. 155, 201, 1971.

Erlandsen, S.L., O.D. Hegre, J.A. Parsons, R.C. McEvoy, R.P. Elde. Pancreatic islet cell hormones. Distribution of cell types in the islet and evidence for the presence of somatostatin and gastrin within the D-cell. J. Histochem. Cytochem. 24: 883-897, 1976.

Erspamer, V., P. Melchiorri. Active polypeptides of the amphibian skin and their synthetic analogues. Pure and Applied Chemistry 35: 463-494, 1973.

F

Fahrenkrug, J., O.B. Schaffalitzky de Muckadell. Plasma secretin concentrations in man. Effect of intraduodenal glucose, fat, amino-acids, ethanol, HCl, or ingestion of a meal. Eur. J. Clin. Invest. 7: 201-203, 1977.

Fajans, S.S., J.C. Floyd, Jr., R.F. Knopf, J.W. Conn. Effects of amino acids and proteins on insulin secretion in man. Rec. Prog. Horm. Res. 23: 617-656, 1967.

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

Feldman, M., J.H. Walsh, I.L. Taylor. The effect of naloxone and morphine on gastric acid secretion and on serum gastrin and pancreatic polypeptide concentrations in humans. Gastroenterology 79: 294-298, 1980.

Ferreira, S.H., J.R. Vane. Half-lives of peptides and amines in the circulation. Nature 215: 1237-1240, 1967.

Feurle, G.E., A. Wirth, C. Diehm, M. Lorenzen, G. Schlierf. Exercise-induced release of pancreatic polypeptide and its inhibition by propranolol: evidence for adrenergic stimulation. Eur. J. Clin. Invest. 10: 249-251, 1980.

Feyrter, F. Über diffuse endokrine epitheliale Organe. Zentralbl. innere Med. 545: 31-34, 1938.

Fisher, U., H. Hommel, E. Schmid. Dynamics of canine pancreatic blood flow and of insulin secretion during an intravenous glucose load. Pflugers Arch. 358: 89-100, 1975.

Flaten, O., J. Myron. Adrenergic modulation of the release of pancreatic polypeptide after intraduodenal and oral glucose in man. Scand. J. Gastroenterol. 16: 781-787, 1981.

Floyd, J.C. Jr., R.E. Chance, M. Hayashi, N.E. Moon, S.S. Fajans. Concentrations of a newly recognized pancreatic islet polypeptide in plasma of healthy subjects and in plasma and tumors of patients with insulin-secreting islet cell tumors. Clin. Res. 23: 535a, 1975.

Floyd, J.C. Jr., S.S. Fajans. A newly recognised pancreatic islet polypeptide: concentrations in healthy subjects and in patients with diebetes mellitus. Diabetes 25 Suppl. 1: 330, 1976.

Floyd, J.C. Jr., S.S. Fajans, S. Pek. Regulation in healthy subjects of the secretion of a newly recognized pancreatic islet polypeptide. Clin. Res. 24: 485A, 1976.

Floyd, J.C. Jr., S.S. Fajans, S. Pek, R.E. Chance. A newly recognized pancreatic polypeptide: plasma levels in health and disease. Rec. Prog. Horm. Res. 33: 519-570, 1977.

Floyd, J.C. Jr., S. Pek, R.F. Knopf, R. Crowther, S.S. Fajans. Effects of adrenergic regulation on plasma levels of pancreatic polypeptide in man. Clin Res. 25: 621A, 1977.

Foá, P.P., L. Santamaria, S. Berger, J.A. Smith, H.R. Weinstein. Effects of the hypoglycaemic-glycogenolytic factor (HGF), epinephrine and insulin in normal and depancreatised dogs. Proc. Soc. Exp. Biol. Med. 80: 635-639, 1952.

Forssmann, W.G., V. Helmstaedter, R.E. Chance. Ultrastructural and immunohistochemical demonstrations of pancreatic polypeptide-containing F-cells in the stomach and pancreas of Tupaia belangeri. Cell Tis. Res. 177: 481-492, 1977.

Forssmann, W.G., V. Helmstaedter, J. Metz, J. Greenberg, R.E. Chance. The identification of F-cell in the dog pancreas as the pancreatic polypeptide producing cell. Histochemistry 50: 281-290, 1977.

Friesen, S.R., J.R. Kimmel, T. Tomita. Pancreatic polypeptide as screening marker for pancreatic polypeptide apudomas in multiple endocrinopathies. Am. J. Surg. 139: 61-72, 1980.

Frigerio, B., M. Ravazola, S. Ito, R Buffo, C. Capella, E. Solcia, L. Orci. Histochemical and ultrastructural identification of neurotensin cells in the dog ileum. Histochemistry 54: 123-131, 1977.

Frohman, L.A., L.L. Bernardis. Effect of hypothalmic stimulation on plasma glucose, insulin and glucagon levels. Am. J. Physiol. 221: 1596-1603, 1971.

Fuxe, K. Cellular localization of monoamines in the median eminence and in the infundibular stem of some mammals. Acta Physiol. Scand. 58: 383-384, 1963.

G

Gates, R.J., N.R. Lazarus. The ability of pancreatic polypeptides (APP and BPP) to return to normal the hyperglycaemia, hyperinsulinaemia and weight gain of New Zealand obese mice. Hormone Res. 8: 189-202, 1977.

George, D.T., E.J. Rayfield. L-dopa induced plasma glucagon release. J. Clin. Endocrinol. Metab. 39: 618-621, 1974.

Gepts, W., J. De Mey. M. Marichal-Pipeleers. Hyperplasia of "pancreatic polypeptide" - cells in the pancreas of juvenile diabetics. Diabetologia 13: 27-34, 1977.

Gerich, J.E., J.H. Karam, P.H. Forsham. Reciprocal adrenergic control of pancreatic alpha- and beta-cell function in man. Diabetes 21: 332-333, 1972.

Gingerich, R.L. Suvey of potential pancreatic polypeptide secretogogues. Diabetes 26: Suppl 1, 375, 1977.

Gingerich, R., M. Greider, R. Chance, M. Johnson. Secretion of canine pancreatic polypeptide (CPP) in vitro. Diabetes 25: suppl. 1, 329, 1976.

Gingerich, R.L., R.C. Hickson, J.M. Hagberg, W.W. Winder. Effect of endurance exercise training on plasma pancreatic polypeptide concentration during exercise. Metabolism 28: 1179-1182, 1979.

Gingerich, R.L., P.E. Lacy, R.E. Chance, M.G. Johnson. Regional pancreatic concentration and in-vitro secretion of canine pancreatic polypeptide, insulin and glucagon. Diabetes 27: 96-101, 1978.

Giustina, G., M. Peracchi, E. Reschini, A.E. Pancrai, M. Pinto. Dose-response study of the inhibiting effect of somatostatin on growth hormone and insulin secretin in normal and acromegalic patients. Metabolism 24: 807-815, 1975.

