Studies on the Control of Exocrine Pancreatic Secretion in the Dog

A thesis presented in partial
fulfilment of the requirements for
the degree of Doctor of Philosophy
at Massey University

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2002

ABSTRACT

Conventional wisdom has held that, despite the close apposition of the proximal duodenum with the pancreas, the gut communicates with the pancreas only via "long" pathways involving the systemic arterial circulation, the central nervous system or a combination of both. This thesis examines the possibility of *local* avenues of communication, venous or neural, which take advantage of the developmental proximity of the pancreas to the gut and the veins of the hepatic portal system.

After carefully studying the anatomy, a venous latex casting technique was employed to more closely examine the venous drainage patterns of the canine pancreas in its area of close apposition with the duodenum. No clear evidence was found for a local vascular pathway of communication between the two organs, however tributaries of duodenal origin were sometimes observed to coalesce first with veins draining the pancreas prior to entering the cranial pancreaticoduodenal vein. An interesting observation concerned the presence of valves throughout the hepatic portal system, generally identified adjacent to primary, secondary and tertiary branch points relative to the main portal trunk. In all cases, the valve imprints directed blood towards the portal vein.

The results obtained from *histological* examination of the duodeno-pancreatic area were consistent with the results obtained from the latex casting study. Veins leaving the duodenum coursed between rather than through pancreatic lobules, and converged with progressively larger rather than smaller tributaries within interlobular septa. An unexpected observation was the presence of distinct inward-projecting folds in the walls of the veins of the portal vasculature that in some cases bore histological resemblance to sensory organelles. While these structures did not exhibit immunoreactivity to sensory neuropeptides, positive immunoreactivity was identified at the level of the endothelium in some of these veins. It was hypothesized that sensory structures in the portal vasculature might be involved in reflex regulation of the pancreas.

In order to test the hypothesis that components of a sensory mechanism exist within the hepatic portal system of the dog, and that such a sensory mechanism plays a role in the

control of exocrine pancreatic function, the latency of the pancreatic fluid secretory response to a bolus of secretin injected into the aorta was compared to that of secretin injected into selected veins of the portal system and systemic circulations in anaesthetized dogs. Overall, the results of two experiments suggested that the differences in latency between sites generally reflected expected differences due to circulation time. Additionally, the portal vein threshold dose was determined. The threshold dose was the smallest dose of secretin which elicited a detectable pancreatic response following injection into the portal vein site. When administered at each of the remaining non-portal vein sites, the threshold dose elicited a response in 7 of 16 cases. Collectively, the results of these studies provided no clear support for a sensory role for the portal vein or liver in the pancreatic response to secretin. The possibility that other local forms of communication exist that exert control over the exocrine pancreas await further investigation.

Dedicated to my grandmother, Ida Mills, who died of pancreatic carcinoma at Orillia Hospital in Ontario, Canada, August 17, 1977

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge my primary supervisor, Professor David Mellor, for the guidance and assistance provided me over the course of this PhD. In addition to his role as a graduate research mentor, he maintained not only a constant high level of enthusiasm for my research, but also a great deal of confidence in my abilities. Through our many discussions, both scientific and philosophical, I learned to ask questions, to design and execute hypothesis-based research activities, and to then critically evaluate the results. Most of all, I learned to pay careful attention to detail. Professor Mellor was also supportive of my decision to complete the writing of my thesis overseas, and, without his patience, understanding and respect for the responsibilities and commitments I had toward my family, this thesis would have never been completed. Finally, thanks to Professor Mellor, I am now not only a better scientist, but also a better veterinarian.

I would also like to extend my thanks and appreciation to Dr Gordon Reynolds. Dr Reynolds played an integral role in the experimental phases of my PhD. His experience and expertise in gastrointestinal physiology allowed me to acquire the necessary surgical skills, and assisted me not only with the design and set-up of the experiments, but also with interpretation of the results. I am also very much appreciative of the critical evaluations provided by Dr Reynolds during the final stages of the writing of this thesis.

Sincere appreciation is also extended to Mr Mervyn Birtles for his generous provision of laboratory facilities, supplies and technical support, and for his guidance with histology, immunocytochemistry and photomicroscopy. I will be forever indebted to him for his patience, the many long hours he so graciously donated to my project, his generous commitment of funding, and importantly, for allowing me the opportunity to work in the friendly environment of his laboratory. Mr Birtles continued to assist me during the writing stages of the PhD by helping me with the descriptive and interpretive aspects of the histology and immunocytochemistry, and by assisting me in deciding which photomicrographs should be included in the thesis.

I would like to thank Dr Grant Guilford for contributing a veterinary perspective to my research.

I am also extremely grateful to Mr Allan Nutman, a brilliant anatomy technician, whose skills in animal specimen preparation, particularly latex casting, are superseded by few. His precision and attention to detail facilitated a more thorough understanding of the descriptive anatomy of the hepatic portal system of animals. Mr Nutman also graciously donated his time and expertise to the preparation of seven vascular casts of the hepatic portal system of the dog. For this, and the many hours he spent teaching me the procedures, I cannot possibly show my full appreciation. As a veterinarian, I am also extremely appreciative of the renewed awareness I have as a result of witnessing the respect that Mr Nutman shows for the animals who so innocently give their lives for teaching. I also thank Mr Nutman for the many hours he spent critically evaluating and commenting on the details and accuracy of Chapters 2 and 3, for his wisdom, and, importantly, for his friendship. Mr Nutman is nothing short of a perfectionist, and I am very fortunate and grateful to have had the opportunity to work with and learn from such a talented man.

The physiological experiments performed during the course of this PhD could not have been accomplished without the assistance of a number of other people. Thank you to Jill Hogan, Mary-Jane Taylor, Pauline McGuigan and Megan Walker for the care they provided to my research dogs, and for their assistance with physical examinations, blood collections and induction of anaesthesia. Thank you also to Dr Roz Machon, a veterinary anaesthesiologist, and to Mr. Andrew Scuffham, the anaesthesia technician, for their assistance with anaesthesia and equipment. A very special thanks is extended to Mr Brett Guthrie, my research technician. In addition to ensuring that all equipment was available and in proper working order for each experiment, Mr Guthrie spent many long hours (frequently outside of normal working hours) assisting with anaesthesia, surgery and data collection. His efforts were greatly appreciated.

Acknowledgement of research funding goes to the Palmerston North Medical Research Foundation and to the Department of Physiology and Anatomy. Graduate stipend support

was provided through a Massey University PhD scholarship.

I am also grateful to the following members of the academic staff at the Atlantic Veterinary College at the University of Prince Edward Island (Canada) for assisting me with: 1) interpretation of electromyographic recordings - Dr Tarek Saleh, Department of Anatomy and Physiology; 2) interpretation of dose-response curves - Dr Alastair Cribb, Department of Anatomy and Physiology; 3) interpretation of pH measurements - Dr Brian Wagner, Department of Chemistry, and; 4) descriptive anatomy - Dr Susan Dawson, Department of Anatomy and Physiology.

Finally, and most importantly, this dissertation could have *never* been completed without the love and support of my family, my Mom, and especially my Dad who passed away before its completion. To my dear husband, Jeff, whose technical and interpretive skills were invaluable, I thank him from the bottom of my heart for his undying patience, his optimism, his never-ending kindness and understanding. To Jocelyn and Nicola, my daughters, I thank them for loving me throughout.

And to the dogs I came to know.....

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A	aorta
AUC	area under the curve
BSA	bovine serum albumin
CaVC	caudal vena cava
CCK	cholecystokinin
CGRP	calcitonin gene-related peptide
CNS	central nervous system
CMV	cranial mesenteric vein
EMG	electromyography
HR	heart rate
ID	internal diameter
IU	international units
GLM	general linear model
GLP-1	glucagon-like peptide-1
GRP	gastrin-releasing peptide
HCl	hydrochloric acid
HCO_3^-	bicarbonate
MABP	mean arterial blood pressure
MMC	migrating motor complex
MV	mesenteric vein
NaCl	sodium chloride
NKA	neurokinin A
OD	outer diameter
pH	potency of hydration
PDV	cranial pancreaticoduodenal vein
PP	pancreatic polypeptide
PV	portal vein
PYY	peptide YY
RR	respiratory rate
SAS	Statistical Analysis System
SD	standard deviation
SDMV	superficial dorsal metatarsal vein
Sub P	substance P
T	body temperature
TRH	thyrotropin-releasing hormone
VIP	vasoactive intestinal peptide

Chapter One

CONTROL OF EXOCRINE PANCREATIC FUNCTION: AN INTEGRATIVE APPROACH

SUMMARY

This review examines the primary mechanisms involved in the control of exocrine pancreatic function during both the interdigestive and postprandial states. In order to reveal the dynamic nature of control as well as the complex interplay between the central nervous system (CNS) and the organs of digestion, a bolus of food is followed on a journey through the upper gastrointestinal tract. Using this integrative approach, it is shown how the activities of the gut, liver and CNS play a regulatory role in the pancreatic response. Moment-to-moment updates on the digestive status are received and translated both centrally and peripherally, and by way of complex neural reflex pathways and circulating hormones, information regarding the status of the digestive system is continuously relayed back and forth between the organs of digestion. The secretions of the pancreas and liver are modified, both qualitatively and quantitatively, while positive and negative feedback mechanisms put into place a 'check-and-balance' system which ensures that specific needs are met without an inappropriate use of resources. The final pancreatic response is dependent upon the sum of all stimulatory and inhibitory messages arriving from all sources at any given point in time. Although the CNS and circulating hormones play an important role in pancreatic function, many questions regarding the 'link' between cause and effect remain unanswered. For example, one might question why circulating hormones and metabolites need to first pass through the liver and enter the general circulation only to be re-circulated back to the pancreas which is in such close anatomical proximity to the small intestine. There is substantial embryological and anatomical evidence to support the idea that the unique position of the pancreas adjacent to the proximal descending limb of the duodenum has functional significance. Information regarding the digestive status of the animal may be communicated *locally* to the pancreas, and in this manner, may then exert some degree of local regulation on the exocrine pancreas. Potential means by which such a transfer of information could take place is via a direct vascular or neural relay from the small intestine to the pancreas. These specific mechanisms do not appear to have been considered, and while difficult to study, warrant further investigation.

INTRODUCTION

This chapter reviews our current understanding of exocrine pancreatic function and its control, in particular as it relates to the dog. Neural and humoral control mechanisms are examined together in both the fasted and fed states. During the postprandial period, a bolus of food is followed on a journey through the gut. The simultaneous contributions of the gut and nervous system as they relate to regulation of pancreatic function are

discussed. Towards the end, questions are posed and finally, new ideas are presented. Using this integrated approach, it is hoped that the reader will gain a better understanding, not only of the central role of the pancreas in the digestive process and the difficulties involved with studying such a complex system, but also of the areas requiring further study.

INTERDIGESTIVE PANCREATIC SECRETION

The interdigestive period is characterized by regular and slowly repeating cycles of gastrointestinal motor activity to which the secretory activities of the stomach, liver, pancreas and duodenum are tightly linked (Boldyreff, 1911; Szurszewski, 1969; DiMagno *et al.*, 1979; Vantrappen *et al.*, 1979; Keane *et al.*, 1980; Konturek and Thor, 1986). The combination of these cyclical motor and secretory activities is believed to assist in removal of residual undigested food, sloughed mucosal cells and secretions which might otherwise accumulate between meals (Code and Schlegel, 1973). These activities, which also lead to the release of immunoglobulin A from the gastric and duodenal mucosae (Fandriks *et al.*, 1995; Mellander *et al.*, 1997), may also reduce the risk of bacterial overgrowth in the small intestine (Vantrappen *et al.*, 1977). Pancreatic secretory rates and composition of pancreatic fluid during fasting, while highly variable among species, are dependent upon both the absence of intestinal stimuli and the phase of motor activity during which they are measured (DiMagno and Layer, 1993).

THE MIGRATING MOTOR COMPLEX (MMC)

The secretory activities of the exocrine pancreas during the interdigestive period are tightly coupled with gastrointestinal motility, an observation originally described by Boldyreff in 1911 and since then revisited by DiMagno, *et al.*and Vantrappen, *et al.* in 1979. The migrating motor complex, or MMC, is a recurring band of motor activity which begins in the lower oesophageal sphincter (Cantor *et al.*, 1986) and stomach (Code and Marlett, 1975), and migrates distally along the length of the small bowel. Its electrical correlate was first described by Szurszewski in 1969, and by convention, the phases of exocrine pancreatic secretion have been labelled to correspond to these events. Each cycle consists of four phases and recurs every 60-120 minutes. During phase I, myogenic slow waves migrate down the small intestine and terminate in the ileum. This phase represents a

period of motor and secretory quiescence. Lower oesophageal sphincter tone is low (Itoh *et al.*, 1978a) and upper gastrointestinal motility is undetectable at this time. Duodenal fluid absorption predominates over secretion (Sjövall *et al.*, 1990; Mellander *et al.*, 1995), exocrine pancreatic and gastric output are minimal, and bile does not enter the small intestine (DiMagno and Layer, 1993).

Phase II, beginning approximately 30 minutes after the onset of phase I, is characterized by intermittent action potentials superimposed upon the myogenic slow waves. These spike bursts result in progressive increases in gastrointestinal motility and secretion. Irregular motor activity during this period is associated with a switch to net secretion (Sjövall *et al.*, 1990; Mellander *et al.*, 1995) into the lumen of the small bowel, a process which promotes duodenogastric reflux activity (Keane *et al.*, 1981). Lower esophageal sphincter contractility, pepsin levels and gastric acid secretion increase while bile and pancreatic juice begin to enter the duodenum (Itoh *et al.*, 1978a; DiMagno *et al.*, 1979; Vantrappen *et al.*, 1979). Peak secretory outputs by the stomach, liver and pancreas occur late in duodenal phase II or early antral phase III of the MMC which coincides with the appearance of intense regular contractile activity in the stomach and small intestine (DiMagno *et al.*, 1979; Vantrappen *et al.*, 1979). Coincident with these peaks in motor and secretory activities are peaks in circulating levels of the hormones motilin and pancreatic polypeptide (PP) (Keane *et al.*, 1980; Chen *et al.*, 1983).

Phase III is characterized by a short period of 1:1 phase locking between muscular contractions and intestinal smooth muscle slow waves (Sarna, 1985; Szurszewski, 1969). Unlike phase II, this coordination between electrical and mechanical events favours flow of residual digesta in an *aborad* direction. Contractions are intense and regular during phase III, and close examination of motility traces during this period reveal periodic variations in intensity every 1-2 minutes superimposed on the underlying pattern. These periodic increases in contractile activity, each preceded by a very short period of inactivity, appear first in the lower oesophageal sphincter and migrate distally through the stomach into the duodenum (Hall *et al.*, 1982). As phase III progresses, both the frequency of periodic contractions and the secretory activities of the stomach, liver and exocrine pancreas gradually decline. The decrease in secretory activity continues into phase IV

which is characterized by a very short period of irregular contractile activity (DiMagno and Layer, 1993). The entire cycle, having lasted 60 minutes or more, continues to recur in the same orderly manner until the beginning of the next meal.

Control of the MMC and interdigestive pancreatic secretion

A variety of factors have been shown to participate in regulating the MMC. It has been suggested for example, that an intact enterohepatic re-circulation of bile salts may be necessary for regular cycling of duodenal MMCs in both man and dogs (Ozeki *et al.*, 1992; Kajiyama *et al.*, 1998), and in both species, the *regularity* of the MMC appears to be governed, at least in part, by duodenal pH (Itoh *et al.*, 1981; Woodtli and Owyang, 1995). The roles of hormones and nerves in the control of the MMC, have perhaps received the most attention over recent years. Motilin, a hormone released from the upper small intestinal mucosa, possibly in response to the presence of bile (Nilsson *et al.*, 1993; Qvist *et al.*, 1995; Kajiyama *et al.*, 1998), is believed to play an important role in the genesis of the MMC (Peeters *et al.*, 1980) and may participate in the initiation of cyclic activity of pancreatic secretion in dogs (Lee *et al.*, 1986). In man and in dogs, both motilin and the inhibitory hormone, PP, fluctuate cyclically with the MMC (Itoh *et al.*, 1978b; Owyang *et al.*, 1983; Lee *et al.*, 1986) while plasma concentrations of gastrin, secretin and cholecystokinin (CCK) tend to remain fairly stable and do not show periodicity (Rees *et al.*, 1982; Lee *et al.*, 1986; Dale *et al.*, 1989). Plasma levels of the inhibitory hormone, somatostatin, reportedly also cycle with the MMC in dogs (Aizawa *et al.*, 1981), and recently it was shown in man that *intraduodenal* levels, but not *plasma* levels, of the neuropeptides, vasoactive intestinal peptide (VIP), somatostatin, and substance P (Sub P) also vary cyclically during the interdigestive period (Naslund *et al.*, 1998). The roles of these hormones and neuropeptides in the contractile and secretory activities of the duodenum and pancreas have not been established.

Despite the cyclical increases of motilin and PP roughly 'in-phase' with the motor and secretory events of the MMC, a direct causal relationship between hormone release and pancreatic secretion cannot be assumed. Interestingly in dogs, when the pancreas is autotransplanted to the pelvis, amylase activity continues to cycle, albeit at a faster rate than the cycle length of the duodenum (Zimmerman *et al.*, 1992). Plasma motilin

concentrations also cycle in-phase with the duodenal MMC, but bear no relationship to peaks in amylase output by the autotransplanted pancreas. Fasting levels of PP remain low and show no relationship to the phases of the MMC, peaks in amylase output or cycles of plasma motilin concentration. Similarly, it has been demonstrated that trypsin secretion continues to cycle, although out of phase with the MMC, following duodenectomy in dogs, a procedure which significantly reduces plasma concentrations of both motilin and PP and abolishes their cyclical activities (Malfertheiner *et al.*, 1989). The results of these two studies provide us with important information: first, the exocrine pancreas possesses inherent rhythmic secretory capabilities; second, the exocrine pancreas is capable of cycling *independently* of both motilin and PP; and third, a neurally intact system is necessary for *full* coordination of the MMC with the secretory activities of the pancreas. Pancreatic polypeptide and other circulating, non-cycling hormones and peptides might play a role in modulating the intrinsic neural activities of the pancreas and/or the activities of the extrapancreatic nerves.

While the sympathetic nervous system also participates in control of interdigestive motility and exocrine pancreatic secretion (see Di Magno & Layer, 1993, for a review) the role of the parasympathetic nervous system has received a great deal more attention. In general, cold vagal blockade disrupts, but does not totally abolish, the synchronous changes in duodenal motility and pancreatic secretion (Hall *et al.*, 1982; Zabielski *et al.*, 1993). Cold vagal blockade abolishes periodic activity in the lower oesophageal sphincter and stomach, and results in the replacement of duodenal phase II activity with phase I quiescence, while duodenal phase III activity (so-called 'vagal-independent complexes') continues to cycle at expected intervals (Hall *et al.*, 1982). Pancreatic secretion is similarly reduced by lidocaine vagotomy, but continues to show periodicity with peaks coinciding with duodenal phase III electrical activity (Magee and Naruse, 1983). Studies have also demonstrated that atropine (You *et al.*, 1980; Magee and Naruse, 1983; Hall *et al.*, 1984; Lee *et al.*, 1986; Layer *et al.*, 1993; Katschinski *et al.*, 1995), a non-selective muscarinic inhibitor, and more recently, telenzepine (Nelson *et al.*, 1996), a selective M_1-receptor antagonist, alter antroduodenal motility in a manner remarkably similar to that achieved with vagotomy, significantly reduce pancreatic enzyme output during all phases of the MMC and decrease plasma motilin and PP concentrations while abolishing their cyclical

patterns. These studies show that, although vagal/cholinergic mechanisms play an important role in overall control of the MMC, they are not primarily responsible for *coordinating* the motor activities of the duodenum with the secretory activities of the pancreas.

The above studies provide us with important clues regarding the mechanisms of control of pancreatic function during the fasting period (Figure 1). We know for example, that plasma levels of motilin and PP cycle in-phase with the MMC, but that the exocrine pancreatic secretion can cycle independently of them. We know that an intact vagus nerve is required for normal periodic activity in the lower oesophageal sphincter and stomach, and for phase II irregular activity in the small bowel. We know from the results of several studies that the regular contractile activities of the duodenum and secretory activities of the pancreas characteristic of phase III are tightly coupled, but vagally-independent. We also know that cholinergic mechanisms regulate interdigestive cycling of pancreatic enzymes and PP, and mediate components of the gastric MMC and phase II activity throughout the gut. Motilin release and/or action also appears to utilize muscarinic cholinergic pathways to initiate antral, but not duodenal phase III activity.

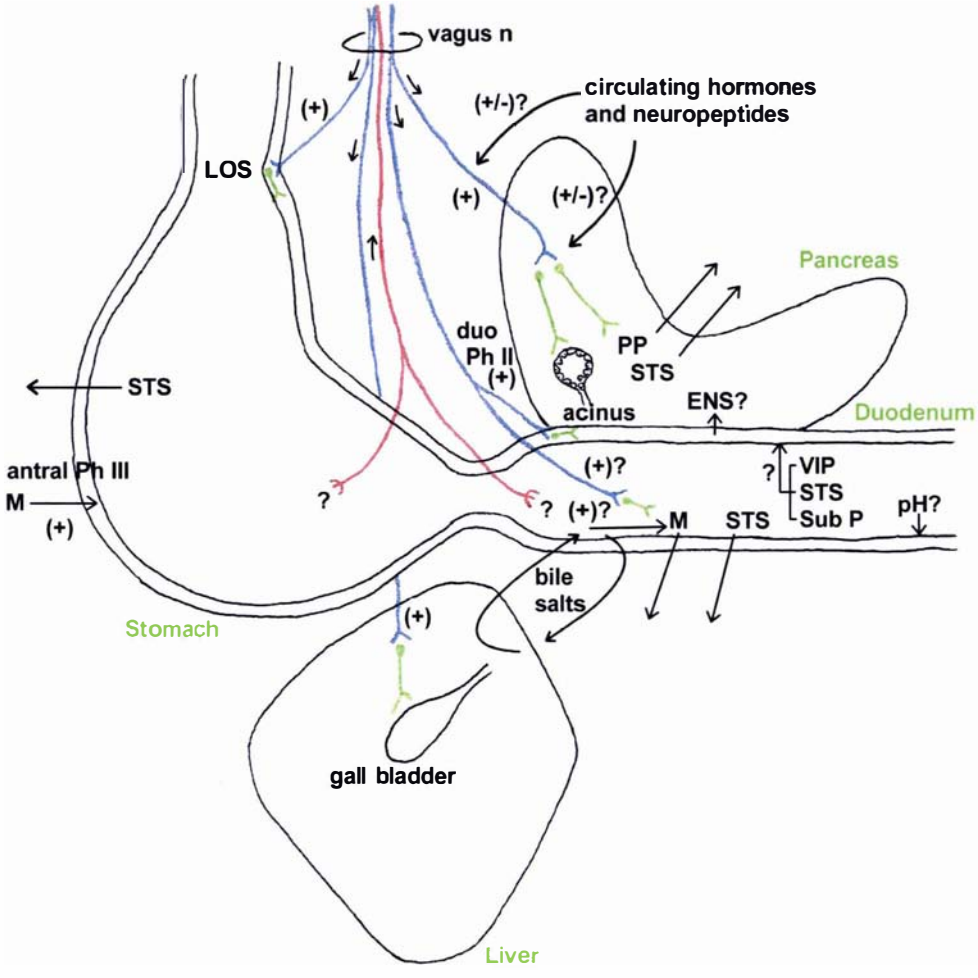

Figure 1. Neurohormonal factors involved in the control of the MMC and associated exocrine pancreatic secretion. An intact enterohepatic re-circulation of bile salts may be necessary for regular cycling of duodenal MMCs, while duodenal pH may influence the regularity of the cycle. Plasma levels of motilin (M), pancreatic polypeptide (PP) and somatostatin (STS), and intraduodenal levels of vasoactive intestinal peptide (VIP), substance P (Sub P) and somatostatin have been shown to fluctuate cyclically with the MMC, however their precise roles in the control of the MMC and pancreatic secretion are unknown. An intact vagus nerve is required for normal periodic activity in the lower oesophageal sphincter (LOS) and stomach, for phase II irregular activity in the small bowel and, quantitatively speaking, for normal secretion by the exocrine pancreas. Duodenal phase III activity and associated peaks in pancreatic secretion are vagally-independent. The enteric nervous system (ENS) may play an important role in 'coupling' the contractile activities of the duodenum with the secretory activities of the pancreas. Vagal afferents are shown in red; pre-ganglionic vagal efferents are shown in blue; post-ganglionic nerve fibres are shown in green; (+) stimulates; (-) inhibits; (?) contribution unknown

Nevertheless, many questions remain unanswered. We do not know what mechanisms are involved with hormonal release; whether hormonal release is a cause or a consequence of neural activation and pancreatic secretion; how the extrinsic nerves specifically interact with other neural elements, hormones or neuropeptides; what controls the vagal-independent complexes; and most importantly, what 'couples' the motor events of the small intestine with the secretory activities of the pancreas. To date, no single manipulation has been able to inhibit, abolish or otherwise alter pancreatic output in the (anatomically) intact animal without concurrently disrupting other elements of the MMC. The possibility that the enteric nervous system may serve this coupling role is suggested by the recent demonstration that enteric neurons project to the pancreas (Kirchgessner and Gershon, 1990). It has also been demonstrated that the gallbladder is innervated by neurons, the cell bodies of which are located in the myenteric plexus of the duodenum (Mawe and Gershon, 1989). These observations provide strong support for a role for the enteric nervous system in coordinating the activities of the duodenum with the secretory activities of both the liver *and* the pancreas. This area warrants further investigation.

POSTPRANDIAL PANCREATIC SECRETION

The interdigestive pattern of pancreatic secretion is interrupted and converted to the postprandial pattern by the thought, sight, smell, taste and swallowing of appetizing foods. These stimuli set into motion a complex set of regulatory events which lead ultimately to effective digestion, absorption and assimilation of nutrients. In the following section, an integrative approach is used to follow a bolus of food through the digestive tract of a dog. In conventional style, the postprandial pattern of secretion is discussed in phases: cephalic, gastric and intestinal, and, due to an increase in exocrine pancreatic activity which is observed in dogs eight to twelve hours following ingestion of a meal, an additional 'delayed postprandial' phase has been included in the discussion. Although there is some degree of temporal order to these phases due to the aboral progression of food through the digestive system, it should be remembered that significant overlap and interactions occur. It is these interactions which are so important for a full understanding of the integrated response to a meal.

'THE CEPHALIC PHASE'

Prior to and immediately following the first bite of a standard meat meal, neural reflexes are activated which initiate the early (but relatively short-lived) cephalic phase of digestion in the dog. This anticipatory phase, which is composed of visual, olfactory, gustatory and tactile stimuli, alerts and prepares the gastrointestinal tract centrally for incoming nutrients. The net result of such stimuli is strong parasympathetic stimulation leading to profuse salivation, altered gastrointestinal motility and increased secretory activities by the stomach, liver and pancreas.

Cephalic phase stimulation elicits strong efferent vagal activity to the organs of digestion (Figure 2). In the salivary glands, cephalic stimulation results in the production of a profuse watery secretion (Pavlov, 1910). Simultaneously with these events, PP concentrations in plasma rise (Taylor *et al.*, 1978; Schwartz *et al.*, 1979; Konturek *et al.*, 1990), gastrin is released from the antral mucosa (Pe Thein and Schofield, 1959; Sjostrom *et al.*, 1980; Riquet *et al.*, 1989; Konturek *et al.*, 1990), while somatostatin release is inhibited (Riquet *et al.*, 1989). Gastric acid is secreted (Pavlov, 1910; Nilsson *et al.*, 1972; Riquet *et al.*, 1989), pepsin levels rise (Riquet *et al.*, 1989), gastric emptying is inhibited (Pappas *et al.*, 1988), neural reflexes interrupt and convert the interdigestive pattern of motility to a postprandial pattern (Hall *et al.*, 1986; Chung and Diamant, 1987), the gall bladder contracts (Pavlov, 1910; Furukawa and Okada, 1991; Scott and Tan, 1993) and pancreatic blood flow increases (Inoue *et al.*, 1993). Vagal stimulation of the pancreas leads to the release of insulin (Pavlov, 1910; Hommel *et al.*, 1972; Bergman and Miller, 1973; Fischer *et al.*, 1976) as well as to the release of pancreatic fluid (Pavlov, 1910; Anrep, 1916; Harper and Vass, 1941; Thomas and Crider, 1947; Eisenberg and Orahood, 1971; Bergman and Miller, 1973; Kaminski *et al.*, 1975), although the release of pancreatic fluid may also be indirectly mediated by the vagal release of antral gastrin (Preshaw and Grossman, 1965; Preshaw *et al.*, 1966a; Rosenberg *et al.*, 1976). These anticipatory responses which are facilitated by the autonomic nervous system are also subjected to inhibitory influences which may result from distaste or other unpleasant sensations associated with feeding (Solomon, 1987).

