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Dietary and hormonal regulation of pancreatic digestive enzymes

Hirschi, Karen Kemper, Ph.D.

The University of Arizona, 1990

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DIETARY AND HORMONAL REGULATION OF PANCREATIC DIGESTIVE ENZYMES

by

Karen Kemper Hirschi

A Dissertation Submitted to the Faculty of the

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Karen Kemper Hirschi

entitled Dietary and Hormonal Regulation of Pancreatic Digestive Enzymes

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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SIGNED: Karen K. Hirschi

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DEDICATION

This dissertation is dedicated to my husband, Kendal D. Hirschi, for his unfaltering love and support, and for his contagious appreciation of laughter and life.

This work is also dedicated to my parents, Robert and Lucy Kemper, in appreciation of their continued support and encouragement, without which my academic pursuits would not have been realized.

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LIST OF ABBREVIATIONS

A1	amylase 1
A2	amylase 2
A3	amylase 3
ANOVA	analysis of variance
BAPNA	Nα-benzoyl-DL-arginine p-nitroanilide
BSA	bovine serum albumin
C	chymotrypsinogen
CAM	calmodulin
CCK	cholecystokinin
CPM	cycles per minute
CV	condensing vacuoles
2D	two-dimensional
DAG	diacylglycerol
DEX	dexamethasone
ECM	extracellular matrix
EGF	epidermal growth factor
FFA	free fatty acids
GAPNA	glutaryl-L-phenylalanine p-nitroanilide
GC	golgi complex
HBSS	Hank's balanced salt solution

LIST OF ABBREVIATIONS--Continued

HCl	hydrochloric acid
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid
HF	high-fat diet
HG	high glucose
HI-CS	heat-inactivated calf serum
IEF	isoelectric focusing
IEP	isoelectric point
IP_3	inositol-1,4,5-triphosphate
L	lipase
LF	low-fat diet
LG	low glucose
LSD	least significant differences
Mr	estimated molecular weight
MW	molecular weight
NP	non-purified diet
ODC	ornithine decarboxylase
PBS	phosphate-buffered saline
PC A	procarboxypeptidase A
PC B	procarboxypeptidase B
PE	proelastase

LIST OF ABBREVIATIONS--Continued

PI-4,5P	phosphoinositol-4,5-bisphosphate
PK	protein kinase
PK-A	cyclic AMP-dependent protein kinase
PK-C	phospholipid-dependent protein kinase
PMSF	phenylmethyl-sulfonyl flouride
PP	pancreatic polypeptide
RER	rough endoplasmic reticulum
SA	specific activity
SAPNA	N-acetyl-L-alanyl-L-alanyl-L-alanine p-nitroanilide
SDS	sodium dodecyl sulfate
SEC	secretin
SF	serum-free
STI	soybean trypsin inhibitor
T	trypsinogen
TCA	trichloroacetic acid
VIP	vasoactive intestinl peptide
ZG	zymogen granules

ABSTRACT

The activity, synthetic rate and mRNA levels of pancreatic digestive enzymes adapt to changes in their respective dietary substrates. Previous studies in vivo implicate ketones and secretin in lipase adaptation and cholecystokinin (caerulein) in protease and, possibly, amylase adaptation. These studies were undertaken to determine the direct role(s) in vitro of these nutrient and hormonal factors on digestive enzymes in cultured rat pancreatic acinar cells. Ketones, intermediates of triglyceride metabolism, did not affect lipase activity in cells isolated from rats fed commercial non-purified diet, whether cultured in media containing high or low concentrations of glucose, a competitive energy substrate. Beta(OH)-butyrate also had no affect on lipase activity in cells from high-fat fed rats in which lipase was already elevated, but did increase lipase activity dose-dependently in cells isolated from rats fed a purified low-fat diet. Importantly, lipase levels were higher in cells from rats fed high fat diet, compared to those of low-fat fed rats, demonstrating that dietary adaptation persisted in culture. The role of ketones on lipase regulation appeared to be more complex than originally proposed, and they may not be the sole mediator. Direct effects of secretin and caerulein on the activity, secretion and relative synthesis of lipase were studied. Secretin significantly increased media lipase activity biphasically after 12 h, but had no effect on cellular lipase activity. After 24 h, secretin had no effect on lipase activity or relative synthesis of pancreatic enzymes (amylase, lipase, chymotrypsin, trypsin, elastase). In

cultured cells, enzyme activities decreased over time, while media activities increased. Amylase relative synthesis decreased over time, while that of the proteases increased. Caerulein further decreased cellular content of all enzymes and increased media amylase and lipase activities. Caerulein, however, increased the relative synthesis of trypsin ($p<0.05$) and chymotrypsin ($p<0.06$), supporting its proposed role in protease, but not amylase, adaptation. These studies did not, however, support definitive roles of ketones or secretin in lipase regulation; perhaps they have interactive effects, or act in conjunction with other potential regulators, in the dietary adaptation of lipase.

CHAPTER 1

INTRODUCTION

The exocrine pancreas plays a key role in the digestion of foodstuffs in the gastrointestinal tract, a process which necessarily precedes absorption and utilization of nutrients in the body. One of the major exocrine pancreatic functions is to synthesize and secrete a number of enzymes that aid digestion. The acinar cells of the exocrine pancreas have the ability to adapt the production and secretion of these digestive enzymes to accommodate their dietary substrates. That is, consumption of a diet high in protein, fat or carbohydrate increases proteases, lipase and amylase, respectively. These dietary adaptations are the result of alterations in enzyme content, synthesis and mRNA levels. Although the exact cellular mediators are not defined, various hormones and nutrients have been implicated in pancreatic adaptation to diet.

One gastrointestinal hormone, cholecystokinin (CCK), is proposed to mediate the dietary adaptation of the proteases, via feedback regulation of its release from the small intestine. In vivo infusion of CCK or its analogue caerulein increases pancreatic protease content, synthesis and, in some cases, mRNA levels. However, these studies have been done, predominantly, in vivo. Therefore, it is difficult to determine if the effects are caused directly by CCK or by its potential interactions with other hormones or regulators.

CCK has also been proposed to regulate pancreatic adaptation of amylase to dietary carbohydrate because its infusion causes a small decrease in the synthesis of this enzyme. However, there are no effects of CCK infusion on amylase mRNA levels. Therefore, it may be that CCK is involved in translational regulation or may interact with other potential regulators circulating in the bloodstream, such as insulin or glucose. There is a preponderance of evidence suggesting that adaptation of amylase to dietary carbohydrate is mediated through changes in plasma glucose and insulin levels, so that the role of CCK in this adaptation is controversial.

Another gastrointestinal hormone, secretin, is proposed to regulate the adaptation of pancreatic lipase to dietary fat because its infusion into rats increases the relative synthesis of lipase. However, secretin does not alter mRNA levels; whereas, dietary fat increases lipase content, synthesis and mRNA levels. Therefore, secretin may either be involved in translational control of lipase or interact with other hormones or metabolites, such as ketones, in the regulation of lipase.

Blood ketones, intermediates in triglyceride metabolism, are positively correlated with high-fat diets, as is pancreatic lipase activity. Furthermore, continuous infusion of β(OH)-butyrate into rats increases lipase activity. The direct effects of secretin or ketones, however, are difficult to elucidate from in vivo studies because of potential homeostatic responses, and have not been examined under controlled in vitro conditions. Thus, the roles of these two potential mediators of pancreatic adaptation to dietary fat remain unclear.

The studies presented herein examined the direct effects of ketones, secretin and caerulein and the interactive effects of secretin and caerulein on pancreatic digestive

enzyme content, secretion and synthesis, using an in vitro system of primary acinar cells. These cells are maintained in a serum-free, hormonally defined medium and are viable for four to five days. They retain differentiated function as evidenced by the presence of zymogen granules and abundant rough endoplasmic reticulum, hormonal responsiveness to insulin and epidermal growth factor, ability to secrete amylase in response to secretagogues, and the ability to synthesize amylase de novo for four days in culture. Such studies allow the evaluation of the direct action of these proposed mediators of pancreatic dietary adaptation (ketones, secretin, cholecystokinin) on acinar cell content, secretion and synthesis. Eliminated is the possibility of interaction with other potential regulators which exists in vivo.

CHAPTER 2

LITERATURE REVIEW

Digestion and Absorption

Most foodstuffs ingested by humans, and other mammals, are in forms that are not readily available to satisfy nutrient needs. They cannot be absorbed from the gastrointestinal tract until they have been broken down mechanically and chemically into smaller molecules. This disintegration of naturally occurring foodstuffs into assimilable forms constitutes the process of digestion (1,2).

Salts, crystalloids in general and water can be absorbed unchanged. However, starches, proteins and fat must be hydrolyzed to monosaccharides, amino acids, and monoacylglycerols, glycerol and fatty acids before they can be absorbed and utilized by the body. In the course of these digestive reactions, the vitamins and minerals contained in the foodstuffs also become more assimilable.

Digestion begins in the oral cavity, continues in the stomach, and is completed in the intestine in the presence of pancreatic and biliary secretions. Resulting nutrients are then ready for absorption from the small intestine into the body's circulatory system.

The Oral Cavity

The oral cavity contains the first fluid with which ingested food comes into contact - the saliva, or the secretion discharged from various glands including the parotid, mandibular and sublingual glands (2). Saliva consists of approximately 99.5% water with a pH of about 6.8, and its most important functions are the moistening of the oral mucosa and the ingested food which aids in its mechanical breakdown (mastication), lubrication for swallowing and germicidal protection (3).

Saliva also contains enzymes such as salivary amylase (ptyalin), ribonuclease and lingual lipase, which begin the hydrolytic degradation of food particles (4). The role of salivary amylase, as well as ribonuclease, is limited in the oral cavity. Although salivary amylase is capable of hydrolyzing starch and glycogen to maltose, this is ultimately of little significance in the body because of the short time it is actually in contact with food in the oral cavity. Also, its digestive action ceases once swallowed into the stomach's acidic environment because salivary amylase is readily inactivated at pH 4.0 and below. Pancreatic amylase, which has the same action and specificity as salivary amylase, completes starch digestion in the small intestine. In many animals, a salivary amylase is entirely absent (4).

The role of lingual lipase is not as limited as that of salivary amylase in the digestion of foodstuffs. Lingual lipase is secreted from serous glands (von Ebner's glands) on the dorsal portion of the tongue (5) and is distinctly different from pancreatic lipase (6). It is active in a broad pH range (2-8) and, therefore, continues to hydrolyze triglyceride in the acidic gastric environment (7). This enzyme preferentially hydrolyzes medium-chain triglycerides at rates five- to eight-fold faster than long-chain triglycerides

and does not have position specificity, as does pancreatic lipase (8). Lingual lipase activity is inhibited in the presence of bile salt micelles in the small intestine, and this inhibition is not prevented by colipase (7), as is the case with pancreatic lipase. Therefore, lingual lipase is ineffective in the later intestinal stage of adult digestion, but does play an important role in the neonatal period when pancreatic function is immature. It is responsible for 30-50% of lipid digestion in newborns (9,10).

After foodstuffs are sufficiently broken down mechanically and carbohydrates and lipids are partially hydrolyzed in the oral cavity, ingested materials proceed, via the esophagus, to the stomach. Here, protein digestion begins, lipid digestion continues, and a limited amount of absorption occurs.

The Stomach

The stomach is an enlarged, yet collapsible, segment of the digestive tract between the esophagus and small intestine that serves to store, as well as process, food for absorption by the intestine (11). Although absorption is not a major function of the stomach, some substances are absorbed in limited quantities into the bloodstream, such as ethanol and short- and medium-chain triglycerides (12).

Much of the luminal surface of the stomach is lined with stratified squamous, nonglandular epithelia, which do not have significant secretory activity (11). This gastric mucosa has, embedded within it, three distinct types of gastric exocrine glands: the cardiac, pyloric, and oxyntic glands. Both the cardiac and pyloric glands, near the esophageal orifice and duodenum, respectively, produce a secretion rich in mucus which forms a protective, lubricative layer around the stomach. The third and most distinctive

of these glands, the oxyntic, occupies most of the fundus and corpus of the stomach (13) and contains numerous oxyntic or parietal cells, which are the site of gastric hydrochloric acid (HCl) secretion (14). A second cell type also characteristic of this gland is the chief cell, which synthesizes and secretes gastric pepsinogen (11). The mixed secretion of these two cell types, known as gastric juice, is normally a clear, pale yellow fluid containing 0.2-0.5% HCl and 97-99% water, with a pH of about 1-2. The juice also contains variable amounts of mucin, inorganic salts and pepsinogen (2).

The release of gastric juice in the stomach is caused by circulating levels of the gastrointestinal peptide gastrin (15). Gastrin originates in the gastric antrum and is stimulated by the presence of peptides, amino acids and calcium in the gastric lumen through the activation of neural reflexes, by circulating catecholamines and by another gastrointestinal peptide, bombesin (16). Once food, particularly protein, enters the stomach and gastric juices are released, protein digestion begins.

As a result of contact with HCl, proteins are denatured - their tertiary structure is lost via destruction of hydrogen bonds (2). The polypeptide chain unfolds, making it more accessible to the actions of proteolytic enzymes. Pepsinogen is activated at the same time that dietary proteins are denatured, also by the action of HCl, which splits off a polypeptide to form the smaller, active endopeptidase, pepsin. Pepsin, in turn, autocatalyzes other pepsinogen molecules to their active form and subsequently hydrolyzes peptide bonds within the main polypeptide structure of dietary proteins, rather than those adjacent to the N- or C-terminal residues (17). Thus, dietary proteins are digested into peptides and amino acids in the stomach.

Lipid digestion in the stomach occurs to a lesser extent than that of protein. It is continued by lingual lipase and aided, in part, by gastric lipase, which is released by the gastric mucosa and preferentially hydrolyzes short-chain triglycerides (10). Carbohydrates, in contrast, are not digested to any appreciable extent; but all food in the stomach, in general, is converted to a semiliquid mass (chyme) by the actions of HCl, pepsin and churning motions of the stomach. The stomach subsequently empties its contents gradually through the pyloric sphincter, via peristaltic contractions, into the small intestine where digestion is completed. Pepsin, which is active only in the acidic gastric environment, is inactivated in the alkaline medium of the intestine (17). Protein digestion, as well as that of lipid and carbohydrate, is continued in the small intestine by pancreatic and intestinal enzymes.

The Intestine

In the small intestine, the breakdown of food begun in the mouth and stomach is completed. The resulting nutrient molecules are then absorbed from the digestive tract into the circulatory system of the body, from which they are delivered to individual cells where needed.

Anatomically, the small intestine is characterized by circular folds in the submucosa; numerous microscopic fingerlike projections, villi, on the mucosa; and tiny cytoplasmic projections, microvilli, on the surface of individual epithelial cells (17). All of these features lend to the large surface capacity of the small intestine (300 square meters), which is, on average, six meters long. The upper 25 centimeters, known as the

duodenum, is the most active in the digestive process; the remaining portions, ileum and jejunum, are largely concerned with absorption of nutrients (17).

As the acidic chyme of the stomach is introduced into the duodenum, responses are elicited not only from the intestine, but also from the pancreas and gall bladder. The secretions from all of these organs are involved in intestinal digestion. Also, each of the different components of chyme (acid; amino acids, peptides and proteins; fatty acids, monoglycerides and fats; and glucose, maltose and starch) elicits a unique and specific response from these organs (2,18).

The presence of gastric acid in the duodenum stimulates the release of secretin, a gastrointestinal hormone. Although secretin is produced in the duodenum itself, its major role is stimulation of the pancreas to secrete large volumes of pancreatic juice rich in bicarbonate, which is necessary for neutralizing acidic chyme (19). The resulting shift in pH is needed for optimum activity of the enzymes involved in intestinal digestion (18). Biliary secretions from the gallbladder are also provoked by secretin, as well as intraduodenal acidity, and their alkaline nature aids in this neutralization (20). Although there is general agreement that acidification of the duodenum causes release of secretin sufficient to explain stimulation of pancreatic bicarbonate secretion, controversies exist as to whether factors other than acid, such as dietary fat, stimulate secretin release (19). Fat added to a protein meal does not cause a significant increase in secretin, nor does the protein meal itself (21). However, intraduodenal infusion of a 10% (22) or 20% (23) fat solution causes a significant increase in plasma secretin levels. Duodenal infusion of oleic acid (22,24) also increases plasma secretin levels sufficient to account for parallel increases in pancreatic bicarbonate secretion.

Although the primary biological action of secretin is to stimulate pancreatic secretion of bicarbonate-rich fluid, it is also a weak stimulant of pancreatic enzyme secretion (19). Secretin may, in fact, play a specific role in the secretion of the digestive enzyme pancreatic lipase, which is responsible for the degradation of triglycerides in the duodenum (25). Also, secretin interacts with another gastrointestinal hormone, cholecystokinin (CCK) to influence pancreatic secretion. A threshold or subthreshold dose of secretin increases the enzyme and bicarbonate responses to exogenous or endogenous CCK more than can be explained by simple additive effects of the two stimulants (26).

Cholecystokinin is also released from the duodenum, but in response to intraduodenal peptides and amino acids, as well as fat (27). CCK then causes gall bladder contraction and further stimulates the release of bile provoked by secretin (28). CCK is also a strong stimulant of pancreatic enzyme secretion in vivo (19), stimulating secretion of all pancreatic enzymes. The major digestive enzymes secreted in the rat, with respective molecular weight(s) and isolelectric point(s) are (29,30): amylase (53,000 & 55,000; 8.6 & 8.9), lipase (50,000; 6.8), elastase (26,000 & 28,500; 4.9 & 9.2), ribonuclease (14,000; 9.2), and the proteases - trypsin (21,000 & 21,000 & 22,000; 4.3 & 4.4 & 8.0), chymotrypsin (25,000 & 25,000; 4.8 & 9.0) and carboxypeptidase (A= 49,000; 4.4 - B= 47,000 & 47,000 & 47,000; 4.3 & 4.5 & 4.6) (27). Cholesterol esterase and phospholipase A_2 are also secreted in response to CCK, and as stated earlier, secretin potentiates this secretory effect.

Carbohydrate. Pancreatic amylase (E.C. 3.2.1.1) has a similar action to salivary amylase, hydrolyzing α-1,4 bonds of starch and glycogen to maltose, maltotriose and a

mixture of branched (1:6) oligosaccharides (α-limit dextrins). These dextrins are not digested because amylase has very little activity against α-1,4 bonds near the α-1,6 branching point (31). The end-products of pancreatic amylase are potential inhibitors of the enzyme, but they are rapidly hydrolyzed by intestinal brush border enzymes and the products removed from the intestinal lumen (31). These brush border carbohydrases (with their respective substrates) include: lactase (lactose); sucrase (sucrose, α-dextrins with 1,4 end links); isomaltase (α-dextrins of three or four glucose molecules with 1,6 links); α-limit dextrinase (α-dextrins of five or six glucose molecules with 1,6 links); glucoamylase sucrase (glucosyloligosaccharides); and trehalase (trehalose). The monosaccharides that result from this extensive digestive process (glucose, galactose and fructose) are all absorbed from the intestinal lumen at rates that exceed those expected from passive diffusion into the portal blood. Saturable, and presumably carrier-mediated, active transport systems have been demonstrated for all three monosaccharides (32).

Lipid. Pancreatic lipase (E.C. 3.1.1.3), with a pH optimum of 6 to 8, acts at the oil-water interface of the finely emulsified lipid droplets formed by mechanical agitation in the gut (33). It hydrolyses triglyceride at the 1 and 3 positions, and yields fatty acids and 2-monoglycerides (34). The emulsification of fats in the intestine is aided by bile salt micelles, which are present in biliary secretions. However, these micelles occupy the oil-water interface, thus, displacing lipase from the surface of the emulsions and reduce its lipolytic activity (35). This inhibitory effect of bile salt micelles is, however, reversed by colipase, a protein cofactor present in pancreatic secretions (36). Colipase is secreted as procolipase (102-107 amino acids), which is proteolytically cleaved to its active form

(96 amino acids with MW=10,000; 37). Colipase binds to lipase in a 1:1 ratio, and also has binding sites for bile salt micelles (35,38). It acts as an anchor that displaces micelles from the interface and brings lipase closer to its site of action, allowing complete hydrolysis of triglycerides (39).

The 2-monoacylglycerols and fatty acids produced from this hydrolysis leave the oil phase of the lipid emulsion and diffuse into mixed micelles consisting of bile salts, lecithin and cholesterol (40). The micelles transport the products of digestion across the unstirred water layer between the aqueous environment of the intestinal lumen to the brush border of the mucosal cells where they are absorbed into the intestinal epithelium. The bile salts pass on to the ileum, where most are absorbed into the enterohepatic circulation.

Phospholipids of dietary or biliary origin (i.e. lecithin) are hydrolyzed by pancreatic phospholipase A_2 to fatty acids (predominantly from the two position) and lysophospholipids (1-acyl glycerophosphatides), which are also absorbed from the micelles. Cholesteryl esters are hydrolyzed by pancreatic cholesterol esterase and the free cholesterol, together with most of the biliary cholesterol, is absorbed through the brush border after transportation in the micelles (40).

Within the intestinal wall, 2-monoacylglycerols are further hydrolyzed to produce free glycerol and fatty acids by a lipase, which is distinct from pancreatic lipase. These products are then utilized to resynthesize triacylglycerols in the intestinal mucosa. The absorbed lysophospholipids, together with much of the absorbed cholesterol, are also reacylated to regenerate phospholipid and cholesteryl esters. The triacylglycerols, phospholipids and cholesteryl esters produced are incorporated into lipid-protein

complexes, known as chylomicrons, that are secreted into the lymph and then enter the bloodstream.

Protein. Pancreatic proteases digest proteins and peptides in the intestine to small peptides (two to six amino acids) and single amino acids (41). Unlike pancreatic amylase and lipase, these proteases [trypsin (E.C. 3.4.4.4), chymotrypsin (E.C. 3.4.4.5), elastase and carboxypeptidase] are not secreted by the pancreas in their active form, but rather as proenzymes (trypsinogen, chymotrypsinogen, proelastase and procarboxypeptidase, respectively) which must be activated by proteolytic cleavage. Enterokinase, a brush border enzyme, which is stimulated by trypsinogen itself and released from the membrane by the action of bile acids, selectively cleaves a hexapeptide from the amino terminus of trypsinogen to produce trypsin (42). Trypsin then activates the other pancreatic proproteases and autocatalytically releases more trypsin from trypsinogen (31). The result is a mixture of endopeptidases (trypsin, chymotrypsin and elastase) and exopeptidases (carboxypeptidase A and B). The endopeptidases hydrolyze protein and peptones to polypeptides. Trypsin specifically cleaves peptide bonds between basic amino acids, whereas chymotrypsin is specific for peptides containing uncharged amino acid residues such as aromatic amino acids (2,33). The exopeptidases, carboxypeptidase A and B, further hydrolyze polypeptides at their carboxyl terminal peptide bonds, liberating single amino acids (2,33).

The final stages of protein digestion are carried out by a wide array of brush border and cytoplasmic peptidases in the intestinal enterocytes. Peptides larger than three amino acids are hydrolyzed extracellularly by brush border enzymes (43), while tripeptides are hydrolyzed by both brush border and cytoplasmic amino peptidases. Most

of these enzymes cleave one amino acid at a time from the N-terminus (31. After luminal and brush border protein hydrolysis, the products are transported across the intestinal wall, amino acids are degraded, metabolized to other amino acids, incorporated into proteins, or released unaltered into portal blood (31).

Intestinal digestion, and subsequent absorption, would be impossible without the enzymes secreted from the exocrine pancreas. The work of Pavlov in 1902 (44) revealed that the content of these enzymes in secreted pancreatic juice adapts to changes in dietary constituents. For example, consumption of a diet high in protein results in an increase in the protease content of pancreatic juice. The exact mechanisms of these dietary adaptations are currently unknown, but the proposed dietary and hormonal regulatory factors will be reviewed following a more in-depth introduction to the pancreas.

Pancreas

The pancreas is a retroperitoneal organ that appears distinctly lobulated (45). It has a mass of 85 ± 15 g in the human female and 90 ± 16 g in the male. The adult pancreas is 14-18 cm long, 2-9 cm wide and 2-3 cm thick, and is divided into four regions: head, neck, body and tail (45,46). It has a rubbery consistency and contains 71% water, 13% protein and 3-20% fat (47).

Functionally, the pancreas is a mixed exocrine-endocrine gland, with the exocrine portion comprising 84% of its total volume. Ductular cells and blood vessels constitute 4%, while endocrine cells occupy only 2% of total volume. The remaining 10% is made up of extracellular matrix (46).

The endocrine pancreatic cells, or the islets of Langerhans, are responsible for the synthesis and secretion of various peptide hormones including insulin and glucagon and, therefore, play an integral part in carbohydrate metabolism and blood glucose regulation (48). These islets are unevenly distributed among the exocrine pancreatic cells which secrete both bicarbonate-rich fluid (ductal cells) and digestive enzymes (acinar cells) through the pancreatic duct into the intestinal lumen where needed for intestinal digestion (48). The relationship and interactions among these cell types are complex. Therefore, the morphology and function of the endocrine and exocrine portions of the pancreas will be reviewed independently. Their functional inter-relationship will be considered in subsequent sections that discuss pancreatic regulatory factors.

Endocrine

The endocrine pancreas constitutes a small but important cell population of the pancreas. It is comprised of islets of Langerhans, varying in size from 100 to 200 μ, that are surrounded by a connective tissue capsule that serves to isolate them from surrounding exocrine tissue (49). There are approximately one to two million islets in the adult human pancreas, and each islet contains one to two thousand cells.

The β cell is the most abundant endocrine cell type in the islets of Langerhans (65%), and is responsible for the production and secretion of insulin. The islets also contain smaller numbers of glucagon-producing α cells (15%), pancreatic polypeptide (PP)-producing cells (10%), and somatostatin-producing D cells (5%; 49). The β cells are usually clustered together in the central part of the islets with α, PP and D cells more toward the periphery. In most mammals, PP-producing cells are more abundant in

the head, or duodenal portion, of the pancreas, whereas α cells are more prevalent in the tail, or splenic, region (50).

Insulin. Insulin is an important anabolic hormone that acts on a variety of tissues including liver, fat and muscle (51). It is synthesized in the β cells initially as a single polypeptide chain, known as proinsulin that is converted to insulin by removal of a linking polypeptide of 33 amino acids. The resulting active molecule consists of two peptide chains (A and B) connected by disulfide bridges, with an approximate molecular weight of 5734 (52).

The secretion of insulin from the β cells is stimulated mainly by high levels of plasma glucose, although glyceraldehyde, glutamine and leucine also act as secretagogues (53). In addition to increasing β-cell exocytosis, and therefore insulin release, glucose exerts important intracellular effects that influence the production of insulin (54). These effects likely represent manifestations of the enhanced metabolism of glucose in the β cell and perhaps may also reflect glucose-stimulated elevations in cAMP levels (55). Glucose appears to regulate insulin biosynthesis by at least three mechanisms (54): 1) selective increase in the rate of transcription of the insulin gene; 2) selective stabilization of preformed insulin mRNA; and 3) rapid and select stimulation of the rate of insulin mRNA translation. The first two mechanisms probably account for a 5-10 fold increase in total insulin mRNA levels and would occur over a period of several hours to several days. In contrast, glucose stimulation of insulin mRNA translation leads to a virtually instantaneous 10-20 fold increase in insulin biosynthesis (54). The underlying mechanisms of these various effects are complex and not well understood.

The primary role of insulin, produced and secreted in response to glucose, is to regulate blood glucose metabolism. When blood glucose levels are elevated, insulin acts to facilitate glucose transport from the bloodstream into muscle and adipose tissue (50). Insulin also acts directly on these cells, and hepatocytes, to stimulate glucose utilization and decrease protein and lipid catabolism by modifying the activity of a variety of enzymes (56).

The actions of insulin at the cellular level are initiated by insulin binding to its plasma receptor (57). This receptor is present on virtually all mammalian tissues, although the concentration varies from 40 receptors on erythrocytes to more than 200,000 receptors on adipocytes and hepatocytes (56). Insulin binding to its receptor produces a transmembrane signal that alters intracellular metabolism and mediates the action of the hormone (56).

Insulin enhances glucose utilization by positively regulating enzymes involved in glycolysis, such as glucokinase, pyruvate kinase and glyceraldehyde-3-phosphate-dehydrogenase (58). At the same time, insulin decreases glucose production by negatively regulating phosphoenol-pyruvate carboxykinase, which is involved in gluconeogenesis (57). These effects of insulin on the various enzymes aforementioned are transcriptional (58). Furthermore, because sequence-specific binding to DNA by insulin or the insulin receptor has not been found, it is likely that insulin is exerting its affects via other nuclear proteins (58).

Besides its affects on glucose utilization, insulin also decreases protein catabolism and urea production in liver cells. Also, in adipocytes, insulin increases lipid synthesis and decreases lipolysis by supressing the increase in cAMP caused by either epinephrine

or glucagon (59). Overall, insulin is considered an anabolic hormone because it promotes the uptake and storeage of nutrients and, at the same time, inhibits the utilization of existing energy stores.

Insulin-Acinar Interactions. The islets of Langerhans are dispersed throughout the pancreatic exocrine tissue, which allows hormones secreted by endocrine cells (i.e. insulin) to interact with exocrine acinar cells (48). In support of a direct effect of insulin on the exocrine pancreas, several studies have identified and characterized saturable insulin receptors on pancreatic acinar cells (60,61). Binding data indicate that these receptors have a high affinity for insulin and there are about 10,000 per acinar cell (62,63). This ability of insulin, produced in the endocrine pancreas, to act on acinar cells of the exocrine pancreas has been described as a paracrine effect. Simply, a paracrine messenger (insulin) is released by an effector (β cell) and traverses the interstitial space between it and adajcent target cells (acinar cells) to influence their action (64).

There is histological evidence for a regulatory role of islet-cell hormones on pancreatic acinar cells, based on the existence of a capillary portal circulation within the exocrine pancreas (65). The majority of the arterial blood flow (75%) goes directly to the exocrine pancreas, while 25% flows to the islets. Venous blood carrying hormones from the islets then enters the capillaries surrounding the acinar cells enroute to the heart. The result is considerably higher concentrations of islet hormones interacting with acinar cells than those found in the peripheral circulation (69). The high hormone concentrations may exert trophic effects on the acinar cells, which would explain why those adjacent to islets are larger, have larger nuclei and nucleoli, and contain more

zymogen granules (48,67). These, so called, periinsular acini are distinguished by histochemical staining for digestive enzymes, appearing as dark rings or "haloes" surrounding the paler islet cells (67).