Glaser, B., A.I, Vinik, A.A. Sive, J.C. Floyd, Jr. Plasma human pancreatic polypeptide responses to administered secretin: Effects of surgical vagotomy, cholinergic blockade, and chronic pancreatitis. J. Clin. Endocrinol. Metab. 50: 1094-1099, 1980.

Goldfine, I.D., C. Abraira, D. Grunewald, M.S. Goldstein. Plasma insulin levels during imaginary food ingestion under hypnosis. Proc. Soc. Exp. Biol. Med. 133: 274-276, 1970.

Grace, S.C., W.D. Davidson, D. State. Renal mechanisms for removal of gastrin from the circulation. Surg. Forum. 25: 323-325. 1974.

Greenberg, G.R., R.F. McCloy, V.S. Chadwick. T.E. Adrian, J.H. Baron, S.R. Bloom. Effect of bovine pancreatic polypeptide on basal pancreatic and biliary outputs in man. Dig. Dis. Sci. 24: 11-14, 1979.

Greenberg, G.R., P.Mitznegg, S.R. Bloom. Effect of pancreatic polypeptide on DNA-synthesis in the pancreas. Experientia 33: 1332-1333, 1977.

Grossman, M.I. and others. Candidate hormones on the gut. Gastroenterology 67: 730-755, 1974.

Guzman, S., J. Lonovics, J.A. Chayvialle, D.J. Hejtmancik, P.L. Rayford, J.C. Thompson. Gastrin-mediated release of somatostatin and pancreatic polypeptide. Surg. Forum 30: 374-376, 1979.

Guzman, S., J. Lonovics, J.A. Chayvialle, K.E. Hejtmanicik, P.L. Rayford, J.C. Thompson. Effects of gastrin on circulating levels of somatostatin, pancreatic polypeptide, and vasoactive intestinal peptide in dogs. Endocrinology 107: 231-236, 1980.

Guzman, S., J. Lonovics, P.G. Devitt, K.E. Hejtmancik, P.L. Rayford, J.C. Thompson. Hormone-stimulated release of pancreatic polypeptide before and after vagotomy in dogs. Am. J. Physiol. 240: G114-121, 1981.

H

Hällgren, R., G. Lundqvist, R.E. Chance. Serum levels of human pancreatic polypeptide in renal disease. Scand. J. Gastroenterol. 12: 923-927, 1977.

Harvey, R.F., L. Dowsett, M. Hartog, A.E. Read. A radioimmunoassay for cholecystokinin-pancreozymin. Lancet ii: 826-828, 1973.

Hazelwood, R.L., S.D. Turner, J.R. Kimmel, H.G. Pollack. Spectrum effects of a new polypeptide (third hormone?) isolated from chicken pancreas. General and Comparative Endocrinol. 21: 485-497, 1973.

Hedo, J.A., M.L. Villanueva, J. Marco. Influence of plasma free fatty acids on pancreatic polypeptide secretion in man. J. Clin. Endocrinol. Metab. 49: 73-77, 1979.

Heitz, Ph.U., G. Klöppel, W.H. Häcki, J.M. Polak, A.G.E. Pearse. Nesidioblastosis: the pathologic basis of persistent hyperinsulinemic hypoglycaemia in infants. Diabetes 26: 632-642, 1977.

Heitz, Ph., J.M. Polak, S.R. Bloom, T.E. Adrian, A.G.E. Pearse. Cellular origin of human pancreatic polypeptide (HPP) in endocrine tumors of the pancreas. Virchows Arc. B. Cell Path. 21: 259-265, 1976a.

Heitz, Ph., J.M. Polak, S.R. Bloom, A.G.E. Pearse. Identification of the D1-cell as the source of human pancreatic polypeptide (HPP). Gut 17: 755-758, 1976.

Hermansen, K., T.W. Schwartz. The influence of calcium on the basal and acetylcholine-stimulated secretion of pancreatic polypeptide. Endocrinology 105: 1469-1474, 1979.

Hermansen, K., T.W. Schwartz. Differential sensitivity to somatostatin of pancreatic polypeptide, glucagon and insulin secretion from the isolated perfused canine pancreas. Metabolism 28: 1229-1233, 1979.

Hertelendy, F., L. Machlin, D.M. Kipnis. Further studies on the regulation of insulin and growth hormone secretion in the sheep. Endocrinology 84: 192-199, 1969.

Hickman, R., S.J. Saunders, J. Terblanche. Liver function in the pig: total hepatic and portal flow values in vivo. S. Afr. Med. J. 48: 1197-1200, 1974.

Hoffman, W.S. A rapid photo-electric method for the determination of glucose in blood and urine. J. Biol. Chem. 120: 51-55, 1937.

Hökfelt, T., J.M. Lundberg, L. Terenius, G. Jancso, J. Kimmel. Avian pancreatic polypeptide (APP) immunoreactive neurons in the spinal cord and spinal trigeminal nucleus. Peptides 2: 81-87, 1981.

Hollander, F. Insulin test for presence of intact nerve fibres after vagal operations for peptic ulcer. Gastroenterolgy 7: 607-614, 1946.

Hornnes, P.J., C. Kuhl. Decreased plasma pancreatic polypeptide levels in pregnancy. J. Clin. Endocrinol. Metab. 52: 605-607, 1981

Hunter, W.M., F.C. Greenwood. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature 194: 495-496, 1962.

I

Iversen, L.L. Dopamine receptors in the brain. Science 188: 1084-1089. 1975.

Iversen, J., S.R. Bloom, T.E. Adrian, K. Hermansen. Control of glucagon, insulin and PP secretion in the isolated pancreas. Acta Endocrinol. Suppl. Vol. 85: 8, 1977.

Itaya, K., M. Ui. Colorimetric determination of free fatty acids in biological fluids. J. Lipid Res. 6: 16-20, 1965.

J

Jackson, W.P.U., A.I. Vinik. Does hyperglycaemia mean diabetes? Med. Proc. 16: 362-365, 1970.

Jaffe, B.M., D.F. Kopen, K. De Schryver-Kecskemeti, R.L. Gingerich, M. Greider. Indomethicin-sensitive pancreatic cholera. N. Engl. J. Med. 297: 817-821, 1977.

Järhult, J., L.O. Farnebo, B. Hamberger, J. Holst, T.W. Schwartz. The relation between catecholamines, glucagon and pancreatic polypeptide during hypoglycaemia in man. Acta Endocrinol. 98: 402-406, 1981.

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

Johnson, L.R. Gastrointestinal hormones and their functions. Ann. Rev. Physiol. 39: 135-158, 1977.

Johnson, R.H., J.M.K. Spalding. Disorders of the autonomic nervous system. Blackwell 54: 252, 1974.