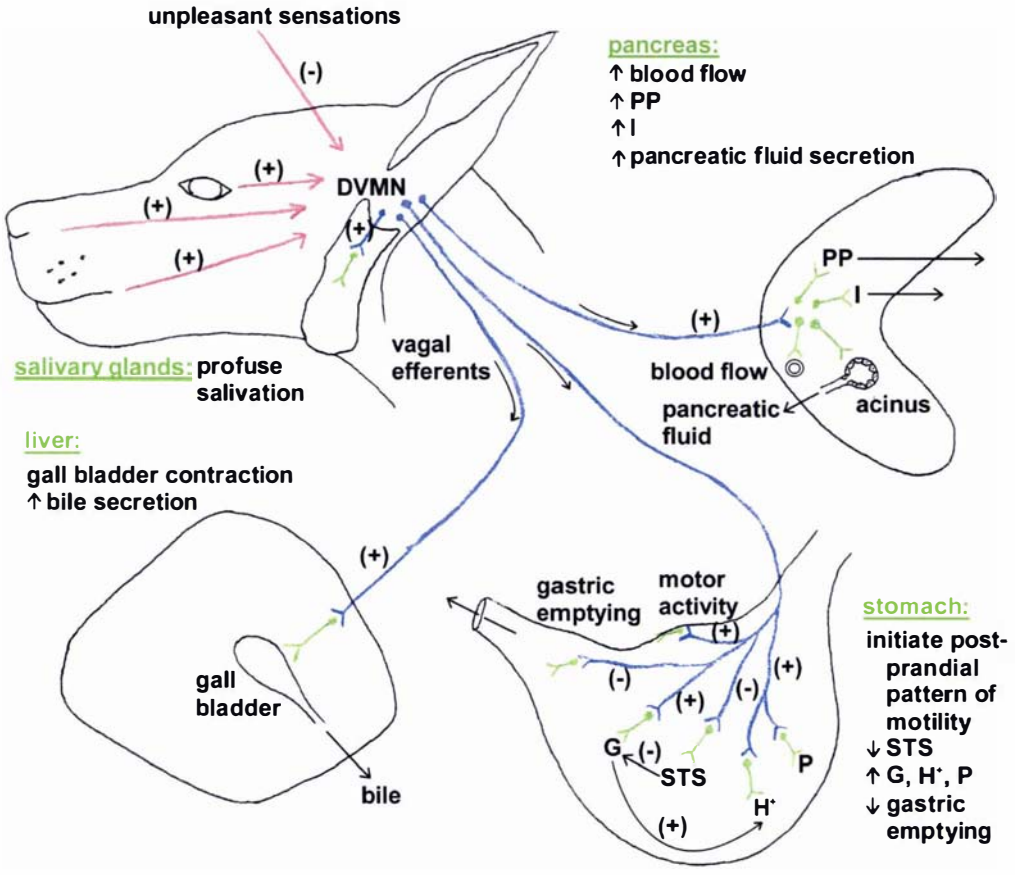

Figure 2. Effects of cephalic phase stimulation on the salivary glands, the stomach, liver and exocrine pancreas. Cephalic phase stimulation results from the sight, smell, taste, thought or swallowing of an appetizing meal. Visual, olfactory and gustatory impulses travelling within their respective cranial nerves to the brain stem, elicit strong efferent vagal nerve activity to the stomach and ancillary organs of digestion. Parasympathetic stimulation leads to profuse salivation, inhibits the release of somatostatin (STS) which normally inhibits gastrin release, increases the rate of secretion of gastrin (G), gastric acid (H^+) and pepsin (P) by the stomach, stimulates the release of bile into the duodenum, increases pancreatic polypeptide (PP) release and pancreatic blood flow, and increases the release of insulin (I) as well as exocrine pancreatic fluid by the pancreas. In the stomach, vagal stimulation also initiates the postprandial pattern of motility and decreases gastric emptying. These activities can also be subjected to inhibitory influences which may result from distaste or other unpleasant sensations associated with feeding. Afferent fibres carrying visual, olfactory and gustatory impulses to the brain stem are shown in red; vagal pre-ganglionic efferent fibres are shown in blue; post-ganglionic fibres are shown in green; (DVMN) dorsal vagal motor nucleus; (+) stimulates; (-) inhibits

Overall, the net early exocrine pancreatic response to both the anticipatory phase as well as the presence of food in the mouth is the production of pancreatic fluid which is low in volume and high in protein (Preshaw *et al.*, 1966a).

'THE GASTRIC PHASE'

The gastric phase of pancreatic secretion commences when food first enters the stomach (Figure 3). Mechanical stimuli (distention) activate both long (vago-vagal) and short (local) intramural reflex arcs (Grossman, 1967) which initiate contractile activity and secretion in the stomach and at all levels of the small bowel. Parasympathetic stimulation facilitates gall bladder emptying (Admirand and Way, 1973; Scott and Tan, 1993) as well as the secretion of gastric acid. Gastric acid secretion is initiated both directly, via stimulation of the oxyntic glands, and indirectly, via the release of antral gastrin (Grossman, 1967). These effects are potentiated by the local release of histamine (Cooke, 1969). Preliminary digestion of protein is promoted by the proteolytic actions of pepsin which has now formed in the acidic environment of the stomach from its inactive precursor, pepsinogen (Schwann, 1836; Langley, 1882). The catalysis of this reaction is further accelerated by the presence of pepsin itself (Herriott, 1938). The resulting smaller peptides and amino acids, via a direct stimulatory effect on antral G cells (DelValle and Yamada, 1990), maintain gastric acid secretion for as long as intact protein is present within the lumen. Digestion of dietary fat also begins in the stomach, where both lingual and gastric lipases initiate hydrolysis of medium- and long-chain triglycerides (Hamosh, 1990).

Irregular motor activity resembling phase II of the interdigestive cycle, having already been initiated during the cephalic phase of digestion, continues, and is now evident at all levels of the stomach and small bowel (Code and Marlett, 1975). In the distal stomach, the intrinsic underlying myogenic slow wave activity, which has to this point operated independently of external neuroendocrine influences, becomes superimposed with spike potentials resulting from food distention, nervous impulses and gastrointestinal hormones (Christensen, 1971; Cooke and Christensen, 1973; Anonymous1973; Cooke, 1975). These spike potentials result in muscle contractions which facilitate mixing of the gastric juice, grinding of gastric solids and propulsion of the contents through the antrum. High antral

pressures generated against a closing pylorus force liquids into the duodenum and propel solid material back into the body of the stomach for further digestion and reduction in particle size (Code and Carlson, 1968; Cooke, 1975). Gastrin release and subsequent acid production may wane as the pH of the luminal contents falls, only to switch on again as the pH rises. The repetitive nature of these motor and secretory events regulates gastric emptying by retaining food until particle size is sufficiently reduced to allow chyme to enter the small intestine.

Simultaneously with the onset of gastric acid and biliary secretion is the secretion of a protein-rich fluid by the pancreas (Blair *et al.*, 1966), the composition of which may vary if chyme has by this time entered the small intestine. Distention of the stomach stimulates the pancreas directly via excitation of long cholinergic vago-vagal gastropancreatic reflexes (White *et al.*, 1960; Blair *et al.*, 1966; Debas and Yamagishi, 1978). Concurrent, ongoing release of the pancreatic inhibitory hormone, PP, in response to vagal stimulation, gastrin, or its releasing peptide (Rose, 1984), further modifies the secretion by *limiting* pancreatic output (Taylor *et al.*, 1979; Beglinger *et al.*, 1984). Although it has been hypothesized that *gastrin* release from the antral mucosa mediates the pancreatic response during the cephalic and gastric phases of digestion (for a review of this topic and a complete list of references, see Solomon, 1987) the physiological significance of this reflex has since been challenged (Kohler *et al.*, 1987; Konturek *et al.*, 1990). The relative contributions of a direct vagally-induced control mechanism (gastropancreatic reflex) versus an indirect vagally-mediated release of antral gastrin are unknown, however, the preponderance of available evidence suggests that vagal cholinergic mechanisms play the dominant role in the gastric phase of pancreatic secretion.

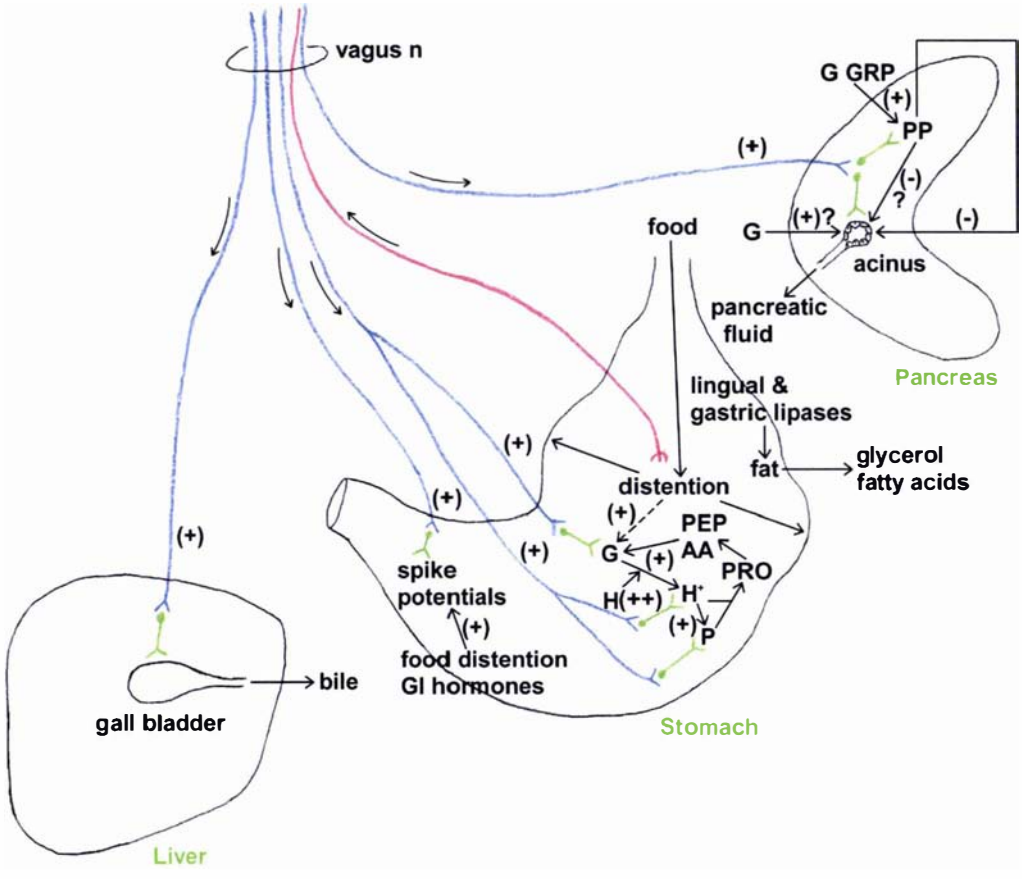

Figure 3. Effects of gastric phase stimulation on the stomach, liver and exocrine pancreas. The gastric phase of exocrine pancreatic secretion commences when food first enters the stomach. Afferent impulses during this phase originate in the stomach and travel via the vagus nerve. The primary stimulus to gastric phase biliary and exocrine pancreatic secretion is distention of the stomach by food. Gastric distention initiates both long (vago-vagal) and short (local intramural)(hashed line) reflexes which lead ultimately to the release of acid from the stomach, bile from the liver and pancreatic juice from the pancreas. Small peptides and amino acids resulting from acid peptic digestion in the stomach act directly on the antral G cells to release more gastrin, and in this manner, maintain gastric acid secretion for as long as intact protein is present within the lumen. Similarly, fat digestion is also initiated in the stomach by the action of lingual and gastric lipases on medium- and long-chain triglycerides. The combination of food distention, circulating gastrointestinal hormones and increased efferent vagal nerve activity lead to increased spiking activity in the stomach and small bowel. A single vagal afferent fibre is shown in red; vagal pre-ganglionic efferents are shown in blue; post-ganglionic fibres are shown in green; (AA) amino acids; (G) gastrin; (GRP) gastrin-releasing peptide; (H) histamine; (H^+) gastric acid; (P) pepsin; (Pep) peptides; (PP) pancreatic polypeptide; (Pro) protein; (+) stimulates; (++) potentiates; (-) inhibits

'THE INTESTINAL PHASE'

The small intestine receives chyme from the stomach. Through coordinated peristaltic waves of contraction, the small intestine begins to propel the contents aborally. The pancreas, as a result of prior and ongoing cephalic and gastric phase stimulation, has been primed by an increased blood flow and enhanced secretion of enzymes. The intestinal phase will now play quantitatively the most important role in the digestive process by further modifying the exocrine pancreatic secretion. Gastric acid as well as the partially digested products of fat and protein which are dissolved or suspended within it, stimulate a variety of endocrine cell types and sensory nerve endings which reside within the mucosa and which are distributed throughout the length of the small bowel. Nervous impulses initiated by the presence of chyme, and hormones released as a result of such stimuli, reflect moment-to-moment fluxes in the luminal contents and determine not only the magnitude of the exocrine pancreatic response, but also the composition of the secreted pancreatic fluid.

Influence of hormones and peptides on exocrine pancreatic secretion

Entry of chyme into the proximal small intestine results in the release of a variety of hormones from specialized endocrine cells within the mucosa of the small bowel (Figure 4). Hormonal mechanisms may be the predominant mediators of the pancreatic response to *high* loads of intestinal stimulants (Niebel *et al.*, 1988; Singer *et al.*, 1989). Endogenous acid potently stimulates the release of secretin (Domschke *et al.*, 1977), as does exposure of the lumen to digestion products of fat or long-chain fatty acids (Watanabe *et al.*, 1986; Faichney *et al.*, 1981). The net effect of such release is an increase in the secretion of bicarbonate and water by the duct cells of the pancreas (Henriksen, 1968; Chey *et al.*, 1979). In the liver, secretin stimulates the secretion of inorganic ions into the bile ductules (Forker, 1977). Secretin also inhibits gastric acid secretion (Greenlee *et al.*, 1957; Itoh *et al.*, 1975), enhances pepsinogen secretion from peptic cells (Magee and Nakajima, 1968; Nakajima *et al.*, 1969; Stening *et al.*, 1969), increases the secretion of enterokinase in the duodenum (Moss *et al.*, 1979) and contracts the pyloric sphincter (Geller and Petrenko, 1980).

Digestion products of both fat and protein (Himeno *et al.*, 1983; Hopman *et al.*, 1985; Backus *et al.*, 1995), particularly the essential amino acids tryptophan and phenylalanine (Konturek *et al.*, 1973; Meyer *et al.*, 1976), stimulate the release of CCK which acts to modify the composition of pancreatic fluid by increasing its enzyme content (Harper and Raper, 1943). Fat-stimulated CCK release is dependent upon the presence of pancreatic enzymes within the lumen (Watanabe *et al.*, 1988), a process which was initiated earlier during gastric phase secretion. At the liver, CCK potently stimulates gall bladder contraction (Ivy and Oldberg, 1928) leading to the secretion of bile, and in this manner supplements the fat digesting and acid neutralizing functions of the pancreas. Cholecystokinin also potentiates the action of secretin on pancreatic bicarbonate secretion (You *et al.*, 1983; Chey *et al.*, 1984), promotes satiety (Gibbs *et al.*, 1973), inhibits gastric emptying (Debas *et al.*, 1975b), exerts a trophic effect on the pancreas (Rothman and Wells, 1967), increases the secretion of enterokinase in the duodenum (Moss *et al.*, 1979), stimulates insulin secretion (Muller *et al.*, 1983) and may enhance the motility of the small intestine (Nakayama and Fukuda, 1966; Dahlgren, 1967; Hedner *et al.*, 1967). Cholecystokinin, in addition, augments contraction of the pyloric sphincter (Grossman, 1974) thereby acting to prevent duodenogastric reflux.

Glucose absorption from the small intestine is followed by a sustained release of insulin from the endocrine pancreas, a response which contributes significantly to stimulation of the exocrine pancreas in the dog and rat at least (Lee *et al.*, 1990; Lee *et al.*, 1995). Circulating insulin potentiates secretin- and CCK-induced pancreatic amylase secretion (Kanno and Saito, 1976), and studies in the rat have demonstrated profound inhibition of these responses following immunoneutralization with specific rabbit anti-insulin serum (Lee *et al.*, 1990). These effects, which have been attributed to a local action of insulin on the pancreatic acini, may be mediated by the 'insulo-acinar' portal system, or possibly, a paracrine mechanism. Similar work has also been done in the dog (Lee *et al.*, 1995), and in that study, suppression of bicarbonate and protein secretion by the anti-insulin serum also coincided with significant increases in the inhibitory hormones, somatostatin and PP, in portal venous effluent. Thus, insulin exerts a stimulatory effect on the exocrine pancreas of the dog through local mechanisms, and the local release of both somatostatin and PP

appears to mediate the suppressive effects of anti-insulin serum on pancreatic secretion.

With the progressive movement of digesta through the small intestine, neurotensin is secreted in response to the presence of fat reaching the ileum (Mashford *et al.*, 1978; Rosell and Rokaeus, 1979; Walker *et al.*, 1985). Neurotensin immunoreactivity, in addition to its presence in the ileum, is also found throughout the acinar cells, in nerve fibres of the myenteric plexus and in muscle layers, within fibres and cell bodies of intrapancreatic ganglia, as well as in nerve processes which project to the pancreatic blood vessels (Schultzberg *et al.*, 1980; Reinecke, 1985). These findings suggest that neurotensin is capable of acting both as a hormone and a neurotransmitter. Circulating neurotensin, in addition to its wide variety of effects on other organs of digestion (Blackburn *et al.*, 1980; Rosell *et al.*, 1980; Calam *et al.*, 1983; Thor and Rosell, 1986; Wood *et al.*, 1988; Siegle and Ehrlein, 1989), *powerfully* stimulates the release of bicarbonate and enzymes from the pancreas (Baca *et al.*, 1982; Khalil *et al.*, 1986; Gullo, 1987) and potentiates the actions of secretin and CCK on the pancreatic secretion of bicarbonate, while having additive effects on the secretion of protein (Baca *et al.*, 1983; Sakamoto *et al.*, 1984).

Concurrent with the release of the stimulatory hormones is the release of *inhibitory* factors which, by virtue of their simultaneous appearances in peripheral blood, would appear to play counter-regulatory roles in the digestive process, thereby preventing excessive or inappropriate 'pro-digestive' activity. Protein and fat in the gut strongly stimulate release of PP (Wilson *et al.*, 1978). Pancreatic polypeptide is also released by secretin, CCK, gastrin and gastrin releasing-peptide (Taylor, 1989). This peptide (PP), unlike other regulatory hormones which originate in the mucosa of the small intestine, originates primarily from the pancreas (within islets and within clusters among exocrine tissue, Larsson *et al.*, 1976), and almost exclusively from that part of the pancreas immediately adjacent to the duodenum (Larsson *et al.*, 1976; Gingerich *et al.*, 1978; Gersell *et al.*, 1979). In contrast to the rapid but short-lived release which occurs during the cephalic phase of digestion, release of PP during the intestinal phase is prolonged. Pancreatic polypeptide inhibits the secretory response of the exocrine pancreas to secretin and CCK

(Lin *et al.*, 1977; Greenberg *et al.*, 1978; Adrian *et al.*, 1979; Taylor *et al.*, 1979; Konturek *et al.*, 1982) and diminishes bile secretion from the liver (Greenberg *et al.*, 1978).

Meal ingestion results in the release of other less well characterized hormones and peptides which have also been shown to inhibit exocrine pancreatic function under certain circumstances. Somatostatin, released from the brain, and from gastric, intestinal and pancreatic D cells as well as from neurons (Hokfelt *et al.*, 1975), inhibits gastrin release from the stomach (Bloom *et al.*, 1974), insulin and glucagon release from the endocrine pancreas (Alberti *et al.*, 1973; Koerker *et al.*, 1974), as well as secretin release and exocrine pancreatic secretion in response to acid in the duodenum (Boden *et al.*, 1975a). Somatostatin also inhibits secretin- and CCK-stimulated pancreatic secretion (Konturek *et al.*, 1976b; Susini *et al.*, 1978; Arnold and Lankisch, 1980). The stimulatory effects of neurotensin on the exocrine pancreas are counter-balanced by the release of the inhibitory hormone, peptide YY (PYY), from endocrine cells in the distal ileum and colon (Lundberg *et al.*, 1982; Taylor, 1985; Greeley, Jr. *et al.*, 1987). Meal ingestion (Taylor, 1985; Greeley *et al.*, 1989), especially intraluminal fat (Greeley, Jr. *et al.*, 1989), is a potent stimulus for its release. In dogs, PYY reduces pancreatic blood flow (Inoue *et al.*, 1988) and inhibits both gastric (Pappas *et al.*, 1985a; Guo *et al.*, 1987) as well as pancreatic secretion (Pappas *et al.*, 1985a; Pappas *et al.*, 1985b; Lluis *et al.*, 1988). Meal ingestion also releases pancreatic glucagon, which inhibits the exocrine pancreatic secretion by reducing volume flow and secretion of bicarbonate and enzymes (Dyck *et al.*, 1969; Nakajima and Magee, 1970; Konturek *et al.*, 1974b; Singer *et al.*, 1978). Pancreastatin, a pancreatic peptide secreted by endocrine cells in the islets of Langerhans, inhibits CCK-stimulated pancreatic exocrine secretion in rats (Funakoshi *et al.*, 1989). In dogs, thyrotropin-releasing hormone (TRH) inhibits secretin-stimulated pancreatic protein and bicarbonate secretion (Yamagishi *et al.*, 1985). It is not clear however, whether TRH acts primarily as a hormone, a paracrine mediator or a neurotransmitter.

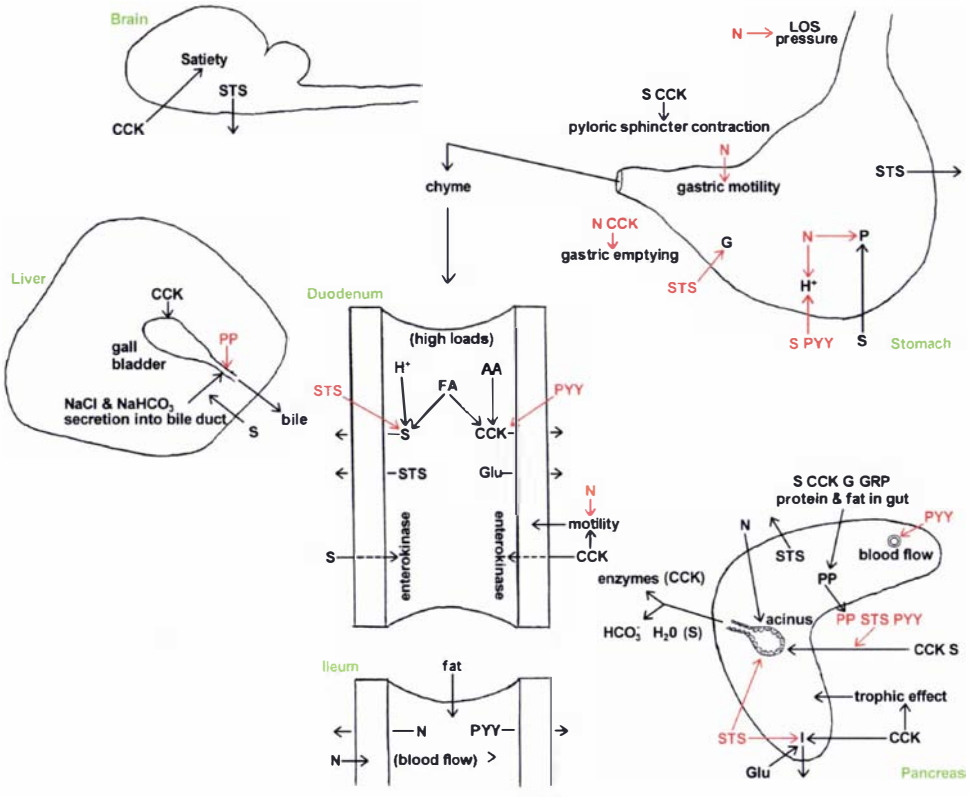

Figure 4. Major effects of intestinal phase stimulation of hormone release on the brain, the stomach, liver and exocrine pancreas. Entry of chyme into the lumen of the proximal duodenum initiates the intestinal phase of pancreatic secretion. High loads of nutrients entering the duodenum stimulate the release of secretin (S) and cholecystokinin (CCK) from the intestinal mucosa. These hormones, as well as neurotensin (N) which is released from the ileum in response to the presence of fat, have a mainly stimulatory effect on the exocrine pancreatic secretion, as well as a wide variety of effects on gastrointestinal motility, bile, and gastric acid secretion. In the brain, CCK promotes satiety. The primary *inhibitors* of pancreatic secretion are *also* released in response to nutrients entering the small intestine. They include pancreatic polypeptide (PP) from the pancreas, somatostatin (STS) from the upper small intestine, and peptide YY (PYY) from the ileum. Some of the effects of insulin (I), STS and PP on the exocrine pancreas may be mediated locally via the insulo-acinar portal system or via a local paracrine mechanism. Hormones exerting a *stimulatory* effect are shown in black; hormones exerting an *inhibitory* effect are shown in red; (AA) amino acids; (FA) fatty acids; (G) gastrin; (GRP) gastrin-releasing peptide; (Glu) glucose; (H^+) gastric acid; (LOS) lower oesophageal sphincter; (P) pepsin

Influence of nerves on exocrine pancreatic secretion

Enteropancreatic reflexes. Concurrent with the release of hormones and other peptides from the small intestinal mucosa, nervous impulses are initiated. Enteropancreatic reflexes are, from a quantitative standpoint, important mediators of the intestinal phase of pancreatic secretion. In the dog, a rich nerve plexus courses via the lesser curvature of the stomach, then the pylorus, and then follows the course of the cranial pancreaticoduodenal vessels to innervate the duodenum and pancreas primarily in the region where the common bile duct and main pancreatic duct enter the intestinal wall (Gayet and Guillaumie M., 1930a; Gayet and Guillaumie M., 1930b; Tiscornia *et al.*, 1976b). This nerve plexus is believed to facilitate a large component of the neurally-mediated 'enteropancreatic' reflex pathway.

Low loads of protein and fat digestion products in the duodenum initiate the early pancreatic *enzyme* response via long, vago-vagal, cholinergic reflexes (Singer *et al.*, 1980a; Singer, 1983; Singer *et al.*, 1989) and it has been estimated that these pathways account for approximately half of the early enzyme response (Solomon and Grossman, 1979; Singer *et al.*, 1980b; Fried *et al.*, 1985) to intestinal stimulation (see Figure 5 for an illustration of this and the following concepts). Although the primary stimulus to pancreatic *fluid and bicarbonate* secretion evoked by hydrochloric acid (HCl) appears to be humoral rather than neural (Singer, 1983), long, vagal cholinergic reflex mechanisms have also been shown to participate in the response to low loads of acid entering the duodenum (Singer *et al.*, 1981; Singer *et al.*, 1985; Niebel *et al.*, 1988). The role of *extravagal* pathways as mediators of enteropancreatic reflexes has been less well studied. The splanchnic nerves are generally inhibitory to exocrine pancreatic secretion (Thomas, 1967), although such inhibitory effects have been difficult to consistently reproduce in dogs (Hayama *et al.*, 1963; Singer *et al.*, 1986; Niebel *et al.*, 1988; Singer *et al.*, 1989). Recently, Kirchgessner and Gershon (1990) demonstrated in rats the presence of thick nerve bundles traversing the duodenum and pancreas, merging in the duodenum with the myenteric plexus, and in the pancreas with nerves entering and exiting intrinsic ganglia.

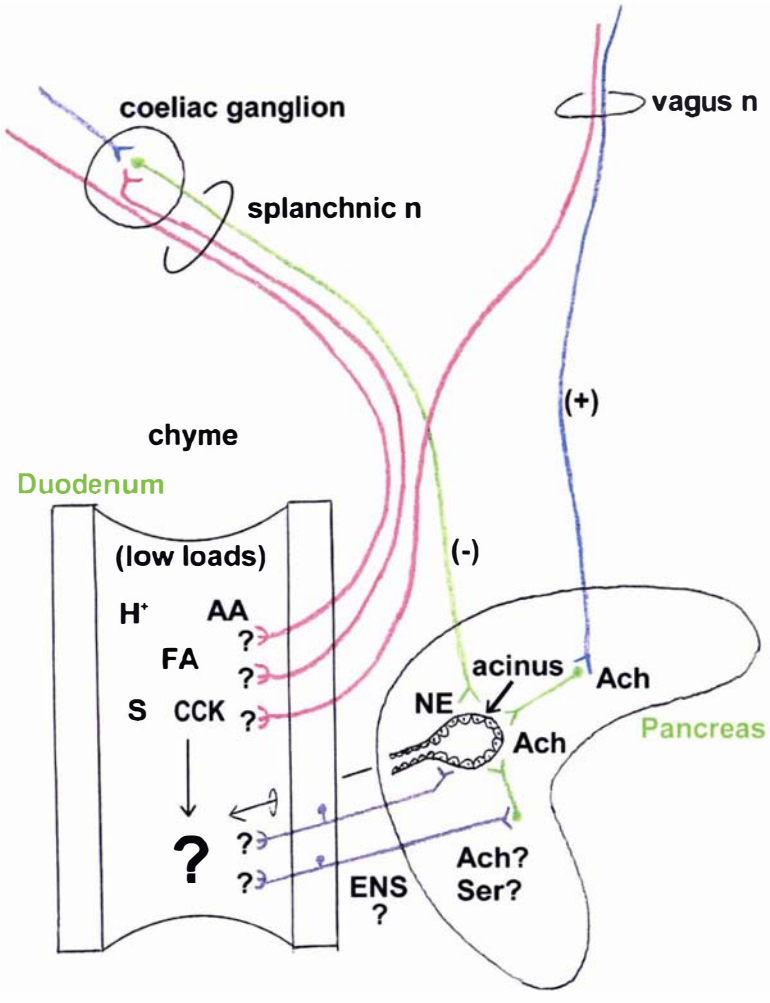

Figure 5. Effects of intestinal phase stimulation of nerves on the exocrine pancreas: enteropancreatic reflexes. Long, vago-vagal enteropancreatic reflexes are the main mediators of the pancreatic enzyme response to low loads of protein/fat digestion products and HCl, and also contribute to pancreatic fluid and bicarbonate secretion in response to low loads of acid entering the duodenum. Although the precise role of the splanchnic nerves in the intestinal phase of pancreatic secretion has been difficult to consistently show, the sympathetic nervous system is generally regarded as inhibitory to exocrine pancreatic function. Recent demonstration in rats of nerve bundles traversing the duodenum and pancreas, merging in the duodenum with the myenteric plexus, and in the pancreas with nerves entering and exiting intrinsic ganglia, suggest that the enteric nervous system (ENS) may also contribute to reflex regulation of the exocrine pancreas. Visceral afferent fibres are shown in red; autonomic preganglionic fibres are shown in light blue; autonomic post-ganglionic fibres are shown in green; enteric nerves are shown in dark blue; (ACH) acetylcholine; (AA) amino acids; (CCK) cholecystokinin; (FA) fatty acids; (H^+) acid; (NE) norepinephrine; (S) secretin; (Ser) serotonin; (+) stimulates; (-) inhibits; (?) contribution unknown

Although neural transmission of signals from gut to pancreas was shown in their studies to be primarily cholinergic, the presence of substantial numbers of serotonergic axons suggested that a more complicated regulatory function also exists. These observations support the possibility that the enteric nervous system may also contribute to reflex regulation of the exocrine pancreas, a belief which was initially held by Thomas (1948). Overall, it would appear from the above studies, that in addition to the effects of circulating hormones, the exocrine pancreatic secretion during intestinal phase stimulation results from continuous and changing interactions between cholinergic, adrenergic and noncholinergic-nonadrenergic (peptidergic) influences.

