STUDIES OF SMALL INTESTINAL AND PANCREATIC
FUNCTION FOLLOWING EXPERIMENTAL EXOCRINE
PANCREATIC INSUFFICIENCY IN THE DOG

By

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Dedicated to

Mr I.G. Munro and the late Mr B.S.H. Potter  Ms.R.C.V.S.
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I declare that this thesis is my own composition
and that I carried out the work described.

The thesis has not been submitted for a degree of another
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CHAPTER 1

GENERAL INTRODUCTION
General Introduction

Gastrointestinal disease in the dog ranks second only to skin disease as a reason for consulting a veterinary surgeon (Burrows, 1983). Despite this high prevalence concerted efforts to uncover the aetiology and pathogenesis of individual disorders have been relatively recent. One such disease, exocrine pancreatic insufficiency (EPI), is associated with changes in the intestinal milieu and in intestinal function. Whether these changes are the direct consequence of lack of pancreatic secretion, or reflect an underlying disease process which precedes EPI is unclear. This thesis examines the effects of experimentally induced EPI on various aspects of small intestinal and pancreatic structure and function. The background to the present study is summarised in the following review of pancreatic exocrine function, naturally occurring EPI and certain aspects of experimentally induced pancreatic dysfunction.

Digestion and absorption of food

Food is composed of large complex molecules which are broken down into smaller molecules to facilitate absorption by specialised intestinal cells and entry into the bloodstream. These digestive and absorptive processes are made possible by the harmonious interaction of the various activities of the pancreas, gastrointestinal tract and liver (Go & Miller, 1983). Disharmony due to dysfunction in one or more of these organs can result in malassimilation of food. The pancreas is the major organ responsible for the enzymatic breakdown of food. It has both an endocrine and an exocrine function, the latter responsible for digestion. The exocrine pancreas is composed of many secretory acini and a duct system, which secretes a fluid rich in enzymes and bicarbonate into the small intestine. The secreted enzymes digest all classes of foodstuffs and can be broadly categorised according to their substrate as lipolytic (lipase), proteolytic (trypsin, chymotrypsin, elastase, carboxypeptidase) or saccharolytic (amylase).
Pancreatic proteolytic enzymes are secreted in an inactive form, ensuring that digestion does not begin until they reach the small intestine where activation by trypsin takes place. Trypsin is formed by both the autocatalysis of trypsinogen at alkaline pH and the conversion of trypsinogen to trypsin by enterokinase, an enzyme situated on the duodenal mucosa. Bicarbonate secretion is principally responsible for neutralising gastric acid entering the duodenum, allowing digestive functions to proceed at optimum pH. Once digestion by pancreatic enzymes has occurred there is further digestion and absorption by specialised small intestinal epithelial cells, enterocytes. Figure 1.1 shows a schematic diagram of an enterocyte. The luminal surface of these enterocytes, the brush border, is composed of numerous rows of small cylindrical processes or microvilli, on which are situated digestive enzymes concerned with the breakdown of nutrients, such as disaccharides and polypeptides. The absorption of nutrients into the enterocyte is a specialised process occurring in different ways for differing dietary components e.g. carrier specific active transport for glucose and galactose. A summary of these digestive and absorptive processes is shown in Figure 1.2.
FIGURE 1.1 Schematic diagram of an enterocyte
Diagrammatic representation of protein (a), fat (b) and carbohydrate (c) digestion and absorption.

(a) Protein is degraded by proteolytic enzymes from the stomach and the pancreas and by brush border peptidases to release dipeptides and amino acids, which can cross the membrane on peptide or amino acid carriers respectively.

(b) Triglycerides are split by pancreatic lipase to release monoglycerides and free fatty acids, which interact with conjugated bile salts and present at the brush border as mixed micelles. The monoglycerides and the free fatty acids then cross the membrane by passive diffusion, while bile salts form further mixed micelles or are reabsorbed in the ileum.

(c) Carbohydrates are split by pancreatic amylase and/or brush border enzymes to release monosaccharides, which can then cross the membrane on glucose or fructose carriers.
Control of exocrine pancreatic function

Exocrine pancreatic function is controlled by complex neural and hormonal mechanisms which integrate the many functions of the gastrointestinal tract, namely, gastric, pancreatic and biliary secretion, and gastric and intestinal motility (Go & Miller, 1983; Greenberg, 1983; Grossman, 1984; Solomon, 1984). This ensures that synthesis and secretion of pancreatic juice into the duodenum is coordinated with the arrival of food, and is sufficient in quantity for its digestion. Neuro-humeral mechanisms also control basal pancreatic secretion which, along with gastric and biliary secretions and rhythmic contractions of the gut, may function as an "intestinal housekeeper" flushing debris and bacteria out of the small intestine (Chadwick, 1983; Itoh & Sekiguchi, 1983). The two major hormones implicated in the control of pancreatic secretion are secretin and cholecystokinin/pancreozymin (C.C.K.). Secretin is released from entero-endocrine cells into the bloodstream in response to acid in the duodenum and causes a marked increase in pancreatic water and electrolyte secretion (Llanos et al., 1977). Cholecystokinin is a hormone present in at least four molecular forms and has a dual localisation in gut and brain. Appetite regulation, trophic effects on the pancreas and stimulation of pancreatic enzyme secretion have all been attributed to C.C.K., but its widely accepted major role is stimulation of pancreatic enzyme secretion (Greenberg, 1983). Cholecystokinin is released in response to chemical stimulation of the small intestine by products of food breakdown, especially the products of protein digestion, amino acids and dipeptides (Meyer, Kelly & Jones, 1976). The release and actions of secretin and C.C.K. are not totally independent as there is some overlap in the factors causing release, and synergism of effects when both are released together (Henriksen & Worning, 1967; Greenberg, 1983). Many other hormones such as glucagon, insulin, gastrin, pancreatic polypeptide and somatostatin may also have a role in the regulation of pancreatic exocrine secretion but as even the actions of secretin and C.C.K. are not fully known it may
be some time before the influence of these hormones is finally understood (Greenberg, 1983; Solomon, 1984). It should also be noted that the endocrine pancreas may have some control over the exocrine pancreas; various endocrine-exocrine interactions have been proposed and impairment of one aspect of pancreatic function may influence the other (Henderson, Daniel & Fraser, 1981). Neural control of the pancreas is by parasympathetic, sympathetic and peptidergic nerves, all part of the autonomic nervous system. Parasympathetic stimulation causes a marked increase in pancreatic secretion, especially enzymes, and is thought to be responsible for most of the secretion which results from cephalic and gastric stimulation (Grossman, 1984). Parasympathetic nerves may also be responsible for proposed duodeno-pancreatic reflexes, i.e. a rapid increase in pancreatic secretion which occurs after intestinal stimulation but before a rise in blood C.C.K. concentrations (Singer, Solomon & Grossman, 1980). The role of sympathetic and peptidergic nerves in the control of pancreatic secretion is less clear than that of the parasympathetic nerve supply, and continues to be assessed (Holst et al., 1983).

**Exocrine Pancreatic Insufficiency**

When primary pancreatic disease is present or there is incoordination or failure of the mechanisms regulating pancreatic secretion, a lack of exocrine pancreatic secretion may result - exocrine pancreatic insufficiency (EPI).

**Clinical aspects**

In the dog primary pancreatic disease is usually due to chronic pancreatitis or pancreatic degenerative atrophy (Anderson & Low, 1965; Hill, Osborne & Kidder, 1971; Szabo et al., 1978; Prentice, James & Wadsworth, 1980; Rimaila-Parnanen & Westermarck, 1982). Chronic pancreatitis has a higher incidence in older female dogs, relapsing bouts of pancreatitis causing fibrosis and acinar destruction which can lead to severe impairment of pancreatic endocrine function.
and result in diabetes mellitus (Anderson & Low, 1965; Rimaila-Parnanen & Westermarck, 1982). Pancreatic degenerative atrophy on the other hand is a disorder of young dogs, especially German Shepherds, in which it is thought to be hereditary (Westermarck, 1980). It is characterised by idiopathic atrophy of the acinar cells with minimal inflammatory changes and little or no impairment of endocrine function (Hill, Osborne & Kidder, 1971; Szabo et al., 1978).

Primary pancreatic diseases are the most commonly diagnosed causes of EPI in the dog. However, certain conditions can result in functional impairment or secondary pancreatic insufficiency. Intra-luminal destruction of pancreatic enzymes may occur in the Zollinger-Ellison syndrome in which intestinal pH is markedly decreased by excess secretion of gastric acid (Jones, Nicholls & Badman, 1976; Straus, Johnson & Yalow, 1977). Diminished secretion of intestinal hormones, e.g. CCK, secondary to small bowel mucosal disease such as Sprue (DiMagno, 1983) may result in inadequate stimulation of pancreatic secretion. Finally, pancreatic enzyme synthesis may be decreased in protein malnutrition (Barbezat & Hansen, 1968; Gyr et al., 1975).

Dogs with EPI usually present with clinical signs of weight loss, polyphagia, increased faecal volume and deterioration in hair coat. These signs are thought to become apparent when the capacity of the pancreas to secrete digestive enzymes is reduced to 10% of normal (DiMagno, Summerskill & Go, 1973; Regan et al., 1977). The reduction in volume, enzyme and bicarbonate content of pancreatic secretion in dogs with primary pancreatic disease was demonstrated by the elegant experiments of Sateri in 1975. However, exocrine secretory changes in secondary pancreatic insufficiency in the dog have been less fully investigated; only indirect methods of pancreatic function testing have been employed, but these suggest a marked reduction in enzyme secretion (Rogers et al., 1980; Batt & Mann, 1981).
Pathophysiology

Figure 1.3 summarises the luminal and intraluminal alterations that occur in EPI. The lack of pancreatic enzyme and electrolyte secretion results in marked changes in the intestinal milieu, viz an increase in the maldigestion of nutrients and a decrease in pH. The digestion of carbohydrates, proteins and fats is affected. Impairment of carbohydrate digestion results in the accumulation of osmotically active polysaccharides, bacterial fermentation of which can produce alcohols and organic acids. These may increase intestinal motility and secretion, further compounding intra-luminal water retention (Moon, 1978; Isaacs & Kim, 1979; Mathias & Clench, 1985). Carbohydrate malabsorption may also be responsible for bacterial overgrowth in the small intestine due to an increase in substrate availability for bacterial multiplication. Impairment of carbohydrate digestion is, however, incomplete as disaccharides and monosaccharides can be digested and absorbed by the small intestine (see Figure 1.2).

Protein digestion is not affected until EPI is almost total because only small amounts of pancreatic proteolytic enzymes are necessary to complete the digestion of proteins following their initial peptic degradation in the stomach. Polypeptides, dipeptides and amino acids (the products of this digestion), can also be assimilated and absorbed at the brush border (see Figure 1.2).

Fat digestion in contrast is very seriously affected by EPI as lipase is produced almost entirely by the pancreas and is irreversibly inactivated at pH < 4 (Heizer, Cleveland & Iber, 1964), such as can occur due to diminished bicarbonate secretion (Dutta, Russell & Iber, 1979). Further impairment of fat absorption is caused by the precipitation of bile salts at low pH, which compromises optimal micelle formation prior to fat absorption (Regan et al., 1979). Medium and short chain triglycerides can be absorbed without prior hydrolysis by lipase.
FIGURE 1.3 Summary of the luminal and intraluminal changes occurring in exocrine pancreatic insufficiency.
Undigested fats and precipitated bile salts may cause additional problems when metabolised in the colon by colonic bacteria to hydroxy fatty acids and to deconjugated and hydroxy bile salts, both of which stimulate colonic secretion, and further aggravate impaired fluid reabsorption (Phillips, 1972; Isaacs & Kim, 1979; Balistreri, Heubi & Suchy, 1983; Mathias & Clench, 1985). Vitamin absorption may also be affected by EPI as the fat soluble vitamins D, E, A and K are lost in the faeces when fat is malabsorbed (Dutta et al., 1982); low serum concentrations of vitamin E have been found in dogs with EPI (Williams, 1985). Water soluble vitamins may also be affected as low serum cobalamin and high folate concentrations have been recorded in clinical cases of EPI (Batt & Morgan, 1982). The reasons for these changes are not clear but may be attributable to the changes that occur in EPI: decreased pH, decreased pancreatic enzyme concentrations and possibly bacterial overgrowth. The discovery that the canine pancreas secretes an "intrinsic factor" (Horodagoda et al., 1986) may have some bearing on cobalamin deficiency in dogs with EPI. Alternatively exocrine pancreatic secretions may not influence the absorption of cobalamin and folate as the observed alterations may have preceded EPI.

The role of the pancreas in preventing bacterial invasion of the small intestine is unclear. The increase in maldigested food in EPI may enable bacterial overgrowth to occur, whilst a bacteriocidal peptide identified in canine pancreatic secretions may be responsible for the maintenance of the relatively sterile environment of the small intestine (Rubinstein et al., 1985). The one reported investigation of the small intestinal bacterial flora in canine EPI indicated that bacterial overgrowth was present in two thirds of the cases studied. This study was undertaken in animals with naturally occurring EPI in whom an antecedent enteropathy could not be excluded (Williams, 1985; Williams, Batt & McLean, 1987).

The abnormal intestinal milieu may also affect the small intestinal mucosa as mild structural and marked biochemical abnormalities have been demonstrated in dogs with naturally occurring EPI (Batt, Bush & Peters, 1979b; Williams, 1985).
Biochemical changes were characterised by increased activities of some jejunal brush border and lysosomal enzymes (Batt, Bush & Peters, 1979b; Williams, 1985). It has been suggested that these changes are due to a decrease in the rate of degradation of brush border enzymes by pancreatic proteases (Arvanitakis & Olsen, 1974), with subsequent stimulation of lysosomal degradative mechanisms (Batt, Bush & Peters, 1979b). A primary enteropathy could not be excluded in these cases, in whom changes were only partially reversed after treatment with pancreatic enzyme supplementation (Williams, 1985).

The many changes in the intra-luminal environment in EPI can precipitate a self-perpetuating state of decompensation with nutrient losses leading to malnutrition, in turn leading to further pancreatic impairment by protein calorie malnutrition and/or diminished release of intestinal hormones that modulate pancreatic function (Rogers, 1983). It is therefore important to diagnose EPI at an early stage and to start treatment before this vicious cycle of debilitation proceeds too far. However, the clinical signs of EPI are non-specific and are produced by other gastro-intestinal diseases for which treatments differ considerably. It is therefore practically important that diagnostic tests which distinguish these conditions are employed.

**Diagnosis of EPI**

Diagnostic tests for EPI are judged by their ability to distinguish between maldigestion (pancreatic disease) and malabsorption (intestinal disease). Tests fall into four main categories - faecal tests, invasive tests, orally administered tests and the measurement of serum enzymes.

Microscopic examination of faeces may reveal undigested food particles (starch grains, fat globules, muscle fibres). Quantification is largely subjective, making this an unreliable diagnostic technique. Quantitative determination of faecal fat by chemical methods is a more objective procedure and allows evaluation of an animal's fat balance. This test requires long collection periods, is unpleasant to
perform and does not distinguish accurately between maldigestion and malabsorption, though dogs with EPI tend to have higher concentrations of faecal fat than occurs in malabsorption (Burrows, Merrit & Chiapella, 1979). Tests of faecal proteolytic activity are widely used and measure residual proteolytic enzymes of pancreatic origin in the faeces (Westermarck & Sandholm, 1980). However, the wide variation in the daily faecal concentrations of proteolytic enzymes of pancreatic origin, even in normal animals, limits the usefulness of these tests in diagnosis (Hill, 1972; Burrows, Merrit Chiapella, 1979).

Invasive tests of EPI are considered to be the most accurate indicators of pancreatic function in man (Lankisch, 1982). These tests involve the placement and maintenance of a tube in the small intestine and the assay of aspirated pancreatic secretion for enzymes and electrolytes. These requirements preclude their use in the dog except on a very limited scale (Sateri, 1975).

Orally administered tests of exocrine pancreatic function include fat and carbohydrate absorption tests, and a test for the \textit{in vivo} estimation of active chymotrypsin, the BT-PABA test. All rely on the detection of the test substance (or part of it) in the bloodstream and this is influenced by gastric emptying time and intestinal transit time. In fat absorption tests a long chain triglyceride is administered orally and the increase in plasma turbidity or triglyceride level is visually or quantitatively assessed (Brobst & Funk, 1972; Simpson & Doxey, 1983). If plasma turbidity or triglyceride concentration is low, the test is repeated with the addition of lipase to the long chain triglyceride; maldigestion is inferred by an increase in turbidity or triglyceride concentration. Quantitative triglyceride determination has only recently been performed and requires further evaluation to define the limits of its usefulness (Simpson & Doxey, 1983). Carbohydrates used in the assessment of EPI include the polysaccharide starch and the monosaccharides glucose and xylose. Measurement of plasma glucose after an oral dose of starch is an unreliable indicator of pancreatic function because starch digestion requires both pancreatic amylase and brush border enzymes, and
differentiation between deficiencies of these enzymes cannot be made. Oral tolerance testing using monosaccharides (which don't have to be digested before absorption) can be useful, a normal test providing evidence of the integrity of the small intestine. However, oral glucose tolerance tests can be affected by alterations in pancreatic endocrine function associated with pancreatic exocrine disease, rendering interpretation of results difficult in cases with concurrent intestinal and pancreatic disease. A better method is to use the monosaccharide D-xylose which is metabolised more slowly than glucose (Wyngaarden, Segal & Foley, 1957) and is not markedly affected by endocrine changes (Merrit, 1980). After oral administration xylose is transported across the small intestine, both passively and actively (Salomon, Allums & Smith, 1961; Levitt, Hakim & Lifson, 1969), where its increase in the blood can be measured. Xylose absorption has become the standard test for assessing the absorptive capacity of the small intestine in dogs and in EPI results are usually within the normal range (Hill, Kidder & Frew, 1970; Stradley, Stern- & Heinhold, 1979). Reduced xylose absorption has also been reported in humans, and dogs with EPI (Helman, Barbezat & Bank, 1978; Rogers et al., 1980; Batt & Mann, 1981).

The synthetic peptide, BT-PABA was devised specifically to replace unreliable faecal 'digest' tests and impracticable duodenal intubation tests (Imondi, Stradley & Wolgemuth, 1972). After oral administration the peptide is split by pancreatic chymotrypsin in the gut, releasing free PABA, which is absorbed across the intestine into the bloodstream where it can be assayed, enabling an in-vivo estimation of chymotrypsin activity to be made. This test has been considered to be a useful and specific test for the detection of EPI (Freudiger & Bigler 1977; Strombeck 1978; Batt, Bush & Peters, 1979a; Rogers et al, 1980; Batt & Mann, 1981). However, its ability to identify partial EPI has been questioned (Imondi, Stradley & Wolgemuth, 1972; Salway & Payne, 1976). Misleading results may also occur in the presence of small intestinal disease due to the malabsorption of free PABA, though concentrations are usually higher than those found in EPI (Batt
& Mann, 1981). The BT-PABA test combined with the Xylose test allows simultaneous evaluation of pancreatic and intestinal function (Stradley, Stern, Heinhold, 1979; Rogers et al., 1980; Batt & Mann, 1981). Combination of these two tests has revealed that many dogs with EPI have a very low PABA and a normal xylose, positively implicating the pancreas as the cause of the malassimilation, though occasionally both a low PABA and low xylose are found (Rogers et al. 1980; Batt & Mann, 1981). The reasons for reduced xylose absorption in dogs with EPI confirmed by other clinical tests and by the PABA test are not clear. It may be that a reduction in xylose absorption could result from intestinal bacterial overgrowth, an increased density of the brush border membrane or a reduction in brush border carriers; whether these are the direct result of EPI or an underlying enteropathy predisposing to pancreatic atrophy is not clear (Hellman, Barbezat & Bank, 1978; Batt & Mann, 1981; Williams, 1985).

Enzymes of pancreatic origin are found in the serum of normal dogs and are thought to enter the bloodstream through small venules and lymphatics draining the pancreas (Waldron et al., 1971; Papp et al., 1980). Serum concentrations of amylase and lipase are increased during acute inflammation of the pancreas and are employed in the diagnosis of pancreatitis (Brobst, Ferguson & Carter, 1970; Mia, Koger & Tierney, 1978). As amylase and lipase are not produced solely by the pancreas (Nothman & Callow, 1971; Hamosh et al., 1975) it has been recommended that amylase and lipase are assayed simultaneously to increase the specificity of a diagnosis of pancreatitis (Brobst, Ferguson & Carter, 1970; Mia, Koger & Tierney, 1978 b). The assay of a pancreas specific enzyme such as trypsin could resolve some of these difficulties, but trypsin is bound to inhibitors in the bloodstream (Ohlsson, 1971), which precludes its use as a test detecting enzymic activity. To overcome this problem a radioimmunoassay has been developed to measure serum trypsin-like immunoreactivity (TLI) (Williams & Batt, 1983). What the TLI test actually measures and the route of entry of TLI into the bloodstream is unclear, but it is suspected that the immunoreactive material
present in normal dog serum is trypsinogen (Borgstrom & Ohlsson, 1980; Williams & Batt, 1983) and that like other pancreatic enzymes it enters the bloodstream by leakage into the pancreatic lymphatic or venous system. Low TLI concentrations have been demonstrated in dogs with exocrine pancreatic insufficiency (Williams & Batt, 1983) and high concentrations reported in dogs with experimentally induced pancreatitis (Borgstrom & Ohlsson, 1980). In addition TLI appears to be pancreas specific as circulating TLI is abolished by pancreatectomy in man (Adrian, 1980; Malvano et al., 1980). The relationship of plasma TLI concentrations to plasma amylase and lipase activity, and the diagnostic usefulness of TLI in acute pancreatitis have not been evaluated.

Clinically it would be useful to know the relationship between the TLI test and the BT-PABA test in varying degrees of EPI in order to determine which is the better indicator of pancreatic atrophy. In human patients with severe EPI there is good correlation between serum TLI and concentrations of pancreatic enzymes in the intestinal lumen (Koop et al., 1980a; Vezzadini et al., 1980; Andriulli et al., 1981), but initial studies in the dog using BT-PABA as an indicator of luminal enzyme activity do not support this finding (Williams & Batt, 1986).
Conclusions

Exocrine pancreatic insufficiency is associated with severe derangement of the assimilation of food which can initiate a vicious cycle leading to progressive debilitation. As naturally occurring EPI only presents when pancreatic dysfunction is severe little is known about the time sequence of changes or their relationship to varying degrees of pancreatic structural abnormality. It is not clear whether the alterations observed in the small intestinal mucosa, the small intestinal bacterial flora, and in the absorption of xylose, cobalamin and folate, in dogs with naturally occurring EPI are due solely to the lack of pancreatic secretion, or whether these changes precede the development of EPI.

There have been few studies of the reversibility of changes associated with EPI by the administration of oral pancreatic enzymes, and none have examined the effects of enzyme replacement on the intestinal flora. The efficacy of pancreatic secretion replacement is not known.

Two tests of pancreatic exocrine function, BT-PABA and TLI, are currently considered useful in the diagnosis of EPI though their relative efficacy in this respect is not clear. Similarly knowledge of the pancreas specificity of amylase, lipase and TLI in the dog is incomplete. Interpretation of any changes observed in plasma amylase, lipase and TLI in dogs with naturally occurring or experimental pancreatitis is presently speculative.

Aims

The major aims of the present study were:

1) to determine the effects of experimental EPI on the structure and biochemical characteristics of the small intestinal mucosa, the bacterial flora of the small intestine, and the absorption of xylose, cobalamin and folate.

2) to assess the reversibility of any abnormalities detected (see 1) above) by the administration of exogenous pancreatic enzymes or by whole pancreatic secretions.
3) to devise, prepare and validate canine models of experimental EPI as a prelude to fulfilling aims 1 & 2.

Subsidiary aims were:
4) to assess the effects of experimentally induced pancreatitis and pancreatectomy on the plasma concentrations of amylase, lipase and TLI.
5) to compare the BT-PABA test and TLI tests in dogs with varying degrees of exocrine pancreatic atrophy.
6) to assess the effects on pancreatic exocrine and endocrine function of removing two thirds of the pancreas in the dog (see also Chapter 4).

The remaining material in this thesis is presented in five chapters. Chapter 2 describes the development and evaluation of a reversible model of EPI which has the potential merits of achieving minimal disruption of pancreatic structure, and ease in the manipulation of pancreatic secretions. Practical problems limited its efficacy in fulfilling the two major aims of the study and it was therefore discarded. Chapter 3 describes the preparation of a pancreatic duct ligated model and the investigations of EPI which were undertaken. The use of this model permitted most of the main aims of the study to be fulfilled. In Chapter 4 sub-total EPI was produced by the removal of two thirds of the pancreas and anastomosis of the pancreas to the bowel. This study which involves defining the degree of exocrine and endocrine pancreatic insufficiency was also relevant to the practical possibilities of organ transplantation in man. In Chapter 5 the effects of complete pancreatectomy on intestinal absorption and on the plasma concentrations of pancreatic enzymes are investigated. In each chapter the methods employed are described and their limitations reported, and the results obtained are reported and discussed. Finally, in Chapter 6 the conclusions for the study as a whole are drawn together, the extent to which the original aims were achieved discussed, and suggestions made for future studies.
CHAPTER 2
DEVELOPMENT AND EVALUATION OF A REVERSIBLE MODEL OF CANINE EXOCRINE PANCREATIC INSUFFICIENCY

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2.4 Discussion
2.1 Introduction

Clinical cases of canine exocrine pancreatic insufficiency (EPI) are reported to have biochemical abnormalities of the small intestinal mucosa largely restricted to brush border enzymes and lysosomes, overgrowth of small intestinal bacteria, and abnormal levels of serum vitamins (Batt, Bush & Peters, 1979b; Batt & Morgan 1982; Williams, 1985; Williams, Batt & McLean, 1987). These changes are thought to be due to a lack of pancreatic secretion or components of this secretion. The biochemical changes are characterised by an increase in the specific activities of the brush border enzymes, particularly the disaccharidases, maltase and sucrase (Batt, Bush & Peters, 1979b; Williams, 1985; Williams & Batt, 1987). It has been suggested that these increased specific activities are due to the absence of pancreatic proteolytic enzymes which regulate the turnover of exposed brush border enzymes (Arvanitakis & Olsen, 1974; Alpers & Tedesco, 1975; Seetharam et al., 1976; Kwong, Seetharam & Alpers, 1978). The small intestinal bacterial overgrowth could be due to malassimilation of food giving an increased substrate availability for bacterial growth. Alternatively it could result from a decrease in antibacterial activity, or a combination of both causes. The altered serum vitamin concentrations indicated by a decrease in cobalamin and an increase in folate could simply be due to a lack of certain ingredients of pancreatic secretion as exogenous pancreatic enzymes have been shown to reverse cobalamin malabsorption in human cases of EPI (Deren, Arora & Toskes, 1973). They could equally be the consequence of secondary bacterial changes (Batt & Morgan, 1982). Treatment of naturally occurring canine EPI with pancreatic enzyme supplements alone reverses the dietary malassimilation but does not restore either the mucosal normality (some abnormalities reverse, some persist and others develop do novo) or the normal concentrations of serum vitamins (Williams, 1985).

One interpretation of these findings is that an underlying enteropathy is a key factor in EPI and directly responsible for the pancreatic atrophy, perhaps through the malabsorption of an essential trace element, vitamin or amino acid (Levenson et al.,...
An enteropathy could also account for the observed alterations in the intestinal bacterial flora and serum vitamin levels.

As naturally occurring clinical cases of EPI are not seen at an early stage it is clearly not possible to study the sequence and the time course of events to determine the relationship between pancreatic and intestinal changes. To determine whether the small intestinal changes described above are due solely to a lack of pancreatic secretion one approach would be to study bowel function in dogs before and after the experimental induction of EPI. Pancreatectomy or pancreatic duct ligation are possible surgical methods of inducing EPI. Unfortunately, pancreatectomy results in diabetes mellitus, which, despite the administration of exogenous insulin is difficult to control, and makes it impossible to tell whether any changes observed are due simply to the lack of the exocrine pancreas. Pancreatic duct ligation on the other hand causes an initial acute pancreatitis which usually leads to chronic pancreatitis and total pancreatic atrophy (Idezuki, Goetz & Lillehei, 1969; Rausis, Choudhury & Ogata, 1970; Kyriakides, Nuttall & Miller, 1979; Gabel et al., 1983). In the short term, the endocrine pancreas is only mildly impaired (mirroring the clinical condition) whereas in the long term, chronic inflammation and pancreatic atrophy result in diabetes mellitus (Idezuki, Goetz & Lillehei, 1969; Rausis, Choudhury & Ogata, 1970). A further disadvantage of both pancreatectomy and duct ligation is that both procedures are irreversible and thus preclude the study of the reversibility of any changes attributable to the removal of exocrine secretion.