The presence of these haloes have been associated with locally high insulin levels. They tend to be more prominent in obese hyperinsulinemic mice and disappear after treatment with alloxan, a β cell toxin (67). Also, in ducks, where α and β cells are topographically separated, the nuclei of acinar cells around the β cells appear to be larger (68). However, Landing (69) found the same proportion of periinsular haloes in the pancreata of normal and diabetic children, and Malaisse-Lagae et al. (70) noted that haloes were unusually prominent in pancreata from rats made diabetic with streptozotocin. Therefore, the haloes could result from effects of other islet cell hormones, as well.

Malaisse-Lagae and coworkers (70) also noted that, besides morphological distinctions, there are also differences in the relative concentrations of pancreatic digestive enzymes in the periinsular , or haloed cells, compared to acinar cells further from the islets, or teleinsular cells. Amylase and chymotrypsin concentrations are markedly reduced in the periinsulinar cells of normal rats. These differences could be due, in part, to the actions of insulin or other islet cell hormones.

Studies examining the effects of insulin on pancreatic acinar cells have been performed in vivo and in vitro and reveal specific effects on digestive enzymes. Kern and Adler (71) found that the infusion of glucose for three days leads to elevated plasma insulin and glucose levels, and increases pancreatic amylase content by 25%, decreases the content of chymotrypsin and trypsin, and increases protein synthesis by pancreatic

lobules by 40%. Although this in vivo study, and others, suggest a role for insulin in acinar cell regulation, infusion studies do not eliminate the possibility of potentiating effects of insulin on other hormones or regulators that may, instead, cause these changes. Therefore, the effects of insulin were examined in vitro. Insulin treatment of isolated pancreatic acini was found to increase glucose uptake (60). In the AR42J pancreatic acini carcinoma cell line, insulin increases cell growth and amylase synthesis (72). Finally, in acini from diabetic rats, insulin increases the synthesis of amylase, lipase and chymotrypsin in parallel with total protein synthesis (73). These effects of insulin on digestive enzymes, therefore, differ from previously mentioned longer-term in vivo effects of insulin to regulate differentially the synthesis of amylase and other enzymes. Perhaps the longer-term effect of insulin is transcriptional and the shorter-term effect is translational. Or perhaps insulin is acting with other regulators, such as glucose, to exert its effects. In any case, it is clear that insulin is acting directly on acinar cells to regulate digestive enzymes and its role in the regulation of pancreatic amylase, specifically, will be discussed later.

Glucagon. Glucagon, conversely to insulin, is a catabolic hormone responsible for the mobilization of energy substrates from storage depots. It is synthesized in the pancreatic α cells, originally as a 160-residue proglucagon molecule (74), but its active form is a single polypeptide chain of 29 amino acids with a molecular weight of 3485 (75).

In contrast to insulin, glucagon secretion is increased in response to low blood glucose levels, whether induced by starvation or insulin. Also, glucagon release is directly inhibited by glucose in vitro (76). The liver appears to be the major target site

for glucagon; within minutes of its presence, liver cAMP levels are increased. Cyclic AMP, in turn, activates a protein kinase cascade, resulting in rapid glycogenolysis and hepatic output of glucose. At the same time, glycogen synthesis is suppressed. There is also enhanced gluconeogenesis that may be mediated by cAMP activation of hepatic and adipose tissue lipases, which produce fatty acid activation of the gluconeogenesis process. Ketogenesis and lipolysis are also increased in response to glucagon secretion. In general, glucagon causes catabolism of glycogen and stored lipids in order to replenish circulating levels of energy nutrients (59).

Somatostatin. Somatostatin, another peptide hormone produced by the endocrine pancreas (D cells), has negative effects on both insulin and glucagon release. Like the other endocrine pancreatic hormones, it is produced as a prohormone consisting of 28 amino acid residues, but exists as a tetradecapeptide in its most active form (77). Although somatostatin was originally located and identified by its inhibitory action on pituitary growth hormone release, it has a wide range of gastrointestinal actions. These include not only inhibition of other pancreatic peptide hormones, but effects on gut digestion, motility and absorption (15).

In the stomach, somatostatin inhibits basal, food-stimulated and neurally stimulated gastrin release (78). It also inhibits meal-stimulated acid secretion at doses below those required to inhibit gastrin release (79). Although somatostatin plays a role in the regulation of digestion, its effects on gastric emptying are not as clearly defined. In some instances, somatostatin increases gastric emptying (80), whereas, in others, it causes a decrease (81). Somatostatin is also thought to decrease stomach, as well as intestinal, motility (82), possibly secondary to its inhibition of basal release of

acetylcholine (83). Somatostatin also affects absorption in the small intestine by inhibiting that of amino acids (84) and increasing ileal mucosal absorption of sodium and chloride (85).

Somatostatin also affects the pancreas, not only the endocrine portion where it is produced, but the exocrine cells, as well. In the exocrine pancreas, somatostatin inhibits enzyme, but not bicarbonate, secretion. This action may be secondary to diminished duodenal release of secretin (86) or CCK (87) in the presence of somatostatin. However, somatostatin receptors have been identified on the plasma membranes of pancreatic acinar cells (88). In the endocrine pancreas, as stated earlier, somatostatin decreases both insulin and glucagon associated with low blood sugar levels (89). Somatostatin also inhibits DNA, RNA and protein synthesis in the pancreas, in general, and chronic administration causes pancreatic hypoplasia (90). Concurrent administration of caerulein, which increases pancreatic growth, with somatostatin antiserum produced greater pancreatic growth than caerulein alone, supporting a role of somatostatin as a growth inhibitory factor in the pancreas (91).

Pancreatic Polypeptide. Another endocrine pancreatic hormone exerting some paracrine effects on the exocrine pancreas is pancreatic polypeptide (PP). This hormone consists of 36 amino acids; and in its preprohormone state, it is a 95-amino acid molecule containing a 29-amino acid signal peptide and a carboxyl-terminal extension (92). As stated, it exerts biological action on the exocrine pancreas; but, unlike somatostatin, it affects both pancreatic protein and bicarbonate secretion - inhibiting both to a similar degree (93). PP also increases DNA synthesis in the rat pancreas, but does not alter the stimulation by CCK (94).

Other gastrointestinal effects of PP include increasing motility in the stomach and intestine, increasing gastric emptying of a meal, and increasing the rate of intestinal transit (95). PP is also a very weak stimulant of basal gastric secretion in the stomach (95). In general, although PP has no effect of insulin or glucagon release, it seems to have biologic effects on the gastrointestinal tract antagonistic to those of somatostatin; however, both hormones tended to decrease pancreatic exocrine secretion.

Exocrine

Anatomy. The exocrine pancreas is proportionally much larger than the endocrine pancreas, occupying 84% of the total pancreatic volume, compared to only 2% containing endocrine cells (46). The basic subunit of the exocrine pancreas is the acinus, which is bound by a connective tissue matrix continuous with that surrounding the ductular epithelium. The acinus consists mostly of secretory acinar cells with a smaller number of centroacinar cells marking the beginning of the ductular system of the gland (46). The supporting matrix, or basal lamina, of the pancreas consists of types IV and V collagen, laminin and heparan sulfate proteoglycan, whereas types I and III collagen and fibronectin occupy the adjacent extracellular spaces surrounding the ducts and blood vessels (96).

Ultrastructure. The acinar cells that make up the acini constitute >80% of the pancreas. They are polarized and pyramidally shaped with their apices facing the lumen of the acini (46,97). The main subcellular organelles involved in the formation and storage of proteins for export are arranged in a highly polarized fashion in these secretory cells, reflecting their functional and temporal inter-relationships (Figure 1).

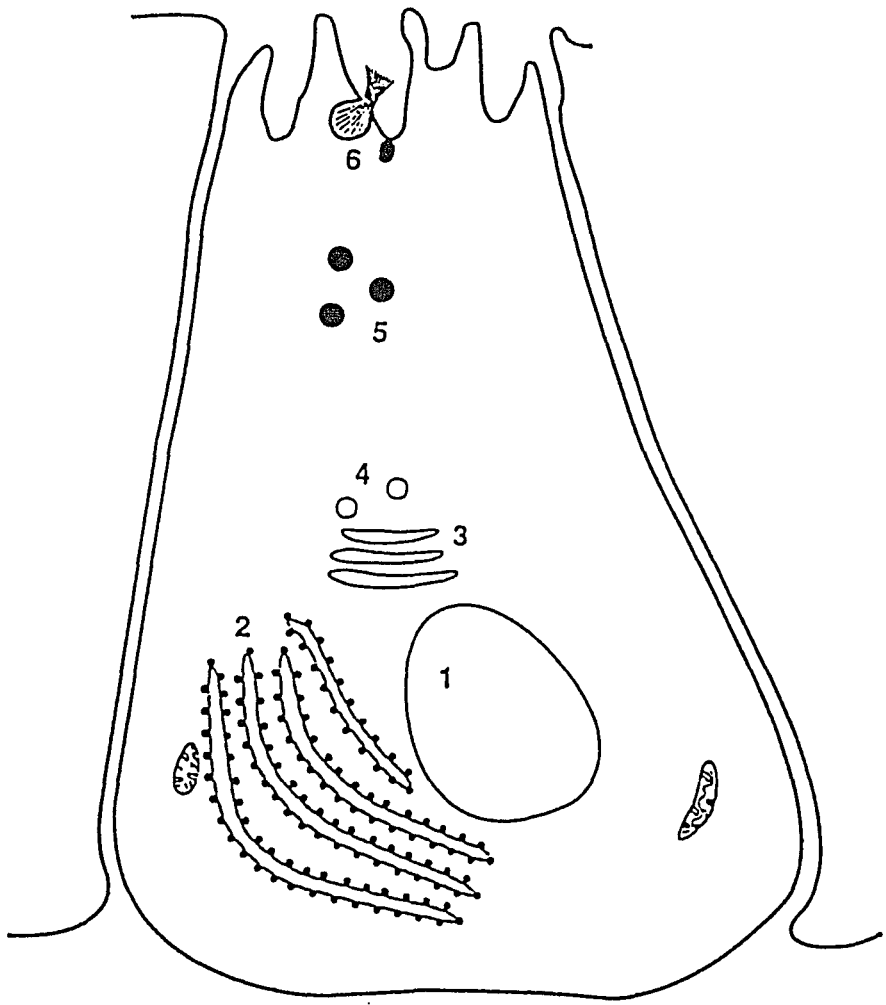

Figure 1: SCHEMATIC OF A PANCREATIC ACINAR CELL

A typical acinar cell is shown with the nucleus (1) and rough endoplasmic reticulum (RER; 2) localized in the basal portion of the cell and the Golgi complex (GC; 3), condensing vacuoles (CV; 4) and zymogen granules (ZG; 5) located in the apical pole of the cell. Upon appropriate stimulation, the ZGs fuse with the apical membranes, releasing their contents into the lumen. Schematic is taken from: Brannon, P.M. (1990) Copper and the exocrine pancreas: implications for lipid digestion. In: Role of Copper in Lipid Metabolism, (Lei, K.Y., ed.) pp.137-160, CRC Press, Boca Raton.

The nucleus of an acinar cell is basally located, with the paranuclear regions of the cytoplasm being occupied by the rough endoplasmic reticulum (RER;97). The RER is a highly convoluted membrane system studded on its cytosolic surface by attached polysomes and is the site of secretory protein synthesis (46). The supranuclear region of the cell is occupied by the Golgi complex, which consists of stacks of flattened cisternae of varying size and numerous small, smooth-surfaced vesicles. In the Golgi complex, ER-synthesized secretory proteins are post-translationally modified and then packaged into condensing vacuoles (the initial, immature stage of zymogen granules; 98). Secretory proteins are concentrated at this level of immature granules, which allows the storage, in aggregate form, of large quantities of secretory protein within a minimum of intracellular granule space (99). These vacuoles gradually move, by undefined mechanisms, to the apical portion of the acinar cell; the concentration process continues as they move. Upon reaching the apical region, the condensation is complete and these now mature zymogen granules remain at the cellular apex until the appropriate neurohormonal stimulus triggers exocytosis. These mature protein storage sites, or zymogen granules, vary in number depending on the stage of cellular development (100) and the state of neurohormonal stimulation (101).

The membrane surrounding the acinar cells, or plasmalemma, is also specialized, structurally and functionally, depending on its location on the cell. The apical plasmalemma possesses short microvilli (102) and has a relatively thick glycocalyx on its outer surface (103). Within the core of each microvillus is a bundle of filaments likely composed of actin, myosin, tropomyosin and α-actin (104). Also found in these microvilli is villin, known to bundle reversibly actin filaments in a calcium-dependent

manner (104). It is thought that these microvilli participate in the active transport and movement of secretory granules during stimulated secretion (97).

The basolateral membrane of the pancreatic acinar cell has few lateral interdigitations, although this region contains small, smooth-surfaced vesicular invaginations (46). These structures may reflect pinocytosis activity known to take place in this membrane region (105). There are also hormone receptors, such as those for CCK, exclusively localized on the basolateral plasmalemma, further emphasizing the specializations of the apical and basolateral plasmalemmal domains in stimulus-secretion coupling (106).

Gap junctions are prominent specializations of the lateral plasmalemma in pancreatic acinar cells (107). Intramembranous particles in gap junctions are composed primarily of a single protein species (with an apparent molecular weight of 26,000), which forms a pore structure across the membrane bilayer (97). The inner diameter of the gap junction pore allows only small molecules (500-1000 daltons) to traverse between the cells (108). The gap junctions also provide channels for intercellular movement of ions and other small, metabolically important molecules and are thought to be the sites of electrical communication between cells (109).

Synthesis of Digestive Enzymes. The major functions of the exocrine pancreas are to synthesize and secrete various enzymes and a fluid rich in bicarbonate, both of which aid in the gastrointestinal digestion of foodstuffs. The specific functions of the digestive enzymes (amylase, lipase, cholesterol esterase, phospholipase A_2, ribonuclease, procarboxypeptidase, trypsin and chymotrypsin) have been discussed; now the synthetic and secretory regulators of some of them (amylase, lipase and the proteases) will be

reviewed. The synthesis of the digestive enzymes occurs through a common pathway that will also be reviewed. As outlined by Palade (110), there are six successive steps in the synthesis and secretion of proteins in the exocrine pancreatic acinar cell: synthesis, segregation, intracellular transport, concentration, intracellular storage, and discharge. This is an orderly process that proceeds from the basal to apical region of the cell.

To begin this process, translation of RNAs for all proteins is initiated on polysomes free in the cytosol. However, proteins intended to be exported possess a special sequence on the 3' side of the AUG initiation codon, which is translated into an amino terminal extension, or signal peptide (111). Upon emergence of this signal peptide, ribosomal translation is temporally halted because the signal peptide interacts with a large-molecular-weight protein-RNA complex, the signal recognition particle, which is associated with the large ribosomal subunit. At this time, proteins not possessing a signal peptide complete translation in the cytosol where they reside (112). Translation-arrested polysomes, however, find their way to the ER membrane, where they interact with a membrane protein, or docking protein (113). At this point, the SRP and docking protein detach from the ribosome and can reenter the cycle; both molecules are thought to act catalytically in the targeting process (114). The ribosome-nascent chain complex engages a functional ribosome membrane junction. As the nascent polypeptide emerges from the ribosome, it is translocated across the ER membrane, a process driven by nucleoside triphosphate hydrolysis (114). The ribosomal subunits and RNA then dissociate from the ER and are available for another round of translation beginning in the cytosol. Of importance in this process is the requirement for the signal recognition particle to complex with the docking protein in order for translation of the

signal-bearing RNA to be completed. This provides a mechanism for initial sorting, or segregation, of nonexportable proteins from those which must be synthesized and subsequently processed by the ER-Golgi export pathway (115).

As the secretory proteins move from the ribosomes to the cisternae of the ER, several modifications occur. Besides cleavage of the signal peptide, there is formation of disulfide bridges (116) and initial glycosylation (117) of some exportable proteins. There are also conformational changes as proteins assume their tertiary or quaternary structure as a result of disulfide bond formation. These structural changes are thought to account for irreversible segregation of proteins within the RER.

Within 20-30 min of their synthesis, secretory proteins move from the cisternae of the RER to the Golgi complex. Further post-translational modification also occurs in the Golgi (97). These include terminal glycosylation, sulfation of proteoglycans and further proteolytic cleavage of propeptides to their final secretory form. This transfer is supposedly mediated by vesicles arising from pinched-off transition elements of the RER that act as carriers for the secretory proteins (118). Then, secretory proteins are concentrated into condensing vacuoles which later become mature storage granules (98). Finally, the Golgi complex serves the important function of sorting and routing ER-synthesized proteins to their final destination (i.e., lysosomal hydrolases to lysosomes or secretory proteins to storage granules). There is also a sorting of signals, yet unknown, of secretory proteins that will be released in response to neurohormonal stimulation and those secreted without intervention of a intracellular messenger system (i.e., constitutive secretory pathway; 119).

Secretion and Its Regulation. Zymogen granules, following their formation, move by unknown mechanisms to the apical portion of the acinar cell. They serve as mature storage sites waiting to be released in response to an appropriate neurohormonal stimulus. Their release, or the exocytotic event, consists of: movement of the zymogen granule toward the apical membrane (possibly by brownian motion); recognition by the zymogen granule of the appropriate fusion partner (possibly aided by a calcium-dependent protein cofactor); and fusion and fission of the zymogen granule membrane with the plasmalemma at the site of exocytosis (119). The contents of the zymogen granules are released into the relatively alkaline environment of the acinar lumen. Production of a fluid rich in bicarbonate by nearby centroacinar cells is thought to be necessary for final dissolution of the zymogen granule contents (120). This exocytosis occurs in response to receptor-mediated stimuli on the basolateral membrane of the acinar cells.

Pancreatic secretagogues, neurotransmitters and gastrointestinal hormones that stimulate secretion of digestive enzymes, bind to specific receptors on the basolateral membrane of the acinar and ductal cells (106). The steps following receptor occupancy that lead to secretion of either proteins or ions are generally referred to as stimulus-secretion coupling (121). This process involves intracellular messengers, which are informational molecules (ions, cyclic nucleotide, phospholipids) that convey a message from the plasma membrane to the inside of the cell. These messengers then activate effectors (protein kinases, phosphatases, adenosine triphosphatase), which bring about the secretory process. The end result, for protein secretion by the acinar cell, is the directed fusion of the zymogen granule with the luminal plasma membrane. For fluid

secretion by the ductal cells, the end result is net translocation of Na+, Cl- and other osmotically active solutes into the lumen of the acinus with the passive movement of water (122).

The exact intracellular messengers and effectors involved in any stimulus-secretion response within the acinar cell differ depending of the secretagogue eliciting the response (Figure 2) but two major classes of secretagogues exist. For the first class, phosphotidyl inositol and cytoplasmic Ca^{2+} are the major intracellular messengers as exemplified by CCK, acetylcholine and bombesin (123). The second class, exemplified by secretin and vasoactive intestinal peptide (VIP), act through cyclic AMP (cAMP; 124).

The secretory-stimulus pathway for CCK is initiated by interaction of CCK with its receptor. Radiolabelled CCK binds to two different classes of binding sites (125,126). One class of binding sites has a low capacity but high affinity for CCK, and the other has a high capacity but a low affinity for CCK (125,126). There is also recent evidence for a third class of CCK binding sites on acinar cells, but the biological activity associated with its occupancy is yet unknown (127). The primary event engendered by occupancy of the high and low affinity receptor sites is an increased hydrolysis of phosphatidylinositol-4,5-bisphosphate (PI-4,5P) in the plasma membrane by associated phospholipase C hydrolysis to produce diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP_3; 128). The onset of this response after CCK-receptor interaction is very rapid, occurring within 15 sec of agonist exposure (128). The breakdown products of PI-4,5P subsequently initiate independent intracellular affects.

One product of PI-4,5P hydrolysis, IP_3, appears to be involved in cellular Ca++ mobilization from a nonmitochondrial store, probably the RER or calcisomes, which are

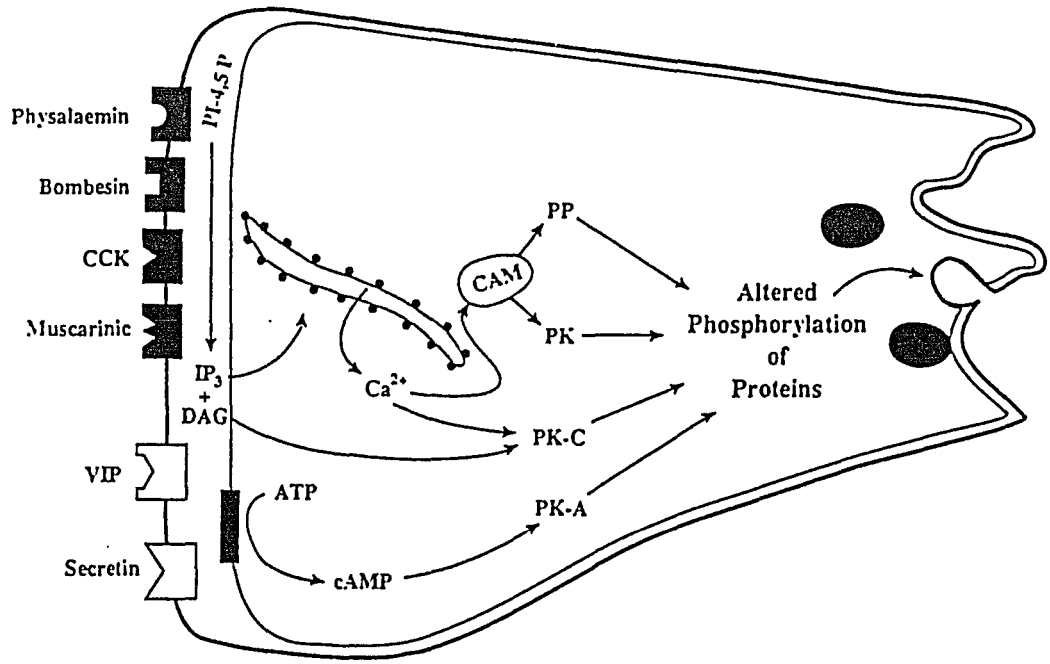

Figure 2: STIMULUS-SECRETION COUPLING IN THE PANCREATIC ACINAR CELL

Abbreviations: CCK, cholecystokinin; VIP, vasoactive intestinal peptide; PI-4,5P, phosphoinositol-4,5-bisphosphate; IP_3, inositol-1,4,5-triphosphate; DAG, diacylglycerol; CAM, calmodulin; PP, protein phosphatase; PK, protein kinase; PK-C, phospholipid dependent protein kinase; PK-A, cyclic AMP-dependent protein kinase. Schematic is taken from: Brannon, P.M. (1990) Copper and the exocrine pancreas: implications for lipid digestion. In: Role of Copper in Lipid Metabolism, (Lei, K.Y., ed.) pp.137-160, CRC Press, Boca Raton.

non-ER membrane enclosed Ca^{2+}-storeage vessicles (129,130). The hydrolysis of PI-4,5P, itself, may play a role in intracellular calcium release. The Ca^{2+} bound at the inner plasma membrane surface to the negatively charged phosphoinositol headgroups are thought to be released directly upon hydrolysis of the phospholipid, and thereby, contribute to the large increase in intracellular Ca^{2+} seen in acinar cells within a few seconds after receptor occupation (131). The released Ca^{2+} then has several receptors, or effectors, with which it binds, including calmodulin (CAM) - a ubiquitous acidic protein (132). In most cases, the Ca^{2+}-CAM complex then binds to and activates enzymes such as protein kinase and protein phosphatase (133). Diacylglycerol, the other hydrolytic product of PI-4,5P, is a physiologic activator of protein kinase C (134). All of these activated enzymes play a role in phosphorylation of distinct cellular proteins, with functions not yet clearly defined (135), and may play a direct role in regulation of acinar cell exocytosis. However, most of the specific enzymes or regulatory proteins involved in exocytosis or ion transport have not yet been identified.

Secretin, like cholecystokinin, binds to a specific receptor on the basolateral membrane of acinar cells (19,122). It, too, provokes a stimulus-secretion response, but mainly via a different intracellular messenger - cAMP. Upon secretin-receptor interaction, there is a large increase in cAMP ranging from 8- to 30-fold (136), which results from activation of adenylate cyclase in acinar cell plasma membranes (137). Cyclic AMP then binds to and activates protein kinase A, which phosphorylates intracellular proteins. The protein substrates for cAMP-activated protein kinase are unknown, but are thought to play a role in exocytosis and ion transport, leading to

secretion of enzymes by acinar cells and an isotonic, NaCl- and bicarbonate-rich fluid by ductal cells (122).

Some evidence also suggests that secretin, at high concentrations (>10^{-8}M), stimulates secretion of acinar cells via the phosphoinositide second messenger system (138), similarly to CCK. Secretin receptors of both high and low affinity are proposed to exist (138). Occupation of the high affinity receptors in rat pancreatic acini leads to an increase in cAMP levels and a low rate of enzyme secretion. By contrast, occupation of the low-affinity receptors appears to cause a more marked increase in enzyme secretion via a cAMP-independent mechanism, the phosphoinositide pathway, which leads to increased inositol triphosphate, intracellular calcium and diacylglycerol (138). Therefore, secretin may, indeed, function via both messenger systems to regulate enzyme release.

Tissue-Specific Gene Expression. Exocrine pancreatic acinar cells synthesize a group of hydrolytic enzymes involved in the digestion of food, as stated earlier. These enzymes in the pancreas increase markedly during pancreatic development, largely due to an increased rate of transcription (139). Transcriptional enhancers have been identified that appear to be responsible for the expression of these digestive enzymes. A conserved sequence has been identified in the enhancers by sequence comparisons of the 5'-flanking regions of several of these genes, including amylase, elastase, chymotrypsin, trypsin, ribonuclease and carboxypeptidase (140,141). Subsequent functional studies showed that fragments containing this consensus sequence act as pancreas-specific enhancers in cultured cells (141,142,143) and in transgenic mice (144,145).

Competition for binding in gel mobility-shift experiments and methylation interference studies suggest that the pancreas-specific enhancer element contains two distinct binding motifs (142,146). One motif contains the sequence, CAGCTGTG, and the other, TTTTCCC. Deletion of these sequences in the rat chymotrypsin B enhancer abolishes enhancer function (141). Similarly, mutations in related CAGCTGTG and CACCTGTG motifs in the rat amylase 2A (141) and rat elastase I (143) enhancers, repectively, lead to severely decreased gene expression. These studies suggest a critical role for this enhancer region in the control of pancreas-specific gene expression.

Recent research has focused on the identification of pancreatic nuclear proteins that interact with the enhancer regions of digestive enzyme genes to increase their expression in the exocrine pancreas. Nelson and coworkers (147) have cloned two cDNAs that encode proteins (Pan-1 and Pan -2) that bind to the consensus sequence $CA^{C}/_{G}CTG^{T}/_{G}$, the CACCTGTC motif, which is present in transcriptional control elements of exocrine pancreatic genes, and increase expression of such genes. However, both Pan-1 and Pan-2 bind to related motifs present in the rat insulin enhancer, as well as a DNA segment containing the SV40 AP-4 element. Therefore, it is not likely that these proteins are solely responsible for acinar-specific expression of digestive enzymes. It is possible, however, that the Pan proteins interact with other factors that bind to the CACCTGTC element or the contiguous TTTTCCC motif. The affinities and specificities of the potential interacting factor(s) may then determine the functional competence of the complex (147).

Howard and coworkers (148) recently identified a nuclear protein with specific affintiy for the enhancer region of the mouse Amy 2.2 gene. Protein binding was

characterized by gel retardation, DNase I footprinting and G-methylation interference and appears to be required for amylase enhancer activity. The same protein was also found to bind the enhancer of the elastase gene, despite the difference in sequence organization within the enhancer region. The two reported consensus sequences are separated by 17 bp in the mouse amylase 2.2 gene, but are continuous in the rat elastase I gene, indicating that the amylase enhancer comprises a single functional unit (148). This rat pancreatic protein appears to be the same as a protein isolated from AR42J cells, which was designated PTF1 (142). Cockell and colleagues (142) demonstrated that PTF1 interacts with the mouse Amy 2.1 gene, as well as that of trypsin, chymotrypsin and carboxypeptidase. Mutations in the enhancers of these genes lead to concomitant loss of protein binding and enhancer activity, which provides functional evidence for a positive effect of protein binding on gene expression. However, future isolation of pure PTF1 or cloning of the PTF1 gene is necessary to confirm this proposed function. The possibility that PTF1 protein interacts with Pan-1 and Pan-2 in acinar-specific gene expression also needs to be examined in the future.

Dietary Regulation of the Exocrine Pancreas

As stated earlier, Pavlov (44),in the early 1900s, first reported the adaptation of the exocrine pancreas secretions to changes in nutritional substrates in the diet. Since then, many studies have confirmed and extended these findings. Pancreatic cellular content, as well as secretion (149), of the major digestive enzymes (proteases, lipase, amylase) changes with alterations in their respective substrates (protein, triglyceride, starch; 150,151). This adaptation occurs through changes in enzyme synthesis (152-

154) and mRNA levels (155-158). For one adaptation, pancreatic lipase to dietary fat, the change in mRNA levels occurs through altered transcription (159); but, for the remaining adaptations, transcriptional regulation has not been definitively established. These mechanisms of pancreatic adaptation to dietary substrates and their possible mediators will be discussed in detail in this, and the following, sections.

Protein

Early studies (160,161) investigating the effects of various quantities of dietary protein on the pancreatic proteases (chymotrypsinogen, trypsinogen) demonstrate that beyond a minimal level of protein (10% wt/wt) in the diet, individual proteases are regulated in direct proportion to the amount of their substrate. Later studies confirm this dose-dependent effect of dietary protein from 10-80% casein (162-164). These changes in proteolytic activity (80-600%) parallel (152) changes in protease synthetic rates (250-1800%), which tend to precede the changes observed in tissue levels of enzymes (160,161). Changes in acinar cell content of proteases begin within 24 h after administration of a high- protein diet, and continue for five to seven days, after which time protease levels plateau (149,158). However, changes in protease synthetic rates occur within two hours of dietary change and continue to increase slowly for up to nine days (152).

These observed changes in protease enzyme content and synthesis are mediated by similar changes in their mRNA levels (156); protease mRNA levels increase 200-400%. However, it is yet unclear whether the dietary adaptation of chymotrypsinogen

and trypsinogen mRNA levels result from changes in gene transcription or mRNA stability.

