# THE EFFECT OF TOTAL PARENTERAL NUTRITION ON PANCREATIC AND GASTRIC ENDOCRINE SECRETION

## Ву

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## ABSTRACT

Total parenteral nutrition (TPN) provides an experimental situation where adequate nutrition is provided intravenously, bypassing the gastrointestinal tract. Under these conditions the importance of orally ingested nutrients in the control of gastric and pancreatic endocrine secretion can be assessed. The objectives of this thesis were two-fold. First, to examine the effects of TPN on the enteroinsular axis component of insulin secretion. Second, to study the importance of orally ingested nutrients in the regulation of gastric hormone secretion using the TPN rat model.

In order to carry out these objectives, techniques for TPN and enteral feeding (TEN) of the rat were first developed. A dietary regimen for use in TPN and TEN rats was formulated from commercially-available, human TPN components. Under most circumstances, the TPN/TEN regimen met or exceeded the nutritional requirements for growing rats, as determined by the National Research Council (1978). Hematological analysis revealed few side effects of intravenous or intragastric feeding. Parenterally and enterally-fed animals demonstrated comparable weight gain to that of a control group (ORAL) fed a rat chow (#5012, Ralston Purina) diet <u>ad libitum</u>. In addition, both TPN and TEN animals appeared healthy after the 7-day infusion period. These studies indicated that the infusion formulation was suitable for chronic intravenous and intragastric feeding.

In the first series of experiments, the effects of TPN and TEN on the hormonal component of the enteroinsular axis were studied. TPN animals exhibited hyperinsulinemia and mild hyperglycemia. Conversely, TEN animals exhibited normal plasma glucose and immunoreactive insulin (IRI) concentrations. These data suggested that enterally delivered nutrients were assimilated with greater efficiency than intravenously administered nutrients. It was hypothesized that gut factors normally released by oral food intake facilitated the disposal of nutrients by hepatic and/or peripheral tissues.

During the infusion period, TPN animals exhibited chronically depressed circulating IRgastric inhibitory polypeptide (GIP) levels, in contrast to TEN animals where IR-GIP was elevated. Seven days of TPN or TEN resulted in no change in fasting plasma IRI or IR-GIP levels. However, an exaggerated insulin response to an oral glucose challenge (OGC) occurred after TPN, while the glucose response was reduced. The insulin response from the perfused pancreata of TPN animals to a GIP gradient was 20% and 40% greater than from ORAL and TEN pancreata respectively. Shorter periods of TPN (3 and 5-day periods) indicated that the hypersensitivity of the pancreas to GIP was a progressive condition, increasing with longer periods of infusion. Immunocytochemical and morphometric analysis revealed no differences in the jejunal GIP-cell population after chronic (7-day) intravenous or intragastric feeding. In addition, these routes of feeding had no effect on pancreatic islet area or endocrine cell composition of the islets. Based on these results, it was hypothesized that the increased B-cell sensitivity to GIP may have been causally related to the exposure of the pancreas to chronically low plasma GIP levels during the infusion period.

To further test this hypothesis, chronically depressed plasma GIP levels, observed during TPN, were elevated by exogenous GIP infusion to levels seen in TEN rats. Chronic GIP - treatment in TPN animals (TPN-GIP) resulted in normalization of the insulin response to an OGC and in the <u>in vitro</u> insulin response of the isolated pancreas to GIP. These data were taken as further evidence that B-cell sensitivity to GIP was affected by ambient plasma GIP levels, and it was hypothesized that changes in sensitivity may be mediated by alteration at the receptor or post-receptor level.

The effect of TPN on nutrient and neuronally mediated insulin release was also investigated. During TPN, metabolites and neuronal elements provided the main stimulus for insulin release, since hormonal components of the enteroinsular axis remained inactive. The present experiments indicated that the B-cell was hypersensitive to glucose, vagal stimulation and the cholinergic agonist methacholine, but normally sensitive to vasoactive intestinal polypeptide (VIP) and the insulinotropic amino acid arginine. These results indicated that TPN was associated with an increased B-cell sensitivity to specific hormonal, nutritive and neuronal stimuli. It was hypothesized that an increased B-cell sensitivity to these specific stimuli contributed to hyperinsulinemia observed in TPN animals during the infusion period, and to the exaggerated insulin response observed after an oral glucose challenge.

Total parenteral nutrition also provided an experimental situation in which to study the importance of gastric nutrients in the regulation of GI-hormone secretion. TPN resulted in a rapid and progressive depletion of circulating gastrin levels. G-cell secretory activity in vivo under basal and stimulatory conditions was also reduced by TPN. This condition persisted in vitro in the isolated stomach. The antral G-cell population was shown to decrease progressively with longer TPN periods, but G-cell hypoplasia and reductions in antral gastrin content were less dramatic than reductions in G-cell secretory activity. It was hypothesized that reductions in Gcell secretory activity were in part causally related to antral G-cell hypoplasia. The present data further suggested, however, that mechanisms which control synthesis and/or secretion within G-cells may have also been impaired, since various stimulants of gastrin release could not reverse gastrin hyposecretion observed during basal periods. Gastrin hyposecretion also could not be reversed by chronic bombesin administration, but was reversed by a 6-day period of oral - refeeding, indicating that the presence of nutrients in the gastric lumen was the primary regulator of tissue gastrin levels and G-cell secretory activity. The gastric D-cell was much less affected by the absence of nutrients in the gastric lumen than was the G-cell, and antral somatostatin hypersecretion may have contributed to G-cell hyposecretion.

The experiments presented in this thesis indicated that total parenteral nutrition had marked effects on both B- and G-cell secretory activity. These studies clearly demonstrated the importance of enteral feeding in the maintenance of normal pancreatic and gastrointestinal endocrine secretion.

# TABLE OF CONTENTS

	PAGE
ABSTRACT	ii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
ACKNOWLEDGEMENTS	xvii
GENERAL INTRODUCTION	
I) INSULIN	1
<ul> <li>A) Nutrient Regulation of Insulin Release</li> <li>B) Intra-Islet Modulation of Insulin Release</li> <li>C) Nervous Control of Insulin Release</li> <li>D) The Enteroinsular Axis and Insulin Secretion</li> <li>E) Long-Term Regulation of Insulin</li> <li>F) Rationale for Present Experiments</li> </ul>	2 4 5 8 13 14
II) GASTRIN	17
<ul> <li>A) Actions</li> <li>B) Release</li> <li>C) Long-Term Regulation</li> <li>D) Rationale for Present Experiments</li> </ul>	17 19 22 24
GENERAL METHODS AND MATERIALS	
I) PEPTIDE QUANTIFICATION	25
<ul> <li>A) Radioimmunoassay (RIA) <ol> <li>Calculations</li> <li>Gastrin <ol> <li>Iodination</li> <li>Assay Buffer</li> <li>Antisera</li> <li>Standards</li> <li>Controls</li> <li>Sample Preparation</li> <li>Non-Specific Binding (NSB)</li> <li>Protocol</li> <li>Charcoal Extracted Plasma (CEP)</li> <li>Separation</li> </ol> </li> </ol></li></ul>	25 25 25 25 25 27 27 27 28 28 28 28 28 28 28 28 28 28 28 28 28

3) Gastric Inhibitory Polypeptide (GIP) 29 29 a) Iodination b) Assay Buffer 30 c) Antiserum 30 d) Standards 30 e) Controls 31 f) Protocol 31 g) Separation 31 4) Glucagon 31 a) Iodination 31 b) Assay Buffer 31 c) Antiserum 32 d) Standards 32 e) Protocol 32 f) Separation 32 5) Insulin 33 a) Iodination 33 b) Assav Buffer 33 c) Antiserum 34 d) Standards 34 e) Controls 34 f) Sample Preparation 34 g) Protocol 34 h) Separation 35 6) Somatostatin 35 a) Iodination 35 b) Assay Buffer 36 c) Antibody 36 d) Sample Preparation 36 e) Standards 37 f) Assay Protocol 37 g) Separation 37 II) **ANIMAL STUDIES** 37 A) TPN-TEN Methodology 37 1) Animals 37 2) Surgical Procedures 38 a) Intravenous Catheterization for TPN Animals 38 b) Intragastric Catheterization for TEN Animals 38 c) Preparation of the Infusion Apparatus 39 d) Diet Delivery System 39 3) Intravenous (TPN)/Intragastric (TEN) Solutionsa) TPN/TEN Solution I 42 42 b) TPN/TEN Solution II 45 c) Orally Fed Animals 45 B) Isolated Perfused Organ Preparations 45 1) Solutions and Reagents 45 a) Krebs Concentrate 45 b) Krebs-Ringer Bicarbonate Perfusate 45 c) Drugs and Peptides 46 d) Electrical Stimulation 47

vii

		۷
C)	<ol> <li>Perfusion Apparatus</li> <li>Surgical Preparation         <ul> <li>a) Anaesthesia</li> <li>b) Isolated Perfused Pancreas</li> <li>c) Isolated Perfused Rat Stomach</li> <li>d) Isolated Perfused Pancreas-Stomach Preparation                 <ul></ul></li></ul></li></ol>	47 47 47 50 50 51 51 54 54 54 57 57 57 57 58 58 58
III)	IMMUNOCYTOCHEMISTRY (ICC)	58
A) B)	<ul> <li>Rationale</li> <li>Procedures</li> <li>Tissue Fixation</li> <li>Dehydration</li> <li>Embedding</li> <li>Cutting Sections</li> <li>Dewaxing and Blocking</li> <li>Immunostaining <ul> <li>a) First and Second Layer Antibodies</li> <li>i) Indirect method</li> <li>ii) Avidin biotin method</li> </ul> </li> <li>Peroxidase Reaction</li> <li>Counterstain</li> <li>Quantification <ul> <li>a) Antrum</li> <li>b) Pancreas</li> <li>c) Intestine</li> </ul> </li> </ul>	58 59 59 59 59 60 60 60 60 60 60 60 60 60 60 60 60 60
IV)	STATISTICAL INTERPRETATION	63
CHA	PTER ONE - NUTRITIONAL ADEQUACY	
I)	INTRODUCTION	64
II)	METHODS	65
III)	RESULTS	65

.

.

	<ol> <li>Comparisons of an Oral Control Diet and TPN/TEN Diet with NRC Recommendations         <ul> <li>a) Nutritional Requirements</li> <li>b) Amino Acid Requirements</li> <li>c) Mineral Requirements</li> <li>d) Vitamin Requirements</li> <li>d) Vitamin Requirements</li> </ul> </li> <li>Evaluation of Nutritional Adequacy         <ul> <li>a) Survival</li> <li>b) Changes in Body Weight During the Infusion Period</li> <li>c) Fasting Body Weight</li> <li>d) Blood and Urine Evaluation</li> </ul> </li> </ol>	65 67 67 70 70 70 70 70 70
IV)	DISCUSSION	74
CHA COM	APTER TWO - THE EFFECT OF TPN ON THE HORMONAL MPONENT OF THE ENTEROINSULAR AXIS	70
L)	INTRODUCTION	79
II)	METHODS	82
III)	RESULTS	82
	<ol> <li>Plasma Glucose, Insulin and GIP Levels in TPN, TEN and ORAL Animals During a 7-Day Experimental Period         <ul> <li>a) Body Weight</li> <li>b) Plasma Glucose, Insulin and GIP Levels on Days 2, 4, and 6 in TPN, TEN and ORAL Animals</li> <li>c) Plasma Glucose, Insulin and GIP Levels in TPN, TEN and ORAL Animals During a 24-Hour Period</li> </ul> </li> <li>The Effects of 7-Day Total Parenteral Nutrition and Intragastric Feeding on Glucose and Insulin Responses to an Oral Glucose Challenge (OGC)         <ul> <li>a) TPN vs. TEN Animals</li> <li>b) TPN vs. ORAL Animals</li> </ul> </li> </ol>	82 82 83 83 90 90 90
	<ul> <li>3) The Effects of 7-Day Total Parenteral Nutrition and Intragastric Feeding on the Insulin Response of the Isolated Perfused Pancreas</li> <li>a) The Effect of Glucose and GIP on Insulin Release from the Perfused Pancreas of TPN and TEN Animals</li> <li>b) The Effect of Glucose and GIP on Insulin Release from the Perfused Pancreas of TPN and ORAL Animals</li> <li>c) The In Vitro Insulin Response of TEN and ORAL Animals</li> </ul>	91 98 98 98 98
	<ul> <li>d) Immunocytochemical Quantification of B-Cell and K-Cell Populations</li> <li>4) The Effect of a 7-Day GIP Infusion on Insulin Release in TPN Animals <ul> <li>a) Body Weight</li> <li>b) The Effect of TPN and TPN-GIP on Plasma Glucose, Insulin and GIP Levels</li> <li>c) The Effect of TPN-GIP on Oral Glucose Challenge</li> <li>d) The Effect of Glucose and GIP on Insulin Release from the Isolated Dependence of TPN CIP on Oral AL Animals</li> </ul> </li> </ul>	99 107 107 107 107
	Pancreas of TPN, TPN-OF and UKAL Animals	108

viii

,

	e) The Effect of TPN and TPN+GIP on Somatostatin Release from the Isolated Paparas	100
5	The Effect of 3. 5 and 7-Day TPN on the In Vitro Insulin Response to	108
	Glucose and GIP	117
	a) Body Weight	117
	b) The In Vitro Insulin Response to Glucose and GIP after a 3-Day	
	Infusion Period in TPN and ORAL Animals	117
	c) The In Vitro Insulin Response to Glucose and GIP after 5-Day TPN	117
	d) The In Vitro Insulin Response in TPN and ORAL Animals to Glucose	
	and GIP after 7-Day TPN	117
6	The Effects of CCK and Glucose on Insulin Release from the Perfused	
	Pancreas after 7-Day TPN	123
	a) Body Weight	123
	b) The In Vitro Insulin Response to Glucose and CCK	123
IV)	DISCUSSION	126

# CHAPTER THREE - THE EFFECT OF TPN ON NUTRIENT AND NEURONAL COMPONENTS OF THE ENTEROINSULAR AXIS

I)	INTRODUCTION	139
II)	METHODS	141
III)	RESULTS	141
	<ol> <li>The Effect of 7-Day Total Parenteral Nutrition on Glucose Stimulated Insulin Release         <ul> <li>a) Body Weight</li> <li>b) Plasma Glucose and Insulin Levels on Days 2, 4, and 6</li> <li>c) The In Vitro Insulin Response to a Glucose Gradient</li> <li>d) The In Vitro Insulin Response to 300mg/dl Glucose: TPN vs. ORAL Animals</li> <li>e) The In Vitro Insulin Response to Arginine and Glucose: TPN vs. ORAL Animals</li> <li>f) The In Vitro Insulin Response of Wistar Rats to 300mg/dl Glucose and TPN Solution</li> </ul> </li> <li>The Effect of TPN on Islet Morphology         <ul> <li>a) Pancreatic Morphology</li> <li>c) The Effect of TPN on Neurally-Mediated Insulin Release</li> <li>a) Body Weight</li> <li>b) The Effects of MCh and Vagal Stimulation on Glucose-Stimulated Insulin Release, TPN vs. ORAL Animals</li> <li>c) The Effects of Vagal Stimulation and MCh on Glucagon and Gastrin Release: TPN vs. ORAL Animals</li> <li>d) The Effects of VIP and Glucose on Insulin Release from the Pancreas Preparation of TPN and ORAL Animals</li> </ul> </li> </ol>	141 141 141 142 142 142 143 150 150 155 155 155 155 155
IV)	DISCUSSION	163

# CHAPTER FOUR - THE EFFECT OF TPN ON GASTRIN RELEASE

I)	INTRODUCTION	. 171
II)	METHODS	174
III)	RESULTS	174
	<ol> <li>The Effect of 7-Day TPN on Gastrin Release in the Rat         <ul> <li>a) Plasma Gastrin Levels during the 7-Day Test Period</li> <li>b) The Gastrin Response to an Oral Peptone Challenge</li> <li>c) The Effect of Vagal Stimulation on Gastrin Release from the Perfused Stomach</li> <li>d) The Effect of 7-Day TPN on Antral Mucosal Height and G-Cell Number</li> </ul> </li> <li>The Effect of TPN Duration on Gastrin and Somatostatin Release from the Perfused Stomach         <ul> <li>a) Body Weight</li> <li>b) The Effect of Vagal Stimulation and MCh on Basal Gastrin and Somatostatin Release after 3-Day TPN</li> <li>c) The Effect of Vagal Stimulation and MCh on Basal Gastrin and Somatostatin Release after 7-Day TPN</li> <li>c) The Effect of Vagal Stimulation and MCh on Basal Gastrin and Somatostatin Release after 7-Day TPN</li> <li>d) Antral G-Cell Quantification and Antral Gastrin Concentration</li> </ul> </li> <li>The Effect of Re-feeding on Gastrin and Somatostatin Secretion         <ul> <li>a) Body Weight</li> <li>b) The Plasma Gastrin Response to Oral Peptone</li> <li>c) In Vitro Gastrin and Somatostatin Release, TPN vs. ORAL Animals</li> <li>d) Antral G and D-Cell Quantification</li> </ul> </li> <li>The Effect of 7-Day TPN on Bombesin Stimulated Gastrin and Somatostatin Release from the Isolated Stomach</li> <li>b) The Effect of IV-Bombesin and TPN on Gastrin Release</li> <li>a) The Effect of IV-Bombesin and TPN on Gastrin Release</li> <li>j) Plasma Gastrin Levels on Days 3 and 5</li> <li>ii) The Gastrin Response to Oral Peptone (TPN vs. TPN-Bbs)</li> <li>iii) The IN Vitro Gastrin Response to MCh and Bombesin (TPN vs. TPN-Bbs Animals)</li> </ol>	174 174 174 175 175 181 181 181 181 182 182 187 187 187 187 187 187 193 193 193 193 193 193
IV)	DISCUSSION	199
SUM	IMARY AND CONCLUSIONS	208
REF	ERENCES	212

# **APPENDICES:**

Ι.	CHEMICAL SOURCES	.229
II	LIST OF ABBREVIATIONS	231
ш	COMPOSITION OF TPN/TEN SOLUTION I	232
IV	ANTIBODY SOURCES (First Layer)	233
v	ANTIBODY SOURCES (Second Layer)	234

# LIST OF TABLES

.

PAGE

Ι	Nutrient Requirements	66
II	Amino Acid Requirements	68
Ш	Mineral Requirements	69
IV	Vitamin Requirements	71
V	Fasting Body Weight of TPN, TEN and ORAL Animals	73
VI	Blood Chemistry	75
VII	Body Weight of TPN, ORAL and TPN-GIP Rats	85
VIII	Quantification Results	105
IX	Body Weight of TPN, ORAL and TPN-GIP Rats	110
X	Body Weight of TPN and ORAL Rats During a 3, 5 and 7-Day TPN Period	119
XI	Body Weight of TPN and ORAL Rats	124
XII	Body Weight of TPN, TEN and ORAL Rats	144
XIII	Islet Size and Composition in TPN and Control Rats	151
XIV	Body Weight of TPN and ORAL Rats	157
XV	Body Weight of TPN and ORAL Rats	183
XVI	Body Weight of ORAL and TPN'R' Rats	189

1

# LIST OF FIGURES

	-	PAGE
1.	Summary of Mechanisms Controlling Insulin Release.	16
2.	Sample Standard Curve for Gastrin Radioimmunoassay.	26
3.	Total Parenteral Nutrition Infusion Apparatus.	40, 41
4.	Apparatus for Diet Delivery.	43
5.	Housing Unit for TPN Infusion.	44
6.	Perfusion Apparatus.	48
7.	The Insulin Response of the Isolated Perfused Pancreas or Pancreas-Stomach to Glucose and Arginine.	52
8.	The Gastrin Response of the Isolated Perfused Stomach or Pancreas-Stomach to Glucose and Arginine.	53
9.	The Insulin Response of the Perfused Pancreas-Stomach Preparation to 160mg/dl Glucose and Vagal Stimulation.	55
10.	The Insulin Response of the Perfused Pancreas-Stomach Preparation to Glucose and Vagal Stimulation With or Without Atropine.	56
11.	Illustration of Indirect-Peroxidase and Avidin-Biotin Staining Methods.	61
12.	Body Weight Changes During the TPN Infusion Period.	72
13.	Plasma Glucose, Insulin and GIP Levels on Days 2, 4 and 6 of a 7-Day TPN Infusion Period.	86
14.	Plasma Glucose, Insulin and GIP Levels During a 24hr Period Between Days 5 and 6 of the TPN Period.	88
15.	The Plasma Glucose and Insulin Responses to Oral Glucose in TPN and TEN Animals.	92
16.	Integrated Plasma Glucose and Insulin Responses to Oral Glucose in TPN and TEN Animals.	93
17.	The Plasma Glucose and Insulin Responses to Oral Glucose in TPN and ORAL Animals.	94
18.	Integrated Plasma Glucose and Insulin Responses to Oral Glucose in-TPN and ORAL Animals.	95
19.	The Plasma Glucose and Insulin Responses to Oral Glucose	

in TEN and ORAL Animals. 96 Integrated Plasma Glucose and Insulin Responses to Oral 20. Glucose in TEN and ORAL Animals. 97 21. The Insulin Response of the Isolated Perfused Pancreas of TPN and TEN Animals to Glucose and a GIP Gradient. 100 22. The Insulin Response of the Isolated Perfused Pancreas of TPN and ORAL Animals to Glucose and a GIP Gradient. 102 The Insulin Response of the Isolated Perfused Pancreas of 23. ORAL and TEN Animals to Glucose and a GIP Gradient. 104 106 24. B-Cell Quantification. Plasma Glucose, Insulin and GIP Levels on Day 5 of a 7-Day 25. Infusion Period in TPN, ORAL and TPN-GIP Animals. 111 26. Plasma Glucose and Insulin Responses to Oral Glucose in TPN, TPN-GIP and ORAL Animals. 113 27. The Integrated Plasma Glucose and Insulin Responses to Oral Glucose. 114 The Insulin Response of the Isolated Perfused Rat Pancreas to 28. Glucose and a GIP Gradient in TPN, ORAL and TPN-GIP Animals. 115 The SLI Response of the Isolated Perfused Pancreas of TPN. ~29. ORAL and TPN-GIP Animals to a GIP Gradient and Glucose. 116 30. The Insulin Response of the Isolated Perfused Pancreas of TPN and ORAL Animals to Glucose and a GIP Gradient After a 3-Day TPN Period. 120 The Insulin Response of the Isolated Perfused Pancreas of 31. TPN and ORAL Animals to Glucose and a GIP Gradient After a 5-Day TPN Period. 121 32. The Insulin Response of the Isolated Perfused Pancreas of TPN and ORAL Animals to Glucose and a GIP Gradient After a 7-Day TPN Period. 122 The Insulin Response of the Isolated Perfused Pancreas of 33. TPN and ORAL Animals to Glucose and a CCK Gradient. 125 The Plasma Glucose and Insulin Levels on Days 2, 4 and 6 of 34. a 7-Day TPN Period in TPN, TEN and ORAL Animals. 145 35. The Insulin Response of the Isolated Perfused Pancreas of TPN, TEN and ORAL Animals to a Glucose Gradient (80-225mg/dl). 146 xiv

36.	The Insulin Response of the Isolated Perfused Pancreas of TPN and ORAL Animals to 300mg/dl Glucose.	147
37.	The Insulin Response of the Isolated Perfused Pancreas to Arginine of TPN vs. ORAL Animals	148
38.	The Insulin Response of the Isolated Perfused Pancreata of Wistar Rats to the TPN Solution.	149
40.	The Insulin Response of the Isolated Perfused Pancreas- Stomach Preparation of TPN and ORAL Animals to Vagal Stimulation and MCh Infusion.	158
41.	The Integrated Insulin Responses to Vagal Stimulation and MCh.	160
42.	The Glucagon and Gastrin Responses to Vagal Stimulation and MCh from the Isolated Perfused Pancreas-Stomach Preparations of TPN and ORAL Animals.	161
43.	The Insulin Response of the Isolated Perfused Pancreas of TPN and ORAL Animals to a VIP Gradient in the Presence of 300mg/dl Glucose.	162
44.	Plasma Gastrin Levels on Days 2, 4 and 6 of a 7-Day TPN Period in TPN and ORAL Animals.	176
45.	Plasma Gastrin Response to Oral Peptone in ORAL and TPN Animals.	177
46.	The Gastrin Response of the Isolated Perfused Stomach of ORAL and TPN Animals to Vagal Stimulation.	178
47.	Antral G-Cell Number and Mucosal Height, ORAL and TPN Animals.	179
48.	The Gastrin and Somatostatin Responses of the Isolated Perfused Stomachs of TPN and ORAL Animals to Vagal Stimulation and MCh after a 3-Day TPN Period.	184
49.	The Gastrin and Somatostatin Responses of the Isolated Perfused Stomachs of TPN and ORAL Animals to Vagal Stimulation and MCh after a 7-Day TPN Period.	185
50.	Antral G-Cell Quantification and Gastrin Concentration.	186
51.	The Gastrin Response of TPN and TPN'R' Animals to an Oral Peptone Challenge.	190
52.	The Gastrin and Somatostatin Responses of the Isolated Perfused Stomachs of TPN and TPN'R' Animals to MCh and Vagal Stimulation after a Refeeding Period.	191

53.	Quantification of Antral G-Cell Number after a Refeeding Period.	192
54.	The Gastrin and Somatostatin Responses of the Isolated Perfused Stomachs of TPN and ORAL Animals to Bombesin.	195
55.	Plasma Gastrin Levels on Days 2 and 5 of a 7-Day Period in TPN and TPN-Bbs Animals.	196
56.	The Plasma Gastrin Responses of TPN and TPN'R' Animals to an Oral Peptone Challenge.	197
57.	The Gastrin and Somatostatin Responses of the Isolated Perfused Stomachs of TPN and TPN-Bbs Animals to MCh and Bombesin.	198

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## **GENERAL INTRODUCTION**

Total parenteral nutrition (TPN) can be defined as the administration of nutrients via the intravascular route, when given as the primary source of nutritional support. This method of feeding is used extensively in clinical situations where enteral feeding cannot meet the body's nutritional demands. TPN also provides a unique experimental situation because nutrients are delivered intravenously, bypassing the gastrointestinal tract. Under such conditions the importance of luminal nutrients on gastrointestinal hormone secretion can be studied. In addition, the importance of gastrointestinal hormones on pancreatic endocrine secretion can be assessed.

A parenterally-fed rat model was developed in 1972 by Steiger et al. In the time since its development, however, only a limited amount of information has been obtained dealing specifically with the effect of TPN on pancreatic or gastrointestinal hormone secretion in the rat. Furthermore, this model has rarely been used to study the importance of gastrointestinal (GI) hormones (normally released in response to luminal nutrients) on insulin secretion. The intent of this thesis was to specifically explore the effects of TPN on gastrin secretion, and to study the importance of GI-hormones in the regulation of insulin secretion.

The purpose of this general introduction is two-fold. First, to discuss briefly the factors which regulate insulin and gastrin release in the rat. Second, to rationalize the use of TPN as an experimental model to gain further insight into the mechanisms responsible for the control of insulin and gastrin secretion in the rat.

#### I) INSULIN

Insulin is one of the most important hormones involved in the regulation of body fuel metabolism and homeostasis. It is not surprising, therefore, that a deficient or excessive secretion results in severe metabolic disturbances such as those encountered in diabetes mellitus, obesity and in hypoglycemic syndromes.

The control of insulin release is a complex phenomenon involving both short and long-term

1

regulation. Factors which exert a direct and immediate effect upon insulin release include circulating nutrients, hormones, neurotransmitters and paracrine substances (Berthoud, 1984; Edwards, 1984; Gerich et al., 1976). Such an interaction between the B-cell and these secretagogues would be expected in response to a meal and followed by a rapid discharge of insulin which efficiently meets the metabolic demands of the body. The secretory behavior of the B-cell can also be influenced in a delayed fashion by nutritional and endocrine factors (Malaisse, 1984). Chronic interactions of the B-cell with these nutritive or hormonal factors can modulate the acute responsiveness of B-cells to a variety of stimuli (Malaisse, 1972).

#### A) Nutrient Regulation of Insulin Release

D-glucose is the main physiological regulator of B-cell function and insulin secretion. Hormonal, neuronal and other nutrient factors also stimulate insulin release, but with few exceptions they cannot stimulate insulin release in the absence of 'threshold' concentrations of glucose (Gerich et al., 1976; Hedeskov, 1980). The relationship between the extracellular glucose concentration and insulin release has been demonstrated <u>in vivo</u> (Perley and Kipnis, 1967) and <u>in <u>vitro</u> using the isolated perfused pancreas preparation (Grodsky et al., 1963), isolated islets (Ashcroft et al., 1972; Hopcroft et al., 1985), dispersed islets (Hopcroft et al., 1985) and isolated purified B-cell preparations (Pipeleers et al., 1982). With respect to the isolated perfused pancreas (Pederson and Brown, 1978) and isolated islets (Ashcroft et al., 1972) this relationship was sigmoidal, with the greatest increase in insulin secretion occurring between 90mg/dl and 300mg/dl glucose. These results, as well as those reported by others (Gerich et al., 1976; Ozawa and Sand, 1986), are in general agreement that the threshold for glucose-induced insulin release <u>in vitro</u> is approximately 100mg/dl.</u>

The pattern of insulin release from the perfused rat pancreas and isolated islets in response to glucose has been studied extensively (Gold and Grodsky, 1984; Hopcroft et al., 1985; Pipeleers et al., 1982). There is general agreement that when glucose is presented as a continuous (square wave) stimulus the insulin response is multiphasic. Stimulation with glucose results in an immediate burst of insulin lasting 2-5 min, followed by a nadir and then a second ascending

(linear) secretion, which reaches plateau levels with time. A third phase of insulin release, namely a slow decrease after prolonged exposure to hyperglycemic conditions, has recently been described in the isolated perfused rat pancreas and isolated islets (Grodsky et al., 1986; Bolaffi et al., 1986). In order to explain the phasic pattern of insulin release, several mathematical models have been developed. They fit into one of two basic conceptual categories (O'Connor et al., 1980; Gold and Grodsky, 1984). First, there are those based upon compartmentalization of stored insulin (storage limited models). They hypothesize that there are two insulin pools, one available for immediate release and responsible for the first phase of release, and a much larger pool responsible for the second phase of release. Second, there are those based upon the interaction between intracellular stimulatory and inhibitory signals (signal limited models).

The precise mechanisms responsible for glucose-induced insulin release have not been elucidated. The view presently held, however, (known as the 'fuel hypothesis') suggests that the process of nutrient-induced insulin release is caused by an increased rate of nutrient utilization in the B-cell (Malaisse et al., 1979; Sener and Malaisse, 1984). Although the precise intracellular mechanism which connects nutrient metabolism with insulin secretion is yet unknown, key metabolites and/or cofactors (NADH and ATP) generated by nutrient catabolism are believed to stimulate the release of intracellular Ca<sup>2+</sup> and cAMP. It is proposed that increases in cAMP and intracellular Ca<sup>2+</sup> activate cAMP- and Ca-dependent protein kinases, which in turn stimulate secretion (Harrison et al., 1984).

Other nutrients, including both amino acids and free fatty acids, have been shown to be involved in the regulation of insulin secretion (Gerich et al., 1976). Individual amino acids differ in their ability to stimulate insulin secretion and there are some species differences as well. In the dog, Rocha et al. (1972) demonstrated that tryptophan, leucine, aspartate and isoleucine were the most potent stimulators. In man, however, arginine, lysine and leucine were found to be the most potent stimuli (Fajans and Floyd, 1972). In vitro, using the isolated perfused rat pancreas, Pederson and Brown (1978) and Gerich et al. (1974) have demonstrated the glucose dependency of arginine on insulin release. At 80mg/dl glucose, arginine was a weak stimulant of insulin release, but, in the presence of graded increases in glucose concentration from 80 to 300mg/dl the

3

insulin response to arginine was potentiated. Free fatty acids (FFA) have also been shown to affect insulin secretion. In the dog, FFA infusion as oleate or triglyceride emulsion elevated plasma insulin levels (Crespin et al., 1969). In man, FFA infusions had little effect on fasting insulin levels but augmented insulin responses to subsequent stimulation with glucose (Balasse and Ooms, 1973).

#### **B)** Intra-Islet Modulation of Insulin Release

Pancreatic islets contain a mixture of four cell types responsible for the secretion of glucagon (A-cells), insulin (B-cells), somatostatin (D-cells) and pancreatic polypeptide (PP-cells) (Samols et al., 1986). Rat islet cells have a unique morphological relationship, in that the islets consist of a central core of B-cells surrounded by a mantle of A-, D- and PP-cells (Orci, 1984; Smith and Davis, 1983). The proportion of A-cells and PP-cells depends on the location of the islets within the pancreas, the head containing a proportionately larger PP-cell population and the tail containing proportionately more A-cells (Orci, 1984). The morphology of the islet also suggests a functional relationship between islet cells. The presence of gap junctions and desmosomes between islet cells suggests the possibility of cell to cell communication (Orci et al., 1975). In keeping with this theory, electrical coupling and passage of small molecules between islet cells have been demonstrated (Pipeleers, 1984; Orci, 1984).

Islets also have a unique microvasculature. Bonner-Weir and Orci (1982) examined the organization of the microvasculature in rat islets. It was concluded that different vessels entered the islets, sending their capillaries through the central B-cell core, then perfusing the non-B-cell mantle. These observations suggest that the influences of glucagon and somatostatin on B-cells would most likely occur in a paracrine, rather than an endocrine, fashion. The anatomical relationship between these three cell types is supported by a functional relationship. <u>In vitro</u>, glucagon stimulates biphasic B- and D-cell secretion (the response being dose-dependent), while somatostatin rapidly inhibits both A- and B-cell secretion (Samols et al., 1986). Further support for a functional relationship is the presence of high affinity binding sites for both glucagon and somatostatin on B-cell plasma membrane preparations (Pipeleers, 1984). Although direct evidence

has not been obtained to confirm B-cell secretory modulation by pancreatic somatostatin and glucagon, the possibility should be considered when discussing the control of insulin release.

#### C) Nervous Control of Insulin Release

The pancreatic islets are supplied with a rich innervation of cholinergic, catecholaminergic and peptidergic nerves (Ahren et al., 1986; Edwards, 1984; Smith and Davis, 1983). Preganglionic cholinergic nerves originate in the dorsal motor nucleus and terminate within pancreatic ganglia or directly within islets (Miller, 1981). Catecholaminergic fibers from splanchnic nerves pass through the celiac ganglion and enter the pancreas as part of the mixed autonomic nerve. They are associated with individual endocrine cells within islets, or with the vasculature supplying islets (Miller, 1981). In recent years a large number of intrapancreatic peptide-containing nerves have also been identified. Although there appears to be a high degree of variability among species, VIP, GRP, NPY, substance P and enkephalin-containing nerve fibers have been found in close association with islet cells and the vasculature supplying islets (Ahren et al., 1986). The morphological relationship of adrenergic, cholinergic and peptidergic nerves with the endocrine pancreas suggests that nerves are involved in the physiological regulation of islet function.

A role for the parasympathetic nervous system in the control of glucose metabolism was alluded to as early as 1925, when Britton demonstrated that hypoglycemia in anesthetized cats could be provoked by vagal stimulation. This response was attributed to the release of insulin. The hypothesis that vagal stimulation leads to insulin release has more recently been confirmed. Electrical stimulation of the vagus nerve elicited a rapid insulin response <u>in vivo</u> from a variety of species, including dog (Frohman et al., 1967; Kaneto et al., 1967), calf (Bloom and Edwards, 1981) and pig (Holst et al., 1981). Vagally-induced insulin release has also been demonstrated <u>in vitro</u> in dog (Bergman and Miller, 1973) and rat (Nishi et al., 1987), suggesting a direct effect of the vagus nerve on the endocrine pancreas. The <u>in vitro</u> insulin response to vagal stimulation in the rat is partially blocked by atropine and completely blocked by hexamethonium (Nishi et al., 1987). These results suggest that, in the rat, post-ganglionic, cholinergic and non-cholinergic (possibly peptidergic) nerves may function as the final mediators of vagally-induced insulin

secretion.

The parasympathetic, post-ganglionic neurotransmitter acetylcholine has been shown to induce insulin secretion <u>in vitro</u> in the perfused rat pancreas (Loubatieres-Mariani et al., 1973) and isolated rat islets (Sharp et al., 1974). The addition of atropine blocked these responses, suggesting direct stimulation of muscarinic receptors on the B-cells by acetylcholine. The presence of high affinity binding sites for the cholinergic antagonist <sup>3</sup>H-methylscopolamine in isolated islets has been demonstrated (Grill and Ostenson, 1983), providing further evidence for a direct cholinergic effect on B-cells.

The possibility that the insulin response to vagal stimulation is, at least in part, the result of modulation by other islet hormones cannot be ruled out. Glucagon is released during electrical stimulation and by exogenous acetylcholine administration both in vivo and in vitro (Ahren et al., 1986). Furthermore, somatostatin secretion is inhibited by vagal stimulation in vitro in rat (Nishi et al., 1987) and by vagal stimulation and acetylcholine in pig (Holst et al., 1983). A combination of increased glucagon and decreased somatostatin secretion could augment the insulin response observed during vagal stimulation (Edwards, 1984).

Although the mechanism for acetylcholine stimulation of insulin release remains unresolved, the glucose-dependency of this process is generally accepted. In the absence of glucose, acetylcholine is ineffective. When glucose is presented in physiological concentrations (4-8mM), however, acetylcholine potently stimulates insulin secretion from the isolated pancreas of dog and rat (Iversen, 1973; Loubatieres-Mariani et al., 1973) and from isolated islets (Sharp et al., 1974). These studies are indicative of the permissive nature of glucose on non-nutrient stimulation of insulin release.