A third approach would be to divert the pancreatic secretion externally and numerous studies have developed techniques for the collection of pancreatic secretion from dogs. However, such studies have not been concerned with any changes in intestinal function which might arise. Collection techniques such as immobilisation of small bowel, bowel resection and/or the insertion of large metal cannulae into the small bowel (Thomas, 1959; Fawcett, 1970) have been employed which would almost certainly interfere with bowel motility and function, and perhaps
also predispose to bacterial overgrowth and mucosal damage (King & Toskes, 1979; Mathias & Clench, 1985).

In the light of the limitations of these "collection" methods it was decided to cannulate the main pancreatic duct without entering the duodenum and to exteriorise the cannula, a technique similar to that of Routley and co-workers (Routley et al., 1952). To ensure that all the pancreatic secretion flowed through the cannula the accessory pancreatic ducts would be ligated. Reversibility could be achieved by applying a modified Routley's technique (Routley et al., 1952) and inserting the free end of the cannula into the duodenum via the pancreatic papilla. A loop would then be exteriorised from which secretions could be collected externally as shown in Figure 2.1. Restoration of flow would easily be achieved by rejoining the cannulae. Because secretions are allowed to flow freely, it was anticipated that the subsequent pancreatitis and effects on pancreatic endocrine function would be minimal.

The feasibility of creating such a model was investigated using canine cadavers. These studies showed that it was possible to locate and cannulate the main pancreatic duct without entering the duodenum with minimal trauma to pancreas and duodenum. The successful creation of this model would allow some of the original aims (see Chapter 1) to be addressed.

**Aims**

The principal aim given success of the experimental model was to determine the effects of experimentally induced EPI on the structure and biochemistry of the small intestine, the bacterial flora of the small intestine, the absorption of xylose, the plasma concentrations of cobalamin and folate, and to assess whether any such changes were reversible following restoration of exocrine secretion.

A subsidiary aim was to measure plasma concentrations of trypsin-like immunoreactivity (TLI), amylase and lipase before and after diversion of pancreatic secretions to determine whether the plasma concentrations of these enzymes
resulted from leakage from the pancreas, or absorption from the gut following pancreatic secretion (Malvano et al., 1980; Williams & Batt, 1983).

As long term diversion of pancreatic secretion causes dehydration and death (Gamble & McLver, 1928; McCaughan, 1931) a balance had to be struck between allowing time for intestinal changes to occur and avoiding the lethal consequences of long term removal of secretion. It was decided therefore to collect secretion for only four days and to monitor the dogs carefully during this collection period.

**Protocol**

![Protocol Diagram]

Figure 2.2 is a summary of the protocol. Exocrine pancreatic function testing and estimation of plasma amylase, lipase, glucose, cobalamin and folate concentrations were undertaken in the seven days pre-operatively. Small intestinal biopsies and duodenal juice were obtained at the time of pancreatic duct cannulation. There followed a one week recovery period, during which time blood was taken daily for the estimation of amylase, lipase and glucose. At the end of this recovery period exocrine pancreatic function was assessed and blood was obtained for the estimation of cobalamin and folate concentrations. Pancreatic secretion was then diverted for four days and blood was taken daily for the estimation of amylase, lipase, TLI, cobalamin, folate, packed cell volume and bicarbonate. Pancreatic function tests were carried out on the second day of this period and biopsies of the
small intestine and duodenal juice were obtained at the end of this period. Finally, pancreatic secretion was redverted into the duodenum for seven days. Blood was then taken for the estimation of amylase, lipase, TLI, cobalamin and folate concentrations and pancreatic function tests were repeated. At the end of this period intestinal biopsies and duodenal juice were obtained under terminal anaesthesia and a post-mortem examination was performed.

2.2 Materials and Methods

2.2.1 Patients
Seven adult Greyhounds (5 male, 2 female) were housed in a controlled environment and received a standard diet (Pedigree Chum and mixer, Pedigree Petfoods, Melton Mowbray, Leicestershire.) for at least one month prior to, and throughout, the study. Dogs were acclimatised to the jackets, bags and harnesses used for the collection of pancreatic secretion.

2.2.2 Anaesthesia
Prior to surgery the dogs were fasted overnight and premedicated with acepromazine (ACP, 0.05mg/kg ; C-Vet Ltd.) and chlorpheniramine (Piriton,1mg/kg ; Allen & Hanbury Ltd., Greenford, Middlesex.) twenty minutes before induction of anaesthesia with alphaxalone and alphadolone (Saffan,1.2mg/kg ; Glaxovet Ltd., Middlesex). Anaesthesia was maintained with halothane (1-2 %), nitrous oxide and oxygen (2:1) during the operative period when dogs were mechanically ventilated.

2.2.3 Surgery
The operation was performed through a midline abdominal incision. The edge of the body of the pancreas was separated from the duodenum by blunt dissection and the pancreatic ducts were located with minimal trauma to both the pancreas and the duodenum. Haemorrhage was controlled with ligatures and diathermy. Any accessory ducts were ligated. An incision was made in the main duct and a cannula (FG 8, infant feeding cannula 50cm,Vygon,95440 Ecouen, France.) inserted into the
main duct 2-4mm towards the pancreas and secured with two ligatures (Neurolon, 3/0 ; Ethicon Ltd.). A second cannula was then inserted into the duodenal end of the cut pancreatic duct and fed into the duodenum. This cannula was modified, to allow easy flow of secretion, by making several holes in the terminal 2-3cm that would lie in the duodenum, and was secured in place with two ligatures. To ensure the cannulae remained in place each was secured to the duodenum with a vicryl suture. An incision was then made in the right flank and the free ends of the two cannulae tunneled out through the body wall into the incision. The cannulae were secured to the body wall with sutures and joined together with a short connecting piece (see Figure 2.1). To confirm that the cannulae remained patent and that the ligatures had not been tied too tightly the flow of pancreatic secretion was observed following stimulation with secretin (Boots Ltd., 1U/kg; injected intravenously over thirty seconds). Finally, the abdominal incision and the small skin incision in the flank were closed. A prolene loop was used to close the abdominal muscle layer (Ethilon, 0 ; Ethicon Ltd.), chromic catgut for the subcutaneous tissue (2/0 ; Ethicon Ltd.) and monofilament polyamide for the skin (2/0; Supramid, B Braun Melsungen A.G., Germany.).

2.2.4. Post-operative care
Post-operatively the dogs were placed in a heated recovery area, and given an intravenous dextrose saline drip for twenty four hours. Buprenorphine (Temgesic, 0.03-0.1mg/kg ; Reckitt & Colman Ltd., North Humberside.) was administered for three days to ensure post-operative analgesia. Oral alimentation was restricted to balanced electrolyte solution (Lectade; Beecham Animal Health) for forty-eight hours post-operation, thereafter solid food was reintroduced.

Each dog wore a jacket for the duration of the study and the duodenal end of the cannula was flushed with sterile saline twice daily.

2.2.5 Pancreatic function tests
*Combined BT-PABA/xylose absorption test*
FIGURE 2.1 Schematic diagram of the reversible model of EPI pancreatic juice flows along the cannula inserted into the main pancreatic duct, through the duodenal cannula and into the duodenum. Juice is collected by removing the joining piece and attaching a collection bag to the pancreatic cannula.
Exocrine pancreatic function and small intestinal absorption were assessed by use of the chymotrypsin labile substrate N-benzoyl-L-tyrosyl-p-aminobenzoic acid (BT-PABA) combined with the pentose sugar xylose (Batt & Mann, 1981). A solution containing BT-PABA (Fluorochem Ltd., Glossop, Derbyshire) (16.5mg/kg body weight) and D-xylose (BDH Chemicals, Poole, Dorset) (0.5g/kg body weight) in 10 ml/kg of water was administered by stomach tube after an overnight fast. Venous blood samples were taken into heparin immediately before, and at thirty minute intervals for three hours after administration of the test solution. Plasma samples were assayed for PABA (Batt, Bush & Peters, 1979a) and xylose (Trinder, 1975) and the results adjusted by subtracting the pre-administration concentrations of these substances.

**Amylase, lipase and trypsin-like immunoreactivity assays.**

Amylase (EC 3.2.1.1) was assayed in heparinised plasma samples by use of an enzymic colorimetric test with p-nitrophenyl-α,D-maltoheptaoside as substrate (α-Amylase PNP; Boehringer Corporation London Ltd.). Lipase (EC 3.1.1.3) was measured using a turbidimetric method with triolein as substrate (Lipase, UV turbidometric, Boehringer Corporation London Ltd.). Changes in absorbance were measured on a spectrophotometer (LKB 4050, LKB Instrument Ltd., Croydon), 405nm for amylase and 365nm for lipase. Samples were assayed immediately after collection.

Plasma for the assay of trypsin like immunoreactivity was stored at -20°C until measured by radioimmunoassay (Williams & Batt, 1983).

**Glucose assay**

Endocrine pancreatic function was assessed by measuring fasting blood glucose concentrations. Blood glucose was measured immediately by use of a glucose analyser (Reflocheck, Boehringer Corporation London Ltd.).
2.2.6. Collection of pancreatic secretion

The duodenal end of the cannula was blocked off with a small plastic spigot and the pancreatic end attached to a sterile collecting bag (Simpla leg bag, Simpla Plastics Ltd., Cardiff.) which was secured underneath the dog with straps. The bags were changed twice daily at 9am and 5pm, and the volume of secretion was recorded before freezing at -20°C. To monitor the effects of removing secretion on fluid and electrolyte balance, packed cell volume (PCV) (an index of hydration) and bicarbonate concentration were measured in venous blood samples taken throughout the drainage period. Bicarbonate was measured on a blood gas analyser (ABL2, Radiometer Ltd., Denmark) and PCV determined by centrifugation in microhaematocrit tubes for five minutes. Secretion was restored to the duodenum by re-connecting the two ends of the cannulae.

2.2.7 Vitamin assays

Plasma was separated from heparinised blood samples taken after an overnight fast and then stored at -20°C until vitamin assays were carried out.
Plasma cobalamin and folate concentrations were measured by use of a commercially available radio-immunoassay system (Becton & Dickinson Ltd.) which had previously been validated in the dog giving results comparable with those obtained by microbiological assays (Batt, 1984, unpublished observations).

2.2.8 Collection and examination of duodenal juice

Duodenal juice was collected at laparotomy (prior to biopsy of the small intestine) with a sterile catheter (Abocath 14G) inserted obliquely through the wall of the duodenum into the lumen. The juice was then transferred into two sterile Bijou bottles which were stored on ice until examination. One sample was examined directly by light microscopy for the presence of *Giardia* spp. and the other cultured for aerobic and anaerobic bacteria.
**Culture of duodenal juice**

Duodenal juice (0.1 ml) was serially diluted with sterile distilled water to give dilutions of 10^-2, 10^-3, 10^-4, 10^-5 and 10^-6.

**Aerobic culture**

Each dilution (0.1 ml) was applied in duplicate to plates of Nutrient agar, Mann Rogosa Sharp agar (MRS), Maconkey's agar, Sabarau'ds agar and blood agar base containing 5% horse blood. These plates were inoculated using a glass hockey stick (which was flamed between inoculations), and then incubated at 35°C for 24 hours and examined for bacterial colonies. The number and type of colonies on each medium at each dilution was recorded for all dilutions. Each representative colony was then Gram stained and tested for catalase. The addition of a drop of dilute hydrogen peroxide (25 %) to a suspension of bacteria with the production of gas bubbles constituted a positive result for catalase.

**Anaerobic culture**

Each dilution (0.1 ml) was applied in duplicate to plates of blood agar base containing 5% horse blood. The plates had been pre-gassed with hydrogen and carbon dioxide for 24 hours before inoculation. After inoculation (as for aerobic plates) plates were placed in an anaerobic jar containing a hydrogen and carbon dioxide generator (BBL, Gaspack) and an anaerobic indicator strip, and incubated at 35°C for 72 hrs. Counting, staining and catalase testing were then performed as described above.

**Classification of bacteria**

Bacteria were categorised according to the media on which they grew, the Gram stain result, the cell morphology and whether catalase positive or negative. The criteria for classification are shown in Table 2.1. Total counts for aerobic colonies were calculated by summing the number of colonies that grew on nutrient agar and
the number growing on MRS. Total anaerobe counts are the sum of the colonies that grew on the anaerobic plates.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gram</th>
<th>Catalase</th>
<th>Spores</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus/Micrococcus spp.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>coccus</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>coccus</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>bacillus</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>bacillus</td>
</tr>
<tr>
<td>Coryneform spp.</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>pleomorphic</td>
</tr>
<tr>
<td>Bacteroides spp.*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>rod</td>
</tr>
<tr>
<td>Clostridium spp.*</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>rod</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>rod</td>
</tr>
</tbody>
</table>

TABLE 2.1 Classification of bacteria
* = obligate anaerobe

2.2.9 Intestinal biopsy

Biopsies of small intestinal mucosa were obtained at laparotomy under general anaesthesia as described previously (2.2.2-4). The multiple suction biopsy apparatus (Quinton Instrument Co. Ltd., Seattle, USA) used to obtain the biopsies consists of a firing mechanism with a long tube and a capsule attached to the end. The tube and capsule are passed down the oesophagus through the stomach and into the duodenum. A vacuum is applied which sucks the intestinal mucosa into a hole in the capsule, a high pressure water jet is fired down the tube and hits a small knife blade inside the capsule, cutting off a sample of mucosa. Water then carries the biopsy to the proximal end of the tube where it is collected. This apparatus allows multiple biopsies to be taken without having to continually withdraw and reposition the tube.

Biopsies were taken from the distal duodenum: three were placed in buffered formal saline for histological examination (the tissue was embedded in paraffin wax, sectioned and stained with haematoxylin and eosin) and one into each of 3ml distilled water and 3ml SVE medium for assays of brush border enzymes. (SVE
medium was composed of sucrose (0.3 mol/l) containing EDTA (1 mmol/l) pH 7.4 and ethanol (22 mmol/l)). Biopsies taken into water were stored at -20°C pending assay of disaccharidases. Those collected into SVE were kept frozen to enable further investigation arising from the results of the disaccharidase assays.

2.2.10 Enzyme assays
The biopsies collected in 3ml distilled water were disrupted in a Dounce homogeniser (Kontes Glass Co., Vineland, New Jersey, USA) with twenty strokes of a tight fitting pestle (type B) and the homogenates were assayed for lactase (EC 3.2.1.23), sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) (Peters, Batt, Heath & Tilleray, 1976). Table 3.1 (Chapter 3) gives details of substrates and assay conditions. Fluorescence was measured using a Perkin-Elmer 2000 fluorescence spectrophotometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire).

The protein content of each homogenate was determined by the method of Schacterle & Pollack (1973) with bovine serum albumin (Armour Pharmaceutical Co., Eastbourne, Sussex) as the standard. Enzyme specific activities were expressed as mU/mg protein. Absorbance was determined by use of a Beckman DU-8 spectrophotometer (Beckman Scientific Instruments, High Wycombe, Buckinghamshire).

2.2.11 Post-mortem examination
A post-mortem examination was undertaken in each dog to ascertain the route of pancreatic secretion into the intestine and the nature of the pancreatic duct system. The left and right lobes of the pancreas were cannulated and a blue dye (methylene blue) and a radiopaque dye (Hypaque, Winthrop Ltd.) injected to allow visual and radiographic examination.
2.3 Results

2.3.1 Post-operative recovery
Three animals developed complications in the first three days post-operatively. One became severely jaundiced, was euthanased; post-mortem showed a ruptured bile duct. The remaining two had no secretion flowing from their pancreatic cannulae: one had come out of the pancreatic duct and the other had become blocked at the proximal (pancreatic) end. Of the remaining four animals one chewed at the exteriorization site and pulled the cannula out of the duodenum during the drainage procedure. The effects of drainage and reversibility was therefore studied in only three animals.

2.3.2 Pancreatic function
Figure 2.3 shows the results of the BT-PABA/xylose test. It can be seen that in all dogs plasma PABA concentrations decreased slightly post-operatively, fell dramatically after diversion of secretion and were partially restored to normal by the re-introduction of secretion into the duodenum. Plasma xylose concentrations fell in all animals post-operatively and were unaffected by the removal of secretion in dogs 2 & 3, but decreased further in dog 1. Re-introduction of secretion restored the level in dog 1, increased it in dog 3, but decreased it in dog 2.
The increases in plasma amylase and lipase in each dog post-operatively followed similar patterns. However, the timing and magnitude of these increases varied among dogs, but all increases had subsided by the end of one week (Figure 2.4).
FIGURE 2.3 BT-PABA/ Xylose absorption tests.
Figure 2.4 Post-operative plasma amylase and lipase activity.

After diversion of secretion a marked increase in amylase and lipase activity occurred to a greater extent than post-operatively. After the re-introduction of secretion there was rapid restoration of pre-drainage values (Figure 2.5).

TLI concentrations increased dramatically during diversion, peaked rapidly, after one day and returned to pre-diversion levels after the reintroduction of secretion (Figure 2.5).

Blood glucose concentrations were within the normal range (3.2 - 6.6mmol/l) in all dogs throughout the study period (Table 2.2).

### 2.3.3 Drainage procedure

In dogs 1 & 2 pancreatic secretion was collected for four days, but in dog 3 secretion stopped flowing after two days. This dog was operated on immediately. Intestinal biopsies were obtained and a blockage in the pancreatic cannula was cleared and the flow of juice restored. All dogs were slightly depressed by the end of the diversion period. Table 2.2 shows the amount of secretion collected and the effect on venous bicarbonate and packed cell volume (PCV). Bicarbonate values tended to drop and then stabilise but PCV did not change noticeably during diversion.
FIGURE 2.5. The effects of the external diversion of pancreatic secretion on plasma concentrations of amylase, lipase, TLI and cobalamin.
**TABLE 2.2 Results of pancreatic juice diversion**

<table>
<thead>
<tr>
<th>DOG</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>bicarbonate (mmol/l)</td>
<td>26</td>
<td>21</td>
<td>15</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>62</td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td>pancreatic juice (ml)</td>
<td>-</td>
<td>850</td>
<td>950</td>
</tr>
<tr>
<td>plasma folate (µg/l)</td>
<td>3.9</td>
<td>6.6</td>
<td>4.8</td>
</tr>
<tr>
<td>blood glucose (mmol/l)</td>
<td>4.1</td>
<td>3.8</td>
<td>4.2</td>
</tr>
</tbody>
</table>

### 2.3.4 Mucosal changes

There were no marked gross histological changes in biopsies throughout the study. Figure 2.6 gives the specific activities of maltase, sucrase and lactase. After diversion of secretion, dogs 1 & 3 had reductions in the specific activities of maltase, sucrase and lactase which were partially restored towards normal by the re-introduction of secretion. In dog 2 following diversion, the specific activity of lactase was reduced but the specific activities of sucrase and maltase were increased. Re-diversion of secretion reversed the increases in sucrase and maltase, but lactase decreased further.

### 2.3.5 Vitamin concentrations

When secretion was removed for four days (dogs 1 & 2), especially dog 2, plasma cobalamin decreased rapidly during diversion and increased toward normal after the restoration of secretion. This is illustrated in Figure 2.5. Plasma folate concentrations did not vary greatly post-operatively (Table 2.2).

### 2.3.6 Examination of duodenal juice

Examination of duodenal juice for *Giardia* spp was invariably negative.

After diversion of secretion there was a massive increase in the numbers and types of aerobic and anaerobic bacteria isolated.
Figure 2.6. The effects of diversion of pancreatic secretion on disaccharidase activity and reversibility following rediversion of secretion into the duodenum.
Table 2.3 details these findings. In all three dogs there were increases in the aerobic or facultatively anaerobic bacteria *Lactobacillus* spp., *Streptococcus* spp. and *Bacillus* spp., and in the obligate anaerobic bacteria, *Clostridium* spp. (dogs 2 & 3) and *Bacteroides* sp. (dog 1). Re-diversion of secretion into the intestine had little effect on the quantity, although there were qualitative changes: *Lactobacillus* spp. were replaced by *Streptococcus* spp. on MRS in all dogs and *Bacteroides* sp. and *Clostridium* spp. disappeared in dogs 1 and 2 respectively (Figure 2.7 and Table 2.3).

### 2.3.7 Post mortem examination

Only a few omental adhesions to the pancreas were found surrounding the cannulae. The pancreas was fairly normal in appearance in dogs 1 & 3, but in dog 2 the right lobe had atrophied and the left was hard, nodular and firm.

Where the left and right lobes of each pancreas had been incised to enable a cannulae to be inserted for the injection of dye, the main ducts were dilated and the duct of the atrophied right lobe of dog 2 contained a thick exudate. When dye was injected, it flowed out of the pancreatic cannula in all dogs, but in dogs 1 & 3 some escaped around the edge of the duodenal cannula into the duodenum. Figure 2.8 shows a radiograph taken after the injection of radiopaque dye into the pancreatic...
<table>
<thead>
<tr>
<th>Time</th>
<th>pre-diversion</th>
<th>diversion</th>
<th>rediversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>AER</td>
<td>ANAER</td>
<td>AER</td>
</tr>
<tr>
<td><strong>DOG 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staph/Micro</td>
<td>3</td>
<td>6.48</td>
<td>7.78</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>5</td>
<td>6.18</td>
<td>7.48</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
<td>7.78</td>
<td></td>
</tr>
<tr>
<td>Bacillus</td>
<td>4</td>
<td>6.68</td>
<td>7.48</td>
</tr>
<tr>
<td>Coryneform</td>
<td>4.3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Bacteroides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobact.</td>
<td></td>
<td>6.78</td>
<td>8.48</td>
</tr>
<tr>
<td>yeast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+ve cat+/-</td>
<td></td>
<td>6.0+</td>
<td>7.7-</td>
</tr>
<tr>
<td>G-ve cat+/-</td>
<td></td>
<td>4.0+</td>
<td>6.95+</td>
</tr>
<tr>
<td>TOTAL</td>
<td>4</td>
<td>6.36</td>
<td>7.59</td>
</tr>
</tbody>
</table>

| **DOG 2**    |     |       |     |       |     |       |
| Staph/Micro  |     |       |     |       |     | 7.48  |
| Streptococcus|     | 5.3   | 7   |       |     |       |
| Lactobacillus|     | 6.78  |     |       |     |       |
| Bacillus     |     | 6     | 7.48|       |     |       |
| Coryneform   |     | 3.48  |     | 7.2   |     |       |
| Bacteroides  |     |       |     |       |     |       |
| Clostridia   | 4   | 7     | 7.6 |       |     |       |
| Enterobact.  |     | 7     | 7.7 | 7.7   |     |       |
| yeast        |     |       |     |       |     |       |
| G+ve cat+/-  |     | 7.9-  |     | 6.3-  |     |       |
| G-ve cat+/-  |     | 7.7-  | 6.84+| 7.7+ &-|     |       |
| TOTAL        | <3  | 4.11  | 7.41| 8.23  | 7.78| 8.28  |

| **DOG 3**    |     |       |     |       |     |       |
| Staph/Micro  | 3   | 4.3   | 5   | 6.78  | 7.48|       |
| Streptococcus| 4   | 5     | 6.78| 7.48  |     |       |
| Lactobacillus|     | 6.58  |     |       |     |       |
| Bacillus     | 3.3 | 6     | 7.3 | 6.48  |     |       |
| Coryneform   |     | 5     | 5   |       |     |       |
| Bacteroides  |     |       |     |       |     |       |
| Clostridia   |     | 6.48  |     |       |     |       |
| Enterobact.  |     |       |     |       |     |       |
| yeast        |     |       |     |       |     |       |
| G+ve cat+/-  |     | 7.0-  | 6.78+| 6.78-| 7.95+|       |
| G-ve cat+/-  |     | 3.3   | 6.69| 7.12  | 8.15| 8.18  |
| TOTAL        | <3  | 3.3   | 6.69| 7.12  | 8.15| 8.18  |

**TABLE 2.3** Number (log10) and type of bacteria in duodenal juice  
cat = catalase ; Staph = Staphylococcus spp ; Micro = Micrococcus spp ;  
Strep = Streptococcus spp ; Enterobact. = Enterobacteriaceae.  
AER = aerobic, ANAER = anaerobic
FIGURE 2.8
Radiograph of pancreas following the intraductal injection of radiopaque contrast medium (dog 1).
dc = duodenal cannula, pc = pancreatic cannula, arrow indicates contrast medium on the duodenal mucosa.

FIGURE 2.9
Fistula between the pancreas and the duodenum (dog 1)
d = duodenum, p = pancreas
cannula of dog 1. The ducts draining the right and left lobes connected in dogs 1 & 3 but, in dog 2 dye did not leave the pancreatic cannula, or flow into the left lobe when the right lobe was injected.

Figure 2.9 shows a dissection of the main pancreatic duct where the cannula entered the duodenum (dog 1). It can be seen that a fistula has formed between the pancreas and duodenum, and this was responsible for the leakage of dye into the duodenum observed in dogs 1 & 3.

Histological examination of the pancreases revealed only minor changes in dogs 1 & 3, viz, mild chronic inflammation of the pancreatic ducts and little parenchymal damage. Chronic inflammation and severe exocrine atrophy of the right lobe and mild chronic interstitial inflammation with mild exocrine atrophy of the left lobe were observed in dog 2. Normal clumps of islet cells were observed in all three dogs.

2.4 Discussion

Clinical Outcome

Despite careful preliminary studies reversible EPI was achieved experimentally in only three of seven animals. The technique was successful only in the short term for three weeks after cannulation (at post-mortem) two dogs (1&3) had developed a fistula between the pancreas and the duodenum. These results are similar to those of Routley et al., (1952) who reported functioning cannulae in the "majority" of dogs (numbers not given) for periods of around 3 to 4 weeks before either fistula formation around the cannula or formation of a new drainage channel allowed pancreatic juice to enter the duodenum.

The "depression" in the three dogs towards the end of the collection period could have resulted from fluid and electrolyte imbalance and/or acid base disturbance. The animals were not dehydrated clinically. All three were moderately acidaemic as indicated by the reduced bicarbonate levels during the early part of the collection period, though this effect did not result in obvious hyperventilation. Bicarbonate stabilised, presumably due to enhanced renal reabsorption, at a lower than normal level. Although serum electrolyte values were not measured it is likely
that all the dogs suffered some degree of sodium and potassium depletion. Their rapid recovery following re-introduction of secretion and the absence of alternative explanation for their symptoms such as infection, supports the explanations offered.

**Plasma amylase, lipase and TLI**

In the post-operative period, amylase and lipase activities rose and fell in unison, but to a varying degree for individual dogs. These increases were presumably caused by the inevitable trauma to the pancreas at surgery and the increased resistance to the flow of pancreatic juice caused by the cannula. The parallel increases in amylase and lipase are in accord with studies of induced acute pancreatitis in dogs (Brobst, Ferguson & Carter, 1970; Mia, Koger & Tierney, 1978b), and with findings in man, where cannulation of the pancreatic duct for collection of secretion and injection of radiopaque contrast media results in elevation of amylase and lipase (Figarella et al., 1978; Okuno et al., 1985). [It is unlikely that the use of heparinised plasma samples for the determination of lipase affected the results. Heparin is known to activate lipoprotein lipase and hepatic triglyceride lipase (Fielding, 1970; Greten et al., 1974), but preliminary studies using heparinised and non-heparinised plasma samples from the dogs in this study failed to reveal significant differences in the estimated levels of both amylase and lipase. Perhaps the main danger is in heparinising the animal and activating lipoprotein lipase *in vivo*.]

The increase in amylase, lipase and TLI following diversion of secretion were unexpected as the flow of secretion had not been interrupted (free drainage of large volumes of secretion into the collection bags was observed throughout). It may have resulted from increased flow resistance causing back pressure, or haemoconcentration resulting from the loss of pancreatic secretion. The latter explanation seems unlikely as PCV remained remarkably constant in each dog. A further possibility is an increase in pancreatic enzyme synthesis due to impairment of intestinal feedback mechanisms which control synthesis. Feedback inhibition systems have been described in the rat and man and are thought to act by
decreasing concentrations of CCK (Green & Lyman, 1972; Louie et al., 1986; Owyang, Louie & Tatum, 1986). However, studies in the dog using trypsin inhibitors and diversion of pancreatic secretion have failed to demonstrate any such feedback inhibition system (Sale et al., 1977).

