THE TEST MEAL

AND

EXOCRINE PANCREATIC FUNCTION

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by

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THIS THESIS IS DEDICATED TO MY PARENTS, TEACHERS AND FRIENDS

"BIOLOGICAL PHENOMENA HAVE A COMPLEXITY

NOT FOUND AMONG THE WORLD

OF THE PHYSICIST"

T.D.Sterling.

(Fed.Proc.24.5.1965.

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S E C T I O N I

Chapter I

INTRODUCTION

The pancreas is a very inaccessible organ situated in the retroperitoneal space. Study of its physiology and function was first confined to animals, where it was possible to construct a pancreatic fistula and thus collect pure pancreatic juice. In addition, pancreatic tissue from animals was also obtainable relatively easily.

Early attempts to study the pancreas in man was restricted to patients with pancreatic fistulae, usually the result of surgical procedures. These investigations were on the whole unsatisfactory as the conditions of the experiment were not truely physiological and the juice soon became contaminated and infected.

Another method employed later used the intraduodenal tube to collect duodenal contents. The aspirate consisted of a mixture of gastric acid and contents, duodenal juice, bile and succus entericus. Various meals were used to stimulate the pancreatic secretion. Other authors have emphasized the importance of preventing contamination of duodenal contents with gastric juice, and by inserting a second tube or double lumen tube to aspirate the acid from the stomach this was attained. Meal stimulation was now no longer possible so that various drugs and later the two hormones, secretin and pancreozymin, were used to stimulate pancreatic secretion. These two hormones very soon completely replaced any other method of pancreatic stimulation. The intravenous injection or infusion of secretin and/or pancreozymin is, however, not a physiological procedure. The initial enthusiasm aroused by this method was tempered due to the varied results obtained by different workers.

More recently, Lund (253) has used the test meal to stimulate

pancreatic secretion. This technique has been replicated by numerous authors, with promising results reported by all. These reports are characterized by the varied nature of the test meal used, the different position of the intraduodenal tube, the type of suction employed, the period of collection, the length of the test and the type of enzymes estimated.

The purpose of the present study is to investigate the physiological responses to the test meal, to define its value in the investigation of pancreatic function and to try and standardise the procedure to obtain optimal pancreatic stimulation.

Chapter 2

PHYSIOLOGICAL STUDIES OF THE EXOCRINE PANCREAS

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This chapter reviews the present knowledge on the structure and function of the pancreatic exocrine cells, the secretions of these cells and the hormones which influence this secretion.

A. THE PANCREATIC EXOCRINE CELL AND ENZYME SYNTHESIS

1. Histology: The acinar cell is a fairly large cell with a well developed nucleus and nucleolus. The cell wall has two or three layers with an irregular apical portion and microvilli projecting into the lumen. These villi may arise partly as a result of fusion of the zymogen granules with the cell wall and the discharge of its contents into the lumen.

The cytoplasm has a granular appearance but its most characteristic structural component is made up of membranous structures (364, 366). Its other main components include the mitochondria, ribosomes, golgi apparatus and the zymogen granules (190). Between the cytoplasmic membranes, which are arranged between the zymogen granules in the apex of the cell and around the nucleus in the basal two thirds, is the ground substance or matrix of the cell.

The cytoplasmic membranes or cytomembranes (365), as they are sometimes called, consist of a double membrane arranged in pairs and bounding a narrow space. This is the appearance on cross section, but it has been proposed that it may, in fact, be a single continuous membrane bounding a space (308, 312).

A large number of fairly uniform sized particles are present in the matrix. The majority are attached to the external surface of the cytoplasmic membranes and give to this part the name of "rough surface" cytomembranes, as compared to "smooth surface" cytomembranes (313)

where no particles are attached. The rest are scattered in the cytoplasmic matrix. These particles have been found to be rich in ribonucleic acid (357) and have been referred to as RNA particles or ribosomes. Palade and Siekevitz (312) have found continuity between the smooth and rough surface membranes in some areas confirming their opinion that these are not unrelated structures. The term "microsome" (311) refers to that portion of the cell fraction separating out during fractionation which contains elements of the endoplasmic reticulum together with some attached ribosomes.

In the golgi region (centrosphere) located in the distal half of the cell, just above the nucleus, is a system of "smooth surface" membranes (i.e., without any ribosomes attached). These membranes are arranged in pairs with component membranes, either closely packed or separated by a vacuolar space of varying width. There are a number of granules of various shapes and sizes in the golgi region.

The zymogen granules (190) are present in the apical segment of the cell and are surrounded by a smooth membrane. They are rich in pancreatic enzymes but contain only traces of ribonucleic acid and phospholopids. Their diameter may range from 0.5 to 1.5 microns, and in the starved animal the granules may occupy a considerable part of the volume of the cell. The role of the zymogen granules in storage of the digestive enzymes will be discussed later.

The mitochondria of the acinar cell appear to be no different to that of other cells.

2. Enzyme Synthesis: It seems apparent as the result of a great deal of experimental work that two essential processes are taking place in the acinar cell resulting eventually in the secretion of the pancreatic enzymes. The first is the synthesis of the enzymes and the second is the transport of these enzymes from the site of synthesis

to the surface membrane of the cell, from where it may be discharged into the lumen. The study of this pathway has been made possible by the process of differential centrifugation (189, 190) in which the different elements of the acinar cell are separated and examined for their enzyme content. These have also been labelled with radioactive amino acids (6). This formation together with the known chemical properties of the various structures have contributed to an understanding of the whole process.

Synthesis of the pancreatic enzymes probably takes place in the microsomal fraction (356, 360). Radioactivity is highest in this fraction soon after the injection of the labelled amino acids (242, 243). Acid extracts of this fraction has shown the presence of a number of enzymes known to be stable to acid; viz., trypsin, chymotrypsinogen A and B, ribonuclease and deoxyribonuclease (225). Some authors have stated that the nuclear and mitochondrial fractions may synthesis protein (7, 32) but there is no conclusive evidence for this.

3. Enzyme Transport: The enzyme must be transported from the microsome fraction to the zymogen granules, where they are stored. Transportation seems to be an intrinsic function of the membranes within the exocrine cell, although the actual steps involved are still somewhat unclear. As previously stated, the ribosomes are attached to the endoplasmic reticulum and this constitutes the "rough" or cytomembranes. It would not, therefore, be out of place to assume that these membranes are involved in the first stage of enzyme transport. Palade (307) has shown the presence of intracisternal granules within the cavities formed by these membranes and has suggested that the enzymes synthesized by the attached ribosomes are transported across the membranes into the cavity. He also demonstrated that the specific radioactivity of chymotrypsinogen in the membrane fraction rises immediately behind that of the corresponding ribosomal fraction.

Morris and Dickson (291), however, found that the specific radioactivity was highest in the supernatant fluid soon after that which occurred in the microsomal fraction. They suggested that ribonuclease, which they investigated, may be transferred to the soluble portion of the cell before reaching the zymogen granule. Similar results have been obtained by Redman (335) for amylase. He found that a very large percentage of the amylase in the active pancreas of pigeons was in soluble form. The amylase secreted is thought to be derived mainly from this soluble form. He therefore felt that the amylase remained mainly in the cytoplasm and was not aggragated into the zymogen granules. Two routes have thus been postulated from the ribosome fraction - the one across the membrane into the cavity of the endoplamic reticulum (307) and the other from the ribosome into the cell matrix (243).

It would seem that both routes are probably utilised because, as Lin and Grossman (247) have pointed out, continuous stimulation of the pancreas with parasympathomimetic drugs while depleting the zymogen content of the cells still stimulates a high enzyme secretion from the pancreas.

Whatever route is initially involved, the next region of high activity of enzymes appears to be the golgi area. There is good microscopic evidence to support the view that this area immediately preceeds the zymogen granules in enzyme transport and is possibly intimately connected with the formation of the zymogen granule (310, 63,). Electron-microscopic studies (367) have demonstrated that the membranes of the zymogen granule are derived from the golgi membranes and that the enzymes are transported directly across the granule membranes. Morris and Dickson (291) and other authors (65) have shown the presence of granules within the golgi region which may represent different stages in the formation of the zymogen granule from minute granules to the well defined zymogen granule. Thus it seems that the golgi apparatus provides

the membranes for the formation of the zymogen granules and that these membranes actively accumulate the enzymes within their cavities, possibly coalescing to form the well formed and matrue granule (365, 368).

4. Enzyme Storage: Present evidence suggests that the zymogen granule probably has nothing to do with enzyme synthesis as administered radioactivity appears later in this fraction of the cell. Its main role seems to be the storage and release of enzymes. The zymogen granules have been separated by differential contrifugation (189, 190) and found to have the same qualitative and quantitative enzymic content as that of the pancreatic juice (trypsinogen, chymotrypsinogen A and B, procarboxypeptidase A and B)(70, 225). Similar results have been found for ribonuclease A and B (140). When the pancreatic juice contains a high enzyme content as the result of some stimulation, it is noted that the zymogen granules decrease (175) rapidly in number although they never disappear completely. If stimulation is continued over any length of time, the pancreatic juice secreted still contains a high content of enzymes despite the fact that the granules are now few in number. Thus it seems that the ribosomes (microsomes) are the site of synthesis and the zymogen granules the site of storage of the pancreatic enzymes.

5. Enzyme Secretion: When secretion takes place, the zymogen granule attaches itself to the plasma membrane of the cell and discharges its contents into the lumen. Palade (309) has suggested that this membrane from the zymogen granule now becomes part of the cell membrane and that an equal portion must be removed from the cell membrane. This removed portion is returned to the intracellular membranous structures - a process referred to as "reversed phagocytosis" by Hokin (191).

6. Time required for Synthesis, Transport and Secretion of the Enzymes: The time during which the digestive enzymes are synthesized within the acinar cell, stored and transported through the cell to appear in the pancreatic juice, is certainly much shorter than first indicated by earlier authors. The synthesis of protein molecules is very rapid (249) and takes only a minute or two. Various authors have indicated that the synthesis of the pancreatic enzymes takes less than three minutes (168, 291), while the transport through the cell takes less than one hour (168). Transport along the excretory ducts to the duodenum is very rapid (168, 221).

B. PANCREATIC ENZYMES

1. Introduction: The pancreatic juice contains a number of enzymes which play an important part in digestion; amylase for the digestion of starch; at least four proteolytic enzymes for the digestion of protein, namely, trypsin, chymotrypsin and carboxypeptidases A and B; ribonuclease and deoxyribonuclease for nucleic acid digestion; one phosphatidase for phosphatides; lipase for glycerol esters; and cholesterol esterase for cholesterol esters. The above enzymes are all synthesized within the acinous cells (226) of the pancreas and are finally stored within the zymogen granule (190, 312) to be discharged into the duct lumen (358) after secretion is stimulated.

The storage of active enzymes like lipase and amylase within the zymogen granules possibly has a protective value (98) to the cell and prevents intracellular digestion. As protein material is present within the zymogen granule, the proteolytic enzymes are present in inactive precursor forms which only become active once the enzymes are discharged into the small bowel.

2. Amylase: Human pancreatic amylases are usually referred to as α

or endoamylase. Alpha amylases are glycosidases, i.e., they hydolyse glycoside links in polysaccharides. This action takes place only at the 1,4, glucosidic bonds (415) in a supposedly random fashion (46). Two stages are apparent in the degradation of these bonds; the first stage is rapid, with the formation of maltose and maltotetroase, while in the second slower stage the latter product is converted to glucose and maltose.

Calcium is a component of amylase and plays an important role in stabilizing the enzyme against alkaline pH values (125), proteolytic enzymes (373) and elevated temperature. Calcium free amylases require the presence of calcium before they become catalytically active (197). Chloride ion and other anions activate amylase and each has a varying effect on the pH activity curve. The optimum pH of amylase activity is 6.7 to 7.2.

Amylase is secreted from the pancreas in active form (237). In the laboratory the most commonly used substrate to assay amylase activity is starch (69). The first product formed in the hydrolysis of starch are dextrins, giving a red colour when combined with iodine and changing to a blue colour when sufficient maltose is formed.

3. Trypsin: In 1932, Northrop and Kunitz (301) working together first isolated, in crystalline form, two proteolytic enzymes, trypsin and chymotrypsin, and their precursors from pancreatic extracts (302). As indicated by earlier authors, only the inactive form of trypsin-trypsinogen is present in the pancreatic juice and the spontaneous activation of trypsinogen to trypsin in pancreatic juice does not occur (118) in the normal pancreas. Activation of trypsinogen to trypsin begins immediately after the pancreatic juice enters the duodenum, through the action of a mucosal enzyme enterokinase and also autocatalytically by the action of the trypsin thus formed. The enterokinase acts by

splitting a hexapeptide, H_2N-Asp_4-Lys-CooH, from the trypsinogen (299). The pH optimum for the reaction is 7-9 for bovine trypsinogen (244).

The trypsin formed has a molecular weight of 23,8000 and it acts by hydrolysing peptides, amides and esters at the L-arginine or L-lysine linkages of these amino acids (300). The action of trypsin is modified both by the presence of inhibitors and the presence (or absence) of certain factors which effect its stability. These pancreatic trypsin inhibitors (PTI)(156, 223, 234) are present in the pancreatic juice and it has been suggested that, should spontaneous activation of trypsinogen to trypsin occur, it would be immediately neutralised by the PTI (118). It is unknown whether the inhibitor has any function other than this presumed function in the pancreatic juice. Recently Wohlman et al (428) have suggested that trypsin inhibitors from the pancreatic juice are still active in the intestine and may account for the fall off in activity lower down the intestine. They have not ruled out the possibility of the destruction of the enzyme proteins by the proteolytic activity per se. Calcium, magnesium and bile (117) have been reported to increase trypsin stability. Calcium ions protect trypsin from autodigestion, the greater amount of calcium ions, the greater activation (180). There is no decrease in activity of the enzyme in 7 hours if stored at 0 - 4°C. (252), but incubating at body temperature results in a rapid fall off of activity. Lyophilized preparations are stable for years if kept dry and cool.

The tests for assaying "trypsin" activity, such as those employing gelatin, haemoglobin, casein or X-Ray film as substrate (163) are not specific measurements of tryptic activity, but are a measure of overall proteolytic activity, including that of chymotrypsin and elastase. Recently a substrate has been synthesized called N-Benzoyl-L-Arginine ethyl ester (BAEE)(350) which, in the absence of thrombin, plasmin and kallidrein, is a specific substrate for the measurement of trypsin

activity (105, 300). Titration (181, 421) and spectrophotometric (350) methods using BAEE as a substrate are in common use.

4. Chymotrypsin: Chymotrypsin, like trypsin, is an endopeptidase and acts on peptide bonds (296). Like trypsin, too, it catalyses the hydrolysis of amide and ester bonds (300), but has no action on bonds involving carboxyl groups of lysine or arginine. The amino acid composition (423) of chymotrypsin has been worked out and more recently Mathews et al has constructed a 3-dimensional electron density map (276) for the enzyme. Its molecular weight is thought to be about 25,100 (424). A crystalline derivative (66) of chymotrypsinogen has been prepared.

Chymotrypsin is synthesized in the RNA particles (359) attached to the endoplastic reticulum. It is secreted into the pancreatic juice in its inactive form, chymotrypsinogen A. and B. Only when the pancreatic juice reaches the intestine does the enzyme become activated. The activation of chymotrypsin (45, 51, 113, 298) occurs in the presence of trypsin, chymotrypsin and enterokinase. This activation is influenced by trypsin concentration (306, 100), pH, temperature and presence of calcium (439, 141). The position of cleavage has been determined by numerous workers and the conditions for optimum activation have been studied.

The assay of chymotrypsin is relatively simple using a synthetic substrate such as tyrosine ethyl ester (TEE) or acetyl-tyrosine ethyl ester (ATEE).

5. Lipase: Lipase acts exclusively on emulsified esters and not on esters in solution. This action occurs in the interphase (346) of the emulsion and not in the oil or water phase.

Lipase is secreted by the pancreas in its active form. An inactive

"prolipase" (429) has been found by some workers, but not substantiated by others. The complete hydrolysis of triglycerides proceeds in a stepwise fashion, from the Tri-di-mono-glycerol fraction, at a decreasing rate of the reaction. Contrary to the findings of some earlier workers (101), Constantin et al (80) has shown that lipolysis may be complete.

Bile salts, pH (54) and calcium ions (426) are important for providing optimum conditions for lipase activity. Bile salts, besides their role in the emulsification of fats in the duodenum (254), also increase the rate and time of lypolysis (345). This may be accomplished by the bile salts preventing the inhibition of lipase activity which occurs in the presence of fatty acids (99). The effect of bile salts in shifting the optimal pH from 8 to 9 down to 6.0 to 6.5 (98) is of physiological importance. Purified lipase preparations have been prepared (30). Little was lost if the enzyme was stored following lyophilization for 90 days at 5°C. The presence of albumin, trypsin (157) and phospholipids (238) are all thought to influence lipase activity.

Esterases, which are also present in pancreatic juice, hydrolyse the esters of lower alcohols and fatty acids (100, 417). In contrast to lipase, this reaction can take place in solution.

6. Ribonuclease: Ribonuclease activity is present in the zymogen granule (140) but, unlike the proteolytic enzymes, the pathway to the granule may be through the cell matrix (291) rather than via the cell membranes.

The ribonucleases break down ribonucleic acid into its smaller components. The enzyme is acid stable and has an optimum pH for activity at 7.7. It is also reported to be magnesium independent (443) despite some reports to the contrary (246).

7. Carboxypeptidases: The carboxypeptidases are exopeptidases as they act on the terminal bonds of proteins and peptides. More specifically, they catalyze the hydrolysis of peptide bonds adjacent to the terminal carboxyl group of these substances. Two forms of carboxypeptidases are present, A (14) and B (129). The substrate specificity of these two forms are complementary. In the pancreas, these two carboxypeptidases are present in inactive zymogen forms, designated procarboxypeptidase A and B.

Procarboxypeptidase A is present in two forms (55, 57): The one form has three subunits (55, 441), one unit being the zymogen for carboxypeptidase A, while another has chymotrypsin-like endopeptidase activity and can hydrolyse ATEE. A number of crystalline bovine pancreatic carboxypeptidase A (5, 14) have been prepared, all of which appear to have the same specific enzyme activity (297). Carboxypeptidase A acts preferentially on peptide bonds adjacent to terminal amino acids residues with a 6-carbon ring side group, e.g., tyrosine, tryptophan, phenylalanine.

Carboxypeptidase B has also been prepared in crystalline form. Activation of procarboxypeptidase B is mediated by trypsin (86). The active enzyme selectively catalyzes the hydrolysis of those peptide bonds adjacent to terminal residues whose side groups end in NH_2, e.g., lysine or arginine.

The optimum pH for carboxypeptidase activity is 7.5. Zinc is firmly bound to the enzyme and appears to play an important part in the enzyme's activity (400). Other metals can, however, be substituted for zinc (71).

C. ENZYME INHIBITORS

In 1936, Kuniitz (234) crystallized a polypeptide trypsin inhibitor from bovine pancreas. This also inhibited chymotrypsin (440), kallikrein and thrombin (124). A second trypsin inhibitor was isolated from the bovine pancreas and was also later found to be present in the pancreatic juice. This inhibitor had no effect on chymotrypsin (59).

The exact role of these inhibitors is not completely clear. It is generally assumed that within the pancreas they combine with any free trypsin that may be present (180). A decrease in trypsin inhibitor activity in the post-operative state is thought to be responsible for some cases of pancreatitis. Trasylol is reported to raise the level of the trypsin inhibitor (289) and thus possibly modifies an attack of pancreatitis.

Kalser (223) has suggested that the trypsin inhibitor is secreted together with the enzymes from the acinar cells, the amount of inhibitor increasing with the increase of proteolytic acitivity in the pancreatic juice. When the pancreatic juice reaches the intestine, the trypsin inhibitor is inactivated by an extract of duodenal mucosa which was not enterokinase (223). The trypsin inhibitor has also been found to be inactivated by trypsin and chymotrypsin (156). Thus it seems that a trypsin inhibitor is present within the pancreas, possibly in the zymogen granules (142), and is secreted from the acinar cells together with the pancreatic enzymes into the pancreatic juice, where it combines with any free trypsin. Once in the duodenum, where its action is no longer needed, it is inactivated.

Naturally occuring trypsin inhibitors have been found to be present in soya (233) and lima beans (130, egg albumin, blood plasma and colustrum (245). An excess amount of uncooked soya bean in the diet of rats (255) and chicks (344) is reported to result in hypertrophy of

the pancreas and increased output of enzymes. It is thought to be due to a compensatory mechanism as consequence of trypsin inhibition in the duodenum. Similar experiments in man have produced equivocal results (24). In the test meals used in our project, trypsin inhibitors were not thought to be present.

D. BICARBONATE

Bicarbonate is an important constituent of the pancreatic juice, providing optimum conditions for enzyme action by neutralizing the acid from the stomach. The presence of carbonic anhydrase in the duct cells (40) of the pancreas has suggested that these cells are responsible for bicarbonate formation and secretion. This is supported by the effect of carbonic anhydrase inhibitors (109) in blocking the volume and bicarbonate output of the pancreas to secretin. More recent evidence, using micropuncture (331) techniques, excludes involvement of the ducts of the extralobular collecting system in bicarbonate secretion. Janowitz and Dreiling (210) point out that the presence of carbonic anhydrase may only be important in providing a critical intracellular pH and not directly involved in bicarbonate production. No definite proof is available as yet, but the general feeling is that bicarbonate is secreted by the centro-acinar and intralobular duct cells, with a possible contribution by the acinar cells of the pancreas.

Early investigations in dog (78) and man (210) have suggested that the concentration of bicarbonate in the pancreatic juice increases with increasing flow rates. More recently, however, Wormsley (431), using increasing doses of secretin has found that bicarbonate concentration decreases linearly with increasing rates of pancreatic secretion, except under certain specific conditions (430). These conditions include weak stimulation of the pancreas during the early and late phases of secretin

stimulation and when stimulation is prolonged.

E. ELECTROLYTES

The principle anions in pancreatic juice are bicarbonate and chloride. Sodium, potassium and calcium are the main cations. Chloride ion bears an inverse relationship to that of bicarbonate (237, 177, 110) and increases in association with increasing flow rates. The bicarbonate-chloride ratio, however, remains the same and is in the order of about 155 mEq/Litre in man (240).

The concentration of sodium and potassium in the pancreatic juice is similar to the concentration in plasma and varies with changes in blood plasma. The total output of sodium and potassium from the pancreas increases as the volume flow increases. The concentration of calcium is less than that present in the serum.

F. THE PANCREATIC HORMONES

Secretin and pancreozymin have been used extensively in the investigation of pancreatic physiology and function. The ingestion of a meal stimulates the secretion of endogenous gastrin from the gastric mucosa and secretin and pancreozymin from the duodenal mucosa. An understanding of the properties and function of these hormones is, therefore, important in the interpretation of the results produced by these hormones.

1. Secretin: Secretin has been found to be a basic linear polypeptide with 27 amino acid residues (292) and has a minimum molecular weight of 3,200 to 3,500. Its chemical structure has recently been worked out by Jorpes and Mutt (218, 293). The final

isolation of pure secretin was found to have an activity of 400,000 Hammasten cat units/mg. (218) compared to earlier preparations with only 1,500 cat units/mg. (215).

Secretin may be prepared in a dry form (404) and is then stable for many years. In this form it is rapidly soluble in water, dilute acids and alkalis. A crystalline (143) and synthetic preparation (50, 399) of secretin is available.

The intravenous injection of secretin in animals or man stimulates the pancreas to produce an abundant volume of clear, highly alkaline fluid low in enzymes. The latent period from the time of injection to commencement of a response is about 20 seconds. The volume rate increases to maximum within a few minutes, but may be delayed for as long as 13 minutes (404), and then decreases. With high doses, secretion may go on for a number of hours. Following the first period of maximum flow, Voegtlin et al (404) noted a secondary peak between the 7th and 25th minute in about 35% of their patients. The first few drops have a high enzyme content and this is thought to be due to a "wash out" effect (410) of the juice within the pancreatic ducts. This initial high enzyme content, however, decreases rapidly (404) to a low level and remains at this low level despite repeated injections (282) of secretin and changes in the flow of the pancreatic juice. This suggests that there is a basal rate of enzyme liberation which is unaffected by secretin. Other authors have, however, reported an initial transitory rise after a second dose of secretin given some time after the first dose. Friedman (132) suggests this may be due to the secretion of previously resting elements, as it is recognized that not all the zymogen granules discharge at once. Earlier reports (228, 375) of the enzyme-stimulating effect of secretin were almost certainly due to the fact that these preparations were impure and probably contained some pancreozymin. The work of Harper and Mackay (174) supported

this view. Histological examination of the pancreas after prolonged stimulation with secretin revealed that diminution in zymogen granules of the acinar cells did not occur. The existing evidence suggests that secretin does not cause an enzyme response but rather a "wash out" effect of released enzymes. The total volume of juice secreted (146, 155) and HCO_3 response is closely related to the dose of secretin injected. Increasing the dose of secretin will increase both the volume and bicarbonate response until a threshold is reached. This threshold appears to be higher with a constant intravenous infusion in comparison with a rapid intravenous injection (431).

Besides its secretory and bicarbonate effect on the pancreas, secretin has also been reported to stimulate bile flow (161, 213, 416). Earlier work suggested that the concentration of solids, pigments and bile salts (2, 4) was also increased following a secretin injection. This, however, was not confirmed by later investigators (161, 283) who reported a stimulating effect on bile flow only. The choleretic action of secretin is relatively slight in dogs (213, 416) and human subjects (161), although in the isolated pig liver (169) the bile produced following secretin stimulation had a concentration of bicarbonate and chloride as high as that in pancreatic juice.

The action of secretin on Brunner's glands is unclear, as some preparations of secretin appear to stimulate secretion (127) while others (154, 369) have no effect.

Secretin has an inhibitory effect on gastric acid secretion (134, 147, 214, 432) stimulated by food, antral irrigation and gastrin. There appears to be a maximal inhibitory dose of secretin producing about 70% inhibition of gastrin-stimulated acid secretion. More recently, Johnson and Grossman (211) have suggested that secretin is the only enterogastrone released by acid in the duodenum. Secretin is

reported to release insulin from the pancreas both in vitro and in vivo. This may be related to its structural similarity to glucogon (293).

2. Pancreozymin: With increasing purification of the original crude secretin extract, it soon became apparent that some other substance, possibly hormonal, was present which accounted for the enzyme stimulation occuring in these early preparations. Harper and Raper (173) were the first to separate this substance and found its action to be almost identical to that of vagal stimulation, i.e., it stimulated the pancreas to secrete a juice rich in enzymes but low in volume and bicarbonate. The action is resistant to atropine (173), unlike vagal stimulation which is inhibited by atropine. The experiments of Harper and Vass (172) suggested that this substance was a hormone. This was confirmed by Wang and Grossman (411), who observed the effects of a variety of excitants, administered by way of the intestine, on the secretin-stimulated secretion of pancreatic transplants.

The isolation of pancreozymin (87) has lagged behind that of secretin although it now seems possible that the final stage has been reached. Jorpes and Mutt (219, 292) have isolated pancreozymin with an activity of 18,000 Crick, Harper and Raper units/mg. These authors also stressed the parallel changes in activity of pancreozymin and cholecystokinin through all stages of purification, suggesting that this is one hormone and not two. Other authors have reported pancreozymin preparations (48, 144) without any cholecystokinin activity and have separated these two hormones electrophorectically. Besides the cholecystokinin effect (216), preparations of pancreozymin have also been shown to contain stimulants of pepsin secretion and intestinal muscle motility (183). This latter substance, which is probably

substance P (405), has been separated from pancreozymin, but more recent evidence suggests that this action on the small intestine is due to the hormone itself (183).

Repeated or prolonged stimulation of the acinar cell by pancreozymin or vagal stimulation results in the depletion of zymogen granules within the cell (96, 174, 190, 243). The enzyme content of pancreatic juice, however, remains high (174, 222, 247), suggesting that pancreozymin plays a role in enzyme synthesis. At present there is insufficient evidence as to the direct effect of pancreozymin on enzyme synthesis. It has been demonstrated that the hormone stimulates cellular respiration and augments enzyme secretion by the transfer of the enzymes across the acinar cell membrane (93, 104, 193). There seems, however, to be no uptake of amino acid by the cell (192), indicating that pancreozymin has no effect on enzyme synthesis. Other workers have found that in the rat and pigeon, pancreozymin augments the synthesis of exportable protein (339). Pancreozymin causes vasodilatation (187) and increases the blood flow (212) to the pancreas; this may have an important bearing on its action.

The dose-response curve (167) for pancreozymin has been worked out. Some suppression of enzyme output seems to occur at high doses, probably as a result of impurities in the material.

Pancreozymin appears to have the same distribution in the intestine as that of secretin. This varies in different animals. In the dog, for example, equal amounts are present both in the upper and lower half of the small intestine, while in the pig the lower half has no pancreozymin activity. No pancreozymin activity was found in the stomach of the dog, pig or cat (173).

Pancreozymin is reported to have more side effects than secretin. These include headache, nausea, vomiting, abdominal cramps and venospasm during injection. Two of our own patients developed acute

shock-like state, preceeded by an unbearable itch, within minutes of the injection. In Burton's series (60) only 1% of the tests had to be discontinued because of untoward reaction, while 13% of all their patients had some reaction. Skin testing does not seem to be helpful. These reactions can be minimized if the hormone is well diluted and injected slowly.

3. Gastrin: The name gastrin was first used by Edkins (115) for a substance present within extracts of antral mucosa which, when injected intravenously into anaesthetixed cats, stimulated gastric secretion. This substance was at first thought to be histamine, but later investigators (149, 229) were able to prepare histamine-free extracts from antral mucosa which still had a marked effect on gastric secretion. It was not until 1963, however, that Gregory and Tracy (150) were able to finally isolate and characterize gastrin (154) from hog antral mucosa and later from human antral mucosa (43, 152). Gastrin occurs in two peptide forms; gastrin 1 and gastrin 11. Gastrin 1 has no sulphate radicle, making it less acidic and it can therefore be separated by chromatography and electrophoresis. All gastrins so far studied in different species, including man, have the same carboxyl terminal tetrapeptide amide sequence, Try-Met-Asp-Phe-NH_2, suggesting that this is the active radical (158) responsible for the biological action of this hormone. This is supported by the action of synthetic peptides (397) in which it is found that only those peptides containing the C-terminal tetrapeptide have the same physiological action as gastrin (290). All the physiological actions of gastrin can be produced by this active tetrapeptide, only the potency tends to be less (397). Synthetic gastrin is now available for clinical use (12, 38).

Gastrin has been found to have a large number of actions and affects on other organs besides the stomach:-

a) Stimulation of gastric acid secretion - Gastrin is very much more

effective than histamine in stimulating gastric secretion; at least 500 times more effective if compared on molar basis (268). The response to subcutaneous gastrin is rapid, the peak response occuring after about half an hour and the response lasts about three hours (266). No side effects have been noted. The response to an intravenous injection of gastrin is immediate, with the peak secretory response observed in the second 5 minute period.

b) Stimulation of gastric pepsin secretion.

c) Stimulation of pancreatic secretion - It has been shown by the use of endogenous (48, 322) and exogenous (323, 324) and synthetic gastrin (433), that this hormone has a pancreozymine-like action on the pancreas. Some increase in volume and bicarbonate output has been reported, but the maximum response to gastrin appears to be less than one fourth of that of secretin (323).

d) Stimulation of hepatic biliary flow and bicarbonate secretion - This has been found under conditions in which the entero-hepatic circulation has been interrupted (294, 442) and also when it has been maintained by a constant infusion of sodium taurocholate (348).

The above are generally regarded as the physiological actions of gastrin. In very large doses given intravenously, however, gastrin has some additional effects which cannot be reproduced by endogenously released gastrin. These include stimulation of gastric tone (42) and motility, stimulation followed by inhibition of small intestine motility inhibition of gastric secretion of acid (135), very strong stimulation of gastric pepsin secretion (120) and occasionally some effect on blood pressure (248). It should be noted that some of the latter actions have not necessarily been reproduced in man. Inhibition of gastric

secretion in large doses occurs in dogs but does not seem to occur in man (267). Some of the other actions have not yet been tested in man.

Chapter 3

REGULATION OF EXOCRINE PANCREATIC SECRETION

Chapter 3 REGULATION OF EXOCRINE PANCREATIC SECRETION

A. INTRODUCTION

With the discovery of secretin it was thought that all problems relating to pancreatic stimulation had been resolved. Stimulation of the vagus nerve produced a juice high in enzymes, while secretin produced a pancreatic juice high in volume. Recent research has revealed that there are more complex factors involved. Pancreozymin has been isolated and the role of gastrin in pancreatic secretion suggested. The nervous influence no longer appears quite as simple with the discovery of a gastro-pancreatic reflex, cephalic stimulation, sympathetic innervation, inhibitory fibers and local intestinal-pancreatic reflexes. Finally, the pancreas is no longer seen as an isolated organ lying in the retroperitoneal space, but as part of a gastro-duodenal-pancreatic cycle. Whatever occurs in one part of the cycle must influence the other. This is a relatively new concept of pancreatic physiology and thus in discussing the regulating factors involved in pancreatic secretion, we must include the gastric and duodenal influence.

B. REGULATING FACTORS

1. HORMONES: Three hormones have been implicated in the secretion of the pancreas. The first was secretin, later came the discovery of pancreozymin and, more recently, there have appeared numerous papers in the literature of the role of gastrin in pancreatic secretion

a) Secretin: In January, 1902, Bayliss and Starling (37) proved

the existence of secretin. The path to this discovery had, however, been traced by earlier authors who found that acid in the duodenum stimulated pancreatic secretion even after section of the vagi and splanchnic nerves; destruction of the medulla and spinal cord; removal of the coeliac ganglia and transection of the pylorus. It was also noted that atropine did not affect the response to acid in the duodenum. Local reflexes of a sympathetic nature were thought to be responsible until Starling injected an acid extract of jejunum into the jugular vein of his animals and produced a marked pancreatic response. This experiment was soon followed by the demonstration of secretin-like activity in the venous blood draining a loop of intestine into which acid had been added. Later, experiments with the transplanted pancreas and cross circulation experiments, in which the only connection was by was of the blood, appeared to confirm that a humoral mechanism was involved. From the crude extract used by Starling (209) we have reached a stage to-day when it would appear that we have a pure secretin preparation (217).

Secretin not only affects the pancreas directly, but also influences pancreatic secretion indirectly by its effect on gastric acid secretion. The presence of acid in the duodenum has a strong inhibitory influence on gastric acid (9) secretion. Earlier workers (147) showed that extracts of intestinal mucosa, containing secretin, inhibited gastric secretion. More recently (211), with the availability of purified secretin, it has been shown conclusively that this hormone is the inhibitory agent present in mucosal extracts. Greenlee et al (147) showed that injected secretin was able to inhibit the gastric secretion produced by the presence of food in the antrum. Secretin, however, had no effect on the gastric secretion produced by vagal stimulation (214) (insulin hypoglycaemia or psychic stimulation). Greenlee et al (147) has suggested that secretin, in

some way, prevents the release of gastrin from the antrum. This work agrees essentially with that of Jordon and Peterson (214) who also found that secretin inhibited the intestinal phase of gastric secretion. Wormsly and Grossman (432) found that secretin was able to produce a maximum reduction of ± 70% on gastric secretion. Johnson and Grossman have obtained even greater inhibition. A further increase in the dose of secretin produces no further increase in inhibition. The dose of secretin which produces this "maximum inhibition" on gastric secretion, also produces about 75 - 90% of the maximal pancreatic response.

Gillespie (134) reported that cholecystokinin was able to produce a greater inhibition of gastric secretion than their secretin preparation. It seems, however, that their cholecystokinin extract had a number of impurities.

b) Pancreozymin: The discovery of pancreozymin by Harper and Vass (172) came much later. They were involved in investigating the pancreatic response of anaesthetized cats to various food substances placed in the intestine. A continuous flow of pancreatic juice was obtained by repeated intravenous injections of secretin. They noted, following section of all the extrinsic nerves to the intestine, that the food still produced a good enzyme response. They therefore prepared an extract of duodenal mucosa and found on intravenous injection into cats that a very good enzyme response was obtained without significant change in volume rate. Like secretin, pancreozymin is also reported to be a potent inhibitor of gastric acid secretion and of motility of the denervated stomach (295). This may be related to the fact that the C-terminal peptapeptides of both pancreozymin and gastrin are identical.

c) Gastrin: The isolation and preparation of gastrin by Gregory and Tracy (151) have made possible a systematic investigation of this hormone's effect on pancreatic secretion. Earlier, Blair et al (48) had found that the presence of various stimulants in the pyloric gland area of the anaesthetized cat caused on increase in pancreatic enzyme secretion, after the vagus nerves and splanchnic nerves were cut. These investigations and other (162) suggested that stimulation of the pyloric gland area of the stomach results in the release of a humoral stimulus for pancreatic secretion. Gregory noted that the gastrin preparation provided a large increase in enzyme output, without an increase in volume flow, when a gackground flow of pancreatic juice was provided by an intravenous injection of secretin. This persisted despite an intravenous injection of atropine. This action of gastrin has been confirmed by the experiments of Preshaw, Cooke and Grossman (323) who showed that acidification of the antrum inhibited the secretory response of the pancreas to antral stimulation. The release of gastrin from the pyloric area has been shown to be inhibited by acidification. Thus it has been demonstrated that the pancreas responds both to exogenous (323) and endogenous gastrin (48, 323), the latter released by antral stimulation. This suggests that this hormone is in fact a physiological stimulant, not only to gastric secretion, but also to pancreatic secretion.

Although the antrum is the main source of gastrin, some gastrin activity has been found in extracts of duodenal mucosa (241, 398) of the cat, dog, hog and man. The first part of the duodenum has the highest gastrin content; up to 10% of that found in the antrum. It is unclear at present whether this duodenal gastrin is the same as the gastrin released from the gastric antrum. The release of

gastrin from the antral mucosa may be affected by nervous (vagal)(277), mechanical (162) or chemical (83, 338) stimuli. The nervous mechanisms are supported by the decreased gastrin activity following vagotomy, antroneurolysis, with ganglionic blocking agents and by local anaesthesia.

2. NERVOUS REGULATION OF PANCREATIC SECRETION: A great deal of controversy is centred around the role of the nervous connections to the pancreas in man. The literature on animal experiments is plentiful but experimental results of different animals are confusing due to the varied response of the pancreas to nervous stimulation. Some of the earlier work was probably at fault due to the lack of knowledge of the anatomy of the nervous connection to the pancreas.

a) Anatomy: The pancreas receives an abundant nerve supply from both right and left vagi and the splanchnic nerves. The vagus contains the parasympathetic supply to the pancreas and the fibres pass through the coeliac plexus and end in the intrinsic ganglia of the pancreas. From here the post-ganglionic fibres pass to the acinar cells, islet cells and some motor fibres to the smooth muscles of the ducts. The nerve fibres reach the pancreas via nerve plexuses surrounding arteries, with the majority (80%) passing along the lesser curvature of the stomach, to cross the pylorus and descend into the duodenal wall before entering the pancreas. The remainder traverse the hepatic flexure and join the pancreas in the region of the pylorus (164). The splanchnic secretory fibres, containing the sympathetic supply to the pancreas, also pass via the coeliac ganglion to the pancreas, although some authors have shown the presence of splanchnic secretory fibres which do not synapse in the coeliac ganglion (20).

At present, it is unclear whether, in fact, the splanchnic nerve supply has true secretory fibres (384) or whether they exert an apparent secretory action (20, 35, 235) indirectly through their effect on the blood vessels (337). Visceral afferent fibres are also present in splanchnic nerves (337). The presence of the intrinsic ganglion of the pancreas has given rise to some interesting speculation regarding its relationship, if any, to the ganglionic plexus in the intestine. Thomas (386) has suggested that there are local reflexes from the intestine to the pancreas.

b) Function:

(i) Stimulation - Pavlov was the first to show that the vagus is the secretory nerve to the pancreas. Early observation in man showed an increased pancreatic response to sham feeding, but the gastric juice was not prevented from entering the duodenum. Alphin and Lin (8) attributed this cephalic response to the passage of acid into the duodenum. Unlike the acid response, however, when gastric juice was prevented from entering the duodenum, sham feeding (91, 148) caused a scanty flow of pancreatic juice rich in enzymes. In patients with pancreatic fistulae (362), pancreatic secretion to a cephalic response could not be demonstrated. More recently, however, Sarles et al (347), using a double lumen tube and balloon to prevent acid contamination of the duodenum, showed to their satisfaction, that there was a definite cephalic phase of pancreatic secretion in man. They found that the pancreatic juice secretion started within 2 to 4 minutes of stimulation (sight and smell of food) and almost always preceeded gastric secretion. Of particular

interest was the duration of secretion, which lasted at least one hour for a stimulus of 5 minutes. Preshaw, Cooke and Grossman (325) have suggested that this effect is due to the vagal release of gastrin from the antrum as the pancreatic response is inhibited by acidification of an innervated pouch of the pyloric gland area. Acidification of the antrum effectively inhibits vagal release of gastrin (316). Sarles (347) disagrees that a gastrin mechanism is involved, as the gastric and pancreatic secretion in their subjects did not have a parallel course. These workers have suggested that the vagus has a direct effect on the pancreas.

The gastropancreatic distension reflex (418, 419) is further evidence of the neutral effect on pancreatic secretion, although Harper (176) felt that the early return of the reflex, following vagotomy, might indicate involvement of a hormonal rather than a nervous mechanism. Distension of the fundus of dogs resulted in a reflex increase in volume and enzyme output.

Despite contradictory evidence, most workers agree (18) that stimulation of the secretory nerves to the pancreas results in a modest increase in output of fluid and a substantial increase in output of enzymes from the pancreas. In the rabbit (35), output of fluid and enzymes is increased by either vagal or splanchnic stimulation, and in the pig, stimulation of the vagus cuases a profuse flow of juice with a high amylase content and bicarbonate concentration (72, 263). The stimulus was still effective after the removal of the entire small intestine and pyloric antrum (186). There is some histological evidence (72) to suggest that the pancreatic response to vagal stimulation in man should be similar to that in the pig, suggesting a fairly

prominent role of nervous stimulation in pancreatic secretion, unlike that in the dog and cat.

Vagal stimulation also potentiates the secretory response to a constant intravenous infusion of secretin (56, 172). This may be due to a vasodilator (187) effect of the vagus on the blood vessels of the pancreas.

(ii) Inhibition - Although, in general, stimulation of the vagus appears to increase pancreatic secretion, some authors have noted an inhibitory effect. This inhibition may, however, be secondary to sphincteric or ductal spasm and not as a result of inhibition on the acinar cell. Von Anrep noted an increase in pancreatic weight during vagal stimulation and porposed that this was due to sphincteric spasm and retention of secretion. Other investigators (230) have excluded the sphincteric mechanism for their system and have noted an increase in ductal resistance on stimulation of the vagus. Further evidence for a vagal mediated inhibition of secretion is the effect of atropine which decreases this duct pressure (285). The experiments of Guillaumie (164) did not altogether support the above findings. She found that contraction of the ducts was not a constant feature and suggested that any evidence of inhibition may be due to contraction of muscles of the duodenum.

Sympathetic stimulation appears to have a more definite inhibitory effect (136), although, again, the mechanism involved is unclear. Some authors suggest a direct inhibitory effect on the acinar cell, while others emphasize the role of increased ductal pressure and vasoconstriction. Splanchnic section appears to increase enzyme output under a variety of conditions.

Even when the decrease in blood flow to the pancreas was prevented by the administration of phenoxybenzamine, sympathetic stimulation using adrenaline or nor-adrenaline still causes a decrease in pancreatic secretion (29).

More recently, Gilsdorf (136) and his co-workers experimenting on dogs, found that stimulation of the posterior hypothalamus cuased a decrease of pancreatic secretion and an immediate rise in pancreatic duct pressure. Stimulation of the anterior hypothalamus, however, caused a slow, steady increase in secretion and a very small fall in ductal pressure. They also noted that stimulation of the coeliac ganglion cuased a rise in pancreatic ductal pressure, while direct vagal stimulation caused a pronounced fall. While some of these results appear to be confusing, it is well to remember that under normal physiological conditions neither system acts on its own.

(iii) Effect of Nerve Section or Blocking - The effects of sectioning the various nerves, or appropriate drug administration, while not resolving all the problems, does confirm some of the above work.

The cephalic reflex is dependent on an intact vagus nerve and the gastro-pancreatic reflex disappears immediately after vagotomy (420) but reappears partially at least with time. Division of both the vagi and the pslanchnic nerves, however, results in a permanent disappearance of the reflex.

Following vagotomy there appears to be no significant change in basal secretion (31, 138, 381), although some authors (182) have noted some decrease in the volume and amylase output of daily pancreatic secretion after selective vagotomy, and a

further decrease after truncal vagotomy. Local anaesthetization of the vagi in the neck of dogs which have fasted reduce the resting volume of pancreatic juice in the pancreatic fistulae of the dogs by as much as 62%. This prompted the investigators to suggest that the vagi contribute substantially to the resting pancreatic secretion (182).

The response of the pancreas following vagotomy to secretin or food appears to vary in different series of experiments. An increase in volume flow and enzyme output, but no concentration, was noted with secretin stimulation in a vagotomised animal (340), but this does not agree with the findings in man where after vagotomy the secretin response remained unchanged (108, 355). Crider and Thomas (90) found that, although there appeared to be a temporary absence of a response to a peptone solution in the intestine following section of the vagi, the volume response returned to near normal but the protein output remained considerably less than the control amount. The response to acid was similar, but that to soap in the intestine was normal. Pincus (319) and his group found that there was a decrease of as much as 80% in the protein output from the pancreas of dogs with chronic vagotomy, contrary to the view of Routley (340) and others. Govaerts (138) described two phases in the pancreatic response to a meal and suggested from his results that the early phase was under the control of the vagus as this was not abolished by selective vagotomy but was abolished by total vagotomy. Studies on pancreatic secretion in man following vagotomy are few, especially with regard to the long-term effect. Controlled studies (24) performed some years after vagotomy have shown no increase, or even decrease, in volume, enzyme concentration or

output in the duodenal contents following secretin stimulation. Oesophagogastrectomy, in patients in whom a complete vagotomy is unquestionable, resulted in a normal bicarbonate output and volume response to secretin stimulation, but the enzyme content of the pancreatic juice was decreased.

The pancreozymin response following vagotomy has not been well documented but, again, some authors (340) have shown a significant increase in volume flow and enzyme output, while others(31) have reported no significant change.

It should perhaps be remembered that vagotomy alone, without a drainage procedure, increases the gastric emptying time (281) and results in gastric distension (327) in most animals. This may significantly influence the pancreatic response to various stimuli following vagotomy and account for the disagreement amongst the various authors.

Important evidence implicating cholinergic control in pancreatic secretion is the effect of atropine and other anti-cholinergic drugs which cause a marked inhibition of enzyme secretion under almost all conditions of stimulation except pancreozymin (175). Although atropine paralyzes the secretory endings of the vagus, this affect is not obtained with the therapeutic doses used in human subjects (275), so that information on humans as to the effect of anticholinergics does not always coincide with the findings of animal studies. In patients with an external pancreatic fistula, the basal secretion was not altered by atropine (11, 119, 264, 287) in some cases, while others (196, 288, 362, 372) reported a decrease in basal outflow. The secretin-stimulated pancreatic juice was slightly reduced by anticholinergics.

3. THE ROLE OF THE STOMACH IN PANCREATIC SECRETION: As indicated in the previous section, it would seem that pancreatic secretion begins before food or acid actually enters the duodenum (347). The main portion of pancreatic secretion must, however, occur concurrently with the passing of the gastric contents into the duodenum. The gastric influence on pancreatic secretion, and the rate and character of gastric emptying, while not of particular importance in secretin-pancreozymin studies, are of particular interest when studying the influence on pancreatic secretion of foods and other substances taken orally.

a) The gastro-pancreatic reflex (418, 419): Pancreatic secretion can be stimulated by distension of the fundus of the stomach in dogs. White et al (418, 419) found similar results in man and suggested that a neutral mechanism was involved. Harper (176), however, noted that the reflex returned fairly quickly after vagotomy and felt that this may indicate an hormonal rather than a nervous influence.

b) Gastric emptying: The stomach is a reservoir for food but it also titrates chyme into the duodenum (203), thus allowing the smooth passage of food and facilitating the whole process of digestion. The action of the gastric pump is stimulated by the volume (208) of its contents. There are a number of controlling factors which influence the rate of gastric emptying and these include the type of food ingested, the osmolarity of the food and the acid in the duodenum.

Many methods have been used to determine the rate of gastric emptying. These include radiological (353, 401), radio-active chromium (153) and surface scanning, but perhaps the best known method is that of Hunt (207) which requires repeated nasogastric intubation over a period of several days. More recently, George (133) has introduced

his "double sampling test meal" which can be completed at one sitting. All methods are reported to be reproducible.