Acetylcholine released from parasympathetic vagal nerve terminals is one of the most potent neural mechanisms for stimulation of insulin release (Edwards, 1984). The importance of this mechanism in the control of glucose homeostasis is, however, unclear. There is general agreement that the vagus nerve is activated by the so-called 'cephalic phase' of food ingestion. This phenomenon has been clearly demonstrated in numerous studies and is the result of parasympathetic stimulation of insulin release, although the final mediator may be peptidergic in

6

some species (Berthoud, 1984; Edwards, 1984; Berthoud and Jeanrenaud, 1982; Louis-Sylvestre, 1976). Less clear, however, is the role for parasympathetic nervous control of insulin release during hyperglycemia. The presence of peripheral glucoreceptors in the pancreas, liver and duodenum, sensitive to changes in glucose concentration and anatomically associated with the vagus nerve, suggest that an afferent limb of a feedback loop exists (Edwards, 1984). There is also compelling evidence to suggest that glucose is continuously monitored centrally by glucosensitive cells in the ventromedial hypothalamus which orchestrate a wide range of neurally mediated responses upon change in peripheral or central glucose concentration (Edwards, 1984).

The presence of an efferent limb of this system can only be speculated upon at the present time. In sympathectomized calves, pretreatment by atropine virtually abolished the release of insulin which would otherwise occur in response to hyperglycemia (Edwards, 1984). Furthermore, in rat, sectioning of the vagus nerve had no effect on fasting insulin levels, but significantly reduced the response to exogenously administered glucose (Håkanson et al., 1971). These studies support the existence of neuronal (vagal) pathways which function to modulate insulin release in response to hyperglycemia.

Of the large number of peptides in peptidergic nerves associated with the endocrine pancreas, only VIP and GRP appear to have insulinotropic properties (Ahren et al., 1986). The insulinotropic nature of VIP has been demonstrated in various species (Schebalin et al., 1977; Kaneto et al., 1977; Adrian et al., 1978; Szecowka et al., 1983). The glucose-dependency and potentiating effects of VIP on glucose-stimulated insulin release have also been demonstrated (Szecowka et al., 1983). GRP stimulates the release of insulin <u>in vitro</u> and <u>in vivo</u> in various species, potentiating glucose-induced insulin release (Ahren et al., 1986). A mechanism that releases these neuropeptides in response to hyperglycemia has yet to be established. Both VIP and GRP can, however, be released in response to vagal stimulation (Fahrenkrug et al., 1979; Holst et al., 1984; Knuhtsen et al., 1985). These peptides could, therefore, function as the non-cholinergic final mediators hypothesized to be involved in vagally-mediated insulin release in the rat (Nishi et al., 1987).

The sympathetic nervous system has generally been thought of as a key component mediating

the catabolic responses to stress. The glucagonotropic action of either sympathetic nerve stimulation or adrenoreceptor agonists is in keeping with this hypothesis (Ahren et al., 1986). The action of the sympathetic nerves on the anabolic hormone insulin, however, does not appear as straightforward. Electrical stimulation of splanchnic or mixed pancreatic nerves inhibited the release of insulin in response to glucose in dogs (Marliss et al., 1973) and calves (Bloom and Edwards, 1978), an inhibition reversed by the alpha-adrenergic antagonist phentolamine. Basal insulin release was also decreased by sympathetic nerve stimulation in dog (Ahren et al., 1986). These data suggest that sympathetic nerves to the pancreas may have a tonic inhibitory role and/or inhibit glucose-stimulated insulin release. But this hypothesis has been questioned with the observation that a beta-adrenergic stimulatory component on insulin release exists (Porte, 1967). The physiological significance of both a stimulatory and inhibitory component of sympathetic nerves remains unresolved, but the actions of catecholamines may depend on other neuronal, hormonal or nutritive influences.

### D) The Enteroinsular Axis and Insulin Secretion

Unger and Eisentraut (1969) introduced the term 'enteroinsular axis' to describe a proposed regulatory mechanism in which the hormones of the gastrointestinal (GI) tract exerted an influence on the secretion of pancreatic islet hormones. More recently, this definition has been modified to include the effects of hormones, neural components and nutrients (Creutzfeldt, 1979). Historically, the possibility of an association between the GI-tract and the internal secretion of the pancreas evolved from the observations of Moore et al. (1906). It was demonstrated that duodenal mucosal extracts administered to diabetic patients could reduce glucosuria. In a series of experiments some twenty years later, it was demonstrated that this hypoglycemic factor was not the exocrine pancreatic stimulant secretion (Zunz and Labarre, 1929; Labarre and Still, 1930). Labarre (1932) postulated the existence of a hormone 'incretin', released from the gastric mucosa by glucose, which influenced insulin secretion. Further support for the incretin concept came with the development of a radioimmunoassay (RIA) for insulin. Elrick et al. (1964) observed a significantly greater insulin response to glucose when given orally rather than intravenously. In the same year, McIntyre et al. (1964) observed a similar effect with intrajejunal compared to

intravenous glucose administration, despite achieving lower plasma glucose levels via the intrajejunal route. Perley and Kipnis (1967) estimated that gastrointestinal insulinotropic factors account for half of the insulin secreted in response to oral glucose. The observation that orally administered glucose elicited a greater insulin response than intravenously administered glucose formed the basis for the 'incretin concept'.

Certain criteria have been established which must be fulfilled before a gut endocrine factor can be considered an incretin (Creutzfeldt, 1979). First, it must be released by luminal nutrients, especially carbohydrates. Second, it must stimulate insulin secretion in the presence of glucose if exogenously infused in amounts not exceeding blood levels achieved after food ingestion. Various GI hormones, including CCK, gastric inhibitory polypeptide (GIP), gastrin and secretin, have been tested for their insulin releasing properties. Only GIP fulfilled both criteria for incretins (Creutzfeldt, 1979; Creutzfeldt and Ebert, 1985).

CCK and gastrin did not fulfill these criteria but their insulinotropic properties still suggest that they had a physiological function in the control of insulin secretion (Dupré et al., 1969). Exogenous gastrin augmented glucose-induced insulin release in concentrations attained after a protein meal, but not after oral glucose (Rehfeld and Stadil, 1973). CCK was observed to stimulate insulin release from the isolated perfused pancreas in the presence of high glucose (300mg/dl) at doses between 0.2-5.0 ng/ml (Pederson and Brown, 1979; Mueller et al., 1983). Although plasma CCK levels have not been unequivocally ascertained, Mueller et al. (1983) demonstrated that CCK could stimulate insulin release at levels submaximal to that for pancreatic exocrine secretion. Thus, the possibility that both CCK and gastrin contribute to insulin secretion observed after a meal cannot be discounted.

Gastric inhibitory polypeptide, a 42-amino acid linear peptide (Jornvall et al., 1981) was isolated from hog duodenal-jejunal mucosal extracts (Brown et al., 1969; Brown et al., 1970). GIP was originally postulated to be an enterogastrone because of its ability to inhibit gastrin and histamine-stimulated gastric acid secretion from vagally and sympathetically denervated stomach pouches. Recently, it has been suggested that the acid inhibitory action of GIP may be mediated in part by the local release of somatostatin (McIntosh et al., 1981). It was further suggested that GIP may function in conjunction with other neural and hormonal factors released by digestion to produce inhibition of acid secretion.

The insulinotropic activity of GIP was first demonstrated by Dupré et al. (1973). Simultaneous intravenous infusion of GIP and glucose in man improved glucose tolerance and increased insulin release when compared to the infusion of glucose alone. The insulinotropic nature of GIP has also been established in other species, including rat and dog (Pederson and Brown, 1976; Pederson et al., 1975). In both rat and man the actions of GIP on insulin release were glucose-dependent, and there was a threshold glucose concentration below which GIP had no insulinotropic effect. In the isolated perfused rat pancreas this threshold level was found to be 100mg/dl, above which GIP potentiated glucose-induced insulin release in a concentrationdependent manner (Pederson and Brown, 1976). The concentration-dependent nature of GIP was clearly shown in the isolated rat pancreas when GIP was presented as a gradient (0-1ng/ml) in the presence of 300mg/dl glucose (Pederson et al., 1982). GIP was found to augment insulin release in a concentration-dependent manner above levels of 350pg/ml. In man, Elahi et al. (1979) - employed the glucose clamp technique to demonstrate that plasma glucose must rise above fasting levels (90mg/dl) before the potentiating effect of GIP on insulin release could be observed. It was further shown that the potentiating effect of GIP on insulin release was greater during moderate hyperglycemia (225mg/dl) than during mild hyperglycemia (145mg/dl).

When GIP was presented as a 'square-wave' stimulus, insulin release was biphasic in nature (Pederson and Brown, 1976), similar to that of the other insulinotropic agents, arginine and glucose (Pederson and Brown, 1978; Gerich et al., 1974). When GIP was presented as a linear gradient (0-1ng/ml), however, the insulin response was monophasic (Pederson et al., 1982). Brown et al. (1980) stated that the biphasic insulin release observed with a 'square-wave' GIP stimulus was not a physiological phenomenon, suggesting that a gradient was a more appropriate way to present GIP to the isolated pancreas.

A radioimmunoassay procedure developed by Kuzio et al. (1974), and the availability of several antisera raised against porcine GIP (EGIII) (Jorde et al., 1985), have permitted the measurement of serum immunoreactive (IR) GIP in several species. Fasting IR-GIP levels in man

were estimated at 237pg/ml, rising to 1,200pg/ml following a test meal (Kuzio et al., 1974). IR-GIP was also shown to be released in response to oral glucose in several species, including man (Cleator and Gourlay, 1975), dog (Pederson et al., 1975) and rat (Pederson et al., 1982). In rat, fasting IR-GIP levels were found to be 450pg/ml, rising to approximately 2,000pg/ml in response to oral glucose. Intravenously administered glucose, however, was found not to stimulate IR-GIP above basal levels (Cataland et al., 1974; McCullough et al., 1983). Triglycerides have also been demonstrated to release IR-GIP in dog (Pederson et al., 1975) and man (Brown, 1974; Cleator and Gourlay, 1975). Although protein given as meat extract (Brown, 1974) did not produce an IR-GIP response, release was observed in response to mixed amino acids (Thomas et al., 1978). These studies demonstrated that IR-GIP was released in response to luminal carbohydrates and other nutrients. They also demonstrated that plasma GIP levels attained during carbohydrate stimulation were similar to those necessary to induce insulin secretion <u>in vitro</u> in the isolated rat pancreas (Pederson et al., 1982). GIP, therefore, fulfilled both criteria necessary for being an incretin.

GIP requires the presence of one or more effector molecules in order to exert an insulinotropic effect. The glucose-dependent nature of GIP in vitro and in vivo supports this observation (Pederson and Brown, 1976; Elahi et al., 1979). How glucose and GIP interact, however, remains unclear. The observation that mannoheptulose blocked GIP potentiated, glucose-stimulated insulin release from the isolated rat pancreas suggested that glucose metabolism may have been a prerequisite (Mueller et al., 1982). A step distal to fructose phosphorylation has been implicated, because GIP potentiated D-glyceraldehyde stimulated insulin release (Dahl, 1984). These studies indicated that metabolism of glucose was required for GIP to exert its potentiating effect on insulin release, and not the presence of glucose per se. Thus, GIP can be placed in a category with other secretagogues which require the presence of one or more 'exciter molecules' such as glucose to exert its insulinotropic effect on the B-cell.

The possibility that GIP acts indirectly through the release of other islet hormones was not supported. GIP, when presented to the perfused rat pancreas, stimulated glucagon release only at glucose concentrations below the threshold for insulin secretion (Pederson and Brown, 1978); in

man, GIP had no effect on glucagon secretion under euglycemic or hyperglycemic conditions (Elahi et al., 1979). Moreover, GIP has not demonstrated any stimulatory or inhibitory effect on pancreatic somatostatin secretion at doses considered physiological.

More evidence exists in support of a direct effect on the B-cell. The presence of specific high affinity binding sites for GIP has been demonstrated on the membranes of insulin secreting hamster B-cell tumors (Maletti et al., 1984), an insulin secreting B-cell line (In III) (Amiranoff et al., 1984) and a human benign insulinoma (Maletti et al., 1987). In the B-cell line, GIP produced an insulin response with a concomitant rise in cAMP (Amiranoff et al., 1984). Glucagon, a peptide sharing sequence homology with GIP, has also been shown to stimulate cAMP production from B-cells (Maletti et al., 1987). It has been postulated that cAMP induces insulin release by activation of the appropriate protein kinase(s), which influence(s) several variables of islet function, such as the intracellular redistribution of  $Ca^{2+}$  leading to the amplification of the secretory response of the B-cell to nutrients (Malaisse and Malaisse-Lagae, 1984). At present, however, GIP receptors have not been observed on normal B-cells and only pharmacological doses of GIP - have been effective in releasing insulin from freshly isolated islets (Schauder et al., 1975; Fujimoto, 1981). Possible explanations for this apparent lack of GIP receptors and sensitivity to GIP in isolated islets have been proposed by Brown et al. (1980). First, damage to the B-cell receptor may occur during collagenase digestion of the islets. The receptors may be susceptible to enzymatic damage during the isolation procedure. Since B-cell tumors, human insulinoma and the B-cell line In III possess receptors and are not subjected to digestion, this is a possible explanation. A second possibility suggested by Brown et al. (1980) was that these B-cell preparations contain a higher density of specific receptors than found in normal tissue. The inability to demonstrate receptors in normal pancreatic, liver or stomach preparations lends support to this possibility. A third explanation is that GIP, when iodinated, is structurally altered so that binding to its specific receptor is prevented. The iodination of Tyr-10, and oxidation of Met-14 and Trp-25 could create this problem since all are in the mid portion of GIP, an area stated to be essential for insulinotropic actions (Brown et al., 1980; Maletti et al., 1986). A final explanation is that isolated islets or B-cell preparations lack the appropriate environment to facilitate the action of

GIP. The isolation procedure not only destroys neural and vascular components of the islets, but also alters the flow characteristics and compartmentalization of the islet interstitial space (Pipeleers, 1984). In the isolated perfused pancreas, where GIP is a potent insulinotropic hormone (Pederson and Brown, 1976), intrapancreatic nerves and the macro- and microvasculature of the islets are left intact. At the present time, therefore, the perfused pancreas model and <u>in vivo</u> studies appear to be the best methods for studying the physiological effects of GIP on insulin release.

#### E) Long-Term Regulation of Insulin Release

The control of insulin release is a complex phenomenon involving both short- and long-term regulatory mechanisms. Long-term regulatory mechanisms include the direct and indirect chronic interactions between the B-cell and nutritive and hormonal factors, which can modulate the acute insulin responsiveness of the pancreas. Alterations in the responsiveness of the pancreas to a variety of stimuli can be demonstrated, for example, after extended periods of excessive caloric intake or food deprivation. In addition, chronic hormonal hyper- or hypo-secretion can affect the responsiveness of the pancreas to a number of stimuli (Malaisse, 1972; Hedeskov, 1980).

The effects of prolonged food deprivation on insulin secretion are well documented. In man and rat, food deprivation leads to a diminished level of circulating insulin, and a reduced responsiveness of the B-cell to subsequent stimulation (Malaisse, 1972). <u>In vitro</u> studies in the rat and mouse demonstrated that fasting (24-72h) did not reduce pancreatic insulin content, but impaired both glucose-stimulated insulin release and glucose metabolism in B-cells (Buchanan et al., 1969; Hedeskov and Capito, 1974; Zawalich et al., 1979). Hedeskov (1980) proposed that the prolonged lack of a glycemic stimulus during starvation caused a diminished capacity for glucose utilization in islets. Impaired glucose metabolism in turn prevented activation of intracellular coupling factors, which are required for stimulation of insulin release by glucose and other insulin secretagogues.

In contrast to nutrient deprivation, chronic excessive caloric intake can contribute to hyperinsulinism. Obesity, for example, is often attributed to the prolonged ingestion of calories in excess of the individual's energy requirements, and hyperinsulinism is a common denominator in many cases (Bray, 1978). The cause of elevated plasma insulin in obesity has not been determined. However, studies in obese man and rats suggested that hyperinsulinemia may be linked in part to the exaggerated insulin responses of the pancreas to a variety of nutrient, hormonal and neuronal stimulants of insulin release (Creutzfeldt et al., 1978; Chan et al., 1984). Although excessive caloric intake has contributed to hyperinsulinism, so have carbohydrate-rich normocaloric diets (Blasquez and Lopez-Quijada, 1969). In addition, prolonged feeding of carbohydrate-rich diets in the rat resulted in the development of hypertrophied islets (Wissler et al., 1949), which could contribute to the observed hyperinsulinemia associated with this type of diet. Thus, abnormal insulin secretory activity could result from chronic modifications in the normal qualitative or quantitative intake of nutrients.

In addition to the effects of nutrients on long-term regulation of insulin, many hormones secreted in excessive or insufficient amounts can affect islet function (Malaisse, 1972). For example, growth hormone hypersecretion and hyposecretion are associated with increased or decreased B-cell sensitivity respectively (Malaisse et al., 1968). Similarly, glucocorticoid deficiency, produced by adrenalectomy, led to reduced B-cell sensitivity, while pharmacological doses of synthetic glucocorticoids markedly enhanced the secretory response of the pancreas (Malaisse et al., 1967; Perley and Kipnis, 1966). Chronic changes in thyroid hormones can also alter the responsiveness of islets to stimulation (Malaisse, 1972). Although the effects of these hormones on insulin release have been studied in detail, no information regarding the effects of gastrointestinal hormone hypersecretion or insufficiency on islet function is currently available.

#### F) Rationale for the Present Experiments

Insulin secretion from the pancreatic B-cells is controlled by three major factors: nutrients, the nervous system and hormones. The effects of these factors can be modulated by intra-islet (paracrine and neural) interactions (fig. 1). Glucose is the most important physiological regulator of B-cell function, probably acting both directly on the B-cell and indirectly through the activation of hormonal and nervous components. The vagus nerve is activated during the cephalic phase of insulin release and possibly during hyperglycemia. Acetylcholine released from the

parasympathetic vagal nerve terminals is the most potent neural mechanism for stimulation of insulin secretion. The observation that oral glucose produces a larger insulin response than an intravenous (IV) glucose load clearly demonstrates the importance of the enteroinsular axis during a meal. GI-hormones, termed incretins, are released by nutrients and stimulate insulin release in a classical endocrine fashion. Inhibition of insulin secretion may involve the direct effects of low glucose concentrations and both the direct and indirect effects of the sympathetic nervous system acting in concert with other catabolic hormones.

Numerous <u>in vivo</u> and <u>in vitro</u> models, many of which have been mentioned in this introduction, have been employed to study the relative contribution of nutritive, hormonal and neuronal influences on insulin secretion. In general, these models can be grouped into one of two categories. First, there are those which are used to study short-term regulation of insulin release (i.e. mechanisms activated in response to a meal). Second, there are those which are used to investigate the long-term modulation of insulin release (i.e. the effect of nutrients and hormones on the chronic regulation of islet function).

The technique of chronic intravenous feeding in unrestrained rats, first developed by Steiger et al. (1972), provides a unique opportunity for the study of long-term regulation of insulin secretion. Total parenteral nutrition is a unique method because all nutrients are supplied to the animal by the intravascular route, bypassing the GI-tract. Thus, with minimal surgical intervention, gastrointestinal factors (incretins) normally contributing to the regulation of insulin release, remain chronically suppressed (fig. 1). The effects of total parenteral nutrition on the enteroinsular axis component of insulin release in the rat have not been critically examined.

The purpose of the studies presented in chapter two was to specifically examine the effects of TPN on the enteroinsular axis in the rat. The impetus for these experiments came from preliminary experiments by Pederson et al. (1985), where it was demonstrated that 7-days of TPN leads to altered B-cell secretory activity. It was hypothesized that this alteration was the result of a modification in the enteroinsular axis component of insulin release. To further explore this possibility a TPN rat model was developed in this laboratory. The effects of TPN on the enteroinsular axis were first assessed, in vivo, using oral glucose tolerance experiments, and then



# Fig. 1

Summary of mechanisms controlling insulin release.

in vitro using the isolated perfused pancreas, where the secretory response of the pancreas to specific components of the enteroinsular axis (i.e. glucose and GIP) could be examined.

Total parenteral nutrition involves the intravenous delivery of a highly concentrated amino aciddextrose solution. Thus, the pancreas is continuously presented with stimulants of insulin release. The effects of continuous systemic nutrient delivery on pancreatic islet morphology has not been examined. In addition, the effects of TPN on the acute secretory response of the pancreas to nutrient and to neuronal stimulation have not been examined. The purpose of the studies presented in chapter three was first to examine the effects of TPN on the insulin responsiveness of the B-cell to glucose or to neuronal stimulation and, second, to assess the effects of TPN on islet endocrine cell morphology. Thus, the work presented in chapters two and three addresses several aspects concerning the effects of total parenteral nutrition on pancreatic endocrine secretion.

#### II) GASTRIN

Gastrin was extracted from antral tissue by Edkins in 1905. It was not until 1964, however, that Gregory and Tracy accomplished the purification and chemical characterization of this hormone. Subsequent to this, and aided by a radioimmunoassay for gastrin, several molecular forms other than the heptadecapeptides have been identified (reviewed in Walsh, 1987). The most prominent forms are G-17 and G-34. In the antrum, G-17 is the most abundant gastrin, while in the duodenum, G-34 represents the major molecular form (Lamers et al., 1982).

#### A) Actions

Gastrin is associated with a wide range of activities throughout the gastrointestinal tract. Its primary physiological role, however, is one of stimulating acid secretion (McGuigan et al., 1971; Walsh and Grossman, 1975; Feldman et al., 1978). Acid production is observed only for forms of gastrin that contain the C-terminal amide sequence, and G-17 and G-34 are equipotent stimulants (Eysselein et al., 1984). The action of gastrin on the parietal cell is mediated through specific membrane receptors, which bind the carboxyl end of the molecule, the region which

contains the biological activity of the hormone (Soll et al., 1984).

Interactions of gastrin with acetylcholine and histamine are important determinants of the overall gastric secretory response (Soll and Walsh, 1979; Soll, 1982). Specific histamine  $H_2$ -receptor antagonists, such as cimetidine, inhibit gastric acid secretion in response to gastrin as well as histamine (Grossman, 1978). Furthermore, cephalic stimulation enhances the effect of gastrin on acid secretion, which is abolished by atropine or by vagotomy (Walsh, 1987). In addition, synergistic interactions between these three secretagogues of gastric acid secretion, as demonstrated by Soll (1982), futher support a strong interdependence.

A second potentially important physiological role for gastrin is the control of mucosal growth, particularly in the acid secreting oxyntic gland mucosa of the stomach (Walsh, 1987; Johnson et al., 1979). The demonstration that pentagastrin stimulated protein synthesis in oxyntic gland mucosa (Johnson et al., 1969), led to the hypothesis that gastrin was a trophic hormone acting on the stomach. In follow-up studies this hypothesis was expanded to include the trophic effects of gastrin on the oxyntic gland, small intestine, colon and pancreas (Johnson, 1977). Although pentagastrin was able to produce trophic effects in the gut and pancreas, there was some question as to whether exogenous and/or endogenous gastrin were capable of producing such effects. It was shown, however, that both sulfated and non-sulfated G-17 and G-34 were capable of trophic action in the oxyntic gland, G-34 being twice as potent as G-17 (Johnson and Guthrie, 1976). Furthermore, continuous intravenous infusion of G-17 II, at doses that were submaximal for gastric acid stimulation, produced significant increases in DNA synthesis in the stomach (Ryan et al., 1978).

The trophic effects of endogenous gastrin were also studied by experimentally depressing serum and antral gastrin levels. Antrectomy provided a condition where serum gastrin levels were chronically reduced in the fasted and fed state (Håkanson et al., 1986). Under this condition, the oxyntic glandular portion of the stomach, and the pancreas, duodenum and colon, were found to atrophy; this could, however, be partially prevented by the administration of pentagastrin (Dembinski and Johnson, 1979). TPN was also effective in reducing serum gastrin levels, and was associated with atrophy of the GI-tract and pancreas (Johnson et al., 1975a). In a follow-up
study pentagastrin treatment prevented this atrophy (Johnson et al., 1975b). Although this evidence appears convincing, recent studies which support trophic effects for gastrin in the stomach, do not support any effects in the intestine and pancreas (Håkanson et al., 1986). They suggest that CCK is a more likely candidate for trophic control of the pancreas and small intestine, and further suggest a third class of gastrin/CCK-like peptides for the control of the distal small and large intestine.

Other biological activities have been associated with gastrin based on studies where the doses of gastrin used were considered to be submaximal for acid secretion. These include: regulation of glucose-stimulated insulin release (Rehfeld and Stadil, 1973); and stimulation of pancreatic enzyme secretion (Valenzuela et al., 1978). Gastrin also causes contraction of the lower esophageal sphincter, but this appears to be a pharmacological effect (Jensen et al., 1978).

#### **B)** Release

Gastrin secretion is controlled by a complex set of interactions between inhibitory and stimulatory factors. The anatomical location of antral G-cells in the mucosa of the antral glands suggests that gastrin secretion can be regulated by luminal, vascular and neural routes. The anatomical relationship with these elements is supported by a functional relationship. Gastrin release is controlled by luminal nutrients, neuronal components and by hormones (Walsh, 1987).

The primary stimulants of gastrin release are small peptides and amino acids presented luminally (Elwin, 1974). The amino acids phenylalanine and tryptophan are the most effective (Taylor et al., 1982). When these nutrients are presented parenterally, however, gastrin release is not affected (McArthur et al., 1983). Luminal pH can also greatly affect gastrin secretion. Maintenance of gastric pH greater than 3 potentiated the gastrin response observed when amino acids were ingested (Walsh and Grossman, 1975), while a pH less than 3 inhibited postprandial gastrin release (Walsh et al., 1975). The addition of calcium, as contained in dairy products, and some calcium-containing antacids, also stimulated gastrin release (Levant et al., 1973). These studies clearly point out the importance of luminal factors which control the appropriate gastrin response to a meal. It is now recognized that adrenergic mechanisms may be involved in the stimulation of gastrin release. Intravenously administered adrenaline released gastrin in man, an effect suppressed by ß-adrenergic blockade (Stadil and Rehfeld, 1973). More recently, ß-adrenergic agonists were shown to stimulate gastrin release (Koop et al., 1982; Koop et al., 1983). These studies suggested that adrenergic agents may stimulate gastrin release by neuronal and hormonal pathways.

The role of the parasympathetic nervous system in the control of gastrin release appears to be complex. Part of the complexity arises from the dual role exhibited by the vagus nerve (stimulatory and inhibitory), and to differences which exist among species. In the rat, truncal vagotomy produced hypergastrinemia, which persisted in the isolated perfused stomach preparation (Pederson et al., 1981; Pederson et al., 1984). These experiments suggested the removal of an inhibitory mechanism, since immunocytochemical studies did not reveal any alterations in the G-cell population. It was also shown that atropine abolished the elevated <u>in vitro</u> gastrin secretion, suggesting that a muscarinic cholinergic mechanism was involved. On the other hand, electrical activation of the vagus nerve also enhanced gastrin secretion from the isolated perfused stomach (Nishi et al., 1985; Pederson et al., 1981), an effect that could be partially blocked by atropine and completely blocked by hexamethonium (Pederson et al., 1981). A similar effect with atropine was observed when the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP) was used as a stimulus (Schubert et al., 1982). These experiments suggest that both cholinergic and noncholinergic, post-ganglionic intramural neurons are responsible for neurally mediated gastrin release.

In the rat antral mucosa, somatostatin-containing cells have cytoplasmic processes that come in close contact with gastrin-containing cells (Larrson et al., 1979), suggesting an anatomical linkage between the two cell types. Evidence for a functional linkage between the two cell types receives further support from release studies. <u>In vitro</u>, somatostatin infusion inhibited basal gastrin release (Saffouri et al., 1980), and the addition of somatostatin antiserum to the perfusate produced a significant increase in gastrin secretion (Saffouri et al., 1979; Koop et al., 1988). In a similar study, an antisomatostatin monoclonal antibody (Soma 10), when infused concurrently with

somatostatin, neutralized the inhibitory effect of somatostatin on basal gastrin release (Tang, 1988). These experiments suggest that somatostatin can exert a tonic inhibitory effect on gastrin secretion, possibly through an endocrine or paracrine interaction. In contrast to the observations of these investigators, Chiba et al. (1981) found that their antisomatostatin antiserum was only effective in modulating gastrin release from antral mucosal scrapings, not from the isolated perfused stomach. They suggested that these results were more in line with a true paracrine effect for somatostatin.

In vitro studies in the rat have demonstrated that secretion of gastric somatostatin was inhibited by electrical stimulation of the vagi (McIntosh et al., 1981; Nishi et al., 1985) and by vascular perfusion of parasympathomimetics (Saffouri et al., 1980; McIntosh et al., 1981). In the same experiments, a concomitant increase in gastrin secretion was observed. These studies indicated that both gastrin and somatostatin secretion were in part regulated by vagal cholinergic mechanisms. A possible stimulatory pathway for gastrin secretion may, therefore, include direct cholinergic and noncholinergic intramural neurons. A second possibility is that through - noncholinergic or cholinergic influences on somatostatin, the restraint on gastrin release is eliminated, thus contributing to the gastrin response indirectly (Saffouri et al., 1980). Studies using atropine and hexamethonium in the rat perfused stomach have added to this complexity. Atropine infused during vagal stimulation reversed the inhibition of somatostatin and a stimulatory component became dominant (Nishi et al., 1985). Hexamethonium, however, reversed the inhibition without any stimulation (McIntosh et al., 1981). In has been postulated that vagal stimulation likely activated a post-ganglionic muscarinic inhibitory pathway and a non-muscarinic stimulatory pathway (McIntosh et al., 1985). In the rat, therefore, gastrin and somatostatin secretion are regulated, in part, by intramural neurons that are non-cholinergic and stimulatory.

The primary candidate for neuronally mediated atropine-resistant stimulation of gastrin and somatostatin secretion is gastrin releasing peptide (GRP). When infused vascularly into the perfused rat stomach, bombesin, the amphibian counterpart to GRP, caused the release of both gastrin and somatostatin (DuVal et al., 1981; Martindale et al., 1982). In addition, electrical stimulation of the vagal trunks <u>in vitro</u> in the rat resulted in GRP release with a concomitant

increase in gastrin and decrease in somatostatin release, the later response being reversed by atropine (Nishi et al., 1985). Atropine was shown not to have an effect on bombesin-stimulated gastrin or somatostatin release in the rat (DuVal et al., 1981). In the dog, GRP infusion during vagal blockade elevated postprandial gastrin secretion (Greenberg, 1987). Furthermore, Schubert et al. (1985) demonstrated that a major portion of the gastrin response to either DMPP or vagal stimulation could be reduced by antibombesin sera. These experiments were in support of a role for GRP in the control of gastrin and somatostatin secretion, acting directly and/or indirectly as an intramural excitatory neurotransmitter. Further support was gained by immunocytochemical studies, which demonstrated GRP immunoreactivity in neurons associated with the myenteric plexus, and nerve fibers in the mucosa of the corpus and antrum (reviewed in Walsh, 1987).

Elevated plasma gastrin and gastrin release from the perfused stomach have been demonstrated without any alteration in somastostatin levels (Pederson et al., 1981). Furthermore, it has been shown that somatostatin and gastrin secretion from the gastrointestinal tract can be differentially regulated (Martindale et al., 1982). In the dog, gastrin release by insulin-induced hypoglycemia and by feeding has been observed, both independent of any changes in plasma somatostatin (Greenberg, 1987). These experiments demonstrate that a functional linkage between somatostatin and gastrin does not always occur.

**C)** Long-Term Regulation

As previously mentioned, gastrin has at least two physiological roles, one in the control of gastrointestinal function, the other as a regulator of gastrointestinal growth and maintenance. In light of these important functions, it is not surprising that abnormalities in gastrin homeostasis can be accompanied by severe gastrointestinal disturbances (Mulholland and Debas, 1988). It is of importance, therefore, to gain an understanding of not only those factors which acutely regulate gastrin secretion, but also those which contribute to the long-term control of tissue and serum gastrin levels.

In the adult rat, fluctuations in gastrin secretion and tissue content are primarily related to the feeding state of the animal (Johnson and Guthrie, 1983). Early studies by Lichtenberger et al.

(1975) demonstrated that a 4-day fast dramatically reduced serum and antral gastrin concentrations when compared to an orally fed control group. Subsequent studies by other investigators, employing food deprivation techniques, confirmed these observations (Johnson and Guthrie, 1983; Koop et al., 1982; Schwarting et al., 1986). The population of G-cells has also been shown to be reduced in fasted rats (Lichtenberger et al., 1975; Bertrand and Willems, 1980). This reduction was shown to correlate well with decreases in antral gastrin levels during the course of starvation, but did not parallel changes in serum gastrin (Lichtenberger et al., 1975). Upon refeeding, serum gastrin levels had normalized by the sixth day; antral gastrin and G-cell number, however, did not recover for a further three days. These studies suggested that the secretory response of G-cells was not intimately linked to G-cell number or tissue gastrin content. A similar finding was made by Koop et al. (1982). In that study it was observed that a 3-day fast was associated with reduced gastrin secretion from the isolated perfused rat stomach under basal and stimulated conditions. It was also observed that the gastrin output from the perfused stomach was more reduced than antral gastrin concentration. Taken together, these studies suggest that, in the - rat, the presence of food in the GI-tract is necessary for the preservation of normal serum and antral gastrin concentrations. They also demonstrate that the dynamic responsiveness of G-cells does not simply reflect tissue gastrin content.

Starvation studies have provided valuable information regarding the importance of food in the preservation of normal gastrin tissue and serum levels, as well as in regulating appropriate gastrin responses to specific stimuli. Results from these studies must be viewed with caution, however, as starvation is also associated with a multitude of metabolic changes. Total parenteral nutrition provides an experimental situation that facilitates the study of changes in hormone release and gut morphology in the absence of luminal stimulation, while providing adequate nutritional support.

TPN has been shown to result in hypoplasia of the stomach, small bowel and pancreas in the rat (Johnson et al., 1975a, b). These studies also demonstrated reduced serum and antral gastrin levels in these animals. Similar findings have been made in other TPN studies on the rat (Track, 1980; Ryan et al., 1979). In those by Johnson et al. (1975b) and Ryan et al. (1979), the relative decreases in antral gastrin were only half as great as in serum gastrin. As in the situation of

starvation, these results suggested that G-cell secretory activity was more dramatically affected by the absence of nutritional stimuli than was antral gastrin tissue concentration.

#### D) Rationale for the Present Experiments

Currently there is little information available regarding the effect of TPN on gastrin secretion in response to specific stimuli in vivo or in vitro. Studies in man have demonstrated normal fasting and postprandial gastrin secretion after 25-days of TPN (Greenberg et al., 1981). In the dog, however, 3-weeks of TPN was associated with reduced fasting plasma gastrin levels, and a blunted response to a test meal (Odachi and Koga, 1987). These studies clearly point out that the effects of TPN on gastrin secretion are species dependent. No such studies regarding the effects of TPN on gastrin secretion during fasting or in response to specific stimuli have been reported for the rat.

Chapter four consists of studies designed specifically to examine the effects of TPN on gastrin secretion in the rat. First, the effects of 7-day TPN on gastrin release in response to oral peptone <u>in vivo</u>, and to electrical activation of the vagus nerve <u>in vitro</u>, were studied. Second, the effects of 3- and 7-days of TPN on gastrin secretion were compared to determine if gastrin hyposecretion was a progressive condition. Third, it was determined if G-cell hyposecretion associated with TPN was reversible. This was accomplished by refeeding TPN animals. Finally, the effects of bombesin on TPN-induced gastrin hyposecretion were examined. The purpose of this experiment was to determine if stimulants of gastrin release, when administered chronically, could prevent hyposecretion.

# **GENERAL METHODS AND MATERIALS**

# I) PEPTIDE QUANTIFICATION

# A) Radioimmunoassay (RIA)

Radioimmunoassay, a competitive protein binding assay, was employed to measure peptides in plasma and perfusate samples. This method, based on binding ligand to specific antibodies, was capable of measuring physiological levels of peptide hormones.

#### 1) <u>Calculations</u>

In all assays, separation of bound and free <sup>125</sup>I-peptide was accomplished using a charcoal separation method, and only free <sup>125</sup>I-peptide counted. Calculation of the percentage <sup>125</sup>I-peptide bound was done according to the following formula:

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

where C = counts per min; NSB = non-specific binding.

A standard curve was then constructed (fig. 2A) and the peptide content of the unknown samples was determined by reading percentage-bound from the standard curve to obtain peptide concentrations. Alternatively, a data reduction software package was used to transform data to a logit-log plot, and the concentrations of unknown samples were calculated given the counts per min per assay tube (fig. 2B).

#### 2) <u>Gastrin</u>

Immunoreactive gastrin (IRG) was measured using an RIA procedure described by Jaffe and Walsh (1978).

## a) <u>Iodination</u>:

The iodination procedure for synthetic human gastrin-1 (SHG-1) was a modification of the procedure by Stadil and Rehfeld (1972). Human synthetic gastrin-1 (Research Plus) was dissolved in 0.4M phosphate buffer (0.4M Na<sub>2</sub>HPO<sub>4</sub>, titrated to pH 7.4 with 0.4M NaH<sub>2</sub>PO<sub>4</sub>)



# Fig. 2

A: Gastrin standard curve %B vs. [Gastrin]. B: Data from the gastrin standard curve transformed to Logit-log plot.

to give a final concentration of  $0.5\mu g/\mu l$ . Synthetic human gastrin-1 was then incubated for 1 min with 0.2 mCi Na<sup>125</sup>Iodine and 10 $\mu$ l chloramine T (0.5 mg/ml in 0.04M phosphate buffer). The oxidation reaction was stopped by the addition of 10 $\mu$ l sodium metabisulphite (0.5 mg/ml in 0.04M phosphate buffer). The iodination mixture was then buffered by the addition of 500 $\mu$ l of imidazole buffer (0.05M, pH 7.5).