The increases in amylase, lipase and TLI, suggest a common route into the bloodstream, perhaps via lymphatics or pancreatic venules (Waldron et al., 1971). Since pancreatic secretion was diverted externally these enzymes could not have been reabsorbed from the intestine. Conceivably an increase in back pressure or increased enzyme synthesis during diversion could mask an intestinal route. In the normal dog a dual route of entry into the bloodstream may exist as the small intestine is permeable to large protein molecules such as trypsinogen (Gotze & Rothman, 1975; Liebow & Rothman, 1975).

**Exocrine pancreatic function and intestinal absorption**

The reduction in plasma xylose and PABA concentrations post-operatively, prior to diversion of pancreatic secretion is probably due to bacterial overgrowth. The utilisation of xylose by bacteria has been suggested as a mechanism to explain the association of bacterial overgrowth and low xylose absorption (Goldstein et al., 1970). Alternatively (or additionally) the bacteria, their products, or intraluminal metabolites e.g. deconjugated bile salts may have damaged enterocytes and impaired xylose absorption (Donaldson, 1967; Gracey et al., 1971; Gianella, Rout & Toskes, 1974; Gracey, Houghton & Thomas, 1975; Toskes et al., 1975). The concurrent reduction in PABA might also have resulted from impaired absorption by damaged enterocytes or by the metabolism of free PABA by bacteria.

The further reduction in PABA (dogs 1 & 2) following diversion of pancreatic juice is consistent with the observations of Williams (1985) in dogs with naturally occurring EPI and testify to the successful exclusion of pancreatic juice from the intestine during this period. The less dramatic fall (dog3) after diversion, to within the reported range for dogs with EPI, (Williams, 1985) may indicate that early fistulation had taken place allowing some pancreatic juice to enter the duodenum.
In all three dogs rediversion of secretions into the intestine caused an increase in PABA demonstrating reversibility of the model. However pre-operative concentrations of PABA were never fully restored.

Vitamin concentrations

The reduction in plasma cobalamin during diversion of secretion in dogs 1 & 2 and the increases observed after the re-introduction of secretion was an unexpected finding. Were the situation similar to that in man (Grasbeck & Salonen, 1976; Toskes, 1980), one would predict that it would have taken many months to deplete body stores so as to reduce the plasma cobalamin concentration. The possible interpretations of this finding are discussed in Chapter 3. The rapid fall in plasma cobalamin suggests that in the dog its turnover is fast.

Bacteriology

The techniques employed did not allow the determination of the numbers of strictly anaerobic bacteria and therefore the number of anaerobic colonies includes organisms which grow both aerobically and anaerobically i.e. facultative anaerobes. The persistently elevated numbers of bacteria post-operatively and the reduced absorption of PABA and xylose post-operatively prior to diversion of secretion, suggest that something other than reduced pancreatic secretion was responsible for the overgrowth. Perhaps an alteration in intestinal motility following surgery was partly responsible for the overgrowth, as decreased intestinal motility which impairs the ability of the small intestine to clear bacteria has been shown to be an important factor in allowing bacterial proliferation in the intestine (King & Toskes, 1979; Mathias & Clench, 1985). Post-surgical bacterial overgrowth is a well recognised condition in man and investigators have sought to define the role of bacteria in the causation of the malabsorption and diarrhoea that occurs in this situation. Some studies have used experimental animals with a surgically created self-filling intestinal blind loop which retains food and bacteria and allows overgrowth of bacteria. The increased numbers of bacteria and the reduced
monosaccharide absorption in the present study are similar to those reported in the experimental blind loop (Gracey et al., 1971; Gianella, Rout & Toskes, 1974). Certain qualitative changes may however be attributable to the effects of EPI per se e.g. the increase in *Lactobacillus* spp. following the diversion of pancreatic secretion, due probably to a decrease in intestinal pH and an increase in unabsorbed carbohydrates following diversion. The replacement of *Lactobacillus* spp. by *Streptococcus* spp. after the restoration of secretion was probably due to a decrease in substrate availability and an increase in the intraluminal pH. *Lactobacillus* spp. have not been consistently recorded in clinical cases of EPI. This might be explained by the use of a selective medium (MRS) in the present study, and not in the clinical study (Williams, 1985).

**Mucosal Changes**

Histological examination of biopsies revealed few changes in structure but biochemical derangement was severe. Pancreatic proteolytic enzymes are thought to regulate the turnover of exposed proteins on the brush border, such as disaccharidases (Arvanitakis & Olsen, 1974) and in dog 2 the increased activities of sucrase and maltase, reversed by the reintroduction of secretion, are consistent with reduced degradation of these proteins as a consequence of decreased pancreatic protease activity within the gut lumen. The observed increases in the activities of sucrase and maltase are also in agreement with previous findings - in dogs with naturally occurring EPI (Batt, Bush & Peters, 1979; Williams, 1985; Williams & Batt, 1987), in human patients with EPI due to chronic pancreatitis (Arvanitakis & Olsen, 1974), in hamsters with ligated pancreatic ducts (Senegas-Balas et al., 1981), in rats after sub-total pancreatectomy (Alpers & Tedesco, 1975) and in the CBA/J mouse with EPI (Kwong, Seetharam & Alpers, 1978). Reversal of increased sucrase and maltase activity was also found in previous studies in dogs after supplementation with pancreatic enzymes (Williams, 1985).
The decrease in lactase activity following diversion of juice contrasts with the increases in sucrase and maltase and may reflect an increase susceptibility of lactase to residual proteinases of pancreatic or bacterial origin (see Chapter 3).

In marked contrast to the increases found in dog 2 and in previous studies of EPI, the activities of maltase, sucrase and lactase in dogs 1 & 3 decreased after diversion of pancreatic secretion. These findings are however similar to those reported in dogs with EPI plus obligate anaerobic overgrowth (Williams, 1985; Williams & Batt, 1987). The massive increase in the number and type of bacteria after diversion of secretion may be of particular importance in causing the reduced activities of the disaccharidases. *Clostridium* spp., *Bacteroides* spp. and facultative anaerobic *Streptococcus* spp. have been implicated in the causation of reduced disaccharidases in the experimental blind loop syndrome (Jonas, Flanagan & Forstner, 1977), and extracts of these species have been found to have a disaccharidase releasing ability, similar to that of pancreatic proteolytic enzymes and papain (Alpers & Tedesco, 1975; Louvard et al., 1975; Seetharam et al., 1976), which is maximally inhibited by an elastase inhibitor (Jonas, Krishnan & Forstner, 1978). In addition bacteria, their secreted products or intraluminal metabolites, can inactivate brush border enzymes in-situ (Gracey, Houghton & Thomas, 1975; Riepe, Goldstein & Alpers, 1980). In the present study marked decrease in disaccharidase activities coincided with large increases in *Streptococcus* spp. and *Bacteroides* sp in dog 1 and *Streptococcus* spp. and *Clostridium* spp. in dog 3. By contrast, in dog 2,*Clostridium* spp. were present before the diversion of pancreatic juice. Following diversion large increases in facultative anaerobic *Streptococcus* spp. did not occur. Interestingly, the marked increase in the disaccharidase activities in dog 1 after the reintroduction of secretion coincided with the disappearance of *Bacteroides* sp. suggesting that bacterial species may be an important determinant of the effects on disaccharidase activity.

Reduced activities of disaccharidases have not been found in clinical cases of canine anaerobic bacterial overgrowth (Batt, Carter & Peters, 1984; Batt & McLean,
greater numbers of bacteria in this study, comparable with the experimental blind loop syndrome, could explain this difference, particularly as disaccharidase activities are often normal in the less contaminated segments of intestine (Jonas, Flanagan & Forstner, 1977). An additional factor which might explain the maintenance of normal disaccharidase activities in the less contaminated segments in the experimental blind loop syndrome and in canine cases of anaerobic overgrowth is increased synthesis of brush border proteins. As decreased rates of synthesis of high molecular weight proteins has been demonstrated in partially pancreatectomised rats (Alpers & Tedesco, 1975) and dogs with EPI (Williams, Batt & McLean, 1985) this factor may be of key importance in explaining the decreased activities of disaccharidases found in dogs with EPI.

In this study alterations in disaccharidase activities probably reflect a balance between the numbers of bacteria, their products and intraluminal metabolites, the activity of pancreatic proteinases and the rate of protein synthesis within the individual dog.

Summary
The severe bacterial overgrowth, which failed to be reversed by the re-introduction of pancreatic secretion was probably due to interference with intestinal motility resulting from the initial surgical procedure. This made it impossible to assess the effects of a lack of pancreatic secretion per se. However, the model allowed the combined effects of EPI and bacterial overgrowth to be examined. The decrease in disaccharidase activities found in two dogs supports previous findings in three clinical cases of anaerobic overgrowth in EPI. The effects of diverting secretion on plasma amylase, lipase and TLI were also very interesting, levels becoming more elevated than during the post-operative period. If the back pressure during diversion was not elevated, this could indicate a feedback effect of pancreatic enzyme depletion in the dog. The findings do not support a purely enteric route of entry of TLI into the bloodstream. The rapid decrease in plasma cobalamin after the
diversion of pancreatic secretion was remarkable and prompted further experiments on cobalamin absorption in the dog, described and discussed in Chapter 3. Due to the limited success of this model and the severe bacterial overgrowth which occurred I decided to evaluate an alternative model of EPI, namely ligation of the pancreatic ducts, in pursuit of my original aims.
CHAPTER 3

INVESTIGATION OF EXPERIMENTAL EXOCRINE PANCREATIC INSUFFICIENCY: DUCT LIGATED MODEL

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3.2.3 Pancreatic function tests

3.2.4 Cobalamin absorption

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3.3.7 Examination of jejunal mucosa

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3.4 Discussion
3.1 Introduction

The severe post-surgical bacterial overgrowth in the reversible model of exocrine pancreatic insufficiency (EPI) (Chapter 2) made it impossible to assess the effects of the lack of pancreatic secretion *per se* on the small intestinal mucosa and flora. An alternative model of EPI, created by ligating the pancreatic ducts involves less invasive surgery and hopefully less propensity to bacterial overgrowth. This would facilitate more precise assessment of the effects of a lack of pancreatic secretion on intestinal structure and function. Reversibility of any changes which occurred could be studied following the administration of exogenous pancreatic enzymes. As ligation of the pancreatic ducts can, in the long term, impair pancreatic endocrine function (Idezuki, Goetz & Lillehei, 1969; Rausis, Choudhury & Ogata, 1970) studies would have to be carried out within a relatively short time period and endocrine function monitored simultaneously.

As discussed previously (Chapter 2), studies undertaken in dogs with naturally occurring EPI provide strong evidence that pancreatic enzymes play a role in the degradation of certain microvillar membrane proteins. The effects of a lack of pancreatic enzymes on the disaccharidases lactase, sucrase and maltase were described in Chapter 2. However, mucosal enzyme changes in dogs with naturally occurring EPI are not restricted to these disaccharidases. In addition to finding increased specific activities of maltase and sucrase, Batt, Bush & Peters (1979b) found that the specific activities of the brush border enzymes Zn$^{2+}$-resistant α-glucosidase and γ-glutamyl transferase and the lysosomal enzymes, N-acetyl β-glucosaminidase and α-mannosidase were increased; Williams (1985) found that the specific activities of the lysosomal enzymes acid phosphatase and α-mannosidase were increased whereas the specific activity of alkaline phosphatase (brush border) decreased. As well as the changes in the specific activity of enzymes, the proportion of microvillar protein in bands corresponding to a molecular weight >200kDa was up to 20 times greater than normal in dogs with naturally occurring EPI (Sørenson et al., 1987). Treatment of naturally occurring
EPI with pancreatic enzyme supplements reversed the dietary malassimilation but did not restore mucosal normality (some abnormalities reverse, others persist, or develop *do novo*) (Williams, 1985; Sørenson, 1987).

In addition to allowing repetition and expansion of the mucosal studies described in Chapter 2, the pancreatitis which follows duct ligation should allow comparison of the plasma concentrations of the pancreatic enzymes amylase, lipase and trypsin-like immunoreactivity. Serum concentrations of amylase and lipase are increased during acute inflammation of the pancreas and are used in the diagnosis of pancreatitis (Brobst, Ferguson & Carter, 1970; Mia, Koger & Tierney, 1978a). However, amylase and lipase are not produced solely by the pancreas (Nothman & Callow, 1971; Hamosh et al., 1975; Stickle, Carlton & Boon, 1980; Jacobs, Hall & Rogers, 1982; Murtaugh & Jacobs, 1985), an increase in circulating activities of amylase is also found in intestinal obstruction (Hiatt, 1958) and in lipase after steroid administration (Parent, 1982). To increase the specificity of a diagnosis, it has been recommended that amylase and lipase are assayed simultaneously (Brobst, Ferguson & Carter, 1970; Mia, Koger & Tierney, 1978b). However, increased activities of both these enzymes have also been found in hepatic and renal disease (Strombeck, Farver & Kaneko, 1981; Polzin et al., 1983).

To overcome these problems methods have been developed to detect increases in enzymes solely of pancreatic origin, such as pancreatic iso-amylase and phospholipase A$_2$ (Stickle, Carlton & Boon, 1980; Jacobs, Hall & Rogers, 1982; Westermarck & Rimaila-Parnanen, 1983; Murtaugh & Jacobs, 1985). Unfortunately, there are conflicting reports about the presence of a pancreas specific iso-amylase in the serum of normal dogs and the exact source of amylase in each peak on electrophoresis has not been established (Stickle, Carlton & Boon, 1980; Jacobs, Hall & Rogers, 1982; Murtaugh & Jacobs, 1985) although increased iso-amylase concentrations have been demonstrated in dogs with acute pancreatitis (Jacobs, Hall & Rogers, 1982; Murtaugh & Jacobs, 1985). Furthermore phospholipase A$_2$ was only elevated in just over 50% of dogs with acute
pancreatitis, although an increase tended to indicate a severe necrotizing pancreatitis (Westermarck & Rimaila-Parnanen, 1983).

The assay of serum trypsin and/or trypsinogen, pancreas specific enzymes, might resolve some of these difficulties. However circulating trypsin is bound to inhibitors (Ohlsson, 1971) preventing any enzymic assay. A radioimmunoassay has been developed which detects both trypsin and trypsinogen (Williams & Batt, 1983). Preliminary studies in the dog have indicated that the immunoreactive species present in the dog is probably trypsinogen, but until this is confirmed the immunoreactive species has been called "trypsin-like immunoreactivity" (TLI) (Williams & Batt, 1983). Low TLI concentrations have been demonstrated in dogs with exocrine pancreatic insufficiency (Williams & Batt, 1983) and circulating TLI is abolished after pancreatectomy in man (Adrian, 1980 ; Malvano et al., 1980), suggesting that the pancreas is the sole source of TLI in man. These findings suggest that the assay of circulating TLI would be useful in the diagnosis of acute pancreatitis. Indeed high concentrations of TLI have been reported in dogs with acute pancreatitis induced experimentally by the injection of bile salts (Borgstrom & Ohlsson, 1980). However these dogs were only studied for ten hours after the induction of pancreatitis and the interrelationships of circulating TLI, amylase and lipase in acute pancreatitis have not been determined. In addition, mild oedematous pancreatitis is the form of pancreatitis most commonly encountered in naturally occurring cases, not the severe necrotizing pancreatitis induced by the intra-pancreatic injection of bile salts (Coffin & Thordal-Christensen, 1953).

In comparison with the pancreatic juice diversion model of EPI (Chapter 2) the duration of EPI is not limited to four days in the duct ligated model. This allows the long term effects of EPI on the absorption of cobalamin and folate to be examined. Unfortunately, determination of the plasma concentrations of cobalamin and folate enables only indirect assessment of their absorption, as factors such as the mobilisation of body stores can mask any decrease in absorption. However, using a cobalamin absorption test it should be possible to determine directly the effects
of EPI on the absorption of cobalamin. Cobalamin absorption is a specialized process which, in many mammalian species, is facilitated by the binding of cobalamin to intrinsic factor of gastric origin with subsequent attachment of this intrinsic factor-cobalamin complex to specific receptors in the microvillar membrane of ileal enterocytes (Grassbeck & Salonen, 1976; Marcoulis & Nicholas, 1983). Absorption is then thought to occur by receptor mediated endocytosis (Shephard, Jenkins & Jewell, 1984; Robertson & Gallagher, 1985). Initially it was thought that intrinsic factor was absent in the dog and that cobalamin absorption was by simple or facilitated diffusion (Yamaguchi, 1969), but there is now convincing evidence that the dog produces intrinsic factor and that absorption of cobalamin occurs after binding of the cobalamin-intrinsic factor complex to receptors in the ileum (Marcoulis & Rothenberg, 1981; Batt & Horadagoda, 1986). However, the canine stomach reportedly produces only small amounts of intrinsic factor (Marcoulis & Rothenberg, 1981) and gastrectomised dogs do not appear to malabsorb cobalamin (Abels, Kapel & Lindemans, 1977). These findings, together with the observation that serum cobalamin concentrations are reduced in dogs with EPI (Batt & Morgan, 1982) suggest that the pancreas might play an important role in the absorption of cobalamin in the dog. Subsequent studies have identified a pancreatic intrinsic factor, suggesting that both gastric and pancreatic intrinsic factors play a role in promoting absorption of cobalamin by receptor mediated endocytosis in the dog (Batt & Horodagoda, 1986; Horadagoda et al., 1986). However, the precise role of the canine pancreas in the absorption of cobalamin awaits clarification.
Aims

The aims of this study were threefold. The first was to determine the effects of experimentally induced EPI on the structure and biochemistry of the small intestinal mucosa, the bacterial flora of the small intestine, the plasma concentrations of cobalamin and folate, and to assess whether any such changes were reversible following pancreatic enzyme therapy. The second was to determine the degree of elevation of trypsin-like immunoreactivity and the temporal relationship of any such increase to increases in circulating amylase and lipase activity in pancreatitis following pancreatic duct ligation. The third aim was to determine directly the effect of EPI on cobalamin absorption in the dog and to investigate whether any such influence was mediated by pancreatic enzymes.

Protocol

![Diagram of Protocol]

**FIGURE 3.1 Summary of Protocol**

CPJ = canine pancreatic juice, DL = pancreatic duct ligation
Numbers indicate time (weeks) after pancreatic duct ligation.

Figure 3.1 summarizes the protocol for this study.

Absorption of $^{58}\text{Co}$ cyanocobalamin and pancreatic function tests (BT-PABA/xylose, diet assimilation, fat balance) were performed on four occasions: prior to surgery (pre-operatively), in the third week after surgery (post-operatively), in the fourth week after surgery (Pancrex), when both diet and function tests were supplemented with bovine pancreatic enzymes, and in the fifth week after surgery when only the $^{58}\text{Co}$ cyanocobalamin solution was supplemented with canine
pancreatic juice (CPJ). Blood samples for the measurement of amylase, lipase and TLI were taken at 0, 1, 2, 3, 4, 5 and 14 days post-operatively. A final sample for TLI was taken on the morning of the terminal biopsy. Plasma cobalamin and folate concentrations were measured pre-operatively and at 2, 3, 4, 5 and 8 weeks post-operatively. Endocrine pancreatic function was assessed pre-operatively and at 3, 8 and 20 weeks post-operatively.

Intestinal biopsies and duodenal juice were obtained at the time of pancreatic duct ligation and at 6, 8 (Greyhounds) and 20 weeks (Beagles) after duct ligation.

3.2 Materials and methods

3.2.1 Animals

Eight dogs (4 Beagles and 4 Greyhounds) of both sexes were housed in a controlled environment and received a standard diet for at least six weeks prior to, and throughout, the study. This standard diet consisted of a dried food (Diet A, SDS Ltd., Witham, Essex) and tinned meat (Kennel pack, Winalot-Spillers, London), fed twice daily. For the first, second and fourth weeks post-operatively the diet was supplemented with a commercial pancreatic enzyme preparation (Pancrex veterinary powder, Paines and Byrne Ltd., Middlesex) at a dose of 4g per meal for Beagles and 8g per meal for Greyhounds. Supplementation was stopped for the fifth and sixth week, reintroduced in the seventh week (after intestinal biopsy) and continued until the end of the study.

3.2.2 Anaesthesia, surgery and post-operative care

Anaesthesia was that described in Section 2.2.2.

The operation was performed through a midline abdominal incision. Pancreatic ducts (usually two) were located and ligated and then omentum was secured between the pancreas and the duodenum. Abdominal closure and post-operative care were the same as previously described in Section 2.2.3 & 4.
3.2.3 Pancreatic function tests

*Plasma amylase/lipase/ TLI*

Plasma samples for the estimation of amylase, lipase and TLI were stored at -20°C until they were assayed as described in section 2.2.5.

*BTPABA/xylose*

The combined BT-PABA/xylose test was performed as described in Section 2.2.5. In the fourth week after surgery the test was supplemented with bovine pancreatic enzymes at a dose of 4g for Beagles and 8g for Greyhounds (Pancrex veterinary powder).

*Dietary assimilation*

The wet weight of faeces collected during the 48hr period after the administration of 58Co cyanocobalamin was recorded and expressed as a percentage of the dietary intake.

*Fat balance studies*

The faeces collected during the 48hrs after administration of 58Co cyanocobalamin were homogenized manually and stored at -20°C until determination of the fat content (Van de Kamer et al., 1949). Three samples of the diet were assayed for fat, and fat excretion was expressed as a percentage of the dietary intake.

*Glucose tolerance and insulin response*

After an initial blood sample 1ml/kg body weight of a solution of 500g/l dextrose (Evans Medical, Speke, Merseyside) was injected into the cephalic vein over a 30 second period. Blood was collected subsequently from the other cephalic vein at 5, 10, 15, 20, 30, 45, and 60 minutes. A blood spot was immediately applied to a glucose test strip and the remaining blood was collected into heparinised tubes for insulin assay. Glucose concentration was measured using a glucose analyser.
(Reflocheck, B.C.L. Ltd., London) and insulin was assayed by radioimmunoassay (Wellcome Diagnostics Ltd., Beckenham, Kent).

Intravenous glucose tolerance tests were performed pre-operatively and at 3, 8 (Greyhounds) and 20wks post-operatively (Beagles). Blood for the assay of insulin was taken during the 8 and 20wk tolerance tests.

The glucose half time, glucose fractional turnover rate, basal plasma insulin and insulin peak response above basal level were calculated as described by Kaneko and co-workers (Kaneko et al., 1977).

### 3.2.4 Cobalamin absorption

Cobalamin absorption was assessed by the oral administration of 0.5µg/dog of ^58^Co cyanocobalamin (Amersham International plc.) with subsequent measurement of radioactivity in blood, urine and faeces. Cobalamin was administered in distilled water (10ml H₂O/kg body weight) by stomach tube and each dog was placed in a metabolism cage for 48hrs to facilitate collection of urine and faeces. In the fourth week post-operatively the cobalamin solution was supplemented with bovine pancreatic enzymes (Pancrex veterinary powder, 4g/Beagle, 8g/ Greyhound) and in the fifth week with canine pancreatic juice (150ml/Beagle, 250 ml/ Greyhound). Venous blood samples (10ml) were taken from the jugular vein and transferred into heparinised tubes at 0, 3 and 6 hours after oral dosing. A standard meal containing 2.5g of the non-absorbable marker chromic oxide was fed 2hrs after oral dosing. During the 48 hour collection period three normal meals were fed and water was provided ad-libitum.

Radioactivity was measured with a gamma counter (LKB 1217, LKB Instruments, Croydon) in duplicate samples of blood and urine and in four samples of homogenized faeces. As a measure of cobalamin absorption faecal excretion was calculated as the percentage of the administered oral dose which was recovered in faeces.
3.2.5 Assay of cobalamin and folate in plasma, and cobalamin in pancreatic juice.

Plasma concentrations of cobalamin and folate were measured by a radioimmunoassay as described in Section 2.2.7. The cobalamin concentration of pancreatic juice samples collected from dogs with a pancreatic fistula (Chapter 2) was measured by bioassay using the Z strain of *Euglena gracilis* as the test organism (Anderson, 1964).

3.2.6 Assay of pancreatic juice and enzyme supplement for trypsin.

Pancreatic juice collected from the dogs undergoing pancreatic juice diversion was used in this study (Chapter 2). Juice and enzyme supplement were assayed for trypsin after activation with porcine enteropeptidase (Sigma) using the specific substrate benzoyl arginine ethyl ester (Schwert & Takenaka, 1955).

3.2.7 Collection and examination of duodenal juice.

Duodenal juice was collected at laparotomy, prior to intestinal biopsy, and was examined and cultured as described in Section 2.2.8.

3.2.8 Collection and examination of intestinal biopsies.

Per-oral jejunal biopsies were obtained at laparotomy, approximately 10cm distal to the duodenal-jejunal flexure using a hydraulic multiple biopsy instrument as described in Section 2.2.9.

Three biopsies were placed into buffered formal saline for routine histology, three into chilled 3% gluteraldehyde (pH 7.3) fixative and stored at 4°C, and three into a dry collection tube and frozen at -20°C for electrophoresis. One biopsy was placed into each of 3ml distilled water, 3ml SVE medium and 3ml MOPS medium (sucrose 0.3mol/l, ethanol 220mmol/l and 3-(N-Morpholino) propane-sulphonic acid 2mmol/l adjusted to pH 7.4) for enzyme assays and stored at -20°C until assay.
Morphological examination

The biopsies taken into formalin were embedded in paraffin wax, sectioned and stained with Haematoxylin and Eosin. Using a linear micrometer eyepiece, calibrated with a graticule, in a binocular microscope, three features were examined. First villous height was measured from the crypt mouth to the tip. Secondly, villous width was measured at the widest point above the base. Thirdly, enterocyte height was measured in the mid-villous region. These measurements were performed on at least three of the tallest villi on each biopsy and mean readings were recorded in microns. The individual results for each dog were then grouped and differences between the mean pre-operative, post-operative and Pancrex supplemented values were examined using a Students paired 't' test.

The biopsies taken into gluteraldehyde were transferred after three hours into a sucrose buffer (0.2mol/l sucrose, pH 7.3) and stored at 4°C until secondary fixation with 2% osmium tetroxide (1hr at 4°C). They were then dehydrated, embedded in Epon and sectioned. Sections were taken from the mid-villous region, stained with uranyl acetate and lead citrate and examined on a transmission electron microscope (Jeol 100CX).

Enzyme assays

The biopsies collected into distilled water were disrupted in a Dounce homogeniser and were assayed for lactase, sucrase and maltase using the conditions and substrates summarised in Table 3.1. The biopsies collected into SVE and MOPS were disrupted in a Dounce homogeniser with ten strokes of a loose fitting pestle (Type A) followed by twenty strokes of a tight fitting pestle (Type B). The SVE homogenates were assayed for the enterocyte organelle marker enzymes using the conditions and substrates summarised in Table 3.1. The MOPS homogenates were assayed for aminopeptidase N (see Table 3.1). Where appropriate, SVE medium, MOPS medium or distilled water was used as a diluent and blank in the enzyme assays.
<table>
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<tr>
<th>ENZYME</th>
<th>EC No.</th>
<th>SUBSTRATE</th>
<th>pH</th>
<th>INCUBATION MEDIUM</th>
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<tbody>
<tr>
<td>Zn²⁺-resistant α-glucosidase</td>
<td>3.3.1.20</td>
<td>4-Methylumbelliferyl-α-D-glucopyranoside</td>
<td>6.2</td>
<td>Sodium phosphate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Maltase</td>
<td>3.2.1.20</td>
<td>Maltose</td>
<td>6.0</td>
<td>Sodium maleate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Sucrase</td>
<td>3.2.1.48</td>
<td>Sucrose</td>
<td>6.0</td>
<td>Sodium maleate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Lactase</td>
<td>3.2.1.23</td>
<td>Lactose</td>
<td>6.0</td>
<td>Sodium maleate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>3.1.3.1</td>
<td>4-Methylumbelliferyl-phosphate</td>
<td>10.2</td>
<td>Diethanolamine-HCl buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Aminopeptidase N</td>
<td>3.4.11.2</td>
<td>Leucyl-2-naphthylamide</td>
<td>7.2</td>
<td>Sodium phosphate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>γ-Glutamyl transferase</td>
<td>2.3.2.2</td>
<td>Glutamyl-2-naphthylamide</td>
<td>8.0</td>
<td>Ammonium-HCl buffer (0.05 mol/l)</td>
</tr>
<tr>
<td>N-acetyl-B-glucosaminidase</td>
<td>3.2.1.30</td>
<td>4-Methylumbelliferyl-2-acetamido-2-deoxy-B-D-</td>
<td>5.7</td>
<td>Sodium acetate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>3.2.1.24</td>
<td>4-Methylumbelliferyl-α-D-mannopyranoside</td>
<td>4.6</td>
<td>Sodium acetate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>3.1.3.2</td>
<td>4-Methylumbelliferyl phosphate</td>
<td>4.0</td>
<td>Sodium acetate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>5' nucleotidase</td>
<td>3.1.3.5</td>
<td>3H Adenosine 5'-monophosphate</td>
<td>9.0</td>
<td>Piperazine-HCl buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Tris-resistant α-glucosidase</td>
<td>3.2.1.20</td>
<td>4-Methylumbelliferyl-α-D-glucopyranoside</td>
<td>8.0</td>
<td>Sodium acetate buffer (0.1 mol/l)</td>
</tr>
<tr>
<td>Catalase</td>
<td>1.11.1.6</td>
<td>Hydrogen peroxide</td>
<td>7.0</td>
<td>Imidazole (20 mmol/l); bovine serum albumin (1 mg/ml)</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>1.1.1.37</td>
<td>Oxaloacetate; NADH</td>
<td>7.4</td>
<td>Sodium phosphate buffer (0.1 mol/l)</td>
</tr>
</tbody>
</table>

Each incubation medium, except for assay of maltase, sucrase and lactase, contained TritonX-100: (a) 0.1%(w/v); (b) 0.25%(w/v); (c) 0.01%(w/v)


**TABLE 3.1 Summary of conditions and substrates for enzyme assays**
The protein content of each homogenate was determined as described in Section 2.2.10 and enzyme specific activities were expressed as mU/mg protein. Fluorescence was measured by use of a Perkin-Elmer 2000 fluorescence spectrophotometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire), absorbance was determined using a Beckman DU-8 spectrophotometer (Beckman Scientific Instruments, High Wycombe, Buckinghamshire) and radioactivity was counted using a Packard Tricarb 460 CD liquid scintillation counter (Packard Instruments Ltd., Caversham, Berkshire).