(i) Volume: As early as 1898, Marbaix (270) had shown that the rate of gastric emptying was, to a large extent, dependant on the volume of the gastric contents, the weight of the contents being less important. His results indicated that gastric emptying occurred in an exponential manner, i.e., the rate of emptying of a meal was a constant proportion of the volume of the meal within the stomach. This has been confirmed by numerous authors (200, 205). Within minutes of being swallowed, food begins to leave the stomach. The larger the original volume ingested, the more rapid the initial rate of gastric emptying, but, within a short while the rate of gastric emptying becomes constant and a fixed fraction of the volume of the test meal remaining in the stomach empties per minute (205). This pattern of gastric emptying appears to apply to all types of meals, whatever the viscosity, including liquid meals (199). There appears to be no correlation between body weight and gastric emptying (52).

(ii) Osmotic pressure (15, 116, 206, 280, 353, 354): An osmoreceptor is thought to be present in the duodenum (200). Substances like water and solutes which pass easily through the membrane of the osmoreceptor cell, causing it to swell, increases the rate of gastric emptying by reducing the inhibitory signals. Other solutes which absorb water slow gastric emptying. Elias (116) found a similar response to the products of the hydrolysis of dissacharides, the degree of slowing being proportional to the concentration of the solute.

(iii) Acid - Acid has a strong inhibitory effect on gastric emptying (201, 251, 354, 314, 401) and the mechanism involved must play an important role in maintaining a fairly constant duodenal pH (239). It is generally thought that a second duodenal receptor is present which responds to either pH change or hydrogen ion concentration. The work of Hunt and Knox (201) suggests that the latter is the important controlling factor. The pH threshold for the inhibition of gastric emptying is 6.

(iv) Fat - The presence of fat in the duodenum has an inhibitory effect on gastric motility (64, 123, 170, 204, 251, 328, 329, 394). Fat in the stomach has no such action (329). Quigley and Mesham (328), noted that fatty acids and soaps were more effective than neutral fats in slowing gastric emptying. Hunt and Knox (204) have concluded from their studies that those fatty acids with a chain length of 12 - 18 carbons are about four times more effective than the shorter chain group in slowing gastric emptying. This confirms the earlier work of Harkins (170) et al. The mechanism, however, is unclear. A third receptor may be present in the duodenum and some experimental work (123) suggests that an hormonal influence may be involved. Calling into question a pure hormonal involvement is the fact that vagotomy (406) in man reduces the inhibitory effect of fat on gastric emptying.

(v) Position - Hunt et al (202) found that when individuals were put in the head-down position, there was no appreciable difference in the rate of gastric emptying for meals containing glucose, while meals containing hydrochloric acid or trisodium citrate slowed the emptying rate of the gastric contents. Hunt

suggests that any substance with a strong inhibitory action on gastric emptying will have a similar effect to that of glucose in the head-down position. This is due to the removal of inhibitory factors in this position.

(vi) Mixed meal - Only a few studies have been reported on the effects of a mixed meal on gastric emptying. These reports suggest that the fat content of the meal is the controlling factor. The fat phase separates out and floats to the top, while the lower liquid phase, poor in fat, empties first and most rapidly from the stomach (53, 64, 422). Gastric emptying slows down when the fat phase starts to pass into the duodenum. Thus, fats and carbohydrates leave the stomach at independent rates (170).

c) Gastric Acid Secretion: The very same factors which influence the gastric emptying rate also have a similar influence on gastric acid secretion. Thus, hydrochloric acid (88, 95, 318), hypertonic solutions, carbohydrates (94) and fats (139, 231), which all slow gastric emptying, also decrease acid secretion. Sircus (363) has indicated that two receptors are probably present in the duodenum, the one being an osmoreceptor with a humoral mechanism as it is not influenced by local anaesthesia of the duodenal mucosa, and the second a pH sensitive receptor effective through neutral pathways. This latter mechanism has been contested by other authors (9) who postulate a humoral mechanism.

Diversion of pancreatic juice not only increases the volume and bicarbonate output of the pancreas in response to a meal stimulus, but also increases, very significantly, the acid output from Heidenhaim pouches (85).

C. SUMMARY OF THE PHYSIOLOGICAL FACTORS REGULATING PANCREATIC SECRETION

The ingestion of food stimulates the production of acid by the oxyntic cells. This stimulation is mediated by the interplay between the hormone (gastrin) and the parasympathetic nervous system. Gastrin has a stimulatory effect on the pancreas, so that it is likely that pancreatic secretion is commenced even before the pancreatic hormones begin to influence pancreatic secretion.

The presence of gastric contents within the duodenum is the most important factor stimulating the secretion of pancreatic juice. The rate of gastric emptying is, therefore, an important controlling factor. This is regulated, in turn, by a number of intraduodenal factors which depend upon the presence of a number of receptors within the duodenal mucosa. These factors include duodenal acidity, osmolarity of the duodenal contents and the presence of fat.

With the passage of the acid gastric contents into the duodenum, the specific pancreatic hormones, secretin and pancreozymin, are secreted from the duodenal mucosa into the portal circulation and exert their characteristic effect on the pancreas. Secretin produces a volume response of highly alkaline fluid low in enzymes; pancreozymin stimulates a small volume of highly viscus fluid rich in enzymes. It is highly unlikely, however, that either hormone is secreted alone after a meal. Therefore, the pancreatic juice secreted will have a varied volume, alkalinity and enzyme content, all of which may depend on the type of stimulus applied to the duodenal mucosa.

The role of the nervous system on pancreatic secretion in man is unclear. Vagal stimulation appears to potentiate the effect of secretin on pancreatic secretion. This so called "vagal tone" may be important in individual responses to an intravenous injection of

secretin. This effect does not, of course, rule out the possible direct role of the vagus on pancreatic secretion.

Chapter 4

THE RESPONSE OF THE PANCREAS TO A MEAL

I. DUODENAL pH

a) Fasting pH: The pH in the duodenum (16, 73, 121, 224, 227, 341, 343, 383) has been of great interest to many workers, especially those concerned with the pathogenesis of duodenal ulceration. Two methods of measuring duodenal pH are popular: (a) an aspiration technique (16, 73) to collect the duodenal juice, or (b) an intraduodenal probe (126, 342) which can record the pH directly. Both methods are controversial (79) and have inherent problems, although the direct method is more advantageous and accurate than the aspiration method in experienced hands. One of the main problems is to be sure of the position of the tube throughout the period of the experiment, as this may have an important bearing on the pH.

Whatever the mechanism involved, the end pH at a particular point will be the result of the mixing of gastric contents and duodenal juice (pancreatic juice, bile and succus entericus). In the normal individual, the pH in the stomach is decidedly acid - pH 2.4 (Rovelstad)(341), pH 1.5 - 2.0 (Maxwell et al)(278), pH 1.6 (King et al)(227). The amount of titratable acidity varies within fairly wide limits after histamine, and this is probably the most important factor influencing the duodenal pH. Individuals with anacidity (224), for example, have a fasting duodenal pH of about 7.16 and if gastric juice is aspirated continuously, the duodenal pH was found by King (227) to range between 7.53 and 7.83. In the normal fasting patient without gastric aspiration, the pH remains fairly stable between 5 and 7, but sudden drops in pH (referred to as "acid waves" by some workers (73, 278), do occur. This usually lasts for a few minutes only, but may be present for as long as

twenty minutes. The pH remains fairly stable within these limits whether the juice is collected from the first (44) or second part of the duodenum (224), collected for a one hour period (227) or using a glass electrode (121). The pH of pure pancreatic juice in healthy dogs was found to vary within a narrow range of pH 8.0 - 8.3 (177).

b) The pH following a meal: After the ingestion of a meal, the pH in the duodenum (343) tends to fluctuate much more than during the fasting state, especially in the duodenal bulb. The gastric acidity, the buffering action of the food, the rate of gastric emptying and the neutralizing capacity of the pancreatic juice, bile and succus entericus, are all responsible in some way for the variations in the duodenal pH.

Gastric acidity tends to be highest about 1 - 1½ hours after eating and during this time, the pH in the duodenum fluctuates relatively widely and fairly frequently. In dogs, Mann and Bollman (269) found the pH to be about 5.5 but it frequently fell to as low as 4.6 during this time, while King (227) et al found the mean duodenal pH to be 5.53. No mention was made of the position of the tube in the duodenum. Thomas (383) found the pH in the first part of the duodenum to vary between 3.0 and 4.8 after the ingestion of food, and very rarely higher or lower, with only a few exceptions. Rovelstad and Maher (343) noted that the pH here may be as low as 1.5 at times. Lower down in the duodenum, the pH varied between 4.2 and 5.8, while in Kearney's series (224) in the second part of the duodenum, it varied between pH 5 and 7. Thomas (383) was impressed with the rapidity with which the pH changed from the antrum of the stomach to the duodenum. They suggested that the mechanism involved may be the "receptive relaxation" first described by Joseph and Meltzer (220) in which there is loss of duodenal tone and activity.

This, together with a massive outpouring of duodenal juices, would dilute the gastric juice and raise the pH.

The further the distance from the pylorus at which the pH is measured, the higher the pH tends to be (383). Similar results were found in man by Atkinson and Henley (16). They measured the pH in the first part of the duodenum during a twenty four hour period while the individual was allowed to eat his usual meals and walk around. In two thirds of the recordings the pH in the first part of the duodenum was 3.5 or more, while in the second part of the duodenum it was already much higher. More recently, Anderson and Grossman (10), using an intraluminal glass electrode, found the postbulbar pH to be between 6.5 and 7.5, with very little fluctuation. They found no correlation between the pH there and the gastric pH, whereas there was some correlation between the bulbar and gastric pH.

Tomenius and Williams (396) made the interesting observation during measurement of the gastric pH with the glass electrode, that the position of the patient was important and would have some influence in the pH.

c) Mechanism of pH stability: The pH in the duodenum is the result of the mixing of gastric contents (acid) with the duodenal secretions (alkaline). As has been pointed out, the pH fluctuates fairly widely in the first part of the duodenum, and is still in the acid range although higher than the pH in the gastric antrum. Further down the duodenum the pH increases rapidly and remains steadier, with occasional "acid waves" (73, 278), but seldom does it become decidedly alkaline.

(i) Bicarbonate - It is generally accepted that the alkaline pancreatic juice is a necessary part of the neutralizing process within the duodenum. The work of a number of authors is

interesting in this connection. Hoerner (188), for example, showed that even when the pancreatic juice was excluded from the duodenum, there was a significant degree of neutralization of acid from the stomach. Similar results were obtained by Pincus et al (319), using dogs fitted with gastric and pancreatic duct fistulae. They found that, although the pH in the duodenum tended to be higher when pancreatic juice was present, this was not marked and they concluded "that the pancreatic juice exerts only a moderate effect on the pH of the intestinal contents," More recently, Preshaw et al (326), using dogs with pancreatic and gastric fistulae, found that the bicarbonate response of the pancreas to duodenal acidification was insufficient to neutralize the acid content of the duodenum which had elicited the response. This work agrees essentially with previous findings (324) of the maximum secretory capacity of the canine pancreas for bicarbonate being much less (4 times) than the maximum secretory capacity of the stomach.

Lagerlof et al (239) have made a few interesting observations: They found that the volume of the bile and enteric juice in the post-secretin duodenal juice was just less than half of the volume of the pancreatic juice, indicating that any increase in pH further down the duodenum or jejunum, is probably due to secretion of enteric juice. They have also suggested that the absorption of hydrogen ions through the duodenal mucosa may play an important part in the neutralizing mechanism.

(ii) Acid: The weight of evidence to date suggests that the presence of acid in the duodenum has a definite influence on

pancreatic secretion (239, 326, 351, 387), certainly in the case of bicarbonate and water secretion. Whether it has a similar influence on pancreatic enzyme secretion is still a matter of controversy. This pattern of response would suggest that the presence of gastric acid in the duodenum stimulates the secretion of secretin, which produces a large volume of highly alkaline fluid rich in bicarbonate, but poor in enzymes. There may be an initial high enzyme output ("wash out effect"). Pincus (320), however, could find no correlation between pancreatic volume and pH and Crider and Thomas noted that section of both vagi decreased the pancreatic response to acid in the duodenum. The work of earlier authors, however, using cross circulation experiments, showed that injection of acid into the duodenum of the one animal produced a profuse secretion of pancreatic juice in the second animal. This work suggests that the mechanism of acid stimulation of pancreatic secretion is humoral in origin. Confirmation of this work has come from other authors using an auto- (209) or homo-transplanted pancreas with or without a transplanted loop of the upper small bowel. The instillation of acid into the transplanted bowel or duodenum in situ, produced a flow of pancreatic juice in the transplanted pancreas. Thomas and Crider (387) reported a pH threshold below which acid was able to stimulate pancreatic secretion. They found that this threshold varied between 3 and 5, but suggested that in dogs, at least, the "practical threshold" was probably about pH 4.

(iii) Acid-bicarbonate relationship: Lagerlof (239) was able to show that the rate at which acid passed from the stomach into

the duodenum was directly proportional to the bicarbonate secretion. Preshaw et al (326) confirmed these findings in dogs with pancreatic and gastric fistulae. They reported a direct relationship between the amount of titratable acid introduced into the duodenum per unit time and the bicarbonate output from the pancreas.

As pancreatic enzymes appear to be inactivated in an acid environment, it is, therefore, not surprising that there exists some definite relationship between acid in the duodenum and pancreatic bicarbonate secretion. Wang and Grossman (411) found, nonetheless, that the enzyme output of the transplanted pancreas was higher when acid was injected into the duodenum than when secretin was given intravenously. The work of Preshaw, Cooke and Grossman (326) supported this finding, although they noted that the amount of protein secreted by the pancreas eventually fell to basal levels, despite the fact that the flow rate and bicarbonate output remained high. In contrast to the above findings, Lagerlof (239) found that the amount of enzymes secreted did not differ significantly to that found in the secretin test.

2. VOLUME AND ENZYME RESPONSE TO THE INGESTION OF A MEAL

Almost all the work in this field has been performed on animals because of the relative ease in obtaining pure pancreatic juice. A few studies performed on humans with a pancreatic fistula have served to emphasize the difficulty of transposing the results of animal experiments to humans.

a) Basal secretion: Baxter (36) showed in rabbits that there was a continuous pancreatic secretion, small in quantity but, in some cases, containing normal amounts of enzymes. In the fasting dog, resting secretion varied between 1 to 2 cc. per hour (19) with increasing amounts noted during periods of hunger contractions.

In man it is obviously difficult to examine the basal pancreatic secretion under normal physiological conditions. It is difficult to be sure that some gastric contents have not passed into the duodenum and, as Annis and Hallenbeck (13) have pointed out, the diverting of pancreatic juice to the exterior will increase pancreatic secretion. In general, however, it appears that there is a continuous secretion from the pancreas in man. Babkin (19) suggests that this is probably less than 12 cc. per hour, which agrees essentially with the rate found by Sinclair (362). When gastric contents were not prevented from entering the duodenum, the fasting secretion was as much as 66 cc. (404) and 105 cc. per hour (68).

b) Water stimulation: The effect of water on pancreatic secretion should be mentioned first as many food substances are first dissolved in water during test conditions. Crider and Thomas (89) noted that in dogs with gastric and duodenal fistulae, water produced a brief and rapid flow of pancreatic juice containing a relatively high nezyme content (392). Isotonic solutions, however, had little effect. Similar results were found by Wang and Grossman (411). They suggest that the stimulatory effect of water may be due to its relative hypotonicity.

c) Carbohydrate stimulation: The earlier work of Babkin and Savich (21) showed that the addition of carbohydrate to the acid

solution introduced into the stomachs of dogs produced a higher tryptic activity than acid alone. This was confirmed by the work of Thomas and Crider (39), who gave their animals a constant infusion of secretin during the control period and after the injection of carbohydrate into the intestine. Wang and Grossman (411) point out, however, that this pancreatic response to various carbohydrates is small, both in volume and enzyme output. This agrees essentially with Comfort and Osterberg's experiments on humans (75).

d) Protein stimulation: Protein has been indicated to stimulate a pancreatic juice higher in enzyme content than carbohydrate by various authors. Thomas and Crider (389) tested the effects of various products of protein digestion and some commercial protein preparations. They found that these products produced a moderate secretion of pancreatic juice with a high nitrogen content, but there were fairly wide variations between the products. These authors suggested that the protein, requiring greater digestion, stimulated greater enzyme secretion. In the cat, Harper and Vass (172) noted a good enzyme response to a casein meal with a 50% average increase in volume. Wang and Grossman (411) found that the peptone solutions in 2 out of 3 experimental dogs produced a greater pancreatic enzyme response than intravenously injected pancreozymin. They also noted that the peptone and amino acid solutions used, produced almost identical responses. Other authors (387) have shown, however, that certain amino acids have been ineffective. Peptones cause depletion of zymogen granules in the acinar cells of normal dogs (330).

In man (362), protein (casilan or casein) stimulates a response of moderate volume. Sinclair reported that the enzyme

response was slight but definite, while Comfort and Osterberg (75) found similar results.

e) Fat stimulation: Fat is thought to produce a small volume (385) of pancreatic juice with good enzyme output. (257). The enzyme output shows a slow increase while the stimulatory effect of fat is continuing (411). Very inadequate reports are available of the response of the human pancreas to the presence of fat or its digestive products. Some authors have reported very little response (76, 362), while others have merely noted that the secretion rate is increased (279). Comfort and Osterberg (76) have reported that olive oil produced a similar response to that of casein.

3. THE PROBLEM OF ADAPTATION AND PARALLELISM

In 1897, Walther (409), working in Pavlov's laboratory, carried out some experiments, the results of which suggest that different types of stimuli applied to the pancreas may promote the secretion of different enzyme patterns. Pavlov then published his theory of "purposive adaptation" of the individual enzymes to the kind of food ingested. Babkin (17) later repeated the work of Walther and published his own theory of "parallel secretion" of the pancreatic enzymes in which he stated that, no matter what the stimulus, the three main pancreatic enzymes were still secreted in a parallel fashion, although quantitatively the total enzyme content may vary. This problem has not yet been settled despite a vast literature on the subject. Many authors show good evidence for parallel secretion (173) while others show equally good evidence to support the theory of adaptation. Earlier workers were at some disadvantage because of their poor methods of enzyme estimations and varied experimental

procedures. Baxter (34) showed that in rabbits there was good parallelism between the concentration of amylase, lipase and trypsin. Harper et al (172) came to the same conclusion with cat preparations, and Magee (206) with dogs. More recently, experiments in man have shown a marked parallelism in the secretion of lipase and amylase induced by food stimulation (195), while earlier on, Lagerlof (236) had shown similar results using secretin to stimulate pancreatic secretion. All these above results followed acute stimulation of the pancreas. Lagerlof (236) also found that, in contrast to the effect of secretin, stimulation of the pancreas by certain digestive products may result in a dissociated enzyme production as described earlier by Pavlov. Other workers, however, have shown a similar dissociation of enzymes using secretin (102, 404). Guth et al (165), following acute stimulation with a test meal in dogs, and using elaborate statistical methods provided results which conflicted with the other two theories. Still other authors have presented evidence for parallelism of enzyme secretion following stimulation with hexamethonium (195), secretin and pancreozymin (61).

The theory of adaptation is perhaps best illustrated, not so much by acute experiments (of which there are few studies reported in the literature) but by the offers of different diets, in animals and man, on enzyme secretion. Magee and Hong (261) studied the daily output of pancreatic juice in chronic pancreatic fistulae dogs and the influence on this, of different diets given for periods of three days. They noted, for example, that the addition of soya flour to the standard diet increased both the volume and amylase significantly, but had no effect on protease. Reboud et al (334) studied the adaptation of pancreatic amylase and chymotrypsin in rats, to a starch or protein rich diet, using labelled valine. They found that there was a 8 - 9 fold increase in the rate of amylase synthesis and a two

fold decrease in the rate of chymotrypsin synthesis in those rats on the starch diet. Michelson (286) is quoted to have placed a number of normal people on special diets for periods of 10 - 14 days and noted adaptation to the diet of trypsin and lipase content of the pancreatic juice, but not of amylase. Abramson (1) on the other hand, found a decrease of all enzymes in people on diets deficient in eggs, fish and meat. Interesting work carried out by Grossman et al (159) showed that in rats kept on a constant diet for periods of three weeks, there was an adaption of their enzymes to the diet. Thus, for example, those rats on a high carbohydrate diet, developed very high amylase and low trypsin levels and the lipase remained unchanged. A high protein diet produced an increased trypsin level and a high fat diet, for some reason, depressed the amylase content of the pancreas. Similar results were found by Beamer (39). Other work by Rebound (333) et al on rats fed balanced, starch rich, casein rich or fat rich diets, showed that homogonates of the pancreata of rats fed the casein rich or starch rich diets, had an increased activity of chymotrypsin and amylase respectively. The specific activity of trypsinogen remained constant on all the diets, while lipase appeared to show some differences on different diets, but the results were not consistent with the theory of adaptation to the main components of the diet. These authors (332) showed further that the quantitative amount of chymotrypsinogen and trypsinogen in the homogenates of rats fed high protein diets was two to three times higher than an equivalent amount of homogenates from rats fed high carbohydrate diets. Ben Abdeljlil (41), working in the same laboratory, extended the investigations to the pancreatic juice of these rats which showed the same type of adaptation to the diet ingested. The adaptation started immediately after the diets were changed and

appeared to be related to the products formed during digestion, since both dextrose and starch in the same concentrations induced the same effect. Bovine pancreatic juice is low in amylase and lipase activity (226), and this may be the result of their particular digestive habits. Magee and his co-workers (258, 259) also found an adaptation of the pancreatic enzymes to different diets and suggested that the important factor regulating pancreatic enzyme secretion, even under conditions of deficient diet, is an adequate duodenal stimulus. Magee concludes that the manufacture of amylase is "independent of the other pancreatic enzymes." Hong and Magee (194) in later experiments have noted that different dietary amino-acids influence the individual pancreatic enzymes in different ways when fed to rats over three weeks. Grossman et al (160), as a result of their work on rats, suggested that amylase output may be mediated by some humoral mechanism, while trypsin may be influenced by a nervous reflex factor. More recently, Magee and White (262) has shown evidence disproving their original hypothesis of the intensity of the duodenal stimulus regulating enzyme production from the pancreas. They found similar amounts of proteolytic enzymes per pancreas tissue of rats fed both high protein diets and rats fed diets alternating between high and low protein.

Snook and Meyer (370,371) have made the very interesting suggestion that dietary proteins slow down enzyme breakdown in the intestinal lumen by competing with the enzyme as a substrate for proteolysis. This would not explain the high trypsin content of homogenates of the pancreas in rats fed high protein diets, as suggested in the work of Magee. They also note that the high trypsin inhibitor content of certain diets may influence the pancreatic enzyme response (371).

The results of partial and complete cell fractionations have not entirely helped in solving the problem of parallelism versus adaptation. This method shows that the enzyme content of the acinar cell is found predominantly in the zymogen and microsome fractions. Palade (312) has suggested that about 70% of the enzyme activity is found in the zymogen granule fraction of starved animals, with the remainder of the microsome and their supernatant fraction. Only chymotrypsin, ribonuclease and amylase have been carefully studied with respect to their intracellular distribution, but even with these enzymes there is some disagreement. Hanson (169), for example, found the proteolytic and amylase fraction to be higher in the zymogen fraction than any other cell fraction, while no lipase was found in the zymogens. Desnuelle (96), however, found that a large part of the lipase content was, in fact, within the zymogen granule. The supernatant fluid which is said to have a high amylase (243) and lipase content, had no proteolytic activity (169). The chromatographic studies of Kellef and Cohen (225) showed the presence of trypsinogen, chymotrypsinogen A, ribonuclease in the same relative proportions as pancreatic juice, while the proportion of chymotrypsinogen B was low. In general, however, it seems that, with regard to the zymogen fraction, there is a close parallelism between its enzymes and that of pancreatic juice.

Chapter 5

THE INVESTIGATION OF NORMAL AND ABNORMAL EXOCRINE PANCREATIC FUNCTION

Chapter 5 THE INVESTIGATION OF NORMAL AND ABNORMAL EXOCRINE PANCREATIC FUNCTION

A. ASSESSMENT OF PANCREATIC FUNCTION WITH DUODENAL INTUBATION

1. WITHOUT PANCREATIC STIMULATION: Attempts to measure pancreatic function directly in humans were first made by inserting a tube in the duodenum near the ampulla of Vater, and collecting duodenal contents (77, 131, 393). No attempt was made to stimulate the pancreas, but gastric juice was not prevented from entering the duodenum. This seemingly crude attempt to assess pancreatic function did not meet with much success, as samples contaminated with gastric juice had to be discarded. As Comfort and Osterberg (77) noted, only when the pancreatic enzymes were completely absent, could the results be regarded as abnormal, due to the very considerable range of variation in enzyme activity of the duodenal contents of normal humans. Waldron (408), in experiments on animals, noted that the mere presence of the duodenal tube acted as a mechanical stimulus to pancreatic secretion.

2. WITH PANCREATIC STIMULATION:

a) Hormonal stimulation: Since the discovery of secretin by Bayliss and Starling (37), and later the discovery of pancreozymin by Harper and Raper (173), these two hormones have been used by numerous authors (3, 74, 102, 103, 107, 236, 404) in the investigation of pancreatic function and disease. The earlier authors used secretin alone, but the enthusiasm of these investigators diminished when the

difficulties of the diagnosis of moderate or minimal disease of the pancreas became apparent. To increase the diagnostic significance of the secretin test, later authors either increased the dose of the secretin (179, 240), or introduced pancreozymin (114, 379) into the secretin test. Before the use of pancreozymin, some authors had used some other stimulant of pancreatic secretion, such as mecholyl or insulin together with the secretin. These drugs produced a pancreozymin-like response by their effect on the vagus nerve.

The technique of intubation, the number of tubes used (double lumen(3, 102, 137) or a separate gastric and duodenal tube (27)), the dose of secretin and pancreozymin, the method of injection (single injection or slow infusion), duration of collections, the number and type of enzymes measured and interpretation of results, varies in different centres. Pancreozymin may be given before (377), very soon after (60) or some time after secretin (26, 272). Some authors, however, still feel that pancreozymin adds little to the diagnostic value of the secretin test (111), and leave this additional injection out. Similarly, enzyme estimation in the secretin test alone is thought to be of little value (60, 137). Sun and Shay (378) choose to omit these estimations from their secretin test. When pancreozymin is used together with secretin,however, enzyme estimations are reported to be of diagnostic importance by most workers. In general, only one enzyme is measured and this has been amylase in most reported series.

b) Test meal stimulation: Recently Lund (253) has reintroduced interest in the use of the standard test meal in the investigation of pancreatic enzyme secretion and as a test of pancreatic disease.

The general method of the test meal used by most authors, is to pass a tube through the pylorus into the lower duodenum (Table 7). After collecting the basal juice for about 20 minutes, a liquid meal is given consisting of a mixture of carbohydrate, fat and protein. The duodenal contents which may be aspirated by suction or simple siphonage, contains a mixture of gastric contents (meal + acid), succus entericus, bile and pancreatic juice. The pH and volume of the aspirates are recorded and the concentration of one or more of the pancreatic enzymes estimated.

c) Drug stimulation: Various drugs, such as nitrates (145), methyl and dimethyl guanadine, mecholyl and other parasympathomimetic drugs (75), stimulate pancreatic secretion and have been used, usually together with secretin. Their responses are variable and unpredictable, however, and their side effects have made the use of any of these agents unjustifiable. Hypoglycaemia (132, 236, 390), induced by insulin, has also been used to stimulate the pancreas in man. The response is thought to be due to vagal stimulation as is the case with the parasympathomimetic drugs.

B. ASSESSMENT OF PANCREATIC FUNCTION WITHOUT INTUBATION

The normal and abnormal pancreas has been studied by various means, e.g., simple duodenal aspiration, secretin, pancreozymin, the test meal and various drugs. The numerous variations on all of these tests, serve to indicate that no one test is perfect or even near perfect, especially with regard to moderate or minimal pancreatic disease. A host of other tests have, therefore, been used in the investigation of pancreatic disease.

1. Pancreatic enzyme estimations: Pancreatic enzymes have been measured in the sreum (61, 376, 379), urine (58), pleural and peritoneal aspirate and faeces, in various forms of pancreatic disease. Some authors have claimed the results of these tests to be superior to any other test of pancreatic disease but most workers feel that the above mentioned are unreliable parameters, although they may be useful in certain specific cases.

2. Other laboratory procedures: During the acute attack of pancreatitis, the presence of glycosuria, hyperglycaemia, bilirubinaemia, raised alkaline phosphatase (111), hyperlipaemia (412), methaemalbuminaemia (305) and other enzymes (22) have been estimated by various workers and thought useful in the detection of disease. Serum calcium (413) may also be reduced in severe cases.

In the subacute phase or settling phase of pancreatitis, other investigations may be included: The glucose tolerance test (274), microscopic examination of duodenal contents for cholesterol crystals and biliary pigments and the provocative enzyme test.

In chronic disease of the pancreas, some of the above tests may be useful and also other tests which indicate pancreatic insufficiency. These include tests of faecal fat excretion, starch tolerance test, measurement of stool amino nitrogen, gelatin tolerance test and the plasma proline test.

Silberberg and Hadorn (361) have described a relatively new and interesting technique in assessing pancreatic malfunction. They separated the pancreatic enzymes in the duodenal juice by micro-electrophoresis and then identified them using histiochemical stains. Abnormally low enzyme activity was easily observed.

3. Radiology: The presence of pancreatic calcification on radiology of the abdomen is one of the few diagnostic criteria of pancreatic disease. Any other form of radiology attempts to define disease of the pancreas by its effect on surrounding tissues and organs. Thus, the presence of inflammation in the pancreatic area may manifest itself in the presence of a local ileus or sentinal loop, the colon "cut off sign" and fluid levels.

An increase in the size of the pancreas may be indicated by external pressure defects of the stomach, the duodenal cap (122), the duodenal loop and the colon. The preceeding are outlined by air in the acute attack, or barium in subacute or chronic disease of the pancreas. More recently, highly specialized radiological techniques (232) have been used and these include hypotonic duodenography (33), frontal tomography following combined retro-peritoneal and peritoneal air insufflation and coeliac and superior mesenteric selective arteriography to demonstrate filling defects in the pancreas.

4. Pancreatic scanning: The use of ^{75}Se-selenomethionine (49) as an agent for pancreatic scanning, has provided a useful additional tool in the diagnosis of some cases of pancreatic disease. An "unequivocally normal pancreatic scan is strong evidence that the gland is normal." (284).

S E C T I O N 2

Chapter 6

AIMS OF INVESTIGATION

The aims of the present study were to investigate the following:

1. The exocrine function of the pancreas in response to a test meal in the normal control subject: The "normal" individuals consisted of hospitalized patients without any gastro-intestinal or pancreatic disorders previous to, or at the time of the investigation. In the main, they were patients from the surgical wards of Groote Schuur Hospital. The test meal used in this study was one described by previous workers, consisting of a mixture of carbohydrate, fat and protein with an added flavouring. The ingestion of the test meal stimulated pancreatic secretion which was aspirated via an intra-duodenal tube. The volume, pH and enzyme content (amylase, trypsin, chymotrypsin and lipase) was measured.

2. The effect of varying the volume, quality (with regard to carbohydrate, fat and protein) and osmolarity of the test meal on pancreatic function:

a) Volume: The effect of volume of the test meal on pancreatic enzyme output was only tried on one patient, who, during the course of one week, received 300 ml., 350 ml., 450 ml. and 550 ml. of test meal. The quality remained the same throughout.

b) Quality: An attempt was made to assess the pancreatic response to different meals consisting of carbohydrate only, fat only, protein only and also different combinations of each. In almost all cases the types of food used and the quantities were the same as for

the test meals. All the meals were prepared the morning of the test in the Diet Kitchen of Groote Schuur Hospital.

c) Osmolarity: The osmolarity of all sample test meals was performed, and the attempt made to see whether there was any correlation between this and pancreatic secretion of enzymes.

3. The exocrine function of the pancreas in response to a test meal in patients with pancreatic disease and other disorders which may affect pancreatic function: These patients were divided up into 2 groups:

a) Patients with proven acute, subacute and chronic pancreatitis. Besides the test meal study, all these patients had a complete pancreatic work-up, which included a secretin-pancreozymin pancreatic function test, serum enzyme studies during this latter test, glucose tolerance test, faecal fat studies, liver function studies, a barium meal, hypotonic duodenography in some cases and sweat tests.

The majority of patients had recurrent or chronic pancreatitis, but a test meal was performed on a few patients as soon as they started taking liquids following an acute attack.

b) Patients with gastrointestinal and other disorders which may affect pancreatic function. Test meals were performed on patients with the following disorders: Peptic ulcers, porphyria, starvation regime, infectious hepatitis and lymphoma of the bowel.

4. A comparison between the exocrine response of the pancreas to the test meal and that produced by secretin-pancreozymin stimulation in health and disease: Where possible, the two tests were performed during the same week. When this was not done, the period between the two tests is indicated in the text. The secretin-pancreozymin test was that used routinely in our laboratory, consisting of a 60 minute collection post-secretin and a 20 minute collection post-pancreozymin injections. Gastric and duodenal tubes were used throughout.

5. Problems of the test meal: The various problems raised by other workers have already been mentioned. These include the effect of varying test volume, quality and osmolarity.

Two additional problems of the test meal will be raised; (a) Gastric acidity and its effect on the test meal and (b) the quantity of duodenal contents lost during aspiration following the test meal. To assess the amount lost, radio-active Rose Bengal was added to the meals in a number of tests. The amount of radio-activity present in the duodenal aspirate and the amount present in the stomach contents aspirated at the end of the test were measured, and in this way it was possible to calculate the amount of duodenal contents lost during the procedure.

Chapter 7

MATERIAL

A. THE SUBJECTS

1. Normal controls: The majority of controls were selected from the surgical wards, and a few from the medical wards of Groote Schuur Hospital. None of the patients had abdominal disease or symptoms. None had a history of an excessive amount of alcohol consumption. When permission was obtained from the patient, a secretin-pancreozymin test was performed as well. A total of 18 test meals were performed on 16 normal controls. All the subjects were informed of the nature of the investigation and their consent was a mandatory prerogative for continuing with the study.

2. Patients with pancreatic disease: Any patients without definitive evidence of pancreatic disease was excluded from this group. The criteria (273) adopted as evidence of pancreatic disease were those generally accepted at the Gastrointestinal Unit of Groote Schuur Hospital, consisting of the following:

(a) Laparotomy evidence of pancreatitis, or

(b) Grossly abnormal pancreatic function test (secretin-pancreozymin), or

(c) Serum amylase greater than 1000 units/ml. during an attack, or

(d) Radiological evidence of pancreatic calcification, or

(e) A good history of pancreatitis plus an abnormal pancreatic function test and a serum amylase greater than 300 units/ml, or

(f) A good history, plus abnormal glucose tolerance test, plus a positive provocative enzyme test, plus a suggestive barium meal picture, plus one criteria from (e).

3. Patients with other diseases: These included patients with diseases that may involve the pancreas, such as porphyria, hypo-proteinaemia or conditions that could influence the duodenal aspirate, such as gastric surgery or lymphoma of the bowel

B. THE TEST MEAL AND OTHER MEALS USED IN THE INVESTIGATION

a) The test meal: The indredients used were the following:

Soya bean oil	18 Gram	
Casilan	15 Gram	
Glucose	40 Gram	
Bingo	20 Gram	(Flavouring)

Water added to make a total volume of 300 ml.

This mixture has a protein content of 5%, carbohydrate of 15% and fat of 6%. The osmolarity of the test meal was 898 milliosmols per litre and the pH was 6.9. The meal was prepared on the day of the test in the Diet Kitchen of Groote Schuur Hospital by one of the qualified dieticians.

b) The water meal: This consisted of 300 ml. of tap water, without added flavouring, with a pH of 8.7 and osmolarity of 1.0 milliosmols per litre.

c) The saline meal: This was prepared by adding sodium chloride to ordinary tap water, so that the final osmolarity was as close as possible to that of the test meal. The final mixture was rather

salty. The osmolarity was 790 milliosmols/litre and the pH was 8.7.

d) Protein meal: This consisted of 15 Grams of Casilan mixed with water, so that the final volume was 300 ml. To make the meal more palatable, a neutral flavouring was added (Sucaryl tablet, a diabetic sweetener). The osmolarity was 48.0 milliosmols/litre and the pH 6.3.

e) Carbohydrate test meal: This consisted of 40 Grams of glucose made up to 300 ml. with water. No flavouring was neccesary. The osmolarity was 835 milliosmols/litre and the pH was 6.3.

f) Fat test meal: This consisted of 18 Grams of soya bean oil with the addition of Sucaryl as a flavouring. Water was added to make a total volume of 300 ml. This meal was prepared by the Senior Dietician, who took great care mixing was as thorough as possible. The osmolarity was 1.0 milliosmols/litre and the pH 7.3.

g) Other meals used in the study: In a number of tests, the test meal, as described in (a) was given, but one of the main ingredients was ommitted. Thus, the individual received a carbohydrate-free, fat-free or low protein meal. The total volume was still 300 ml. Sucaryl was added to sweeten the meal when necessary.

Chapter 8

METHODOLOGY

A. THE STANDARD TEST MEAL

1. Period of fasting: All patients fasted from 10 p.m. the night before the test. The test was usually started at 9 a.m. the following morning.

2. Apparatus:

(a) Radio-opaque Number 16 Rusch duodenal tube, with an internal diameter of 4 mm. and an external diameter of 6 mm. The tube had 6 perforations at its distal end, spaced 4 cm. apart and covering a total distance of 14 cm. from the end.

(b) Continuous steam vacuum suction of 5 lbs/square inch was used. In a number of cases a Stedman suction pump was used, providing a similar pressure.

(c) A large plastic bath containing two rows of test tubes surrounded by ice. The duodenal aspirate was collected in a large measuring cylinder surrounded by ice. At the end of each collection period, the juice was poured into a test tube and left in the ice bath.

3. Intubation and Screening: The duodenal tube was inserted via the nasal passage into the stomach, using fluoroscopic control. The subject then lay on his right side and the tip of the tube was again manoeuvred, under fluoroscopic control, until it had entered the duodenum. At this stage, the subject lay on his back and the tube

was advanced until its tip reached the duodeno-jejunal flexure. The external end of the tube was fixed to the patient's face with tape. During the course of the test, the position of the tube was constantly checked and adjusted when necessary.

4. Suction: The suction was constantly monitored and the pressure relieved every few minutes, thus allowing efficient flow. When the flow of duodenal juice appeared to be blocked or considerably slowed down, a few centimeters of air was syringed down the tube to re-establish patency.

5. Position of the patient: In almost all the cases the subject lay on a bed at an angle of about 60° to the horizontal. In the occasional patient, when gastric emptying was obviously minimal and insufficient duodenal aspirate was present, the subject lay horizontally on the bed and was turned slightly on his right side. When the duodenal aspirate increased, it was usually possible to reassume the reclining position.

6. Collections: With the Rusch tube in position, suction was started and the basal secretion collected for a minimum of 15 minutes. If there was a fair amount of secretion after this time, the collection was continued, but for a maximum of 25 minutes. The subject was then given the warm test meal, and instructed to drink this slowly over about three minutes. Suction was resumed immediately after the ingestion of the meal. The time was noted and the duodenal aspirate collected for 30 minutes. The suction was then stopped, while the juice was poured from the measuring cylinder, into one of the labelled test tubes. Three further 30 minute collections were made. At the

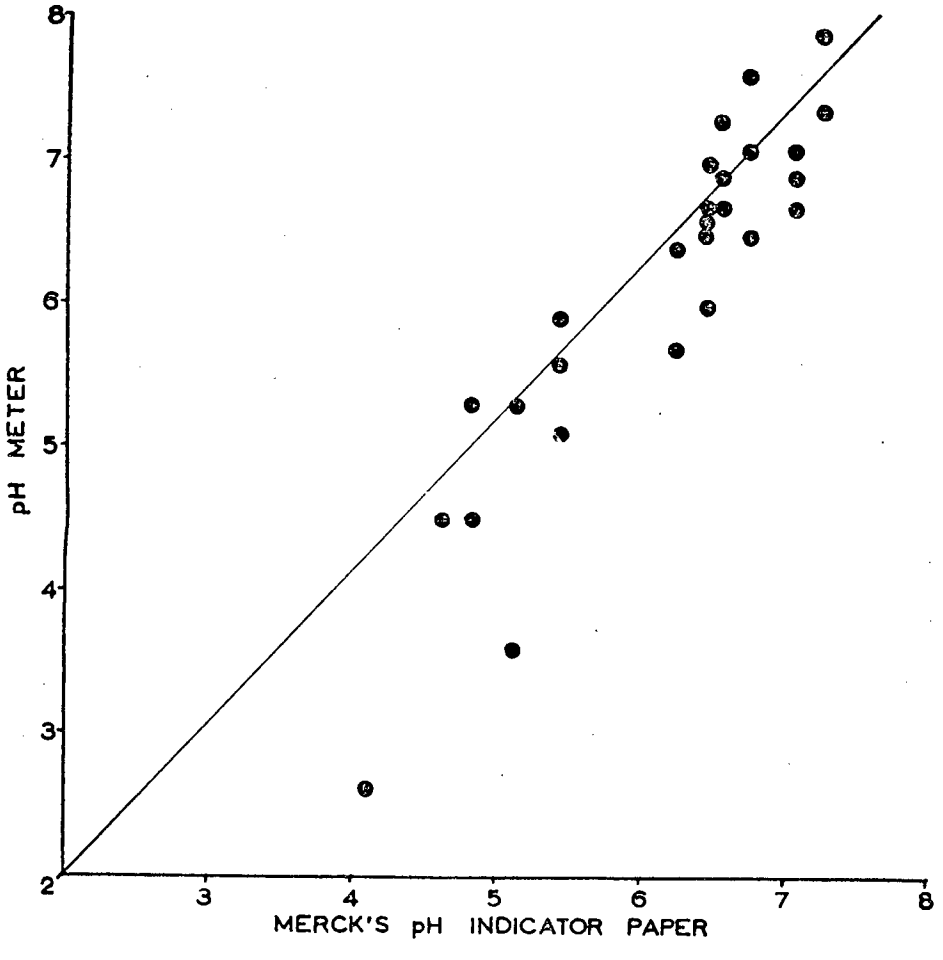

FIG. 1: pH PAPER VERSUS pH METER

end of test, 5 samples had been collected - 1 basal and four 30 minute collections. A number of tests wer performed using eight 15 minute collections.

The duodenal aspirate was surrounded by ice during all stages, from the time of its collection until the time of its measurement.

7. Recordings:

(a) Volume: For this purpose a 100 millilitre measuring cylinder was used.

(b) pH: The pH was measured by the investigator, using Merck's pH indicator paper. When compared with the pH meter (Radiometer), this paper was found to be sufficiently accurate for our purposes (Fig. 1). In accordance with the instructions the pH was measured immediately after a drop of duodenal juice was applied to the paper.

B. DIFFICULTIES ENCOUNTERED DURING THE STANDARD TEST MEAL

1. The meal: No real problems were encountered with the test meal. An occasional subject was unable to drink the meal during the allotted three minutes, and in such cases, further time was given them. None of the subjects to whom the test meal was administered found it impossible to ingest the meal with the tube in situ, although a few complained that it was slightly uncomfortable.

2. Intubation: With experience, not much difficulty was encountered in manipulating the tube through the pylorus. This took an average of about 10 to 20 minutes and very occasionally as long

as 60 minutes. Once through the pylorus, there was usually no difficulty in advancing the tube up to the duodeno-jejunal flexure. If vomiting was problematic either during the intubation or ingestion periods, the test was abandoned.

3. Suction or siphonage: In the first few test meals simple siphonage without suction was attempted. The collecting flask was placed about 60 centimeters below the duodenum level. This method was useful only when large quantities of duodenal aspirate were present. With a low volume of duodenal contents, this method required a great deal of attention and proved to be far inferior to suction.

4. Duodenal aspirate: A number of problems were encountered relating to the aspirate:

(a) pH: The pH of the duodenal aspirate was monitored more frequently when the pH fell below 6 or when the aspirate appeared to be heavily contaminated with gastric juice. When the pH fell below 5.0, however, aspiration was stopped for about 1 minute. In the majority of cases, the pH increased towards neutrality spontaneously. Duodenal juice was not discarded even when the pH was below 5.0.

(b) Volume: During the early part of the research programme, an inadequate volume of aspirate was one of the main problems encountered in both normal and abnormal individuals. It soon became clear, however, that turning the subject slightly on his right side facilitated gastric emptying and a good volume was obtained in all cases.

(c) Gastric contamination of aspirate: Heavy contamination of duodenal aspirate with gastric contents (food and acid) occurred very infrequently. Filtering of these samples of duodenal juice was resorted to early in the research programme but was found to be unnecessary. Filtering was thus confined to those very rare samples where the juice was too thick to be readily used for enzyme estimations.

C. THE SECRETIN-PANCREOZYMIN TEST (26)

After an overnight fast, a Number 14 or 16 Rusch radio-opaque duodenal tube was passed into the duodenum, and the tip was adjusted under fluoroscopic control, to lie at the junction of the second and third part of the duodenum. A second tube was then passed and positioned in the most dependent part of the stomach. The fasting duodenal contents were aspirated and discarded, and the collections were continued for a further 10 minutes. Secretin (Boots), 2 units/Kg. body weight, diluted in 20 ml. of normal saline, was then injected intravenously. The duodenal contents were aspirated for 60 minutes and pooled in two samples, those collected during the first 20 minutes and those during the subsequent 40 minutes. At the end of one hour pancreozymin (Boots), 1.5 units/Kg body weight, diluted in 20 ml. normal saline was slowly injected intravenously and the duodenal contents aspirated for a further 20 minutes. Vacuum suction was used as described above. The volume of all the samples was measured. All collections were made under ice.

D. ENZYME ESTIMATIONS

1. General: The duodenal juice was collected in test tubes

surrounded by ice. Aliquots of these samples were transferred to the laboratory, still surrounded by ice, until the time of enzyme estimation. All estimations were performed on the day of the test. The tryspin assay was started as soon as the first sample becams available. Lipase and amylase assays could only be started once the test meal had been completed. No attempt was made to precipitate out the protein (252) and thus separate enzyme protein from the rest of the juice, or to filter unless the juice was heavily contaminated with food. The former method is reported to substantially decrease enzyme activity (23, 28). Details of all methods used can be found in the Apendix, page 149.

2. Amylase: A modified amyloclastic method described by Pimstone (317) was used. Starch is the substrate in this procedure. The principle of the method is that the duodenal juice containing the enzymes is added to the starch solution and incubated for a specific time. The reaction is then stopped with acid, iodine is added and the breakdown of starch is measured by the decrease in blue colour which has occurred as compared to a standard solution to which acid was added before incubation.

(a) Apparatus: A Klett-Sommerson photoelectric colorimeter used for the final readings, using a number 62 red filter.

(b) Method: A full description of the method may be found in the Appendix, page 149. All samples were prepared in duplicate. The 200 ml. flask into which the 1 ml. of duodenal juice is pipetted, is not immersed in ice as amylase is stable at room temperature sor some hours. By the time that the amylase assay was started, a number of trypsin results were available and it was possible to decide what incubation period would be necessary for the

amylase procedure.

3. Trypsin: The spectrophotometric method described by Schwert and Takenaka (350) was used as a basis for the trypsin estimations. The principle of the method is based on the hydrolysis of the synthetic substrate, N-benzoyl-l-arginine ethyl ester hydrochloride (BAEE) by trypsin. The change in wave length which occurs during the reaction is recorded on the recording apparatus of the spectrophotometer. The actual method used was basically similar to that employed in the Seravac (352) laboratories and modified by Barbezat (28).

(a) Apparatus: A model DB Beckman spectrophotometer with a recording apparatus. A Colora Ultra-thermostat apparatus was attached so that the cuvette chamber could be kept at a constant temperature (25° C)

(b) Method: The dilutions used varied from 1/5 to 1/80, depending on whether high or low enzyme concentrations were present. This had to be determined by trial and error. The presence of food in the duodenal juice appeared to have surprisingly little effect on the recordings. The line on the graph was rather ragged, but nevertheless straight, and duplicate samples were not usually more than 5% different.

(c) Definition of activity: One unit of activity is defined as "that activity which causes a decrease in optical density at 253 mμ of 0.001/minute."

4. Chymotrypsin: The method used for assaying chymotrypsin activity was similar to that employed in the Seravac (352) laboratories and modofied by Barbezat (28). This is based on a method first

described by Schwert and Takenaka (350). As in the case of trypsin, a synthetic substrate N-acetyl-L-tyrosine ethyl ester monohydrate (ATEE) was used. Chymotrypsin hydrolyses this substrate and the change in wave length which occurred during the reaction is recorded on the recording apparatus of the spectrophotometer.

(a) Apparatus: As for trypsin.

(b) Method: In general, the dilutions were similar to those of the trypsin method but not infrequently, higher dilutions were necessary. A slight rise was noted on the graph before the line proceeded to fall. No obvious reason for this was apparent.

