FAECAL PANCREATIC ELASTASE 1 - A USEFUL NON INVASIVE MEASURE OF EXOCRINE PANCREATIC FUNCTION

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DECLARATION

THE WORK DESCRIBED IN THIS REPORT WAS CARRIED OUT BY ME AT THE DEPARTMENT OF CHEMICAL PATHOLOGY, UNIVERSITY OF GHANA MEDICAL SCHOOL, KORLE-BU UNDER THE SUPERVISION OF PROF. V. RANA AND DR. SAMUEL Q. MADDY.

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TO THE GLORY OF GOD AND THE PATIENCE AND ENCOURAGEMENT BY MY WIFE MARGARET AND CHILDREN RICHARD, MARGARET AND TETTEH-OTU
I wish to express my appreciation to all who contributed towards the successful completion of this work.

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<td>µL</td>
<td>microlitre</td>
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<td>mL</td>
<td>milliliter</td>
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<td>ng</td>
<td>nanogram</td>
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<td>µg</td>
<td>microgram</td>
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<td>gram</td>
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<td>Kg</td>
<td>Kilogram</td>
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<td>%</td>
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<td>°C</td>
<td>degree Celsius</td>
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<td>ABTS</td>
<td>[2,2-Azino-bis-(3-ethylbenzothiazolin-6 sulphonlic acid) diammonium salt]</td>
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<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>El</td>
<td>Pancreatic elastase 1</td>
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<td>ELISA</td>
<td>Enzyme linked immuno sorbent assay</td>
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<td>ERCP</td>
<td>Endoscopic retrograde cholangiopancreatography</td>
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<td>NBT-PABA</td>
<td>N-benzoyl-L-tyrosyl-Para amino benzoic acid</td>
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<td>PLT</td>
<td>Pancreolauryl test</td>
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<td>POD</td>
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ABSTRACT
The major objective of this work was to establish the assay of faecal elastase 1 in spot stool samples as an exocrine pancreatic function test for the Korle-Bu teaching hospital.

The assay of faecal elastase 1 was carried out in spot stool samples from apparently healthy persons and subjects with various pancreatic and non pancreatic diseases. The result obtained confirmed the diagnostic efficiency of pancreatic elastase 1 determination in stool as a standard test of exocrine pancreatic function.

The results of the faecal elastase 1 test were able to characterise the degree of severity of exocrine pancreatic insufficiency into, (1) the mild to moderate group with elastase concentration between 100 and 200 μg/g stool and (2) the severe pancreatic insufficiency group with elastase concentration of <100 μg/g stool.

Elastase 1 activity in spot stool samples from apparently healthy group ranged from 165 to 870 μg/g with a (mean ± SEM) of 379 ± 41 μg/g, the enzyme activity in patients with various gastroduodenal diseases but non pancreatic in origin ranged from 85 to 1171 μg/g, with a (mean ± SEM) of 479 ± 41 μg and the range of 20-285 μg/g with a (mean ± SEM) of 112.9 ± 11.6 μg/g in the pancreatic disease group.

The pancreatic elastase 1 was found to be stable in faeces for several weeks when stored frozen and offer the possibility of saving cost to the patient when determinations are done in batches.

The results show that the determination of faecal pancreatic elastase 1 concentration in spot stool samples of patients with epigastric pain provides a reliable differential diagnosis of exocrine pancreatic insufficiency.
1.1 INTRODUCTION

The pancreas is a soft, flattened, elongated gland which lies behind the peritoneum of the posterior abdominal wall. The head of the pancreas is on the right side and lies within the “C” shaped concavity of the duodenum at the level of the body of the second lumbar vertebra. The tail of the gland lies to the left and located at the level of the body of the first lumbar vertebra.

The pancreas has an arterial supply of blood from the common hepatic, splenic and other arteries and venous drainage to the splenic, superior mesenteric and portal veins, (Moosa and Mulholland 1991) (Fig. 1).

In 90% of individuals the main pancreatic duct joins the common bile duct to enter the duodenum as a single duct. The pancreas contains both exocrine and endocrine components with emphasis on the exocrine function. The acinar cell (80%), is the dominant cell type of the pancreas and constitutes by far the most important for the exocrine function, whiles the duct cell comprises 18% with endocrine tissue comprising 2% (Moosa and Mulholland 1991).

The exocrine pancreas secretes a fluid containing such electrolytes like sodium, chlorides and bicarbonates as well as a large number of functionally important pro-enzymes like trypsin, proteases, lipase, amylase, elastases, carboxypeptidases, chymotrypsins, co-lipases and phospholipase.
FIG. 1  The pancreas: (a) anterior view; (b) posterior view.

Meal induced pancreatic secretion is assumed to be the most important exocrine function of the pancreas, because total lack of pancreatic secretion results in maldigestion and poor absorption of several nutrients. Nevertheless, the vast majority of studies on regulation of pancreatic secretion deal with stimulated pancreatic secretion.

The most important control of acinar enzyme and fluid secretion is exerted by the hormone cholecystokinin (CCK), with secretin and bombesin as other hormones available for exocrine secretion.

Exocrine pancreatic function which includes the hydrokinetic function of duct cells and the exocrine function of acinus cells are best estimated with the secretin–cholecystokinin test which is the gold standard of pancreatic function. This test described about half a century ago had seen numerous modifications and standardisations to remain acceptable for use, Niederau and Grendell 1985.

The secretin–cholecystokinin test is one of the direct function tests which are frequently used in Northern America but are uncommon in Europe because of practical disadvantages: They are invasive, time consuming, expensive, uncomfortable to the patient, require fluoroscopic tube placement and are not internationally standardised, (Walkowiak J. et al. 1999; Loser C. et al. 1996; and Katschinski M. et al. 1997). Due to these disadvantages the secretin-choleystokinin test is regarded unsuitable for routine application in Europe and therefore confined to few academic centres only (Loser C 1997).

Duodenoscopy and endoscopic cannulation of the papilla of vater with visualisation of the biliary tree and pancreatic duct using endoscopic retrograde cholangiopancreatography is invasive, expensive and available only in referral centres in Europe and USA.
Although this is the gold standard test of the imaging procedures it is not available here in Ghana.

Lundh's test, also a direct measure of pancreatic function requires exogenous substrates to stimulate the pancreas is also invasive as it involves the intubation of the patient.

For the same reasons as above neither the secretin-cholecystokinin or the Lundh test is performed here in the Korle Bu teaching hospital. It is therefore imperative that a useful laboratory based pancreatic function test has to be employed which will be helpful to support clinical findings in the diagnosis and treatment of patients who have recurrent abdominal pain and whose imaging studies are equivocal to support clinical findings.

The differential diagnosis of chronic pancreatitis present two major challenges: the first involves identifying the patients with abdominal pain who have chronic pancreatitis but the results of whose imaging procedures reveal minimal or no morphologic changes. In these cases, pancreatic function tests may be of value (Steer et al., 1995); the second challenge involves identifying the patients who have symptoms or findings suggestive of chronic pancreatitis but in fact have pancreatic cancer.

Ductal obstruction can be caused by lesions that interfere with the flow of pancreatic juice into the duodenum resulting in pancreatitis; attacks of pancreatitis may occur as a result of pancreatic, ampullary or duodenal tumours, pancreatic duct strictures and sphincter of Oddi dysfunction (Steer et al., 1995). Persons with pancreas divisum, an anatomic variant which occurs when the dorsal and ventral pancreatic segments fail to fuse, may have a relative obstruction to juice flow, since most of the pancreatic juice must pass through the small orifice of the lesser papilla, (Cotton et al 1980).
In the past, pancreatic function tests that require gastrointestinal intubation to obtain exocrine pancreatic secretion have played a prominent role in the diagnosis of pancreatic cancer. Low outputs of pancreatic enzymes after exogeneous stimulation of the pancreas are shown to occur in 90% of patients with cancer of the head of the pancreas (DiMagno et al. 1977).

Several laboratory based tests have been used in the past which are referred to as indirect tests of pancreatic function which do not involve the intubation of the patient for the purposes of obtaining duodenal fluid for biochemical assay of electrolytes and enzymes. They include fluorescein dilaurate and N-benzoyl- L-tyrosyl-Para amino benzoic acid (NBT-PABA) tests which describe the exogenous stimulation of the pancreas with synthetic peptides. These tests lack the sensitivity and specificity to diagnose patients with chronic pancreatitis (Steer et al. 1995; Lankisch, 1982), the latter test is no longer available in most European countries, (Soldan et.al. 1997).

Serum pancreatic enzymes have been used over many years in the diagnosis of pancreatic diseases and their measurements have been based on clinical and experimental evidence of the relationship between the variations in their levels and damage to the exocrine pancreas, (Ventrussi et al 1989).

The pancreatic enzymes routinely measured in the laboratory are amylase, pancreatic iso amylase, lipase and trypsinogen. Generally these tests lack the specificity and sensitivity to diagnose patients with chronic pancreatitis (Steer et al 1995 and Lankisch 1982).
Discrepancies concerning the relative diagnostic value of these laboratory tests have resulted in confusing opinions expressed by clinicians and which are attributable to variability and absence of standardisation of chemical substrates used in different centres, (Clavien et al 1989).

For many years, methods proposed to measure tryptic activity in stool have been ineffective and misleading because they lack the sensitivity and specificity for the substrates in the measure of trypsin and chymotrypsin, (Delmar et al 1979) and (Haverback et al 1963).

Munch and Amman (1992) used a faecal immunoreactive lipase as a new tubeless test in the diagnosis of pancreatic exocrine insufficiency but despite its excellent diagnostic specificity it has a very low sensitivity. Although faecal chymotrypsin determination is widely accepted as a valuable screening method to detect exocrine pancreatic insufficiency, it is beset with few problems which include enzyme inactivation during intestinal passage, the effect of dietary protein content on proteolytic enzymes and the effect of faecal pH on methodology (Riedel et al., 1991).

Faecal fat analysis is used as a measure of steatorrhoea, which is a clinical sign of progressive severe exocrine pancreatic insufficiency. This test is however both insensitive and non-specific in the diagnosis of mild to moderate degrees of chronic pancreatitis and is also unpopular among laboratory staff, (Stein et al., 1996).

Breath tests using stable isotopes of carbon or hydrogen have been established for the evaluation of various gastrointestinal functions including measurement of exocrine pancreatic insufficiency, but despite several promising studies, current available breath tests are not sufficiently validated for clinical application (Loser et al., 1998).
Breath tests which are based on the detection of decreased lipolytic activity of the pancreas cannot sufficiently detect mild to moderate forms of exocrine pancreatic insufficiency. Gastric emptying of the tracer, mucosal absorption, hepatic circulation, and metabolism among others, are the factors that affect these tests (Loser et al.; 1997 and 1998).

All of the above mentioned indirect pancreatic function tests proved to have limited sensitivity especially in mild and moderate forms of exocrine pancreatic insufficiency and most of them are affected by gastrointestinal operations, faecal pH and drugs which lower their specificity.

In the last ten years, an enzyme linked immunoassay (ELISA) for human pancreatic elastase I has been developed for measuring the exocrine capacity of the pancreas. Pancreatic elastase I is present in both human pancreatic secretions and faeces (Sziegoleit et al., 1989), and the first promising results of the faecal elastase I has been reported (Soldan et al 1997). Faecal elastase I is a highly sensitive proteolytic, pancreas specific enzyme. It is present in human pancreatic juice at a concentration of between 170 and 360μg/ml (Sziegoleit A 1984). During intestinal passage elastase I is bound to bile acids and has been shown to be a transport protein for cholesterol.

In contrast to most of the other pancreatic enzymes, elastase I is stable during intestinal passage and it is not degraded. Its concentration in stool is found to be five to six times those determined in pancreatic juice and thus reflect pancreatic function (Sziegoleit et al 1989).

Elastase I is determined immunologically with a new “sandwich” type enzyme immunoassay (Scheefers- Borschel et al 1992).
Human faecal elastase I is immunologically specific; the test is not affected by enzyme replacement therapy, it is quite stable between the passage of stool and time of analysis as there is no loss of immunoreactivity in samples (Stein et al., 1996). Samples can be stored at least for a week at room temperature, or frozen for several months (Loser et al., 1997; and Stein et al., 1996).

Faecal elastase - I has both high diagnostic sensitivity and specificity compared with faecal chymotrypsin and faecal lipase, (Stein et al., 1996).

Loser 1997, compared the sensitivity and specificity of elastase -I and chymotrypsin in faeces of patients with mild, moderate and severe exocrine pancreatic insufficiency according to results of secretin-caerulin test on those patients and reported that for a cut-off concentration for faecal elastase -I of 200µg/g of stool and a cut-off of chymotrypsin activity of <3 u/g of stool.

Faecal elastase sensitivity for mild exocrine pancreatic insufficiency was 63% and for both moderate and severe forms the sensitivity was 100% and specificity of 93% in contrast with chymotrypsin whose sensitivity for mild was 25%, 50% for moderate and 86% for severe exocrine pancreatic insufficiency and a specificity of 89%.

Faecal elastase- I determination as evaluated on the basis of the secretin-caerulin test as the “gold standard” of pancreatic functioning test is highly superior compared to faecal chymotrypsin activity (Loser et al., 1996).

The superiority of the faecal elastase-I over the faecal chymotrypsin in the diagnosis of exocrine pancreatic insufficiency has been pointed out by several studies (Dominguez-Munoz et al., 1995; Glasbrenner et al., 1996; Loser et al., 1996 and Gullo et al., 1999)
Laboratory based tests as well as imaging studies are used to support clinical findings in the diagnosis of patients with pancreatic disease, however due to lack of sensitivity and specificity of the old laboratory tests, as discussed so far, to characterise the degree of severity of patients with pancreatic disease, it has become necessary to employ tests that are organ specific and whose methods are sensitive. These criteria are achieved by using faecal elastase-I which uses a simple spot stool from a patient for the assay.
AIMS AND OBJECTIVES

The present study was aimed at establishing the assay of faecal elastase-I as a laboratory diagnostic tool for assessment of pancreatic diseases including chronic pancreatitis and carcinoma of the pancreas at the Korle-Bu hospital. The assay may be used to evaluate the pancreatic function in patients with various gastrointestinal problems associated with recurrent upper abdominal pain and who are being treated at the Korle-Bu hospital.