The procedure for purification of the <sup>125</sup>I-SHG-I was a modification of the method described by Brown et al. (1976). A DEAE Sephadex A25 column was prepared as follows: amino ethyl cellulose beads were washed and swollen in 0.05M imidazole buffer overnight. A 0.9 cm X 13 cm column was then poured and further equilibrated with the imidazole buffer. The iodination mixture was applied to the column and eluted with imidazole buffer (0.05M pH 7.5) and a 0-1M NaCl gradient at a flow rate of 1.0 ml/min. Fractions were collected at 1 min intervals, counted and an elution profile plotted. The 4 fractions after the second radioactive peak (purest<sup>125</sup>-SHG-I) were then tested in an RIA. Fractions producing acceptable standard curves were then pooled, dispensed as 2.0ml aliquots and stored at -20°C for use in the assay. On the day of the assay, <sup>125</sup>I-SHG-I aliquots were diluted with assay buffer to give a final concentration of approximately 2,000 cpm/100µl.

# b) Assay Buffer:

The stock assay buffer consisted of sodium barbital (0.02M) and RIA grade BSA (0.5% w/v). Concentrated HCl was used to adjust the buffer pH to 8.4.

c) Antisera:

Rabbit anti-SHG-I serum was obtained from Dr. G.J. Dockray, Liverpool, U.K. This antiserum (L2) contains a C-terminal directed antibody which binds G-17-I and II in equimolar amounts, and G-34 to a lesser extent (Dockray, et al., 1977). L2 did not cross react with physiological concentrations of CCK-8.

The lyophilized antiserum was dissolved in distilled water, diluted (1:500) with assay buffer and stored at -20°C as 1.0ml aliquots. For use in the assay 1 aliquot was thawed and further diluted in assay buffer to achieve a final concentration of 1:250 X  $10^3$ . At this dilution a zero binding of 40-50% was achieved and the assay was capable of measuring immunoreactive gastrin concentrations within a range of 6.25 to 400 pg/ml.

## d) Standards:

Synthetic human gastrin-1 was stored at -20°C in 200µl fractions at a concentration of 100 ng/ml. For use in the assay 1 fraction was diluted to 50 ml in assay buffer, then serially diluted to produce standards ranging from 6.25 pg/ml to 400 pg/ml. The standards were stored at -20°C as 1.0ml fractions, thawed and used in the assay for generation of a standard curve.

#### e) <u>Controls</u>:

Synthetic human gastrin-I was dissolved in assay buffer to give a final concentration of 100 pg/ml. Fractions were then stored as 1.0ml aliquots at -20°C. Gastrin controls were assayed in duplicate at the beginning and end of each assay to monitor intra-assay and interassay variability. Deviation in control value of  $\pm$  25 pg/ml from expected value (100 pg/ml) invalidated the assay.

# f) Sample Preparation:

Test assays were routinely done in order to determine if perfusate samples required dilution, i.e. concentrations greater than 400 pg/ml. An automatic diluting system (LKB Model 2075, Sweden) was used to dilute samples with assay buffer. When collected for gastrin radioimmunoassay, antral tissue samples were prepared according to the method described by Lichtenberger et al. (1975). Briefly, antral tissue was obtained after perfusion, frozen in liquid nitrogen and stored at -70°C until assayed for antral gastrin content. For the assay samples were weighed and then boiled in 2.0ml distilled water for 15 min. Tissue was then homogenized (5 min) and centrifuged (5 min, 1000Xg). The supernatent was diluted appropriately and assayed for gastrin using the same method described for perfusate samples.

#### g) <u>Non-Specific Binding (NSB)</u>:

Each assay contained non-specific binding tubes included to measure the binding of radioactive tracer to elements other than the antibody. Non-specific binding for each assay was accounted for in the calculation described in section I)A)1).

#### h) <u>Protocol</u>:

For each assay a separate standard curve was constructed using values calculated from total counts, NSB, standard and zero binding tubes in triplicate. Sample and control tubes followed

the standard curve and were done in duplicate. The composition of standard, sample and control tubes was 700 $\mu$ l diluent buffer, 100 $\mu$ l sample, standard or control, 100 $\mu$ l of antiserum and 100 $\mu$ l of <sup>125</sup>I-SHG-1 (2,000 cpm/100 $\mu$ l). The assays were then incubated for 48 h at 4°C before separation.

## i) Charcoal Extracted Plasma (CEP):

Outdated human plasma (Canadian Red Cross, Vancouver) was filtered (Size 4 filter paper, Whatman), then stirred with 1% activated charcoal (Norit) for 1 h. This mixture was centrifuged at 10,000 rpm for 30 min at 4°C and filtered twice before being dispensed into fractions. CEP was stored for up to 2 months at -20°C.

#### j) <u>Separation</u>:

Separation of bound and free peptide was accomplished by adsorption of free  $^{125}$ I-Gastrin to the dextran-coated charcoal matrix and exclusion of the larger antibody-antigen complexes from this matrix. Gastrin charcoal (12.5g activated charcoal, 2.5g dextran T-70 made up to 1.01 in 0.04M phosphate buffer, pH 6.5) was stirred overnight at 4°C. On the following day 7% CEP was added to the charcoal solution and the mixture stirred for an additional 1h before adding 200µl to each tube. The assays were centrifuged for 30 min at 3,000 rpm, decanted, and each tube counted for 3 min. The gastrin content of each tube was determined using the calculation described in section I)A)1).

# 3) Gastric Inhibitory Polypeptide (GIP)

The radioimmunoassay for GIP was a modification of the method previously described by Kuzio et al. (1974). To prevent adsorption of GIP to glassware, all glass tubes used in the iodination and assay were siliconized.

# a) <u>Iodination</u>:

Porcine GIP (15µg) was dissolved in 20µl 0.4M phosphate buffer (0.4M Na<sub>2</sub>HPO<sub>4</sub> titrated to pH 7.4 with 0.4M NaH<sub>2</sub>PO<sub>4</sub>) and added to 1mCi Na <sup>125</sup>I and 5µl chloramine T (0.5 mg/ml) for 20 sec. The oxidation reaction was stopped by the addition of 10µl sodium metabisulphite (200 mg/ml).

Purification of the <sup>125</sup>I-GIP was accomplished by gel filtration on a G-25 Sephadex column

(0.9 X 10 cm). The column was prepared and equilibrated with 0.2M acetic acid containing 0.5% bovine serum albumin (BSA) and 2.0% Trasylol (proteolytic enzyme inhibitor). The iodination mixture was applied to the column and eluted at a flow rate of 24 ml/h. Fractions of 400µl volume were collected and 10µl aliquots of each fraction were counted for 0.1 min on a 1285 gamma-spectophotometer (Searle Inst., Ill.). The column profile was plotted: counts/10µ1/0.1 min against fraction number. Two peaks were obtained; the first peak contained the <sup>125</sup>I-GIP fraction and the second <sup>125</sup>Iodine. A damage assay was then performed across the first peak fractions. In this assay, samples were diluted to 5,000 cpm/100µl and 100µl added to 900µl of diluent buffer (0.04M PO<sub>4</sub> pH 6.5, 1.5% trasylol and 5% charcoal extracted plasma). Charcoal separation was carried out by the addition of 200µl of dextran coated charcoal (1.25g activated charcoal, 0.25% dextran T-70 in assay buffer with 5% CEP) followed by centrifugation (3,000 rpm, 10°C, 30 min). The supernatant was decanted and counted along with the pellet. The fractions exhibiting the best <sup>125</sup>I-GIP adsorption to charcoal were first pooled, then diluted 1:1 with eluant buffer and acid ethanol (1,500 ml ethanol; 500 ml H<sub>2</sub>O; 30 ml concentrated HCl) - so that a final activity 2.5 X 10<sup>6</sup>cpm/100µl was obtained. The <sup>125</sup>I-GIP was stored at -20°C for up to 6 weeks.

# b) Assay Buffer:

A stock solution of 0.4M phosphate buffer was prepared from  $0.4M \text{ NaH}_2\text{PO}_4$  titrated to pH 6.5 with 0.4M Na<sub>2</sub>HPO<sub>4</sub>. On the day of the assay, the stock solution was diluted to 0.04M containing 5% CEP and 1.5% trasylol.

# c) Antiserum:

Rabbit anti-GIP serum (Gö5, 28/4/75) was raised against porcine GIP conjugated to BSA by the carbodiimide method and injected as an emulsion with Freund's complete adjuvant. The antiserum was stored lyophilized in 200µl aliquots. Fractions were diluted in assay buffer to give a final dilution of 1:30 X 10<sup>-3</sup> yielding a zero binding of 25-30%. The assay was capable of measuring IR-GIP within the range of 0.125 to 4.0 ng/ml.

# d) <u>Standards</u>:

Porcine GIP (purified according to the methods of Brown et al., 1970, and Brown, 1971)

was weighed on a Cahn-25 microbalance (Cahn Instruments, California) and dissolved in 0.2M acetic acid with 0.5% BSA and 2.0% trasylol. This mixture ( $1\mu g/100\mu l$  GIP) was lyophilized and stored at -20°C. For use in the assay 1 fraction was dissolved in 12.5 ml assay buffer, and then serial dilutions were made to provide standards from 0.125 to 4.0 ng/ml

## e) Controls:

Controls, which were included in each assay, were prepared by dissolving gut extract in phosphate buffer (0.4M), pH 6.5 containing 20,000 KIU trasylol per 100 ml and 0.5% plasma to a concentration of 1,000 pg/ml. Fractions of this extract were stored at -20°C. Duplicate control tubes were inserted in each assay as a measure of intra- and interassay variability. Deviation of more than 10% from expected control values invalidated the assay.

#### f) <u>Protocol</u>:

Each assay was composed of total counts, NSB, zero binding and standard tubes in triplicate. The control and sample tubes were assayed in duplicate. The composition of standard, control and sample tubes was: 700µl diluent buffer, 100µl standard, control or sample, 100µl antiserum, and 100µl <sup>125</sup>I-GIP (5,000 cpm/100µl). Assays were incubated for 48 h at 4°C.

#### g) <u>Separation</u>:

Charcoal separation was as previously described for gastrin radioimmunoassay in section I)A)2)j).

# 4) Glucagon

# a) Iodination:

Iodinated pancreatic glucagon (<sup>125</sup>I-glucagon) was purchased from NEN Research Products and stored at 4°C upon arrival. The <sup>125</sup>I-glucagon was in solution with n-propanol, Tris buffer containing bovine serum albumin, trasylol and glycine and for use in the assay <sup>125</sup>I-glucagon was diluted with assay buffer so that 100µl contained 2,000 cpm.

# b) Assay Buffer:

A stock solution of 0.6M phosphate buffer pH 7.4 was prepared by titrating 0.6M NaH<sub>2</sub>PO<sub>4</sub> against 0.6M Na<sub>2</sub>HPO<sub>4</sub>. For use in the assay EDTA (0.30%, w/v), BSA (0.30% w/v), and

Trasylol (5.0% v/v) were added and the buffer diluted to 0.06M with distilled water.

#### c) Antiserum:

An antiserum (R-50) raised in a rabbit against porcine glucagon was used in this assay and was a gift from Dr. S.R. Bloom, London. This antiserum possessed C-terminal specificity reacting with whole porcine glucagon but not with N terminal fragments. There was slight cross reactivity (2%) with human intestinal glucagon-like immunoreactivity (GLI) but not with other structurally related peptides of the secretin family. The serum was obtained in lyophilized form reconstituted in distilled water and stored in 10 $\mu$ l aliquots. Antiserum was then diluted (1:1,200) with assay buffer, to achieve a final dilution in the assay of 1:12,000. Zero binding of this antibody was typically 35-40%. At such a dilution IR-glucagon could be detected within the range of 12.5pg/ml to 400pg/ml.

# d) <u>Standards</u>:

Pure pancreatic glucagon (Sigma) was dissolved in assay buffer and stored at -20°C as fractions containing 1 ng/vial. To construct a standard curve the frozen fractions were diluted appropriately with assay buffer to give a range of concentrations from 12.5 to 400 pg/ml.

## e) <u>Protocol</u>:

Each assay consisted of total counts, NSB, zero binding and standard tubes in triplicate, and sample and control tubes in duplicate. The composition of the standard, sample or control tubes was 700 $\mu$ l diluent buffer, 100 $\mu$ l sample, control or standard and 100 $\mu$ l antiserum. Assay tubes were incubated for 48 h at 4°C. <sup>125</sup>I-glucagon (2,000 cpm/100 $\mu$ l) was then added to all the tubes and incubation continued for an additional 48 h.

# f) Separation:

Separation of bound from free <sup>125</sup>I-glucagon was by the dextran-coated charcoal method. Charcoal (12.5g activated charcoal, 2.5g dextran T-70 made up to 1.01 in 0.04M phosphate buffer, pH 6.5) was stirred overnight. Prior to use, 5% CEP was added to the mixture and stirred for an additional 30 min, and 500µl added to each assay tube. The assays were centrifuged at 3,000 rpm, 4°C for 30 min, decanted, and each tube counted for 3 min. The immunoreactive glucagon content of each sample was calculated as described in section I)A)1).

# 5) Insulin

#### a) <u>Iodination</u>:

In preparation for the iodination, all glass tubes were siliconized to prevent adsorption of the peptide to the glass. Phosphate buffer was prepared from a 0.4M stock solution (0.4M  $Na_2HPO_4$  titrated to pH 7.5 with 0.4M  $NaH_2PO_4$ , stored at 4°C) for use in the insulin iodination. Porcine insulin (Novo Pharmaceuticals) was dissolved in 10µl 0.01M HCl, then diluted to a final concentration of 5µg/10µl in 0.2M phosphate buffer.

Ten microliters of the insulin were added to a reaction vessel along with 1mCi <sup>125</sup>Iodine and 25µl chloramine T (4 mg/ml in 0.2M phosphate buffer). The reaction was allowed to proceed for 10 sec at which time 100µl sodium metabisulphite (2.4 mg/ml in 0.2M phosphate buffer) was added to stop the oxidation reaction. After 45 sec, 50µl of sodium iodide (10 mg/ml in 0.2M phosphate buffer) was added, and the reaction mixture was further diluted with 1.8ml 0.04M phosphate buffer.

Purification of <sup>125</sup>I-insulin was by adsorption onto 10 mg of microfine silica (QUSO G-32) in a total volume of 2.0ml 0.04M PO<sub>4</sub> pH 7.5. The mixture was mixed thoroughly and centrifuged (1,000 rpm). The <sup>125</sup>I-insulin was adsorbed onto the silica while the free iodine in the supernatant was discarded. The pellet was washed with 3.0ml distilled water (3 times) to ensure complete removal of the <sup>125</sup>I. Iodinated insulin was eluted from the silica using 3.0ml acid ethanol (1,500 95% ethanol, 500 ml distilled water, 30 ml concentrated HCl) and was stored at -20°C following addition of a further 2.0ml acid ethanol and 1.5ml of distilled water. The incorporation of the <sup>125</sup>Iodine into the peptide was calculated by counting 10µl fractions of the dilute <sup>125</sup>I-insulin, the QUSO pellet and the original iodination mixture in QUSO prior to centrifugation. The following formula was used to measure % incorporation:

# % Incorporation = $\frac{\text{Acid ethanol supernatant} + \text{OUSO pellet}}{\text{Total}^{125}\text{I}}$ (supernatant 1, 2, 3) + pellet

## b) Assay Buffer:

The stock buffer was 0.4M PO<sub>4</sub> pH 7.5 made as described in section I)A)5)a). Stock buffer was diluted 1:10 with distilled water and 5% charcoal extracted plasma for use in the assay.

#### c) Antiserum:

Guinea pig anti-insulin serum (GPO1) was raised against unconjugated porcine insulin in this laboratory. Lyophilized 100µl fractions of 1:10 diluted serum were made up to 50 ml in assay buffer and stored at -20°C as 1.0ml fractions (1:5,000). For use in the assay these fractions were further diluted 1:20 so that the final dilution in the assay was 1:1 X 10<sup>6</sup>, giving a zero binding of 40-55%. The antiserum at this dilution detected IR-insulin within a range of 2.5  $\mu$ U/ml to 160  $\mu$ U/ml (0.125 ng/ml-7.5ng/ml).

#### d) <u>Standards</u>:

Pure rat insulin (100µg) was obtained from Novo Pharmaceuticals (Denmark) in lyophilized form. Insulin was dissolved in distilled water and made up to a concentration of 200 ng/ml in phosphate buffer, pH 7.5 (5.77g Na<sub>2</sub>HPO<sub>4</sub>, 1.05g NaH<sub>2</sub>PO<sub>4</sub>, 60g BSA, 6.0g sodium merthiolate, 6.0g NaCl diluted to 1.0 l with distilled water) and stored at -20°C as 1.0ml fractions. For use in the assay 1 fraction was diluted with assay buffer to achieve a final concentration of 160  $\mu$ U/ml and refrozen. To construct a standard curve serial dilutions of the fractions were made to achieve a concentration range from 5  $\mu$ U/ml to 160  $\mu$ U/ml.

#### e) <u>Controls</u>:

Control tubes were included at the beginning and end of each assay to assess intra- and interassay variability. Controls were prepared from perfusate pooled from a perfused rat pancreas experiment in which the stimulus was 300 mg/dl glucose and 10 mM L-arginine-HCl. The perfusate was diluted in assay buffer to give a final concentration of 40  $\mu$ U/ml or 60  $\mu$ U/ml then stored as 1.0ml fractions at -20°C. Any deviation in the control values by ±10  $\mu$ U/ml resulted in invalidation of the assay results.

# f) Sample Preparation:

Test assays were done for each set of experiments to determine if dilution of plasma or perfusate samples was required. All samples assayed were within the standard curve range (5-160  $\mu$ U/ml).

## g) Protocol:

Each assay consisted of total counts, NSB, zero binding, standard and sample tubes.

Samples were assayed in duplicate except for the standard curve which was routinely done in triplicate. The composition of the standard, sample and control tubes was 700 $\mu$ l assay buffer, 100 $\mu$ l of antiserum (1:1 X 10<sup>6</sup> final). Assays were incubated for 24 h at 4°C at which time 100 $\mu$ l of <sup>125</sup>I-insulin (10,000 cpm/100 $\mu$ l) was added to each tube and the incubation continued for an additional 24 h.

# h) Separation:

Dextran-coated charcoal (5.0g dextran T-70, 50g activated charcoal) was made up to 1.01 in 0.04M phosphate buffer pH 7.5. This mixture was stirred overnight at 4°C then added to each assay tube (200µl), centrifuged for 30 min at 10°C and the supernatant decanted The pellet was dried overnight then counted on a Tracor 1290 gamma counter and results calculated as previously described in section I)A)1).

# 6) <u>Somatostatin</u>

# a) Iodination:

The iodination of somatostatin was based on the procedure described by McIntosh, et al. (1978). Synthetic tyr-1-somatostatin (Peninsula) was dissolved in 10µl of 0.5M phosphate buffer, pH 7.4. To this mixture 1mCi<sup>125</sup>I and 10µl chloramine T (20 mg/ml in 0.05M phosphate buffer, pH 7.4) were added. The reaction was allowed to proceed for 30 sec and stopped by the addition of 10µl sodium metabisulphite (5 mg/ml in 0.05M phosphate buffer).

Primary purification was accomplished as follows: the iodination mixture was added to 20 mg QUSO in 1.0ml CEP, vortexed and centrifuged for 3 min. The supernatant was discarded and the pellet washed with 1.0ml distilled water and centrifuged; this step was carried out twice. The <sup>125</sup>I-somatostatin was eluted from the pellet by addition of 1.0ml acetic acid / acetone (100µl glacial acetic acid, 3.9ml acetone, in 4.0ml of distilled water) and centrifuged for 20 min. Incorporation of <sup>125</sup>I into somatostatin was determined by the same method described for insulin in section I)A)5)a).

The supernatant was diluted to 1 X 10<sup>7</sup> cpm/100µl with 0.1M acetic acid containing 0.1% BSA (previously tested for interference in the assay) and dispensed into 100µl aliquots. Fractions were lyophilized and stored at -20°C. Secondary purification was done on the day of the assay to remove fragments of <sup>125</sup>I-somatostatin. A Sephadex CM-52 (Pharmacia) column (0.9 X 10 cm) was washed with the elution buffer (0.2M ammonium acetate, pH 4.6) and then equilibrated with 0.002M ammonium acetate pH4.6 at a flow rate of 1 ml/min. One aliquot of <sup>125</sup>I-somatostatin was dissolved in 0.002M ammonium acetate buffer and applied to the column. The column was then washed with the equilibration buffer for 20 min. The <sup>125</sup>I-somatostatin was then eluted from the column with the elution buffer. Two milliliter fractions were collected, counted and an elution profile plotted. For the assay, the peak fraction was taken and neutralized in 2.0M NaOH before diluting to 3,000 cpm/100µl with assay buffer.

## b) Assay Buffer:

A stock solution of somatostatin RIA buffer containing 4.9g sodium barbital (24mM), 0.32g sodium acetate (4.0mM), 0.1g merthiolate (0.25mM) and 2.6g sodium chloride (44mM), pH 7.4, was prepared and stored at 4°C. For use in the assay, 0.5% BSA (Miles) and 1% Trasylol were added to the buffer.

#### c) <u>Antibody</u>:

A monoclonal antibody, SOMA 03, was used in the somatostatin RIA. Soma 03 was raised against cyclic somatostatin 14 (Buchan, et al., 1985) and was used in this assay as described by McIntosh et al. (1987). Crude ascites containing Soma 03 was stored at -20°C. When required, a fraction of the ascites was thawed, passed through a sterile  $0.45\mu$ m filter (Millipore Corp., Bedford, Mass.), diluted 1:1 in a diluent solution (0.9% saline, 0.1% sodium azide, 0.1% BSA) and stored at -70°C as 50 $\mu$ l aliquots. On the day of the assay Soma 03 was diluted in assay buffer and used at a final dilution of 1:5 X 10<sup>6</sup>. Under these conditions somatostatin-like immunoreactivity (SLI) could be measured within the range of 3.9 to 500 pg/ml. Soma 03 recognizes cyclic somatostatin 14, linear somatostatin 14 (SS-14) and somatostatin-28 (McIntosh, et al., 1987) and is directed against antigenic determinants in the central region of SS-14. Soma 03 does not cross react with gastrin, motilin, or GIP (Buchan, et al., 1985).

#### d) <u>Sample Preparation</u>:

One milliliter samples for SLI determination were collected in chilled tubes containing 1,000 KIU Trasylol. Tubes were stored at -20°C and thawed at 4°C on the day of the assay.

# e) Standards:

Synthetic cyclic somatostatin (Peninsula) was dissolved in 0.1M acetic acid containing 0.05% BSA and dispensed as  $50\mu l$  ( $10\mu g$ ) fractions, lyophilized and stored at  $-20^{\circ}$ C. On the day of the assay 1 fraction was dissolved in 1.0ml assay buffer and serial dilutions made to obtain standards ranging from 3.9 pg/ml to 250 pg/ml.

# f) Assay Protocol:

Each assay consisted of total counts, non-specific binding and standard tubes assayed in triplicate and sample and control tubes in duplicate. Standard, control, and sample tubes contained 100µl of assay buffer, 100µl of standard, control or sample, 100µl of antiserum and 100µl <sup>125</sup>I-somatostatin. The assay was incubated at 4°C for 72 h.

## g) Separation:

Charcoal was prepared by stirring 0.25% dextran T-70 and 1.25% activated charcoal in 0.05M phosphate buffer pH 7.5 for 1 h, at which time CEP was added (100µl CEP /100ml charcoal solution) and the mixture stirred for an additional 30 min. Each assay tube received 1.0ml of the charcoal solution and was centrifuged for 30 min at 3,000 rpm. The supernatant was discarded and each tube counted for 3 min on a Tracor 1290 gamma counter. Concentrations of somatostatin-like immunoreactivity were calculated using the formula described in section I)A)1).

# II) ANIMAL STUDIES

## A) TPN-TEN Methodology

# 1) Animals

Male Wistar rats (Charles River or Animal Care, U.B.C.) weighing 200-325g were used in all experiments. Animals were initially housed in metal cages (5-6 rats/cage) in a light controlled (12 h cycle), temperature controlled (24°C) room. During the 1 week acclimation period, all animals were allowed free access to water and rat chow (#5012, Ralston Purina). Prior to surgery animals were fasted for 12 h.

# 2) Surgical Procedures

The surgical procedures were designed to produce as little trauma as possible and were done under strict aseptic conditions promoting rapid recovery and minimal infection.

# a) Intravenous Catheterization for TPN Animals:

Central intravenuous catheterization provides an avenue for the safe delivery of a highly concentrated diet Rats were anesthetized intraperitoneally (IP) with pentobarbital (30mg/kg) following a 12 h fast and placed on a heating pad set at 38°C. The neck and midscapular areas were shaved and sterilized with a topical antiseptic (Proviodine<sup>®</sup>). Instruments and materials used in this procedure were first boiled and then placed in 70% ethanol prior to surgery. A small incision (1 cm) was made just above the right clavicle; the external jugular vein was carefully isolated and was doubly ligated with 000 silk (Ethicon<sup>®</sup>) 1.0 cm above the superior vena cava. A venotomy was performed between the 2 ligatures and a sterilized catheter (10cm, Silastic<sup>®</sup>) medical grade tubing, 0.635 mm ID X 1.19m OD) filled with heparinized saline (0.9% sodium chloride, Travenol<sup>®</sup>, 50 U/ml heparin ) was inserted and advanced 2.0-2.5cm inferiorly into the superior vena cava. The final catheter placement was approximately 0.5cm from the heart and was secured by the two separate ligatures. The rat was turned onto its abdomen and a small incision made in the skin at the midscapular region. A subcutaneous tunnel was made using fine point hemostats and the free end of the catheter was brought through the tunnel and exteriorized in the midscapular region. Tissue glue (Histocryl<sup>®</sup>) was then used to further secure the fixed end of the catheter, the ventral wound was sprayed with a topical antibiotic (Neosporin<sup>®</sup>) and closed with wound clips (Autoclip<sup>®</sup>, Clay Adams). The free end of the catheter was passed through a sterile stainless steel anchor button (Instech Laboratories) which was secured under the skin by wound clips after the incision area was sprayed with topical antibiotic.

# b) Intragastric Catheterization for TEN Animals:

The midscapular and abdominal areas were shaved and sterilized with a topical antiseptic. The abdomen was opened along the mid-line (linea alba) and the stomach carefully isolated. A small (2mm) incision was made in the non-glandular fundic portion of the stomach and a 15cm catheter (Silastic<sup>®</sup> medical grade tubing, 0.635mm ID X 0.94mm OD with a 1cm bumper made from P.E. tubing) was placed into the lumen of the stomach and secured with a purse string suture (Ethicon<sup>®</sup> 3.0 mersilene). The free end of the catheter was tunneled subcutaneously using fine-point hemostats to an incision made in the midscapular region. Before the abdominal wound was closed, the catheter was further secured to the stomach with tissue glue and the wound sprayed with a topical antibiotic. The muscle layer was sutured with 3.0 mersilene (Ethicon<sup>®</sup>) and the skin incision closed with Autoclips<sup>®</sup>. The animal was then turned onto its abdomen and the free end of the catheter passed through an anchor button as described in section IIA2a.

# c) <u>Preparation of the Infusion Apparatus</u> (Figs. 3A and B):

The infusion apparatus provided a method for infusing TPN/TEN solutions and withdrawl of blood samples from unrestrained rats. After IV or intragastric (IG) catheter placement, the external end of the catheter was connected to a heavier grade tubing (0.51mm ID X 1.5mm OD polyvinyl tubing, Cole Parmer) by a stainless steel connector (20 gauge). The catheter was then passed through a flexible stainless steel spring catheter shield (Instech Laboratories) attached to the anchor button, and secured by a small stainless steel collar (Instech Laboratories).

The flexible spring guard and the catheter tubing were connected to a rotary swivel (model 375/20, Instech Laboratories, Philadelphia, PA) mounted on top of a wire mesh cage designed to prevent coprophagia (23 X 23 X 28cm). The swivel allowed the animal to move freely about the cage. A 1.0ml syringe filled with heparin-saline was attached to a 22 gauge needle (Lure- lock, Becton Dickson) and connected to the swivel by 10cm polyvinyl tubing (0.51mm ID X 1.5m OD, Cole Parmer). While the animals were recovering from surgery (1-2 days) the catheters were kept patent with heparinized saline (50U/ml). During the recovery period rats were allowed free access to water and rat chow (#5012). The animal room was on a 12 h light cycle (700h-1900h) and kept at 22°C.

d) Diet Delivery System (Figs. 4 and 5):

Intravenous and intragastric solutions (described in detail in section II)A)3)a) and b)) were added to a sterile 100 X 60cm non-vented Soluset<sup>®</sup> burette (Abbott Ireland Ltd., Donegal, Ireland). This burette was equipped with two injection ports for the introduction of other agents,





Infusion apparatus for intragastric and intravenous feeding.



bacteria retentive air filters, and a diaphragm which prevented air from entering the circuit. The burette was attached to an I.V. extension set (Size "B" Chamber, model 0210, Critikon Inc., Tampa, FL) equipped with a peristaltic pump chamber, a Y injection site, and a male lure lock adapter.

Through the adapter, the system was connected to an I.V. filter (#0460, Critikon Inc.) which was equipped with a vent system to remove air bubbles from the TPN/TEN solutions and a 0.22 µm filter which prevented bacteria from entering into the system. Before the diet delivery system was connected to the infusion apparatus, the system was filled with the TPN/TEN solution and all air bubbles were removed. The pump chamber was then attached to a Holter infusion pump (model 903, Extracorporeal Medical Specialties, King of Prussia, PA ) and the free end of the system connected to the swivel.

# 3) Intravenous (TPN)/Intragastric (TEN) Solutions

The TPN/TEN solutions were designed specifically for the rat to promote modest weight gain and maintain the animal in a proper nutritional state over the infusion period. The solutions used -- in this study were a modification of a solution system designed and used by Innis and Boyd (1983).

a) <u>TPN/TEN Solution I</u> (See Appendix III):

Solution I contained all the essential amino acid, carbohydrate, vitamin and mineral requirements for the animals. This solution was prepared using strict aseptic techniques under a laminar flow hood in the following manner. First, 50 ml of dextrose solution was removed from a DW50 partial fill Viaflex<sup>®</sup> bag. To the remaining portion, 375 ml of 10% Travasol (amino acids), 0.3ml Trace-4<sup>®</sup> (minerals) and 3.0ml M.V.C. 9+3 (vitamins) were added. After thorough mixing, calcium gluconate (10 ml), sodium chloride (8.0ml), magnesium sulfate (0.2ml), potassium phoshate (5.0ml) and potassium chloride (35 ml) were added. If solution I was to be stored for more than 1 week, the vitamins were added just prior to use. The pH of the solution was 6.1 with an osmolality of 1600 mOsm/kg. Fresh TPN/TEN solution I was added to burretes each day and delivered to the animals at a flow rate of 9.2 ml/kg/hr for the length of the infusion period.



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## b) TPN/TEN Solution II:

Solution II (20% Soyacal<sup>®</sup>) provided the essential free fatty acid requirements to the animals. The solution had an osmolality of 315 mOsm/kg and a pH of 7.1 and was administered as a bolus injection (2.0ml) twice per day (1100h and 1700h).

c) Orally Fed Animals:

Animals fed orally (ORAL) were surgically prepared and harnessed in an identical manner as TPN or TEN animals. ORAL animals were fed rat chow (#5012, Ralston Purina, 100g/kg/day) ad libitum and, like TPN and TEN animals, were allowed free access to fresh water.

#### **B)** Isolated Perfused Organ Preparations

The isolated vascularly perfused rat pancreas and stomach provided <u>in vitro</u> models to access the effects of total parenteral nutrition on pancreatic and gastric hormone release.

1) Solutions and Reagents

a) Krebs Concentrate:

A concentrated stock solution (10X) of the following composition was prepared and stored at 4°C for up to 1 month:

285.0 ml	KCl	(154.0 mM)
243.0 ml	CaCl <sub>2</sub>	(103.0 mM)
78.0 ml	MgSO <sub>4</sub> ·7H <sub>2</sub> O	(154.0 mM)
97.0 ml	KH <sub>2</sub> PO <sub>4</sub>	(154.0 mM)

b) Krebs-Ringer Bicarbonate Perfusate:

The perfusate provided a substitute for whole blood so that basal peptide release from isolated organs could be assessed. Dextran (3%) and BSA (0.2%) were first dissolved in saline (154 mM) and stirred overnight at 4°C. On the day of the perfusion, NaHCO<sub>3</sub> (13.0g dissolved in  $11 H_2O$ ) and Krebs concentrate (Sec. IIB1a) were added in the appropriate volumes to obtain the desired final concentration in the perfusate.

A stock solution of glucose was prepared from a commercial 50% dextrose solution (55 ml in 500 ml  $H_2O$ ) and the desired amount added to give the appropriate final glucose concentration.

The perfusate was made up to its final volume by the addition of saline. The glucose concentration of the perfusate was then checked (Beckman Glucose Analyzer II, Beckman Instrument Inc., Fullerton, Ca.). Osmolality and pH of the solution were periodically checked and found to be 280-285 mOsm/kg and pH 7.4 respectively. The final composition of the perfusate was as follows:

KCI	4.4 mM	
CaCl <sub>2</sub>	2.5 mM	
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.2 mM	
KH <sub>2</sub> PO <sub>4</sub>	1.5 mM	
NaHCO <sub>3</sub>	25.0 mM	
Dextran	3.0 %	
BSA	0.2 %	
NaCl	120.0 mM	
Glucose	80.0, 160.0 or 300.0 mg/dl	

c) Drugs and Peptides:

Physiological and pharmacological agents were either directly added to the perfusion medium or introduced just before entry into the organ preparation by sidearm infusion. Peptides were dissolved in 0.01M acetic acid and made up to the desired concentration with distilled water. Agents introduced via sidearm infusion were diluted from stock solutions in perfusate buffer. The final desired concentration was determined given the infusate (0.2 ml/min) and the perfusate (3-4 ml/min) flow rates. Sidearm infusions were delivered by a Harvard syringe infusion pump (model 22, Ealing Scientific, St. Laurent, Quebec).

Square wave changes in perfusate glucose (and peptides) delivered to the pancreas were accomplished using a dual channel peristaltic pump (Holter model 903) and flasks containing differing concentrations of glucose. While the perfusate from one flask passed through the pancreas, the perfusate from the other flask was recirculated. A change in glucose concentration was then achieved rapidly by interchanging the two input lines at the level of the bubble trap. Glucose and peptides were also introduced to the pancreas as a linear gradient. A gradient was produced by adding the glucose/peptide to the distal flask of two flasks equal in cross-sectional area, connected in series.

#### d) Electrical Stimulation:

The vagus nerve at the level of the esophagus has branches which innervate both the stomach and the pancreas. Through this nerve, neuronal stimulation of the <u>in vitro</u> preparations was accomplished. Bipolar electrodes (Harvard Sci.) were placed around the anterior vagal trunks which were then electrically stimulated (10 Hz, 7V, and 5msec. duration) using a Grass stimulator (Model S6C, Grass Instrument Co., Quincy, Mass.).

# 2) <u>Perfusion Apparatus</u>

The perfusion apparatus is illustrated in figure 6. The perfusate was stirred and continuously gassed with 95%  $O_2$  and 5%  $CO_2$  which was saturated with water vapour by bubbling through a water reservoir. The perfusate was delivered to a heating block by a peristaltic pump (Holter, model 921). The heating block maintained the perfusate at 37.5°C by a servo-feedback control mechanism. A temperature probe was used to ensure proper maintenance of the perfusate temperature. A filter bubble trap device removed air bubbles and particulate matter from the perfusate before being introduced into the organ preparation. The perfused organ was maintained at 37°C by the heating block, aided by a 60 watt desk lamp situated over the preparation. Plastic wrap was placed over the isolated organ preparation to prevent temperature fluctuations caused by circulating air, and moisture loss.

The perfusion pressure was monitored and maintained between 40-60 mmHg. If the pressure was above this level and could not be normalized the preparation was not used.

# 3) Surgical Preparation

Isolation of the vascularly perfused rat pancreas was a modification from Penhos et al. (1969).

#### a) Anesthesia:

Animals were first anesthetized by either an intraperitoneal or intravenous injection with sodium pentobarbital (60 mg/kg, Somnitol<sup>®</sup>).

b) Isolated Perfused Pancreas:

After the appropriate level of anesthesia was achieved, a midline incision was made along the <u>linea alba</u> from the sternum to the pelvis. The left renal artery and vein and the adrenal artery





were doubly ligated and sectioned. The abdominal aorta and inferior vena cava were located and the associated connective tissue around these vessels cleared. Two loose ligatures were placed around the aorta inferior to the superior mesenteric artery and one above the celiac artery. A loose ligature was then placed around the portal vein and a second more rostral around the portal vein, artery and bile duct. A drainage tube was inserted into the duodenum inferior to any pancreatic connections, adjacent to the ligament of Treitz. The mesenteric blood vessels and lymphatics were doubly ligated from the duodenum to the ileo-cecal junction. The anterior mesenteric artery and vein at the level of the cecum was singly ligated. The colon was then doubly ligated caudal to the cecum and the isolated intestine removed.

The connective tissue around the spleen was removed and the vascular connections between the spleen and pancreas were singly ligated. After removal of the spleen, the esophagus and associated artery were doubly ligated and the vascular connections between the stomach and pancreas along the greater curvature were singly ligated. A double ligature was placed around the duodenum just below the pylorus and the stomach was removed. The right adrenal artery, renal vein and artery were singly ligated.

In preparation for cannulation, the aorta was clamped below the superior mesenteric artery, allowing vascular perfusion of the pancreas to continue. An arterial cannula (PE 190) filled with heparinized saline was placed in the abdominal aorta and advanced to the level of the superior mesenteric artery. The cannula was secured by the two arterial ligatures and the arterial clamp removed. The remaining loose ligature above the celiac artery was tied and 1.0ml of heparinized saline was injected into the remaining vasculature causing a blanching of the pancreas.

The animal was hemi-sectioned at the level of the diaphragm and the portal vein cannulated. The arterial cannula was connected to the perfusion apparatus and flow began immediately. Perfusion of the pancreas was via the superior mesenteric and celiac arteries and venous drainage collected from the portal vein into chilled test tubes.