(c) Definition of activity: One unit of activity is defined as " that activity which causes a decrease of optical density at 237 mμ of 0.001/minute."

5. Lipase: The method used was that described by Weber (414). The natural substrate for pancreatic lipase is triglyceride. In the method described, lipase is incubated for 2 hours with an olive oil emulsion and the resulting fatty acid (oleic acid) liberated is measured by titrating with sodium hydroxide. The presence of bile salts prevents the inhibition of lipase activity so that larger amounts of fatty acids are liberated and the rate of hydrolysis remains constant for relatively longer periods.

E. RADIO-ACTIVE STUDIES

I^{131} Rose Bengal, obtained from the Radio Chemical Centre (Amersham) was used. This substrate is a non-absorbable marker (256) comparable to PEG (polyethylene glycol) or any other marker not absorbed

through the mucosa. Twenty five micro-curies was made up to 100 ml. in water and this was added to 200 ml of the test meal being used on that day. The subject thus ingested the usual 300 ml. of the meal. Each sample was placed in a 6 G.M. tube ring counter connected to a conventional scaling unit and the radio-activity calculated as follows:

Standard radio-activity = y

Number of counts in sample of duodenal juice = S

Number of counts given = y x 98

$$\% \text{ total radio-activity present in sample} = \frac{S \times 100}{y \times 98}$$

F. ENZYME ACTIVITY AT DIFFERENT pH VALUES

In order to simulate test meal conditions as closely as possible, hydrochloric acid was added to relatively clear pancreatic juice collected during a secretin-pancreozymin test. Gastric contamination of the duodenal contents was prevented by gastric aspiration. The test tubes were surrounded by ice throughout the procedure except during the time of actual pH readings.

0.1 N hydrochloric acid was added slowly to fixed aliquots of the relatively pure pancreatic juice until the pH was at the required reading. Enzyme estimations were performed inthe usual way. Suitable corrections were made for the dilution factor.

Chapter 9

THE STANDARD TEST MEAL IN NORMAL CONTROLS

RESULTS PART I

The results of the test meal in 2 groups of control subjects will be discussed. The groups are as follows:

Group A: 18 normal individuals in whom duodenal collections were made for four 30 minute periods after the ingestion of the test meal

Group B: 10 normal individuals in whom collections were made for eight 15 minute periods after the ingestion of the test meal.

Chapter 9 THE STANDARD TEST MEAL IN NORMAL CONTROLS

A. THE NAKED EYE APPEARANCE OF THE DUODENAL ASPIRATE

The duodenal aspirate was surprisingly clear of gastric contents throughout the period of the test meal. It was never as clear as pure pancreatic juice, but neither was it as murky as the basal aspirate before the test meal was given. A change in character of the duodenal aspirate from the murky, bile-stained, mucoid solution in the basal collection to a watery, less bile-stained solution without mucus was evident soon after the meal was administered. Occasionally one or two collections contained large quantities of the test meal, but this did not seem to interfere with the laboratory estimations. It must be assumed, however, that the accuracy of these samples was not high as with the clearer collections. All specimens remained fairly equally bile-stained for each subject during the period of the test.

B. VOLUME OF DUODENAL ASPIRATE *

The volume of the duodenal aspirate is, at best, only a very rough estimate of the total volume of duodenal contents passing the end of the radio-opaque tube. Table 1. shows the percentage radio-activity of the Rose Bengal in the duodenal aspirate during the test and the gastric washings remaining at the end of the test after the administration of a fixed dose. The percentage duodenal aspirate

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* See Appendix page 158 for individual results.

TEST NO.	TEST MEAL		I^{131} ROSE BENGAL		
	Type	Volume of Aspirate ml	% in Duodenal Aspirate	% in Gastric Aspirate	% Recovered
62	Standard	78	47.8	10.0	46.3
92*	Carbohydrate	137	7.9	21.0	10.0
112	Standard	122	12.9	19.8	16.1
101*	Saline	210	29.7	1.1	28.2
69	Standard	254	28.5	28.5	39.8
57	Standard	268	47.0	20.6	59.2
83	Standard	326	31.5	0.2	31.6
58	Standard	246	39.0	1.8	39.7
59	Standard	53	3.1	14.1	3.6
84	Standard	24	1.7	15.9	2.0
70	Standard	49	4.32	6.4	5.2
113	Standard	51	2.8	10.13	3.1
114°	Standard	269	34.0	20.0	42.5
107°	Low Protein	161	19.6	25.4	26.2
61	Standard	65	6.5	14.7	7.6
37	Standard	258	19.2	55.3	43.0
108	Casilan	54	6.3	30.8	9.1
93°	Fat	96	7.2	3.8	7.5
22†	Standard	369	18.2	15.0	24.3
95†	Fat	67	8.7	4.8	9.1
97†	Water	439	44.0	0.8	44.4
99°	Saline	388	27.6	0.5	27.7
32	Standard	198	15.9	2.4	16.2
65	Standard	163	5.7	10.9	6.4
	MEANS		19.6	13.6	22.8

TABLE I

*, °, † } Tests with the same symbols were performed on the same subject

recovered could be calculated from the radioactivity, assuming that there was thorough mixing in the duodenum prior to aspiration.

Mt = total amount of radioactive marker in meal.

Ms = amount of marker left in stomach.

then Md = Mt - Ms = amount of marker that entered duodenum.

Mr = amount recovered in duodenal aspirate.

then $\frac{Mr}{Md} \times 100$ = percentage recovered.

The percentage recovered ranged from 2% to 59.2% with a mean of 22.8%.

NOTE:

1. The wide range of aspirate recovered with repeated test meals, even in the same patient.
2. The fairly good relationship between the volume aspirated and its radioactivity.

The results of the radioactive studies suggest that the volume results mentioned hereafter represent only a fraction of the actual amount of duodenal juice passing the end of the tube.

(i) Basal volume: In group A the mean 15 minute basal volume was 24 ml. while in group B it was 15.2 ml. (Fig. 2 and 3) In two patients this period was extended for nine and four 15 minute periods respectively. In the first patient the volumes were: (15 minute collections in ml.) 10, 40, 2, 0, 0, 0, 0, 0, 0. and in the second subject (15 minute collections in ml.) 42, 50, 5, 0.

Eight of the 28 individuals in groups A and B had no basal aspirate for the 15 minutes before the test meal was given.

(ii) Volume following ingestion of the standard test meal: Figures 2 and 3 show the volumes during the collection periods in the control groups A and B. Although there was no duodenal

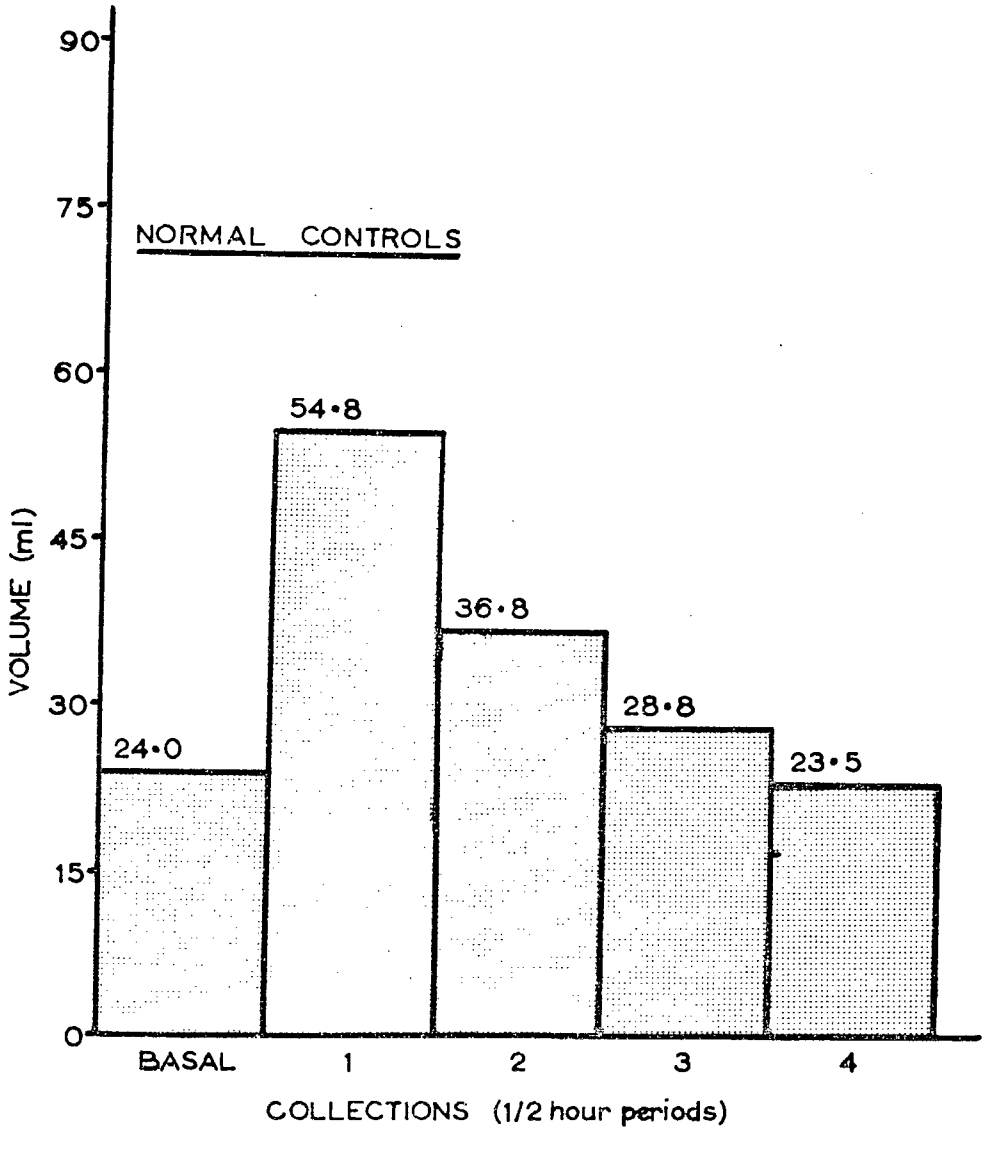

FIG. 2: VOLUME OF ASPIRATE
FOUR 30 MINUTE SAMPLES

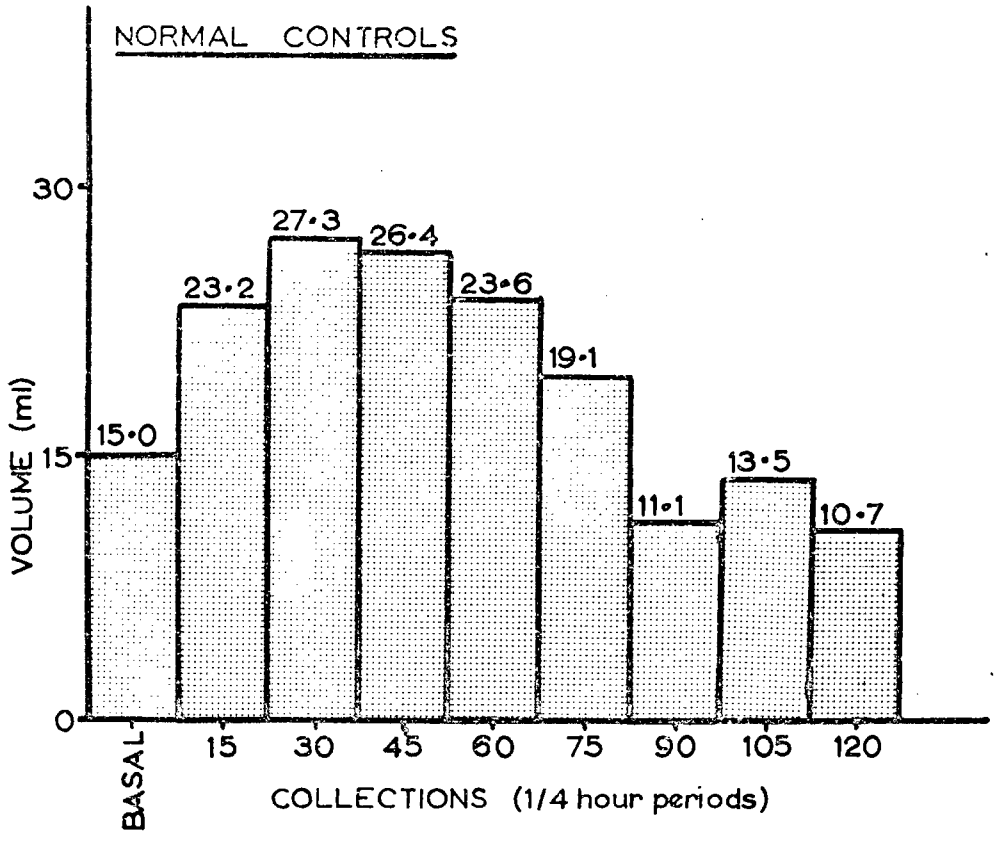

FIG. 3: VOLUME OF ASPIRATE
EIGHT 15 MINUTE SAMPLES

aspirate during the first half hour period (unless the patient was turned on his side, as was the case with a few patients), the majority showed a sudden increase in aspirate about 5 - 10 minutes after the meal. Occasionally 5 - 10 ml. of fluid could be aspirated after the administration of a few mouthfuls of the meal, followed by a short period of about 10 minutes, during which time there was no aspirate. Note that in Fig. 2 there was a sudden, marked increase in volume during the first half hour collection and in Fig. 3 the 15 minute samples show a more gradual rise and a slow fall in the duodenal aspirate.

The difference in volume of the four half hour periods in these normal subjects was not statistically significant ($P > 0.05$). Although the first half hour period showed the highest mean volume, this was not always apparent in the individual cases. Cases 2 and 16, for example, had the highest volumes in the 4th half hour period. The volume of the 3rd half hour period in Case 17 was more than 5 times that of the first sample. When the test meal was repeated on the same subject, the total volume of the aspirated juice was often considerably different as shown in tests 11 and 12, where the volumes were 29 cc. and 90 cc. respectively, and tests 14 and 15 with volumes of 22 cc. and 93 cc. respectively.

C. pH OF THE DUODENAL ASPIRATE

(i) Basal pH: As indicated in Fig. 4, the mean basal pH was less than 6. This was very obviously due to fairly heavy contamination with basal gastric contents, as the sample

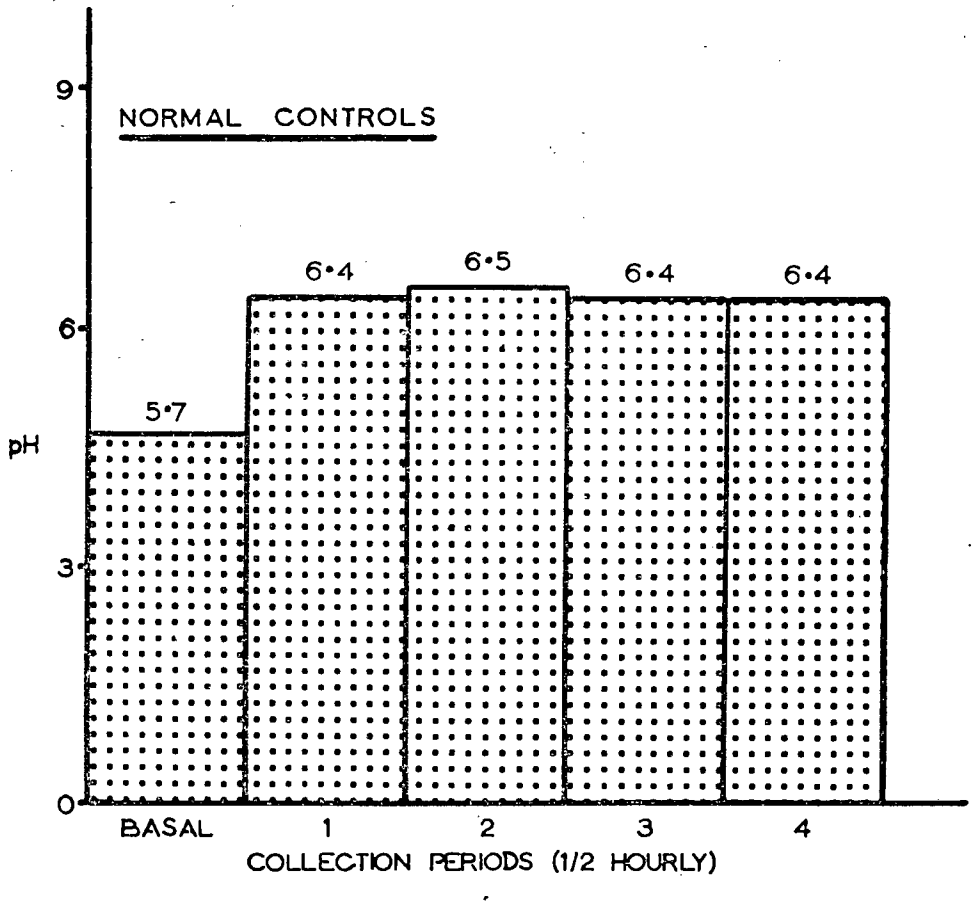

FIG. 4: pH OF ASPIRATE
FOUR 30 MINUTE SAMPLES

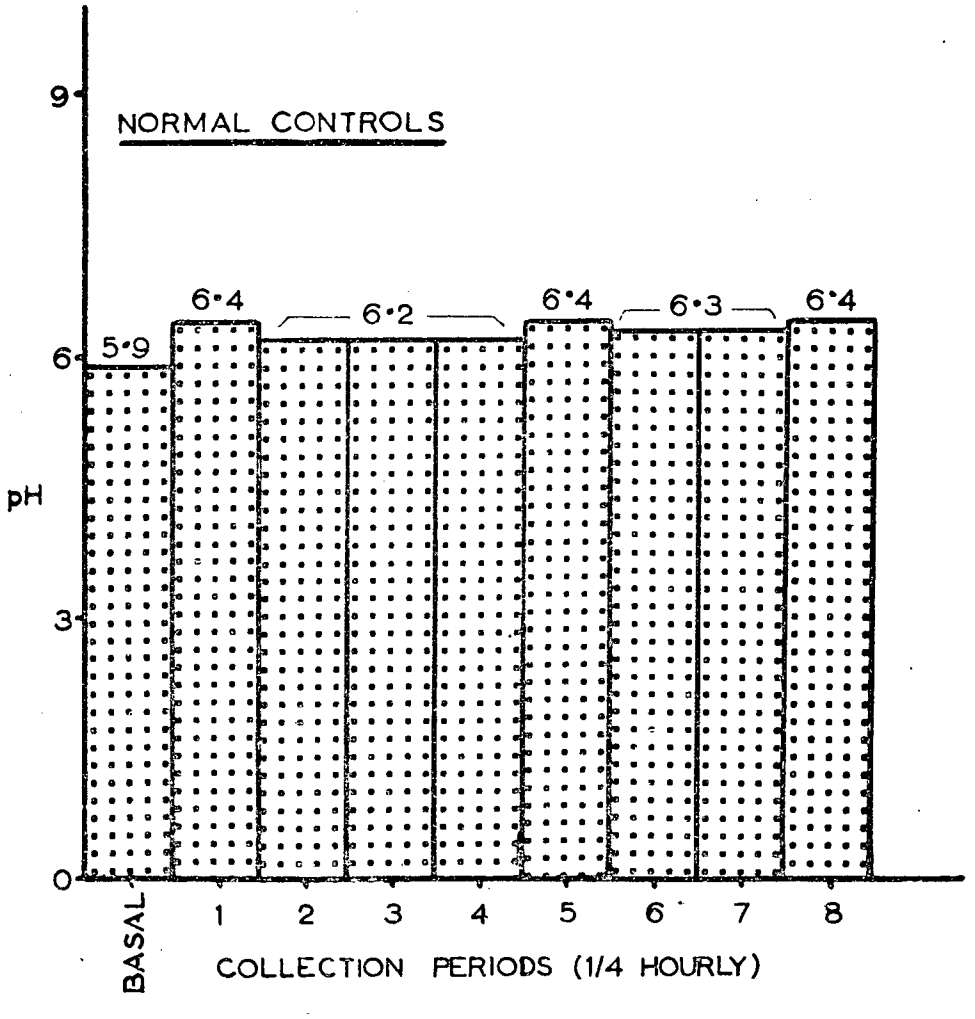

FIG. 5: pH OF ASPIRATE
EIGHT 15 MINUTE SAMPLES

TEST NO.	AUGMENTED HISTAMINE TEST mEq/hour		TEST MEAL		Number of Samples with pH < 6.0
	B.A.O.	M.A.O.	Number of Samples	Mean pH	
8.	1.2	21.3	4	6.2	0
72, 76	0.5	19.0	8	6.5	0
115	1.2	13.2	8	6.3	0
114	0.6	11.8	4	6.6	0
116	1.7	16.6	4	6.4	0
26	4.6	7.3	8	6.5	0
70	0	2.2	8	6.6	0
27	4.6	7.3	8	6.4	0

TABLE 2

B.A.O. = Basal acid output
M.A.O. = Maximum acid output

contained a large amount of mucoid material.

(ii) pH following ingestion of the test meal (Fig. 4 and 5): Following the ingestion of the test meal, there was usually a rise in the pH over that of the basal pH. Where the basal pH was low, e.g. pH 3, 5.4 and 4.8, it rose in the first sample to a pH of 7, 6.2 and 6.3 respectively. Isolated pH readings of the aspirate during the test meal showed infrequent falls in pH to as low as pH 5. Within a matter of seconds, and not usually longer than one minute, the pH had risen to the pH before the fall. The volume of the aspirate appeared to have no effect on the pH, which remained above 6 despite very large volumes, as in test 1, 2, 9 and 17. The pH remained steady throughout the two hour period of the test. Statistically, there was no difference in the pH between the half hour samples ($P > 0.05$).

(iii) Effect of gastric acidity on duodenal pH: Table 2 shows the results of the augmented histamine tests, performed on a different day, on a number of subjects without disease. Column 4 indicates the number of samples of duodenal aspirate taken during the course of a test meal in these patients, and Column 5 the mean pH of these samples. The last column indicates the number of pH readings below 6. Unfortunately, none of the subjects recorded in the Table had a very high maximum acid output (MAO), the highest being 21.3 mEq/hour. All the four half hour samples following the ingestion of the meal had a pH above 6.0 in this subject. The pH never fell below 6.0 in the other subjects so tested.

Table 3: TRYPSIN (UNITS/ML) (FOUR 30 MINUTE COLLECTIONS)

Sample	Range	Mean (M)	S.D.
Basal	812 - 14,233	3035	3417
1	1900 - 8933	4922	2094
2	2133 - 9600	4767	2025
3	2366 - 6350	4174	1135
4	2933 - 6337	5137	1788
MEAN	3547 - 7200	4770	957

Lower limit of normal (M - 2 S.D.) = 2800 units/ml

D. ENZYMES IN THE DUODENAL ASPIRATE [*]

1. TRYPSIN (See Table 3)

(a) Basal values: The trypsin concentration in the basal secretion ranged from 812 to 14, 233 units/ml. with a mean of 3,035 units/ml. The basal trypsin concentration in test 2 can be disregarded because of the very low pH. In 9 out of 13 tests, where a basal sample was obtainable, the trypsin concentration was below the calculated normal value after stimulation (vide infra).

(b) Trypsin concentration following ingestion of the standard test meal:

GROUP A (Four 30 minute collections): Following the ingestion of the meal, there was a very sudden rise in the trypsin concentration in all tests, except when the basal enzyme concentration was very high. The elevated trypsin concentrations were maintained throughout the 2-hour period of the test, and there was a good correlation between the first, second, third and fourth half hour collections ($P > 0.05$). The result of the first half hour collection was, therefore, highly significant and representative of all the samples. In fact, except for test 1, where a dilution effect may have been responsible for the low trypsin concentration in the first half hour sample, and test 2 where the pH interfered, all the other first half hour samples contained normal trypsin concentrations.

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[*] See Appendix page 158 for individual results.

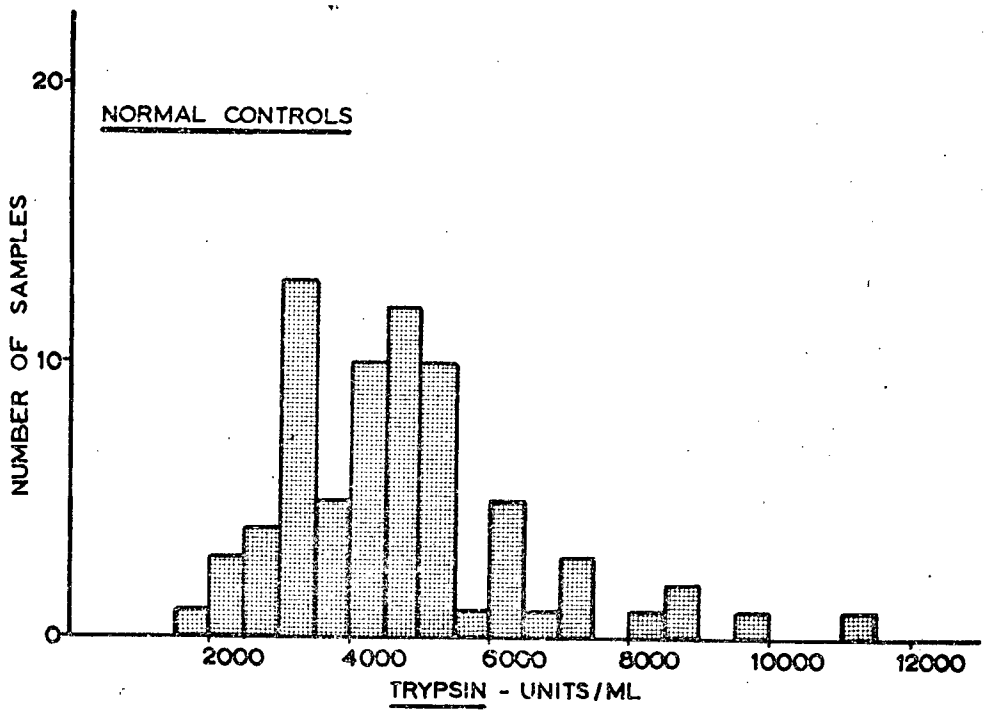

FIG. 6: DISTRIBUTION OF TRYPSIN CONCENTRATION

Table 3 shows the range of trypsin concentrations for each half hour period, together with means for each period and the standard deviations. The lower limit of normal for trypsin was taken as the mean of all samples minus twice the standard deviation which was found to be 2,800 units/ml. Fig. 6 was constructed from the trypsin concentrations in the individuals half hour periods, and shows that the majority of these values fell between 3,000 - 5,500 units/ml. (68%). Only 8.2% of trypsin concentrations were less than 2,800 units / ml. When the mean of the four half hour periods was taken, the trypsin concentration was never less than 3,500 units/ml.

GROUP B (Eight 15 minute collections): These tests were performed with a view to comparing the first 15 minute period to that of the first half hour period in the standard test meal, and to obtain a better picture of the pattern of enzyme response following a test meal.

All 10 subjects in this group had a normal trypsin concentration in the first 15 minute sample following the ingestion of the meal. Statistically, there was no difference between this sample and the first half hour sample following the standard test meal ($P > 0.05$). Sample 1 and 2 almost invariably had a high trypsin concentration. If the test, however, is prolonged for four or five 15 minute periods, the overall mean may fall into the abnormal range because of the sharp fall which occurs following the first peak, e.g. test 20 and 24. Eight 15 minute samples incorporates both enzyme peaks.

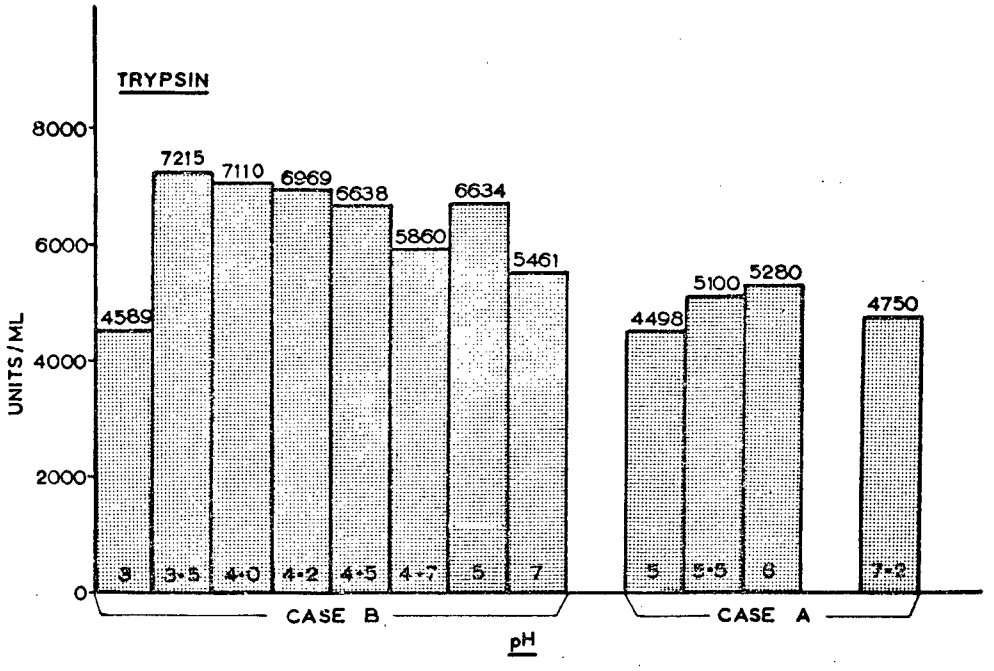

FIG. 7: EFFECT OF pH ON TRYPSIN ACTIVITY

c) Effect of pH on trypsin activity (Fig. 7): The work quoted below must be regarded purely as a preliminary study. In the pancreatic juice of subject A, the trypsin activity remained fairly stable between the pH of 7.2 amd 5.0. A small increase was, however, noted between pH 7.2 and 6.0. As the pH fell from 7.0 to 3.5 in the pancreatic juice of subject B, there was a progressive increase in trypsin activity from 5466 units to 7215 units / minute - a rise of 1749 units/minute. At a pH of 3.5 the critical point appeared to be reached, and there was a sudden fall in trypsin activity. It would appear the pH 3 is the point at which trypsin is inactivated.

2. AMYLASE

a) Basal values: This ranged from 0.46 - 16.42 units/ml with a mean of 8.66 units/ml. Three out of 11 basal samples were in the abnormal range, while the results of a further 3 samples can be disregarded because amylase appears to be inactivated at the pH of these samples. Note the very high standard deviation of this sample.

b) Amylase concentration following ingestion of the standard test meal:

GROUP A (Four 30 minute collections): Following the ingestion of the meal, there was usually a rise in the amylase concentration of the first 30 minute sample. Samples of the second, third and fourth 30 minutes maintained this high level, so that the mean amylase concentrations for the 4 samples did not differ significantly from each other ($P > 0.05$).

Table 4 shows the range, means and standard deviations of

Table 4: AMYLASE ($x10^3$ UNITS/ML) (FOUR 30 MINUTE COLLECTIONS)

Sample	Range	Mean (M)	S.D.
Basal	0.46 - 16.42	8.66	5.1
1	4.25 - 15.71	9.16	2.7
2	4.71 - 14.06	8.36	2.7
3	3.50 - 13.01	8.21	3.0
4	5.25 - 11.36	9.03	2.1
MEAN	5.12 - 11.20	8.40	1.9

Lower limit of normal (M - 2 S.D.) = 4.6 u/ml.

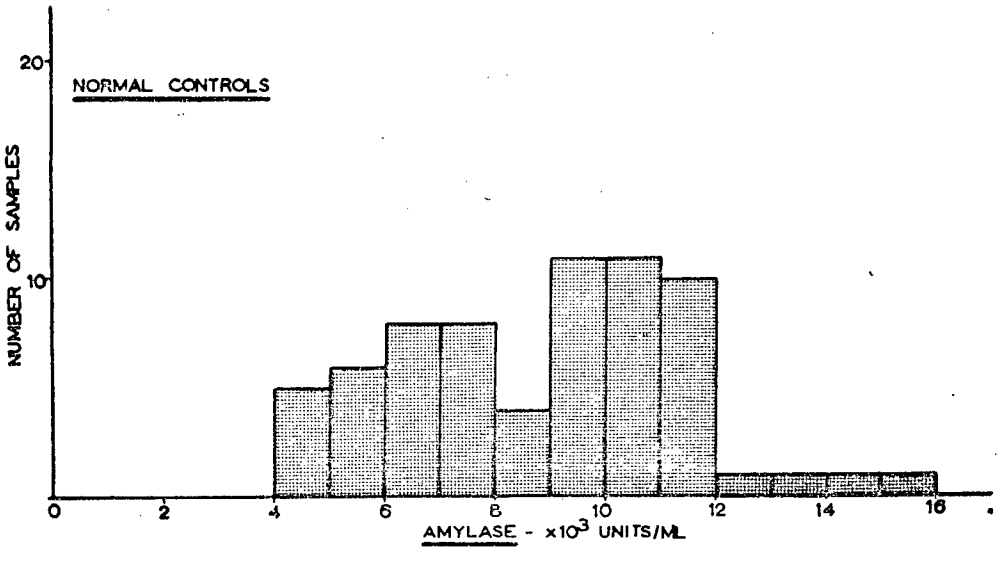

FIG. 8: DISTRIBUTION OF AMYLASE CONCENTRATIONS

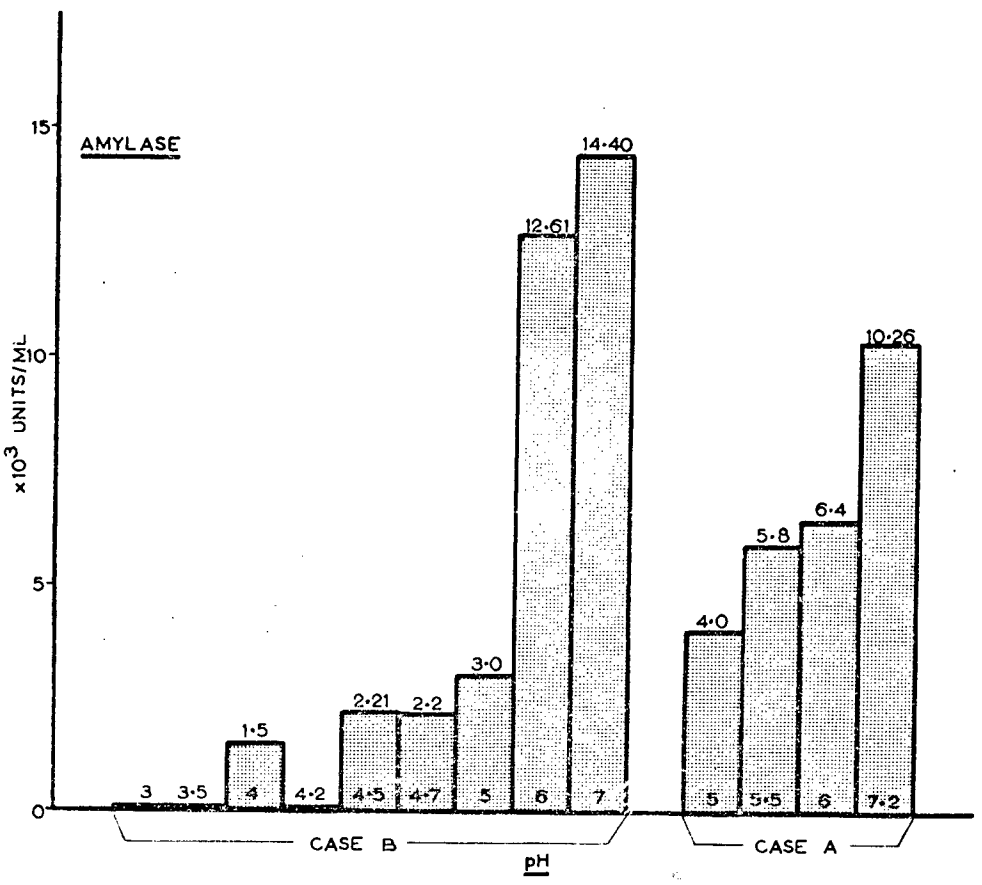

FIG 9: EFFECT OF pH ON AMYLASE ACTIVITY

the basal and four 30 minute periods following stimulation with the test meal. The lower limit of normal amylase concentration was taken as the mean of all the samples minus twice the standard deviation (Mean - 2 SD), which was found to be 4.6 units/ml. Only one result of the first half hour smaples (test 16) fell in the abnormal range, while 7.5% of all the individual samples during the test were below 5.0 units/ml. (Fig. 8). Fig. 8 also shows the distribution of these results with the majority occuring between 4 - 12 units/ml. All the means of the individual tests were above 5.0 units/ml.

GROUP B (Eight 15 minute collections): Following the ingestion of the meal, the mean amylase concentrations rose from 5.84 units/ml in the basal to 9.5 units/ml. in the first 15 minute sample. Only one result in this first sample was in the abnormal range. Statistically, there was a good correlation between this first 15 minute sample and the first half hour sample of the standard test meal ($P > 0.05$).

c) Effect of pH on amylase activity (Fig 9): In subject A and B (see figure) there was a progressive fall off in amylase activity from that present in the relatively pure pancreatic juice. A moderate fall occurred from pH 7.0 to pH 6.0, while in case B there was a marked fall in activity from pH 6.0 to pH 5.0. There was a mean fall of 8.8 amylase units/ml. in both cases, as the pH fell to 5.0. Below pH 4.0 there was no amylase activity at all.

Table 5: CHYMOTRYPSIN (UNITS/ML) (FOUR 30 MINUTE COLLECTIONS)

Sample	Range	Mean(M)	S.D.
Basal	637 - 2820	2117	1414
1	641 - 6682	2731	1518
2	1108 - 4966	2228	1226
3	699 - 6900	2027	1363
4	808 - 5400	2194	1223
MEAN	1290 - 4316	2286	923

Lower limit of normal (M - 2 S.D.) = 450 units/ml

3. CHYMOTRYPSIN

a) Basal values: This basal chymotrypsin value ranged from 637 to 2820 units/ml. with a mean of 2117 units/ml. The basal chymotrypsin of test 2 and 16 may be disregarded because of the low pH values of these samples. Although the standard deviation was high, none of the basal values fell in the abnormal range.

b) Chymotrypsin concentration following ingestion of the standard test meal:

GROUP A (Four 30 minute collections): Following the ingestion of the meal, there was a rise in the chymotrypsin concentration in 7 samples, but this was not quite as marked as with the other enzymes. Like the other enzymes, however, the mean chymotrypsin concentration for each 30 minute period remained within fairly narrow limits. Statistically, there was no difference in the chymotrypsin concentration between the first, second, third and fourth half hour periods ($P > 0.05$).

Table 5 shows the range, mean and standard deviation of the basal period, and each half hour period following the ingestion of the meal. The lower limit of normal was taken as the mean of all the samples minus twice the standard deviation. This was found to be 450 units/ml. All the individual chymotrypsin results were greater than 500 units/ml. (Fig. 10) and 94.4% were greater than 1000 units/ml.

GROUP B (Eight 15 minute collections): Following the ingestion of the meal there was a rise in the mean chymotrypsin concentration

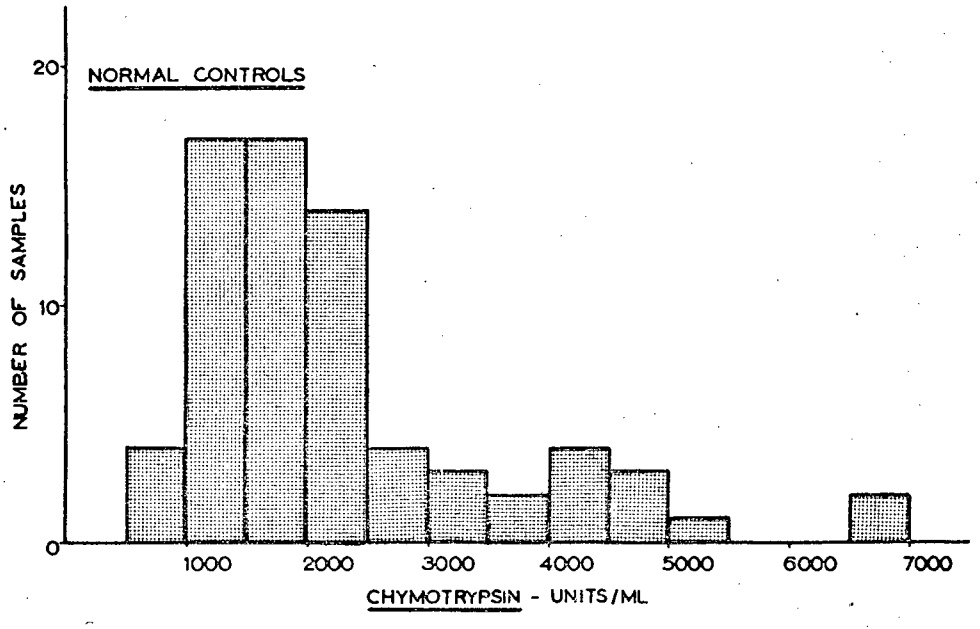

FIG. 10: DISTRIBUTION OF CHYMOTRYPSIN CONCENTRATIONS

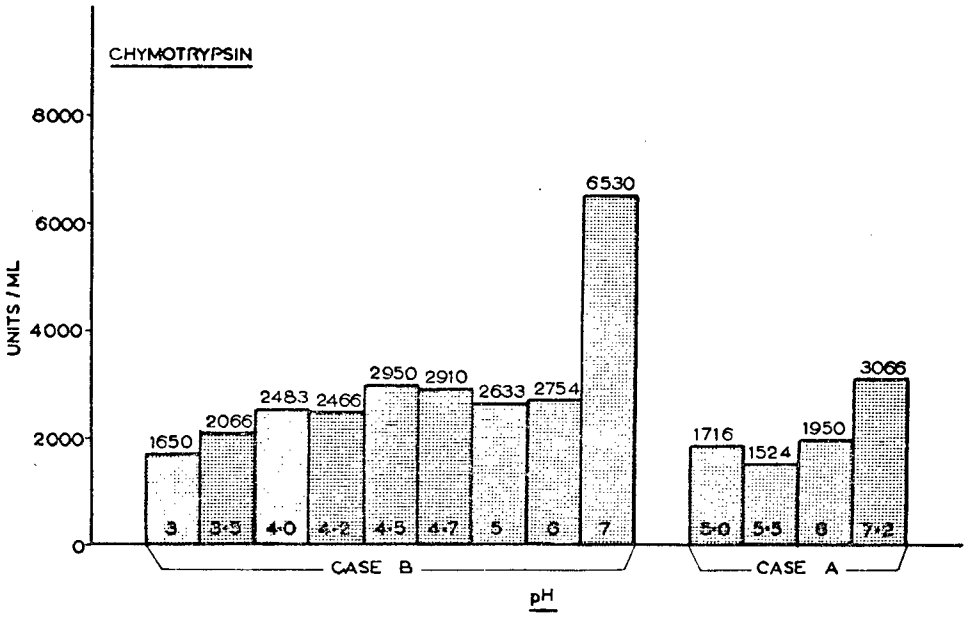

FIG. 11: EFFECT OF pH ON CHYMOTRYPSIN ACTIVITY

from 1827 units/ml. to 3316 units/ml in the first 15 minute sample. All the chymotrypsin concentrations in the first sample were within the normal range. Statistically there was no difference in the chymotrypsin concentration between the first 15 minute sample of this test meal and the first half hour sample of the standard test meal ($P > 0.05$).

c) The effect of pH on chymotrypsin activity (Fig. 11): As the pH decreased from 7.2 or 7.0 to a pH of 6.0, there was a very dramatic fall off in chymotrypsin activity. In Case A, chymotrypsin activity fell 35% of the activity present in the relatively pure pancreatic juice, while in Case B the activity fell 57%. Between pH 6.0 and 3.5 the chymotrypsin activity remained relatively stable and with further reduction in pH, there was a gradual further fall in activity.

4. LIPASE

a) Basal values: The basal lipase concentration ranged from 250 to 511 International Units (I.U.) per litre with a mean of 3771 I.U./litre. Four out of the 6 basal readings were below the normal range.

b) Lipase concentration following ingestion of the test meal:

GROUP A (Four 30 minute collections): Following stimulation with the test meal, there was a significant rise in lipase concentration in the duodenal aspirate. As indicated in Table 6, the mean concentration remained high throughout the 2-hour period of the test. Collections 2 and 3 had a mean

Table 6: LIPASE (I.U./LITRE) (FOUR 30 MINUTE COLLECTIONS)

Sample	Range	Mean (M)	S.D.
Basal	250 - 511	377	93
1	432 - 1131	659	230
2	271 - 969	522	231
3	349 - 1094	542	225
4	510 - 917	722	129
MEAN	457 - 785	574	108

Lower limit of normal (M - 2 S.D.) = 350 I.U./Litre

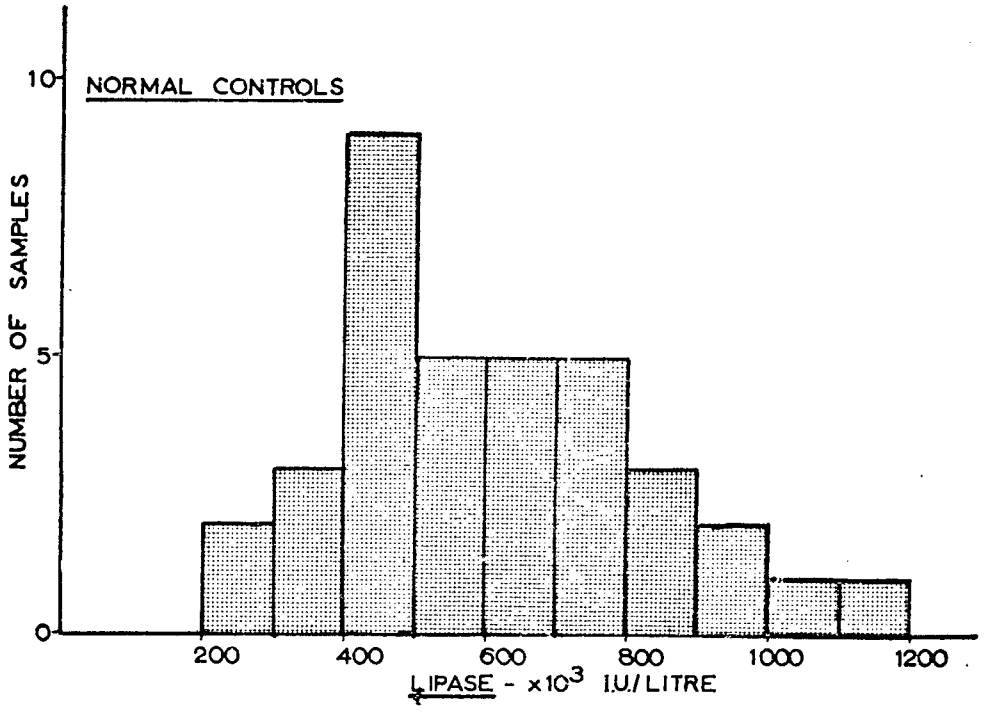

FIG. 12: DISTRIBUTION OF LIPASE CONCENTRATIONS

lipase concentration lower than the first and fourth collections, but this difference was not statistically significant ($P > 0.05$).

Table 6 shows the range of lipase concentrations for each 30 minute period, together with the means for each period and the standard deviations. The lower limit of normal was 350 I.U./litre (Mean - 2SD). The standard deviation of the means of each test was more than half that of the first sample. 91.7% of all the samples were in the calculated normal range (Fig. 12). When the means of each test was taken, this never fell below 400 I.U./litre. All the lipase concentrations in the first 30 minute samples were well within the normal range, and, as there was no difference between the samples ($P > 0.05$), the first sample was representative of the lipase values in the whole test. Fig. 2 indicates the distribution of various samples.

GROUP B (Eight 15 minute collections): Following the ingestion of the meal, there was a very sudden rise in the lipase concentration from a basal mean of 359 I.U./litre to a mean of 646 I.U./litre in the first 15 minute sample.