Neurohormonal interactions. The interactions of nervous and hormonal mechanisms which participate in the control of exocrine pancreatic function are complex. Many peptide hormones have been shown to act via vagal cholinergic mechanisms to modulate pancreatic secretion, thus rendering the separation of neural versus hormonal control of pancreatic secretion somewhat artificial. Vagal cholinergic activity for example, is the most powerful stimulus and appears to be the final common pathway for release of PP, irrespective of the initiating factor. The actions of CCK and other peptides on the release of PP appear to act either through a cholinergic mechanism or against a background of permissive cholinergic tone (Schwartz, 1980). In contrast, the release of secretin and CCK from the small intestinal mucosa is under neither cholinergic nor splanchnic control (Niebel *et al.*, 1988; Singer *et al.*, 1989). In fact, peptides such as CCK, bombesin and neurotensin, in addition to their roles as gut hormones, also serve as neurotransmitters or neuromodulators in both the peripheral and central nervous systems (Hökfelt *et al.*, 1980). These peptides may activate receptors on nerve cells which eventually stimulate the PP cell directly (Schwartz, 1980). Similarly, the presence of CCK receptors on vagal afferent neurons in the rat (Zarbin *et al.*, 1981) suggest that CCK (released from nerves or endocrine cells) may be capable of activating the vagus nerve in this manner, and indeed, endogenously released CCK at 'physiological' levels has been shown to mediate pancreatic enzyme secretion by stimulating vagal afferent pathways that originate in the gastroduodenal mucosa (Figure 6) (Li and Owyang, 1994).

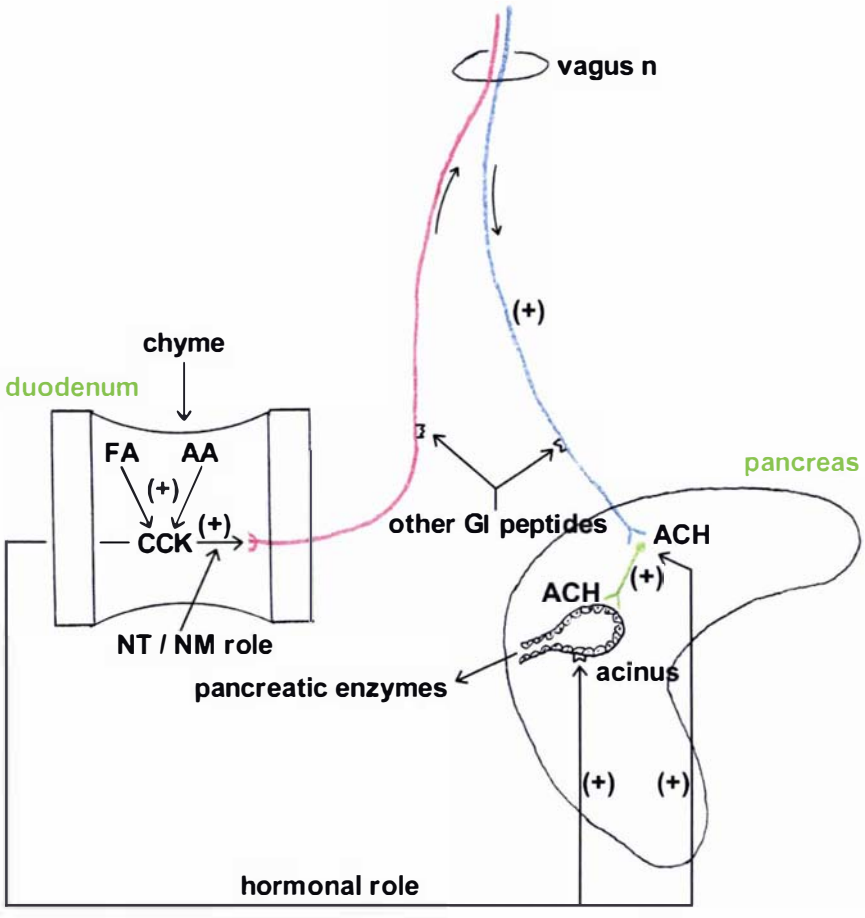

Figure 6. Examples of neurohormonal interactions during intestinal phase stimulation of the exocrine pancreas. A number of gastrointestinal peptides have been shown to act via vagal cholinergic pathways to influence the exocrine pancreatic secretion. Cholecystokinin is an example of a peptide which serves not only as a classical endocrine hormone, but also as a neurotransmitter/neuromodulator in both the peripheral and central nervous systems. (AA) amino acids; (CCK) cholecystokinin; (FA) fatty acids; (NT / NM) neurotransmitter/neuromodulator; (+) stimulates; (-) inhibits

Higher (presumably 'supra-physiological') levels of CCK appear to act on intrapancreatic neurons and, to a lesser extent, on pancreatic acini directly (Li and Owyang, 1993). Overall, it would appear that multiple neural mechanisms which also involve humoral/hormonal agents operate simultaneously to control exocrine pancreatic function. These observations not only revolutionize our current understanding of the mechanisms of action of hormones on the exocrine pancreatic secretion, but also illustrate the complex hormonal-neurotransmitter-neuromodulator functions which characterize many of the gastrointestinal peptides.

Peptidergic nerves that influence exocrine pancreatic secretion. Activation of nerve fibres in the pancreas releases the classical neurotransmitter, acetylcholine, along with a variety of neuropeptides which have also been shown to influence pancreatic secretion. Pancreatic neurons have been found to contain not only CCK (Larsson, 1979) and neurotensin (Reinecke, 1985), but also VIP (Larsson, 1979), gastrin-releasing peptide (GRP) (Price *et al.*, 1984), galanin (Dunning *et al.*, 1986), Sub P (Larsson, 1979), calcitonin gene-related peptide (CGRP) (Seifert *et al.*, 1985), enkephalins (Larsson, 1979) and neuropeptide Y (Lundberg *et al.*, 1983). Of these, documentation exists for the release of VIP (Holst *et al.*, 1984), GRP (Knuhtsen *et al.*, 1985) and galanin (Dunning *et al.*, 1990). Vasoactive intestinal peptide-containing neuronal cell bodies are scattered throughout the exocrine pancreas and a moderate number of VIP-containing nerves are found in the stroma both around and within the vascular wall (Larsson *et al.*, 1978; Bishop *et al.*, 1980). A dense network of VIP nerve fibres also has been demonstrated in the pancreatic duct wall (Bishop *et al.*, 1980). In dogs (Konturek *et al.*, 1976a; Makhlouf *et al.*, 1978) and in man (Domschke *et al.*, 1977), VIP is a partial agonist of pancreatic exocrine secretion. Gastrin-releasing peptide, originally isolated from porcine intestinal and non-antral gastric tissue (McDonald *et al.*, 1979), was shown to have striking similarities to bombesin at its COOH-terminal portion. In man, the highest concentrations of bombesin-like immunoreactivity is found in nerves of the stomach and pancreas (Price *et al.*, 1984). When given exogenously to dogs, GRP releases CCK but not secretin (Miyata *et al.*, 1978), leading to the secretion of fluid and protein from the pancreas.

Neuropeptides released upon activation of nerve fibres in the pancreas also *inhibit* exocrine pancreatic function. Calcitonin gene-related peptide is widely distributed throughout the central, peripheral and enteric nervous systems (Rosenfeld *et al.*, 1983; Springall *et al.*, 1983; Mulderry *et al.*, 1985; Feher *et al.*, 1986) and has been found to suppress gastric acid secretion (Tache *et al.*, 1984) as well as pancreatic protein and bicarbonate secretion (Nealon *et al.*, 1986) when given exogenously. The effect of CGRP on the exocrine pancreas is indirect and appears to be mediated by the release of somatostatin (Helton *et al.*, 1989). The opioid peptides, enkephalin and morphine, inhibit the pancreatic responses to both exogenous and endogenous stimulants (Konturek *et al.*, 1978), and met-enkephalin significantly suppresses the release of secretin stimulated by duodenal acidification or by ingestion of a mixed meal (Chey *et al.*, 1980). As in the case of CGRP, endogenous opioids are widely distributed in the gastrointestinal tract within endocrine cells (Polak *et al.*, 1977) as well as within neurons of the myenteric plexus (Polak *et al.*, 1977) and pancreas (Polak *et al.*, 1977; Bruni *et al.*, 1979).

Feedback control of pancreatic function

The pancreatic response to the presence of food in the proximal small intestine of the dog plays an important role in feedback regulation of the pancreas (Figure 7). The release of CCK and secretin are under the control of specific 'releasing peptides' which may be of intestinal or pancreatic origin. In addition, 'monitor peptide', a peptide of pancreatic origin, appears to function as a specific positive enhancement for CCK release (Herzig, 1998). Food (particularly amino acids and fats), in the presence of pancreatic juice, competes with 'releasing peptides' as substrates for pancreatic proteases. Having avoided proteolytic inactivation by the pancreatic proteases in this manner, the secreted releasing peptides are now freely available to signal gut endocrine cells to release hormones which lead ultimately to an increase in exocrine pancreatic secretion.

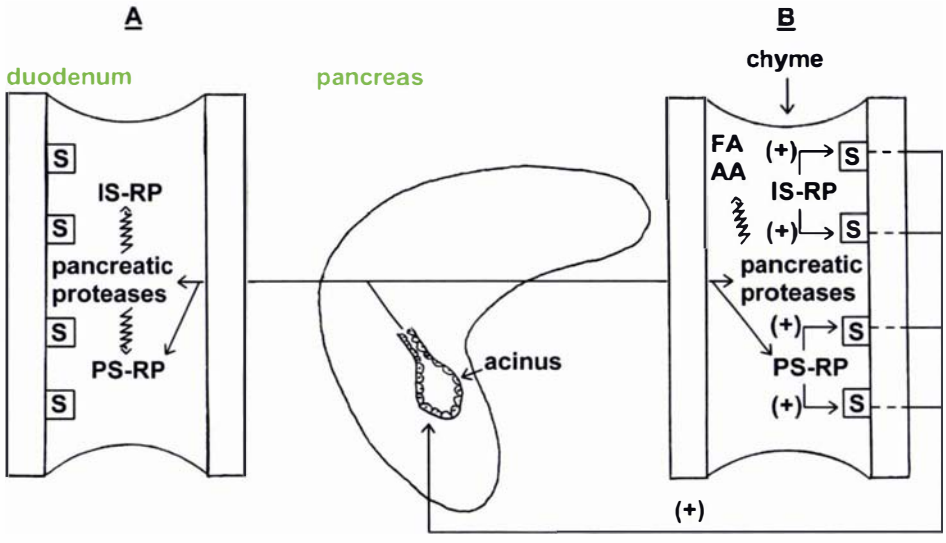

Figure 7. Negative feedback regulation of the exocrine pancreatic secretion by pancreatic proteases in the dog. A. During the fasted state, secretin release is inhibited by the proteolytic actions of pancreatic proteases on secretin-releasing peptides; B. During the fed state, intraduodenal nutrients (particularly fats and amino acids) compete with secretin-releasing peptides as substrates for pancreatic proteases. Having avoided proteolytic inactivation in this manner, the luminally-secreted releasing-peptides are now freely available to signal the secretin-producing cells to release secretin. A similar mechanism may operate to release neurotensin from the ileum. (AA) amino acids; (FA) fatty acids; (S) secretin-producing cell; (IS-RP) secretin-releasing peptide of intestinal origin; (PS-RP) secretin-releasing peptide of pancreatic origin; (+) stimulates; zig-zag lines denote proteolytic breakdown.

In the dog, secretin and neurotensin rather than CCK appear to mediate this feedback regulation. We know this because, although the postprandial pancreatic hypersecretion induced by diversion of pancreatic juice is accompanied by significantly higher plasma concentrations of CCK *as well as* secretin (Shiratori *et al.*, 1989; Imamura *et al.*, 1993) and neurotensin (Nustede *et al.*, 1990), the suppression of augmented pancreatic secretion by re-instillation of pancreatic juice coincides with a significant suppression of plasma secretin and neurotensin alone. Cholecystokinin levels are unaffected. Recent identification and characterization of a trypsin-sensitive 'secretin-releasing peptide' in canine pancreatic juice suggests that a factor in pancreatic fluid also exists for the release of secretin which has a positive feedback effect on exocrine pancreatic secretion (Song *et al.*, 1999; Li *et al.*, 2000). Whether the release of neurotensin is also mediated by a releasing peptide remains to be determined. The involvement of neural mechanisms in the release and/or actions of secretin and its releasing peptide is also presently unclear, however it has been suggested that cholinergic mechanisms are involved in the dog (Schafmayer *et al.*, 1993) and that vagal afferent pathways and β-adrenoceptors are involved in the rat (Li *et al.*, 1995). Overall, these observations indicate that pancreatic juice in the upper gut lumen controls the release of at least two gut hormones during the intestinal phase of pancreatic secretion in the dog, and that neural mechanisms are probably also involved.

As digestion proceeds, substrate availability for the pancreatic proteases slowly wanes, shifting the proteolytic activities of the pancreas back towards the releasing factors. The net result is a gradual decline in hormone release and consequent suppression (negative feedback control) of pancreatic secretion.

The pancreatic response to food in the upper small intestine of the dog is also largely determined by the output of bile by the liver (Figure 8). Fat and amino acids in the presence of bile, exert a predominantly inhibitory effect on pancreatic protein secretion (Thomas and Crider, 1943) by inhibiting the release of both CCK (Gomez *et al.*, 1986; Gomez *et al.*, 1988; Nustede *et al.*, 1993) and neurotensin (Gomez *et al.*, 1986; Nustede *et al.*, 1993) from the lumen of the small bowel.

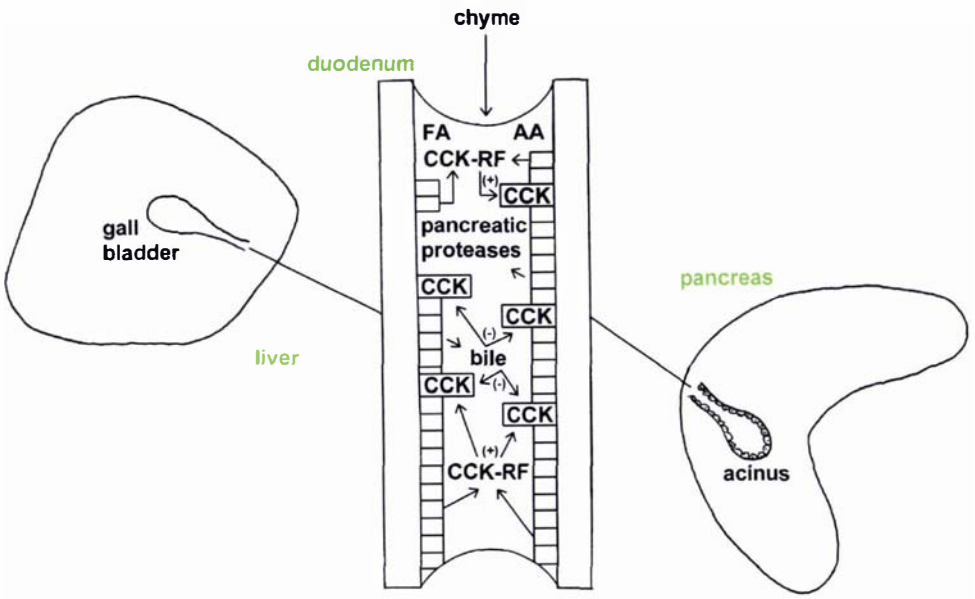

Figure 8. Proposed mechanism by which the exocrine pancreatic response to food in the dog is in part determined by the output of bile by the liver. Fat and amino acids in the presence of bile, exert a predominantly inhibitory effect on pancreatic protein secretion by inhibiting the release of CCK from the lumen of the small bowel. This negative feedback relationship between the postprandial intestinal bile acid content and the secretion of CCK and pancreatic enzymes is independent of any interaction between pancreatic proteases and bile acids. Despite the presence of freely available, tonically-secreted (intestinal) CCK-releasing factors (and potentially CCK releasing-factors of pancreatic origin), CCK-producing cells are under constant suppression by intraduodenal bile acids. A similar mechanism may operate to inhibit the release of neurotensin from the ileum. (AA) amino acids; (CCK) CCK-producing cell; (CCK-RF) CCK-releasing factor; (FA) fatty acids; (+) stimulates; (-) inhibits

In a manner similar to that by which pancreatic juice diversion augments stimulated hormone and pancreatic secretion, total diversion of bile in the dog significantly increases amino acid-stimulated release of both CCK and pancreatic protein secretion. Replacement of the bile salt pool completely reverses these enhanced effects. This negative feedback relationship between the postprandial intestinal bile acid content and the secretion of regulatory peptides and pancreatic enzymes is independent of any interaction between pancreatic proteases and bile acids (Nustede *et al.*, 1993). The mechanism(s) by which luminal bile salts inhibit the release of CCK is not known in dogs. In man however, it has been demonstrated that, despite the presence of (tonically-secreted) CCK-releasing factors in the small intestine, the CCK-producing cells are under constant suppression by intraduodenal bile acids, since removal of duodenal bile acids by inhibition of gall bladder contraction removes the suppression, leading to a dramatic increase in plasma CCK levels (Koop *et al.*, 1996). Whether a similar mechanism exists in the dog remains to be determined. Taken together, the results of the above studies provide strong support for a physiological role for pancreatic proteases and bile salts in the negative feedback regulation of pancreatic secretion in dogs.

Post-duodenal control of exocrine pancreatic function (distal intestinal phase of pancreatic secretion)

Passage of nutrients from the duodenum distally affects both upper gut function as well as the composition and net output of the exocrine pancreatic secretion. Whereas the duodenum and, to a large extent, the jejunum (Niebel *et al.*, 1991; Singer *et al.*, 1997) control the upper gut and exocrine pancreatic secretion mainly via *stimulatory* mechanisms, the ileum and colon generally induce *net inhibition* (the ileal and colonic "brakes") during both the interdigestive and postprandial periods. In this respect, the influence of post-duodenal sites of digestion may also be looked upon as a form of negative feedback control. The specific controlling mechanisms mediating the inhibitory effects of nutrients in the distal small bowel and colon are poorly understood. Introduction of a mixed isotonic nutrient solution into the ileum or colon of dogs delays phase III of the MMC in the duodenum, inhibits ileal motility and increases plasma concentrations of PYY and glucagon-like peptide-1 (GLP-1) (Wen J. *et al.*, 1995). Intra-ileal amino acids induce an "ileal brake" on the hormonally-stimulated pancreatic HCO_3^- and protein response

(Niebergall-Roth *et al.*, 2000), whereas perfusion of the ileum with *carbohydrates* delays gastric emptying (Shinagawa *et al.*, 1994; Tohno *et al.*, 1995), prolongs small intestinal transit time (Shinagawa *et al.*, 1994; Tohno *et al.*, 1995), increases bile acid delivery into the duodenum (Tohno *et al.*, 1995) and *augments* (relatively) pancreatic exocrine secretion (Shinagawa *et al.*, 1994; Tohno *et al.*, 1995; Sarr *et al.*, 1997) while increasing the plasma concentration of PYY (Tohno *et al.*, 1995). These opposing effects on the gastrointestinal tract and exocrine pancreas are presumably protective mechanisms which act to slow transit of intraluminal chyme in the upper gut and increase pancreatic secretion to augment intraluminal digestion (Sarr *et al.*, 1997). Extrinsic denervation of the jejuno-ileum abolishes all of the aforementioned carbohydrate-induced changes in upper gut and pancreatic function, as well as the increase in plasma PYY (Sarr *et al.*, 1997). It is unknown whether these effects occur through neural pathways or from the release of an unidentified regulatory hormone under the control of extrinsic neural innervation. Overall, a great deal of work needs to be done to define the specific pathways involved in the control of upper gut and pancreatic function in response to various concentrations of nutrients present at post-duodenal sites.

'THE DELAYED POSTPRANDIAL PHASE'

Eight to twelve hours following ingestion of the meal, a delayed, *second* phase of pancreatic secretion is observed in the dog (Itoh *et al.*, 1980). In contrast to the first peak of secretion which is rich in enzymes, the second peak of secretion is rich in bicarbonate, and its secretory volume is significantly greater than that achieved during the first peak (40 ml/hr versus 25 ml/hr). Little is known about the factors controlling this delayed hypersecretion, or its function. During this time, the pH of the intraduodenal contents drops to values approaching 4.5, however, in contrast to the immediate postprandial period, no increase in circulating secretin is observed (Huertas *et al.*, 1991). Similarly, no increases in plasma levels of VIP or gastrin have been found to correlate with this phase of postprandial pancreatic secretion (Huertas *et al.*, 1993b). The vagus nerves also do not play a role in the genesis of the late hypersecretory phase because truncal vagotomy only increases the *latency* of the *late* pancreatic response while totally suppressing the early pancreatic response (Huertas *et al.*, 1992). What is known, however, is that the mechanism(s) inducing the late hypersecretory phase involve the distal ileum because

distal ileum resection completely abolishes this pancreatic response (Huertas *et al.*, 1993a). By 16 hours after food ingestion, the exocrine pancreatic secretion has returned to the basal level of output (Itoh *et al.*, 1980) which characterizes the interdigestive period. Here it remains until ingestion of the next meal.

NEW PERSPECTIVES

Although significant advances have been made in our understanding of the neurohumoral/neurohormonal mechanisms involved in the control of exocrine pancreatic function, many questions still remain unanswered. The interplay between the nervous system and the gut endocrine system is complex and while it is clear that nervous stimuli as well as circulating hormones play a central role in regulating pancreatic responses, specific details of the mechanisms of their interactions are still largely unknown. Whereas it has been possible to identify the 'cause' of a pancreatic response, and an 'outcome' on the pancreas in response to a variety of manipulative procedures, neither the mechanisms underlying stimulation and release of these mediators, nor the precise pathways *linking* 'cause' and 'effect' in the intact animal are known. The remainder of this review focuses on the latter, the 'link' between cause and effect, and examines this issue from a new perspective. Two hypotheses are put forward: first, that the location of the pancreas relative to the small intestine has functional significance with respect to the linking mechanism(s), and second, that the linking mechanism(s) involves *direct* nervous and/or humoral communication between the small intestine and/or the hepatic portal system[1] and the pancreas. These ideas are examined separately.

It should be noted that, although the ideas presented below are primarily directed at control of the exocrine pancreas, such 'short-loop' regulation may also, or *only*, operate for pancreatic *endocrine* function.

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[1]Technically, the hepatic portal system is composed of both an extra-hepatic component (portal vein and it's tributaries) and an intra-hepatic component (branches of the portal vein in the liver). In this thesis, however, the term 'hepatic portal system' is used to refer only to the extra-hepatic system of veins.

FUNCTIONAL SIGNIFICANCE OF THE LOCATION OF THE PANCREAS

Fundamental to our understanding of the role of the exocrine pancreas in digestion is a thorough knowledge of not only the physiological mechanisms involved in its regulation, but also the embryological and anatomical relationships of the pancreas with the very organ to which it is physiologically linked – the small intestine. Embryologically, the pancreas of mammals, birds, reptiles and amphibians develops similarly from the primitive foregut (Slack, 1995), from two buds developing on the duodenum. The dorsal bud arises dorsally from the cranial duodenum while the ventral bud arises ventrally from the main stem of the hepatic diverticulum near its origin. As the stomach and duodenum rotate, the ventral pancreatic diverticulum and hepatopancreatic orifice become repositioned onto the dorsal surface of the duodenum where the two pancreatic diverticuli contact and ultimately fuse (Noden and deLahunta, 1985). Fusion in such a location explains the unique anatomical positioning of the mature pancreas immediately adjacent to the proximal descending limb of the duodenum.

The observations

Despite similarities in the embryology, it is interesting to note the variability that exists among species, not only in the *closeness* with which the pancreas approaches the duodenum, but also in the manner in which the pancreas becomes associated with the veins of the hepatic portal system in the adult. These anatomical relationships are described in more detail in Chapter 2, where specific references for each species can be found. In species such as ruminants and pigs for example, the pancreas either partially or completely encircles the portal vein, while in other species, i.e., man and dogs, the most striking feature of the anatomy is the intimate relationship of the pancreas with the duodenum. In all species, the body of the pancreas and descending duodenum are provided with a common blood supply, the cranial pancreaticoduodenal artery and vein. These vessels course deeply between the two organs. Close examination by the author of this area in the dog, presented in more detail in Chapter 3, also reveals a dense network of veins emanating from the duodenal wall, features which, subjectively, are not as prominent in adjacent segments of small intestine where pancreatic contact is lacking. The significance of these observations is presently unknown, however the anatomical and vascular features

described may provide important information regarding the links between cause and effect in the overall control of exocrine pancreatic function.

The hypothesis

Situated within the curve of the proximal small intestine and within the confines of the hepatic portal system, the pancreas receives signals, which by convention, arrive via 'long' circuits, either endocrine or nervous, or a combination of both. In such a position however, it would seem that the pancreas is also ideally situated for receiving information via local pathways, that is, information regarding perhaps the nutrient concentrations in intraluminal chyme or intraportal plasma, the hormones which these substances release and/or the nervous impulses which they initiate, independently that is, of the systemic circulation or the CNS (Figure 9).

One might question why humoral agents, the origins of which are the small intestine, need to first circulate throughout the entire systemic circulation before finally affecting the pancreas which is in such close anatomical proximity. Such a requirement would seem to be an inefficient method of control - one which does *not* take advantage of the fortuitous proximity of the pancreas to the gut. When hormones, for example, first pass into the systemic circulation to affect the pancreas, is it not likely that these signals might become dampened by the effects of dilution and perhaps metabolism by the liver (e.g. short fragments of CCK) and/or other body tissues (e.g gastrin, secretin, long fragments of CCK) (Doyle *et al.*, 1984; Temperley *et al.*, 1971; Clendinnen *et al.*, 1973; Lehnert *et al.*, 1974; Meyer and Jones, 1974)? A local influence of the gut on the exocrine pancreas would seem to be a more sensitive means by which the pancreas could respond to subtle changes in the digestive process, unless, of course, tissue metabolism of the agent is an absolute requirement for its pancreatic effect. To illustrate how delivery of a peptide hormone into the systemic circulation via the liver may alter the final pancreatic response, consider the following: small peptides of gastrin and CCK are progressively cleared during hepatic transit in rats, whereas peptides longer than seven amino acids traverse the liver without significant degradation (Doyle *et al.*, 1984). Hepatic inactivation would largely preclude any potential contribution of these smaller peptides to gastrointestinal function if they must reach their target tissue via the systemic circulation. As some small fragments

of gastrin and CCK appear to be important biologically (Gregory, 1974; Rehfeld *et al.*, 1980), it is interesting to speculate that they, like the longer fragments, may also play a role in pancreatic regulation, but that they exert their effects *locally*, rather than systemically.

The hypothesis proposed here is that local avenues of communication may exist which take advantage of the developmental proximity of the pancreas to the gut and hepatic portal system.