K

Kalk, W.J., A.I. Vinik, S. Bank, K.D. Buchanan, P. Keller, W.P.U. Jackson. Glucagon responses to arginine in chronic pancreatitis: possible pathogenetic significance in diabetes. Diabetes 23: 257-263, 1974.

Kalk, W.J., A.I. Vinik, S. Bank, J.R. Hayes, J Ardill, K.D. Buchanan, P. Keller, W.P.U. Jackson. Plasma gastrin responses to arginine in chronic pancreatitis. Diabetes 23: 264-267, 1974a.

Kalk, W.J., A.I. Vinik, M. Paul, P. Keller, W.P.U. Jackson. Immunoreactive glucagon responses to intravenous tolbutamide in chronic pancreatitis. Diabetes 24: 851-855, 1975.

Kaneto, A., T. Kaneko, H. Kajinuma, K. Kosaka. Effect of vasoactive intestinal polypeptide infused intrapancreatically on glucagon and insulin secretion. Metabolism 26: 781-786, 1977.

Kansal, P.C., M.G. Buse. Effect of adrenergic blocking agents on plasma insulin and blood glucose during urethan and epinephrine induced hyperglycaemia. Metabolism 16: 548-556, 1967.

Karam, J.H., S.G. Grasso, L.C. Wegienka, G.M. Grodsky, P.H. Forsham. Effect of selected hexoses, of epinephrine and of glucagon on insulin secretion in man. Diabetes 15: 571-578, 1966.

Katz, A.I., A.H. Rubenstein. Metabolism of proinsulin, insulin and C-peptide in the rat. J. Clin. Invest. 52: 1113-1121, 1973.

Kayasseh, L., W.H. Häcki, K. Gyr, G.A. Stalder, W.W. Ritter, F. Halter, J. Girard. The endogenous release of pancreatic polypeptide by acid and meal in dogs. Effect of somatostatin. Scand. J. Gastroenterol. 13: 385-391, 1978.

Kimmel, J.R., L.J. Hayden, H.G. Pollock. Isolation and characterization of a new pancreatic polypeptide hormone. J. Biol. Chem. 250: 9369-9376, 1975.

Kimmel, J.R., H.G. Pollock, R.L. Hazelwood. Isolation and characterization of chicken insulin. Endocrinology 83: 1323-1330, 1968.

Kimmel, J.R., H.G. Pollock, R.L. Hazelwood. A new pancreatic polypeptide hormone. Fed. Proc. 30: 1318 Abs., 1971.

Klaff, L.J., A.I. Vinik, M. Berelowitz, W.P.U. Jackson. Circulating antibodies in diabetics treated with conventional and purified insulins. S. Afr. Med. J. 54: 149-153, 1978.

Knopf, R.F., J.W. Conn, S.S. Fajans, J.C. Floyd, E.M. Guutsche, J.A. Rall. Plasma growth hormone response to intravenous administration of amino acids. J. Clin. Endocrinol. Metab. 25: 1140-1144, 1965.

Kobayashi, S., T. Fujita. Fine structure of mammalian and avian pancreatic islets with special reference to D cells and nervous elements. Z. Zellforsch. 100: 340-363, 1969.

Kolts, B.E., J.E. McGuigan. Radioimmunoassay measurements of secretin half-life in man. Gastroenterology 72: 55-60, 1977.

Korn, E.D. Clearing factor, a heparin-activated lipoprotein lipase. II Substrate specificity and activation of coconut oil. J. Biol. Chem. 215: 15-26, 1955.

Kuzio, M., J.R. Dryburgh, K.M. Malloy, J.C. Brown. Radioimmunoassay for gastric inhibitory polypeptide. Gastroenterology 66: 357-364, 1974.

L

Langslow, D.R., J.R. Kimmel, H.G. Pollock. Studies of the distribution of a new avian pancreatic polypeptide and insulin among birds, reptiles, amphibians and mammals. Endocrinology 93: 558-565, 1973.

Lantigua, R.A., U. Lilavivathana, R.G. Campbell, L.S. Jacobs. Adrenergic modulation of pancreatic polypeptide secretion. Metabolism 29: 787-792, 1980.

Lantigua, R.A., W.F. Streck, D.H. Lockwood, L.S. Jacobs. Glucocorticoid suppression of pancreatic and pituitary hormones: pancreatic polypeptide, growth hormone, and prolactin. J. Clin. Endocrinol. Metab. 50: 298-303, 1980.

La Rossa, J.T., R. Agrin, C.J. Melby. Apomorphine-stimulated growth hormone release. Am. J. Med. 63: 909-913, 1977.

Larsson, L-I. Human pancreatic polypeptide, vasoactive intestinal polypeptide, and watery diarrhoea syndrome. Lancet i: 149, 1976.

Larsson, L-I. Corticotropin-like peptides in central nerves and in endocrine cells of gut and pancreas. Lancet ii: 1321-1323, 1977.

Larsson, L-I, J.F. Rehfeld, F. Sundler, R. Hakanson. Pancreatic gastrin in foetal and neonatal rats. Nature 262: 609-610, 1976.

Larsson, L-I., T. Schwartz, G. Lundqvist, R.E. Chance, F. Sundler, J.F. Rehfeld, L. Grimelius, J. Fahrenkrug, O. Schaffalitzky de Muckadell, N. Moon. Occurrence of human pancreatic polypeptide in pancreatic endocrine tumours. Am. J. Pathol. 85: 675-684, 1976.

Larsson, L-I., F. Sundler, R. Hakanson. Immunohistochemical localization of human pancreatic polypeptide (HPP) to a population of islet cells. Cell Tiss. Res. 156: 167-171, 1975.

Larsson, L-I., F. Sundler, R. Hakanson. Pancreatic polypeptide - a postulated new hormone: identification of its cellular storage site by light and electron microscopic immunocytochemistry. Diabetologia 12: 211-226, 1976.

Larsson, L-I., F. Sundler, R. Hakanson, H.G. Pollock, J.R. Kimmel. Localization of APP, a postulated new hormone to a pancreatic endocrine cell type. Histochemistry 42: 377-382, 1974.

Lassmann, V., P. Vague, B, Vialettes, M.C. Simon. Low plasma levels of pancreatic polypeptide in obesity. Diabetes 29: 428-430, 1980.

Laurentz, D.A., R.L. Hazelwood. Does the third pancreatic hormone (APP) play a trophic role in the growth of the embryonic chick proventriculus? Proc. Soc. Exp. Biol. Med. 160: 144-149, 1979.

Lazarus, N.R., R.A. Gutman, L. Recant. A method for electrophoretic characterization on polyacrylamide gel of circulating insulin immunoreactive substances. Anal. Biochem. 40: 241-246, 1971.