Each experiment began with a 10 min equilibration period in the presence of basal glucose (80 mg/dl). The equilibration period ensured minimal effects of surgical trauma on secretory rates, but was also a period where minor adjustments in flow rate and temperature were made if

necessary. Under ideal conditions the flow rate was 4 ml/min, and 1 min fractions were collected using a fraction collector (model 7000, L.K.B. Inc., Sweden).

## c) Isolated Perfused Rat Stomach:

The isolated vascularly perfused rat stomach preparation was a modification of a procedure used by Lefebvre and Luyckx (1977). The left renal artery and vein and the adrenal artery were doubly ligated. Three loose ligatures were placed around the abdominal aorta, two inferior and one superior to the celiac artery. The descending colon was doubly ligated and freed from connective tissue to the level of the superior mesenteric artery. A loose ligature was placed around the portal vein and a second more rostrally around the portal vein, and bile duct. The vessels between the spleen and pancreas were ligated and the spleen removed. Vessels between the stomach and pancreas were doubly ligated to the level of the gastro-epiploic arteries. A drainage tube was inserted into the pylorus after the duodenum had been doubly ligated. The vagi were isolated sub-diaphragmatically at the level of the esophagus and marked with a loose ligature. The right renal artery and vein, the adrenal artery and the superior mesenteric artery were ligated. The pancreas, small bowel and colon were then removed. An arterial cannula was placed into the aorta, advanced to the level of the celiac artery and secured. The animal was hemi-sectioned at the level of the diaphragm, the portal vein cannulated, and the isolated stomach blanched with heparin-saline. The organ was perfused via the celiac artery at a flow rate of 3 ml/min and the venous drainage collected every min using a fraction collector. A 20 min equilibration period preceded the experimental period at which time minor adjustments in flow rate and temperature were made.

## d) Isolated Perfused Pancreas-Stomach Preparation:

The isolated perfused pancreas-stomach preparation, a novel <u>in vitro</u> model, was based on the perfused rat pancreas preparation described by Penhos et al. (1969). This preparation was used for two specific reasons. First, it provided an experimental situation whereby parasympathetic nervous control (vagus nerve) of insulin secretion could be studied. In the perfused rat pancreas model it is extremely difficult to identify vagal branches; in the pancreasstomach preparation however vagal trunks can easily be identified and electrically stimulated at

the level of the esophagus. Second, the perfused pancreas-stomach preparation allowed both gastric and pancreatic hormone release to be studied simultaneously.

The procedure for surgical isolation was as follows. The left renal artery and vein were doubly ligated. Three loose ligatures were placed around the aorta, two inferior to the superior vena cava and one placed cephalad to the celiac artery. The descending colon and associated artery were singly ligated. The duodenum was doubly ligated inferior to the pancreas and a drainage tube was inserted. The mesenteric arcade vessels and lymphatics were doubly ligated from the level of the duodenum to the ileo-cecal junction. The mesenteric artery and vein at the level of the cecum were singly ligated. The colon was then doubly ligated caudal to the cecum and the isolated bowel removed. The vessels between the spleen and pancreas were ligated and the spleen removed. A loose ligature was placed around the portal vein and a second around the portal vein, artery and bile duct. An arterial cannula was inserted into the aorta, advanced cephalad to the level of the superior mesenteric artery, and then secured. The animal was hemisectioned and the portal vein cannulated. The <u>in vitro</u> pancreas-stomach preparation was perfused via the celiac and superior mesenteric arteries at a flow rate of 4 ml/min and and venous drainage collected in 1 min. fractions. A 20 min equilibration period preceded the experimental period at which time perfusate containing 80 mg/dl glucose was infused.

The following experiments were performed to validate this novel preparation.

i) <u>In vitro</u> insulin response of the perfused pancreas vs. pancreas-stomach preparation (Fig. 7):

The isolated pancreas (PANC) or pancreas-stomach (PANC-STOM) were perfused with 80mg/dl glucose or 300mg/dl glucose in the presence of 10mM arginine from 6-25 min. Insulin release from the perfused pancreas-stomach and pancreas preparations under basal conditions or in the presence of 10mM arginine and high glucose (300mg/dl) were equivalent (fig. 7).

ii) A comparison of the gastrin response to arginine from the perfused pancreasstomach and stomach preparations (Fig. 8):

The isolated stomach (STOM) or pancreas-stomach (PANC-STOM) were perfused with 10mM arginine from 11-30 min in the presence of 80mg/dl glucose. Mean basal gastrin levels



# Fig. 7

The insulin responses of the isolated perfused pancreas-stomach (PANC-STOM, n=4) and pancreas (PANC, n=4) preparations to 80mg/dl glucose, 300mg/dl glucose and 10mM arginine. No significant differences were observed.



Fig. 8

The gastrin responses of the isolated perfused pancreas-stomach (PANC-STOM), n=4) and stomach (STOM, n=4) to 10mM arginine in the presence of 80mg/dl glucose, p<0.05 using unpaired Student's t-test compared to STOM preparation.

during 0-10 min and 31-40 min were significantly higher in the perfused pancreas-stomach than in the stomach alone  $(271 \pm 38 \text{ vs. } 159 \pm 41 \text{pg/min}, \text{p} < 0.05)$ (fig. 8). Arginine infusion resulted in a 2.3-fold increase in gastrin release from both preparations. The mean gastrin response to arginine appeared higher in pancreas-stomach ( $612 \pm 184 \text{pg/min}$ ) than stomach ( $361 \pm$ 80 pg/min) preparations but the difference was not significant.

# iii) The effect of vagal stimulation on in vitro insulin release:

The vagi of the isolated pancreas-stomach preparation were electrically stimulated using the parameters 7V,  $10H_2$  and a 5 msec duration from 11-20 min in the presence of 160mg/dl glucose. The insulin response to vagal stimulation (Vagal) was then compared to a control group receiving 160mg/dl glucose alone (fig. 9). Stimulation resulted in a 4.7-fold increase in insulin secretion above basal levels. The mean insulin response to vagal stimulation (582 ± 231uU/min) was significantly higher than to 160mg/dl alone (94 ± 26uU/min, p<0.05).

iv) The effect of atropine on vagally stimulated insulin release (Fig. 10):

Following a 10 min perfusion of the perfused pancreas-stomach preparation with 160mg/dl glucose, the vagal trunks were electrically stimulated (7V,  $10H_2$ , 5 msec) from 16-50 min in the absence or presence of 1 X 10<sup>-5</sup>M atropine sulfate (26-35 min). Vagal stimulation produced a significant rise in insulin secretion above basal levels (11-15 min) that was maintained for the entire perfusion period (p<0.05) (fig. 10A). Insulin secretion was significantly reduced by atropine treatment (fig. 10B). This experiment demonstrated that electrical stimulation of the vagus resulted in a biphasic insulin response that was primarily mediated through muscarinic receptors.


Fig. 9

The insulin responses of the perfused pancreas-stomach preparation to 160mg/dl glucose in the absence (control, n=4) or presence (VAGAL, n=4) of vagal stimulation, \*p<0.05 using an unpaired Student's t-test compared to control group.





# The insulin responses of the pancreas-stomach preparation to vagal stimulation (A) or vagal stimulation and $10^{-5}$ M atropine sulfate (B). Insulin release expressed per 5 min period during vagal stimulation and atropine were compared to basal conditions (11-15 min), \*p<0.05 using Student's t-test for unpaired data.

#### C) Blood Sampling and In Vivo Tests

#### 1) **Blood Sampling**

Blood samples were collected from animals during TPN/TEN infusion and, intravenous glucose, oral glucose, and peptone tolerance tests. Samples were collected from either the superior vena cava or the tail vein. The route of sampling was dependent on the experiment. Intravenous samples were collected as follows:

- Animals were removed from the diet delivery system and the infusion line to the animal washed with 300  $\mu$ l of saline.

-  $750 \,\mu\text{l}$  of blood was withdrawn and the first 250  $\mu\text{l}$  discarded.

- The IV line was then cleared with 200  $\mu$ l of heparin-saline (50U/ml)

- Blood samples were placed in chilled 1.5ml Eppendorf<sup>®</sup> tubes (Sybron Canada Ltd., Rexdale, Ontario) containing heparin (14U).

Tail vein samples were collected from unrestrained animals using Natelson<sup>®</sup> heparinized blood collecting tubes (Fisher Scientific) and placed in chilled Eppendorf<sup>®</sup> tubes.

Plasma was separated from whole blood using a Savant<sup>®</sup> high speed centrifuge (Savant Instruments, Model HSC-10K, Hicksville, NY) at 9500 rpm, 4°C for 5 min. Samples were stored at -20°C until assayed.

## 2) Oral Glucose Tolerance Test (OGC)

Before an oral glucose challenge, animal harnesses were removed. Inhalation anesthesia provided a rapid induction and withdrawal from anesthesia. Induction of anesthesia was accomplished using 4% halothane in 100%  $O_2$  delivered by an anesthetic machine. After induction, the appropriate level of anesthesia could be maintained with 2% halothane delivered by a nose cone. The entire procedure for removal of the harness took less than 1 min and animals were allowed at least 30 min to recover.

After recovery from the anesthetic animals were placed on an inverted metal cage kept warm with a 60 watt desk lamp positioned over the cage. A basal blood sample was taken from the tail vein using Natelson<sup>®</sup> blood collecting tubes then a 1g/kg, 40% glucose solution was administered by syringe and feeding tube. Blood samples (250µl) were obtained at 10, 20, 30 and 60 min following glucose administration. Plasma was stored at -20°C until assayed.

Plasma glucose levels were measured using a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). This analyzer employs the glucose oxidase method for determining glucose concentration. Ten microliter plasma samples were assayed in duplicate.

#### 3) Amino Acid Tolerance Test

Oral peptone challenge (OPC) is a modification of the technique described for OGC. A basal blood sample was collected followed by the administration of a 10% peptone solution (0.5 ml/100g body weight) by a feeding tube. Blood samples (400µl) were collected at 10, 20, 30, 60, 90 and 120 min after the oral peptone administration. Plasma samples were stored at -20°C for subsequent IR-gastrin determination.

#### 4) **Blood Chemistry**

Plasma samples (400µl) were obtained for blood chemistry analysis and stored at -20°C until assayed. Samples, assayed in duplicate were thawed and loaded into a Kodak E700 multi analyzer for determination of plasma: Ca<sup>++</sup>, K<sup>+</sup>, Na<sup>+</sup>, PO<sub>4</sub>, cholesterol, total protein and -- triglyceride.

#### 5) Clotting Time

Whole blood samples were drawn up into an un-heparinized capillary tube. The end of the tube was broken off at 10sec intervals until the blood had clotted. This time was recorded.

#### III) IMMUNOCYTOCHEMISTRY (ICC)

#### A) Rationale

Immunocytochemistry (ICC) provides a method whereby tissue specific antigens can be identified in cells. The exact location of these cells can be determined and cells can also be quantified. In general, immunoperoxidase staining methods employ these four basic steps:

i) Tissue fixation (antigen determinants are preserved in the fixed tissue);

ii) Immunostaining (antigen specific antibodies are incubated with the tissue and form antigen-antibody complexes);

- iii) 2nd layer antibody; and
- iv) Peroxidase reaction (antigen-antibody complexes are identified by a colour change).

#### **B)** Procedures

#### 1) **Tissue Fixation**

During surgical procedures and isolated organ perfusion, the following tissues were obtained for fixation: intestine (jejunum), pancreas (tail portion) and stomach (antrum). Samples were immediately fixed in Bouin's solution (75 ml saturated picric acid, 25 ml formaldehyde) for 2 h, then transferred to 70% ethanol. An additional ethanol wash was completed the following day to remove residual picric acid. Samples could be stored in 70% ethanol at room temperature for up to 1 year.

#### 2) Dehydration

Samples were cut to give proper orientation and size before being loaded into cassettes (Fisher Scientific) for tissue processing. All dehydration steps were done in a Histomatic<sup>®</sup> – tissue processor (Model 166, Fisher Scientific) using the following protocol:

-	80% alcohol	30 min
-	90% alcohol	30 min
-	100% alcohol	30 min X 3
-	Xylene	30 min X 2
-	Paraffin	60 min X 2

#### 3) Embedding

Processed samples were removed from the tissue processor and embedded in paraffin (60°C) at a tissue embedding centre (Model 166, Sybron Canada, St. Laurent, Quebec). The paraffin blocks were set for two hours at 4°C before being stored at room temperature until cut.

#### 4) <u>Cutting Sections</u>

Paraffin blocks were trimmed and cooled (-10°C) before 5µm sections were cut using a rotary microtome (Model 1130, Reichert-Jung). The sections were first floated on 50% alcohol then transferred to a heated water bath (50°C) to flatten before being mounted on slides. The

sections were air dried for 24-48 h prior to immunostaining on a heating block (37°C).

#### 5) Dewaxing and Blocking

Paraffin was removed in xylene (5 min X 2) and sections were dried in petroleum ether (1.5 min). To prevent non-specific staining, sections were pretreated by incubating with 0.3% H<sub>2</sub>O<sub>2</sub> in phosphate buffered saline (PBS)-azide for 30 min. at room temperature.

#### 6) <u>Immunostaining</u>

#### a) First and Second Layer Antibodies:

Various first layer antibodies were used to stain intestine, stomach and pancreas. The antibody source specificity and dilutions are listed in Appendix IV. Before application of the first layer, slides were washed in PBS-azide then antibodies applied to sections and incubated at 4°C overnight in a humidity chamber. Two second layer antibody systems were available for use in immunostaining and the method chosen depended on the experiment.

#### i) Indirect method (Fig. 11A):

Second layer antibodies were chosen depending on the source of the primary antibody.
 Before application of the second layer, slides were thoroughly washed in PBS-azide and the second layer applied at the appropriate dilution (see Appendix V) for 1 h at room temperature.

#### ii) Avidin biotin method (Fig. 11B):

The biotinylated second layer was applied at the appropriate dilution (see Appendix V) for 1 h at room temperature. Slides were then washed in PBS-azide and the third layer (avidin-biotin complex, 1:200, Vector) applied for 1 h.

b) Peroxidase Reaction:

Sections were first washed in PBS-azide and then developed in diaminobenzidine (DAB 25  $\mu$ g/100ml PBS) and H<sub>2</sub>O<sub>2</sub> (0.025%). The color reaction was allowed to develop for 8-12 min, then the slides were counterstained.

#### 7) <u>Counterstain</u>

After development of the colour reaction, slides were counterstained, dehydrated and cleared by the following procedure:

#### 





Peroxidase enzyme

Avidin

Biotin

# Fig. 11

Immunostaining methods.

Hematoxylin	1 sec
Rinse (tap water)	
Acid ethanol	1 sec
Rinse (tap water)	
Lithium chloride	1 sec
Rinse (tap water)	
70% Ethanol	1 min
90% Ethanol	1 min
100% Ethanol	5 min
Xylene I	5 min
Xylene II	5 min

Sections were removed from Xylene II and immediately coverslipped with Permamount<sup>®</sup>.

#### 8) **Ouantification**

equipped with a videocamera.

a) Antrum:

Antral sections were stained for immunoreactive gastrin containing (G) cells. The number of G-cells per mm mucosa was obtained using the following formula:

#Cells/mm = #Cells/[Area mm<sup>2</sup>(10 antral mucosa areas)] X depth mm mucosa (epithelium)

b) Pancreas:

The average total area/islet ( $\mu$ m<sup>2</sup>) was measured. In addition, the percentage of the islet occupied by each cell type (glucagon, insulin, pancreatic polypeptide and somatostatin) was calculated for each islet (5 per section) from specifically stained serial sections.

c) Intestine:

The jejunum was immunostained for GIP. The number of immunoreactive cells per 20 villus crypt profiles was obtained for each section. The data was expressed as cell number/20 villus

crypt profiles.

## IV) STATISTICAL INTERPRETATION

Data was presented as mean  $\pm$  SEM and statistical significance (p<0.05) determined using either a Student's t-test for unpaired data (2 groups) or one-way analysis of variance (ANOVA, 3 groups). Groups yielding significant F values were compared using the Newman-Keuls test.

# CHAPTER ONE NUTRITIONAL ADEQUACY

#### I) INTRODUCTION

In 1972, Steiger, Vars and Dudrick introduced a technique for chronic intravenous feeding in rats. The technique involved the development of an infusion apparatus, surgical procedures and a nutrient solution to allow chronic intravenous infusion of adequate nutrients into unrestrained animals. The surgical procedure involved placement of an intravenous catheter into the superior vena cava and exiting in the mid scapular area. This placement allowed for the infusion of a highly concentrated solution containing glucose, amino acids, minerals and vitamins, while causing no damage to the vein by the hypertonic solution. The infusion apparatus consisted of a specially designed harness to secure the catheter to the animal, and a swivel-pump assembly allowing the animal to move freely about the cage while being continuously infused.

The surgical techniques and infusion apparatus described by Steiger et al. (1972), with minor modifications, have been used extensively by many investigators for both intravenous (Innis and Boyd, 1983; Popp et al., 1982a and b; Goodgame et al., 1978) and intragastric (Track et al., 1984; Baer and Dupré, 1985) infusion and is presently used in this laboratory. The current technique, which is a modification of that described above, has few inherent problems and allows freedom of movement, normal sleeping posture and grooming activity of the harnessed rats. Goodgame et al. (1978) and Burt et al. (1980) reported similar weight gain and nitrogen retention in harnessed rats when compared to those held in standard laboratory cages.

Although the infusion technique is extensively used and the specific nutrient requirements of rats well documented (NRC, 1978; AIN, 1977), there is considerable variation in the TPN formulations used. Goodgame et al. (1978) used a regimen consisting of only dextrose and amino acid solutions. Innis and Boyd (1983) and Lanza-Jacoby (1986) used a full complement of commercially available TPN components for use in humans, both formulations being designed to meet NRC recommendations for the rat. Track et al. (1984) combined separate vitamins,

minerals, glucose and amino acids, suggesting that diets derived from commercially available solutions did not support adequate growth. There is also considerable variability in the caloric balance and volume of TPN solutions delivered daily. Popp et al. (1982a) infused 38ml of solution with a mean caloric value of 226kcal/kg/day, while Goodgame et al. (1978) infused 85ml of solution providing 318kcal/kg/day. These differences in energy delivered per day, as well as the differences in diet composition, most likely reflect the lack of literature available on intravenous nutrient requirements for rats.

In the current investigation, a TPN/TEN regimen was formulated using available commercial human TPN solutions. The regimen was first evaluated for its ability to meet with the NRC's recommendations for the rat (NRC, 1972, 1978), both quantitatively with respect to gross energy, amino acids, vitamins and minerals, and qualitatively with reference to energy supplied in the form of carbohydrate, fat and nitrogen. The TPN/TEN diet was also compared to a laboratory rat chow (#5012 Ralston Purina). Second, the nutritional adequacy of the TPN/TEN diet was evaluated by monitoring the growth pattern of animals over a 7-day infusion period and — comparing blood chemistry parameters of TPN, TEN and orally fed animals.

#### II) METHODS

All methods were previously described in General Methods and Materials.

#### III) RESULTS

# 1) <u>Comparison of an ORAL Control Diet and TPN/TEN Diet with NRC</u> <u>Recommendations</u>

a) Nutritional Requirements (Table I):

The nutritional requirements for rats in the weight range 200-300g were obtained from the NRC publications for nutrient requirements of rats (NRC,1972, 1978). When necessary, data were converted to nutrient intake per rat per day given a daily food intake of 20g. Nutrient intake

	Oral Recommended*		Oral Experimental**		TEN-TPN†			
	Daily	% Dry Weight	Daily	% Dry Weight	Daily	% Dry Weight	% Energy	
Carbohydrate	>1.6g	>8.0%	5.7g	25.3%	14.0g	80.0%	72%	
Fat	1.0g	5.0%	1.0g	4.5%	0.8g	5.0%	14%	
Protein/Amino Acid	2.5g	12.0%	7.9g	35.0%	2.4g	14.0%	14%	
Calories/day (kcal)	76.0		75.0		66.0			
Gross energy	3.8kcal/g		3.34kcal/g		1.10k	cal/ml		
Ration	20.0g		22.5g (assumi 10% caj	ng a ge loss)	55.0m 4.0m	l TPN #1 l TPN #2		

## TABLE I NUTRIENT REQUIREMENTS

- \* Based on values recommended by NRC, Food and Nutrition Board, for growing rats (1972, 1978).
- \*\* Values approximated for a 200-300g rat based on values obtained for rat chow #5012 (Ralston Purina Co.).
- † Values approximated for a 200-300g rat fed IV or IG.

of experimental rats fed rat chow #5012 (ORAL) was estimated from literature available from Ralston Purina. A conversion was made from nutrient content in the diet to nutrient intake per rat per day assuming a daily intake of 22.5g. Daily intake by TPN/TEN animals was calculated from data available on each specific dietary component assuming a cumulative infusion rate (either intragastric-TEN or intravenous-TPN) of 59ml/250g.

The recommended daily fat requirement for growing rats (200-300g) was 1.0g/day, equivalent to that given ORAL animals (1.0g/day) and slightly greater than that given to TPN/TEN animals (0.8g/day). The carbohydrate intake for TPN/TEN animals was approximately 2.5 times greater than that given ORAL animals. Both TPN/TEN and ORAL diets exceeded the least requirement for carbohydrate of 1.6g/day or 8.0% of the dry weight of the diet recommended by the NRC (1978). The recommended protein requirement was given as 2.5g/day, comprising 12% of the diet. ORAL animals received 7.9g/day, while TPN/TEN animals received 2.4g/day. The daily recommended caloric intake of growing rats is 76kcal, assuming a caloric density of approximately 3.8kcal/g of food. The gross caloric density of rat --chow #5012 is 3.34kcal/g and, therefore, ORAL animals ingested approximately 75kcal/day, similar to that recommended. TPN/TEN animals received 66kcal/day, a value approximately 12% below that listed by the NRC.

#### b) Amino Acid Requirements (Table II):

The recommended daily intake of essential amino acids from growing rats was estimated from values obtained from the NRC (1978). Values for ORAL animals were based on information available on diet #5012 (Ralston Purina) assuming a daily intake of 22.5g. Values for TPN/TEN animals were based on information available for 10% Travasol (Travenol, Canada). With the exception of asparagine, both ORAL and TPN/TEN diets supplied the recommended daily intake of essential amino acids. Under most circumstances, the amount supplied in these diets met or exceeded that recommended by the NRC (1978).

#### c) Mineral Requirements (Table III):

The recommended daily mineral requirements and daily intakes of ORAL and TPN/TEN animals are listed in Table III. Minerals were supplied to TPN animals from chemically defined

67

<u></u>	Oral Recommended*	Oral Experimental**	TEN-TPN†
Amino Acid	mg/day	mg/day	mg/day
l-alanine oo	_	<u> </u>	484
l-arginine	120	320	242
l-asparagine	80	-	- · -
l-glutamic acid	800	-	484
l-histidine	60	130	103
1-isoleucine	100	274	112
1-leucine	150	416	145
1-lysine	140	310	135
1-methionine	120	98	136
l-phenylalanine	160	240	145
1-proline	80	-	97
1-threonine	100	203	91
l-tryptophan	29	61	42
1-tyrosine	-	150	9
l-valine	120	267	108

#### TABLE II AMINO ACID REQUIREMENTS

- $\infty$  Non-essential amino acid.
- \* Based on values recommended by NRC, Food and Nutrition Board, for growing rats (1978).
- \*\* Data approximated for a 200-300g rat based on values obtained for rat chow #5012 (Ralston Purina Co.).
- † Values approximated for a 200-300g rat fed IV or IG.

TABLE III	MINERAL REQUIREMENTS
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<u></u>	Oral Recommended*	Oral Experimental**	TEN-TPN†	
Mineral	mg/day	mg/day	mg/day	
Calcium	85.0	227.0	58.0	
Chloride	8.6	97.0	244.0	
Magnesium	6.9	48.0	1.7	
Phosphorus	69.0	166.0	29.0	
Potassium	62.0	243.0	243.0	
Sodium	8.5	64.0	45.0	
Sulfur	5.1	-	1.25	
Chromium	0.005	0.065	0.0002	
Copper	0.086	0.45	0.019	
Fluoride	0.017	1.4	-	
Iodine	0.0026	0.026	-	
Iron	0.6	6.0	-	
Manganese	0.9	1.6	0.009	
Selenium	0.0017	0.00045	-	
Zinc	0.21	1.6	0.075	

\* Based on values recommended by NRC, Food and Nutrition Board, for growing rats (1978).

\*\* Values approximated for a 200-300g rat based on data obtained for rat chow #5012 (Ralston Purina Co.).

† Values approximated for a 200-300g rat fed IV or IG.

solutions of electrolytes and trace metals (Trace-4, IMS Ltd.). The ORAL diet was found to contain all daily requirements for minerals in the appropriate amounts with the exception of sulfur. The TPN/TEN diet supplied most minerals in adequate amounts with the exceptions of fluoride, iodide, iron and selenium. It was assumed that fluoride would be obtained from drinking water and iodide from the topical antiseptic (Proviodine) applied to the animals on day 0.

#### d) Vitamin Requirements (Table IV):

Daily intake values for vitamins supplied to TPN/TEN animals were obtained from information available on M.V.C. 9+3 (Lyphomed Inc.). The TPN/TEN diet contained all recommended vitamins with the exception of vitamin K. The ORAL diet contained all recommended vitamins with the exception of Folic acid. Vitamin K was supplied in trace amounts. Under most circumstances, both ORAL and TPN/TEN diets supplied adequate amounts of each vitamin required.

#### 2) Evaluation of Nutritional Adequacy

a) <u>Survival</u>:

Of 15 animals surgically prepared to receive TPN, 13 survived (80%) a 7-day infusion period. No animals receiving IG infusion (TEN) or fed orally (ORAL) were lost during the infusion period.

b) <u>Changes in Body Weight During the Infusion Period (Fig. 12)</u>:

Mean changes in body weight of TPN, TEN and ORAL animals from day 2 through day 6 are shown in figure 12. These data represent body weight changes during oral feeding or nutritional support. ORAL animals demonstrated significant weight gain in each day of the study, with the greatest gain on day 2. Weight change stabilized in the ORAL group by day 4. TPN animals showed significant weight gain from day 3 onward, with the greatest daily increase of  $4.6 \pm 1.2g$  on day 6. Intragastrically fed animals demonstrated significant weight increases from day 4 onward. By day 6 weight increases were comparable in all 3 groups.

c) Fasting Body Weight (Table V):

Mean initial and final body weights listed in table V represent fasted body weight prior to and

## TABLE IVVITAMIN REQUIREMENTS

	Oral	Oral	TEN-TPN†	
Vitamin Re	commended*	Experimental**		
Ascorbic acid (mg)	-	-	1.87	
Biotin (mg)	-	0.007	0.013	
Folic acid (mg)	0.02	-	0.025	
Niacin (mg)	0.40	1.35	0.74	
Pantothenic acid (mg)	0.16	0.28	0.28	
Pyridoxine (mg)	0.12	0.10	0.075	
Riboflavin (mg)	0.60	0.10	0.070	
Thiamin (mg)	0.80	0.25	0.055	
Vitamin À (ĨŰ)	80.0	45.0	62.0	
Vitamin $B_{12}$ (µg)	1.0	0.0045	0.925	
Vitamin D (IU)	8.5	67.0	3.7	
Vitamin E (IU)	0.60	0.63	0.20	
Vitamin K (µg)	1.0	-	-	

- \* Based on values recommended by NRC, Food and Nutrition Board, for growing rats (1978).
- \*\* Values approximated for a 200-300g rat based on values obtained for rat chow #5012 (Ralston Purina Co.).
- † Values approximated for a 200-300g rat fed IV or IG.





Body weight change during days 2 through 6 of nutritional support in TPN (n=13), TEN (n=16) and ORAL (n=23) animals.

72

## TABLE V

# FASTING BODY WEIGHT (X±SEM) OF TPN, TEN AND ORAL ANIMALS

	INITIAL (g)	FINAL (g)	DAILY (g/day)
TPN	195.4 <u>+</u> 3.7	211.9 <u>+</u> 3.6	2.35 <u>+</u> .28
ORAL	198.9 <u>+</u> 2.9	216.6 <u>+</u> 2.5	2.50 <u>+</u> .29
TEN	286.6 <u>+</u> 3.7	302.4 <u>+</u> 3.6	2.32 ± .51

after a 7-day TPN period. All 3 experimental groups demonstrated significant weight increase during the infusion period (p<0.05). The estimated daily increases in weight for TPN, TEN and ORAL animals were  $2.4 \pm 0.3$ ,  $2.3 \pm 0.5$  and  $2.5 \pm 0.30$ g respectively.

d) <u>Blood and Urine Chemistry (Table VI)</u>:

Blood samples were collected from TPN (n=16), TEN (n=7) and ORAL (n=7) animals on day 5 of a 7-day study for measurement of various blood and urine parameters listed in Table VI. No significant differences in hematocrit or whole blood clotting time were observed among TPN, TEN or ORAL animals. Plasma Ca<sup>2+</sup>, PO<sub>4</sub> and Na<sup>+</sup> concentrations were similar in all 3 groups, while plasma K<sup>+</sup> was significantly elevated in TEN compared with ORAL or TPN animals (p<0.05). No differences in plasma triglyceride or total cholesterol concentrations were observed, however, plasma total protein was significantly lower in TPN compared with ORAL and TEN animals (p<0.05). Plasma glucose was significantly higher in TPN (179 ± 7mg/dl) compared to ORAL (136 ± 8.0mg/dl) or TEN (151 ± 6.0mg/dl) animals, however, urine glucose was undetectable in all 3 groups as determined using a Beckman glucose analyzer II. Urinary -osmolality was significantly higher in ORAL compared to TPN or TEN animals (p<0.05). These observations, along with the general observation of increased urine flow in TPN and TEN animals, indicate excretion of large amounts of dilute urine.

#### IV) DISCUSSION

Maintenance of normal body composition by total parenteral nutrition requires that adequate quantities of energy substrate be provided to and utilized by the animal. The present study was performed to evaluate the nutritional adequacy of a diet (TPN/TEN) formulated in this laboratory for chronic intragastric or intravenous feeding.

The estimated daily energy intake of TPN/TEN animals of approximately 250g was 66kcal, an energy level slightly below that of 76kcal recommended by the NRC (1972) for rats of comparable weight. TPN and TEN animals were administered a lower caloric load for the following reasons. First, Popp et al. (1979, 1982b) determined the daily energy expenditure of

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## TABLE VI BLOOD CHEMISTRY

	ORAL	TEN	TPN
Hematocrit (%)	42.1 ± 1.1	$41.2 \pm 1.1$	37.8 ± 1.8
Clotting time (min)	$2.2 \pm .27$	2.4 ±.17	$2.8 \pm .53$
$Ca^{2+}$ (mmol/l)	2.51 ±.03	$2.42 \pm .02$	$2.43 \pm .22$
K <sup>+</sup> (mmol/l)	4.97 ± .20	$6.58 \pm .10^{*}$	$5.12 \pm .11$
Na <sup>+</sup> (mmol/l)	$141.7 \pm .87$	$136.1 \pm .57$	$142.6 \pm .66$
$PO_4$ (mmol/l)	$2.56 \pm .16$	$2.19 \pm .06$	$2.35 \pm .16$
Glucose (mg/dl)	$136.0 \pm 8.0$	151.4 ± 6.0	179.7 ± 2.5*
Cholesterol (mg/dl)	57.7 ± 3.9	$51.9 \pm 2.7$	49.9 <u>+</u> 2.5
Triglyceride (mg/dl)	55.8 ± 6.2	54.9 ± 7.9	42.5 <del>±</del> 8.9
Total Protein (g/dl)	$5.3 \pm .03$	$5.1 \pm .80$	$4.72 \pm 0.10^{*}$
Urine Glucose (mg/dl)	-	-	-
Urine Osmolality (mOsm)	$2100 \pm 320*$	575 ± 85	$500 \pm 190$

\*  $p \le 0.05$  comparing groups for each parameter using ANOVA and Newman-Keuls tests.

TPN rats of similar size range to those used in this study to be approximately 38kcal/day. Any energy in excess of 38kcal/day should therefore be adequate to promote growth. Second, intravenous infusion of caloric loads in excess of 75kcal/day has been associated with the development of hepatic steatosis (Chang and Silvas, 1974); this observation was supported by Goodgame et al. (1978) and Kronevi and Roos (1976) who reported the development of fatty liver within 10 days in TPN rats infused 80kcal/day and 70kcal/day respectively. Conversely, Innis (1986a) and Popp et al. (1982a), using caloric loads below 60kcal/day, reported no liver abnormalities. These data suggest that one causal factor of fatty liver development may be infusion of excessive nutrients that cannot be efficiently processed. Third, there does not appear to be a positive correlation between energy intake and weight gain above 70kcal/day. Similar weight gain was observed in TPN animals by Popp et al. (1982a) and Goodgame et al. (1978) at 57kcal/day and 80kcal/day respectively. Finally, TPN and TEN animals used in this study demonstrated significant weight gain at 66kcal/day, with average daily increases similar to those observed by other investigators using diets of the same composition and daily caloric loads –(Johnson et al., 1975; Martins et al., 1985; Baer and Dupré, 1985).

TPN/TEN solutions delivered 14g of carbohydrate as glucose per day which contributed to 72% of the diet's gross energy. This percentage, although high compared to that of rat chow and that recommended by the NRC (1978), was comparable to AIN-76<sup>TM</sup>, a diet formulated by the American Institute of Nutrition (AIN), which delivered 65% of its gross energy as carbohydrate. AIN-76<sup>TM</sup> produced similar growth with no observable side effects when compared to other diets low in carbohydrate (AIN, 1977). The carbohydrate content of the diet used by other investigators (Lanza-Jacoby, et al., 1987; Popp et al., 1982; Innis, 1986b) was in agreement with the TPN/TEN diet used in this study. TPN animals did exhibit elevated plasma glucose levels when compared to ORAL or TEN rats. This hyperglycemia may be attributed to the route of administration, rather than the carbohydrate load, since the solution delivered intragastrically did not produce a significant elevation in glucose when compared to a control group fed rat chow.

Fat is considered an optional component of the rat diet except as a source of essential free

fatty acids (EFA) and for its role in the utilization of dietary fat-soluble vitamins (NRC, 1978). The properties of intravenous fat emulsions, being calorically dense yet isosmotic with respect to blood, also make them an ideal source of non-nitrogen calories. For this reason Soyacal<sup>®</sup> was given as an intravenous bolus twice per day, providing approximately 0.8g fat. This value exceeds that necessary to fulfill daily EFA requirements of 0.1g/day (Innis and Boyd, 1983), providing an extra source of calories. The daily fat intake of TPN/TEN animals was similar to that of ORAL animals and met the NRC requirement for daily fat consumption. Infusion of the TPN/TEN solutions either intravenously or intragastrically did not significantly alter plasma triglycerides or total cholesterol when compared to ORAL animals. Similar observations have been reported by Innis (1986a) for parenterally maintained rats and Lanza-Jacoby et al. (1987) for TPN and IG fed rats.

The recommended daily combined protein/amino acid requirement for growing rats was estimated at 2.5g (NRC, 1972). ORAL and TPN/TEN animals received 8.0g/day and 2.3g/day respectively. The daily protein intake of TPN/TEN animals was slightly lower than the recommended value. However, the NRC diet contained considerably less non-nitrogen calories, increasing the requirement for protein. A more important consideration than total protein is the requirement for specific essential amino acids. Both ORAL and TPN/TEN diets were found to meet or exceed recommended daily requirements of all essential amino acids with the exception of asparagine. Prolonged absence of even one essential amino acid can lead to typical protein deficiency syndrome (Rogers, 1979). Short experimental periods, such as those employed in this study (7-days), would not, however, be long enough for deficiency symptoms to develop (Rogers, 1979). The amino acid composition and intake of 2.4g/day in this study was equivalent to that of other TPN regimens formulated to meet NRC recommendations (Lanza-Jacoby, 1987, 1982; Popp et al., 1982; Innis and Boyd, 1983; Pederson et al., 1985). Plasma total protein was found to be significantly lower in TPN animals compared to either ORAL or intragastrically fed (TEN) groups. The significantly reduced plasma total protein in TPN animals is most likely the result of central intravenous infusion, rather than an inadequacy in the diet, since TEN animals exhibited normal plasma protein levels.

The TPN/TEN diet fulfilled all vitamin requirements with the exception of vitamin K, a fat soluble vitamin involved in the synthesis of clotting factors. TPN/TEN animals demonstrated normal whole blood clotting times (Ringler and Dabich, 1979) suggesting that symptoms of vitamin K deficiency did not develop during the infusion period. Plasma electrolyte concentrations in TPN/TEN and ORAL animals were also considered to be within normal ranges (Ringler and Dabich, 1979) reflecting adequate mineral levels in both diets. TPN and TEN animals had extremely dilute urine compared with the ORAL group, which was expected considering the large volume of solution infused daily. Although osmotic clearance was not determined, the fact that the urine was dilute and devoid of measurable quantities of glucose suggested metabolic utilization of infused solutes.

The present study was performed to evaluate the nutritional adequacy of a TPN/TEN diet formulated in this laboratory. The TPN/TEN diet was found to be comparable to other formulations known to successfully maintain the health of TPN and intragastrically fed animals, and met with most recommendations set out by the NRC (1978). Furthermore, the TPN/TEN diet promoted weight gain in TPN and TEN animals while producing few side effects as indicated by hematological data. These results support the conclusion that the TPN/TEN diet was suitable for short term chronic intravenous or intragastric feeding.

# CHAPTER TWO THE EFFECT OF TPN ON THE HORMONAL COMPONENT OF THE ENTEROINSULAR AXIS

#### I) INTRODUCTION

The term 'enteroinsular axis' was first used by Unger and Eisentraut (1969) to describe a proposed regulatory system where nutrients in the gastrointestinal (GI) tract, through the release of GI-hormones, would exert influences on pancreatic endocrine secretion. This definition has more recently been expanded to include the actions of nutrients, hormones and neural components of the axis on pancreatic islet hormones (Creutzfeldt, 1979). Gastrointestinal factors which are released by luminal nutrients (especially glucose), and which augment glucose-stimulated insulin release, are termed 'incretins'. While all established GI-hormones have been tested for their insulin releasing properties, only gastric inhibitory polypeptide (GIP) fulfills the – criteria of an incretin (Creutzfeldt, 1979; Creutzfeldt and Ebert, 1985).