All the lipase concentrations in the first sample were within the normal range. Statistically, there was no difference between this sample and the first half hour sample of the standard test meal.

c) Effect of pH on lipase activity (Fig. 13): Lipase showed a very interesting response to pH change. In the pancreatic juice of Case A, there was a progressive fall in lipase activity as the pH fell

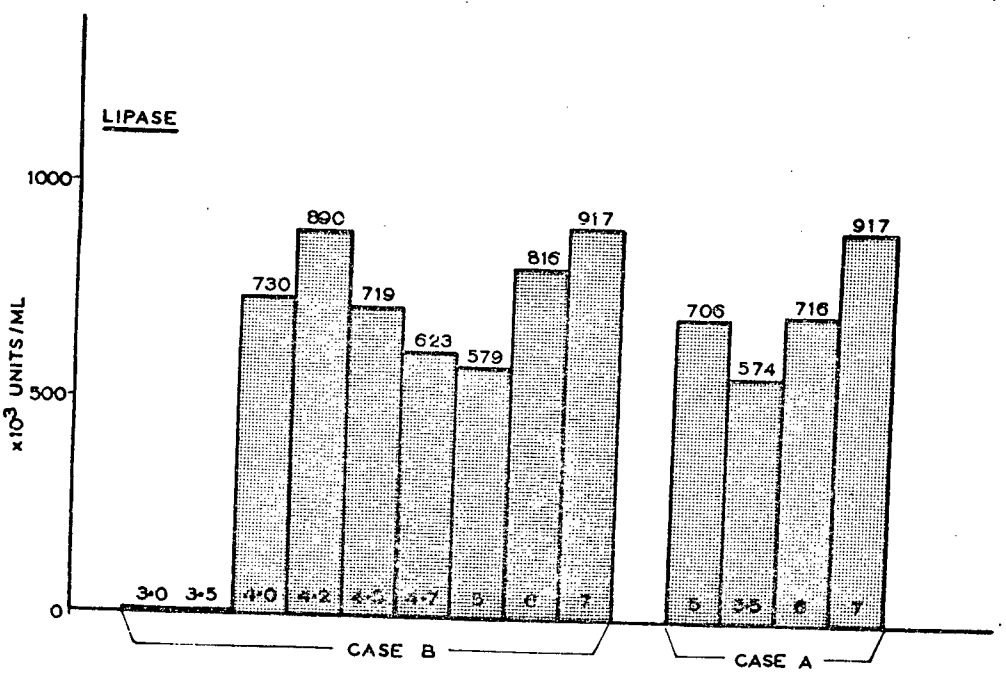

FIG. 13: EFFECT OF pH ON LIPASE ACTIVITY

to a value of 5.5. At pH 5.0, however, lipase activity has started to increase again.

In the pancreatic juice of Case B, a similar response occurred. Lipase activity fell until the pH had reached 5.0. As the pH fell further, there was a progressive increase in lipase activity until it had almost reached a point where the activity at pH 4.2 was that of the relatively pure pancreatic juice. The lipase activity then began to fall until the critical point at pH 4.0 had been reached, at which stage the activity fell to zero.

E. GENERAL PATTERN OF ENZYME RESPONSE OVER THE TWO HOUR PERIOD* OF THE TEST (FIG. 14 - 22)

When the results of the four enzyme concentrations in the aspirate from the individual subjects was superimposed on one another, the following patterns were observed:

a) For each subject, the enzymes appeared to be secreted in a parallel fashion. The peaks and falls tended to occur in the same sample of juice aspirated for all four enzymes studied.

b) Quantitatively, the enzyme concentrations tended to vary from subject ot subject. In one subject trypsin clearly had the highest curve (Test 22), while in another ingestion the same meal, the trypsin curve was considerably lower than both the lipase and amylase curves (Test 20).

c) The individual enzymes tended to have a double peak in their concentrations during the two hours of the test. The peaks for all four enzymes tended to occur in the first and last 30 minutes of the

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* See Appendix, page 159 for individual sample results.

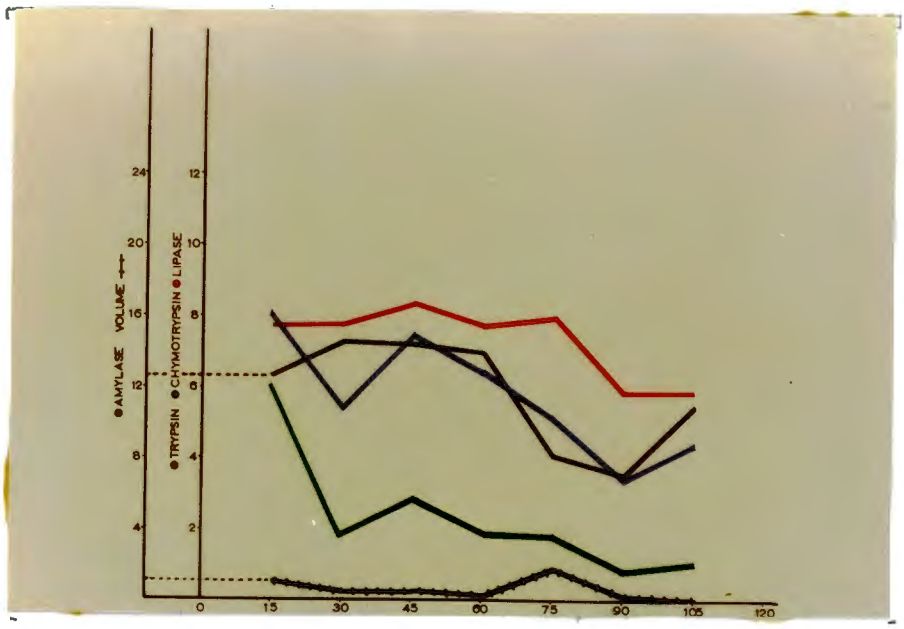


FIG 14

Blue = Trypsin
Red = Lipase
Green = Chymotrypsin
Black = Amylase.

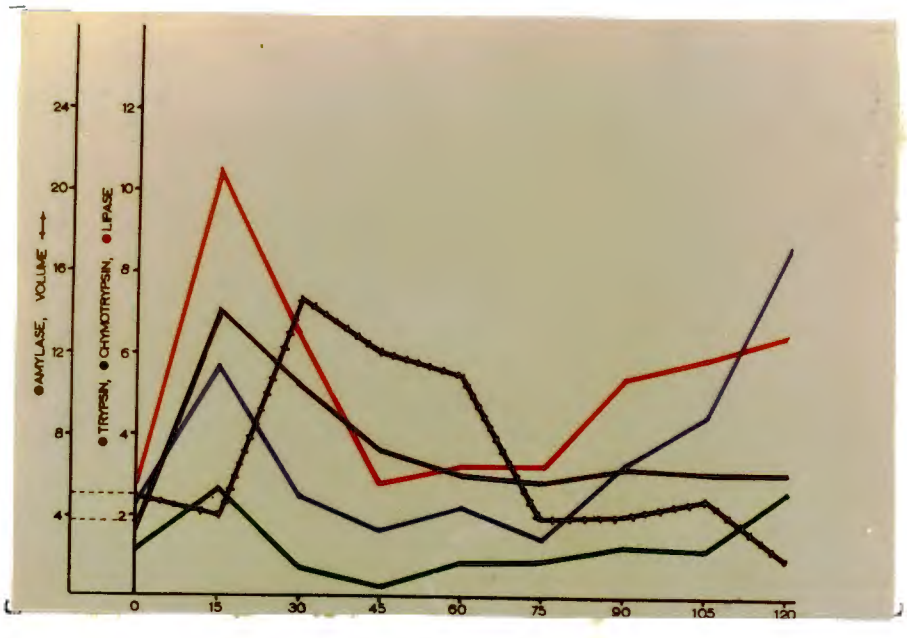


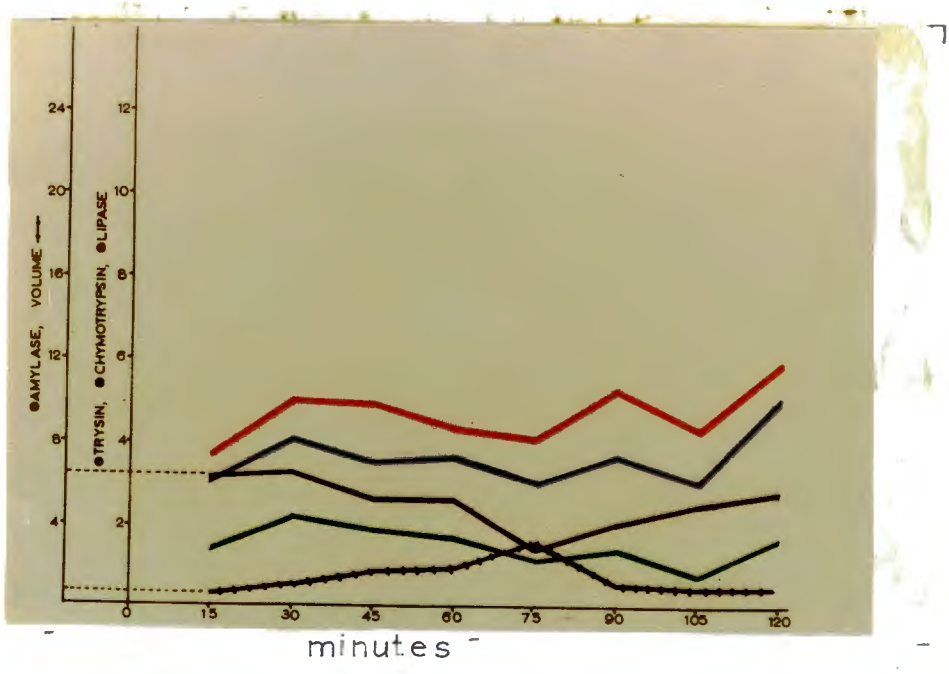
FIG. 15

Fig. 14 (Test 19)

No basal collection is present so that the first enzyme peak is not well demonstrated. Trypsin and chymotrypsin, however, show a fairly marked fall in the second collection. The concentration of all four enzymes gradually fall during the latter half of the test with a very small rise in the last collection.

Fig. 15 (Test 20):

The first enzyme peak occurred during the first 15 minutes of the test, and the enzyme concentrations then fell as the volume of the duodenal juice aspirated increased. Fourty five minutes after the ingestion of the meal, the enzyme concentrations reached basal levels. Lipase and trypsin concentrations began to rise again towards the latter part of the test.


●AMYLASE, VOLUME
●TRYSIN, ●CHYMOTRYPSIN, ●LIPASE
24
20
16
12
8
4
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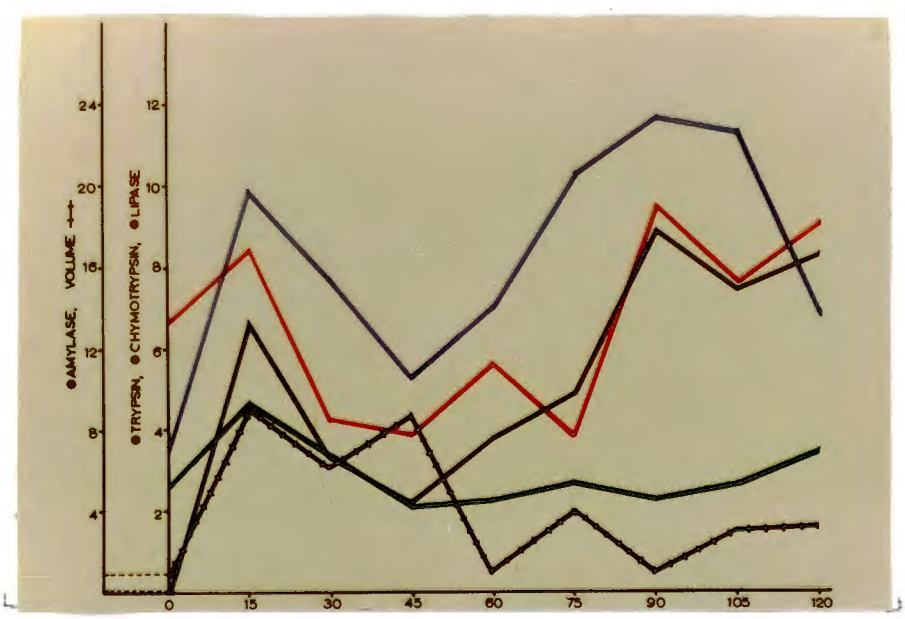
8
6
4
2
0
15
30
45
60
75
90
105
120
minutes

FIG. 16


●AMYLASE, VOLUME
●TRYPSIN, ●CHYMOTRYPSIN, ●LIPASE
24
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12
8
4
12
10
8
6
4
2
0
15
30
45
60
75
90
105
120

FIG. 17

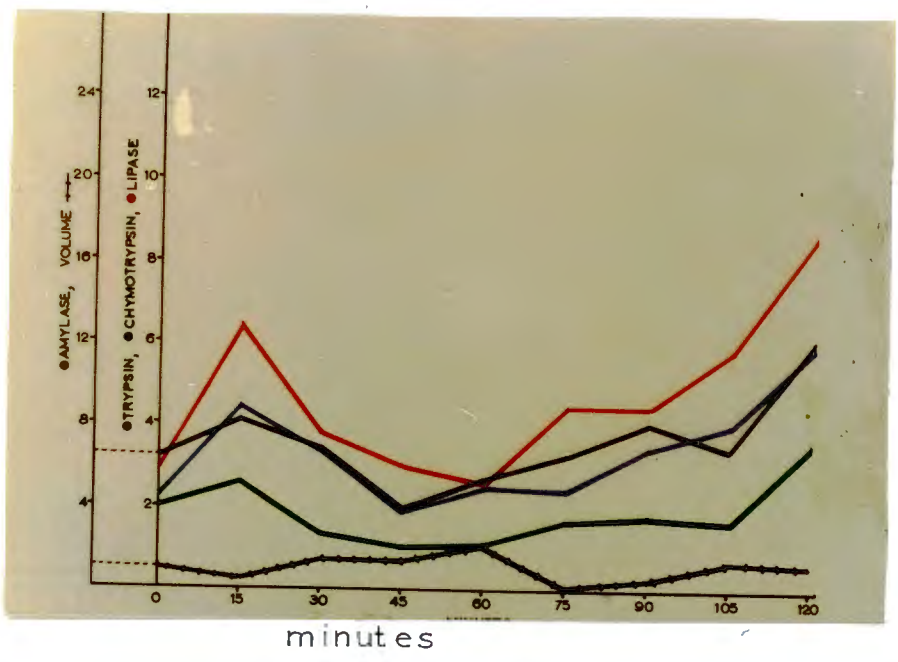
Fig. 16 (Test 21):

The two peaks are not well demonstrated in this test but it should be noted that even small changes in enzyme activities in each aspirate sample, during the 2 hours of the test, occurred simultaneously for all 4 enzymes.

Blue = Trypsin.
Red = Lipase.
Green = Chymotrypsin.
Black = Amylase.

Fig. 17 (Test 22):

The early peak followed by a sudden fall is well illustrated. The volume of aspirate was high during the first half of the test, and low during the second jalf. Despite this, the enzyme concentrations showed the same pattern as in the tests in which the volume remained constant throughout. Note again, that although trypsin, amylase and lipase had a very good concentration during the latter half of the test, chymotrypsin showed very little rise. In most of the other graphs, the lipase curve is greater than that of the other enzymes. In this graph, however, the trypsin curve is highest.


●AMYLASE, VOLUME ↔
●TRYPSIN, ●CHYMOTRYPSIN, ●LIPASE
24
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12
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15
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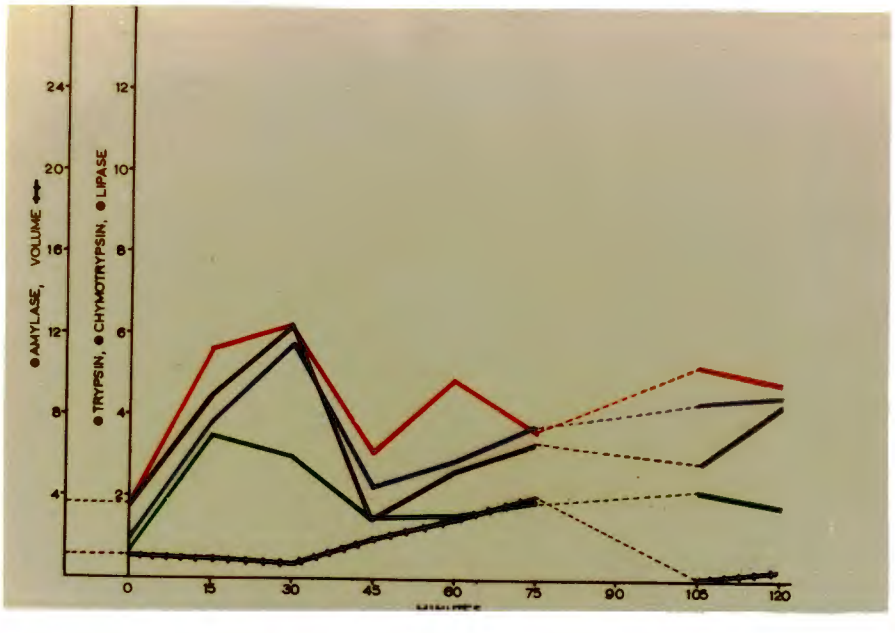
minutes

FIG 18


●AMYLASE, VOLUME ↔
●TRYPSIN, ●CHYMOTRYPSIN, ●LIPASE
24
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16
12
8
4
12
10
8
6
4
2
0
15
30
45
60
75
90
105
120

FIG. 19

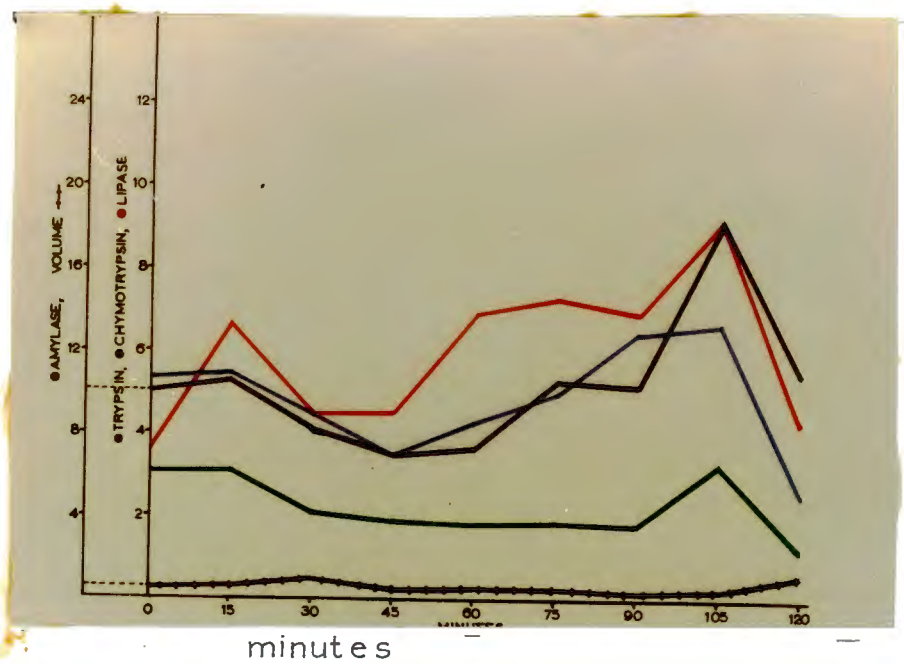
Fig. 18 (Test 24):

A good parallelism can be observed between all 4 enzymes. The first peak is seen in the first sample with a gradual rise to the second peak in enzyme concentrations, starting about 1 hour after the ingestion of the meal and still increasing after 2 hours. The volume remained relatively low throughout the test, yet the enzyme concentrations showed wide variations.

Blue =Trypsin.
Red =Lipase.
Green =Chymotrypsin.
Black =Amylase.

Fig. 19 (Test 25):

The parallel secretion of all four enzymes is particularly well demonstrated in this test. The first peak for all enzymes (except chymotrypsin) was reached in the second 15 minute collection and not the first collection as demonstrated in the other tests.


● AMYLASE, VOLUME ←→
● TRYPSIN, ● CHYMOTRYPSIN, ● LIPASE
24
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16
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15
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105
120
minutes

FIG. 20

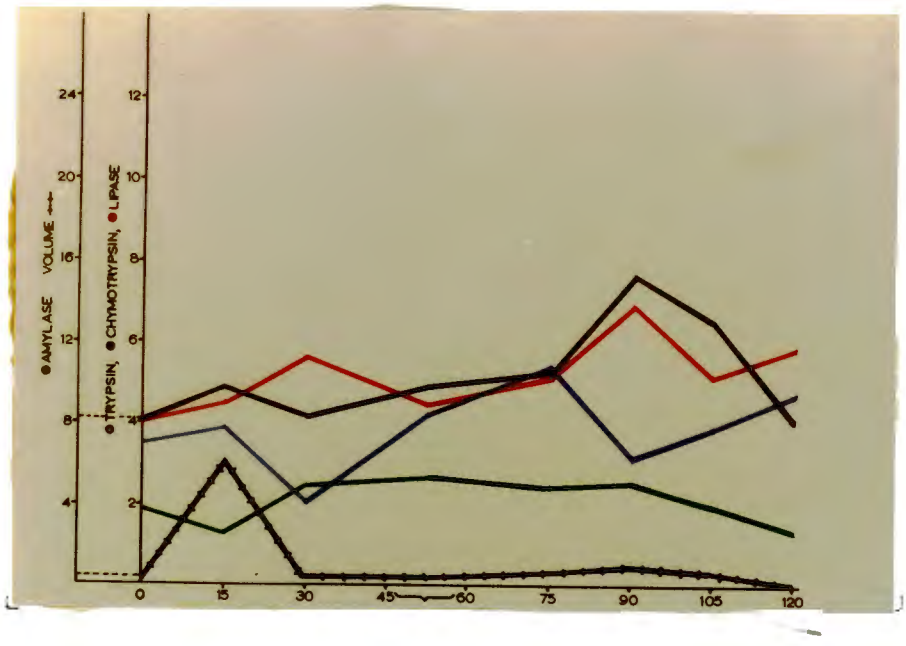
● AMYLASE VOLUME ←→
● TRYPSIN, ● CHYMOTRYPSIN, ● LIPASE
24
20
16
12
8
4
12
10
8
6
4
2
0
15
30
45
60
75
90
105
120

FIG. 21

Fig. 20 (Test 26):

There was little variation in the chymotrypsin concentrations throughout the 2 hour test period. The trypsin and amylase activity showed an almost point-for-point parallelism. A small first peak was followed by a gradual rise in enzyme activity beginning about 45 minutes after the ingestion of the meal. The volume of the aspirate was low throughout the test and the enzyme concentration varied widely.

Blue =Trypsin.
Red =Lipase.
Green =Chymotrypsin.
Black =Amylase.

Fig. 21 (Test 27):

Tests 26 and 27 were performed on the same subject. The pattern of response tends to be similar, with a very small first peak in the first 30 minute collection and a large second peak in the fourth 30 minute collection.

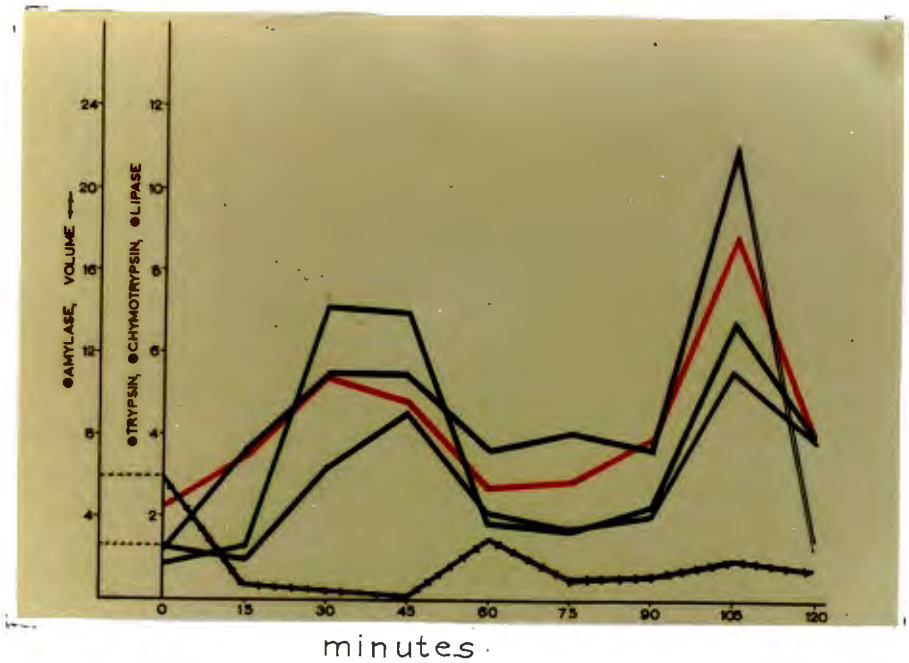
●AMYLASE, VOLUME
●TRYPSIN, ●CHYMOTRYPSIN, ●LIPASE
24
20
16
12
8
4
12
10
8
6
4
2
0
15
30
45
60
75
90
105
120
minutes

FIG. 22

Fig. 22 (Test 28):

The two enzyme peaks are clearly shown in the graph. The first peak shows a rather gradual rise, to reach the highest concentration 45 minutes after the ingestion of the meal. This is followed by a sudden fall in enzyme concentration. The second peak is characterized by a very sharp rise for all enzymes, including chymotrypsin. The volume has remained relatively constant throughout. This graph demonstrates a very close parallelism between the enzymes.

Blue = Trypsin.

Red = Lipase.

Green = Chymotrypsin.

Black = Amylase.

2 hour period.

d) The highest peak enzyme concentration was found to occur in either the first or second peak.

e) There appeared to be no definite correlation between enzyme concentration and the volume of the aspirate.

F. SUMMARY OF RESULTS: THE TEST MEAL IN NORMAL SUBJECTS

1. Volume: Only an average of 22.8% of the duodenal contents passing the end of the tube was aspirated.

a) Basal volume: Basal aspirate decreased with time, and 28% of the normal subjects had no basal aspirate for 20 minutes.

b) Volume following test meal: The volume increased rapidly soon after ingestion of the meal and there was a good correlation between the four 30 minute periods ($P > 0.05$).

2. pH:

a) Basal pH: The mean basal pH was less than 6.0 due to contamination with gastric contents in most of the tests.

b) pH following stimulation with the test meal: There was a constant rise in pH following ingestion of the meal, especially if the basal pH was less than 6.0. The pH remained relatively constant during the four 30 minute periods ($P > 0.05$), with an occasional

short-lived fall to lower levels in a few tests.

3. Enzymes:

a) Basal enzymes: Basal enzyme values showed a wide range from calculated normal to abnormal concentrations.

b) Enzymes following the test meal:

(i) Trypsin activity was relatively stable between 7.2 and 3.5, and lipase between 7.0 and 4.0. Chymotrypsin activity showed a mean fall of 46% between pH 7 and 6 and amylase, the most unstable enzyme at low pH values, showed a progressive fall below pH 7.0.

(ii) A good correlation was present between the first, second, third and fourth 30 minute period in the trypsin, amylase, chymotrypsin and lipase concentrations ($P > 0.05$).

(iii) A good correlation was present between the first 15 minute sample of the eight 15 minute sample test and the first 30 minute sample of the standard test meal. ($P > 0.05$).

(iv) Two peaks in enzyme activity noted, were during the first and fourth 30 minute periods.

(v) The enzymes are secreted in a parallel fashion following the ingestion of a test meal.

G. COMMENT ON THE RESULTS OF TEST MEALS ON CONTROL SUBJECTS

The basal aspirate appeared to be of no particular value in the test procedure. The pH was often low, and the enzyme concentration varied a great deal and is often in the abnormal range.

Following the ingestion of a standard meal, there is a sudden increase in the volume of the aspirate and the concentration of trypsin, lipase, amylase and chymotrypsin. If the pH was low during the basal period, this also increased. Associated with this, the character of the aspirate changed, becoming more watery, less mucoid and more evenly bile-stained.

The pH tended to vary within narrow limits, and was usually above a pH of 6. An occasional reduction to lower levels occurred for very short periods only. A relatively high MAO (maximum acid output) did not appear to affect the intraduodenal pH. A large volume of acid gastric contents passing into the duodenum did not appear to affect the pH substantially as the pH remains constant despite a wide range of duodenal aspirate volumes during the test procedure. The rate of gastric emptying was probably an important controlling factor preventing an excessive amount of acid contents from passing out of the stomach, which cannot be neutralized by a corresponding secretion of alkaline juice from the pancreas. A fine balance is thus present between the rate of gastric emptying and the rate of pancreatic secretion.

The volume of the aspirate did not appear to be a useful measurement in the test meal studies. This was almost certainly due to the very varied amount of duodenal contents which were aspirated from the duodenum. Markers can be used to calculate the total volumes of

duodenal contents and pancreatic secretion, but as the rate of flow through the duodenum is unknown and, as the distribution of the marker throughout the duodenal contents may not be equal, the results may, in fact, be misleading (434). Some markers may also be bound to the mucosa (256).

Within minutes of swallowing food, there was a marked increase in the concentration of pancreatic enzymes, reaching its first peak within the first 15 minutes. This was followed by a fairly sudden fall. Lower enzyme concentrations were maintained for about 45 minutes, and then a second peak was observed during the last half hour of the test. Chymotrypsin did not demonstrate this double peak quite as well as the other enzymes. This may be due to the effect of pH on chymotrypsin activity, which decreases quite markedly when the pH changes from 7 to 6.

Statistically, there was no difference between the four half hour samples for all four enzymes ($P > 0.05$). There was also a good correlation between the enzyme concentrations in the first 15 minute sample of the eight sample test meals and the first half hour sample of the standard test meal ($P > 0.05$). Thus, while eight 15 minute samples were probably useful in physiological studies on pancreatic secretion for the purpose of diagnosing pancreatic insufficiency, only one 15 minute sample may be sufficient. This one 15 minute sample technique may be used as a screening test for pancreatic function, provided that the pH in the aspirate is not very acid. This will be discussed in a later section in more detail.

Although no statistical confirmation is available, it seems, from a close study of the graphs presented, that for each subject the enzymes are secreted in parallel fashion following the ingestion of the meal. Quantitatively, however, the different enzymes appear to vary in

different subjects. The enzyme apparently showing the highest concentrations in one case may have the lowest concentrations in another.

Trypsin and lipase appear to be least affected by a decrease in pH. Trypsin, in particular, seems to increase its activity as the pH falls. At a pH of 4, there is a very sudden decrease in activity in the case of lipase, and a slower fall in the case of trypsin. Amylase and chymotrypsin are very much more sensitive to pH falls. Chymotrypsin would appear to be of little use in test meal procedures where the pH ranges, on the average, between 6 and 7. The double peak shown by the other enzymes is also not as prominent in the case of chymotrypsin and, as shown in Section 3, there is no statistical difference between the chymotrypsin results of normal and abnormal subjects. With the methods used in this laboratory, trypsin would seem to be the best single enzyme to measure; this enzyme gives good, consistently high enzyme activity, it is least affected by changes in pH and its activity can be measured rapidly.

H. DISCUSSION

The study of pancreatic secretion in humans under normal physiological conditions has always been difficult. The use of secretin and pancreozymin in studies on pancreatic physiology, while providing useful information, are certainly not ideal, as the circulating level of these hormones during normal digestion are not known and the doses injected are, therefore, purely arbitrary. The collection of duodenal aspirate following the ingestion of a meal, under the condition described in this section, would approximate optimum physiological conditions more than any other procedure.

TABLE 7 - TEST MEAL STUDIES

Author	No. of Cases	Type of Tube	Position of tip	Type of Suction	Period of Collections	Enzymes Measured	Test Meal	F = Fat P = Protein C = Carbohydrate
Lund, 1962 (253)	56	Polyvinyl Inner diam. 1.9 mm.	Duodenum upper jejunum	Simple siphonage	10 Min. samples for 1hr., then ½hr. samples for 1 hr.	Trypsin	Dried milk Vegetable oil Dextrose Water	F=6% P=5% C=15%
Ventzke et al (1964) (403)	75	Polyvinyl Inner diam. 2.0 mm.	100 cm. from incisors		2 hours	Chymotrypsin Trypsin Lipase	Liquid	
Thaysen et al 1964 (382)	158	Polyvinyl Inner diam. 2.1 mm.	Duodenojejunal flexure	Simple siphonage	Basal and 4 20 minute collections (80 min. total)	Amylase	Skimmed milk powder Corn oil 45 G Glucose 20 G Water 300 ml	P=10G F=17G C=35G
Zieve et al 1966 (444)	17	Polyvinyl Inner diam. 1.7 mm.	90-100 cm. from incisors		2 hours	Amylase Lipase Phospholipase A Trypsin Chymotrypsin Carboxypeptidase	Corn oil 14 G Dextrose 15 G Skimmed milk 12G Choc. syrup 8 G	P=12G F=14G C=35G
Worning and Mullertz 1966 (436)	77	Polyvinyl Inner diam. 2.1 mm.	Duodenojejunal flexure	Simple siphonage	Basal (20 min.) 4, 20 minute collections (80 min. total)	Lipase Amylase Trypsin Chymotrypsin	Skimmed milk powder Corn oil 45 G Glucose 20 G Water 300 ml	P=10G F=17G C=35G
Hartley et al 1966 (178)	81	Double lumen Dreiling tube	90 cm. from incisors	Pumps	Basal ± 10min. 1, 45min. per. 1 30min. per. (75 min. total)	Amylase Trypsin	Lund's test meal (above) Volume, 300 ml.	P=6% F=5% C=15%
Cook et al 1967 (84)	44	12 Fr gauge radio-opaque tube	Duodenojejunal flexure	Siphonage	4 - 30 minute periods (total 2hrs)	Trypsin	Corn or Soya bean oil (18G) Casilan 15G Glucose 40 G Crusha syrup 15 G Hot water to 300 ml	P=5% C=15% F=6%

Table 7 summarizes the experimental method and type of test meal used in the various reports on similar studies. The majority of these reports compared the results of the test meal performed on normal subjects and patients with pancreatic disease (84, 253, 403, 438) or the test meal compared to the secretin-pancreozymin test in assessing for pancreatic function (178, 382, 444). Worning and Mullertz (436) studied the pH and enzyme concentrations in the duodenal aspirate of 77 healthy subjects following the ingestion of a standard meal. They made no attempt, however, to vary the time periods, or change the quality and quantity of the meal. Four 20 minute collections were performed following the ingestion of the meal compared to the four 30 minute or eight 15 minute collections in our procedure.

Three problems present themselves in the initial stages of the study; firstly, the possible stimulating effect of an intraduodenal tube; secondly, the occasional small volumes of aspirate which may have been insufficient for laboratory enzyme estimations; and thirdly, the inhibitory effect of low duodenal pH values on enzyme responses. The presence of the intraduodenal tube has been reported to act as a mechanical stimulus (408) to pancreatic secretion. Although this may occur initially and account for the occasional large basal collections, in the present study, in which basal collections were prolonged, the duodenal aspirate was reduced to a few millimeters. Voegtlin et al (404) and Christiansen (68) reported basal secretions of 16 ml. and 26 ml. per 15 minutes in their subjects even when no gastric aspiration was attempted. In those tests in which the duodenal aspirate was very small, it was found that turning the subject on his right side for short periods was often sufficient to start gastric emptying with a corresponding secretion of pancreatic juice.

As gastric emptying occurs in an exponential manner (200, 205), whatever the volume of the food or the position(202) of the subject, a relatively continuous flow of aspirate, large at first and decreasing in quantity as the test procedure continues, might be expected. This in fact seemed to occur. The various duodenal factors described in the introduction, exert their effect once the food starts to pass through into the duodenum, and these factors may or may not slow gastric emptying. These duodeno-gastric reflexes probably play an important role helping to maintain the relative constancy of the duodenal pH and thus provide the necessary alkalinity for enzyme estimations in nearly all samples. The presence of acid in the duodenum also slows gastric emptying (251). The acid stimulates the pancreas, liver (161) and glands of Brunner (128) to secrete an alkaline fluid, which then neutralizes the acid contents from the stomach. The mechanisms involved here are very efficient (239) under normal physiological conditions, so that the pH seldom falls to low levels. The latter did, however, occur, and the occasional sample had to be discarded because of a very low pH, which inactivated the enzymes to a varying degree. A fall in pH during the test procedure could probably occur under a number of conditions: Gastric hypersecretion of acid; very rapid gastric emptying exceeding the rate of neutralization; or a decreased response of the pancreas to an acid load. This latter effect has been reported in patients with duodenal ulcers (239, 425), but there is no reason to think that it may occur under normal physiological conditions. The most obvious cause for sudden fall in pH would appear to be a sudden increase in the rate of gastric emptying (239), occuring at a time when inhibitory stimuli from the duodenum are at a minimum. This is supported by the observation that those samples in which the pH was low usually contained

more food than those samples with the higher pH. It seems that little can be done for the cases in which the pH remains persistently low, except perhaps to turn the subject on his left side, which may slow the rate of gastric emptying. The important factor, however, is to be aware of the occasional drop in pH by constant pH estimations during the test. With vigilance, the pH factor is seldom a major problem.

The constancy of the duodenal pH found in the majority of our tests agress essentially with that found by other authors. Worning et al (436) reported a significant decrease in the pH during the four 20 minute collections and noted a significantly lower pH in males than females with normal gastric acid secretion. The lowest mean pH in this series for both males and females during all four 20 minute periods was 5.674, while the lowest recorded single sample had a pH of 4.71. The pH was significantly higher in some samples from subjects with hypochlorhydria than in subjects with normal gastric secretion. Thaysen et al (382) also recorded a fall in the mean pH from about 6.5 in the first sample to about 5.9 in the fourth sample. During the 2 hours following the test meal in our subjects, there was very little change in the mean pH. The lowest recorded mean pH was 6.2, while the lowest single reading was 4.5. There was no apparent decrease in the pH with time.

The volume of the aspirate varied markedly in the normal subjects, and even in the same subject with repeat tests in the present study. As indicated from our results using radio-active Rose Bengal, only about 25% of the volume passing the end of the tube is aspirated, so that any result of pancreatic enzyme secretion based on a quantitative analysis without correcting for these losses must be regarded with suspicion. The loss into the duodenum would be even greater if thin polyvinyl tubes were used to aspirate. Thaysen

et al (382) collected from 10 to 50% of the total amount of duodenal contents in different experiments and Zeive et al (444) reported the recovery of about 40% of the amount passing the end of the intraduodenal tube. Worning and Amdrup (435) reported a yield of 86 to 100% using three tubes to aspirate jejunal contents. When only one tube was used, however, the yield varied between 6 to 63%. Cook (81), using ^{51}chromium as a marker, found the recovery of duodenal juice to vary between 10 to 25%.

Few systematic investigations seem to have been carried out on enzyme inactivation under test meal conditions. Lagerlof (239) merely noted that a high degree of inactivation occurred due to the acid reaction, especially with regard to amylase. Any sample with a pH of 5.5 or less was discarded. Thaysen et al (382) found amylase to be quite stable, except at pH values below 5.0. Trypsin is reported to be most stable at a pH of 4.5 (250), and becomes increasingly unstable towards neutral and basic reactions (303).

Temperature seems to affect the enzymes differently. Amylase activity of intestinal contents is fairly stable with little decrease in activity when stored at 37°C. for 2 hours. (92). Trypsin and chymotrypsin, however, show a marked decline in activity when incubated at 37°C., but not when incubated at 4°C. (252). Choi et al (67) noted that freeze storage decreases the activity of trypsin by 50%, while after refrigeration storage there were only small losses of up to 15%.

The double peak occuring for all enzymes during the two hours of the test is interesting, and requires some explanation. The only author to report a similar pattern was Lund (253), who measured only trypsin concentration. The early peak occurs almost exclusively during the first 15 minutes following the ingestion of the meal. It is generally assumed that this high enzyme concentration during the

early part of the test is due to a "wash out " of the enzymes (74, 410) which have been concentrated within the pancreatic tubules during the resting phase. The effect of secretin on pancreatic enzyme secretion has been cited in support of this theory. An intravenous injection of secretin causes, in some cases, a high concentration of enzymes in the first sample asperated. This concentration soon falls to basal levels (404). A repeat injection of secretin produces no further rise in the enzyme concentration (282) although Friedman reported a small secondary rise. The evidence for a "wash out" effect is, we believe, not conclusive, and, in fact, some evidence counters this theory. It is perhaps fair to assume that the enzymes may become concentrated in the resting pancreas but gastric "waves" (73, 278) have been reported in fasting conditions and this passage of acid into the duodenum must stimulate pancreatic secretion. The passage of the tube into the stomach and the manipulation through the pylorus into the duodenum often causes varying degrees of retching, increased gastric secretion and the passage of gastric contents into the duodenum. Basal collections are often high and even before these collections are started, a large amount of gastric contents have probably passed down into the duodenum and stimulated the pancreatic secretion. By the time the test procedure is started, any enzymes which may have been concentrated in the pancreatic ducts have long since been secreted in the alkaline fluid which passes into the duodenum and neutralizes the acid contents from the stomach. Another mechanism is, therefore, suggested to account for this high peak early during the test. The zymogen granules are the storehouses (190, 312) for the pancreatic enzymes, but enzyme secretion can continue at a high level even when the granules have been depleted by continuous pancreozymin stimulation (174). It is suggested, therefore, that the zymogen granules provide a readily

accessible source of large amounts of enzymes for rapid secretion, while simultaneously the mechanisms of enzyme synthesis are being adapted to provide further sources of enzymes. The whole process of synthesis, transport and secretion of enzymes from the acinar cells is reported to take less than one hour. Any initial stimulus to the acinar cells of the pancreas, be it neural or hormonal (pancreozymin), stimulates the zymogen granules (174) to discharge a large quantity of enzymes. The number of granules discharging during the time of the initial stimulus will depend on the strength of the stimulus. The very rapidity with which the high enzyme output occurs in the test meal procedures is in favour of a neural mechanism as suggested by Govaerts (138). Pancreatic secretion is sometimes noted to have started at the time of ingestion, and when pancreozymin stimulation must be minimal. Various gastro-pancreatic (418, 419) or vagal reflexes may be responsible for the early response occuring following the ingestion of the meal. Following this, the zymogen granules continue to secrete enzymes at a slower rate.

The role of gastrin, which has a pancreozymin-like effect on the pancreas (48, 322, 323, 324) is unclear. Any effect it may have on pancreatic secretion should occur during the early phase of the test meal, when gastrin stimulation is maximal. Gastrin stimulation may contribute to the high enzyme concentration during the first peak. It may also help to maintain a good enzyme output between the neural effect and that of pancreozymin, which begins to accumulate in the blood as soon as food starts to pass through the pylorus and comes into contact with the duodenal mucosa.

How does secretin fit into the early response? Most of the authors describing the "wash out" phenomenon have used a secretin

preparation which contained traces of pancreozymin. Following the injection of secretin and the very rapid secretion of large quantities of highly alkaline fluid into the pancreatic ducts, there is a concurrent rapid discharge of a large number of granules while the acinar cells adjust to this change of environment. The granules that discharge their enzyme contents during this early response are those that have accumulated at the tip of the cell during the fasting period.

The second peak, occuring consistently during the last 30 minutes of the test procedure, may be due to a number of factors: (a) The passage of the fat content of the meal into the duodenum, (b) the effects of increased hormone secretion and the effects of increased enzyme synthesis and (c) the increase in enzyme concentration with a decrease in volume of aspirate. As shown by various authors, mixed foods in the stomach tend to separate out, with fat floating to the top and, therefore, leaving the stomach last (64, 422). In the following chapter it will be shown that fat is a strong stimulus to enzyme secretion. It is possible that fat does contribute to this late peak by leaving the stomach 'late,' but other factors must be involved, as this second peak will even occur following the ingestion of meals without fat. While the synthesis of the pancreatic enzymes by the ribosomes within the acinar cell is very rapid, (168, 291) the transport of these enzymes through the cell, and until they appear in the pancreatic juice, may take up to an hour (168). Thus, it is possible that, at about the time that the second peak occurs, the enzymes synthesized at the time of maximum pancreozymin stimulation (during the first hour following ingestion of the meal) have reached their maximum secretory rate. As the secretin

stimulation has decreased by this time, some concentration of enzymes has probably occurred as well, in the smaller volume of juice secreted.

Chapter 10

THE EFFECT OF VARYING THE VOLUME, QUALITY AND OSMOLARITY OF THE TEST MEAL ON PANCREATIC FUNCTION

RESULTS PART 2

Chapter 10 THE EFFECT OF VARYING THE VOLUME, QUALITY AND OSMOLARITY OF THE TEST MEAL ON PANCREATIC FUNCTION

A THE RESPONSE OF THE PANCREAS TO DIFFERENT VOLUMES OF THE STANDARD TEST MEAL

A normal control subject received, during the course of a few days, varying volumes of the standard test meal. Ordinary tap water was added to the meal to make up volumes of 300, 350, 450 and 550 ml. The subject was able to swallow all the meals without feeling any discomfort.

a) Volume response: The volume totals of duodenal aspirate after the four test meals were as follows:

300 ml.	test meal	- 198 ml.
350 ml.	"	- 49 ml.
450 ml.	"	- 105 ml.
550 ml.	"	- 448 ml.

The 300 ml. test meal produced a larger volume of aspirate than the 450 ml. meal. A fairly marked difference is present between the two meals with similar volumes.

b). pH: The pH remained remarkably stable in all the tests. No pH below 6.4 was recorded in any sample, even following the 550 ml. test meal and despite fairly large volumes of the aspirate.

c) Enzyme response: Reference to Table 8 shows that, despite the fact that the total volume of the aspirates following the 550 ml. test meal was almost nine times that following the 350 ml. test meal, the mean concentrations for each enzyme (except chymotrypsin)

TEST MEAL VOLUME	MEAN pH	VOLUME ASPIRATES (ml)	$\times 10^3$ UNITS/ml AMYLASE	(UNITS/ml) TRYPSIN	(UNITS/ml) CHYMOTRYPSIN	$\times 10^3$ (I.U./LITRE) LIPSE
300	6.5	198	10.93	4081	2756	628
330	6.7	49	11.87	5831	2382	728
450	6.4	105	13.19	7112	4193	376
550	6.4	448	13.76	4702	8333	757

Table 8: MEAN pH, ENZYME CONCENTRATIONS AND TOTAL VOLUME ASPIRATES FOLLOWING DIFFERENT VOLUMES OF TEST MEALS.

was very similar. The highest mean amylase, chymotrypsin and lipase concentrations occurred following the 550 ml. test meal, while the highest mean trypsin concentration occurred following the 450 ml. test meal.

The pattern of enzyme response was similar for all test meals, and followed the pattern described in the previous section. A high peak activity occurred during the first 15 minutes following the ingestion of all the meals, with a second higher peak occuring during the last 30 minutes of the test.

Statistically, there was no difference between the different test meals with regard to the concentrations of amylase, trypsin, chymotrypsin and lipase in the duodenal aspirate following the ingestion of these meals ($P > 0.05$).

B. THE RESPONSE OF THE PANCREAS FOLLOWING THE INGESTION OF MEALS * OF DIFFERENT COMPOSITION AND A GASTRIN INJECTION

Seven normal subjects received a minimum of three of the following meals: Standard test meal, protein meal, carbohydrate meal, fat meal, water meal, saline meal, carbohydrate-free meal, low protein meal, fat-free meal, saline meal plus gastrin injection and gastrin injection without any meal. These meals are fully described under the section headed "Material." All collections following the meal were made during 15 minute periods. Fig. 23 shows the individual pH readings of all the samples; Fig. 24 the total volumes aspirated and Fig. 25 - 28 the mean enzyme concentrations in the aspirate following the different meals. The results following each meal were analysed together to give final results quoted in Table 9.