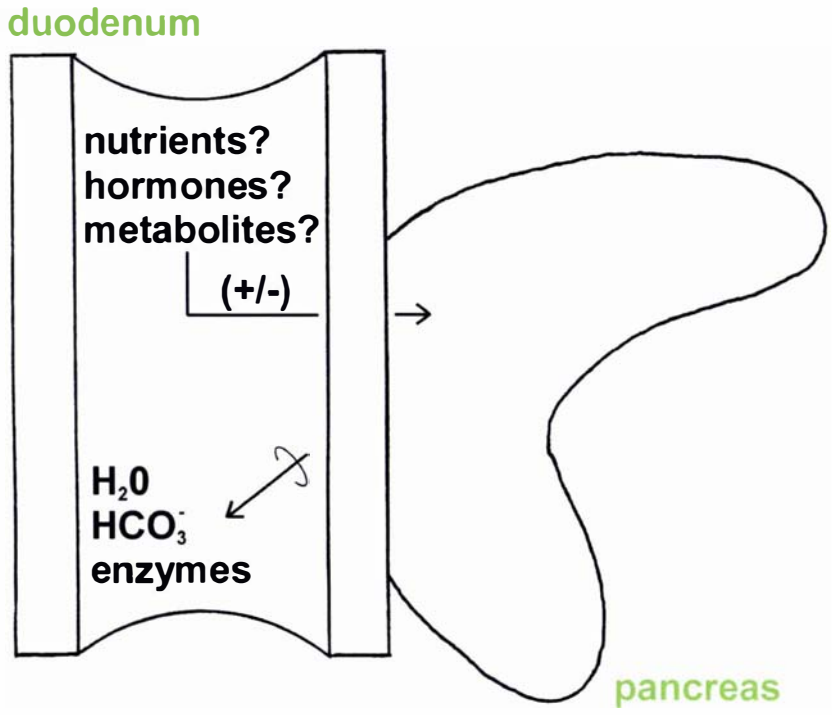

Figure 9. The hypothesis. Situated adjacent to the proximal descending limb of the duodenum, the canine pancreas is in a prime location for receiving information regarding the digestive status of the animal. In addition to the more conventional forms of control, nutrients, metabolites and hormones may influence the exocrine pancreas via 'local' mechanisms which operate independently of the systemic circulation or central nervous system. (+/-) stimulates or inhibits

Support for the hypothesis

Several neuroanatomical features have been described which lend support to the above hypothesis. In the early 1900s, Pavlov and his co-worker, Popielski, speculated that a 'peripheral reflex' passed directly from the duodenal mucosa to the body of the pancreas where a large collection of ganglion cells was observed close to the upper border of the hepatogastroduodenal ligament (Bodanszky *et al.*, 1973). Thomas (1943) later suggested that a local reflex pathway, if it indeed existed, probably consisted of connections between the intrinsic ganglia of the pancreas and the enteric nerve plexus. The presence of such nervous connections has since been confirmed in the rat by Kirchgessnor & Gershon (1990) as mentioned earlier. In 1971, Thambugala and Baron suggested that the CNS, via sympathetic and parasympathetic input, acted on intrapancreatic and/or myenteric ganglia to facilitate (or inhibit) a local duodenopancreatic reflex, and other investigators have proposed that a combination of short (duodenopancreatic), medium (relaying in the coeliac ganglia) and long (vago-vagal) arc reflex pathways may form the basis of the intestinal phase of pancreatic secretion (Tiscornia *et al.*, 1976a). Since this time, gross anatomical dissections in dogs have demonstrated large numbers of nerves running between the duodenum and pancreas (Tiscornia, 1977), and the presence of fibres penetrating the pancreas directly from the duodenum has been confirmed histologically in rats (Anglade *et al.*, 1987). From these observations, several things have become clear: first, that the neuroanatomy in this region is complex; second, that direct nervous connections between the duodenum and pancreas are present at least in the rat and dog, and finally, that the *potential* exists for a local, neurally-mediated, direct relay of information from the duodenum to the pancreas - one which is perhaps capable of functioning independently of the CNS.

In the dog, the existence of a *short* duodenopancreatic reflex mechanism has been proposed but never demonstrated in a physiological sense, owing in part to the difficulties associated with isolating this segment of the duodenum and pancreas from the influence of the arterial circulation and autonomic nerves. Pavlov's and Popielski's original suggestion that a 'peripheral reflex' mechanism may exist was based on the observation that the exocrine pancreatic secretion was still elicited by intraduodenal hydrochloric acid following division of both vagus nerves, bilateral section of the splanchnic nerves,

destruction of the spinal cord and extirpation of the solar plexus (Pavlov, 1910). Today, we know that the response of the pancreas to acid instilled into the duodenum can largely be explained by the hormonal action of secretin. Since that time however, indirect evidence to suggest that short duodenopancreatic reflexes may indeed play a role in the control of exocrine pancreatic function has accumulated. In 1979, Solomon and Grossman observed that the transplanted canine pancreas was significantly less sensitive to intestinal stimulants than the intact pancreas, and Barzilai *et al.* (1987) showed that pancreatic bicarbonate secretion in response to intraduodenal fat and amino acids is dependent upon an intact *local* duodenopancreatic nervous connection. Perhaps the most important contribution was made by Kirchgessner and Gershon (1990) who not only demonstrated that the enteric nervous system innervates the pancreas, but also showed for the first time that these neurons are capable of modifying pancreatic exocrine as well as endocrine activity. Today, as a result of the observations made by Tiscornia (1977), Anglade (1987) and Kirchgessner and Gershon (1990), strong anatomical support exists for such a reflex, and while a physiological role for these observations remains to be clearly demonstrated, importantly, a short limb reflex, be it neurological or other, has never been *disproved.*

Evidence against the hypothesis

Having cited a number of examples of earlier work which support our hypothesis, there have been reports which, while not disproving the possibility of short neural reflex or vascular pathways, offer little *direct* support for one. The predominating role of a long vago-vagal cholinergic reflex mechanism for CCK-stimulated pancreatic enzyme secretion was suggested from 'latency of response' studies in conscious dogs. Singer *et al.* (1980a) demonstrated that the latency of the pancreatic amylase response to intraduodenal tryptophan or oleate was significantly less than the latency of response to intraportal CCK. Atropine and truncal vagotomy increased the latency to tryptophan and oleate 10-fold but had no effect on the latency to intraportal CCK. These results led these investigators to conclude that a (long) vago-vagal cholinergic mechanism mediates the early pancreatic enzyme response to intestinal stimulants. While it would appear from this study that the sum of all effectors of the pancreatic response, minus the influence of the long vago-vagal reflex on that response, leaves only the influence of hormones, this may not necessarily be the case. Singer and colleagues even commented that it was possible that short vascular

pathways exist between the intestine and pancreas but that, although they could not disprove this possibility, it seemed unlikely. Interestingly, the possibility of a similar short *nervous* reflex pathway between the intestine and pancreas which may be vagally 'driven' was not acknowledged.

In another series of elegant experiments, Li and Owyang (1993; 1994) demonstrated that CCK, at "physiological" concentrations, stimulates pancreatic enzyme secretion in anaesthetized rats via a capsaicin-sensitive afferent vagal pathway, a pathway which originates in the gastroduodenal mucosa (Li and Owyang, 1993). An interesting aspect of this experiment is that the pancreatic response to *exogenously-administered* CCK was shown to be heavily dose-dependent, and these investigators went to considerable trouble to define a "physiological" concentration of CCK. Although one could question the approach used whereby "physiological" was determined from plasma concentrations of CCK collected via cardiac puncture rather than from portal blood, these authors later provided compelling evidence that *endogenous* CCK released in response to an intraduodenal infusion of casein *also* stimulates pancreatic enzyme secretion via a long, vago-vagal afferent pathway (Li and Owyang, 1994). The observation that both vagotomy and perivagal application of capsaicin[2] completely *abolished* the increase in pancreatic protein secretion virtually rules out the possibility of a significant local reflex pathway for CCK, at least in anaesthetized rats. It is perhaps also important to note here that not *all* studies have shown that vagotomy significantly reduces pancreatic enzyme secretion in response to CCK or nutrient stimulation (Henriksen, 1969; Konturek *et al.*, 1972; Konturek *et al.*, 1974a; Debas *et al.*, 1975a; Solomon and Grossman, 1979). Li and Owyang (1994) however, attribute these contradictory findings to the use of "supraphysiological" doses of CCK as well as to differences in the length of time between vagotomy and the start of the experiment.

[2] Capsaicin is a neurotoxin specific to thin, unmyelinated, primary afferent sensory neurons. At low doses, capsaicin is able to stimulate their normal function, whereas at high doses, it produces a long-lasting depolarization and an inappropriate opening of calcium channels resulting in functional inactivation of these fibres.

Taken together, the results of these studies point to the importance of long vago-vagal pathways in the control of pancreatic secretion following nutrient stimulation, at least where CCK is involved. The possibility of *local* reflex pathways were not specifically tested however, and the inability to demonstrate a local reflex involving CCK does not negate the possibility that such a mechanism exists for other hormones or nutrients.

POTENTIAL MEANS BY WHICH THE SMALL INTESTINE AND HEPATIC PORTAL SYSTEM MAY COMMUNICATE WITH THE EXOCRINE PANCREAS

It is appropriate here to discuss some of the mechanisms by which a direct, 'local' pathway between the duodenum and pancreas could exist, and because of the species differences noted above, to extend this concept to also include potential means by which the *hepatic portal system* may communicate with the pancreas. In this section, we will focus on conventional forms of control, i.e., humoral and nervous mechanisms, but will examine their potential involvement from a different perspective.

The first means by which the gut could potentially communicate with the pancreas is directly via the blood, in a manner similar to that in which the *endocrine* pancreas communicates with the *exocrine* pancreas (for reviews of the 'insulo-acinar' portal system, see Williams and Goldfine, 1985, and Bonner-Weir, 1993). The basis of this arrangement would be the extension of efferent capillaries, venules or veins from the gut, or from the cranial pancreaticoduodenal vein, directly into pancreatic acinar tissue (Figure 10). Thus, as blood passes from the gut, it becomes enriched with high concentrations of hormones, nutrients and metabolites. These then diffuse through highly permeable capillary beds into pancreatic acinar tissue prior to entering (or re-entering) the general hepatic portal system. In other words, the pancreas would be 'portal' to the *small intestine*, but on a scale smaller than that seen in the liver. This type of circulation might also require some form of directed blood flow to ensure blood preferentially enters one or both of these vascular routes to enter a given segment of the pancreas prior to emptying into (or returning to) the cranial pancreaticoduodenal vein. In those species in which the pancreas either partially or completely encircles the portal vein, blood may be *similarly* diverted, but from the portal vein directly through an 'in-contact' portion of pancreas. Although there is no

contemporary anatomical or histological evidence to support the existence of such a circulation, these possibilities do not appear to have been thoroughly explored.

Another means by which the gut could conceivably communicate with the pancreas via the blood is through a local countercurrent exchange mechanism (Figure 11). In sheep, cows and other species, luteolysis is attributed to the uterine synthesis and secretion of $PGF_{2\alpha}$ (Goding, 1974; Horton and Poyser, 1976) and its subsequent transport to the ovary (McCracken *et al.*, 1972; Hixon and Hansel, 1974; McCracken *et al.*, 1981). By *local* circulation, $PGF_{2\alpha}$ is transferred from the uterine vein to the ovarian artery via diffusion across the walls of both vessels. Evidence also exists for uterine $PGF_{2\alpha}$ being transferred locally from uterine lymphatic vessels to the adjacent ovary and ovarian vein (Heap *et al.*, 1985), suggesting the possibility of yet *another* means of 'short route' communication in our proposed model. That such forms of local communication between veins, arteries and lymphatics exist in other parts of the body justifies our quest and allows us to speculate further that similar non-conventional mechanisms may operate in the dense network of the vasculature both in and around the duodeno-pancreatic area.

A third means by which the gut could directly communicate with the pancreas is via a local nervous mechanism. A direct relay of information from the small intestine to the pancreas may occur through local extensions of the enteric nervous system (Figure 12), as mentioned previously. It is tempting to speculate that the enteric nervous system, in addition to coordinating the motor activities of the small bowel, also serves the 'coupling' role between the duodenum and pancreas during the interdigestive period, or relays signals to the pancreas describing the local situation in the stomach and duodenum during the postprandial period. Although there is presently not a great deal of evidence to support such hypotheses, chemical activation of enteric neurons *has* been shown to increase cytochrome oxidase activity in intrapancreatic neurons and acinar cells (Kirchgessner and Gershon, 1990). More recently, a physiological role for enteric neurons was proposed by Gicquel *et al.* (1994) who used capsaicin to determine if local stimulation of mucosal sensory nerve endings would produce measurable effects on pancreatic secretion. These researchers found that low doses of intraduodenal capsaicin stimulated pancreatic secretion in anaesthetized rats, and interestingly, this effect was found to be independent of the

vagus nerves, nicotinic synapses, acetylcholine release and adrenoceptors. One explanation put forward to account for these observations was that intraduodenal capsaicin stimulates the release of sensory neuropeptides from capsaicin-sensitive primary afferent nerve endings, which in turn stimulate the pancreas indirectly via the enteric nervous system. More work in this area is needed to ascertain what role, if any, the enteric nervous system plays in the control of exocrine pancreatic function. This information may provide important clues about pancreatic function and its control during both the interdigestive and postprandial periods of pancreatic secretion.

Finally, during the histological studies (the results of which are presented in Chapter 4), a fourth potential mechanism of local communication between the duodenum and pancreas was postulated. This hypothesis, although not envisioned during the planning stages of the thesis, emerged from the unexpected observations of positive immunoreactivity to three sensory neuropeptides along the endothelium of the portal vasculature. The possibility that these structures may have a 'chemosensory' function, sensing hormones or metabolites in portal blood, led to the hypothesis that these or other heretofore unrecognized structures in the portal vasculature might be involved in reflex regulation of the pancreas (Figure 13). Such a mechanism might utilize local, or even central, processing of information. Although this concept of control of pancreatic function is unprecedented and highly speculative, examples do exist of highly specific visceral receptors which sense changes in the internal environment (e.g., arterial baroreceptors, carotid body chemoreceptors, lung inflation and deflation receptors, atrial volume receptors) and assist in the homeostatic control of visceral function (Cervero and Foreman, 1990).

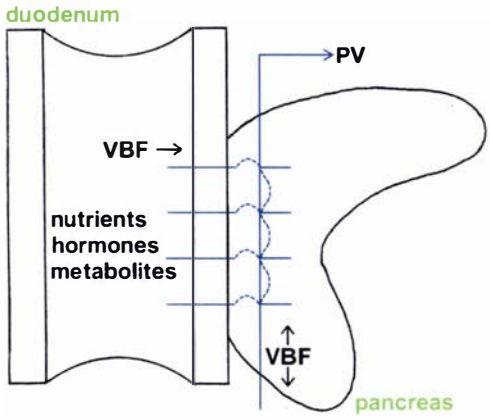

Figure 10. Direct vascular communication between the duodenum and exocrine pancreas. In this hypothetical situation, the pancreas is 'portal' to the small intestine. Venous blood, enriched with high concentrations of nutrients, metabolites and hormones, passes from the gut directly through pancreatic acinar tissue *before* entering the portal vein (PV). Hashed lines represent points at which blood is diverted through the pancreas. (VBF) venous blood flow

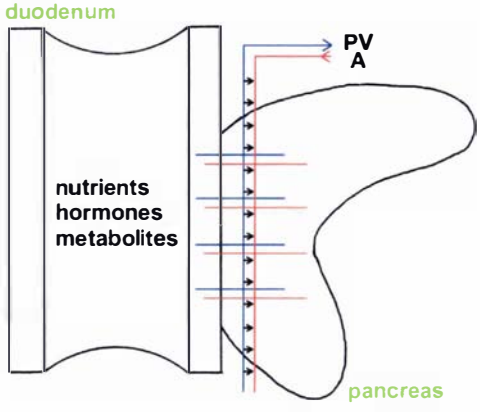

Figure 11. Local countercurrent exchange between the duodenum and exocrine pancreas. In this hypothetical situation, chemical messages are transferred from the gut to the pancreas via local circulation. Nutrients, metabolites and hormones are transferred from either veins or lymphatics to an adjacent artery across their apposed walls, and then enter the pancreas directly via the arterial circulation. (A) aorta; (PV) portal vein; Arrows point in direction of transfer from vein (or lymphatic) to artery.

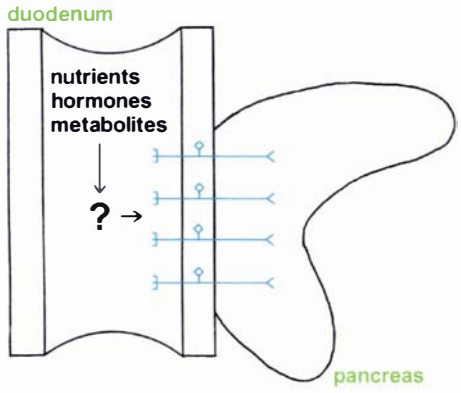

Figure 12. Direct nervous communication between the duodenum and exocrine pancreas. In this hypothetical situation, information regarding the digestive status of the animal is relayed directly to the pancreas via the enteric nervous system. Enteric nerves are shown in blue.

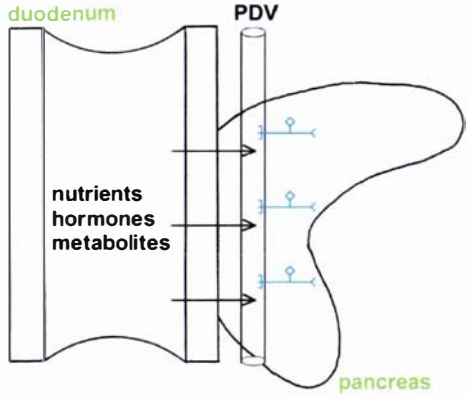

Figure 13. Communication between the duodenum and exocrine pancreas involving a neural relay of information from the portal vasculature. In this hypothetical situation, the endothelium of the portal vasculature is assigned a chemosensory function. Information derived from nutrients, hormones or metabolites entering portal blood is relayed to the pancreas either directly (as shown) or indirectly via the CNS. In this example, a reflex involving only the pancreaticoduodenal vein (PDV) is illustrated, but similar mechanisms could exist for other portal vessels. Nerves are shown in blue.

Difficulties associated with studying the hypothesis

From a practical standpoint, demonstration of the existence of any form of locally-mediated reflex is difficult at best. As mentioned earlier, the difficulties of isolating the duodenal-pancreatic segment from the systemic arterial circulation and the influence of both medium arc and long arc reflexes in conscious animals are real, and indeed, any attempts at abolishing the influence of the extrinsic nerves may reduce or even nullify the anticipated response if an underlying autonomic tone forms its foundation. Thus, experiments designed to evaluate the effects of either vagotomy or splanchnicectomy on pancreatic function, as well as experiments which isolate the pancreas via autotransplantation may not be valid for such purposes. In transplantation studies, not only is the extrinsic innervation abolished, but the anatomical relationship of the pancreas to the small intestine is disrupted. Pancreatic response studies in which the effects of drugs on the autonomic nervous system are used to draw conclusions regarding the relative contributions of the parasympathetic and sympathetic nervous systems also must be carefully examined. For example, interpretation of studies in which the effects of vagotomy and atropine on a given preparation are used to describe and to isolate the role of the parasympathetic nervous system may be complicated by the existence of other non-vagal pathways which also utilize cholinergic transmission. That is to say that the potential influence of an unrecognized, cholinergic, *extra-vagal* nervous pathway may not be controlled for in the experimental design.

Finally, conclusions based on studies in which *exogenous* secretagogues are used to stimulate the pancreas may be similarly misleading. There may be inherent dangers in extrapolating information regarding the effects of *endogenously* released hormones from experiments based on their *exogenous* administration. For example, portal vein concentrations of gastrointestinal hormones are generally higher than those of peripheral veins (Boden *et al.*, 1974; Boden *et al.*, 1975b; Hocking *et al.*, 1982; Wolfe and McGuigan, 1984), an effect which is at least partly attributable to hormone-eliminating mechanisms ('first-pass' effect) in the liver and to dilution in the systemic circulation. If indeed a local form of regulation exists, then the concentration of the hormone at (or near) the site at which pancreatic stimulation is *initiated* may be more relevant than the concentration of the hormone in the systemic circulation. To illustrate this, Pierzynowski

et al. (1993) demonstrated that, following temporary ligation of the cranial pancreaticoduodenal artery so that the CCK injected did not directly reach the pancreas, low doses of CCK-33 administered directly into the gastroduodenal arterial circulation, significantly increased all of the pancreatic parameters studied, whereas the same dose did not stimulate secretion when administered into a jugular vein. The authors concluded that the stimulatory action of CCK is at least in part regulated by a mechanism mediated locally in the gut. While this may very well be the case, the authors ignored the possibility of a local role for the hepatic portal system. Similarly, other studies have failed to show an effect of CCK on exocrine pancreatic function following exogenous administration, even when blood levels reached those achieved postprandially (Cuber *et al.*, 1989; Dale *et al.*, 1989). These studies exemplify how careful one must be drawing conclusions regarding the 'link' between cause and effect when studying pancreatic function and its control, and further illustrate how assumptions based on conventional wisdom often preclude consideration of other plausible, but heretofore, unrecognized pathways of regulation.

OUTLINE OF THESIS

While great advances have been made in our understanding of exocrine pancreatic function and its control, the specific mechanisms by which the gut communicates with the pancreas remain obscure. In this thesis, novel avenues of communication that may take advantage of the developmental proximity of the pancreas to the gut and hepatic portal system are explored.

First, gross anatomical relationships of the pancreas to the gut and hepatic portal system will be described in detail. This will consist of a thorough review of the currently available anatomical literature with facts confirmed, and expanded upon (where the literature was not sufficiently specific) by the author's own observations of post mortem specimens from a variety of species. This will form the subject of Chapter 2.

In the dog, the body of the pancreas is intimately associated with the proximal descending limb of the duodenum, and in this area of close apposition, the blood supply is complex. A direct venous communication between the duodenum and the pancreas was initially considered by the author as a likely route of communication in this species. In Chapter 3,

the results of a detailed study of latex-injected vascular casts of the gut and hepatic portal system of the dog will be presented in an attempt to provide evidence for such a route of communication.

Gross inspection of latex venous casts, while a useful technique, does not allow observation of microscopic venous communications between the duodenum and pancreas, nor does it allow for an assessment of potential functional communication between veins and arteries (e.g. a countercurrent exchange system) should they exist. Thus the microscopic features of the veins of the hepatic portal system and the duodenum and pancreas in their area of close apposition will be the subject of Chapter 4.

Little evidence was obtained from the work to be presented in Chapters 2-4 to support the hypothesis that a venous communication between the duodenum and pancreas or a local countercurrent exchange mechanism exists. At this point of the thesis, examination of the enteric nervous system, the last of the three originally hypothesized routes of communication, was set aside because of unexpected developments stemming from the microanatomical studies. These histological observations, to be presented in Chapter 4, will raise the question of whether a sensory role for the portal vasculature exists. An important and previously unexplored hypothesis, the possibility that the hepatic portal system plays a supplementary role in the neural control of exocrine pancreatic function, were examined through physiological experimentation using an acute canine model. These experiments will be described in Chapters 5 and 6.

In Chapter 7, an overview of the research outcomes will be presented, with a discussion of the strengths and weaknesses of the approaches taken. Suggestions for future research will conclude the thesis.

Chapter Two

COMPARATIVE TOPOGRAPHICAL ANATOMY OF THE PANCREAS

SUMMARY

Preliminary observations by the author of the digestive tracts of several mammalian species indicated that the pancreas is variably associated with both the proximal descending limb of the duodenum and the hepatic portal system of veins. These anatomical associations, which have not previously received a great deal of attention, prompted this investigator to question their biological significance. In this Chapter, a review of the standard human and animal anatomy textbooks is presented, augmented by the author's own observations of the digestive tracts of the dog, ox, pig, horse and chicken. Currently available information concerning the comparative anatomy of the pancreatic, duodenal and hepatic portal areas is examined from a new perspective. It was observed that differences between species exist in the *closeness* with which the body and/or right lobe of the pancreas approach the duodenum in the adult. In the dog and in man, the body, or head, respectively, of the pancreas appears to embed itself in the wall of the duodenum, while in the chicken, both the dorsal (left) and ventral (right) pancreatic lobes lie tightly enclosed between the descending and ascending limbs of the duodenum. In the ox, pig and horse, such a close association of the pancreas with the duodenum is less apparent. In these species, the pancreas is more loosely associated with the small intestine, approaching the duodenum closely only in the areas of their pancreatic ducts. Equally variable among species are the relationships of the adult pancreas with the veins of the hepatic portal system. In the ox and in man, the pancreas *partially* surrounds the portal vein, while in the pig and horse, the pancreas completely surrounds the portal vein. In the dog and chicken, such close relationships with the portal vein itself are not apparent to the same extent. Rather, in these and other species in which the pancreas closely apposes the proximal small intestine, both the pancreas and duodenum are related to the cranial pancreaticoduodenal vein, which courses between the two organs, providing common drainage to both. These observations led the author to speculate that the duodenum and/or the veins of the hepatic portal system may play a local role in the control of exocrine pancreatic function. Possible means by which the gut and/or portal system may communicate with the exocrine pancreas are discussed, and include humoral mechanisms, neural mechanisms, or a combination of both. The hypothesis that some form of local communication exists between the gut or the hepatic portal system and the pancreas, and that this communication plays a supplementary role in the control of exocrine pancreatic function, is the subject of the remainder of this thesis.

INTRODUCTION

Preliminary observations by the author of the digestive tract from post mortem specimens of several mammalian species indicated that the pancreas is variably associated with both the proximal aspect of the descending limb of the duodenum and, more significantly, with

the veins of the hepatic portal system. These specific anatomical associations, which have not received a great deal of attention in general anatomy textbooks, prompted this investigator to question their biological significance. In this chapter, the anatomy of the pancreatic, duodenal and hepatic portal areas is further explored through a review of the standard anatomy textbooks, augmented where the literature was not sufficiently specific, by the author's own observations of post mortem specimens from a number of species (dog, ox, pig, horse and chicken). The purpose of this chapter is to therefore examine, from a novel perspective, what is known about the comparative anatomical relationships of the pancreas with the duodenum and hepatic portal system of veins.

Spatial associations between organs are often best understood by first examining the origin of the tissue or tissues of interest. This chapter begins by examining the embryological development of the mammalian pancreas, and then, using the dog as a model for comparison, cites the anatomical differences which exist between adult members of representative species. The greater part of the descriptive anatomical accounts are compiled from general anatomy references. Where the author's own observations are presented, they are so indicated. Principal references consulted are: **for the dog** – Getty (1975), Adams (1986), Miller (1993), Evans & deLahunta (2000); **for ruminants, pig and horse** – Getty (1975), Constantinescue (1991), Dyce *et al.* (1996); **for birds** – Getty (1975), Nickel *et al.* (1977), Dyce *et al.* (1996); **for man** – Langebartel (1977), Gray (1985), Hall-Craggs (1985), Bockman (1993), Gray (1995), Rosse and Gaddum-Rosse (1997). Where possible, the terminology used to describe topographical details and vascular anatomy was adapted to conform to Nomina Anatomica Veterinaria (Anonymous, 1994), Nomina Embryologica Veterinaria (Anonymous, 1994) and Nomina Anatomica standards (Anonymous, 1989).

The three-dimensional topography of the area under study is extremely complex; the simplified illustrations from textbooks have therefore been used to depict the general relationships as they are currently understood. These illustrations have been reprinted with permission from the author or publisher.

EMBRYOLOGY OF THE PANCREAS

Embryologically, the pancreas of mammals, birds, reptiles and amphibians develops similarly from the primitive foregut (Slack, 1995). The left lobe of the pancreas and its associated ductal system originate from the dorsal pancreatic diverticulum, which arises as an endodermal out-pocketing on the dorsal aspect of the cranial duodenum. The dorsal pancreatic diverticulum grows into the primitive dorsal mesentery (the mesoduodenum) and the adjacent dorsal mesogastrium which later becomes the deep portion of the greater omentum. The right lobe of the pancreas and *its* system of ducts are derived from the ventral pancreatic diverticulum which arises ventrally from the main stem of the hepatic diverticulum near its origin (Figure 1A). As the stomach and duodenum rotate, the hepatic diverticulum and its associated pancreatic anlage come to lie on the dorsal aspect of the duodenum where the dorsal and ventral pancreatic diverticuli fuse to form the left and right pancreatic lobes, respectively (Figures 1B and 1C). This site of fusion is commonly referred to as the 'body' of the pancreas in domestic animals. Such fusion also results in an anastomosis of the two ductal systems, which enables the exocrine pancreatic secretions to pass into the duodenum either by way of the pancreatic duct (which originates in the ventral pancreatic diverticulum) or by way of the accessory pancreatic duct (which originates in the dorsal pancreatic diverticulum). The pancreatic duct (also known as the Duct of Wirsung) and the accessory pancreatic duct (also known as the Duct of Santorini) open into the duodenum at the major and minor duodenal papillae, respectively (Figure 1D)[3]. This developmental pattern, which is shared among species, accounts for the unique anatomical positioning of the pancreas adjacent to the proximal descending limb of the duodenum in the adult animal.

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[3] Because the pancreatic ducts are often confused with the duodenal papillae upon which they open, the following points, as stated by Shively (1984), are useful for clarification. First, the bile duct always opens at the major duodenal papilla. Since all domestic animals have a bile duct, they also have a major duodenal papilla. Second, the main pancreatic duct (if present) also opens into the major duodenal papilla. Third, the accessory pancreatic duct (if present) always opens at the minor duodenal papilla. Finally, species which lack an accessory pancreatic duct (small ruminants and most cats) do not have a minor duodenal papilla.