Leblanc, H., L.A. Rigg, S.S.C. Yen. The response of pancreatic and pituitary hormones to pulses and constant infusion of somatostatin. J. Clin. Endocrinol. Metab. 41: 1105-1109, 1975.

Lebovitz, H.E., J.M. Feldman. Pancreatic biogenic amines and insulin secretion in health and disease. Federation Proc. 32: 1797-1802, 1973.

Lefebvre, P.J., A.S. Luyckx, A.H. Niezet. Renal handling of endogenous glucagon in the dog: comparison with insulin. Metabolism 23: 753-761, 1974.

Levin, A.B. A simple test of cardiac function based upon the heart rate changes induced by the valsalva manoeuver. Am. J. Cardiol. 18: 90-99, 1966.

Levitt, N.S., A.I. Vinik, A.A. Sive, L.J. Klaff. Hormonal regulation in diabetic autonomic neuropathy. A. Afr. J. Sci. 73: Suppl. p vi, 1977.

Levitt, N.S., A.I. Vinik, A.A. Sive. S. van Tonder, A. Lund. Impaired pancreatic polypeptide responses to insulin-induced hypoglycaemia in diabetic autonomic neuropathy. J. Clin. Endocrinol. Metab. 50: 445-449, 1980.

Lin, T-M., R.E. Chance. Gastrointestinal actions of a new bovine pancreatic peptide (BPP). In: Endocrinology of the gut. P 143-145. Eds. W. Chey, P. Brooks. C.B. Slack, Inc., New Jersey, 1974.

Lin, T-M., R.E. Chance. Spectrum of gastrointestinal actions of bovine PP. In: Gut Hormones. P.242-246. Ed. S.R. Bloom. Churchill Livingstone, 1978.

Lin, T-M., D.C. Evans, R.E. Chance, G.F. Spray. Bovine pancreatic peptide: action on gastric and pancreatic secretion in dogs. Am. J. Physiol. 232: E 311-315, 1977.

Lonovics, J., P. Devitt, P.L. Rayford, J.C. Thompson. Actions of VIP, somatostatin, and pancreatic polypeptide on gallbladder tension and CCK-stimulated gallbladder contraction in vitro. Surg. Forum 30: 407-409, 1979.

Lonovics, J., S. Guzman, P. Devitt, K.E. Hejtmancik, R.L. Suddith, P.L. Rayford, J.C. Thompson. Release of pancreatic polypeptide in humans by infusion of cholecystokinin. Gastroenterology 79: 817-822, 1980.

Lonovics, J., S. Guzman, P.G. Devitt, K.E. Hejtmancik, R.L. Suddith, P.L. Rayford, J.C. Thompson. Action of pancreatic polypeptide on exocrine pancreas and on release of cholecystokinin and secretin. Endocrinology 108: 1925-1930, 1981.

Loo, S.W., H.J. Hirsch, K.H. Gabbay. Human pancreatic polypeptide: portal peripheral gradient in man. Diabetes 26: Suppl. 1, 214, 1977.

Loren, I., J. Alumets, R. Hakanson, F. Sundler. Immunoreactive pancreatic polypeptide (PP) in the central and peripheral nervous system: preliminary immunocytochemical observations. Cell Tiss. Res. 200: 179-186, 1979.

Lorenzi, M., E. Tsalikian, N.V. Bohannan, J.E. Gerich, J.H. Karam, P.H. Forsham. Differential effects of L-Dopa and apomorphine on glucagon secretion in man: evidence against central dopaminergic stimulation of glucagon. J. Clin. Endocrinol. Metab. 45: 1154-1158, 1977.

Lovgren, N.A., J. Poulsen, T.W. Schwartz. Impaired pancreatic innervation after selective gastric vagotomy. Reduction of the pancreatic polypeptide response to food and insulin hypoglycaemia. Scand. J. Gastroenterol. 16: 811-816, 1981.

Luft, R., S. Efendic, T. Hökfelt. Somatostatin - both hormone and neurotransmitter. Diabetologia 14: 1-13, 1978.

Luyckx, A.S., P.J. Lefebvre. Arguments for a regulation of pancreatic glucagon secretion by circulating plasma free fatty acids. Proc. Soc. Exp. Biol. Med. 113: 524-528, 1970.

M

Madison, L.L., N.M. Kaplan. The hepatic binding of ^{131}I labelled insulin in human subjects during a single transhepatic circulation. J. Lab. Clin. Med. 52: 927, 1958.

Madison, L.L., W.A. Seyffert Jr., R.H. Unger, B. Barker. Effect of plasma free fatty acids on plasma glucagon and serum insulin concentrations. Metabolism 17: 301-304, 1968.

Mahoney, C., J.M. Feldman. Species variation in pancreatic islet monoamine uptake and action. Diabetes 26: 257-261, 1977.

Malaisse-Lagae, F., J-L. Carpentier, Y.C. Patel, W.J. Malaisse, L. Orci. Pancreatic polypeptide: a possible role in the regulation of food intake in the mouse. Experientia 33: 915-917, 1977.

Marco, J., J.A. Hedo, M.L. Villanueva. Inhibitory effect of somatostatin on human pancretic polypeptide secretion. Life Sciences 21: 789-792, 1977.

Marco, J., J.A. Hedo, J. Castillo-Olivares, M.L. Villanueva. Human pancreatic polypeptide secretion in conditions of exogenous and endogenous hyperglycaemia. Diabetologia 19: 418-422, 1980.

Marco, J., J.A. Hedo, M.L. Villanueva. Control of pancreatic polypeptide secretion by glucose in man. J. Clin. Endocrinol. Metab. 46: 140-145, 1978.

Marco, J., M.A. Zulueta, I. Correas, M.L. Villanueva. Reduced pancreatic polypeptide secretion in obese subjects. J. Clin. Endocrinol. Metab. 50: 744-747, 1980.

Materia, A., I.M. Modlin, D. Albert, A. Sank, R.F. Crochelt, B.M. Jaffe. The effect of somatostatin and 16,16-dimethyl-prostaglandin E_2 on bombesin-stimulated canine gastric acid, plasma gastrin and pancreatic polypeptide secretion. Regul. Pept. 1: 297-305, 1981.

McCullum, R.W., J.R. Sowers, J.M. Hershman, R.A.L. Sturdevant. Metoclopramide stimulates prolactin secretion in man. Clin. Res. 23: 479A, 1975.

McGuigan, J.E., B.M. Jaffe, W.T. Newton. Immunochemical measurement of endogenous gastrin release and circulation. Gastroenterology 56: 1181, 1969.

Minuto, F., M. Marugo, M. Giusti, M. Baiardi, G. Giordano. Effect of metoclopramide on prolactin secretion in man. Horm. Metab. Res. 9: 492-494, 1977.

Mitznegg, P., S.R. Bloom, W. Domschke, S. Domschke, E. Wuensch, L. Demling. Pharmacokinetics of motilin in man. Gastroenterology 72: 413-416, 1977.