Several experimental models have been developed to study the role of GIP in the enteroinsular axis. In man, glucose-clamping techniques were used to demonstrate the insulinotropic nature and glucose-dependency of GIP on insulin secretion (Elahi et al., 1979). In rat, the isolated perfused pancreas was employed to demonstrate the glucose-dependent insulinotropic actions of GIP in vitro (Pederson and Brown, 1976). The relative contribution of GIP to the incretin effect has been demonstrated using anti GIP serum (Ebert and Creutzfeldt, 1982; Lauritsen et al., 1981). GIP antiserum partially reduced the insulin response to an oral glucose load when injected intravenously in rat. High affinity binding sites for GIP have been identified on cells of insulin secreting tumors (Maletti et al., 1984; Maletti et al., 1987) and the B-cell line In 111 (Amiranoff et al., 1984) suggesting a direct effect of GIP on the B-cell. The ability of pure porcine GIP to stimulate insulin release from In 111 cells, with a concomitant rise in cAMP production, further suggested a physiological role for GIP in insulin secretion (Amiranoff et al., 1984).

In concentrations considered to be physiological GIP is a potentiator of glucose-induced insulin release in vivo (Elahi et al., 1979) and in vitro from the isolated perfused pancreas (Pederson et al., 1982). This phenomenon, however, has not been demonstrated in freshly isolated islets. The apparent insensitivity of isolated islets, which respond only to pharmacological concentrations of GIP (Fujimoto, 1981; Schauder et al., 1975), may be related to the isolation procedure, as GIP receptors have not been identified in normal islet tissue. Brown et al., (1980), suggested that GIP receptors may be damaged during the isolation procedure, being particularly susceptible to enzymatic digestion by collagenase. Although islets maintained in culture do appear to regain their sensitivity to physiological doses of GIP (Siegel and Creutzfeldt, 1985), they are still relatively unresponsive compared to the isolated perfused pancreas (Pederson et al., 1982). The inherent problems associated with this preparation necessitate the use of other models to study the physiology of GIP.

Two rat models have been developed to access the effects of chronically elevated or depressed plasma GIP levels on the enteroinsular axis (Pederson et al., 1982; Pederson et al., 1985). Jejunoileal bypass (JIB) in rats, where the blind loop was left intact, resulted in high fasting GIP levels (Pederson et al., 1982). JIB rats also demonstrated a reduced insulin response to glucose during an oral glucose challenge, and the insulin responsiveness of the perfused pancreas to glucose and GIP was reduced when compared to a control group. In a follow-up study, resection of the blind loop resulted in normalization of fasting GIP levels and the <u>in vitro</u> insulin response to glucose and GIP (Buchan et al., 1983). These experiments demonstrated that the blind loop was the source of high circulating GIP levels in JIB rats and that the hyperGIPemia was causally related to the decreased insulin response in these animals. Buchan et al. (1983) postulated that the blunted insulin response was due to the "down regulation" of GIP receptors on B-cells, resulting from chronic hyperGIPemia.

Contrary to the hyperGIPemia observed in JIB rats, animals fed by total parenteral nutrition (TPN) exhibited chronically suppressed plasma GIP levels (Pederson et al., 1985). In TPN rats an exaggerated insulin response to oral glucose was observed in vivo, and the insulin response of the isolated perfused pancreas to glucose and GIP was significantly higher in TPN compared

to control animals. The apparent increase in B-cell sensitivity to GIP was suggested to be causally related to chronically low GIP levels observed during TPN. It was postulated that chronically low plasma GIP levels led to an increase in GIP receptor density on B-cells and/or an increased receptor sensitivity to GIP (Pederson et al., 1985).

The primary purpose of the present experiments was to further examine the effects of TPN on the enteroinsular axis. In the first series of experiments, animals were placed on TPN, administered the TPN solution intragastrically (TEN), or fed a rat chow diet (ORAL), for a period of 7 days. The rationale for including an intragastrically fed group was two-fold. First, it provided a more suitable control group, as animals were fed the exact regimen administered to TPN animals, eliminating the possibility that differences between TPN and orally fed control animals, observed by Pederson et al. (1985), were due to a diet effect. Second, it provided an experimental situation opposite to TPN, in that the GI-tract was continuously presented with nutrients, suggesting that the enteroinsular axis is chronically active.

A number of parameters of the enteroinsular axis were studied. Blood samples were obtained during the infusion period and during one 24-h period for plasma glucose, insulin and GIP determination. The plasma glucose and insulin responses to an 8 h fast and to an oral glucose challenge (OGC) were determined after the 7-day infusion period. The effect of glucose and GIP on insulin release from the isolated perfused pancreas of TPN, TEN and ORAL animals was then investigated to determine if chronic intravenous or intragastric feeding altered the B-cell sensitivity to these secretagogues. Finally, immunocytochemical methods were employed to examine the effects of the different methods of diet delivery on pancreatic islet B-cell composition.

In a second set of experiments the possibility of a causal relationship between circulating GIP levels and the B-cell sensitivity to GIP was further explored. The purpose of this study was to determine whether the increased sensitivity observed in TPN animals was related to chronic hypoGIPemia. Animals (TPN-GIP) placed on TPN were infused with pure porcine GIP EG III), presented with the TPN medium, to raise circulating GIP levels within the range observed during intragastric feeding. The insulin responses to glucose in vivo and to glucose and GIP in

vitro from the isolated perfused pancreas were compared to those of animals receiving TPN alone and ORAL animals after the 7-day infusion period.

A third series of experiments was performed to determine the rapidity of onset of the increased B-cell sensitivity to GIP. Animals were exposed to TPN for a period of 3, 5 or 7 days. The insulin response of the perfused pancreas to GIP and glucose was then assessed.

Some uncertainty exists regarding a role for CCK in the enteroinsular axis, however, the presence of CCK receptors on B-cells (Sakamoto et al., 1985), and the ability of CCK to stimulate insulin release in vivo (Dupré et al., 1969) and in vitro (Pederson and Brown, 1979; Sakemoto et al., 1982; Zawalich et al., 1987) support a role. The observation that CCK release was not stimulated by parenterally administered fat or amino acids (Fried et al., 1982), and the apparent CCK cell hypoplasia of the small bowel associated with TPN (Buchan et al., 1985), suggest that CCK levels may remain depressed during TPN. Since it was hypothesized that chronically low GIP levels during TPN were causally related to an increased B-cell sensitivity to GIP (Pederson et al., 1985), such a phenomenon may also develop for CCK. In a final study, -- therefore, the in vitro insulin response to CCK-octapeptide (CCK-8) was analyzed in TPN and control animals after a 7-day infusion period. The purpose of this study was to provide further evidence in support of a role for CCK in the enteroinsular axis.

#### II) METHODS

All methods were previously described in General Methods and Materials.

#### III) **RESULTS**

# 1) <u>Plasma Glucose. Insulin and GIP Levels in TPN, TEN and ORAL</u> <u>Animals During a 7-Day Experimental Period</u>

a) Body Weight (Table VII):

TPN, TEN and ORAL animals demonstrated significant weight gain (p<0.05) over the 7-day

82

infusion period. The mean daily weight gain in TPN, TEN and ORAL animals was  $1.4 \pm 0.5$ ,  $2.3 \pm 0.4$  and  $1.7 \pm 0.3g$  respectively.

b) <u>Plasma Glucose</u>, Insulin and <u>GIP Levels on Days 2, 4, and 6 in TPN, TEN and</u> <u>ORAL Animals (Fig. 13)</u>:

Plasma glucose, insulin and GIP levels were measured on days 2, 4 and 6 of the experimental period. Glucose levels were found to be consistently higher in TPN animals but were not significantly different from TEN or ORAL animals (fig. 13A). Plasma insulin levels were significantly different (p<0.05) among the 3 groups on days 2 and 4. On day 6, plasma insulin remained elevated in the TPN group ( $94 \pm 12\mu$ U/ml) but dropped in TEN animals ( $52 \pm 6\mu$ U/ml) to levels similar to those observed in the ORAL group ( $40 \pm 9\mu$ U/ml) (fig. 13B). Plasma GIP levels were significantly higher (p<0.05) in TEN animals throughout the infusion period when compared to levels observed in TPN or ORAL animals (fig. 13C).

c) <u>Plasma Glucose</u>, Insulin and <u>GIP Levels in TPN</u>, <u>TEN and ORAL Animals</u> <u>During a 24-h Period (Fig. 14)</u>:

Blood samples were collected over a 24-h period on day 5 for plasma glucose, insulin and GIP determination. Plasma glucose remained elevated in TPN animals throughout the 24-h period, with the highest levels observed at 2300h (fig. 14). The plasma glucose profile of ORAL animals paralleled that of the TPN group and was not found to differ significantly over the 24-h period. The glucose profile of TEN animals did not parallel that of TPN or ORAL animals, peaking earlier (1900h) and then falling to significantly lower levels (p<0.05) than observed in either the TPN or ORAL groups between 2400 and 0700h. Plasma insulin was significantly higher (p<0.05) in TPN when compared to ORAL and TEN animals over the entire 24-h period (fig. 14B). The plasma GIP profile during the 24-h period is shown in fig. 14C. GIP levels remained elevated in TEN animals throughout the sampling period, being significantly higher than in the TPN group at all time periods (p<0.05). GIP levels remained consistently depressed in TPN animals over the 24-h period. In the ORAL group, however, a peak GIP response was observed at 2300h, at which point TEN and ORAL animals demonstrated significantly higher GIP levels than the TPN group (p<0.05). The peak GIP response corresponded to the peak

glucose and insulin levels observed in ORAL animals. GIP levels in TEN and TPN animals did not parallel their respective insulin or glucose profiles.

## TABLE VII

## BODY WEIGHT (X±SEM) OF TPN, ORAL AND

# TEN RATS

	INITIAL (g)	FINAL (g)	DAILY (g/day)
TPN	267.4 ± 5.0	277.0 ± 5.7	1.37 ± .50
ORAL	296.8 ± 5.3	308.8 ± 3.9	1.71 ± .30
TEN	291.0 ± 4.6	307.1 ± 4.6	$2.30 \pm 0.4$

Fig. 13

A: Plasma glucose levels in intraveneously fed (TPN n=12), intragastrically fed (TEN, n=12) and orally fed (ORAL, n=12) animals during the TPN period (7 days). No significant differences were observed among the groups. In this and subsequent figures, error bars represent  $\pm$  SEM.

B: Plasma insulin levels in TPN (n=12), TEN (n=12) and ORAL (n=12) animals during the TPN period. All groups displayed significant differences on days 2 and 4 using ANOVA and Newman-Keuls tests, TPN vs. ORAL (\*p<0.05), TPN vs. TEN (†p<0.05), and TEN vs. ORAL (p<0.05). On day 6, TPN vs. TEN and ORAL groups (\*p<0.05).

C: Plasma GIP levels in TPN (n=6), TEN (n=6) and ORAL (n=6) animals during the TPN period, \*p<0.05 using ANOVA and Newman-Keuls tests comparing TPN to TEN and ORAL groups.



Fig. 14

Plasma glucose (A) (TEN vs. TPN and ORAL groups, \*p<0.05), insulin (B) (TPN vs. TEN and ORAL groups,  $\Delta p$ <0.05) and GIP (C) (TEN vs. TPN and ORAL groups, \*p<0.05; TEN vs. TPN animals,  $\delta p$ ,0.05; TEN and ORAL vs. TPN, †p<0.05) levels during a 24 h period between days 5 and 6 in TPN (n=6), TEN (n=6) and ORAL (n=6) animals, using ANOVA and Newman-Keuls tests.







#### a) TPN vs. TEN Animals (Figs. 15 and 16):

The glucose and insulin responses to 1g/kg oral glucose were measured in fasted TPN and TEN animals after a 7-day infusion period. Fasting plasma glucose levels in TPN animals (139  $\pm$  5mg/dl) were not significantly different from TEN animals (136  $\pm$  6mg/dl) (fig. 15A). The peak glucose response to OGC occurred at 10 min in both groups, but was significantly higher (p<0.05) in TEN (234  $\pm$  12mg/dl) compared to TPN (204  $\pm$  9mg/dl) animals. Plasma glucose levels remained higher in TEN animals at 20 and 30 min (p<0.05). The integrated glucose responses to OGC are shown in fig. 16A. TPN animals demonstrated a significantly reduced (10%) total glucose response to OGC when compared to the TEN group (p<0.05).

Fasting plasma insulin levels were not significantly different between TPN ( $39 \pm 6\mu U/ml$ ) and TEN ( $30 \pm 2\mu U/ml$ ) animals (fig. 15B). Following an OGC, plasma insulin levels rose to a peak of  $119 \pm 14\mu U/ml$  at 20 min in TPN animals. In contrast, TEN rats achieved a significantly lower peak insulin response ( $79 \pm 10\mu U/ml$ ) at 10 min and remained significantly lower during the 20 and 30 min periods (p<0.05). The integrated insulin response to OGC demonstrated a significantly greater response (25%) to glucose in TPN animals (p<0.05). The insulinogenic index (integrated insulin / integrated glucose) was significantly greater in the TPN group (0.44 ± 0.04 vs. 0.27 ± 0.03\mu U·dl·mg<sup>-1</sup>·ml<sup>-1</sup>, p<0.05).

#### b) TPN vs. ORAL Animals (Figs. 17 and 18):

An oral glucose tolerance test was performed on fasted TPN and ORAL animals after a 7-day TPN period. Fasting plasma glucose levels appeared higher in ORAL compared to TPN animals  $(145 \pm 7 \text{ vs.} 130 \pm 4 \text{mg/dl})$  but the difference was not found to be statistically significant. The peak glucose response to OGC was achieved at 20 min in both groups and was significantly higher in the ORAL animals  $(208 \pm 11 \text{ vs.} 182 \pm 4 \text{mg/dl})$ . Plasma glucose levels remained higher during the 30 and 60 min periods in ORAL animals. The integrated glucose response to an OGC was significantly reduced (18%, p<0.05) in TPN animals (fig. 18A). Fasting plasma insulin levels were similar in TPN and ORAL animals  $(24 \pm 2 \text{ vs.} 21 \pm 3\mu\text{U/ml})$  (fig. 17B).
Following an OGC, plasma insulin levels rose to a peak of  $78 \pm 10\mu$ U/ml at 10 min in TPN animals. ORAL rats exhibited a significantly lower peak insulin response ( $52 \pm 10.7\mu$ U/ml) at 20 min, however, post-peak insulin levels were similar in both groups. The total insulin response to OGC was greater in TPN animals compared to the ORAL group, but the difference was not significant (fig. 18B). The insulinogenic index was significantly higher (24%) in TPN animals ( $0.25 \pm 0.02$  vs.  $0.19 \pm 0.01\mu$ U·dl·mg<sup>-1</sup>·ml<sup>-1</sup>, p<0.05).

### c) TEN vs. ORAL Animals (Figs. 19 and 20):

The glucose and insulin responses to a 1g/kg oral glucose challenge were measured in fasted ORAL and TEN rats after a 7-day infusion period. Fasting plasma glucose levels were similar in ORAL ( $114 \pm 5$ mg/dl) and TEN ( $119 \pm 7$ mg/dl) animals (fig. 19A). The peak glucose responses occurred at 20 min in both groups and were not significantly different. The integrated glucose responses to an OGC were similar in ORAL and TEN animals (fig. 20A).

Fasting plasma insulin levels were elevated in TEN ( $28 \pm 5\mu$ U/ml) compared to ORAL ( $19 \pm 4\mu$ U/ml) animals but the difference was not statistically significant. Following an OGC, plasma insulin levels rose to a peak of  $69 \pm 10\mu$ U/ml in TEN animals, and to a peak of  $73 \pm 11\mu$ U/ml in ORAL animals. The post peak responses of TEN and ORAL animals were similar. The integrated insulin responses (fig. 20B) were similar in TEN and ORAL animals, as were the insulinogenic indices ( $0.29 \pm 0.03$  vs.  $0.26 \pm 0.02\mu$ U·dl·mg<sup>-1</sup>·ml<sup>-1</sup>).

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Plasma glucose (A) and insulin (B) responses to a 1g/kg an OGC in TEN (n=7) and TPN (n=7) animals after the TPN period (7 days). 'G' represents the administration the glucose solution at 0 min. A Student's t-test for unpaired data was used to compare responses at each time interval (\*p<0.05). Error bars represent  $\pm$  SEM.



Integrated glucose (A) and insulin (B) responses to an OGC, p<0.05 using a Student's t-test for unpaired data. The formula used for the calculation of integrated responses was:

$$X_{I} = \frac{(X_{t0} + X_{t1})(t1-t0) + (X_{t1} + X_{t2})(t2-t1) \dots - t_{n-1}(X_{t0})}{2}$$

where  $X_I$  = integrated release of the measured parameter and X = concentration of measured parameter at time = t.



Plasma glucose (A) and insulin (B) responses to a 1g/kg OGC in ORAL (n=8) and TPN (n=9) animals after the TPN period (7 days), \*p<0.05 using a Student's t-test for unpaired data.



Integrated glucose (A) and insulin (B) responses to an OGC, \*p<0.05 using a Student's t-test for unpaired data.



Plasma glucose (A) and insulin (B) responses to a 1g/kg OGC in TEN (n=14) and ORAL (n=12) animals after the TPN period (7 days), \*p<0.05 using a Student's t-test for unpaired data.



Integrated glucose (A) and insulin (B) responses to an OGC. No significant differences were observed.

## 3) <u>The Effects of 7-Day Total Parenteral Nutrition and Intragastric</u> <u>Feeding on the Insulin Response of the Isolated Perfused Pancreas</u>

a) <u>The Effect of Glucose and GIP on Insulin Release from the Perfused Pancreas</u> of TPN and TEN Animals (Fig. 21):

After completion of the oral glucose challenge experiment, the pancreata of TPN and TEN animals were surgically isolated and perfused with a perfusate containing 300rmg/dl glucose in the presence of a 0-1ng/ml linear GIP gradient. The insulin response in both groups was biphasic (fig. 21A). The first phase consisted of a rapid increase in insulin secretion, reaching a plateau which was maintained until 6 min. The second phase (7-40 min) consisted of a more linear increase in insulin secretion present for the duration of the perfusion. At all points during the perfusion the insulin response from the pancreata of TPN animals was greater than in the TEN group. The integrated insulin response represents the total insulin response during each phase (fig. 21B). The phase I and II responses were significantly greater in TPN compared to TEN animals ( $5.0 \pm 0.5$  vs.  $3.4 \pm 0.4$ mU;  $135.0 \pm 8.0$  vs.  $82.0 \pm 8.0$ mU, p<0.05).

b) <u>The Effect of Glucose and GIP on Insulin Release from the Perfused Pancreas</u> of TPN and ORAL Animals (Fig. 22):

GIP was presented to the pancreas as a linear gradient from 0-1ng/ml in the presence of perfusate containing 300mg/dl glucose. The insulin response was biphasic in both TPN and ORAL groups, a plateau being reached and maintained until 7 min, followed by a linear increase in insulin (fig. 22A). At 8 min the insulin response of the 2 groups diverged and the insulin secretion rate of TPN animals was greater than the ORAL group. Integration of the responses revealed a significantly greater (p<0.05) insulin response during phase II (fig. 22B).

c) The In Vitro Insulin Response of TEN and ORAL Animals (Fig. 23):

The response of the isolated perfused pancreas of TEN and ORAL animals after a 7-day infusion period is shown in fig. 23A. The insulin responses to glucose (300mg/dl) and GIP (0-1ng/ml) were similar between groups. Integration of the phase I (1-7 min) and II (8-40 min) responses revealed no significant differences in total insulin secretion (fig. 23B).

d) Immunocytochemical Quantification of B-Cell and GIP-Cell (K-cell) Populations (Table VIII and Fig. 24):

No significant differences were observed in the islet area and percentage of insulin cells in the islets, nor in the number of immunoreactive GIP cells in the jejunum, among the 3 groups.

A: The insulin response of the isolated perfused pancreas of TPN (n=11) and TEN (n=13) animals to 300mg/dl glucose in the presence of a GIP gradient from 0-1ng/ml after a 7-day TPN period. In this and subsequent figures of data collected from the isolated perfused pancreas studies in which either glucose or a peptide was infused as a gradient, the concentration of the infusate was assumed to increase in a linear fashion, The apparatus used in these experiments was previously shown to deliver infusates in a linear fashion (Pederson et al., 1982; Pederson et al., 1985).

B: The integrated response to 300mg/dl glucose and 1ng/ml GIP. Phase I represents the initial insulin response and the proceeding plateau (periods 1-6 min). Phase II represents the second more progressive rise in insulin release (periods 7-40 min). \*Denotes significance (p<0.05) comparing TEN to TPN animals during each phase using an unpaired Student's t-test. The formula used for the calculation of integrated responses was:

$$X_{I} = \frac{(X_{t0} + X_{t1})(t1 - t0) + (X_{t1} + X_{t2})(t2 - t1) \dots - t_{n-1}(X_{t0})}{2}$$

where  $X_I$  = integrated release of the measured parameter and X = concentration of measured parameter at time = t.





A: The insulin response of the isolated perfused pancreas of TPN (n=10) and ORAL (n=8) animals to 300mg/dl glucose in the presence of a GIP gradient from 0-1ng/ml after the TPN period (7 days). B: The integrated insulin response to 300mg/dl glucose and 0-1ng/ml GIP, \*p<0.05 comparing TPN to ORAL during each phase (phase I, 1-7 min; phase II, 8-40 min) using an unpaired Student's t-test.





B.

IR - INSULIN (mU)







A: The insulin response of the isolated perfused pancreas of ORAL (n=11) and TEN (n=12) animals to 300mg/dl glucose in the presence of a GIP gradient from 0-1ng/ml after the TPN period (7 days). B: The integrated phase I (1-7 min) and II (8-40 min) responses to 300mg/dl glucose and 0-1ng/ml. No significant differences were observed.

### TABLE VIII

PANCREAS	TPN (n=6)	TEN (n=6)	ORAL (n=6)
Mean Islet Area (µm <sup>2</sup> ) % Insulin	12917 ± 1250 66%	11667 ± 2917 71%	$15000 \pm 5417$ 74%
GUT	TPN	TEN	ORAL
- GIP Cells ††	6.7 ± 2.2	9.4 ± 3.6	9.1 ± 2.8

## **QUANTIFICATION RESULTS**

†† Number of immunoreactive GIP cells per 20 villus/crypt profiles/rat.







Insulin-immunoreactive cells in islets from TPN (A), TEN (B) and ORAL (C) animals (magnification X 240).

# 4) <u>The Effect of a 7-Day GIP Infusion on Insulin Release in TPN</u> <u>Animals</u>

Two TPN experimental groups were used in this study, one receiving TPN solution alone, the other receiving TPN solution supplemented with pure porcine GIP (TPN-GIP). A stock solution of GIP was prepared as described for other peptides in the General Introduction. It was prepared each day and added to fresh TPN solution. The final concentration of GIP in the TPN solution ( $10\mu g/55ml$ ) was capable of raising circulating IR-GIP (fig. 25C) to levels within the range observed in TEN animals (fig. 13C).

a) Body Weight (Table IX):

The mean initial and final body weights ( $\pm$ SEM) of rats receiving parenteral alimentation (TPN), parenteral alimentation plus exogenous GIP (TPN-GIP) and rats being orally fed (ORAL), are shown in Table IX. TPN, TPN-GIP and ORAL groups demonstrated significant weight gain over the study period ( $2.6 \pm 0.4$  vs.  $3.3 \pm 0.7$  vs.  $3.4 \pm 0.4$ g/day).

b) <u>The Effect of TPN and TPN+GIP on Plasma Glucose, Insulin and GIP Levels</u> (Fig. 25):

On day 5 of the infusion period, blood samples were collected from TPN, TPN-GIP and ORAL animals for plasma glucose, insulin and GIP determinations. The mean plasma glucose concentration in ORAL animals ( $164 \pm 14$ mg/dl) was significantly lower than observed in TPN ( $206 \pm 13$ mg/dl) and TPN-GIP ( $199 \pm 12$ mg/dl) animals (p<0.05) (fig. 26A). Plasma insulin levels were significantly different among all groups (TPN-GIP,  $167 \pm 12$  vs. TPN,  $133 \pm 4$  vs. ORAL,  $67 \pm 11\mu$ U/ml) (p<0.05) (fig. 25B). The addition of GIP to the parenteral infusate resulted in significantly higher plasma IR-GIP levels ( $1,037 \pm 122$ pg/ml) when compared to TPN ( $493 \pm 74$ pg/ml) and ORAL ( $487 \pm 71$ pg/ml) animals (p<0.05) (fig. 25C).

c) <u>The Effect of TPN and TPN+GIP on the Insulin Response to an OGC (Figs. 26</u> and 27):

The glucose and insulin responses to a 1g/kg oral glucose challenge were measured in TPN, TPN-GIP and ORAL animals after a 7-day infusion period (fig. 26A). Fasting plasma glucose levels were similar in all 3 groups. The glucose response was reduced in TPN and TPN-GIP animals when compared to the ORAL group, the total glucose response being significantly higher in ORAL animals (p<0.05) (fig. 27A). Mean fasting plasma insulin levels were not significantly different among the 3 groups. The peak insulin response in TPN animals ( $67\mu$ U/ml) was higher than in TPN-GIP or ORAL groups, peaking at  $67\mu$ U/ml at 20 min (fig. 26B). The peak insulin responses of TPN-GIP ( $58 \pm 13\mu$ U/ml) and ORAL ( $59 \pm 11\mu$ U/ml) animals occurred at 10 min and 30 min respectively. The integrated insulin response in TPN animals ( $3,581 \pm 288\mu$ U/ml·60 min) was significantly higher than the total response of the TPN-GIP group ( $2,417 \pm$  $246\mu$ U/ml·60 min, \*p<0.05) but not significantly higher than that of ORALS ( $2,920 \pm$  $250\mu$ U/ml·60 min, fig. 27B). The insulinogenic index was significantly greater (p<0.05) in TPN compared with TPN-GIP and ORAL animals ( $0.40 \pm 0.04$  vs.  $0.25 \pm 0.06$  vs.  $0.28 \pm$  $0.06\mu$ U·dl·mg<sup>-1</sup>·ml<sup>-1</sup>).

d) <u>The Effect of Glucose and GIP on Insulin Release from the Isolated Pancreas of</u> <u>TPN, TPN-GIP and ORAL Animals (Fig. 28)</u>:

The isolated pancreata of TPN, TPN-GIP and ORAL animals were perfused with 300mg/dl –glucose in the presence of a 0-1ng/ml GIP gradient. The insulin response of the TPN group was consistently higher than the response observed in TPN-GIP or ORAL animals. The integrated response during phase I (1-7 min) was greater in TPN (7.4  $\pm$  1.5mU) compared to ORAL (5.9  $\pm$  1.3mU) and TPN-GIP (4.9  $\pm$  1.2mU) animals but the difference was not statistically significant (fig. 28B). The phase II response (8-39 min) was significantly higher in TPN (201  $\pm$  16mU) compared to ORAL (147  $\pm$  17mU) and TPN-GIP (119  $\pm$  13mU) animals (p<0.05).

e) <u>The Effect of TPN and TPN+GIP on Somatostatin Release from the Isolated</u> Pancreas (Fig. 29):

The isolated pancreata of TPN, TPN-GIP and ORAL animals were perfused with 300mg/dl glucose in the presence of a 0-1ng/ml GIP gradient. Perfusion samples were collected and pooled during 5 min intervals for somatostatin determination. GIP in the presence of glucose failed to stimulate somatostatin release in any group (fig. 29A). The somatostatin response, however, was consistently higher in ORAL animals throughout the perfusion period. When the responses were integrated (fig. 29B), total somatostatin release was significantly higher

(p<0.05) in the ORAL group  $(2,733 \pm 529$ pg) compared only to TPN-GIP animals  $(1,637 \pm 255$ pg). The response of TPN animals  $(2,229 \pm 350$ pg) was not significantly different from the ORAL group.

## TABLE IX

## BODY WEIGHT (X±SEM) OF TPN, ORAL AND

## TPN-GIP RATS

	INITIAL (g)	FINAL (g)	DAILY (g/day)
TPN	247.7 ± 4.4	265.9 ± 3.2	$2.6 \pm 0.4$
ORAL	$253.0 \pm 4.5$	276.0 ± 2.7	3.3 ± 0.7
TPN-GIP	249.3 ± 5.3	$274.2 \pm 6.0$	$3.4 \pm 0.4$

A: Plasma glucose levels on day 5 of a 7-day infusion period in TPN (n=8), ORAL (n=7) and TPN-GIP (n=8) animals, \*p<0.05 using ANOVA and Newman-Keuls tests comparing levels in each group.

B: Plasma insulin levels on day 5. Significant differences in plasma insulin were observed among groups (TPN-GIP vs. ORAL, \*p<0.05; TPN-GIP vs. TPN, p<0.05; ORAL vs. TPN, p<0.05).

C: Plasma GIP levels on day 5 (TPN-GIP vs. TPN and ORAL, \*p<0.05).





Plasma glucose (A) and insulin (B) responses to a 1g/kg OGC in TPN (n=6), ORAL (n=6) and TPN-GIP (n=6) animals after the 7-day infusion period. 'G' represents the administration of the glucose solution at 0 min.



A: The integrated glucose respose to an OGC, ORAL vs. TPN-GIP and TPN groups, \*p<0.05 using ANOVA and Newman-Keuls tests. B: The insulin responses to an OGC where \* denotes a significant (p<0.05) difference between TPN-GIP and TPN groups,  $\diamond$  denotes no significant difference between TPN-GIP and ORAL groups, and † denotes no significant difference between TPN and ORAL groups using ANOVA and Newman-Keuls tests.



A: The insulin response of the isolated perfused pancreas of TPN (n=8), ORAL (n=7) and TPN-GIP (n=12) animals to 300mg/dl glucose in the presence of a GIP gradient from 0-1ng/ml, after the 7-day TPN period. B: The integrated phase I (1-7 min) and II (8-39 min) responses to glucose and GIP, \*p<0.05 comparing TPN-GIP and TPN groups, p<0.05 comparing TPN and ORAL groups, and  $\Diamond$  indicates no significant difference between TPN-GIP and ORAL groups using ANOVA and Newman-Keuls tests.



A: The SLI response to 300mg/dl glucose in the presence of a 0-1ng/ml GIP gradient in TPN (n=7), ORAL (n=6) and TPN-GIP (n=8) animals. B: The integrated response to glucose and GIP, \*p<0.05 using ANOVA and Newman-Keuls tests comparing the TPN-GIP to ORAL group. There was no significant difference (†) between TPN-GIP and TPN groups, or between TPN and ORAL groups ( $\diamond$ ).

5) <u>The Effect of 3-, 5- and 7-Day TPN on the In Vitro Insulin Response</u> to Glucose and GIP

a) <u>Body Weight (Table X)</u>:

The mean initial and final body weights of rats receiving TPN for 3, 5 and 7 days are listed in Table X. Both TPN and ORAL groups demonstrated significant weight gain during 3, 5 and 7-day infusion periods.

b) <u>The In Vitro Insulin Response to Glucose and GIP after a 3-Day Infusion</u> Period in TPN and ORAL Animals (Fig. 30):

The insulin responses of the isolated pancreata of TPN and ORAL animals to 300mg/dl glucose and a 0-1ng/ml GIP gradient are shown in fig. 30A. The insulin secretion rates of TPN and ORAL animals were equivalent throughout the perfusion. When the insulin responses from TPN and ORAL pancreata were integrated, no significant differences during phase I and II were observed ( $6.5 \pm 1.2$  vs.  $6.7 \pm 2.2$ mU;  $116 \pm 11$  vs.  $105 \pm 23$ mU) (fig. 30B).

c) The In Vitro Insulin Response to Glucose and GIP after 5-Day TPN (Fig. 31): Upon completion of the 5-day infusion period the pancreata of TPN and ORAL animals were surgically isolated and perfused with 300mg/dl glucose and a 0-1ng/ml GIP gradient. The insulin responses of TPN and ORAL animals were equivalent until 30 min when a divergence occurred, the insulin response from the TPN group being greater for the remainder of the perfusion period (fig. 31A). The integrated insulin responses from TPN and ORAL pancreata, however, were not different during phase I or phase II ( $3.4 \pm 0.6$  vs.  $2.9 \pm 0.7$ mU;  $136 \pm 9$  vs.  $122 \pm 10$ mU) (fig. 31B).

d) <u>The In Vitro Insulin Response in TPN and ORAL Animals to Glucose and GIP</u> after 7-Day TPN (Fig. 32):

The pancreata of TPN and ORAL animals were perfused with glucose (300mg/dl) and GIP (0-1ng/ml) after a 7-day TPN period. From period 11 through the remainder of the perfusion, the insulin secretion rate was consistently higher in TPN animals (fig. 31A). The integrated insulin response during phase I was greater in the TPN group, but this difference was not significant (7.3  $\pm$  0.7 vs. 6.2  $\pm$  0.9mU). The response during phase II, however, was

significantly greater (23%) in TPN animals ( $174 \pm 5$  vs.  $134 \pm 8$ mU, p<0.05).

## TABLE X

## BODY WEIGHT (X±SEM) OF TPN AND ORAL

## <u>RATS</u>

		INITIAL (g)	FINAL (g)	DAILY (g/day)
3-DAY TPN ORAI	TPN	197.0 ± 2.1	211.4 ± 0.9	4.8 ± 0.8
	ORAL	$199.8 \pm 4.3$	$215.6 \pm 5.0$	$5.3 \pm 0.6$
~				<i>'</i>
5-DAY	TPN	$223.6 \pm 4.8$	$240.0 \pm 3.9$	$3.3 \pm 0.5$
	ORAL	$221.4 \pm 4.2$	$247.0 \pm 6.2$	$5.1 \pm 0.7$
7-DAY	TPN	267.4 ± 5.0	277.8 ± 5.6	$1.5 \pm 0.4$
	ORAL	296.9 ± 5.3	308.9 ± 3.9	$1.7 \pm 0.6$



Fig. 30

A: The insulin response of the isolated perfused pancreas of TPN (n=5) and ORAL (n=4) animals to 300mg/dl glucose and a 0-1ng/ml GIP gradient after a 3-day TPN period. B: The integrated insulin response to glucose and GIP during phases I and II. No significant differences were observed.



Fig. 31

A: The insulin response of the isolated perfused pancreas to 300mg/dl glucose and a 0-1ng/ml GIP gradient in TPN (n=10) and ORAL (n=8) animals after a 5-day infusion period. B: The integrated phase I and II responses to GIP and glucose. No significant differences were observed.



A: The insulin response of the isolated perfused pancreas to 300 mg/dl glucose and a 0.1 ng/ml GIP gradient in TPN (n=10) and ORAL (n=8) animals after a 7-day infusion period. B: The integrated phase I and II responses to GIP and glucose, \*p<0.05 using an unpaired Student's t-test.

# 6) <u>The Effects of CCK and Glucose on Insulin Release from the</u> <u>Perfused Pancreas after 7-Day TPN</u>

### a) Body Weight (Table XI):

TPN and ORAL animals demonstrated significant weight gain over the 7-day TPN period (2.3  $\pm$  0.3 vs. 2.6  $\pm$  0.3g/day).

#### b) The In Vitro Insulin Response to Glucose and CCK (Fig. 33):

Following a 7-day TPN period, the pancreas of TPN and ORAL animals were perfused with 300mg/dl glucose in the presence of a 0-1ng/ml CCK-8 gradient. The pattern of the insulin response was biphasic in nature, similar to that observed with glucose and GIP (fig. 32). The insulin secretion rate was consistently higher in TPN animals for the entire perfusion period. The integrated insulin responses during phase I and II (fig. 33B) were 48% and 26% greater respectively in TPN compared to ORAL animals ( $10 \pm 1.3$  vs.  $5.3 \pm 0.6$ mU;  $173 \pm 10$  vs.  $129 \pm 9.2$ mU, p<0.05).

## TABLE XI

## BODY WEIGHT (X±SEM) OF TPN AND ORAL

# <u>RATS</u>

INITIAL (g)	FINAL (g)	DAILY (g/day)
$205.0 \pm 1.2$	218.6 ± 1.8	$1.9 \pm 0.2$
$205.4 \pm 0.9$	$220.0 \pm 2.2$	$2.1 \pm 0.2$
	INITIAL (g) 205.0 ± 1.2 205.4 ± 0.9	INITIAL (g) FINAL (g)   205.0 $\pm$ 1.2 218.6 $\pm$ 1.8   205.4 $\pm$ 0.9 220.0 $\pm$ 2.2



Fig. 33

A: The insulin response of the isolated perfused pancreas of TPN (n=10) and ORAL (n=9) animals to 300mg/dl glucose in the presence of a CCK gradient from 0-1ng/ml, after a 7-day TPN period. B: The integrated response during phases I and II, p<0.05 using an unpaired Student's t-test.

#### IV) DISCUSSION

Total parenteral nutrition (TPN) provides a unique experimental situation. The gut endocrine system is bypassed while adequate nutrition is provided via the intravascular route. In contrast to TPN, total enteral nutrition (TEN) delivers the same nutrient load to the GI-tract, but is continuously presenting a stimulus to the GI-endocrine system. The present studies provided the opportunity to observe the effects of chronic intravenous or intragastric feeding on the enteroinsular axis in the rat.

TPN animals demonstrated elevated plasma glucose and insulin levels throughout the 7-day infusion period, while plasma GIP levels resembled those of fasted rats (fig. 13). Intragastric (IG) feeding resulted in an initial elevation of plasma insulin. By day 4, however, levels had stabilized, approaching those of ORAL animals. This initial elevation of insulin in IG fed rats has been observed by others (Baer and Dupré, 1985; Track et al. 1984) and may reflect a period of adaptation to the continuous nutrient load. Similar differences in plasma GIP levels between - IV and IG fed rats have also been demonstrated over a 6-day infusion period (Baer and Dupré, 1985). Chronically elevated or depressed plasma GIP levels could be explained by the presence or absence of luminal nutrients, which are necessary for stimulation of GIP release (Cataland et al., 1974; Pederson et al., 1975). More difficult to explain, however, is the apparent lack of correlation between plasma GIP and insulin levels observed in TPN and TEN animals, most evident during the later stages of the infusion period. Although GIP levels were elevated in the TEN group, it is possible that the insulin response to circulating glucose and GIP levels was appropriate. The glucose-dependent nature of GIP in the rat (Pederson and Brown, 1976; Chan, 1985) may have prevented any further elevation in plasma insulin despite elevated circulating GIP.