Consumption of diets high in predigested proteins, composed of small peptides, affect the acinar cell content and synthesis of chymotrypsinogen and trypsinogen to the same extent as intact protein (164); but, amino acids, provided either intravenously or orally, fail to increase pancreatic protease activities (165,166). Therefore, the adaptation of the proteases to dietary protein does not involve their digestive end products. Instead, intact dietary protein or small peptides regulate the proteases. These proteins and peptides have been proposed to increase the release of CCK from the duodenum, which may then cause the increase in pancreatic proteases. This suggested hormonal regulation will be discussed in detail in the next section.

The dietary adaptation of the proteases is affected not only by the amount of protein in the diet, but also by its quality. Consumption of diets containing large amounts of high-quality protein, such as casein or fish protein, increases chymotrypsinogen, whereas consumption of low-quality protein, such as gelatin or zein, does not, unless the diets are supplemented with their limiting amino acids (167). Furthermore, high-protein diets with high biological value, such as those containing whole-egg protein, increase chymotrypsinogen more than casein-containing diets unless, again, the casein is supplemented to improve its biological value (168).

Severe protein malnutrition also affects dietary adaptation of the proteases. When a nitrogen-free diet is consumed by rats, producing protein malnutrtion, chymotrypsinogen and trypsinogen content, as well as that of amylase, decrease (169). A protein-free diet also decreases amylase and cationic protease synthesis; however, the

synthesis of the anionic proteases increases (163). A general decrease in enzyme synthesis in protein malnutrition may reflect limited essential amino acids and an attempt to spare amino acids, in general, for other tissues. The unexpected increase in anionic proteases is proposed as a protective mechanism to ensure digestion of any consumed proteins (163) and represents a paradoxical increase compared to the overall pancreatic response.

Triglyceride

Early studies investigating the effects of high-fat diets on pancreatic lipase found no dietary adaptation of this enzyme (150,170). After the identification of colipase and its addition to assays for lipase activity, however, a profound adaptation of lipase content (170-800%; 151,154,171,172,173,174) and synthesis (200%; 154) in response to high-fat (41-75% kcal) diets was uncovered. This adaptation of lipase to high-fat diets begins immediately and continues for five days (172). Lipase mRNA levels are also increased (200-380%) with increasing dietary fat content (154,156,159). Recently, Wicker and Puigserver (159) reported a progressive stimulation of lipase gene transcription over a period of adaptation to a lipid-rich diet. Therefore, the stimulation of lipase transcription may entirely explain lipase message accumulation during dietary adaptation.

The adaptive response of lipase is the same whether dietary fat is increased at the expense of protein (172,173) or carbohydrate (154,174), suggesting that dietary fat, alone, is responsible for the adaptation. Dietary fatty acids also increase pancreatic lipase levels (171,172), as does intravenous infusion of emulsified triglyceride (165).

Therefore, it appears that oral consumption of, or enteral response to, intact fat is not required for lipase adaptation. However, the role of fatty acids post-digestion in lipase adaptation is unclear because infusion of ketones, which increase lipase content, causes a decrease in serum fatty acids (175).

Lipase adapts not only to changes in total amount of dietary fat, but to types of fat, as well. However, some controversy exists as to whether differences in lipase adaptation seen with consumption of different types of fat are caused by variation in chain length or degree of saturation of the dietary fats. Deschodt-Lanckman et al. (172) and Bazin et al. (171) report that unsaturated fats stimulate lipase to a greater extent than do saturated fats. However, Saraux et al. (176) report that degree of saturation of fats does not influence lipase, but rather chain length of the fatty acids in dietary triglycerides regulate lipase adaptation; long-chain triglycerides stimulate lipase more than medium-chain triglycerides. Sabb et al. (174) report no difference in lipase adaptation to saturated and unsaturated fats and to long-chain and medium-chain triglycerides when diets contain 67% kcal as fat. However, when dietary fat content is reduced to 40% kcal, consumption of only highly unsaturated diets (containing safflower oil; P/S ratio = 7.9) leads to increased lipase content. Therefore, composition of dietary fat is more influential at levels of dietary fat where lipase adaptation is not maximally induced. Such is the case with moderate fat diets, where unsaturated fat increases lipase more than saturated fat.

Byproducts of triglyceride digestion, ketones and fatty acids, also affect and may mediate lipase adaptation. In fact, both lipase activity and blood ketone levels are positively correlated with high-fat diets (171). Furthermore, continuous infusion of

β(OH)-butyrate into rats results in an increase in lipase activity equal to that caused by a 40% fat diet (171). However, the direct effects of ketones on pancreatic acinar cells cannot be inferred from in vivo infusion studies. Therefore, to determine if ketones do, indeed, regulate pancreatic lipase, it is necessary to examine their affects in a totally in vitro system of cultured acinar cells, in which interaction with other potential regulators is eliminated.

There may also be other mediators of pancreatic lipase, such as secretin (to be reviewed), involved in this dietary adaptation. The affects of secretin, either alone or in conjunction with the above mentioned nutrients, also need to be addressed in an in vitro model system.

Carbohydrate

Much like the responses of proteases and lipase to dietary protein and triglyceride, amylase content (150,153,172,177,178) and synthesis (153,154,156,179) increase (50-500% and 200-800%, respectively) in response to high-carbohydrate (65-73% kcal) diets. These diets must, however, contain greater than 10% protein (163) to provide essential amino acids or amylase content is decreased. Amylase mRNA levels also increase (900%) with increases in dietary carbohydrate (155). This adaptation occurs whether carbohydrate is increased at the expense of protein (163) or fat (154,178), indicating carbohydrate is primarily responsible for the adaptation. However, various carbohydrates affect pancreatic amylase differently.

Although orally administered glucose affects pancreatic amylase similarly to dietary starch (172,173,180), sucrose and fructose have lesser effects (180).

Furthermore, lactose and galactose do not affect amylase content (172). Intravenously administered glucose also increases amylase content (165), supporting a role of starch digestive end-products in this dietary adaptation. Further support is provided by the fact that inhibition of starch digestion decreases amylase content (181). Blood glucose, whether infused or stimulated by dietary carbohydrates (starch, sucrose or fructose), is associated with increased amylase adaptation. However, because high blood glucose concentrations stimulate insulin release, it is unclear whether glucose is a sole mediator or acts in conjunction with this hormone (to be discussed in next section).

Glucocorticoids that antagonize many biological functions of insulin also seem to regulate pancreatic amylase activity. The relationship between dietary carbohydrate and glucocorticoids is unclear, but glucocorticoid depletion, following adrenalectomy, decreases pancreatic amylase content (182), synthesis and mRNA (183). Insulin and glucose levels also decrease after adrenalectomy (183), but repleting these with oral glucose does not increase amylase activity. These results strongly suggest a direct influence of glucocorticoids on acinar cells. However, it is still unknown whether the long-term regulation of amylase in the pancreatic adaptation to dietary carbohydrate is mediated by glucocorticoids and insulin separately , or whether their action on the pancreas is dependent on the presence of both insulin and glucocorticoids.

Hormonal Regulation of the Exocrine Pancreas

As stated in the previous section, there are several hormones (CCK, secretin, insulin) which have been implicated as mediators of the adaptation of pancreatic enzymes (proteases, lipase, amylase) to their dietary substrates. The secretion and

general biological actions of CCK, secretin and insulin have been briefly described previously. Herein their non-secretory effects on pancreatic acinar cells, underlying mechanisms, and their proposed regulatory roles will be described.

Insulin

Insulin is a proposed regulator of amylase, specifically, in its adaptation to dietary carbohydrate (184). In fact, certain pancreatic amylase genes, including rat Amy-2 and mouse Amy-2.2, seem to require insulin for expression (58). Diabetes in rats (alloxan- or streptozotocin-induced), which decreases insulin production, also decreases amylase content (172,185), synthesis (186) and mRNA levels (187) in rats. Replacement of insulin in diabetic rats restores amylase content (185,188), synthesis (186) and mRNA levels (187). Furthermore, Osborn and coworkers (189,190) have localized insulin-responsive sequences on the mouse Amy-2.2 gene and determined that transcription of amylase is repressed in diabetic mice.

Glucose may, however, interact with insulin in the regulation of amylase, although the mechanisms are not clearly defined. Importantly, the reversal of the effects of diabetes with insulin treatment appears to be dependent upon increased glucose utilization because insulin is ineffective in restoring amylase in diabetic rats fed a high-fat diet (185). Furthermore, fasting in mice, which decreases plasma glucose and insulin, decreases amylase content (172) and synthesis (191); however, glucose administration restores them. If diazoxide, which inhibits glucose-induced insulin release, is also administered, amylase content and synthesis are not restored (191). These studies suggest that insulin is an important regulator of pancreatic amylase.

However, it remains to be determined whether glucose stimulates the release of insulin which then regulates the transcription of amylase alone, or whether glucose-stimulated insulin enhances the utilization of glucose by the acinar cells which interacts with insulin in the regulation of amylase. In either case, the regulatory scheme would account for amylase adaptation to orally and intravenously administered glucose, as well as ingested starch.

Secretin

Besides its role in pancreatic secretion, secretin is proposed to regulate the long-term adaptation of pancreatic lipase to dietary fat. Its in vivo infusion into rats increases the relative synthesis of lipase (192-194), but does not alter mRNA levels (195). Because consumption of high-fat diets increases lipase mRNA levels, as well as its content and synthesis; it is unlikely that secretin, alone, regulates this pancreatic adaptation. It is possible, however, that secretin is involved in a translational regulation of lipase either alone or in conjunction with other regulators.

The duodenal infusion of oleic acid, a hydrolytic product of triglycerides, increases plasma secretin levels (196), providing further support for its role in lipase adaptation to both oral triglycerides and fatty acids. However, there is no evidence to support a role of secretin in lipase adaptation to intravenous triglycerides, because the release of secretin has been demonstrated with only luminal fatty acids. Possibly, secretin interacts with other hormones or metabolites, such as ketones, in the regulation of pancreatic lipase. In order to determine the exact role of secretin in pancreatic lipase regulation, it is necessary to examine its direct effects on acinar cells in an in vitro

culture system. In this way, the effects of secretin, alone, can be determined, as well as its interactive effects with other potential regulators (ketones, fatty acids).

Cholecystokinin

In addition to immediate postprandial secretory actions, CCK is also involved in the long-term regulation of the pancreas including growth and adaption to dietary substrates - specifically protein (197,198). The mechanisms involved in long-term action of CCK are not well understood, but may be mediated, in part, by regulation of pancreatic gene expression.

One adaptive action of CCK is the stimulation of pancreatic growth (increased pancreatic weight and DNA content), that is promoted, in part, by consumption of high-protein diets (198,199). This CCK-induced pancreatic growth probably involves induction of ornithine decarboxylase (ODC), which catalyzes the rate-limiting steps in the biosynthesis of the polyamines putrescine, spermidine and spermine (200). Polyamines have been shown to facilitate many aspects of DNA, RNA and protein synthesis and have been implicated in the regulation of growth and proliferation (201). Recently, CCK infusion was shown to increase pancreatic ODC mRNA levels two-fold (197), which correlates well with the two-fold increase in ODC immunoreactivity seen in rat pancreas after treatment with caerulein, a potent CCK analogue (202). Therefore, CCK is thought to exert pretranslational regulation of ODC, which then mediates, in part, the pancreatic growth reported with CCK treatment (197).

CCK is also implicated in the long-term adaptive changes in pancreatic digestive enzyme production in response to dietary protein. Consumption of a high-protein diet

by rats for seven days results in increased plasma CCK levels (198). Furthermore, CCK or caerulein treatment causes an increase in pancreatic protease content (203) and trypsinogen 1 and 2 synthesis (204,205). Its effects on protease mRNA levels, however, are not clear. Renaud et al. (203) report that caerulein injection increases chymotrypsinogen and trypsinogen mRNA levels. Similarly Lewis et al. (197) report that infusion of CCK at physiologic levels increases the mRNA levels of trypsinogen 1 and chymotrypsinogen B. Wicker et al. (206) also found that caerulein infusion increases protease mRNA levels. However, Steinhilber et al. (204) report that caerulein infusion does not change anionic trypsinogen mRNA levels.

Despite these conflicting reports of its regulation of protease mRNA levels, CCK remains the likely mediator of pancreatic protease adaptation. It appears that CCK controls this adaptation to dietary protein through feedback regulation of its secretion from the small intestine (195). Active trypsin in the intestinal lumen decreases the release of CCK by degrading a CCK-releasing factor produced in the intestinal mucosa or pancreas (207). However, when protein or peptides are present in the lumen, as with high-protein diets, the trypsin molecules become occupied with their hydrolysis. Therefore, CCK-releasing factor remains active, CCK is released and proceeds to the pancreatic acinar cells where it exerts its effects on protease content, synthesis and, possibly, mRNA levels. This proposed role of CCK accounts for the fact that only dietary protein and peptides, which require trypsin hydrolysis, lead to pancreatic adaptation; whereas, amino acids, intravenous or oral, do not. However, because the effects of CCK on pancreatic proteases have been examined predominantly with in vivo infusion of injection studies, it is not known whether CCK, itself, is causing these effects, or whether

CCK is potentiating the effects of other hormones or regulators in circulation. Therefore, it is necessary to examine the direct effects of CCK on acinar cells in culture to determine its exact role in protease regulation.

In some studies of dietary adaptation of the pancreas, consumption of high-protein diets also decreases amylase mRNA levels (155,157). Although in vivo elevation of plasma CCK has no effect of amylase mRNA levels (197), and infusion of caerulein causes a relatively small decrease in amylase synthesis (203,204,206); direct effects of CCK on pancreatic amylase in vitro have not been examined. It is possible, however, that the decrease in amylase mRNA levels associated with consumption of high-protein diets is provoked by the low levels of dietary carbohydrate frequently needed to achieve a diet high in protein. Therefore, changes in amylase gene expression could be mediated by a mechanism sensitive to decreased dietary carbohydrate content, possibly involving insulin or glucocorticoids (208), which were discussed earlier.

In Vitro Model Systems of the Exocrine Pancreas

Many different model systems have been used to study exocrine pancreatic function and regulation. A major advance is this endeavor came with the development of in vitro cell systems. Early in vitro studies used either gland slices, fragments (i.e. lobules) or whole tissue from young animals (209). Although significant data were obtained, major problems existed, such as tissue viability, representational sampling, diffusional barriers, and the presence of multiple cell types (209). The development of

techniques allowing isolation of individual acinar cells and intact acini from the pancreas has alleviated some of these problems.

Reproducible sampling of a purified cell type has allowed the study of receptors, second messengers and effector systems that constitute the cellular regulatory pathways. Furthermore, because isolated cells can be maintained in primary culture, the study of longer-term cellular regulation such as that of protein synthesis and gene regulation is now possible.

The in vitro model systems currently used to study exocrine pancreatic function and regulation can be divided into three major groups: cultured acinar cells; cultured acini; and AR42J cells, an exocrine pancreatic tumor cell line. These models will be discussed individually in terms of their characteristics, advantages and limitations.

Acinar Cell Culture

The first isolated dispersed acinar cells were prepared by Amsterdam and Jamieson (210) by enzymatic digestion of rat pancreatic tissue with crude collagenase (contaminated with other proteases) and hyaluronidase, divalent cation chelation and mechanical shearing. Subsequently, purified collagenase and chymotrypsin replaced the crude enzyme treatment in order to obtain more reproducible results (211). In these preparations, collagenase acts to digest the basement membrane and connective tissue stroma; calcium chelation separates desmosomes and tight junctions; and mechanical shearing completes the cell separation (211). This isolation procedure yields >95% acinar cells, as expected from proportions of cells in the pancreas, with few endocrine or

duct cells. Initial studies used these isolated cells acutely (within 4-6 h of isolation); subsequently, longer term cultures (4-10 d) of these cells have been developed.

These isolated cells (212,213,214), have been used acutely for a variety of studies measuring receptors, cyclic nucleotides, and ion fluxes. By contrast, secretion of macromolecules is reduced variably in the dispersed acinar cell preparation and requires higher concentrations of secretagogues (210,214,215). For example, Kondo and Schulz (176) found that amylase release from perfused rat pancreas is increased 8-20 fold with CCK treatment; whereas, the same CCK treatment stimulates only a two-fold increase in amylase release from dispersed acinar cells. This altered secretion may be due to changes in the plasma membrane, either luminal (209) or lateral (216,217), caused by cell dispersal.

The luminal plasma membrane is believed to be involved in exocytosis, and its distinct nature is lost when tight junctions are split by calcium chelation (218). Disruption of the lateral plasma membranes, upon cell dispersal, causes dissociation of gap junctions. Bruzzone and coworkers (216,217) have found a two-fold increase in basal secretion by acini upon disruption of gap junctions by heptanol; such disruption would necessarily occur upon dispersal of individual acinar cells. Increased basal secretion rate of dispersed acinar cells may influence the secretagogue-stimulated secretion pathway. Perhaps availability of enzymes for secretion becomes a problem if enzyme synthesis is not increased proportionally to secretion. In any case, this altered secretion rate of dispersed acinar cells does not make them the best candidate for secretion studies. Although information about enzyme secretion is readily obtainable,

secretion studies, in general, may be better performed in acini which have unaltered luminal and lateral plasma membranes.

Despite the inherent complication of membrane alterations seen in isolated acinar cells, their use has distinct advantages. As previously mentioned, they remain in suspension, making reproducible sampling easier, they have no diffusion barriers, and they can be maintained in culture for several days (219,220). Therefore, it is extremely beneficial to develop an optimal acinar cell model for the study of exocrine pancreatic function, and many have contributed to this endeavor since the original attempts of Amsterdam and Jamieson (210). Of the methods developed for primary culture of pancreatic acinar cells, each produces cells with unique characteristics. This may reflect differences in species, culture techniques, and the medium in which they are maintained (219).

Rat pancreatic acinar cells have been isolated and cultured in serum-containing medium either with (221,222) or without (220) components of extracellular matrix (ECM). Those cultured without ECM are maintained up to 10 days and exhibit normal acinar cell morphology, but are unresponsive to hormones (223). Of the two acinar cell preparations cultured with ECM, one produces cells that reaggregate into acini-like structures (220), and the other, cells that form monolayers and proliferate (221). A primary culture of mouse acinar cells also exists (224). These cells are cultured on collagen substrates, on which they attach and proliferate. They form monolayers of cells with recognizable apical and basal surfaces, but with a reduced content of secretory granules that differ in composition from normal zymogen granules (209). They also

maintain some degree of hormonal-responsiveness (224) to caerulein; however, its effects are primarily trophic.

The primary acinar cell cultures (detailed elsewhere) used for the studies described herein is one developed by Brannon and colleagues (223,225). This procedure yields individual acinar cells by sequential enzymatic digestion (collagenase and hyaluronidase) and chelation (EDTA) of rat pancreatic tissue as modified from the original procedure of Amsterdam and Jamieson (211,220,223). The cells are then maintained in a serum-free (SF), hormonally-defined medium, and are viable (80-85%) for four to five days in culture. They exhibit many characteristics of differentiated acinar cells (223). Morphologically, they appear rounded, which is typical of dispersed acinar cells. Examination by electron microscopy reveals zymogen granules and abundant RER, characteristic of differentiated ultrastructure.

Functionally, these acinar cells retain hormonal responsiveness to insulin (223) and epidermal growth factor (EGF;225). They also retain ability to secrete enzymes in response to various secretagogues such as carbamyl choline (223), secretin (25) and caerulein (226), demonstrating that they do have functional hormone receptors. This, coupled with the fact that these acinar cells do synthesize enzymes (i.e. amylase) de novo for up to four days in culture (223,227), make this system particularly useful for studying the regulatory effects of these secretagogues on cellular enzyme content and synthesis. However, as mentioned earlier, although these cells do secrete enzymes in response to stimuli, they are not the best model for secretion studies.

This model system is also useful for examining the dietary adaptation of pancreatic digestive enzymes. More specifically, the increase in amylase and lipase that

occurs in vivo in response to their dietary substrate, persists in acinar cells cultured from rats fed high-carbohydrate (227) and high-fat (228) diets, respectively. This model system is, therefore, ideal for examining interactive effects of antecedent diet with hormones and metabolites. Because the cells are cultured in a defined medium without serum, the presence of unwanted, confounding variables is limited.

There are, however, inherent complications and limitations of in vitro acinar cell model systems, in general. For example, the enzyme content of acinar cells tends to decrease over time in culture (223,224). This may be due, in part, to increased basal secretion of dispersed acinar cells, as discussed previously. Other possible explanations, such as altered enzyme synthesis and stability in culture, have not been ruled out. It is therefore important to consider system limitations when designing experiments.

Acini Culture

In order to overcome the poor secretory response of isolated acinar cells, the original preparation (209) was modified by Williams et al. (229) to produce intact acini units. The calcium chelation, noncollagenase protease treatment and hyaluronidase treatment were eliminated. Thus, isolated acini were produced by incubation of pancreatic tissue with only purified collagenase, followed by mechanical shearing. Such acini consist of 10-30 acinar cells and retain normal luminal and lateral structure, including apical tight junctions and lateral gap junctions (229). Enzyme secretion by the pancreatic acini in response to secretagogues is greater in both magnitude and sensitivity than that of isolated acinar cells, and equal to that of perfused glands or dissected

lobules (209,230). This increased responsiveness probably results from preservation of cell-to-cell coupling or of apical structures thought to be required for exocytosis (209).

With these acute preparations of acini (229,231), it has been possible to localize secretagogue receptors and study their relation to protein secretion (232,233,234). However, more than one cell type may be present in acini preparations, such as a small number of centroacinar cells that are an integral component of the unit (209). Therefore, it is necessary to account for the contribution of these cells in the secretory process.

Long-term studies of cellular regulation have been limited in acini by their short lifetime (three to five hours). Attempts have been made, therefore, to culture acini for longer periods of time in an environment that would preserve differentiated function. Logsdon and Williams (235) have developed a suspension culture of acini that is viable for up to 24 h. These acini, cultured in the presence of 20% fetal calf serum, maintain normal morphology, responsiveness to CCK and carbachol, and the ability to synthesize proteins. Additionally, EGF, insulin and carbachol increase the responsiveness of these acini to stimuli; therefore, it is thought that acini must receive a certain level of stimulation in culture in order to maintain their differentiated state over time (235). This does not seem to be the case for isolated acinar cells (223,225). It is important to note that any factor added to culture media becomes a confounding experimental variable. Also of note, investigators have been unable to culture these acini units for longer that 24 h, making it virtually impossible to study longer-term regulation in this model system. Further, enzyme content decreases similarly in this 24 h in cultured acini and acinar cells

AR42J Cell Line

At the time of introduction of the AR42J cell line, the study of biogenesis, packaging and discharge of secretory proteins had been examined effectively in pancreatic acinar cells and acini. Although many of the steps leading to the discharge of digestive enzymes and proenzymes had been identified (110), the details of the release mechanism were still obscure. Also unknown, were the relationship between cytodifferentiation and cellular processing on hormonal discharge of secretory proteins. There was a need for a long-term culture in which the role of tissue organization and its influence upon cell function could be studied.

Jamieson and coworkers, interested in this problem, set out to characterize, morphologically, a rat pancreatic acinar cell tumor that had been first described by Reddy (236) and Rao (237). These tumor cells, induced in rats by dietary treatment with nafenopin (236), are highly cytodifferentiated (238). Although they contain abundant RER, elements of the Golgi complex and secretory granules, they also undergo mitosis in a manner similar to that seen in the developing pancreas. However, the secretory granules are smaller than normal zymogen granules and may not behave identically.

Cells in the parenchyma to the tumor grow as disarrayed cords and sheets, are randomly oriented with respect to each other, and do not form acini structures (238). However, those cells that abut on blood vessels undergo polarization and form oriented epithelial layers in which the nuclei of the cells face the vessel wall and the secretory granules orient in the opposite direction (238). This may be due to the presence of the basal lamina components (laminin, collagen type IV, fibronectin), which may serve as

organizing sites to align the acinar tumor cells. Only in this area of the tumor, is a basal lamina present that underlies the basal plasmalemma. The tumor cells in the parenchyma appear, via electron microscopy, to have extensive disruption of tight junctions, infrequent gap junctions, and no cellular polarization (238).

Although the acinar tumor cells contain the cellular machinery for the production and packaging of secretory proteins, it was unknown whether the lack of polar organization of the plasmalemma compromised their secretagogue-responsiveness. Therefore, further studies were performed by Iwanij and Jamieson (239) to investigate the tumor cell functional capacity. Dispersed tumor cells discharge secretory proteins in vitro when incubated with hormones that act via four different classes of receptors: carbamylcholine, caerulein, secretin-VIP, and bombesin (239). With all hormones tested, maximal discharge was about 50% of that seen in pancreatic lobules with the same dose optima, except for secretin, whose dose optimum was 10-fold higher. Biochemical analysis of the tumor cell secreted proteins compared to those of normal cells, revealed a reduction or absence of the following enzymes in tumor cell secretion: proelastase, basic trypsinogen, basic chymotrypsinogen, ribonuclease and lipase. In contrast, tumor cells secreted a higher amount of acidic chymotrypsinogen (239). Therefore, although the acinar tumor cells are highly differentiated cytologically and express functional receptors, they show quantitative and qualitative differences compared to normal acinar cells with regard to their production and secretion of secretory proteins (239).

Because secretory proteins are markers for acinar cell differentiation, Iwanij and Jamieson (240) sought to establish whether the secretory profile of the tumor cell was unique to the transformed cell or whether it resembled that of a stage of normal

pancreatic development. The secretory pattern of the tumor cells reflect that of day-19 normal embryonic acinar cell differentiation with respect to the phenotypic expression of secretory proteins. They propose, therefore, that the cells mirror a stage of normal pancreatic development (240) and they suggest this system provides an opportunity to examine the factors involved in the regulation of gene expression in normal development and in neoplastic transformation of the exocrine pancreas.

Subsequent to these studies, Logsdon and coworkers (241) took advantage of the immature stage of differentiation in the rat tumor cell line AR42J, derived from the exocrine pancreas, to demonstrate that glucocorticoids play a role in acinar cell differentiation. In AR42J cells, glucocorticoids directly increase both the number of secretory granules, and the levels of mRNA for amylase. These changes, then, lead to an increase in the synthesis, content and secretion of this enzyme. The glucocorticoid treatment of these cells also leads to a decrease in replication. It is concluded, then, that glucocorticoids have pleiotropic effects on these pancreatic acinar cells, and these effects lead to a more differentiated phenotype.

For studies such as the one described above, this AR42J cell line is particularly useful. It is also beneficial for the study of pancreatic development and its relation to cellular function. However, because these cells are not fully differentiated and lack several major digestive enzymes (lipase, procarboxypeptidase B, basic proteases), it would be impractical to examine dietary or hormonal regulation of the exocrine pancreas using this model system.

CHAPTER 3

EFFECTS OF DIET AND KETONES ON PANCREATIC LIPASE

Summary

The activity, synthesis rate and mRNA level of pancreatic lipase increase with dietary fat intake. Ketones, intermediates of lipid metabolism, have been proposed to mediate this change. Therefore, we investigated their direct effect on cultured pancreatic acinar cells, and examined, as well, their possible interactive effects with glucose and dietary fat. Beta-(OH)-butyrate (0.01 to 2 mmol/L) did not affect lipase activity in cells isolated from rats fed commercial non-purified diet (NP) and cultured in high (HG, 27.8 mmol/L) or low glucose (LG, 6.9 mmol/L) media. The effects of ketones were also examined in acinar cells isolated from rats fed purified high fat (HF, 67% J (kcal) fat) or low fat (LF, 11% J (kcal) fat) diet. Cellular lipase was significantly higher in cells from HF-fed rats at both 24 and 48 h (264% and 145% of LF values, respectively; $p<0.0001$). Beta-(OH)-butyrate significantly increased ($p<0.04$) lipase activity in LF cells at 48 h, in a dose-dependent manner with 0.05 to 2.0 mmol/L ß(OH)-butyrate, but did not affect lipase activity in HF cells. These studies suggest that ketones may be involved in the regulation of pancreatic lipase in rats fed a LF diet, but their role is complex and interactive with dietary carbohydrate and fat.

Introduction

Pancreatic lipase (E.C. 3.1.1.3) activity is specifically enhanced by triglycerides regardless of their origin: exogenous (diet, 151,174,176,242) or endogenous (adipose tissue - in starvation and streptozotocin-induced diabetes; 171,185). Lipase synthesis and mRNA levels also adapt to high dietary fat levels, increasing 3.1- and 3.9-fold, respectively (158). Bazin and coworkers (171) propose that ketones, intermediates in triglyceride metabolism, are responsible for the adaptive response because both lipase activity and blood ketone levels are positively correlated with HF diets, fasting and diabetes. Furthermore, continuous infusion for 5 days of ß(OH)-butyrate into rats results in a 40% increase in lipase activity (171). However, direct effects of ketones on pancreatic acinar cells have not been examined, and cannot be inferred from infusion studies which may result in interactive effects of ß(OH)-butyrate with other metabolites or hormones.

In order to examine direct effects of ketones on pancreatic lipase, it is necessary to employ an experimental system in which only ketones can be varied without homeostatic hormonal response, such as the primary culture of pancreatic acinar cells that has been developed (223,225). These cells are maintained in a defined serum-free (SF) medium and are viable for 4-5 days. They retain differentiation, as evidenced by the presence of zymogen granules (223); hormonal responsiveness to insulin (223) and epidermal growth factor (EGF; 225); ability to secrete amylase in response to secretagogues (223); and the ability to synthesize amylase de novo throughout 4 days in culture (227). The present study, therefore, examined the direct effects of ß(OH)-butyrate on pancreatic lipase in vitro in these cultured acinar cells. To determine if

ketones and glucose interact in regulating pancreatic lipase, the effects of ß(OH)-butyrate were also examined in acinar cells cultured in LG medium. Ketone bodies act as energy substrates, but are secondary to glucose. Possibly, cells in a HG media would not utilize ketones readily. Therefore, the effects of ketones were examined in cells cultured in both LG and HG media to evaluate ketone and glucose interaction. Finally, the interactive effects of antecedent diet and ß(OH)-butyrate were examined in acinar cells cultured from rats fed HF or LF diets, because cells from HF-fed rats may have maximally stimulated lipase and be unable to respond further to ketones.

Materials and Methods

Animals and Diets

Male, weanling Sprague-Dawley rats (40-100 g; Harlan, Indianapolis, IN, USA) were housed individually, maintained on a 12 h light-dark cycle and fed ad libitum for 7 days either a NP diet (Continental Grain Co., Chicago, IL, USA) that contained 24% crude protein, 4% crude fat and 4.5% crude fiber, or a purified diet: HF or LF (Appendix A; 4) that contained 20% casein, 11.3 or 65% starch, and 28.9 or 5% corn oil. The purified diets were isocaloric and isonitrogenous, but varied in cellulose content, a non-energetic dietary component that has no effect on pancreatic enzyme content (243).