The specific objectives are to

1. Evaluate pancreatic function in the patients with cancer of the head of pancreas.
2. Characterise the degree of severity of chronic pancreatitis using cut off levels established by the study.
3. Define levels of faecal elastase-1 in a control group comprising
   (a) Apparently healthy Ghanaians
   (b) Patients with gallstones without bile duct obstruction.
   (c) Peptic ulcer patients and
   (d) Irritable bowel syndrome patients.
1.2. **LITERATURE REVIEW**

1.2.1 **Diagnostic test of pancreatic disease**.

Several tests of exocrine pancreatic function have evolved over the past 50 years, since the pioneer work of Largerlof in 1942 on the evaluation of secretin test in pancreatic function and disease.

Diagnostic tests of pancreatic diseases have been divided into two main categories as shown in Tables 1 and 2. Tests of pancreatic morphology and tests of pancreatic function. Pancreatic functions tests are sub divided into direct and indirect tests. These are direct tests which stimulate pancreatic flow, bicarbonate and enzyme secretion. (Table 2)

A. Direct tests of pancreatic secretory capacity involves the collection of duodenal or pancreatic juice after pancreatic stimulation with exogenous hormones such as secretin, cholecystokinin, and caerulin and combinations of the secretagogues secretin-cholecystokinin or secretin-caerulin, this is reported as the gold standard pancreatic function test (Steer *et al.*, 1995).

B. Indirect tests which utilise nutrients to stimulate pancreatic enzyme secretion and act as substrates for the determination of proteolytic, lipolytic or amylolytic enzymes. These include Lundh test, N-benzoyl-L-tyrosyl-para amino benzoic acid test (NBT-PABA) and Pancreolauryl test.

Faecal tests which include faecal fat, and faecal chymotrypsin, faecal elastase 1 and faecal trypsin; and also serum and urine enzymes like amylase and serum lipase, are all classified as indirect tests.
Indirect pancreatic function tests are a practicable alternative to direct pancreatic function tests for diagnosing exocrine pancreatic insufficiency.

They allow staging of the disease and thereby facilitate comparison of different studies, and involve neither side effects, risks, nor complications for patients as well as investigators, and laboratory staff alike Lankisch 1993.
**Table 1 - Diagnostic tests of pancreatic disease: Test of pancreatic morphology.**

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<td>Radioisotopic pancreatic scan</td>
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<td>Endoscopic Retrograde Cholangiopancreatography (ERCP)</td>
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<td>Cytological studies</td>
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### Table 2 Diagnostic Tests of pancreatic disease: Test of pancreatic function

**A. Direct tests**

- Secretin test
- Secretin-Pancreozymin test
- Secretin - Bombesin test
- Radio selenium test
- Secretin-terulean test

**B. Indirect tests:**

- Lundh tests
- NBT-PABA tests
- Pancreolauryl tests
- Serum Enzymes in response to pancreatic stimulation

**(Faecal tests)**

- Chymotrypsin
- Trypsin
- Elastase-1
- Lipase

**Serum and urine enzymes**

- Amylase
- Lipase
1.2.2 Imaging procedures

Various imaging tests have been developed to help with the diagnosis of pancreatic diseases, these include, ultrasound, computed tomography and endoscopic retrograde cholangiopancreatography (ERCP)

ULTRASOUND

This is achieved with a scan mode instrument. Echo patterns are displayed and recorded photographically as cross sectional and longitudinal images. Ultrasound is an imaging method which has become a less technical investigative procedure which is used to visualise the pancreas. As the most readily available and least expensive of the imaging modalities, it is useful in the detection of pancreatic tumours as well as evaluation of the extent of disease. Ultrasound is less sensitive in the identification of resectable disease, mainly due to undetected vascular involvement (Mittelstaedt 1991).

Ultrasound has a reported sensitivity of up to 70% with a specificity of 90%.

Results of the scan are usually interpreted as Normal or abnormal.

ENDOSCOPIC RETROGRADE CHOLANGIO PANCREATOGRAPHY (ERCP)

Duodenoscopy and endoscopic cannulation of the papilla of vater with visualization of the biliary tree and pancreatic duct was first described in 1968 followed by reports on ERCP in early 1970s (McCune et al., 1968).

ERCP is indicated in patients with chronic pancreatitis, only when ultrasonography is ambiguous, when preoperative mapping is required or when endoscopic therapy is being considered (Di Magno et al., 1977).
In chronic pancreatitis, ductular abnormalities range from normal to severe destruction. New classifications of ductular changes in chronic pancreatitis was suggested during an international workshop in 1983 (Axon et al., 1984). The researchers suggested that the pancreatogram in chronic pancreatitis should be divided into normal, equivocal, mild, moderate and severe alterations, which are either diffuse or localized. In patients with pancreatic carcinoma, pancreatograms from ERCP show five major types including complete obstruction, ductal tapering and cavity formation (Axon et al., 1984).

ERCP is considered intermediate in both cost and degrees of patient discomfort, clinical evaluation including history and physical examination and routine laboratory tests are considerably less expensive and ease on the patient than ERCP (Huibregtse et al., 1991).

ERCP is achieved by passing a duodenoscope to visualize the ampulla and then passing a teflon catheter into the papillary orifice.

Findings of localized narrowing, obstruction or displacement of the pancreatic duct were considered indicative of pancreatic cancer, where as irregular ductal dilation and stricture were indicative of pancreatitis (Di Magno et al., 1977).

RADIOISOTOPIC PANCREATIC SCAN is achieved with a Rh-Gamma Camera providing a scan after injection of (75 g) methionine. Decrease uptake of the isotope, non visualisation and solitary defects cause the scan to be interpreted as abnormal (Di Magno et al., 1977).
In **THERMOGRAPHY**, the pictorial recording of natural infra red emission of the body is performed. Anterior and posterior scans are made with the recording of increased heat corresponding to the surface anatomy (Di Magno *et al.*, 1977).

**ARTERIOGRAPHIC VISUALISATION** of the pancreas is achieved by a separate single and sequential injections of the celiac and superior mesenteric arteries. Arterial occlusion and narrowing and venous abnormalities, such as compression and occlusion are manifestations of pancreatic cancer (Di Magno *et al.*, 1977).

**COMPUTED TOMOGRAPHY** (CT)

Since the first in vivo application of computed tomography in 1971, computed tomography has become the imaging method of choice under most circumstances for suspected pancreatic abnormalities. CT is highly accurate in staging the extent of pancreatic carcinoma and has been shown to be more accurate than angiography in demonstrating tumour involvement of major peripancreatic vessels. A false positive CT diagnosis of pancreatic carcinoma has been reported in 8% of patients.

Computed tomography has a reported sensitivity of up to 90% and a specificity of 85%. The changes in chronic pancreatitis which can be seen in 85% of cases include parenchymal atrophy, parenchymal and ductal calcifications, pancreatic ductal dilatations and findings of biliary obstruction (Baron 1991).
1.2.3. **Test of pancreatic function**

Bicarbonate concentration in pancreatic secretion has been accepted as accurate measure of exocrine pancreatic function, because the principal physiological function measured for the exocrine pancreas is maximal bicarbonate output which is related to the functional mass of the tissue,(Steer et al., 1995). It is easy to measure and the concentration in normal subjects after stimulation with secretin is well defined i.e. above 80 mmol/L during an 80min collection period (Arvanitakis et al., 1978; Lankisch 1982; Niederau and Grendell 1985).

Pancreatic bicarbonate concentration however depends on factors such as plasma bicarbonate concentration, flow rate of pancreatic juice, and dilution of pancreatic secretion by bile and gastric juice in the duodenum(Arvanitakis et al., 1978).

Pancreatic enzyme secretion in response to direct or indirect stimulation is considered a less accurate measurement of functioning acinar cells because of the wide variation in normal subjects (Wormsley 1978), and this is complicated by the fact that intraduodenal concentration of pancreatic enzymes does not reflect concentration in pancreatic secretion due to differences in the activation rate of pro enzymes, the dilution of the enzymes by other secretions and the effect of luminal pH (Wormsley 1978).

The collection of duodenal juice is technically difficult and invasive and requires the constant attention of well trained physician or technician to be properly performed (Moeller et al., 1972). The collection of duodenal juice remains a technical problem. The volume of duodenal juice secreted must be completely aspirated (there must be no loss into the jejunum and no duodenogastric reflux and duodenal juice should not be contaminated by gastric juice).
Balloons which have been developed for occlusion of both the pylorus and the distal duodenum have not been generally accepted (Niederau and Grendell 1985), because the balloons may provide painful sensations and could affect intestinal motility, which may result in an alteration of pancreatic secretion. However, pure pancreatic juice has been collected after endoscopic cannulation of the pancreatic duct. This technique however does not ensure complete collection of pancreatic juice, so that only concentration but not the outputs of enzymes and bicarbonate could be determined. (Niederau and Grendell 1985).

**Direct Tests**

In the direct tests of exocrine pancreatic function, there is considerable controversy about which secretory stimulus or combination of secretory stimuli to use to improve the sensitivity of the test. Secretin has been used alone as well as combination with cholecystokinin, caerulein and bombesin as secretory stimuli for the exocrine pancreas.

The secretin test is based on the assumption that a decrease in the capacity to secrete fluid, bicarbonate and enzymes indicates pancreatic damage, and that the dose of stimulant which provides maximal stimulation to a normal gland also exerts the same effect on the diseased pancreas (Wormsley 1978).

After secretin administration, only a correct evaluation of the hydrokinetic function of the pancreas may be expected, cholecystokinin was therefore added to the test procedure in order to assess the ecbolic pancreatic function (Burton et al., 1960).

The secretory stimulants have been administered at different doses, by different routes, and in different orders of administration (Arvanitakis et al., 1978; Niederau and Grendell 1985 and Lankisch 1982).
Other direct tests of pancreatic function include the administration of radio-labelled amino acids (75 se-methionine) given intravenously and rapidly taken up by pancreatic acinar cells and incorporated into pancreatic digestive enzymes. This test which requires duodenal intubation, as well as pancreatic stimulation and administration of radioisotope, is considered even more invasive than the gold standard secretin-cholecystokinin test (Niederau and Grendell 1985).

The pancreozymin-secretin test has been standardised, and discrete diagnostic parameters for diagnosing pancreatic exocrine insufficiency have been given. In their studies Mueller et al., 1972 adhered closely as possible to the original methodology for the pancreozymin -secretin test whereby a Dreilling double-lumen tube was positioned fluoroscopically after at least an 8 hour fast. The tip of the Dreilling tube was positioned slightly proximal to the ligament of Treitz to allow for the expected effect of pancreozymin on intestinal motility, as the tube was advanced.

Both tubes were connected to intermittent suction with 70 mm Hg intermittent negative pressure. After a 20 minute baseline collection, pancreozymin was given intravenously and, four consecutive 20 minute collections were obtained. All samples were collected on ice.

Analytical determination in duodenal juice include the volume of secretion and bicarbonate concentration as well as the measurement of the activities of trypsin, amylase and lipase.

The Indirect Pancreatic Function Tests

Lundh Test- Pancreatic stimulation by a test meal was introduced as a diagnostic procedure by Lundh in 1962, the test is widely used in Europe but infrequently applied in the Americas (Klaus et al., 1975).
The test involves the assessment of the pancreatic secretory response to a test meal and thus to the endogenous release of hormones. After intubation of the duodenum with a tube, a test meal (300 ml) is given which contains 6% Fat, 5% protein and 15% carbohydrate to the patient. Duodenal contents are aspirated either in four consecutive 30 min fractions or a single 2 hour collection. Trypsin which is measured in the duodenal aspirate is less sensitive to changes of pH but more discriminating than lipase and phospholipase in exocrine pancreatic dysfunction (Lankisch 1982), although additional determination of lipase or amylase has been recommended to improve the diagnostic sensitivity of the Lundh’s test (Ihse et al, 1977).

Most investigators who favour the Lundh test stress that it is a simple, physiological and inexpensive test that is possible to perform in most hospitals. Since the direct tests of exocrine pancreatic function are impracticable and results difficult to interpret, and since the busy clinician needed a simple test of pancreatic function with a reasonable diagnostic success rate, the Lundh test was likely to be more useful in clinical practice (Waller 1975). However there are certain disadvantages which limit the usefulness of the test (DiMagno and Summerskill 1972; Niederau and Grendell 1985). Since the test is based on the endogenous release of secretin and cholecystokinin, the test may not be valid where there is mucosal damage as in coeliac disease, because of impaired hormonal release; hence the test cannot differentiate between malabsorption of pancreatic or non pancreatic origin.

Since the release of hormones depend on the integrity of gastroduodenal anatomy, the Lundh test is not applicable in the evaluation of pancreatic function in patients with vagotomy or Bilroth II subtotal gastrectomy (Arvanitakis and Cooke 1978), neither does it provide information regarding the volume and bicarbonate secretory capacity of the pancreas (Arvanitakis and Cooke 1978; Niederau and Grendell 1985).
The results are influenced by several non pancreatic factors, such as gastric emptying, intraduodenal pH, and endogenous release of hormones. The endogenous stimulation has been proposed to be more physiological than the exogenous stimulation by secretin or cholecystokinin (Niederau & Grendell 1985).

In a progress review by James, it was reported that the Lundh test could not distinguish between carcinoma of the pancreas and chronic pancreatitis (James 1973).

**Tubeless Tests**

In tubeless pancreatic function tests usually a compound is given which is split into two or more parts by pancreatic enzymes in the duodenum. One of these products is readily absorbed in the small intestine, conjugated in the liver and excreted in the urine. The recovery rate within a given time is taken as an index of pancreatic function.

a) N-Benzoyl-L-Tyrosyl-P-Aminobenzoic Acid (NBT-PABA) is a synthetic tripeptide that is specifically cleaved by the pancreatic enzyme chymotrypsin only in the duodenum leading to the liberation of para amino benzoic acid (PABA), (Imondi et al., 1972).

This oral pancreatic function test is essentially an indirect test of chymotrypsin secretion. The peptide is applied together with a test meal which is usually a standard breakfast. Furthermore chymotrypsin specificity of benzoyl-L-tyrosyl-PABA lies in the conversion of the substrate to only two products PABA and N-benzoyl-L-tyrosine.

During the incubation with intestinal mucosal homogenate of rats and guinea pigs, Yamato and Kinoshita, 1978 found that the resultant products were rapidly absorbed from the small intestine and excreted in the urine and that the absorption rate correlated with the intestinal chymotrypsin activity.