Electrophoresis

Microvillar membrane vesicles were isolated from jejunal biopsies by the CaCl\textsubscript{2}-precipitation method described by Schmitz and co-workers (Schmitz et al., 1973). Biopsies were homogenised for 4 min. on ice in 1.5 ml 0.05 M mannitol, 0.002 M Tris-HCl pH 7.2 by use of a motor driven Dounce homogeniser, then CaCl\textsubscript{2} was added to a final concentration of 0.01 M. Each sample was left on ice for 10 min. before centrifugation at 1200 \( \times \) g for 10 min. The resultant supernatants were centrifuged at 2500 \( \times \) g for 50 min. Each pellet, which contained the membrane vesicles, was resuspended in 0.15 ml dissociation buffer (0.12 M Tris-HCl pH 6.8 containing 0.001 M EDTA, 10% w/v SDS, 20% v/v glycerol, 2.5% mercaptoethanol and 0.1% w/v bromphenol blue) and then boiled for 4 min.

SDS-polyacrilamide gels were run using a discontinuous system (Laemmli, 1970) with a 5-15% polyacrylamide gradient. After staining with Coomassie Brilliant Blue, the gels were scanned at 570 nm on a Beckmann DU-8 spectrophotometer (Beckman Scientific Instruments, High Wycombe, Buckinghamshire). The molecular weights were calculated by linear regression using BioRad (BioRad laboratories Ltd., Watford) and Pharmacia (Pharmacia House, Milton Keynes) high molecular weight standards.
3.2.9 Post-mortem examination
A post-mortem examination was carried out on all dogs after their final biopsy. To ascertain whether all the pancreatic ducts were ligated the right and left lobes of the pancreas were cannulated and dye (methylene blue) was injected. The pancreas was then removed, weighed and placed in buffered formal saline for histological examination. The histological findings were graded according to the degree of exocrine pancreatic atrophy as either normal, mild, moderate, severe or very severe (no exocrine tissue present).

3.2.10 Statistical methods
The significance of differences between the means for the results of pancreatic function tests, cobalamin absorption, microvillar electrophoresis and mucosal enzyme studies at different time intervals were assessed using the Student's paired 't' test. The significance of differences between the means for fasting insulin and insulin response in the control and experimental groups were assessed by "Fisher's test" and then an unpaired 't' test where "Fisher's test" was not significant, or by a 'z' test where "Fisher's test" was significant. The relation between the trypsin activity and cobalamin concentration of pancreatic juice, the amount of trypsin administered and the reduction in cobalamin excretion, and the terminal concentration of plasma TLI and pancreatic weight were assessed using Spearman's rank correlation coefficient. The interrelationships of post-operative plasma concentrations of amylase, lipase and TLI were determined by regression analysis after checking the normality of the data with a Kolmogarov-Smirnov one sample test. Differences with a P value less than 0.05 were considered significant.
3.3 Results

3.3.1 Post-operative recovery
All dogs were slightly "depressed" for the first two days after surgery but recovered uneventfully with no complications when solid food was re-introduced. It was not possible to perform cobalamin absorption tests with pancreatic juice added in dogs 1 and 8 because of high temperature and nasal discharge in the former and severe weight loss in the latter. Dog 8 was maintained on Pancrex until undergoing a terminal biopsy (with Pancrex added) in week 6. Dog 1 recovered and underwent a terminal biopsy in week 20 (no Pancrex added).

3.3.2 Exocrine Pancreatic function

Plasma amylase, lipase and TLI
Plasma values for TLI, amylase and lipase in control dogs (clinically healthy Beagles and Greyhounds) and following surgery are shown in Table 3.2 and Figure 3.2. Duct ligation was followed by rapid increases in TLI, amylase and lipase. TLI concentrations peaked before those of amylase and lipase in 6 dogs and had fallen to within the normal control range in dogs (1 & 3) by the third day post-operatively. Peak TLI concentrations ranged from 52 to 171 µg/l with a mean relative increase of 16.5x the basal value (Table 3.2). Fourteen days post-operatively TLI concentrations in all 8 dogs were within the normal range.

By contrast, the lipase and amylase concentrations remained above the normal range in all dogs for the five day study period. Plasma amylase and lipase rose in unison. Peak concentrations were recorded on the same day in seven of the eight dogs. Levels also fell in unison except in two dogs (6 & 7), in whom plasma amylase fell more abruptly than plasma lipase. The peak concentration for amylase ranged from 3,439-11,396 IU/l with an increase of the mean of 13.7x over basal values, and for lipase 4,519-13,455 IU/l with a mean increase of 25.6x over basal values (Table 3.2). On the fourteenth day after surgery plasma amylase activity was within the control range in 6 dogs whereas lipase activity was above
FIGURE 3.2 Post-operative plasma concentrations of amylase, lipase and TLI
the control range in 6 dogs. Regression analysis demonstrated a correlation
between post-operative values of amylase and lipase \((r = 0.74, P < 0.001)\), amylase
and TLI \((r = 0.53, P < 0.001)\) and lipase and TLI \((r = 0.67, P < 0.001)\).

<table>
<thead>
<tr>
<th>DOG</th>
<th>TLI basal peak day of (µg/l)</th>
<th>RI* peak</th>
<th>AMYLASE basal peak day of IU/l</th>
<th>RI* peak</th>
<th>LIPASE basal peak day of IU/l</th>
<th>RI* peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.6 10.6 2</td>
<td></td>
<td>796 4.7 2</td>
<td></td>
<td>397 23 2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.8 18.8 4</td>
<td></td>
<td>565 20.2 5</td>
<td></td>
<td>331 25.8 5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.8 14.1 1</td>
<td></td>
<td>590 15.8 2</td>
<td></td>
<td>398 23.5 2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.1 27.2 4</td>
<td></td>
<td>359 29.3 5</td>
<td></td>
<td>359 37 5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.8 24.9 1</td>
<td></td>
<td>661 10.4 3</td>
<td></td>
<td>493 27.6 3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>13.9 5.8 2</td>
<td></td>
<td>616 13.1 3</td>
<td></td>
<td>516 21.3 5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9.6 17.8 3</td>
<td></td>
<td>618 11.9 3</td>
<td></td>
<td>356 32 3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.2 11.9 1</td>
<td></td>
<td>796 4.3 2</td>
<td></td>
<td>318 14.2 2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.5 ±0.98 (47)</td>
<td>638 ±35 (29)</td>
<td>265 ± 28 (29)</td>
<td>(5.1-42)</td>
<td>(294-1232)</td>
<td>(68-560)</td>
</tr>
</tbody>
</table>

**TABLE 3.2** The effect of duct ligation on plasma concentrations of TLI, amylase and lipase.

RI* = Relative Increase, peak activity : basal activity
Control data presented as the mean ± standard error with the number of observations and the range in brackets.

**BT-PABA/xylose**

Table 3.3 gives the results for BT-PABA/xylose tests, dietary assimilation and fat balance. Compared with pre-operative values, peak plasma PABA concentrations following oral BT-PABA were significantly decreased at all three time points after surgery. However, after the addition of Pancrex, PABA concentrations were significantly greater than those measured without supplementation in the third (post-op) and fifth week (CPJ) after surgery.

Xylose absorption was not significantly altered throughout the study (Table 3.3).
**Dietary assimilation**

Table 3.3 shows the results for dietary assimilation. Faecal mass expressed as a percentage of dietary intake was significantly elevated at all time points after surgery. However, Pancrex supplementation caused a significant decrease in faecal mass compared with other values recorded after surgery.

**Fat balance**

Faecal fat excretion was also significantly increased at all time points after surgery (Table 3.3). In addition, fat excretion was significantly decreased by enzyme supplementation compared with other values recorded after surgery.

<table>
<thead>
<tr>
<th>pk. PABA (μmol/l)</th>
<th>DIET ASSIMILATION (% intake excreted)</th>
<th>FAT EXCRETION (% intake excreted)</th>
<th>pk. XYLOSE (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE-OP. 42.3 ± 2.3</td>
<td>33.6 ± 2.6</td>
<td>19.8 ± 2.8</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td>POST-OP. 13.9 ± 3.0***</td>
<td>77.7 ± 6.7***</td>
<td>78.7 ± 4.9***</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>PANCREX 35.5 ± 1.3*#</td>
<td>42.1 ± 3.8*#</td>
<td>50.4 ± 3.9***#</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>CPJ 16.6 ± 1.1***</td>
<td>83.4 ± 5.8***</td>
<td>76.7 ± 4.4***</td>
<td>□3.8 ± 0.2</td>
</tr>
</tbody>
</table>

**TABLE 3.3 RESULTS OF FUNCTION TESTS**

□ = administered prior to canine pancreatic juice (CPJ)
Results are expressed as the mean ± standard error of the mean
pk. = peak plasma concentrations after an oral dose
Significance ; compared with pre-op., *= P <0.05, **= P <0.01, ***= P <0.001 ; compared with post-op. # = P <0.01 ; compared with CPJ □ = P <0.01.

**Glucose tolerance and insulin response**

The results of glucose tolerance tests and insulin responses are presented in Table 3.4. Fasting concentrations of blood glucose were not significantly altered in any dog at any time post surgery. However, glucose tolerance, assessed by glucose half time and fractional clearance rate, was significantly impaired at 3 and 20 weeks after surgery. Fasting insulin and insulin peak response were lower than
normal in the 3 Greyhounds at 8 weeks and in the 4 Beagles at 20 weeks, although the decrease was only significant in the Beagles (Table 3.4).

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre-op</th>
<th>3 Weeks</th>
<th>8 Weeks</th>
<th>20 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.56 ± 0.12</td>
<td>4.93 ± 0.31</td>
<td>4.96 ± 0.75</td>
<td>4.78 ± 0.24</td>
</tr>
<tr>
<td>Glucose half time (minutes)</td>
<td>13.1 ± 0.9</td>
<td>28.1 ± 3.6**</td>
<td>23.2 ± 4.9</td>
<td>31.1 ± 4.2**</td>
</tr>
<tr>
<td>Fractional turnover (%/ minute)</td>
<td>5.5 ± 0.4</td>
<td>2.7 ± 0.3**</td>
<td>3.4 ± 0.9</td>
<td>2.4 ± 0.3*</td>
</tr>
<tr>
<td>Fasting Insulin (μU/ml)</td>
<td>$7.03 ± 2.2$ (7.5-26.3)</td>
<td>ND</td>
<td>9.80 ± 1.6</td>
<td>7.25 ± 2.7*</td>
</tr>
<tr>
<td>Insulin peak response (μU/ml)</td>
<td>$70.2 ± 8.1$ (39-116)</td>
<td>ND</td>
<td>39.8 ± 21.2</td>
<td>30.9 ± 7.3*</td>
</tr>
</tbody>
</table>

**TABLE 3.4 Pancreatic endocrine function after duct ligation**

$\bar{3}$ = control group (n = 9), Figures between parenthesis indicate the range ; $a$ = all dogs ; $b$ = 3 Greyhounds ; $c$ = 4 Beagles ; ND = not determined ; Significance, * = P <0.05, ** = P <0.01, compared with pre-op. or control values.

### 3.3.3 Cobalamin absorption

Following oral administration of $^{58}$Co cyanocobalamin no significant increase over background radioactivity was detected in samples of blood or urine pre-operatively or at the three time points after surgery. Much of the faeces collected in the metabolism cages was green in colour but had reverted to a normal brown colour before the collection period was completed, indicating the passage of the chromic oxide and therefore any unabsorbed $^{58}$Co cyanocobalamin through the gut. Figure 3.3 shows the faecal excretion of radioactivity after an oral dose of $^{58}$Co cyanocobalamin. Faecal cobalamin excretion, expressed as the percentage of the oral dose of radioactivity recovered in faeces, rose considerably in the third week after surgery (post-op). Supplementation with Pancrex had no significant effect on post-operative faecal excretion of cobalamin. However, supplementation with canine pancreatic juice (CPJ) resulted in a significant decrease in post-operative faecal excretion of cobalamin (49 ± 2.7) in the six dogs studied compared with their
unsupplemented post-operative values (59.92 ± 4.5) and also with their Pancrex supplemented (69.83 ± 4.82) values; excretion however, was still significantly higher than pre-operatively (33.67 ± 5.43).

FIGURE 3.3 Faecal excretion of radioactivity following an oral dose of $^{58}$Co-cyanocobalamin.
Significance; compared with PRE-OP * = P <0.05, *** = P <0.001.
Compared with POST-OP # = P <0.05.
Compared with PANCREX ¢ = P <0.05.
CPJ = canine pancreatic juice.

3.3.4 Plasma concentrations of cobalamin and folate
Figure 3.4 shows the plasma concentrations of cobalamin and folate before and after pancreatic duct ligation. Following duct ligation, plasma cobalamin concentrations fell steadily and were significantly reduced in the eighth week after duct ligation. In contrast, plasma folate concentrations were significantly elevated (Students paired 't' test) in the sixth and eighth week after pancreatic duct ligation.
3.3.5 Trypsin content of pancreatic juice and enzyme supplement, and cobalamin concentrations in pancreatic juice

Table 3.5 shows the trypsin activity and cobalamin concentration of eleven samples of pancreatic juice collected from the dogs with pancreatic fistulae (Chapter 2). The amount of trypsin and cobalamin contained in individual samples of juice varied widely. However, there is a significant correlation (Spearman $p = 0.806, P < 0.01$) between the activity of trypsin and the concentration of cobalamin contained in the juice.

<table>
<thead>
<tr>
<th>Juice sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>cobalamin (ng/l)</td>
<td>65</td>
<td>90</td>
<td>110</td>
<td>575</td>
<td>255</td>
<td>1020</td>
<td>255</td>
<td>210</td>
<td>265</td>
<td>135</td>
<td>190</td>
</tr>
<tr>
<td>trypsin (mU/l)</td>
<td>27.0</td>
<td>20.1</td>
<td>28.8</td>
<td>51.6</td>
<td>45.8</td>
<td>137.7</td>
<td>29.6</td>
<td>32.5</td>
<td>30.6</td>
<td>33.8</td>
<td>29.8</td>
</tr>
</tbody>
</table>

TABLE 3.5 Cobalamin concentration and trypsin activity of pancreatic juice

Table 3.6 shows the amounts of trypsin contained in the pancreatic juice and enzyme supplement added to the $^{58}$Co cyanocobalamin solution (expressed as
mU/ kg of body weight). The assay of canine pancreatic juice and Pancrex for trypsin revealed that the amount of pancreatic juice given to each dog contained more trypsin than the powdered supplement. There was no significant correlation (Spearman $p = 0.156$) between the amount of trypsin administered as Pancrex and the reduction of cobalamin excretion. There was however a significant correlation (Spearman $p = 0.943$, $P < 0.01$) between the trypsin activity of canine pancreatic juice and the reduction in cobalamin excretion.

<table>
<thead>
<tr>
<th>Trypsin (mU/kg body weight)</th>
<th>Dog No. pancreatic juice</th>
<th>Pancrex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
<td>0.18</td>
</tr>
<tr>
<td>1</td>
<td>1.35</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>1.63</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>0.31</td>
<td>0.19</td>
</tr>
<tr>
<td>5</td>
<td>0.49</td>
<td>0.16</td>
</tr>
<tr>
<td>6</td>
<td>0.34</td>
<td>0.20</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>0.24</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± se</td>
<td>0.85 ± 0.23</td>
<td>0.20 ± 0.01</td>
</tr>
</tbody>
</table>

TABLE 3.6. Amount of trypsin administered with $^{58}$Co cyanocobalamin

ND = not determined

3.3.6 Examination of duodenal juice

Examination of duodenal juice for *Giardia* spp was always negative. Table 3.7 and Figure 3.5 show the results of bacteriology. Pancreatic duct ligation was followed by a large increase in the numbers of colonies and types of bacteria. The median number ($\log_{10}$ colony forming units, range in brackets) of aerobes increased to 6.0 (<3-8.48) and anaerobes to 7.15 (4.08-8.92) from pre-ligation values of <3 (<3-5.45) aerobes and approximately 3 (<3-6.8) anaerobes. The addition of Pancrex to the diet caused a marked decrease in the post-ligation values to 4.0 (<3-3.62) aerobes and 4.85 (3.81-6.85) anaerobes. There were also marked qualitative changes in the flora after duct ligation, with increases in the number of *Lactobacillus* spp, *Streptococcus* spp (dogs 1,2,4,5,6,7), and strictly anaerobic bacteria, *Clostridium* spp (dogs 1,6,7) and *Bacteroides* sp isolated (dog 4). The addition of Pancrex to the diet reduced the numbers of *Lactobacillus*,
FIGURE 3.5 Number of bacteria in duodenal juice
<table>
<thead>
<tr>
<th>DOG No.</th>
<th>ONE</th>
<th>TWO</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME</td>
<td>preEPI</td>
<td>postEPI</td>
</tr>
<tr>
<td>BACTERIA</td>
<td>AER</td>
<td>ANAER</td>
</tr>
<tr>
<td>Staph/Micro</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>7</td>
<td>7.18</td>
</tr>
<tr>
<td>Lactobacillus spp</td>
<td>6.78</td>
<td>5</td>
</tr>
<tr>
<td>Bacillus spp</td>
<td>4</td>
<td>5.78</td>
</tr>
<tr>
<td>Coryneform spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+ve cat+/-</td>
<td>7.0+</td>
<td></td>
</tr>
<tr>
<td>G-ve cat+/-</td>
<td>6.3+</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DOG No.</th>
<th>THREE</th>
<th>FOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME</td>
<td>preEPI</td>
<td>postEPI</td>
</tr>
<tr>
<td>BACTERIA</td>
<td>AER</td>
<td>ANAER</td>
</tr>
<tr>
<td>Staph/Micro</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus spp</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Coryneform spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteroides spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yeast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+ve cat+/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-ve cat+/-</td>
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<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

**TABLE 3.7** Numbers (log 10/ ml) and types of bacteria in duodenal juice.
cat = catalase ; Staph = Staphylococcus spp ; Micro = Micrococcus spp ; AER = aerobic, ANAER = anaerobic
### Table 3.7 (contd.) Numbers (log 10/ml) and types of bacteria in duodenal juice.

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>FIVE</th>
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<th>SEVEN</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>preEPI</td>
<td>postEPI</td>
<td>PANCREX</td>
<td>preEPI</td>
<td>postEPI</td>
<td>PANCREX</td>
</tr>
<tr>
<td></td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
</tr>
<tr>
<td>Staph/Micro</td>
<td>3.7 4.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>3 7</td>
<td>4.48</td>
<td>4.3 8.6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus spp</td>
<td>6 3</td>
<td>7.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bacillus spp</td>
<td>4</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coryneform spp</td>
<td>5.28 3</td>
<td>3.48</td>
<td></td>
<td></td>
<td></td>
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<td>Bacteroides spp</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium spp</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Enterobact.</td>
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<td>6.34</td>
<td>4.7</td>
<td></td>
<td></td>
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<tr>
<td>yeast</td>
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<tr>
<td>G+ve cat+/-</td>
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<td></td>
<td>5.7+</td>
<td>4.78-</td>
<td></td>
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<tr>
<td>G-ve cat+/-</td>
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<td>4.0+ 3.9+</td>
<td>4.3+ 4.0+</td>
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<td></td>
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<tr>
<td>TOTAL</td>
<td>4 5.34 6 7</td>
<td>4.04 4</td>
<td>4.56 5.75</td>
<td>7.7 8.61</td>
<td>4.85 4.85</td>
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<td>postEPI</td>
<td>PANCREX</td>
<td>preEPI</td>
<td>postEPI</td>
<td>PANCREX</td>
</tr>
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<td></td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
<td>AER ANAER</td>
</tr>
<tr>
<td>Staph/Micro</td>
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<td>3 3.3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>7.48 8.78</td>
<td>4</td>
<td>4.3 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus spp</td>
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<td></td>
<td>6.32</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Bacillus spp</td>
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<td></td>
<td>4</td>
<td></td>
<td></td>
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<tr>
<td>Coryneform spp</td>
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<td></td>
</tr>
<tr>
<td>Bacteroides spp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium spp</td>
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</tr>
<tr>
<td>Enterobact.</td>
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<td>3.85</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G+ve cat+/-</td>
<td>5.7+ 5.78+</td>
<td></td>
<td>6.3-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-ve cat+/-</td>
<td>3.9+</td>
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<tr>
<td>TOTAL</td>
<td>5.48+</td>
<td>6.8 8.48</td>
<td>8.92 4 3.48</td>
<td>&lt;3 &lt;3 ND</td>
<td>ND 6.32</td>
<td>6.85</td>
</tr>
</tbody>
</table>

cat = catalase; Staph = Staphylococcus spp; Micro = Micrococcus spp; AER = aerobic, ANAER = anaerobic
*Streptococcus* and *Clostridium* spp, but *Bacteroides* sp. were still isolated from dog 4. The time of sampling did not appear to influence the results as findings were similar in dogs sampled at 8 and 20 wks. after pancreatic duct ligation.

### 3.3.7 Examination of jejunal mucosa

**Morphological examination**

Table 3.8 gives the results for measurements of villous height and width, and enterocyte height. Neither the post EPI values nor the Pancrex supplemented values differed significantly (Students paired 't' test) from those obtained before duct ligation.

<table>
<thead>
<tr>
<th>DOG</th>
<th>Pre-ligation</th>
<th>Post-ligation</th>
<th>Pancrex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>v.h. v.w. e.h.</td>
<td>v.h. v.w. e.h.</td>
<td>v.h. v.w. e.h.</td>
</tr>
<tr>
<td></td>
<td>(µm) (µm) (µm)</td>
<td>(µm) (µm) (µm)</td>
<td>(µm) (µm) (µm)</td>
</tr>
<tr>
<td>1</td>
<td>615.3 180.9 25.4</td>
<td>762.6 163.1 23.7</td>
<td>ND ND ND</td>
</tr>
<tr>
<td>2</td>
<td>829.9 164.9 30.25</td>
<td>822.5 180.9 27.2</td>
<td>691.6 186.2 30.2</td>
</tr>
<tr>
<td>3</td>
<td>677.5 212.8 23.7</td>
<td>601.2 164.9 26.3</td>
<td>811.8 177.4 22.8</td>
</tr>
<tr>
<td>4</td>
<td>670.3 202.2 17.5</td>
<td>702.2 177.4 21.8</td>
<td>702.2 212.8 22.8</td>
</tr>
<tr>
<td>5</td>
<td>819.3 216 28.9</td>
<td>762.6 195 28.1</td>
<td>691.6 191.5 28.9</td>
</tr>
<tr>
<td>6</td>
<td>699 219.7 24.5</td>
<td>865 223.4 29.7</td>
<td>684.2 198.7 25.4</td>
</tr>
<tr>
<td>7</td>
<td>727 219.9 25.4</td>
<td>883.1 216 24.5</td>
<td>723.5 198.7 28.1</td>
</tr>
<tr>
<td>8</td>
<td>734.2 188 26.3</td>
<td>ND ND ND</td>
<td>928.9 202.2 29.8</td>
</tr>
<tr>
<td>mean</td>
<td>721.6 200.6 25.2</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>±26</td>
<td>±7.3 ±1.4</td>
<td>- - -</td>
<td>- - -</td>
</tr>
</tbody>
</table>

**TABLE 3.8 Results of morphological studies of jejunal mucosa.**  
* v.h = villous height; v.w. = villous width; e.h. = enterocyte height

Figure 3.6 is representative of the general ultrastructural appearance of mid-villous enterocytes before pancreatic duct ligation. Following duct ligation marked ultrastructural alterations were observed in mid-villous enterocytes in sections taken from 4 dogs (3,4,5 &6). Not all enterocytes in a section were affected. Affected cells showed little if any microvillar damage, the changes being largely restricted to intracellular organelles, especially mitochondria. Figure 3.7 shows the large numbers of swollen mitochondria found after duct ligation. Following Pancrex
FIGURE 3.6 Ultrastructure of the jejunal mucosa (x5,000)
FIGURE 3.7
Ultrastructure of the jejunal mucosa following pancreatic duct ligation.
Electron dense cell (x3,300)
arrow indicates microvillar damage

Concentric arrangements of parallel membranes (x6,600)

FIGURE 3.7 (continued)
supplementation swollen mitochondria were present in enterocytes from the four previous dogs (3,4,5&6) and also those from dog 8. In addition electron dense cells, areas of severe microvillar damage and concentric arrangements of parallel membranes were found in sections from dog 5 (see Figure3.7).

**Enzymology**

Figure 3.8 and Appendix 1 gives the results of the mucosal enzyme assays. Prior to duct ligation there was a striking difference in the specific activities of the brush border enzyme aminopeptidase N, assayed in both SVE and MOPS media, between three of the Beagles (dogs 1,2 &4) and the other dogs. After duct ligation quantitative enzymologic examination of biopsies revealed decreased activities of lactase (brush border), catalase (peroxisomal marker enzyme) and Tris resistant- $\alpha$ - glucosidase (endoplasmic reticulum marker enzyme). There were no significant alterations in any of the other enzymes. However, there was some individual variation as can be seen from Figure 3.8. Following duct ligation the activities of the brush border enzymes maltase, sucrase and Zn$^{2+}$-resistant- $\alpha$ - glucosidase increased in all but one dog (dog 4). Following treatment with Pancrex the specific activity of sucrase was higher than both pre-and post-ligation values. In addition, the post-operative decreases in the activities of lactase, catalase and Tris-resistant-$\alpha$ - glucosidase were reversed, activities increasing to pre-ligation values. No significant alterations were observed for any of the other marker enzymes.
FIGURE 3.8 Results of mucosal enzyme assays

- = dog 4. Significance, compared with PRE: * = P < 0.05, ** = P < 0.01; POST: o = P < 0.05, oo = P < 0.01; PANCREX: x = P < 0.05, xx = P < 0.01.
FIGURE 3.8(continued) Results of mucosal enzyme assays
FIGURE 3.8 (continued) Results of mucosal enzyme assays
**Electrophoresis**

The effect of pancreatic duct ligation (and treatment with Pancrex) on the microvillar membrane was examined in 5 dogs (3, 4, 5, 6 and 7). The results of gel scans were pooled and the mean proportion of protein in seven bands, 40kDa in width, was calculated. The results are shown in Figure 3.9. Pre-operatively only a small proportion of membrane protein was found in bands corresponding to molecular weight > 200kDa. After pancreatic duct ligation there was a significant increase in the proportion of protein found in the bands corresponding to 200-240kDa (mean proportion of protein in band 200-240kDa = 3.3±4%(range 0-9.5%) pre-operatively, 13.6±7.2%(range 2.5-20.5%) post-operatively). As well as the increase in the proportion of protein in the 200-240kDa band 4 dogs had increases in the band corresponding to 120-160kDa and 2 dogs had increases in the band corresponding to 80-120kDa. Pancrex supplementation caused a significant reduction in the proportion of protein in bands corresponding to molecular weight 200-240kDa (6.9±5.2%(range 0-12.8%)).
FIGURE 3.9 Results of brush border electrophoresis
Data expressed as the mean ± se.
Significance compared with, PRE-OP * = P <0.05, PANCREX ## = P <0.01
3.3.8 Post-mortem examination

In each dog the pancreas was smaller, firmer and more nodular than normal with some adhesions of the pancreas to the small intestine and the liver. Pancreatic atrophy was more severe in the dogs culled at 20wks than those culled at 8 wks. Following injection of dye into the right and left pancreatic lobes none entered the duodenum demonstrating the ducts had been ligated successfully. Table 3.9 gives the pancreatic weights and terminal concentrations of plasma trypsin-like immunoreactivity; a significant correlation (Spearman \( r = 0.810, P < 0.02 \)) was found between them. Histological examination revealed moderate exocrine pancreatic atrophy and duct dilation in dogs 5-8, and severe exocrine atrophy and duct dilation in dogs 1-4. Residual islet cells were seen scattered throughout the pancreas in several dogs, irrespective of the time of culling.