Acinar Cell Culture

Two rats per treatment group were decapitated and the pancreata were removed aseptically and pooled. Pancreatic acinar cells were isolated and cultured according to the method of Brannon et al. (223,225). Pancreata were minced into 1-2 mm pieces and

incubated with 20 ml Hank's Balanced Salt Solution (HBSS) with 1 mmol/L EDTA for 15 min at 37° C while shaking 120 cycles per min (cpm). (All media were purchased from Gibco, Grand Island, NY, USA.) The chelated mixture was centrifuged (IEC Centra-7, International Equipment Co., Needham Heights, MA, USA) for 2 min at 500 xg. The resulting supernatant was discarded; the pellet was rinsed with 20 ml Ham's F-12 medium and centrifuged for 2 min at 500 xg; the supernatant was again discarded. The tissue pellet was digested with 20 ml of 9.5 μmol/L collagenase type II and 16.3 μmol/L hyaluronidase (Worthington Biochemical Corp., Freehold, NY, USA) with 1% heat-inactivated calf serum (HI-CS) in Ham's F-12 medium at 37° C for 20 min at 120 cpm in a shaking water bath. After centrifugation at 500 xg for 2 min, the supernatant was discarded; the pellet was rinsed with 20 ml of HBSS. The chelation and digestion were repeated in sequence as described. After the second digestion and centrifugation, the pellet was rinsed with 20 ml Ham's F-12 medium with 5% HI-CS, centrifuged for 2 min at 500 xg and resuspended in 10 ml Ham's F-12/5% HI-CS. The suspension was filtered through 500 and 25 μm Nytex filters, layered onto 40 ml 5% HI-CS/6% Ficoll and centrifuged for 10 min at 200 xg. The pellet was rinsed with 20 ml Ham's F-12/5% HI-CS then with 20 ml Waymouth's medium 752/1 (with 25 mmol/L HEPES buffer, 2.4 mmol/L glutamine, 0.01% soybean trypsin inhibitor (STI) and 1X antibiotic-antimycotic) and the pellet was resuspended in 10 ml Waymouth's medium. Cell number and viability were determined with trypan blue dye (0.1% final concentration). Cells were plated in 24-well petri clusters (Costar, Van Nuys, CA,USA) at a density of $1X10^6$ cells per well in 2.0 ml Waymouth's serum-free (SF) medium with 0 to 2.0 mmol/L ß(OH)-butyrate (Sigma Chemicals, St. Louis, MO, USA) and incubated (Model #3187, Forma Scientific,

Marietta, OH, USA) at 37° C with 5% humidified CO_2 for up to 48 h. SF medium contained 0.17 mmol/L bovine serum albumin (BSA; Sigma Chemicals, St. Loius, MO, USA), $1X10^{-8}$ mol/L dexamethasone (DEX; Sigma Chemical, St. Louis, MO, USA) and 42 pmol/L epidermal growth factor (EGF; Sigma Chemicals, St. Louis, MO, USA), and 27.8 mmol/L glucose (HG) or 6.9 mmol/L glucose (LG). Cells were harvested in triplicate and an aliquot taken from each well for the determination of viability. Cells were centrifuged (Model J6-B, Beckman, Palo Alto, CA, USA) at 1800 xg at 4° C for 2 min; the media supernatant was discarded; and cell pellets were washed with 1 ml ice-cold phosphate buffered saline (PBS) and frozen at -80° C for subsequent analysis.

Cell Analyses

Frozen cell pellets were homogenized in 1 ml phosphate buffered saline (PBS) by sonication (Ultrasonic Dismembrator, Artek Systems Corp., Farmingdale, NY, USA) on ice for 7-10 s. Aliquots of homogenates were assayed for cellular protein by the Lowry method (244) with BSA as the standard and for lipase activity by an autotitrimetric (Autotitrator, Radiometer, Westlake, OH, USA) method with olive oil as the substrate in the presence of excess colipase (245). Colipase was partially purified from hog pancreas by the method of Ouagued et al. (246). Lipase activity was expressed as units (μmoles FFA released/min) per mg protein. In some experiments, lipase activity was also expressed as a percentage of the freshly-isolated control (LF cells) value for ease of comparison.

Statistical Analyses

Data, expressed as mean ± SEM, were analyzed by one- or two-way analysis of variance (ANOVA) and least significant differences (LSD;247). Significant differences were denoted by $p<0.05$.

Results

In acinar cells from rats fed NP diet, ß(OH)-butyrate did not significantly affect lipase activity when cells were cultured 24 or 48 h (Table 1). Lipase activity was, however, significantly lower (70%) in acinar cells cultured 48 h than those cultured 24 h. Similarly, ß(OH)-butyrate did not significantly affect lipase activity of acinar cells cultured 24 or 48 h in LG medium (Figure 3). Lipase activity was, again, significantly lower in cells cultured in LG or HG medium at 48 h than at 24 h. Further, lipase activity tended ($p<0.30$) at 48 h to be lower (38%) in cells cultured in LG medium than in HG medium.

Beta-(OH)-butyrate did not significantly affect the protein content of cells from NP-fed rats cultured in media containing LG at 24 h (Table 2). However, 0.25, 0.50 and 1.0 mmol/L B(OH)-butyrate significantly increased ($p<0.00001$) the protein content of cells in LG media for 48 h. Media glucose did not affect protein content or viability of cells. Beta-(OH)-butyrate also had no affect on the viability of cells cultured in LG media for 24 or 48 h; however, the cellular viability for all cells was significantly lower (11%) at 48 h compared to that at 24 h ($p<0.00001$; data not shown).

Antecedent diet and ß(OH)-butyrate significantly affected lipase activity (Figure 4) in acinar cells isolated and cultured from rats fed for 1 wk HF or LF diet. Overall, HF

Table 1: EFFECT OF β(OH)-BUTYRATE ON LIPASE ACTIVITY IN CULTURED ACINAR CELLS[1]

β(OH)-BUTYRATE (mmol/L)	PANCREATIC LIPASE (U/MG PROTEIN)[2] 24 h	48 h
0.00	38.01 ± 14.31[a]	10.51 ± 6.32[b]
0.01	34.37 ± 19.20[a]	9.85 ± 6.09[b]
0.05	27.97 ± 7.91[a]	9.50 ± 5.48[b]
0.50	22.17 ± 5.84[a]	6.69 ± 3.67[b]
2.00	28.16 ± 2.96[a]	7.81 ± 9.07[b]

[1]Cells were isolated from rats fed 1 wk commercial nonpurified diet and cultured 24 or 48 h in serum-free medium with various concentrations of β(OH)-butyrate.

[2]Values represent mean ± SEM of triplicate samples from three experiments except for 2.00 mmol/L β(OH)-butyrate, which represents triplicate samples from one experiment.

[a,b]Values in a row or column not sharing a superscript differed significantly by 2-way ANOVA and LSD ($p<0.05$). There was no significant independent effect of β(OH)-butyrate and no interactive effect of time and β(OH)-butyrate. There was a significant ($p<0.0001$) independent effect of culture time (24 h > 48 h).

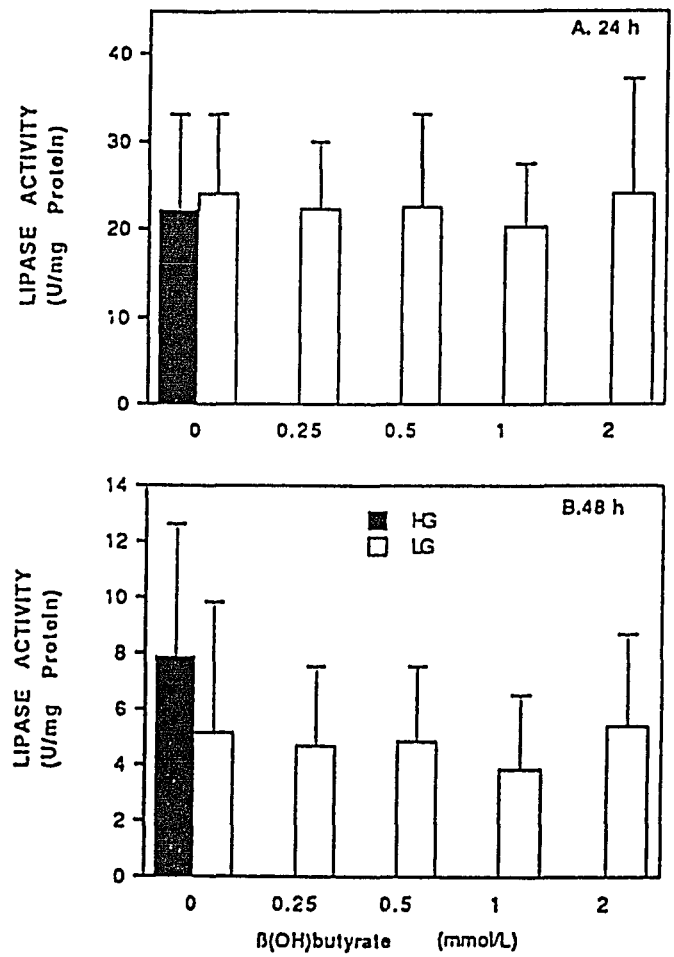

Figure 3: EFFECT OF β(OH)-BUTYRATE ON PANCREATIC LIPASE ACTIVITY IN ACINAR CELLS CULTURED IN HIGH GLUCOSE (HG) OR LOW GLUCOSE (LG) MEDIA FOR 24 (A) AND 48 (B) H.

Cells were isolated from rats fed 1 wk commercial non-purified diet and cultured in HG (27.8 mmol/L) or LG (6.9 mmol/L) serum-free medium for 24 (A) or 48 (B) h with various concentrations of β(OH)-butyrate. Each value represents mean ± SEM of triplicate samples from three experiments. Data were analyzed by 2-way ANOVA and LSD ($p<0.05$).

There was a significant ($p<0.00001$) independent effect of culture time on lipase activity (24 h > 48 h). There was no significant independent effect of β(OH)-butyrate and no significant interactive effect of time and β(OH)-butyrate.

Table 2: EFFECT OF β(OH)-BUTYRATE ON PROTEIN CONTENT OF ACINAR CELLS CULTURED IN HIGH GLUCOSE (HG) OR LOW GLUCOSE (LG) MEDIA FOR 24 AND 48 H[1]

		CELLULAR PROTEIN (MG/ML)[2]	
β(OH)-BUTYRATE (mmol/L)		24 h	48 h
HG:	0.00	0.19 ± 0.05[ab]	0.11 ± 0.02[d]
LG:	0.00	0.19 ± 0.02[ab]	0.10 ± 0.02[d]
	0.25	0.19 ± 0.02[ab]	0.16 ± 0.04[bc]
	0.50	0.18 ± 0.02[ab]	0.16 ± 0.03[bc]
	1.00	0.21 ± 0.02[a]	0.15 ± 0.02[bc]
	2.00	0.18 ± 0.02[ab]	0.13 ± 0.02[cd]

[1]Cells were isolated from rats fed 1 wk commercial nonpurified diet and cultured in HG (27.8 mmol/L) or LG (6.9 mmol/L) serum-free medium for 24 or 48 h with various concentrations of β(OH)-butyrate.

[2]Values represent mean ± SEM of triplicate samples from three experiments.

[abcd]Values in a row or column not sharing a superscript differed significantly be 2-way ANOVA and LSD ($p<0.05$). There was no effect of media glucose on protein content of cells cultured for 24 or 48 h. Although β(OH)-butyrate had no effect on cells cultured for 24 h, 0.25, 0.5 and 1.0 mmol/L β(OH)-butyrate significantly increased ($p<0.0001$) the protein content of cells in LG media after 48 h.

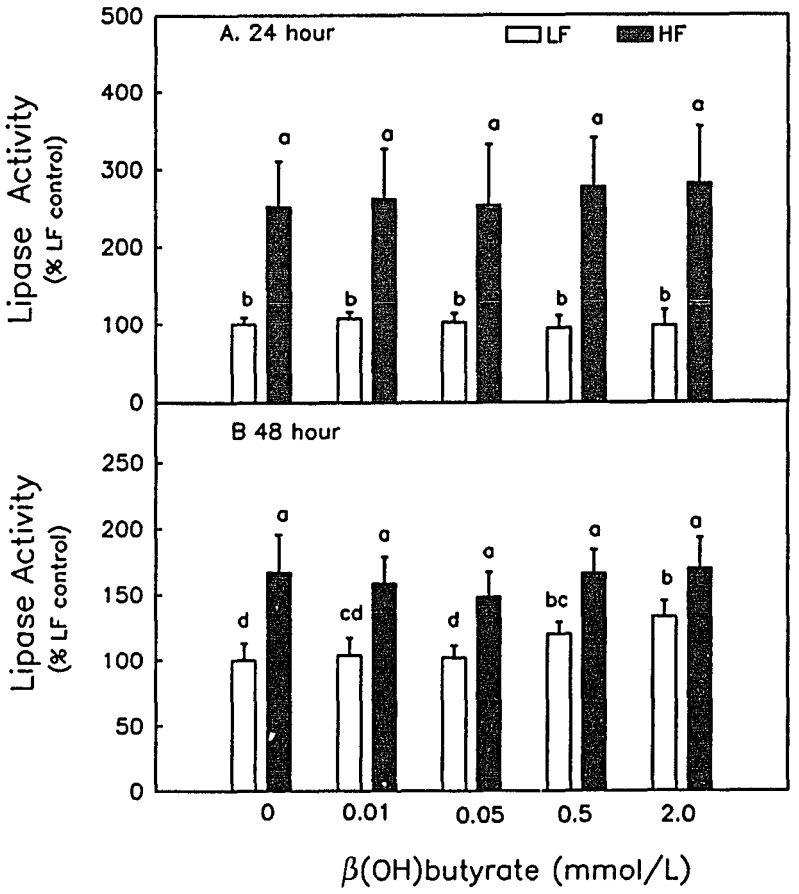

Figure 4: EFFECT OF β(OH)-BUTYRATE AND ANTECEDENT DIET ON PANCREATIC LIPASE ACTIVITY IN ACINAR CELLS CULTURED 24 AND 48 H.

Cells were isolated from rats fed 1 wk HF (67% J (kcal) fat) or LF (11% J (kcal) fat) diet and cultured in HG medium for 24 h (A) or 48 h (B) with various concentrations of β(OH)-butyrate. Each bar represents mean ± SEM of at least triplicate samples from three experiments.

^{abcd}Values (expressed as a % of LF control values: 24 h = 13.21 ± 2.28 or 48 h = 5.27 ± 0.84) at 24 or 48 h not sharing a superscript differed significantly by 2-way ANOVA and LSD ($p<0.05$). At 24 h, there was a significant independent effect of diet (HF>LF), but no independent effect of β(OH)-butyrate and no interactive effect of β(OH)-butyrate and diet. At 48 h, there were significant independent effects of diet ($p<0.0001$; HF>LF) and β(OH)-butyrate ($p<0.035$; 2.0>0.5>0.05=0.01 mmol/L), and no significant interactive effect of diet and β(OH)-butyrate.

cells had significantly ($p<0.0001$) greater lipase activity at both 24 (2.6-fold) and 48 h (1.5-fold) than LF cells. Beta-(OH)-butyrate significantly affected ($p<0.04$) lipase activity in only LF cells at 48 h, causing a dose-dependent increase in lipase activity with 0.5 to 2.0 mmol/L ß(OH)-butyrate. Neither 0.01 nor 0.05 mmol/L ß(OH)-butyrate affected lipase activity. In contrast, ß(OH)-butyrate did not affect lipase activity in HF cells after 24 or 48 h in culture.

Beta-(OH)-butyrate did not significantly affect the protein content of HF or LF cells at either 24 or 48 h (Table 3). However, the protein content of all HF cells was significantly decreased at 48 hr vs. 24 h ($p<0.04$). LF cells, in general, had significantly higher protein content compared to HF cells at 24 h ($p<0.03$) and 48 h ($p<0.001$). The viability of cells from both HF- and LF-fed rats was not significantly changed by β(OH)-butyrate or time in culture (data not shown).

Table 3: EFFECT OF β(OH)-BUTYRATE AND DIET ON PROTEIN CONTENT OF ACINAR CELLS CULTURED 24 AND 48 H[1]

	β(OH)-BUTYRATE (mmol/L)	CELLULAR PROTEIN (MG/ML)[2] 24 h	48 h
LF:	0.00	0.42 ± 0.07[a]	0.38 ± 0.06[a]
	0.01	0.42 ± 0.07[a]	0.38 ± 0.06[a]
	0.05	0.43 ± 0.05[a]	0.39 ± 0.07[a]
	0.50	0.41 ± 0.06[a]	0.40 ± 0.08[a]
	2.00	0.44 ± 0.07[a]	0.38 ± 0.08[a]
HF:	0.00	0.37 ± 0.09[b]	0.30 ± 0.08[c]
	0.01	0.37 ± 0.09[b]	0.30 ± 0.08[c]
	0.05	0.38 ± 0.09[b]	0.30 ± 0.08[c]
	0.50	0.35 ± 0.09[b]	0.30 ± 0.08[c]
	2.00	0.36 ± 0.08[b]	0.30 ± 0.08[c]

1Cells were isolated from rats fed 1 wk HF (67% J (kcal) fat) or LF (11% J (kcal) fat) diet and cultured in HG medium for 24 or 48 h with various concentrations of β(OH)-butyrate.

2Values represent mean ± SEM of triplicate samples from three experiments.

[abc]Values in a row or column not sharing a superscript differed significantly by 2-way ANOVA and LSD ($p<0.05$). β(OH)-butyrate did not significantly affect the protein content of HF or LF cells at either 24 or 48 h. The protein content of all HF cells, however, was significantly decreased at 48 h ($p<0.04$). LF cells, in general, had significantly higher protein content compared to HF cells at 24 h ($p<0.03$) and 48 h ($p<0.001$).

Discussion

Beta-(OH)-butyrate affected lipase activity only in cells isolated from rats fed LF diet, where circulating levels of ketones are typically low. In contrast, rats fed HF diet have high levels of blood ketones (171) and an enhanced level of lipase synthesis (154). Therefore, additional ß(OH)-butyrate may not alter lipase regulation in cells isolated from HF-fed rats. Higher levels of lipase synthesis in the HF-fed rats would also explain their higher cellular lipase activity compared to cells of LF-fed rats at 24 and 48 h in culture, despite the fact that cells from LF-fed rats had, in general, higher protein content. A similar persistence of dietary carbohydrate regulation on amylase activity and synthesis has also been demonstrated in cultured pancreatic acinar cells (227).

Why ß(OH)-butyrate did not affect cellular lipase activity in rats fed NP diet with 10% J (kcal) fat remains unclear. Although it is difficult to compare the results from experiments using rats fed purified diets to those using rats fed NP diet, it is interesting to note that the lipase activity in cells from NP-fed rats was intermediate when compared to lipase activities in cells isolated from rats fed purified HF or LF diet. Antecedent diet does affect the hormonal responsiveness of these cultured cells to EGF (225) and may affect metabolic regulation as well. Cell maintenance may also be affected by antecedent diet, as evidenced by a decreased viability (11%) of cells from NP-fed rats after 48 h while that of cells from LF- or HF-fed rats was virtually unchanged. There was also greater variance in lipase activity in cells from rats fed NP diets (40% of mean) than purified diets (21%), although there is no apparent reason for higher variance with NP diets. In light of other experiments (171), one would expect ketones to affect these cells similarly to cells from LF-fed rats (11% J (kcal) fat). There were composition differences

among these purified and non-purified diets that may have affected the response to ketones, such as differences in types of fiber. Although the content was similar (NP=4.5%; LF purified=5.0%), the purified diet contains cellulose which has been shown not to affect the pancreatic content of lipase, while the NP diet contains a variable mixture of wheat and corn bran, whose affects on pancreatic lipase are not clearly defined. Isaksson et al. (248) found no difference in lipase activity (U/mg tissue) after wheat bran supplementation. However, Stock-Damge et al. (248) found significant decreases in lipase secretion and Sheard and Schneeman (250) reported elevated pancreatic lipase content with wheat bran supplemented diets. Wheat bran feeding has also lead to changes in cholesterol and bile salt content of bile secretions in rats (251), which may alter lipid metabolism. The protein content of the diets was also similar, but, again, the sources differed between NP and purified diets. The purified diets contain only casein, while the NP diet contain a combination of animal and vegetable protein sources. The quality of dietary protein does affect the adaptive response of pancreatic proteases (167,252), and may also affect that of pancreatic lipase.

An interactive effect of ketones and media glucose on lipase activity was not found in these studies; however, β(OH)-butyrate increased the protein content of cells in LG media after 48 h in culture. Possibly, ketones have interactive effects with other metabolites or hormones in the regulation of lipase, and perhaps other enzymes. In conditions where lipase is enhanced (diabetes, starvation, high-fat feeding), ketone bodies (171,185) and free fatty acids (FFAs; 253) are both elevated. Therefore, FFAs may play a role, either alone or in conjunction with ketones, in the regulation of lipase. However, infusion of ketones into humans for 5 min causes a decrease (30%) in serum

fatty acids (175). Therefore, the direct effects of FFAs on lipase activity needs to be examined in cultured acinar cells, as well as possible interactive effects of FFAs with ketones.

Other possible regulators of pancreatic lipase include secretin, which when infused into rats causes a 4-fold increase in lipase synthesis (194). Secretin has been shown to be elevated after intraduodenal infusion of 20% fat (254). However, secretin infusion does not affect lipase mRNA, whereas dietary fat increases lipase content, synthesis and mRNA (195). Further, we found that secretin, in vitro, does not affect lipase content of cultured cells, even though these cells retain secretory responsiveness to secretin (25).

These findings demonstrate that the effects of dietary fat on lipase activity persisted in cultured acinar cells, even though the total cellular activity decreased with culture time. This change in cellular lipase activity may be due to an increased basal secretion rate secondary to disruption of acini gap junctions in the isolated acinar cells (216) and decreased synthetic rate in culture. These results also support a role of ketones in the regulation of pancreatic acinar cells, although the exact role of ketones appears to be more complex than originally hypothesized (171). Further, the magnitude of the regulation by ketones appears to be relatively small. The role of ketones in the regulation of lipase synthesis and mRNA levels needs to be examined. A modest increase in lipase synthesis could, over a greater period of time, lead to a greater accumulation of lipase content. However, these results do not support ketones as the sole mediator of the dietary regulation of pancreatic lipase.

CHAPTER 4

EFFECTS OF CAERULEIN AND SECRETIN ON AMYLASE AND LIPASE

Summary

We examined the effects of secretin (0 to 200 nM) and caerulein (0 to 100 nM) on rat pancreatic acinar cells cultured 0 to 48 h in serum-free medium. The effects of 100 nM secretin with 1 nM caerulein were also studied because secretin may potentiate the effects of caerulein. Cellular and media (secreted) lipase and amylase were analyzed as were cellular DNA and protein content. Cellular lipase and amylase activities significantly decreased ($p<0.0001$) over time in all treatment groups, while media amylase and lipase significantly increased ($p<0.0001$). Neither secretin nor caerulein affected cellular lipase or media amylase. However, secretin significantly increased ($p<0.04$) and caerulein tended to increase ($p<0.08$) media lipase in a dose-dependent manner. At 12 h, 10 nM secretin maximally increased media lipase (58%), suggesting that cultured acinar cells remain responsive to secretin in vitro. Caerulein, at all concentrations, significantly decreased ($p<0.001$) cellular amylase, but exhibited a dose-dependent effect only at 24 h when 100 nM caerulein maximally decreased cellular amylase (34%). Secretin (100 nM) did not alter these effects of caerulein. These results support the proposed role of caerulein in the regulation of amylase, but not a direct role of secretin in the regulation of lipase.

Introduction

The activity, synthesis rate and mRNA level of lipase adapt to changes in its dietary substrate, triglyceride, increasing 2.4-, 3.1- and 3.9-fold, respectively, in response to a high fat diet (158). Similarly, amylase activity (161), synthesis rate (170) and mRNA levels (155) increase in response to a high carbohydrate diet. The cellular mediators of these pancreatic adaptations remain unknown, but various hormones and nutrients have been proposed as mediators of this dietary adaptation. One hormone, secretin, is proposed to regulate lipase, specifically, because its intravenous infusion for 24 h into rats stimulates lipase synthesis (4-fold; 194). Another gastrointestinal hormone, cholecystokinin, is proposed to regulate amylase and the proteases. Caerulein, a cholecystokinin analog, decreases amylase synthesis (205) without parallel changes in its mRNA levels (206) when infused continuously.

Although these results suggest secretin and caerulein may regulate pancreatic digestive enzymes, interactive effects of these hormones with other hormones or nutrients cannot be eliminated with in vivo infusion studies. To examine the direct effects of each hormone and their possible interactive effects on acinar cells, this present study used an in vitro system - primary cultures of pancreatic acinar cells (223). These cells are maintained in a defined serum-free (SF) medium and are viable for 4-5 days. They retain differentiated characteristics, secretagogue-stimulated secretion and hormonal responsiveness (225) and synthesize amylase de novo (227) for 4 days in culture.

Materials and Methods

Animals and Diet

Male, weanling Sprague-Dawley rats weighing 40-100 g (Harlan, Indianapolis, IN, USA) were housed individually, maintained on a 12 h light-dark cycle and fed ad libitum a commercial non-purified diet that contained 24% crude protein, 4% crude fat and 4.5% crude fiber (Continental Grain Co., Chicago, IL, USA).

Acinar Cell Culture

Two rats per cell preparation were decapitated and the pancreata were removed aseptically and pooled. Pancreatic acinar cells were isolated and cultured according to the method of Brannon et al. (223,225). Briefly, pancreata were minced into 1-2 mm pieces and incubated with 20 ml HBSS containing 1 mM EDTA for 15 min at 37° C while shaking 120 cpm. (All culture media were purchased from Gibco, Grand Island, NY, USA.) The chelated mixture was centrifuged for 2 min at 500 xg. The resulting supernatant was discarded; the pellet was rinsed with 20 ml Ham's F-12 medium and centrifuged for 2 min at 500 xg; the supernatant was again discarded. The tissue pellet was digested with 20 ml of 1 mg/ml collagenase type II and 1 mg/ml hyaluronidase (Worthington Biochemical Corp., Freehold, NY, USA) with 1% HI-CS in Ham's F-12 medium at 37° C for 20 min at 120 cpm in a shaking water bath. After centrifugation at 500 xg for 2 min, the supernatant was discarded; the pellet was rinsed with 20 ml of HBSS. The chelation and digestion were repeated in sequence as described. After the second digestion and centrifugation, the pellet was rinsed with Ham's F-12 medium with 5% HI-CS, centrifuged for 2 min at 500 xg and resuspended in 10 ml Ham's F-12/5% HI-

CS. The suspension was filtered through 500 and 25 μm Nytex filters, layered onto 40 ml 5% HI-CS/6% Ficoll and centrifuged for 10 min at 200 xg. The acinar pellet was rinsed with 20 ml Ham's F-12/5% HI-CS then with 20 ml Waymouth's medium 752/1 with 25 mM HEPES buffer, 2.4 mM glutamine, 0.01% STI and 1x antibiotic-antimycotic. The pellet was then resuspended in 10 ml Waymouth's medium. Cell number and viability were determined by trypan blue dye exclusion using a cell suspension containing 0.10% trypan blue dye. Then cells were plated in 24-well petri clusters (Costar, Van Nuys, CA, USA) at a density of $1X10^6$ cells per well in 2.0 ml Waymouth's SF medium with 0 to 200 nM secretin (Sigma Chemicals, St. Louis, MO, USA), 0 to 100 nM caerulein (Sigma Chemicals, St. Louis, MO, USA) or 1.0 nM caerulein plus 100 nM secretin. Cells were incubated at 37° C with 5% humidified CO_2 for up to 48 h. SF medium contained 10 mg/ml BSA, $1X10^{-8}$ M DEX and 42 pM EGF (all purchased from Sigma Chemicals, St. Louis, MO, USA). In initial studies, secretin did not have any affects after 24 h; in vivo secretin affected lipase within 24 h. Therefore, we only cultured cells with secretin for up to 24 h, but cultured cells with caerulein for 48 h in the absence of preliminary studies excluding later effects.

Cells were harvested (three wells per treatment) after 6, 12, 24 or 48 h incubation, and an aliquot was taken from each well for the determination of viability. Cells and media were then separated by centrifugation at 1800 xg for 2 min at 4° C. The media (supernatant) were removed and frozen at -80° C for subsequent analysis, as were cell pellets after one rinse with 1 ml ice-cold PBS.

Cell Analyses

Frozen cell pellets were homogenized in 1 ml PBS by sonication on ice for 7-10 s. Aliquots of homogenates were assayed for cellular DNA (256), cellular protein with BSA as the standard (244), lipase activity by an autotitrimetric method with olive oil as the substrate in the presence of excess colipase (245) and amylase activity by the Phadebus blue starch method (255). Frozen media samples were also assayed for lipase and amylase activity. All enzyme activities were expressed as units per mg cellular protein and as a percentage of the freshly-isolated control (no treatment) value.

Statistical Analyses

Data, expressed as mean ± SEM, were analyzed by one- or two-way analysis of variance and least significant differences (247). Significant differences were denoted by $p<0.05$.

Results

Secretin did not affect cellular lipase activity (Figure 5), but significantly increased media lipase ($p<0.04$) in a biphasic manner. At 12 h, 10 nM secretin maximally increased media lipase (58%). Secretin did not, however, affect cellular or media amylase (Figure 6).