It has been hypothesized that the lower plasma insulin and glucose levels observed in IG compared to IV fed rats may be the result of an increased peripheral sensitivity to insulin (Baer and Dupré, 1985). Higher plasma insulin and glucose levels in TPN rats may, on the other hand, reflect a decreased efficiency in nutrient disposal, due to the development of insulin
resistance. Hyperinsulinemia and hyperglycemia are often observed in fasted or fed obese humans and rodents, which also display various degrees of insulin resistance (Olefsky, 1981). Therefore, higher plasma insulin and glucose levels in IV fed animals could be due to the development of insulin resistance. In the present study, however, fasting glucose and insulin levels were similar in all 3 groups (figs. 15, 17 and 19). In addition, TPN animals demonstrated a superior glucose tolerance to an oral glucose load (i.e. glucose response was reduced compared to ORAL animals) (figs.17 and 18). These results are not in support of the development of insulin resistance in TPN animals.

An alternative explanation for the apparent decreased peripheral efficiency with which the nutrient load was assimilated by TPN animals may be related to the absence of stimulation of gastrointestinal factors. It is generally accepted that oral glucose tolerance is superior to intravenous glucose tolerance (Perley and Kipnis, 1967; McIntyre et al., 1964; Elrick et al., 1964). Gastrointestinal hormones or incretins augment insulin release, contributing to this difference (Ebert and Creutzfeldt, 1982). There is also considerable evidence to suggest that GI-factors released by luminal stimulation augment insulin-induced nutrient uptake in hepatic and peripheral tissues in man and rat (Chisholm et al., 1972; Lickley et al., 1975; Livingston and Moxley, 1982; Arner et al., 1983; Bolinder et al., 1985). Livingston and Moxley (1982) determined that rats fed glucose orally not only demonstrated an increased insulin receptor affinity in hepatocytes and adipocytes (at 3 h post-glucose ingestion), but also an increased insulin-mediated conversion of glucose to lipid. Similarly, in man it was shown that oral glucose ingestion was associated with increases in both affinity of the insulin receptor and the cellular sensitivity of adipocytes for insulin (Arner et al., 1983; Bolinder et al., 1985). These effects could not be demonstrated after intravenous glucose administration (Bolinder et al., 1985).

The precise nature of these putative GI-factors remains to be determined, however, there is evidence to suggest that GIP may augment insulin-induced carbohydrate and fat uptake by peripheral tissues (Krarup, 1988). Infusion of porcine GIP facilitated insulin-mediated supression of hepatic glucose production and diminished glucagon-stimulated hepatic glucose production in the dog and the rat (Anderson et al., 1984; Hartmann et al., 1986). Enhanced chylomicron triacylglycerol removal was demonstrated in the dog administered porcine GIP (Wasada et al., 1981), while Eckel et al. (1979) showed increased lipoprotein lipase activity in the presence of GIP in cultured preadipocytes. More recently, Hansen et al. (1985) demonstrated that GIP enhanced insulin receptor affinity and cellular sensitivity in adipocytes, and Beck and Max (1983) showed an enhanced insulin effect on fatty acid incorporation into adipose tissue in the rat. From these studies it can be suggested that hyperinsulinemia associated with systemic delivery of nutrients may have been, in part, due to the absence of GI-factors which normally augment insulin-induced nutrient disposal. Therefore, higher circulating insulin levels may have been required to rectify tissue insensitivity to insulin.

Although alterations in tissue sensitivity could explain the necessity for high circulating insulin levels, other factors could contribute to this condition. First, elevated plasma insulin levels may in part be due to an increased B-cell sensitivity to insulin secretagogues such as glucose or amino acids. An altered sensitivity may result to compensate for the loss of the enteroinsular axis component controlling insulin release. Second, the continuous systemic delivery of the highly insulinotropic dextrose-amino acid solution (fig. 38), coupled with the mild hyperglycemia in TPN animals, could lead to hypertrophy or hyperplasia of pancreatic islets, with normal sensitivity to insulin secretagogues. Karam et al. (1974) suggested that excess intake of B-cell cytotrophic factors, such as carbohydrates and amino acids, could contribute to islet cell hyperplasia observed in obese humans. In addition, rats fed a high carbohydrate diet and which exhibited mild hyperglycemia, developed B-cell hyperplasia within 30 days (Wissler et al., 1949). These subjects will be dealt with in more detail in a later section.

Circadian rhythms for insulin and GIP have been described in the rat when fed <u>ad libitum</u> (Pederson et al., 1985; Rubin et al., 1986). In the present study, circadian rhythmicity was observed for insulin in all 3 experimental groups (fig. 14B). A rhythmicity for GIP, however, was only observed in the ORAL group, with levels remaining chronically elevated and depressed in TEN and TPN animals respectively (fig. 14C). Circadian fluctuations have also been observed for insulin in IV and IG fed animals (Lanza-Jacoby et al., 1982), but no rhythm for GIP was demonstrated in starved animals (Rubin et al., 1986). These results indicated that despite continuous intragastric or intravenous feeding, daily fluctuations in plasma insulin were present. The GIP cell, however, required the direct contact with and the absorption of luminal nutrients for release, plasma levels fluctuating only in response to meal feeding.

Intravenous or intragastric feeding for 7 days was found not to affect fasting plasma insulin or glucose levels, as no significant differences among the 3 groups were observed (fig. 15, 17, and 19). With respect to TPN animals, these findings are in agreement with those of other studies (Pederson et al., 1985). Intragastric feeding was also associated with normal insulin and glucose responses to an OGC, as no difference between ORAL and TEN animals was observed. These data suggest that although GIP levels were chronically elevated in the TEN animals, no visible alterations in the hormonal component of the enteroinsular axis occurred. It has been shown that plasma GIP levels can rise to 2,000pg/ml in the rat following an OGC (Pederson et al., 1982), a level approximately 35% greater than mean levels observed in TEN animals during the infusion period (fig. 13C). Therefore, a modification of the enteroinsular axis (i.e. 'down regulation' of GIP receptors on B-cells) may require higher circulating GIP levels or a longer --- duration of hyperGIPemia.

In previous experiments, a reduced glucose and exaggerated insulin response to an OGC were observed after 6-day TPN (Pederson et al., 1985). In the present study, a reduced plasma glucose response to an OGC was observed after TPN and was consistent with these previous experiments (figs. 15-20). The total insulin response, however, was only significantly greater in TPN when compared to TEN animals. Another way to interpret these results was to assess the insulin response in relation to the plasma glucose response. The insulinogenic index, the ratio between total insulin and glucose responses, provided this comparison and has been employed by others when interpreting glucose challenge experiments (Perley and Kipnis, 1967; Ebert and Creutzfeldt, 1982). The insulinogenic index was significantly greater in TPN when compared to plasma glucose was exaggerated in TPN animals. The exaggerated insulin response in relation to plasma glucose feeding led to a modification of the enteroinsular axis. Although the specific modification cannot be elucidated from these studies, a normal plasma GIP

response to an OGC in TPN animals has been observed (Pederson et al., 1985), suggesting that the GI-endocrine component of the axis is normal and that the modification is distal to the release of GIP.

Studies using the <u>in vitro</u> isolated perfused pancreas were employed to further investigate the effects of IV and IG feeding on the enteroinsular axis and the involvement of GIP. The <u>in vitro</u> insulin response to glucose and GIP was characteristically biphasic in all 3 groups (figs. 21-23). It has been shown that the threshold concentration of GIP during hyperglycemia (300mg/dl glucose) for potentiation of glucose-stimulated insulin release <u>in vitro</u> was approximately 250-350pg/ml (Chan, 1985; Pederson et al., 1985). The present study is in agreement with these findings. A GIP threshold concentration of approximately 300-375pg/ml was observed, with no visible differences between TPN, TEN or ORAL animals. The phase I response, representing insulin secretion during the initial stages of the perfusion (0-7 min), was attributed to the insulinotropic actions of glucose alone. The phase II response represented GIP-potentiated insulin secretion.

The phase I responses were found to be higher in TPN animals compared with TEN or ORAL animals. These results are similar to those of Pederson et al. (1985) where it was shown that insulin secretion from TPN pancreata was consistently higher during the initial stages of the perfusion. Based on these studies it is difficult to say with certainty whether TPN affects glucose stimulated insulin release, since a GIP stimulus was also present. However, higher phase I responses in TPN animals are indicative of an increased pancreatic sensitivity to glucose. Studies designed to further investigate this possibility are found in chapter three.

The <u>in vitro</u> insulin response during phase II (8-40 min) was 20% greater in TPN when compared to ORAL animals (fig. 22). Such a finding was consistent with previous experiments in which a 6-day TPN period was employed (Pederson et al., 1985). In addition to these observations, the present study also showed that the insulin response during phase II was greater from the pancreata of TPN compared to TEN animals (fig. 21). These results demonstrated that the increased <u>in vitro</u> insulin response to glucose and GIP in TPN animals was not due to the administration of the infusion solution itself, since TEN animals received the identical formulation. The divergence of the insulin curves between TPN and ORAL or TEN animals during the second phase of the perfusion were indicative of an increased B-cell sensitivity to GIP.

Immunocytochemical studies of the pancreas have demonstrated normal islet size and B-cell mass after 7-day intravenous or intragastric feeding (table VIII) (fig. 24). In the obese Zucker rat, which is hyperinsulinemic and exhibits an exaggerated in vitro insulin response to GIP, it was shown that pancreatic islets were enlarged and contained a greater proportion of B-cells (Chan et al., 1984). Hyek and Woodside, (1979), demonstrated that enlarged islets from these rodents were capable of secreting greater amounts of insulin in vitro under basal and stimulated conditions. These studies suggested that a causal relationship between hypertrophic islets and the exaggerated insulin response of obese rodents existed. In TPN and TEN rats, however, normal islet size and B-cell mass suggested that hypertrophy of existing B-cells and/or hyperplasia of B-cells did not occur. A more plausible explanation for the greater insulin response from TPN pancreata is, therefore, an increased B-cell sensitivity to GIP. However, a - larger insulin response may have also been attributed to an increased pancreatic islet population, or an increased, readily-releasable insulin pool within B-cells. Determining the source of the larger insulin response to GIP would require measurement of the total pancreatic insulin content and total pancreatic islet volume. It would further require an in vitro islet preparation to assess the effects of GIP. Although measurement of total insulin content is not a difficult procedure (Lake et al., 1987), the well-established pancreatic atrophy associated with TPN (presumably of the exocrine pancreas) demonstrated by Johnson et al. (1975a, 1977), may have led to ambiguous results. Another approach would be to isolate pancreatic islets and undertake release studies with GIP, measuring insulin release in proportion to total islet insulin content. The inherent problems associated with islet isolation render such preparations inappropriate for use in the present study. They include: a long incubation period for islets to recover from the isolation procedure (Siegel and Creutzfeldt, 1985); and the relative insensitivity of such preparations to physiological doses of GIP (Siegel and Creutzfeldt, 1985; Ishizuka et al., 1988), when compared to the perfused pancreas (Pederson et al., 1985). Although an increased islet number

or B-cell content of insulin cannot be discounted, the fact that the insulin response curves of TPN pancreata diverge from TEN or ORAL pancreata in the presence of a GIP gradient supports an increased sensitivity of B-cells to GIP.

The present study revealed that the GIP-containing cell number of the jejunum was similar in all experimental groups (table VIII). It has been reported that 7-days of TPN had little effect on mucosal depth or on the enteroendocrine cell populations of the small bowel, with the exceptions of CCK and neurotensin (Buchan et al., 1985). The CCK cell number was found to dramatically decrease in both the duodenum and jejunum, while the number of neurotensin-containing cells in the ileum increased. Similarly, 7-days of TPN in the rat was also associated with hypoplasia of the antral G-cell population (fig. 47). These results indicate that although certain enteroendocrine cell types are susceptible to alteration during TPN (presumably due to lack of enteral nutritional stimulus), the GIP cell population is not affected. The observation that prolonged fasting (36 h) in man failed to affect basal and stimulated GIP secretion lends further support to this hypothesis (Andrews et al., 1983). Continuous intragastric feeding, which led to elevated circulating GIP levels, also had no effect on the GIP-cell population of the jejunum. Similar results have been obtained in obese rodents which were shown to be hyperphagic (Chan et al., 1984). These studies suggested that the GIP-cell population was also resistant to change during chronic stimulation.

In a second study, the role of GIP in the development of the exaggerated <u>in vivo</u> and <u>in vitro</u> insulin responses to glucose and GIP in TPN animals was investigated. To examine whether alterations in the enteroinsular axis were the result of chronically suppressed plasma GIP levels, exogenous porcine GIP (EG III) was added to the TPN medium and infused at a continuous rate along with the TPN solution.

Infusion of porcine GIP with the TPN solution produced plasma GIP levels (fig. 25C) comparable to those observed during TEN (fig. 13C). The elevated plasma GIP in TPN-GIP animals was associated with even higher plasma insulin than that observed with TPN alone, but did not result in a significant reduction in plasma glucose (fig.25). Such findings are consistent with previous TPN studies where exogenous porcine GIP was infused (Baer and Dupré, 1985).

These experiments raise some interesting possibilities regarding the role of exogenous porcine GIP and the mode of nutrient delivery.

First, they suggest that the full biological actions of porcine GIP may be different from endogenously released rat GIP, stimulating insulin release but not facilitating nutrient disposal. Although the structure of rat GIP has not been confirmed, differences between rat and porcine GIP are thought to exist (Amland et al., 1984; Walsh, 1987).

Another possibility is that GIP may contribute directly to fat metabolism, as suggested by various investigators (Eckel et al., 1979; Wasada et al., 1981; Beck and Max, 1983; Salera et al., 1983), but play only an indirect role on glucose metabolism by stimulating insulin release. Thus, it has been suggested that other physiological mechanisms, initiated by nutrients in the GI-tract, may augment the actions of insulin on glucose metabolism in peripheral tissue (Baer and Dupré, 1985). Studies showing enhanced insulin effectiveness on glucose uptake in adipocytes after ingestion of an oral glucose load (Arner et al., 1983; Bolinder et al., 1985), but not after intravenous glucose infusion (Bolinder et al., 1985), certainly suggest that such unknown gut factors may contribute to nutrient metabolism. The absence of stimulation of these factors during intravenous feeding may partially explain the apparent decreased sensitivity of peripheral tissues to insulin.

Other factors could also contribute to the hyperglycemia in TPN-GIP animals, including: the development of insulin resistance causally related to the hyperinsulinemia; impaired hepatic extraction of glucose; and the presence of catabolic hormones such as glucagon. The possibilities of insulin resistance and impaired hepatic handling of glucose were not assessed in this study. Normal fasting plasma insulin and glucose levels, and a superior glucose tolerance in TPN-GIP over ORAL animals do not, however, support these possibilities (fig. 26). Glucagon secretion is stimulated by GIP and amino acids (Pederson and Brown, 1978), but a role for glucagon in the maintenance of hyperglycemia is unlikely since it has been demonstrated that glucagon is not significantly elevated during TPN, with or without the addition of exogenous GIP (Baer and Dupré, 1985). These present studies do not reveal the primary cause of hyperinsulinemia and mild hyperglycemia in TPN-GIP rats. They do, however, point

out important differences between enteral and parenteral feeding which cannot be completely explained by the presence or absence of the incretin GIP.

Oral glucose challenge studies performed after the 7-day infusion period revealed a reduced glucose response in TPN and TPN-GIP animals, while the insulin response was greatest in the TPN group (fig. 26 and 27). The insulinogenic index demonstrated that the insulin response was comparable in TPN-GIP and ORAL groups but exaggerated in the TPN group. These results indicated that the infusion of porcine GIP 'normalized' the insulin response to an oral glucose load and ensuing plasma glucose levels. This further supports a role for chronically low plasma GIP levels in the development of B-cell hypersensitivity during TPN.

It was interesting to note that both TPN and TPN-GIP animals exhibited a reduced glucose response to an OGC. It was originally suggested that the reduced plasma glucose response in TPN animals was brought about by the exaggerated insulin response (Pederson et al., 1985). This may be partly true since the total glucose response was lowest in the TPN group, however, the blunted glucose response in TPN-GIP animals suggested that other factors were involved. Similar blunted responses have been observed in JIB and small bowel resected rats (MSBR) where the absorptive area of the gut was significantly reduced (Pederson et al., 1982; Buchan et al., 1983). While no change in mucosal depth after 7-day TPN has been reported (Buchan et al., 1985), others have demonstrated decreased gut mass, mucosal cell hypoplasia and reduced brush border enzyme activity after TPN (Johnson et al., 1975a, b). In another study, the wet weight of the GI-tract from TPN animals was reduced by 60% (Innis, 1986). It is quite possible that TPN caused atrophy of the small bowel, which led to a decreased surface area for glucose absorption. This would partly explain the blunted glucose response in TPN and TPN-GIP animals.

The <u>in vitro</u> insulin responses to glucose and GIP are shown in fig. 28. TPN animals demonstrated a 25% greater second phase insulin response than observed in ORAL and TPN-GIP animals. Results of this experiment indicated that infusion of GIP for a period of 7 days, which raised plasma levels 2-fold higher than observed in the TPN group, resulted in a 'normal' <u>in vitro</u> insulin response to GIP. It can be implied, therefore, from both <u>in vivo</u> and <u>in vitro</u> studies, that the exaggerated insulin response of TPN animals was causally related to chronically depressed circulating GIP levels.

The possibility that alterations in pancreatic somatostatin secretion (i.e. a decrease) may have contributed to the exaggerated insulin response of TPN pancreata to GIP was investigated. However, simultaneous measurement of insulin and SLI output from the pancreata of TPN, TPN-GIP and ORAL animals revealed no correlation between SLI secretion and insulin output in response to GIP (fig 29). It can be suggested from these results that increased B-cell activity associated with TPN was not the result of altered pancreatic SLI secretion.

To determine the rapidity of onset of the increased <u>in vitro</u> insulin response to glucose and GIP observed after 7-days of TPN (fig. 32), animals were exposed to TPN periods of shorter duration. Slight increases in insulin secretion rate above that observed in ORAL animals were apparent after 3 and 5-days of TPN (fig 30 and 31). It was evident, however, that significant hypersensitivity of the B-cell to GIP and glucose, whether it be through a modification at the receptor or post-receptor level, required an extended period of intravenous alimentation and chronically depressed plasma GIP levels.

The inability of parenterally administered amino acids and fats to stimulate CCK release (Fried et al., 1982) and the apparent CCK cell hypoplasia of the small bowel associated with TPN (Buchan et al., 1985), suggested that circulating CCK levels remained depressed during TPN. The <u>in vitro</u> insulin response to CCK-8 presented as a gradient was significantly higher from TPN pancreata (fig. 33). As has been proposed for GIP (Pederson et al., 1985), a causal relationship between ambient CCK levels and the B-cell sensitivity to CCK may, therefore, exist. Thus, low circulating CCK levels may lead to changes in B-cell sensitivity to CCK, possibly reflecting alterations at the receptor or post-receptor level. The presence of CCK receptors associated with B-cells, as visualized using autoradiography (Sakamoto et al., 1985), and the ability of CCK to stimulate insulin release from perifused islets (Zawalich et al., 1987), certainly indicate that such an alteration in sensitivity may be due to a receptor modification. In addition, the ability of CCK-8 to stimulate insulin release in a concentration range of 0-1ng/ml in the present study, further supports a role for CCK in the enteroinsular axis and therefore in the control of insulin release.

The present series of experiments allowed for several conclusions. First, the observation that TPN led to chronically depressed circulating GIP levels, in contrast to the effects of intragastric feeding, indicated that the presence of luminal nutrients was required to maintain plasma GIP levels. Second, fluctuations in GIP levels over the course of a 24-h period were only observed in ORAL animals, indicating that a circadian rhythm for GIP is primarily the result of intermittent feeding. Third, the GIP-cell population of the jejunum is not susceptible to alteration by TPN and thus the chronic absence of luminal nutrients, in contrast to the CCK-cell population of the jejunum or the G-cell population of the antrum. Fourth, TPN animals exhibit hyperinsulinemia and mild hyperglycemia, a condition not observed in TEN animals. These results indicated that intravenously delivered nutrients were assimilated with less efficiency than intragastrically administered nutrients. Such a phenomenon could be attributed to the presence of gut factors released by luminal nutrients, which facilitated nutrient uptake by hepatic and peripheral tissues. The present data did not support the development of insulin resistance.

TPN animals demonstrated an exaggerated insulin response to an oral glucose load <u>in vivo</u> --and to GIP and glucose <u>in vitro</u>. It was hypothesized that this condition was the result of an increased B-cell sensitivity to glucose, brought about by chronically low GIP levels (Pederson et al., 1985). The present experiments add further support to this hypothesis. First, infusion of the TPN solution intragastrically did not alter pancreatic sensitivity to glucose or GIP, suggesting that continuous delivery of the infusion solution was not responsible for B-cell hypersecretion. Second, this condition could not be explained by hyperplasia of the GIP-cell population in the gut or by GIP-cell hypersecretion. Third, insulin hypersecretion was not the result of an increased B-cell mass. Fourth, the divergence of the insulin curves between TPN and TEN or ORAL animals <u>in vitro</u> in the presence of GIP (0-1ng/ml) was indicative of an increased B-cell sensitivity to GIP. Finally, the addition of GIP to the TPN solution, raising circulating plasma GIP levels to the equivalent of those observed in TEN animals, prevented B-cell hypersecretion <u>in vivo</u> and <u>in vitro</u>. These experiments indicated that the B-cell sensitivity to GIP is affected by ambient plasma GIP levels, and that changes in sensitivity may have been mediated by alteration at the receptor or post-receptor level.

An increased receptor density on the B-cell could account for the increased pancreatic insulin response to GIP observed in the present study and by Pederson et al. (1985). A number of peptide hormones can regulate their respective receptor densities. With few exceptions, an increase in ambient hormone concentration leads to a decreased density of their homologous receptors (Catt et al., 1979). This phenomenon is known as 'down regulation'. As suggested by Pederson et al. (1985), it is possible that in the TPN situation low circulating GIP levels led to an increased receptor density, or 'up regulation' of GIP receptors,. Although an attractive explanation for the increased insulin response to GIP after TPN, this phenomenon at present has only been described for insulin where reduced insulin secretion was associated with an increased receptor number in liver tissue of hamster and rat (Hepp et al., 1975; Davidson and Kaplan, 1977). 'Up regulation' has also been described for both gastrin (Takeuchi et al., 1980) and prolactin (Shiu and Friesen, 1981). In both situations, however, greater receptor density was associated with increased, not decreased, ambient hormone levels. The time required for de novo synthesis of membrane receptors can be from hours to tens of hours (Hollenburg and Goren, 1985). Thus the significantly longer time required for the manifestation of the increased insulin response (7 days) may reflect a more complex phenomenon than simply an increased GIP-receptor density on B-cells.

Although receptor number often correlates well with the magnitude of the cellular response (Catt et al., 1980), other factors at the receptor level can influence these responses. In addition to down regulation of receptor number, increases in ambient hormone levels can lead to receptor desensitization through direct inactivation of receptors, decreased affinity of receptors (negative cooperativity), and/or alteration in receptor organization and distribution, making binding of receptor and ligand more difficult (Jaret and Smith, 1985). All of these possibilities could lead to a reduced cellular response, not necessarily altering receptor number. The degree of reduction in the response would depend on the magnitude of increase in ambient hormone levels. In the TPN situation, therefore, the increased B-cell response may reflect an increase in receptor number, or an increased receptor sensitivity, where the number of 'active' receptors is greater. In addition to potential changes in cell surface receptors, post-receptor changes may also account for the

increased sensitivity.

High ambient hormone levels are also associated with changes in post-receptor sensitivity to hormones. It has been demonstrated <u>in vivo</u> in morbidly obese human (Olefsky, 1981), and <u>in vitro</u> from cultured rat adipocytes (Garvey et al., 1986), that high ambient insulin levels are not only associated with insulin receptor down regulation, but also with post-receptor desensitization. In contrast, it is possible that chronically low circulating GIP levels, as observed during TPN, may lead to receptor and/or post-receptor modifications of B-cells, accounting for the increased sensitivity to GIP.

Different methodology, such as that employing isolated islet preparations maintained in culture, could provide further insight into the role of ambient GIP levels in the acute responsiveness of B-cells to GIP and other insulin secretagogues. This preparation, coupled with an RIA for insulin and a radio-receptor assay for GIP, could be used to determine if conditions resembling chronic hypo- or hyperGIPemia alter: 1) receptor density or sensitivity; 2) post receptor events leading to insulin release and/or insulin content of the B-cell population; and -3) islet volume of the pancreas. Such techniques should be considered for future studies on the role of GIP in the enteroinsular axis.

# CHAPTER THREE THE EFFECT OF TPN ON NUTRIENT AND NEURONAL COMPONENTS OF THE ENTEROINSULAR AXIS

### I) INTRODUCTION

Insulin secretion from the pancreatic B-cell is regulated by 3 major factors: metabolites, hormones and neurotransmitters (Berthoud, 1984; Edwards, 1984; Malaisse, 1984). The complex interactions between these factors and the B-cell allows for an appropriate insulin response under various metabolic conditions. A variety of circulating nutrients can stimulate insulin release, however, D-glucose appears to be the primary regulator of insulin secretion (Gerich et al., 1976; Hedeskov, 1980). D-glucose exerts a direct effect on the B-cell and an indirect effect, modulating insulin release by other insulinotropic agents. The vagus nerve constitutes the primary stimulatory pathway of the autonomic nervous system, being activated by the cephalic phase of food ingestion and during hyperglycemia (Berthoud, 1984; Edwards, 1984). Classic neurotransmitters, such as acetylcholine, and the peptidergic neurotransmitter VIP, have been implicated as final mediators of vagally-induced insulin secretion (Ahren et al., 1986). The GI-hormones gastrin, GIP and CCK all demonstrate insulinotropic activity in physiological concentrations (Rehfeld and Stadil, 1973; Pederson et al., 1982; Meuller et al., 1983) and, at least with respect to GIP, constitute major hormonal components of the enteroinsular axis (Creutzfeldt, 1979; Creutzfeldt and Ebert, 1985).

Early studies by McIntyre et al. (1964) and Elrick et al. (1964), demonstrating that oral glucose elicited a greater insulin response than intravenous glucose, confirmed the presence of gut factors which potentiated glucose-stimulated insulin release. The relative contribution of these gastrointestinal factors, including GIP, has been the subject of investigation in man and rat (Perley and Kipnis, 1967; Ebert and Creutzfeldt, 1982). From these studies it has been estimated that approximately 50% of the insulin secreted after enterally administered glucose was released by GI-factors. The ability of an anti-GIP serum to reduce this insulin response

attributed to gut factors clearly demonstrated the involvement of GIP (Ebert and Creutzfeldt, 1982; Lauritsen et al., 1981). Together, these studies demonstrated that the enteroinsular axis represents a major control mechanism for insulin release and, therefore, body fuel homeostasis.

During TPN, the nutrient load is delivered systemically, bypassing the GI-tract and, presumably, the hormonal component of the enteroinsular axis. Studies which show that circulating GIP levels remain low during TPN confirm that at least a major part of the hormonal component of the axis is inactive (Baer and Dupré, 1985; Pederson et al., 1985) (fig. 14). In the TPN situation, therefore, insulin secretion is controlled by neuronal and nutritive inputs to the Bcell, as well as possible interactions with other islet hormones. The relative contribution of these remaining components, which ensure an adequate insulin response for assimilation of incoming nutrients, must, therefore, increase. If this did not occur, TPN animals would be severely hyperglycemic, hypoinsulinemic and would lose weight, a situation which does not develop (Track et al., 1984; Baer and Dupré, 1985; Lickley et al., 1978).

The purpose of the present study was to examine the effects of TPN on insulin secretion both - <u>in vivo</u> and <u>in vitro</u>. It was previously demonstrated that 7-day TPN led to a modification of the enteroinsular axis, attributed to an increased B-cell sensitivity to GIP (Pederson et al., 1985). TPN may also lead to modifications in other components of the axis (nutrient and neuronal components), since nutrients and neural influences provide the main stimulus for insulin release during TPN. The present series of experiments was designed to test this hypothesis.

In the first study, animals were fed intravenously for a period of 7 days. Blood samples were collected from TPN and control animals during the infusion period to assess the effects of TPN on circulating glucose and insulin levels. After the 7-day period, the pancreata of TPN and control animals were isolated and perfused with glucose presented as a gradient or as a constant infusion, to determine if TPN altered the pancreatic sensitivity to glucose.

In a second study, the <u>in vitro</u> perfused stomach-pancreas preparation was employed. This preparation was used to examine the insulin response to vagal stimulation and to the cholinergic agonist acetyl-ß-methylcholine (MCh) after 7-day TPN. Both gastrin and glucagon demonstrate insulinotropic activity (Rehfeld and Stadil, 1973; Samols et al., 1986), so it is possible that they

contributed to the exaggerated <u>in vivo</u> insulin response to glucose observed after TPN (Pederson et al., 1985). The perfused stomach-pancreas preparation allowed for simultaneous measurement of these peptides, along with insulin. In addition, the effect of TPN on the <u>in vitro</u> pancreatic response to VIP, an insulinotropic neuropeptide, was examined.

#### II) METHODS

All methods were previously described in General Methods and Materials.

#### III) **RESULTS**

# 1) <u>The Effect of 7-Day Total Parenteral Nutrition on Glucose Stimulated</u> <u>Insulin Release</u>

a) Body Weight (Table XII):

TPN, TEN and ORAL animals demonstrated significant (p<0.05) weight gain over the 7-day infusion period with average daily increases of  $2.9 \pm 0.6$ ,  $3.7 \pm 0.7$  and  $3.1 \pm 0.6$ g respectively.

b) Plasma Glucose and Insulin Levels on Days 2, 4, and 6 (Fig. 34):

Blood samples were obtained for glucose and insulin determination on days 2, 4 and 6 from non-fasted animals. Plasma glucose levels (fig. 34A) were significantly higher in TPN animals on day 2 compared to TEN or ORAL animals Plasma glucose levels were also higher in TPN animals on days 4 and 6, but this difference was not significant. Plasma insulin levels were found to differ significantly (p<0.05) among all groups on day 2, with TPN animals exhibiting the highest concentration (fig. 34B). Circulating levels remained significantly higher (p<0.05) in TPN animals on days 4 and 6; levels in TEN and ORAL animals being equivalent.

c) The In Vitro Insulin Response to a Glucose Gradient (Fig. 35):

Glucose was presented to the perfused pancreas of TPN, TEN and ORAL animals as a gradient from 80 to 225mg/dl. The insulin response to glucose is shown in fig. 35A. In TPN animals insulin secretion did not rise above basal levels until min 13 of the perfusion

(corresponding to a perfusate glucose concentration of 145mg/dl glucose). In ORAL and TEN animals, insulin did not rise significantly above basal values until min 17, corresponding to 160mg/dl glucose. The threshold for insulin secretion above basal levels was, therefore, lower in TPN compared to TEN or ORAL animals; above this level, insulin secretion was consistently higher for any given concentration of glucose in the TPN group. The integrated insulin response to glucose (fig. 35B) was significantly higher in TPN (8.8  $\pm$  1.6mU) compared with TEN (4.5  $\pm$  0.5mU) or ORAL (3.6  $\pm$  0.5mU) animals (p<0.05).

d) <u>The In Vitro Insulin Response to 300mg/dl Glucose: TPN vs. ORAL Animals</u> (Fig. 36):

The isolated pancreas of TPN and ORAL animals was perfused with 300mg/dl glucose. The insulin response to glucose in both groups was biphasic; first a rapid increase in insulin secretion followed by a nadir (0-7 min), then a linear increase for the remainder of the perfusion. The insulin response was consistently higher in TPN animals for the entire perfusion period. The integrated insulin response to glucose for phase I was significantly higher (p<0.05) in TPN (6.5  $-\pm 0.6$ mU) compared to ORAL (4.7  $\pm 0.6$ mU) animals. The phase II insulin response was higher in TPN compared to ORAL animals, but the difference was not significant (fig. 36B).

e) <u>The In Vitro Insulin Response to Arginine and Glucose: TPN vs. ORAL</u> <u>Animals (Fig. 37)</u>:

The isolated pancreata of TPN and ORAL animals were perfused with 10mM arginine from 6-15 min in the presence of glucose presented as a gradient from 200-300mg/dl. During the period 1-5 min, insulin secretion was consistently higher in the TPN group, the total response being significantly greater from TPN pancreata  $(2.0 \pm 0.15 \text{ vs. } 1.2 \pm 0.18 \text{ mU}, \text{ p} < 0.05)$  (fig. 37B). Arginine produced a biphasic response in both groups. Integration of the data revealed no significant differences in responses between TPN and ORAL animals  $(21.8 \pm 2.5 \text{ vs. } 21.8 \pm 1.2 \text{ mU})$ .

f) <u>The In Vitro Insulin Response of Wistar Rats</u> to 300mg/dl Glucose and TPN <u>Solution (Fig. 38)</u>:

The isolated pancreata of Wistar rats were perfused with perfusate containing 300 mg/dl glucose. From periods 13-22 min, TPN solution #1 was introduced by side arm infusion. The TPN solution demonstrated high insulinotropic activity, with the insulin response during this time period being significantly elevated (p<0.05) over that observed with glucose alone.

### TABLE XII

## BODY WEIGHT (X ± SEM) OF TPN, TEN, AND ORAL RATS

	INITIAL (g)	FINAL (g)	DAILY (g/day)	
TPN	247.0 ± 3.2	267.0 ± 2.2	$2.9\pm0.6$	
TEN	$249.0 \pm 4.6$	$275.0 \pm 1.0$	$3.7 \pm 0.7$	
ORAL	$238.0 \pm 3.0$	$260.0 \pm 3.0$	$3.1 \pm 0.6$	



A: Plasma glucose levels during the 7-day TPN period of TPN (n=12), TEN (n=12) and ORAL (n=13) animals, \*p<0.05 using ANOVA and Newman-Keuls tests, comparing groups at each time period.

B: Plasma insulin levels were found to differ significantly among all three groups on day 2 (TPN vs. ORAL, \*p<0.05; TPN vs. TEN,  $\dagger p$ <0.05; and TEN vs. ORAL,  $\diamond p$ <0.05). On days 4 and 6 insulin levels were found to be significantly higher in TPN compared to ORAL and TEN groups (\*p<0.05).



A: The insulin response of the isolated perfused pancreas to a glucose gradient from 80-225mg/dl in TPN (n=11), TEN (n=9) and ORAL (n=8) animals. B: The integrated response to glucose, p<0.05 using ANOVA and Newman-Keuls tests.

146



A: The insulin response of the isolated perfused pancreas of TPN (n=7) and ORAL (n=7) animals to 300mg/dl glucose after a 7-day TPN period. B: The integrated insulin response to glucose, p<0.05 using Student's t-test for unpaired data.

147



A: The insulin response of the isolated perfused pancreata of TPN (n=7) and ORAL (n=7) animals to 10mM arginine in the presence of glucose presented as a gradient (200-300mg/dl).

B: Integrated responses during a period of glucose infusion alone (1-5 min) and with 10mM arginine introduced by side arm infusion. \*Denotes a significant difference (p<0.05) using a Student's t-test for unpaired data.



The insulin response of the isolated perfused pancreata of Wistar rats (n=4) to the TPN solution #1 delivered as a side arm infusion in the presence of 300mg/dl glucose. \*Denotes significance (p<0.05) using a Student's t-test for paired data, comparing insulin secretion during TPN solution infusion with the mean response to 300mg/dl glucose alone.

149

#### 2) The Effect of TPN on Islet Morphology

a) Pancreatic Morphology (Table XIII):

After a 7-day infusion period, TPN and ORAL animals were sacrificed and pancreatic tissue obtained for immunocytochemical identification and quantification of the islet cell population. Results from these studies revealed that insulin cells always formed the core of the islets, with glucagon, pancreatic polypeptide and somatostatin-containing cells occupying the periphery (fig. 39). The mean total islet area, and the percentage of the islet occupied by each cell type, were similar among groups. Quantitative analysis of the islets of TPN and pancreatic control animals is shown in Table XIII.

## TABLE XIII

## ISLET SIZE AND COMPOSITION IN TPN AND CONTROL RATS

	ISLET SIZE	ISLET COMPOSITION			(% ± SEM)	
	(µm²)	Insulin	Glucagon	PP	Soma	
TPN	18,259	73.8	17.6	7.3	5.7	
(n=50)	± 1,394	± 1.2	± 0.7	± 0.4	± 0.6	
CONTROL	17,361	71.7	17.1	6.8	5.3	
(n=50)	± 1,269	± 1.0	± 0.6	± 0.3	± 0.4	

\* No significant differences were observed.

Immunocytochemical examination of pancreatic islets of TPN and ORAL animals after a 7-day period of infusion (magnification X 240).

- A: TPN, insulin;B: TPN, pancreatic polypeptide;C: TPN, glucagon;
- D: TPN, somatostatin;
- E: ORAL, insulin;
- F: ORAL, pancreatic polypeptide; G: ORAL, glucagon; and H: ORAL, somatostatin.

B.

C.

D.



153

E.