The results of the first human studies showed that patients with pancreatic exocrine insufficiency had consistently lower excretion rates of PABA than healthy controls or
patients with non pancreatic diseases (Arvanitakis et al., 1976 and Gyr et al., 1976).

The test procedure is not completely standardised and results from previous studies show inconsistencies (Lankisch 1982).

While the sensitivity of the test is good in severe and moderate exocrine pancreatic insufficiency, it is not fully established in cases with only slight impairment of pancreatic function (Lankisch 1982; Niederau and Grendell 1985); whilst false positive results have been reported with patients having non pancreatic diseases such as small bowel disease, renal insufficiency and chronic liver disease.

The NBT-PABA test is no longer available in most European countries (Soldan et al., 1997).

Pancreolauryl Test

Like the NBT-PABA test the pancreolauryl test is a simple and non invasive tubeless function test, the test compound, fluorescein dilaurate is a synthetic, poorly water soluble ester which is hydrolysed by specific aryl esterases from pancreatic juice into lauric acid and water soluble fluorescein. It is readily absorbed from the small intestine, conjugated in the liver and excreted in the urine. The test capsules are given orally in the middle of a standard breakfast, (50g white bread, 20g butter and one cup of tea). Although this test has been standardised and available in some countries commercially,( Niederau and Grendell 1985) several reports of a variety of false positive results similar to those of the NBT PABA - test has been reported, whilst treatment with pancreatic enzymes or vitamin B_{12} interfere with the fluorescein measurement and should be discontinued five days before the test (Niederau and Grendell 1985).
Breath Tests

In general, breath tests are based on the detection of decreased lipolytic activity of the pancreas, but cannot sufficiently detect mild to moderate forms of exocrine pancreatic insufficiency and are valid tests for pancreatic steatorrhoea only.

Besides the duodenal activity of lipolytic pancreatic enzymes, gastric emptying of the tracer, mucosal absorption, hepatic circulation, metabolism, endogeneous CO\textsubscript{2} production and pulmonary excretion are factors that might contribute to the reduction of sensitivity and specificity of breath tests (Loser \textit{et al.}, 1998).

Faecal Tests

72 hours faecal fat analysis remains the standard test for diagnosing and quantifying fat malabsorption in chronic pancreatitis, even though it is both insensitive and non specific in the diagnosis of chronic pancreatitis (Hoffman \textit{et al.}, 1985). Furthermore, faecal fat analysis is also time consuming and unpopular among laboratory staff.

The approach to assess exocrine pancreatic function by analysing a single specimen of stool has attracted much attention, assays determining faecal activities of trypsin, chymotrypsin and recently immunoreactive lipase have been developed but the main problem with these assays is the considerable overlap between data collected from healthy subjects and data from patients with impaired pancreatic function, clearly reflecting a deficit in sensitivity (Katschinski \textit{et al.}, 1997).
The development of synthetic low molecular substrates and specific titrimetic estimation of trypsin and chymotrypsin in stool have constituted the main faecal enzyme estimation for exocrine pancreatic insufficiency. Patients with the malabsorption syndrome with aetiologies other than pancreatic exocrine insufficiency had normal stool trypsin and chymotrypsin values and most patients with functionally manifest chronic pancreatitis and pancreatic cancer consistently revealed low faecal chymotrypsin activity (Amman et al., 1968).

For many years, many methods proposed to measure tryptic activity in stool have been ineffective due to lack of sensitivity required to detect small amounts of activity (Haverback et al., 1963) and also the lack of substrates specific for trypsin and chymotrypsin, due to hydrolysis by proteolytic enzymes produced by intestinal bacteria and proteolytic enzymes produced by the succus entericus. Haverback described a method utilising the substrates p-toluene sulfonyl-l-arginine methyl ester (TAME) and N-acetyl-L-tyrosine ethyl ester (ATEE) for measurement of trypsin and chymotrypsin in human faeces respectively and showed that these substrates were not affected by proteases in the intestine or succus entericus (Haverback et al., 1963).

However in patients with chronic pancreatitis and malabsorption on the basis of exocrine insufficiency of the pancreas, the chymotrypsin determination appear to be a better index for pancreatic insufficiency than trypsin values because chymotrypsin was much more stable in human intestinal juice than was trypsin (Wohlman et al., 1962).

It is recognised that stool enzymes will reflect the amounts of proteolytic enzymes that the pancreas secretes into the intestine and follows therefore that patients with less pancreatic damage and consequently a lesser degree of pancreatic insufficiency will have stool enzyme values which will be more difficult to interpret.
The range of stool trypsin and chymotrypsin values was rather wide among these patients taking pancreatic substitution therapies (Haverback et al., 1963), hence to accurately assay the basal levels of proteolytic enzymes in stool in a patient on pancreatic substitution therapy, the replacement therapy must be discontinued for a period of at least 3 or 4 days. At the end of this time period, stool enzymes usually have reverted to their basal levels (Haverback et al., 1963).

To the question as to whether quantitation of the stool enzymes over a period of 24 to 72 hours was a better guide than spot stool, a comparative study by (Haverback et al., 1963), showed good agreement for both control subjects and patients with pancreatic exocrine insufficiency.

The direct pancreatic function tests described earlier such as the secretin -cholecystokinin test have the highest sensitivity and specificity for the detection of exocrine pancreatic insufficiency and have been described as the ‘gold standard’ for testing pancreatic function (Dominguez-Munoz et al., 1995; Loser et al., 1996). Direct pancreatic function tests however, have various practical disadvantages: they are time consuming, invasive, expensive, uncomfortable, not standardised and require fluoroscopic tube placement (Dominguez-Munoz et al., 1995; Loser et al., 1996; Stein et al., 1997 and Soldan et al., 1997). The secretin - cholecystokinin test is unsuitable for routine application and is therefore confined to a few academic centres in Europe (Loser et al 1996).

Several simple indirect pancreatic function tests for clinical practice, such as the NBT-PABA test, fluorescein dilaurate, faecal chymotrypsin and different breath tests
established have been shown to have limited sensitivities in mild and moderate exocrine pancreatic insufficiency (Loser et al., 1996; Soldan et al., 1997 and Loser et al., 1998). These tests are interfered with by some drugs including enzyme replacement therapies, which necessitate their withdrawal days before the test. Diarrhoea, pH and gastrointestinal operations lower their specificities (Niederau and Grendell, 1985).

In general, these indirect pancreatic function tests have been found to be unreliable for clinical practice (Soldan et al., 1997; Gullo et al., 1999 and Loser et al., 1997).

**Pancreatic elastase 1**

In the last ten years pancreatic elastase had been isolated and further characterised as a human and pancreas specific enzyme that is not degraded during intestinal passage and which is five to six fold enriched in faeces compared with duodenal juice (Sziegoleit and Linder, 1991 and Sziegoleit et al., 1989).

Furthermore, a highly sensitive enzyme linked immunosorbent assay (ELISA) for human faecal and duodenal elastase-1 determination using two specific monoclonal antibodies is commercially available (Szigoleit et al., 1989).

Early clinical studies gave promising results in patients with exocrine pancreatic insufficiency for determination of faecal elastase-1 concentration in comparison with the fluorescein dilaurate test, (Dominguez-Munoz et al., 1995 and Glasbrenner et al., 1994).

The serum pancreolauryl test, although a simple test has a high accuracy for the diagnosis of moderate to severe chronic pancreatitis, but with limited specificity in the presence of intestinal malabsorption, cholestasis as well as gastrectomy cases (Niederau and Grendell, 1985).
Dominguez-munoz et al., 1995, showed that patients with chronic pancreatitis had a significantly decreased faecal elastase 1 excretion compared with patients with gastrointestinal and hepatic diseases. On the contrary serum pancreaolauryl tests results were not different between patients with chronic pancreatitis and patients with gastrointestinal diseases that interfere with normal digestion and/or absorption (Dominque-Munoz et al., 1995).

Andreas Sziegoleit described the properties of a novel pancreatic proteinase previously isolated as a cholesterol-binding protein with an intrinsic proteolytic activity and having an apparent molecular weight of 28,000. The protein was shown to be stable towards the actions of trypsin and chymotrypsin. It’s proteolytic activity was demonstrated by its ability to hydrolyse casein and staphylococcal anti-toxin. The cholesterol binding pancreatic proteinase was detected by fused rocket immunoelectrophoresis with specific antiserum after large scale purification and extraction from necrobiotic human pancreas organs. Sziegoleit observed that the enzyme failed to hydrolyse the substrates for trypsin and chymotrypsin and suggested that the proteolytic action of the cholesterol binding pancreatic proteinase discriminates from trypsin and chymotrypsin (Sziegoleit et al., 1985).

The cholesterol bound pancreatic proteinase was stable at 4°C over several months without any loss of enzymic activity but lost its esterolytic activity at a rate of 8% per week at room temperature with complete inactivation at 56°C within 5min (Sziegoleit et al., 1985).

Autoradiographs revealed binding of the pancreas protein to two serum proteins namely alpha 1 - antitrypsin and alpha 2 macroglobulin with the peptide bond at the carboxy side of alanine residues being one of the favoured sites of proteolytic attack, a property in common with porcine elastase (Mallory and Travis, 1975).
Although the cholesterol-binding pancreatic proteinase was discovered immunologically by its deoxycholate binding capacity, its proteolytic activity was found as an additional function, whose specificity indicated a relationship to the elastases (Sziegoleit et al., 1985).

Sziegoleit 1985 demonstrated that both the cholesterol-binding pancreatic proteinase and human pancreatic elastase 1 give identical molecular weights by SDS/polyacrylamide gel electrophoresis and both proteins show the same electrophoretic mobilities as well as showing strong cross reactivity between each other indicating that both proteins are immunologically identical.

The cleavage patterns of human pancreatic elastase 1, and cholesterol-binding pancreatic proteinase are very similar, they both split peptide bonds adjacent in neutral amino acids with hydrophobic residues like Alanine, Valine and Leucine (Sziegoleit et al., 1985). Previously Feinstein 1974 isolated two elastases from human pancreas and demonstrated that elastase 1 has elastolytic activity common with porcine elastase (Feinstein et al., 1974). Mallory and Travis also isolated pancreatic protease E with characteristics similar to that of elastase 1, which hydrolyses the synthetic substrate [n-acetyl-l-alanyl-l-alanyl-l-alanine methyl ester ac(Ala)J one] (Mallory and Travis, 1975).

The elastases have the ability to hydrolyse elastin (Feinsten et al, 1974), as well as the synthetic substrates Suc-Ala-Ala-Ala-NH-Np for the elastase 1 and the cholesterol binding pancreatic proteinase; thus it was suggested that cholesterol binding pancreatic proteinase/elastase 1 be given its own characteristic name (Sziegoleit et al., 1985).

It is clear from the above that pancreatic elastase 1 is a proteolytic enzyme which had been isolated and given various names by different workers.
1.2.4. Pancreatic elastase 1 (Pancreato peptidase E, EC 3.4.21.11)

Elastase-1 is a serine protease which is pancreas specific. The enzyme rapidly hydrolyses the scleroprotein elastin, (Largman et al., 1976). Because of its unique specificity in degrading elastin, pancreatic elastase-1 has been implicated in emphysema and has also been shown to produce the vascular injury observed in acute pancreatitis (Largman et al., 1976).

The enzyme is quite stable at neutral or slightly alkaline pH, but unstable at pH <4.0. Its molecular weight has been estimated to be 30,000 + 1000 Daltons (Sziegoleit 1985, Feinstein et al., 1974 and Mallory and Travis 1975), and its co-efficient of extinction reported as 24.5.

Human pancreatic elastase 1 is extremely stable in vivo, and detectable in pancreatic juice as well as in faeces by quantitative immunoreactions (Sziegoleit et al., 1989).

In the gut, it combines with cholesterol and bile acids. The unbalanced intestinal absorption of lipolytic products notably bile acids and cholesterol results in the elastase 1 neutral sterol complex found in faeces (Sziegoleit et al., 1991).

Compared with values found in pancreatic juice the concentrations of elastase-1 is five to six times greater in stool, leading to the conclusion that the enzyme is not essentially degraded during intestinal passage thus the enzyme in stool reflect pancreatic function (Sziegoleit et al., 1989). It was also observed that storage of stool samples at room temperature for 3 days does not influence the enzyme concentration indicating the stability of the enzyme (Stein et al., 1996).

A Sandwich-type enzyme linked immuno sorbent assay for human pancreatic elastase 1 has been described (Sziegoleit et al., 1989). Using highly purified reagents, the test seems to be sensitive and specific for the detection of elastase 1 (Sziegoleit et al., 1989).
For the diagnosis of acute pancreatitis the serum levels of elastase 1 seems to be superior to that of trypsin, pancreatic lipase or amylase and that the enzyme linked immuno sorbent assay for elastase 1 has an advantage over the radioimmuno assay method due to the limited life time of radio labelled reagents and the need of special equipment (Sziegoleit et al., 1989).

The diagnosis of chronic pancreatitis is hampered by the absence of easily available histological confirmation and is therefore based on the morphology and functional variables (Niederau and Grendell, 1985 and Steer et al. 1995).

The diagnostic sensitivity and specificity of faecal elastase-1 has been studied by various workers (Loser et al., 1996; Dominique-Munoz et al., 1995 and Glasbrenner et al., 1996); and the results shown in table 3:
Table 3: Comparison of sensitivity of fecal elastase 1 concentration (μg/g) with a cut off of <200μg/g, faecal chymotrypsin activity (u/g) with a cut off of <3u/g and serum pancreolauryl test concentration (μg/ml) with a cut off of <4.5μg/ml by various workers.

<table>
<thead>
<tr>
<th>Exocrine Pancreatic Insufficiency</th>
<th>Sensitivity</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elastase 1</td>
<td>Chymotrypsin</td>
</tr>
<tr>
<td>Mild</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>25</td>
</tr>
<tr>
<td>Moderate</td>
<td>100</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>79</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Severe</td>
<td>100</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

N/A = Not applicable

PLT = Pancreeolauryl test
The faecal elastase test has a 100% sensitivity in the diagnosis of moderate to severe chronic pancreatitis as well as very high specificity compared with other indirect function tests in indicating primary pancreatic dysfunction in patients with gastric resection, diffuse intestinal disease and malabsorption (Dominguez-Munoz et al., 1995).