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* See Appendix page 162-172 for individual sample results of pH, volume and enzyme concentrations

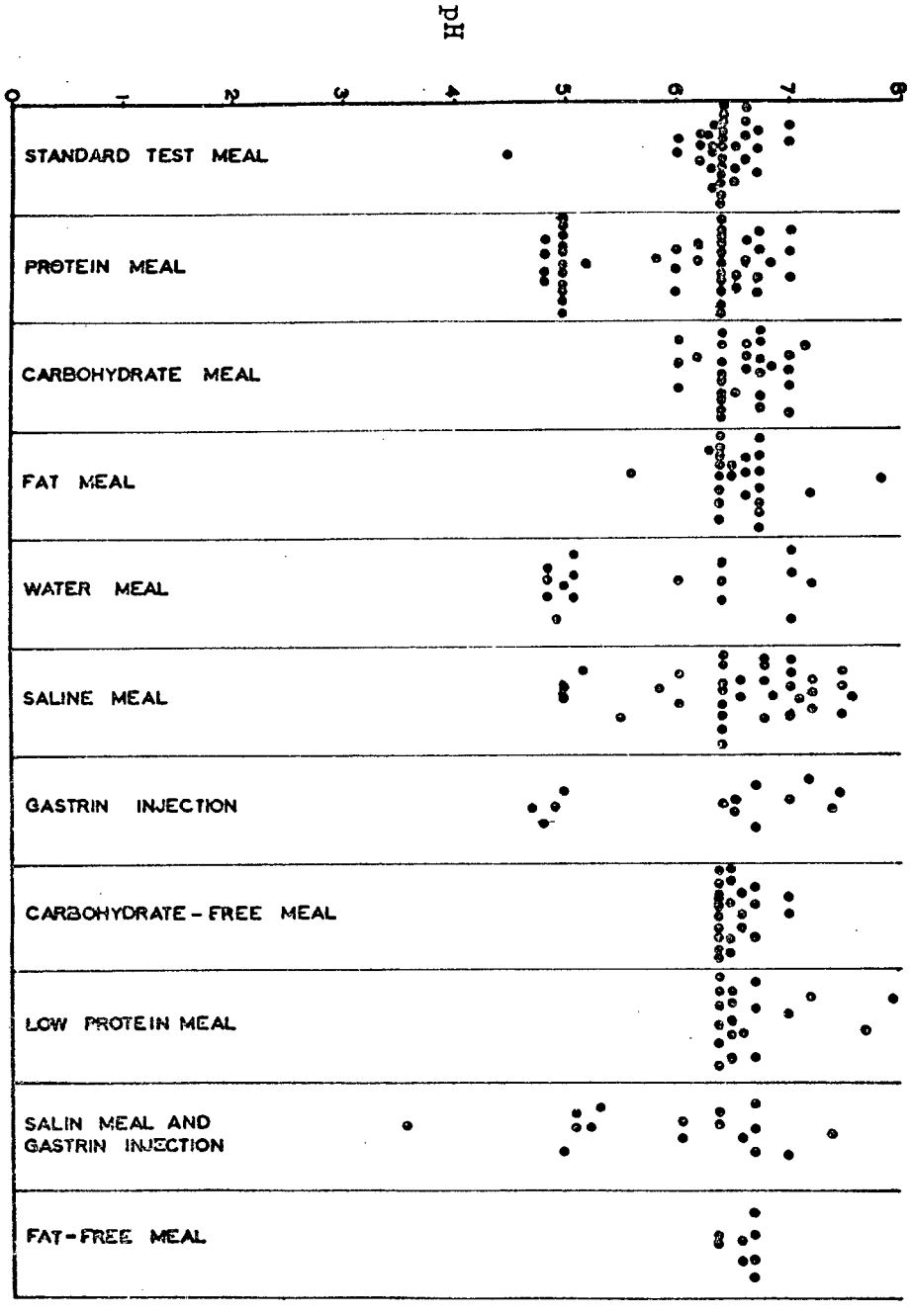

FIG. 23: pH READINGS FOLLOWING DIFFERENT TEST MEALS

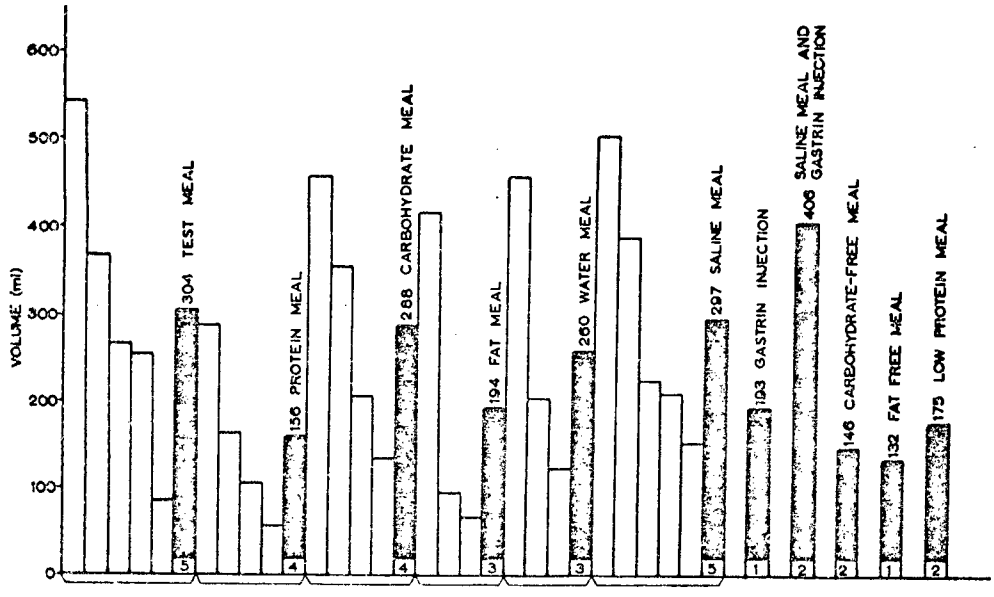

FIG. 24: VOLUMES OF ASPIRATE FOLLOWING INGESTION OF DIFFERENT TEST MEALS

The shaded columns refer to the mean of the total volumes, indicated in some tests by the preceding non-shaded columns, of each meal.

TABLE 9.

Mean and range of Enzyme Concentrations following different test meals.

Meal*	TRYPSIN (UNIT/ML)		AMYLASE ($X10^3$ UNITS/ML)		CHYMOTRYPSIN (UNITS/ML)		LIPASE (I.U/LITRE)	
	Range 11	Mean 4	Range 13	Mean 5	Range 11	Mean 4	Range 9	Mean 4
Standard Test Meal (4)	3741-8783	5882	8.04-11.44	9.87	1358-2858	2255	503-657	577
Protein T.M. (4)	3957-8016	5748	7.07-10.45	8.75	1966-3616	2747	580-1117	719
Carbohydrate T.M. (4)	2400-4028	3373	5.17-12.64	7.75	1723-3014	2189	344-599	440
Fat T.M. (3)	4151-11,127	6568	10.65-11.91	11.40	1457-5458	3031	637-1114	823
Water T.M. (3)	1797-5284	3713	2.79-14.37	10.41	1178-2636	2249	173-510	326
Saline T.M. (5)	1536-4601	3350	1.96-10.86	6.70	1050-2656	2208	174-748	425
Gastrin Injection (1)	3786	3786	10.58	10.58	2068	2068	478	478
Carbohydrate Free T.M. (2)	5785-6695	6239	12.44-14.43	13.44	1735-3405	2570	817-956	886
Low Protein T.M. (2)	4637-6997	5817	9.62-15.46	12.55	2224-4336	3280	509-720	615
Saline and Gastrin (2)	647-2702	1743	1.99-2.16	2.10	541-2664	1673	56-241	155
Fat Free (1)	3965	3965	10.31	10.31	3061	3061	630	630

* No. in brackets refers to the number of tests performed in each group. (Refer to appendix page for individual results.)

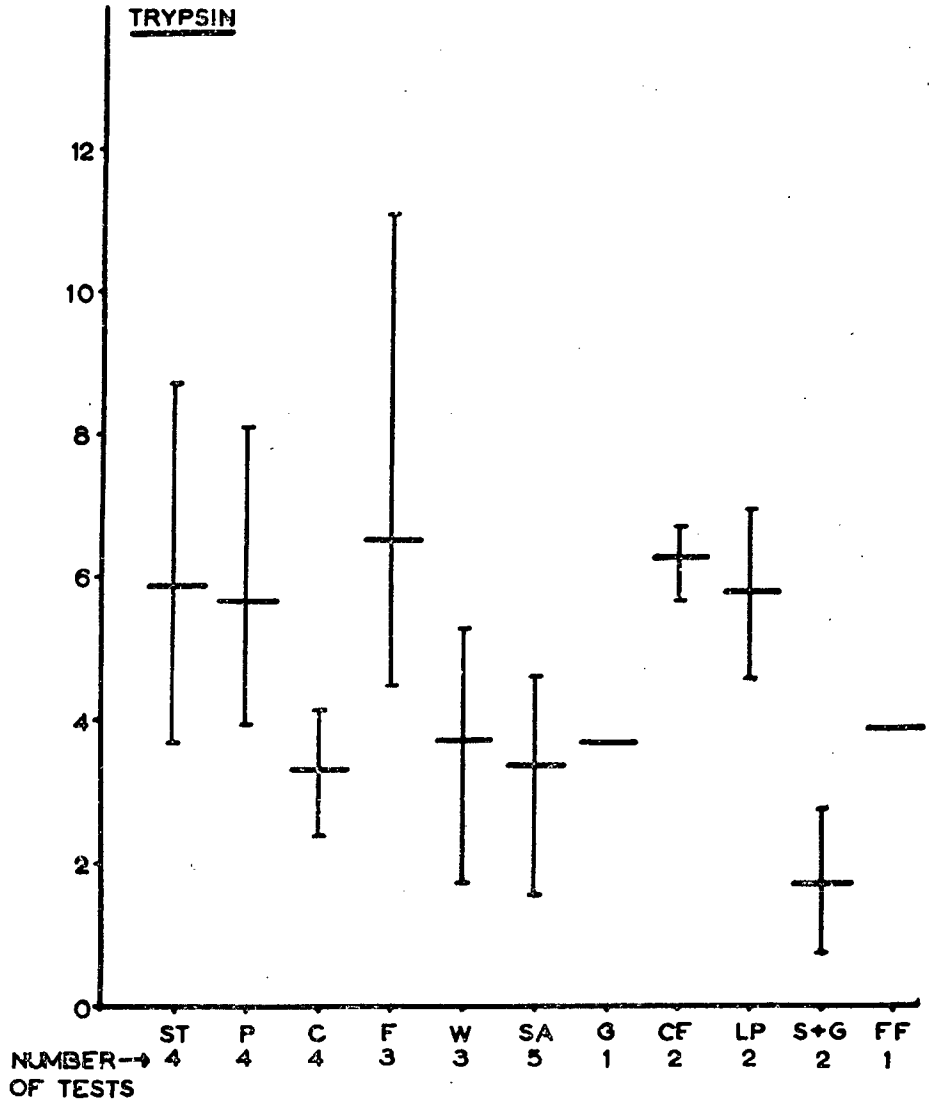

FIG. 25: THE MEAN AND RANGE OF TRYPSIN CONCENTRATIONS FOLLOWING INGESTION OF DIFFERENT TEST MEALS

ST = Standard Test meal
P = Protein Test Meal
C = Carbohydrate Test Meal
F = Fat Test Meal
W = Water Test Meal
SA = Saline Test Meal
G = Gastrin Injection
CF = Carbohydrate-free Test Meal
LP = Low Protein Test Meal
S+G = Saline Meal + Gastrin Injection
FF = Fat-free Test Meal

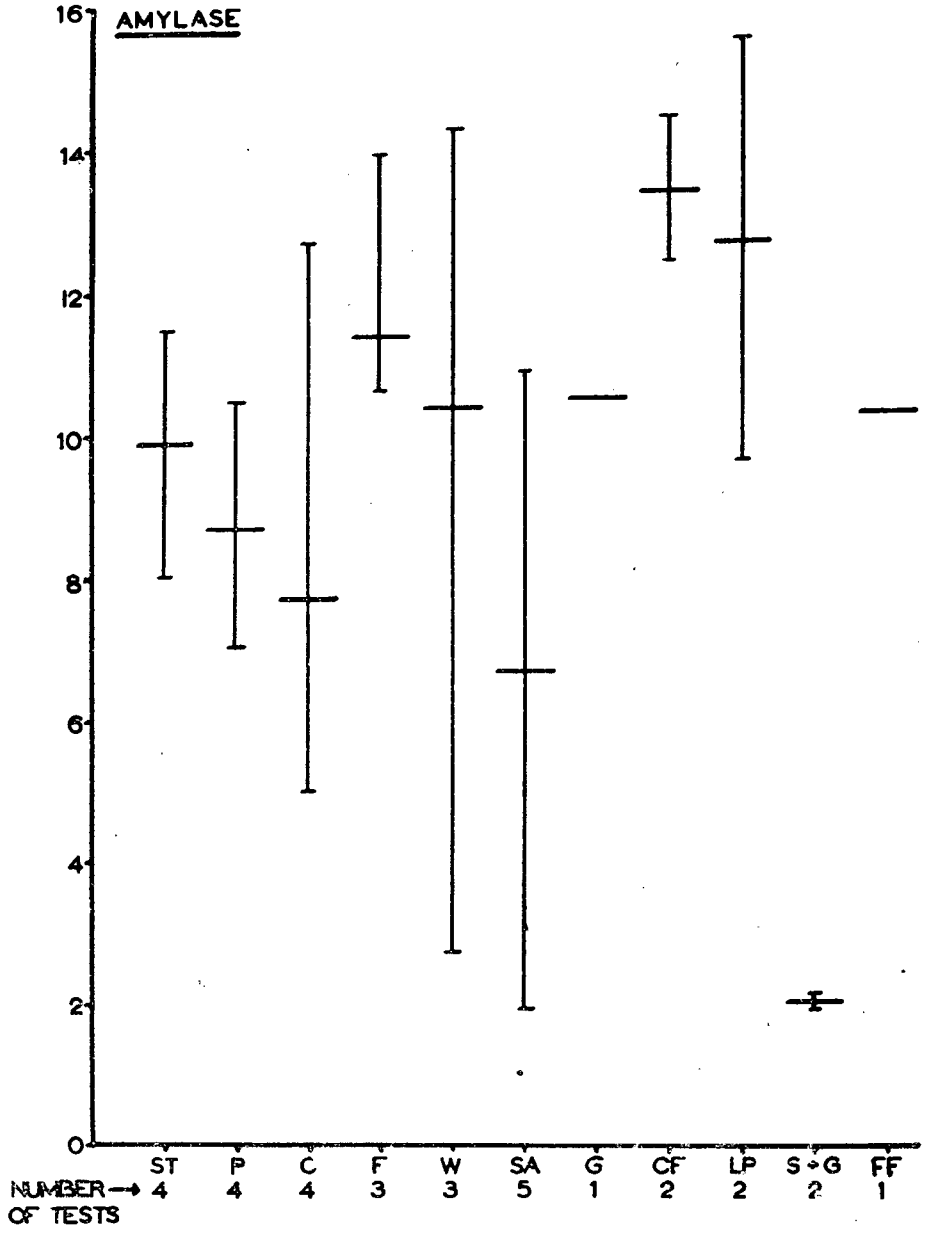

FIG. 26: THE MEAN AND RANGE OF AMYLASE CONCENTRATIONS FOLLOWING THE INGESTION OF DIFFERENT TEST MEALS

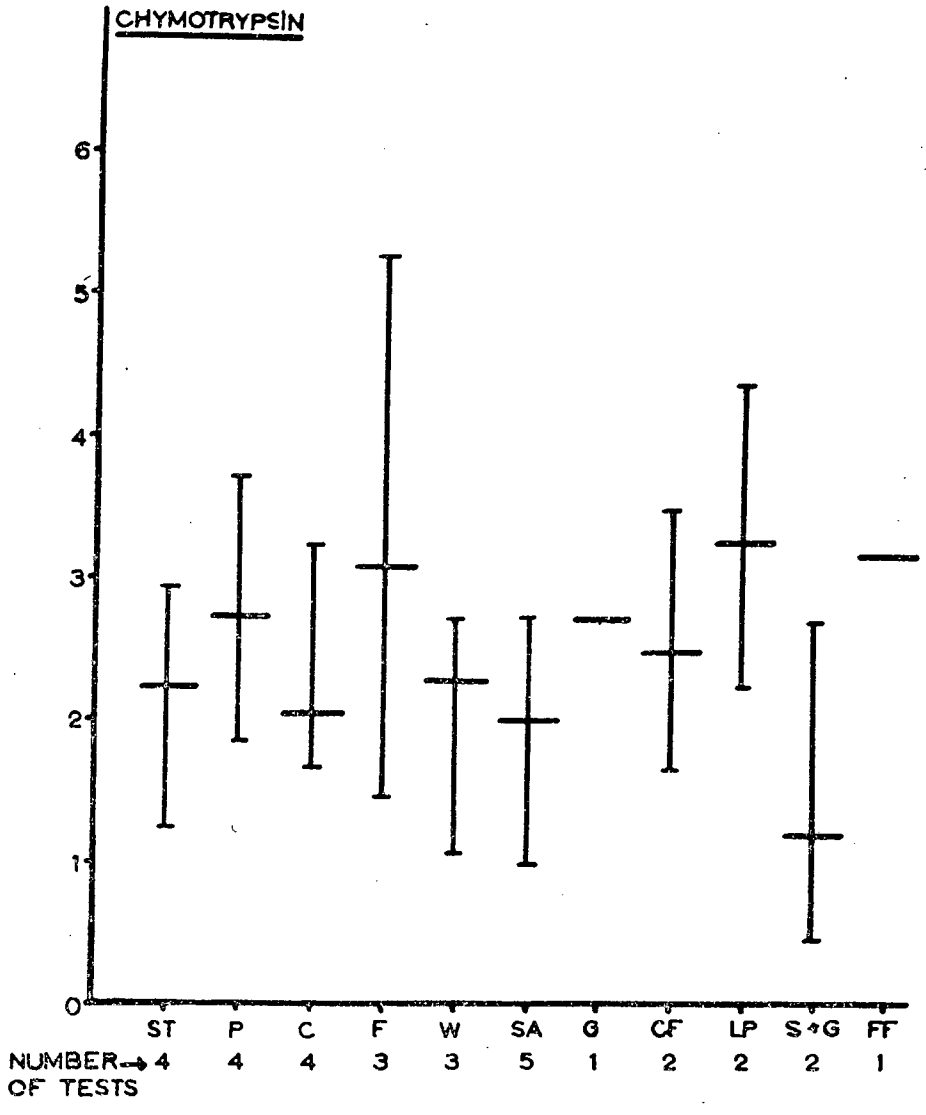

FIG. 27: THE MEAN AND RANGE OF CHYMOTRYPSIN CONCENTRATIONS FOLLOWING THE INGESTION OF DIFFERENT TEST MEALS

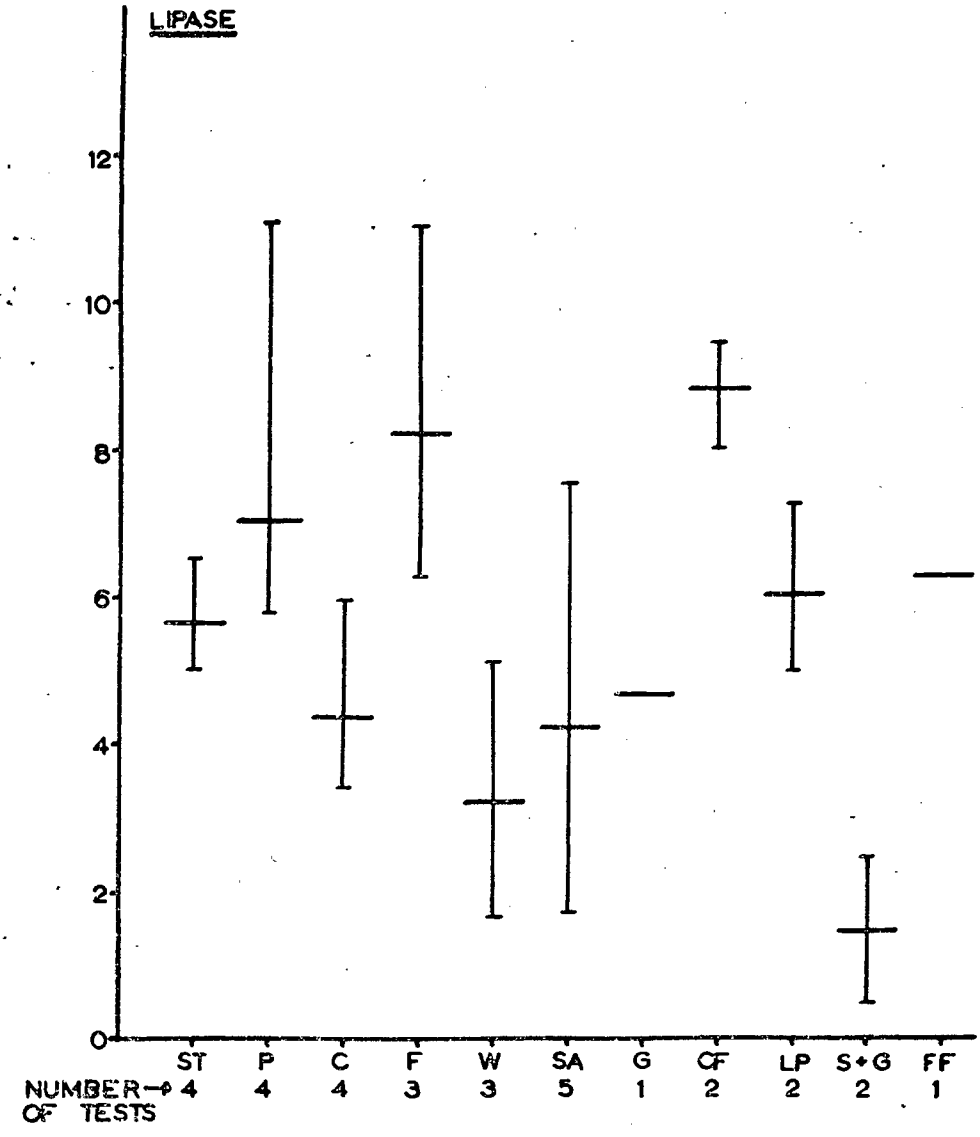

FIG. 28: THE MEAN AND RANGE OF LIPASE CONCENTRATIONS FOLLOWING THE INGESTION OF DIFFERENT TEST MEALS

1. Standard test meal: (4 tests) This has been reported in the first section. In the 4 test meals included in this section, the pH was 6.0 or more in all samples; the mean volume was high (304 ml.) and all the enzyme concentrations were well within the normal limits.

2. Protein test meal (4 tests): Thirty six percent of the pH readings were below 6.0. One subject was tested twice with casilan meals (Test 88 and 120), and low pH readings were recorded on both occasions, whereas a saline meal in the same subject had high pH values in the aspirate. Similar recording were made in another subject where the casilan meal was the only meal to have more than one low pH reading (See Appendix page 163 , Table F).

The mean volume of the aspirates was only half that recorded following the test meal (152 ml). There appears to be no correlation between the pH and the volume.

ENZYMES

(a) Trypsin: The mean trypsin concentration was 5748 units/ml and the range 3957 to 8016 units/ml. As indicated in Fig. 25, this was a good response, although better responses were recorded by the standard and fat test meal .

(b) Amylase: The mean amylase concentration was 8.8 units/ml and the range 7.07 to 10.45 units/ml. Again, the standard and fat meal produced a better response.

(c) Chymotrypsin: The mean concentration was 2747 units/ml with a range of 1966 to 3616 units/ml. These results were slightly better than those following the test meal.

(d) Lipase: The mean concentration was 719 I.U./litre and the range 580 to 1117 I.U./litre. Only the fat and carbohydrate-free meals produced a better lipase response.

3. Carbohydrate test meal (4 tests): The pH was 6.0 or more in all the aspirates. The mean volume of all the test meals was 288 ml (Fig 24) which was considerably higher than the fat and protein meals and about equal to the test meal. Note also that the carbohydrate-free meals had a mean volume of the aspirates, half that of the carbohydrate meal. Despite these very large volumes, ranging up to 160 ml in a single quarter hour sample (Test 90, Sample 3), the pH did not fall below 6.0. It should also be noted that the last sample, collected 2 hours after the ingestion of the meal, did not have a very small volume. This suggests that, had the test been prolonged after 2 hours, further collection would have been possible as a result of apparently prolonged carbohydrate stimulation.

ENZYMES

(a) Trypsin: The mean concentration was 3373 units /ml with a range of 2400 to 4028 units/ml. Only the water meal and the combination of a saline meal with gastrin produced a smaller trypsin response. A fair number of readings (47%) were below the normal range standardized by the test meal.

(b) Amylase: The mean concentration was 7.75 units/ml and the range 5.17 to 12.64 units/ml. The mean value was less than that of the protein meal and higher than the saline meals. Test 92, with the highest mean amylase concentration, also had the smallest

volume of aspirate, suggesting that a dilution affect may be partly responsible for the low enzyme concentration seen with the other carbohydrate meals.

(c) Chymotrypsin: The mean concentration was 2189 units/ml and the range 1723 to 3014 units/ml. The mean concentration was not significantly different from that of the other meals.

(d) Lipase: The highest lipase concentration was less than the lowest lipase concentration following the fat meal and only slightly more than the lowest concentration following the protein meal (Table 9). The mean concentration was about equal to that following the water and saline meal.

4. Fat test meal (3 tests): Only one pH reading was less than 6.4. This low reading was probably the result of very rapid gastric emptying during a short period of time. Two out of the three fat test meals had small volumes of aspirates, 96 ml. and 67 ml. respectively, while the third meal produced a volume of 418 ml. In this latter case, it was still less than that following the ingestion of the standard and carbohydrate test meal.

ENZYMES

(a) Trypsin: The mean concentration was 6568 units/ml and the range 4151 to 11,127 units/ml. The mean trypsin concentration following the fat meal was the highest mean concentration of all the meals, and it was 3195 units higher than the mean concentration following the carbohydrate meal.

TABLE 10 (15 MINUTE SAMPLE)

Sample		Vol	pH	% Radioactivity
Basal	1	42	5	-
"	2	50	5	-
"	3	5	6.4	-
"	4	0	-	-
	1	250	4.8	15.30
	2	85	6	4.20
	3	36	6.4	1.40
	4	20	5.1	0.59
	5	38	4.8	13.96
	6	20	4.9	2.16
	7	10	4.8	0.46
	8	10	4.9	5.81

The Volume, pH and percentage radioactivity of the duodenal samples following the ingestion of a water meal containing I^{131} Rose Bengal.

(b) Amylase: The mean concentration was 11.40 units/ml and the range 10.65 to 11.91 units/ml. Note the very small range of amylase activity, compared to the side range for trypsin. The one abnormally low amylase reading (Test 94, Sample 3) was probably the result of a dilution affect and a pH of 5.4.

(c) Chymotrypsin: The mean concentration was 3031 units/ml, with a range of 1457 to 5458 units/ml. Only the low protein meal caused a higher mean chymotrypsin concentration in the aspirate.

(d) Lipase: The mean concentration was 823 I.U./litre with a range of 637 to 1114 I.U./litre. This mean concentration was considerably more than that following the standard and carbohydrate meal, and was almost 200 I.U. more than the mean concentration following the fat-free meal.

5. Water test meal (3 tests): Fifty percent of the pH readings were below 6.0. Test 97 illustrates a number of interesting points (Table 10). The last column shows the percentage radioactivity of I^{131} Rose Bengal present in each aspirate. At the end of the test, 0.82% radioactivity was left in the stomach indicating a loss of 55.20% of the duodenal contents past the tube. Before the ingestion of the meal, the pH varied between 5.0 and 6.4 in the three basal samples. After the ingestion of the meal, there was a similar wide variation in the pH. Water was present in all the samples as indicated by the presence of radioactivity in all these samples.

ENZYMES

(a) Trypsin: The mean concentration was 3713 units/ml, and

the range 1797 to 5284 units/ml. This mean concentration was about equal to that following the carbohydrate meal, and considerably less than that following the standard, fat and protein meals.

(b) Amylase: The mean concentration was 10.40 units/ml with a range of 2.79 to 14.37 units/ml. This high mean amylase concentration was almost equal to that following the fat meal.

(c) Chymotrypsin: The mean concentration was 2249 units/ml, with a range of 1178 to 2636 units/ml. These results were not significantly different from the other test meals ($P > 0.05$).

(d) Lipase: The mean concentration of the 3 meals was 326 I.U./litre with a range of 173 to 510 I.U./litre. This mean concentration was the second lowest recorded of all the test meals, and was significantly lower than that of the mean lipase concentration following the fat meal.

6. Saline test meal (5 tests): Fourteen percent of the individual samples had a pH below 6.0, which was considerably better than the pH of the samples following the water meal. Even when the volume of the aspirate was high (120 ml. in Test 99, Sample 3), the pH could be as high as 7.0.

ENZYMES

(a) Trypsin: The mean concentration was 3350 units/ml with a range of 1536 to 4601 units/ml. This mean concentration was the second lowest recorded of all the different test meals.

	SAMPLE	VOLUME	pH	AMYLASE $x10^3$ Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE $x10^3$ I.U./litre
GASTRIN →	B	10	7.1	17.56	2066	1074	417
	1	8	7.0	10.87	5716	2950	990
	2	20	6.7	14.25	7700	4583	105
	3	30	6.5	17.07	6133	3299	844
	4	60	4.9	13.77	1483	708	188
	5	7	6.4	3.72	2799	1615	511
	6	18	5	4.32	2583	1299	386
	7	37	4.8	1.05	1699	741	271
GASTRIN →	8	13	6.0	6.98	2174	1350	531
	9	4	7.2		2600		
	10	22	6.7		4666		
	11	10	6.5		2249		
	12	32	4.7		2050		

Table 11: TOTAL VOLUME MEAN pH AND ENZYME CONCENTRATIONS FOLLOWING TWO INJECTIONS OF GASTRIN SPACED TWO HOURS APART.

(b) Amylase: The mean concentration was 6.70 units/ml, with a range of 1.96 to 10.86 units/ml. Like the trypsin results, this was the second lowest mean amylase concentration of all the different meals.

(c) Chymotrypsin: The mean concentration was 2208 units/ml, the range being 1060 to 2656 units/ml. This mean concentration, although low, was about equal to the mean concentration following the water meal.

(d) Lipase: The mean concentration was 425 I.U./litre and the range from 174 to 784 I.U./litre. This mean concentration was about equal to that of the lipase concentration following the carbohydrate meal.

7. Gastrin injection (Table 11): Thirty three percent of the samples had a pH below 6.0. For the first 45 minutes the pH remained high, then there was a sudden fall associated with an increase in the volume of the aspirate. Following the second injection of gastrin, the pH remained high for a further 45 minutes before falling to 4.7 in the last sample, again associated with a higher volume than the previous three aspirates.

ENZYMES

(a) Trypsin: The mean trypsin concentration was 3786 units/ml. for the first 8 samples. There was a very definite rise in the trypsin concentration of the first sample following the gastrin injection. Following the second injection, a rise did occur, but

only in the second quarter hour after the injection. The mean concentration of the first 8 samples was equal to that following the water meal.

(b) Amylase : The mean concentration was 10.58 units/ml. This excludes the amylase concentration in 3 samples because of the low pH recorded in these samples. The relatively high mean concentration is about equal to the amylase concentration following the water meal.

(c) Chymotrypsin: The mean concentration was 2068 units/ml.

(d) Lipase: The mean concentration was 478 I.U./litre. There was a marked rise in the lipase concentration in the first 15 minutes. The mean concentration was similar to that following the carbohydrate and saline meals.

8. Carbohydrate-free test meal (2 tests): All the pH values of the individual aspirates were 6.4 or more. The mean volume of the two meals was 146 ml, compared to the carbohydrate meals which had a mean volume of 288 ml, and the standard test meals with a mean volume of 304 ml.

ENZYMES

(a) Trypsin: The mean concentration of the 2 meals was 6239 units/ml, and only the fat meal produced a higher concentration.

(b) Amylase: The mean concentration was 13.44 units/ml. This was the highest mean concentration of all the different meals, and was 5.69 units higher than the mean concentration following the carbohydrate meal.

(c) Chymotrypsin: The mean concentration was 2570 units/ml, which is not very different to the mean chymotrypsin concentration following the carbohydrate meal (2189 units/ml).

(d) Lipase: The mean concentration was 886 I.U./litre with a range of 817 to 956 I.U./litre. Like that for amylase, the mean lipase concentration following this meal was the highest concentration of this enzyme of all the different meals.

9. Low protein meal:(2 tests): All the aspirates had a pH of 6.4 or more. The mean volume of the 2 meals was 175 ml.

ENZYMES

(a) Trypsin: The mean concentration was 5817 units/ml. This was very slightly more than the mean trypsin concentration following the protein meal.

(b) Amylase: The mean concentration was 12.6 units/ml. This was 3.85 units more than the mean amylase concentration following the protein meal. Only the carbohydrate-free meal produced a higher mean amylase concentration.

(c) Chymotrypsin: The mean concentration was 3280 units/ml, which was the highest chymotrypsin concentration of all the different meals.

(d) Lipase: The mean concentration was 615 I.U./litre. This is almost a 100 I.U./litre less than the mean lipase concentration following the protein meal.

10. Saline test meal + Gastrin injection (2 tests): Thirty three percent of the samples had a pH of less than 6.0. One sample with a

pH of 3.6 was the lowest ever recorded pH in our series. This low pH was associated with a very high volume. The mean volume of the two tests was 406 ml, compared to the mean volume following the saline meals of 297ml.

ENZYMES

(a) Trypsin: The mean concentration of the two meals was 1743 units/ml, which was very much less than the mean trypsin concentration following any other meal.

(b) Amylase: The mean concentration was 2.1 units/ml, which again was very considerably less than the mean amylase concentration following any other meal. Amylase concentrations associated with low pH values were not included.

(c) Chymotrypsin: The mean concentration was 1673 units/ml. This was the only mean concentration less than 2000 units/ml.

(d) Lipase : The mean concentration was 155 I.U./litre. Like the other enzymes, this was very considerably less than the mean lipase concentrations following the other test meals, and significantly less than the fat and protein meals (P 0.01).

11. Fat-free meal (1 test): All the pH values in the aspirates were 6.4 or more. The total volume of the aspirates was 132 ml.

ENZYMES

(a) Trypsin: The mean concentration of the aspirates was 3965 units/ml. This was higher than the carbohydrate meal, but considerably less than the protein meal.

(b) Amylase: The mean concentration was 10.31 units/ml. This was more than the amylase concentration following the standard, protein and carbohydrate meals, but less than that following the fat meal.

(c) Chymotrypsin: The mean concentration was 3061 units/ml.

(d) Lipase: The mean concentration was 630 I.U./litre. This is more than the concentration following the standard and carbohydrate meals, but less than that following the protein and fat meals.

STATISTICS

The S. method of multiple comparisons [*] used to analyse the results of this section tended to favour acceptance of the hypothesis that there was no difference between groups. This, together with the very wide range of enzyme concentration found during a single meal, accounts for the fact that a statistical difference between the meals was only found in the few meals described below.

(a) Trypsin: The only significantly difference in trypsin concentration was found between the fat meal and saline meal + gastrin injection.

(b) Amylase: A significantly different amylase concentration was found between the fat and saline meal + gastrin injection, and between the carbohydrate-free meal and saline meal + gastrin injection.

(c) Chymotrypsin: No statistically significant difference was found between any of the meals.

TABLE 12.

Enzyme concentrations following different meals.

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I.U./litre
STANDARD TEST MEAL (Test 117)	B	50	6.4	3.79	2394	1200	281
	1	41	6.6	14.21	5716	2666	1052
	2	148	6.7	10.49	2508	7999	657
	3	122	6.6	7.33	1641	316	281
	4	109	6.4	7.16	2208	842	323
	5	40	6.4	5.85	1458	924	323
	6	41	6.4	6.57	3333	1349	542
	7	50	6.4	6.34	4433	1316	594
	8	20	6.4	6.34	8633	2649	657
CASILAN MEAL (Test 86)	B	8	6.4	10.57	2516	1791	563
	1	6	6.5	10.50	6550	3383	688
	2	10	6.5	8.63	5116	1566	740
	3	8	6.4	6.91	3566	1366	500
	4	5	6.4	3.68	2041	2349	354
	5	4	6.0	3.71	1499	1500	292
	6	5	6.4	8.16	5866	1550	750
	7	10	6.4	5.03	2983	1483	479
	8	4	6.4	9.94	5033	2533	834
CARBOHYDRATE MEAL. (Test 90)	B	64	6.4	-	3033	1633	375
	1	44	6.4	7.15	2166	1833	365
	2	14	6.0	4.48	3041	1475	375
	3	160	6.4	1.88	1766	958	219
	4	124	6.0	3.03	2374	1108	239
	5	40	6.4	3.52	1550	1108	198
	6	22	6.0	7.62	2258	2366	469
	7	32	6.4	9.53	2308	1933	584
	8	22	6.4	9.5	3744	3000	729
FAT MEAL (Test 94)	B	20	6.4	8.94	2383	1658	479
	1	100+	6.6	4.38	846	408.	343
	2	36	6.4	10.11	7866	883	782
	3	12	5.4	1.49	2299	1008	313
	4	56	6.4	7.50	2849	1025	323
	5	10	6.4	13.35	4916	2483	865
	6	34	6.4	20.83	5450	2083	990
	7	42	6.4	10.63	4599	2249	438
	8	20	6.4	14.84	4383	1516	1042

(c) Lipase: A statistically significant difference was found between the test meal and saline meal + gastrin; between the fat meal and carbohydrate, water, saline and saline meal + gastrin; between the protein meal and water, saline and saline meal + gastrin.

C. THE PATTERN OF RESPONSE OF pH, VOLUME OF ASPIRATE AND THE ENZYMES, AMYLASE, TRYPSIN, CHYMOTRYPSIN AND LIPASE, TO DIFFERENT MEALS - AN EXAMPLE IN ONE SUBJECT (Table 12).

The subject described in this section was an asymptomatic European male of 35 years. During the course of six days, he was subjected to 4 test meals - the standard test meal, casilan, carbohydrate and fat test meals. All the tests were performed under the standard conditions as described under methodology. Collections were made during 15 minute periods.

(a) Volumes: The volumes of the duodenal aspirates, following all the meals, except the casilan meal, were high. Although the very large volumes did seem to dilute the enzymes in some samples, e.g. sample 1 of the fat meal, this was not so in all cases, e.g. sample 2 of the test meal. It should also be noted that, despite the fact that the volume of the duodenal aspirate following the fat meal was 5 times that of the casilan meal, the concentration of all enzymes following this fat meal was still higher than that in the casilan meal.

(b) pH: The pH fell below 6 in only one out of 36 samples. This sample (sample 3 in the fat meal) had a very high volume and it must be assumed that gastric emptying was particularly rapid, associated with an inadequate stimulus to pancreatic secretion.

(c) Enzymes: Table 12 and Fig. 29 to 32 show the enzyme patterns for the different meals. It can be seen that the double

TEST MEAL	OSMOLARITY M.OSM/LITRE	MEAN OF TOTAL VOLUMES OF ASPIRATE
Standard Test Meal	898	304
Protein	36	152
Carbohydrate	835	288
Fat	1.0	194
Water	1.0	260
Saline	719	297
Carbohydrate Free	10	146
Low Protein	960	175
Fat Free	850	132

Table 13: MEAN OSMOLARITIES OF DIFFERENT TEST MEALS AND TOTAL VOLUME OF ASPIRATES

peak observed during the standard test meal was present following the ingestion of all the other meals as well.

For each meal, the four enzymes rise and fall together and appear to be secreted in a parallel fashion.

There appears to be no acute adaptation to the different meals i.e. the fat meal evokes not only a high secretion of lipase, but of all the enzymes, and the carbohydrate meal does not appear to stimulate a higher amylase response than the other enzymes.

The pattern of enzyme response is similar for all the meals, only the height of the peaks appear to vary.

D. THE OSMOLARITY OF THE DIFFERENT TEST MEALS.

Table 13 indicates the osmolarity of the different test meals and the mean of the total volumes in each group of meals.

There appeared to be a relationship between the osmolarity of the test meal and the volume of the duodenal contents. The standard test meal, carbohydrate and saline test meals with relatively high osmolarities had the highest mean volumes of aspirates. The fat, protein and carbohydrate-free meals with the very low osmolarities had a correspondingly small volume of aspirate. A few exceptions were seen, notably the small volume of aspirate following the fat-free meal, but as only one subject received this meal, the results may not be meaningful.

E. SUMMARY

(A) Different volumes of the same test meal, although perhaps altering the volume of the duodenal aspirate, appeared to make little difference to the pH, amylase, trypsin, chymotrypsin and lipase

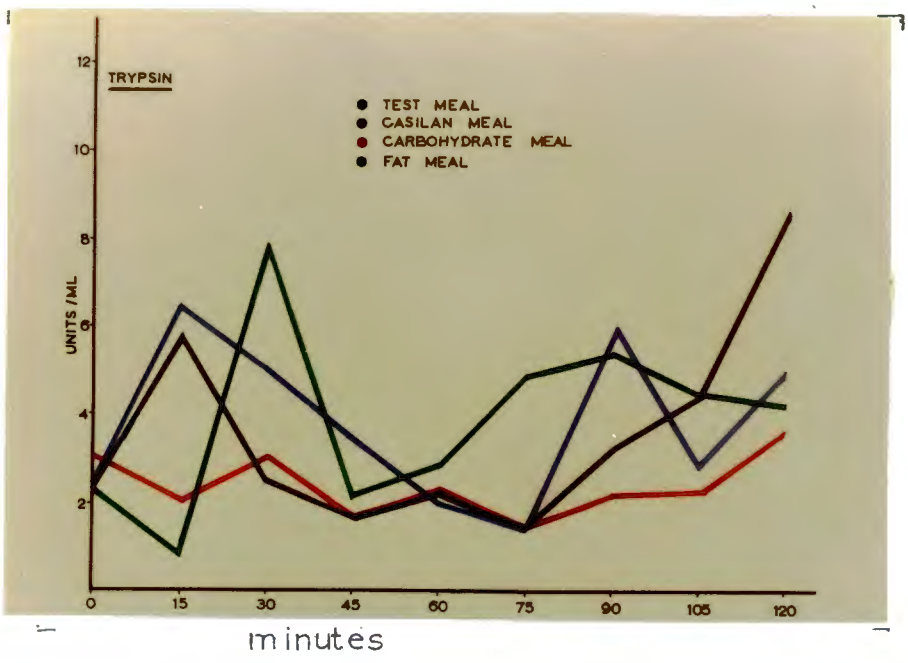
TRYPSIN
TEST MEAL
CASILAN MEAL
CARBOHYDRATE MEAL
FAT MEAL
UNITS /ML
12
10
8
6
4
2
0
15
30
45
60
75
90
105
120
minutes

FIG. 29

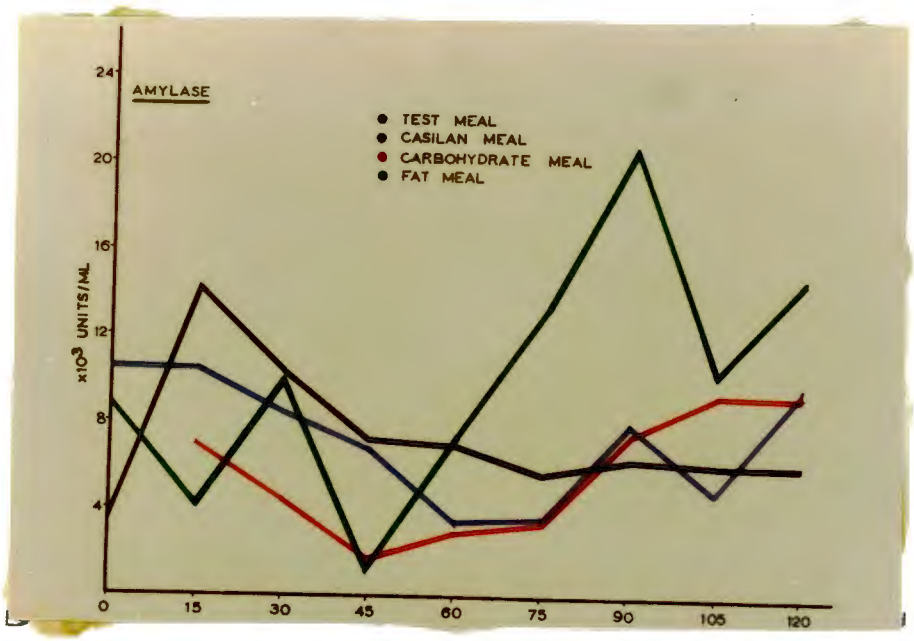
AMYLASE
TEST MEAL
CASILAN MEAL
CARBOHYDRATE MEAL
FAT MEAL
x10³ UNITS/ML
24
20
16
12
8
4
0
15
30
45
60
75
90
105
120

FIG. 30

Fig. 29:

This shows the trypsin concentration in the aspirates during the 2 hours following the ingestion of a standard test, casilan, fat and carbohydrate meals. Despite the fact that the tests were performed on different days, the pattern of trypsin concentration was remarkably similar. The double peak observed in the standard test meal is present with all the other meals. The main difference between the meals appears to be the peaks reached by the enzyme concentrations during the 2 hours. Following the first rise and fall, the enzyme concentrations reach a plateau and remain at almost the same level for all the meals during the 3rd, 4th and 5th samples.

Black = Test Meal
Blue = Casilan Meal
Red = Carbohydrate Meal
Green = Fat Meal

Fig. 30:

The 2 enzyme peaks are again observed. It should be noted that the carbohydrate meal does not stimulate a high amylase concentration in the aspirates.

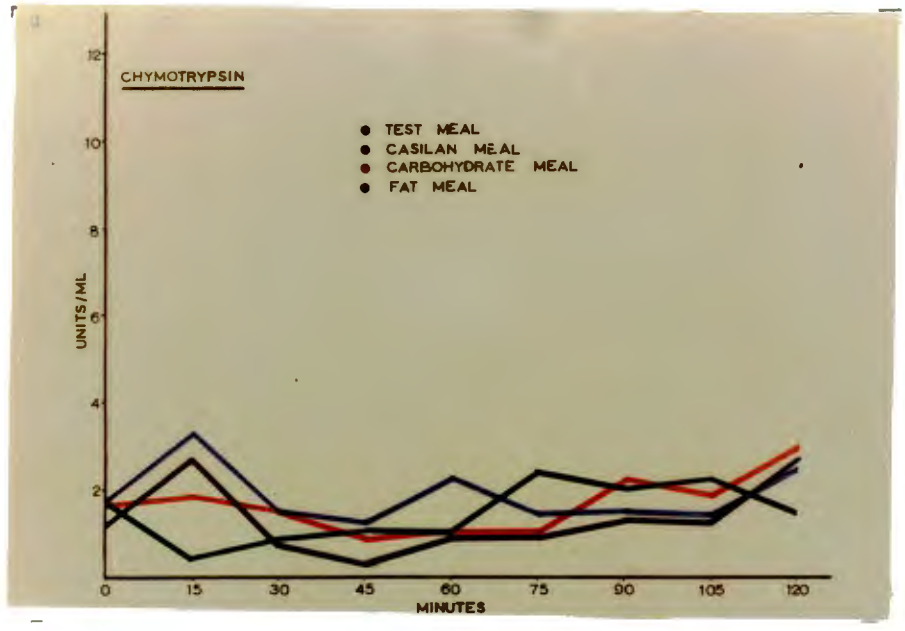
CHYMOTRYPSIN
TEST MEAL
CASILAN MEAL
CARBOHYDRATE MEAL
FAT MEAL
UNITS/ML
12
10
8
6
4
2
0
15
30
45
60
75
90
105
120
MINUTES

FIG. 31

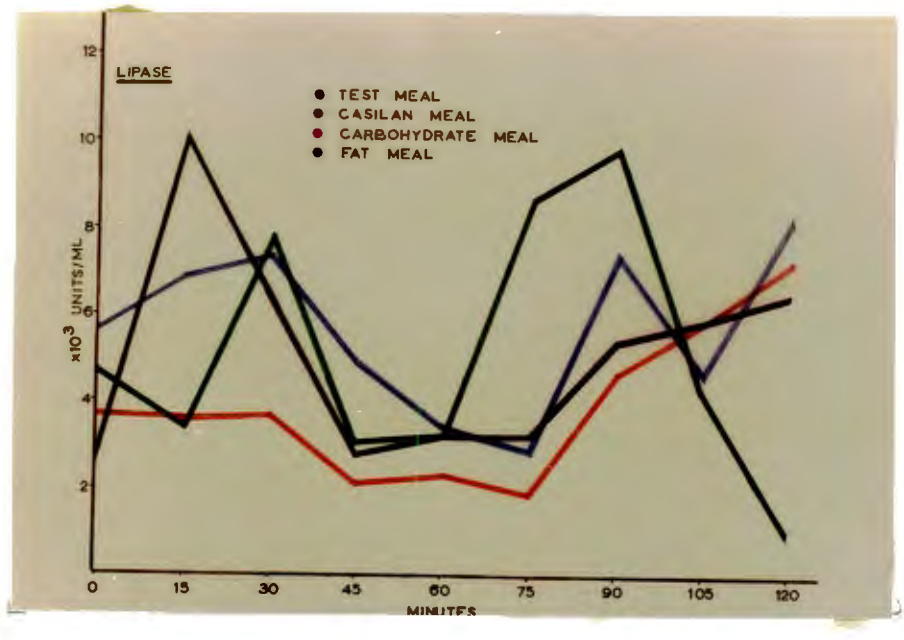
LIPASE
TEST MEAL
CASILAN MEAL
CARBOHYDRATE MEAL
FAT MEAL
x10³ UNITS/ML
12
10
8
6
4
2
0
15
30
45
60
75
90
105
120
MINUTES

FIG 32

Fig. 31:

The chymotrypsin concentration following the ingestion of the different meals shows very little variation, although an early peak may still be observed and there is a tendency for the enzyme concentrations to rise during the latter half of the test.

Black = Test Meal
Blue = Casilan Meal
Red = Carbohydrate Meal
Green = Fat Meal

Fig. 32:

The pattern of lipase response is very similar to that of trypsin. The very low pH in the first sample following the ingestion of the fat meal probably explains the reason for the delayed first peak following this meal. Again, all the enzyme concentrations for the different meals are at a lower level during the 3rd, 4th and 5th samples. The close similarity between the enzyme patterns of response is again observed.