#### 3) The Effect of TPN on Neurally-Mediated Insulin Release

#### a) Body Weight (Table XIV):

TPN and ORAL animals demonstrated significant (p<0.05) weight gain during the TPN period. The average daily weight increase for TPN and ORAL animals was  $2.5 \pm 0.3$  and  $2.6 \pm 0.3$ g respectively.

b) <u>The Effects of MCh and Vagal Stimulation on Glucose-Stimulated Insulin</u> <u>Release, TPN vs. ORAL Animals (Figs, 40-41)</u>:

Following a 7-day TPN period, the pancreas and stomach were isolated and perfused with 160mg/dl glucose. In the presence of glucose, the vagi were stimulated subdiaphragmatically (7V, 10Hz, 5msec) during 11-20 min, and  $5\times10^{-5}$ M MCh was infused via a sidearm infusion during 31-40 min. Glucose alone was able to stimulate insulin secretion above basal levels (fig. 40). The integrated insulin response to 160mg/dl glucose during periods 0-10 min and 21-30 min did not reveal any significant differences between TPN and ORAL animals (fig. 41A, B). Vagal stimulation produced a rapid and significant biphasic increase of insulin above levels – produced by glucose alone (fig. 40B). The integrated insulin response in TPN animals was found to be significantly greater (25%) than in ORAL animals (9.2 ± 0.6 vs. 6.9 ± 1.1mU) (p<0.05). MCh produced a biphasic insulin response similar to that observed with vagal stimulation but the response was 4-fold greater with MCh (fig. 40C). The integrated insulin response to MCh was 23% greater in TPN (44 ± 2mU) than ORAL (34 ± 1mU), the difference being significant (p<0.05).

c) <u>The Effects of Vagal Stimulation and MCh on Glucagon and Gastrin Release:</u> <u>TPN vs. ORAL Animals (Fig. 42)</u>:

The stomach-pancreas preparations were perfused with 160mg/dl glucose alone or in the presence of vagal stimulation (7V, 10Hz, 5msec, periods 11-20 min) or MCh (periods 31-40 min). Mean basal glucagon levels were similar in TPN and ORAL animals ( $178 \pm 44$  vs.  $140 \pm 25$ pg/ml). Vagal stimulation failed to elicit a response above basal levels in either group. MCh, however, produced an average 2-fold increase in glucagon secretion in both groups ( $341 \pm 50$  vs.  $377 \pm 54$  pg/ml).

Basal gastrin levels from the perfused stomachs of ORAL animals were 4-fold higher than from TPN animals ( $249 \pm 32$  vs.  $58 \pm 11$ pg/min). Vagal stimulation produced a 4-fold increase in gastrin secretion above basal levels in both groups, however, the mean gastrin response was significantly higher in ORAL animals ( $1,071 \pm 144$  vs.  $262 \pm 57$  pg/min). MCh produced a transient increase in gastrin secretion in both groups, but the increases above basal levels were not significant.

d) <u>The Effects of VIP and Glucose on Insulin Release from the Pancreas</u> <u>Preparation of TPN and ORAL Animals (Fig. 43)</u>:

Following a 7-day infusion period, pancreata of TPN and ORAL animals were isolated and perfused with glucose (300mg/dl) and VIP (0-3ng/ml) presented as a gradient. The insulin response to glucose and VIP (fig. 43A) was biphasic in both groups. The integrated phase I insulin response appeared greater in TPN animals but the difference was not found to be statistically significant. The phase II responses were similar in TPN and ORAL rats (fig. 43B).

# TABLE XIV

7

# BODY WEIGHT (X ± SEM) OF TPN AND ORAL RATS

	INITIAL (g)	FINAL (g)	DAILY (g/day)	
TPN	252.8 ± 6.6	270.0 ± 8.6	$2.5 \pm 0.3$	
ORAL	$258.9 \pm 5.2$	$277.2 \pm 8.7$	$2.6 \pm 0.3$	

The insulin response of the isolated perfused stomach-pancreas preparation to (B) vagal stimulation (7V, 10Hz, 5msec) and (C)  $5X10^{-5}M$  MCh in the presence of 160mg/dl glucose in TPN (n=12) and Oral (n=8) animals after a 7-day TPN period.







The integrated insulin response to (A) vagal stimulation (7V, 10Hz, 5msec) and (B)  $5X10^{-5}M$  MCh in TPN and ORAL animals. \*Denotes significance (p<0.05) using an unpaired Student's t-test.



The (A) glucagon and (B) gastrin responses of the isolated perfused stomach-pancreas preparation to vagal stimulation (7V, 10Hz, 5msec) and MCh ( $5X10^{-5}M$ ) in the presence of 160mg/dl glucose in TPN (n=8) and ORAL (n=8) animals after 7-day TPN. Gastrin secretion from the stomachs of ORAL animals was significantly higher during the entire infusion period, while no significant differences were observed between TPN and ORAL animals for glucagon release.

161



A: The insulin response of the isolated perfused pancreas to a 0-3ng/ml VIP gradient in the presence of 300mg/dl glucose in TPN (n=7) and ORAL (n=6) animals after a 7-day TPN period. B: The integrated insulin response to glucose and VIP during phase I and II. No significant differences were observed.

162
#### IV) DISCUSSION

The present study provided the opportunity to observe the effects of chronic intravenous feeding (TPN) on insulin release in vitro and in vivo. Previous reports dealing with the metabolic effects of TPN, specifically with reference to circulating insulin and glucose levels, have produced differing results. Baer and Dupré (1985) demonstrated elevated plasma insulin and glucose levels in TPN compared with IG-fed animals during a 6-day study. Track et al. (1984) reported similar results during the first 2 days of a 12-day study, but then the circulating glucose levels in TPN animals dropped to below those of IG-fed animals. Insulin levels were similar in the 2 groups throughout this study. In contrast to the above experiments, Lickely et al. (1978) showed insulin and glucose to be elevated in IG-fed rats on day 8, but by day 14 no differences were observed. These discrepancies suggest that the length of study and the TPN regimen used are important factors to consider when interpreting results dealing with metabolic factors.

The results of the present study measuring insulin and glucose levels during a 7-day TPN period were in agreement with Baer and Dupré (1985). The initial 48 h of IV or IG infusion was representative of a period of adaptation, most clearly evident in the TEN group where insulin levels were elevated. Beyond this period, steady state conditions were observed, with stable glucose and insulin levels, insulin being dramatically elevated in TPN animals. As discussed in the previous chapter, various factors could contribute to the hyperinsulinemia observed in TPN animals. One such factor is that of an increased B-cell sensitivity to metabolites such as glucose or neurotransmitters with insulinotropic activity.

The <u>in vitro</u> perfused pancreas preparation was used to examine the effects of TPN on glucose-stimulated insulin release. Results from these glucose gradient experiments may reflect an increased B-cell sensitivity to glucose in TPN animals (fig. 35). Insulin was found to rise above basal levels at a lower corresponding perfusate glucose concentration and insulin secretion beyond this point was greater from TPN compared to TEN or ORAL pancreata. The 'normal' insulin response from TEN animals suggests that the increased insulin response in TPN animals was causally related to the route of nutrient administration, rather than to the nutrient load <u>per se</u>. The significant differences observed in circulating insulin, despite modest differences in plasma glucose observed during the TPN period (fig. 34), therefore, may be the result of an increased B-cell sensitivity to glucose. Although it is difficult to compare the present study with those of others, due to differences in experimental protocols, it is possible that the observed hyperinsulinemia of IV compared to IG-fed rats may have reflected the development of peripheral insensitivity to insulin as suggested by Baer and Dupré (1985), and/or an increased pancreatic Bcell sensitivity to glucose, as suggested by the present study.

In a previous TPN study (Pederson et al., 1985), it was shown that TPN pancreata exhibited an exaggerated <u>in vitro</u> insulin response to glucose (300mg/dl) in the presence of a GIP gradient (0-1ng/ml). At GIP levels below that necessary for potentiation of insulin secretion, insulin release was higher from TPN compared to control pancreata, but the differences were not significant. This is a similar finding to that observed in studies from the previous chapter (figs. 21 and 22). In the current study, conditions of the pancreatic perfusions were identical to those of the previous studies, with the exception of the absence of a GIP stimulus. In the absence of GIP, the first phase insulin response was significantly greater from TPN pancreata. The insulin curves converged during the second phase (fig. 36), as insulin secretion from TPN and ORAL pancreata were equivalent. These studies confirmed the presence of an initial exaggerated response (phase I) which can be attributed to glucose. The larger second phase response observed from TPN pancreata in the presence of GIP (figs. 21 and 22) and glucose was more likely attributed to a difference in the B-cell sensitivity to GIP.

The larger first phase insulin response in TPN animals may be explained using 'storage limited' or 'signal limited' models for insulin secretion kinetics, as described by O'Connor et al. (1980). Using the storage limited model as an explanation, the B-cells of the TPN pancreata may contain a larger glucose-labile insulin compartment(s) sensitive to 300mg/dl glucose, thus causing the greater first phase response. The rate at which the compartment(s) is refilled, however, may not be altered by TPN, explaining the similarity in the second phase response to that of the ORAL group. Employing the signal limited model, a glucose stimulus may result in

the release or generation of a greater quantity of intracellular stimulants in B-cells of TPN animals, which in turn give rise to a greater insulin response. The magnitude of the nadir is equivalent in both curves (fig. 36), suggesting that the feedback inhibition of insulin release is intact in TPN animals. The identities of exciters or inhibitors are presently unknown, but could include metabolites, cofactors or ions, particularly calcium and potassium (O'Connor et al., 1980; Gold and Grodsky, 1984).

The underlying factors responsible for the increased B-cell response to glucose observed in vitro after TPN can only be speculated upon. Hypersensitivity to glucose may reflect alterations in intracellular mechanisms which regulate glucose-stimulated insulin release. Such alterations may be brought about by the continuous systemic delivery of the hypertonic dextrose-amino acid solution. In man, continuous (12 h) intravenous infusion of glucose, resulting in hyperglycemic conditions (200mg/dl), was associated with an exaggerated insulin response to a subsequent period of hypoglycemia (Dimitriadis et al., 1985). It has also been shown in acute in vitro studies in the rat that prolonged stimulation by glucose produced a time-dependent sensitization of B-cells to subsequent stimuli (O'Connor et al., 1980; Grill, 1981), a phenomenon referred to as the B-cell 'memory'.

In contrast to these studies, fasting (24-72 h) was associated with a reduced B-cell sensitivity to glucose, resulting in a blunted insulin response (Hedeskov and Capito, 1974; Zawalich et al., 1979; Bosboon et al., 1983). It was also shown that the ability of B-cells to metabolize glucose was diminished. Impaired glucose metabolism, due to the reduced activity of glycolytic intermediates or other potential factors involved in stimulus-secretion coupling, was proposed to be the causal factor responsible for the blunted response (Hedeskov, 1980; Levy et al., 1976). It is quite possible that during TPN the continuous delivery of nutrients and concomitant demand for their disposal caused the B-cell to be chronically 'activated'. Under such circumstances, the opposite to starvation, the B-cell's ability to metabolize glucose may have been increased, owing to an increased cellular response.

Although the present data supports an increased B-cell sensitivity to glucose, a similar effect was not observed with arginine (fig. 37), suggesting that glucose and this insulinotropic amino

165

acid stimulate different intracellular mechanisms leading to insulin release. Elahi et al. (1982) hypothesized that GIP and arginine stimulate insulin release through similar, possibly common, mechanisms. This hypothesis was based on studies in man (Elahi et al., 1982) and the rat (Pederson and Brown, 1978), where it was shown that arginine and GIP demonstrated a competitive interaction in the augmentation of glucose-stimulated insulin release. The present data, however, suggests that arginine and GIP do not stimulate insulin release via a common mechanism, since the B-cell only appeared hyperresponsive to GIP.

The effect of TPN on islet morphology in the rat has not been examined. Previous studies on the rat have demonstrated that rats fed carbohydrate-rich diets exhibited mild hyperglycemia, and developed extensive islet cell hyperplasia (Wissler et al., 1949). In addition, excessive carbohydrate intake has been implicated in the development of islet cell hyperplasia (Karam et al., 1974) and hyperinsulinemia in man (Malaisse, 1972). The present study, however, indicates that chronic intravenous infusion of a carbohydrate-rich diet which was clearly insulinotropic (fig. 37), did not alter islet size or the area occupied by each endocrine cell type within islets - (table XIII). Thus, the exaggerated insulin response observed from TPN pancreata could not be attributed to changes in islet morphology, lending support to the hypothesis of an increased Bcell sensitivity to glucose.

Electrical stimulation of the vagus nerve below the diaphragm elicited a biphasic insulin response from the perfused pancreas-stomach preparation of TPN and control animals (figs. 40A and B). The integrated insulin responses (fig. 41A) revealed that vagal stimulation potentiated glucose-stimulated insulin release in both groups, but the response was 25% greater from TPN pancreata. Nishi et al. (1987) demonstrated that atropine partially blocked vagally stimulated insulin release <u>in vitro</u> in the rat, and that hexamethonium completely blocked the insulin response. It was suggested from these studies that preganglionic vagal fibers, via ganglionic nicotinic receptors, stimulated intrapancreatic cholinergic and non-cholinergic neurons which, in turn, stimulated insulin secretion. With this in mind, the larger insulin response from TPN pancreata may have reflected alterations at various levels of this neuronally mediated stimulatory pathway.

In the present study, the cholinergic agonist acetyl-ß-methylcholine (MCh) also potentiated glucose-stimulated insulin release (figs. 40C and 41B). Similar to that of vagal stimulation, the insulin response was biphasic in both groups, but the total insulin response was 23% greater from TPN pancreata. The possibility that MCh acted directly at the level of the B-cell is supported by the presence of high affinity muscarinic receptors on B-cell membranes (Grill and Ostenson, 1983) and the ability of atropine to reduce the peak insulin response to vagal stimulation in vitro by 70% (Nishi et al., 1987). In addition, it has been demonstrated that atropine completely blocked the effects of acetylcholine (ACh) stimulated insulin release from the perfused rat pancreas (Loubatieres-Mariani et al., 1973), while in another study the blockade was substantial but incomplete (Verchere, 1987). These studies suggest that, in the rat, ACh is the main postganglionic neurotransmitter controlling insulin secretion, and that the majority of the insulin response observed with MCh in the present study was due to a direct effect on pancreatic B-cells. However, studies by Nishi et al. (1987), demonstrating a non-cholinergic neuronal component of insulin release, and the ability of MCh to stimulate glucagon release in the present study, suggest that a portion of the insulin response observed with MCh was mediated through stimulation of intrapancreatic nerves and/or islet hormones. The increased insulin response with MCh in TPN pancreata may, therefore, have reflected an altered B-cell sensitivity to MCh, attributed to an increased receptor number (or sensitivity), or a post-receptor modification. Another possibility is that the increased B-cell response was due to a less well established mechanism involving modifications in pancreatic ganglia or in paracrine interactions due to chronic TPN.

VIP is one of the best characterized pancreatic neuropeptides with insulinotropic activity (Ahren et al., 1986). VIP was released into the pancreatic vasculature by vagal stimulation, as demonstrated in the pig (Holst et al., 1984), and, at concentrations greater than 1ng/ml, was a potent stimulant of insulin release in the isolated perfused pancreas of TPN and ORAL animals (fig. 43). The lack of a divergence of insulin curves during the second phase of insulin release between TPN and ORAL animals suggested that the B-cell response to VIP was not affected by TPN.

It appeared that the exaggerated insulin response observed with vagal stimulation in TPN pancreata was more likely due to a post-ganglionic effect. Evidence in support of this hypothesis stems from the observation that a similar exaggerated response was exhibited by the cholinergic agonist MCh. The possibility of a post-ganglionic effect through non-cholinergic (possibly peptidergic) nerves cannot be discounted, but VIP, a potent insulinotropic neuropeptide (fig. 43), did not elicit an exaggerated insulin response from TPN pancreata.

Glucagon potentiates glucose-stimulated insulin release (Samols et al., 1965; Samols et al., 1966) and receptors for glucagon have been associated with membranes of insulin secreting tumors (Bhathena et al., 1982) and purified B-cells (Pipeleers, 1984). It was stated by Samols et al. (1986) that pancreatic glucagon acts through paracrine effects rather than endocrine effects on the B-cell. The possibility, therefore, that glucagon contributes to the exaggerated response from TPN pancreata, cannot be confirmed or discounted based on the present studies. The ability of vagal stimulation to elicit the exaggerated response in the absence of a significant rise in glucagon, however, is not in support of a role for glucagon (fig. 42A). Thus, the most likely —site of the alteration is at the level of the B-cell.

In a study using isolated islets, Grill and Ostenson (1983) concluded that muscarinic receptors associated with islet tissue were subject to alteration by the long-term glucose environment. Islets kept in culture for a period of 96 h, in a medium containing high glucose (200mg/dl), exhibited a greater binding of [<sup>3</sup>H]-methylscopolamine (a specific muscarinic antagonist) than islets cultured in low glucose (60mg/dl). In a follow-up study using rats rendered hyperglycemic by streptozotocin treatment (STZ) and rats with normoglycemia, islet binding characteristics were analyzed (Ostenson and Grill, 1987). Binding of [<sup>3</sup>H]-methylscopolamine was enhanced in islet tissue of STZ rats. In addition, cholinergically induced insulin release in relation to either islet volume or islet insulin content was greater from STZ rats. The authors concluded that the level of glycemia was causally related to the number of muscarinic receptors on B-cells. They further stated that the increased B-cell sensitivity to carbamylcholine was due to the increased number of muscarinic receptors. It was interesting to note from these studies that STZ islets secreted considerably more insulin under basal conditions (31mg/dl

glucose) when expressed per islet insulin content, but not in the presence of 122mg/dl glucose, suggesting that STZ islets were hyperresponsive to glucose levels considered to be hypoglycemic (Ostenson and Grill, 1987).

Based on the above studies by Grill and Ostenson, it is possible that the slight but consistent elevation in plasma glucose observed in TPN rats (especially in the first 2/3 of the TPN period) may have contributed to the exaggerated response observed <u>in vitro</u> with vagal stimulation and MCh. Higher circulating glucose levels may have led to an increased muscarinic receptor density or sensitivity, owing to the greater B-cell response to parasympathetic stimulation. It cannot, however, be ruled out that the exaggerated response with MCh or vagal stimulation may have reflected a non-specific intracellular effect. A higher metabolic activity of the B-cell, due to the continuous infusion of nutrients and the demand for high circulating insulin levels, may have been the rate-limiting factor. Because glucose is the obligatory permissive factor for stimulation of insulin release (its presence being required for stimulation by other secretagogues), it is not possible from the present studies to separate the effects of glucose and cholinergic agonists — entirely.

Previous studies have indicated that ACh and electrical activation of the vagus nerve are stimulants of gastrin release from the perfused rat stomach (Pederson et al., 1984; Pederson et al., 1981). The combined perfusion of both stomach and pancreas in the present study provided an opportunity to study the effect of TPN on gastrin, as well as on insulin and glucagon secretion. Gastrin secretion from the stomachs of TPN animals was dramatically reduced (75%) compared to secretion from control stomachs (fig. 42B). In a previous TPN study, it was shown that both serum and antral gastrin concentrations were severely depressed compared to orally fed animals (Johnson et al., 1975a, b). The present study suggests that the secretory activity of the G-cell under basal and stimulated conditions <u>in vitro</u> is also affected by TPN. It can be inferred from these studies that gastrin, which exhibits insulinotrophic activity (Rehfeld and Stadil, 1973), most likely does not contribute to elevated circulating insulin levels of TPN animals, nor to the exaggerated insulin response observed during an OGC.

In conclusion, the present series of experiments have demonstrated that 7-day TPN resulted in insulin hypersecretion in vivo. Quantitative and morphological analysis of the islets suggested that such a condition was not due to alterations in the endocrine cell composition of the islets. In vitro studies, using the isolated perfused pancreas or pancreas-stomach preparations, indicated that TPN resulted in a modification in the responsiveness of the pancreas to specific insulin secretagogues (glucose and ACh). These data suggest that 7-days of TPN led to alterations in the nutrient and neuronal components of the enteroinsular axis, possibly reflecting modifications at the level of the B-cell. In contrast, TPN resulted in no alteration in A-cell secretory activity and was associated with G-cell hyposecretion under the conditions studied. An increased B-cell sensitivity may be brought about as compensation for the loss of the hormonal component of the enteroinsular axis which, under normal feeding conditions, is essential in controlling insulin release and, therefore, regulating body fuel metabolism. An increased sensitivity may also reflect an adaptation to a continuous nutrient load and the reduced efficiency with which this load is assimilated when presented systemically. Elevated insulin levels in TPN animals may, in part, - be due to the reduced peripheral disposal of nutrients. However, higher circulating levels may also reflect an increased B-cell sensitivity to glucose and the neurotransmitter acetylcholine, which provide the main stimulus for insulin release during TPN.

# CHAPTER FOUR THE EFFECT OF TPN ON GASTRIN RELEASE

#### I) INTRODUCTION

Prolonged food deprivation in the rat leads to dramatic decreases in both serum and antral gastrin concentrations (Koop et al., 1982; Schwarting et al., 1986; Lichtenberger et al., 1975). Food deprivation is also associated with a reduction in the population of gastrin-containing G-cells (Lichtenberger et al., 1975; Bertrand and Willems, 1980). Studies by Lichtenberger et al. (1975) demonstrated that serum gastrin levels were more severely affected by starvation than was antral gastrin concentration or G-cell number. A similar trend was observed after the resumption of feeding, where serum gastrin levels recovered more rapidly than tissue levels. These studies suggest that the gastrin secretory function is not intimately linked to tissue hormone levels or gastrin cell number. Similar observations have been made <u>in vitro</u>. A 72 h fast reduced the gastrin secretion rate under basal conditions and during periods of stimulation with acetylcholine or bombesin (Koop et al., 1982), however, gastrin secretor was more affected than antral tissue content. It can be suggested from these studies that the presence of food is required to maintain normal circulating gastrin levels and secretory responses of the G-cell to specific stimuli. Such studies are difficult to interpret with certainty, however, due to the multitude of metabolic changes that accompany food restriction.

An alternative and more physiological model used to study the effects of nutrient exclusion from the gastrointestinal tract is the parenterally-fed rat. Notwithstanding adequate nutrition, however, TPN rats exhibited stomach, small bowel and pancreatic atrophy, and a significant reduction in antral and serum gastrin concentration (Johnson et al., 1975a, b). These studies suggested that the absence of nourishment was not responsible for the depressed serum and antral gastrin levels associated with food restriction. Similar findings regarding gastrin and TPN have been shown by other investigators (Ryan et al., 1979; Track, 1980). But these studies did not examine the gastrin response to stimulation either <u>in vivo</u> or <u>in vitro</u> from the isolated stomach, nor did they compare fasting gastrin levels in TPN compared to orally fed rats. Possible alterations in the G-cell population due to intravenous feeding have also not been investigated. Therefore, the purpose of the present study was to examine the effects of TPN on gastrin release in vivo and in vitro under conditions resembling those of fasting and feeding.

In the first study, gastrin release after 7-day TPN was investigated. These experiments were run in parallel with control animals (ORAL) fed rat chow <u>ad libitum</u>. Blood samples were obtained on days 2, 4 and 6 of the infusion period to monitor the pattern of gastrin release in these animals. Following the infusion period, the gastrin response to an oral peptone challenge (OPC) was examined. The effect of vagal stimulation on gastrin secretion from the isolated perfused stomachs of TPN and ORAL animals was also studied. Finally, antral sections were obtained for subsequent identification and quantification of gastrin-containing G-cells.

In a previous study by Track (1980), it was shown that TPN led to a dramatic depression of plasma and antral gastrin levels by the sixth day of infusion; on day 2, however, only plasma gastrin levels were depressed. The purpose of the second series of experiments, therefore, was - to compare the effects of 3 and 7-days of TPN on the antral G-cell population, and on gastrin secretion from the isolated stomach.

Schwarting et al. (1986) demonstrated that food restriction had less of an effect on the antral D than G-cell population. In addition, antral somatostatin concentrations were shown to increase after food deprivation (Schwarting et al., 1986; Shulkes et al., 1979). <u>In vitro</u> studies performed after a 72 h fast revealed no significant alteration in the responsiveness of D-cells to inhibitors or stimulants of somatostatin release (Koop et al., 1982). These experiments suggest that D-cell population and D-cell secretory activity are more resistant to starvation than are G-cells. It has not been determined if TPN has any effect on gastric somatostatin secretion. In addition to gastrin release studies performed after 3 and 7-days of TPN, somatostatin secretion under basal conditions, and during vagal stimulation or MCh infusion, was studied.

Re-feeding after long-term food deprivation led to a more rapid recovery of serum than antral gastrin concentrations (Lichtenberger et al., 1975). A dramatic increase in the population of G-cells and antral gastrin content occurred between days 6 and 9 of a re-feeding period, attaining

levels comparable to control animals. Serum gastrin levels, however, had recovered by day 6. The effects of oral re-feeding on gastrin and somatostatin secretion, after a period of TPN, have not been examined in the rat, nor have the effects of re-feeding on the G-cell population. The purpose of a third study, therefore, was to examine the effects of oral re-feeding (after 7-days of TPN) on gastrin and somatostatin release. Following re-feeding, the gastrin response to an OPC was examined. Subsequent to this, the effect of methacholine and vagal stimulation on gastrin and somatostatin secretion from the isolated perfused stomachs of re-fed animals (TPN'R'), and control animals, was determined.

The mechanisms responsible for the long-term regulation of antral and serum gastrin concentrations remain unclear. Adequate nutrition delivered intravenously, as mentioned, does not prevent reductions in G-cell secretory activity associated with food deprivation (Johnson et al., 1975a), suggesting that the presence of nutrients in the gastric lumen is necessary. However, various elemental liquid or solid diets, and non-nutritive bulk diets, also fail to maintain antral and serum gastrin (Lichtenberger et al., 1975; Ryan et al., 1979; Sircar et al., - 1980), suggesting that specific nutrients and/or hormones released by these nutrients maintain gastrin levels (Lichtenberger, 1982). The inability of pentagastrin to normalize serum or antral gastrin, when given along with TPN, suggests that gastrin per se is not a stimulant for the synthesis or release of antral gastrin (Johnson et al., 1975b). Bombesin, the amphibian counterpart of GRP, however, when administered 3 times per day I.P. into starved or fed animals, resulted in a significant increase in serum and antral gastrin levels (Johnson and Guthrie, 1983). It was suggested from these studies that GRP, not luminal nutrients per se, regulates tissue and antral gastrin levels. Studies by Lehy et al. (1983) supported this hypothesis: bombesin administered twice daily, S.C., for 1 week, led to antral gastrin cell hyperplasia in orally fed rats. If bombesin is the main regulator of tissue gastrin levels, chronic bombesin administration in TPN rats should prevent decreases in serum gastrin levels and maintain normal G-cell secretory activity in response to stimulants of gastrin release.

This hypothesis was tested in a final series of experiments. Bombesin was administered to TPN animals (TPN-Bbs) I.V., 3 times per day during the infusion period. Blood samples were

collected on days 3 and 5 of a 7-day TPN period to determine if bombesin infusion prevented hypogastrinemia. Following TPN, the <u>in vivo</u> gastrin response to an oral peptone challenge, and the <u>in vitro</u> gastrin response to MCh and bombesin, were compared between TPN and bombesin-treated rats.

#### II) METHODS

All methods were previously described in General Methods and Materials.

#### III) **RESULTS**

#### 1) The Effect of 7-Day TPN on Gastrin Release in the Rat

a) Plasma Gastrin Levels during the 7-Day Test Period (Fig. 44):

On each sampling day, plasma gastrin levels in TPN animals were significantly lower than - controls (p<0.05). Control rats displayed plasma gastrin levels 3-fold higher than those of TPN animals as early as day 2 of the TPN period ( $159 \pm 32 \text{ vs.} 52 \pm 17 \text{pg/ml}$ ) (fig. 44). This pattern of release continued during the test period with the difference increasing to 7-fold by day 6 (151  $\pm 23 \text{ vs.} 21 \pm 11 \text{pg/ml}$ ).

#### b) The Gastrin Response to an Oral Peptone Challenge (Fig. 45):

TPN and ORAL animals were administered 0.5ml/100g of a 10% peptone solution following an overnight fast upon completion of the 7-day infusion period. Fasting plasma gastrin levels (fig. 45A) were 40% higher in ORAL ( $64 \pm 9pg/ml$ ) compared to TPN ( $34 \pm 5pg/ml$ ) animals. Fasting gastrin levels in ORAL animals were found to be half those observed on day 6 of the test period, while TPN animals had levels similar to those on day 6. Following oral peptone administration, plasma gastrin levels rose to a peak of  $143 \pm 28pg/ml$  at 20 min in ORAL animals. TPN animals demonstrated a reduced (40%) and delayed peak response ( $88 \pm 30pg/ml$ ) occurring at 60 min. The total gastrin response to oral peptone (fig. 45B) was significantly higher in ORAL ( $14.8 \pm 2.4ng/ml \cdot 120$  min) compared to TPN ( $8.9 \pm 2.7ng$  /ml·120 min) animals (p<0.05).

c) <u>The Effect of Vagal Stimulation on Gastrin Release from the Perfused Stomach</u> (Fig. 46):

After completion of a peptone challenge, the stomachs of TPN and ORAL animals were isolated and perfused with perfusate containing 80mg/dl glucose (basal conditions). During periods 11-30 min the anterior vagus was electrically stimulated (7V, 10Hz, 5msec). Mean basal gastrin levels from TPN stomachs ( $70 \pm 17$ pg/min) were significantly lower (p<0.05) than levels observed from the stomachs of control animals ( $142 \pm 21$ pg/min) (fig. 46). Vagal stimulation resulted in 2.4-fold and 4.0-fold increases in gastrin secretion from the stomachs of TPN and ORAL animals respectively. The mean gastrin response to vagal stimulation was significantly reduced (70%) in TPN animals ( $166 \pm 47$ pg/min) when compared to ORAL animals ( $581 \pm 97$ pg/min) (p<0.05). After removal of the stimulus, gastrin secretion returned to basal levels.

d) <u>The Effect of 7-Day TPN on Antral Mucosal Height and G-Cell Number (Fig.</u> – <u>47</u>):

Immunocytochemical identification and quantification of the antral G-cell population revealed a significant reduction (22%) in the number of gastrin-containing G-cells in TPN stomachs compared to stomachs of control animals ( $72 \pm 5.0$  vs.  $97 \pm 8.0$  cells/mm mucosa) (p<0.05). Antral mucosal height was also significantly reduced (16%) compared to control animals ( $184 \pm$ 7.0 vs.  $220 \pm 14.0 \mu$ m).



Plasma gastrin levels on days 2, 4 and 6 of the TPN period (7 days) in ORAL (n=13) and TPN (n=9) animals. Error bars represent  $\pm$  SEM and \* denotes significance (p<0.05) using a Student's t-test for unpaired data.

176



A: Gastrin release from ORAL (n=12) and TPN (n=8) animals in response to a 10% peptone solution (0.5ml/100g b.wt.). 'P' represents the oral administration of peptone at 0min. B: Integrated gastrin response to peptone. \*Denotes significance (p<0.05) using a Student's t-test for unpaired data.



Gastrin secretion from the isolated perfused stomach of ORAL (n=13) and TPN (n=7) animals during basal conditions (80mg/dl glucose) and during vagal trunk stimulation (7V, 10Hz, 5msec).



A: Quantification of antral G-cell population of ORAL (n=10) and TPN (n=9) animals. B: Mean mucosal height of antral tissue, p<0.05 using an unpaired Student's t-test. Gastrin immunoreactive cells in the antral glands of (C) TPN and (D) ORAL animals (magnification X 320).





## 2) <u>The Effect of TPN Duration on Gastrin and Somatostatin Release</u> from the Perfused Stomach

a) <u>Body Weight (Table XV)</u>:

TPN and ORAL animals demonstrated significant weight gain (p<0.05) during either 3 or 7 day infusion periods. Mean daily increases in body weight of TPN and ORAL animals during 3-day  $(3.0 \pm 0.4 \text{ vs. } 3.5 \pm 1.0 \text{g})$  and 7-day  $(2.5 \pm 0.4 \text{ vs. } 3.3 \pm 0.4 \text{g})$  were similar.

b) <u>The Effect of Vagal Stimulation and MCh on Basal Gastrin and Somatostatin</u> <u>Release after 3-Day TPN (Fig. 48)</u>:

The stomachs of TPN and ORAL animals were perfused with perfusate containing 80mg/dl glucose in the presence of 5X10<sup>-5</sup>M MCh (6-15 min) or vagal stimulation (7V, 10Hz, 5msec, 26-35 min). Mean basal gastrin secretion (fig. 48A) from the stomachs of TPN animals was significantly lower (45%) than observed in ORAL animals ( $34 \pm 7$  vs.  $62.7 \pm 20$  pg/min). MCh, introduced by sidearm infusion, resulted in comparable increases in gastrin release from the stomachs of TPN and ORAL animals. The mean gastrin response to MCh was, however, significantly greater in ORAL (291  $\pm$  74pg/min) compared to TPN (94  $\pm$  18pg/min) animals. Gastrin secretion returned to basal levels when MCh infusion was discontinued. Vagal stimulation resulted in a 6-fold increase in gastrin secretion from both TPN and ORAL animals. Again, however, the mean gastrin response from the stomachs of ORAL animals was significantly higher (p<0.05) (572  $\pm$  107 vs. 225  $\pm$  50pg/min) (fig. 48A). During basal conditions, somatostatin secretion from the stomachs of TPN and ORAL rats did not differ significantly  $(131 \pm 27 \text{ vs. } 209 \pm 44 \text{ pg/min})$  (fig. 48B). MCh infusion resulted in an average 30% reduction in somatostatin secretion in TPN ( $85 \pm 15$  pg/min) and ORAL ( $119 \pm 22$  pg/min) animals when compared to basal levels. Somatostatin secretion returned to basal levels in both TPN (134  $\pm$  10pg/min) and ORAL (207  $\pm$  30pg/min) animals when MCh infusion was discontinued. Electrical stimulation of the vagi produced a transient decrease in somatostatin secretion when compared to basal levels (21-25 min) in TPN ( $123 \pm 16$  pg/min) and ORAL (180  $\pm$  35pg/min) animals. After removal of the stimulus, a rebound effect was observed in TPN and ORAL groups (198  $\pm$  20 vs. 292  $\pm$  60pg/min).

c) <u>The Effect of Vagal Stimulation and MCh on Basal Gastrin and Somatostatin</u> <u>Release after 7-Day TPN (Fig. 49)</u>:

The stomachs of TPN and ORAL animals were perfused with perfusate containing 80mg/dl glucose in the absence (basal conditions) or presence of  $5\times10^{-5}$ M MCh (6-15 min) and vagal stimulation (7V, 10Hz, 5msec, 26-35 min). Mean gastrin secretion under basal conditions (fig. 49A) was reduced by 70% in TPN (68 ± 10pg/min) compared to ORAL (210 ± 61pg/min) animals (p<0.05). MCh infusion resulted in comparable increases in gastrin release from the stomachs of ORAL and TPN rats, however, the mean gastrin response to MCh was significantly greater in ORAL animals (446 ± 81 vs. 156 ± 32pg/min) (p<0.05). Electrical stimulation of the vagi produced significant increases in gastrin release from the perfused stomachs of TPN and ORAL rats. The mean gastrin response during stimulation was greater (60%) from ORAL (752 ± 108pg/min) than from TPN (326 ± 52pg/min) stomachs (p<0.05).

Mean basal somatostatin levels were similar in ORAL and TPN animals (266 ± 45 vs. 193 ± 41pg/min) (fig. 49B). MCh reduced mean basal somatostatin secretion by approximately 75%
in both TPN and ORAL groups (51 ± 10 vs. 53 ± 7pg/min). Vagal stimulation reduced basal levels (21-25 min) by approximately 50% in TPN (110 ± 22pg/min) and ORAL (102 ± 21 pg/min) animals. After removal of the stimulus, a rebound effect was observed in both groups.

d) Antral G-Cell Quantification and Antral Gastrin Concentration (Fig. 50):

Immunocytochemical identification and quantification of the antral G-cell population revealed a 14% reduction in the number of gastrin-containing G-cells of TPN compared to ORAL rats after 3-day TPN, but the difference was not significant (99.3  $\pm$  9.0 vs. 85.1  $\pm$  13.0 cells/mm mucosa). After 7-day TPN, a 25% reduction in the G-cell population of TPN rats was observed; the difference was significant (98.3  $\pm$  3.2 vs. 72.2  $\pm$  5.1 cells/mm mucosa, p<0.05). The antral tissue gastrin concentration was also found to be reduced (34%) after 7-days of TPN, however, the difference was not significant (3.98  $\pm$  0.41 vs. 6.01  $\pm$  1.4µg/g tissue wet wt.).

## TABLE XV

## BODY WEIGHT (X ± SEM) OF TPN AND ORAL RATS

N 22	6±4	235 ± 3	$3.0 \pm 0.5$
AL 22	4 ± 4	$235 \pm 4$	$3.5 \pm 1.0$
N 21	7 ± 3.0	$234 \pm 3.1$	$2.5 \pm 0.4$
AL 22	$0 \pm 3.7$	$243 \pm 2.0$	$3.3 \pm 0.4$
]	N 22 AL 22 N 21 AL 22	N $226 \pm 4$ AL $224 \pm 4$ N $217 \pm 3.0$ AL $220 \pm 3.7$	N $226 \pm 4$ $235 \pm 3$ AL $224 \pm 4$ $235 \pm 4$ N $217 \pm 3.0$ $234 \pm 3.1$ AL $220 \pm 3.7$ $243 \pm 2.0$



The effects of  $5X10^{-5}M$  MCh and vagal stimulation (7V, 10Hz, 5msec) on (A) basal gastrin secretion and (B) basal SLI secretion from the isolated perfused stomach of TPN (n=12) and ORAL (n=11) animals after a 3-day TPN period, \*p<0.05 using an unpaired Student's t-test.



The effects of  $5X10^{-5}M$  MCh and vagal stimulation (7V, 10Hz, 5msec) on (A) basal gastrin secretion and (B) basal SLI secretion from the isolated perfused stomach of TPN (n=9) and ORAL (n=8) rats after a 7-day TPN period, \*p<0.05 comparing responses at each time period using an unpaired Student's t-test.



A: Quantification of the antral G-cell population of ORAL and TPN animals (n=7) after a 3-day TPN period. B: Quantification of the G-cell population and antral gastrin concentration (n=7) in ORAL and TPN animals (n=7) after a 7-day TPN period. \*Denotes significance (p<0.05) using a Student's t-test for unpaired data.