<table>
<thead>
<tr>
<th>DOG</th>
<th>week</th>
<th>TLI (( \mu g/l ))</th>
<th>pancreas wt. (g)</th>
<th>exocrine atrophy</th>
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<tr>
<td>1</td>
<td>20</td>
<td>1.6</td>
<td>4.9</td>
<td>severe</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>0.7</td>
<td>3.3</td>
<td>severe</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.7</td>
<td>4.3</td>
<td>severe</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>1.1</td>
<td>3.26</td>
<td>severe</td>
</tr>
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<td>7.6</td>
<td>8.62</td>
<td>moderate</td>
</tr>
<tr>
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<td>8</td>
<td>3.7</td>
<td>8.76</td>
<td>moderate</td>
</tr>
<tr>
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<td>8</td>
<td>4.4</td>
<td>9.98</td>
<td>moderate</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>14.7</td>
<td>12.37</td>
<td>moderate</td>
</tr>
</tbody>
</table>

TABLE 3.9 Pancreatic weight, morphology and TLI after duct ligation.
3.4 Discussion

*Plasma amylase, lipase and TLI*

The results indicate that following duct ligation there is a dramatic increase in the plasma activities of amylase, lipase and TLI and that the trend is similar for post-operative values of amylase, lipase and TLI. The parallel increases of amylase and lipase confirm previous findings in experimental pancreatitis in dogs (Brobst, Ferguson & Carter, 1970; Mia, Koger & Tierney, 1978). Parallel increases of serum amylase, lipase and TLI have also been observed in humans with pancreatitis (Ventrucci et al., 1983) and following endoscopic retrograde cholangiopancreatography (ERCP) (Okuno et al., 1985). These parallel increases in enzyme activity after duct ligation, ERCP and naturally occurring pancreatitis probably reflect the passage of pancreatic juice through lymphatics and venules draining the pancreas into the circulation (Waldron et al., 1971; Papp et al., 1980).

TLI tended to peak before amylase and lipase and cleared more rapidly with values returning to within the control range in two dogs by three days, and every dog by fourteen days. This rapid return to normal could be attributed to the binding of trypsin to inhibitors and macroglobulins (Ohlsson, 1971), thereby reducing the immunoreactivity of trypsin and/or increasing the rapidity of its elimination from the bloodstream (Borgstrom & Ohlsson, 1980). On the other hand this may not adequately explain the findings in the present study, as trypsin-inhibitor complexes have not been found following the intravenous injection of pancreatic juice in dogs, or ERCP in humans (Borgstrom & Ohlsson, 1978; Borgstrom & Ohlsson, 1980). Pancreatic duct ligation has been likened to giving an intravenous injection of pancreatic juice (Hiatt & Warner, 1969) and the absence of trypsin-inhibitor complexes following pancreatic juice injection and ERCP is probably because these procedures cause an increase in circulating trypsinogen (not trypsin) which is not bound by proteinase inhibitors (Borgstrom & Ohlsson, 1978; Borgstrom & Ohlsson, 1980).
It may be that TLI is eliminated from the bloodstream by a different route from that taken by amylase or lipase. In man the kidney is the main route for the elimination of trypsinogen (Borgstrom & Ohlsson, 1978; Koop et al., 1980b), whereas the liver appears to be more important in the turnover of amylase (Rosenblum, Rabb & Alpers, 1982). This may also be the case in the dog, as trypsinogen is thought to be cleared by the kidney (Borgstrom & Ohlsson, 1980) and basal amylase activity is increased by hepatectomy (Nothman & Callow, 1971) but not by functional nephrectomy (Hudson & Strombeck, 1978). However, the main sites of turnover for amylase, lipase and TLI in the dog remain to be clarified.

It should be emphasised that this study made use of a surgical model of pancreatitis which differed in aetiology and severity from clinical cases; interpretation of the diagnostic significance of changes observed have to be made with care. Nevertheless, the interstitial oedema, focal lymphangitis and disruption of the acinar cell plasma membranes which occur shortly after duct ligation (Hiatt & Warner, 1969; Bockman et al., 1973) compare with naturally occurring mild oedematous pancreatitis (Coffin & Thordal-Christensen, 1953). In addition, the long term effects of pancreatic duct ligation, exocrine degeneration, fibrosis and impairment of pancreatic endocrine function (Idezuki, Goetz & Lillehei, 1969; Heptner, Neubauer & Schleyerbach, 1974; Bewick et al., 1983) are similar to those of chronic pancreatitis (Anderson & Low, 1965; Rimaila-Parnanen & Westermark, 1982).

Because the serum concentrations of amylase and lipase can increase 2-3 fold in dogs with non-pancreatic diseases (Wagner & Macy, 1982; Polzin et al., 1983) increases of around 3-4 times the upper normal serum concentrations are considered necessary to support a diagnosis of acute pancreatitis (Strombeck, 1979). Serum lipase is a more reliable indicator of naturally occurring pancreatitis than serum amylase (Strombeck, Farver & Kaneko, 1981; Westermark & Rimaila-Parnanen, 1983). In the present study the much higher relative increases in serum lipase also made it a more useful indicator of pancreatitis than serum amylase.
However, TLI is thought to be pancreas specific therefore increases above the control range support a diagnosis of pancreatitis. Indeed, when both lipase and amylase activity were below 3-4x normal in two dogs (4 & 8) on the first and third day respectively, the TLI concentration was above the normal limit. This suggests that TLI is a useful indicator of pancreatic disease in cases where both amylase and lipase are not markedly elevated. The rapid increases in TLI (peak activities were recorded before amylase and lipase in six of the eight dogs) suggest TLI is a useful early indicator of disease. Plasma lipase was elevated for longer than TLI and in two dogs the TLI was within the control range by three days after pancreatic duct ligation, suggesting that a normal TLI may not rule out pancreatitis. A normal TLI in association with elevated activities of amylase and lipase may be a sign of decreasing disease. Further evaluation of TLI in clinical cases with pancreatic and non-pancreatic disease is necessary before firm recommendations on its usefulness as a diagnostic test can be made.

The correlation between TLI and pancreatic weight at termination of the experiment appeared also to indicate TLI reflects the severity of exocrine atrophy observed histologically. However, the chronic pancreatic inflammation which accompanied exocrine atrophy in this study is not seen in naturally occurring pancreatic atrophy; it may therefore be unwise to equate TLI with a specific weight of pancreas in naturally occurring cases of EPI. The potential usefulness of TLI in assessing exocrine pancreatic mass is further investigated in Chapters 4 & 5.

**Pancreatic endocrine function**

The changes in pancreatic endocrine function (increased $T_{1/2}$, decreased fractional turnover rate, and decreased fasting insulin and insulin peak response) after duct ligation are similar to those reported in previous studies of dogs with ligated pancreatic ducts (Heptner, Neubauer & Schleyerbach, 1975; Verschoor et al., 1975) and probably reflect islet cell damage following the acute pancreatitis, fibrosis and atrophy (Heptner, Neubauer & Schleyerbach, 1975). However,
despite the reductions in endocrine function, fasting blood glucose concentrations remained remarkably constant and no dogs became overtly diabetic.

**Cobalamin absorption**

The significant decrease in cobalamin absorption following the successful induction of EPI (marked increase in faecal weight, severe steatorrhoea and reduced plasma PABA concentrations following an oral dose of BT-PABA) may be due to several factors. In man, pancreatic proteolytic enzymes are thought to degrade R proteins, which normally bind cobalamin but do not permit its absorption. Degradation of R proteins thus allows the transfer of cobalamin to intrinsic factor (Allen et al., 1978a; Allen et al., 1978b) and hence the subsequent absorption of the intrinsic factor cobalamin complex after binding to specific receptors in the ileum (Hooper et al., 1973) (see Figure 3.10). Supplementation with pancreatic enzymes can restore cobalamin absorption in human cases of exocrine pancreatic insufficiency (Deren, Arora & Toskes, 1973; Toskes et al., 1973). However in this study in dogs, despite the effectiveness of the pancreatic extract (Pancrex) in reducing faecal weight and steatorrhoea, and increasing BT-PABA absorption (see Table 3.3), cobalamin excretion was not decreased by its addition. Furthermore, in spite of the *in vitro* findings that trypsin is the most potent pancreatic enzyme in degrading R proteins (Allen et al., 1978a) there was no correlation between the amount of trypsin administered in Pancrex and the observed decrease in cobalamin excretion. Interestingly, in man no correlation has been found between the concentration of pancreatic enzymes in the small intestine and cobalamin absorption and it has been reported that rapid transfer of cobalamin from R to intrinsic factor can take place despite very low enzyme activities (Steinberg, Currington & Toskes, 1979).

Low plasma cobalamin may lead to further impairment of cobalamin absorption (Lindenbaum, 1974) but this seems unlikely in the present study as plasma cobalamin concentrations were not significantly reduced during the absorption
Figure 3.10 Schematic diagram of cobalamin absorption

Cbl = cobalamin  
R = R protein  
IF = intrinsic factor  
TC2 = transcobalamin 2
tests. The present findings suggest that pancreatic enzymes do not play an important role in the normal absorption of cobalamin in the dog.

Perhaps the component of pancreatic juice causing the partial restoration of cobalamin absorption is the intrinsic factor found in canine pancreatic juice (Horadagoda et al., 1986). Moreover, the significant correlation between the trypsin concentration of pancreatic secretion, not Pancrex, and the reduction in cobalamin excretion may reflect the presence of pancreatic intrinsic factor, which is secreted in parallel with trypsin (Batt et al., 1987). Indeed, pancreatic intrinsic factor may be of particular significance in the dog since the dog has relatively low levels of gastric intrinsic factor and cobalamin absorption is not reduced by gastrectomy (Abels, Kapel & Lindemans, 1977; Marcoulis & Rothenberg, 1981). It has also been demonstrated that pancreatic intrinsic factor promotes the absorption of cobalamin by the ileum in the dog (Batt & Horodagoda, 1986) but further investigation is required to determine the relative importance of gastric and pancreatic intrinsic factors in the normal absorption and turnover of exogenous and endogenous cobalamin in the dog.

Alternatively the decreased cobalamin absorption might involve the lack of pancreatic bicarbonate secretion and/or the increased secretion of gastric acid reported in this model of EPI (Greenlee, 1961; Chey & Lorber, 1967). This consequent reduction in small intestinal pH could hinder the transfer of cobalamin to intrinsic factor (Allen et al., 1978a). Indeed there was a significant decrease in cobalamin excretion when administered with canine pancreatic juice, but it is likely that the buffering capacity of bicarbonate contained in the pancreatic juice was lost before the exit of stomach contents into the duodenum.

A further possibility is that bacteria in the small intestine impaired cobalamin absorption by binding cobalamin and intrinsic factor-cobalamin complexes, or by metabolising cobalamin (Gianella, Broitman & Zamcheck, 1971); the increase in small intestinal bacteria found after the induction of EPI may have occurred by the time of testing post-operative cobalamin absorption. However supplementation
with Pancrex decreased the bacterial numbers markedly at two widely different
time points (8 & 20wks) but did not restore plasma cobalamin concentrations or
reduce cobalamin excretion after an oral dose. Reduction in intestinal bacteria
does not therefore appear to be responsible for the increase in cobalamin
absorption with pancreatic juice supplementation.
The presence of substantial amounts of cobalamin in canine pancreatic juice is
further evidence of the importance of the pancreas in the turnover of cobalamin
and explains the extremely rapid but reversible drop in plasma cobalamin
concentrations in two dogs following diversion and re-diversion of pancreatic
secretion (see Chapter 2). Finding cobalamin in canine pancreatic juice was
unexpected, as it had been assumed that, like man, the major route of cobalamin
turnover would be its excretion in bile bound to R proteins (Toskes, 1980). The
relative amounts of cobalamin excreted in bile and pancreatic juice in the dog are
not known but the rapid drop in plasma cobalamin following diversion of
pancreatic juice, suggest that the pancreas is a major route of turnover in the dog.
A schematic representation of cobalamin absorption in the dog is shown in Figure
3.11. Further studies using labeled cobalamin should enable the major route and
the time scale of cobalamin turnover to be established.

Plasma concentrations of cobalamin and folate
The progressive alterations in the plasma concentrations of cobalamin and folate
after the induction of EPI offer an explanation for the high folate and low
cobalamin concentrations found in naturally occurring cases of EPI (Batt &
Morgan, 1982; Williams, 1985) and suggest that an underlying enteropathy is an
unlikely cause. However, comparable plasma concentrations of folate and
cobalamin to those found in EPI have been documented in dogs with bacterial
overgrowth without EPI (Batt & Morgan, 1982; Batt, Carter & Peters, 1984). In the
present study the plasma concentrations of cobalamin and folate showed no
tendency to return to pre-ligation values after the large reduction in bacterial
FIGURE 3.11 Schematic diagram of cobalamin absorption in the dog.

Cbl = cobalamin
R = R protein
IF = intrinsic factor
TC2 = transcobalamin 2
numbers following Pancrex supplementation, suggesting that the increased bacterial flora was not solely responsible for the altered plasma vitamin concentrations in EPI. These results are in agreement with findings in dogs with naturally occurring EPI in which low plasma cobalamin concentrations and high plasma folate concentrations persist despite the successful treatment of the diarrhoea and weight loss with pancreatic enzyme supplement (Williams, 1985). The likely causes of the reduced cobalamin absorption have been previously discussed and perhaps the most probable cause of the increased plasma folate concentration is the increased jejunal absorption of folate at low pH, the consequence of absence of pancreatic bicarbonate (Russell, Dutta & Iber, 1979).

**Xylose absorption**

Despite the successful induction of EPI (and alterations in the bacterial flora) xylose absorption remained remarkably constant throughout the study. This contrasts with the marked post-operative reduction in xylose absorption observed in the reversible model of EPI (Chapter 2). As EPI was successfully induced in both studies the unaltered concentrations found here are perhaps attributable to the less severe bacterial overgrowth. The effects of EPI on xylose absorption are in agreement with previous studies of dogs with naturally occurring and experimentally induced EPI (Hill, Kidder & Frew, 1970; Stradley, Stern & Heinhold, 1979). Other investigators have found reduced xylose absorption in dogs with naturally occurring EPI (Rogers et al., 1980; Batt & Mann, 1981). An explanation for these apparent discrepancies is proposed in Chapter 5.

**Changes in the bacterial flora of the small intestine**

The quantitative bacteriological findings prior to the induction of EPI (6 of the 8 dogs with \( \leq 10^4 \) aerobes/ml duodenal juice and 5 of the 8 with \(< 10^3 \) anaerobes/ml duodenal juice) agree with three previous studies, that reported mean viable counts of \(< 10^4 \) organisms/ml of juice from the proximal jejunum
(Gelbart et al., 1976; Greenlee et al., 1977) and duodenum (Batt, Needham & Carter, 1983) in normal dogs. The types of bacteria identified were also similar in these studies - various mixtures of Enterobacteriaceae, Staphylococcus, Streptococcus and Lactobacillus spp. and few, if any, obligate anaerobic species.

After duct ligation the marked increase in the numbers and types of bacteria, especially Lactobacillus and Streptococcus spp., and the strictly anaerobic bacteria, Clostridium and Bacteroides spp. may be due to both the effects of surgery and the consequences of EPI. However, it seems unlikely that deranged intestinal motility caused by surgery or pancreatitis is responsible for the bacteriological changes in the present study, because at six, eight and twenty weeks after duct ligation (when duodenal juice was sampled) there was no evidence of acute pancreatitis (absence of clinical signs, firm and nodular pancreases with no evidence of oedema, petechiae or haemorrhage), or impaired intestinal motility (relatively few adhesions, normal contraction of the duodenum and jejunum when the biopsy instrument was inserted).

As alterations in the luminal environment are of particular relevance in determining specific bacterial species (Hoskins & Boulding, 1976), the consequences of EPI for example, the maldigestion of food, seem a more likely explanation of the bacterial overgrowth. Intestinal pH is also likely to be reduced by the absence of pancreatic secretion (Dutta, Russell & Iber, 1979), and increased secretion of gastric acid following pancreatic duct ligation (Greenlee, 1961; Chey & Lorber, 1967). Increased acidity would favour the growth of acid tolerant organisms, and explain the large numbers of Lactobacillus and Streptococcus spp. both of which are acid tolerant (Simon & Gorbach, 1984). The lack of bacteriostatic factors present in canine pancreatic juice, such as bacteriostatic peptides (Rubinstein et al., 1985) may also have favoured bacterial colonisation. In addition, the weight loss and nutritional changes following the induction of EPI may have facilitated the overgrowth of bacteria, as intestinal local
defence systems, such as IgA secretion (Chandra, 1975 ; McMurray et al., 1977), cellular immune function (Gross & Newberne, 1980) and mucous production (Sherman et al., 1985) are impaired by malnutrition. Diminished defences could also have increased the vulnerability of enterocytes to bacterial damage. Overgrowth of bacteria in malnourished rats with blind loops has been shown to cause the more rapid development of disaccharidase deficiencies than in normal rats with blind loops (Sherman, Wesley & Forstner, 1985).

These changes in the luminal environment could predispose to further changes, such as an increase in the concentrations of bacterial metabolites, e.g. volatile fatty acids, which would further reduce the intra-luminal pH. The utilisation of intraluminal oxygen by the large numbers of aerobic and facultative anaerobic bacteria may promote the growth of strict anaerobes (Simon & Gorbach, 1984). A further consequence of bacterial overgrowth is deranged gut motility (Justus et al., 1983) favouring further overgrowth of bacteria.

The numbers and types of bacteria isolated from duodenal juice after duct ligation in this study compare with findings in dogs with naturally occurring EPI (Williams, 1985 ; Williams, Batt & McLean, 1987). However, in the present study larger numbers of the obligate anaerobic bacteria Clostridium and Bacteroides spp. were isolated (present study Clostridium spp.,10\(^7\) - >10\(^8\) and Bacteroides sp 10\(^5\) colony forming units/ml, compared with Clostridium spp.10\(^3\)-10\(^5\) colony forming units/ml and no Bacteroides spp., Williams, 1985 ; Williams, Batt & McLean, 1987).

The dramatic fall in the numbers and types of bacteria following treatment with Pancrex may reflect increased dietary assimilation (decreased faecal weight, reduced steatorrhoea, increased fat absorption and increased PABA), and, as all animals gained weight on Pancrex, the reversal of any effects of malnutrition. In addition, trypsin, lipase and pancreatic extract inhibit the growth of V. cholerae in vitro and pancreatic extract is reported to decrease the susceptibility of protein deficient monkeys to V. cholerae infection (Felsenfeld & Gyr, 1970 ; Gyr,
Felsenfeld & Zimmerlining, 1978). Although the reasons for these antibacterial properties are unclear, pancreatic extract may have had a similar effect on the bacteria in the present study.

**Mucosal changes**

**Structure**

The absence of consistent histologic changes in either villous architecture or epithelial cell structure on light microscopic examination, following the induction of and treatment of EPI, agrees with the findings in dogs with naturally occurring EPI (Batt, Bush & Peters, 1979b ; Williams, 1985). However, ultrastructural abnormalities were found after the induction of EPI and after treatment with pancreatic extract, which is in contrast with the normal mucosal ultrastructure in humans with naturally occurring EPI (Shimoda et al., 1974). These ultrastructural abnormalities are similar to those reported in rats and humans with small intestinal bacterial overgrowth (swollen mitochondria, electron dense cells, concentric layers of parallel membranes) (Ament et al., 1972 ; Gracey, Papadimitriou & Bower, 1974; Toskes et al., 1974). However, despite the marked increase in the bacterial flora in the present study, ultrastructural abnormalities were found only in biopsies from four dogs, including dog 3 which had the smallest increase in intestinal bacteria. Only a proportion of enterocytes in these biopsies were affected, as in previous studies of bacterial overgrowth where the mucosal lesions have been described as patchy and readily missed by intestinal biopsy (Ament et al., 1972 ; Gracey, Papadimitriou & Bower, 1974 ; Toskes et al., 1974). It seems probable that the ultrastructural changes observed following pancreatic duct ligation are at least partially due to an increase in intestinal bacteria.

**Biochemistry**

The increase in the proportion of microvillar proteins of molecular weight >200kDa following the induction of EPI compares with findings in dogs with naturally
occurring EPI (Sørenson et al., 1987). More specifically, the effect of duct ligation on the mean proportion of total protein with a molecular weight between 200-240kDa, is virtually identical to the increases in the band corresponding to 218kDa in naturally occurring cases of EPI (0.5-1.8% control; 8-16% EPI). There was individual variation in the degree of increases in the 200-240kDa band and dog 4 in particular (which had overgrowth with Bacteroides sp.) showed a very small increase to 2.5% of total protein. In contrast to dogs with naturally occurring EPI the increase in the proportion of proteins of high molecular weight in the present study was not confined to bands corresponding to a molecular weight > 200kDa; an increase was also observed in bands with an approximate molecular weight of 150-160kDa (dogs 3, 5, 6 & 7) and bands with an approximate molecular weight of 90-100kDa (dogs 5 & 6). The decrease in the high molecular weight bands following pancreatic enzyme supplementation in the present study is also similar to the effects of enzyme supplementation reported in a dog with naturally occurring EPI (Sørenson et al., 1987), where the proportion of protein corresponding to 218kDa was reduced but not restored to normal. The present study confirms changes reported in dogs with naturally occurring EPI; their absence pre-operatively precludes the possibility that an underlying mucosal abnormality is responsible for the greater than normal proportion of microvillar membrane proteins of high molecular weight present in both treated and untreated EPI. These findings in the dog are consistent with previous in vitro, and in vivo studies in other species (Hauri, Quaroni & Isselbacher, 1979; Kenny & Maroux, 1982; Sørenson et al., 1982; Sjostrom et al., 1983), showing that sucrase-isomaltase and maltase-glucosamylase are synthesised in the endoplasmic reticulum, modified in the Golgi apparatus and initially inserted into the microvillar membrane as large single polypeptide chain forms (pro-forms) of high molecular weight, which can be split in vitro by chymotrypsin, elastase or trypsin to the two polypeptide chain forms of lower molecular weight normally found in the membrane (see Figure 3.12). It has been suggested that the 218kDa band found
FIGURE 3.12
Summary of the synthesis and degradation of brush border enzymes

deployment

synthesis

pro-form cleavage

cleavage of hydrophobic anchor

inactivation/degradation

Golgi apparatus

endoplasmic reticulum

deep seated enzyme
e.g. alkaline phosphatase

pro-form of superficial enzyme
with hydrophobic anchor
e.g. sucrase-isomaltase

*italics* = action of pancreatic enzymes
in dogs with naturally occurring EPI represents single chains of both pro-sucrase-
isomaltase and pro-maltase-glucoamylase. Therefore it seems likely that the large increases in bands between 200-240kDa in the present study represent an accumulation of the pro-forms of these enzymes. The variable increases in bands of lower molecular weights may indicate the accumulation of the pro-forms of other enzymes.

In addition to an increased proportion of pro-forms in the microvillar membrane, increased specific activities of certain brush border enzymes e.g. sucrase and maltase have been demonstrated in dogs and other species (see Chapter 2) with exocrine pancreatic insufficiency (Batt, Bush & Peters, 1979b; Williams, 1985; Williams, Batt & McLean, 1987). These increased specific activities have been attributed to a decrease in the degradation of exposed brush border enzymes in the absence of pancreatic enzymes. As well as modifying enzyme pro-forms, pancreatic enzymes are thought to cleave exposed enzymes from the brush border (Arvanitakis & Olsen, 1974; Alpers & Tedesco, 1975; Kwong, Seetharam & Alpers, 1978) and to inactivate or degrade brush border enzymes in situ (Seetharam et al., 1976).

The susceptibility of brush border proteins to pancreatic proteolytic enzymes appears to depend on how they are anchored to the cell membrane; the carbohydrases, maltase, sucrase, lactase and Zn\(^{2+}\)-resistant α-glucosidase are thought to be anchored to the membrane by relatively exposed hydrophobic anchors (Kenny & Maroux, 1982), whereas alkaline phosphatase, which is relatively resistant to proteinases (Alpers & Tedesco, 1975; Louvard et al., 1975), is strongly associated with lipid (Nayudu & Hercus, 1974) (see Figure 3.12).

As dogs with naturally occurring EPI also have increased specific activities of lysosomal enzymes (Batt, Bush & Peters, 1979; Williams, 1985) and a decreased rate of protein synthesis in the jejunal mucosa (Williams, Batt & McLean, 1985), the effects of pancreatic secretion do not appear to be restricted to the brush border. Indeed, lysosomes may play a role in the turnover of integral brush border proteins.
(Schimke & Doyle, 1970), and it has been suggested that the increased specific activities of lysosomal enzymes in dogs with EPI occurs by virtue of the excessive accumulation of brush border proteins or macromolecules in lysosomes as a consequence of failure to degrade brush border proteins or luminal macromolecules (Batt, Bush & Peters, 1979b).

In the present study, despite the evidence of decreased degradation of the microvillar membrane by pancreatic enzymes (increased proportion of proteins of high molecular weight), the activities of maltase, sucrase and Zn$^{2+}$-resistant α-glucosidase, although increased, were not significantly different from those recorded pre-operatively. Moreover, in marked contrast to naturally occurring EPI, the activities of lysosomal enzymes were not increased and there was a significant decrease in the activity of the brush border enzyme lactase, peroxisomal catalase and the endoplasmic reticulum enzyme Tris-resistant α-glucosidase.

It seems likely that the large increase in the numbers and types of bacteria which followed the removal of pancreatic secretion was responsible for some of the differences between the present study and those in dogs with naturally occurring EPI. Figure 3.13 summarises the effects of bacteria (aerobes and anaerobes) on the intestinal mucosa. Bacterial proteinases and glycosidases can cleave proteins and glycoproteins from the brush border (Jonas, Krishnan & Forstner, 1977; Jonas, Krishnan & Forstner, 1978; Prizont, 1981) and bacteria, their secreted products or intraluminal metabolites can inactivate brush border enzymes in-situ (Gracey, Houghton & Thomas, 1975; Riepe, Goldstein & Alpers, 1980). In addition to these brush border effects, experimental studies in humans, rats and dogs have demonstrated that bacteria, their secreted products or intraluminal metabolites may damage intracellular organelles (Ament et al., 1972; Gracey, Papadimitriou & Bower, 1974; Toskes et al., 1974; Batt & McLean, 1987).

The effects of bacteria on intestinal mucosa are not uniform but appear to depend on: the numbers and types of bacteria present e.g. anaerobic bacteria in large numbers are specifically implicated in the disaccharidase deficiencies observed in
FIGURE 3.13 Summary of the postulated effects on the mucosa of EPI with bacterial overgrowth

- mitochondrion
- peroxisome
- ** = (dog4) Bacteroides sp. ??