Modlin, I.M., D. Albert, R. Crochelt, A. Sank, B.M. Jaffe. Evidence for an intestinal mechanism of pancreatic polypeptide release. Dig. Dis. Sci. 26: 587-590, 1981.

Modlin, I.M., B.E. Ehrlich, C.B. Lamers, J.M. Diamond, J.H. Walsh. Variable effect of lithium on canine pancreatic polypeptide and gastrin release by a meal or bombesin. Digestion 22: 177-184, 1981.

Modlin, I.M., C.B. Lamers, B.M. Jaffe. Evidence for cholinergic dependence of pancreatic polypeptide release by bombesin - a possible application. Surgery 88: 75-85, 1980.

Münger, B.L., F. Caramia, P.E. Lacy. The ultrastructural basis for the identification of cell types in the pancreatic islets. II Rabbit, dog and opposum. Z. Zellforsch. 67: 776-798, 1965.

Murphy, W.A., J.L. Fries, C.A. Meyers, D.H. Coy. Human pancreatic polypeptide inhibits insulin release in the rat. Biochem. Biophys. Res. Commun. 101: 189-193, 1981.

Mutt, V., J.E. Jorpes. Structure of porcine cholecystokinin-pancreozymin: clearance with thrombin and trypsin. Europ. J. Biochem. 6: 156-162, 1968.

N

Neil, E. Catecholamines and the cardiovascular system. In: Handbook of Physiology, Section 7, Vol. VI, P. 473-489. Section Eds. R.O. Greep, E.B. Astwood. American Physiological Society, Washington, D.C., 1975.

Nelson, R.L., F.J. Service, D.M. Ilstrup, V.L. Go. Are elevated pancreatic polypeptide levels in patients with insulinoma secondary to hypoglycaemia? Lancet ii: 659-661, 1980.

Nickerson, M., B. Collier. Drugs inhibiting adrenergic nerves and structures innervated by them. In: The Pharmacological Basis of Therapeutics, 5th edition. P. 541-543. Eds. L.S. Goodman, A. Gilman. MacMillan Publishing Co. Inc., 1975.

Ohneda, A., E. Aguilar-Parada, A.M. Eisentraut, R.H. Unger. Control of pancreatic glucagon secretion by glucose. Diabetes 18: 1-10, 1969.

Olschowka, J.A., T.L. O'Donohue, D.M. Jacobowitz. The distribution of bovine pancreatic polypeptide-like immunoreactive neurons in rat brain. Peptides 2: 309-331, 1981.

Orci, L., D. Baetens, M.P. Dubois, C. Rufener. Evidence for the D-cell of pancreas secreting somatostatin. Horm. Metab. Res. 7: 400-402, 1975.

Orci, L., R.H. Unger. Hypothesis: functional subdivisions of islets of Langerhans and possible role of D-cell. Lancet ii: 1243-1244, 1975.

P

Pappas, T.N., J.G. Yovos, E.C. Ellison, T.M. O'Dorisio, S. Cataland, W.G. Pace, L.C. Carey. Pancreatic polypeptide in acute pancreatitis and small-bowel infarcation in dogs. Dig. Dis. Sci. 26: 1013-1018, 1981.

Parks, D.L., R.L. Gingerich, B.M. Jaffe, B. Akande. Role of pancreatic polypeptide in canine gastric acid secretion. Am. J. Physiol. 236: E488-494, 1979.

Pavlov, I.P. Conditioned reflexes. New York Oxford, 1927.

Pearse, A.G.E. 5-Hydroxytryptophan uptake by dog thyroid C cells and its possible significance in polypeptide hormone production. Nature 211: 598-600, 1966.

Pearse, A.G.E. The cytochemistry and ultrastructure of polypeptide hormone producing cells of the APUD series, and the embryologic, physiologic and pathologic implications of the concept. J. Histochem. Cytochem. 17: 303-313, 1969.

Pearse, A.G.E. The diffuse neuroendocrine system and the APUD concept: related "endocrine" peptides in brain, intestine, pituitary, placenta and anuran cutaneous glands. Medical Biology 55: 115-125, 1977.

Pearse, A.G.E., J.M. Polak. Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. Gut 12: 783-788, 1971.

Pearse, A.G.E., J.M. Polak, S.R. Bloom. The newer gut hormones - cellular sources, pathology and clinical aspects. Gastroenterology 72: 746-761, 1977.

Pederson, R.A., H.E. Schubert, J.C. Brown. Gastric inhibitory polypeptide: its physiologic release and insulinotropic action in the dog. Diabetes 24: 1050-1056, 1975.

Pelletier, G. Identification of four cell types in the human endocrine pancreas by immunoelectron microscopy. Diabetes 26: 749-756, 1977.

Pelletier, G., R. Leclerc. Immunohistochemical localization of human pancreatic polypeptide (HPP) in the human endocrine pancreas. Gastroenterology 72: 569-571, 1977.

Peringer, E., P. Jenner, C.D. Marsden. Effect of metoclopramide on turnover of brain dopamine noradrenalin and 5-hydroxytryptamine. J. Pharm. Pharmac. 27: 442-444, 1975.

Peterson, W., M. Feldman, I. Taylor, M. Bremer. The effect of 15(R)-15-methyl prostaglandin E2 on meal-stimulated gastric acid secretion, serum gastrin, and pancreatic polypeptide in duodenal ulcer patients. Dig. Dis. Sci. 24: 381-384, 1979.

Pimstone, N.R. A study of the starch-iodine complex: a modified colorimetric microdetermination of amylase in biologic fluids. Clinical Chemistry 10: 891-906, 1964.

Pimstone, B.L., M. Berelowitz, S. Kronheim. Somatostatin, 1976. S. Afr. Med. J. 50: 1471-1474, 1976.

Pimstone, B.L., M. Berelowitz, S. Kronheim, B. Shapiro, D. Kranold. Presence of immunoreactive somatostatin in normal human and rat serum: physiologic response to nutrient stimuli. S. Afr. J. Sci. 73: Suppl. p iv, 1977.

Pitts, J.E., J.A. Jenkins, I.J. Tickle, T.L. Blundell, S.P. Wood. Structural studies on avian pancreatic polypeptide. Biochem. Soc. Trans. 5: 1119-1120, 1977.

Polak, J.M., S.R. Bloom, T.E. Adrian, Ph. Heitz, M.G. Bryant, A.G.E. Pearse. Pancreatic polypeptide in insulinomas, gastrinomas, vipomas, and glucagonomas. Lancet ii: 328-330, 1976.

Polak, J.M., S.R. Bloom, S. Hobbs, E. Solcia, A.G.E. Pearse. Distribution of a bombesin-like peptide in human gastrointestinal tract. Lancet i: 1109-1110, 1976.