In view of the wide variations in diagnostic sensitivities of the indirect function tests of exocrine pancreatic insufficiency, the evaluation of a novel function test should be based on the best established functional variable which is the secretin cholecystokinin test (Loser et al. 1996).

Significant correlations between faecal as well as duodenal elastase 1 concentration and duodenal lipase, amylase, trypsin and bicarbonate proves that secretion patterns of elastase are similar to those of other pancreatic enzymes (Loser et al., 1996).

Furthermore, the correlation of faecal elastase 1 concentration is highly significant to duodenal elastase 1 concentrations, confirming the suggestion that measurements of faecal elastase 1 is representative of pancreatic secretion of elastase 1 (Sziegoleit et al., 1989; Loser et al., 1996).

In a well documented analysis by Linderau and Grendell 1985, the overall sensitivity of various indirect pancreatic test for mild and moderate chronic pancreatitis when compared to secretin-cholecystokinin test were: fluorescein dilaurate 39%, NBT-PABA 46% and faecal chymotrypsin 49%.

For severe chronic pancreatitis the corresponding values were 79%, 71% and 85% respectively. In contrast when a similar comparison was done between pancreatic elastase and secretin-cholecystokinin in patients with cystic fibrosis, the sensitivity for moderate and severe pancreatitis were 89.3% and 100% respectively.
Furthermore, statistically significant correlation between faecal elastase1 and duodenal volume, bicarbonate concentration, amylase, lipase and trypsin secretion in all cases was found (Walkowiak et al., 1999).

1.2.4. CLASSIFICATION OF PANCREATITIS

The terminology surrounding the classification of inflammatory disease of the pancreas is confusing and based on both clinical and morphologic criteria (Axon et al., 1984). Over the last four decades, multiple classifications have been proposed (Table..4). Differentiation by aetiology allows one to predict responsible factors for pancreatitis but gives little information on potential overlapping pathophysiologic mechanisms (Owyang and Levitt, 1991).
Table 4  Classification of pancreatitis - First Marseille symposium

<table>
<thead>
<tr>
<th>Clinico Pathologic</th>
<th>Etiologic</th>
<th>Clinical</th>
<th>Pathologic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute relapse</td>
<td>Alcohol</td>
<td>Acute Attack</td>
<td>Haemorrhagic</td>
</tr>
<tr>
<td></td>
<td>Tropical/Malnutrition</td>
<td>Fulminant</td>
<td>Necrotic</td>
</tr>
<tr>
<td></td>
<td>Hyperparathyroidism</td>
<td>Severe</td>
<td>Edematous</td>
</tr>
<tr>
<td></td>
<td>Hereditary</td>
<td>Mild</td>
<td>Protein Plugs</td>
</tr>
<tr>
<td></td>
<td>Obstruction</td>
<td></td>
<td>Postnecrotic scarring</td>
</tr>
<tr>
<td>Non Relapse</td>
<td>Trauma</td>
<td></td>
<td>Diffuse Fibrosis</td>
</tr>
<tr>
<td></td>
<td>Mucoviscidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pancreas divisum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic relapse</td>
<td></td>
<td>Persistent pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Painless</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pancreatic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insufficiency</td>
<td></td>
</tr>
</tbody>
</table>

In 1963 a symposium on pancreatitis was held in Marseilles, in which a clinical classification of pancreatitis was proposed and has since been widely used (Sarner and Cotton 1984).
According to the Marseille classification, pancreatitis can be divided into acute and chronic, with both types having relapsing and non-relapsing varieties.

The distinction between acute relapsing and chronic relapsing pancreatitis is usually made on grounds of function (Sarner and Cotton 1984). Some minor modifications of the original Marseille classifications were proposed to enable clinicians to distinguish clearly acute and chronic forms of pancreatitis at a second conference (Owyang and Levitt 1991).

The second symposium of Marseille proposed to distinguish obstructive chronic pancreatitis from other forms of chronic pancreatitis. Obstructive chronic pancreatitis is said to be characterised by dilatation of the ductal system, diffuse atrophy of the acinar parenchyma and uniform fibrosis. In contrast to other forms of chronic pancreatitis, intraductal plugs or stones usually are rare or absent, and both structural and functional changes may improve when the obstruction is relieved (Owyang and Levitt 1991).
The current recommended classification of pancreatitis is shown in figure 2.

Fig. 2 Current recommended classification of pancreatitis.

Modified from the second symposium of Marseilles, Owyang and Levitt 1991
1.2.5. **CAUSES OF EXOCRINE PANCREATIC INSUFFICIENCY**

- Chronic relapsing pancreatitis associated with chronic alcoholism
- Primary hyperparathyroidism associated with trauma.
- Hereditary pancreatitis.
- Haemochromatosis
- Cystic Fibrosis
- Neoplasms
  - Adenocarcinoma of the pancreas
  - Islet cell carcinomas
- Benign Pancreatic tumours causing pancreatic ductal obstruction
- Duodenal tumours occluding sphincter of Oddi
- 95 percent pancreatectomy for chronic pancreatitis
- Severe protein depletion and malnutrition
- Kwashiorkor
- \( \text{Alpha}_1 \) - antitrypsin deficiency.
Clinically, it is well known that obstruction of the pancreatic duct—whether by cancer, ampullary or pancreatic calculi or inflammation—is a cause of pancreatic insufficiency and malabsorption; with lesions arising in the head of the gland more likely to cause such a complication.

1.2.5.1. Ductal Adenocarcinoma

Carcinoma of the pancreas is the most common cause of malignancy and biliary obstruction which produces obstruction of the intra pancreatic or supra pancreatic portion of the common bile duct (Freeny 1981).

Ductal pancreatic carcinoma is a deadly carcinoma and at the time of diagnosis 90 percent of the tumour have already spread beyond the confines of the pancreas and cannot be resected. Diagnosis of the tumour often occurs too late because signs and symptoms are non-specific and occur after advanced disease is present and because a simple reliable screening method is not available (Di Magno 1991).

Incidence.

Data obtained from surgical specimens and autopsies performed at Memorial Hospital in New York over the fifty year period from 1935 to 1985 showed that the incidence of pancreatic adenocarcinoma increased from less than 5 per 100,000 to between 11 and 12 per 100,000 of the male population and slightly less for females (Di Magno 1991).

The morphological patterns of pancreas carcinoma could be delineated as shown in table 5.
<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duct Cell adenocarcinoma</td>
<td>76%</td>
</tr>
<tr>
<td>Giant Cell carcinoma</td>
<td>5%</td>
</tr>
<tr>
<td>Microadenocarcinoma</td>
<td>4%</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>2%</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>2%</td>
</tr>
<tr>
<td>Cystadenocarcinoma</td>
<td>1%</td>
</tr>
<tr>
<td>Acinar cell carcinoma</td>
<td>1%</td>
</tr>
<tr>
<td>Unclassified</td>
<td>9%</td>
</tr>
</tbody>
</table>

Table 5: Distribution of non endocrine human pancreas carcinoma.

(Silverberg and Lubera 1988)
Most pancreatic adenocarcinomas are moderately well differentiated mucinous carcinomas arising from the cuboidal epithelium of the pancreatic duct.

**Risk factors**

Variables that appear to affect the development of cancer of the pancreas include diet high in fat and/or cholesterol and excessive cigarette smoking (Wynder et al., 1975), and variables which play no role in the development of cancer of the pancreas. Wynder attributed an increase of pancreas cancer in immigrant Japanese and Jews to lifestyle and hence preventable, as compared to the findings that pancreas cancer is relatively rare in Japan. Tobacco or its metabolically active forms can reach the pancreas either by reflux from the bile duct or through the blood stream and therefore a risk of pancreas cancer in cigarette smokers (Wynder et al., 1975).

Eighty percent of pancreatic cancers occur between the ages of 60 and 80 and the disease is unusual in persons younger than the age of 40 (DiMagno et al., 1977; Grant and Wosornu 1974 and Quartey-Papafio and Archampong 1977).

Pancreatic cancer occurs more commonly in males and among urban residents (DiMagno 1991). Diseases such as diabetes and pancreatitis are associated with the development of pancreatic cancer and this occurs more commonly among female diabetics and among those with hereditary forms of pancreatitis, (Kestrel 1970).
PATHOLOGY

Ductal adenocarcinoma predominantly occurs in the head of the gland (60 percent to 70 percent), and most arise in the part of the head of the pancreas anlage close to the intrapancreatic portion of the common bile duct. The remaining tumour of the head of pancreas arise in the central pancreatic head behind the ampulla of vater.

The three sites of tumours of the head of the pancreas are associated with different early features of malignancy. Tumours that arise in the dorsal pancreas obstruct the common bile duct and cause jaundice whereas tumour arising near the ampulla of vater or uncinate pancreas obstruct the main pancreatic duct and cause pancreatic insufficiency and obstructive chronic pancreatitis.

Tumours of the head of pancreas are relatively large when first diagnosed and tumour of the body and tail of the pancreas are larger since they are detected later than tumours of the head of pancreas (Di Magno 1991).

Prevalence

In the 5 year study by Grant and Wosornu 25 cases of cancer of pancreas were recorded out of which 15 were confirmed at laparotomy and 10 at autopsy.

About 80% of patients diagnosed as having cancer of the pancreas are aged 40 years and older (Grant and Wosornu 1974; Quartey Papafio and Archampong 1977; Di Magno et al., 1977). The signs and symptoms presented were not different in the series.

The clinical presentation of hepatomegaly with ascites in the absence of other signs mimics the advanced stage of carcinoma of the pancreas (Grant and Wosornu, 1974).

The presentation and diagnosis of the neoplasias of the pancreas must precede the onset of jaundice if exploratory surgery should play a meaningful role (Quartey Papafio and Archampong, 1977). Although jaundice was present in 100% of their subjects, laparotomy
finding of a distended gall bladder was a helpful diagnostic feature in differentiating between intrahepatic cholestasis and carcinoma of the pancreas

Clinical Manifestation

The signs and symptoms of pancreatic cancer are non specific. A variety of non pancreatic diseases as well as pancreatitis may cause features identical to those experienced by patients with pancreatic cancer.

Signs and symptoms suggestive of pancreatic cancer are shown in table 6.

Pain

Pain occurs during the course of pancreatic cancer in up to 90% of patients with the disease and is the presenting symptom in 79% of patients. It is observed that both mechanical compression and invasion of the perineural spaces stimulate nociceptors that cause pain (Di Magno, 1991). Aspirin and other non steroidal analgesics afford effective pain relief giving credence to the hypothesis that the neoplastic invasion of the perineural spaces releases bradykinin or prostaglandins, both substances that stimulate nociceptors, (DiMagno 1991).

Jaundice

Jaundice is a presenting symptom in 68% to 100% of patients with cancer of the head of pancreas (Di Magno et al., 1977; Quartey Papafio and Archampong 1977; Grant and Wosornu 1974).

Weight loss

Weight loss of more than 10% ideal body weight is an almost universal finding in patients with pancreatic cancer and the weight loss was due to decreased calorie consumption as a
result of decreased food intake and malabsorption due to reduced pancreatic secretion caused by obstruction of the pancreatic duct by the tumour.

**Diabetes Mellitus**

Diabetes is a significant finding in pancreatic cancer patients, with diabetic patients developing pancreatic cancer and other patients developing hyperglycaemia secondary to pancreatic cancer (Di Magno *et al.*, 1977).

**Other clinical features**

Signs and symptoms such as nausea, vomiting, weakness and anorexia occur frequently but are rather non-specific.
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<tbody>
<tr>
<td>Weight loss</td>
<td>92</td>
<td>95</td>
<td>52</td>
</tr>
<tr>
<td>Jaundice</td>
<td>82</td>
<td>100</td>
<td>68</td>
</tr>
<tr>
<td>Pain</td>
<td>72</td>
<td>85</td>
<td>80</td>
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<tr>
<td>Anorexia</td>
<td>64</td>
<td></td>
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<tr>
<td>Dark Urine</td>
<td>63</td>
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<tr>
<td>Light Stools</td>
<td>62</td>
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<td></td>
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<tr>
<td>Nausea</td>
<td>45</td>
<td></td>
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</tr>
<tr>
<td>Vomiting</td>
<td>37</td>
<td>32</td>
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<tr>
<td>Weakness</td>
<td>35</td>
<td>36</td>
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<tr>
<td>Pruritis</td>
<td>24</td>
<td>75</td>
<td>28</td>
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<tr>
<td>Palpable gall bladder</td>
<td>55</td>
<td>44</td>
<td></td>
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<tr>
<td>Ascites</td>
<td>25</td>
<td>20</td>
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</tbody>
</table>
Treatment Regimen of pancreatic cancer

Chemotherapy

Neither single nor combined agents have been shown to prolong life or enhance the quality of life in patients with pancreatic cancer. 5-Fluoracil, Mitomycin-C and Streptozotocin have been shown to have effect in reducing tumour size but not survival (Di Magno 1991).

Radiation treatment

Improvements in radiation treatment have been the addition of intraoperative electron beam radiation. Although this may limit the progression of the tumour, there has been no significant change in survival (Roldan et al., 1988; Di Magno 1991).

Surgery

Surgery is the only chance for a cure. Unfortunately only approximately 10% of patients with ductal tumours have resectable tumours (Edis et al., 1980). Pancreaticoduodenectomy, or the Whipple procedure is the surgical treatment of choice for carcinoma of the head of pancreas with high operative mortality rates due mainly to anastomotic leakage and haemorrhage at the pancreaticojejunostomy (Schapiro 1975).

Prognosis

Neoplasias of the pancreas generally have a poor prognosis, an occasional ampullary lesion may give rise to jaundice at a relatively early stage but in general growth of the pancreas and periampullary region remain until a very late stage (Di Magno, 1991). No patient has survived for more than nine months after surgery although there was no mortality in the immediate post-operative period (Quartey Papafio and Archampong 1977).
1.2.5.2. PATHOLOGICAL ANATOMY OF CHRONIC CALCIFYING PANCREATITIS.

According to the definition of the Marseille Symposium (Sarner and Cotton 1984), chronic pancreatitis is characterised by lasting damage, whether or not the cause is removed. In calcifying pancreatitis the lesion is characterised by sclerosis and disappearance of the exocrine parenchyma in which pancreatic calculi are visible on X-ray (Sarles 1974).