Caerulein significantly decreased cellular amylase activity ($p<0.001$) at all concentrations after incubation for 12 and 24 h (Figure 7). However, a dose-dependent effect occurred at only 24 h when 100 nM maximally decreased cellular amylase activity

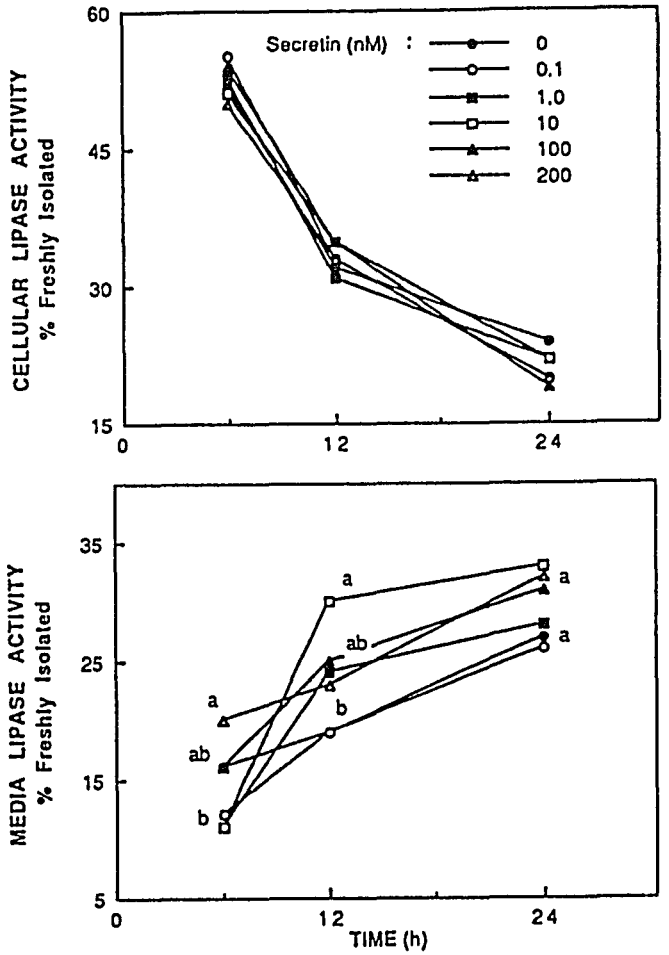

Figure 5: EFFECT OF SECRETIN ON CELLULAR AND MEDIA LIPASE ACTIVITY IN CULTURED ACINAR CELLS.

Cells were isolated from rats fed commercial unpurified diet and cultured in serum-free medium for 6, 12 or 24 h with various concentrations of secretin. Each point represents the mean of triplicate or quadruplicate samples from three experiments. The average freshly-isolated cellular lipase activity was 20.93 ± 4.94 (mean ± SEM).

There was a significant decrease over time ($p<0.001$) in cellular lipase activity (6h>12h>24h) and a significant increase ($p<0.0001$) in media lipase activity (6h<12h<24h), but no interactive effect of secretin with culture time. Secretin increased media lipase ($p<0.04$), but did not affect cellular lipase activity.

[ab]Values at each time point not sharing a superscript differed significantly by 1-way ANOVA ($p<0.04$).

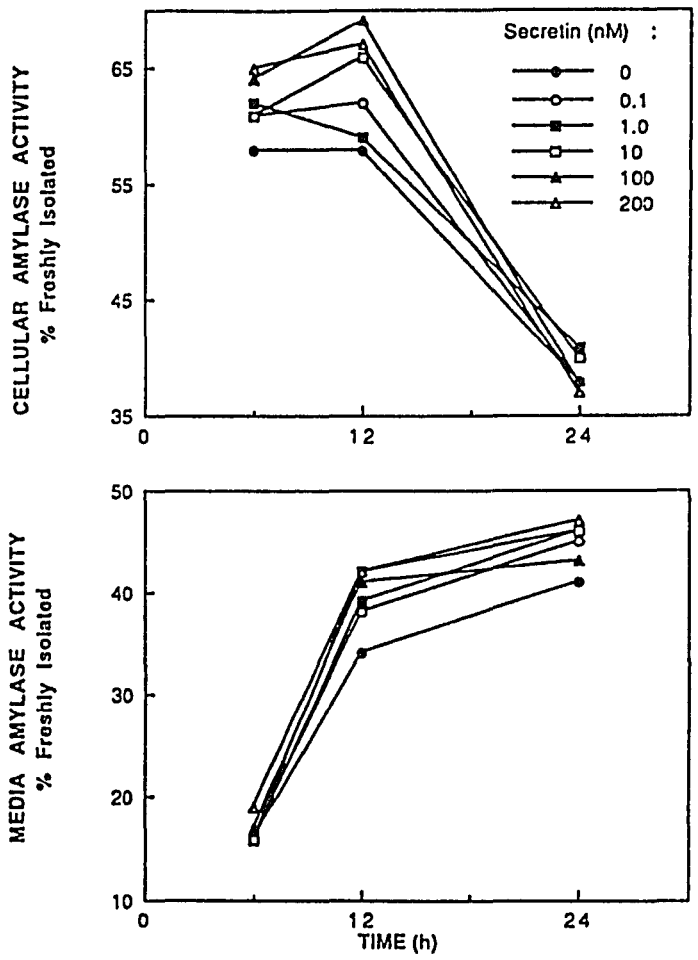

Figure 6: EFFECT OF SECRETIN ON CELLULAR AND MEDIA AMYLASE ACTIVITY IN CULTURED ACINAR CELLS.

Cells were isolated from rats fed commercial unpurified diet and cultured in serum-free medium for 6, 12 or 24 h with various concentrations of secretin. Each point represents the mean of triplicate or quadruplicate samples from three experiments. The average freshly-isolated cellular amylase activity was 84.27 ± 10.40 (mean ± SEM).

There was a significant decrease ($p<0.0001$) in cellular amylase activity after 12 h in culture (6h=12h>24h), and a significant increase ($p<0.0001$) in media amylase activity over time (6h<12h<24h), but no interactive effect of secretin with culture time. At any given time, there was no significant effect of secretin.

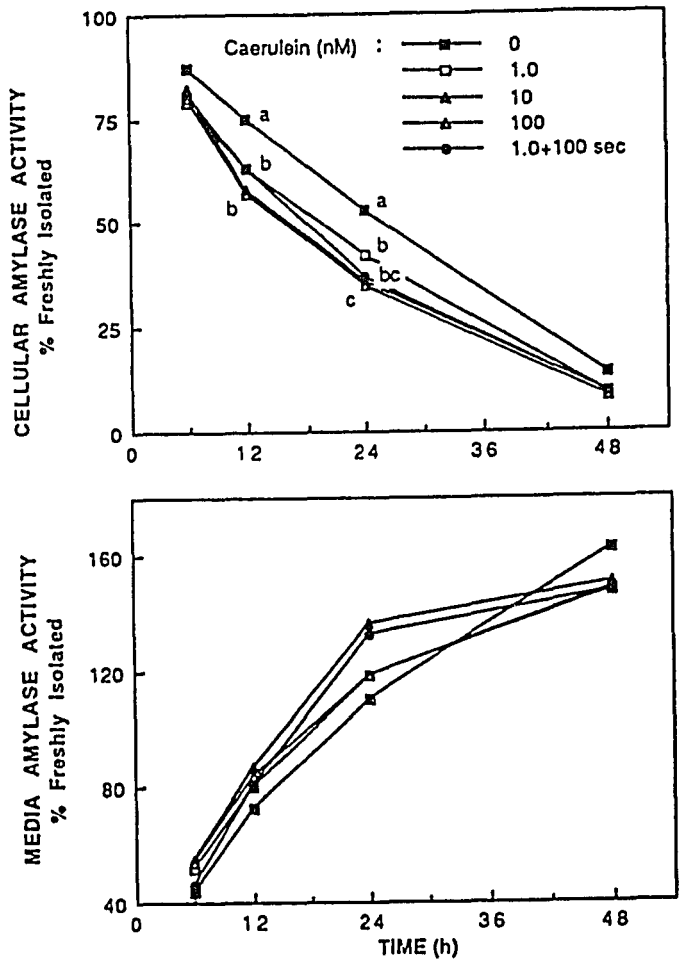

Figure 7: EFFECTS OF CAERULEIN ON CELLULAR AND MEDIA AMYLASE ACTIVITY IN CULTURED ACINAR CELLS.

Cells were isolated from rats fed commercial unpurified diet and cultured in serum-free medium for 6, 12, 24 or 48 h with various concentrations of caerulein or caerulein and secretin. Each time point represents the mean of triplicate samples from three or four experiments. The average freshly-isolated cellular amylase activity was 107.50 ± 7.29 (mean ± SEM).

There was a significant decrease in cellular amylase activity (6h>12h>24h>48h; $p<0.001$) and a significant increase in media amylase activity (6h<12h<24h<48h; $p<0.001$) over time, but no interactive effect of caerulein with time. Caerulein significantly decreased cellular amylase activity ($p<0.001$), but did not affect media amylase.

^{abc}Values at each time point not sharing a superscript differed significantly by 1-way ANOVA ($p<0.05$).

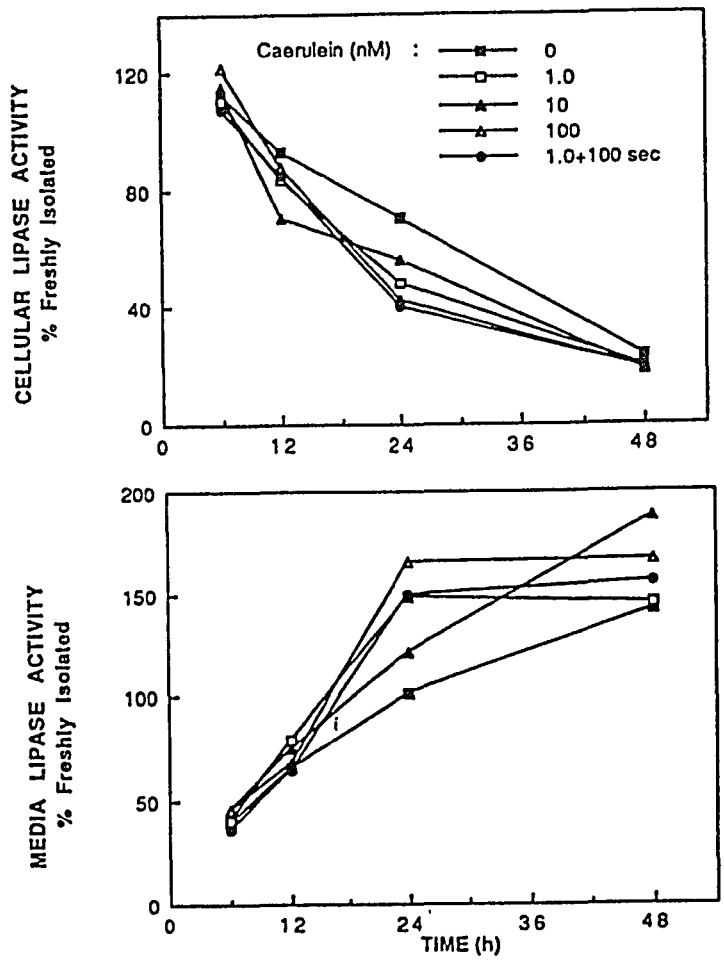

Figure 8: EFFECTS OF CAERULEIN ON CELLULAR AND MEDIA LIPASE ACTIVITY IN CULTURED ACINAR CELLS.

Cells were isolated from rats fed commercial unpurified diet and cultured in serum-free medium for 6, 12, 24 or 48 h with various concentrations of caerulein or caerulein and secretin. Each point represents the mean of triplicate samples from three or four experiments. The average freshly-isolated cellular lipase activity was 19.10 ± 0.89 (mean ± SEM).

There was a significant decrease in cellular lipase activity (6h>12h>24h>48h; $p<0.001$) and a significant increase in media lipase activity over time (6h<12h<24h<48h; $p<0.001$), but no interactive effect of caerulein with culture time. There was no effect of caerulein on lipase activity.

by 34%. Caerulein did not affect media amylase (Figure 7) or cellular lipase (Figure 8), but tended to increase media lipase activity ($p<0.08$). Secretin (100 nM) did not potentiate the effects of caerulein (1 nM) on cellular and media lipase (Figure 8) or amylase (Figure 7) activities.

In all cultured cells, cellular lipase and amylase activity significantly decreased ($p<0.0001$) with time in culture, while media lipase and amylase activity significantly increased ($p<0.0001$) (Figures 5-8). Overall, there were no effects of secretin or caerulein on total (cellular plus media) lipase and amylase activities in cultured acinar cells (data not shown), but total amylase activity increased 25% at 12 and 24 h ($p<0.001$). Cellular viability decreased slightly (4 to 9%) after 12 h in culture in all cells (Tables 4 and 5), but neither secretin nor caerulein affected cellular viability at any concentration.

Table 4: EFFECT OF SECRETIN ON VIABILITY OF CULTURED PANCREATIC ACINAR CELLS[1]

	AVERAGE VIABILITY (%)[2]		
SECRETIN (nM)	6 h[a]	12 h[ab]	24 h[b]
0	92.7 ± 1.0	86.3 ± 2.9	90.7 ± 0.7
0.1	91.5 ± 0.7	86.4 ± 3.1	85.5 ± 2.4
1.0	91.2 ± 2.6	89.6 ± 1.7	86.5 ± 1.4
10	87.5 ± 1.0	87.5 ± 0.5	86.2 ± 3.6
100	90.6 ± 0.8	92.1 ± 0.7	88.1 ± 2.4
200	89.0 ± 2.7	88.7 ± 0.8	85.7 ± 1.3

[1]Cells were isolated from rats fed commercial unpurified diet and cultured 6, 12 or 24 h in serum-free medium with varied concentrations of secretin. Viability was determined by trypan blue exclusion. At isolation, the viability was 88.4 ± 1.0.

[2]Values represent mean ± SEM of triplicate or quadruplicate samples from three experiments.

[ab]By 2-way ANOVA, there was a main effect of time on viability ($p<0.02$; 6 h - 90.4 ± 1.1[a]; 12 h - 88.4 ± 1.3[ab]; and 24 h - 87.1 ± 1.2[b], but no difference among individual time-treatment groups.

Table 5: EFFECT OF CAERULEIN ON VIABILITY OF CULTURED PANCREATIC ACINAR CELLS[1]

	AVERAGE VIABILITY (%)[2]			
CAERULEIN (nM)	6 h[a]	12 h[a]	24 h[b]	48 h[b]
0	87.0 ± 3.3	85.2 ± 2.4	77.3 ± 3.3	76.0 ± 4.0
1.0	85.8 ± 2.5	83.8 ± 4.3	75.0 ± 4.7	79.3 ± 1.8
10	86.8 ± 4.3	85.8 ± 2.8	75.0 ± 5.7	76.3 ± 3.5
100	85.5 ± 3.6	84.2 ± 2.5	79.3 ± 0.3	78.0 ± 0.6
1.0 + 100 nM Secretin	86.0 ± 3.8	84.0 ± 2.4	80.7 ± 3.5	79.7 ± 2.9

[1]Cells were isolated from rats fed commercial unpurified diet and cultured 6, 12, 24 or 48 h in serum-free medium with varied concentrations of caerulein or caerulein and secretin. Viability was determined by trypan blue dye exclusion. At isolation, the viability was 89.3 ± 3.3%.

[2]Values represent mean ± SEM for triplicate samples from three or four experiments.

[ab]By 2-way ANOVA, there was a main effect of time on viability ($p<0.0001$): 6 h - 86.2 ± 3.2[a]; 12 h - 84.6 ± 2.7[a]; 24 h - 77.5 ± 3.6[b]; 48 h - 77.9 ± 2.5[b]; but no difference among individual time-treatment groups.

Discussion

Secretin increased media lipase activity biphasically without changing cellular lipase activity, which suggests that the secretory processes in these cultured cells respond to this hormone via functional receptors. However, these results do not support a direct cellular role of secretin in the regulation of lipase, contrary to findings in a previous study by Rausch et al. (194). They found that intravenous infusion of secretin into rats for up to 24 h stimulates a 4-fold increase in lipase synthesis. Since their experiments were done in vivo, it is possible that secretin did not directly influence lipase synthesis. Instead, secretin may have stimulated or interacted with other hormones or metabolites upon infusion to alter lipase synthesis.

Caerulein, on the other hand, decreased cellular amylase activity in vitro similarly to its effect on amylase synthesis in previous in vivo infusion studies (205), supporting the proposed role of this hormone in the regulation of amylase synthesis. However, the extent of decrease in amylase cellular content in the present in vitro study (34%) is much less than the decrease in amylase synthesis observed in the previous in vivo study (86%). Other hormones or metabolites may have interacted with and potentiated the effects of caerulein.

Besides its effects on amylase synthesis in vivo, caerulein treatment also increases pancreatic protease content and trypsinogen synthesis (204). However, its effects on protease mRNA levels remain unclear. Caerulein injection leads to increased chymotrypsinogen and trypsinogen levels (203), whereas caerulein infusion does not change anionic trypsinogen mRNA levels (204). Although not examined in the present study, future in vitro experiments will be designed to investigate the direct effects of

caerulein on pancreatic proteases. Again, other hormones or metabolites may have interacted with caerulein upon in vivo administration.

Green et al. (254) suggested that secretin may potentiate the effects of caerulein in vivo. However, we found no additive effects of secretin when cells were cultured in the presence of both hormones. Other hormones (insulin, glucagon) and metabolites (glucose or ketones) may interact with and potentiate the effects of caerulein and secretin on the regulation of these enzymes, but they have not yet been examined. Previous work has suggested a role of glucose (257) and ketones (171) in regulating amylase and lipase.

Regardless of treatment, both cellular lipase and amylase activities decreased over time in culture, while media enzyme activities increased. However, total (cellular plus media) amylase and lipase changed relatively little, as did cellular viability. Total amylase activity ranged from 70.4 - 87.1 U/mg protein in the secretin studies and 360 - 450 U/mg protein in the caerulein studies. Total lipase activity ranged from 10.7 - 13.7 U/mg protein in the secretin studies and 43.3 - 48.3 U/mg protein in the caerulein studies. This suggests that, while these cultured cells maintained differentiated function, they synthesized less and secreted more pancreatic enzymes. This may be due in part to an increased basal secretion due to disruption of acinar gap junctions in the isolated acinar cells. Bruzzone et al. (216) have shown that disruption of gap junctions in acini and the resulting blockage of cell-to-cell communication lead to an increased (two- to three-fold) basal release of amylase. Although it has not been measured directly in isolated acinar cells, the basal secretion may be increased compared to cells in vivo.

Cultured acinar cells maintained their responsiveness to caerulein and exhibited changes in enzyme content that were consistent with reported in vivo regulation. Future experiments are required to examine the effects of caerulein on enzyme synthesis and mRNA levels directly to determine its mechanism of action. In contrast to caerulein response, secretin did not affect in vitro acinar enzyme content, but did stimulate secretion. Future experiments are needed to examine the effects of other hormones and nutrients, in combination with secretin, on pancreatic enzyme regulation. In conclusion, this model of hormonally-responsive cultured acinar cells can be used to study the independent and interactive effects of hormones in the regulation of digestive enzymes. However, some responses are apparently blunted, perhaps because of desensitization of the regulatory process by independently increased basal secretion rates from the isolated cells.

CHAPTER 5

EFFECTS OF CAERULEIN AND SECRETIN ON DIGESTIVE ENZYME SYNTHESIS

Summary

Caerulein is proposed to regulate the synthesis of pancreatic proteases and amylase. Similarly, secretin is implicated in the regulation of pancreatic lipase synthesis. Evidence of these regulations is predominantly from in vivo studies. We, therefore, examined the effects of caerulein and secretin directly on acinar cells to eliminate possible interactions with other regulatory factors. Cellular and media enzyme activities and relative synthesis were measured after 24 h hormonal treatment. Cells were incubated with ^{14}C-amino acids and then subjected to two-dimensional gel electrophoresis to separate individual acinar proteins for subsequent determination of incorporated radioactivity and relative synthesis. In general, all enzyme activities decreased (33%; $p<0.02$) over time in culture and media enzyme activities increased (370%; $p<0.00001$) in all treatment groups. Caerulein further decreased cellular content of all enzymes ($p<0.002$) and increased media amylase and lipase activities ($p<0.02$). Caerulein, however, significantly increased the relative synthesis of trypsin (28%) and tended to increase that of chymotrypsin (25%; $p<0.06$), which supports its proposed role in protease regulation. Secretin, on the other hand, did not significantly affect the cellular or media activities or the relative synthesis of any pancreatic enzyme

evaluated; therefore, this study does not support the proposed role of secretin in lipase regulation.

Introduction

The activity, synthetic rate and mRNA levels of amylase, lipase and proteases adapt to changes in their respective dietary substrates - carbohydrate, fat and protein (150,151,153,155,156,170,258). The cellular mediators of these pancreatic adaptations to diet remain unknown, but various hormones and nutrients have been implicated.

One gastrointestinal hormone, cholecystokinin, is proposed to regulate the proteases and amylase. Caerulein, a cholecystokinin analog, increases pancreatic protease content (203) and synthesis (205); however, its effects on protease mRNA levels remain unclear. When injected, caerulein increases chymotrypsinogen and trypsinogen mRNA levels (203), as does infused CCK-8 and endogenous CCK (stimulated by soybean trypsin inhibitor, STI; 197,259). However, infused caerulein either slightly increases or does not change trypsinogen mRNA levels (204,206). Caerulein also decreases amylase synthesis (204,205) without parallel changes in its mRNA levels (204,206) when infused continuously. In vitro, caerulein decreases amylase cellular activity in a dose-dependent manner after 24 h of hormonal treatment; the maximally-effective dose was 100 nM (226).

Another hormone, secretin, is proposed to regulate pancreatic lipase. When infused into rats, it causes a 4-fold increase in lipase synthesis (194). Secretin has also been shown to be elevated after intraduodenal infusion of 20% fat (23). However, secretin infusion does not affect lipase mRNA, whereas dietary fat increases lipase

content, synthesis and mRNA (195). Further, we found that secretin, in vitro, does not affect the activity of lipase in cultured cells, even though these cells retain secretory responsiveness to secretin; the maximally-effective dose was 10 nM at 12 h (25). However, its effects on enzyme relative synthesis in vitro have not yet been examined.

The results described above suggest caerulein and secretin may regulate pancreatic digestive enzymes. However, their direct effects on enzyme relative synthesis have not been examined and cannot necessarily be inferred from infusion or injection studies because of potential interaction with other hormones or regulators in vivo. Therefore, this study examines the effects of caerulein and secretin on the relative synthesis, as well as cellular content and secretion, of pancreatic enzymes in an in vitro system - primary cultures of pancreatic acinar cells (223,225). These cells are maintained in a defined serum-free medium and are viable for four to five days. They retain differentiated characteristics, secretagogue-stimulated secretion and hormonal responsiveness (225), and synthesize amylase de novo for four days in culture (227).

Materials and Methods

Animals and Diet

Male, weanling Sprague-Dawley rats weighing 40-100 g (Harlan, Indianapolis, IN, USA) were housed individually, maintained on a 12 h light-dark cycle and fed ad libitum a commercial non-purified diet that contained 24% crude protein, 4% crude fat and 4.5% crude fiber (Continental Grain Co., Chicago, IL, USA).

Acinar Cell Culture

Two rats per cell preparation were asphyxiated with CO_2 and the pancreata were removed aseptically and pooled. Pancreatic acinar cells were isolated and cultured according the method of Brannon et al. (223,225). Pancreata were minced into 1-2 mm pieces and incubated with 20 ml HBSS without Ca^{2+} and Mg^{2+} containing 1 mM EDTA for 15 min at 37° C while shaking 120 cpm. (All culture media was purchased from Gibco, Grand Island, NY, USA) The chelated mixture was centrifuged for 2 min at 500 xg. The resulting supernatant was discarded; the pellet was rinsed with 20 ml Ham's F-12 medium and centrifuged for 2 min at 500 xg; the supernatant was again discarded. The tissue pellet was digested with 20 ml of 1 mg/ml collagenase type II and 1 mg/ml hyaluronidase (Worthington Biochemical Corp., Freehold, NY, USA) in Ham's F-12 medium at 37° C for 30 min at 120 cpm in a shaking water bath. After centrifugation at 500 xg for 2 min, the supernatant was discarded; the pellet was rinsed with 20 ml of HBSS. The chelation and digestion were repeated in sequence as described. After the second digestion and centrifugation, the pellet was rinsed with Ham's F-12 medium, centrifuged for 2 min at 500 xg and resuspended in 10 ml Ham's F-12. The suspension was filtered through 500 and 25 μm Nytex filters, layered onto 40 ml 6% Ficoll and centrifuged for 7 min at 200 xg. The pellet was rinsed with 20 ml Ham's F-12 then with 20 ml Waymouth's medium 752/1 with 25 mM HEPES buffer, 2.4 mM glutamine, 0.01% STI and 1x antibiotic-antimycotic. The pellet was then resuspended in 10 ml Waymouth's medium.

Cell number and viability were determined by trypan blue dye exclusion using a cell suspension containing 0.1% trypan blue dye. Then cells were plated in 24-well petri

clusters (Costar, Van Nuys, CA, USA) at a density of 1 x 10^6 cells per well in 2.0 ml Waymouth's SF medium with 100 nM caerulein, 10 nM secretin (both from Sigma Chemicals, St. Louis, MO, USA) or no treatment. These hormonal concentrations had the greatest effects on pancreatic enzyme secretion and activity in previous studies (25,226) and were therefore used in the present study. Cells were incubated at 37° C with 5% humidified CO_2 for 24 h which was a time period shown effective previously (194,226). SF medium contained 10 mg/ml BSA, 1 x 10^{-8} M DEX (Sigma Chemicals, St. Louis, MO, USA), and 42 pM EGF (Bethesda Research Labs, Gaithersburg, MD, USA).

Some cells from each preparation were harvested (2 wells per sample) either after a brief (<1 h) rest period following isolation or after 24 h in culture. Cells and media were then separated by centrifugation at 1800 xg for 4 min at 4° C. The media (supernatant) were removed and frozen at -80° C for subsequent analysis, as were cell pellets after one rinse with 1 ml ice-cold PBS. Other acinar cells from the same isolation were used for protein synthesis studies either immediately following preparation or after 24 h in culture.

Cell Analyses

Frozen cell pellets were homogenized in 1 ml PBS by sonication on ice for 7-10 s. Aliquots of homogenates were assayed for cellular protein with BSA as the standard (244), lipase activity by an autotitrimetric method with olive oil as the substrate in the presence of excess colipase (174,245), amylase activity by the Phadebus blue starch method (255), trypsin activity using Nα-benzoyl-DL-arginine p-nitroanilide (BAPNA) as the substrate (260), chymotrypsin activity using glutaryl-L-phenylalanine p-nitroanilide

(GAPNA) as the substrate (261), and elastase activity using N-acetyl-L-alanyl-L-alanyl-L-alanine p-nitroanilide (SAPNA) as the substrate (262). Media samples were also assayed for lipase and amylase activity, but proteolytic activities could not be measured because of the STI in the media. All enzyme activities were expressed as units per mg cellular protein.

Protein Synthesis

Acinar cells that were treated with 100 nM caerulein, 10 nM secretin or no hormone (control) were incubated with 3 μCi per 2 ml of a mixture of 15 ^{14}C-amino acids (algal protein hydrolysate, Amersham CFB25, 0.25 Ci/ml, Amersham Corp., Arlington Heights, IL) for 0 or 90 min for analysis of cellular protein synthesis. The incorporation of ^{14}C-amino acids into TCA-precipitable acinar cell proteins was found, in preliminary experiments, to be linear for up to 120 min in freshly-isolated and cultured cells (data not shown). Cells were harvested (6 wells per sample) and centrifuged at 1800 xg for 5 min at 4° C. Supernatants were discarded and cell pellets were then rinsed once with 1 ml ice-cold PBS and stored at -80° C until analyzed.

Rates of synthesis of individual exocrine proteins were measured by evaluating the incorporation of radiolabeled amino acids into proteins separated by two-dimensional electrophoresis (263; for validation of method see Appendix B). Proteins were first separated by isoelectric focusing (IEF) in 4% polyacrylamide in the presence of 1.0% pH 4-6, 1.0% pH 5-8, 1.0% pH 9-11, 2.5% pH 3.5-10 Ampholines (LKB Instruments, Inc., Gaithersburg, MD), 9.0 M urea, 0.28 M sucrose and 0.2 mM PMSF. The resulting IEF tube gels were soaked for 5 min in 62.5 mM Tris buffer, pH 6.8,

containing 2% sodium dodecyl sulfate (SDS) and 0.01% bromophenol blue dye. Proteins were then separated by molecular weight in a 10-20% polyacrylamide gradient gel containing 0.1% SDS in the second dimension. Resulting protein spots were stained with 0.06% Coomassie Blue dye, excised from gels with a scalpel and digested overnight in 1 ml 30% hydrogen peroxide at 60° C. Radioactivity incorporated into individual protein spots was determined by counting digested second dimension gel spots in a scintillation counter (Tri-Carb 460 CD, Packard, Downers Grove, IL).

Relative synthesis of individual exocrine proteins was calculated by dividing the amount of radioactivity incorporated into one protein spot by the total amount of radioactivity incorporated into all protein spots. This analysis eliminated the complicating variable of differing intracellular or tRNA specific activities (SA) among the treatments, which we have shown previously varies with hormonal (264) and antecedent dietary treatment (225). Therefore, data were not analyzed as total incorporation, because intracellular SA were not determined.

Statistical Analyses

Data, expressed as mean ± SEM, were analyzed by analysis of variance (ANOVA) and least significant differences (247). Two-way ANOVA was also used to assess the independent effects of culture (comparing freshly-isolated - 0 h - versus 24 h cultured independent of hormone treatment) and hormonal treatment (comparing caerulein or secretin treated cells versus untreated cells independent of culture time) as well as interactive effects of culture and hormonal treatment. Significant differences were denoted by $p<0.05$.

Results

Effects of Culture

There were significant independent effects of culture on cellular and media enzyme activities, as well as interactive effects of culture with hormonal treatment. In the caerulein studies, average cellular amylase, lipase, elastase, chymotrypsin and trypsin activities (Table 6) decreased over 24 h in culture (19%, $p<0.0001$; 38%, $p<0.04$; 36%, $p<0.00001$; 36%, $p<0.00001$; 33%, $p<0.00001$; respectively), but these decreases were due to changes in activities in caerulein treated cells after 24 h. In the control cells, amylase activity actually increased (12%; $p<0.00001$) with time in culture, while that of lipase and chymotrypsin remained unchanged. Media amylase and lipase activities (Table 7), in general, increased (811%, $p<0.00001$; 505%, $p<0.00001$; respectively) over time in culture, but increased even more in caerulein treated cells. In the secretin studies, cellular activities decreased for amylase (19%; $p<0.001$), lipase (33%; $p<0.002$), chymotrypsin (24%; $p<0.001$), elastase (15%; $p<0.02$) and trypsin (15%, $p<0.01$; Table 9), while media activites increased for amylase (85%; $p<0.00001$) and lipase (81%, $p<0.00001$; Table 10) over time in culture.