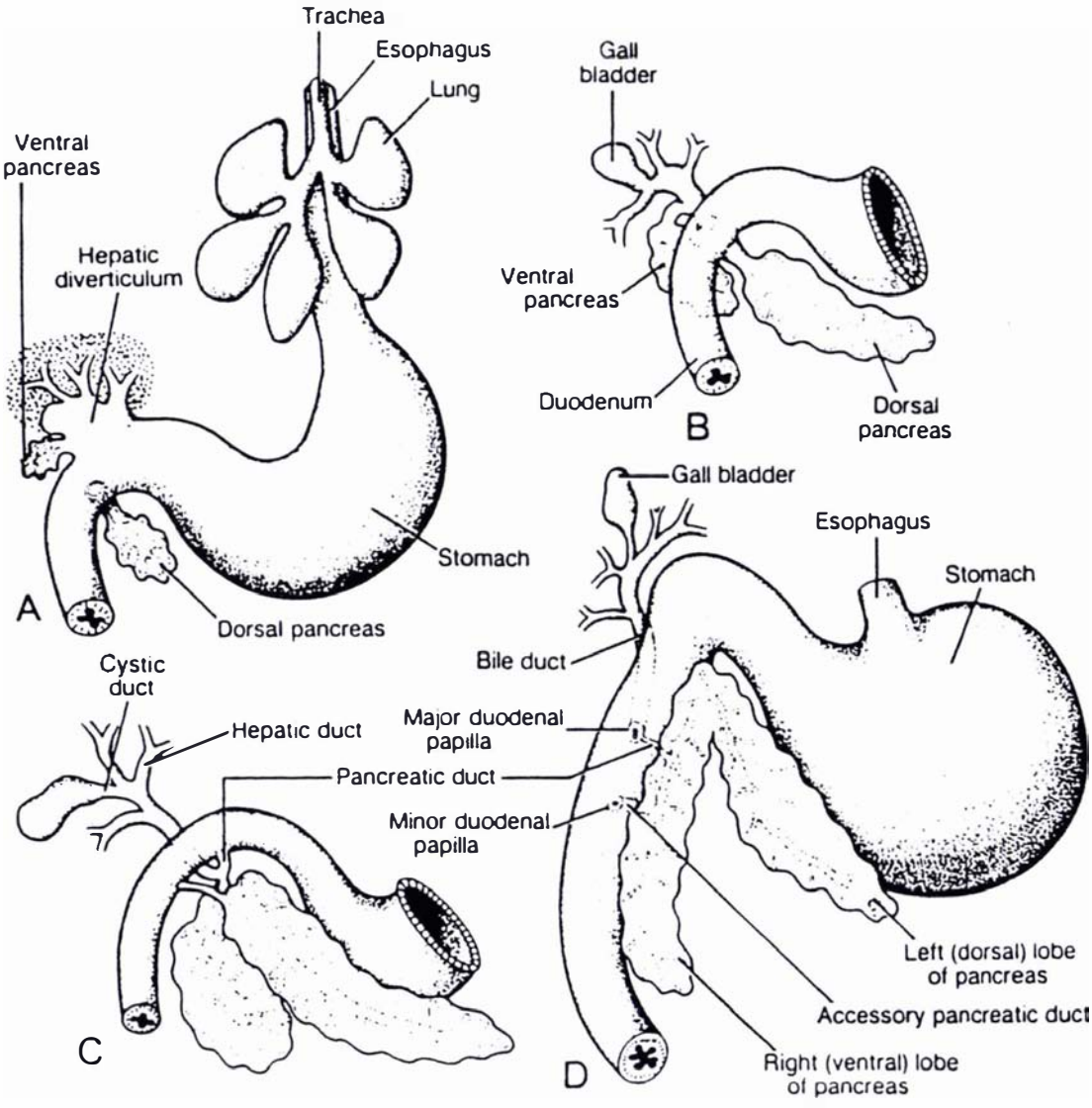

Figure 1. Ventral views of the canine foregut illustrating the changes in location of the lobes of the pancreas and associated ducts. A was drawn from a 9-mm embryo; D was drawn from an adult; B and C are intermediate stages. **(Reprinted from The Embryology of Domestic Animals; Developmental Mechanisms and Malformations, Noden & de Lahunta, p 297, 1985, by permission of the current holder of this title, WB Saunders Company)**

COMPARATIVE TOPOGRAPHICAL ANATOMY OF THE ADULT PANCREAS

DOG (Canis familiaris)

Relations and Blood Supply

The canine pancreas is a compound, 'V'-shaped, tubuloacinar gland. The left lobe, which is positioned in a deep portion of the greater omentum, lies dorsal to the greater curvature of the stomach, while the right lobe, enclosed within the mesoduodenum, lies medial to the descending limb of the duodenum (Plate 1). The long and narrow left and right lobes of the pancreas meet at an acute angle caudal to the pylorus. The right lobe of the pancreas begins at the caudate lobe of the liver and extends caudally, ending usually a short distance caudal to the right kidney. In all canine specimens dissected by the author, it was observed that the lateral border of the proximal right lobe of the pancreas is very closely associated with the mesenteric border of the proximal descending limb of the duodenum (Plates 1 & 2). In this region (the so-called 'body'), the pancreas is relatively fixed to the small intestine compared to the more distal aspect of the right lobe, which is relatively loose and free-floating within the mesoduodenum. This zone of apparent 'contact' between the right lobe of the pancreas and duodenum spans the distance between the two pancreatic ducts and encloses within it a complex network of arteries, veins and nerves.

The common hepatic artery (a branch of the coeliac artery) and gastroduodenal vein, which arise from the abdominal aorta and portal vein respectively, course from their more dorsal origins ventrally to enter the pancreas at its angle (Figures 2 & 3). Within the pancreas, these vessels divide into the right gastroepiploic artery and vein and cranial pancreaticoduodenal artery and vein. The gastroepiploic vessels emerge ventrally, distal to the pylorus, and course cranially along the greater curvature of the stomach. In contrast, the cranial pancreaticoduodenal artery and vein continue caudally between the pancreas and duodenum. Near the middle of the right lobe of the pancreas, the close apposition of the pancreas to the duodenum terminates. The distal half of the pancreas looses immediate contact with the duodenum (not shown in Figures 2 & 3). The cranial pancreaticoduodenal artery and vein again split, this time into pancreatic and duodenal branches, which continue their respective courses caudally through the pancreas and along the mesenteric border of the duodenum. Both the *cranial* pancreaticoduodenal artery and vein form arterial-arterial

and venous-venous anastomoses, respectively, with the corresponding *caudal* pancreaticoduodenal artery and vein. The latter pair arises from their parent vessels, the cranial mesenteric artery and vein.

The left lobe of the pancreas resides within the deep portion of the greater omentum (Plate 1). It extends caudally and to the left from the body of the pancreas toward the spleen passing between the visceral surface of the stomach and the transverse colon. Its left extremity lies near the cranial pole of the left kidney. The splenic artery, a branch of the coeliac artery, and the splenic vein, a branch of the portal vein, each contribute branches to, or receive tributaries from, respectively, adjacent segments of the pancreas (Figures 2 & 3). The splenic artery and the splenic vein also form arterial-arterial and venous-venous anastomoses, respectively, with other vessels supplying/draining different segments of the stomach, spleen, duodenum and pancreas (see Chapter 3).

Pancreatic Ducts

The pancreatic duct enters the proximal duodenum at the body of the pancreas at the major duodenal papilla, cranial and slightly dorsal to the accessory pancreatic duct (Plate 3). The pancreatic duct joins perpendicularly with the wall of the duodenum and the bile duct. In this location, the bile duct is deeply embedded in the gut wall, having approached and entered the duodenum obliquely from the liver. The accessory pancreatic duct enters the duodenum at the minor duodenal papilla, 2-4 cm caudal to the major duodenal papilla. This duct leaves the pancreas and enters the duodenal wall at a proximo-distal angle. In the dog, the accessory pancreatic duct is always present and is larger than the pancreatic duct, which is absent in 24% of cases examined (Sherman and Lindenmuth, 1969). This is in contrast to the domestic cat in which the pancreatic duct persists, while the accessory pancreatic duct is retained in only 20% of cases examined (Schummer *et al.*, 1979).

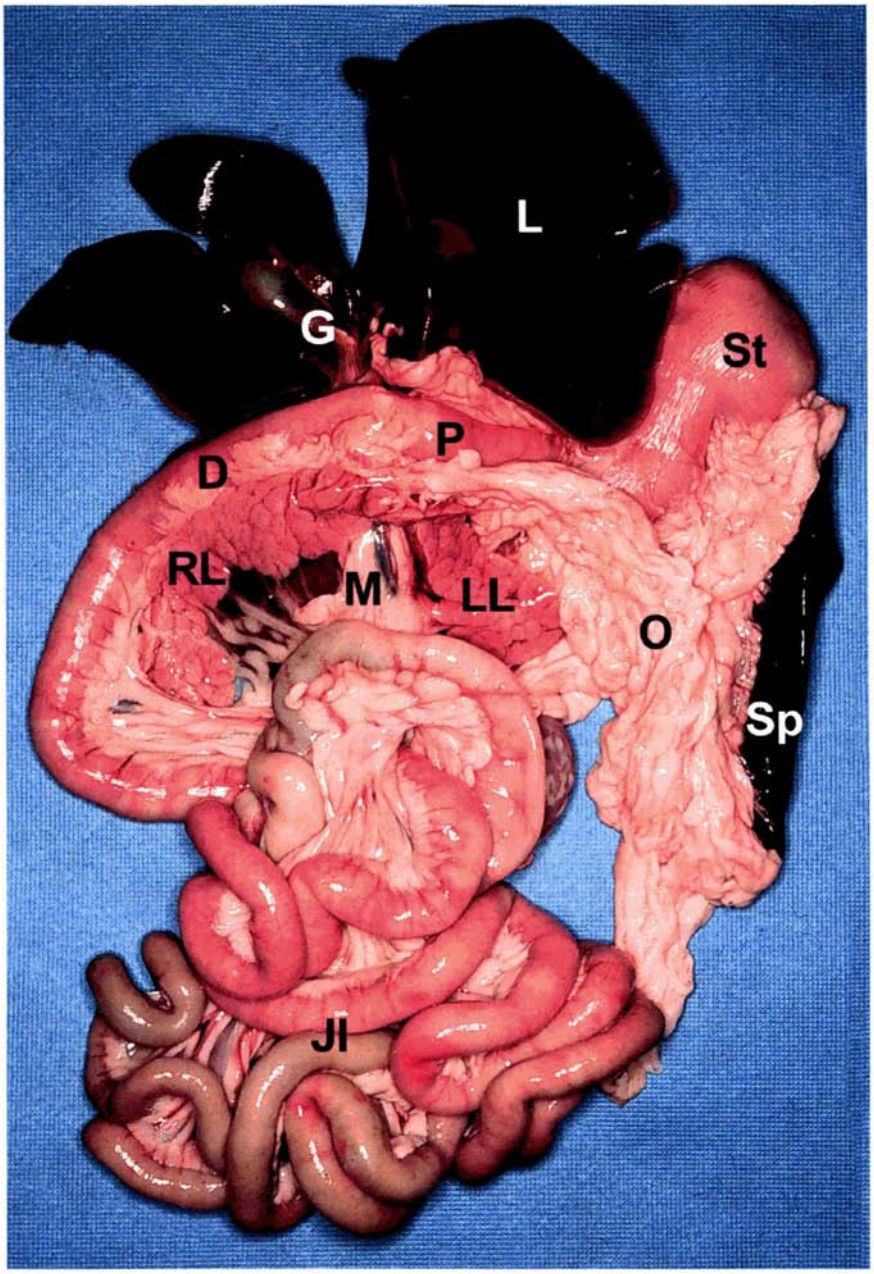

Plate 1. Ventral aspect of the pancreas in relation to the gastrointestinal tract of an adult dog. The greater omentum has been reflected to the left, the liver reflected cranially and the remaining intestinal tract pulled caudally to facilitate visualization of the pancreas. D descending duodenum; G gall bladder; JI jejuno-ileum; L liver; LL left lobe of pancreas; M mesoduodenum; O greater omentum; P pylorus; RL right lobe of pancreas; Sp spleen; St stomach. (Photograph by Angus Fordham)

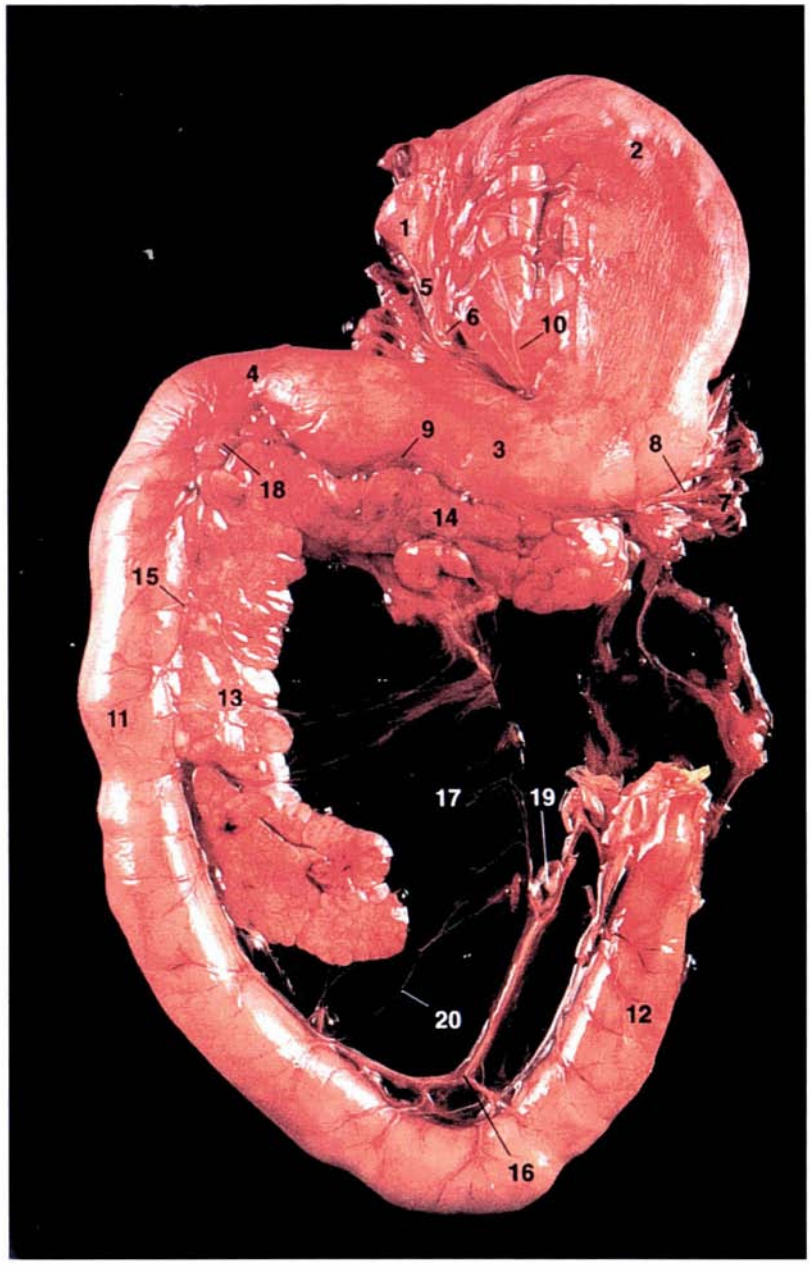

Plate 2. Ventral aspect of the stomach, duodenum and pancreas of a dog. Surrounding viscera have been removed. Note the relationship of the proximal right lobe of the pancreas with the proximal descending limb of the duodenum. 1 cardia; 2 fundus; 3 pyloric antrum; 4 pylorus; 5 lesser omentum; 6 left gastric artery; 7 greater omentum; 8 left gastroepiploic artery; 9 right gastroepiploic artery; 10 lymphatic vessels; 11 descending duodenum; 12 ascending duodenum; 13 right lobe of pancreas; 14 left lobe of pancreas; 15 cranial pancreaticoduodenal artery (from coeliac artery); 16 caudal pancreaticoduodenal artery (from cranial mesenteric artery); 17 mesoduodenum; 18 duodenal lymph node; 19 mesenteric lymph node; 20 gastroduodenal branches of autonomic nerve fibres. (Reprinted from A Colour Atlas of Clinical Anatomy of the Dog and Cat, Boyd & Paterson, p 133, 1991, by permission of the publisher, Mosby)

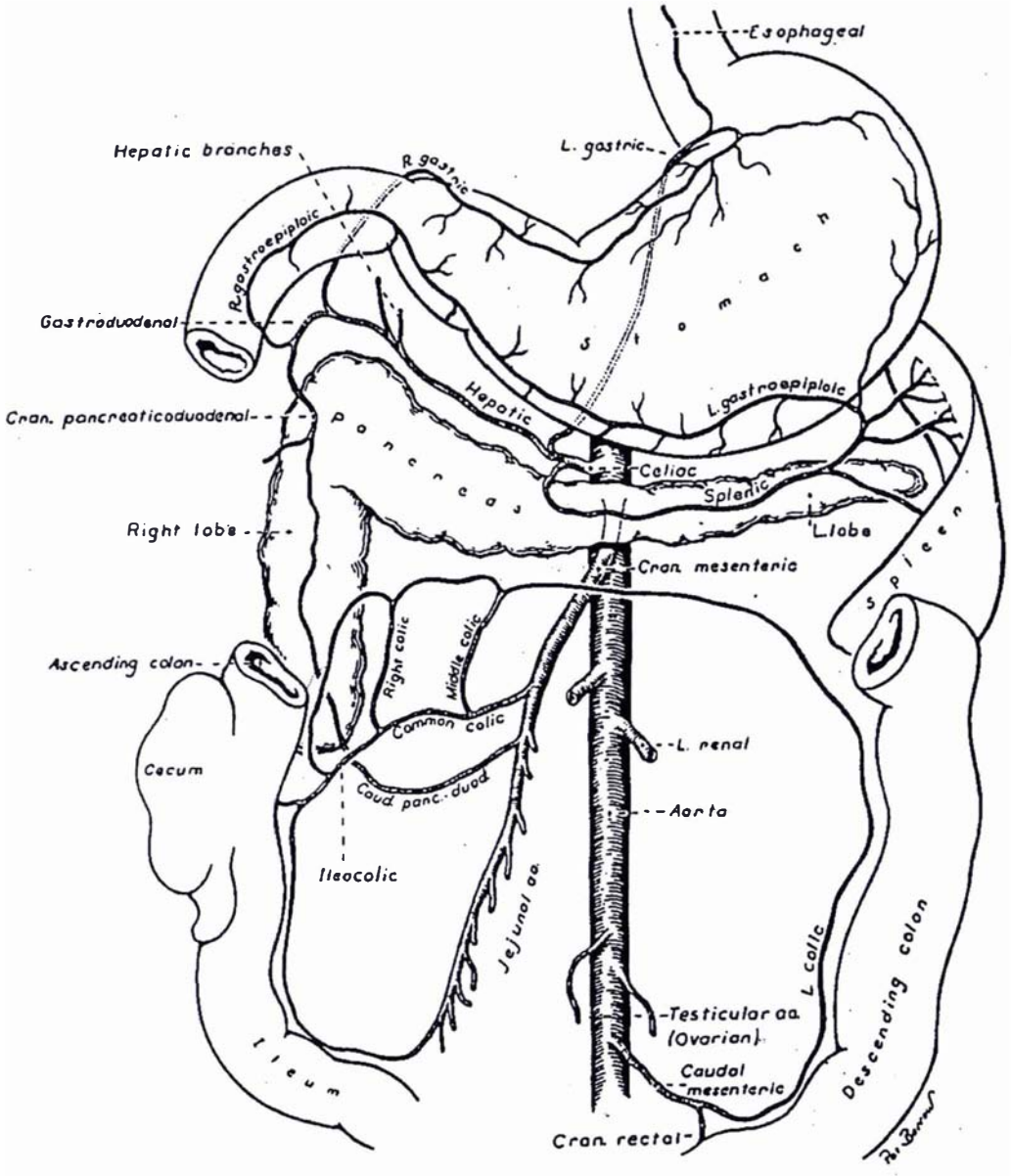

Figure 2. Ventral view of the branches of the coeliac and cranial mesenteric arteries of the dog. **(Reprinted from Miller's Guide to the Dissection of the Dog, Evans & de Lahunta, p 204, 1996, by permission of the publisher, WB Saunders Company)**

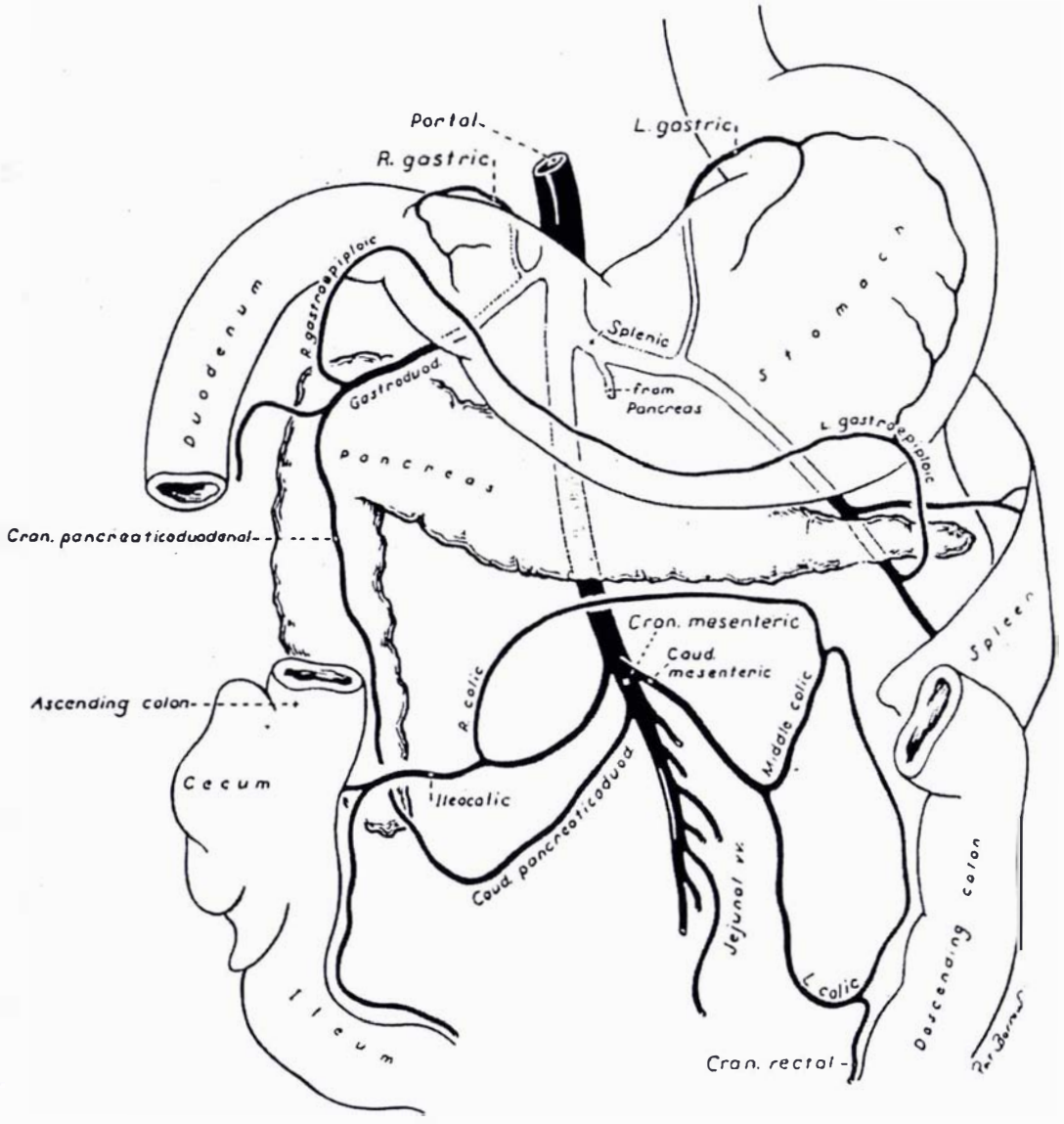

Figure 3. Ventral view of the tributaries of the portal vein of the dog. **(Reprinted from Miller's Guide to the Dissection of the Dog, Evans & de Lahunta, p 208, 1996, by permission of the publisher, WB Saunders Company)**

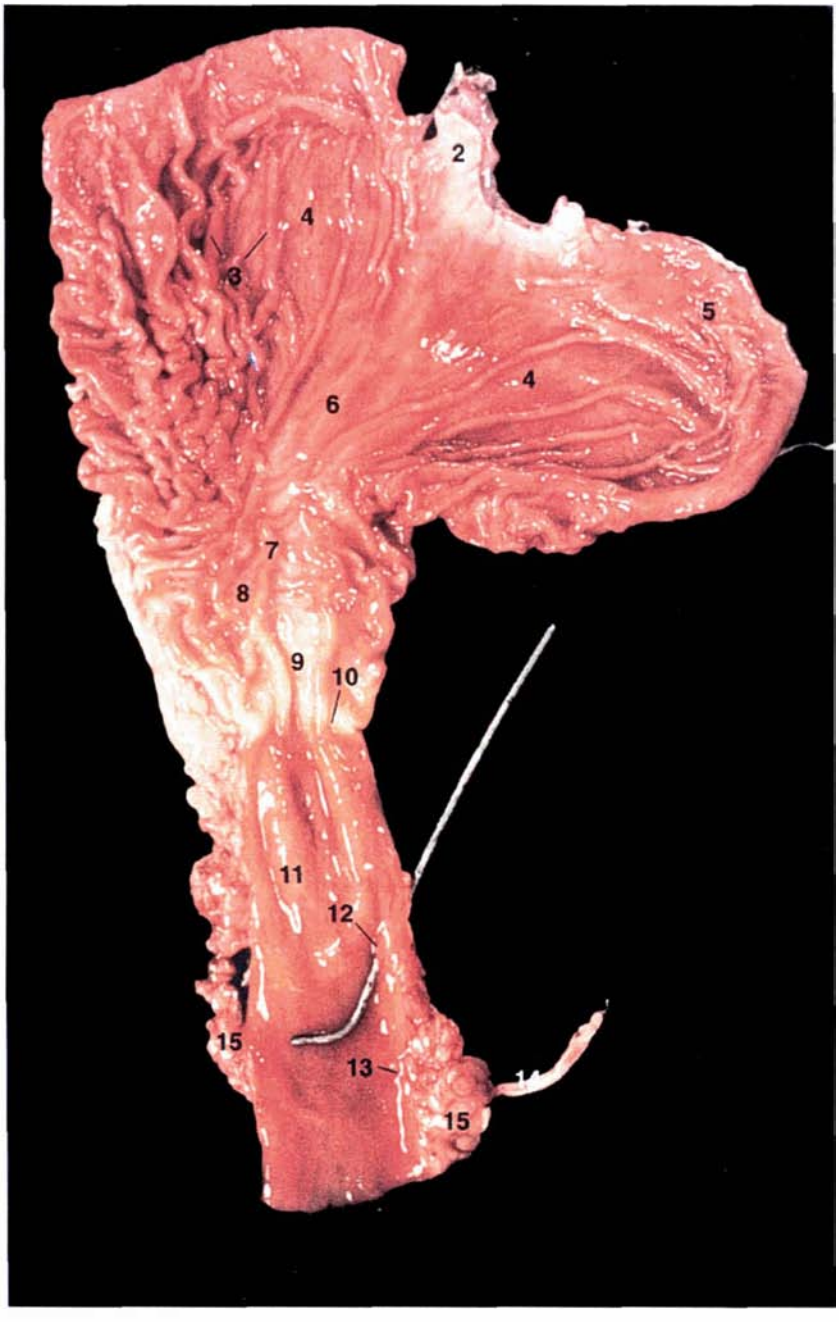

Plate 3. Opened stomach and duodenum of a dog. The stomach and duodenum have been cut along their long axes to reveal the mucosal linings. The probe occupies the entry of the major duodenal papilla. 1 cardia; 2 region of cardiac glands; 3 gastric folds; 4 region of gastric glands (proper); 5 fundus; 6 body; 7 region of pyloric glands; 8 pyloric antrum; 9 pyloric canal; 10 pylorus; 11 duodenum; 12 major duodenal papilla; 13 minor duodenal papilla; 14 accessory pancreatic duct; 15 pancreatic tissue. (Reprinted from A Colour Atlas of Clinical Anatomy of the Dog and Cat, Boyd & Paterson, p 133, 1991, by permission of the publisher, Mosby)

OX (*Bos taurus*)

Relations and Blood Supply

The pancreas of the ox is a soft, lobulated gland of irregular form. In this species, it is located almost entirely to the right of the median plane. The pancreas consists of a large right lobe and a smaller, wider left lobe which join cranially and on the right side of the hepatic portal vein (Figure 4). This junction, referred to as the'body' or *corpus pancreatis*, is adherent dorsally to the liver and to the common bile duct and duodenum. Within the body and separating the right and left pancreatic lobes is a deep notch (the *incisura pancreatis*) through which the cranial mesenteric artery and hepatic portal vein pass.

The right lobe of the pancreas extends caudally along the descending duodenum within the mesentery (mesoduodenum). Its dorsolateral surface is attached cranially to the liver, passes ventrally to the right kidney and comes into contact caudally with the wall of the abdomen at the lumbocostal angle. The ventromedial surface is in contact with the rumen and is to some extent adherent to the colon. The left lobe extends across the abdomen enclosed within the dorsal attachment of the greater omentum at the root of the mesentery. The dorsal surface is free from the liver between the hepatic portal vein and the caudal vena cava. The left extremity of the left lobe turns dorsally and inserts itself between the crura of the diaphragm and the dorsal sac of the rumen. At this location, the left lobe is retroperitoneal. The dorsal surface is also related to the coeliac, hepatic and cranial mesenteric arteries and the splenic vein.

Pancreatic Ducts

In the ox, the entire pancreas is usually drained by the accessory pancreatic duct which opens into the lumen of the descending duodenum at a point 20-30 cm distal to the point of entry of the common bile duct at the major duodenal papilla (Figure 4). Occasionally however, a portion of the left lobe is drained by a small duct which joins with the common bile duct in the substance of the gland. This is in contrast to sheep and goats in which the pancreatic duct persists while the accessory pancreatic duct regresses. It joins the bile duct 5-7 cm from the duodenum.