Sarles reported on the pathological differences in chronic pancreatitis associated with calcification and that due to obstruction of the main pancreatic duct and observed the features of calcified pancreatitis as a lobular distribution of lesion where an affected lobule is surrounded by normal ones. In calcifying pancreatitis, there is dilation of the acini and the pancreatic ducts, and also atrophy and disappearance of the ductal epithelium with the ducts filled with protein plugs which subsequently calcify with formation of calculi. The main pancreatic duct is normal at the beginning of the disease but later becomes dilated as a result of an impediment to the flow of the pancreatic juice which takes the form of stenosis in the region of the head of the gland and as a result of accumulation of calculi (Sarles et al., 1965).

Ammann et al., 1984 observed that the median intervals from onset of alcohol induced chronic pancreatitis to pancreatic calcification, severe exocrine insufficiency and diabetes were 5.12, 5.65, 5.75 years respectively and showed that there is a correlation of pancreatic function parameter with the appearance of pancreatic calcification.

Calcifying pancreatitis is found in a number of circumstances.

a) chronic alcoholic pancreatitis in countries with temperate climates.

b) Idiopathic pancreatitis

c) Familial infantile pancreatitis.
d). Chronic pancreatitis where Kwashiorkor is prevalent.
e.) Chronic Pancreatitis in hyperparathyroidism.

Tarso and co workers, found that the acinar cells of patients with chronic calcifying pancreatitis contain a decreased number of mature zymogen granules with an increase in the population of immature prozymogen granules with the rough endoplasmic reticulum and the Golgi apparatus significantly more dilated than in normal population (Tarso et al., 1973).

Incidence and prevalence:
The only prospective study on the incidence and prevalence of chronic pancreatitis was performed in Copenhagen in 1978 and 1979 (Owyang and Levitt 1991). It showed an incidence of 8.2 new cases per 100,000 inhabitants per year and a prevalence of 26.4 cases per 100,000 inhabitants. The study also showed enormous differences in incidence rates between different areas collected during the same period with alcohol consumption being the major factor in the development of chronic pancreatitis. (Owyang and Levitt 1991).

Aetiology and Geographical distribution of calcifying pancreatitis
A geographical pathology survey which related data obtained in 21 centres including, Europe, Latin America, and Africa concluded that in Countries where malnutrition is unknown chronic calcifying pancreatitis is rare. In these countries, the average per capita alcohol consumption is low. However chronic calcifying pancreatitis is frequent in countries or places where the consumption of alcohol is great (Sarles 1974).

Sarles showed that calcified pancreatitis is quite distinct from pancreatitis secondary to an obstruction in the main pancreatic duct and that the first characteristic feature of calcified
Pancreatitis is the lobular distribution of the lesion where there is dilation of the acini and ducts leading to the formation of rounded cavities, atrophy and disappearance of the ductal epithelium (Sarles 1974).

Chronic calcifying pancreatitis is characterised by the formation of intra ductal protein plugs or precipitates and calcified stones in ducts. These stones largely consisted of calcium carbonate 95% and a major protein called stone protein which had been shown histologically as eosinophilic precipitates previously (Guy et al., 1983).

The precipitates have been found in the pancreatic juice of alcoholic subjects as well as patients with chronic calcifying pancreatitis. The presence of this protein in precipitates can stagnate for many years in pancreatic ducts resulting in the impediment to the flow of pancreatic juice which takes the form of stenosis in the region of the head of the gland and associated with accumulation of calculi (Guy et al., 1983).

Sarles (1974), demonstrated that patients with chronic calcific pancreatitis had a mean daily ethanol intake of approximately 179g/day, and that chronic consumption of alcohol leads to pancreatitis in man.

The incidence of alcoholism as the major etiologic factor in chronic relapsing pancreatitis range from a low of 2 percent in Dublin to a high of 86 percent in a United States series.

Alcohol- The risk for alcohol induced pancreatitis is higher in the high fat, high protein diets subjects (Gastard et al., 1973). The mechanism by which alcohol produces chronic pancreatitis is not known but inferences can be made from the alcoholic effect on pancreatic secretion. Sarles showed that ingestion of alcohol results in pancreatic secretion secondary to the passage of gastric acid secretion into the duodenum.
In dogs, instillation of 1 g alcohol per kg of body weight into the stomach while the gastric fistula is closed brings about an increase in the output of pancreatic juice and of bicarbonate, protein secretion and cholecystokinin (Sarles 1974), while chronic alcoholism also causes an increase in basal secretions of proteases, amylase and lipase, and a decrease in trypsin inhibition in rats. Furthermore, an increased responsiveness of the pancreas to cholecystokinin stimulation in man has been reported (Renner et al. 1980). It is conceivable therefore that alcohol produces pancreatitis by interfering with the intracellular transport and discharge of digestive enzyme causing colocalisation of digestive and lysosomal hydrolases (Owyang and Levitt 1991).

The Kerala state in India is reported to have the highest chronic calcifying pancreatitis in the world, and the age of onset is 12 years compared to 38 years in other parts of the world. Although alcohol appears to be ruled out low dietary protein intake (35 g to 57 g) per day compared with 72 g in developed countries may play a part in the disease (Sarles 1974).

Incidentally, alcoholic chronic calcifying pancreatitis occurs in patients with high fat and high protein diets (Sarles et al., 1965), thus the disease seems to be particularly related to two main conditions; alcoholism (especially in countries where individuals are fed in high protein, high fat diet) and malnutrition.

**Hyperparathyroidism**

Calcified chronic pancreatitis occurs in untreated hyperparathyroidism. The pathogenesis of this type of pancreatitis is presumed to be related to the effect of hypercalcaemia which is a potent stimulus of human pancreatic enzyme secretion (Layer et al., 1985).
It has therefore being proposed that hyperparathyroidism may cause continuous and excessive stimulation of the acinar cell, which could lead to pancreatitis (Owyang and Levitt 1991). Furthermore chronic hypercalcaemia causes significant increase in pancreatic calcium secretion in patients with hyper parathyroidism (Layer P et al., 1982), resulting in damage to the pancreas and promoting the development of calcified chronic pancreatitis (Owyang and Levitt 1991).

Obstruction.

Obstruction of the main pancreatic duct by tumours and pseudocysts can lead to a distinct form of chronic pancreatitis known as obstructive chronic pancreatitis (Sarles et al., 1965). This is characterised by acinar atrophy and fibrosis and dilatation of the ductal system. In contrast to alcohol induced chronic pancreatitis, intraductal plugs or stones are very rare in obstructive chronic pancreatitis, and both structural and functional changes may improve when obstruction is removed (Triscornia and Dreilling 1966).

Nutritional (tropical) forms

Nutritional forms of chronic pancreatitis have been reported among juveniles and young adults in some African, and Asian countries (Schaper et al., 1960 and Pitchumoni et al., 1984). It is characterised by pancreatic insufficiency, diabetes mellitus, and disseminated pancreatic calcifications, marked emaciation and hair and skin changes resembling Kwashiorkor. Abdominal pain characterises the onset of tropical pancreatitis, and abdominal X-ray show diffuse pancreatic calculi and dilatation within the ducts. Chronic inflammatory cell infiltration, and atrophy of the pancreatic parenchyma are also seen (Owyang and Levitt 1991).
The etiology of tropical pancreatitis is not yet clearly understood, although the common factor appeared to be malnutrition in most cases (Pitchumoni et al., 1984). Recent evidence have suggested that other factors such as toxic products in certain nutritional components like cyanogens in cassava root may be more important (Pitchumoni et al., 1984). Cassava is consumed by the majority of poor people in many Afro-Asian countries. It contains 65mg toxic glycosides/100g. When glycosides react with gastric hydrochloric acid, hydrocyanic acid is liberated. The enzyme rhodanase acts on hydrocyanic acid leading to thiocyanate production in the presence of adequate amounts of methionine and cystine.

Cyanogens impair a number of enzymes including superoxide dismutase, an important scavenger of free radicals, these are presumed to cause cell injury (Pitchumoni et al., 1988 and Braganza et al., 1983).

In Europe, where malnutrition is unknown, chronic calcifying pancreatitis is rare but occurs occasionally in Germany, Denmark, Argentina and Latin America where the average per capita alcohol consumption is low. Chronic Calcifying pancreatitis is more frequent in France, Italy and Switzerland where the consumption of alcohol is great (Sarles 1974).

**Idiopathic Type**

The major form of non alcoholic chronic pancreatitis in North America and Europe is the idiopathic type which account for 10% to 40%. It presents in juveniles where abdominal pain dominates the clinical picture and in a senile group with painless disease. In both groups patients present with exocrine insufficiency, diabetes and pancreatic calcification. (Owyang and Levitt 1991).
Hereditary Pancreatitis.

Comfort and Steinbert first recognised this problem at the Mayo clinic in 1952. Typically, hereditary chronic pancreatitis appears in childhood at a mean age of 10 to 12 years. The disease is inherited through an autosomal dominant gene of incomplete penetrance. Patients frequently experience recurrent attacks of severe upper abdominal pain, and overt diabetes develops 8 to 10 years after the onset of pain in 20% of cases and gross steatorrhoea in 15% to 20% (Comfort and Steinbert 1952).

1.2.5.3. Congenital diseases of the pancreas

Abnormalities in the embryologic development of the pancreas result in its congenital diseases. The pancreas originates from two diverticula of the endodermal lining of the gut: the first is the dorsal bud, which elongates to become the body and tail of the pancreas and the ventral anlage arising from the ventral foregut which becomes the head and uncinate process of the pancreas (Liddle 1991).

Both pancreatic buds develop ductal systems with the dorsal duct growing from the duodenum and the ventral duct draining into the common bile duct. The ventral duct anastomoses with the dorsal duct forming the main pancreatic duct of Wirsung. The proximal portion of the dorsal duct undergoes varying degrees of regression disappearing or remaining as an accessory duct of Santorini.

Pancreas divisum

Pancreas divisum is the most common anomaly of the pancreas and is caused by failure of the ducts of the dorsal and ventral anlagen to fuse during embryologic development. In pancreas divisum, the ventral duct of Wirsung empties into the duodenum through the
major papilla but serves only a small portion of the pancreas (Liddle 1991 and Cotton et al., 1980). Secretions from the tail, body, neck and remainder of the head of the pancreas drain into the duodenum through the minor papilla by way of the duct of Santorini (accessory pancreatic duct).

In a series of patients with pancreatitis a 16% incidence of pancreas divisum is reported (Cotton et al., 1980). It therefore appears as though pancreas divisum predisposes to pancreatitis, and stenosis of either the minor or major papilla has been associated with pancreas divisum in cases in which pancreatitis has occurred (Cotton et al 1980).

Cystic fibrosis

Cystic fibrosis is the most common hereditary disease involving the exocrine pancreas. The pathologic features of cystic fibrosis result from abnormalities in exocrine secretion. Features characteristic of cystic fibrosis include increased viscosity of exocrine secretions and increased electrolyte concentration in sweat and saliva (Liddle 1991).

Chronic pulmonary disease is one of the major concerns in patients with cystic fibrosis and over 80% of patients with cystic fibrosis have pancreatic insufficiency at the time of diagnosis (Liddle 1991). The exocrine pancreatic insufficiency is caused by obstruction of the small ducts by viscous secretions leading to necrosis of the acinar and ductal cells and eventual fibrosis of the pancreatic lobules (Phillips et al., 1999).

Of the 15% of cystic fibrosis patients who do not have the clinical pancreatic exocrine insufficiency, some of them appear to develop recurrent episodes of acute pancreatitis (Shwachman et al., 1975).
Clinical presentations of patients with chronic pancreatitis.

The presenting symptom of the vast majority of patients with chronic pancreatitis is abdominal pain. The pain is epigastric, dull and constant. A characteristic feature of the pain is radiation directly through to the back. The pain is partially relieved by sitting with the trunk bent forward or lying prone.

The pain which is aggravated by food ingestion is characteristic of chronic pancreatitis or carcinoma of the pancreas, whiles ingestion of alcohol may also aggravate the pain (Owyang and Levitt 1991), whiles nausea, vomiting, anorexia and weight loss are common. Diarrhoea, steatorrhoea and azotorrhoea occur when exocrine secretion of pancreatic enzymes is insufficient to maintain normal digestion, while glucose intolerance is common early in the course of chronic pancreatitis (Owyang and Levitt 1991).

Other clinical presentations of chronic pancreatitis include jaundice secondary to common bile duct compression by the pancreas, which may result in ascites or pleural effusion due to leak of pancreatic secretion from a ruptured duct or pseudocyst. The signs and symptoms very suggestive of pancreatic carcinoma (abdominal pain, weight loss or jaundice) are common to those of pancreatitis. See Table: 6 for presenting features of pancreatic cancer.

Pathomechanism of symptoms in chronic pancreatitis

Pain

Abdominal pain and malabsorption are the major symptoms of chronic pancreatitis and possible causes include inflammation of the pancreas and neural inflammation which is related to increased intraductal pressure secondary to continued pancreatic secretion in the face of ductal stones (Owyang and Levitt 1991).
Amman and others observed that the majority of patients with chronic calcific pancreatitis eventually became pain free and that the onset of relief was associated with decreased pancreatic secretion; which suggest that pain in chronic pancreatitis is directly related to the secretory capacity of the pancreas and that with the glandular destruction below a critical level, the substrate for pain vanishes (Amman et al., 1984).

A similar observation was made by Girdwood who reported that 31% of patients with painless pancreatitis had severe pancreatic insufficiency compared with 3% who had painful pancreatitis (Girdwood et al., 1981).

Intra pancreatic neural inflammation is another factor that may play a role in the genesis of pain in chronic pancreatitis. Morphologic studies indicate that the pancreatic nerves appear to be larger and more numerous in chronic pancreatitis with the further observation that the organisation of intra neural organelles such as microtubules, microfilaments and mitochondria are disrupted, whiles significantly there is alteration in the perineurial sheath which shields nerves from surrounding connective tissue (Bockman et al., 1988).

Malabsorption:

Malabsorption of fat and proteins occurs only when pancreatic enzyme outputs were 10% or less of normal. Di Magno observed that 90% of the pancreatic gland must be functionally destroyed or obstructed before steatorrhoea and creatorrhoea occurs. The late appearance of malabsorption in chronic pancreatitis could be due to the large reserve for exocrine pancreatic enzyme secretion (Di. Magno et al., 1973).