EPI
- pancreatic enzymes
- degradation
- synthesis

Bacterial overgrowth
- bacteria
- bacterial products
- intraluminal metabolites
- degradation
- inactivation
- intracellular damage
- synthesis
intestinal segments of experimental blind loops (Jonas, Flanagan & Forstner, 1977; Jonas, Krishnan & Forstner, 1978; Riepe, Goldstein & Alpers, 1980); the susceptibility of mucosal enzymes to the particular flora present e.g. mucosal changes in dogs with aerobic overgrowth are restricted to a decrease in the specific activity of the brush border enzyme alkaline phosphatase and the intracellular marker enzyme for peroxisomes catalase and an increase in the activity of the brush border enzyme γ-glutamyl transferase and the endoplasmic reticulum enzyme Tris-resistant α-glucosidase (Batt & McLean, 1987); the ability of the mucosa to compensate for any damage e.g. brush border enzyme activities are unaltered in dogs with naturally occurring anaerobic overgrowth and animals with experimental blind loops where the numbers of anaerobic bacteria, although higher than normal, are lower than those associated with the pandisaccharidase deficiency (Jonas, Flanagan & Forstner, 1977; Batt, Carter & Peters, 1984; Batt & McLean, 1987).

Bearing in mind that the specific activities of enzymes are a balance between synthesis on one hand and the susceptibility to degradation or inactivation on the other, I shall tentatively attempt to interpret the findings in the present study, where bacterial overgrowth is combined with EPI.

The results of electrophoresis of the microvillar membrane suggest there is a decrease in the degradative effects of pancreatic enzymes, whereas the absence of the expected increases in disaccharidase activity (especially the decrease in the specific activity of lactase), the decrease in the specific activity of catalase (and the ultrastructural findings), suggest an increase in the effects of bacteria. A further indication that bacteria are responsible for limiting the expected increases in disaccharidase activity is provided by findings in dog 4, where decreased specific activities of lactase, sucrase and maltase (and the smallest increase in the % of microvillar protein in the band corresponding to 200-240kDa) were associated with overgrowth of Bacteroides sp. (dog4), an obligate anaerobic bacterium implicated in disaccharidase deficiencies in the blind loop syndrome (Jonas,
Krishnan & Forstner, 1978; Riepe, Goldstein & Alpers). Furthermore, if dog 4 is excluded from the statistical analysis, the increase in maltase activity for the group is significant (P< 0.05; 301.7 ± 37.8mU/mg pre-op; 341.5 ± 58 mU/mg after duct ligation). Figure 3.13 is a summary of the postulated effects on the mucosa of EPI with bacterial overgrowth.

The specific reduction in the activity of lactase suggests that lactase is more susceptible to the combined effects of EPI and bacterial overgrowth than the other brush border enzymes. Indeed, other studies have indicated that lactase is particularly susceptible to inactivation or degradation by proteinases (Seetharam, Perillo & Alpers, 1980) and intestinal bacterial overgrowth (Sherman, Wesley & Forstner, 1985). Moreover, lactase becomes more susceptible to proteinases when its synthesis decreases (Seetharam, Perillo & Alpers, 1980), and a decreased rate of synthesis of high molecular weight proteins has been demonstrated in partially pancreatectomised rats (Alpers & Tedesco, 1975) and dogs with naturally occurring EPI (Williams, Batt & McLean, 1985). As lactase activity is not reduced in dogs with bacterial overgrowth alone (Batt & McLean, 1987), the reduced specific activity of lactase in the present study probably reflects a combination of the susceptibility of lactase to inactivation or degradation and decreased ability to compensate for any loss by increased synthesis.

The reduced activities of the marker enzymes for intracellular organelles, Tris-resistant α-glucosidase (endoplasmic reticulum) and catalase (peroxisomes), may also be a consequence of bacterial overgrowth. Indeed, the specific activity of catalase is reduced in dogs with aerobic overgrowth (Batt & McLean, 1987) and the specific activity of Tris-resistant α-glucosidase is reduced in dogs with naturally occurring EPI with anaerobic overgrowth (Williams, 1985). However, the specific activities of catalase and Tris-resistant α-glucosidase are not decreased in dogs with EPI or anaerobic bacterial overgrowth alone (Batt, Bush & Peters, 1979b, Williams, 1985; Batt & McLean, 1987). These observations may therefore reflect
the effects of an increase in bacteria combined with a decreased rate of protein synthesis.

As bacteria were not adherent to the microvillar membrane (an observation made previously in studies of the experimental blind loop syndrome (Gracey, Papadimitriou & Bower, 1974; Toskes et al., 1975)) and very little damage to microvilli was observed in the present study, any responsibility bacteria may have had for the subcellular changes observed, was probably due to the effects of bacterial products (e.g. proteinases, glycosidases, enzyme inhibitors) or intraluminal metabolites (e.g. deconjugated bile salts, hydroxy fatty acids). Ultrastructural abnormalities similar to those described have been observed in animals fed deconjugated bile salts (Gracey, Papadimitriou & Burke, 1973).

Damage to the brush border by bacterial products (Prizont, 1981; Batt & McLean, 1987) may also increase the permeability of the brush border membrane to bacterial products or to intraluminal metabolites which damage intracellular organelles. Further study is required to determine the precise pathogenetic significance of various intraluminal metabolites and bacterial products.

Even if it is accepted that combinations of the various mucosal changes documented in dogs with naturally occurring EPI (Batt, Bush & Peters, 1979b; Williams, 1985; Williams, Batt & McLean, 1987) and naturally occurring bacterial overgrowth (Batt, Carter & Peters, 1984; Batt & McLean, 1987) explain the mucosal changes in the present study, certain unexplained findings remain; *Clostridium* spp. were associated with reduced activities of sucrase, maltase and lactase in dogs with naturally occurring EPI (Williams, 1985; Williams, Batt & McLean, 1987), whereas the higher numbers of *Clostridium* spp. in the present study were not. The specific activity of alkaline phosphatase was reduced in dogs with naturally occurring EPI and dogs with naturally occurring aerobic overgrowth (Williams, 1985; Batt & McLean, 1987; Williams, Batt & McLean, 1987) but was normal in the present study. Increases in the specific activities of lysosomal enzymes have been observed in dogs with naturally occurring EPI and dogs with
naturally occurring aerobic overgrowth (Batt, Bush & Peters, 1979b; Williams, 1985; Williams, Batt & McLean, 1987); no increases were observed in the present study.

These differences may reflect the longer duration of EPI and/or bacterial overgrowth in naturally occurring cases. Indeed, the pandisaccharidase deficiency which is characteristic of the experimental blind loop at 6 to 8wks after surgery (Gianella, Rout & Toskes, 1974; Jonas, Flanagan & Forstner, 1977) does not become established completely until the 4th post-operative week (Sherman, Wesley & Forstner, 1985); prior to this only lactase may be deficient (Sherman, Wesley & Forstner, 1985). Therefore, the short duration of the present study may not have allowed the full mucosal effects of EPI or bacterial overgrowth to develop.

Vitamin E (tocopherol) deficiency has been documented in naturally occurring EPI (Williams, 1985) and increased activities of a lysosomal enzyme (acid phosphatase) have been found in brain and muscle tissue in experimental tocopherol deficiency in chicks and rats (Desai, Calvert & Scott, 1964; Machlin et al., 1977). It is conceivable that the absence of lysosomal changes in dogs with experimentally induced EPI may reflect the short time available for tocopherol deficiency to develop.

Alternatively, differences in the mucosal findings between the present study and those of naturally occurring EPI and bacterial overgrowth may indicate an underlying intestinal abnormality in the naturally occurring cases. The dogs with EPI studied by Batt, Bush & Peters (1979b), Williams (1985) and Williams, Batt & McLean (1987) were German Shepherd dogs, a breed which is prone to - EPI (Westermarck, 1980); an enteropathy associated with bacterial overgrowth (Batt, Needham & Carter, 1983; Batt, Carter & Peters, 1984); an enteropathy resembling chronic tropical sprue in man (Batt, Bush & Peters, 1983); and a relative deficiency of IgA (Whitbread, Batt & Garthwaite, 1984). Increased activities of lysosomal enzymes have been recorded in all of these conditions (Batt, Bush & Peters, 1983; Batt, Needham & Carter, 1983; Batt, Carter & Peters, 1984). It is
possible therefore that the enteropathy found in German Shepherds with naturally occurring EPI and/or bacterial overgrowth is not solely attributable to a lack of pancreatic secretion and/or bacterial overgrowth.

The extremely low activities of aminopeptidase N in three of the Beagles (dogs 1, 2 & 4) at all three time points is remarkable. Initially it was considered that an increased sensitivity to EDTA in the SVE medium was responsible, but the activities of biopsies taken into MOPS were equally low. Bacteria do not seem to be implicated as enzyme activities could not be correlated with either the number or type of bacteria. It has been proposed that abnormalities in aminopeptidase N may be age related, as low activities were found in eight month old, but not nine month old, Irish Setter littermates with a wheat sensitive enteropathy (Batt, Carter & McLean, 1985; Batt, McLean & Carter, 1987). The effects of age are unlikely to be responsible for the low activities observed in the present study as all four Beagles (dogs 1-4) came from the same litter and were thirteen months old at the time of their first biopsy. Further investigation of Beagle jejunal biopsies by electrophoresis and an antibody to aminopeptidase-N should indicate whether a synthetic defect is present.

**Effects of treatment**

The mucosal changes following treatment can be considered in terms of alterations in the balance between synthesis on one hand and degradation and inactivation on the other. As the decreased protein synthesis found in naturally occurring EPI was restored to normal after treatment with Pancrex (Williams, Batt & McLean, 1985) it seemed probable that any effects of EPI on protein synthesis in the present, more acute study would also be reversed. The dramatic reduction in the bacterial flora, along with the increased dietary assimilation and PABA absorption which followed supplementation with Pancrex, suggests that degradation or inactivation of mucosal enzymes by bacteria would decrease, while
degradation or inactivation by pancreatic enzymes would increase. Indeed, following treatment the mucosal changes thought to be associated with bacterial overgrowth and/or reduced protein synthesis, the reduced specific activities of lactase, catalase and Tris-resistant α-glucosidase, were all restored to pre-operative values. However, an abnormality which is associated with the absence of pancreatic enzymes, the increased proportion of microvillar proteins of high molecular weight, was not fully reversed, and the specific activities of sucrase, maltase, and Zn²⁺-resistant α-glucosidase increased, although only the specific activity of sucrase was significantly greater than pre-operatively. The incomplete reversal of the bacterial overgrowth may have limited any increases in the specific activities of the disaccharidases, particularly when one considers that the specific activities of maltase and Zn²⁺-resistant α-glucosidase are significantly higher than pre-operatively when the dog with Bacteroides sp. present after treatment (dog 4) is excluded from the statistical analysis (maltase, pre-op 276.8±50, Pancrex 348.3±57.4mU/mg ; P <0.05 : Zn²⁺-resistant α-glucosidase, pre-op 5.41±1.4, Pancrex 8.0±1.8mU/mg). The low disaccharidase activities in dog 4, before and after treatment, which coincided with the presence of relatively low numbers of Bacteroides spp, are consistent with the findings discussed in Chapter 2 and suggest that particular bacterial species (not just the combined effects of large numbers of different bacteria) can affect the mucosa.

The ultrastructural abnormalities observed after the induction of EPI did not reverse with treatment and may also reflect the incomplete reversal of the bacterial overgrowth.

The mucosal findings after treatment resemble those in untreated naturally occurring EPI (where the specific activities of maltase, sucrase and Zn²⁺-resistant α-glucosidase and the proportion of microvillar proteins of high molecular weight are higher than normal (Batt, Bush & Peters, 1979b ; Williams, 1985 ; Sørenson et al., 1987)) and suggests that the intraluminal concentrations of pancreatic enzymes after Pancrex supplementation are insufficient to restore normal brush
border degradation. This may well be so, as the addition of Pancrex to the diet and the BT-PABA test improved dietary assimilation and the absorption of PABA but did not restore them to pre-operative values. The failure to restore brush border normality is perhaps not surprising when one considers the frequent, cyclical output of pancreatic juice in the normal animal compared to the relatively infrequent dose of pancreatic enzymes given with a meal. Interestingly, Williams (1985) also found an increase in Zn$^{2+}$-resistant $\alpha$-glucosidase following treatment and this may be due not only to the increased protein synthesis he observed but also to a reduction in intraluminal bacteria, which he was unable to quantitate. In contrast to the effects of pancreatic enzyme supplementation in dogs with naturally occurring EPI the specific activities of N-acetyl-$\beta$-glucosaminidase, $\alpha$-mannosidase, acid phosphatase (lysosomes) and $\gamma$-glutamyl transferase (Brush border) were not significantly different from those pre-operatively. As previously discussed these differences may reflect either the shorter duration of EPI in the present study or an underlying enteropathy in dogs with naturally occurring EPI.
Summary
The successful induction of EPI was followed by an increase in the numbers and types of intestinal bacteria, and structural and biochemical changes of the small intestinal mucosa characterised by mitochondrial swelling, an increase in the proportion of microvillar proteins of high molecular weight (200-240kDa) and a decrease in the specific activities of lactase, catalase and Tris-resistant α-glucosidase. Pancreatic enzyme supplementation improved dietary assimilation, partially reversed the increase in the proportion of microvillar proteins of high molecular weight and the increase in bacteria, reversed the decrease in the specific activities of lactase, catalase and Tris-resistant α-glucosidase, and increased the specific activity of sucrase. Changes in the mucosa before and after treatment with Pancrex are consistent with alterations in the balance between the degradation and inactivation of mucosal enzymes by pancreatic enzymes, the effects of bacteria or their intraluminal metabolites, and alterations in protein synthesis following EPI. The findings suggest that pancreatic enzymes have a role in regulating, the small intestinal bacterial flora, the turnover of microvillar proteins of high molecular weight and the specific activity of certain mucosal enzymes in the dog. The mucosal changes found after the induction of EPI in the present study are consistent with various combinations of the changes demonstrated in dogs with naturally occurring EPI, EPI with anaerobic overgrowth, and naturally occurring bacterial overgrowth. However, while the larger numbers and types of intraluminal bacteria and the shorter duration of EPI may be responsible for the differences between the present study and studies in dogs with naturally occurring EPI, some differences may be due to an underlying enteropathy in dogs with naturally occurring EPI.

The plasma concentrations of amylase, lipase and trypsin-like immunoreactivity rose dramatically after pancreatic duct ligation and there was a good correlation between the post-operative values of these enzymes. The parallel increases in enzyme activity after duct ligation suggest a common route of entry into the
bloodstream, probably through lymphatics and venules draining the pancreas. However, trypsin-like immunoreactivity tended to peak before amylase and lipase and cleared more rapidly, values returning to within the control range before those of amylase and lipase. This rapid return to normal might mean that trypsin-like immunoreactivity is eliminated from the bloodstream by a route different to amylase and lipase. However, the main routes of turnover for amylase, lipase and trypsin-like immunoreactivity in the dog remain to be clarified. As amylase and lipase are not solely of pancreatic origin the larger relative increases in lipase activity suggest that it is a more useful indicator of pancreatitis than amylase and confirms findings in previous studies. However, TLI is thought to be a pancreas specific measurement and increases above the normal range should support a diagnosis of pancreatitis. Further evaluation of TLI in clinical cases with pancreatic and non-pancreatic disease is necessary before firm recommendations on its usefulness can be made.

This study indicates that cobalamin absorption is decreased in exocrine pancreatic insufficiency and partially restored by canine pancreatic juice but not by bovine pancreatic enzymes. These findings suggest that the pancreas has an important role in the normal absorption of cobalamin in the dog which is not attributable to pancreatic enzymes. The presence of substantial amounts of cobalamin in the pancreatic juice is further evidence of the importance of the pancreas in the turnover of cobalamin in the dog and explains the extremely rapid but reversible drop in plasma cobalamin concentrations in two dogs following external diversion and re-diversion of pancreatic secretion (Chapter 2). Further studies using labeled cobalamin are necessary to establish the major routes and the time scale of cobalamin turnover in the dog. The progressive alterations in the plasma concentrations of cobalamin and folate after the induction of EPI offer an explanation for the high folate and low cobalamin concentrations found in dogs with naturally occurring EPI and suggest that it is unlikely that an underlying enteropathy is responsible for these alterations.
CHAPTER 4
PANCREATIC FUNCTION FOLLOWING SUB - TOTAL PANCREATECTOMY : A COMPARISON BETWEEN DRAINAGE OF SEGMENTS OF THE PANCREAS INTO THE STOMACH OR DUODENUM

4.1 Introduction

4.2 Materials and Methods
  4.2.1 Animals
  4.2.2 Assessment of growth
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  4.2.4 Pancreatic function tests
  4.2.5 Estimation of the plasma concentrations of cobalamin and folate
  4.2.6 Plasma protein estimation
  4.2.7 Post-mortem examination
  4.2.8 Statistical methods

4.3 Results
  4.3.1 Clinical outcome
  4.3.2 Growth
  4.3.3 Pancreatic function
  4.3.4 Plasma concentrations of cobalamin and folate
  4.3.5 Post-mortem findings

4.4 Discussion
4.1 Introduction

Although pancreatic transplantation is now technically feasible there are many problems which have restricted its clinical application. Foremost amongst these are the early complications, pancreatitis and thrombosis of both arterial and venous anastomoses (Kyriakides et al., 1979). Pancreatitis is probably a consequence of the composite effects of surgical trauma, impaired blood supply before and following anastomosis, and the method of coping with the exocrine secretion. It can lead to the release of active pancreatic enzymes and other harmful substances (e.g. kallikrein and myocardial depressant factor) associated with local and systemic damage (Bockman et al., 1973; Lasson & Ohlsson, 1984). Local damage to pancreatic blood vessels may lead to thrombosis and further impairment of the pancreatic blood supply. Systemic complications include damage to the lungs and heart, and disseminated intravascular coagulation (Feldman et al., 1981; Lasson & Ohlsson, 1986). To avoid these severe consequences it is important to minimise the initial pancreatitis by ensuring minimal trauma at surgery, good organ preservation and a good vascular anastomosis.

The methods of dealing with the exocrine secretion are also relevant and fall into two main categories: occlusion of the duct or free drainage. Duct occlusion is achieved either by ligation of the pancreatic duct, or by the injection of a polymer into the duct system. Duct ligation has been associated with a high incidence of pancreatitis and vascular thrombosis in the short term (Idezuki, Goetz & Lillehei, 1969; Rausis, Choudhury & Ogata, 1970) and fibrosis and endocrine impairment in the long term (Idezuki, Goetz & Lillehei, 1969; Heptner, Neubauer & Schleyerbach, 1974; Verschoor et al., 1975). Duct obliteration by polymer injection has fewer immediate complications (Baumgartner et al., 1981) but late effects include abscess formation and fibrosis of the pancreas with anatomic distortion of pancreatic islet cells (Dubernard et al., 1980; Kyriakides et al., 1979; Baumgartner et al., 1981) which impairs endocrine function (Gabel et al., 1983). Free drainage of the pancreas, when compared with duct occlusion techniques, has a lower incidence of thrombosis and pancreatitis in the short term, and less severe fibrosis...
and endocrine impairment in the long term (Rausis, Choudhury & Ogata, 1970; Kyriakides et al., 1979; Kyriakides, Nuttall & Miller, 1979; Baumgartner et al., 1981; Gabel et al., 1983). The sites utilised for the free drainage of exocrine secretion include the peritoneum, and the gastrointestinal and urinary tracts. Each of these sites has potential disadvantages. Drainage into the peritoneal cavity has been associated with ascites (Dutoit et al., 1981; Sutherland, Goetz & Najarian, 1981), with gut drainage there is a risk of leakage of gut contents from a pancreaticoduodenal anastomosis (Kyriakides et al., 1979) while drainage into the urinary tract involves nephrectomy and anastomosis of the pancreatic duct to the ureter (Baumgartner et al., 1981). If a safe technique for anastomosing the pancreas to the gut were available it would seem logical to opt for this "more natural" drainage route. Physiologically the duodenum would be the optimal site. Although exocrine pancreatic function is of secondary importance in pancreas transplantation, draining pancreatic secretions into the duodenum would aid the digestion of food. This is of particular relevance to organ transplantation in children, as dietary supplementation with pancreatic extracts, some of which contain high concentrations of purines and bile acids may be harmful (Niessen et al., 1983). However, despite its physiological advantages, drainage into the duodenum is technically more difficult than drainage into the stomach, which lies in direct apposition to a segmental pancreatic graft (Calne, 1984).

There is therefore a need for an effective technique of anastomosis of pancreas to the gut and information on whether the site of this anastomosis is important. By removing the body and uncinate process of the pancreas and draining the tail of the pancreas into the duodenum or stomach a segmental pancreas transplant is simulated which avoids the problems of vessel anastomosis or rejection. Detailed study of pancreatic function and structure would allow the effects of the site of this anastomosis and suitability of the anastomotic technique to be determined. This model would provide an opportunity to compare the BT-PABA and TLI tests in sub-total pancreatic insufficiency and to assess any concomitant effects on the absorption of xylose, cobalamin and folate.
**Aims**

This study had three aims. The first was to devise an effective technique of anastomosis between pancreas and gut. The second was to determine the effects of this technique and the site of anastomosis on growth, pancreatic structure and pancreatic endocrine and exocrine function. The final aim was to compare the BT-PABA and TLI tests, and to determine the effects of partial pancreatectomy on the absorption of xylose, and plasma concentrations of cobalamin and folate.

**Protocol**

![Diagram of protocol](image)

FIGURE 4.1 Summary of protocol

Figure 4.1 is a summary of the protocol for this study. Each animal underwent exocrine pancreatic function testing (BT-PABA/xylose, TLI) pre-operatively and at 1, 3 & 6 months post-operatively. Plasma cobalamin, folate and protein concentrations were also measured at these times. Endocrine pancreatic function was assessed by measuring fasting blood glucose, glucose tolerance and insulin response to intravenous glucose, pre-operatively (except insulin response) and at 3 & 6 months after surgery. A post-mortem and detailed examination of the pancreas were undertaken at the end of the six month period.

**4.2 Materials and Methods**

**4.2.1 Animals**

Ten young Beagles (4 male, 6 female) were paired according to age, housed in a controlled environment, fed a standard diet (Pedigree Chum and mixer, Pedigree Petfoods, Melton Mowbray, Leics.) and received water ad-libitum throughout the
4.2.2 Assessment of growth
Animals were weighed weekly from birth until the end of the study. To allow comparison of the study group with the colony, "normal" Beagle growth curves were prepared from colony records.

4.2.3 Anaesthesia, surgery and post-operative care
Prior to surgery, dogs were fasted overnight and premedicated with acepromazine (0.25mg/kg) half an hour before induction of anaesthesia with thiopentone sodium (10-15mg/kg; Intraval sodium, May & Baker, Dagenham, UK). Anaesthesia was maintained with halothane (1-2%), nitrous oxide and oxygen (2:1) on a ventilator. The operation was performed through a mid-line abdominal incision. The body and uncinate process of the pancreas were excised with care so as to preserve the cranial and caudal pancreatico-duodenal vessels, thereby ensuring the viability of the duodenum (Figure 4.2a). The cut end of the pancreas was pegged at right angles into a slot in the wall of the stomach or duodenum created by a longitudinal myotomy (Figure 4.2b). In this position the duct could be sutured directly to the mucosa over a stent and the margins of the cut end of the pancreas sutured to the muscle rim of the myotomy. To achieve this, a silastic cannula was used as a stent, passed into the pancreatic duct and then secured with prolene sutures (7/0) (Ethicon Ltd.). The sutures were left long and used later to anastomose the duct to the mucosa. Next the back wall of the anastomosis between pancreatic margin and muscle rim was secured with interrupted vicryl (5/0) (Ethicon Ltd.). The free end of the stent was then passed into the lumen of the bowel through a stab incision in the mucosa. This was done by puncturing the bowel lumen near the anastomosis with a wide bore needle (18G). The needle was then advanced further so that it pierced the mucosa from within to without. The free end of the stent could then be passed into the lumen of the needle and the needle withdrawn so that the stent was carried into the lumen of the bowel. The duct and mucosa were stitched on either side of
FIGURE 4.2 Dog's pancreas in situ:

a) UP: uncinate process; H: head; B: body; T: tail; -----: myotomy; _______: line of transection.

b) Anastomosis of the tail of the pancreas to the duodenum:
   backwall of the anastomosis is complete and the stent is in situ.

c) Two prolene sutures anchor stent and draw duct to mucosa.
the stent with the two prolene sutures referred to above (Figure 4.2c). The anastomosis was then completed and wrapped in omentum. Post-operatively dogs were placed in a heated recovery area. Intravenous dextrose saline was maintained for 24hrs and buprenorphine (0.03mg/kg; Temgesic, Reckitt & Colman Ltd.) was administered for three days to provide analgesia. Prophylactic antibiotics were provided for one week using a combination of Benzathine penicillin (11.25mg/kg) and procaine penicillin (15mg/kg) (Duplocillin, Gist Brocades Ltd.), along with a combination of trimethoprim (2.5mg/kg) and sulfadiazine (12.5mg/kg) (Trivetrin, Wellcome).

4.2.4 Pancreatic function tests
Exocrine pancreatic function was assessed by the combined BT-PABA/xylose test and the assay of plasma TLI as described in Section 2.2.5. Endocrine pancreatic function was assessed by glucose tolerance and insulin response to intravenous glucose as described in Section 3.2.3.

4.2.5 Estimation of the plasma concentrations of cobalamin and folate
Plasma samples for the estimation of cobalamin and folate were stored at -20°C until assay by radioimmunoassay (Section 2.2.7.).

4.2.6 Plasma protein
Plasma concentrations of protein were estimated in heparinised samples by use of an autoanalyser (Technicon sequential multiple autoanalyser).

4.2.7 Post-mortem examination
At the end of the study period a post-mortem examination was carried out on all dogs. To ascertain whether the anastomosis between the pancreas and bowel was patent, the free end of the pancreas was incised and a cannula inserted into the main pancreatic duct. A blue dye and radiocontrast medium were then injected and a radiograph was taken. The pancreatic remnant was then weighed and placed in
buffered formal saline for histological examination. The histological findings were graded by a pathologist (Dr. Ian Talbot, Department of Pathology, University of Leicester) according to the degree of exocrine pancreatic atrophy as either normal, mild, moderate, severe or very severe (no exocrine tissue present).

4.2.8 Statistical methods
The significance of the differences between the means within the same group at different time intervals were assessed using Students paired 't' test. The significance of differences between the means of the two different groups at the same time point were assessed using "Fisher's test" and then an unpaired 't' test where "Fisher's test" was not significant, or a 'z' test where "Fisher's test" was significant. The correlations between PABA and TLI, liveweight gain and PABA and pancreatic weight and TLI were assessed using Spearman's rank correlation coefficient. The line of best fit for pancreatic weight plotted against TLI was determined by regression analysis. Differences with a P value less than 0.05 were considered significant.
4.3 Results

4.3.1 Clinical outcome
The animals recovered uneventfully from the surgical procedure and were behaving normally in three to four days.

4.3.2 Growth
The results are presented in Figure 4.3 and Table 4.1. After surgery the mean liveweight gain in the "duodenal" group was 3.56 +/- 1.37kg compared with a weight loss of -0.79 +/- 0.89kg in the "stomach" group. In the three pairs of dogs operated on at 17, 17 and 19 weeks (Figure 4.3 (a), (b) and (d)) the mean liveweight gain for those animals with duodenal drainage was 4.5 +/- 2.8kg and for stomach drainage 0.5 +/- 0.55kg; this difference was significant (unpaired 't' test P<0.05). In two animals (a & b) in the "duodenal" group, normal growth was observed to full maturity. In the third (c) growth was relatively normal, in the fourth (d) poor weight gain could have been associated with severe pancreatic atrophy (the pancreatic duct could not be cannulated at post-mortem) and in the fifth (e), the operation was performed at 34 weeks when growth was already complete. All dogs in the "stomach" group had abnormal growth patterns (Figure 4.3, a-e).