Culturing acinar cells also affected the synthesis of the digestive enzymes, although the effects of culture varied for some enzymes (lipase, procarboxypeptidase and elastase), among experiments. In the caerulein studies, the relative synthesis (Table 8) of amylase decreased significantly over 24 h (43%; $p<0.0003$), whereas that of elastase, chymotrypsin and trypsin significantly increased (46%, $p<0.002$; 181%, $p<0.00002$; and 26%, $p<0.006$; respectively). The relative synthesis of lipase and procarboxypeptidase were not significantly changed over 24 h. In the secretin studies, the relative synthesis

Table 6: EFFECTS OF CAERULEIN ON PANCREATIC ENZYME ACTIVITIES IN CULTURED ACINAR CELLS[1]

	Cultured Cells			
	0 h		24 h	
	0 nM	100 nM Caerulein	0 nM	100 nM Caerulein
	(U/mg cellular protein)[2]			
Amylase	191.4 ± 12.3[b]	196.1 ± 13.0[ab]	216.7 ± 18.8[a]	97.8 ± 11.0[c]
Lipase	13.15 ± 3.53[a]	13.36 ± 1.55[a]	10.07 ± 3.45[a]	6.27 ± 2.42[b]
Chymotrypsin	17.74 ± 1.95[a]	18.68 ± 1.83[a]	16.14 ± 1.65[a]	7.23 ± 2.98[b]
Elastase	0.79 ± 0.05[a]	0.80 ± 0.04[a]	0.66 ± 0.05[b]	0.35 ± 0.04[c]
Trypsin	1211 ± 142[a]	1199 ± 114[ab]	1020 ± 114[b]	582 ± 80.4[c]

[1]Cells were isolated from rats fed commercial unpurified diet and cultured 0 or 24 h in serum-free media with 0 or 100 nM caerulein.

[2]Values represent mean ± SEM of duplicate or quadruplicate samples from three experiments.

[abc]Values for each enzyme activity measured not sharing superscripts differed significantly ($p<0.05$) when analyzed by 2-way ANOVA with LSD. There was a significant effect of culture; average cellular amylase, lipase, elastase, chymotrypsin and trypsin activities were significantly decreased from 0 to 24 h ($p<0.004$). Caerulein significantly decreased the cellular activities of amylase (55%; $p<0.00001$), elastase (47%; $p<0.00001$), chymotrypsin (55%; $p<0.003$) and trypsin (43%; $p<0.002$), and tended to decrease cellular lipase activity (38%; $p<0.12$). There was an interactive effect of culture and caerulein ($p<0.002$) for amylase, chymotrypsin, elastase and trypsin.

Table 7: EFFECTS OF CAERULEIN ON MEDIA ENZYME ACTIVITIES SECRETED BY CULTURED ACINAR CELLS[1]

	Cultured Cells			
	0 h		24 h	
	0 nM	100 nM Caeruelin	0 nM	100 nM Caerulein
	(U/ml/mg cellular protein)[2]			
Amylase	19.2 ± 1.85[c]	15.7 ± 1.63[c]	86.4 ± 12.7[b]	196 ± 19.0[a]
Lipase	0.97 ± 0.28[c]	0.70 ± 0.29[c]	3.85 ± 0.84[b]	4.59 ± 0.23[a]

[1]Cells were isolated from rats fed commercial unpurified diet and cultured 0 or 24 h in serum-free media with 0 or 100 nM caerulein.

[2]Values represent mean ± SEM of six samples from one experiment.

[abc]Values for each enzyme activity measured not sharing superscripts differed significantly ($p<0.05$) when analyzed by 2-way ANOVA with LSD. Media amylase and lipase activities significantly increased over 24 h in culture ($p<0.00001$). Caerulein treatment significantly increased media amylase (227%; $p<0.00001$) and lipase (16%; $p<0.02$) activities after 24 h in culture. There was a significant interactive effect of culture and caerulein ($p<0.02$) on each enzyme.

Table 8: EFFECTS OF CAERULEIN ON THE RELATIVE SYNTHESIS OF PANCREATIC ENZYMES BY CULTURED ACINAR CELLS[1]

	Cultured Cells			
	0 h		24 h	
	0 nM	100 nM Caerulein	0 nM	100 nM Caerulein
	% Relative Synthesis[2]			
Amylase	19.8 ± 2.09[a]	23.9 ± 3.20[a]	13.5 ± 2.61[b]	11.9 ± 1.76[b]
Lipase	2.50 ± 0.94	1.55 ± 0.48	2.00 ± 0.99	1.14 ± 0.43
Procarboxypeptidase	7.63 ± 1.11	6.61 ± 1.10	6.33 ± 1.22	4.20 ± 0.62
Chymotrypsin	24.4 ± 2.07[b]	22.6 ± 2.56[b]	36.1 ± 6.28[a]	48.4 ± 6.71[a]
Elastase	2.06 ± 0.20[b]	2.69 ± 0.04[b]	5.02 ± 1.08[a]	3.98 ± 0.69[a]
Trypsin	15.2 ± 1.02[b]	15.7 ± 1.71[b]	18.1 ± 3.04[b]	25.2 ± 2.54[a]

[1]Cells were isolated from rats fed commercial unpurified diet and cultured 0 or 24 h in serum-free medium with 0 or 100 nM caerulein.

[2]Values represent mean ± SEM of duplicate samples from six experiments.

[abc]Values for each enzyme not sharing superscripts differed significantly ($p<0.05$) when analyzed by 2-way ANOVA with LSD. The relative synthesis of amylase decreased significantly over 24 h in culture (43%; $p<0.0003$), whereas that of elastase, chymotrypsin and trypsin significantly increased (46%, $p<0.002$; 181%, $p<0.0002$; and 26%, $p<0.006$, respectively). Caerulein treatment significantly increased the relative synthesis of trypsin (28%; $p<0.048$) and tended to increase that of chymotrypsin (25%; $p<0.06$). There was a significant interactive effect of culture and caerulein ($p<0.05$) on trypsin relative synthesis.

Table 9: EFFECTS OF SECRETIN ON PANCREATIC ENZYME ACTIVITIES IN CULTURED ACINAR CELLS[1]

	Cultured Cells			
	0 h		24 h	
	0 nM	10 nM Secretin	0 nM	10 nM Secretin
	(U/mg cellular protein)[2]			
Amylase	208.2 ± 22.0[a]	212.7 ± 21.0[a]	171.3 ± 13.1[b]	168.9 ± 13.0[b]
Lipase	26.53 ± 4.64[a]	24.70 ± 4.74[a]	16.67 ± 3.45[b]	17.77 ± 3.21[b]
Chymotrypsin	17.92 ± 1.82[a]	19.16 ± 2.15[a]	13.42 ± 1.89[b]	14.66 ± 2.21[b]
Elastase	0.70 ± 0.08[a]	0.72 ± 0.08[a]	0.60 ± 0.07[b]	0.60 ± 0.06[b]
Trypsin	1161 ± 107[a]	1187 ± 115[a]	996.4 ± 122[b]	990.8 ± 101[b]

[1]Cells were isolated from rats fed commercial unpurified diet and cultured 0 or 24 h in serum-free media with 0 or 10 nM secretin.

[2]Values represent mean ± SEM of triplicate samples from three experiments.

[ab]Values for each enzyme activity measured not sharing superscripts differed significantly ($p<0.05$) when analyzed by 2-way ANOVA with LSD. There was s significant effect of culture on enzyme activities; average cellular amylase, lipase, chymotrypsin, elastase and trypsin activities were significantly decreased after 24 h in culture ($p<0.02$). Secretin did not affect the cellular activity of any of these enzymes.

Table 10: EFFECTS OF SECRETIN ON MEDIA ENZYME ACTIVITIES SECRETED BY CULTURED ACINAR CELLS[1]

	Cultured Cells			
	0 h		24 h	
	0 nM	10 nM Secretin	0 nM	10 nM Secretin
	(U/ml/mg cellular protein)[2]			
Amylase	14.6 ± 4.56[b]	15.4 ± 4.49[b]	91.3 ± 20.2[a]	91.7 ± 13.0[a]
Lipase	1.61 ± 0.27[b]	1.60 ± 0.21[b]	8.17 ± 0.37[a]	8.76 ± 1.33[a]

[1]Cells were isolated from rats fed commercial unpurified diet and cultured 0 or 24 h in serum-free media with 0 or 10 nM secretin.

[2]Values represent mean ± SEM of triplicate samples from three experiments.

[ab]Values for each enzyme activity measured not sharing superscripts differed significantly ($p<0.05$) when analyzed by 2-way ANOVA with LSD. Media amylase and lipase activities significantly increased over 24 h in culture ($p<0.00001$). Secretin did not affect amylase or lipase activity.

Table 11: EFFECTS OF SECRETIN ON THE RELATIVE SYNTHESIS OF PANCREATIC ENZYMES BY CULTURED ACINAR CELLS[1]

	Cultured Cells			
	0 h		24 h	
	0 nM	10 nM Secretin	0 nM	10 nM Secretin
	% Relative Synthesis[2]			
Amylase	18.2 ± 3.14[a]	13.9 ± 2.14[a]	10.8 ± 1.05[b]	10.3 ± 0.96[b]
Lipase	2.42 ± 0.66[a]	1.62 ± 0.66[a]	1.02 ± 0.59[b]	1.20 ± 0.57[b]
Procarboxypeptidase	7.87 ± 0.62[a]	6.67 ± 1.54[a]	3.43 ± 1.03[b]	4.72 ± 0.91[b]
Chymotrypsin	23.4 ± 3.14[b]	29.2 ± 4.76[b]	34.0 ± 4.12[a]	34.9 ± 4.43[a]
Elastase	3.30 ± 0.91	2.92 ± 0.55	3.38 ± 1.01	2.45 ± 0.76
Trypsin	17.1 ± 1.65[b]	17.2 ± 2.42[b]	21.4 ± 3.16[a]	21.3 ± 2.87[a]

[1]Cells were isolated from rats fed commercial unpurified diets and cultured in serum-free media 0 or 24 h with 0 or 10 nM secretin.

[2]Values represent mean ± SEM of duplicate samples from three experiments.

[ab]Values for each enzyme not sharing superscripts differed significantly ($p<0.05$) when analyzed by 2-way ANOVA with LSD. The relative synthesis of amylase, lipase and procarboxypeptidase were significantly decreased over time in culture (34%, $p<0.002$; 45%, $p<0.05$; 43%, $p<0.001$, respectively), whereas the relative synthesis of chymotrypsin and trypsin were significantly increased over 24 h (24%, $p<0.02$; 20%, $p<0.04$, respectively).

(Table 11) of amylase, again, significantly decreased over time in culture (34%; $p<0.002$), as did that of lipase and procarboxypeptidase (45%, $p<0.05$; 44%, $p<0.001$; respectively). Chymotrypsin and trypsin relative synthesis, again, increased (24%, $p<0.02$; 20%, $p<0.04$, respectively), whereas that of elastase did not change over 24 h.

Effects of Caerulein and Secretin

Caerulein affected enzyme content, secretion and synthesis after 24 h of treatment, but had no acute effects. Caerulein, after 24 h, significantly decreased the cellular activities (Table 6) of amylase (55%; $p<0.00001$), elastase (47%; $p<0.00001$), chymotrypsin (55%; $p<0.003$) and trypsin (43%; $p<0.002$), and tended to decrease cellular lipase activity (38%; $p<0.12$). It, concomitantly, increased media (Table 7) amylase (227%; $p<0.00001$) and lipase (16%; $p<0.02$) activities compared to cummulative basal secretion by untreated cells.

Caerulein also significantly increased the relative synthesis (Table 8) of trypsin (39%; $p<0.048$) and tended to increase that of chymotrypsin (34%; $p<0.06$). Caerulein did not, however, significantly affect the relative synthesis of amylase, lipase, elastase or procarboxypeptidase, compared to untreated cells.

Secretin, on the other hand, did not affect, either acutely or after 24 h, the cellular activity (Table 9), media activity (Table 10) or relative synthesis (Table 11) of any pancreatic enzyme evaluated.

Discussion

There were significant independent effects of culture on pancreatic enzyme content, secretion and synthesis of untreated acinar cells, some of which varied between experiments. In both the caerulein and secretin studies, the cellular activities of elastase and trypsin decreased slightly (14-16%). However, the cellular activity of amylase slightly increased (12%) in the caerulein studies, but decreased (17%) in the secretin studies. Also variable between experiments were the cellular activities of lipase and chymotrypsin which were unchanged in the caerulein studies, but decreased in the secretin studies (37% and 25%, respectively). A decrease in total protein content (30%) over 24 h in culture has been shown in previous in vitro studies (224) using cultured mouse pancreatic acinar cells, but changes in individual enzyme content were not examined.

Perhaps the differences in cellular amylase, lipase and chymotrypsin activities over time in culture were a function of their basal secretion rates that varied somewhat between studies. In the secretin studies, the media activities of amylase and lipase increased 6.3- and 5-fold, respectively, over 24 h in culture compared to 4.5- and 4-fold, respectively, in the caerulein studies. Therefore, the higher basal secretion seen in the secretin studies may account for the greater decrease in cellular content over 24 h, if total enzyme synthesis was similar in both studies. The same may also be true for chymotrypsin, but the unavailability of media activity data for the proteases makes it difficult to determine.

There were also changes in enzyme relative synthesis over time in culture. Amylase relative synthesis decreased and that of chymotrypsin and trypsin increased over

time in culture in both secretin and caerulein studies for reasons not yet determined. Previous studies using this in vitro system (227) also found a decrease in amylase relative synthesis after 24 h in culture. Although a different method (affinity adsorption) for the measurement of relative synthesis was used, the range of amylase relative synthesis was similar to that obtained in these studies (6-17% compared to 10-23%). Although variable among experiments, lipase and procarboxypeptidase relative synthesis decreased slightly over time in only the secretin studies and elastase relative synthesis increased in only the caerulein studies. Why the effects of culture would vary between the two studies, or occur at all, is not clear, but may reflect variability among cell isolations and altered regulation of synthesis under these culture conditions.

One other condition in which a similar shift in the pattern of enzyme relative synthesis is seen is in rats fed a protein-free diet (163); amylase relative synthesis decreases while that of the anionic proteases increase. Presumably, neither the rats in vivo nor the cells in culture lack essential amino acids because prior to acinar cell isolation, rats were fed a standard rodent diet containing 24% crude protein and the media contained an ample supply of all essential amino acids. Whether the availability or utilization of media amino acids by cultured acinar cells in altered is unknown, but we have previously shown that the specific activity of the intracellular phenylalanine pool decreases (54%) in acinar cells cultured 48 h (225), suggesting that altered amino acid uptake and utilization may occur in cultured acinar cells. Future studies need to examine how culture conditions affect amino acid utilization. Clearly, however, cultured acinar cells continued to synthesize the major digestive enzymes, but in different relative proportions. Additionally, factors regulating the synthesis of the different enzymes could

be missing or inadequate in this defined culture medium which contains only dexamethasone, albumin and epidermal growth factor.

Even though these cells are secretagogue-responsive, secretin had no effect on enzyme content or secretion after 24 h. In previous studies (25), however, the concentration of secretin used (10nM) promoted significant secretory affects at 12 h. Therefore, the maximal secretory effects of secretin may occur before 24 h and may not persist over time in culture. These studies examined the effects of secretin on enzyme synthesis at 24 h because this was a time period during which secretin affected enzyme synthesis in vivo (194). However, because secretin had no affect on the relative synthesis of any enzyme measured, these studies do not support its proposed role in the regulation of pancreatic lipase (194).

Caerulein exerted effects, independent of culture time, on enzyme content, secretion and relative synthesis. It further stimulated acinar cell secretion of amylase and lipase, and concomitantly decreased their cellular content. This may be a general effect on all enzymes, since the cellular activities of elastase, chymotrypsin and trypsin also decreased with caerulein treatment; however, their media activities could not be measured because the cellular media contained STI. A general, in vivo, effect of CCK injection or infusion on pancreatic enzyme secretion has been reported in the past (29,265).

Besides its general effect on acinar cell secretion, caerulein specifically increased the relative synthesis of trypsin and chymotrypsin, supporting its proposed role in protease regulation in response to dietary protein (198). The magnitude of change in synthesis of the proteases in vitro (34-39%) was less than that seen in previous in vivo

infusion studies (2-3 fold; 204,205) using the same period of hormonal treatment. It is difficult to determine the exact concentration of caerulein that the acinar cells are exposed to in in vivo infusion studies using 0.25 μg/kg/h. However, via calculations based on average weight and blood volume of the rats used, we estimated that the concentration of caerulein (100 nM) present in the cell media during our studies was similar to, or slightly greater than, levels to which acinar cells were potentially exposed. Therefore, it may be that higher concentrations of caerulein are required to stimulate similar changes in enzyme synthesis in dispersed acinar cells. The same may be true for enzyme secretion because the secretory effects stimulated in acinar cells were also smaller than those seen in perfused pancreas or dissected lobules (5-8 fold compared to 8-20 fold; 214,215). This phenomenon of decreased responsiveness by dispersed acinar cells to secretagogues has been described previously (214,215), and attributed to disruption of cell-to-cell communication and membrane structure associated with the dispersal of individual acinar cells.

In addition to these effects of caerulein on enzyme synthesis by cultured rat acinar cells, CCK also affects the growth of mouse acinar cells in culture. Rat acinar cells in this model system, however, do not grow in culture, thus such effects were not observed in our studies (223,225). In vitro, caerulein and CCK-8 increase by 3-fold the incorporation of ^{3}H-thymidine into DNA by cultured mouse acinar cells (208). These in vitro effects of CCK support its role in pancreatic growth which may involve the induction of ornithine decarboxylase (ODC), an enzyme needed for the biosynthesis of polyamines (200). The polyamines putrescine, spermidine and spermine are involved in DNA, RNA and protein synthesis and are, therefore, thought to be linked to the

regulation of cell growth and proliferation (201). The CCK-stimulated increase in pancreatic DNA content is associated with an increase in polyamine content (266) and immunoreactivity (202). Furthermore, in vivo infusion of CCK-8 or endogenous stimulation of plasma CCK (via STI infusion) lead to increased ODC mRNA levels (197,267), which provide further support for a role of CCK in pancreatic cell growth. Despite differences between these two in vitro culture systems, both support a direct role of CCK in pancreatic regulation.

Of note, the enzymatic content of cultured acinar cells does not reflect solely the synthetic rates of the enzymes, as is seen in vivo (163). There were two distinct patterns of association between content and synthesis among the enzymes evaluated, that of the proteases and that of amylase and lipase. The cellular activity of trypsin decreased over 24 h in culture even though its relative synthesis increased, leading to a negative, linear correlation of trypsin content and its relative synthesis (Figure 9). A similar pattern was seen with chymotrypsin and elastase. Both in this study and a previous one (227), however, the cellular content of amylase increased with increasing relative synthesis; but the results from this study revealed that this relationship reached a plateau at 12-14% relative synthesis, after which a continued increase in amylase relative synthesis was not associated with an increase in amylase content (Figure 10). For amylase, in contrast to trypsin, synthesis decreased over time in culture, while content decreased even more. The relationship of lipase content and synthesis was similar to that of amylase. Clearly, other factors besides synthesis also affect enzymatic content; secretion, which may be altered in cultured acinar cells, could influence enzyme content. In fact, disruption of gap junctions in acini, as occurs upon dispersal of individual acinar cells, causes a two-

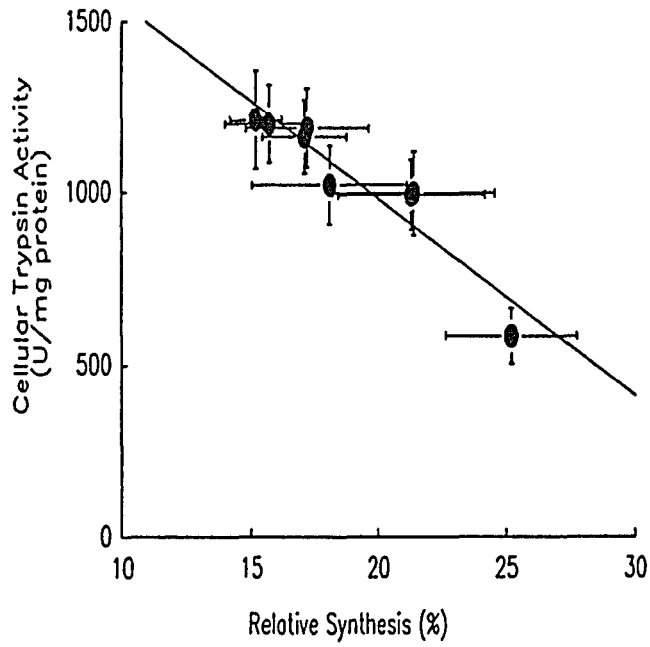

Figure 9: CORRELATION BETWEEN TRYPSIN ACTIVITY AND RELATIVE SYNTHESIS

Cellular trypsin activity (U/mg protein; from Tables 1 & 4) was analyzed as a function of trypsin relative synthesis (%; from Tables 3 & 6) by linear regression analysis (31). Values represent mean ± SEM from cells isolated from rats fed commercial rodent diet and treated with caerulein, secretin or no hormone. ($y = 2122.80 - 57.12x$; $R = 0.938$)

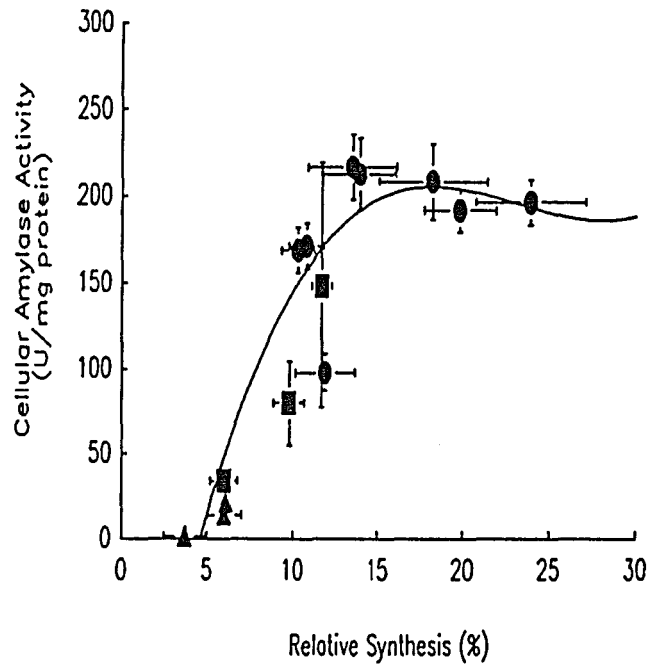

Figure 10: CORRELATION BETWEEN AMYLASE ACTIVITY AND RELATIVE SYNTHESIS

Cellular amylase activity (U/mg protein; from Tables 1 & 4 and Ref. 21) was analyzed as a function of amylase relative synthesis (%; from Tables 3 & 6 and Ref. 21) by curve-fitting regression analysis (31). Values represent mean ± SEM from cells isolated from rats fed commercial rodent diet (●) and treated with caerulein, secretin or no hormone, or from rats fed a high carbohydrate (■) or high fat (▲) diet. ($y = -223.24 + 60.21x - 2.73x^2 + 0.039x^3$; $R = 0.529$)

fold increase in basal secretion (216,217). If such an increase in basal secretion persisted in cultured cells, then enzymatic content would reflect the combined effects of altered synthesis and secretion in culture. In the case of trypsin and the other proteases, a possible increased secretion by these acinar cells in vitro, exceeding even their increased rates of synthesis, could lead to decreases in cellular content, as was found. The larger and more rapid decreases of amylase and lipase content upon culture may result from the combined effects of decreased synthesis and possible increased secretion; only when synthetic rates exceed secretion would enzyme content increase. However, there seems to be a threshold for amylase and lipase but not the proteses, after which synthetic rate does not lead to increased content. Why this plateau would occur is unknown, but further studies examining enzyme secretion, as well as degradation, are needed to elucidate these relationships among pancreatic enzyme content, secretion and synthesis in vitro.

These studies have shown a direct role of caerulein in the regulation of pancreatic protease synthesis, supporting its role in pancreatic adaptation to dietary protein. However, a direct role of secretin in lipase regulation has not been demonstrated in these studies.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The long-range goal of this dissertation research was to evaluate the proposed mediator(s) of pancreatic adaptation to dietary substrates, which had been identified in previous in vivo studies, using a long-term, in vitro cell system. Both ketones, intermediates in triglyceride metabolism, and the gastrointestinal hormone, secretin, are proposed to mediate the pancreatic adaptation to dietary fat. The effects of ketones (β(OH)-butyrate), alone and in conjunction with antecedent diet and media glucose, on pancreatic lipase cellular activitiy were examined in cultured acinar cells. The effects of secretin, and its interaction with CCK, on pancreatic lipase, as well as other digestive enzymes, cellular activity, secretion and synthesis were also examined in vitro. Another gastrointestinal hormone, CCK, is thought to regulate pancreatic adaptation to dietary protein and, possibly, carbohydrate. Therefore, in vitro effects of caerulein (a CCK analogue) on the panceatic proteases, amylase, and other digestive enzyme activities, secretion and synthesis were determined.

Beta-(OH)-butyrate significantly increased lipase activity only in acinar cells isolated from rats fed purified LF diet, where circulating levels of ketones are typically low. In contrast, additional β(OH)-butyrate had no effect on cells isolated from rats fed purified HF diet, where blood ketones and lipase synthesis are endogenously elevated.

Other factors may influence the effects of ketones, because β(OH)-butyrate did not affect lipase activity in cells isolated from rats fed low-fat commercial, non-purified diet even when cultured in low-glucose media to increase ketone utilization. The fiber and protein compositions differed greatly between the purified and non-purified diets and may have influenced the adaptive response of pancreatic lipase. However, lipase synthesis was not measured in any of these cells, and enzyme synthesis does not necessarily parallel cellular activity in vitro. Therefore, it is difficult to draw conclusions about the exact effects of ketones on lipase regulation. Perhaps lipase synthesis is elevated in rats fed both types of low-fat diet, but the lipase secretion is higher in rats fed a commercial, non-purified diet. Although it appears that ketones are involved in pancreatic lipase regulation, their role is still unclear. Future studies need to evaluate the effects of ketones on lipase synthesis and mRNA. Ketones may also act in conjunction with other metabolites such as free fatty acids, which are also elevated in conditions where lipase is enhanced (diabetes, starvation, high-fat feeding) or glucose which is low when ketones are elevated. Further, ketones may also interact with the hormone secretin, which is elevated upon intraduodenal infusion of fat or fatty acids. Thus, the role of ketones as a mediator of pancreatic adaptation to dietary fat is supported by the present results from in vitro studies, but appears to be more complex, and possibly interactive, than initially proposed from results in vivo.

Secretin treatment of acinar cells in vitro did not affect lipase activity or synthesis, although it did increase lipase secretion. These results indicate that secretin, alone, does not regulate pancreatic lipase. This conclusion is also supported by recent in vivo studies in which infused secretin does not alter lipase mRNA. Coupled, the results

from in vivo and in vitro raise doubt about the role of secretin as the mediator of pancreatic adaptation to fat because dietary fat increases both lipase synthesis and mRNA. Rather, secretin may interact with other factors in vivo to influence pancreatic lipase. Its interactive effects with ketones and fatty acids on lipase activity, synthesis and secretion should be examined in order to determine the direct mediator(s) and mechanisms of pancreatic adaptation to dietary fat.

Although the exact regulator(s) of pancreatic lipase in response to dietary fat remains unknown, the studies contained herein support the proposed role of CCK as the mediator of pancreatic adaptation to dietary protein. In vitro caerulein treatment of pancreatic acinar cells specifically increased the relative synthesis of trypsin and chymotrypsin. Further support comes from recent in vivo studies using a CCK-receptor antagonist which showed that CCK acts directly on acinar cells to increase protease mRNA levels (259). Combined these in vitro and in vivo results indicate that CCK, itself, mediates the increase in protease activity, synthesis and mRNA levels associated with consumption of high-protein diets.

Pancreatic adaptation to dietary protein is initiated by the actions of CCK-releasing peptide in the duodenum to stimulate the secretion of CCK, which then exerts its affects on the pancreas. Duodenally-infused soybean trypsin inhibitor increases plasma CCK levels and pancreatic protease mRNA levels. Still unknown is whether CCK increases protease mRNA production or decreases its degradation. Also unknown is whether CCK is internalized by the acinar cell to cause its effects or whether it is acting via second messengers within the acinar cell.

In vitro, caerulein also increased the media activities of amylase and lipase and concomitantly decreased their cellular activities. This may be a general effect of caerulein on all digestive enzymes because all cellular activities were decreased with caerulein treatment. However, because media data were unavailable for the proteases, a general conclusion cannot be made.

Caerulein did not, however, affect pancreatic amylase synthesis. Therefore, these results do not support a role of CCK in pancreatic adaptation to dietary carbohydrate. The effects of insulin, glucose and glucocorticoids on amylase activity, synthesis and mRNA levels in vitro need to be examined to elucidate the mechanism(s) of pancreatic amylase regulation.

In summary, CCK mediates directly pancreatic adaptation to dietary protein, but not dietary carbohydrate. The mediators of adaptation to dietary fat remain to be identified, but appear to include ketones, which play a complex, but perhaps limited, role.

APPENDIX A

Table A.1: COMPOSITION OF PURIFIED DIETS

	Diet	
	LF	HF
Fat (% kcal)	11	67
Component	% Weight	
Casein	20.0	20.0
DL-Methionine	0.3	0.3
Salts[1]	3.5	3.5
Vitamins[2]	1.0	1.0
Choline Bitartrate	0.2	0.2
Cellulose	5.0	34.8
Fat[3]	5.0	28.9
Cornstarch	65.0	11.3

[1]AIN Mineral Mixture-76
[2]AIN Vitamin Mixture-76
[3]Corn Oil

APPENDIX B

VALIDATION OF TWO-DIMENSIONAL GEL ELECTROPHORESIS TECHNIQUE

Two-dimensional (2D) gel electrophoresis, a procedure which separates proteins according to their isoelectric point and molecular weight, was simultaneously developed by Scheele and O'Farrell (263,268). This technique has subsequently been used by many laboratories and is used in the studies described herein to separate pancreatic digestive enzymes to determine their relative synthesis in cultured acinar cells. Given that the digestive enzymes (approximately 20 individual proteins) account for 96-97% of the total acinar protein (269), 2D-gel electrophoresis is an effective means of separating these proteins for analysis of radiolabeled amino acid incorporation and relative synthesis.

Individual exocrine pancreatic proteins separated by 2D gel electrophoresis have been identified and characterized by a number of investigators. Scheele and colleagues used two different methods of protein identification to do so. In one instance, proteins were identified according to the position of enzyme activities in the first-dimensional gel and considerations of molecular weight based on commercially available pancreatic proteins in the second dimension (263). Exocrine proteins were also identified by visualization in the second-dimensional gel with a procedure that avoids chemical fixatives, subsequent elution, renaturation and measurement of biological activity (269).