Concurrent with the reduction in pancreatic enzyme secretion there is also decreased bicarbonate secretion in patients with severe chronic pancreatitis with gastric pH being greater than in healthy controls and consistently low duodenum pH (<4.0) in patients with
chronic pancreatitis 90 minutes after eating (Di Magno 1977). Hence the intra gastric and intra duodenal acidity results in the inactivation of the pancreatic enzymes delivered at the ligament of Treitz. These factors are important to consider in the treatment of pancreatic steatorrhoea.

Complications of chronic pancreatitis

Pseudocyst.

A pseudocyst is a collection of pancreatic juice outside the normal boundaries of the ductal system, which is enclosed by a fibrous tissue membrane.

The development of pseudocyst is preceded by pancreatitis in 90% of subjects and pancreatic trauma in 10% (Sankaran and Walt 1975). The symptoms and signs are noted as pain in upper abdomen and weight loss with chronic ascites. Rupture of the pancreatic pseudocyst in the peritoneal cavity and haemorrhage are the principal causes of death (Sankaran and Walt 1975), and patients with suspected pancreatic pseudocysts should be kept under observation if decision is made to delay surgery, especially in patients with fresh pseudocysts.

Pancratic ascites. This refers to the chronic accumulation of massive amounts of ascitic fluid in the course of acute or chronic pancreatitis, and in pancreatic cancer as a result of metastases in the peritoneal cavity or because of obstruction of the splenic or portal veins by the tumour (Donowitz et al., 1974). It occurs typically in alcoholic cirrhotics who complain of mild to moderate abdominal pain (Sankaran and Walt 1975).

The prognosis of patients with pancreatic ascites depends on the cause of the pancreatitis and the abdominal pathology identifiable (Donowitz et al., 1974).
**Splenic Vein thrombosis**

Splenic vein thrombosis is seen in patients with recurrent pancreatitis secondary to excessive alcohol consumption and those with extrahepatic portal hypertension is a well known complication of chronic pancreatitis (Salam *et al.*, 1973) and diagnosis should be suspected in every patient who presents with variceal bleeding in the absence of signs of liver disease, and a history of pancreatitis.

**Treatment of chronic pancreatitis**

Treatment of chronic pancreatitis is aimed at the control of pain and the correction of malabsorption with adequate pancreatic enzyme replacement.

**Control of pain**

Avoidance of alcohol decreases the frequency and severity of abdominal pain of patients with chronic alcoholic pancreatitis. Trapnell reported that 75% of his patients with chronic alcoholic pancreatitis experienced pain relief when they stopped drinking (Trapnell *et al.*, 1979). This observation is explained by the stimulatory effect of alcohol on pancreatic secretion (Borman *et al.*, 1980).

**Enzyme therapy**

In patients with chronic pancreatitis decreased enzyme secretion may result in hyperstimulation of the pancreas and produce pain, however effective enzyme replacement therapy might reduce pancreas stimulation, decrease intraductal pressure and diminish the pain (Ilse *et al.*, 1977).
Surgical treatment

Surgery is considered when all medical attempts at relieving the pain have failed. The type of surgery should be chosen according to the severity of pain, ductal morphology and the extent of parenchymal disease. Patients who have ductal dilatation have a 70% to 80% chance of obtaining pain relief with either a partial resection with pancreaticejejunostomy or lateral pancreaticojjunostomy, with mortality of less than 2% (DiMagno 1991). Patients with moderate to severe parenchymal disease and no ductal dilatation should be considered for partial pancreatic resection (DiMagno 1991).
CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 The Study Population

Faecal elastase 1 was measured in spot stool samples from 97 subjects who presented between February 1998 and May 2000 at Department of Chemical Pathology, University of Ghana Medical School and were classified under three groups.

Group 1 consisted of 25 apparently healthy persons with no history of alcoholism, diabetes nor evidence of gastroduodenal diseases, were enrolled after giving informed consent. There were 16 males, 9 females with age range of 25-63 years and average age of 43.4 years.

Group 2 consisted of 32 patients with chronic pancreatic insufficiencies (26 males and 6 females between the ages of 23 and 78 years and an average age of 51.4 years. The patients were referred for the test having being diagnosed of various inflammatory conditions of the pancreas by their clinicians. Information on these patients were obtained from request cards and personal communication with the clinician as well as notes from patients folders.

Chronic pancreatic exocrine insufficiencies were due to cancer of the head of pancreas, obstructive jaundice, calcification of the pancreas and idiopathic causes.

The diagnosis of chronic pancreatitis was based on the clinical presentation of patients and findings of the following diagnostic aids; plain radiography, ultrasonography and computerised tomography. Laparotomy was done for both exploratory and treatment of cases requiring surgical intervention.

Group 3 consisted of 40 patients (18 males and 22 females between the ages of 8 and 78 years with an average age of 43.5 years).
All the patients were referred for the test after being diagnosed of various non exocrine pancreatic diseases of gastro-duodenal origin including gall stones, peptic ulcer, diabetes, gastritis and non specific abdominal pain. Personal communication with the Clinicians and Surgeons revealed that diagnoses were made after patients clinical history as well as findings from endoscopy, ultrasonography and computed tomography as well as biopsy findings and routine laboratory tests for stool, urine and blood had been investigated.

2.1.2 Sample Collection and handling

All subjects for the study were given small monowax containers and were asked to bring spot stool samples.

Samples received were labeled with the patients name and date of receipt using adhesive plaster. Samples were stored frozen at -20°C until ready for the assay of the pancreatic elastase 1 enzyme.

2.2 METHODS

2.2.1 Principles of the pancreatic elastase 1 - stool test

An Elisa (enzyme linked immunosorbent assay) plate in the form of wells which are coated with monoclonal antibody and only recognises human pancreatic elastase 1 (E1) binds with E1 from samples and standards and is immobilised in the wells.

A second monoclonal antibody, which is biotinylated binds to E1 during incubation at room temperature. A conjugate of POD (peroxidase) and streptavidin binds to the biotin moiety. The peroxidase oxidises ABTS -the substrate solution-[2,2-Azino-bis- (3-ethylbenzothiazolin-6 sulphonic acid) diammonium salt] which turns dark green.

The concentration of the oxidised substrate is determined photometrically at 405nm.
2.2.2 **Reagents and equipment**

**Reagents:**
1. Pancreatic elastase 1 kit for stool purchased from Schebo.Tech. GmbH, Wettenberg, Germany; consisting of 12 Elisa-strips with 8 wells each coated with monoclonal antibody to human pancreatic elastase 1 (E1).
2. Sample/washing buffer concentrate, (phosphate buffered saline, pH 7.2 with detergent).
3. Extraction buffer concentrate for stool, (Phosphate buffered saline, pH 7.2 with detergent and sodium azide).
5. Control, (human pancreatic elastase 1 in aqueous solution with sodium azide).
6. Second monoclonal antibody to E1, conjugated to biotin, in aqueous solution with sodium azide.
7. POD-Streptavidin (light sensitive) in aqueous solution.
Equipments:

3. Vortex mixer with variable speed from Stuart Scientific, Surrey, U.K.

   Adjustable precision pipettes: 0-50\mu l, 50-200\mu l and 200-1000\mu l

5ml and 10 ml graduated glass pipettes from Labora Ltd. Accra, Ghana.

Polystyrene test tubes

500ml measuring cylinder

Cold room (-20°C)

Pipettor tips, yellow and blue

2.2.3 Preparation

Preparation of working reagents

100ml sample/washing buffer is added to 400ml of distilled water and mixed. This preparation is stable for 6 months at 4-8°C

Preparation of stool specimen

Stool samples were allowed to thaw at room temperature before weighing.

Stool specimen are individually weighed using a Bosch 2000 analytical balance with analytical sensitivity of 100\mu g.

Stool masses between 30mg and 80mg were made by taking a small sample of stool with disposable inoculating loop into plastic bottles, (capable of containing 15ml of solution) accurately weighed and noted.

Extraction buffer was added to the stool sample according to the mass of sample to give a final concentration of 10mg stool/ml extraction buffer; for example for 50mg stool mass was added 5ml of extracting buffer.
Homogenisation and extraction of stool samples

Stool suspension was mixed thoroughly at room temperature using vortex mixer at 6,000 rpm. Each sample was homogenised intermittently over a period of 1 hour. The stools were homogenised to ensure complete extraction of pancreatic elastase 1. The suspension was kept at 4-8°C overnight. Each suspension was re-homogenised for a minute the following morning, and the particles allowed to settle prior to further dilutions of the enzyme.

Dilution of stool extracts (1:500)

A predilution of 1 in 20 was made by pipetting 50μl extracted stool sample into 1ml of sample/washing buffer and mixed by vortex. A final dilution of 1 in 500 was achieved by adding 40μl of the 1 in 20 diluted sample to 1ml of sample/washing buffer.

2.2.4 Determination of pancreatic elastase 1 in stool extracts

Assay procedure

Tests were done in duplicates alongside pancreatic elastase 1 standards and control. The standards had the following concentrations

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (ng/ml)</th>
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<tr>
<td>Standard 1</td>
<td>0.3</td>
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<tr>
<td>Standard 2</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard 3</td>
<td>2.0</td>
</tr>
<tr>
<td>Standard 4</td>
<td>4.0</td>
</tr>
<tr>
<td>Standard 5</td>
<td>10.0</td>
</tr>
<tr>
<td>Control</td>
<td>4.0 ± 10%</td>
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</tbody>
</table>

Samples were done in batches.
Depending on the number of samples, a selected number of strips each with 8 wells are used for the assay i.e. for 20 tests including standards, control and stool extracts, five Elisa strips are arranged on Elisa plate.

Due to the limited volumes of the second monoclonal antibody to E1 conjugated to biotin and the POD-streptavidin solutions 150μl and 50μl respectively, care is taken to avoid waste by using dilutions that can do a number of tests and at the same time not wasting strips by exposure.

a. 50μL of each standard and control was added to each of two adjacent wells and was followed by;

b. 50μL of each extracted 1 in 500 diluted stool specimen to each of two adjacent wells.

c. The wells containing the standards and samples were incubated for 60 minutes at room temperature. At the end of the incubation period the wells were emptied by decanting into the sink and each well was washed three times with 250μl/well of sample/washing buffer.

Any remaining liquid is removed by inverting the plate containing the strips on a soft tissue.

Shortly before use a 1 in 100 dilution of the biotin conjugated second monoclonal antibody was prepared i.e. for a 4 test strip; 20μl of biotin conjugated antibody was added to 2nd of sample/washing buffer and vortex.

50μl/well of the biotin conjugated second monoclonal antibody was added to the wells and incubated for further 30 minutes at room temperature.

The contents of the wells were decanted and each well was washed 3 times with 250μl/well using sample/washing buffer again. The plate was inverted on tissue paper to
A 1 in 400 dilution of the POD-streptavidin solution was prepared immediately before use i.e. for 4 test strips, 5μl POD-streptavidin was added to 2ml of sample/washing buffer. 50μl/well of POD-streptavidin was added and incubated for further 30 minutes in the dark at room temperature.

The wells were emptied and washed three times as done previously.

100μL of ready to use substrate solution was added to each well and incubated for 20 minutes in the dark at room temperature again.

The substrate reaction was stopped at the end of the incubation period by the addition of 100μl of the stop solution.

**Measurement and quantification of Results**

The mean absorbances of all duplicates after the blank value has been subtracted were read at 405nm using the Denley microtitre plate reader. A graph plot of the concentrations of the standards with their corresponding absorbances were plotted on log-log (Regression mode) graph by the reader.

The concentration of the samples were calculated by the reader using the corresponding absorbances.

The concentrations for the stool samples were multiplied by 50 (corresponding to the concentration of extracted stool specimen ie 10mg/ml and the dilution factor of 500) to yield the concentration of pancreatic elastase 1 in ng-EL/mg stool which equals μg EL/g stool.
2.2.5. **Statistics**

Statistical analyses were conducted using Microsoft excel for Windows 98.

Analyses performed included descriptive statistics, comparisons of group means using analysis of variance (ANOVA) or the corresponding non-parametric procedures. Statistics at Square One from the British Medical Journal was consulted for all statistical analyses.

Graphs were plotted with Microsoft excel for Windows 98 version.

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CHAPTER THREE

RESULTS

3.1 In vitro assay

3.1.1 Precision of the method

The intra assay coefficient of variation calculated from assays of 5 aliquots of same faecal sample from an apparently healthy subject gave 3.5% (appendix 2).

The inter assay coefficient of variation, calculated over three months from two different faecal samples ranged from 5.3% to 14.6% (appendix 3).

3.1.2 Enzyme stability in stool with storage.

The effect of storage on the stability of the faecal elastase I was determined using stool samples from an apparently healthy subject and a chronic pancreatitis patient on enzyme (viokase) replacement therapy. Enzyme concentrations for weeks 1 to 4 of storage at -20°C were 385, 375, 375 and 350μg/g respectively for the apparently healthy subject and a concentration of 40, 40, 35 and 35μg/g for weeks 1 to 4 respectively for the subject with chronic pancreatitis.

As shown in Figure 3, there was virtually no loss of enzyme activity due to storage at -20°C over a period of 4 weeks in both samples.

Chart presentation of the faecal elastase I concentration in the groups studied are shown in figures 4 & 5.
Fig 3. Changes in fecal elastase 1 with storage at -20°C in 4 different stools of (a) one patient with exocrine pancreatic insufficiency and (b) one apparently healthy person. E1d refers to pancreatic elastase 1 refer to apparently healthy person. E1h refers to pancreatic elastase 1 of a healthy person.
Fig. 4 Individual values of faecal elastase 1 concentration of the apparently healthy (AH) group and patients with non pancreatic diseases (NPD). The broken line represents the lower limit of normal.
Fig. 5 Individual values of faecal elastase 1 concentration of the apparently healthy (AH) group and patients diagnosed for chronic pancreatitis. Mild to moderate pancreatic insufficiency (MMP) and severe pancreatic insufficiency (SP). The broken line represents the lower limit of normal.
Loss of faecal enzyme activity was 9% at week four of storage at -20°C whereas a decrease of 12.5% on enzyme activity was observed at storage at -20°C by the 4th week in the diseased subject.

It is noted that the high percentage decrease in the activity of elastase 1 may be attributable to the dilution factor of 50 used, (see appendix 4) and not necessary due to storage.