Total plasma protein remained remarkably constant throughout the study with very little difference between the groups at any time point (Table 4.2).
FIGURE 4.3 Growth curves
TABLE 4.1 Pancreatic function and morphology

<table>
<thead>
<tr>
<th>Dog Stomach</th>
<th>L.W.G (kg)</th>
<th>av.PABA (μmol/l)</th>
<th>av.TLI (μg/l)</th>
<th>terminal TLI (μg/l)</th>
<th>PANCREAS weight (g)</th>
<th>exocrine atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0.3</td>
<td>5.0</td>
<td>1.7</td>
<td>1.8</td>
<td>1.0</td>
<td>severe</td>
</tr>
<tr>
<td>b</td>
<td>1.2</td>
<td>13.2</td>
<td>6.1</td>
<td>8.6</td>
<td>4.5</td>
<td>mild</td>
</tr>
<tr>
<td>c</td>
<td>-3.65</td>
<td>7.8</td>
<td>0.9</td>
<td>2.4</td>
<td>1.7</td>
<td>severe (i)</td>
</tr>
<tr>
<td>d</td>
<td>0.2</td>
<td>5.9</td>
<td>1.37</td>
<td>1.2</td>
<td>1.23</td>
<td>severe (i)</td>
</tr>
<tr>
<td>e</td>
<td>-2.0</td>
<td>13.6</td>
<td>3.96</td>
<td>3.14</td>
<td>2.18</td>
<td>mild</td>
</tr>
<tr>
<td>mean</td>
<td>-0.79</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>se</td>
<td>0.89</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>6.6</td>
<td>20.4</td>
<td>4.24</td>
<td>2.9</td>
<td>4.24</td>
<td>mild</td>
</tr>
<tr>
<td>b</td>
<td>5.65</td>
<td>15.5</td>
<td>4.44</td>
<td>2.8</td>
<td>5.48</td>
<td>mild</td>
</tr>
<tr>
<td>c</td>
<td>4.85</td>
<td>15.9</td>
<td>1.27</td>
<td>1.7</td>
<td>2.16</td>
<td>severe (i)</td>
</tr>
<tr>
<td>d</td>
<td>1.3</td>
<td>12.7</td>
<td>1.63</td>
<td>1.3</td>
<td>1.38</td>
<td>3/4severe/1/4normal</td>
</tr>
<tr>
<td>e</td>
<td>-0.6</td>
<td>20</td>
<td>6.42</td>
<td>3.7</td>
<td>7.7</td>
<td>mild / normal</td>
</tr>
<tr>
<td>mean</td>
<td>3.56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.19</td>
<td></td>
</tr>
<tr>
<td>se</td>
<td>1.37</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.14</td>
<td></td>
</tr>
</tbody>
</table>

**4.3.3 Pancreatic function**

Exocrine pancreatic function

Post-operatively, peak plasma PABA concentrations were significantly decreased, compared with pre-operative values, in both groups at all time points (Figure 4.4). Peak PABA values were lower in the "stomach" than in the "duodenal" group at 1, 3 and 6 months and this difference was significant at 1 & 3 months. In the seven growing dogs (pairs a, b and d and one of pair c drained into the duodenum) there was a significant correlation (P < 0.02; Spearman p = 0.893) between the post-operative liveweight gain and the mean post-operative peak PABA value (sum of the 1,3 and 6 month values divided by three), over the 6 month study period (Table 4.1).

Plasma trypsin-like immunoreactivity was markedly reduced in both groups at all time points after surgery, with little difference between groups (Figure 4.4). There was no significant correlation (Spearman's rank correlation) between the mean post-operative liveweight gain and the mean post-operative TLI value (Table 4.1).
FIGURE 4.4 Plasma concentrations of PABA and TLI. Data are presented as the mean ± se. Significance: compared with pre-op, * = P < 0.05, ** = P < 0.01; compared with same time point in duodenal group, # = P < 0.05, ## = P < 0.01.

Figure 4.5 shows individual post-operative plasma TLI and PABA results; relevant control ranges are detailed in Chapter 5, Table 5.1.

Xylose absorption was not impaired at any time point after surgery in either group (Table 4.2).
TABLE 4.2 Results of function tests

<table>
<thead>
<tr>
<th><a href="mmol/l">Xylose</a></th>
<th>SITE</th>
<th>pre-op</th>
<th>1 mo.</th>
<th>3 mo.</th>
<th>6 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>3.7 ± 0.2</td>
<td>3.5 ± 0.2</td>
<td>4.1 ± 0.3</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>4.0 ± 0.3</td>
<td>3.6 ± 0.3</td>
<td>4.0 ± 0.3</td>
<td>4.7 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><a href="ng/l">Cobalamin</a></th>
<th>SITE</th>
<th>283.8 ± 27.2</th>
<th>425.6 ± 38.4</th>
<th>425.6 ± 77.8</th>
<th>415.6 ± 57.2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>337.8 ± 59.6</td>
<td>448.2 ± 28.7</td>
<td>482.2 ± 82.2</td>
<td>379.2 ± 62.7</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>337.8 ± 59.6</td>
<td>448.2 ± 28.7</td>
<td>482.2 ± 82.2</td>
<td>379.2 ± 62.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><a href="%C2%B5g/l">Folate</a></th>
<th>SITE</th>
<th>6.1 ± 0.7</th>
<th>8.1 ± 0.5**</th>
<th>9.6 ± 1.0*</th>
<th>10.1 ± 0.8*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>6.95 ± 0.5</td>
<td>7.7 ± 0.8</td>
<td>9.7 ± 0.9</td>
<td>9.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>6.95 ± 0.5</td>
<td>7.7 ± 0.8</td>
<td>9.7 ± 0.9</td>
<td>9.1 ± 0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><a href="g/l">Protein</a></th>
<th>SITE</th>
<th>50 ± 2.2</th>
<th>49.4 ± 1.3</th>
<th>49.0 ± 1.3</th>
<th>49 ± 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>51 ± 2.3</td>
<td>50.4 ± 1.5</td>
<td>51.2 ± 1.7</td>
<td>53.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>51 ± 2.3</td>
<td>50.4 ± 1.5</td>
<td>51.2 ± 1.7</td>
<td>53.8 ± 1.1</td>
</tr>
</tbody>
</table>

TABLE 4.2 Results of function tests

S = stomach, D = duodenum; * = P < 0.05, ** = P < 0.01, compared with pre-operative values.

FIGURE 4.5 Individual results of post-operative exocrine function tests

Endocrine pancreatic function

Table 4.3 gives the results of glucose tolerance and insulin response to i.v. glucose. Fasting blood glucose remained within normal limits in each dog throughout the study. However, glucose tolerance (judged by fractional turnover rate) was reduced in both groups at 3 and 6 months, although this reduction was only significant in the "stomach" group (P < 0.01) and there were no significant differences between groups. Fasting insulin and the insulin peak response were also markedly decreased at 6 months (compared with the pre-operative control group) with no significant difference between groups.
TABLE 4.3 PANCREATIC ENDOCRINE FUNCTION

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Pre-op</th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting blood glucose</strong> (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>5.18 ± 0.4</td>
<td>4.46 ± 0.2</td>
<td>5.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>5.22 ± 0.3</td>
<td>4.6 ± 0.2</td>
<td>5.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Fractional turnover rate</strong> (%/minute)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>5.0 ± 0.5</td>
<td>2.0 ± 0.4**</td>
<td>2.4 ± 0.2**</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>4.4 ± 1.0</td>
<td>2.7 ± 0.3</td>
<td>3.75 ± 0.8</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting insulin</strong> (µU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>¶17.0 ± 2.2</td>
<td>ND</td>
<td>4.25 ± 1.2**</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>¶17.0 ± 2.2</td>
<td>ND</td>
<td>6.25 ± 2.0*</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin peak response</strong> (µU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>¶70.2 ± 8.1</td>
<td>ND</td>
<td>11.6 ± 3.3**</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>¶70.2 ± 8.1</td>
<td>ND</td>
<td>16.9 ± 3.8**</td>
<td></td>
</tr>
</tbody>
</table>

¶ = control group (9 Beagles) ; ND = not determined ; S = stomach, D= duodenum.
* = P< 0.05, ** = P< 0.01 compared with control/pre-op values

4.3.4 Plasma concentrations of cobalamin and folate

Plasma concentrations of cobalamin were not markedly affected by the drainage procedure and there were no significant differences within or between groups (Table 4.2).

Plasma concentrations of folate were increased at each time interval after surgery for both groups. These increases were only significant in the "stomach" group although no significant differences were observed between "stomach" and "duodenal" groups (Table 4.2).

4.3.5 Post-mortem findings

In each dog the pancreas was surrounded by the omentum wrapped around it at surgery. The size and appearance of the pancreatic remnant was variable, but the small remnants were generally firm and nodular. The duct to mucosa anastomosis was patent in 9 of the 10 dogs (Figure 4.6) ; I was unable to cannulate the remaining dog's pancreatic duct (duodenum, dog d.). Table 4.1 shows the pancreatic weights and terminal concentrations of TLI. It is apparent that those drained into the stomach were on the whole lighter than those drained into the duodenum, but this difference was not significant. There was a significant correlation (Spearman ρ = 0.794, P<0.01) between pancreatic weight and terminal
FIGURE 4.6
Radiograph of duct to mucosa anastomosis following the injection of radiopaque contrast medium
p = pancreas, s = stomach, arrow indicates contrast medium
TLI concentration (Figure 4.7). Regression analysis reveals that this relationship is probably logarithmic ($y = 1.442 \times 0.589; r = 0.71; P, < 0.02$).

![Graph showing the relationship between plasma TLI (µg/l) and pancreatic weight (g).]

**FIGURE 4.7 Terminal TLI and pancreatic weight**

Histological examination of the pancreatic remnants (Table 4.1, Figure 4.8) confirmed the impression on gross examination that the most severe exocrine atrophy was present in small, firm, light pancreases. In addition, TLI values were lowest in the dogs with the most severe exocrine pancreatic atrophy.
FIGURE 4.8 Histology of pancreatic remnants

Normal (x25)

Mild-moderate exocrine atrophy (x25)
Residual islands of exocrine tissue (x10)

Severe exocrine atrophy (x25)

FIGURE 4.8 (continued)
4.4 Discussion

Drainage technique

Draining the tail of the pancreas into the gut after the removal of two thirds of the pancreas avoids the problems of vessel anastomosis and rejection. This enables the effects of the drainage technique on pancreatic structure and function to be studied without these complications.

The absence of complications associated with drainage of the pancreas into the bowel (leakage of intestinal contents and peritonitis) indicates the success of the drainage technique used in this study. The technique - arrived at independently - resembles that of Rausis and co-workers (Rausis, Choudhury & Ogata, 1970) but differs in that the stent was secured by sutures and the pancreas was pegged into a myotomy. A higher rate of pancreatic duct patency was achieved (9 of the 10 animals at six months) than previously described for free drainage into the peritoneum, where spontaneous closure occurred after several days (Sutherland, Goetz & Najarian, 1979; Baumgartner et al., 1981; Bewick et al., 1983), and for drainage into bowel (Rausis, Choudhury & Ogata, 1970; Aquino et al., 1973). This is important as it is thought that occlusion of the pancreatic duct is responsible for the fibrosis and exocrine atrophy in ligated, polymer injected, and free draining intraperitoneal grafts (Idezuki, Goetz & Lillehei, 1969; Kyriakides et al., 1979; Kyriakides, Nuttall & Miller, 1979; Sutherland, Goetz & Najarian, 1979; Baumgartner et al., 1981; Bewick et al., 1983). However, in the present study exocrine atrophy and fibrosis were present in four dogs with patent ducts (dogs; a, c & d stomach; c, duodenum). The reasons for these findings in the absence of duct occlusion are unclear. It seems unlikely that protein deficiency was responsible for the atrophy (Barbezat & Hansen, 1968; Gyr et al., 1975) as plasma protein concentrations remained constant throughout the study. As the TLI values were consistently low in the four dogs at 1, 3 & 6 months and since there is a correlation (for the group as a whole) between pancreatic weight and TLI, it is likely that the atrophy occurred early. This suggests that early factors such as surgical trauma, interruption of pancreatic blood supply or innervation acting singly or in
combination are responsible for the atrophy observed.

**Pancreatic endocrine function**

Despite the severe reduction of pancreatic mass and the significant decreases in fasting insulin in each dog, normal fasting blood glucose concentrations were maintained after surgery. However, fasting blood glucose concentration is a relatively poor indicator of pancreatic endocrine function as blood glucose is maintained within normal limits until β cell function falls below 20% of normal (Turner et al., 1982). Glucose tolerance tests revealed that the glucose fractional turnover rate was significantly decreased in the "stomach" group and that the peak insulin response was significantly reduced in both "stomach" and "duodenal" groups. As decreased insulin release and low fasting insulin concentrations were found in dogs with relatively normal pancreatic histology it is probable that these decreases resulted from the reduction in pancreatic mass. This finding agrees with two previous studies where it was suggested that segmental pancreatic grafts represent an insufficient supply of islet material (Sutherland, Goetz & Najarian, 1979; Bewick et al., 1983). The higher fractional turnover of glucose, the increased fasting insulin and peak insulin responses in the "duodenal" group is perhaps due to better preservation of islet cell groups following the less severe exocrine atrophy and fibrosis than that seen in the "stomach" group. The higher fractional turnover of glucose in the "duodenal" group may also be a consequence of better exocrine function, for in naturally occurring EPI the decreased glucose tolerance and insulin response are restored to within normal limits when the diet is supplemented with exogenous pancreatic enzymes (Williams, Batt & McLean, 1986). The mechanisms by which enzyme supplementation reverses the endocrine defects found in naturally occurring EPI are unclear.

When the endocrine function of the free draining pancreatic segments at six months is compared with that of the duct ligated pancreas at five months (Chapter 3) there are no significant differences (unpaired Students 't' test) in glucose tolerance, basal insulin or peak insulin response. This shows that despite the
removal of a large portion of the endocrine tissue, free draining pancreatic segments have endocrine function similar to that of the whole pancreas after duct ligation.

**Pancreatic exocrine function**

Pancreatic exocrine function (assessed by the BT-PABA test and plasma TLI) was significantly decreased in both groups at 1,3 & 6 months post-operatively. As xylose absorption was not altered in either group post-operatively and no intestinal mucosal abnormalities were found at post-mortem it is likely that reduced plasma PABA concentrations indicate diminished luminal chymotrypsin activity rather than malabsorption (Batt & Mann, 1981), or alternatively bacterial utilisation of free PABA (see Chapter 2). The lower PABA absorption in the "stomach" group is therefore presumably due to the destruction of pancreatic enzymes in the stomach. Destruction of pancreatic enzymes in the stomach would also explain the poor liveweight gain over six months. An analogy can be made with the Zollinger-Ellison syndrome, where secondary pancreatic insufficiency results from the inactivation of pancreatic enzymes by large amounts of gastric acid (Jones, Nicholls & Badman, 1976; Straus, Johnson & Yalow, 1977). These findings indicate that in such cases, where the TLI would probably be normal, the PABA test could indicate a reduction in pancreatic enzyme activity. This cannot be the only possible interpretation as two dogs with stomach drainage and poor weight gain (b & e) had normal PABA absorption (>20μmol/l) at 6 months despite very low values at 1 & 3 months.

The correlation between TLI and terminal pancreatic weight (and degree of exocrine atrophy) is a finding similar to that in dogs with ligated pancreatic ducts (see Chapter 3). The slopes of the regression lines are different, suggesting a logarithmic correlation for free drainage. Interestingly a lower TLI was associated with a higher pancreatic weight in the duct ligated dogs than in the free draining groups studied reflecting perhaps the greater relative amounts of fibrous tissue in duct ligated pancreases and/or impaired entry of TLI into the blood stream. The
free draining pancreas should however bear more relationship to the "normal" situation. Figure 4.7 shows that around 8g of pancreas, representing approximately 1/4 of the normal pancreatic mass (normal mass 33.2 +/- 1.1g (n=6)) is necessary to maintain a normal TLI (> 5μg/l). Dogs with naturally occurring EPI due to acinar atrophy have a TLI below 2.5μg/l, corresponding to about 2.5 g or 8% of pancreatic mass. This ties in closely with the figures quoted for man, where clinical signs of EPI are not usually present until the capacity of the pancreas to secrete digestive enzymes is below 10% of normal (DiMagno, Summerskill & Go, 1973; Regan et al., 1977).

Figure 4.5 shows that normal TLI and BT-PABA test results coincide on only one occasion. Neither the PABA result nor the TLI result identify EPI in this dog (stomach drained dog (dog b) with low weight gain and a low terminal pancreatic weight). Despite a low TLI 1 month after surgery (3.89 μg/l) it also had normal TLI results at 3 months (8.33 μg/l) and terminally (8.6 μg/l). Indeed, the terminal TLI result was much higher than in the other nine dogs. As this study involved pancreatic surgery perhaps the most likely cause of the intermittently normal TLI values was pancreatic inflammation. A similar interpretation may also apply to three other dogs with intermittently normal TLI concentrations (see Figure 4.5) (duodenal drained; dog e, 6mo & dog b,1mo : stomach drained ; dog e, 6mo). The borderline PABA result could also reflect chronic pancreatitis, for increased amounts of pancreatic enzymes can be secreted into the intestine of humans with known pancreatitis (Regan et al., 1981). These experimental findings suggest that in dogs with naturally occurring EPI due to chronic pancreatitis the TLI test may not confirm this diagnosis.

On five separate occasions a normal BT-PABA test result was associated with an abnormal TLI. This finding was surprising for three dogs (Dogs c and d (duodenum) and e (stomach)) had a TLI below 2.5 μg/l (the value below which naturally occurring EPI is reliably diagnosed), pancreatic atrophy and abnormal growth patterns and TLI values of 1.37, 2.1 and 2.05μg/l corresponding to PABA values of 20.4, 22.6 and 30μmol/l). These results are similar to those reported in a
naturally occurring case of EPI where the PABA test was normal (peak 32.4 μmol/l) and the diagnosis EPI was made by measurement of plasma TLI (1.6 μg/l) and confirmed by pancreatic biopsy (Williams & Batt, 1986). The prominent residual islands of exocrine tissue in this naturally occurring case resemble the histological findings in dogs c & d (duodenum). The remaining two dogs (duodenum e & a) with low TLI values (4.2 and 3.29 μg/l) and normal PABA values (26.2 and 25.1 μmol/l) had only minor histological abnormalities; each maintained or gained weight suggesting that the exocrine insufficiency was partial.

The occasionally normal PABA results in these dogs may reflect the release of small amounts of chymotrypsin, which in the case of dogs c and d (duodenum) and e (stomach) were enough to split the small amount of BT-PABA but not to maintain a relatively normal growth pattern. Alternatively, these BT-PABA test results may have been due to the degradation of BT-PABA by chymotrypsin-like enzymes of bacterial or mucosal origin (Gyr, Felsenfeld & Imondi, 1978; Sterchi, Green & Lentze, 1983). If an enzyme of mucosal origin capable of splitting BT-PABA was present one would have expected consistently normal BT-PABA results and not the intermittently normal results in this study.

The occasionally normal PABA results in several dogs with at most one third of their pancreas remaining emphasises that the TLI test can be a more sensitive indicator of decreased exocrine function. Moreover, the results of the BT-PABA test in dogs with naturally occurring EPI overlap those of dogs with malabsorption, whereas there is no such overlap with TLI (Williams, 1985), suggesting that TLI is a more specific indicator of decreased exocrine function than BT-PABA. However, Williams (1985) did not assess whether the low PABA values found in dogs with intestinal malabsorption were due to the defective hydrolysis of BT-PABA or to the impaired absorption of free PABA and claims about the lack of specificity await clarification.

**Plasma concentrations of cobalamin and folate**

In marked contrast to the reduced plasma cobalamin concentrations in dogs with
ligated pancreatic ducts (Chapter 3) and despite two thirds pancreatectomy, plasma concentrations of cobalamin did not alter in either group throughout the study. This could indicate that the small amount of pancreas remaining produced enough juice to allow normal cobalamin absorption. However, which component of the pancreatic secretion is responsible for the absorption of cobalamin? The normal plasma cobalamin in dogs (a,c &d (stomach)) with a severe reduction in luminal chymotrypsin (inferred from very low peak plasma PABA comparable to that in duct ligated dogs and naturally occurring cases of EPI (Williams, 1985)) support the contention that a decrease in pancreatic enzyme activity is not responsible for the development of low plasma concentrations of cobalamin. Perhaps pancreatic intrinsic factor, which is acid resistant, contributed to the maintenance of cobalamin concentrations in the present study. Further speculation is inappropriate as the absorption of cobalamin was not assessed directly and the number and types of intestinal bacteria were not determined.

The post-operative increases in plasma folate concentration, significant in the "stomach" group but not in the "duodenal" group, is similar to that observed in dogs with ligated pancreatic ducts (Chapter 2), and in naturally occurring EPI (Batt & Morgan, 1982; Williams, 1985). These increases probably represent an increase in folate absorption at low pH (Russell et al., 1979), a consequence of decreased pancreatic bicarbonate secretion following partial pancreatectomy and subsequent pancreatic atrophy. However, as the numbers of bacteria in the intestinal lumen were not measured, increased folate production by intraluminal bacterial overgrowth (Batt & Morgan, 1982) cannot be excluded.

Summary
A successful technique was developed in which the tail of the pancreas was drained into the stomach or duodenum after excision of some two thirds of the pancreatic mass. Resting blood glucose remained within the normal range in all dogs post-operatively, though carbohydrate metabolism as judged by glucose tolerance and insulin response to intravenous glucose was impaired. This
impairment in endocrine function even in histologically normal pancreatic tissue suggests that the remaining islet tissue was insufficient to ensure optimal function. However, the degree of endocrine impairment in both groups was comparable to that of a whole pancreas five months after duct ligation suggesting that free drainage causes less endocrine impairment than duct ligation. The lesser impairment in pancreatic morphology and function (endocrine and exocrine) in the duodenally drained group suggests that the duodenum is the drainage site of choice.

Tests of pancreatic exocrine function indicated that pancreatic exocrine function was significantly reduced by the drainage procedures. However, the normal PABA results, observed in two stomach drained dogs with poor weight gain and exocrine atrophy, confirms that the BT-PABA test does not always detect EPI. Similarly normal TLI results in dogs with previously low results, reduced pancreatic mass and abnormal weight gain, reveals the limitations of the TLI test in detecting EPI. Only on one occasion were both TLI and PABA results within the normal control range, suggesting that a combination of the two tests would enable most cases of EPI, whether primary or secondary, to be diagnosed with confidence. However, if one test is to be used the results suggest that in naturally occurring cases of EPI, where EPI is generally a consequence of non-inflammatory acinar atrophy, TLI should be a more reliable indicator of exocrine pancreatic function than BT-PABA.

The normal post-operative plasma cobalamin concentrations in each dog emphasises the need for further investigations of the role of the pancreas in cobalamin absorption. The increases in plasma folate concentrations suggest that increased folate absorption is a direct consequence of EPI, due either to bacterial overgrowth or the decreased intraluminal pH secondary to bicarbonate depletion.
CHAPTER 5
THE EFFECTS OF TOTAL PANCREATECTOMY ON PANCREATIC
FUNCTION TESTS

5.1 Introduction
5.2 Materials and Methods
  5.2.1 Animals
  5.2.2 Pancreatic function tests
5.3 Results
  5.3.1 Clinical outcome
  5.3.2 Pancreatic function tests
5.4 Discussion
5.1 Introduction

Until relatively recently procedures for the diagnosis of EPI in the dog have been based on the detection of undigested food in the faeces and the measurement of faecal proteolytic activity. While these tests are useful in screening for intestinal disease, they do not diagnose EPI reliably (Strombeck, 1978; Burrows, Merrit & Chiapella, 1979). The BT-PABA test, an in vivo assay of intestinal chymotrypsin activity, designed to supersede inaccurate faecal tests (and to avoid duodenal intubation in man) (Imondi, Stradley & Wolgemuth, 1972), has been recommended as a specific test of EPI in the dog (Freudiger & Bigler, 1977; Strombeck, 1978; Batt, Bush & Peters, 1979; Rogers et al., 1980; Batt & Mann, 1981). The combination of the BT-PABA test with the Xylose Absorption test enables the simultaneous evaluation of pancreatic exocrine function and intestinal absorptive function (Rogers et al., 1980; Batt & Mann, 1981), reduced plasma PABA and normal xylose suggesting EPI. However, BT-PABA is expensive, requires multiple blood sampling (or urine collection) and may give misleading results in dogs with small intestinal disease (Batt & Mann, 1981; Williams, 1985). Furthermore, the absorption of xylose is reduced in some dogs with EPI (Rogers et al., 1980; Batt & Mann, 1981) but it is not clear whether this is a direct consequence of EPI or is due to an underlying mucosal abnormality (Batt & Mann, 1981; Williams, 1985).

Recently, the TLI test, based on the leakage of pancreatic trypsin-like immunoreactivity into the bloodstream, was introduced to simplify the diagnosis of EPI. Serum TLI concentrations are uniformly low in naturally occurring EPI (Williams & Batt, 1983) and it has been claimed that TLI is a more specific (Williams, 1985) and more sensitive (Williams, 1985; Williams & Batt, 1986) indicator of EPI than the BT-PABA test. In man, TLI is undetectable in serum following pancreatectomy and is therefore regarded as pancreas specific (Adrian, 1980; Malvano et al., 1980). However, it is not known whether the pancreas is the sole origin of TLI in the dog. In man, there is a correlation between the luminal activity of trypsin and serum TLI (Koop et al., 1980a; Andriulli et al., 1981) but in the dog this possible association has not been investigated.
Aims
The study had five aims. First, to determine whether absorption of BT-PABA occurs when pancreatic exocrine secretion is absent from the intestine. Secondly, to determine whether plasma TLI is solely of pancreatic origin. Thirdly, to compare the effects of pancreatectomy on circulating activities of amylase, lipase and TLI. Fourthly, to examine the relationship between plasma TLI and plasma PABA in dogs with varying degrees of EPI. Finally to determine the effects of pancreatectomy on the absorption of xylose.

Protocol
Five dogs underwent pancreatic function testing (BT-PABA/xylose, TLI, amylase, lipase, glucose) in the week prior to surgery. At surgery the pancreas was removed completely and in 4 dogs their own islet cells were isolated and re-injected into the hepatic portal vein. The operation was followed by a one week recovery period at the end of which time the pancreatic function tests were repeated.

5.2 Materials and Methods
5.2.1 Animals and surgical procedures
Five adult Beagles (2 male, 3 female) were housed in a controlled environment and received a standard diet (Pedigree Chum and mixer, Pedigree Petfoods, Melton Mowbray, Leicestershire) for at least one month prior to, and throughout, the study.
Premedication and anaesthesia have been described in Section 2.2.
The operation was performed through a midline abdominal incision. The right lobe and the body of the pancreas were carefully dissected from the duodenum leaving the pancreatico-duodenal artery intact; the left lobe of the pancreas was then mobilised and the whole pancreas removed. Haemostasis was achieved with ligatures and diathermy. The abdomen was then carefully examined and any extraneous pancreatic tissue was removed. Four of the five dogs (1,2,3 & 4) had purified islet cells from their own pancreases injected into the hepatic portal vein.
The abdominal incision was closed and post-operative care instituted as described in Section 2.2.

5.2.3 Pancreatic function tests

BT-PABA/xylose absorption test
The combined BT-PABA/xylose absorption test was performed as described in Section 2.2.5.

Plasma TLI
Plasma for the estimation of trypsin-like immunoreactivity was stored at -20°C until measurement by radioimmunoassay (Williams & Batt, 1983).

Amylase and lipase assays
Plasma samples were assayed immediately for amylase and lipase as described in Section 2.2.5.

Blood glucose
Blood glucose was estimated immediately after the collection of a fasting sample of venous blood as described in section 2.2.5.

5.3 Results

5.3.1 Clinical outcome
The clinical outcome varied. By the end of the first week each dog had lost weight and had an elevated blood glucose concentration (Table 5.1). Dog 2 vomited several times in the first three days after surgery and dog 5 vomited overnight from the fourth till the seventh day. Dog 5, had undergone total pancreatectomy without injection of islet cells and was euthanased after pancreatic function testing at the end of the first week.
<table>
<thead>
<tr>
<th>Dog</th>
<th>blood glucose (mmol/l)</th>
<th>PABA (μmol/l)</th>
<th>Xylose (mmol/l)</th>
<th>TLI (μg/l)</th>
<th>Amylase (IU/l)</th>
<th>Lipase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre-op</td>
<td>post-op</td>
<td>pre-op</td>
<td>post-op</td>
<td>pre-op</td>
<td>post-op</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>36.7</td>
<td>2</td>
<td>3.5</td>
<td>3.7</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>18.8</td>
<td>53.4</td>
<td>10.35</td>
<td>5.7</td>
<td>4.9</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>19.5</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>6.3</td>
<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>16.7</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>5.9</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>20.8</td>
<td>44.4</td>
<td>4.3</td>
<td>5.2</td>
<td>3.9</td>
<td>7.4</td>
</tr>
</tbody>
</table>

CONTROL:
range 3.2-6.6
n 28
range 36.1 +/- 1.6
n 35
range 4.3 +/- 0.1
n 34

TABLE 5.1 Results of pancreatic function tests, before (pre-op) and at one week after pancreatectomy. Control data presented as the mean +/- se; n = number of dogs; nd = not determined
5.3.2 Pancreatic function tests

The results of the BT-PABA/xylose absorption test are given in Table 5.1. Pancreatectomy resulted in considerable reduction in peak plasma PABA concentrations (there was no overlap with the control range) but it did not abolish them.