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

Porte, D. Jr. Beta-adrenergic stimulation of insulin release in man. Diabetes 16: 150-155, 1967.

Porte, D. Jr., A.L. Graber, T. Kuzuya, R.H. Williams. The effect of epinephrine on immunoreactive insulin levels in man. J. Clin. Invest. 45: 228-236, 1966.

Porte, D. Jr., R.H. Williams. Inhibition of insulin release by norepinephrine in man. Science 152: 1248-1250, 1966.

Q

Quickel, K.E., J.M. Feldman, H.E. Lebovitz. Inhibition of insulin secretion by serotonin and dopamine: species variation. Endocrinology 89: 1295-1302, 1971.

R

Rabinovitch, A., J. Dupré. Effects of gastric inhibitory polypeptide present in impure pancreozymin-cholecystokinin on plasma insulin and glucagon in the rat. Endocrinology 94: 1139-1144, 1974.

Reeder, D.D., E.N. Brandt, L.C. Watson, U.B.E. Hjelmquist, J.C. Thompson. Pre- and posthepatic measurements of mass of endogenous gastrin. Surgery 72: 34-41, 1972.

Regan, P.T., V.L.W. Go, E.P. DiMagno. Comparison of the effects of cholecystokinin and cholecystokinin octapeptide on pancreatic secretion, gallbladder contraction and plasma pancreatic polypeptide in man. J. Lab. Clin. Med. 96: 743-748, 1980.

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

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

Reid, J.L. Dopaminergic pathways and their pathophysiological significance. Clin. Sci. Mol. Med. 53: 303-306, 1977.

Richardson, K.C. The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscle. Am. J. Anat. 114: 173-205, 1964.

Robinson, O.P.W. Metoclopramide side-effects and safety. Postgrad. Med. J. 49: Suppl. 4, 77-88, 1973.

Robinson, D.S., P.M. Harris. The production of lipolytic activity in the circulation of the hind limb in response to heparin. Quart. J. Exp. Physiol. 44: 80-90, 1959.

Rubenstein, A.H., S. Cho, D.F. Steiner. Evidence for proinsulin in human urine and serum. Lancet i: 1353-1355, 1968.

Rubenstein, A.H., L.A. Pottenger, M. Mako, G.S. Getz, D.F. Steiner. The metabolism of proinsulin and insulin by the liver. J. Clin. Invest. 51: 912-921, 1972.

Rubenstein, A.H., I. Spitz. Role of the kidney in insulin metabolism and excretion. Diabetes 17: 161-169, 1968.

S

Sacks, H., B.J. Grant, A.I. Vinik. Metabolism of synthetic human heptadecapeptide gastrin by the isolated perfused rat liver. S. Afr. Med. J. 53: 249-251, 1978.

Said, S.I. Vasoactive intestinal polypeptide (VIP). Gastroenterology 67: 735-737, 1974.

Samols, E., G. Marri, V. Marks. Interrelationship of glucagon, insulin and glucose. The insulinogenic effect of glucagon. Diabetes 15: 855-866, 1966.

Samols, E., J.A. Ryder. Studies on tissue uptake of insulin in man using a differential immunoassay for endogenous and exogenous insulin. J. Clin. Invest. 40: 2092-2102, 1961.

Schaffalitzky de Muckadell, O.B., J. Fahrenkrug, J.J. Holst, K.B. Lauritsen. Release of vasoactive intestinal polypeptide (VIP) by intraduodenal stimuli. Scand. J. Gastroent. 12: 793-799, 1977.

Schlegel, W., S. Raptis, R.F. Harvey, J.M. Oliver, E.F. Pfeiffer. Inhibition of cholecystokinin-pancreozymin release by somatostatin. Lancet ii: 166-168, 1977.

Schwartz, S.S., B. Corkey, J.R. Williamson, A.H. Rubenstein. Effect of bovine pancreatic polypeptide on isolated rat liver cells. Endocrinology 106: 1178-1181, 1980.

Schwartz, T.W., R.L. Gingerich, H.S. Tager. Biosynthesis of pancreatic polypeptide. Identification of a precursor and a co-synthesized product. J. Biol. Chem. 255: 11494-11498, 1980.

Schwartz, T.W., U. Grötzinger, I.M. Schöön, L. Olbe. Vagovagal stimulation of pancreatic-polypeptide secretion by graded distention of the gastric fundus and antrum in man. Digestion 19: 307-314, 1979.

Schwartz, T., J.J. Holst, J. Fahrenkrug, C. Kühl, S. Lindkaer Jensen, J.F. Rehfeld, O.B. Schaffalitzky de Muckadell, F. Stadil, O. Vagn-Nielsen. Vagal, cholinergic regulation of pancreatic polypeptide secretion. Acta Endocrinol. Suppl. Vol. 85: 106, 1977.

Schwartz, T.W., J.J. Holst, J. Fahrenkrug, S. Lindkaer Jensen, O.V. Nielsen, J.F. Rehfeld, O.B. Schaffalitzky de Muckadell, F. Stadil. Vagal, cholinergic regulation of pancreatic polypeptide secretion. J. Clin. Invest. 61: 781-789, 1978.

Schwartz, T.W., J.F. Rehfeld. Mechanism of pancreatic-polypeptide release. Lancet i: 697-698, 1977.

Schwartz, T.W., J.F. Rehfeld, F. Stadil, L-I. Larsson, R.E. Chance, N. Moon. Pancreatic polypeptide response to food in duodenal ulcer patients before and after vagotomy. Lancet i: 1102-1105, 1976.

Schwartz, T.W., B. Stenquist, L. Olbe. Physiology of mammalian PP and the importance of vagal regulation. In Gut Hormones. P 261-264. Ed. S.R. Bloom. Churchill Livingstone, 1978.

Schwartz, T.W., B. Stenquist, L. Olbe. Cephalic phase of pancreatic-polypeptide secretion studied by sham feeding in man. Scand. J. Gastroenterol. 14: 313-320, 1979.

Schwartz, T.W., B. Stenquist, L. Olbe, F. Stadil. Synchronous oscillations in the basal secretion of pancreatic-polypeptide and gastric acid. Depression by cholinergic blockade of pancreatic-polypeptide concentrations in plasma. Gastroenterology 76: 14-19, 1979.

Shand, D.G., E.M. Nuckolls, J.A. Oats. Plasma propranolol levels in adults with observations in 4 children. Clin. Pharmac. Ther. 11: 112-120, 1970.

Sheppard, M.C., B. Shapiro, M. Berelowitz, B.L. Pimstone. Half-life and metabolic clearance rate of exogenously administered somatostatin in man. S. Afr. J. Sci. 73: Suppl. piii, 1977.