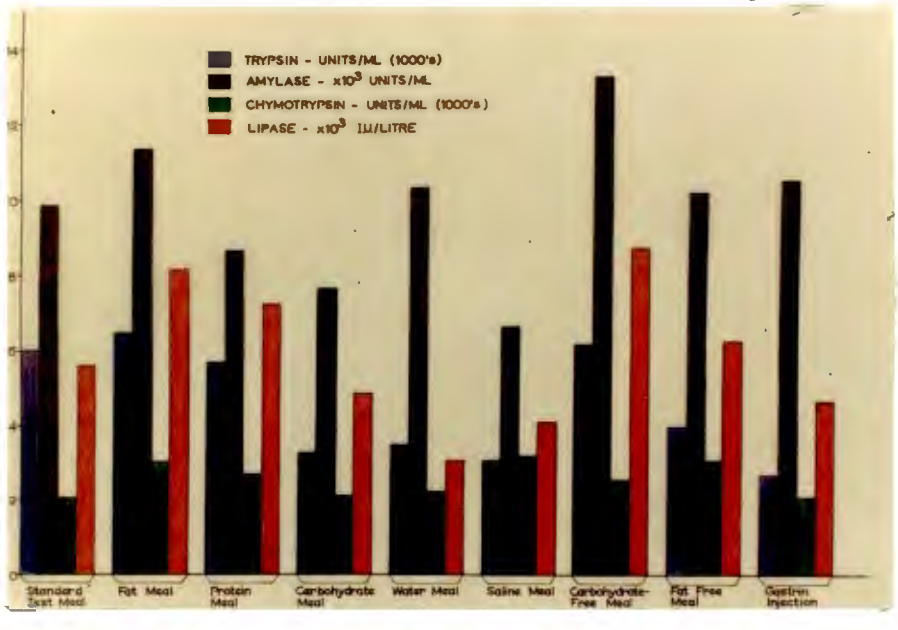

Fig 33: SUMMARY OF THE ENZYME CONCENTRATION FOLLOWING THE INGESTION OF DIFFERENT TEST MEALS

concentrations of the duodenal aspirates.

(B) Fig. 33 attempts to give a comparitive study of the trypsin, amylase, chymotrypsin and lipase concentrations in the duodenal aspirate following the different test meals.

1. The standard test meal: The pH never fell below 6. Only the saline meal with gastrin injection produced a higher volume of aspirate. The response for all enzymes was normal and good.

2. The casilan meal: 36% of the pH readings were below 6. The mean volume response was half that of the test meal. Except for trypsin, the other enzyme concentrations were slightly better than those following the test meal.

3. The carbohydrate test meal: The pH was 6 or above in all aspirates. The volume response was almost equal to that of the test meal. The enzyme concoentrations, except for amylase, approached that of the water meal rather than the test meal.

4. The fat test meal: The pH was 6.4 or more in almost all the aspirates. The mean volume response was slightly better than that following the casilan meal, but considerably less than that following the test meal and carbohydrate meal. Only the carbohydrate-free meal stimulated as high an enzyme response from the pancreas.

5. Water test meal: 50% of the pH readings were below 6. The volume of aspirate was good, but less than that following the test meal or carbohydrate meal. Despite the fact that the enzyme

concentrations were low, they were still within the normal range.

6. Saline test meal: Only 14% of the individual aspirates had a pH below 6. The mean volume of the aspirates was high, being just less than that following the test meal. The mean enzyme concentrations were about equal to those following the water and carbohydrate meal.

7. Gastrin injection: The pH of a third were below 6. The volume response was about equal to that of the fat and protein meal, while the enzyme response approached that of the water and saline meal.

8. Carbohydrate-free test meal: The pH of all the individual samples was 6.4 or more. This meal produced the second smallest volume of aspirate, while the mean enzyme concantrations were about equal to that in the fat meal.

9. Low protein test meal: The pH of all the individual samples was 6.4 or more. The mean volume of the aspirates was slightly more than that following the protein meal.

10. Saline test meal + Gastrin injection: One third of the pH values was below 6. The above combination produced the highest mean volume of aspirate. The mean enzyme concentrations were not only the lowest of all the meals, but were also all below normal levels.

11. Fat-free med: The pH of all the individual samples were 6.4 or

more. The volume of the aspirate was the lowest recorded. The enzyme response was good, especially that of amylase, while the trypsin response appeared less than than of the other three enzymes.

F. COMMENT

Of the four main meals used in our series (standard test meal, fat, carbohydrate and protein meals), only the last caused a considerable depression of the pH in a fair number of the samples. Fat and carbohydrate are known to slow gastric emptying and suppress gastric secretion, while protein stimulates a good acid response. This occurred with gastrin, which also produces a marked acidic response from the stomach, with a low pH in many duodenal samples. While a high acid output may be an important factor in lowering the duodenal pH in some cases, it is not likely to be responsible for the low pH seen in some tests, e.g., following the water and saline meals. These meals do not stimulate a marked acidic response from the stomach, therefore the low pH is probably due to poor stimulation ofthe secretin mechanism. The pH of the duodenal aspirate, in fact, tends to be higher following the saline meal than following the water meal. Hence, the osmolarity of the test meal may be an important factor involved in stimulating the secretion of secretin from the duodenal mucosa. The osmolarity may also be a factor involved in the low pH values following the protein meal, as this meal had a very low osmolarity. This mechanism would explain the normal values and high volume in the aspirates following the ingestion of the standard and carbohydrate test meal, which had high osmolarities. <u>These studies suggest that the presence of acid in the duodenum and the osmolarity of the test meal are important factors stimulating the release of secretin from the mucosa.</u> The relatively large volume of the

aspirate following the intramuscular injection of gastrin and the very large volume following the saline meal + gastrin, is further support in favour of the release of secretin by acid stimulation. A direct effect of gastrin on the pancreas cannot be excluded.

As far as the enzyme response to the different meals is concerned, two factors were observed:

a) For any meal the enzymes appear to be secreted in parallel, rising and falling together.

b) For each enzyme, the pattern of response to the different meals appears to be similar, with only a quantitative difference present. Secretin is not directly involved in enzyme secretion, therefore these enzyme responses are probably mediated by neutral pathways and the stimulatory effect of pancreozymin. It is unlikely that these two mechanisms have a selective action on a particular enzyme but probably influence all the synthetic enzyme processes equally, thus accounting for their parallel secretion. The different meals stimulate the secretion of pancreozymin and the neutral system to varying degrees, thus accounting for the quantitative differences in enzyme response between meals. The studies suggest that carbohydrate would appear to be a poor pancreozymin stimulant (and a strong secretin stimulant), while fat and protein are strong stimulants to this hormone (weaker secretin stimulants).

Although no statistical proof is present, there appears to be no qualitative difference between the meals with regard to the enzyme secretion. A fat meal does not selectively stimulate a higher lipase secretion than the other enzymes, nor does a protein meal selectively stimulate a higher secretion of proteolytic enzymes under the present acute conditions. This fact favours the theory that it is only the

strength of the neutral or hormonal mechanism which stimulates a varying secretion of all the enzymes together.

The effect of different meals on the enzyme response of the pancreas demonstrates again that the high enzyme concentration, seen in the early aspirates, is not due to a "wash out" effect, but to direct stimulation, probably of a nervous nature. As indicated in the various charts and tables, the enzyme concentration in the first sample is not constant, but varied with the type of meal. Thus, following the fat or protein meal, the enzyme concentrations in the first sample tended to be higher than the first peak following the carbohydrate meal. This would be unlikely if a "wash out" effect was involved.

In conclusion, an analysis of all the results using test meals of varying composition suggest that the ideal test meal should contain a high fat and carbohydrate content and a low protein content. The exact volume, while not being of apparent importance, should range between 300 to 350 ml. A high constant osmolarity is important. This test meal would insure good volumes, optimal pH conditions and the maximal response to enzyme production.

G. DISCUSSION

The effect of different food substances on pancreatic secretion in an acute experiment has never been adequately documented in human subjects. Animal experiments have shown an enzyme response to the ingestion of a carbohydrate meal (21, 390), although this response is small both in volume and enzyme output (411). The present study shows a low enzyme concentration following the carbohydrate meals, together with a large volume. Although the

large volume may be thought to have diluted the enzymes, thus lowering the concentration, it should be noted that larger volumes were found following the test meal in which the enzyme concentration was high.

Protein meals, as opposed to the carbohydrate meals, stimulate a good enzyme response in animals (389). Wang and Grossman (411) noted that in some of their experiments this response was even better than that following intravenous pancreozymin. The few experimental studies in humans (75, 362) show only a slight, but definite, enzyme response following a protein meal. The work on animals agrees essentially with that found in our studies in humans, i.e., protein is a good stimulant to enzyme secretion while having only a moderate effect on volume flow.

In animals, fat is reported to stimulate a small volume (385) of pancreatic juice with a good enzyme output (257). The response to fat in humans is confusing, although Comfort and Osterberg (75) have reported a similar response to olive oil and casein. The results in animal experiments and that reported by Comfort and Osterberg has been confirmed in this study. The fat meal, in fact, appears to be an even stronger stimulus than protein to enzyme secretion, while producing only a small volume of pancreatic juice.

Water is reported to stimulate a brief and rapid flow of pancreatic juice containing a high enzyme content (89), in contrast to that produced by isotonic solutions which have no such effect. Wang and Grossman (411) suggested that the osmolarity may be the important factor for this difference. The response to water and saline in our study is difficult to interpret in view of the high volume and low pH values recorded. Both these substances are strong secretin stimulants, as the volumes aspirated during the tests were

very much greater than the volumes of the meals ingested. It should also be remembered that the volume aspirated is only about 25% of the duodenal contents passing the end of the tube. Except for the amylase concentration following the water meal, the other enzyme concentrations are low compared to the standard, fat and protein meals but compare with the mean enzyme concentrations following the carbohydrate meal, suggesting a weak pancreozymin stimulation.

Gastrin is reported to have a pancreozymin-like effect (48, 322, 433) on the pancreas of animals. Little is known of its effects on the human pancreas. Following the gastrin injection in our subjects, there was a good secretin and moderate pancreozymin-like response. As the gastric juice was not aspirated during the test, this response may have been due to presence of acid in the duodenum rather than a direct affect of gastrin on the pancreas.

The results of enzyme concentrations following the different meals support the theory, put forward by Babkin (17) of parallel secretion of the different enzymes, no matter what the stimulus. This is supported by the work of Hong et al (195) in humans. Very little further work in this conneotion has been performed on humans, although parallel secretion of enzymes has been demonstrated in various animals. Lagerlof (236) and others (60), showed parallel secretion of enzymes following an injection of secretin, while many other authors reported a possible dissociation of enzymes following secretin (102). This response with secretin is not surprising in view of the known action of secretin on the secretion of fluid and bicarbonate rather than enzymes. The weight of evidence in the present study, therefore, supports the theory of parallel secretion of enzymes to an acute stimulus, at least with test meal stimulation.

Chapter 11

RESULTS OF THE STANDARD TEST MEAL PERFORMED ON PATIENTS WITH PANCREATIC DISEASE AND OTHER CONDITIONS WHICH MAY AFFECT THE PANCREATIC FUNCTION

RESULTS PART 3

Chapter 11 RESULTS OF THE STANDARD TEST MEAL PERFORMED ON PATIENTS WITH PANCREATIC DISEASE AND OTHER CONDITIONS WHICH MAY AFFECT THE PANCREATIC FUNCTION

These patients may be divided up into 2 groups:

(A) Those patients with definite evidence of pancreatitis, as described under "Material."

(B) Patients with gastrointestinal or other diseases which may affect pancreatic function.

A. PATIENTS WITH PANCREATIC DISEASE

This group consisted of 32 patients, on whom 37 tests were performed, with unequivocal evidence of pancreatic disease (Table 14). The results of the test meals of these patients will be compared with the results of the test meals performed on the 18 normal individuals decribed in Part (1) of the results. Refer to Appendix, page 160 for details of all results reported below.

1. Volume of aspirate (Fig. 34): The mean basal volume was lower than that in the normal group, but rose to a higher level following the ingestion of the meal, and the mean volume remained high throughout the period of the test. This difference may be due to an increased rate of gastric emptying in patients with pancreatic disease. When, however, radio-activily labelled Rose Bengal was used as a marker in the meal, the percentage activity remaining in the stomach of the normal control group was not significantly higher than that remaining in the stomach of the patients with pancreatic disease. In 30% of the pancreatic group there was no basal volume

TABLE 14.

Diagnosis of patients with pancreatic disease.

Test No.	Age.	Sex*	Race**	Diabetes.	Steatorrhea.	Alcoholic History.	Good Clinical History.	Serum Amylase.	Calcification.	Operative Diagnosis.	Abnormal Pft.	DIAGNOSIS
33	43	M	E	@	x	@	@		x	x	@	Chronic Pancreatitis
34	29	M	C	@	@	@	@		@	x	@	Chronic Calcific Pancreatitis
35	68	M	E	@	@	x	x		@	x	@	" " "
36	31	M	E	@	x	@	x		x	x	@	" " "
37	52	M	C	x	x	@	@		@	x	o	" " "
38	40	M	C	@	@	@	x		@	@	@	" " "
39	39	M	C	+	x	@	x		x	@	@	" Pancreatitis
40	See Test (39)											
41	28	M	C	x	x	@	@	300	x	@	@	Acute Relapsing Pancreatitis
42	37	M	C	@	x	@	@		@	x	@	Chronic Calcific Pancreatitis
43	28	M	C	@	@	@	@		@	x	@	" " "
44	39	M	C	@	x	@	@		x	x	@	Chronic Relapsing Pancreatitis
45	38	M	C	x	x	@	@		x	x	@	" " "
46	49	M	C	x	x	@	@	195	x	@	o	" " "
47	52	M	C	x	x	@	@		x	x	@	" Pancreatitis.
48	See Test (39)											
49	72	M	C	@	@	@	@		@	x	@	" Calcific Pancreatitis
50	See Test (34)											
51	42	M	C	x	@	@	@	277	@	@	@	Calcific Pancreatitis
52	40	M	C	@	@	@	@		@	x	@	Chronic Calcific Pancreatitis
53	52	M	C	@	@	@	@		@	x	@	" " "
54	23	M	C	x	x	@	@	1036	x	@	o	Acute Pancreatitis
55	48	M	C	+	x	@	@	600	x	x	@	Acute Relapsing Pancreatitis
56	36	M	E	@	@	@	@		x	@	@	Chronic Pancreatitis
57	40	M	C	x	x	@	@	350	@	@	@	Chronic Calcific Pancreatitis
58	34	M	C			@	@		x	@	@	
59	44	M	C	@	x	@	@	2275	x	@	@	Acute Pancreatitis
60	48	M	C	o	@	@	x		x	@	o	Chronic Pancreatitis
61	30	M	C	x	x	@	@		x	@	@	" "
62	(See Test (53)											
63	58	M	C	@	x	@	@	315	x	x	@	" "
64	54	M	C	@	@	@			@	x	@	" Calcific Pancreatitis.
65	33	M	C	@	@	@	@		x	@	@	Chronic Pancreatitis
83	50	F	E	x	x	x	x	490	x	@	x	Acute Relapsing Pancreatitis
84	37	F	C	x	x	x	x		@	x	x	Calcific Pancreatitis
82	48	F	E	x	x	@	x		@	x	+	Calcific Pancreatitis

@ = YES

x = NO

o = No Result Available

+ = Borderline Result

* M = Male

F = Female

** E = European

C = Coloured

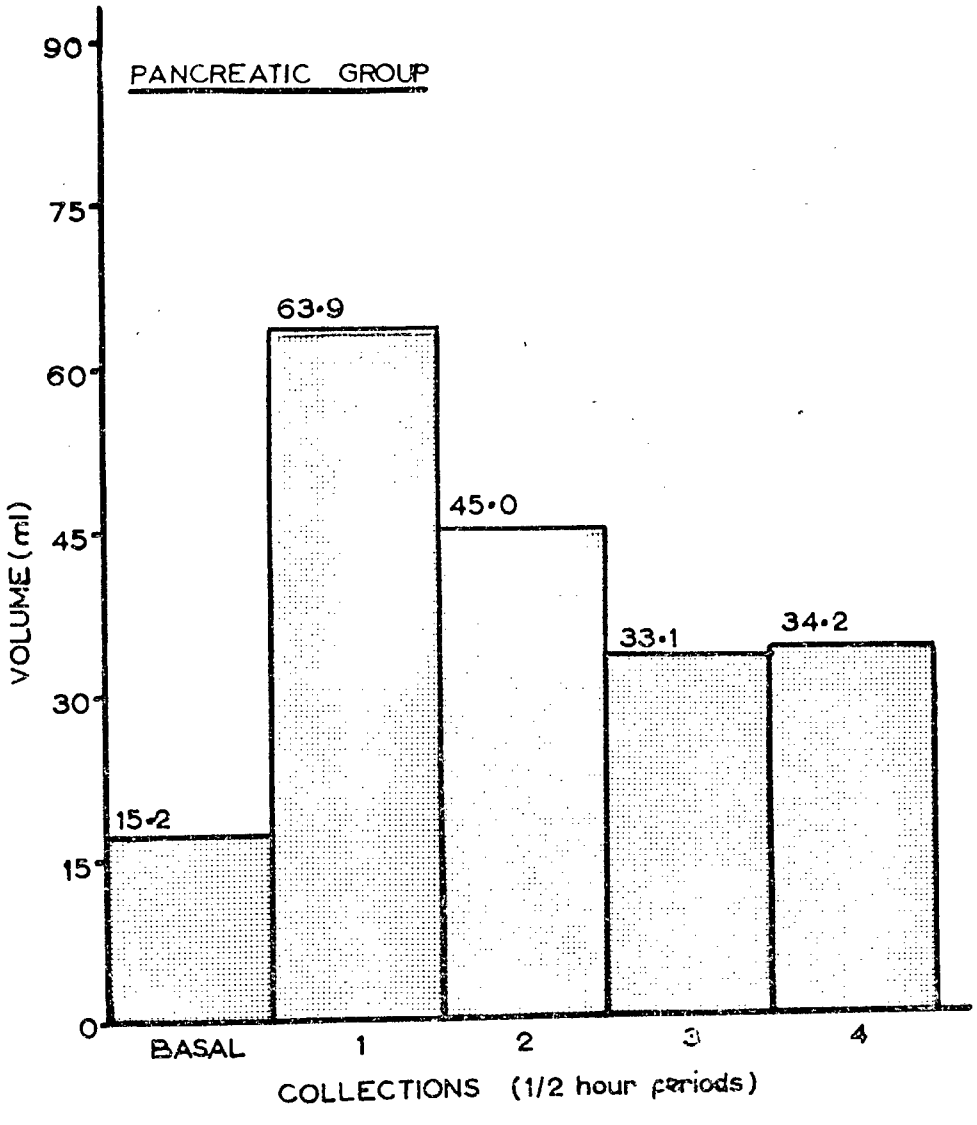

FIG. 34: VOLUME OF ASPIRATE
FOUR 30 MINUTE SAMPLES

SAMPLE	RANGE	MEAN
Basal	4.5 - 7.5	6.1
1	5.0 - 7.0	6.4
2	4.0 - 7.5	6.3
3	3.0 - 7.5	6.3
4	4.7 - 7.5	6.7

Table 15: pH (PANCREATIC GROUP)

after 20 minutes, as compared to 27.7% in the normal group.

Two of the highest recorded volumes aspirated occurred in patients with active inflammatory changes in the pancreas (Test 41 and 83). It should be noted, however, that even patients with severe diabetes and steatorrhoea as a result of chronic pancreatitis often had very high volumes aspirated from the duodenum (Tests 35, 36, 47, 48, 49, 51, 57 and 58). Again, it must be remembered that the volume aspirated is only about 25% of the duodenal contents passing the end of the tube.

2. pH of aspirate: Fig. 35 shows the mean pH of the basal aspirate and for the 4, 30 minute aspirates following stimulation with the test meal. The mean basal pH was slightly higher than in the normal group. In five out of 18 patients the basal pH fell to below 6.0, and in one case to 4.5 (Table 15).

Following the ingestion of the test meal, there was a slight increase in the mean pH of the aspirate, with a more marked increase in the 4th collection. Of particular interest was the fact that even patients with gross pancreatic insufficiency, tended to have a pH of more than 6.0. Tests 38, 50, 52 and 53 are good examples. All four tests were performed on patients who had severe diabetes, moderately severe to severe steatorrhoea and pancreatic calcification; yet the pH in all samples of the aspirate was above 6.0 despite bicarbonate values of 24, 29, 21 and 23 mEq bicarbonate/litre respectively, after the secretin-pancreozymin test. These patients did not necessarily have small volumes of aspirate. The total volumes aspirated during the two hours of the test were 163, 66, 81 and 34 ml. respectively.

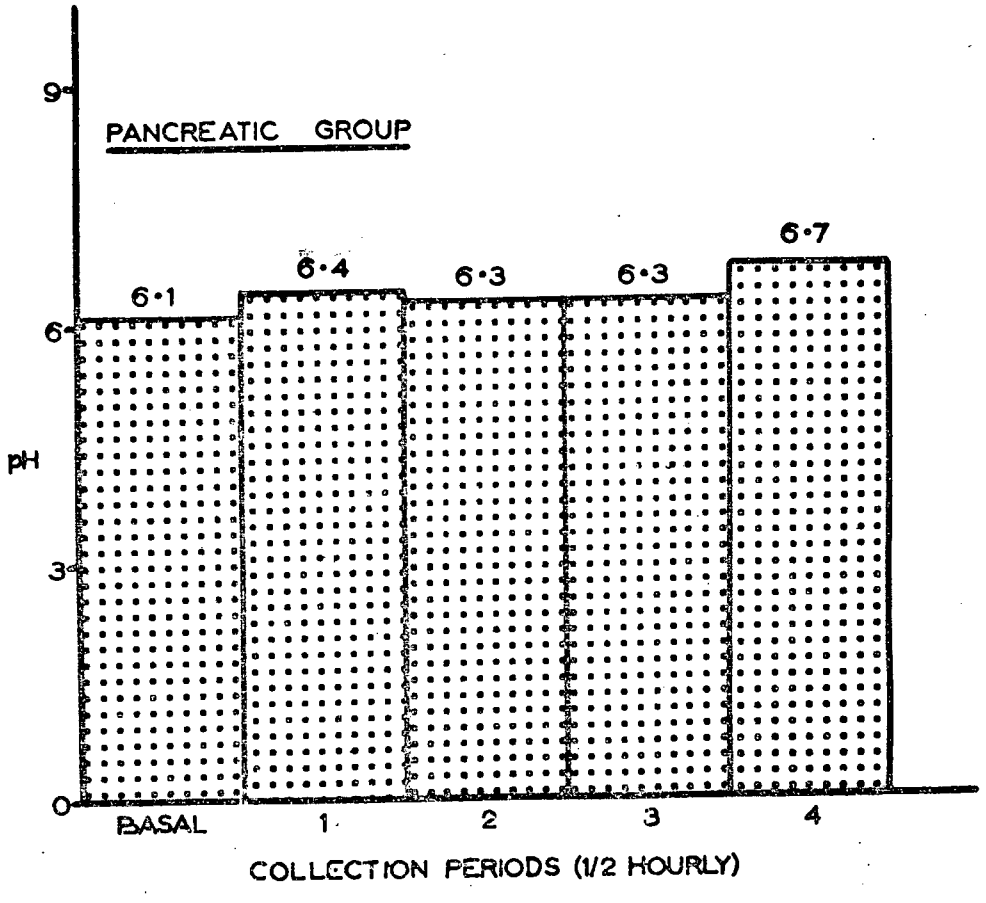

FIG. 35: pH OF ASPIRATE
FOUR 30 MINUTE SAMPLES

TABLE 16.

Effect of acid secretion on duodenal pH in patients with pancreatic disease.

TEST NO	AUGMENTED HISTAMINE TEST. M.Equiv./hour.		DUODENAL pH		
	BAO*	MAO**	Total No. of Samples	Mean	No. of Specimens with pH <6
34	4.9	17.3	3	6.3	None
35	2.3	39.6	4	4.5	3
36	3.6	15.4	-	-	-
38	4.6	33.1	4	6.3	None
39	3.1	8.0	4	7.5	"
46	0	0	4	6.2	"
52	5.8	28.4	4	6.4	"
53	0	3.3	4	7.1	"
55	3.6	14.9	3	5.6	2
56	0	12.6	4	6.4	None
57	0	0	4	6.4	"
58	3.5	12.1	4	6.4	"
64	2.1	14.8	4	5.9	1
65	0	13.9	4	6.4	None
84	5.4	35.6	4	5.4	3
79	1.2	8.2	4	7	None
83	0	6.7	4	6.7	"

* Basal acid output

** Acid output following maximum histamine stimulation.

3. Effect of gastric acidity on duodenal pH in patients with pancreatic disease: Table 16 shows the results of an augmented histamine test performed on 17 patients with pancreatic disease. This table should be compared with Table 2 showing the results of the normal group. Column 4 of Table 16 indicates the number of samples of duodenal aspirate taken during the course of the meal, and Column the mean pH of these meals. The last column indicates the number of pH readings below 6.0. As can be seen from the Table, when the maximum acid output (MAO) was high, as in Test 35 and 84, the majority of the samples had a duodenal pH of less than 6.0. Test 35 was performed on a patient with severe pancreatic insufficiency and Test 84 was performed on a patient who had pancreatic calcification only, but no evidence of exocrine insufficiency. However, not all the cases with high acid outputs had low pH values. Test 38 and 52 were performed on 2 patients who had maximum acid outputs (MAO) of 33.1 and 28.4 mEq/hour respectively, and no sample had a pH below 6.0 after the test meal. Both these latter patients had severe pancreatic insufficiency, with a mean bicarbonate in the aspirate following a secretin-pancreozymin test, of 24 and 21 mEq/litre respectively.

4. Enzyme concentration following ingestion of standard test meal:

a) Trypsin: As indicated in Part(1) of the results, the lower limit of normal for trypsin in the 2 hour test is 2800 units/ml. A result between 2500 and 3000 units/ml may be regarded as a borderline results. Eightly nine percent of the trypsin concentrations in the pancreatic group fell below 3000 units/ml. Four of the 37 patients

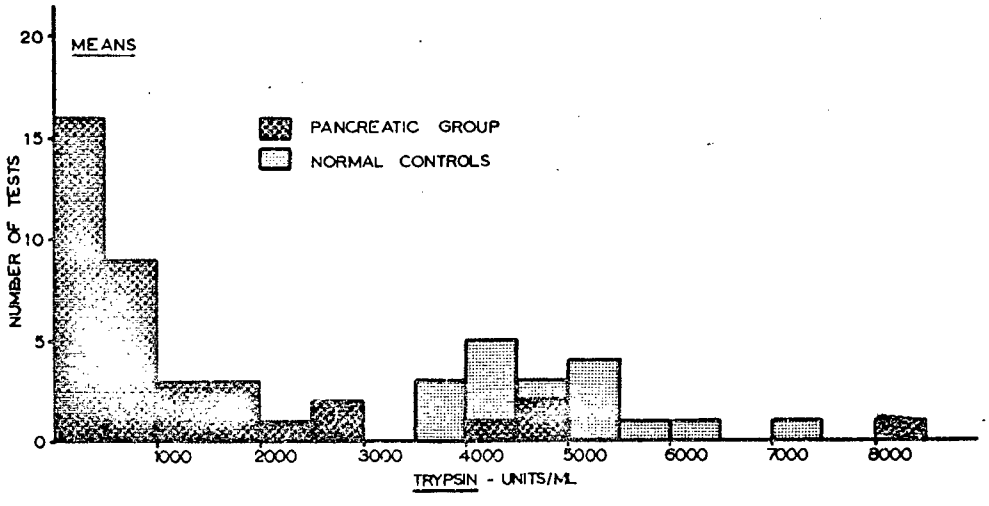

FIG. 36: DISTRIBUTION OF TRYPSIN CONCENTRATIONS

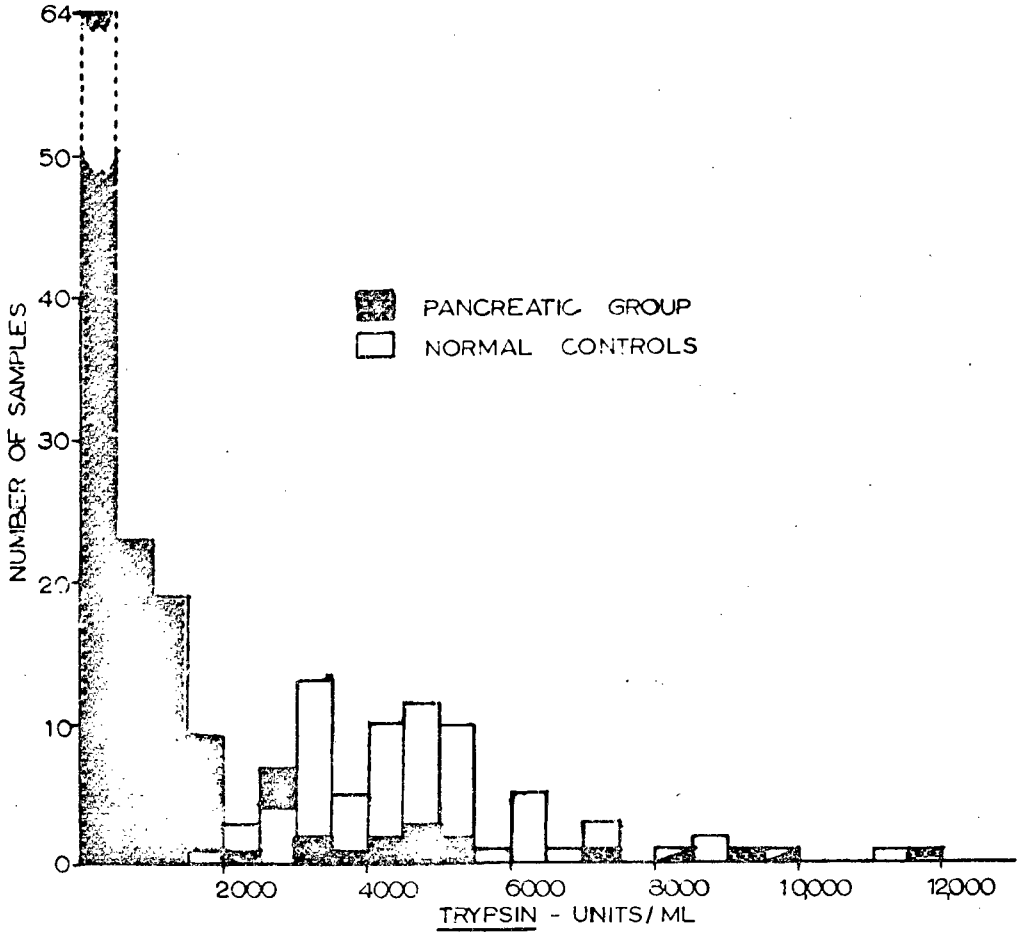

FIG. 37: DISTRIBUTION OF TRYPSIN CONCENTRATIONS
INDIVIDUAL 30 MINUTE SAMPLES

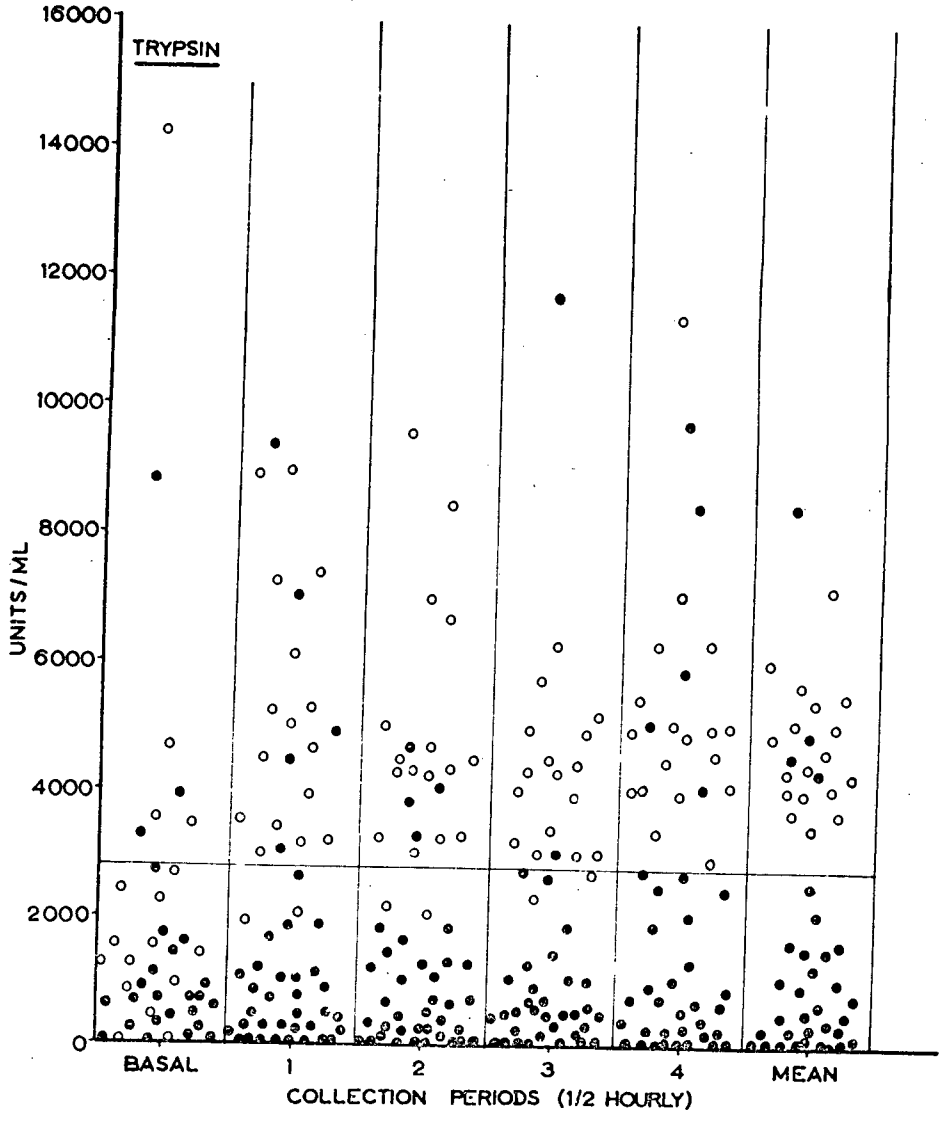

FIG 38: TRYPSIN CONCENTRATIONS - A COMPARISON BETWEEN NORMAL CONTROLS AND THE PANCREATIC GROUP

o = Normal Controls

● = Pancreatic Group

had normal trypsin values. Statistically, there was a very significant difference between the normal control group and the patients with pancreatitis ($P < 0.05$). Fig. 36 shows the number of trypsin results occuring at different concentrations.

In addition, 89% of all the individual results in the patients with pancreatitis were below 3000 units/ml. and almost 47% were between 0 to 500 units/ml (Fig. 37). Fig. 38 indicates the distribution of the individual 30 minute samples in both the normal control and pancreatic groups. The very highest trypsin concentration was present in the third sample of a patient with calcific pancreatitis.

Of those patients with abnormal trypsin results for the 2 hour test, 2 had normal trypsin concentrations in the first sample aspirated; 4517 and 3083 units/ml respectively.

b) Amylase: The lower limit of normal for amylase in the 2 hour test was 4.5 units/ml and therefore any results between 4 and 5 units/ml. may be regarded as borderline. As indicated in Fig. 39, 81% of patients with pancreatic disease had results that were in the abnormal range. Seven of the 37 patients had normal amylase values. Statistically, there was a significant difference between the normal controls and the patients with pancreatic disease. ($P < 0.05$).

Of the individual 30 minute samples of aspirate, 79% had an amylase concentration of less than 5.0 units/m. (Fig.40). The highest recorded amylase concentration again occurred in the one patient with calcific pancreatitis. All the amylase concentrations in the individual samples in both normal and abnormal subjects are shown in Fig. 41. This clearly shows the difference betweem the 2 groups.

Of those patients with abnormal amylase results for the 2 hour test, three had normal amylase concentrations in the first sample aspirated.

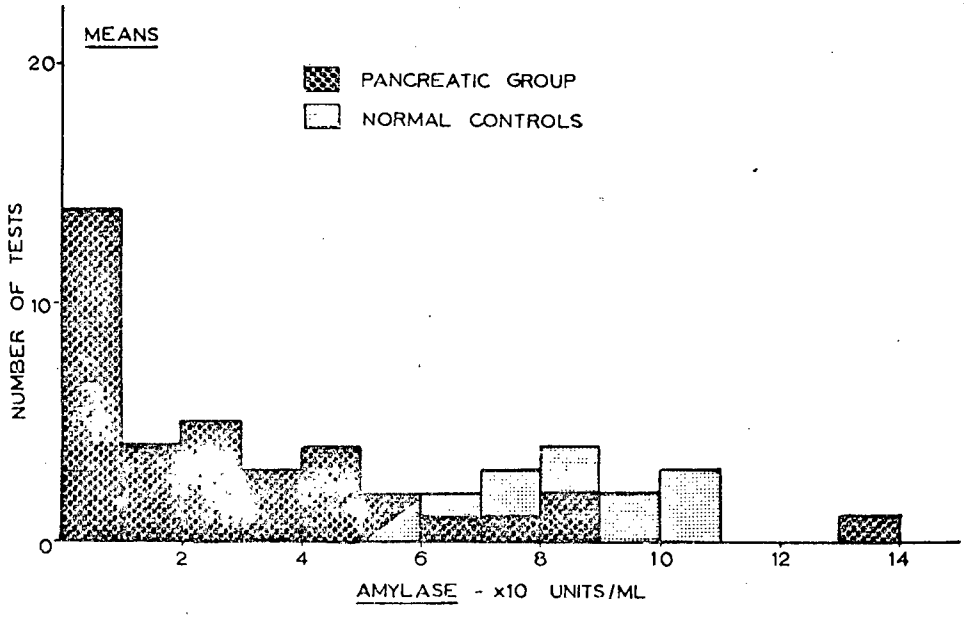

FIG. 39: DISTRIBUTION OF AMYLASE CONCENTRATIONS -

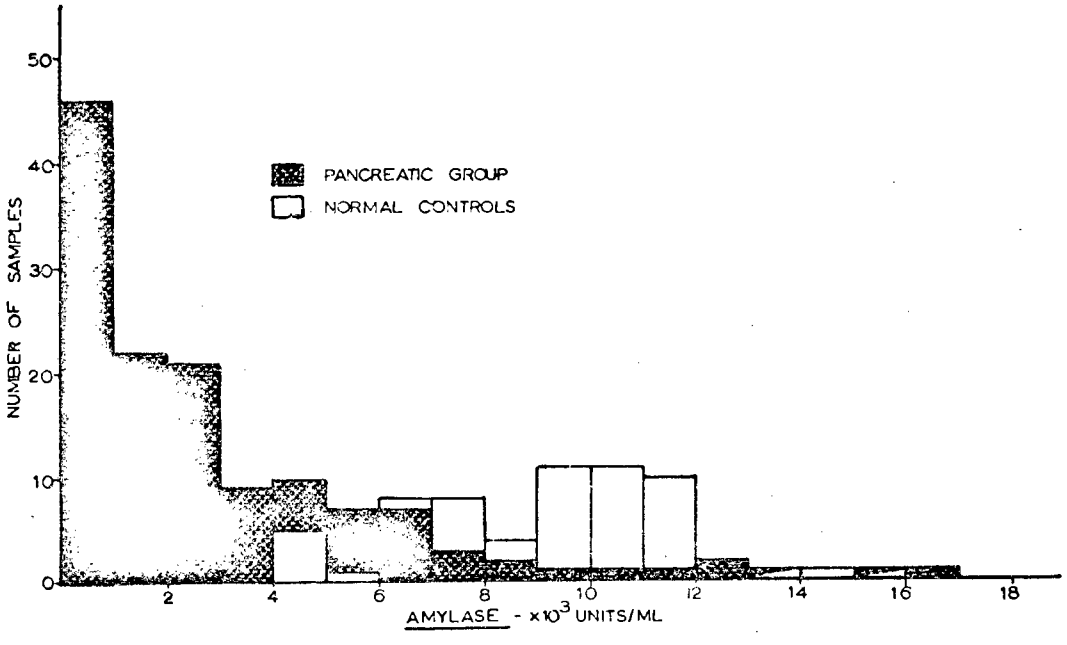

FIG.40: DISTRIBUTION OF AMYLASE CONCENTRATIONS
INDIVIDUAL 30 MINUTE SAMPLES

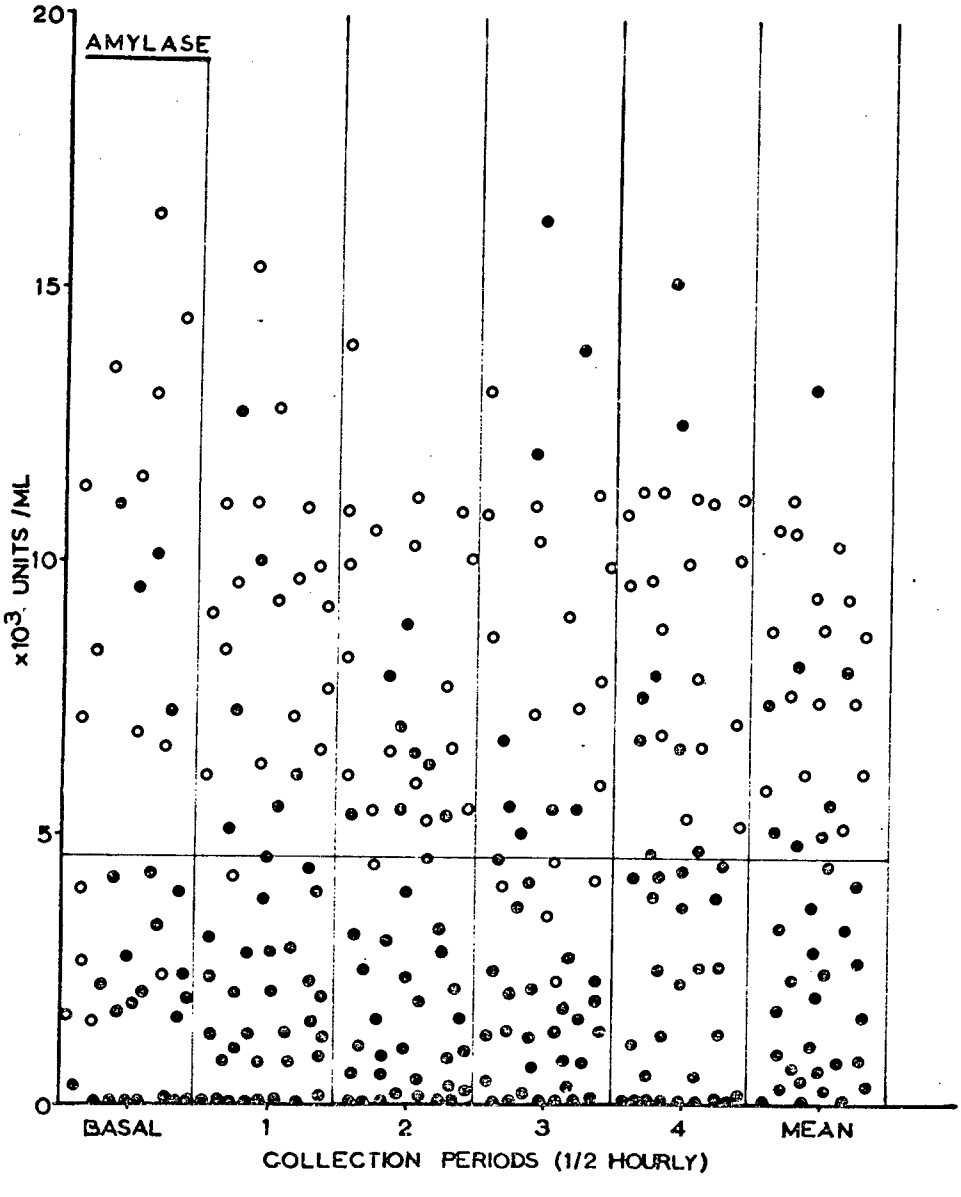

FIG. 41: AMYLASE CONCENTRATIONS – A COMPARISON BETWEEN NORMAL CONTROLS AND THE PANCREATIC GROUP

o = Normal Controls

● = Pancreatic Group

c) Chymotrypsin: The lower limit of normal for chymotrypsin in the 2 hour test was 450 units/ml. Borderline results may therefore be regarded as being between 400 to 500 units/ml. Sixty four percent of the patients with pancreatitis had abnormal chymotrypsin values. Eleven out of 31 patients had a chymotrypsin concentration in the normal range (Fig. 42). The difference between the normal controls and the pancreatic group was not significantly different ($P > 0.05$).

Only 65% of the individual samples in the pancreatic group (Fig. 43) were in the abnormal range. Not a single sample in the normal group had a chymotrypsin concentration below 500 units/ml. so that any value below this can be regarded as definitely abnormal. The highest chymotrypsin concentration was, again, in the one patient with calcific pancreatitis. Fig. 44 shows the distribution of the individual chymotrypsin concentrations in both normal controls and patients with pancreatic diseass.

Of those patients with abnormal chymotrypsin results for the 2 hour test, eight had normal chymotrypsin concentrations in the first sample aspirated. 11/31 + 8

d) Lipase: The lower limit of normal for lipase concentration in the 2 hour test was 350 I.U./litre. Any result between 300 to 400 I.U./litre may be regarded as being borderline. Ninety four percent of the patients with pancreatic disease had abnormal lipase results (Fig. 45). Only 1 our of the 17 patients with pancreatitis had a lipase concentration in the normal range but it should be stressed that the results were only available in 17 patients. Statistically, there was a very significant difference in the lipase concentration between the normal controls and the patients with pancreatic disease ($P < 0.01$).