Using this second procedure, Scheele and coworkers identified the majority of pancreatic exocrine proteins separated by the 2D gel technique. This latter method of protein identification (described below) was used to verify the positions of the major digestive enzymes separated by a modification of Scheele's 2D gel electrophoresis technique used in the studies described herein.

Acinar cells were isolated by the method of Brannon and coworkers (223,225) described previously, and sonicated in deionized water. Total acinar cell protein (300-500 μg) was then subjected to isoelectric focusing (IEF) in 4% polyacrylamide in the presence of 1.0% pH 4-6, 1.0% pH 5-8, 1.0% pH 9-11, 2.5% pH 3.5-10 Ampholines, 9.0 M urea and 0.28 M sucrose. The resulting IEF tube gels (pH gradient 3.5-9) were soaked for 5 min in 62.5 mM Tris buffer, pH 6.8, containing 0.1% SDS and 0.01% bromophenol blue dye. Proteins were then separated by molecular weight in a 10-20% polyacrylamide gradient gel containing 0.1% SDS in the second dimension. Resulting slab gels were then soaked for 1 h in a fixative solution containing 100 mM Tris buffer, pH 9.0, and 5% glycerol. The second dimension gels were then stained with 0.3% coomassie blue G dye in the same Tris buffer for 6 h, shaking at room temperature. Subsequent destaining for 3-4 h was also accomplished in Tris buffer, pH 9.0, with 5% glycerol. The resulting protein spots were excised from the gels with a scalpel and homogenized for 10 sec in 1.0 ml of 100 mM Tris buffer, pH 8.0, containing 100 mM KCl, 0.1 mM $CaCl_2$, 100 μg/ml BSA and 1% Triton X-100. Proteins in the homogenized gel spots were then eluded by shaking overnight at 4° C. The samples were then centrifuged at 3000 rpm at 4° C for 10 min, and enzymatic assays were performed on

the resulting supernatants. All protein spots were assayed for each of the following enzymatic activities: amylase, lipase, trypsin, chymotrypsin and elastase.

The positions of amylase, lipase and trypsin in the second dimension slab gels (Figure B.1) were verified by enzymatic activities (Table B.1) and were in agreement with previous studies (263,269). The positions of chymotrypsin and elastase could not be verified by measurement of biological activity because of the low concentrations in the slab gels relative to the sensitivity of the enzyme assays. Therefore, the positions of these enzymes and procarboxypeptidase, for which no assay is established in our lab, were identified by molecular weight and isoelectric point, as previously established by Scheele and coworkers (263,269) and Schick and coworkers (205). To determine the pH gradient in the first dimensional isoelectric focusing gels, tube gels were cut into 5 mm pieces, and 2.0 ml of D_iH_2O was added to each. The pieces were shaken overnight at room temperature, and the pH of the individual solutions was subsequently measured. The resulting pH gradient was used to determine the isoelectric point of protein spots. The molecular weights of protein spots were determined by plotting their distance migrated in a second dimensional slab gel on a linear graph of the distance migrated of standards with known molecular weights versus the log of their molecular weights.

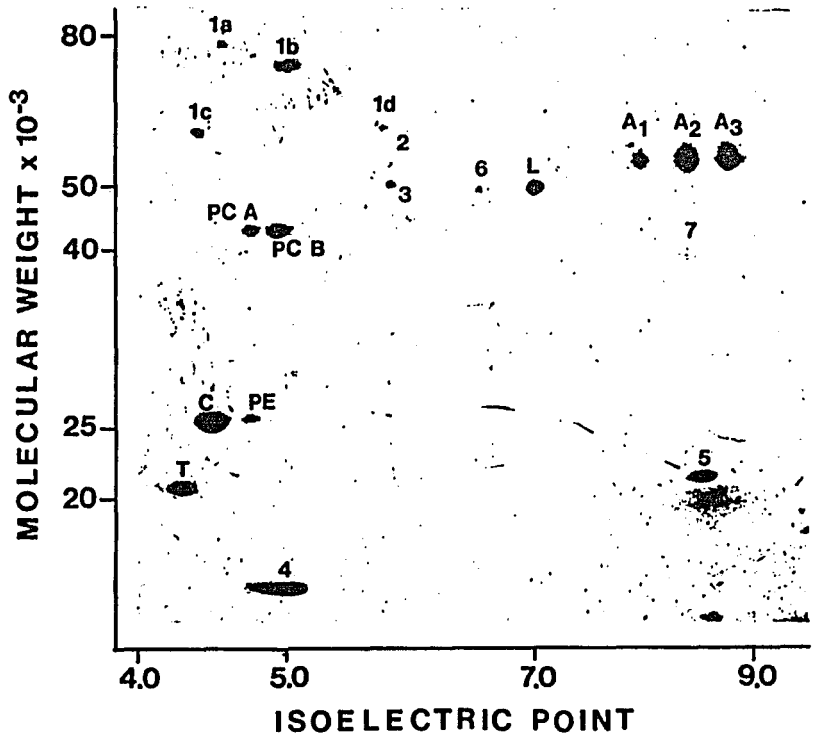

Figure B.1: SEPARATION OF PANCREATIC ACINAR CELL PROTEINS BY 2D GEL ELECTROPHORESIS

Acinar cells were isolated from Sprague-Dawley rats and sonicated in deionized water. Soluble proteins were esparated in the frist dimension by IEF and in the second dimension by SDS gel electrophoresis using a 10-20% acrylamide gradient. Protein spots were stained with Coomassie Blue dye and are identified in the figure by abbreviations described in Table 1.B.

Table B.1: PANCREATIC ACINAR CELL PROTEINS OF SPRAGUE-DAWLEY RATS

				BIOLOGICAL ACTIVITY				
Label	(Pro)Enzyme	IEP[1]	Mr[2]	Amylase	Lipase	Trypsin	Chymotrypsin	Elastase
A_1	Amylase 1	8.0	52,000	+	-	-	-	-
A_2	Amylase 2	8.5	52,000	+	-	-	-	-
A_3	Amylase 3	8.8	52,000	+	-	-	-	-
L	Lipase	7.0	49,500	-	+	-	-	-
T	Trypsinogen	4.3	20,500	-	-	+	-	-
C	Chymotrypsinogen	4.6	25,500	-	-	-	-	-
PE	Proelastase	4.8	27,000	-	-	-	-	-
PC A	Procarboxypeptidase A	4.8	44,500	-	-	-	-	-
PC B	Procarboxypeptidase B	4.9	43,500	-	-	-	-	-
1a-d	Unidentified	4.4-5.8	52,500-77,000	-	-	-	-	-
2	Unidentified	6.0	52,000	-	-	-	-	-
3	Unidentified	5.9	50,000	-	-	-	-	-
4	Unidentified	5.0	14,000	-	-	-	-	-
5	Unidentified	8.6	21,000	-	-	-	-	-
6	Unidentified	6.6	49,500	-	-	-	-	-
7	Unidentified	8.5	41,000	-	-	-	-	-

[1]Estimated isoelectric point
[2]Estimated molecular weight

APPENDIX C

University of Arizona
Tucson, Arizona 85724

VERIFICATION OF APPROVAL OF ANIMAL CARE AND USE BY THE INSTITUTIONAL COMMITTEE

Title: Mechanism of Dietary Adaptation of Pancreatic Function
Principal Investigator: Patsy M. Brannon
Department: Nutrition & Food Sciences
Submission Date: October 27, 1987
Agency: NIH

The University of Arizona University Laboratory Animal Care Committee reviews all sections of proposals to PHS which concern animal care and use. The above proposal has:

(XX) Been reviewed and approved by ULACC and verification of review is attached.

( ) Been reviewed and approval withheld. Verification of review attached.

( ) Will be reviewed within the next 60 days and verification of review will be submitted.

Laurel L. Wilkening
Vice President for Research

Date: October 27, 1987

Assurance of Compliance Pending.
Submitted for Approval to DHHS 12/30/85.
A 1380 (1984)

LIST OF REFERENCES

1. Taeber's Medical Dictionary (1981) 2nd ed., (Thomas, C.L., ed.) F.A. Davis Co., Philadelphia.

2. Mayes, P.A. (1981) Digestion/absorption in the gastrointestinal tract. In: Harper's Review of Biochemistry, 18th ed., (Martin, D.W., Mayes, P.A., Rodwell, V.W., eds.) pp. 527-539, Lange Medical Publications, Los Altos.

3. Tabak, L.A., Levine, M.J., Mandel, I.D. & Ellison, S.A. (1982) Role of salivary mucins in the protection of the oral cavity. J. Oral Pathol. 11:1-17.

4. Young, J.A., Cook, D.I., van Lennep, E.W. & Roberts, M. (1987) Secretion by the major salivary glands. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp. 773-815, Raven Press, New York.

5. Hamosh, M. & Burns, W.A. (1977) Lipolytic activity of human lingual glands (Ebner). Lab Invest. 37:603-608.

6. Hamosh, M., Ganot, D. & Hamosh, P. (1978) Rat lingual lipase: Characteristics of enzyme activity. J. Biol. Chem. 254:12121-12125.

7. Laio, T.H., Hamosh, P. & Hamosh, M. (1984) Fat digestion by lingual lipase: Mechanism of lipolysis in the stomach and upper small intestine. Pediatr. Res. 18:402-409.

8. Hamosh, M. (1978) Rat lingual lipase: Factors affecting enzyme activity and secretion. Am. J. Physiol. 235:E416-421.

9. Saraux, B. & Girard-Globa, A. (1982) Development of pancreatic enzymes in fetal and suckling rats with emphasis on lipase and colipase. J. Dev. Physiol. 4:121-137.

10. Laio, T.H., Hamosh, P. & Hamosh, M. (1983) Gastric lipolysis in the developing rat. Ontogeny of the lipases active in the stomach. Biochim. Biophys. Acta 754:1-9.

11. Susumu, I. (1987) Functional gastric morphology. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp. 817-851, Raven Press, New York.

12. Davenport, H.W. (1977) Physiology of the Digestive Tract, 4th ed.. Yearbook, Chicago.

13. Berger, E.H. (1934) The distribution of parietal cells in the stomach: A histographic study. Am. J. Anat. 54:87-114.

14. Sachs, G. & Berglindh, T. (1981) Physiology of the parietal cell. In: Physiology of the Gastrointestinal Tract, 1st ed., (Johnson, L.R., ed.) pp. 567-602, Raven Press, New York.

15. McGuigan, J., Isaza, J. & Landor, J. (1971) Relationships of gastrin dose, serum gastrin, and acid secretion. Gastroenterology 61:659-666.

16. Walsh, J.H. & Grossman, M. (1975) Medical progress: Gastrin. N. Engl. J. Med. 292:1324-1332.

17. Curtis, H. (1983) Biology. Worth Publishers, New York.

18. Solomon, T.E. (1987) Control of the exocrine pancreatic secretion. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp. 1173-1207, Raven Press, New York.

19. Walsh, J.H. (1987) Gastrointestinal hormones. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp. 181-253, Raven Press, New York.

20. Jones, R., Geist, R. & Hall, A. (1971) The choleretic effects of glucagon and secretin in the dog. Gastroenterology 60:64-68.

21. Rayford, P., Konturek, S. & Thompson, J. (1978) Effect of duodenal fat on plasma levels of gastrin and secretin and on gastric acid responses to gastric and intestinal meals in dogs. Gastroenterology 75:773-777.

22. Rhodes, R.A., Skerven , G., Chey, W.Y. & Chang, T. (1988) Acid-independent release of secretin and cholecystokinin by intraduodenal infusion of fat in humans. Pancreas 3(4):391-398.

23. Green, G.M., Taguchi, S., Friestman, J., Chey, W. & Liddle, R.A. (1989) Plasma secretin, CCK, and pancreatic secretion in response to dietary fat in the rat. Am. J. Physiol. 256:G1016-G1021.

24. Shiratori, K., Wantanabe, S. & Takeuchi, T. (1989) Effect of fatty acid on secretin release and cholinergic dependence of pancreatic secretion in rats. Pancreas 4(4):452-458.

25. Kemper, K.A. & Brannon, P.M. (1988) Effects of secretin on enzymes of cultured pancreatic acinar cells. FASEB J. 2(4): 50359.

26. Chey, W., Lee, K., Chang, T.M., Chen, Y.F. & Millikan, L. (1984) Potentiating effect of secretin on cholecystokinin-stimulated pancreatic secretion in dogs. Am. J. Physiol. 246: G248-252.

27. Stubbs, R. & Stabile, B.. (1985) Role of cholecystokinin in pancreatic exocrine response to intraluminal amino acids and fat. Am. J. Physiol 248:G347-352.

28. You, C., Rominger, J. & Chey, W. (1983) Potentiation effect of cholecystokinin-octapeptide on pancreatic bicarbonate secretion stimulated by a physiologic dose of secretin in humans. Gastroenterology 85:40-45.

29. Mutt, V. & Jorpes, E. (1971) Hormonal polypeptides of the upper intestine. Biochem. J. 125:P57-P58.

30. Schick, J., Kern, H. & Scheele, G. (1984) Hormonal stimulation in the exocrine pancreas results in coordinate and anticoordinate regulation of protein synthesis. J. Cell Biol. 99:1569-1574.

31. Alpers, D.H. (1987) Digestion and absorption of carbohydrates and proteins. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R.,ed.) pp. 1469-1487, Raven Press, New York.

32. Crane, R.K. (1960) Intestinal absorption of sugars. Physiol. Rev. 40:789-825.

33. Sarda, L. & Desnuelle, P. (1958) Action de la lipase pancreatique sur les ester en emulsion. Biochim. Biophys. Acta 30:513-521.

34. Mattson, F.H. & Beck, L.W. (1956) The specificity of pancreatic lipase for the primary hydroxyl groups of glycerides. J. Biol. Chem. 219:735-740.

35. Borgstrom, B. & Erlanson, C. (1973) Pancreatic lipase and colipase interactions and effects of bile salts and other detergents. Eur. J. Biochem. 37:60-68.

36. Morgan, R.G.H. & Hoffman, N.E. (1971) The interaction of lipase, lipase cofactor and bile salts in triglyceride hydrolysis. Biochim. Biophys. Acta 248:143-148.

37. Borgstrom, B., Wieloch, T. & Erlanson-Albertsson, C. (1979) Evidence for a pancreatic pro-colipase and its activation by trypsin. FEBS Lett. 108:407-410.

38. Borgstrom, B. & Donner, J. (1975) Binding of bile salts to pancreatic colipase and lipase. J. Lipid Res. 16:287-292.

39. Borgstrom, B. (1975) On the interactions between pancreatic lipase and colipase and the substrate, and the importance of bile salts. J. Lipid Res. 16:411-417.

40. Shiau, Y.F. (1987) Lipid digestion and absorption. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp. 1527-1556, Raven Press, New York.

41. Gardner, M.G. (1984) Intestinal assimilation of intact peptides and proteins from the diet - a neglected field? Biol. Rev. 59:289-331.

42. Nordstrom, C. (1972) Release of enteropeptidase and other brush border enzymes from the small intestine wall in the rat. Biochim. Biophys. Acta 289:376-377.

43. Nicholson, J.A. & Peters, T.J. (1977) Subcellular distribution of hydrolase activities for glycine and leucine homopeptides in human jejunum. Clin. Sci. Mol. Med. 54:205-207.

44. Pavlov, J.P. (1902) The Work of the Digestive Glands. Griffin, London.

45. Bockman, D.E. (1986) Anantomy of the pancreas. In: The Exocrine Pancreas, (Go, V.L.W., ed.) pp.1-8, Raven Press, New York.

46. Gorelick, F.S. & Jamieson, J.D. (1987) Structure-function relationship of the pancreas. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp. 1089-1108, Raven Press, New York.

47. Heidenhain, M. (1921) Uber die teilungsfahigen druseneinheiten oder adenomeren sowie uber die grundbegriffe der morphologischen systemehre. Arch. Entw. Mech. Org. 49:1-178.

48. Korc, M., Williams, J.A. & Goldfine, I.D. (1980) Pancreatic exocrine function and the islets of Langerhans. Internat. Med. 1(3):20-23.

49. Steiner, D.F., Bell, G.I., Hammer, R.E., Madsen, O.D., Carroll, R.J. & Chan, S.J. (1989) Cellular and molecular biology of the β cell: An overview. In: Perspectives on the Molecular Biology and Immunology of the Pancreatic β Cell, (Hanahan, D., McDevitt, H.O. & Cahill, G.F., eds.) pp.19-36, Cold Spring Harbor Laboratory, New York.

50. Orci, L. (1982) Macro- and micro-domains in the endocrine pancreas. Diabetes 31:4437.

51. Czech, M.P. (1980) Insulin action and the regulation of hexose transport. Diabetes 29:399.

52. Chance, R.E., Ellis, R.M. & Bromer, W.W. (1968) Porcine proinsulin: Characterization and amino acid sequence. Science 161:165.

53. Megalasson, M.D. & Matschinsky, F.M. (1986) Pancreatic islet glucose metabolism and regulation of insulin secretion. Diabetes Metab. Rev. 2:163.

54. Steiner, D.F., Bell, G.I. & Tager, H.S. (1989) Chemistry and biosynthesis of pancreatic protein hormones. In: Endocrinology, (De Groot, L.J., ed.) p. 1263, W.B. Saunders, Philadelphia.

55. Prentki, M. & Matschinsky, F.M. (1987) Ca++, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiol. Rev. 67:1185.

56. Kahn, C.R. & White, M.F. (1988) The insulin receptor and the molecular mechanism of insulin action. J. Clin. Invest. 82:1151-1156.

57. Kahn, C.R. (1985) Current concepts of the molecular mechanism of insulin action. Annu. Rev. Med. 36:429-451.

58. Meisler, M.H. & Howard, G. (1989) Effects of insulin on gene transcription. Annu. Rev. Physiol. 51:701-714.

59. Unger, R.H. & Dobbs, R.E. (1978) Insulin, glucagon and somatostatin: Secretion in the regulation of metabolism. Annu. Rev. Physiol. 40:307.

60. Korc, M., Sankaran, H., Wong, K.Y., Williams, J.A. & Goldfine, I.D. (1978) Insulin receptors in isolated mouse pancreatic acini. Biochem. Biophys. Res. Commun. 84:293-299.

61. Mossner, J., Logsdon, C.D., Goldfine, I.D. & Williams, J.A. (1984) Regulation of pancreatic acinar cell insulin receptors by insulin. Am. J. Physiol. 247:G155-G160.

62. Sankaran, H., Iwamoto, Y., Korc, M., Williams, J.A. & Goldfine, I.D. (1981) Insulin action in pancreatic acini from streptozotocin-treated rats. II. Binding of ^{125}I-insulin to receptors. Am. J. Physiol. 240:G63-G68.

63. Sjodinn, L., Holmberg, K. & Lyden, A. (1984) Insulin receptors on pancreatic acinar cells in guinea pigs. Endocrinology 115:1102-1109.

64. Yamada, T. (1987) Local regulatory actions of gastrointestinal peptides. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp.131-142, Raven Press, New York.

65. Fujita, T. & Murakami, T. (1973) Microcirculation of monkey pancreas with special reference to the insulo-acinar portal system. A scanning electron microscope study of vascular casts. Arch. Histol. Jap. 35:255.

66. Kanazawa, Y., Kuzuya, T. & Ide, T. (1968) Insulin output via the pancreatic vein and plasma insulin response to glucose in dogs. Am. J. Physiol. 215:620.

67. Hellman, B., Wallgren, A. & Petersen, B. (1962) Cytological characteristics of the exocrine pancreatic cells with regard to their position in relation to the islets of Langerhans. Acta Endocrinol. 93:465-473.

68. Wallgren, A. & Hellman, B. (1962) Influence of the islet A and B cells on the exocrine pancreatic tissue in the duck. Acta Anat. 48:137-141.

69. Landing, B.H. (1957) Peri-insular halos in pancreases of diabetic children. Lab. Invest. 6:458-461.

70. Malaisse-Lagae, F., Ravazzola, M., Robberecht, P., Vandermeers, A., Malaisse, W.J. & Orci, L. (1976) Hydrolases content in peri-insular and tele-insular exocrine pancreas. In: Endocrine Gut and Pancreas, (Fujita, T., ed.) pp.313-320, Elsevier Scientific, Amsterdam.

71. Adler, G. & Kern, H.F. (1975) Regulation of exocrine pancreatic secretory process by insulin in vivo. Horm. Metab. Res. 7:290-296.

72. Mossner, J., Logsdon, C.D., Goldfine, I.D. & Williams, J.A. (1986) Regulation of growth and differentiation of pancreatic acinar AR42J cells by insulin and glucocorticoids. Dig. Dis. Sci.

73. Lahaie, R.G. (1984) Translational control of protein synthesis in isolated rat pancreatic acini. Gastroenterology 86:1149.

74. Solcia, E., Capella, C., Buffa, R. Usellini, L., Fiocca, R. & Sessa, F. (1987) Endocrine cells of the digestive system. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johson, L.R., ed.) pp. 111-130, Raven Press, New York.

75. Sasaki, H., Rubalcava, B., Baetens, D., Blazquez, E., Srikant, C., Orci, L. & Unger, R. (1975) Identification of glucagon in the gastrointestinal tract. J. Clin. Invest. 56:135-145.

76. Unger, R., Ohneda, A., Valverde, I., Eisentraut, A. & Exton, J. (1968) Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. J. Clin. Invest. 47:48-65.

77. Pradayrol, L., Jornvall, H., Mutt, V. & Ribet, A. (1980) N-terminally extended somatostatin: the primary structure of somatostatin-28. FEBS Lett. 109:55-58.

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

79. Seal, A., Yamada, T., Debas, H., Hollinshead, J., Osadchey, B., Aponte, G. & Walsh, J. (1982) Somatostatin-14 and -28 clearance and potency on gastric function in dogs. Am J. Physiol. 243:G97-G102.

80. Bloom, S.R., Ralphs, D.N., Besser, G.M. et al. (1975) Effect of somatostatin on motilin levels and gastric emptying. Gut 16:834.

81. Johansson, C., Efendic, S., Wisen, O., Uvnas-Wallenstein, K. & Luft, R. (1978) Effects of short-time somatostatin infusion on the gastric and intestinal propulsion in humans. Scand. J. Gastrenterol. 13:481-483.

82. Poitras, P., Steinbach, J.H., Van Deventer, G., Code, C.F. & Walsh, J.H. (1980) Motilin independent ectopic fronts of the interdigestive myoelectric complex in dogs. Am. J. Physiol. 239:G215-G220.

83. Yau, W., Lingle, P. & Youther, M. (1983) Modulation of cholinergic neurotransmitter release form myenteric plexus by somatostatin. Peptides 4:49-53.

84. Krejs, G.J., Browne, R. & Raskin, P. (1980) Effect of intravenous somatostatin on jejunal absorption of glucose, amino acids, water, and elecrolytes. Gastroenterology 78:26-31.

85. Dharmsathaphorn, K., Binder, H.J. & Dobbins J.W. (1980) Somatostatin stimulates sodium and chloride absorption in the rabbit ileum. Gastroenterology 78:1559-1565.

86. Boden, G., Sivitz, M.C., Owen, O.E., Essa-Koumar, N. & Landon, J.H. (1975) Somatostatin suppresses secretin and pancreatic exocrine secretion. Science 190:163-165.

87. Schlegel, W., Raptis, S., Harvey, R.F. Oliver, J.M. & Pfeiffer, E.F. (1977) Inhibition of cholecystokinin-pancreozymin release by somatostatin. Lancet 2:166-168.

88. Esteve, J., Susini, C., Vaysse, N., Antoniotti, H., Wunsch, E., Berthon, G. & Ribet, A. (1984) Binding of somatostatin to pancreatic acinar cells. Am. J. Physiol. 247:G62-G69.

89. Koerker, D.J., Reul, W., Chideckel, E., Palmer, J., Geodner, C.J., Ensich, J. & Gule, C.C. (1974) Somatostatin: hypothalamic inhibitor of the endocrine pancreas. Science 184:482-484.

90. Morriset, J., Genik, P., Lord, A. & Solomon, T. (1982) Effects of chronic administration of somatostatin on rat exocrine pancreas. Reg. Peptides 4:49-58.

91. Molla, A., Gyr, K., Bardhan, P. & Molla, A. (1984) Effect of intravenous somatostatin on stool output in diarrhea due to vibrio cholerae. Gastroenterology 87:845-847.

92. Schwartz, T. & Tagar, H. (1981) Isolation and biogenesis of a new peptide from pancreatic islets. Nature 294:589-591.

93. Taylor, I., Solomon, T., Walsh, J. & Grossman, M. (1979) Pancreatic polypeptide. Metabolism and effect on pancreatic secretion in dogs. Gastroenterology 76:524-528.

94. Greenberg, G., Mitznegg, P. & Bloom, S. (1977) Effect of pancreatic polypeptide on DNA-synthesis in the pancreas. Experientia 33:1332-1333.

95. Lin, T. & Chance, R. (1978) Spectrum of gastrintestinal actions of bovine PP. In: Gut Hormones, (Bloom, S.R., ed.) pp.242-246, Churchill Livingstone, Edinburgh.

96. Ingber, D.E., Madri, J.A. & Jamieson, J.D. (1985) Neoplastic disorganization of pancreatic epithelial cell-cell relations. Role of basement membrane. Am. J. Pathol. 121:248-260.

97. Kern, H.F. (1986) Fine structure of the exocrine pancreas. In: The Exocrine Pancreas, (Go, V.L.W., ed.) pp. 9-20, Raven Press, New York.

98. Farquhar, M.G. (1985) Progress in unraveling pathways of Golgi traffic. Annu. Rev. Cell Biol. 1:447-488.

99. Scheele, G.A. (1986) Cellular processing of proteins in the exocrine pancreas. In: The Exocrine Pancreas, (Go, V.L.W., ed.) pp.69-85, Raven Press, New York.

100. Doyle, C.M. & Jamieson, J.D. (1978) The development of secretagogue response in rat pancreatic acinar cells. Dev. Biol. 64:11-27.

101. Kallman, F. & Grobstein, C. (1964) Fine structure of differentiating mouse pancreatic exocrine cells in trans filter culture. J. Cell. Biol. 20:399-413.

102. Ekholm, R., Zelander, T. & Edlund, Y. (1962) The ultra-structural organization of the rat exocrine pancreas. I. Acinar cells. J. Ultrastructr. Res. 7:61-72.

103. Maylie'-Pfenninger, M.F. & Jamieson, J.D. (1979) Distribution of cell surface saccharides on pancreatic cells. J. Cell Biol. 80:77-95.

104. Drenckhahn, D. & Mannherz, H.G. (1983) Distribution of actin and the actin-associated proteins myosin, tropomyosin, α-actin, vinculin, and villin in rat and bovine exocrine glands. Eur. J. Cell Biol. 30:167-176.

105. Storrie, B., Pool, R., Sachdeva, M. Maurey, K. & Oliver, C. (1984) Evidence for both prelysosomal and lysosomal intermediates in endocytic pathways. J. Cell Biol. 98:108-115.

106. Rosenzweig, S.A., Miller, L.J. & Jamieson, J.D. (1983) Identification and localization of cholecystokinin binding sites on rat pancreatic plasma membranes and acinar cells: A biochemical and autoradiographic study. J. Cell Biol. 96:1288-1297.

107. Larsen, W.J. (1977) Structural diversity of gap junctions: A review. Tissue Cell 9:373-394.

108. Simpson, I., Rose, B. & Loewenstein, W.R. (1977) Size limit of molecules permeating the junctional membrane channels. Science 195:294-296.

109. Rose, B. & Loewenstein, W.R. (1975) Permeability of cell junctions depends on local cytoplasmic activity. Nature 254:250-252.

110. Palade, G.E. (1975) Intracellular aspects of the process of protein synthesis. Science 189:347-358.

111. Walter, P., Gilmore, K. & Blobel, G. (1984) Protein translocation across the endoplasmic reticulum. Cell 38:5-8.

112. Gilmore, R., Walter, P. & Blobel, G. (1982) Protein translocation across the endoplasmic reticulum: Isolation and characterization of the SRP receptor. J. Cell Biol. 95:470-477.

113. Meyer, D., Krause, E. & Dobberstein, B. (1982) Secretory protein translocation across membranes: The role of the docking protein. Nature 297:647-650.

114. Walter, P. & Lingappa, V.R. (1986) Mechanisms of protein translocation across the endoplasmic reticulum membrane. Annu. Rev. Cell Biol. 2:499-516.

115. Caplan, M.J., Rosezweig, S.A. & Jamieson, J.D. (1986) Processing and sorting of proteins synthesized in the endoplasmic reticulum. In: Physiology of the Membrane Disorders, (Andreoli, T.E., Hoffman, J.P., Fanestil, D.D. & Schultz, S.G., eds.) pp. 273-281, Plenum Publishing Corp., New York.

116. Anfinson, C.B. (1967) The formation of the tertiary structures of protein. Harvey Lect. 61:95-116.

117. Li, E., Tabas, I. & Kornfeld, S. (1978) The synthesis of complex-type oligosaccharides. I. Structure of the lipid-linked oligosaccharide precursor of the complex-type oligosaccharide of the vesicular stomatitis virus G protein. J. Biol. Chem. 253:7762-7771.

118. Jamieson, J.D. & Palade, G.E. (1968) Intracellular transport of secretory proteins in the pancreatic exocrine cell. III. Dissociation of intracellular transport from protein synthesis. J. Cell Biol. 39:580-588.

119. Kelly, R.B. (1985) Pathways of protein secretion in eukaryotes. Science 230:24-32.

120. Ermak, T.H. & Rothman, S.S. (1978) Internal organization of the zymogen granule: Formation of reticular structures in vitro. J. Ultrastr. Res. 64:98-113.

121. Douglas, W.W. (1968) Stimulus-secretion coupling: The concept and clues from chromaffin and other cells. Br. J. Pharmacol. 34:451-474.

122. Hootman, S.R. & Williams, J.A. (1987) Stimulus-secretion coupling in the pancreatic acinus. In: Physiology of the Gastrointestinal Tract, 2nd ed., (Johnson, L.R., ed.) pp. 1129-1146, Raven Press, New York.

123. Schulz, I. & Stolze, H.H. (1980) The exocrine pancreas: The role of secretagogues, cyclic nucleotides, and calcium in enzyme secretion. Annu. Rev. Physiol. 42:127-156.

124. Robberecht, P., Conlon, T.P. & Gardner, J.D. (1976) Interaction of porcine vasoactive intestinal peptide with dispersed pancreatic acinar cells from guinea pig. J. Cell Biol. 251:4635-4639.