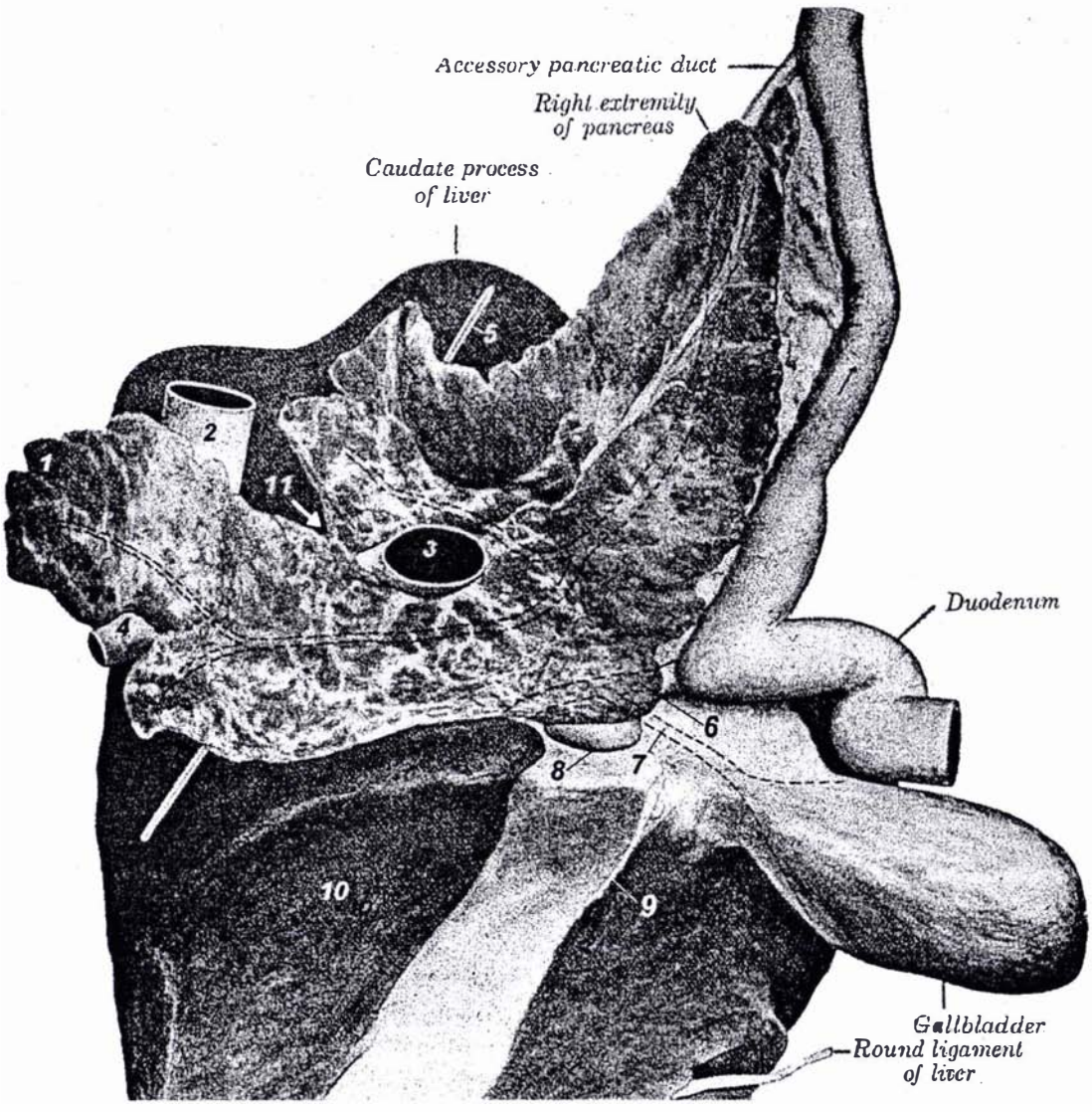

Figure 4. Visceral view of the pancreas and related structures of the ox. 1 left extremity of the pancreas; 2 caudal vena cava; 3 portal vein (the cranial mesenteric artery accompanies the portal vein, but was removed); 4 splenic vein; 5 probe in epiploic foramen; 6 common bile duct; 7 cystic duct; 8 pancreaticoduodenal lymph node; 9 cut edge of lesser omentum; 10 omasal impression on liver; 11 incisura pancreatis. Intraglandular pancreatic ducts are shown by dotted lines. Concealed parts of the common bile duct, cystic duct and neck of the gallbladder are similarly indicated. (Modified an d reprinted from Sisson and Grossman's The Anatomy of the Domestic Animals, 5th edition, Getty, Volume 1, p 914, 1975, by permission of the publisher, WB Saunders Company)

PIG (Sus scrofa)

Relations and Blood Supply

The porcine pancreas is a soft, fleshy, triangular-shaped gland which extends across the dorsal wall of the abdominal cavity caudal to the stomach, sloping in such a manner that the cranial portion of the gland is somewhat more ventral than the caudal portion. Roughly two thirds of the pancreatic tissue lies to the left of the median plane where relationships with the fundus of the stomach, spleen and cranial pole of the left kidney are established (Figures 5 & 6). The right lobe of the pancreas is relatively small and lies within the mesoduodenum near the proximal descending limb of the duodenum. The right lobe makes contact with the liver cranially and may reach the cranial pole of the right kidney caudally. The somewhat more distal extremity of the right lobe is attached to the first curve of the duodenum. The left lobe of the pancreas is related to the left extremity of the stomach along its greater curvature, the dorsal aspect of the spleen and the cranial pole of the left kidney.

The hepatic artery, coursing cranioventrally along the dorsal aspect of the greater curvature of the stomach, sends off several branches to the adjacent cranial segments of the pancreas before sending off its gastroduodenal branch (Figure 6). The gastroduodenal artery and vein vascularize a portion of the right lobe and body of the pancreas. At the angle of divergence of the right pancreatic lobe from the body, both the artery and the vein divide into cranial pancreaticoduodenal and right gastroepiploic branches which then follow similar courses to that found in the dog. The cranial pancreaticoduodenal artery and vein are continuous with the caudal pancreaticoduodenal artery and vein, the latter pair arising from their parent vessels, the cranial mesenteric artery and vein. The splenic and cranial mesenteric arteries each contribute branches to adjacent segments of the pancreas.

In the pig, the pancreas encircles the hepatic portal vein to form a pancreatic ring or *anulus pancreatis* (Figures 5 & 6). The portal vein passes through the pancreas at an acute angle as it courses from the root of the mesentery to the liver.

Pancreatic Ducts

The accessory pancreatic duct opens into the lumen of the descending duodenum at a point 10-12 cm distal to the pylorus at the tip of the right lobe of the pancreas (Figure 5). In the pig, only the accessory pancreatic duct persists.

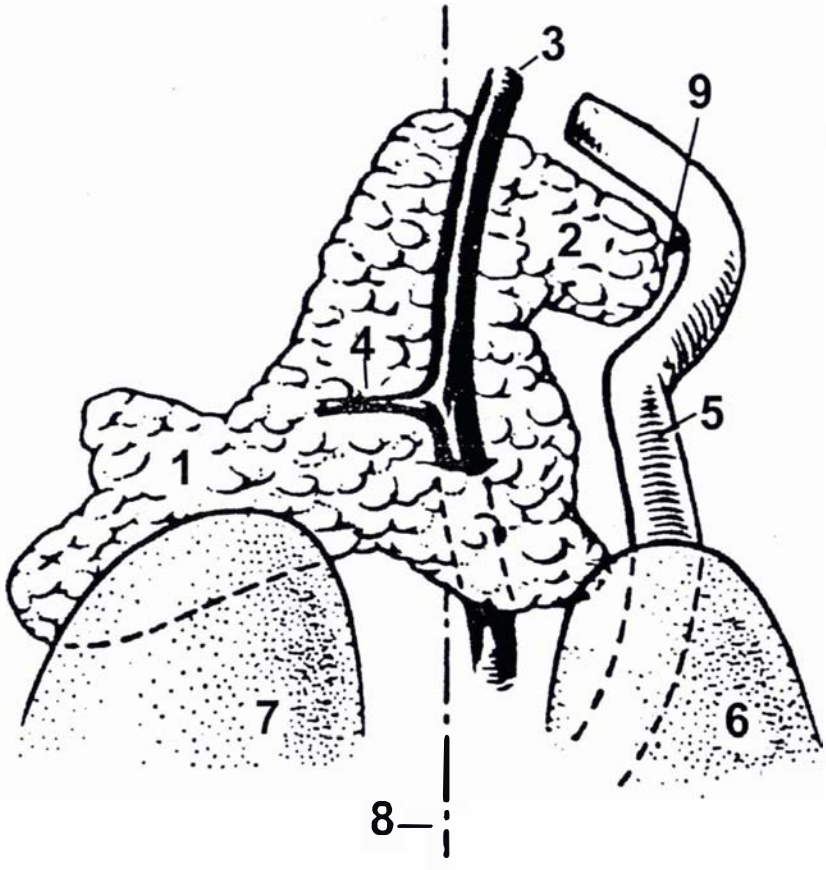

Figure 5. Dorsal view of the porcine pancreas (reconstructed from serial transverse sections). 1 left lobe of pancreas; 2 right lobe of pancreas; 3 portal vein passing through anulus pancreatis; 4 gastrosplenic vein; 5 descending duodenum; 6, 7 cranial poles of right and left kidneys, respectively; 8 approximate position of median plane; 9 accessory pancreatic duct. (Reprinted from Textbook of Veterinary Anatomy, 2nd edition, Dyce, Sack & Wensing, p 796, 1996, by permission of the author, Dr WO Sack)

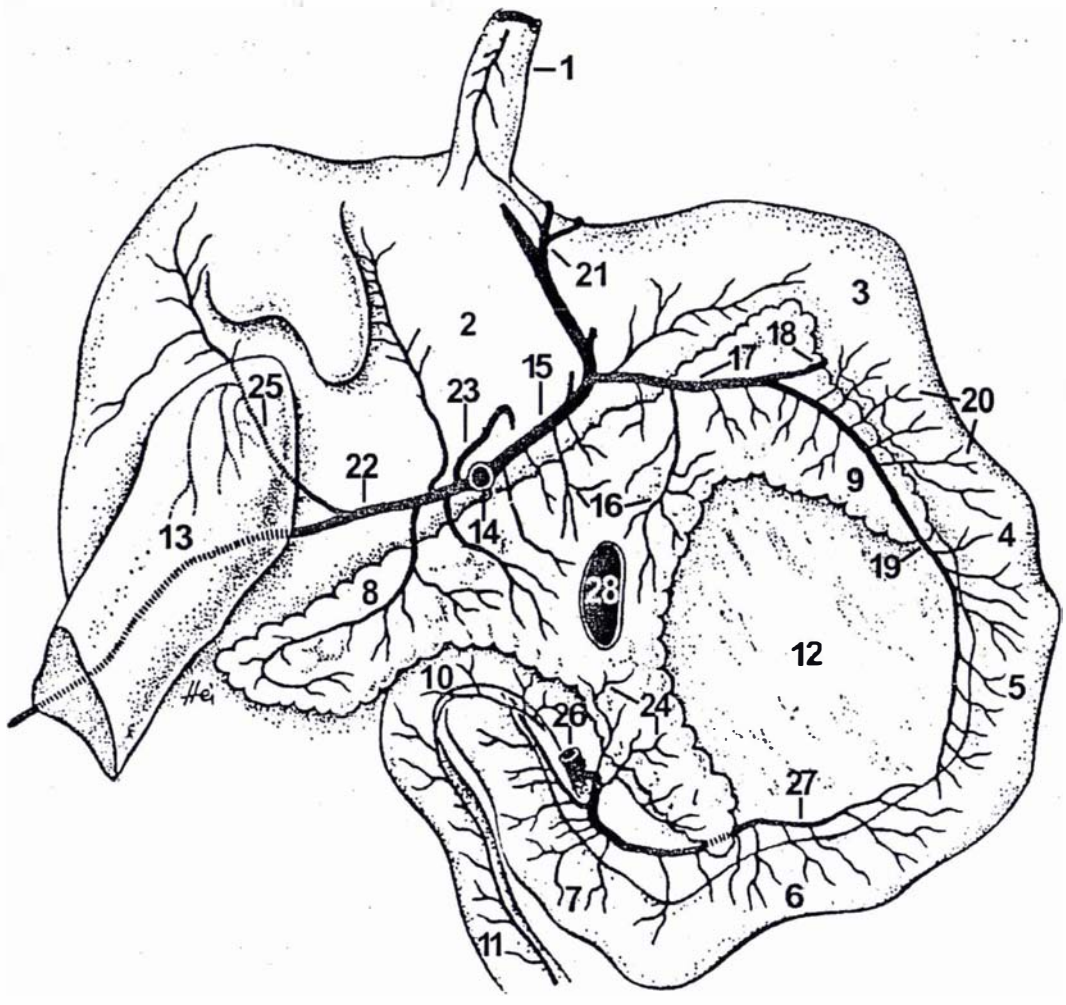

Figure 6. Dorsal view of the stomach, duodenum, pancreas and associated arteries of the pig (semi-diagrammatic). 1 oesophagus; 2 stomach; 3 pylorus; 4 cranial duodenal flexure; 5 descending duodenum; 6 caudal duodenal flexure; 7 ascending duodenum; 8 left lobe of pancreas; 9 right lobe of pancreas; 10 transverse colon 11 descending colon; 12 mesoduodenum; 13 spleen; 14 coeliac artery; 15 hepatic artery; 16 pancreatic branches; 17 gastroduodenal artery; 18 right gastroepiploic artery; 19 cranial pancreaticoduodenal artery; 20 duodenal branches; 21 right gastric artery; 22 splenic artery; 23 left gastric a; 24 pancreatic arteries; 25 gastrosplenic artery; 26 cranial mesenteric artery; 27 caudal pancreaticoduodenal artery; 28 portal vein passing through anulus pancreatis. (Modified and reprinted from The Circulatory System, the Skin, and the Cutaneous Organs of the Domestic Mammals, Volume 3, Schummer, Wilkins, Volmerhaus & Habermehl, p 167, 1981, by permission of the publisher, Springer-Verlag)

HORSE (Equus caballus)

Relations and Blood Supply

The pancreas of the horse is a soft tubuloalveolar gland which is situated transversely on the dorsal wall of the abdomen (Figure 7). It is triangular in overall shape and is composed of a large body, a small right lobe and a long left lobe. The larger portion of the pancreas lies to the right of the median plane.

The dorsal surface of the pancreas faces dorsally and cranially and is in part covered by peritoneum. Its dorsal relationships are the ventral surface of the right kidney and adrenal gland, the caudal vena cava, the hepatic portal vein, the coeliac artery and its branches, the gastrophrenic ligament and saccus cecus of the stomach, the right and caudate lobes of the liver and the gastropancreatic fold. Grooves formed by the coeliac artery (and its branches) and the splenic vein are also present along the dorsal surface of the pancreas. For the most part, the ventral surface of the equine pancreas is concave and faces ventrally and caudally. An obliquely running ridge separates two impressions, the larger of which lies to the left. It is formed by contact of the ventral pancreas with the transverse colon and its junction with the small colon. The smaller impression lies to the right and is formed by contact with the base of the caecum. The ventral aspect of the pancreas usually has no peritoneal covering except over a small area at the duodenal angle.

The right border of the pancreas follows the descending duodenum. The left border is somewhat concave as it lies against the cranial aspect of the duodenum, the stomach and the splenic vessels. A deep notch, the *incisura pancreatis*, is present along the caudal border of the pancreas and is formed by the root of the mesentery. The hepatic portal vein perforates the pancreas obliquely near its caudal border (Figure 7). Dorsal to the vein, a thin bridge of glandular tissue is present which forms a pancreatic (portal) ring or *anulus pancreatis*.

Pancreatic Ducts

The body of the pancreas is attached to the duodenal sigmoid flexure and the adjacent part of the right lobe of the liver. From here, the pancreatic duct and the accessory pancreatic duct (which are both invariably present) leave to enter the duodenum (Figures 7 & 8). The pancreatic duct passes through the cranial margin of the pancreas, enters the wall of the duodenum obliquely, and opens into the hepatopancreatic ampulla alongside the common bile duct. The duct is situated in the pancreatic tissue proper, near its dorsal surface; there is no free part. The accessory pancreatic duct opens on a small papilla at the same level as the major duodenal papilla, but on the opposite wall of the duodenum.

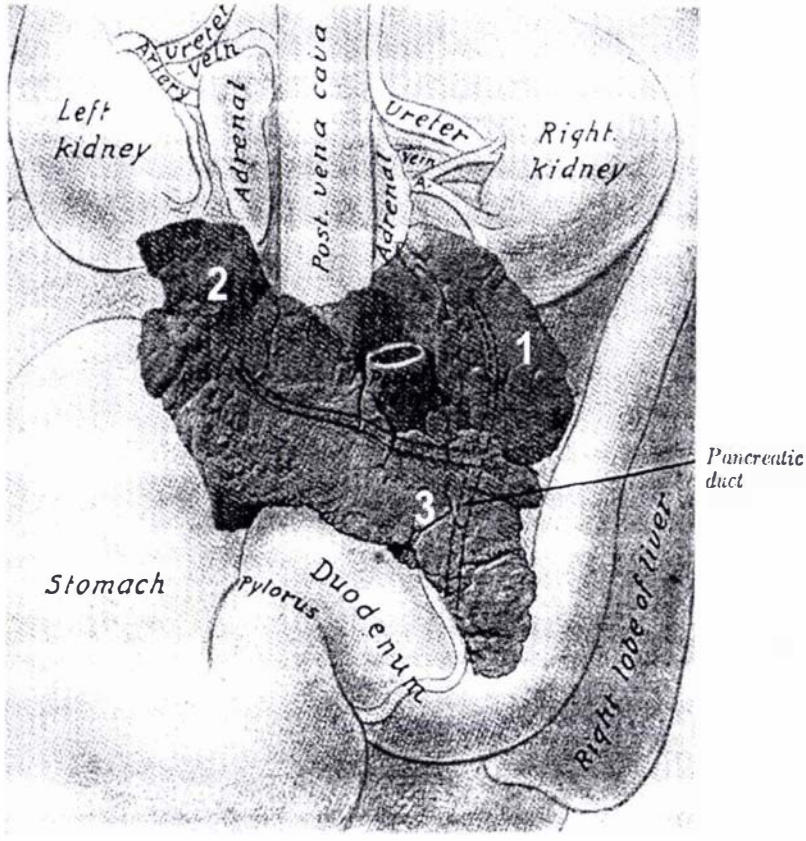

Figure 7. Ventral view of the pancreas and related structures of the horse. 1 right lobe of pancreas; 2 left lobe of pancreas; 3 body of pancreas. The pancreatic duct and its two chief radicles are indicated by dashed lines since they are in the substance of the gland. Post. vena cava = caudal vena cava. (Modified and reprinted from Sisson & Grossman's The Anatomy of the Domestic Animals, 5th edition, Getty, Volume 1, p 491, 1975, by permission of the publisher, WB Saunders Company)

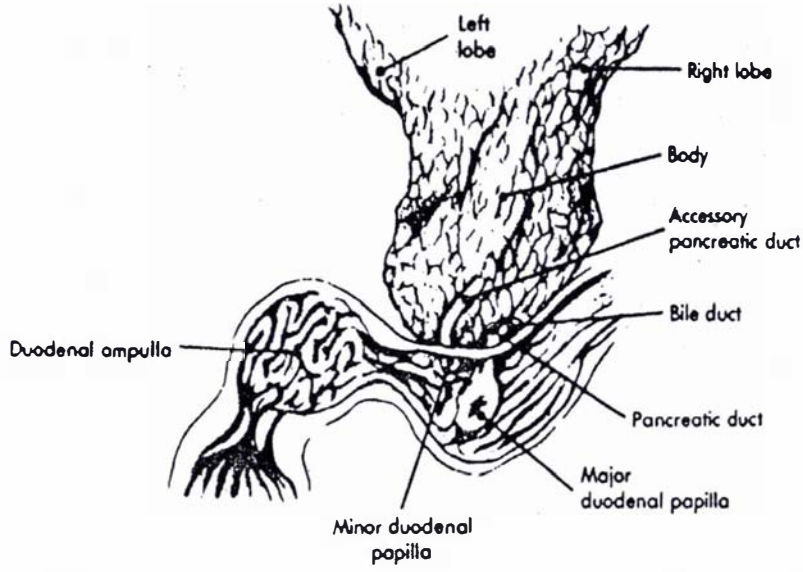

Figure 8. Sites of entry of the pancreatic ducts into the proximal duodenum of the horse. (Reprinted from Clinical Dissection Guide for Large Animals - Horse, Ox, Sheep, Goat, Pig; Constantinescu, p 100, 1991, by permission of the author, Dr GM Constantinescu)

CHICKEN (Gallus gallus)

Relations and Blood Supply

The pancreas of the chicken is long and thin. It is composed of dorsal and ventral pancreatic lobes as well as a very narrow strip of pancreas which courses toward the spleen. The dorsal and ventral pancreatic lobes are continuous with one another and can be readily identified extending longitudinally in the dorsal mesentery sandwiched between the descending and ascending limbs of the duodenum (Figure 9). This tight 'U'-shaped loop of duodenum is distinctive in many members of the avian species and can be found extending caudally along the caudal curvature of the gizzard. Both lobes are enclosed within a pancreaticoduodenal ligament, which is a double sheet of serosa apposing the two intestinal segments. The smaller splenic lobe (reportedly rich in islets of Langerhans) is embedded in adipose tissue, and may in some cases be difficult to see without the use of a microscope.

Differences between species in the relationships of the lobes of the pancreas to each other and with the duodenum have been described by Nickel *et al.* (1977). In the chicken and pigeon, the pancreas almost completely fills the gap between the two limbs of the duodenum, whereas in the duck and goose, the duodenal loop extends well beyond the pancreas. The dorsal and ventral lobes may be connected by a parenchymal bridge as in the chicken, or they may remain independent as in the duck and pigeon. Either may be present in the goose. The splenic lobe is connected with the dorsal lobe in the duck, goose and pigeon, while in the fowl, the splenic lobe joins both the dorsal and ventral main lobes.

Arterial blood reaches the dorsal and ventral lobes of the pancreas via the pancreaticoduodenal artery which is a direct continuation of the right branch of the coeliac artery (Figure 9). The single pancreaticoduodenal artery travels on the dorsal side of the duodenal loop partially embedded in pancreatic tissue. It gives off twelve or more collateral branches to both limbs of the duodenum and ends in the concavity of the duodenal loop (Paik *et al.*, 1969). Each collateral vessel gives off branches to the pancreas prior to reaching the mesenteric border of the duodenum where it then splits, sending branches around each side of the duodenum. The splenic lobe is supplied by the jejunal

and duodenojejunal branches of the coeliac artery. In the chicken, two hepatic portal veins are present (Figure 10). The dominant right portal vein corresponds to the mammalian portal vein. Venous blood passes in the pancreaticoduodenal vein from the dorsal and ventral lobes of the pancreas, and in the splenic vein from the splenic lobe of the pancreas to enter the right hepatic portal vein by way of the common mesenteric vein.

Pancreatic Ducts

Two or three ducts convey pancreatic fluid into the duodenum (Figure 9). In the chicken and pigeon, three pancreatic ducts are generally present. Two of these arise from the ventral lobe and one arises from the dorsal lobe. In the duck and goose, usually only two ducts are present, however three sometime occur. The splenic lobe has no separate duct. In all species, the pancreatic ducts and the bile duct open into the *ascending* duodenum opposite the cranial portion of the muscular stomach.

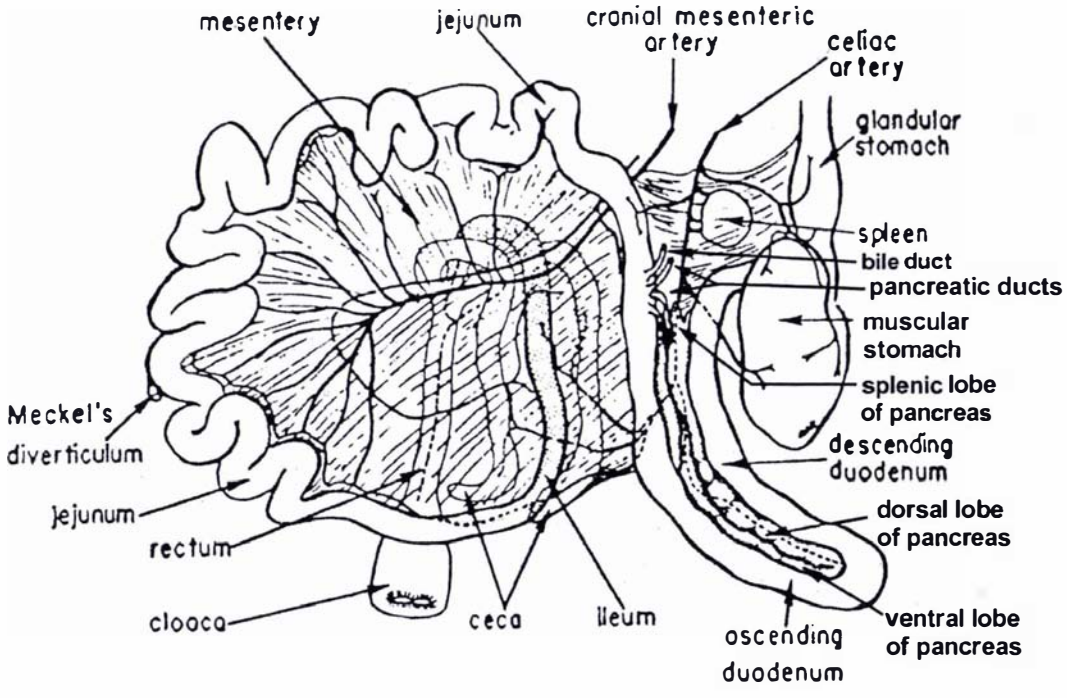

Figure 9. Ventral view of the gastrointestinal tract of the chicken. The dorsal pancreatic lobe is associated with the descending duodenum while the ventral pancreatic lobe is associated with the ascending duodenum. Muscular stomach = gizzard. **(Modified and reprinted from Sisson & Grossman's The Anatomy of the Domestic Animals, 5th edition, Getty, Volume 2, p 1873, 1975, by permission of the publisher, WB Saunders Company)**

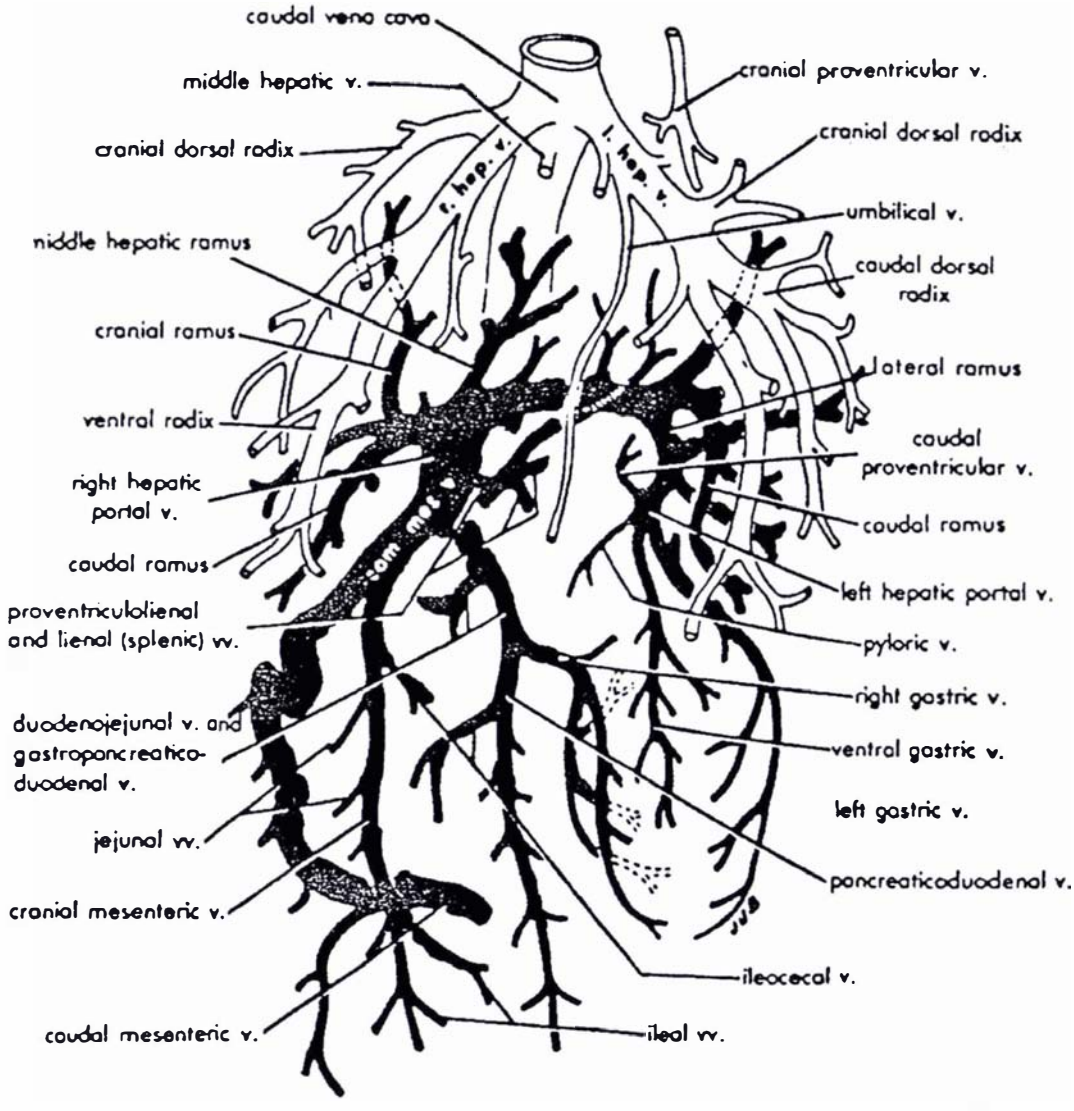

Figure 10. Ventral view of the portal veins and hepatic veins of the chicken showing major tributaries of both systems (drawn from dissections). (Reprinted from Sisson & Grossman's The Anatomy of the Domestic Animals, 5th edition, Getty, Volume 2, p 2005, 1975, by permission of the publisher, WB Saunders Company)

MAN (Homo sapiens)

Relations and Blood Supply

The human pancreas is large and flat, finely lobulated, and pinkish-tan in colour. It extends nearly transversely across the posterior abdominal wall posterior to the stomach from the duodenum to the spleen. In man, the dorsal (left) and ventral (right) lobes of the pancreas are referred to by region rather than by lobe (Figure 11). The greatest mass is concentrated in the *head* of the pancreas, which represents the broad right extremity of the gland and corresponds to the body of the pancreas of lower mammals. It is lodged within the cranial and caudal duodenal flexure and it is here that pancreatic juice is conveyed to the duodenum. From the inferior and left part of the head, there is an extension called the *uncinate process,* which projects superiorly and to the left inserting itself posteriorly between the superior mesenteric vessels (which correspond to the cranial mesenteric vessels in quadrupeds) and the horizontal limb of the duodenum. This process, along with a portion of the head of the pancreas, represents the right or ventral lobe of the pancreas. The head of the pancreas is connected to the *body* by the *neck*[4] which is constricted posteriorly by the superior mesenteric vessels and the hepatic portal vein. These vessels lie in the *pancreatic incisure*, a deep groove lying on the posterior surface of the neck, and are trapped by the neck superiorly and anteriorly, and by the uncinate process inferiorly and posteriorly. The body of the pancreas lies above the duodenojejunal flexure and on the left kidney. Its narrow left extremity forms the *tail* of the pancreas which passes obliquely to the left and slightly posteriorly across the posterior wall of the abdomen terminating at the hilus of the spleen. A portion of the head and the entire neck, body and tail correspond to the left or dorsal lobe of the pancreas. The head and body of the pancreas are retroperitoneal (only their anterior surface is covered with peritoneum), while the tail of the pancreas lies within a portion of the greater omentum which is free and therefore moveable.