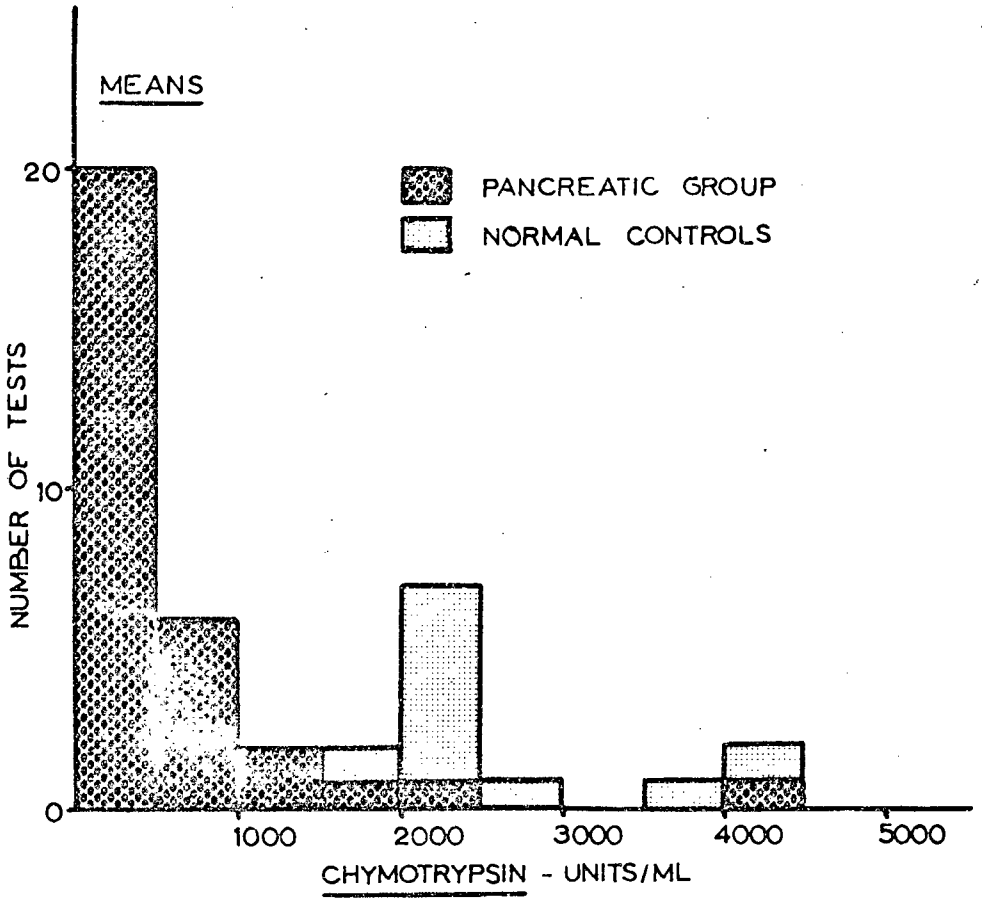

FIG. 42: DISTRIBUTION OF CHYMOTRYPSIN CONCENTRATIONS

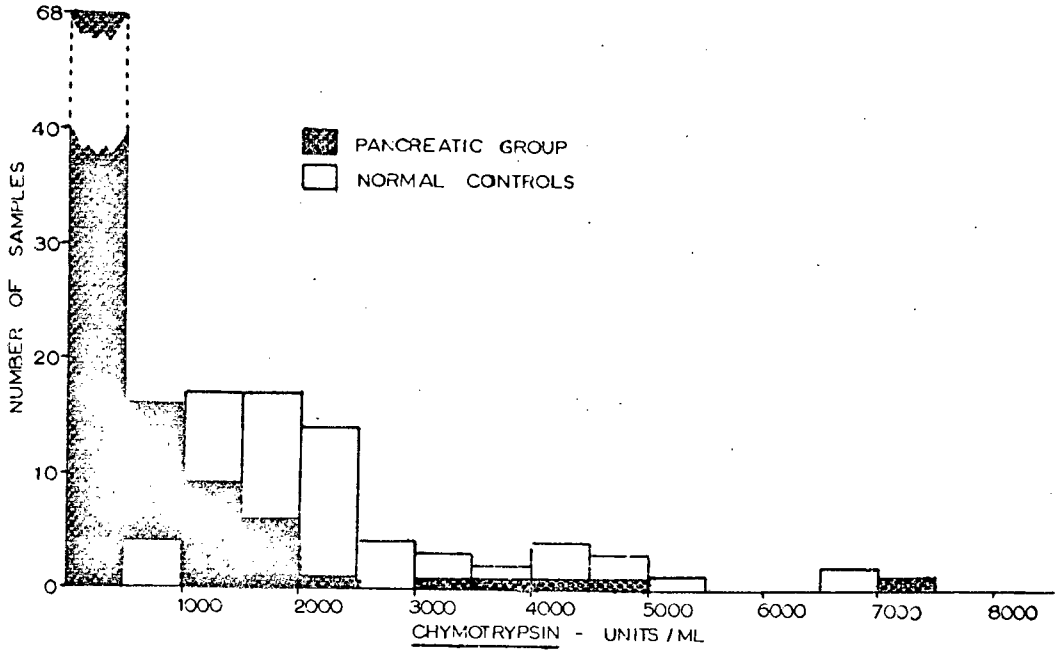

FIG. 43: DISTRIBUTION OF CHYMOTRYPSIN CONCENTRATIONS INDIVIDUAL 30 MINUTE SAMPLES

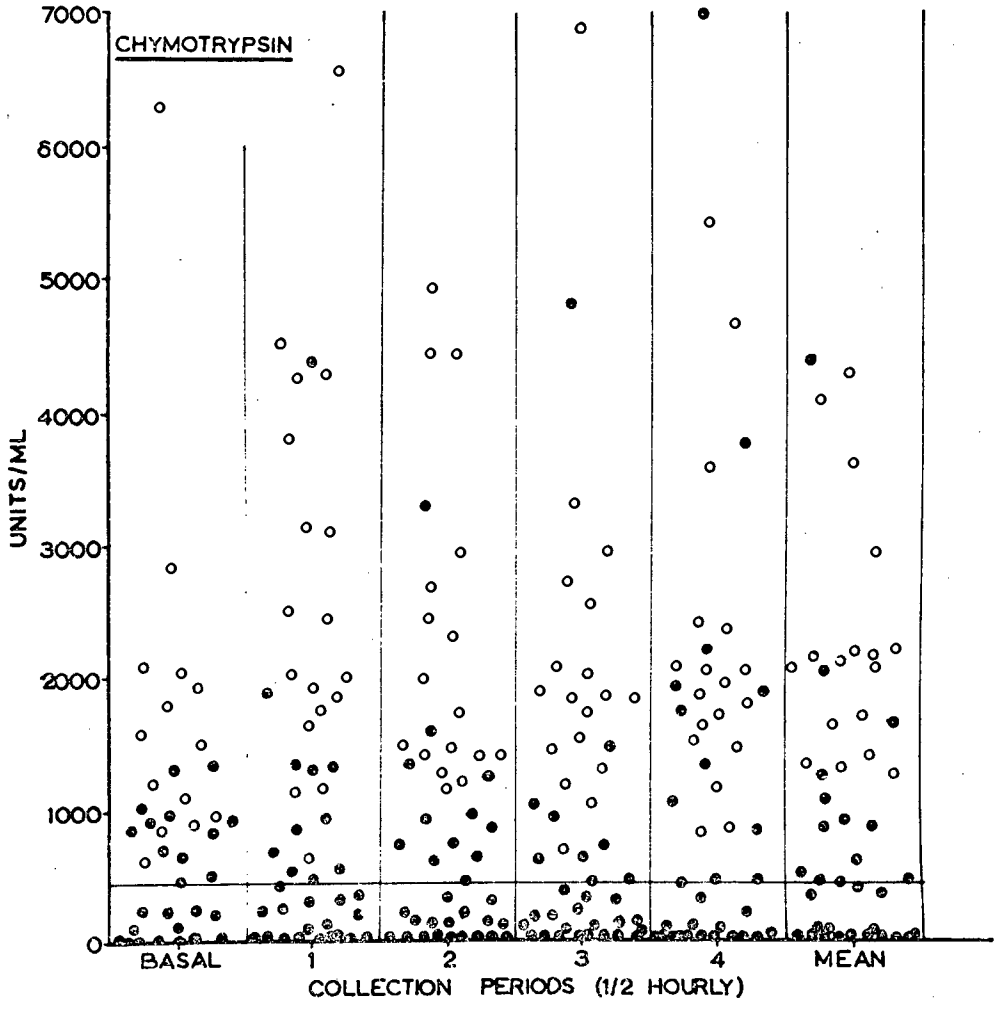

FIG. 44: CHYMOTRYPSIN CONCENTRATIONS – A COMPARISON BETWEEN NORMAL CONTROLS AND THE PANCREATIC GROUP

o = Normal Controls

• = Pancreatic Group

In the individual 30 minute samples a high percentage of the lipase concentration in the pancreatic group were in the abnormal range (92%)(Fig.46). All the very high lipase concentrations in the pancreatic group were from the one patient with calcific pancreatitis. Fig. 47 indicates the distribution of the individual 30 minute samples in both the normal control and pancreatic groups.

All the lipase concentrations in the first sample of aspirate were in the abnormal range in those patients with abnormal results for the 2 hour test.

B. CLINICAL NOTES

1. Acute pancreatitis: A test meal was performed on 4 patients shortly after an acute attack of pancreatitis. All these patients had abnormal test meal results.

Case 1 (Test 41): A 28 year old Coloured male who had been drinking alcohol for many years. The first attack of abdominal pain started 2 weeks before admission with severe exacerbation prior to admission on 4.7.68.

He was apyrexial and not jaundiced. The serum amylase was 300 units. Laparotomy performed on the day of admission showed evidence of early fat necrosis and a markedly swollen pancreas. The patients remained pyrexial for a week post-operatively and then recovered completely.

Test meal performed on 11.7. 68 showed a volume of aspirate which was the highest total volume recorded in any test. The pH of the samples were normal. The mean trypsin and amylase concentrations were in the abnormal range, the latter grossly abnormal. The mean

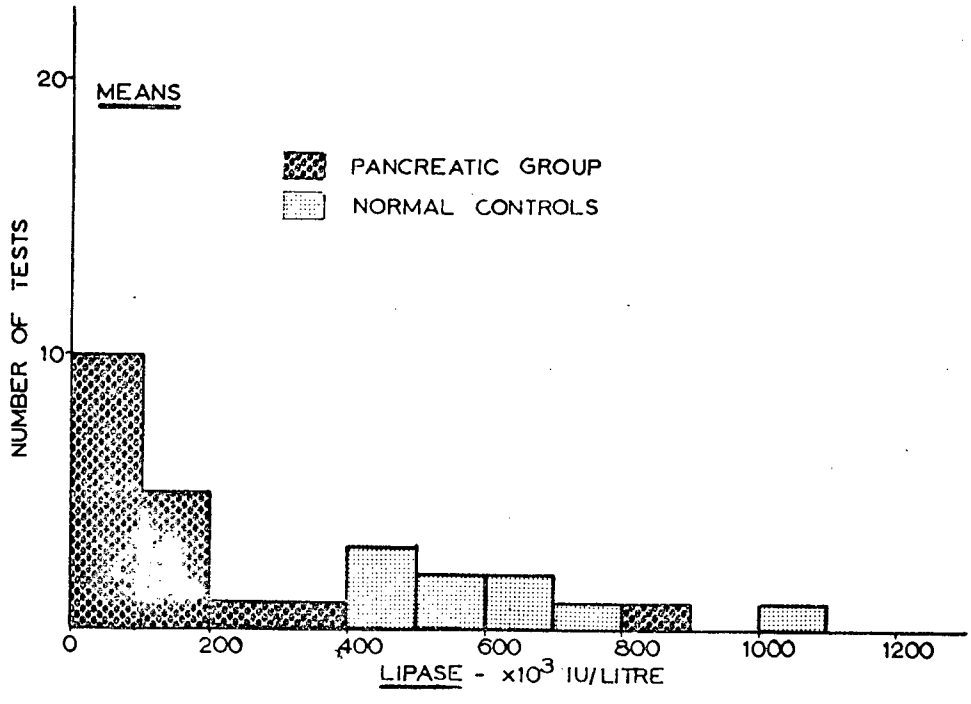

FIG. 45: DISTRIBUTION OF LIPASE CONCENTRATIONS

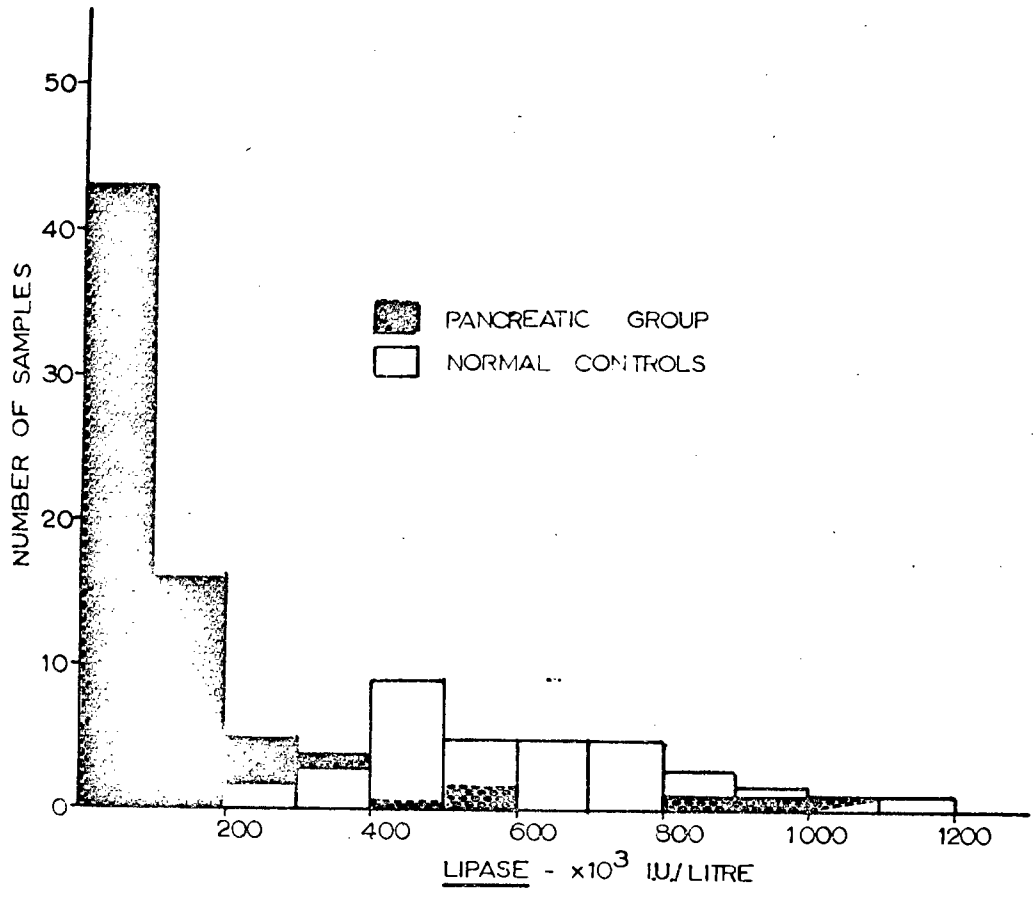

FIG. 46: DISTRIBUTION OF LIPASE CONCENTRATIONS
INDIVIDUAL 30 MINUTE SAMPLES

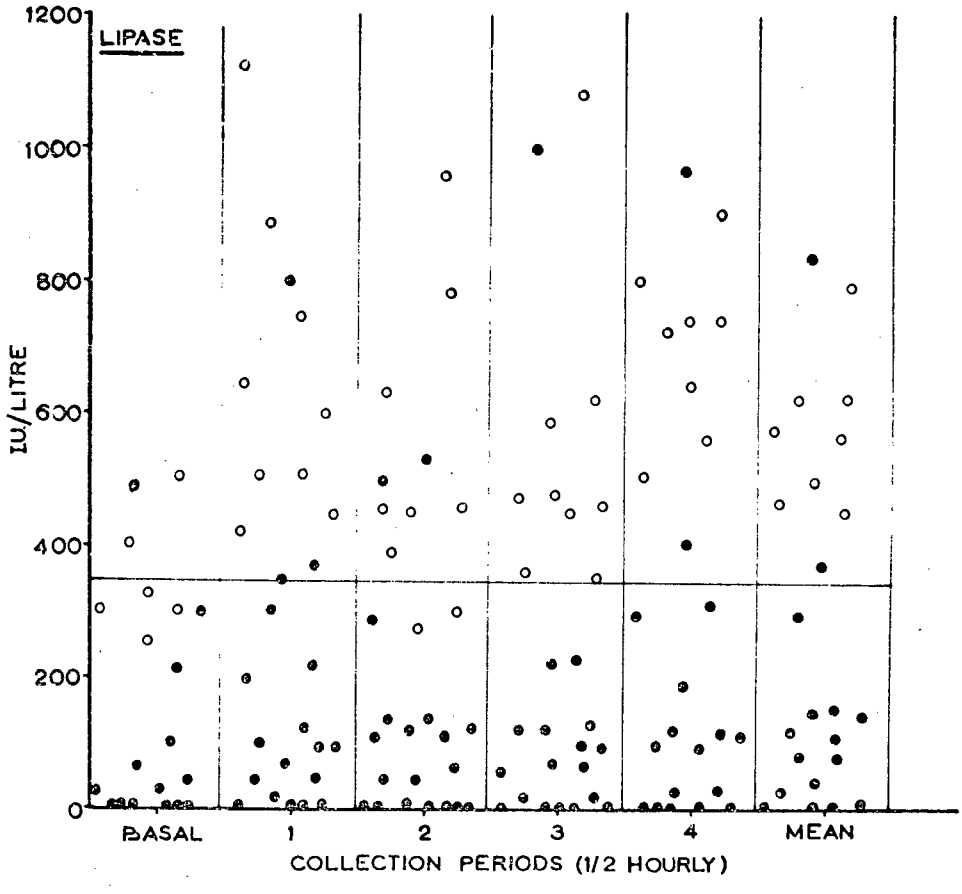

FIG. 47: LIPASE CONCENTRATIONS -
A COMPARISON BETWEEN NORMAL CONTROLS
AND THE PANCREATIC GROUP

o = Normal Controls

● = Pancreatic Group

chymotrypsin concentration was normal.

Comment: The very high volume of aspirate, together with normal pH values, suggests that the mechanisms of fluid and bicarbonate secretion had recovered first, following the attack of pancreatitis. Although the enzymes appeared to be secreted in parallel, they had obviously been affected to different degrees by the inflammatory process.

Case 2 (Test 54): A 24 year old coloured male suffered his first attack of pancreatitis in December, 1967. This was confirmed by laparotomy. He has had recurrent mild attacks of abdominal pain since that time.

The first test meal was performed on 30.7.68. The trypsin, amylase and chymotrypsin concentrations in the duodenal aspirate were normal. Laparotomy performed on 21.8.68 for a possible pancreatic cyst showed no evidence of a cyst, but the pancreas was of a firm consistency.

The patient's second severe attack of abdominal pain started on 27.12.68. The while blood cell count was 15,000/cu.mm. and the serum amylase was 160 units. A test meal performed on 6.1.69 showed a volume of aspirate which was 25% of that following the first test meal. The concentrations of trypsin , amylase and lipase was grossly abnormal with a borderline chymotrypsin result.

Comment: Although the first test was normal, a laparotomy performed seven months after the first acute attack showed no evidence of active disease although the pancreas was clearly abnormal. Performing the test meal soon after the first attack is obviously the preferred procedure to detect active inflammation in the pancreas.

Case 3 (Test 59): A 44 year old Coloured male was admitted severely ill on 25.1.69 with a serum amylase of 3,275 units. Laparotomy on 31.1.69 showed an oedematous and necrotic pancreas. A test meal on 15.2.69 was grossly abnormal with regard to the trypsin and amylase concentrations but the chymotrypsin concentrations was normal.

Comment: In the above case and Case 4 (Test 61), the test meal was performed 2 weeks after laparotomy confirmation of pancreatic disease, and was grossly abnormal in both cases.

Addendum: Since the completion of the graphs, a further test meal, performed on a patient 1 week after an acute attack of pancreatitis, showed all the enzymes to be well within the normal range.

2. Calcific pancreatitis (14 patients): All except two of the patients with calcific pancreatitis had grossly abnormal test meal results. These two exceptions had normal enzyme concentrations in the test meal (Test 82 and84) and in the secretin-pancreozymin test.

Case 1 (Test 82): A 42 year old European male, a chronic alcoholic of 10 years standing. He had never had an acute attack of pancreatitis. The concentration of amylase, trypsin and chymotrypsin following the ingestion of the test meal was the highest recorded enzyme concentration in any group. The mean lipase concentration was also very high.

Comment: The above results suggest that either hypersecretion of the remaining pancreatic tissue is occuring, or that hypertrophy a good regeneration has taken place in a patient with chronic pancreatitis and still drinking alcohol.

Case 2 (Test 84): The presence of calcification in the pancreas of a 35 year old Coloured female was an accidental finding during an investigation for abdominal pain. There was no other evidence of pancreatic disease in the history or clinical investigation. The test meal result showed a normal trypsin and chymotrypsin results were normal while the amylase concentration was borderline.

C. PATIENTS WITH GASTROINTESTINAL AND OTHER DISEASE WHICH MAY AFFECT THE PANCREAS *

Table 17 presents the means of the enzyme concentrations in the duodenal aspirate following stimulation with the standard test meal in 9 patients with various disorders.

1. There was a normal result for all enzymes in one subject (Test 66) some months after a vagotomy and pyloroplasty. The high volume is probably the result of increased rate of gastric emptying secondary to the pyloroplasty. Despite the large volume of the duodenal aspirate, the pH remained high, suggesting a good pancreatic output associated with an increased gastric emptying.

2. Two patients with idiopathic diabetes and diabetes secondary to haemochromatosis (Test 67 and 68) had normal test meal results.

3. The patient with infective hepatitis had a large volume of aspirate and normal enzyme concentrations.

4. The highest pH ever recorded in our series occurred in a patient with gastrointestinal lymphoma extending throughout his small bowel. His enzymes, although low, were within normal range (Test 71).

5. One patient was on a virtual starvation diet consisting of 8 egg whites per day for three months before the test was performed (Test 72).

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* See Appendix page 161 for individual sample results.

TEST NO.	MEAN pH	TOTAL VOLUME	TRYPSIN Units/ml	AMYLASE $x10^3$ Units/ml	CHYMOTRYPSIN Units/ml	LIPASE $x10^3$ I.U./litre	DIAGNOSIS
66	6.8	395	5112	8.29	1337	-	Vagotomy + Pyloroplasty
67	-	107	4658	9.27	1892	-	Diabetic G.T.T.
68	6.2	25	4349	11.18	2075	486	Haemachromatosis
69	6.4	254	4170	8.73	1832	377	Infectious Hepatitis
70	6.6	198	4081	10.93	2756	628	Porphyria
71	8	74	2727	6.31	1330	-	Gastro Intestinal Lymphoma
72	6.4	77	2413	1.30	1374	403	Starvation Diet
73	6.9	67	1587	8.11	546	216	Hypoalbuminaemia
74	6.7	103	1939	1.03	625	111	Hypoalbuminaemia
75	6.3	110	2174	1.52	870	79	Hypoalbuminaemia
76	6.4	120	3962	6.91	2206	469	Test 72 on Normal Diet (1 month)

Table 17: TEST MEAL PERFORMED ON PATIENTS WITH GASTRO INTESTINAL AND OTHER DISORDERS

He had lost about 120 lbs. in weight. His serum albumin was normal. His pancreatic enzymes showed an interesting dissociation. The amylase concentration was grossly abnormal, trypsin concentration was borderline while the chymotrypsin and lipase concentration was normal. The lipase concentration was even high compared to the other enzymes. One month after the patient had returned to a normal diet, the amylase, chymotrypsin, lipase and trypsin in the duodenal aspirate were all normal following a repeat test meal (Test 76).

6. Three test meals (Test 73, 74, 75) were performed on two patients with a serum albumin of 1.7 and 1.8 G% respectively. Both patients had protein-losing enteropathy secondary to tuberculosis of the abdominal lymph nodes. The pH of the duodenal aspirate was normal throughout the test. The concentration of the pancreatic enzymes showed a different pattern in each patient. In Test 73, the pancreatic amylase concentration was normal, while the lipase, trypsin and chymotrypsin concentrations were abnormally low. In Test 74, the concentration of lipase, trypsin and chymotrypsin were again low, but the concentration of amylase was very low. This result was almost exactly reproducible in a repeat test performed a few days later (Test 75).

D. SUMMARY OF RESULTS

1. The mean volume of the aspirate following the ingestion of the meal by the pancreatic group was higher than that found in the normal group.

2. The mean basal pH in the pancreatic group was slightly higher than that found in the normal group. Following the ingestion of the

meal, the mean pH remained above 6 in the majority of samples, even in patients with gross pancreatic insufficiency and/or highish gastric secretion.

3. Enzymes:

(a) Percentage of tests in pancreatic group with enzyme concentrations in abnormal or borderline range:

89% - Trypsin
81% - Amylase
64% - Chymotrypsin
94% - Lipase

(b) Number of tests in pancreatic group with a normal mean enzyme concentration:

4 out of 37 tests for Trypsin
7 " 37 " Amylase
11 " 31 " Chymotrypsin
1 " 17 " Lipase.

(c) The number of tests with a normal enzyme activity in the first 30 minute sample but an abnormal mean enzyme activity for the four 30 minute collections:

2 out of 33 tests for Trypsin
3 " 33 " Amylase
8 " 29 " Chymotrypsin
0 " 15 " Lipase.

(d) Normal test meal results were obtained in patients with ideopathic diabetes, diabetes secondary to haemochromatosis, infectious hepatitis, lymphoma of small bowel and in a patient following vagotomy and pyloroplasty. Abnormal test meal results

were obtained in two patients with very low serum albumin and in a patient on a starvation diet for three months.

E. COMMENT

The patients with pancreatic disease tended to have a higher volume of aspirate than the normal subjects. The reason for this is not readily apparent. Pancreatic secretion is reported to be decreased in patients with pancreatic disease. A large amount of bicarbonate must, therefore, be coming from extra-pancreatic sources in view of the very remarkable stability of the duodenal pH, even in patients with gross pancreatic disease. It can perhaps be speculated, that this extra-pancreatic bicarbonate, together with a large volume of fluid, is secreted by the duodenum and possibly by the liver, in compensation for a decreased bicarbonate content and volume of fluid from the pancreas. This could explain the maintenance of the duodenal pH above 6.0 in the pancreatic group.

The test meal was performed on 7 patients who had suffered an acute attack of pancreatitis. ~~Seven~~ 5 of these patients had abnormal results, and in all these cases the test meal was performed within 3 weeks of the onset of the attack. The ~~one~~ 2 patient with a normal test meal result had the test 1 week 3/12 after his acute attack. These results suggest that the test meal may be very useful in the investigation of acute abdominal pain, if performed as soon as the patient is able to swallow fluids. Positive results may be obtained weeks later, but the diagnostic value will obviously decrease with time as pancreatic tissue regenerates.

All the patients (except two) with chronic pancreatitis (calcific and others) had gross depletion of all the enzymes estimated.

The two exceptions both had calcification present in the pancreas. The very high concentrations of enzymes present in the one patients suggests that even a chronically damaged pancreas is able to regenerate quite actively, and that a certain degree of overcompensation may take place. Sun (376) noted hypersecretion in chronic alcoholics without abdominal pain, and considered this abnormal.

A total of 5 of the 38 patients with pancreatic disease had normal test meal results. One of the ~~seven~~ 5 had suffered an acute attack a few months before the test meal was performed. All other tests, including a secretin-pancreozymin test, were also normal and it is assumed, therefore, that pancreatic function had returned to normality by all available criteria of assessing pancreatic function. Only 2 of the others with normal test meal results had active pancreatic inflammation (Test 79, 83). It should be noted, however, that Test 79 had a border line trypsin concentration in the first sample while in Test 83, the lipase concentration in the first sample was borderline. As indicated in the Results (Part 1), a single 30 minute collection may be sufficient as a screening test in assessing for pancreatic disease. In all the tests performed where the mean enzyme concentrations were abnormal, the first 30 minute collection had normal enzyme activity for trypsin in 2 tests, for amylase in 3 tests, for chymotrypsin in 8 tests and none for lipase. Using a 30 minute collection, therefore, will pick out 94% of those cases in which the complete two hour test would show an abnormal result in the case of trypsin, 91% in the case of amylase, 76% in the case of chymotrypsin and 100% in the case of lipase. For screening purposes, lipase and trypsin would seem to be the most reliable enzymes. When the full 2 hour test is performed, lipase and trypsin again are more reliable than the other two enzymes.

In general, the enzyme concentrations in the pancreatic group

tended to follow the same pattern of parallel secretion as in the normal subjects. With a few exceptions, all four enzymes were depressed in the group of patients with abnormal test meals. Only amylase showed more than one exception, in which the concentration of enzymes were normal. It is difficult to know how much importance should be attached to these exceptions. Experimental error is almost certainly a cause for some of these inconsistencies. The impression is, therefore, that the enzymes are secreted in parallel fashion in the pancreatic group of patients. Pancreatitis, therefore, would appear to depress the synthesis of all enzymes equally. The very definite dissociation between the enzymes, that occurred in the patients with the low protein states and the one patient on a starvation diet, do not contradict this argument. The dissociation indicates that the mechanism for the synthesis of each enzyme may be affected differentially, the limiting factor probably being the different amino-acid requirements for each enzyme.

F. DISCUSSION

The results show that the test meal is useful diagnostically in differentiating between patients with and without pancreatic disease. Although the test meal results were normal in a few patients who were known to have pancreatic disease at the time of the test, or some time prior to the test, this was not a frequent finding. In these few patients other tests of pancreatic function were nearly always normal as well.

A few other reports are present in the literature which compare the test meal in normal and abnormal subjects. Lund (253) also found the test meal to be useful in following a patient's progress

after an attack of pancreatitis and noted that the test meal was abnormal in 6 patients with carcinoma of the head of the pancreas. Ventzke et al (403) reported an abnormal test meal, in all his cases with carcinoma of the pancreas and chronic and calcific pancreatitis, while Cook et al (34) reported one patient with a normal test meal result despite pancreatic calcification. In our series, there were two patients with pancreatic calcification and a normal test meal. Cooke felt that the test meal was particularly useful in differentiating between pancreatic and other types of steatorrhoea. He also recorded abnormal results in 12 our of 13 patients with carcinoma of the pancreas. Besides finding the test meal results abnormal in the majority of patients with chronic pancreatitis and carcinoma of the pancreas, Thaysen et al (382) reported abnormal test meal results in some patients with primary malabsorption, duodenal ulcer, subtotal gastrectomy and patients with hepatic and biliary disease. Worning et al (438), found abnormal enzyme concentrations in all cases of chronic pancreatitis, except one, while 25% of patients with carcinoma of the pancreas had normal enzyme concentrations in all collections.

The functional reserve of the pancreas is great and as the zymogen granules discharge their contents intermittently and not synchronously, it will require a fair amount of distruction of pancreatic tissue before this becomes obvious in the form of decreased enzyme concentration in the duodenal aspirate. In a review of pancreatic gland regeneration, Tiscornia and Dreiling state "that the pancreas has a remarkable ability to regenerate its parenchyma and to recover its exocrine excretory capacity". The test meal, like the secretin-pancreozymin test, only becomes abnormal when a fair degree of destruction of pancreatic tissue has taken place.

It would seem from our results and the work reported above, therefore,

that the test meal is useful in diagnosing malfunction of the pancreas due to chronic pancreatitis, cases of acute pancreatitis and pancreatic carcinoma. It has been used in our series and in the work of Lund (253) to follow the progress of acute pancreatitis. The test meal procedure may be of particular interest in patients with post-gastrectomy syndromes where the gastro-duodeno-pancreatic mechanism is disordered.

An abnormal test meal result, in patients without pancreatic disease was obtained only in those cases where the pH in the aspirate was so low, that enzyme concentrations were suppressed, or when protein metabolism was disturbed. This agrees with the work of Worning et al (437) who measured the concentration of amylase, trypsin, lipase and chymotrypsin in the duodenal juice following a test meal in patients with various intestinal disorders and found that the mean concentrations of pancreatic enzymes were decreased in the majority of the collections. They felt that protein deficiency was the common factor in all their cases and this was, therefore, responsible for the abnormal enzyme results. In their cases, lipase was most markedly reduced, while amylase was more reduced than the proteolytic enzymes. Worning et al (437) and other authors (393), have noted the dissociation that occurs in the enzyme secretion from the pancreas in protein deficiency states. Veghelyi et al (402) have reported that the enzymes tend to disappear in a specific order; lipase first, followed by trypsin, then by amylase. Two patients with protein deficiency states and a third who was on a starvation diet for three months are recorded in our series. In two out of three patients amylase was the enzyme most severely affected and in the third patient amylase was within the normal range while the other enzymes were abnormally low. The patient on a starvation diet had abnormally low amylase and trypsin concentrations in the duodenal

aspirate while the lipase and chymotrypsin concentrations were normal. The dissociation of the pancreatic enzymes which occurs in protein deficiency states (393) and the degree to which the enzymes are affected, may be due to the type of amino-acids deficiency which has occurred in that patient (185). Of all the organs in the body, the pancreas has the highest uptake of amino-acid per gram of tissue weight (62), so that it is not surprising that protein deficiency states should adversely affect the function of this organ.

Chapter 12

A COMPARISON BETWEEN THE STANDARD TEST MEAL AND THE SECRETIN-PANCREOZYMIN TEST FOR ASSESSING PANCREATIC FUNCTION

RESULTS PART 4

Chapter 12 A COMPARISON BETWEEN THE STANDARD TEST MEAL AND THE SECRETIN-PANCREOZYMIN TEST FOR ASSESSING PANCREATIC FUNCTION

Tables 18 and 19 show the mean trypsin, amylase, chymotrypsin and lipase concentrations in the duodenal aspirate following the test meal and during the secretin-pancreozymin test in the normal control and pancreatic groups. The enzyme concentrations in the duodenal aspirate following the secretin injection and then the pancreozymin injections are shown separately, and the final column indicates the mean of both results.

1. Trypsin:

(a) Normal controls: The mean trypsin concentration in the aspirate following the meal stimulus is slightly higher than that following the pancreozymin injection, and considerably higher than that following the secretin injection. Statistically, however, these differences were found not to be significant ($P > 0.05$). Inspection of Table 20 indicates that there were only 2 trypsin results in the abnormal range, and both followed the secretin injection.

(b) Pancreatic group: In the abnormal group, the pancreozymin injection appeared to stimulate a higher trypsin output than the test meal, but this is not a significant increase ($P > 0.05$). There is also no significant difference in the trypsin concentration when the test meal results are compared with the results following a secretin injection ($P > 0.05$).

(c) Disagreement between tests: Tests 41, 44, 59 and 61 show

STIMULUS	TRYPSIN Units/ml	AMYLASE $x10^3$ Units/ml	CHYMOTRYPSIN Units/ml	LIPASE $x10^3$ I.U./litre
Test Meal	4704	9.86	2341	627
Secretin(S)	3170	10.83	2758	498
Pancreozymin(P)	4611	10.47	3644	693
S & P	3799	10.64	3139	595
P Value	> 0.05	> 0.05	> 0.05	> 0.05

Table 18: NORMAL CONTROL GROUP (MEANS)

STIMULUS	TRYPSIN Units/ml	AMYLASE $x10^3$ Units/ml	CHYMOTRYPSIN Units/ml	LIPASE $x10^3$ I.U./litre +
Test Meal	1837	3.83	833	-
Secretin(S)	1600	4.87	1099	-
Pancreozymin(P)	2781	5.44	1762	-
S & P	2213	4.90	1470	-
P Value	> 0.05	> 0.05	< 0.01	-

Table 19: PANCREATIC GROUP (MEANS)

+ Insufficient data

TABLE 20

CONTROL SUBJECTS (6)

Date of Test	Test	TRYPSIN Units/ml				AMYLASE x10^3 Units / ml				CHYMOTRYPSIN Units/ml				LIPASE I. U. /litre				BICARBONATE M. Equiv/litre			VOLUME ml	
		TM	S	P	S+P	TM	S	P	S+P	TM	S	P	S+P	TM	S	P	S+P	S	P	S+P	TM	S+P
20. 2.68 16. 2.68	70	5831	5991	5516	5753	11.87	20.34	14.06	17.20	2382	4908	4233	4570					30	47	38	49	31
3.10.68 1.10.68	11	4018	3708	6083	4895	10.71	10.26	10.61	10.43	2185	3380	4533	3950	797	682	870	776	70	57	63	29	119
5. 6.68 4. 6.68	6	5462	3291	3733	3472	11.20	11.36	10.67	11.13	1732	3174	3700	3349					83	74	80	311	160
1. 7.68. 3. 7.68	8	4526	1401	4450	2417	10.31	10.05	11.05	10.38	2143	1469	3183	2047					103	81	96	194	390
8. 6.68 9. 6.68	9	4045	2583	3583	3085	8.91	8.42	9.27	8.84	1594	1350	2266	1808					97	78	87	35	233
3.12.68 7.12.68	16	4343	2043	4300	3171	6.18	4.55	7.16	5.85	4010	2262	3950	3106	457	313	515	412	81	65	73	106	220

Top Date = date of test meal
Bottom date = date of S + P test
T = Test Meal
S = Secretion
P = Pancreozymin

TABLE 21

PANCREATIC GROUP

Date of Test	Test	TRYPSIN Units/ml				AMYLASE x10^3 Units / ml				CHYMOTRYPSIN Units/ml				LIPASE I.U./litre				BICARBONATE M. Equiv./litre			VOLUME ml	
		TM	S	P	S+P	TM	S	P	S+P	TM	S	P	S+P	TM	S	P	S+P	S	P	S+P	TM	S+P
13. 2.68 25. 7.68	33	1239	2100	1458	1886	2.41	5.03	3.33	4.46	816	1416	1084	1305					39	14	26	115	52
28. 3.68 12.12.67	36	446	995	935	975	1.61	0.69	0.50	0.63	398	1076	805	986					22	28	24	355	54
6. 5.68 4. 5.68	37	670	1539	1442	1490	0.37	7.85	9.41	8.63	234	1408	1162	1285					79	81	80	164	156
16. 7.68 9. 7.68	39	497	712	1048	824	1.78	4.67	5.48	4.95	68	274	521	535					57	35	50	48	179
11. 7.68 22. 7.68	41	1775	2078	2750	2414	2.27	4.02	6.07	5.04	1251	1825	2125	1975					70	64	67	675	250
18. 7.68 17. 7.68	42	275	1929	-	-	4.95	7.10	-	-	0	1166	-	-					16	22	19	37	24
30. 7.68 25. 7.68	43	123	2100	1459	1886	0.45	0.63	0.07	0.44	123	1416	1084	1305					13	19	15	13	48
5. 8. 68 6. 8.68	44	888	2750	4616	3322	0.56	3.31	5.49	4.04	348	1600	1816	1672					62	63	62	86	114
12. 8.68 14. 8.68	45	1005	1272	1495	1347	3.71	2.31	4.70	3.11	495	955	1066	992					36	39	37	109	114
26. 8.68 25. 8.68	47	833	1308	1656	1482	0.80	0.76	1.01	0.88	433	731	1089	911					54	45	49	265	123
17. 9.68 5. 9.68	49	295	176	270	208	0.93	1.95	0.61	1.51	15	256	261	258					37	39	38	283	95
26.11.68 18.11.68	51	1128	1933	2116	1994	4.25	3.97	6.42	4.79	505	1436	1750	1541		130	167	198	39	31	37	588	126
16. 1.69 12. 1.69	56	0	112	-	-	0	0.69	-	-	0	0	-	-					19	-	-	190	82
15. 2.69 17. 2.69	59	1477	2120	3236	2678	2.59	6.80	3.72	5.71	607	1474	1966	1720		453	531	492	66	39	52	53	152
27. 2.69 13. 3.69	61	300	1462	2391	1926	5.66	4.50	3.40	3.95	136	620	1483	1051	109	250	305	277	71	39	55	65	198
11. 4.68 7. 2.68	78	2833	1403	-	-	8.81	5.92	-	-	1322	1175	-	-					57	-	-	139	197
3. 7.68 2. 7.68	79	4770	1536	5660	3598	9.17	4.00	6.97	5.48	2167	1157	3716	2436					84	61	72	92	177
28.11.68 25.11.68	82	8406	1635	4966	3300	13.29	14.83	18.05	16.44	4400	1170	3300	2235	841				63	46	54	98	173
8. 2.69 10. 2.69	83	4300	1741	-	-	8.14	13.47	-	-					372	187			63	-	-	426	46
15. 2.69 14. 2.69	84	4512	2225	6600	4412	4.83	4.91	11.88	8.39	2051	866	2566	1716					78	92	85	24	241

Top Date = Date of Test Meal
Bottom Date = Date of S+P test
T = Test Meal
S = Secretion
P = Pancreozymin

important differences (Table 21). Case 44 had a low trypsin concentration in the aspirate following the test meal as compared to the trypsin concentration following an injection of secretin or pancreozymin when the results were normal. This patient had chronic relapsing pancreatitis, a definite history of heavy alcoholic intake and moderately severe diabetes. Tests 41, 59 and 61 were all performed on patients who were admitted into hospital for acute attacks of pancreatitis. The trypsin concentration in the aspirates following the test meal were definitely abnormal in all three cases, while they were borderline in Tests 41 and 61 and normal in Test 59 following the secretin-pancreozymin test. It is only fair to mention, however, that the test meal was performed as soon as the patient was able to swallow liquids following the onset of an acute attack of pancreatitis, while the secretin-pancreozymin test was performed a few days later when pancreatic function may have improved. Case 59 became diabetic following his attack, and was still fairly ill at the time both tests were performed. The trypsin concentrations in Tests 82, 79, 83 and 84 were normal following stimulation with the test meal and pancreozymin injection, but abnormally low following the secretin injection.

2. Amylase:

(a) Normal controls: The amylase concentration using any one of the tests was very similar ($P > 0.05$). The test meal gave a slightly lower mean amylase concentration than secretin or pancreozymin (Table 19). The higher mean amylase concentration in the aspirate following secretin was an unexpected finding.

Only 1 abnormal amylase result was recorded in the normal group and this followed secretin stimulation.

(b) Pancreatic group: As with the results in normal patients, there was no statistical difference in the amylase concentration following a test meal or one of the other methods of stimulation ($P > 0.05$). The highest mean amylase concentration followed the pancreozymin injection, while the test meal showed the poorest response.

(c) Disagreement between tests: Tests 39 and 44 (Table 21) produced very low amylase concentrations in the aspirate following a test meal, while the results following the pancreozymin injections were borderline or even normal as in Test 51, 37 and 84. The latter three tests all had abnormally low enzymes following the test meal.

3. Chymotrypsin:

(a) Normal controls: Statistically there was no difference between the different tests ($P > 0.05$), although the mean chymotrypsin concentration in the duodenal aspirate following the pancreozymin injection was considerably higher than that following the test meal. Secretin alone also stimulated a higher chymotrypsin concentration than the test meal.

(b) Pancreatic group: Chymotrypsin was the only enzyme to show a significant difference between tests, and only in the abnormal group of patients. The chymotrypsin concentration following the pancreozymin injection was significantly higher than that following the test meal. ($P < 0.01$). As in the normal group,

secretin also stimulated a higher concentration than the test meal.

(c) Disagreement between tests: As shown in Results (Part 1), the chymotrypsin activity falls dramatically from pH 7.0 to 6.0, and as this is the difference between the pH of the relatively pure pancreatic juice obtained following the secretin-pancreozymin injection and that of the duodenal aspirate following the test meal, little significance can be placed on any disagreement between these tests.

4. Lipase:

(a) Normal controls: A comparison was possible in only 2 subjects. Statistically there was no difference between the tests ($P > 0.05$). The test meal and pancreozymin injection showed similar mean lipase concentrations in the duodenal aspirate.

(b) Pancreatic group: In the one patient in whom lipase estimations were performed in all tests, the test meal response was definitely abnormal, and although the pancreozynin response was almost three times as high, the lipase was still in the abnormal range (Table 21).

5. Test meal versus bicarbonate concentrations (Tables 20 and 21):

Case 44, with abnormally low trypsin, amylase and chymotrypsin concentrations following the test meal had normal bicarbonate concentrations in the duodenal aspirate following secretin and pancreozymin injections. This patient suffered

from chronic relapsing pancreatitis and was diabetic as a result of his pancreatic disease. Of the patients with abnormal test meal results following an acute attack of pancreatitis, Case 41, 59 and 61 all had normal bicarbonate concentrations following secretin injection, while the latter two cases had abnormal concentrations following the pancreozymin injection. Case 37, with chronic calcific pancreatitis, had abnormal trypsin, amylase and chymotrypsin concentrations following the test meal, but normal bicarbonate concentrations following secretin and pancreozymin.

In the normal control group, Case 70 had normal enzyme concentrations following the standard test meal and secretin-pancreozymin test, but grossly depleted bicarbonate concentrations following the latter test.

SUMMARY

No statistical difference was found between the test meal, secretin, pancreozymin or secretin-pancreozymin tests with regard to trypsin, amylase and lipase in normal subjects or patients with pancreatitis.

In the pancreatic group, there was a statistically significant difference between the tests with regard to chymotrypsin concentrations. ($P < 0.01$) This was not present in the normal group. Examination of the individual results showed that the enzyme concentrations following the secretin injections were often low in the normal controls. It also showed that the test meal often gave a better indication of the physiological state of the pancreas than secretin and/or pancreozumin when comparing the trypsin, amylase or bicarbonate concentrations in the duodenal aspirate following any of these stimuli.

COMMENT

Although there was little statistical difference between the various tests, individual results showed the test meal to be as good a parameter, or indeed superior to any of the tests using hormonal injections in assessing pancreatic function. The fact that chymotrypsin was the only enzyme to show a statistical difference between the tests is interesting, and would support our results concerning the pH sensitivity of this enzyme. As indicated in the Results (Part 1), there is a marked fall in chymotrypsin activity between pH 7.0 to 6.0. As the pH of the duodenal juice is usually about 7.0, compared to the lower pH following the test meal, this difference would not be surprising.

It may be concluded that the test meal is a relatively simple, reliable and accurate measurement of pancreatic function and has a diagnostic value equal, and possibly even superior, to the tests in current usage with hormonal stimulation. It would certainly seem to be more physiological than the injection of arbitrary amounts of hormones.

DISCUSSION

There are few reports available comparing the secretin-pancreozymin test with the test meal. Hartely et al (179) reported that the augmented secretin test was more reliable than the test meal in diagnosing pancreatic disease and differentiating between chronic pancreatitis and pancreatic carcinoma. Using the secretin test, they found the peak or total bicarbonate output, maximal bicarbonate output, and volume output per kilogram body weight to give more

consistent results than enzyme concentration or output. These authors used four 20 minute collections following ingestion of the test meal. Zieve et al (444), using a 2 hour test, found the test meal to be a more potent stimulus than the secretin-pancreozymin test, although they noted that the latter test may be of special use in diagnosing pancreatic carcinoma where the only abnormality may be a decreased volume of pancreatic secretion. Our results agree essentially with those of Zieve. In the individual cases, the test meal gave a better indication of the patients' pancreatic state than did the secretin-pancreozymin test. The secretin test is still the most popular single test in the measurement of pancreatic function, and the maximum bicarbonate concentration (112, 184) the most reliable single investigation. There are a large number of reasons, however, why any measurement of bicarbonate, whether it be maximum or mean concentration or total bicarbonate output, does not have a very sound basis:

1. The bicarbonate content of the duodenal aspirate during the secretin test is derived from a number of sources (198, 430). The greatest percentage is derived from the pancreatic secretions, but bicarbonate is also present in bile and succus entericus. The choleretic action of secretin is slight but definite in human subjects (161) and dogs (213). In the dog, following an intravenous injection of secretin, the concentration of bicarbonate in the bile may approach 60 mEq/litre compared to the maximum bicarbonate concentration in the pancreatic juice of about 150 mEq/litre. Cholecystectomized subjects with T-tubes in the common bile duct were found to have an average of 46.3 mEq/litre of bicarbonate in the drained fluid following an injection of secretin (407). In a pouch of the duodenum containing a large number of Brunner's glands, the bicarbonate concentration following stimulation with secretin may reach 42 mEq/litre (82). Lagerlof et al (239)

found, in experiments in man, that the bicarbonate concentration in combined enteric and hepatic secretions was on the average about 40 mEq/litre following secretin injection and the volum 50 ml. In the grossly abnormal patient, or in subjects with normal pancreata, this additional bicarbonate will not make a substantial difference to the interpretation of the results. In the borderline cases, however, this extra pancreatic bicarbonate may invalidate the results obtained with the secretin test.

2. Acid contamination from the stomach (427) must occur to some extent in all subjects during the secretin test; tubes draining the stomach become blocked, technicians are not constantly attentive, retching occurs in the occasional patient, or the holes in the gastric portion of the tube may be inadequately placed to drain all the gastric secretion.

3. Recently, interest has been focused on the maximum bicarbonate pancreatic secretion (240, 431) as it is apparent that the dose used by most early investigators was submaximal (74, 106, 112, 237, 427). Increasing the dose of secretin resulted in an increase in the bicarbonate output and concentration. Wormsley (431) noted that the dose of secretin required to give maximal bicarbonate response in the majority of subjects was 10 units/Kg/hour of Boots secretin. A single intravenous dose of 2 units/Kg. produced a response which was just over half of that produced by the constant infusion. In a few subjects, 25 units/Kg/hour was required to produce a maximal bicarbonate response. These results served to illustrate the fact that in almost all recorded secretin test series, submaximal doses of secretin have been used, and that for each individual subject, the

degree of stimulus provided by the particular dose of secretin used will vary.

4. Although Dreiling and Janowitz (112) believed that drainage procedures with a tube in the duodenum produced quantitative aspirations of duodenal contents, this has not been substantiated by most other authors (81, 382, 435, 444). These authors have shown that an average of only about 30% of the duodenal contents is aspirated. Thus, total bicarbonate output can have little value unless corrected for this loss (240), which has not been calculated in most reported series.

5. Secretin has not been standardised, so that differences in strength occur not only between batches, but also between different makes of secretin. (374). Some of the preparations may also contain traces of other intestinal hormones.

6. Transmural fluxes of bicarbonate in the upper small bowel were reported by Swallow and Code (380). This is not likely, however, to influence the results of the secretin test.

All the above factors, while perhaps not being of much importance when the results are grossly abnormal or very obviously normal, will make the interpretation of borderline cases extremely difficult, and will in some cases convert an abnormal test into a normal result and vise versa. Enzyme estimations in the secretin test do not add substantially, if at all, to the diagnostic significance of the test (60). Pancreozymin, besides having a number of unpleasant side effects, adds a substantial amount of alkaline bile as a result of contraction of the gallbladder.

The test meal, therefore, appears to have a number of distinct

advantages over that of the secretin-pancreozymin test:

1. Only one thin tube is required.

2. The test may be performed as soon as the patient is able to swallow liquids.

3. No side effects occur as have been reported with secretin (431), and, especially, with pancreozymin (178).

4. The patient feels more comfortable as he has been given a flavoured drink and may sit in any position and even move around on the bed.

5. If necessary, the test may be shortened to half an hour only.

6. As only trypsin needs to be measured, the laboratory work is cut down to a minimum.

7. As only the concentration of the enzymes is being measured, and not the quantity, a complete aspiration of all the duodenal contents is not necessary. This means that the test meal requires very much less attention and does not need the attention of a doctor in administering the intravenous injections required in the secretin-pancreozymin test.

8. The cost of the test meal is substantially less.

CONCLUSION

Attention has been drawn to a number of fallacies in the secretin test, especially with regard to bicarbonate estimations. Enzyme estimations and the addition of an injection of pancreozymin does

not add substantially to the diagnostic significance of the test. The test meal has a number of advantages over that of the secretin test; it is cheaper, requiring less attention and fewer laboratory procedures, has no side effects, is more comfortable for the patient and the results are equally, if not more reliable than the secretin test with or without pancreozymin.

GENERAL CONCLUSIONS

1. The 2 hour test meal procedure as described under methodology is a very useful, if not the best, method for investigating the physiological exocrine response of the pancreas to food.

2. Eight 15 minute samples are recommended for physiological investigations as this period of time incorporates both enzyme peaks.