# 3) <u>The Effect of Re-feeding on Gastrin and Somatostatin Secretion</u> a) <u>Body Weight (Table XVI)</u>:

Following a 7-day TPN period, animals (TPN'R') were re-fed rat chow for 6 days. ORAL animals were fed rat chow for the entire 13-day test period. Average daily increases in body weight were similar in TPN'R' ( $5.0 \pm 1.0g$ ) and ORAL ( $5.1 \pm 0.6g$ ) animals.

#### b) The Plasma Gastrin Response to Oral Peptone (Fig. 51):

After the test period, animals were administered 0.5ml/100g of a 10% peptone solution following an overnight fast. Fasting plasma gastrin levels (fig. 51A) were not significantly different between TPN'R' (77.5  $\pm$  21pg/ml) and ORAL (87.5  $\pm$  20pg/ml) animals. Plasma gastrin levels doubled in response to oral peptone in both TPN'R' (155  $\pm$  56pg/ml) and ORAL (168  $\pm$  53pg/ml) animals. The total gastrin response to peptone in TPN'R' and ORAL animals were equivalent (11  $\pm$  3 vs. 12  $\pm$  3ng/ml·120 min).

c) In Vitro Gastrin and Somatostatin Release, TPN vs. ORAL Animals (Fig. 52):

The stomachs of TPN'R' and ORAL animals were perfused with perfusate containing 80mg/dl glucose in the absence (basal conditions) or presence of either  $5\times10^{-5}$ M MCh (6-15 min) or vagal stimulation (7V, 10Hz, 5msec, 26-35 min). During basal conditions, the gastrin release from the stomachs of TPN'R' and ORAL animals were not significantly different (195 ± 32 vs. 248 ± 74pg/min). Methylcholine infusion resulted in 2.5-fold increases in gastrin release from the perfused stomachs of both groups. The mean gastrin response to MCh from TPN'R' ( $501 \pm 77pg/min$ ) and ORAL ( $623 \pm 109pg/min$ ) stomachs were not significantly different. Electrical stimulation of the vagus nerve produced 6-fold and 5-fold increases in gastrin secretion from the stomachs of TPN'R' and ORAL animals. The mean gastrin response during vagal stimulation in TPN'R' and ORAL animals was not significantly different (963 ± 114 vs. 1,028 ± 194pg/min). During basal conditions, somatostatin levels in TPN'R' and ORAL animals were equivalent. MCh and vagal stimulation inhibited basal somatostatin by 55% and 25% respectively in both groups.

d) Antral G-Cell Quantification (Fig. 53):

Immunocytochemical identification and quantification revealed no significant differences in

G-cell or D-cell number from the antral tissues of TPN'R' compared to ORAL animals.

## TABLE XVI

## BODY WEIGHT (X ± SEM) OF ORAL AND TPN'R' RATS

	INITIAL (g)	FINAL (g)	DAILY (g/day)
ORAL	256.4 ± 17.8	$308.1 \pm 5.1$	$5.0 \pm 1.0$
TPN'R'	$244.6 \pm 14.8$	297.1 ± 10.3	$5.1 \pm 0.6$



A: Gastrin release from ORAL (n=7) and TPN'R' (n=8) animals in response to 0.5ml/100g oral peptone. 'P' represents the administration at 0 min.

B: Integrated gastrin response to oral peptone. No significant differences in secretion between groups was observed.

190



The effects of  $5X10^{-5}M$  MCh and vagal stimulation (7V, 10Hz, 5msec) on (A) basal gastrin and (B) SLI secretion from the isolated perfused stomach of ORAL (n=7) and TPN'R' (n=8) animals after a 7-day period of infusion followed by 6 days' access to rat chow. No significant differences in secretion between groups was observed.



Quantification of antral G-cell population of ORAL (n=5) and TPN'R' (n=7) animals. No significant differences were observed.

192

#### 4) The Effect of Bombesin on Gastrin Release in TPN Animals

a) <u>The Effect of 7-Day TPN on Bombesin Stimulated Gastrin and Somatostatin</u> <u>Release from the Isolated Stomach (Fig. 54)</u>:

The stomachs of TPN and ORAL animals were isolated and perfused with 80mg/dl glucose alone (basal conditions) and in the presence of bombesin  $(1X10^{-8}M)$  from 16-30 min. Under basal conditions, gastrin release from the stomachs of TPN animals was significantly lower (68%) than from ORAL stomachs (p<0.05) (fig. 54A). Bombesin, introduced by sidearm infusion, produced an average 3.5-fold increase in gastrin release from the stomachs of TPN and ORAL animals, however, the mean gastrin response was significantly higher in the ORAL group (899 ± 199 vs. 271 ± 67pg/min, p<0.05). Basal somatostatin levels were found to be similar in both TPN and ORAL animals (101 ± 15 vs. 110 ± 13pg/min) (fig. 54B). Bombesin infusion resulted in a 30% increase in somatostatin secretion above basal levels in TPN (138 ± 27pg/min) and ORAL (158 ± 26pg/min) animals. Somatostatin secretion returned to preinfusion levels after the infusion was stopped.

b) The Effect of IV-Bombesin and TPN on Gastrin Release:

i) Plasma Gastrin Levels on Days 3 and 5 (Fig. 55):

TPN animals were given either  $20\mu g/kg$  bombesin (dissolved in 0.001N acetic acid and made up to the desired concentration in saline containing 0.2% BSA) or vehicle alone (TPN) as an intravenous injection 3 times per day (0800, 1600 and 2000 h) for a period of 7 days. Blood samples were obtained at 1000 h on days 3 and 5 of the 7-day period. Plasma gastrin levels in TPN and TPN-Bbs animals were similar on days 3 (40 ± 8 vs. 40 ± 5pg/ml) and 5 (29 ± 3 vs.  $33 \pm 6pg/ml$ ).

ii) The Gastrin Response to Oral Peptone (TPN vs. TPN-Bbs) (Fig. 56):

Upon completion of the infusion period, animals were administered an oral peptone challenge (0.5ml/100g of a 10% peptone solution), following an overnight fast. Fasting plasma gastrin levels appeared higher in TPN ( $50 \pm 12pg/ml$ ) than TPN-Bbs ( $36 \pm 4pg/ml$ ) animals but the difference was not statistically significant. Peptone administration resulted in maximum gastrin responses in TPN and TPN-Bbs animals that were not significantly different ( $74 \pm 13$  vs.  $60 \pm 12$  pg/ml). The total gastrin response to peptone in TPN and TPN-Bbs animals was not significantly different (7.5 ± 1.5 vs. 6.6 ± 1.1 ng/ml·120 min).

iii) The In Vitro Gastrin Response to MCh and Bombesin, TPN vs. TPN-Bbs Animals (Fig. 57):

The stomachs of TPN and TPN-Bbs animals were perfused with 80mg/dl glucose. MCh (5X10<sup>-5</sup>M) or bombesin (1X10<sup>-8</sup>M) was introduced by sidearm infusion during periods 11-20 min and 36-45 min respectively. Gastrin secretion during basal and stimulation periods was equivalent in TPN and TPN-Bbs animals.



The effect of  $10^{-8}$ M bombesin on basal (A) gastrin secretion and (B) SLI secretion from the isolated perfused stomach of ORAL (n=5) and TPN (n=6) rats after a 7-day TPN period, \*p<0.05 comparing TPN to control animals at each time period using an unpaired Student's t-test.





Plasma gastrin levels on days 3 and 5 of a 7-day infusion period in TPN animals (n=5) and a group of TPN animals receiving  $20\mu g/kg$  bombesin 3 times per day (TPN-Bbs, n=7). No significant differences were observed.



A: Gastrin release from TPN (n=5) and TPN-Bbs (20µg/kg bombesin 3 times per day, n=7) animals in response to 0.5ml/100g oral peptone. 'P' represents peptone administration at time 0.

B: Integrated response to oral peptone. No significant differences in secretion were observed.



The effects of  $5X10^{-5}M$  MCh and  $10^{-8}M$  bombesin on basal (A) gastrin and (B) SLI secretion from the isolated perfused stomach of TPN (n=5) and TPN-Bbs (n=7) animals after a 7-day TPN period. No significant differences in secretion were observed.

198
#### IV) DISCUSSION

In the first series of experiments, the effects of 7-day TPN on gastrin secretion was investigated. This study demonstrated that total parenteral nutrition resulted in reduced G-cell secretory activity in vivo and from the isolated vascularly perfused stomach of the rat during basal and stimulated conditions. Reduced gastrin secretion occurred rapidly after cessation of oral food ingestion as shown by a reduction in plasma gastrin levels to 1/3 of control values as early as day 2 of the TPN period (fig. 44). It was also shown that this condition increases in severity with prolonged TPN, reaching a 7-fold difference by day 6.

Fasting plasma gastrin concentrations of TPN rats were shown to be 50% of those observed in the ORAL group, and similar to levels observed on day 6 of the TPN period (figs. 44 and 45). These observations confirmed that the TPN solution administered intravenously was incapable of stimulating gastrin release. In addition, the chronic lack of G-cell stimulation appeared to impair normal fasting secretory activity (fig.45A). Hyposecretion of gastrin was also evident during periods of stimulation <u>in vivo</u> by peptone (fig. 45A). The gastrin secretion, in response to orally administered peptone, was reduced by 40% when compared to the ORAL group (fig. 45B).

Hyposecretion of gastrin persisted in the isolated perfused stomachs of TPN animals, with basal gastrin release being 50% that of control animals. Electrical stimulation of the vagus nerve elicited a greatly reduced (70%) gastrin response from the stomachs of TPN animals compared to ORAL animals. These data are in agreement with the results obtained <u>in vivo</u> in that under both basal and stimulatory conditions, gastrin secretion was dramatically reduced after 7-day TPN. <u>In vitro</u> studies also suggested, however, that the gastrin response to nervous stimulation was reduced to a greater degree than the response to luminally administered peptone (70% reduction compared to a 40% reduction). The larger reduction in vagally stimulated gastrin release compared to that with luminally administered protein may indicate that neuronal factors which control gastrin release are affected to a greater extent than mechanisms involved in the direct stimulation of the G-cell by luminal nutrients.

Immunocytochemical quantification of the antral G-cell population demonstrated a 22% reduction in the antral G-cell number of TPN compared to ORAL animals (fig. 47). G-cell hypoplasia was accompanied by a 16% decrease in antral mucosal height. However, the reduction in G-cell number may not have fully accounted for the dramatic reduction in gastrin secretion observed in vivo and in vitro under basal and stimulatory conditions. Similar observations have been made in animals subjected to food restriction (Koop et al., 1982). It was shown that serum gastrin levels, as well as basal and stimulated gastrin output, from the perfused rat pancreas after 3-days of food deprivation, were reduced to a greater extent than antral gastrin content. In addition, in dog, after 3-week TPN, fasting and postprandial gastrin secretion was depressed to a greater extent than antral gastrin content (Odachi and Koga, 1987). It could be argued in the present study that antral G-cell quantification may not truly represent changes in antral gastrin content. In food deprivation studies, however, similar decreases in both tissue and antral gastrin concentrations were observed, suggesting that antral gastrin content was directly linked to the total number of G-cells (Lichtenberger et al., 1975; Schwarting -- et al., 1986).

Results of the present study, as well as those from studies employing food deprivation (Lichtenberger et al., 1975; Koop et al., 1982; Schwarting et al., 1986), suggested that the secretory activity of the G-cell was more susceptible to food withdrawal than either immunohistochemical or tissue gastrin quantification revealed. Based on these results, it is quite possible that, in addition to G-cell hypoplasia, the lack of luminal nutrient stimulation may result in: (1) an altered G-cell sensitivity leading to a decrease in gastrin synthesis and/or secretion by the remaining G-cell population; and/or (2) a reduction in the degree of stimulation received by the G-cell during basal and stimulated conditions. The observation that gastrin hyposecretion persisted during fasting and during stimulatory conditions <u>in vivo</u>, and the finding that the reduction in vagally mediated gastrin release was greater than the reduction observed after oral peptone in TPN animals, support both proposals.

The mechanism responsible for G-cell hypoplasia and the reduced G-cell activity associated with TPN or food deprivation remains to be elucidated. Feeding a high bulk non-nutritive diet

did not prevent decreases in serum and gastrin concentrations, suggesting mechanical distension of the stomach was not a factor (Lichtenberger, 1975). Studies employing liquid diets have produced conflicting results. Lichtenberger et al. (1975) reported that their liquid diet was capable of maintaining antral gastrin levels, while Ryan et al. (1979) showed that a liquid diet did not prevent a decrease in antral gastrin. The issue is further confused by studies using elemental diets in solid or liquid form, which failed to maintain normal levels of antral gastrin (Sircar et al., 1980). Such studies suggest that specific stimuli present in food are necessary for the maintenance of antral gastrin levels. Recent studies have shown that dietary amines resulting from decarboxylation of amino acid precursors are strong stimulants of gastrin release (Lichtenberger et al., 1982b, c; Dial et al., 1986). It was suggested that these dietary amines, present in food or produced by protein metabolism, are partly responsible for maintenance of antral gastrin (Lichtenberger, 1982c). The absence of luminal stimulation from dietary amines in parenteral nutrition may also alter G-cell secretory activity, as suggested by the present study. In addition, other peptides which play important roles in the regulation of gastrin secretion (i.e. - GRP, somatostatin) may be affected by TPN treatment and, in part, may be responsible for gastrin hyposecretion.

In a second series of experiments, the effects of 3 and 7-day TPN on gastrin secretion from the isolated perfused stomach preparation were examined. Data revealed that gastrin hyposecretion was evident as early as day 3; basal secretion being reduced by 45% compared with control levels. Seven-days of TPN led to a further reduction (70%) in basal gastrin levels, indicating that hyposecretion <u>in vitro</u> increased in severity with longer periods of TPN (figs. 48 and 49).

Stimulated gastrin output in response to either methacholine or electrical stimulation of the vagi was also reduced from TPN compared to ORAL stomachs (figs. 48A and 49A). After both 3 and 7-days of TPN, however, the relative incremental increases in gastrin secretion above basal levels from the stomachs of TPN and ORAL animals were comparable. In other words, the differences in gastrin release reflected the prestimulatory (basal) period differential. These data suggested that the afferent preganglionic and postganglionic fibers of the vagus nerve

involved in the stimulation of gastrin release were unaffected by TPN. In addition, the comparable responses of TPN and ORAL stomachs to methacholine suggested that functional ACh receptors on gastrin cells or associated with intramural neurons which stimulated gastrin release were functional, and the G-cell was still responsive to cholinergic stimulation.

Immunocytochemical quantification of the antral G-cell population demonstrated a 14% reduction in G-cell number after 3-days of TPN, but the difference was not statistically significant (fig. 50A). After 7-days of TPN, a 26% and 34% reduction in G-cell number and antral gastrin concentration respectively, were observed (fig. 50B). The differences in G-cell number between 3 and 7-day TPN suggested that hypoplasia was progressive, increasing in severity with longer periods of TPN. These results were similar to those of food deprivation studies (Lichtenberger et al., 1975; Bertrand and Willems, 1980). However, reduction in G-cell number associated with starvation studies was more dramatic than that observed after TPN in the present study, suggesting that the lack of adequate nutrition associated with starvation has a greater effect on the G-cell population than the lack of gastric nutrients alone.

Intravenous feeding in rat for a 2-day period resulted in a 60% decrease in serum gastrin levels while antral gastrin content was unchanged (Track et al., 1980). In the present study, gastrin secretion <u>in vitro</u> was dramatically reduced after 3-days of TPN (45%), yet antral gastrin cell number was only slightly reduced (14%). Similar findings have been made after long-term (3-4 days) starvation studies either <u>in vivo</u> or <u>in vitro</u> in the rat (Lichtenberger et al., 1975; Koop et al., 1982; Schwarting et al., 1986). These studies clearly point out that decreases in gastrin secretion associated with starvation or TPN under basal and stimulatory conditions preclude significant reductions in either antral G-cell number or tissue concentration. Therefore, it can be suggested that the secretory activity of the G-cell is affected more rapidly and to a greater degree than is evidenced by changes in antral endocrine morphology. In TPN studies, however, the lack of correlation between G-cell number and secretory activity was even more apparent than revealed by food deprivation studies.

G-cell hypoplasia most likely contributed to gastrin hyposecretion, as evidenced by further reductions in both G-cell number and gastrin secretion between 3 and 7-day TPN (fig. 50).

However, as previously mentioned, this hypoplasia may not fully account for the dramatic decreases in gastrin secretion. Since the responsiveness of the stomach to vagal stimulation and MCh were similar in TPN and ORAL animals, parasympathetic cholinergic abnormalities were unlikely to be responsible for the gastrin hyposecretion. Other possibilities leading to this condition include impairment of the G-cell secretory process and/or an increased or decreased influence of intrinsic inhibitory (e.g. somatostatin) or stimulatory (e.g. GRP) factors, respectively, on G-cell secretory activity.

In studies by Koop et al. (1982) it was shown that SLI release under a variety of conditions was not affected by a 72 h fast, similar to findings made by other investigators (Tsuda et al., 1981). In the present study, basal SLI secretion appeared reduced in TPN compared to ORAL animals, but the difference was not statistically significant (figs. 48B and 49B). In addition, either vagal stimulation or MCh, both of which inhibited SLI release, produced similar changes in SLI secretion in TPN and ORAL animals. The present results, therefore, support the conclusions made from food deprivation studies, in that the gastric D-cell in rats is less dependent on the presence of food in the gastric lumen than the G-cell.

The physiological significance of somatostatin in the stomach remains to be determined. In vitro and in vivo studies have demonstrated that exogenous somatostatin inhibited gastrin release (Saffouri et al, 1980; Bloom et al., 1974; Raptis et al., 1975). In addition, somatostatin antiserum caused a significant increase in gastrin secretion in vitro (Saffouri et al., 1979; Chiba et al., 1984). Such studies suggested that somatostatin may exert restraint on the G-cell through paracrine or endocrine pathways.

The antral concentration of somatostatin has been shown to increase in 96 h fasted rats, while fundic somatostatin was shown to decrease (Schwarting et al., 1986). Similar increased antral and decreased fundic somatostatin concentrations were observed in 96 h compared with 12 h fasted rats, however, SLI secretion from the perfused stomachs of these animals were equivalent (Koop et al., 1982). Thus, maintenance of normal SLI secretion might be explained by an increased contribution from antral D-cells. If a similar situation exists in TPN animals, increased antral SLI concentration and release may impose a greater restraint of the G-cell and

contribute to gastrin hyposecretion.

In the dog, long-term TPN (3 weeks) resulted in significant decreases in fasting gastrin levels and a reduced gastrin response to a test meal (Odachi and Koga, 1987). After a 7-day period of refeeding, fasting gastrin levels and the response to a subsequent test meal were normalized. The following study was undertaken to determine if a similar reversal in gastrin hyposecretion could be demonstrated in the rat.

After a 6-day refeeding of rats on TPN (7-days), fasting plasma gastrin levels were found to be similar to a group fed orally for the entire 13-day period (fig. 51). Control and TPN'R' animals also demonstrated equivalent responses to orally administered peptone. <u>In vitro</u> gastrin and SLI responses under basal conditions and in the presence of electrical activation of the vagus or MCh were also equivalent in TPN'R' and control animals (fig. 52). These experiments demonstrated a full recovery of G-cell secretory activity during the 6-day refeeding period in TPN animals. These data further demonstrated that the secretory activity of the G-cell was clearly dependent not on the presence of adequate nutrition <u>per se</u>, but on the presence of food in the gastric lumen.

In theory, a decrease in the antral gastrin cell number associated with TPN could be explained by the complete loss of secretory granules from part of the existing G-cell population, rendering them non-immunoreactive and invisible. It has been demonstrated that the number of secretory granules in G-cells diminished in rats fasted for 2 days (Track et al., 1978), suggesting that such a phenomenon was possible. Conversely, during periods of refeeding, these 'invisible' cells may regain their secretory elements and, therefore, their immunoreactivity. In the present study, which employed immunocytochemical methods, such an explanation could have accounted for the apparent G-cell hypoplasia observed after 7-day TPN, and the recovery of cell number after 6-day refeeding (fig. 53). Studies using a combination of immunocytochemistry and autoradiography after injection of tritiated thymidine in starved and refed rats, however, suggest that a portion of G-cells was lost during starvation and replaced by new cells upon refeeding (Bertrand and Willems, 1980). Therefore, 7-day TPN may have led to a partial loss of G-cells that were replaced with a new G-cell population upon refeeding, and not

reflect changes in the immunoreactivity of these cells.

In a final series of experiments, the possible involvement of GRP in gastrin hyposecretion associated with TPN was investigated. In the first study, the effect of 7-days of TPN on bombesin stimulated gastrin and somatostatin release was examined. Prior to bombesin infusion, gastrin secretion from the stomachs of TPN animals was 30% of that observed in ORAL stomachs (fig. 54). Bombesin (1X10<sup>-8</sup>M) produced a 3.5-fold increase in gastrin secretion above basal levels in both groups, however, gastrin secretion from TPN stomachs was still only 30% that of ORAL stomachs. These results suggested that the antral G-cell population was responsive to bombesin after 7-days of TPN. However, bombesin administered acutely <u>in</u> <u>vitro</u> was not capable of normalizing gastrin secretion.

In contrast to gastrin, basal SLI release from the rat stomach was not influenced by TPN. In addition, the SLI responses to bombesin in TPN and ORAL stomachs were equivalent. These results are in agreement with those of food deprivation studies (Koop et al., 1982) and support the notion that food in the gastric lumen is not essential for maintaining the secretory activity of \_\_\_\_\_\_ the gastric D-cell, in direct contrast to the antral G-cell.

Although bombesin administered acutely <u>in vitro</u> (fig. 54) in the isolated perfused stomach did not prevent gastrin hyposecretion, chronic bombesin administration <u>in vivo</u> has been successful. Bombesin administered 3 times per day  $(20\mu g/kg)$  during a period of food deprivation slightly, but significantly, reduced the effects of fasting on serum and antral gastrin tissue levels (Johnson and Guthrie, 1983). In the present study, however, bombesin treatment failed to prevent hypogastrinemia associated with TPN (fig. 55). Bombesin treatment also had no effect on fasting gastrin levels or on gastrin secretion <u>in vivo</u> or <u>in vitro</u> under basal or stimulatory conditions (figs. 56 and 57). These results are not in agreement with those of Johnson and Guthrie (1983).

Differences in the protocol could account for these discrepancies in the results. In the Johnson and Guthrie study, blood samples were collected 1 h after the final bombesin injection, compared with 3 h in the present study. Lehy et al. (1983) demonstrated that a single subcutaneous injection of bombesin ( $10\mu g/kg$ ) has long lasting effects in vivo (2 h) on gastrin

secretion and, in addition, the present study shows that bombesin (10<sup>-8</sup>M) also has long lasting effects <u>in vitro</u> (fig. 54). Therefore, small increases in serum and antral gastrin levels in bombesin treated, starved rats, may reflect the acute synthesis and release of gastrin in response to the final bombesin injection, and not the cumulative effects of 3-day bombesin treatment.

The long-term effects of bombesin treatment in fed rats was clearly demonstrated. In animals fed by liquid diet, bombesin prevented reductions in antral and serum gastrin observed in non-treated rats (Johnson and Guthrie, 1983). In animals fed a normal chow diet, bombesin injected twice per day led to hyperplasia of the antral gastrin cell population (Lehy et al., 1983). Given the results of bombesin treatment in TPN and starved rats compared with the effects of bombesin in fed animals, it would appear that the presence of food in the gastric lumen (or specific constituents of food), which regulates antral gastrin content and/or serum gastrin levels, is of primary importance.

Several conclusions could be drawn from the experiments presented in this chapter. First, TPN resulted in a rapid and progressive depletion of circulating gastrin levels. Second, TPN was associated with gastrin hyposecretion <u>in vivo</u> and <u>in vitro</u> from the isolated perfused stomach under basal (fasting) and stimulated conditions, with the degree of hyposecretion increasing with longer TPN periods. Third, gastrin hyposecretion <u>in vivo</u> or <u>in vitro</u> was completely reversed by subsequent exposure of TPN animals to a period of oral feeding, but was not prevented by chronic injection of bombesin during the TPN period. These results indicated that oral intake of food was a necessary requirement for the maintenance of normal G-cell secretory activity in the rat. Fourth, the gastric D-cell was clearly less dependent upon food in the gastric lumen than the G-cell, gastric SLI secretion under basal conditions and during periods of stimulation or inhibition being equivalent between 7-day TPN and control rats. However, changes in the relative contribution of antral and fundic somatostatin to the total gastric somatostatin secretion cannot be ruled out.

Antral G-cell hypoplasia appears to be causally related to hypogastrinemia <u>in vivo</u> and to gastrin hyposecretion <u>in vitro</u>. Reductions in G-cell number and antral gastrin content, however, may not completely account for the dramatic reductions in the secretion of gastrin

observed after 3 or 7-days of TPN. The inability of various stimulants for gastrin release to reverse gastrin hyposecretion observed under basal conditions suggested that the overall secretory activity of the G-cell was diminished. These observations may be indicative of abnormalities in intracellular mechanisms which lead to a reduced gastrin synthesis and/or secretion. In addition, a greater restraint exerted on the G-cell by somatostatin could contribute to gastrin hyposecretion in TPN animals.

To gain a greater understanding of the effects of TPN on the antral G-cell population and on G-cell secretory activity, different methodology may be required. Combining the techniques of immunocytochemistry and <u>in situ</u> hybridization could be used to determine if the existing G-cell population is actively synthesizing gastrin. Electron microscopy could also be employed to determine if secretory granules within G-cells are diminished during TPN. Such studies could be used to establish if the ability of G-cells to synthesize, store and release gastrin is impaired in these animals. Furthermore, chronic or acute somatostatin antibody (i.e. Soma 10) treatment during TPN, or differential antral and fundic stomach perfusion, would be of use to determine if gastrin hyposecretion is linked to an exaggerated D-cell restraint on gastrin secretion.

### SUMMARY AND CONCLUSIONS

Studies in this thesis were designed to examine the effects of nutrient exclusion from the GItract on the enteroinsular axis component of insulin release, and on the regulation of gastric hormone secretion. These studies were approached using a unique rat model (TPN) in which all nutritional requirements were provided intravenously, bypassing the gastrointestinal endocrine system.

In order to carry out the objectives of this thesis, techniques for parenteral (TPN) and enteral (TEN) feeding of the rat were first developed. A dietary regimen for use in these animals was formulated from human TPN components. Under most circumstances, this regimen met or exceeded the nutritional requirements of the rat as determined by the NRC (1972, 1978). Parenterally and enterally-fed animals demonstrated comparable weight gain to that of an orally-fed control group and animals appeared healthy during the infusion period. In addition, hematological studies revealed few side effects of intravenous or intragastric feeding. It was concluded from studies presented in chapter one, that the infusion regimen provided adequate nutrition to the animals, and that the diet was suitable for chronic intravenous or intragastric feeding.

Studies in chapters two and three were undertaken to examine the effects of TPN on the enteroinsular axis component of insulin release. The effects of TPN on circulating insulin and glucose levels have not been unequivocally ascertained. Results from the present experiments indicated that TPN was associated with hyperinsulinemia and mild hyperglycemia. In contrast to the effects of TPN, TEN animals exhibited normal plasma glucose and insulin levels. Based on these observations, it was concluded that nutrients delivered enterally were disposed of in a more efficient manner than when administered intravenously. Excessive insulin secretion during TPN may have been due to the development of insulin resistance. However, studies in chapter two indicated that fasting insulin levels were normal in TPN rats, and these animals demonstrated a superior tolerance to an OGC. Two alternate hypotheses were presented to explain the hyperinsulinemia in TPN animals. First, it was suggested that GI-mechanisms initiated by

luminal nutrients and which facilitated insulin-induced nutrient uptake by target tissues, were not activated during TPN. Thus, higher circulating insulin levels may be required for nutrient assimilation by TPN animals. A second possibility was that TPN animals developed B-cell hypersensitivity to the intravenously delivered TPN solution. The exaggerated <u>in vitro</u> pancreatic insulin response to glucose and parasympathetic stimulation observed in TPN animals supported this possibility.

During the infusion period, TPN animals exhibited chronically suppressed circulating GIP levels, in contrast to chronically elevated levels in TEN rats. Fluctuations in plasma GIP levels over the course of a 24 h period were only observed in orally fed animals. From these observations, it was concluded that GIP is primarily stimulated by luminal nutrients, and a circadian rhythm for GIP is the result of intermittent oral feeding.

Seven days of TPN resulted in an exaggerated insulin response to an OGC, while the glucose response was reduced. <u>In vitro</u> studies indicated that TPN pancreata were hypersensitive to the incretin GIP, a condition which developed during the course of the 7-day infusion period. It was --suggested that increased B-cell sensitivity to GIP, as demonstrated <u>in vitro</u>, could explain the exaggerated insulin response <u>in vivo</u>. Chronic GIP treatment in TPN animals, raising plasma GIP levels to within a range observed in TEN animals, resulted in normalization of the insulin response to an OGC and the <u>in vitro</u> response of the pancreas to GIP. From these data it was concluded that a causal relationship existed between chronically depressed GIP levels in TPN animals and an increased sensitivity of the pancreas to stimulation by GIP. It was suggested that increased B-cell sensitivity may be mediated by alterations at the receptor or post-receptor level.

The effects of TPN on nutrient and neuronally mediated insulin release were investigated in chapter three. These experiments indicated that the B-cell was hypersensitive to glucose and cholinergic stimulation, but not to the neuropeptide VIP or the insulinotropic amino acid arginine, indicating that a modification of the enteroinsular axis associated with TPN also included selective neuronal and nutritive components. It was suggested that chronic stimulation of the B-cell by the continuous systemic delivery of a highly concentrated amino acid-dextrose solution, causing mild hyperglycemia and hyperinsulinemia, could contribute to the development of this

condition. In contrast to the effects on insulin, under the conditions studied TPN had no effect on the secretion of other islet hormones (glucagon and somatostatin). In addition, TPN had no visible effects on pancreatic islet size, endocrine cell composition of the islets, or on the GIP-cell population of the jejunum. These data were futher supportive of an altered B-cell sensitivity to selective hormonal, nutritive and neuronal components of the enteroinsular axis.

Total parenteral nutrition also provided an experimental situation in which to study the importance of food intake on the regulation of GI-hormone secretion. TPN was associated with a rapid and progressive depletion of circulating gastrin levels. Gastrin hyposecretion was found to persist during fasting and during periods of stimulation <u>in vivo</u> or <u>in vitro</u>. It was concluded that gastrin hyposecretion associated with TPN was, in part, the result of G-cell hypoplasia and a reduced antral gastrin content. However, small reductions in the G-cell population could not fully account for the dramatic reductions in gastrin secretion. Thus, gastrin hyposecretion may also reflect impaired G-cell secretory activity.

Gastrin hyposecretion observed during basal periods could not be reversed acutely, as -demonstrated using various stimulants of gastrin release. In addition, chronic bombesin administration could not reverse the effects of TPN. However, a 6-day period of oral refeeding normalized the G-cell population and the G-cell secretory response under basal and stimulatory conditions. It was concluded that food in the gastric lumen is the primary regulator of the antral gastrin cell population and secretory activity. In contrast to G-cell secretory activity, gastric Dcell secretion was much less affected by the absence of luminal nutrients. However, due to the limitations of the methodology used, it could not be determined if changes in fundic or antral somatostatin secretion had occurred. It is possible, then, that G-cell hyposecretion may, in part, be causally related to a greater restraint from antral somatostatin.

The studies presented in this thesis were designed to examine the effects of total parenteral nutrition on gastric and pancreatic endocrine secretion in the rat. TPN was associated with marked effects on both gastrin and insulin secretion. In the stomach, TPN caused G-cell hypoplasia and hyposecretion under conditions resembling fasting and feeding. In the pancreas, TPN was associated with B-cell hypersecretion in response to specific nutrient, hormonal and

neuronal stimulation, indicating that an alteration in the enteroinsular axis had occurred. These studies clearly indicate the importance of enteral feeding in the maintenance of normal pancreatic and gastrointestinal endocrine secretion.

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#### **APPENDIX I - CHEMICAL SOURCES**

### CHEMICAL

#### SOURCE

Acetic acid (glacial) Acetone Acetyl-B-methyl-choline chloride Ammonium acetate Bacto-peptone Barbital sodium C-IV Bombesin Bovine serum albumin (RIA grade) Calcium chloride Calcium gluconate (clinical grade) Carbon decolourizing neutral (Norit) Chloramine T D-glucose (50% Dextrose) Dextran (clinical grade) Dextran (T-70) Diaminobenzidine Disodium ethylenediamine tetraacetate (EDTA) DW50 (50% Dextrose) Ensure Ethanol, 99% Eosin, aqueous - Fluothane Formaldehyde (histological) Freund's complete adjuvant Gastrin (SHG-1) GIP Glucagon (porcine pancreatic) Glucose oxidase reagent Glucose solution (150mg/dl) Hematoxylin (mercury free) Heparin-sodium Hydrochloric acid Hydrogen peroxide (histological) <sup>125</sup>I-Glucagon Imidazole Insulin (porcine) Insulin (rat) Novo L-+-Arginine monohydrochloride Lithium carbonate Magnesium sulphate (clinical) Neosporin spray Parraffin (paraplast) Pentobarbital sodium Permamount Petroleum ether Picric acid

**BDH BDH** Fisher Baker Difco Fisher **Research Plus** Sigma and Miles Fisher Lyphomed Fisher Eastman Kodak Abbot Sigma Pharmacia BDH Fisher Travenol Ross **Commercial Alcohol** BDH Ayerst Fisher Sigma Sigma, Peninsula RPG Sigma Beckman Beckman Fisher Fisher Fisher Fisher NEN Sigma Novo Fisher Fisher Lyphomed Burroughs Welcome Monoject **MTC** Pharmaceuticals Fisher Fisher

BDH

### APPENDIX I - CHEMICAL SOURCES (Continued)

### CHEMICAL

#### SOURCE

Potassium chloride (clinical) Potassium chloride Potassium phosphate (mono basic) Proviodine (topical antiseptic) Ouso microfine silica, G-32 Sephadex CM-52 Sephadex G-25 Sodium acetate Sodium azide Sodium bicarbonate Sodium chloride Sodium chloride (clinical) Sodium hydroxide Sodium iodide Sodium <sup>125</sup>Iodide in NaOH Sodium merthiolate Sodium metabisulphite Sodium phosphate (dibasic) Sodium phosphate (monobasic) Somatostatin Soyacal (20%) - Trace-4 (multivitamins) Trasylol (aprotinin) Tris-HCl Xylene (histological)

Ayerst Fisher Fisher Rougier Philadelphia Quartz Pharmacia Pharmacia Fisher Baker Fisher Fisher/Baker Lyphomed Fisher Fisher Amersham Eastman Kodak Fisher Fisher Fisher Peninsula Travenol IMS Miles Sigma Fisher

## APPENDIX II - LIST OF ABBREVIATIONS

Bbs	Bombesin
BSA	Bovine Serum Albumin
b.wt.	Body weight
CCK	Cholecystokinin-pancreozymin
CEP	Charcoal extracted plasma
DAB	Diaminobenzidene
GIP	Glucose dependent insulinotropic polypeptide
<u>on</u>	Gastric inhibitory polypeptide
GU	Glucagon-like immunoreactivity
1251	125Indine
	Intraperitoneally
	Immunoreactive
	Immunoreactive inculin
	Infinitutioreactive insulin
	Intragasule
	Intravenous
IVC	Intravenous glucose challenge
MCh	Acetyl-B-methyl choline chloride
NAS	National Academy of Sciences
NRC	National Research Council
NSB	Non-specific binding
OGC	Oral glucose challenge
ORAL	Orally fed animals
PAP	Peroxidase-antiperoxidase
PBS	Phosphate buffered saline
PP	Pancreatic polypeptide
RIA	Radioimmunoassay
SC	Subcutaneous
SHG	Synthetic human gastrin
SLI	Somatostatin-like immunoreactivity
Soma	Somatostatin
TEN	Total enteral nutrition / enterally alimentated
TPN	Total parenteral nutrition / parenterally alimentated
TPN-Bbs	TPN rats infused bombesin
TPN-GIP	TPN rats infused GIP
TPN'R'	TPN rats refed orally

# APPENDIX III - COMPOSITION OF TPN/TEN SOLUTION I

Component	Amount
10% Travasol	375.0 ml
50% DW50	450.0 ml
M.V.C. 9+3	3.0 ml
Trace-4	0.3 ml
Calcium Gluconate (10%)	10.0 ml
Magnesium Sulfate (50%)	0.2 ml
Potassium Chloride (2mEq/ml)	35.0 ml
Potassium Phosphate (3mM/ml)	5.0 ml
Sodium Chloride (4mEq/ml)	<u>8.0 ml</u>
	886.5 ml

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Antigen	Antibody/ Antiserum	Source	Specificity	Dilution
Gastrin	109-21	monoclonal, Walsh, C.U.R.E.	C-terminal	1:100
GIP	3.65h	monoclonal, †RPG, U.B.C.		neat, *tcm.
pGlucagon	23-6-b4	monoclonal, Gregor, Berlin	N-terminal	1:100, tcm.
Insulin	GP01	antiserum, RPG, U.B.C.		1:2000, •ser.
cPancreatic Polypeptide		antiserum	C-terminal	1:2000, ser.
Somatostatin	Soma 08	monoclonal, RPG, U.B.C.	<u>.</u>	1:1000, \$asc

# APPENDIX IV - ANTIBODY SOURCES (First Layer)

† Regulatory Peptide Group\* Tissue culture medium

• serum

♦ acites

# APPENDIX V - ANTIBODY SOURCES (Second Layer)

Secondary Antibody	Source	Dilution
Rabbit anti guinea pig*	Dako	1:200
Rabbit anti mouse*	Dr. Levy, U.B.C.	1:200
Horse anti mouse**	Vector	1:200
Rabbit anti guinea pig**	Zymed	1:200

\* Peroxidase conjugated \*\*Biotinylated