Sherman, B.M., P. Gorden, J. Roth, P. Freychet. Circulating insulin: the proinsulin-like properties of "Big" insulin in patients without islet cell tumours. J. Clin. Invest. 50: 849-858, 1971.

Singer, M.V., W. Niebel, C. Lamers, S. Becker, J. Vesper, W. Hartmann, J. Diemel, H. Goebell. Effects of truncal vagotomy and antrectomy on bombesin-stimulated pancreatic secretion, release of gastrin, and pancreatic polypeptide in the anaesthetized dog. Dig. Dis. Sci. 26: 871-877, 1981.

Sive, A.A., A.I. Vinik, G.O. Barbezat, T.W. Schwartz. Plasma pancreatic polypeptide concentrations in acute pancreatitis. S. Afr. Med. J. 57: 1047-1049. 1980.

Sive, A.A., A.I. Vinik, N. Levitt. Adrenergic modulation of human pancreatic polypeptide (hPP) release. Gastroenterology 79: 665-672, 1981.

Sive, A.A., A.I. Vinik, S.V. van Tonder. Pancreatic polypeptide (PP) response to oral and intravenous glucose in man. Am. J. Gastroenterol. 71: 183-185, 1979.

Sive, A.A., A.I. Vinik, S. van Tonder, A. Lund. Impaired pancreatic polypeptide secretion in chronic pancreatitis. J. Clin. Endocrinol. Metab. 47: 556-559, 1978.

Skare, S., K.F. Hanssen, G. Lundqvist. Increased plasma pancreatic polypeptide (PP) in diabetic ketoacidosis; normalization following treatment. Acta Endocrinol. 93: 446-469, 1980.

Skillman, J.J., W. Silen, H.A. Harper. Role of the liver in secretin inactivation. Am. J. Physiol. 202: 347-348, 1962.

Snedecor, G.W., W.G. Cochrane. Statistical Methods, Sixth Edition. The Iowa State University Press. Ames, Iowa, U.S.A., 1967.

Solcia, E., A.G.E. Pearse, D. Grube, S. Kobayashi, G. Bussolati, W. Creuzfeldt, W. Gepts. Revised Wiesbaden Classification of gut endocrine cells. Rendic. Gastroenterol. 5: 13-16, 1973.

Spitz, I.M., E. Zylber, J. Jersky, D. Le Roith. Atropine suppression of basal and metoclopramide-induced human pancreatic polypeptide secretion in man. Metabolism 28: 527-30, 1979.

Spitz, I.M., E. Zylber, D. Le Roith, M. Shapiro, M. Luboshitsky, J. Jersky, J. Hoffman. The human pancreatic polypeptide response to metoclopramide. Diabetes 27: Suppl. 2, 506, 1978.

Stedman's medical dictionary, twenty-first edition. The Williams and Wilkins Co. Baltimore, 1966.

Stern, A.I., J. Hansky. Secretin stimulated pancreatic polypeptide: a test for chronic pancreatitis. Aust. NZ J. Med. 11: 351-354, 1981.

Stern, A.I., J. Hansky. Pancreatic polypeptide release in gastric ulcer. Dig. Dis. Sci. 26: 289-291, 1981.

Stern, A.I., J. Hansky, M.G. Korman, G. Coupland, J. Waugh. Pancreatic polypeptide. Release following surgery for duodenal ulcer disease. Dig. Dis. Sci. 25: 481-484, 1980.

Sternberger, L.A. Immunocytochemistry P. 129. New Jersey, Prentice-Hall Inc., 1974.

Stevens, A.B., G.J. Mogenson, J.A.F. Stevenson. Blood glucose, insulin and free fatty acids after stimulation and lesions of the hypothalamus. Am. J. Physiol. 222: 1446-1452, 1972.

Strunz, U.T., J.H. Walsh, S.R. Bloom, M.R. Thompson, M.I. Grossman. Lack of hepatic inactiation of canine vasoactive intestinal peptide. Gastroenterology. 73: 768-771, 1977.

Swierczek, J.S., S.J. Konturek, J. Tasler, J. Jaworek, M. Cieszkowski. Pancreatic polypeptide and vagal stimulation of gastric and pancreatic secretion in dogs. Hepatogastroenterology 28: 206-209, 1981.

T

Taylor, I.L. Pancreatic polypeptide release following gastric surgery. Dig. Dis. Sci 25: 481-484, 1980.

Taylor, I.L., M. Feldman, C.T. Richardson, J.H. Walsh. Gastric and cephalic stimulation of human pancreatic polypeptide release. Gastroenteroly 75: 432-437, 1978.

Taylor, I.L., M. Impicciatore, D.C. Carter, J.H. Walsh. Effect of atropine and vagotomy on pancreatic polypeptide response to a meal in dogs. Am. J. Physiol. 235: E443-447, 1978.

Taylor, I.L., G.L. Kauffman, Jr., J.H. Walsh, H. Trout, P. Chew, J.W. Harmon. Role of the small intestine and gastric antrum in pancreatic polypeptide release. Am. J. Physiol 240: G387-391, 1981.

Taylor, I.L., C.T. Richardson, M. Feldman, J.H. Walsh. Mechanisms of human pancreatic polypeptide (HPP) release. Clin. Res. 25: 574A, 1977.

Taylor, I.L., T.E. Solomon, J.H. Walsh, M.I. Grossman. Pancreatic polypeptide. Metabolism and effect on pancreatic secretion in dogs. Gastroenterology 76: 524-528, 1979.

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

Taylor, I.L., J.H. Walsh, D.L. Hogan, J.I.Isenberg. Effect of altered intragastric pH on human pancreatic polypeptide (HPP) release. Clin. Res. 26: 113A, 1978.

Taylor, I.L., J.H. Walsh, J. Rotter, E. Passaro, Jr. Is pancreatic polypeptide a marker for Zollinger-Ellison Syndrome? Lancet i: 845-848, 1978.

Taylor, I.L., J.H. Walsh, J. Wood. P. Chew, D. Carter. Bombesin is a potent stimulant of pancreatic polypeptide (PP) release. Clin. Res. 25: 574A, 1977.

Thomas, F.B., D. Sinar, E.L. Mazzaferri, S. Cataland, H.S. Mektyian, J.H. Caldwell, J.J. Fromkes. Selective release of gastric inhibitory polypeptide by intraduodenal amino acid perfusion in man. Gastroenterology 74: 1261-1265, 1978.

Thompson, J.C., H.R. Fender, N.I. Rasmus, H.V. Villar, P.L. Rayford. Cholecystokinin metabolism in man and dogs. Ann. Surg. 182: 496-504, 1975.

Thompson, J.C., D.D. Reeder, W.D. Davidson, A.C. Charters, W.L. Brückner, C.A.E. Lemmi, J.H. Miller. Effect of hepatic transit of gastrin, pentagastrin, and histamine measured by gastric secretion and by assay of hepatic vein blood. Ann. Surg. 493-505, 1969.