125. Gardner, J.D. & Jensen, R.T. (1987) Secretagogue receptors on pancreatic acinar cells. In: Physiology of the Gastrointestinal Tract, (Johnson, L.R., ed.) pp. 1109-1119, Raven Press, New York.

126. Sankaran, H., Goldfine, I.D., Deveney, C.W., Wong, K.Y. & Williams, J.A. (1980) Binding of cholecystokinin to high-affinity receptors on isolated rat pancreatic acini. J. Biol. Chem. 255:1849-1853.

127. Yu, D.H., Huang, S.C., Wank, S.A., Mantey, S., Gardner, J.D. & Jensen, R.T. (1990) Pancreatic receptors for cholecystokinin: evidence for three receptor classes. Am. J. Physiol. 258:G86-G95.

128. Orchard, J.L., Davis, J.S., Larson, R.E. & Farese, R.V. (1984) Effects of carbachol and pancreozymin (cholecystokinin-octapeptide) on polyphosphoinositide metabolism in the rat pancreas in vitro. Biochem. J. 217:281-287.

129. Streb, H., Bayerdorffer, E., Haase, W., Irvine, R.F. & Shulz, I. (1984) Effect of inositol-1,4,5-trisphosphate on isolated subcellular fractions of rat pancreas. J. Membr. Biol. 81:241-253.

130. Williams, J.A. & Hootman, S.R. (1986) Stimulus-secretion coupling in pancreatic acinar cells. In: The Exocrine Pancreas, (Go, V.L.W., ed.) pp. 123-138, Raven Press, New York.

131. Broeckman, M.J. (1984) Phosphatidylinositol-4,5-bisphosphate may represent the site of release of plasma membrane-bound calcium upon stimulation of human platelets. Biochem. Biophys. Res. Commun. 120:226-231.

132. Cheung, W.Y. (1980) Calmodulin plays a pivotal role in cellular regulation. Science 207:19-27.

133. Tsien, R.Y., Pozzan, T. & Rink, T.J. (1981) Calcium homeostasis in intact lymphocytes: Cytoplasmic free calcium monitored with a new intracellular trapped flourescent indicator. J. Cell Biol. 94:325-334.

134. Nishizuka, Y. (1984) The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature 308:693-698.

135. Burnham, D.B., Munowitz, P., Hootman, S.R. & Williams, J.A. (1985) Regulation of protein phosphorylation in pancreatic acini. Distinct effects of Ca++ ionophore A23187 and 12-O-tetredecanoylphorbol 13-acetate. Biochem. J. 235:125-131.

136. Gardner, J.D., Korman, L.Y., Walker, M.D. & Sutliff, V.E. (1982) Effects of inhibitors of cyclic nucleotide phosphodiesterase on the actions of vasoactive intestinal peptide and secretin on pancreatic acini. Am J. Physiol. 242:G547-551.

137. Long, B.W. & Gardner, J.D. (1977) Effects of cholecystokinin of adenylate cyclase activity in dispersed pancreatic acinar cells. Gastroenterology 73:1008-1014.

138. Trimble, E.R., Bruzzone, R., Biden, T.J. & Farese, R.V. (1986) Secretin induces rapid increases in inositol trisphosphate, cytosolic Ca++ and diacylglycerol as well as cyclic AMP in rat pancreatic acini. Biochem. J. 239:257-261.

139. Rutter, W.J., Przyybyla, A.E., MacDonald, R.J., Harding, J.D., Chirgwin, J.M. & Pictet, R.L. (1978) Pancreas development: an analysis of differentiation at the transcriptional level. In: Cell Differentiation and Neoplasia, pp. 487-508, Raven Press, New York.

140. Swift, G.H., Craik, C.S., Stary, S.J., Quinto, C., Lahaie, R.G., Rutter, W.J. & MacDonald, R.J. (1984) Structure of the two related elastase genes expressed in the rat pancreas. J. Biol. Chem. 259:801-806.

141. Boulet, A.M., Erwin, C.R. & Rutter, W.J. (1986) Cell-specific enhancers in the rat exocrine pancreas. Proc. Natl. Acad. Sci. 83:3599-3603.

142. Cockell, M., Stevenson, B.J., Strubin, M., Hagenbuchle, O. & Wellauer, P..K. (1989) Identification of a cell-specific DNA-binding activity that interacts with a transcriptional activator of genes expressed in the acinar pancreas. Mol. Cell. Biol. 9:2464-2476.

143. Kruse, F., Komro, C.T., Michnoff, C.H. & MacDonald, R.J. (1988) The cell-specific elastase I enhancer comprises two domains. Mol. Cell. Biol. 8:893-902.

144. Osborn, L., Rosenberg, M.P., Keller, S.A. Ting, C.N. & Meisler, M.H. (1988) Insulin response of a hybrid amylase/CAT gene in transgenic mice. J. Biol. Chem. 263:16529-16522.

145. Hammer, R.E., Swift, G.H., Ornitz, D.M., Quaife, C.J., Palmiter, R.D., Brinster, R.L. & MacDonald, R.J. (1987) The rat elastase I regulatory element is an enhancer that directs correct cell specificity and developmental onset of expression in transgenic mice. Mol. Cell. Biol. 7:2956-2967.

146. Meister, A., Weinrich, S.L., Nelson, C. & Rutter, W.J. (1989) The chymotrypsin enhancer core: specific factor binding and biological activity. J. Biol. Chem. 264:20744-20751.

147. Nelson, C., Shen, L., Meister, A., Fodor, E. & Rutter, W.J. (1990) Pan: a transcriptional regulator that binds chymotrypsin, inaulin and AP-4 enhancer motifs. Genes & Devel. 4:1035-1043.

148. Howard, G., Keller, P.R., Johnson, T.M. & Meisler, M.H. (1989) Binding of a pancreatic nuclear protein is correlated with amylase enhancer activity. Nucl. Acid Res. 17(20):8185-8105.

149. Ben Abdeljlil, A., Visani, A.M. & Desnuelle, P. (1963) Adaptation of the exocrine secretion of rat pancreas to the composition of the diet. Biochem. Biophys. Res. Commun. 10:112-116.

150. Grossman, M.I., Greengard, H. & Ivy, A.C. (1942) The effect of dietary composition on pancreatic enzymes. Am. J. Physiol. 138:676-682.

151. Gidez, L.I. (1973) Effect of dietary fat on pancreatic lipase levels in the rat. J. Lipid Res. 14:169-177.

152. Dagorn, J.C. & Lahaie, R.G. (1981) Dietary regulation of pancreatic protein synthesis. I. Rapid and specific modulation of enzyme synthesis by changes in dietary composition. Biochim. Biophys. Acta 654:111-118.

153. Reboud, J.P., Marchis-Mouren, G., Cozzone, A. & Desnuelle, P. (1966) Variation in the biosynthesis rate of pancreatic amylase and chymotrypsinogen in response to a starch-rich or protein-rich diet. Biochem. Biophys. Res. Commun. 22:94-99.

154. Wicker, C. & Puigserver, A. (1987) Effects of inverse changes in dietary lipid and carbohydrate on the synthesis of some pancreatic secretory proteins. Eur. J. Biochem. 162:25-30.

155. Giorgi, D., Bernard, J.P., Lapointe, R. & Dagorn, J.C.. (1984) Regulation of amylase messenger RNA concentration in rat pancreas by food content. EMBO J. 3:1521-1524.

156. Giorgi, D., Renaud, W., Bernard, J.P. & Dagorn, J.C. (1985) Regulation of proteolytic enzyme activities and mRNA concentrations in rat pancreas by food content. Biochem. Biophys. Res. Commun. 127:937-942.

157. Wicker, C., Puigserver, A. & Scheele, G. (1984) Dietary regulation of levels of active mRNA coding for amylase and serine protease zymogens in the rat pancreas. Eur. J. Biochem. 139:381-387.

158. Wicker, C., Scheele, G. & Puigserver, A. (1988) Pancreatic adaptation to dietary lipids is mediated by changes in lipase mRNA. Biochimie 70:1277-1283.

159. Wicker, C. & Puigserver, A. (1990) Expression of rat pancreatic lipase gene is modulated by a lipid-rich diet at a transcriptional level. Biochem. Biophys. Res. Comm. 166(1):358-364.

160. Desnuelle, P., Reboud, J.P. & Ben Abdeljlil, A.. (1962) Influence of the composition of the diet on the enzyme content of rat pancreas. In: Ciba Foundation Symposium on the Exocrine Pancreas, (de Reuck, A.V.S. & Cameron, M.P., eds.) pp. 90-114, Little, Brown, Boston.

161. Grossman, M.I., Greengard, H. & Ivy, A.C. (1943) On the mechanism of the adaptation of pancreatic enzymes to dietary composition. Am. J. Physiol. 141:38-41.

162. Lee, S.S., Nitsan, Z. & Liener, I.E. (1984) Growth, protein utilization and secretion of pancreatic enzymes by rats in response to elevated levels of dietary protien. Nutr. Res. 4:867-876.

163. Schick, J., Verspohl, R., Kern, H. & Scheele, G. (1984) Two distinct adaptive responses in the synthesis of exocrine pancreatic enzymes to inverse changes in protein and carbohydrate in the diet. Am. J. Physiol. 247:G6110616.

164. Temler, R.S., Dormond, C.A., Simon, E., Morel, B. & Mettraux, C. (1984) Response of rat pancreatic proteases to dietary proteins, their hydrolysates and soybean trypsin inhibitor. J. Nutr. 114:270-278.

165. Lavau, M., Bazin, R. & Herzog, J. (1974) Comparative effects of oral and parenteral feeding on pancreatic enzymes in the rat. J. Nutr. 104:1432-1437.

166. Hong, S.S. & Magee, D.F. (1957) Influence of dietary amino acids on pancreatic enzymes. Am. J. Physiol. 197:71-74.

167. Johnson, A., Hurwitz, R. & Kretchmer, N. (1977) Adaptation of rat pancreatic amylase and chymotrypsinogen to changes in diet. J. Nutr. 107:87-96.

168. Snook, J.T. (1969) Factors in whole-egg protein influencing dietary induction of increases in enzyme and RNA levels in rat pancreas. J. Nutr. 97:286-294.

169. Snook, J.T. (1965) Dietary regulation of pancreatic enzyme synthesis, secretion and inactivation in the rat. J. Nutr. 87:297-305.

170. Reboud, J.P., Ben Abdeljlil, A. & Desnuelle, P. (1962) Variations de la teneur en enzymes du pancreas de rat en fonction de la composition des regimes. Biochim. Biophys. Acta 58:326-337.

171. Bazin, R., Lavau, M. & Herzog, J. (1978) Pancreatic lipase and ketogenic conditions. Biomedicine 28:160-165.

172. Deschodt-Lanckman, M., Robberecht, P., Camus, J. & Christophe, J. (1971) Short-term adaptation of pancreatic hydrolases to nutritional and physiological stimuli in adult rats. Biochimie 53:789-796.

173. Robberecht, P., Deschodt-Lanckman, M., Camus, J., Bruylands, J. & Christophe, J. (1971) Rat pancreatic hydrolases from birth to weaning and dietary adaptation after weaning. Am. J. Physiol. 221:376-381.

174. Sabb, J.E., Godfrey, P.M. & Brannon, P.M. (1986) Adaptive response of rat pancreatic lipase to dietary fat: Effect of amount and type of fat. J. Nutr. 116:892-899.

175. Owen, O.E., Reichard, G.A., Markus, H., Boden, G., Mozzoli, M.A. & Shuman, C.R. (1973) Rapid intravenous sodium acetoacetate infusion in man. Metabolic and kinetic responses. J. Clin. Invest. 52:2606-2616.

176. Saraux, B., Girard-Globa, A., Ouagued, M. & Vacher, D. (1982) Response of the exocrine pancreas to quantitative and qualitative variations in dietary lipids. Am. J. Physiol. 243:G10-G15.

177. Ben Abdeljlil, A. & Desnuelle, P. (1964) Sur l'adaptation des enzymes exocrines du pancreas a la composition du regime. Biochim. Biophys. Acta 81:136-149.

178. Snook, J.T. (1971) Dietary regulation of pancreatic enzymes in the rat with emphasis on carbohydrate. Am. J. Physiol. 221:1383-1387.

179. Marchis-Mouren, G., Pasero, L. & Desnuelle, P. (1963) Further studies on amylase biosynthesis by pancreas of rats fed on a starch-rich or casein-rich diet. Biochem. Biophys. Res. Commun. 13:262-266.

180. Howard, F. & Yudkin, J. (1963) Effect of dietary change upon amylase and trypsin activities of the rat pancreas. Br. J. Nutr. 17:281-294.

181. Folsch, U.R., Grieb, N., Caspary, W.F. & Creutzfeldt, W. (1981) Influence of short and long term feeding of an α-amylase inhibitor (Bay e 4609) on the exocrine pancreas of the rat. Digestion 21:74-82.

182. Sesso, A. & Migliornni, R. (1959) Nucleic acid content and amylase activity in the pancreas of the rat following adrenalectomy and cortisone administration. Acta Phsyiol. Lat. Am. 9:5-12.

183. Mossner, J., Bohm, S. & Fishbach, W. (1989) Role of glucocorticoids in the regulation of pancreatic amylase synthesis. Pancreas 4(2):194-203.

184. Williams, J.A. & Golfine, I.D. (1986) The insulin-acinar relationship. In: The Exocrine Pancreas, (Go, V.L.W., ed.) pp. 347-360, Raven Press, New York.

185. Bazin, R. & Lavau, M. (1979) Diet composition and insulin effect on amylase to lipase ratio in pancreas of diabetic rats. Digestion 19:386-391.

186. Palla, J.C., Ben Abdeljlil, A. & Desnuelle, P. (1968) Action de l'insuline sur la biosynthese de l'amylase et de quelques autres enzymes du pancreas de rat. Biochim. Biophys. Acta 158:25-35.

187. Korc, M., Owerbach, D., Quinto, C. & Rutter, W.J. (1981) Pancreatic islet-acinar cell interaction: Amylase messenger RNA levels are determined by insulin. Science 213:351-353.

188. Ben Abdeljlil, A., Palla, J.C. & Desnuelle, P. (1965) Effect of insulin on pancreatic amylase and chymotrypsinogen. Biochem. Biophys. Res. Commun. 18:71-75.

189. Osborn, L.O., Rosenberg, M.P., Keller, S.A. & Meisler, M. (1987) Tissue-specific and insulin-dependent expression of a pancreatic amylase gene in transgenic mice. Mol. Cell. Biol. 7:326-334.

190. Osborn, L.O., Rosenberg, M.P., Keller, S.A., Ting, C.N. & Meisler, M. (1988) Insulin response of a hybrid amylase/CAT gene in transgenic mice. J. Biol. Chem. 263:16519-16522.

191. Danielsson, A., Marklund, S. & Stigbrand, T. (1974) Effects of starvation and islet hormones on the synthesis of amylase in isolated exocrine pancreas of the mouse. Acta Hepato-Gastroenterol. 21:289-297.

192. Rausch, U., Rudiger, K., Vasiloudes, P., Kern, H. & Scheele, G. (1986) Lipase synthesis in the rat pancreas in regulated by secretin. Pancreas 6:522-528.

193. Rausch, U., Vasiloudes, P., Rudiger, K. & Kern, H.F. (1985) In-vivo stimulation of rat pancreatic acinar cells by infusion of secretin. I. Changes in enzyme content, pancreatic fine structure and total rate of protein synthesis. Cell Tissue Res. 242:633-639.

194. Rausch, U., Vasiloudes, P., Rudiger, K. & Kern, H.F. (1985) In-vivo stimulation of rat pancreatic acinar cells by infusion of secretin. II. Changes in individual rates of enzyme and isoenzyme biosynthesis. Cell Tissue Res. 242:641-644.

195. Brannon, P.M. (1990) Adaptation of the exocrine pancreas to diet. Annu. Rev. Nutr. 10:85-105.

196. Faichney, A., Chey, W.Y., Kim, Y.C., Lee, K.Y., Kim, M.S. & Chang, T.M. (1981) Effect of sodium oleate on plasma secretin concentration and pancreatic secretion in dog. Gastroenterology 81:458-462.

197. Lewis, L.D., Rosewics, S., Liddle, R.A. & Logsdon, C.D. (1990) Pancreatic adaptation involves regulation of gene expression by cholecystokinin. In: Gastrointestinal Endocrinology: Receptors and Post-Receptor Mechanisms, (Thompson, J.C., ed.) pp.241-256, Academic Press, New York.

198. Green, G.M., Levan, V.H. & Liddle, R.A. (1986) Interaction of dietary protein and trypsin inhibitor on plasma cholecystokinin and pancreatic growth in rats. Adv. Exp. Med. Biol. 199:123-132.

199. Solomon, T.E., Petersen, H., Elashoff, J. & Grossman, M.I. (1978) Interaction of caerulein and secretin on pancreatic size and composition in the rat. Am. J. Physiol. 235:E714-E719.

200. Luk, G.D. & Yang, P. (1987) Polyamines in intestinal and pancreatic adaptation. Gut 28(S1):95-101.

201. Pegg, A.E. & McCann, P.P. (1982) Polyamine metabolism and function. Am J. Physiol. 243:C212-221.

202. Morisset, J., Sarfati, P. & Grondin, G. (1986) Immunocytochemical demonstration of ornithine decarboxylase in the rat exocrine pancreas using the protein-A-gold technique. Can. J. Physiol. Pharmacol. 64:444-448.

203. Renaud, W., Giorgi, D., Iovanna, J. & Dagorn, J.C. (1986) Regulation of concentrations of mRNA for amylase, trypsinogen I and chymotrypsinogen B in the rat pancreas by secretagogues. Biochem. J. 235:305-308.

204. Steinhilber, W., Poensgen, J., Rausch, U., Kern, H.F. & Scheele, G.A. (1988) Translational control on anionic trypsinogen and amylase synthesis in rat pancreas in response to caerulein stimulation. Proc. Natl. Acad. Sci. USA 85:6597-6601.

205. Schick, J., Kern, H. & Scheele, G. (1984) Hormonal stimulation in the exocrine pancreas results in coordinate and anticoordinate regulation of potein synthesis. J. Cell Biol. 99:1569-1574.

206. Wicker, C., Puigserver, A., Rausch, U., Scheele, G. & Kern, H. (1985) Multiple-level caerulein control of the gene expression of secretory proteins in the rat pancreas. Eur. J. Biochem. 151:461-466.

207. Fushiki, T. & Iwai, K. (1989) Two hypotheses on the feedback regulation of pancreatic enzyme secretion. FASEB J. 3:121-126.

208. Logsdon, C.D. (1986) Stimulation of pancreatic acinar cell growth by CCK, epidermal growth factor, and insulin in vitro. Am. J. Physiol. 251:G487-494.

209. Williams, J.A. (1984) Regulatory mechanisms in pancreas and salivary acini. Annu. Rev. Physiol. 46:361-375.

210. Amsterdam, A. & Jamieson, J.D. (1973) Structural and functional characterization of isolated pancreatic exocrine cells. J. Cell Biol. 69:3028-3032.

211. Amsterdam, A. & Jamieson, J.D. (1974) Studies on dispersed pancreatic exocrine cells. I. Dissociation techniques and morphologic characteristics of separated cells. J. Cell Biol. 63:1037-1056.

212. Christophe, J.P., Conlon, T.P. & Gardner, J.D. (1976) Interaction of porcine vasoactive intestinal peptide with dispersed acinar cells from the guinea pig. Binding of radioiodinated peptide. J. Biol. Chem. 251:4629-4634.

213. Hootman, S.R., Ernst, S.A. & Williams, J.A. (1983) Secretagogue regulation of Na+/K+ pump activity in pancreatic acinar cells. Am. J. Physiol. 245:G339-G346.

214. Kondo, S. & Schulz, I. (1976) Calcium ion uptake in isolated pancreas cells induced by secretagogues. Biochim. Biophys. Acta 419:76-92.

215. Williams, J.A., Cary, P. & Moffat, B. (1976) Effects of ions on amylase release by dissociated pancreatic acinar cells. Am. J. Physiol. 231:1562-1567.

216. Bruzzone, R., Trimble, E.R., Gjinovci, A., Traub, O., Willecke, K. & Meda, P. (1987) Regulation of pancreatic exocrine function: A role for cell-to-cell communication? Pancreas 2:262-271.

217. Meda, P., Bruzzone, R., Knodel, S. & Orci, L. (1986) Blockage of cell-to-cell communication within pancreatic acini is associated with increased basal release of amylase. J. Cell Biol. 103:475-483.

218. Meldolesi, J., Castiglioni, G., Parma, R., Nassivera, N. & De Camilli, P. (1978) Ca++-dependent disassembly and reassembly of accluding junctions in guinea pig pancreatic acinar cells. J. Cell Biol. 79:156-172.

219. Brannon, P.M. (1990) Primary cultures of pancreatic acinar cells. In: Gastrointestinal Endocrinology: Receptors and Post-Receptor Mechanisms, (Thompson, J.C., ed.) pp.199-209, Academic Press, New York.

220. Oliver, C. (1980) Isolation and maintenance of differentiated exocrine gland acinar cells in vitro. In Vitro 16:297-305.

221. Bendayan, M., Duhr, M.A. & Gingras, D. (1986) Studies of pancreatic acinar cells in tissue culture: Basal lamina (basement membrane) matrix promotes three-dimensional reorganization. Eur. J. Biol. 42:60-67.

222. Oliver, C., Waters, J.F., Tolbert, C.L. & Kleinman, H.K. (1987) Growth of exocrine acinar cells on a reconstituted basement membrane gel. In Vitro Cell & Develop. Biol. 23:465-473.

223. Brannon, P.M., Orrison, B.M. & Kretchmer, N. (1985) Primary cultures of rat pancreatic acinar cells in serum-free medium. In Vitro Cell & Develop. Biol. 21:6-14.

224. Logsdon, C.D. & Williams, J.A. (1986) Pancreatic acinar cells in monolayer culture: Direct trophic effects of caerulein in vitro. Am. J. Physiol. 250:G440-G447.

225. Brannon, P.M., Demarest, A.S., Sabb, J.E. & Korc, M. (1986) Dietary modulation of epidermal growth factor action in cultured pancreatic acinar cells of the rat. J. Nutr. 116:1306-1315.

226. Justice, J.D., Kemper, K.A., Kenny, S. & Brannon, P.M. (1988) Effects of caerulein on enzymes of cultured pancreatic acinar cells. FASEB J. 2(4):50358.

227. Justice, J.D. & Brannon, P.M. (1989) Synthesis of amylase by cultured rat pancreatic acinar cells: Effects of antecedent diet. J. Nutr. 119:805-812.

228. Kemper, K.A., Sabb, J.E. & Brannon, P.M. (1987) Effects of diet and ketones on pancreatic lipase in cultured acinar cells. Fed. Proc. 46:1469.

229. Williams, J.A., Korc, M. & Dormer, R.L. (1978) Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am. J. Physiol. 235:E517-E524.

230. Peikin, S.R., Rottman, A.J., Batzri, S. & Gardner, J.D. (1978) Kinetics of amylase release by dispersed acini prepared from guinea pig pancreas. Am. J. Physiol. 235:E743-E749.

231. Bruzzone, R., Halban, P.A., Gjinovci, A. & Trimble, E.R. (1985) A new, rapid method for preparation of dispersed pancreatic acini. Biochem. J. 226:621-624.

232. Sankaran, H., Goldfine, I.D., Bailey, A., Licko, V. & Williams, J.A. (1982) Relationship of CCK receptor binding to regulation of biological functions in pancreatic acini. Am. J. Physiol. 242:G250-G257.

233. Williams, J.A., Sankaran, H., Korc, M. & Goldfine, I.D. (1981) Receptors for cholecystokinin and insulin in isolated pancreatic acini: Hormonal control of secretion and metabolism. Fed. Proc. 40:2497-2502.

234. Williams, J.A., Sankaran, H., Roach, E. & Goldfine, I.D. (1982) Quantitative electron microscope autoradiographs of ^{125}I-cholecystokinin in pancreatic acini. Am. J. Physiol. 243:G291-G296.

235. Logsdon, C.D. & Williams, J.A. (1983) Pancreatic acini in short-term culture: Regulation by EGF, carbachol, insulin, and corticosterone. Am. J. Physiol. 244:G675-G682.

236. Reddy, J.K. & Rao, M.S. (1977) Transplantable pancreatic carcinoma of the rat. Science 198:79-80.

237. Rao, M.S. & Reddy, J.K. (1979) Transplantable acinar cell carcinoma of the rat pancreas. Am. J. Pathol. 94:333-348.

238. Iwanij, V., Hull, B.E. & Jamieson, J.D. (1982) Structural characterization of a rat acinar cell tumor. J. Cell Biol. 95:727-733.

239. Iwanij, V. & Jamieson, J.D. (1982) Biochemical analysis of secretory proteins synthesized by normal rat pancreas and by pancreatic acinar tumor cells. J. Cell Biol. 95:734-741.

240. Iwanij, V. & Jamieson, J.D. (1982) Comparison of secretory protein profiles in developing rat pancreatic rudiments and rat acinar tumor cells. J. Cell Biol. 95:742-746.

241. Logsdon, C.D., Mossner, J., Williams, J.A. & Goldfine, I.D. (1985) Glucocorticoids increase amylase mRNA levels, secretory organelles and secretion in pancreatic acinar AR42J cells. J. Cell Biol. 100:1200-1208.

242. Desnuelle, P. & Figarella, C. (1979) Biochemistry. In: The Exocrine Pancreas. (Howat, H. T. & Sarles, H., eds.), pp. 86-125, W. B. Saunders Co., Ltd., Philadelphia.

243. Schneeman, B.O. & Gallaher, D. (1980) Changes in small intestinal digestive enzyme activity and bile acids with dietary cellulose in rats. J. Nutr. 110:584-590.

244. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

245. Naher, G. (1974) Lipase titrimetric assay. In: Methods of Enzymatic Analysis, (Bermeyer, H. U., ed.) vol. 2, pp. 814-818, Academic Press, New York.

246. Ouagued, M., Saraux, B. & Girard-Globa, A. (1982) A rapid and reliable method for determination of colipase and total lipase in pancreatic tissue. Biochimie 64:301-308.

247. Steel, R.G.O. & Torie, J.H. (1960) Principles and Procedures of Statistics. McGraw Hill Book Co., Inc., New York.

248. Isaksson, G., Lilja, P., Lunquist, I. & Ihse, I. (1983) Influence of dietary fiber on exocrine pancreatic function in the rat. Digestion 27:57-62.

249. Stock-Damge, C., Bouchet, P., Dentinger, A., Aprahamian, M. & Grenier, J. F. (1983) Effect of dietary fiber supplementation of the secretory function of the exocrine pancreas in the dog. Am. J. Clin. Nutr. 38:843-848.

250. Sheard, N.F. & Schneeman, B.O. (1980) Wheat bran's effect of digestive enzyme activity and bile acid levels in rats. J. Food Sci. 45:1645-1648.

251. Lafont, H., Lairon, D., Vigne, J.L., Chanussot, F., Chabert, C., Portugal, H., Pauli, A.M., Crotte, C., Hauton, J.C. (1985) Effect of wheat bran, pectin and cellulose on the secretion of bile lipids in rats. J. Nutr. 115:849-855.

252. Lee, S.S., Nitsan, Z., Liener, I.E. (1984) Growth, protein utilization and secretion of pancreatic enzymes by rats in response to elevated levels of dietary protein. Nutr. Res. 4:867-876.

253. Owen, O.E., Markus, H., Sarshik, S. & Mozzoli, M. (1973) Relationship between plasma and muscle concentrations of ketone bodies and free fatty acids in fed, starved and alloxan-diabetic states. Biochem. J. 134:499-506.

254. Green, G., Friestman, J., Toguchi, S., Chey, W. & Liddle, R. (1987) Role of CCK and secretin in pancreatic response to fat in the rat. Dig. Dis. Sci. 32(10):1167.

255. Ceska, M., Hultman, E. & Ingelman, B.G.A. (1969) A new method for determination of α-amylase. Experientia 25:555-556.

256. Cuttolico, R.A. & Gibbs, S.P. (1975) Rapid filter method for the microfluorometric analysis of DNA. Anal. Biochem. 69:572-582.

257. Soling, H.D. & Unger, K.O. (1972) The role of insulin in the regulation of alpha-amylase synthesis in the rat pancreas. Eur. J. Clin. Invest. 2:199-212.

258. Wicker C. & Puigserver A. (1989) Changes in mRNA levels of rat pancreatic lipase in the early days of consumption of a high lipid diet. Eur. J. Biochem. 180:563-567.

259. Rosewicz S., Lewis, L.D., Wang, X.V., Liddle, R.A. & Logsdon, C.D. (1989) Pancreatic digestive enzyme gene expresion: effects of CCK and soybean trypsin inhibitor. Am. J. Physiol. 256:G733-738.

260. Erlanger, B.F., Kokwsky, N. & Cohen, W. (1961) The preparation of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. 95:271-278.

261. Erlanger, B.F., Edel, F. & Cooper, A.G. (1966) The action of chynotrypsin on two new chromogenic substrates. Arch. Biochem. Biophys. 115:206-210.

262. Bieth, J., Spiess, B. & Wermuth, C.G. (1974) The synthesis and analytical use of a highly sensitive and convenient substrate of elastase. Biochem. Med. 11:350-357.

263. Scheele G. (1975) Two-dimensional gel analysis of soluble proteins; characterization of guinea pig exocrine pancreatic proteins. J. Biol. Chem. 250:5375-5385.

264. Brannon, P.M., Hirschi, K. & Korc, M. (1988) Effects of epidermal growth factor, insulin and insulin-like growth factor I on rat pancreatic acinar cells cultured in serum-free medium. Pancreas 3(1):41-48.

265. Duthie, H.L. & Wormsley, K.G. (1979) Scientific Basis of Gastroenterology. Churchill Livingstone, New York.

266. Morisset, J. & Benrezzak, O. (1984) Polyamines and pancreatic growth induced by caerulein. Life Sci. 35:2471-2480.

267. Rosewicz, S., Lewis, L.D., Liddle, R.A. & Logsdon, C.D. (1988) Effects of cholecystokinin on pancreatic ornithine decarboxylase gene expression. Am. J. Physiol. 255:G818-821.

268. O'Farrell, P.H., Goodman, H.M. & O'Farrell, P.H. (1977) High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133-1142.

269. Scheele, G.A., Pash, J. & Bieger, W. (1981) Identification of proteins according to bioloical activity following separation by two-dimedsional isoelectric focussing/sodium dodecyl sulfate gel electrophoresis: analysis of human exocrine pancreatic proteins. Anal. Biochem. 112:304-313.