3. The following procedure is suggested as a test for abnormal pancreatic function:

   (a) A single 15 or 30 minute sample after the ingestion of the meal may be used as a screening test.

   (b) If the above is found to be grossly abnormal or obviously within normal limits, no further samples are necessary. Borderline, or inconclusive enzyme responses, in the first 15 to 30 minutes necessitates completing the full test for the 2 hour period.

4. Trypsin and lipase were found to be the most reliable of the four pancreatic enzymes to measure.

5. The test meal should consist of fat, and protein for good enzyme stimulation. Carbohydrate should also be added to stimulate a good volume of secretion. A high constant osmolarity is probably also important.

6. The results obtained support the view that (a) all four enzymes are secreted in parallel, (b) parallel depression of the enzymes occurs in pancreatic disease and (c) there is no acute adaptation of the enzymes to the type of food ingested.

APPENDIX.

APPENDIX

I PANCREATIC AMYLASE

REAGENTS:

1. Stock Starch (Analar) 2%

   Weigh 2.000 g. soluble starch accurately.

   Weigh 2.0 g. NaCl roughly.

   (a) Dissolve starch as best as possible in about 60 ml distilled water.

   (b) Put on a low flame and stirring all the time bring slowly to the boil. (Takes about 15 minutes.)

   (c) Now add 2 g. NaCl continuing to stir. Be careful it does not boil over when NaCl is added.

   (d) Pour into a 100 ml. volumetric flask, rinsing container over and over with freshly boiled distilled water immediately, so that 'skin' does not form.

   (e) Fill flask to just below 100 ml. mark. Allow to cool.

   (f) Add 5 drops toluene.

   (g) Fill to 100 ml. mark.

   KEEP IN FRIDGE.

2. Buffer 0.1M pH 7.0

   (a) $Na\ H_2\ PO_4\ 2H_2O$ 15.6 g/1 = 0.1 M

   (b) $Na_2\ H\ PO_4$ 14.2 g/1 = 0.1 M

   Mix the two:
   (a) lowers the pH.
   (b) raises the pH.
   Experiment until the pH is 7.0 exactly.

3. Buffered Starch Solution

   (a) 30 ml. stock starch plus

   (b) 70 ml. buffer 0.1M (pH 7.0)

   Boil for 2 minutes. KEEP IN FRIDGE.

2/.....

4. 5% H_2SO_4

Fill a measuring cylinder with 475 ml. H_2O and add 25 ml concentrated H_2SO_4 carefully in a thin stream.

5. 1.5% NaCl

Weigh 120 g. NaCl on the rough balance and dissolve in 8 litres of H_2O.

6. N/10 Iodine

(a) 12.700 g. I_2 weighed accurately.

(b) 40.000 g. KI weighed accurately.

Dissolve both in 1 litre H_2O and keep in DARK BOTTLE IN FRIDGE.

7. N/100 Iodine

10 ml N/10 iodine made up to 100 ml. Keep in DARK BOTTLE IN FRIDGE.

METHOD

1. Dilute 1 ml. pancreatic juice + 1 ml. glycerine in 200 ml. 1.5% NaCl.
2. Use two 50 ml. tubes per specimen and two for controls.
3. To all tubes add 1 ml. buffered starch. Place in a waterbath at 37°C for $\pm$ 10 minutes.
4. To all except the control add 0.1 ml. diluted pancreatic juice timing it to the second with a stopwatch.
5. Incubate for 15 minutes exactly if normal patient and 30 minutes if low enzymes are expected.
6. To all samples, except the controls, add 2.0 ml. 5% H_2SO_4

Plus: A dash distilled H_2O

1 ml N/100 iodine (accurately)

Distilled H_2O up to the 50ml. mark.

Control:

This does not have to be timed and is usually prepared during the incubation period of test samples. Add the H_2SO_4 first, then the juice, the water, iodine and the water again to the control test tubes in the same quantity as test sample.

3/.....

7. Allow the colour to develop for 30 minutes.

8. Read on the Klett colorimeter set on 3 with distilled water, using a red filter (No. 62).

CALCULATION

$$\frac{C - T}{\frac{C}{6}} \times \frac{X}{1} = \text{Units per ml. in thousands}$$

where X = 2 when the juice is incubated for 30 minutes, and
X = 4 when the juice is incubated for 15 minutes.

We use 0.1 ml. (1.5% NaCl diluted) glycerine - duodenal aspirate mixture for each test.

Since 2 ml. glyc-duodenal-asp. = 200 ml. (1.5% NaCl diluted) glyc-duod-asp. mixture.

Therefore 0.1 ml. (1.5% NaCl diluted) = 0.1/100
= .001 ml. glyc-duod-asp. mixture.

Since the duodenal aspirate is initially diluted by its own volume with glycerine: 0.001 ml. glyc-duod-asp. mixture = 0.0005 ml. duodenal aspirate.

THUS:

Only 0.0005 ml. duodenal aspirate is used for each test solm. Therefore 1 ml. duodenal aspirate digests:

$1/.0005 \times \frac{C - T}{C/6}$ mg. starch in 30 minutes at $37^{o}C$.

Therefore 1 ml. duodenal aspirate digests:

$10^3 \times 2 \times \frac{C - 6}{C/6}$ mg. starch in 30 minutes at $37^{o}C$.

The Unit for duodenal aspirate amylase activity is defined as:

The number of mg. starch digested by 1 ml. duodenal aspirate in 30 minutes at $37^{o}C$.

Thus by definition of a unit, amylase activity of the test solution is:

$$2 \times \frac{C - T}{C/6} \times 10^3 \text{ units/ml.}$$

II LIPASE

REAGENTS:

1. Buffer

   0.05 M. Tris pH 8.0

   6.057 gm Tris (HYDROXMETHYL) METHYLAMINE

   → ± 700 ml dist H_2O

   Add ± 2 ml conc. HCL and make pH 8.0

   Make up to 1 litre with dist H_2O

2. NaOH

   (a) Make 1 N NaOH.

   (b) Daily dilute to 0.05 N (10ml to 200ml).

3. Indicator

   (a) Conc. 2% Thymolpthalein.
   2 gm Thymolpthalein → 100ml with 96% EtOH (Ethanol).

   (b) Working Sol^n

   (i) 40 ml (A) → 500 ml with EtOH.

   (ii) Add 500 mgs phenolpthalein.

4. Substrate

   Weigh 37 gm Gum Acacia
   1 gm Sodium Benzoate.
   Add these with 500 ml dist H_2O to a wide-necked brown reagent bottle. Shake well and hold under hot water. Be careful not to let solids stick to the bottom. Mix for 10 minutes with ultra turrax. Stand for ± 5 minutes to cool.

   Add 500 ml Olive Oil in 3 separate portions, mixing for ± 10 minutes every time and cooling in between. Store in brown bottle in fridge.

5. 20% Sodium Taurocholate.
   200 gm → 1 litre.

METHOD

1. Dilute juice 1/500 with ± 1.5% NaCl.
   (a) First 1/100 (0.1 ml juice + 4.9 ml NaCl).

2/.....

(b) 1/500 1 ml (a) + 4. 5 ml NaCl.
Work on ice and whirligig each dilution.

2. Pippette 0. 2 ml of 1/500 dilution into 2 sample tubes and 2 blank tubes.

3. Add 0. 1 ml Na Taurocholate (20%) to each tube.

4. To every blank add 1 ml Thymolpthalein solution (to stop reaction).

5. Mix substrate and buffer 1 : 1. Ultra turrax for ± 3 minutes. Add 3 ml to every tube. Whirligig.

6. Incubate for 2 hours at 37^{o}C.

7. Stop reaction in SAMPLES with 1 ml Thymolpthalein. Whirligig.

8. Titrate all tubes with 0. 05 N NaOH from white to pink matching each pair of samples and controls in colour.

9. Calculation: Each blank is substracted from its sample (x)

$$x \times \overset{(i)}{2,084} \times \overset{(ii)}{500} = \text{lipase I. U. /litre.}$$

$$- 10^{3} = \text{lipase} \times 10^{3} \text{ I. U. /litre.}$$

$$(i)\quad 2,084 = \frac{5,000 \times 50}{2 \times 60}$$

where 5, 000 is factor of juice used (0. 2ml) expressed as per litre

50 is 50 µMol NaOH in 1 ml NaOH used.

2 X 60 is hours converted to minutes.

(ii) 500 is dilution of juice.

III CHYMOTRYPSIN

REAGENTS:

1. Substrates

   ATEE (N-Acetyl-L-Trysosine ethyl ester monochydrate)
   0.00025 M Solution in 0.05 M phosphate buffer pH 7.
   i.e. 25.2 mg. ATEE/100 ml buffer.

2. 0.001 N. HCL.

3. 0.05 M Phosphate Buffer.

   (i) 7.8 gm $NaH_2PO_4H_2O$ → 1 litre H_2O

   (ii) 7.1 gm Ha_2HPO_4 → 1 litre H_2O

   Mix (i) and (ii) until pH 7.

   NB. Mix (i) and (ii) $\pm$ 50:50.

ASSAY METHOD

1. Dilute juice 1 in 5 to 1 in 40 with 0.001 N HCL , depending on expected enzyme activity.

2. Place the following in the 10 mm cuvettes at 25°C:

	Test	Control	Reference
Substrate	3.0ml	3.0 ml	2.25 ml
0.001 N HCL	-	0.2 ml	0.75 ml
Juice solution	0.2 ml	-	-

3. Set the spectrophotometer at 237 m Put reference cuvette (blank) in the "R" cuvette chamber for the duration of the assay. Adjust absorbance to 0.200 with control cuvette in position. Set recorder.

4. Start graph immediately after adding enzyme. Let run for 3 minutes once a straight line is being recorded.

5. Reset spectrophotometer at 0.200 with control cuvette and adjust recorder between assays.

6. Each assay to be done twice. A 5% error is arbitrarily accepted.

7. A standard with crystalline chymotrypsin of known activity was assayed with each test.

CALCULATION

Expressed as units/ml of juice.
One unit of activity is "that activity which causes a decrease in optical density at 237 m of 0.001/min."

2/.....

e.g Dilution 1/40

Reading over 3 minutes: 235 and 208

235 - 208 = 27

27 x 1/3 = difference in 1 minute

27 x 1/3 x 5 = difference in 1 minute for 1 ml. (0.2 ml enzyme used)

27 x 1/3 x 5 x 40 for dilution = 1800 ATEE u/ml.

IV TRYPSIN

REAGENTS:

1. Substrates

BAEE (N-Benzoyl-L-Arginine ethyl hydrochloride).
0.00025 M solution in 0.05 M phosphate buffer pH 8
i.e. 8.6 mg. BAEE/100 ml buffer.

2. 0.001 N. HCL

3. 0.05 M phosphate Buffer

(i) 7.8 gm $NaH_2PO_4H_2O$ → 1 litre H_2O

(ii) 7.1 gm Ha_2HPO_4 → 1 litre H_2O

Mix (i) and (ii) until pH 8
NB. Mix (i) and (ii) $\pm$ 50:50

METHODS

1. Dilute juice 1 in 5 to 1 in 40 with 0.001 N HCL depending on expected enzyme activity.
2. Place the following in the 10 mm cuvettes at 25°C:

	Test	Control	Reference
Substrate	3.0 ml	3.0 ml	3.00 ml
0.001 N HCL	-	0.2 ml	-
Juice solution ...	0.2 ml	-	-

3. Set the spectrophotometer at 253 m Put reference cuvette (blank) in the "R" cuvette chamber for the duration of the assay. Adjust absorbance to 0.05 with control cuvette in position. Set recorder.
4. Start graph immediately after adding enzyme. Let it run for 3 minutes once a straight line is being recorded.
5. Reset spectrophotometer at 0.05 with control cuvette and adjust recorder between assays.
6. Each assay to be done twice. A 5% error is arbitrarily accepted.
7. A standard with crystalline chymotrypsin of known activity was assayed with each test.

CALCULATION

Expressed as units/ml of juice.
One unit of activity is "that activity which causes a ~~decrease~~ increase in optical density at 253 m of 0.001/min."

2/.....

e.g. Dilution 1/10

Readings over 3 minutes: 130 and 90

130 - 90 = 40
40 x 1/3 = difference in 1 minute
40 x 1/3 x 5 = difference in 1 minute for 1 ml. (0.2 ml. enzyme used).
40 x 1/3 x 5 x 40 for dilution = 466 BAEE u/ml.

TABLE A

STANDARD TEST MEAL - NORMAL CONTROLS (½ Hourly Collection Periods)

Test No.	TRYPSIN Units/ml					AMYLASE x10^3 Units/ml					CHYMOTRYPSIN Units/ml					LIPASE x10^3 I.U./litre					pH					VOLUME ml				
	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4
1	1550	1900	5000	4983	5133	6.96	6.19	10.67	10.78	10.96	1600	641	1500	2000	2400						7	6	7	7	7	48	275	52	28	52
2	475	2633	7066	3050	11400	0.47	9.44	11.00	8.72	11.06	620	2483	2916	1550	4700						3	7	7	7	7	90	54	58	50	68
3	1491	5333	9600	4950	2933	4.23	9.06	10.32	10.48	5.45	1500	1191	1108	1867	1450						6	7	7	7	7	50	90	60	40	30
4	812	7400	4383	3241	4616	0.46	9.90	6.07	7.32	7.08	637	2067	1333	1033	808						6	6	6	7	7	20	50	55	40	25
5	14233	8866	8467	6350	5116	11.41	11.14	10.03	5.79	8.66	6300	6683	4467	1833	1550						-	6	6	5.5	5	-	8	6	6	4
6	2492	7333	4333	5216	4967	11.52	11.15	10.98	11.32	11.36	2820	3190	1445	1450	843											10	88	70	68	85
7	-	8933	4866	4586	3433	-	9.79	5.54	4.11	10.00	-	4300	1400	2083	1966						-	7	7	7	7	-	63	84	32	28
8	3583	3083	3034	5666	6306	6.95	11.01	7.81	11.14	11.27	2100	1666	1400	3366	2140						5.4	6.2	6.2	6.2	6.2	120	104	64	22	4
9	1350	4766	3366	4050		2.68	9.25	8.23	9.09		842	1833	1233	1716							6.0	6.2	6.2	6.2	6.2	8	17	7	12	12
10	2741	5266	6733	4416	3900	13.05	12.85	14.06	-	10.06	1866	3800	4966	6900	3600	313	884	969	1094	833						8	12	9	8	6
11 *	-	5041	4500	2366	4166	-	8.40	6.62	2.27	6.81	-	4583	1400	1038	1816	-	750	636	365	729	-	6	6	5	6	-	12	5	6	6
12 *	3583	3037	4266	4335	4366	8.37	9.10	9.97	13.01	10.77	1933	1900	2700	2583	1733	417	510	458	625	573	-	6.3	6.4	7	6.2	5	57	5	18	10
13	2425	6183	4733	2708	4883	13.52	15.71	10.94	7.88	7.87	1708	3141	2316	1200	2083	334	1131	782	479	750	6	6.3	6.0	5.4	6.2	4	84	29	6	12
14**	-	3567	2133	4333	5100	-	7.19	5.97	9.90	11.10	-	2566	2000	2100	1666	-	604	271	584	813	-	6.4	6.4	6.2	6.2	-	5	7	6	4
15**	2300	3925	2225	3020	5024	6.59	7.57	4.71	7.46	9.53	2117	2000	2483	1783	2399	313	515	292	458	729	6.2	6.0	6.5	6.5	6.7	10	21	37	8	27
16	1358	4553	4541	3941	6337	2.42	4.25	6.72	4.05	9.69	875	4224	4433	1983	5400	250	448	385	349	646	4.8	6.3	6.5	6.4	6.5	50	13	32	25	36
17	4716	3233	3249	3077	5499	16.42	6.52	5.50	4.52	6.60	1983	1149	1299	699	2016	511	656	458	458	917	7	6.4	6.5	6.4	6.5	8	20	52	110	8
18	-	3549	3324	3441	4141	-	6.42	5.33	3.50	5.25	-	1741	1707	1307	1199	-	432	453	474	510	-	6.5	6.5	6	5.6	-	14	32	40	12

* Test 11 and 12 - same subject

** Test 14 and 15 - same subject

TABLE B

STANDARD TEST MEAL - NORMAL CONTROLS (8, 15 Minute Collections)

TRYPSIN

NO.	Basal	1	2	3	4	5	6	7	8
19	-	8081	5470	7533	6499	5200	3466	4566	-
20	2394	5716	2508	1641	2208	1458	3333	4433	8633
21	-	3066	4033	3583	3666	3100	3783	3166	5116
22	3700	9933	7766	5300	7066	10266	11666	11333	6931
23	-	5533	4750	4500		2366	4166	-	-
24	2300	4450	3400	1950	2508	2475	3566	4166	5983
25	991	3800	5666	2283	2866	3800	-	4400	4583
26	5366	5416	4433	3583	4250	4966	6450	6663	2583
27	3583	3916	2158	4266	4266	5433	3248	3933	4800
28	1358	3624	5483	5483	3700	4166	3716	11133	1541

AMYLASE

Basal	1	2	3	4	5	6	7	8
-	12.79	14.69	14.43	14.14	8.28	7.38	11.39	-
3.79	14.21	10.49	7.33	7.16	5.85	6.57	6.43	6.34
-	6.42	6.43	5.36	5.31	2.84	4.18	4.91	5.60
0	13.44	6.73	4.42	7.68	9.85	17.82	14.90	16.65
-	8.39	8.41	6.62	-	2.27	6.81	-	-
6.59	8.28	6.86	4.00	5.42	6.71	8.26	7.00	12.06
3.70	9.15	12.25	3.08	5.43	6.80		5.91	8.78
10.17	10.61	8.39	6.92	7.51	10.62	10.42	18.43	10.84
8.37	9.89	8.41	9.97	9.97	10.65	15.38	13.16	8.39
2.42	2.15	6.35	9.18	4.26	3.75	4.36	11.38	8.00

CHYMOTRYPSIN

Basal	1	2	3	4	5	6	7	8
-	6100	1849	2866	1966	1833	992	1066	-
1200	2666	799	316	842	924	1349	1316	2649
-	1350	2133	1799	1616	1149	1466	749	1649
2683	4633	3299	2099	2266	2766	2333	2633	3433
-	6433	2733	1400		1058	1816	-	-
2117	2600	1400	1100	1383	1733	1833	1733	3066
750	3500	3000	1560	1583	1933	-	2233	1900
3233	3266	2167	1900	1833	1900	1833	3333	1233
1933	1333	2467	2700	2700	2533	2633	2033	1433
875	1283	7166	7000	1866	1700	2266	6800	4000

LIPASE

NO.	Basal	1	2	3	4	5	6	7	8
19	-	761	771	834	771	792	584	584	-
20	281	1052	657	281	323	323	542	594	657
21	-	365	500	469	438	417	531	427	594
22	677	844	438	396	563	386	948	771	907
23	-	750	688	636	636	365	729	-	-
24	313	646	385	313	271	458	458	584	875
25	198	563	604	323	493	375		531	490
26	375	667	479	479	688	729	698	927	438
27	417	458	563	58	58	552	698	552	594
28	250	354	542	490	280	292	396	886	406

VOLUME

Basal	1	2	3	4	5	6	7	8
0	10	5	5	5	3	18	3	0
50	41	148	122	109	40	41	50	20
0	4	10	14	18	30	10	6	6
10	92	62	88	15	40	10	30	32
0	8	14	23	23	2	2	0	0
10	6	15	15	22	2	6	15	12
15	12	8	20	30	42	0	2	6
5	6	10	5	6	6	4	5	12
5	52	5	5	5	8	10	8	2
50	8	5	2	30	12	13	20	16

pH

Basal	1	2	3	4	5	6	7	8
-	6.4	6.7	7.0	7.0	6.7	6.4		
6.4	6.6	6.4	6.7	6.6	6.4	6.4	6.4	6.4
-	6.5	6.5	6.5	6.5	6.4	5.4	5.4	6.0
6.0	6.4	6.4	6.4	6.4	6.0	6.4	6.0	6.4
-	6.0	6.0	5.0	5.0	6.0	6.0	-	-
6.2	6.0	6.0	6.4	6.7	6.5		7.0	6.7
5.4	6.7	7.0	6.2	6.2	6.4	-	6.2	6.7
6.4	6.4	6.4	6.4	6.4	6.2	6.7	6.7	6.4
	6.2	6.4	6.4		7.0	7.0	6.3	6.2
4.8	6.2	6.4	6.5	6.5	6.4	6.4	6.7	6.4

TABLE C

STANDARD TEST MEAL - PANCREATIC GROUP ($\frac{1}{2}$ Hourly Collection Periods)

Test No.	TRYPSIN Units/ml					AMYLASE x10^3 Units/ml					CHYMOTRYPSIN Units/ml					LIPASE x10^3 I.U./litre					pH					VOLUME ml				
	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4
33	575	1075	417	716	2750	0.37	2.47	1.53	1.46	4.20	262	450	161	458	816						6	7	7	7	7	50	48	38	25	4
34**	-	0	0	0	-	_	0.15	0	-	-	0	0	0	0	0						_	6.4	6.0	6.4	6.2	0	25	2	2	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						-	5	4	3	6	6	242	68	38	32
36	-	487	529	421	472	-	0.81	1.96	3.69	0	-	325	150	246	471											0	170	135	35	15
37	-	692	679	608	-	-	1.35	0.35	0.48	-	-	325	231	145	-						-	6	5	6	-	0	116	36	12	0
38	771	0	125	106	121	0.24	0.81	0.31	0.24	0	274	-	-	-	-						7.5	7.5	7.5	7.5	7.5	12	20	10	5	13
39*	412	341	408	406	314	2.98	2.22	4.63	5.65	7.65	237	108	162	158	0						6.7	6.2	6.4	6.2	6.4	7	65	10	38	50
40*	904	564	258	558	608	1.87	1.59	0.44	2.54	2.54	483	75	62	0	134						6.7	6.2	6.5	6.5	6.5	19	13	15	4	8
41	1183	1992	1700	1325	2083	1.70	2.40	1.66	1.23	3.80	1333	1383	1316	930	1374						6.5	6.5	6.5	6.5	6.5	45	95	198	218	164
42	-		283		268	-		5.52		4.38	-		0		0						-	-	6.2	-	6.0	0				30
43	133	229	92	100	71	2.26	0.91	0	0.26	0.64	133	229	92	100	71											10	47	46	18	24
44	1454	345	1858	1041	308	1.90	1.28	1.08	0	0	875	271	683	167	270						6.2	6.2	6.2	6.2	6.2	20	15	20	15	36
45	658	1750	658	688	924	3.35	4.43	3.85	2.04	4.51	800	933	233	387	429											12	22	40	20	15
46	-	-	792	595	733	-	-	6.32	4.04	9.30	-	-	166	171	183						-	6.0	6.2	6.2	6.4	0	0	32	39	38
47	1633	1216	1333	462	320	4.55	1.45	0.97	0.69	0.10	1025	700	620	155	158											10	22	55	100	88
48*	-	300	175	307	708	-	2.98	0.65	1.77	2.58						-	88	54	62	109						0	78	45	78	12
49	-	92	150	276	662	-	0	1.07	1.38	1.26						-	0	10	20	31	-	6.0	7.0	7.5	-	0	112	100	53	18
50**	0	0	0	-	0	2.15	2.00	0.93	-	0.29	0	0	0	-	0	21	21	0	-	0		6.4	6.2	-	6.4	10	22	32	0	12
51	675	1116	1012	1020	1364	4.39	3.94	2.82	2.45	3.80	933	576	767	325	345	73	130	118	78	120	6	6.3	6.7	5.3	6.3	36	152	106	145	225
52	-	271	412	758	450	-	0.74	1.06	0.76	0.58	-	162	158	200	0		52	42	104	42	-	6.4	6.4	6.4	6.4	0	43	4	2	32
53	58	0	0	0	0	1.74	0.17	0.25	0.79	0	0	0	0	0	0	0	0	0	0	0		7	7	7.2	72	10	5	8	15	6
54	579	1038	437	541	1166	0	0.09	0.54	0.72	1.45	500	645	342	345	495	0	94	63	0	125	4.6	6.4	6.4	6.4	6.4	52	94	25	8	38
55	166	875	1338	1196	-	0	2.84	3.30	1.58	-	154	308	950	729	-	0	94	130	130	-	4.5	6.4	5.8	4.8	-	22	8	3	2	0
56	167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	6.7	6.4	6.2	6.5	6.4	18	98	46	6	40
57	674	679	150	366	929	2.41	3.09	3.46	2.16	2.58						42	52	0	21	94	7.2	6.4	6.4	6.5	6.4	8	90	86	58	34
58	3333	4517	1258	1812	2750	3.97	5.13	0.18	1.32	2.36	666	866	133	666	1900	302	313	125	83	114	6.7	6.4	6.4	6.4	6.4	8	110	104	24	8
59	704	1129	1283	966	2533	0	1.31	2.63	2.78	3.70	704	487	461	470	1016						5.4	5.8	6.7	6.7	6.7	20	3	20	24	6
60	-	1840	1499	447	2525	-	7.30	5.41	6.74	12.66	-	383	741	87	2124	-	219	146	0	198	-	6.4	6.0	6.4	6.4	0	22	52	32	62
61	-	487	262	0	500	-	2.13	5.47	13.97	1.07	-	245	300	0	0	-	198	42	62	136	-	7	6.4	6.4	6.4	0	22	22	18	3
62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.0	6.4	6.5	-	6.5	50	50	20	2	6
63	1691	1837	1154	-	5183	7.42	1.16	2.44	4 ;65	8.00	931	533	176	1033	1716	104	73	115	125	281	6.5	6.4	6.4	6.4	6.4	40	160	60	6	8
64	0	129	0	0	0	0	0	0.58	1.90	0	0	0	0	0	0	0	0	0	0	0	5.4	6.4	6.4	6.0	4.7	4	110	64	14	10
65	241	3083	1808	1458	1999	0	4.66	2.20	1.37	4.77	275	1366	824	-	-	0	365	292	229	302	5.1	6.4	6.4	6.4	6.4	34	28	10	35	90
80	8833	9466	3300	2600	4000	11.02	10.69	6.51	5.53	6.58	966	1900	1250	1660	1883											10	8	18	15	4
82	2733	7033	4691	11766	9733	9.50	12.75	8.85	16.40	15.16	1333	4400	3300	4800	7100	498	807	531	1056	969						10	25	35	13	15
83	3966	4883	4033	2900	5383	10.16	6.12	7.92	11.86	6.66	-	-				218	354	500	229	406	7	6.4	6.4	6.4	6.4	8	32	110	154	130
84	-	2650	3833	3099	8466	-	2.69	6.88	5.04	4.71	-	1341	1616	1450	3799						-	5.1	5.1	4.8	6.40	0	4	4	4	10

Test 39, 40, 48 _ same patient
*Test 34 and 50 - same patient.

TABLE D

STANDARD TEST MEAL (½ Hourly Collections)

Test No.	TRYPSIN Units/ml					AMYLASE x10^3 Units/ml					CHYMOTRYPSIN Units/ml					LIPASE x10^3 I. U./litre					pH					VOLUME ml				
	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4	Basal	1	2	3	4
66	675	5500	7300	4550	3100	3.84	10.19	9.87	4.62	8.47	2100	1450	1300	1275	1325						7	7	7	7	6	126	108	146	110	31
67	-	1800	4066	4100	4666	-	6.06	9.97	10.77	10.29	-	400	2417	2267	2483															
68	-	4100	3433	4733	5133	-	12.00	10.94	9.84	11.94	-	2867	1667	1933	1833	-	498	406	531	510	-	7	6.7	7.2	7.2		8	8	6	3
69	3416	3658	3350	3674	5999	1.94	7.47	6.10	8.16	13.19	2833	1533	1033	2603	2158	417	451	315	393	350	4.8	6.5	6.4	6.4	6.4	22	118	42	64	30
70	2291	2278	3607	8149			7.42	6.92	11.07	18.30		641	820	1412	8150		478	401	563	1068		6.6	6.5	6.6	6.7	-	38	63	39	58
71	2775	4133	3033	1827	1916	10.83	9.90	5.50	5.00	4.83	2500	1766	1388	550	1615						-	8		8		10	22	12	15	25
72	2358	2315	2499	2327	2511	1.0	1.34	1.76	2.92	0.18	2035	1083	1358	1749	1308	281	453	479	380	302		6.4	6.2	6.4	6.4	40	38	16	15	8
73	-	917	1916	1333	2183	-	6.69	11.32	6.42	8.00	-	683	500	400	600		240	240	167	219		7	7	7	6.7	-	10	2	5	50
74	-	2774	1570	1453	1958	-	1.36	0.97	0.61	1.20	-	839	603	324	733	-	166	109	52	119	-	6.5	6.7	6.5	7	-	12	34	33	24
75	-	1430	2315	2566	2387	-	2.13	1.52	1.68	0.77	-	1045	833	891	712	-	47	83	94	94	-	6.0	6.4	6.5	6.4	-	12	38	32	28
76	991	4733	2824	3800	4491	3.70	10.70	4.25	6.86	5.84	750	3250	1574	1933	2066	198	583	408	375	511	5.4	6.8	6.2	6.4	6.4	15	20	50	42	8

DETAILED RESULTS OF DIFFERENT TEST MEALS

Each Table refers to a number of tests performed on a single subject.

TABLE E

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I. U./litre
TEST MEAL 300 ml	1	24	6.6	9.29	2741	758	615
	2	14	6.7	5.56	1841	524	344
	3	10	6.7	7.49	2516	1016	386
	4	53	6.4	6.36	2041	625	417
	5	24	6.4	10.33	3049	824	490
	6	15	6.7	11.82	4166	2000	636
	7	52	6.7	18.87	7299	8500	1032
	8	6	6.7	17.74	9000	7800	1105
TEST MEAL 350 ml	1	10	6.4	12.79	8081	6100	761
	2	5	6.7	1469	5470	1849	771
	3	5	7	14.43	7533	2866	834
	4	5	7	14.14	6499	1966	771
	5	5	6.7	8.28	5200	1833	792
	6	18	6.4	7.38	3466	992	584
	7	3	6.4	11.39	4566	1066	584
TEST MEAL 450 ml	1	8	6.4	12.03	4767	1233	438
	2	6	6.4	7.62	3833	1416	448
	3	16	6.4	7.71	3200	1333	344
	4	20	6.4	11.32	4233	1099	386
	5	44	6.4	14.03	5633	2400	615
	6	6	6.5	13.79	10800	6800	542
	7	5	6.5	19.51	12266	9633	122
TEST MEAL 550 ml	B	20	7.2	20.96	4533	3033	490
	1	62	6.4	16.88	4616	1183	657
	2	42	6.5	6.18	2333	1283	354
	3	84	6.4	7.34	2167	666	406
	4	152	6.4	5.85	2150	684	365
	5	10	6.4	13.85	2684	849	1125
	6	26	6.5	21.24	8266	27000	1021
	7	50	6.4	20.69	8733	9000	1105
	8	22	6.5	18.07	6666	26000	1021

TABLE F

	SAMPLE	VOL (ml)	pH	AMYLASE x10³ Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10³ I. U./litre
STANDARD TEST MEAL (test 115)	B	10	6.0	0	3700	2683	644
	1	92	6.4	13.44	9933	4633	844
	2	62	6.4	6.73	7766	3299	438
	3	88	6.4	4.42	5300	2099	396
	4	15	6.4	7.68	7066	2266	563
	5	40	6.0	9.85	10266	2766	386
	6	10	6.4	17.82	11666	2333	948
	7	30	6.0	14.90	11333	2633	771
	8	32	6.4	16.65	6931	3433	907
CASILAN MEAL (test 87)	B	34	4.9	0	3500	1716	104.2
	1	32	6.0	4.65	3716	1666	328
	2	38	5.0	2.26	2333	749	219
	3	5	5.0	5.74	2433	1766	365
	4	18	4.8	4.74	5583	3115	625
	5	22	5.0	17.91	18266	8600	990
	6	10	4.8	8.74	9466	5699	729
	7	6	6.4	19.55	15333	-	980
	8	32	4.8	7.89	7000	-	636
CARBO-HYDRATE MEAL (test 91)	B	50	5.1	0	1749	883	0
	1	54	6.5	4.88	9249	1900	323
	2	8	6.4	7.13	2699	2166	323
	3	18	6.6	1.87	2216	1600	229
	4	23	6.6	9.14	5100	3083	500
	5	24	6.4	7.32	4016	2433	354
	6	30	6.4	6.24	4233	2166	479
	7	20	6.4	4.33	2794	1749	417
	8	10	6.5	0.46	1916	1083	125
FAT MEAL (test 95)	B	38	4.8	2.24	2874	2133	281
	1	15	6.5	7.27	3566	2216	417
	2	7	6.4	8.12	4100	1915	438
	3	7	6.5	17.57	7533	2966	823
	4	8	6.4	13.65	7416	3066	948
	5	12	6.6	13.65	24000	11933	1740
	6	10	6.7	13.33	19666	10733	1646
	7	3	6.6	6.13	13533	6666	1552
	8	5	6.4	5.52	9200	4166	1344

Cont./.....

TABLE F (Cont.)

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I.U./litre
	B1	42	5	2.03	3450	2699	406
	B2	50	5	0	2166	2666	104
	B3	5	6.4	2.54	2366	1666	142
	B4	0	-	4.24	3538	2581	259
	1	250	4.8	0	2133	2149	42
WATER MEAL	2	85	6	2.46	3100	1433	219
(test 97)	3	36	6.4	6.03	4215	2299	406
	4	20	5.1	0	3166	2266	396
	5	38	4.8	0	4050	2949	261
	6	20	4.9	1.06	3983	3149	344
	7	10	4.8	0	4233	3333	271
	8	10	4.9	0	3416	3066	136
	B	30	5	3.84	3308	2416	104
	1	300	5.5	1.85	800	799	625
	2	114	6	3.43	2700	1258	261
	3	25	5.8	6.83	5283	2583	511
SALINE MEAL	4	35	5	12.00	5383	2916	625
(test 100)	5	19	6.4	5.65	6183	3599	636
	6	3	6.4	11.59	4633	2799	417
	7	2	7.5	10.09	3533	1900	292
	8	6	6.8	20.54	8294	4773	657

TABLE G

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I. U. /litre
STANDARD TEST MEAL (test 116)	B	22	4.8	1.94	3416	2833	417
	1	68	6.5	9.49	3533	1216	485
	2	50	6.5	5.45	3783	1850	417
	3	30	6.4	5.56	3600	2000	339
	4	12	6.4	6.65	3100	2066	292
	5	24	6.4	9.80	4366	3166	568
	6	40	6.4	6.53	2983	2041	219
	7	20	6.4	11.56	5333	1633	309
	8	10	6.4	14.83	6666	2683	391
CARBO-HYDRATE FREE MEAL (test 106)	B	6	6.4	20.71	4599	4766	657
	1	44	6.4	16.18	4916	2583	886
	2	37	6.4	4.06	1345	1983	354
	3	20	6.4	12.00	5083	2083	563
	4	17	6.4	16.00	6266	2533	938
	5	18	6.5	18.89	10666	6794	1198
	6	46	6.4	15.62	7399	5599	791
	7	28	6.4	17.37	5033	2933	948
	8	22	6.5	15.31	5566	2733	854
LOW PROTEINS MEAL (test 108)	B	30	6.7	23.38	2692	7533	500
	1	8	6.7	23.72	8400	5367	782
	2	12	6.5	21.62	9266	5133	646
	3	42	6.5	7.28	4500	2183	293
	4	33	6.4	9.35	4000	2200	438
	5	12	6.4	24.58	13000	13133	103
	6	26	6.4	10.93	9150	1483	531
	7	22	6.5	11.88	4733	1555	490
	8	22	6.4	14.38	6433	3633	792
FAT FREE MEAL (test 11)	B	8	7.5	15.58	1966	2083	917
	1	24	6.4	11.03	1666	1325	656
	2	20	6.6	10.84	4766	3666	625
	3	3	6.7	12.17	4883	3416	709
	4	6	6.7	9.67	4183	3300	625
	5	8	6.4	8.18	2458	1633	500
	6	48	6.7	13.41	5433	4416	729
	7	8	6.7	7.03	3583	2783	531
	8	15	6.6	10.15	4749	3950	667

TABLE H

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I. U./litre
STANDARD TEST MEAL (test 114)	1	22	6.7	14.11	2333	1716	625
	2	12	6.7	9.52	2999	2149	625
	3	10	6.5	7.94	2583	1833	594
	4	12	6.6	7.57	2499	1816	511
	5 & 6	31	6.7	11.00	3666	2216	667
	7	56	6.4	5.16	2199	1516	427
	8	25	6.4	9.06	3183	1944	636
CASILAN MEAL (test 85)	B	100	7	7.94	1866	1133	240
	1	100	6.4	4.51	1316	-	552
	2	36	6.8	16.38	10499	3499	1636
	3	24	7	15.60	6799	2283	1542
	4	30	6.6	9.89	6550	2266	1229
	5	20	6.7	9.68	7633	2433	1219
	6	24	6.7	12.64	8666	2333	1208
	7	32	6.6	7.83	8200	2666	917
	8	19	6.7	7.03	4783	1199	636
CARBO-HYDRATE MEAL (test 89)	B	28	7.4	11.69	1441	999	52
	1	72	7.0	12.16	3483	2583	502
	2	18	6.7	8.98	2633	2216	406
	3	8	7.0	7.93	3500	2299	219
	4	17	6.8	6.84	3333	2000	479
	5	72	6.6	4.72	2383	1649	490
	6	112	6.7	7.08	1966	1233	281
	7	14	7.2	8.98	5466	2449	604
	8	42	6.7	2.03	2666	1533	375
FAT MEAL (test 93)	B	35	7	12.54	2391	1749	281
	1	4	7.2	14.79	2966	1666	365
	2	4	7.8	10.74	2655	1333	448
	3	44	6.7	5.12	3116	1649	521
	4	8	6.7	11.53	4750	2550	834
	5	16	6.7	9.54	4349	1466	771
	6	8	6.7	10.18	3399	2299	552
	7	6	6.7	18.21	6799	2466	1125
	8	6	6.7	15.16	7383	4000	1125

Cont. / .....

TABLE H (Cont.)

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I. U./litre
WATER MEAL (test 96)	B	44	7	16.12	2583	1066	229
	1	96	7	3.91	1741	883	156
	2	47	7.2	6.39	2050	1049	250
	3	7	8	11.56	3449	1966	490
	4	11	7.2	15.37	4700	3016	584
	5	27	7	23.88	7783	4016	761
	6	5	7	18.20	8833	3849	719
	7						
	8	8	7	21.22	8433	3666	614
SALINE MEAL (test 99)	B	12	6.4	5.82	1674	1141	313
	1	30	6.7	11.75	4099	2866	750
	2	76	7.2	8.62	2966	2016	604
	3	120	7	6.76	2074	1391	427
	4	82	7	6.24	1633	1166	438
	5	48	7.2	9.75	2883	1783	698
	6	18	7.1	12.06	4416	3116	771
	7	10	7	15.20	4799	3383	1105
	8	4	7.6	16.49	5183	3594	1188
GASTRIN INJECTION (test 104)	B	10	7.1	17.56	2066	1074	417
	1	78	7	10.87	5716	2950	990
	2	20	6.7	14.25	7700	4583	105
	3	30	6.5	17.07	6133	3299	844
	4	60	4.9	13.77	1483	708	188
	5	7	6.4	3.72	2799	1615	511
	6	18	5	4.32	2583	1299	386
	7	37	4.8	1.05	1699	741	271
	8	13	6	6.98	2174	1350	531
	9	4	7.2	10.58	2600		
	10	22	6.7		4666		
	11	10	6.5		2249		
	12	32	4.7		2050		

Cont. / .....

TABLE H (Cont.)

	SAMPLE	VOL (ml)	pH	AMYLASE $x10^3$ Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE $x10^3$ I.U./litre
CARBO-HYDRATE FREE MEAL (test 89)	B	18		4.97	1137	466	83
	1	5	6.4	11.31	4660	1233	655
	2	4	6.6	15.25	8066	2133	1073
	3	3	6.6	9.05	6733	1886	1063
	4	7	6.6	9.97	3833	950	614
	5	6	6.7	11.88	6733	1416	938
	6	12	6.7	8.23	4683	1433	782
	7	4	7	16.78	9533	2016	1406
	8	20	7	17.06	9316	2833	1115
LOW PROTEIN MEAL (test 107)	B	10	6.7	9.47	2799	3366	417
	1	42	6.6	16.91	7266	3666	959
	2	10	6.7	8.97	6166	2033	802
	3	5	6.7	10.62	5700	2066	1063
	4	12	6.5	11.50	4766	1999	875
	5	44	6.4	8.25	2933	1933	386
	6	40	6.5	6.00	3033	2499	400
	7	10	6.4	7.45	3166	1899	584
	8	10	6.4	7.28	4066	2000	688

TABLE I

	SAMPLE	VOL (ml)	pH	AMYLASE $x10^3$ Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE $x10^3$ I.U./litre
STANDARD TEST MEAL (test 28)	B	50	4.8	2.42	1358	875	250
	1	8	6.2	2.15	3624	1283	354
	2	5	6.4	6.35	5683	7166	542
	3	2	6.5	9.18	5183	7000	490
	4	30	6.5	4.26	3700	1866	280
	5	12	6.4	3.75	4166	1700	292
	6	13	6.4	4.36	3716	1266	396
	7	20	6.7	11.38	11133	6800	886
	8	16	6.4	8.00	1541	4000	406
SALINE MEAL (test 103)	1	120	6.4	1.13	1192	1133	20
	2	58	6.4	0	942	817	31
	3	38	6.5	0.97	2050	1667	146
	4	2	6.7	1.60	1717	1050	198
	7	4	7	3.16	4133	3667	511
	8	4	6.7	4.92	6333	4833	573
SALINE MEAL & GASTRIN INJECTION (test 111)	B	60	7.3	3.04	3000	3267	365
	1	30	5.3	2.12	1750	2067	219
	2	200	3.6	-	0	0	-
	3a	80	6.0	1.00	1700	1333	83
	3b	36	6.7	2.21	1900	1383	156
	4	64	7.0	1.09	2783	1350	167
	5	64	6.7	1.09	2583	1917	198
	6	40	6.6	1.90	2867	3333	333
	7	33	6.7	3.08	3300	3933	313
	8	12	7.4	6.80	4733	6000	458

TABLE J

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I.U./litre
STANDARD TEST MEAL (test 118)	B	5	-	8.37	3583	1933	417
	1	52	6.2	9.89	3916	1333	458
	2	5	6.4	8.41	2158	2467	563
	3 & 4	5	6.4	9.97	4266	2700	458
	5	8	7.0	10.65	5433	2533	552
	6	10	7.0	15.38	3248	2633	698
	7	8	6.3	13.16	3933	2033	552
	8	2	6.2	8.39	4800	1439	594
PROTEIN MEAL (test 88)	B	4	6.0	15.63	2366	2500	698
	1 & 2	4+5	5.5	3.19	3900	3333	625
	3	5+5	6.7	9.54	4600	3900	782
	4	12	7	7.57	4550	3666	688
	5	14	5.8	4.74	3133	2666	458
	6	16	6.4	2.58	2933	2833	406
	7	25	7	8.68	4150	3500	427
	8	20	6.4	17.86	3733	3000	646
CARBO-HYDRATE MEAL (test 92)	B	5	5.4	15.71	4950	3900	469
	1	41	6.2	11.66	4366	3166	886
	2	62	6.4	7.25	2716	2000	417
	3	7	7	12.31	3466	3000	625
	4	20	6.7	10.00	3249	2890	594
	5	7	7	17.06	5100	3633	625
	6	4	9	17.02	4966	3533	646
	7	10	6.7	10.82	3766	2833	521
	8	6	6.7	15.02	3450	3100	479
SALINE MEAL (test 101)	B	30	5.1	0	1700	1575	146
	1	80	6.5	2.03	1891	1300	219
	2	68	6.4	4.65	2850	1658	281
	3	38	6.4	4.71	3500	2300	229
	4	10	6.4	4.12	5500	3500	354
	5	2	7.5	9.39	5500	3900	615
	6	4	7.5	9.35	4233	3033	698
	7	6	6.7	8.52	5633	3550	490
	8	2	6.4	5.82	2850	2000	365

TABLE J (CONT.)

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I.U./litre
	1	88	5	2.10	1245	578	240
	2	20	5	4.68	2874	1800	490
	3	2	6.2		1925	1000	333
CASILAN MEAL	4	4	5	18.67	8233	7833	1292
	5	10	5	1.52	4100	3133	229
	6	2	5	1.17	2333	1625	156
	7	4	5	0.41	3250	1783	229
	8	8	6.2	0.18	4760	1392	217

TABLE K

	SAMPLE	VOL (ml)	pH	AMYLASE x10^3 Units/ml	TRYPSIN Units/ml	CHYMOTRYPSIN Units/ml	LIPASE x10^3 I.U./litre
STANDARD TEST MEAL (test 119)	B	10	6.4	9.50	2733	1333	498
	1	15	6.4	9.93	5000	4600	552
	2	10	6.4	15.58	9067	4200	1063
	3	30	6.4	9.65	6016	1933	604
	4	5	6.4	8.26	5367	4667	458
	5	5	6.7	19.72	12933	5067	1427
	6	8	6.5	13.09	10600	4533	685
	7	10	6.5	12.33	11627	9000	729
	8	5	6.7	18.00	8200	5333	1209
WATER MEAL (test 98)	B	30		6.33	2283	2300	83
	1	20	5.1	5.24	3197	1466	208
	2	50	5.1	1.15	537	475	125
	3	44	5	2.03	1833	1333	188
	4	4	6.4	1.29	2108	1583	208
	7	4	6.4	4.24	1308	1033	135
SALINE MEAL (test 102)	1	68	6.4	0	375	250	10
	2	22	6.4	4.57	833	733	115
	3	18	6.4	0.75	750	708	94
	4	5	6	6.06	1042	1162	188
	5	18	6.4	8.11	2917	2000	333
	6	18	6.4	2.93	2167	1067	229
	7	3	7.2	6.08	2667	1650	250
SALINE MEAL & GASTRIN INJECTION (test 110)	B	54	5.1	3.85	2833	2233	240
	1a	34	6.4	1.43	1020	649	104
	1b	5	6.0	1.07	525	374	21
	2	74	5.1	0.14	579	608	21
	3	76	5.1	0	504	550	10
	4	40	5	0	362	262	31
	5	26	5.2	0	312	250	31
	6	8	6.4	3.38	1229	1091	172

STATISTICAL METHODS

1. The t-test: This test was used to test the quality of means of 2 groups of data. A probability level of 0.05 was used throughout. The majority of comparisons were performed with aid of an electronic computer.

2. The one way layout (349): This can be considered as a multivariate t-test, as it only tests the hypothesis of equality of means of a number of groups of data.

Model:

$$Y_{ij} = \mu + \alpha_i + e_{ij} \qquad \begin{matrix} i = 1 \ldots I \\ j = 1 \ldots J_i \end{matrix} ; \; n = \sum_{i=1}^{I} J_i$$

where I = number of groups
J_i = number of observations in the ith group.
e_{ij} = a random error which is assumed to be normally distributed.

The hypothesis is $H_0 : \alpha_1 = \alpha_2 \ldots\ldots = \alpha_I$

The test statistic is

$$F = \frac{\sum_{i=1}^{I} J_i \left(y_{i\cdot} - \bar{y}\right)^2 / I-1}{\sum_{i=1}^{I} \sum_{j=1}^{J_i} \left(y_{ij} - y_{i\cdot}\right)^2 / n - I}$$

where $y_{i\cdot} = \frac{1}{J_i} \sum_{j=1}^{J_i} y_{ij}$ i.e. the mean of the ith group

$\bar{y} = \frac{1}{n} \sum_{i=1}^{I} y_{i\cdot}$ i.e. the overall mean of all observations.

The above stastic has the F distribution with I-1 and n-I degrees of freedom. If $F > F^{.05}_{I-1,n-I}$ then reject the hypothesis. If $F < F^{.05}_{I-1,n-I}$ then accept hypothesis.

3. S-method of multiple comparisons (349): This method is used if the above hypothesis is rejected and we now wish to acertain which means are different. The method rests on the following probability statement:

$$P\left[\sum_{i=1}^{I} c_i \mu_i \; t\left[\sum_{i=1}^{I} a_i y_i \pm \left[(r-1) F^{.05}_{r-1,\,N-r}\right]^{\frac{1}{2}} S \left(\sum_{1}^{r} \frac{c_i}{n_i}\right)^{\frac{1}{2}}\right] V(c_1 \cdots c_I) \quad \text{such that } \sum_{i=1}^{I} c_i = 0\right]$$

If the confidence interval includes zero, then no significant difference is present between the groups tested.

e.g. Fat meal versus carbohydrate meal (Lipase)

Mean lipase concentration in fat meal = 822.7

Mean lipase concentration in carbohydrate meal = 439.9

$\therefore$ Difference = 382.8

Confidence interval for carbohydrate meal = 320.9

$\therefore$ Confidence limit for carbohydrate does not include zero,

$\therefore$ There is a significant difference between these two meals.

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