Thorner, M.O. Dopamine is an important neurotransmitter in the autonomic nervous system. Lancet i: 662-664, 1975.

Track, N.S., L.M. Watters, J. Gauldie. Motilin, human pancreatic polypeptide (HPP) and gastrin plasma concentrations in fasting subjects. Clin. Biochem. 12: 109-117, 1979.

Trinder, P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogen chromogen. J. Clin. Path. 22: 158-161, 1969.

Tsuda, K., H. Sakurai, Y. Seino, S. Seino, K. Tanigawa, H. Kuzuya, K. Mori, H. Imura. Abnormal pancreatic polypeptide response to oral glucose loading in diabetes mellitus. Clin. Endocrinol. 13: 425-429, 1980.

Tsuda, K., Y. Seino, H. Sakurai, S. Seino, J. Takemura, H. Kuzuya, H. Idachi, H. Imura. Cerulein-induced pancreatic polypeptide secretion. Its inhibition by atropine and its possible role in regulating gallbladder relaxation. Am. J. Gastroenterol. 74: 335-358, 1980.

U

Unger, R.H., E. Aguilar-Parada, W.A. Müller, A.M. Eisentraut. Studies of pancreatic alpha cell function in normal and diabetic subjects. J. Clin. Invest. 49: 837-848, 1970.

Unger, R.H., A.M. Einsentraut, M.S. McCall, L.L. Madison. Measurement of endogenous glucose in plasma and the influence of blood glucose concentration upon its secretion. J. Clin. Invest. 41: 682-689, 1962.

Unger, R.H., Ketterer, J. Dupré, A.M. Eisentraut. The effects of secretin, pancreozymin and gastrin on insulin and glucagon secretion in anaesthetised dogs. J. Clin. Inves. 46: 630-645, 1967.

Uys, C.J., S. Bank, I.N. Marks. The pathology of chronic pancreatitis in Cape Town. Digestion 9: 454-468, 1973.

V

Valenzuela, J.E., I.L. Taylor, J.H. Walsh. Pancreatic polypeptide response in patients with chronic pancreatitis. Dig. Dis. Sci. 24: 862-864, 1979.

Van Hoorn, W.A., A.I. Vinik, R. van Hoorn-Hickman. The metabolic clearance of endogenous immunoreactive glucagon and exogenous glucagon in pancreatectomized and sham-operated pigs. Endocrinology 103: 1084-1089, 1978.

Van Middlesworth, L. Metabolism and excretion of thyroid hormones. Handbook of Physiology. Section 7: Endocrinology Vol. III Thyroid P. 215-231. Section eds. R.O. Greep and E.B. Astwood. American Physiological Society Washington D.C., 1974.

Vendsalu, A.A. Studies on adrenaline and noradrenalin in human plasma. Acta Physiol. Scand. 49 (Suppl. 173): 33-35, 1960.

Villanueva, M.L., J.A. Hedo, J. Marco. Heterogeneity of pancreatic polypeptide immunoreactivity in human plasma. FEBS Letters 80: 99-102, 1977.

Vinik, A.I., A. Hardcastle. Structure antigenicity relationship of glucagon and related peptides. Horm. Metab. Res. 6: 395-399, 1974.

Vinik, A.I., R. Hickman, B.J. Grant. Endogenous and exogenous heptadecapeptide gastrin transport across the pig liver. S. Afr. Med. J. 53: 759-765, 1978.

Vinik, A.I., W.P.U. Jackson. Glucagon in the pathogenesis of diabetes. Lancet i: 694-695, 1975.

Vinik, A.I., W.J. Kalk, J.L. Botha, W.P.U. Jackson, K.C.H. Blake. The inexhaustible beta cell. Diabetes 25: 11-15, 1976.

Vinik, A.I., W.J. Kalk, P. Keller, P. Beumont, W.P.U. Jackson. Overactivity of the entero-insular axis in maturity-onset diabetes. Lancet ii: 183-185, 1973.

W

Walsh, J.H. Gastrin heterogeneity: biologial significance. Fed. Proc. 36: 1948-1951, 1977.

Walsh, J.H., H.T. Debas, M.I. Grossman. Pure human big gastrin: immunochemical properties, disappearance half time, and acid-stimulating action in dogs. J. Clin. Invest. 54: 477-485, 1974.

Walsh, J.H., M.I. Grossman. Gastrin. New Engl. J. Med. 292: 1324-1334, 1975.

Waterworth, M.W., G.O. Barbezat, A.I. Vinik, S. Bank, P. Child. Glucagon levels in acute pancreatitis. S. Afr. Med. J. 51: 377-378, 1977.

Way, L.W., R. Johnson, M.I. Grossman. Comparison of pancreatic response to portal and systemic venous administration of secretin, cholecystokinin, and caerulein. Fed. Proc. 28: 274, 1969.

Weir, G.C., E. Samols, S. Loo, Y.C. Patel, K.H. Gabbay. Somatostatin and pancreatic polypeptide secretion: effects of glucagon, insulin and arginine. Diabetes 28: 35-40, 1979.

Wilson, R.M., G. Boden, O.E. Owen. Pancreatic polypeptide responses to a meal and to intraduodenal amino acids and sodium oleate. Endocrinology 102: 859-863, 1978.

Woods, S.C., D. Porte, Jr. Neural control of the endocrine pancreas. Physiol. Rev. 54: 596-617, 1974.

Wu, Z.C., T.M. O'Dorisio, S. Cataland, H.S. Mekhjian, T.S. Gaginella. Effects of pancreatic polypeptide and vasoactive intestinal polypeptide on rat ileal and colonic water and electrolyte transport in vivo. Dig. Dis. Sci. 24: 625-630, 1979.

Y

Yalow, R.S. Heterogeneity of peptide hormones. Rec. Prog. Horm. Res. 30: 597-633, 1974.

Yalow, R.S., S.A. Berson. Assay of plasma insulin in human subjects by immunological methods. Nature 184: 1648-1649, 1959.

Yalow, R.S., S.A. Berson. Radioimmunoassay of gastrin. Gastroenterology 58: 1-14, 1970.

Yalow, R.S., S.A. Berson. And now "big big" gastrin. Biochem. Biophys. Res. Commun. 48: 391-395, 1972.

Yamamura, T., K. Mori, M. Tatsumi, Y. Kotoura, K. Yoshiya, N. Itoh, Y. Seino. Availability of plasma pancreatic polypeptide measurement in diagnosis of chornic pancreatitis. Scand. J. Gastroenterol. 16: 757-761, 1981.

Z

Zipf, W.B., R.P. Kelch, J.C. Floyd, N.J. Hopwood. Growth hormone suppression of pancreatic polypeptide secretion. J. Clin. Endocrinol. Metab. 53: 574-579, 1981.