[4] The term 'neck' of the pancreas is not recognized by the Nomina Anatomica, however it is found in certain standard human anatomy textbooks (Figure 11 of this chapter for an example). It is used in this thesis to describe that area of the human pancreas that lies between the head and the body.

Most of the anterior surface of the pancreas faces into the omental bursa and, through the bursa, is related to the stomach. The anterior surface of the body is concave and is covered with peritoneum, as is the anterior surface of the neck which supports the pylorus with a portion of the omental bursa intervening. The anterior border of the head of the pancreas lies inferior to, or is slightly overlapped by, the superior aspect of the duodenum, while the right and left borders are very closely related to the descending and ascending limbs of the duodenum, respectively (Figure 12). Sometimes a small part of the head of the pancreas is actually embedded in the wall of the descending limb of the duodenum. On the right side, the boundary between the head and the neck is marked by a groove formed by the gastroduodenal artery. The gastroduodenal and the superior pancreaticoduodenal arteries descend in front of the gland to the right side of the junction of the neck with the head (see below). Inferiorly and to the right of the neck, the anterior surface of the pancreas is in contact with the transverse mesocolon, the two surfaces being separated only by fat. Below this, the pancreas is lined with peritoneum, which is continuous with the inferior layer of the transverse mesocolon and also makes contact with a coil of the jejunum. The uncinate process is crossed anteriorly by the superior mesenteric vessels as previously mentioned.

The posterior surface of the head of the pancreas is associated with the hilum of the right kidney and the terminal parts of the renal veins, the inferior vena cava and the right crus of the diaphragm. The bile duct lies either in a groove on the superior and lateral aspect of the posterior surface of the head or in a canal in the parenchyma of the gland (Figure 13). Also running along the posterior aspect of the neck and within the *pancreatic incisure* are the superior mesenteric vessels and the hepatic portal vein. The posterior surface of the body of the pancreas is devoid of peritoneum and makes contact with the aorta and the origin of the superior mesenteric artery, the left crus of the diaphragm, the left adrenal gland and the left kidney and its vessels, particularly the left renal vein. It is also related to the splenic vein, which courses from left to right, and separates the pancreas from the above mentioned structures. The left kidney is further separated from the pancreas by the perirenal fascia and fat. The dorsal surface of the tail of the pancreas is associated with the spleen.

The pancreas and neighbouring duodenum are supplied with arterial blood by branches of the coeliac trunk and the superior mesenteric artery. Venous drainage, in general, follows the pattern of the arterial supply. Veins draining the pancreas join the hepatic portal vein, the splenic vein and the superior mesenteric vein (Figure 14). Whereas in the dog, the caudal mesenteric vein joins the cranial mesenteric vein, in man, the equivalent of this vessel i.e., the inferior mesenteric vein, joins the splenic vein instead (compare Figures 3 & 15).

The coeliac trunk originates from the aorta at the upper margin of the pancreas. The common hepatic artery, one of the coeliac artery's three main branches, runs to the right along the upper margin of the neck and head and gives off the gastroduodenal artery before coursing superiorly towards the liver. The duodenum and the head of the pancreas are served by two parallel arcades of vessels which are analogous to the single cranial and caudal pancreaticoduodenal artery and vein in the dog. They are the *anterior* and *posterior superior* pancreaticoduodenal arteries and veins, and the *anterior* and *posterior inferior* pancreaticoduodenal arteries and veins, which are located in the concavity of the duodenum on the surface of (or embedded in) the head of the pancreas (Figure 16). The posterior superior pancreaticoduodenal artery and vein run inferiorly along the left side of the common bile duct. Pancreatic branches emerge from these arcades as well as from the coeliac trunk directly and form multiple anastomoses with pancreatic vessels originating from the splenic artery and vein.

The splenic artery, which supplies the remainder of the pancreas, runs to the left along the upper margin of the body before coursing to the anterior surface of the tail (Figure 14). The splenic vein lies along the posterior aspect of the body and tail of the pancreas. The convergence of the splenic vein with the superior mesenteric vein forms the hepatic portal vein on the posterior surface of the pancreas (Figure 13).

Pancreatic Ducts

The main pancreatic duct (Duct of Wirsung, 'major' duct or 'chief' duct) extends transversely through the substance of the pancreas from left to right through the body, receiving tributaries from the entire tail, body, neck and from the posterioinferior portion

of the head, including the uncinate process (Figure 17). Upon reaching the neck of the pancreas, it turns posteriorly and to the right where it becomes associated with the common bile duct, which lies on its right side. In the head, the common bile duct penetrates the pancreatic substance or lies in a groove on the posterior surface. The two ducts penetrate the duodenal wall obliquely in parallel fashion and may unite in the duodenal wall to form the hepatopancreatic ampulla (of Vater). The common bile duct and the main pancreatic duct join into a common channel (the ampulla of Vater) in approximately 80% of cases, and open into the duodenal lumen separately in approximately 18% of cases. A functional main pancreatic duct is missing in the remainder (Hand, 1963). The main pancreatic duct and the bile duct empty into the duodenal lumen at the major duodenal papilla (of Vater). This papilla is located along the medial aspect of the descending duodenum 8-10 cm below the pylorus[5]. The accessory pancreatic duct (Duct of Santorini) may anastomose with the main pancreatic duct and its branches. While not always functional, the accessory pancreatic duct drains the anterosuperior aspect of the head emptying approximately 2 cm superior to and slightly anterior to the major duodenal papilla. In approximately 40% of adults, the accessory pancreatic duct has no patent connection with the main pancreatic duct; in approximately 7% of adults, the accessory duct is as large as or larger than the main pancreatic duct. On occasion, the accessory pancreatic duct does not connect with the duodenum at all, but instead drains into the main pancreatic duct.

[5] Whereas in domestic animals the major duodenal papilla and its associated ducts are located cranial to the minor duodenal papilla, the situation is reversed in man.

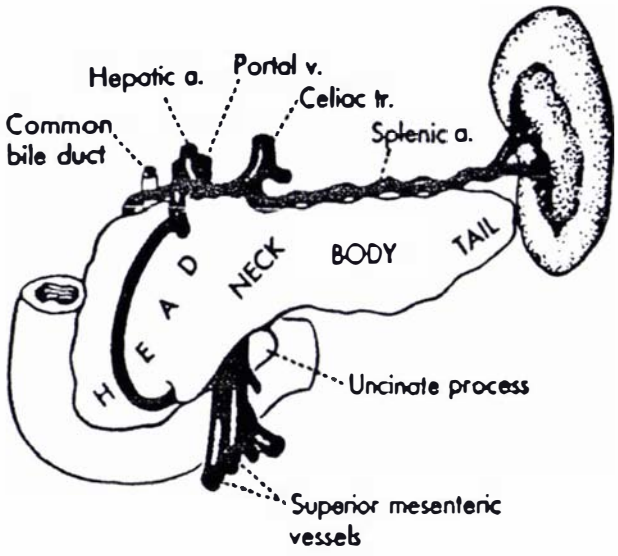

Figure 11. Parts of the human pancreas (anterior view). The head of the pancreas corresponds to the body of the pancreas in lower mammals. The uncinate process along with a portion of the head represent the ventral (right) lobe of the pancreas. The remainder of the head, and the entire neck, body and tail correspond to the dorsal (left) lobe of the pancreas. (Reprinted from Hollinshead's Textbook of Anatomy, 5th edition, Rosse & Gaddum-Rosse, p 563, 1997, by permission of the author, Dr C Rosse)

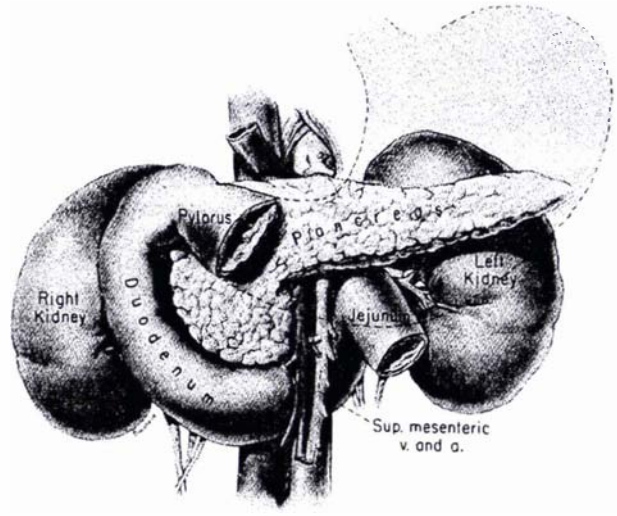

Figure 12. Anterior relations of the human pancreas. Except for part of the pylorus, the stomach is indicated only in outline. The uncinate process is obscured by the superior mesenteric vessels. Note the intimate relationship of the body of the pancreas with the duodenum. (Reprinted from Hollinshead's Textbook of Anatomy, 5th edition, Rosse & Gaddum-Rosse, p 560, 1997, by permission of the author, Dr C Rosse)

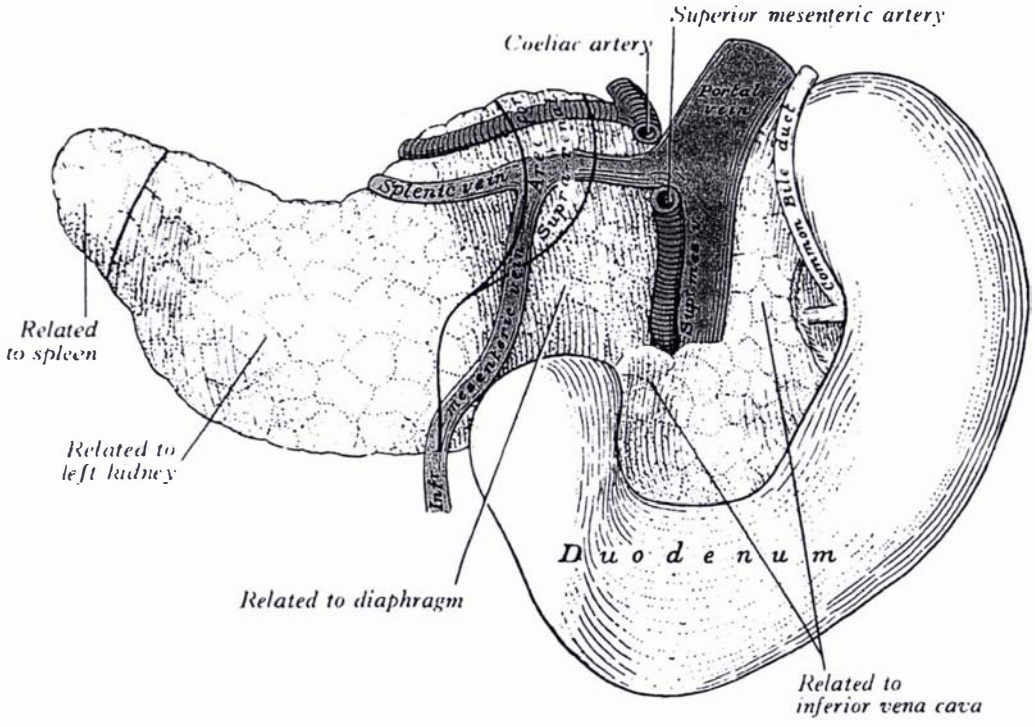

Figure 13. Posterior aspect of the human pancreas and duodenum. Note how the portal vein is sandwiched between the posterior aspect of the neck and the anterior aspect of the uncinate process (within the pancreatic incisure). (Reprinted from Gray's Anatomy. The Anatomical Basis of Medicine and Surgery, 38th edition, Williams, et al., p 1790, 1995, by permission of the publisher, Churchill Livingstone)

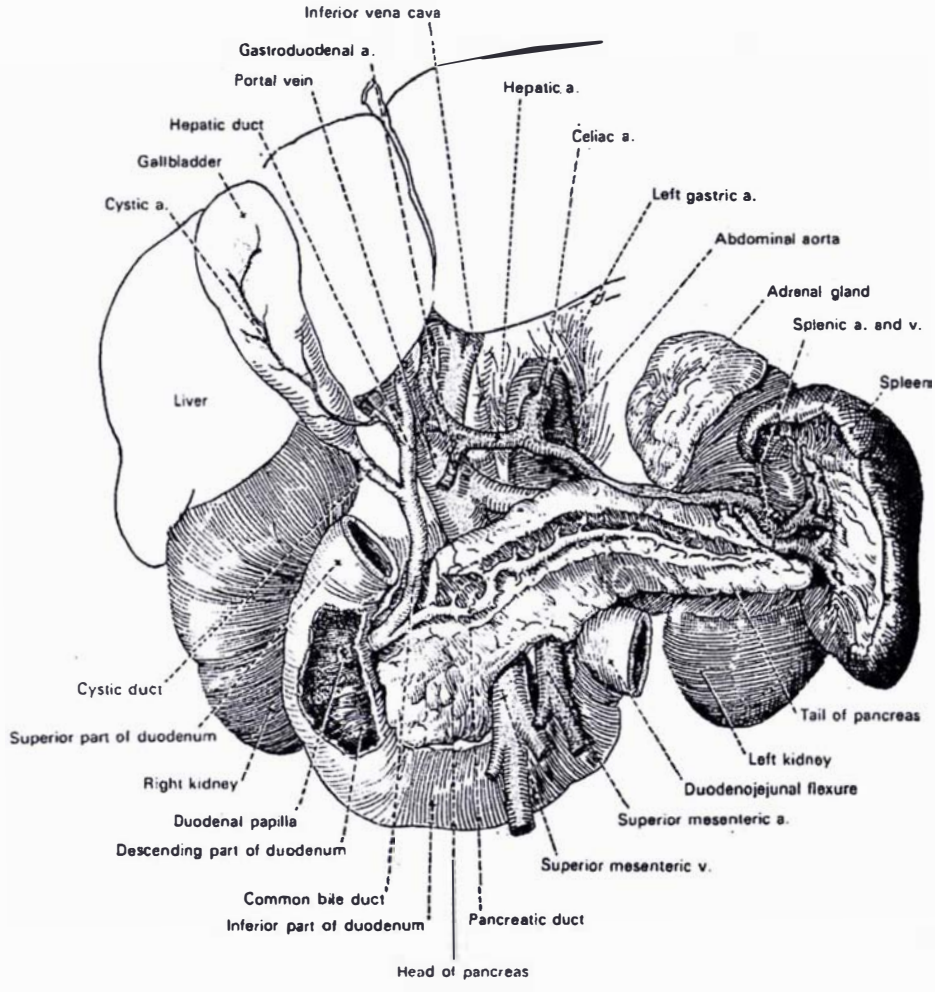

Figure 14. The major arteries and veins of the human pancreas and their relations to surrounding structures (anterior view). The arterial blood supply to the pancreas originates from the coeliac and superior mesenteric arteries. Venous drainage is provided by the portal vein, the splenic vein and the superior mesenteric vein. **(Reprinted from Anatomy of the Human Body, 30th edition, Gray, p 1505, 1985, by permission of the publisher, Lea & Febiger)**

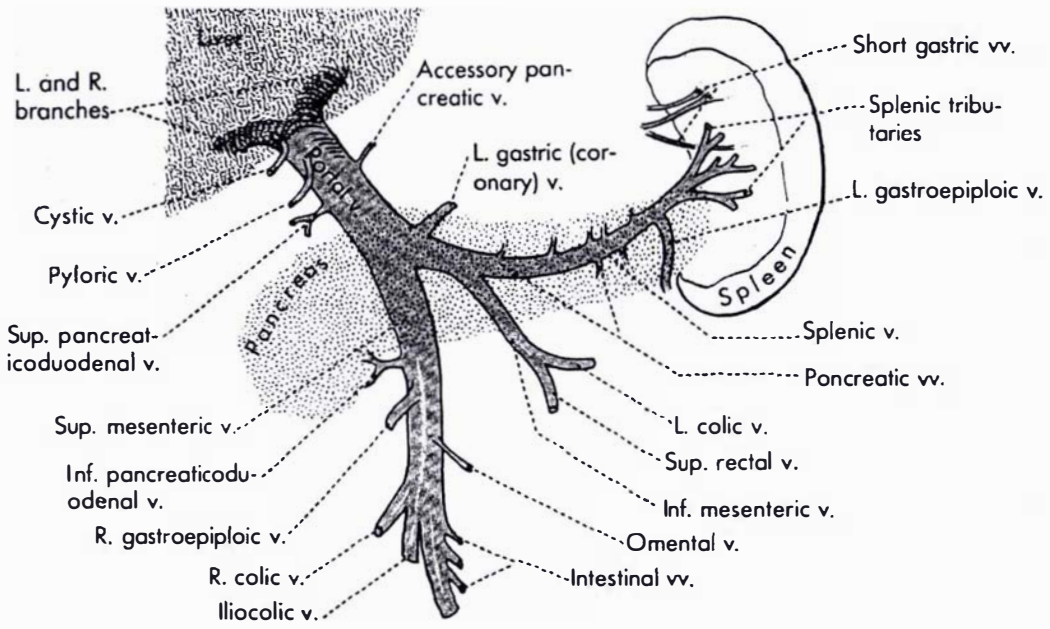

Figure 15. Layout of the human portal vein and its tributaries (anterior view). Note how the inferior mesenteric vein (analogue of the caudal mesenteric vein in quadripeds) drains into the splenic vein rather than the superior mesenteric vein in this species. Compare with Figure 3. (Reprinted from Hollinshead's Textbook of Anatomy, 5th edition, Rosse & Gaddum-Rosse, p 577, 1997, by permission of the author, Dr C Rosse)

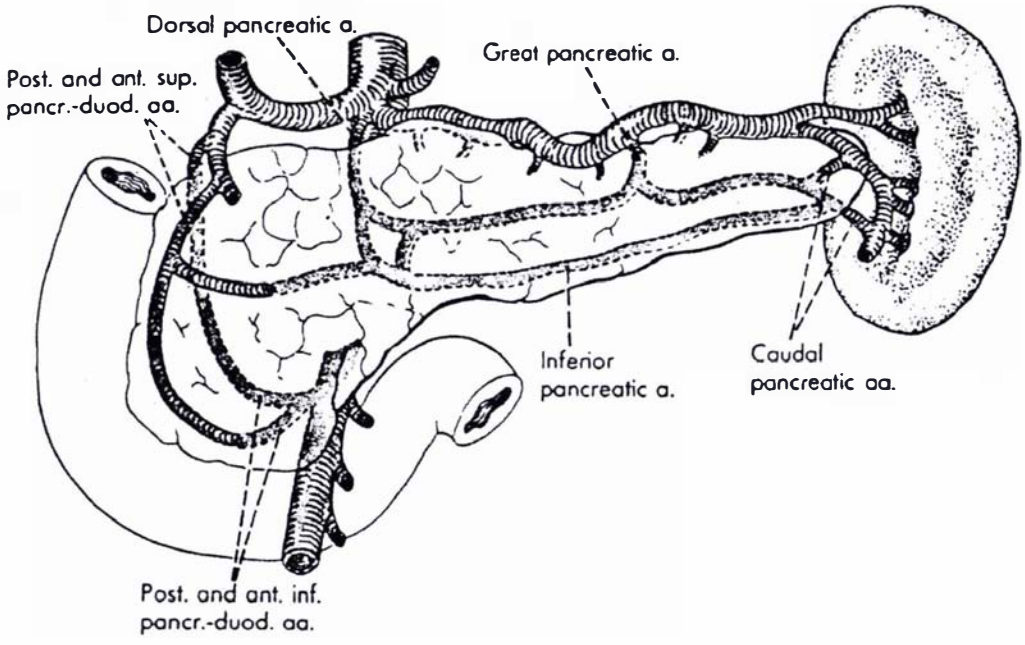

Figure 16. The chief arteries of the human pancreas and their anastomoses (anterior view). Note how the superior and inferior pancreaticoduodenal arteries (and veins - not shown) are paired in man. (Reprinted from Hollinshead's Textbook of Anatomy, 5th edition, Rosse & Gaddum-Rosse, p 568, 1997, by permission of the author, Dr C Rosse)

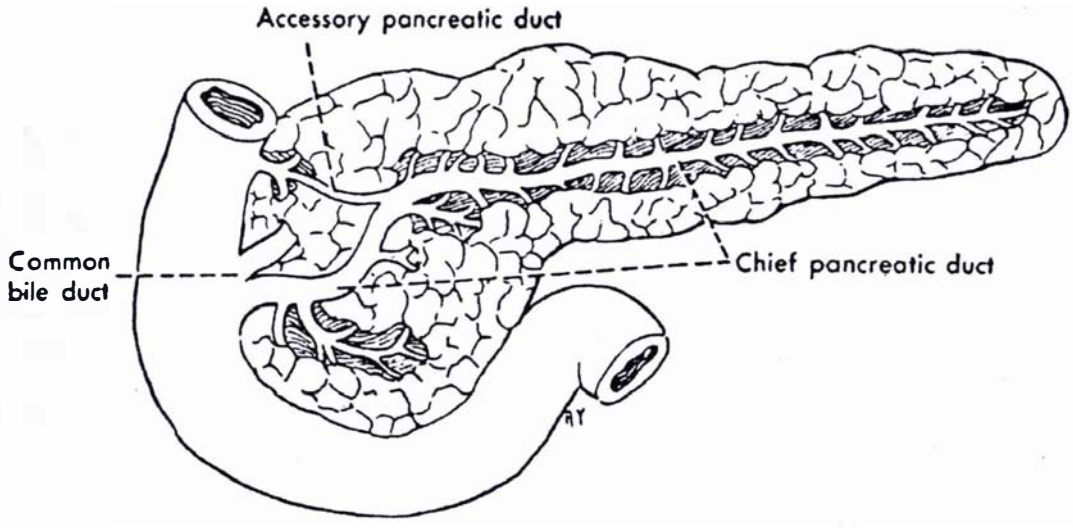

Figure 17. The arrangement of the pancreatic ducts in man (anterior view). Note that the accessory pancreatic duct enters the duodenum cranial to the (chief) pancreatic duct in man. (Reprinted from Hollinshead's Textbook of Anatomy, 5th edition, Rosse & Gaddum-Rosse, p 565, 1997, by permission of the author, Dr C Rosse)

SUMMARY OF SPECIES DIFFERENCES

A summary of the species differences with respect to which pancreatic duct persists in the adult is presented in Table 1. This information is derived from the textbooks of anatomy cited in the introduction to this chapter.

A summary of the differences in the relationships of the pancreas to the duodenum and hepatic portal system is presented in Table 2. The information provided is derived primarily from the textbooks of general anatomy cited in the introduction to this chapter, however, the descriptions of the *closeness* and *degree* of contact between the pancreas and duodenum (in all species except man) have arisen in part from the author's own observations of post mortem specimens (see footnotes to Table 2).

Table 1. Differences between species in pancreatic duct retention

	Persistence of ventral primordium (Pancreatic duct)	**Persistence of dorsal primordium (Accessory pancreatic duct)**
Dog	+ (occasionally -)	+
Cat	+	- (occasionally +)
Ox	- (occasionally +)	+
Sheep & Goat	+	-
Pig	-	+
Horse	+	+
Chicken	+ (2 ducts)	+ (1 duct)
Man	+	+ (occasionally -)

Table 2. Summary of the relationships of the pancreas to the duodenum and the hepatic portal system of veins

	Pancreas-Duodenum	Pancreas-Hepatic Portal System
Dog	**Body of pancreas very closely associated[1] with proximal descending limb of duodenum (close contact zone)**	**Close contact zone houses dense network of veins which provide common drainage to pancreas and duodenum**
Ox	**Pancreas loosely associated[2] with proximal descending limb of duodenum**	**Pancreas partially surrounds hepatic portal vein (*annulus pancreatis*)**
Pig	**Pancreas loosely associated[2] with proximal descending limb of duodenum**	**Pancreas completely surrounds hepatic portal vein (*anulus pancreatis*)**
Horse	**Pancreas loosely associated[2] with proximal descending limb of duodenum**	**Pancreas completely surrounds hepatic portal vein (*incisura pancreatis*)**
Chicken	**Pancreas tightly enclosed[3] between descending and ascending limbs of duodenum**	**Pancreas houses dense network of veins which provide common drainage from pancreas and duodenum**
Man	**Right and left borders of head of pancreas very closely associated with descending and ascending limbs (respectively) of duodenum (close contact zones)**	**Close contact zones house dense networks of veins which provide common drainage from pancreas and duodenum; Pancreas partially surrounds hepatic portal vein (*incisura pancreatis*)**

[1] pancreas appears to 'contact' or 'embed' itself in the wall of the small intestine (author's observation based on 24 dissections)

[2] direct 'contact' of the pancreas with the duodenum is lacking; pancreas approaches duodenum closely only in the area(s) of the pancreatic duct(s) (author's observation based on dissections of four oxen, 16 pigs and two horses)

[3] pancreas enclosed within loop of duodenum, but direct or apparent contact is lacking (author's observation based on four dissections)

DISCUSSION

This chapter describes the embryology and comparative aspects of the topographical anatomy of the pancreas of selected birds and mammals. Particular emphasis is placed on the relationships of the pancreas with the gut and hepatic portal system of veins. The information on the general relationships is derived primarily from the textbooks of anatomy cited in the introduction to this chapter, augmented by the author's own observations from post mortem specimens (Table 2).

In all species described with the exception of avian, in which the pancreas is relatively accessible lying along the ventral body wall, the pancreas is located deep within the abdomen, centred roughly over the midline near the dorsal body wall. It is bordered by the aorta (and its branches) and caudal vena cava dorsally, the liver cranially and dorsally, the greater curvature of the stomach cranially and ventrally, the right kidney dorsally and to the right, the left kidney and spleen to the left, and the intestinal mass caudally and ventrally. The pancreas is always associated, albeit to varying degrees, with the descending limb of the duodenum and the veins of the hepatic portal system.

This review reveals several interesting relationships between the pancreas, the gut and the veins of the hepatic portal system (Table 2). In terms of location, the body and right lobe of the pancreas lie adjacent to the descending limb of the duodenum, and in fact, this consistent relationship in the species described, facilitates immediate identification of the pancreas upon opening the abdomen. However, differences between species exist in the *closeness* with which the body of the pancreas is apposed to the duodenum. In the dog and in man, the body (or head) of the pancreas is very closely associated with either the descending limb or both the descending and ascending limbs of the duodenum; it appears to 'contact' or 'embed' itself in the wall of the small intestine in these species. In the chicken, a further variant of this relationship is observed in that the pancreas lies close to the duodenum along its entire length. In addition, both the dorsal and ventral lobes of the pancreas are tightly enclosed between the descending and ascending limbs of the duodenum (respectively) forming a distinctive 'U'-shaped loop, which extends caudally from the gizzard. In the ox, pig and horse, direct or apparent 'contact' of the pancreas with the duodenum is lacking. Rather, the pancreas of these species is more loosely associated

with the small intestine, lying within the mesoduodenum, and approaching the duodenum closely only in the area(s) of their pancreatic duct(s).

The relationships of the pancreas with the portal vein and its tributaries also differ between the species mentioned. In both the pig and horse, the portal vein can be seen to *perforate* the pancreas en route from gut to liver causing the formation of a ring or anulus (the *anulus pancreatis*). Alternatively, the pancreas can be said to completely surround the portal vein. This association is also evident, but to a lesser extent, in species such as the ox and man, where the portal vein *indents* rather than *perforates* the pancreas, forming a deep notch or incisure (*incisura pancreatis*) near its body. In these species, the pancreas can be said to partially surround the vein. While a similar relationship of the pancreas with the portal vein itself does not appear to exist in the dog and chicken, it is of interest to note that in these and other species in which a portion of the pancreas is consistently found to contact and/or closely approximate the duodenum, the blood supply to the area is complex. At these sites, common drainage between the duodenum and pancreas is accomplished by way of the pancreaticoduodenal veins.

While the above mentioned anatomical relationships have been fairly well described in most textbooks of general anatomy, there has been little attention paid to the comparative aspects of the associations between the pancreas and duodenum, portal vein and its tributaries. Consequently, no questions appear to have been raised regarding the significance of these observations. It is conceivable that biological reasons exist for the anatomical relationships described. For example, one could question why absorbed nutrients, hormones and metabolites must first pass through the liver and enter the systemic circulation before affecting the activities of the pancreas which lies in such close anatomical proximity to the very organ to which the exocrine pancreas responds. The location of the pancreas adjacent to the proximal descending limb of the duodenum and within the confines of the hepatic portal system, would seem to place it in a prime anatomical position for receiving signals regarding the digestive status of the animal, and hence, for exerting some degree of local regulation on the pancreas. One could argue that all of the anatomical associations drawn between the pancreas and the gut and veins of the hepatic portal system can be explained simply by their embryological origins, and that

there exists no other biological or functional reason(s) for their ultimate positions within the body. After all, both the dorsal and ventral lobes of the pancreas originate from a site high in the primitive gut tube, an area which ultimately develops into the duodenum. One could argue that the very close relationship of, for example, the body of the pancreas with the proximal duodenum in the dog, is simply to facilitate introduction of pancreatic and bile secretions into the upper duodenum. This would ensure that digestion was complete or almost complete at the end of the small intestine. Alternatively, this very close anatomical relationship may protect the pancreatic ducts from injury. One could argue that the reason why the pancreas of some species either partially or completely wraps itself around the portal vein is due to the manner in which the developing circulatory system 'invades' the gut. In this scenario, the pancreas may simply be "in the way". The final relationships and positions of these structures close to the dorsal body wall could also conceivably exist for economy of space within the abdomen, or may occur subsequent to elongation and maturation of both the gut and its supporting mesentery. In these respects, the relationships observed would simply reflect developmental changes occurring over time.