There were no significant alterations in peak plasma xylose concentrations after pancreatectomy (Table 5.1).

The results of TLI, amylase and lipase assays are also given in Table 5.1. The plasma amylase and lipase concentrations were variably reduced by pancreatectomy but overlapped with the control range (Table 5.1, Figure 5.1). In contrast to amylase and lipase, plasma concentrations of TLI were significantly reduced by pancreatectomy (paired 't' test, P < 0.001) and there was no overlap with the control range (Figure 5.1). However, small concentrations of plasma TLI were detected in each dog, even in dog 5 who did not have an injection of purified islets.

As the measurement of plasma PABA after an oral dose of BT-PABA and the measurement of plasma TLI appear to be the most specific tests of exocrine pancreatic function (no normal dogs with abnormal test results) in these pancreatectomised dogs, a comparison of TLI and peak plasma PABA concentrations in dogs with total EPI (pancreatectomised dogs) is shown in Figure 5.2 together with data for partial EPI (duodenal group, Chapter 4), and controls. There is a significant correlation between peak PABA and plasma TLI (Spearman \( \rho = 0.537 ; P < 0.001 \)) and this relationship is logarithmic (\( y=9.411 x^{0.555} \); \( r = 0.75; P < 0.001 \)).
FIGURE 5.1. The effect of pancreatectomy on plasma concentrations of pancreatic enzymes.
FIGURE 5.2 The relation between plasma TLI and peak plasma PABA
Lines indicate lower limit of control range
5.4 Discussion
The peak plasma concentrations of PABA following pancreatectomy are similar to those found in dogs with ligated pancreatic ducts (Chapter 3) and dogs with pancreatic juice diverted from the small intestine (Chapter 2). Small increases in plasma or urinary PABA after an oral dose of BT-PABA have previously been reported in pancreatic duct ligated animals (Imondi, Stradley & Wolgemuth, 1972; Stradley, Stern & Heinhold, 1979) and pancreatectomised humans (Lankisch & Lembcke, 1984), but the results of the present study precluded the possibilities that the leakage of chymotrypsin from a partially duct ligated pancreas (Stradley, Stern & Heinhold, 1979) or the failure to discontinue pancreatic enzyme therapy early enough (Lankisch & Lembcke, 1984) were responsible for the increases observed. Therefore, the presence of small amounts of PABA in plasma after pancreatectomy indicates that BT-PABA can be cleaved by a non-pancreatic enzyme or randomly hydrolysed and/or that BT-PABA can be absorbed intact. Indeed, as BT-PABA is cleaved in vitro by certain bacteria (Gyr, Felsenfeld & Imondi, 1978) as well as by an intestinal mucosal peptidase (Sterchi, Green & Lentze, 1983) similar hydrolysis might also occur in vivo. Bacterial hydrolysis is the more likely explanation, as bacterial overgrowth is found in both experimental (Chapter 3) and naturally occurring EPI (Williams, 1985; Williams, Batt & McLean, 1987). However, the absorption of intact BT-PABA is also possible as almost 10% of an oral dose of BT-PABA was recovered intact in the urine of rats and guinea pigs with ligated pancreatic ducts (Yamato & Kinoshita, 1978).

The marked decreases in plasma TLI following pancreatectomy, with no overlap with control values, indicate that TLI is a more sensitive (all pancreatectomised animals had a lower TLI than the controls) and specific (no control dogs had similar concentrations to pancreatectomised dogs) indicator of pancreatic exocrine function than the measurement of plasma amylase or lipase. This finding is not surprising as previous studies have shown that amylase and lipase production is not restricted to the pancreas (Nothman & Callow, 1971; Hamosh et al., 1975; Stickie, Carlton & Boon, 1980; Jacobs, Hall & Rogers, 1982; Murtagh & Jacobs,
1985). However, although the TLI concentrations were markedly reduced by pancreatectomy they were not abolished. This was surprising as TLI is not detectable in pancreatectomised humans (Adrian, 1980; Malvano et al., 1980). There are several possible explanations for this finding. First, despite careful removal of all the pancreatic tissue at surgery, some viable remnants may have remained, although none was found following careful scrutiny at post mortem. Secondly the islet cell purification technique may not have excluded all exocrine tissue. This is unlikely as the completely pancreatectomised dog without an islet cell injection (dog 5) had a higher concentration of TLI than two dogs with injected islets. Thirdly TLI circulating at the time of the operation may still have been present. This does not seem plausible as TLI is rapidly cleared from the circulation, probably by degradation in the kidney (Borgstrom & Ohlsson, 1978). Fourthly it is possible that the assay does not specifically measure plasma trypsin or trypsinogen. Thus the rabbit anti-dog TLI antibody used in the assay may have bound to something other than canine trypsin/trypsinogen, resulting in less binding of the labeled TLI and a higher TLI. This possibility is currently being investigated. Evaluation of pancreatectomised dogs over a longer period than in the present study is necessary to pursue the other possibilities outlined and to exclude the possibility that TLI is produced by organs other than the pancreas.

A correlation was found between plasma TLI and peak plasma PABA which is probably logarithmic. Interestingly, correlations between TLI and the luminal activities of trypsin (Koop et al, 1980a; Andriulli et al.,1981), and between plasma TLI and chymotrypsin (Vezzadini et al., 1980) have been demonstrated in man. Whether TLI reflects luminal concentrations of trypsin and/or chymotrypsin in dogs with natural or experimental EPI remains to be determined. In this study small increases in TLI were generally associated with large increases in PABA (see Figure 5.2) and the findings in Chapter 4 suggest that where pancreatitis can be excluded, TLI is a more sensitive indicator of pancreatic atrophy than BT-PABA. The lower sensitivity of the BT-PABA test may be explained by the absorption of PABA in the absence of pancreatic chymotrypsin extending a previous observation
that relatively small amounts of chymotrypsin can be associated with a normal PABA result (Salway & Payne, 1976). The ability of minimal amounts of chymotrypsin to cleave BT-PABA has been recognised in man; increasing the dose of BT-PABA from 100mg to 500mg increases the sensitivity of the test (Toskes, 1984). Perhaps an increased dose of BT-PABA in the present study would have increased its sensitivity in the dog. An alternative approach utilising a chymotrypsin inhibitor (raw egg white) led to a better separation between normal controls and animals with surgically induced pancreatic insufficiency (Imondi & Wolgemuth, 1979); This modified BT-PABA test has not been evaluated in naturally occurring EPI in the dog.

The relative constancy of xylose absorption after surgery (also found in dogs with EPI induced by duct ligation (Chapter 3) and after partial pancreatectomy (Chapter 4)) agrees with studies in experimental (Stradley, Stern & Heinhold, 1979) and some studies of naturally occurring EPI (Hill, Kidder & Frew, 1970). In other studies conducted in dogs with naturally occurring EPI (Batt, Bush & Peters, 1979; Rogers et al., 1980; Batt & Mann, 1981) xylose absorption was reduced. The dogs in the present study had diabetes which can increase the uptake of monosaccharides (Olsen & Rosenberg, 1970; Nakabou et al., 1980) and mask any effect of EPI. In duct ligated and partially pancreatectomised dogs, where endocrine and exocrine function remains comparable with that in naturally occurring EPI, xylose absorption also remained constant. Perhaps the differences between studies are due to the much lower xylose absorption in normal Beagles than in other normal breeds (Table 5.2) (the mean pre-operative value for Leicester Beagles is below the lower limit of the control range in "normal"dogs (Batt & Mann, 1981)). The study of Hill, Kidder & Frew (1970) compared the results of xylose absorption in naturally occurring EPI in German Shepherd dogs with a normal range established in Beagles, and that of Stradley, Stern & Heinhold (1979) compared xylose absorption in Beagles after pancreatic duct ligation with a Beagle control range. Hence the low xylose absorption in the Beagle prior to EPI may mask any effects of EPI on xylose absorption. The possible causes of low xylose absorption in the Beagle include
<table>
<thead>
<tr>
<th>Normal Dogs</th>
<th>Breed</th>
<th>Peak</th>
<th>60mins</th>
<th>90mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leicester</td>
<td>Beagles</td>
<td>4.1 ± 0.15 (3.2 - 5.7) (24)</td>
<td>3.9 ± 0.15</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>a. Hill</td>
<td>Beagles</td>
<td>4.3 ± 0.1 (3.9 - 4.8) (12)</td>
<td>4.2 ± 0.15</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>b. Stradley</td>
<td>Beagles</td>
<td>ND</td>
<td>4.3 ± 0.4</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>c. Batt</td>
<td>mixed</td>
<td>5.5 ± 0.16 (4.5-6.4) (15)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>d. Rogers</td>
<td>mixed</td>
<td>5.9 ± 1.5 (5)</td>
<td>5.8 ± 0.6</td>
<td>5.3 ± 0.45</td>
</tr>
<tr>
<td>Epi Dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Hill</td>
<td>mixed</td>
<td>4.3 ± 0.3 (5)</td>
<td>3.45 ± 0.6</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>b. Stradley</td>
<td>Beagles</td>
<td>ND</td>
<td>4.1 ± 0.2</td>
<td>3.4 ± 0.2 (5)</td>
</tr>
<tr>
<td>c. Batt</td>
<td>mixed</td>
<td>4.0 ± 0.4 (6)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>d. Rogers</td>
<td>mixed</td>
<td>4.1 ± 1.5 (5)</td>
<td>4.1 ± 0.5</td>
<td>3.9 ± 0.4</td>
</tr>
</tbody>
</table>

**Table 5.2** Xylose absorption in normal dogs and dogs with EPI.

Xylose concentrations presented as the mean ± se, with the range and number of dogs in brackets.
a = Hill, Kidder & Frew, 1970; b = Stradley, Stern & Heinhold, 1979; c = Batt & Mann, 1981; d = Rogers et al., 1980; ND = not determined.
luminal and intraluminal factors such as mucosal abnormalities and bacterial overgrowth. However as xylose absorption is low in Beagles with and without bacterial overgrowth (Chapter, 3) a luminal factor seems the more likely. Indeed, the very low activities of aminopeptidase-N in normal Beagles (Chapter 3) suggests that a mucosal abnormality is present which may also affect xylose absorption. Further investigation is required to determine why xylose absorption is lower in the Beagle than the general dog population and to determine the effects of EPI on the absorption of xylose in non-Beagle dogs.

Summary
Pancreatectomy reduced, but did not abolish plasma PABA and TLI concentrations. The absorption of PABA in the absence of exocrine pancreatic secretion indicates that something other than pancreatic chymotrypsin can cleave BT-PABA and/or that BT-PABA can be absorbed intact. The small amounts of TLI in plasma following pancreatectomy are interesting, but a source of TLI other than the pancreas has yet to be identified. A correlation between plasma TLI and peak plasma PABA concentrations was found and regression analysis suggests that the line of best fit is logarithmic. Whether the plasma concentrations of TLI correlate with pancreatic trypsin or chymotrypsin secretion has yet to be investigated. The results indicate that plasma levels of amylase and lipase are not derived solely from the pancreas and that plasma TLI is a more sensitive and specific indicator of exocrine pancreatic mass than either of these measurements. Xylose absorption was not significantly affected by pancreatectomy perhaps as a result of diabetes or abnormally low xylose absorption peculiar to Beagles. The effect of EPI on the absorption of xylose of other breeds of dog remains to be determined.
CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS
This thesis presents a comprehensive study of the effects of experimental canine EPI on the structure, biochemistry and bacterial flora of the small intestine and on the absorption of cobalamin, xylose and folate. The studies described also provide new information on the effects of 'reversing' EPI by the administration of pancreatic enzymes or pancreatic secretion on the luminal and intraluminal environment of the small intestine and on the absorption of cobalamin, folate and xylose. New insights are gained on the influence of pancreatitis and pancreatic atrophy on conventional tests of pancreatic function, and on the functional consequences of sub-total pancreatectomy combined with pancreas/bowel anastomosis.

To what extent were the initial aims (Chapter 1, p.14) achieved?

The main aims were:

To determine the effects of experimental EPI on the structure and biochemical characteristics of the small intestinal mucosa, the bacterial flora of the small intestine, and the absorption of xylose, cobalamin and folate.

To assess the reversibility of any abnormalities detected above by the administration of exogenous pancreatic enzymes or by whole pancreatic secretions.

To devise, prepare and validate canine models of experimental EPI as a prelude to fulfilling the above aims.

Considering these aims together the results obtained permit the following conclusions:

1. Experimental models of EPI

To fulfill the above aims canine models of EPI were devised and validated. Four such models were studied - 'reversible', pancreatic duct ligated, two thirds pancreatectomised and totally pancreatectomised. The induction of EPI was confirmed in each model by tests of pancreatic function (BT-PABA and TLI). Post-mortem examination of the pancreas and its duct system revealed that structural changes (exocrine and endocrine) were least in the reversible model, of varying severity in partially pancreatectomised dogs, and uniformly severe following
pancreatic duct ligation. Endocrine function was most affected by pancreatectomy (with and without islet cell injection) and impaired in the partially pancreatectomised and duct ligated models. The reversible and pancreatectomised models were of limited usefulness on account of technical problems in the former and the occurrence of diabetes mellitus in the latter.

2. Structure and biochemistry of the small intestinal mucosa

Two models were used in studying these effects - the 'reversible' model and the pancreatic duct ligated model of EPI.

The 'reversible' model of EPI was created successfully in only 3 dogs whereas duct ligation was achieved in 8. Light microscopy did not reveal structural abnormalities of the small intestinal mucosa in either model at any time following exclusion of pancreatic secretions from the small intestine whereas electron microscopy, undertaken in the duct ligated dogs, showed changes in the ultrastructure of the enterocytes of the small intestine (swollen mitochondria). The results of disaccharidase assays obtained in the reversible model were not amenable to statistical analysis as only 3 dogs were studied. In these the specific activities of lactase were decreased whereas the specific activities of maltase and sucrase varied. A more detailed investigation of mucosal biochemistry was undertaken in the duct ligated group. The specific activities of lactase and the intracellular enzymes, catalase (peroxisomes) and Tris-resistant α-glucosidase (endoplasmic reticulum) were reduced following the exclusion of pancreatic secretion from the small intestine. No significant alterations were found in the specific activities of the other brush border (sucrase, maltase, alkaline phosphatase, aminopeptidase-N, γ-glutamyl transferase, Zn-resistant α-glucosidase) or intracellular (malate dehydrogenase, acid phosphatase, N-acetyl β-glucosaminidase, α-mannosidase and 5' nucleotidase) enzymes studied. Electrophoresis of the microvillar membrane revealed that the proportion of microvillar proteins of molecular weight corresponding to 200-240kDa increased following pancreatic duct ligation.
Following the administration of pancreatic enzymes the ultrastructural changes described above did not reverse, and in one dog new abnormalities (electron dense cells, concentric arrangements of parallel membranes, microvillar damage) were detected. Re-introduction of pancreatic secretion into the duodenum restored the specific activity of lactase in 1 dog and the specific activities of maltase and sucrase in 2 others. Oral administration of pancreatic enzymes to the duct ligated dogs restored the specific activities of lactase, catalase and Tris-resistant α-glucosidase, but the specific activity of sucrase increased beyond that observed pre-operatively. The mean proportion of microvillar proteins of molecular weight corresponding to 200-240kDa decreased but remained slightly above pre-operative values.

3. Bacterial flora of the small intestine
The effects of EPI on the bacterial flora of the small intestine were studied in the reversible and the duct ligated models. The exclusion of pancreatic secretion from the small intestine by either method was followed by a large increase in the numbers and types of bacteria. Re-introduction of pancreatic secretion in the reversible model did not reduce the number of intestinal bacteria, but qualitative changes were observed. In each duct ligated dog oral supplementation with pancreatic enzymes reduced bacterial counts and altered the bacterial flora. In both models of experimental EPI Bacteroides spp. were associated with reduced activities of maltase, sucrase and lactase.

4. Absorption of xylose, cobalamin and folate

xylose
The effects of EPI on the absorption of xylose were studied in all four models of EPI. Xylose absorption was reduced post-operatively in the 'reversible' model prior to the diversion of pancreatic secretion making it impossible to assess the effects of removing pancreatic secretion. The absorption of xylose was not significantly affected by the induction of partial or total EPI in the the duct ligated, the partially
pancreatectomised and the totally pancreatectomised models. The oral administration of pancreatic enzymes to duct ligated dogs did not alter the absorption of xylose.

**cobalamin**

The effect of experimental EPI on the absorption of cobalamin was studied in the reversible, duct ligated and partially pancreatectomised models of EPI. In these models cobalamin absorption was assessed indirectly by measuring the plasma concentrations of cobalamin. Cobalamin absorption was measured directly in the duct ligated model using an oral dose of $^{58}$Co cyanocobalamin. The plasma concentrations of cobalamin were decreased one day after the diversion of pancreatic secretion and eight weeks after pancreatic duct ligation, but were unaffected by partial pancreatectomy with drainage of the exocrine pancreas to the gut. The absorption of $^{58}$Co cyanocobalamin was markedly reduced by pancreatic duct ligation.

Re-introduction of pancreatic juice into the small intestine partially restored plasma concentrations of cobalamin. High concentrations (mean 290ng/l) of cobalamin were found in pancreatic secretion ('reversible' model). Supplementation of the $^{58}$Co cyanocobalamin solution with canine pancreatic juice partially restored its absorption in duct ligated dogs. The addition of pancreatic enzymes to the $^{58}$Co cyanocobalamin solution and the diet did not restore the absorption of $^{58}$Co cyanocobalamin or increase plasma cobalamin concentrations.

**folate**

The effect of experimental EPI on the absorption of folate was studied in the reversible, duct ligated and partially pancreatectomised models of EPI. In each the absorption of folate was determined indirectly by measuring plasma folate concentrations. Plasma folate concentration did not alter significantly within four days of the diversion of pancreatic secretion, but increased significantly five weeks after pancreatic duct ligation and four weeks following partial pancreatectomy with anastomosis of the pancreas to the stomach.
Dietary supplementation with pancreatic enzymes did not reduce plasma folate concentrations in pancreatic duct ligated dogs.

**Interpretation of findings**

The structural and biochemical changes of the intestinal mucosa following the induction of EPI are probably due to the combined effects of absence of pancreatic secretion and an increase in bacterial flora. The failure of pancreatic secretion to completely restore mucosal and bacterial changes, and the reduced absorption of PABA and xylose prior to the diversion of pancreatic secretion suggest that in the 'reversible' model intestinal function was affected by the pancreatic cannulation procedure. The almost complete reversal of biochemical changes by pancreatic enzymes in the duct ligated group probably reflects the direct effects of pancreatic enzymes on the mucosa and on the bacterial flora. The failure to reverse structural changes, and the increase in the specific activity of sucrase, suggests that the intraluminal concentrations of pancreatic enzymes were subnormal, and that bacterial overgrowth was incompletely reversed. The constancy of xylose absorption in all but the reversible model suggest that it's absorption is not decreased by EPI. However, the low xylose absorption demonstrated in normal Beagles may have masked any effects of EPI. The findings indicate that turnover of cobalamin in the dog is rapid and suggest that the pancreas has a major role in both cobalamin turnover and absorption. Pancreatic enzymes do not appear to play an important role in the absorption of cobalamin and folate.

**Subsidiary aims were:**

a) to assess the effects of experimentally induced pancreatitis and pancreatectomy on the plasma concentrations of amylase, lipase and TLI.

Structural (pancreatic atrophy and fibrosis) and functional (impaired endocrine function) changes induced by pancreatic duct ligation were consistent with those of chronic pancreatitis. Following duct ligation the activities of amylase, lipase and
TLI increased. TLI peaked before amylase and lipase in 6 of 8 dogs with values returning to within the control range in two of eight dogs by three days and in all dogs by fourteen days. Peak activities of amylase and lipase were recorded on the same day in 7 of 8 dogs. Their activities fell in unison except in two dogs in whom plasma amylase fell more abruptly than plasma lipase. On the fourteenth day after duct ligation plasma amylase activity was within the control range in 6 of 8 dogs whereas lipase activity was above the control range in 6 of 8 dogs. The mean peak increases were 16.5 x basal, 13.7x basal and 25.6 for TLI, amylase and lipase respectively.

One week after pancreatectomy plasma concentrations of TLI were lower than pre-operatively and there was no overlap with the control range. By contrast the plasma activities of amylase and lipase activity were not significantly different from pre-operative values and overlapped the control range. These findings suggest that TLI is more pancreas specific than amylase and lipase and is a more reliable indicator than either of pancreatitis and pancreatic atrophy.

b) to compare the BT-PABA test with the TLI test in dogs with varying degrees of exocrine pancreatic atrophy.

BT-PABA and TLI tests were compared in all four models of EPI. PABA absorption was reduced following diversion of pancreatic secretion, pancreatic duct ligation, two thirds pancreatectomy with drainage into the gut and total pancreatectomy. The PABA concentrations in dogs with two thirds pancreatectomy and duodenal drainage, were higher than those of dogs with two thirds pancreatectomy and stomach drainage, suggesting that the PABA test can can indicate differing degrees of EPI. The PABA test was a useful indicator of the efficacy of the pancreatic extract used to treat the duct ligated dogs. The plasma concentrations of TLI increased following the diversion of pancreatic secretion and returned to normal after its re-introduction. Plasma TLI concentrations increased following duct ligation, peaked within one week and decreased steadily until the conclusion of the experiment. The plasma TLI
concentrations following two thirds pancreatectomy were equally reduced in both "stomach" and "duodenal" groups. The terminal TLI concentrations in duct ligated dogs and two thirds pancreatectomised dogs correlated with pancreatic weight at post-mortem. TLI values were lowest in the dogs with the most severe exocrine atrophy.

The normal PABA results observed in 2 of 5 stomach drained dogs with poor weight gain and exocrine atrophy, and the normal TLI results in 4 of 10 dogs with previously low results, reduced pancreatic mass and abnormal weight gain, reveal that both the TLI and BT-PABA test do not always detect EPI. Only once however were both TLI and PABA results within the control range, suggesting that a combination of both tests would enable the diagnosis of most cases of EPI, whether primary or secondary, to be made with confidence.

Pancreatectomy reduced but did not abolish plasma PABA and TLI concentrations. The absorption of PABA in the absence of exocrine pancreatic secretion indicates that something other than pancreatic chymotrypsin can cleave BT-PABA and or that BT-PABA can be absorbed intact. The small amounts of TLI in plasma following pancreatectomy raises the possibility that a source of TLI other than the pancreas exists and has yet to be identified.

Comparison of TLI and peak plasma PABA concentrations in dogs with total pancreatic atrophy (pancreatectomised dogs) together with data for partial pancreatic atrophy ("duodenal" group) and controls (32 normal dogs) revealed a correlation between the two variables and regression analysis suggested that the line of best fit was logarithmic.

c) To assess the effects on pancreatic exocrine and endocrine function of removing two thirds of the pancreas in the dog. To devise an effective technique of anastomosis between pancreas and bowel.

A successful technique was developed in which the tail of the pancreas was drained into the stomach or duodenum after excision of some two thirds of the pancreatic mass. Patency of the pancreatic duct to gut mucosa anastomosis was
demonstrated in 9 of 10 dogs six months after surgery. Fasting blood glucose concentrations remained within the control range throughout the study. Exocrine pancreatic function (as judged by TLI and BT-PABA) was lower than normal in both sub-groups. Endocrine pancreatic function (assessed by fasting insulin concentrations and the insulin response to intravenous glucose) was also impaired. Pancreatic mass, exocrine pancreatic function (judged by the BT-PABA test and liveweight gain) and endocrine pancreatic function (judged by glucose tolerance tests) were more severely affected in the 'stomach' sub-group than in the 'duodenal' sub-group. The degree of endocrine impairment in both sub-groups was comparable to that of a whole pancreas five months after duct ligation suggesting that free drainage causes less endocrine impairment than duct ligation. This study indicates that anastomosis of the pancreatic duct to the gut allows long term free drainage of pancreatic secretions and that the duodenum is the site of choice for this anastomosis.

Future directions
The studies described cover a wide range of topics and highlight the need for further research. Areas of future interest include:

(1) Mucosal studies
These should be designed to determine:

a) whether the differences between the present studies and those in dogs with naturally occurring EPI and/or naturally occurring bacterial overgrowth are due to the effects of time or to an underlying intestinal abnormality.

b) the nature of specific bacteria, specific bacterial products and/or specific intraluminal metabolites responsible for changes in the mucosal structure and biochemistry.

c) the mechanisms whereby specific bacteria, specific bacterial products and specific intraluminal metabolites cause mucosal damage.

d) the effect on intestinal permeability of bacterial overgrowth or EPI.
e) the suitability of the Beagle for the evaluation of pharmaceuticals or other chemicals in light of low activities of aminopeptidase N and impaired absorption of xylose.

(2) Cobalamin turnover
A complete study of cobalamin absorption, metabolism and excretion in the dog is required to determine:

a) whether intrinsic factor-cobalamin complexes are absorbed intact or whether only cobalamin is absorbed.
b) whether cobalamin is transported in the dog's bloodstream bound to transcobalamin 2.
c) the major routes of cobalamin excretion in the dog.
d) whether pancreatic intrinsic factor is the component of pancreatic juice responsible for the partial restoration of cobalamin absorption in dogs with EPI.

(3) Pancreatic function
Further studies are needed to determine:

a. the mechanism whereby pancreatic duct ligation impairs pancreatic endocrine function.
b. whether free drainage of a transplanted pancreas into the duodenum conserves pancreatic function more than other techniques.
c. whether plasma concentrations of trypsin-like immunoreactivity are affected by non-pancreatic conditions in the dog.
d. the relative contribution of different organs to the synthesis and degradation of amylase, lipase and trypsin-like immunoreactivity in dogs.
APPENDICES
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<td>2.23±0.39</td>
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**APPENDIX 1.** Specific activities of the mucosal enzymes in Chapter 3. (mean ± s.d. μU/mg of protein) Significance: * = P < 0.05, ** = P < 0.01.


Robertson, J. & Gallagher, N.D., (1985). In vivo evidence that cobalamin is absorbed by receptor mediated endocytosis in the mouse. Gastroenterology 88, 908-912


Steinberg, W., Currington, D. & Toskes, P.P., (1979). Evidence that a failure to degrade R binder is unimportant in the pathogenesis of cobalamin malabsorption in patients with chronic pancreatitis. Gastroenterology 76, 1255.


ABSTRACT

TITLE: STUDIES OF SMALL INTESTINAL AND PANCREATIC FUNCTION FOLLOWING EXPERIMENTAL EXOCRINE PANCREATIC INSUFFICIENCY IN THE DOG

AUTHOR: KENNETH W. SIMPSON

The pathogenesis of pancreatic dysfunction and intestinal changes commonly observed in dogs with chronic diarrhoeal disorders is incompletely understood. The present study was therefore undertaken to examine the effects of experimentally induced exocrine pancreatic insufficiency (EPI) on small intestinal and pancreatic function. Four models of EPI were created surgically to assess the effects of EPI on the structure and biochemistry of the jejunal mucosa, the small intestinal bacterial flora, the absorption of cobalamin, folate and xylose, and their reversibility by canine pancreatic secretion or exogenous pancreatic enzymes. The effects of the surgical procedures on pancreatic exocrine and endocrine function were also monitored and a comparison made of two indirect tests of pancreatic function.

EPI was followed by an increase in the proportion of microvillar proteins of high molecular weight, a reduction in the specific activities of lactase, catalase and Tris-resistant a-glucosidase, alterations in bacterial flora, a decrease in cobalamin absorption and an increase in folate absorption. Treatment with pancreatic enzymes, largely reversed the biochemical and bacterial changes but did not reverse structural changes or affect cobalamin and folate absorption. Intraduodenal and oral administration of canine pancreatic secretion (shown to contain cobalamin) partially restored cobalamin absorption.

Pancreatic duct ligation was followed by parallel increases in plasma amylase, lipase and trypsin-like immunoreactivity (TLI). Pancreatectomy reduced circulating TLI but not amylase and lipase. Significant relations between TLI and pancreatic mass, and between TLI and peak plasma PABA were observed. Pancreatic endocrine function was similarly reduced by partial-pancreatectomy and pancreatic duct ligation.

These studies confirm that EPI affects intestinal function and suggest that mucosal changes are due to the absence of pancreatic secretion and to an increase in intestinal bacteria. The pancreas may also have an important role in the turnover and absorption of cobalamin. Finally TLI is a precise indicator of exocrine pancreatic function.