3.1.3 Faecal elastase 1 concentration in subjects studied

The results were expressed as μg faecal elastase 1/g stool. The faecal elastase 1 concentration in the apparently healthy group is summarised in table 7.

The minimum and maximum concentrations of faecal elastase 1 measured in the apparently healthy group is 165 and 870μg/g respectively with a Mean ± SEM of 379 ± 41μg/g. Using a cut off value of 200μg/g stool, 23 out of the 25 apparently healthy subjects had values greater than 200μg/g giving a specificity of 92%.

Table 8 shows faecal elastase 1 values of the patients with various gastro-duodenal diseases which are non pancreatic in origin. The values ranged from 85 to 1171μg/g with a mean ± SEM of 479 ± 41μg/g.

Out of forty subjects with various non pancreatic diseases studied thirty five had values above the cut off value of 200μg/g and therefore constitute 87.5% of the subjects in that group.
Table 7 - Characteristics of the apparently healthy group

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Health Status</th>
<th>El (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P A</td>
<td>M</td>
<td>53</td>
<td>Apparently healthy control</td>
<td>165</td>
</tr>
<tr>
<td>E M</td>
<td>M</td>
<td>32</td>
<td>Apparently healthy control</td>
<td>195</td>
</tr>
<tr>
<td>A A</td>
<td>M</td>
<td>39</td>
<td>Apparently healthy control</td>
<td>200</td>
</tr>
<tr>
<td>C B</td>
<td>M</td>
<td>25</td>
<td>Apparently healthy control</td>
<td>205</td>
</tr>
<tr>
<td>Y S</td>
<td>M</td>
<td>s</td>
<td>Apparently healthy control</td>
<td>210</td>
</tr>
<tr>
<td>D H</td>
<td>F</td>
<td>53</td>
<td>Apparently healthy control</td>
<td>220</td>
</tr>
<tr>
<td>S A</td>
<td>M</td>
<td>49</td>
<td>Apparently healthy control</td>
<td>235</td>
</tr>
<tr>
<td>M D</td>
<td>M</td>
<td>45</td>
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<td>240</td>
</tr>
<tr>
<td>M N</td>
<td>M</td>
<td>60</td>
<td>Apparently healthy control</td>
<td>240</td>
</tr>
<tr>
<td>P R</td>
<td>F</td>
<td>47</td>
<td>Apparently healthy control</td>
<td>252</td>
</tr>
<tr>
<td>M A</td>
<td>F</td>
<td>42</td>
<td>Apparently healthy control</td>
<td>265</td>
</tr>
<tr>
<td>P S</td>
<td>M</td>
<td>51</td>
<td>Apparently healthy control</td>
<td>270</td>
</tr>
<tr>
<td>E L</td>
<td>F</td>
<td>48</td>
<td>Apparently healthy control</td>
<td>295</td>
</tr>
<tr>
<td>E A</td>
<td>M</td>
<td>27</td>
<td>Apparently healthy control</td>
<td>340</td>
</tr>
<tr>
<td>S Q M</td>
<td>M</td>
<td>63</td>
<td>Apparently healthy control</td>
<td>350</td>
</tr>
<tr>
<td>V O</td>
<td>F</td>
<td>54</td>
<td>Apparently healthy control</td>
<td>355</td>
</tr>
<tr>
<td>J D</td>
<td>M</td>
<td>26</td>
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<td>395</td>
</tr>
<tr>
<td>K O-A</td>
<td>M</td>
<td>47</td>
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<td>430</td>
</tr>
<tr>
<td>D N</td>
<td>F</td>
<td>45</td>
<td>Apparently healthy control</td>
<td>505</td>
</tr>
<tr>
<td>C T D</td>
<td>F</td>
<td>54</td>
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<td>530</td>
</tr>
<tr>
<td>A M</td>
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<td>570</td>
</tr>
<tr>
<td>D D</td>
<td>M</td>
<td>30</td>
<td>Apparently healthy control</td>
<td>600</td>
</tr>
<tr>
<td>R S</td>
<td>M</td>
<td>48</td>
<td>Apparently healthy control</td>
<td>765</td>
</tr>
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<td>G R</td>
<td>F</td>
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<td>782</td>
</tr>
<tr>
<td>P K</td>
<td>F</td>
<td>51</td>
<td>Apparently healthy control</td>
<td>870</td>
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</tbody>
</table>

Mean = 379 µg/g
Table 8 - Characteristics of the patient with various gastro-duodenal diseases

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age(yrs)</th>
<th>gastro-duodenal diseases</th>
<th>E1(µg/g)</th>
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</thead>
<tbody>
<tr>
<td>B.A</td>
<td>M</td>
<td>45</td>
<td>Duodenitis</td>
<td>85</td>
</tr>
<tr>
<td>M F</td>
<td>F</td>
<td>72</td>
<td>Diverticular dx.of transverse colon</td>
<td>120</td>
</tr>
<tr>
<td>K.W</td>
<td>F</td>
<td>43</td>
<td>Peptic ulcer disease</td>
<td>129</td>
</tr>
<tr>
<td>O I</td>
<td>M</td>
<td>71</td>
<td>Transient enteritis</td>
<td>170</td>
</tr>
<tr>
<td>R A</td>
<td>F</td>
<td>24</td>
<td>Irritable bowel disease</td>
<td>194</td>
</tr>
<tr>
<td>R. B</td>
<td>M</td>
<td>32</td>
<td>Duodenal ulcer</td>
<td>215</td>
</tr>
<tr>
<td>J.A</td>
<td>M</td>
<td>33</td>
<td>Abd. Gases</td>
<td>220</td>
</tr>
<tr>
<td>J K</td>
<td>F</td>
<td>51</td>
<td>Peptic ulcer disease</td>
<td>246</td>
</tr>
<tr>
<td>P. L</td>
<td>M</td>
<td>38</td>
<td>Abd. Pain recurrent heart burn</td>
<td>250</td>
</tr>
<tr>
<td>M. A</td>
<td>F</td>
<td>22</td>
<td>Peptic ulcer disease</td>
<td>270</td>
</tr>
<tr>
<td>O. E.</td>
<td>F</td>
<td>66</td>
<td>duodenal ulcer</td>
<td>275</td>
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<td>A. D</td>
<td>F</td>
<td>76</td>
<td>Hepatocellular Ca</td>
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<td>D. N</td>
<td>F</td>
<td>55</td>
<td>Irritable bowel syndrome</td>
<td>280</td>
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<tr>
<td>Y T</td>
<td>M</td>
<td>29</td>
<td>Amoebic dysentery</td>
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<tr>
<td>E. C</td>
<td>F</td>
<td>74</td>
<td>Gall stones</td>
<td>320</td>
</tr>
<tr>
<td>S. L</td>
<td>F</td>
<td>60</td>
<td>Biliary obstruction</td>
<td>320</td>
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<tr>
<td>S. H A C</td>
<td>M</td>
<td>54</td>
<td>Non specific colitis</td>
<td>354</td>
</tr>
<tr>
<td>B. D</td>
<td>F</td>
<td>0</td>
<td>Peptic ulcer</td>
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<td>W. B</td>
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<td>Choledocholithiasis</td>
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<td>F</td>
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<td>Irritable bowel disease</td>
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<td>M</td>
<td>24</td>
<td>Chronic diarrhoea.</td>
<td>506</td>
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<tr>
<td>R. Q</td>
<td>M</td>
<td>48</td>
<td>Pseudocyst</td>
<td>520</td>
</tr>
<tr>
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<td>M</td>
<td>26</td>
<td>Aerophagia</td>
<td>531</td>
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<td>J. A O</td>
<td>M</td>
<td>44</td>
<td>Gastritis</td>
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<td>583</td>
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<td>Non specific abd. Pain</td>
<td>625</td>
</tr>
<tr>
<td>O. A</td>
<td>F</td>
<td>29</td>
<td>Gall stones</td>
<td>635</td>
</tr>
<tr>
<td>T. J</td>
<td>M</td>
<td>36</td>
<td>Non specific abd. Pain</td>
<td>635</td>
</tr>
<tr>
<td>A. A</td>
<td>F</td>
<td>47</td>
<td>Gall stones</td>
<td>645</td>
</tr>
<tr>
<td>E. A</td>
<td>F</td>
<td>31</td>
<td>Gall stones</td>
<td>645</td>
</tr>
<tr>
<td>J. Y</td>
<td>F</td>
<td>41</td>
<td>Gall stones</td>
<td>655</td>
</tr>
<tr>
<td>I</td>
<td>M</td>
<td>0</td>
<td>Non specific abd. Pain</td>
<td>655</td>
</tr>
<tr>
<td>F. O</td>
<td>F</td>
<td>8</td>
<td>Chest infection</td>
<td>730</td>
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<td>F</td>
<td>56</td>
<td>Gall stones/Duodenitis</td>
<td>782</td>
</tr>
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<td>F. A. M.</td>
<td>M</td>
<td>42</td>
<td>Epigastric pain and heart burn.</td>
<td>782</td>
</tr>
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<td>M</td>
<td>40</td>
<td>Peptic ulcer</td>
<td>805</td>
</tr>
<tr>
<td>I. N</td>
<td>M</td>
<td>78</td>
<td>Gastritis</td>
<td>870</td>
</tr>
<tr>
<td>C. A</td>
<td>M</td>
<td>40</td>
<td>Abd. Mass and diarrhoea</td>
<td>990</td>
</tr>
<tr>
<td>A. Q</td>
<td>F</td>
<td>20</td>
<td>Peptic ulcer</td>
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</tr>
</tbody>
</table>

Mean = 479µg/g
The difference between the apparently healthy control group and patients with non pancreatic diseases was not statistically significant, (0.02 > P > 0.01).

The range for faecal-elastase 1 concentration in the patients with various pancreatic diseases was 20 to 285 µg/g stool as shown in table 9.

A mean ± SEM for the pancreatic diseases group was 112.9 ± 11.6 µg elastase1/g stool, as compared to the mean ± SEM of the apparently healthy control group of 379 ± 41 µg/g. The difference between the means of the apparently healthy control group and patients with pancreatic disease was statistically significant, (P << 0.001).

Whereas the majority of the patients with non pancreatic diseases gave a faecal elastase level greater than 200µg/g (35 out of 40; giving 87.5%), the majority of the patients with pancreatic disease (29 out of 32; 90.6%), gave faecal elastase1 values less than 200µg/g.

Faecal elastase 1 concentration of 100µg/g and less was observed in 18 of the 32 (56%) patients with pancreatic disease whiles 11 out of 32 (34%) for the same group of patients had values >100 and < 200µg elastase1/g stool; as shown in figure 5.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Pancreatic diseases</th>
<th>E1 (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.B</td>
<td>M</td>
<td>39</td>
<td>Chr. Pancreatitis, D.M.</td>
<td>20</td>
</tr>
<tr>
<td>E.A</td>
<td>M</td>
<td>31</td>
<td>Chr. Pancreatitis</td>
<td>40</td>
</tr>
<tr>
<td>K-J C</td>
<td>M</td>
<td>60</td>
<td>Duodenitis recurrent abd. Pain</td>
<td>45</td>
</tr>
<tr>
<td>S.L</td>
<td>M</td>
<td>34</td>
<td>Chr. Pancreatitis with steatorrhea</td>
<td>46</td>
</tr>
<tr>
<td>A.A</td>
<td>M</td>
<td>39</td>
<td>Chr. Pancreatitis</td>
<td>46</td>
</tr>
<tr>
<td>D.D</td>
<td>M</td>
<td>48</td>
<td>Obstructive jaundice</td>
<td>55</td>
</tr>
<tr>
<td>J.A</td>
<td>M</td>
<td>59</td>
<td>Calcified pancreas, Chr. Pancreatitis</td>
<td>63</td>
</tr>
<tr>
<td>F.K.S</td>
<td>M</td>
<td>70</td>
<td>Chr. Pancreatitis</td>
<td>65</td>
</tr>
<tr>
<td>N.K.A</td>
<td>M</td>
<td>39</td>
<td>Chronic pancreatitis</td>
<td>70</td>
</tr>
<tr>
<td>W.K</td>
<td>M</td>
<td>70</td>
<td>Non specific abd. Pain</td>
<td>70</td>
</tr>
<tr>
<td>O.A</td>
<td>M</td>
<td>32</td>
<td>Chronic pancreatitis with steatorrhea</td>
<td>70</td>
</tr>
<tr>
<td>G.D</td>
<td>M</td>
<td>32</td>
<td>D.M. 2°C to Chr. Pancreatitis</td>
<td>70</td>
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<tr>
<td>T.F</td>
<td>F</td>
<td>59</td>
<td>Obstructive jaundice/Ca gall bladder</td>
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</tr>
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<td>M</td>
<td>40</td>
<td>Chr. Pancreatitis</td>
<td>74</td>
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<td>M</td>
<td>78</td>
<td>Ca. Bile duct</td>
<td>76</td>
</tr>
<tr>
<td>M.B.O</td>
<td>F</td>
<td>64</td>
<td>Ca head of pancreas/Obstr. Jaundice</td>
<td>80</td>
</tr>
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<td>M.I</td>
<td>M</td>
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<td>Obstructive jaundice</td>
<td>90</td>
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<td>M</td>
<td>63</td>
<td>Ca head of pancreas/Obstr. Jaundice</td>
<td>100</td>
</tr>
<tr>
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<td>F</td>
<td></td>
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</tr>
<tr>
<td>H.E</td>
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<td>58</td>
<td>Diabetes mellitus</td>
<td>125</td>
</tr>
<tr>
<td>A.S</td>
<td>M</td>
<td>41</td>
<td>Non specific abd. Pain</td>
<td>140</td>
</tr>
<tr>
<td>C.A</td>
<td>F</td>
<td></td>
<td>Non specific abd. Pain</td>
<td>140</td>
</tr>
<tr>
<td>J.B</td>
<td>M</td>
<td>43</td>
<td>Chr. Pancreatitis</td>
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</tr>
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<td>M</td>
<td>55</td>
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<td>D.N</td>
<td>M</td>
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<td>180</td>
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<td>M</td>
<td>64</td>
<td>Obstructive jaundice</td>
<td>195</td>
</tr>
<tr>
<td>R.B</td>
<td>M</td>
<td>32</td>
<td>Duodenal ulcer</td>
<td>215</td>
</tr>
<tr>
<td>M.D</td>
<td>M</td>
<td>28</td>
<td>Diabetes mellitus</td>
<td>250</td>
</tr>
<tr>
<td>B.A</td>
<td>M</td>
<td>55</td>
<td>Diabetes mellitus</td>
<td>285</td>
</tr>
</tbody>
</table>

Mean = 112.9 µg/g
In accordance with these results a sub classification of patients with pancreatic disease was made as follows:

(a) Mild to moderate pancreatic insufficiency (MMPD) with faecal elastase 1 values between 100 and 200µg/g stool, and

(b) Severe pancreatic insufficiency (SPD) with faecal elastase 1 values up to 100µg/g stool.

The range for the faecal elastase 1 concentration in the patients with mild to moderate pancreatic insufficiency was 120-285µg/g with a Mean ± SEM of 175.8 ± 12.6µg/g while patients classified as having severe pancreatic insufficiency (SPD) with faecal elastase 1 values up to 100µg/g stool had a Mean ± SEM values of 63.9 ± 4.5 µg/g with a range of 20 - 100µg/g.
CHAPTER FOUR

DISCUSSION AND CONCLUSION

The ultimate aim of this study was to establish the assay of faecal elastase 1 as a laboratory based diagnostic test for chronic exocrine pancreatic insufficiency at the Korle-Bu Teaching Hospital. The assay of faecal elastase 1 as a diagnostic tool was to help in the differential diagnosis of patients presenting with upper abdominal pain and other abdominal disturbances for which results of imaging tests as well as the clinicians finding were equivocal.

The diagnostic sensitivity and specificity of faecal elastase 1 had previously been determined by (Stein et al. 1996; Loser et al. 1996) who compared the results of faecal elastase 1 with the secretin-cholecystokinin test- the gold standard pancreatic function test. In those studies both groups found a strong correlation between the duodenal secretion of elastase and that of amylase, lipase and trypsin which are the enzymatic outputs in pancreatic juice after cholecystokinin or pancreozymin stimulation of the pancreas.

They both reported a high diagnostic sensitivity and specificity of 93% and 93% respectively for the detection of exocrine pancreatic insufficiency.

The reference concentration for normal is quoted as 200 to >500\(\mu g\)E1/g stool by (Scheerers-Borchel et al., 1992), manufacturers of the kit.

(Scheerers-Borchel et al., 1992), proposed a cut off value of 200\(\mu g\) elastase 1/g stool for normal cases; however (Stein et al., 1996) showed that faecal elastase 1 assay achieved optimal discrimination at a cut off value of 175 \(\mu g/g\) stool, yielding a specificity of 94%
and a sensitivity of 93%, and in spite of the high cut off value of 200μg/g, the specificity of the faecal elastase determination was reported to be excellent when compared to healthy controls (Glasbrenner et al., 1996).

The stability of the enzyme has been discussed elsewhere by previous researchers who observed that the enzyme was stable at room temperature for up to three days therefore, it can be mailed to diagnostics centres (Stein et al., 1996), and stable for months at -20°C (Szegoleit, 1984). The results of this study confirm that faecal elastase 1 stored frozen at -20°C was highly stable as there was no appreciable loss of enzymic activity over periods ranging from one to four weeks. It did not matter whether the sample for storage was obtained from an apparently healthy person or a subject with pancreatic disease. In Ghana where foreign currency is hard to come by to purchase reagent kits, storage of stool samples to enable batch analysis to be made is essential, as this will help reduce cost to the patient whiles at the same time the stability of the enzyme is not compromised.

As the results indicated there was very low variation of faecal elastase 1 concentration measured in aliquots of same stool sample in determining the precision of the method indicating that the measurement of the enzyme in a small faecal sample was valid for diagnosis.

The intra assay coefficient of variation of 3.5% at a mean concentration of 346μg/g stool was in agreement with previous work done by (Phillips et al., 1999 and Loser et al., 1996), whiles the inter assay coefficient of variation from this study ranged from 5.3% to 14.6% as compared to a range of 4.1% to 10.2% reported by (Scheerers-Borchel et al., 1992), manufacturers of the kit.
The enzyme is not degraded during its passage in the gut whereas it is five to six times enriched in human faeces compared with pancreatic juice whose concentration was showed to be between 170 and 360\(\mu\)g/ml under physiological conditions, (Szegoleit et al., 1989).

The result of the test was not affected by enzyme replacement therapy since one of the subjects with chronic pancreatitis and steatorrhoea had been on the following enzyme supplements prolipase, viokase and pancreatin for the past 2 years. This supports the finding that faecal elastase1 results are not affected by pancreatic enzyme replacement therapy (Stein et al., 1996), which is in contrast with faecal chymotrypsin whose measurement has widely been accepted as a useful indirect test for pancreatic function.

The ability of a simple non invasive but sensitive and specific test of pancreatic function to diagnose or exclude exocrine pancreatic insufficiency or pancreatic involvement in abdominal pain has led to the widespread use of faecal elastase1 as a follow up study of patients with mild, moderate and severe pancreatic insufficiencies.

The specific objective of this study was to evaluate the pancreatic function in the patients with exocrine pancreatic insufficiencies caused by pancreatic cancer and chronic pancreatitis, and compare with apparently healthy persons, and patients with gall stones without duct obstruction, peptic ulcer and other non pancreatic diseases, which constitute a control group.
The results of the study showed that all but 2 of the 25 apparently healthy control subjects enrolled for the study had faecal elastase value of 200μg/g or more giving a 92% specificity. One of the subjects whose enzyme concentration was 165μg/g could not be contacted for a repeat of the test, whereas the second subject had a value of 195μg/g, could be considered to have a normal enzyme concentration when compared with the cut off level of 190μg/g, reported by (Gullo et al., 1999), in their 53 healthy control subjects studied.

The faecal elastase test has proven to be satisfactory as far as the specificity is concerned. In 40 patients with non pancreatic but various gastroduodenal disease cases studied all except 5 had faecal elastase values greater than 200μg/g.

It is possible that three out of the five subjects who had been diagnosed differently for diverticular disease of the transverse colon (by ultrasound), duodenitis, and peptic ulcer by upper gastrointestinal endoscopy may have exocrine pancreatic insufficiencies as well. The concentrations of their faecal elastase which were 85, 120 and 129μg/g respectively, could be classified as mild to moderate degrees of pancreatic insufficiency.

The results were found to be statistically significant (P>0.001) compared with values obtained for the apparently healthy group.

However the faecal elastase concentration of 170 and 194μg/g stool obtained for the subjects with transient enteritis and irritable bowel syndrome respectively are candidates for a repeat test but the patients could not be reached. Diarrhoea may be a possible cause of the reduction of elastase levels and this may be an area for further research, that is the effect of diarrhoea on elastase levels.
Glasbrenner et al. 1996 have suggested that patients with small bowel diseases may have a moderate reduction of faecal elastase1 concentration in the range of 100-200µg/g especially in the presence of diarrhoea.

A false abnormal faecal elastase1 concentration has been reported to be due to the degradation of the elastase in patients with bacterial overgrowth. (Sziegoleit and Linder 1991; Katschinski et al 1997).

With a cut off point of 200µg/g stool, the sensitivity of faecal elastase 1 was 89.3% in all patients with cystic fibrosis studied by (Walkowiak et al 1999), and a sensitivity of 93% was reported by (Loser et al 1996) for all their subjects with exocrine pancreatic insufficiency classified according to the secretin-cholecystokinin test.

The results obtained from this study showed a sensitivity of 90.6% on all the subjects with the pancreatic diseases. This was in agreement with published work mentioned above, thus indicating that faecal elastase 1 determination is a sensitive test for the detection of decreased exocrine pancreatic function.

The test result also shows that patients with chronic pancreatitis proven by imaging methods like computed tomography had decreased faecal elastase1 concentration indicating the severity of their conditions. One subject whose faecal elastase value was 20µg/g had reported to the clinician with an ERP result performed in the U.K., indicating chronic pancreatitis.
The subjects within the pancreatic disease group diagnosed as chronic pancreatitis with steatorrhoea all had elastase 1 concentrations < 100µg/g stool suggesting that faecal elastase 1 may not be able to differentiate pancreatic steatorrhoea from the steatorrhoea of intestinal malabsorption (Katschinski et al 1997).

Steatorrhoea, as part of the malabsorption syndrome, occurs at the absorptive stage in chronic pancreatic disease and also after massive resection of the gut or severe disease of the small intestine (Baron, Whicher and Lee Eds. 1989).

The concentration of faecal elastase 1 in the subjects with the cancer of the head of pancreas suggest severe exocrine pancreatic insufficiencies which may be due to obstructive processes as well as destruction of pancreatic tissue resulting from the tumour; this may play an important role in diminished secretion of the pancreas.

In any case, the secretory capacity is concentrated largely in the head of the gland and up to 85% of the pancreas can be removed surgically in some patients without severe impairment of enzyme secretion (Di Magno 1991).

The secretion patterns of elastase are similar to those of other pancreatic enzymes such as lipase, amylase and trypsin as highly significant correlations was found between faecal elastase and duodenal elastase concentration, duodenal lipase, amylase and trypsin by (Loser et al., 1996), stressing the fact that faecal elastase 1 concentration reflects the exocrine pancreatic capacity.

In their studies on the relationship between pancreatic ductal obstruction and pancreatic secretion Di Magno et al., 1979 found that duodenal volume and bicarbonate concentration were decreased for all stimuli in patients with pancreatic cancer or pancreatitis when compared with control subjects. Moreover the mean length of the
pancreatic duct was shorter in patients with pancreatitis or pancreatic cancer due to atrophy, overall shortening and decrease in pancreatic mass (Di Magno et al 1979).

In chronic pancreatitis, obstruction of the pancreatic duct by calculi, or an inflammatory mass can be a contributing factor in diminished pancreatic secretion, which could lead to lower enzyme levels in faeces.

The underlying reason for the good agreement of the direct pancreatic function tests with the faecal elastase 1 concentration is the intestinal stability of pancreatic elastase 1.

In summary, several imaging procedures with reported diagnostic sensitivities of up to 80% and specificities up to 90% are usually the first diagnostic step when pancreatic disease is suspected. Pancreatic function tests with similar diagnostic efficiency are used to directly or indirectly assess pancreatic exocrine function.

A major diagnostic problem is presented by the patient whose abdominal pain is caused by pancreatic insufficiency but whose imaging studies were normal in the absence of the endoscopic retrograde pancreatography which is the gold standard imaging procedure for diagnosing chronic pancreatic insufficiency in the country.

Pancreatic function tests are of value in characterising the severity of pancreatic insufficiencies but due to the limited sensitivities and specificities of the older tubeless methods, their uses had been limited. However, the gold standard pancreatic function test is invasive, difficult and not pleasant to the patient with the intravenous administration of secretin with or without cholecystokinin not to mention the possible side effects and the passage of tube into the duodenum.
Faecal elastase 1 which is pancreas specific has been found to have high diagnostic sensitivity and specificity values comparable to the secretin - cholecystokinin test.

The results of the faecal elastase 1 test clearly distinguishes patients with pancreatic insufficiencies from patients whose diseases were of non-pancreatic origin.

Faecal elastase is reported to be highly stable during its passage in the gut (Sziegoleit et al 1989), and the test was not affected by enzyme supplements.

In conclusion, morphological and functional changes of the exocrine pancreas in the patient with pancreatic duct obstruction require both imaging tests as well as sensitive and specific pancreatic function tests like the faecal elastase 1 for differential diagnosis in diseases of the exocrine pancreas.

The assay of the faecal elastase 1 in spot stool samples has been used as a laboratory diagnosis or exclusion of chronic pancreatic insufficiencies with the results of the test showing positive for diseases which can lead to pancreatic duct obstruction and negative for non pancreatic diseases.

Parts of this work was accepted for free paper presentation at the Conference on Pancreatic Disease - Controversies and Challenges in Chandigarh India, held on October 8-10, 1999.
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APPENDIX 1

Probability related to multiples of Standard deviations.

<table>
<thead>
<tr>
<th>Number of Standard deviations</th>
<th>Probability of observation showing at least as large a deviation from the population mean</th>
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</thead>
<tbody>
<tr>
<td>0.674</td>
<td>0.5</td>
</tr>
<tr>
<td>1.0</td>
<td>0.317</td>
</tr>
<tr>
<td>1.645</td>
<td>0.100</td>
</tr>
<tr>
<td>1.960</td>
<td>0.05</td>
</tr>
<tr>
<td>2.000</td>
<td>0.046</td>
</tr>
<tr>
<td>2.576</td>
<td>0.01</td>
</tr>
<tr>
<td>3.00</td>
<td>0.0027</td>
</tr>
<tr>
<td>3.291</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Adapted from appendix table A of statistics at square one by TDV Swinscow, printed by Latimer Trend and Company Ltd. Plymouth.
APPENDIX 2

Intra assay precision of faecal elastase 1 test

<table>
<thead>
<tr>
<th>Stool mass</th>
<th>Extraction buffer</th>
<th>Elastase concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>ml</td>
<td>µg/g</td>
</tr>
<tr>
<td>42.4</td>
<td>4.24</td>
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</tr>
<tr>
<td>47.8</td>
<td>4.78</td>
<td>350</td>
</tr>
<tr>
<td>38.0</td>
<td>3.80</td>
<td>350</td>
</tr>
<tr>
<td>30.3</td>
<td>3.00</td>
<td>350</td>
</tr>
<tr>
<td>37.3</td>
<td>3.73</td>
<td>325</td>
</tr>
</tbody>
</table>

APPENDIX 3

Inter assay precision from two different faecal samples

<table>
<thead>
<tr>
<th>Elastase conc. for A</th>
<th>Elastase conc. for B</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/g</td>
<td>µg/g</td>
</tr>
<tr>
<td>114</td>
<td>343</td>
</tr>
<tr>
<td>124</td>
<td>352</td>
</tr>
<tr>
<td>125</td>
<td>325</td>
</tr>
<tr>
<td>85</td>
<td>340</td>
</tr>
<tr>
<td>120</td>
<td>375</td>
</tr>
</tbody>
</table>
APPENDIX 4

The concentrations for the stool samples were multiplied by 50 (corresponding to the concentration of extracted stool specimen i.e. 10mg/ml and the dilution factor of 500) to yield the concentration of pancreatic elastase 1 in ngEl/mg stool which equals μg El/g stool.