On the other hand, the contents of the gastrointestinal tract (or blood draining the gastrointestinal tract) would appear to be an ideal 'sampling' medium for the pancreas. The anatomical relationships described could facilitate a local relay of information from the gut or portal system to the pancreas, and in this manner, could supplement the more conventional forms of pancreatic control. Mechanisms by which such forms of communication could exist between the gut (or portal system) and the pancreas include local vascular pathways, neural mechanisms, or a combination of both. Stimulation of the exocrine pancreas via local vascular pathways may result from a direct interconnection of veins between the duodenum and the pancreas (dog, chicken, man), or by a shunting of blood from the cranial pancreaticoduodenal vein (dog, chicken, man) or from the portal vein (pig, horse, ox, man) *through* the pancreas. Nervous mechanisms would most likely involve direct stimulation of the pancreas by enteric neurons (possibly all species). Such arrangements, be they humoral, neural or otherwise, would provide for an early and continuous update on the digestive status of the animal, and would allow for early modifications of pancreatic output i.e., *before* absorbed nutrients, hormones and metabolites reach the pancreas by way of the systemic circulation. Although the primary

focus of this thesis is exocrine pancreatic function, such local forms of control may also, or exclusively, exist for endocrine pancreatic function, as mentioned in Chapter 1. If indeed any such forms of communication exist, it would appear from the cited differences in the anatomy, that the predominant mechanism may differ between species.

A more thorough understanding of the anatomical relationships described in this chapter may add significantly to our knowledge of mechanisms which control the exocrine pancreas. The biological significance of the spatial associations between the pancreas and its surrounding organs is questioned. The hypothesis that some form of local or direct communication exists between the gut or hepatic portal system and the pancreas, and that this communication plays a supplementary role in the control of exocrine pancreatic function, is the subject of the remainder of this thesis.

Chapter Three

MACROSCOPIC INVESTIGATION OF THE HEPATIC PORTAL SYSTEM OF THE DOG: A STUDY USING LATEX VASCULAR CASTS

SUMMARY

In the dog, the body of the pancreas is closely associated with the proximal descending limb of the duodenum, and in this area of close apposition, the venous anatomy is complex. The possibility that an interconnection of veins between the duodenum and pancreas exists which may allow some degree of local regulation on the pancreas has been postulated by the author, but has not been investigated. The primary objective of this study was to examine this area of the hepatic portal system for evidence of a direct venous communication between the duodenum and/or veins of the hepatic portal system and the pancreas. Vascular casts of the portal vein and its tributaries were prepared using nine canine specimens, each of which was subjected to varying degrees of dissection and maceration. The results showed that the general arrangement of the portal vein and its tributaries is consistent with our current understanding of the portal system of the dog. That is to say that the primary branches of the portal vein, the gastroduodenal, splenic and caudal pancreaticoduodenal veins, together with the ileocolic, cranial mesenteric, caudal mesenteric, and jejunal veins, feed into the main portal trunk. Anastomoses are commonly observed. In areas where the duodenum and pancreas are closely apposed, a distinction was easily made between capillary beds of pancreatic origin and those of duodenal origin. Tributaries of duodenal origin were sometimes observed to coalesce first with veins draining the pancreas prior to entering the cranial pancreaticoduodenal vein. In two areas in one specimen, duodenal tributaries were observed to closely approach capillary beds of pancreatic origin, however the relationships of these vessels with one another could not be clearly determined. Also observed were valve imprints throughout the portal system. Symmetrically bi-lobed, 'V'-shaped flap impressions were identified adjacent to primary, secondary and tertiary branch points relative to the main portal trunk. In all cases, the valve imprints indicated blood flow in the direction of the portal vein. In conclusion, there was no firm evidence to suggest that a direct interconnection of veins exists between the duodenum and pancreas, however the possibility cannot be ruled out on the basis of this study alone. The results of this investigation also showed that blood flow within the portal system is directed by valves, and that these valves direct flow toward the portal vein, rather than toward the pancreas. Further insight into the relationships of the duodenum with the pancreas in their area of close apposition may be provided by examining the *microscopic* features of this area in the dog.

INTRODUCTION

The hepatic portal system is a unique collection of veins which drain the spleen, stomach, pancreas, small intestine and a major part of the large intestine. These veins converge into a common trunk, the hepatic portal vein, prior to entering the liver. Whereas the arteries supplying the above mentioned organs originate from the abdominal aorta, the corresponding veins do not drain directly into the abdominal caudal vena cava. Instead, they form an isolated collection system which effectively diverts blood from the gut to the liver for detoxification and other processing prior to delivery into the systemic venous system. In addition, an important function of the hepatic portal system is the transport of gut-derived nutrients, metabolites and hormones into the systemic circulation. The re-circulation of many of these absorbed factors back to the pancreas plays a critical role in the regulation of digestive processes (Chapter 1).

Earlier observations by the author regarding the topographical anatomy of the pancreas of a variety of species revealed that the pancreas is not only closely associated with the proximal duodenum, but is also anatomically associated with the veins of the hepatic portal system (Chapter 2). These anatomical relationships have been found to exist to varying degrees in different species and it has been proposed by the author that they may have functional significance in terms of the control of pancreatic function. It is conceivable that some form of local vascular communication exists between the gut or hepatic portal system and the pancreas (a 'duodenum-to-pancreas' venous communication), which effectively 'signals' the pancreas regarding the digestive status of the animal. Such communication could take the form of a direct interconnection of veins between the duodenum and pancreas, whereby blood draining the proximal duodenum would first pass through pancreatic tissue prior to draining into the down-stream vessel. Alternatively, one or more vessels may divert some portion of blood from the cranial pancreaticoduodenal vein (which also drains the duodenum) through the pancreas, prior to drainage back into the portal system.

As described in Chapter 2, the body of the pancreas in the dog is closely associated with the proximal descending limb of the duodenum, and in this area of close apposition, the blood supply is complex. The cranial pancreaticoduodenal vein (as well as the

corresponding artery) is enveloped by the body of the pancreas on three sides, and is enclosed by the medial wall of the proximal descending limb of the duodenum on the other. This circumstance precludes a direct view of either the parent vessel or its branches which emanate from the pancreas and duodenum, and obscures from direct observation, any potential 'portal' interactions which may exist between the two organs. If indeed some form of local vascular communication exists between the gut and pancreas, it would seem logical to examine this area in more detail.

The argument that a portal system exists between the gut and the exocrine pancreas (Chapter 1) would be strengthened by evidence which would suggest that blood flow is indeed 'directed' in such a manner. Directed flow of blood through the pancreas would allow the pancreas to effectively 'sample' high concentrations of gut-derived nutrients and hormones prior to their delivery into the systemic circulation. Physical evidence which would support the presence of directed blood flow in veins may be indicated by the presence of venous valves. While it is generally accepted that blood always flows in the direction of the portal vein, it is conceivable that the presence (or lack thereof) of valves at strategic locations, may effectively shunt blood (or fail to impede its flow) through some part or parts of the pancreas for such 'sampling' to occur.

Intact anatomical specimens, while providing useful information regarding topography, provide little opportunity to study the details of the vascular anatomy in relation to surrounding structures. Vascular casts provide an alternative means by which such information can be obtained. The advantages of using vascular casts are that the pattern of the arterial supply and venous drainage of an organ can be visualized macroscopically, and depending upon the degree of removal of surrounding soft tissues, their detailed anatomy and three-dimensional organization both within and around the viscera may be examined. These techniques were utilized in the present study in order to clarify further the details of the drainage of venous blood from the canine gut and pancreas.

This study was undertaken to obtain the following information: first, general information regarding the overall arrangement of the veins of the hepatic portal system, particularly those veins providing a common drainage from the duodenum and pancreas; and second,

evidence which might support the existence of directed blood flow. From this information, it was anticipated that preliminary conclusions might be drawn regarding the likelihood of a direct duodenum-to-pancreas venous communication.

MATERIALS AND METHODS

Nine specimens of the canine gut were examined, seven of which were prepared for the present study (specimens #3 to #9) and two of which were previously prepared for other reasons (specimens #1 & #2). The latter included arterial as well as venous latex casts, however, for the purposes of this investigation, only the protocols for the *venous* casting of these specimens are described. All specimens used in the vascular cast studies were obtained from dead animals which originated from either private owners who gave their permission for the euthanasia and later use of their dog(s) for anatomical study, or from a public pound, the euthanasia and later use of cadavers for anatomical study being authorized by a certified Animal Control Officer. Massey University Animal Ethics approval was obtained for all procedures prior to their being carried out.

ANAESTHESIA AND SPECIMEN PREPARATION

Seven lean, adult dogs of various breeds were sedated by intramuscular injection of ACP (0.03-0.125 mg/kg body weight; Acepromazine, 2 mg/ml, C-Vet Ltd, Suffolk) before being anaesthetized with an intravenous injection of Nembutal (28 mg/kg body weight, to effect; Pentobarbitone sodium, 60 mg/ml, Techvet Laboratories Ltd, Auckland). An anticoagulant, heparin (1000 IU/kg body weight; Heparin sodium, 25,000 IU/ml, Leo Pharmaceutical Products, Denmark), was administered to all dogs intravenously following induction. Anaesthetized animals were then subjected to procedures according to one of two protocols.

Summary of protocol 1 (specimens #1 & #2)

Cannulae (Biolab Scientific NZ, Ltd)[6] were inserted into a common carotid artery (in cranial and caudal directions), an external jugular vein (in cranial and caudal directions) and a femoral vein (in proximal and distal directions). Anaesthetized dogs were then euthanased by exsanguination. Following death, the vasculature of the cadaver was flushed with saline infused into the common carotid artery (50-200 ml saline/min; 0.15 M NaCl solution at 37° C; laboratory prepared; Masterflex peristaltic pump and controller, Cole Parmer Instrument Co, USA) with drainage from the open ends of cannulae inserted into the external jugular and femoral veins. The gastrointestinal tract (with liver, spleen and diaphragm) was excised from the cadaver along with the attached segments of aorta and caudal vena cava (see procedures for removal of gastrointestinal tract described below). Specimens were submerged in saline and placed in a chiller which was maintained at 4° C for 24 hours to allow time for arrest of smooth muscle activity.

Summary of protocol 2 (specimens #3 to #9)

Anaesthetized animals were euthanased by an intravenous injection (lethal overdose) of magnesium sulfate (saturated solution; laboratory prepared) administered via a cephalic vein. As with protocol 1, the gastrointestinal tract (with liver, spleen and diaphragm) was excised from the cadaver along with the attached segments of aorta and caudal vena cava (see below). Each specimen was then submerged in saline, its blood vessels were cannulated and the vasculature was flushed (see procedures for flushing the isolated gastrointestinal tract below). Finally, the specimens were placed in a chiller at 4° C for 24 hours.

Summary of procedures for removal of gastrointestinal tract (specimens #1 to #9)

Each of the euthanased animals were placed in right lateral recumbency and the left abdominal wall was removed (Figure 1). The abdominal aorta was clamped 3 cm cranial to and 3 cm caudal to the origins of the coeliac and cranial mesenteric arteries,

[6] All cannulae were made of clear polyethylene in sizes ranging from 2 mm OD to 12 mm OD. Each cannula was held in place in the vessel by a ligature where the knot was fixed firmly with glue (Superglue; Loctite #424, Loctite Australia Pty Ltd).

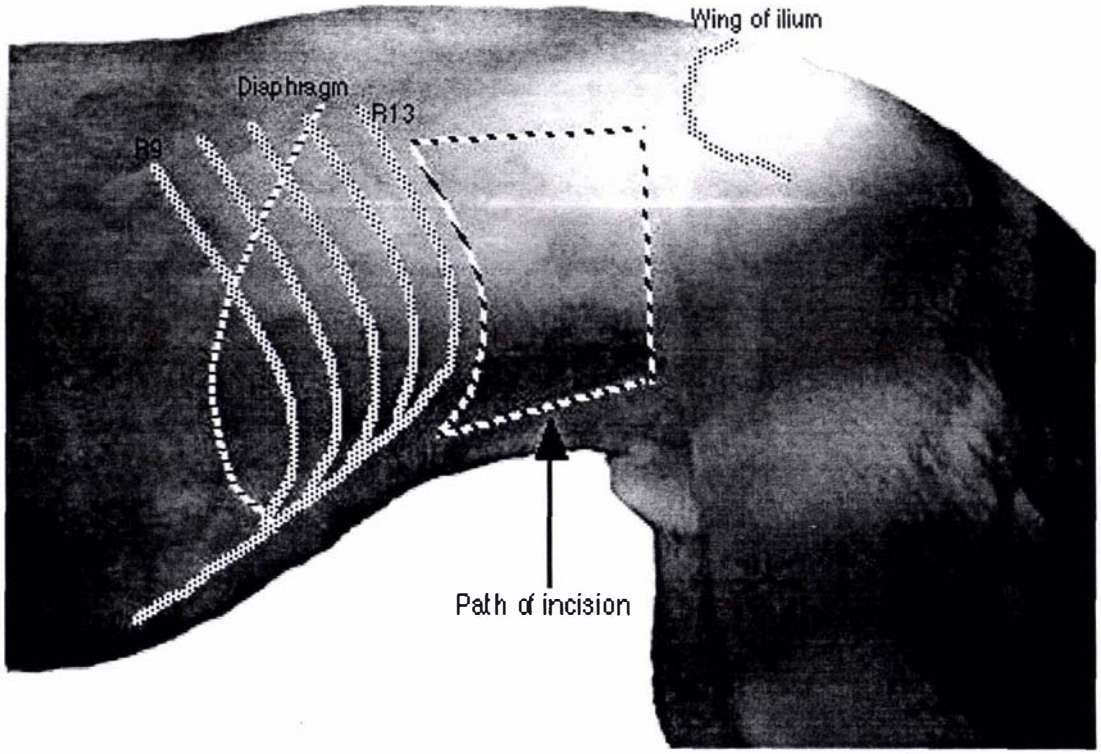

Figure 1. Site for removal of part of left abdominal wall. (Figure by Allan Nutman)

respectively. The intervening section of vessel was cut free from the parent vessel outside the clamp points. The lumbar spinal branches originating from this segment of the abdominal aorta were ligated and then cut distal to the ligatures before the segment of aorta was excised. The diaphragm was cut free from its attachments to the thoracic wall and crura, and the oesophagus was cut three centimetres cranial to the oesophageal hiatus. The caudal vena cava was clamped three centimetres cranial to the caval foramen, and also caudally at the level of the cranial pole of the right kidney. The intervening section of vein was then excised. The descending colon was cut at the level of the pelvic inlet. The gastrointestinal tract, spleen, liver and diaphragm were removed *en bloc* after first severing their mesenteric attachments to the abdominal wall.

Summary of procedures for flushing the isolated gastrointestinal tract with saline (specimens #3 to #9)

With specimens submerged in saline, the clamp was removed from the caudal end of the segment of aorta. A cannula (primed with saline and connected to an infusion line and pump) was inserted into its lumen and tied in place. The clamp was then removed from

the cranial end of the caudal vena cava (that portion remaining at the thoracic surface of the diaphragm). This vessel was also cannulated, but in retrograde direction and then secured in place with a ligature. The clamp at the caudal segment of the vena cava was left in place around the vessel, and the caudal mesenteric vein was ligated. Physiological saline was infused into the aortic cannula at a rate of 30-75 ml/min via a peristaltic pump. Drainage occurred through the thoracic vena cava. The criteria used to judge the endpoint of the saline infusion included the following: 1) fluid flowing from the open end of the cannula inserted into the thoracic vena cava was clear and colourless; 2) gut tissue was pale in colour; and 3) fluid in all vessels draining the gut tissue was clear in colour. Following completion of the flushing procedure, clamps were placed around the free ends of cannulae inserted into the aorta and caudal vena cava.

LATEX PREPARATION AND CASTING TECHNIQUE

Preparation of latex and equipment for infusion

A commercially prepared aqueous suspension of blue rubber latex particles (Skellerup Moulding Latex, Batavian Rubber Co Ltd, Featherston, NZ) was filtered through a domestic grade filter (256 pores/cm^2) to remove small clumps of latex. The viscosity of the latex was then lowered by adding distilled water until a milk-like consistency was produced. The latex was loaded into a 2 litre reservoir and suspended above the specimen. The infusion line from the latex reservoir was primed with latex and its end clamped. For those specimens destined for maceration of their soft tissues after solidification of injected latex (specimens #4, #5 and #6), insect repellant (Shoo Liquid, Sharland and Co Ltd, NZ) was added to the liquid latex (5% V/V) prior to infusion to deter beetles and their larvae from damaging the casts.

Cannulation of jejunal veins

A cannula (primed with saline) was inserted in an orthograde direction into each of four jejunal veins, secured using ligatures, and connected to the infusion line (primed with latex) via three Y-piece plastic adaptors.

Cannulation of portal vein

A cannula for drainage of the portal venous system was inserted into a branch of the portal vein (at the porta of the liver) and secured using ligatures (Figure 2). A second cannula was inserted and fixed into an adjacent branch of the portal vein. This cannula was connected to a mercury manometer for measurement of fluid pressure in the portal vein during infusion of the latex.

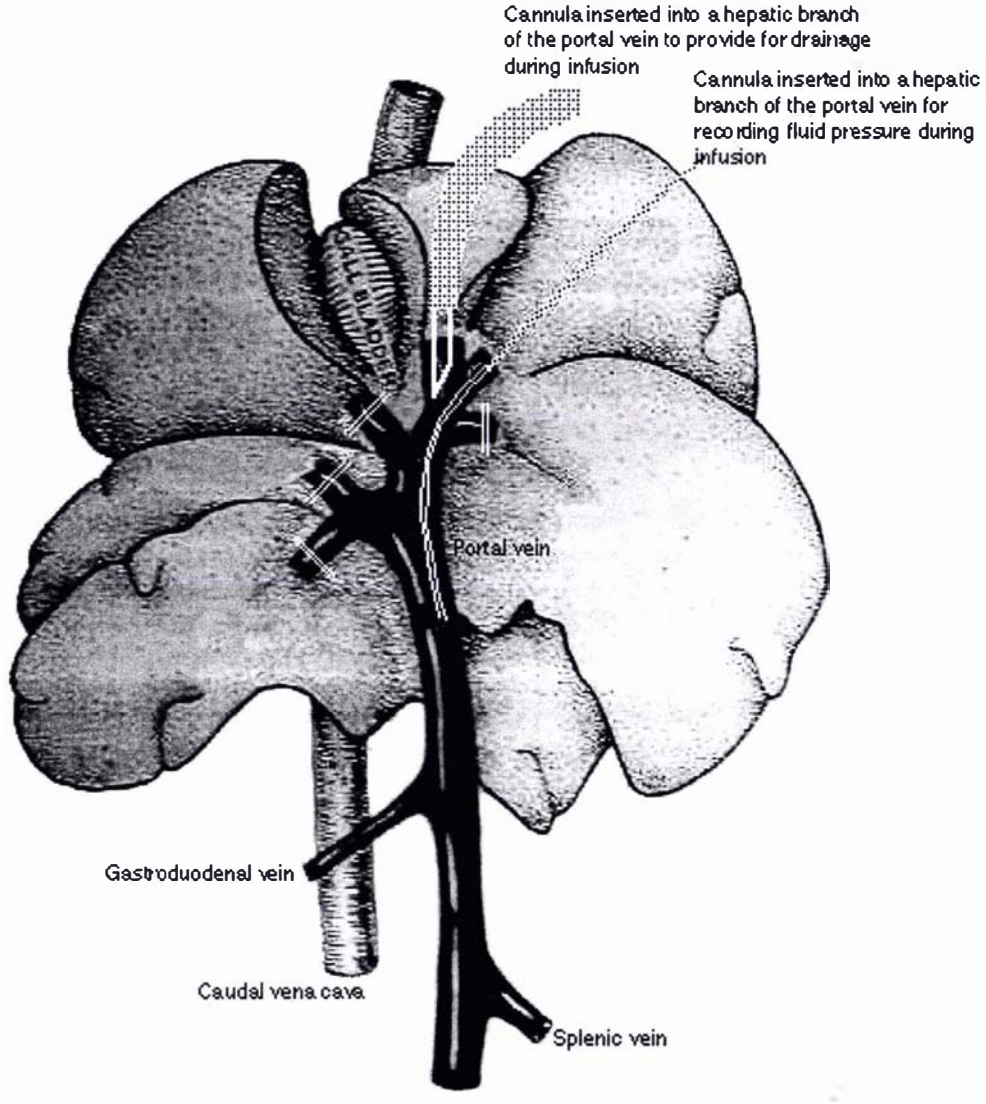

Figure 2. Arrangement of cannulae and ligatures applied to branches of the hepatic vein viewed in relation to the visceral surface of the liver. Ligatures indicated by a double white line. **(From Canine Anatomy - A Systemic Study, Adams, Figure 16.25, p 320, Iowa State University Press, 1986; modified by Allan Nutman)**

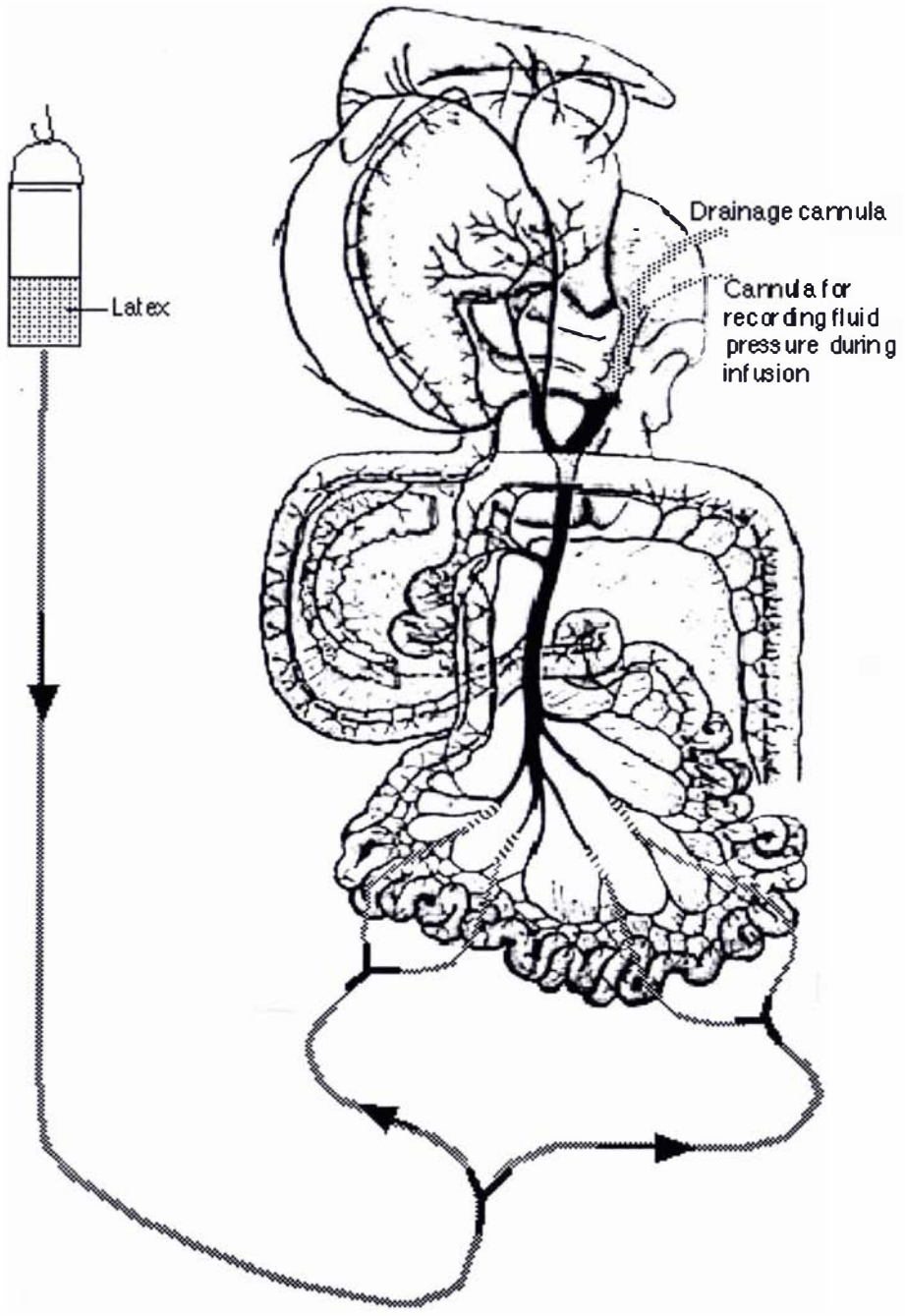

Figure 3. Arrangement of cannulae inserted in jejunal veins and their connection to infusion apparatus. Arrows indicate direction of flow of latex. **(From The Anatomy of the Domestic Animals, Vol 3, Nickel, Schummer & Seiferle, Illustration 207, p 262, Verlag, Paul Parey, 1981; modified by Allan Nutman)**

Infusion of latex into venous system of specimen

Once the cannulation procedures were complete and cannulae inserted into the jejunal vessels were connected to the infusion line, the infusion of latex into the specimen was begun (Figure 3). The level of the latex reservoir above the specimen was adjusted so that the fluid pressure in the lumen of the portal vein was maintained between 20-40 mm Hg. Once saline was displaced from the veins of the portal system and the latex flowing out of the drainage cannula was latex of a similar viscosity to that originally prepared for infusion, the free end of the drainage cannula (portal vein) was clamped. The height of the latex reservoir above the specimen was then re-adjusted to maintain post-infusion latex pressures between 50-60 mm Hg for a period of 3-4 hours. Specimens #4, #5 and #6 were then placed in a chiller at 4° C for 7-10 days to expedite solidification of the latex. Specimens #1, #2, #3, #7, #8 and #9 were immersed in 5% formalin and placed in the chiller to preserve surrounding soft tissue structures.

MACERATION OF SOFT TISSUES USING AN INSECT COLONY

Soft tissues from specimens #4, #5 and #6 were removed using a population of beetles and their larvae, *Dermestides maculatus*. The colony of insects were held in an enclosure which was maintained at 23° C. Humidification was provided by the presence of the specimen. The soft tissues of each specimen were progressively removed over the course of 2-3 weeks.

Intact specimens were removed from the chiller, allowed to equilibrate to room temperature, and debulked of excess or unwanted tissues prior to being placed in the enclosure which housed the beetles. The removal of the soft tissues of a specimen was facilitated by frequent repositioning of the specimen. At the desired stage of maceration, specimens were removed and placed in either 5% formalin solution or water, depending upon the presence or absence of residual soft tissue. Both macerated and preserved specimens were maintained at 4° C until examination.

METHODS OF STUDY

Information regarding the general arrangement of the hepatic portal system was obtained from observations made from each of the nine specimens, however three of the preserved specimens (specimens #7, #8 & #9) were incompletely fixed and were therefore not further examined. Preserved specimen #2 was partially dissected in order to reveal the gross anatomy of the cranial pancreaticoduodenal vein, while specimen #4 was examined when not completely macerated to determine the relationships of smaller vessels with the duodenum and pancreas. Specimens #1 to #6 were examined for the presence of valves. Unless otherwise indicated, all valves described are parietal valves (those which are situated *adjacent* to the entrances of tributaries) as opposed to ostial valves (those which are situated *at* the entrance of smaller veins with larger veins). Veins were studied using a hand-held, illuminated, 6X optical magnifying glass (Schweizer Optik, Germany).

The gross arrangement of the portal vein and its tributaries was studied first by examining intact anatomical specimens, then specimens which were partially dissected, and finally, specimens in which surrounding soft tissues had been progressively removed. The patterns of venous drainage from the pancreas were studied, and are initially described, by following primary, secondary and then tertiary branches of the portal vein to the level of the pancreas, rather than by beginning at the level of the end organ. While this approach may seem unconventional in terms of direction of blood flow, descriptions of specimens and their patterns of venous drainage from superficial to deep and from larger to smaller, allow for a clearer understanding of the (highly variable) three-dimensional anatomy, particularly in the area of close apposition between the duodenum and pancreas. This approach has been used previously to describe the portal system of domestic animals (Getty, 1975).

RESULTS

Observations concerning the general layout of the hepatic portal system are presented first with emphasis placed on the veins supplying the pancreas and their patterns of anastomoses. Then, a detailed description of the venous anatomy in the area of close apposition between the pancreas and the duodenum is presented, followed last by evidence for the presence of directed blood flow.

GENERAL ARRANGEMENT OF THE HEPATIC PORTAL SYSTEM

Plate 1 shows the general arrangement of the hepatic portal system of the dog and is representative of freshly prepared and fully preserved intact specimens in which selective venous casting techniques were applied. In all intact preparations, the major vessels providing drainage from the pancreas were clearly visible, consisting of gastroduodenal, splenic and caudal pancreaticoduodenal branches feeding into the main portal trunk[7] (Plates 2 & 3; to assist with orientation, see Chapter 2, Figure 3). In some specimens, the jejunal veins and/or portions of the ileocolic and/or caudal mesenteric veins were also present.

Anastomoses were a prominent feature throughout the portal system in all specimens examined. Large continuous loops could be traced from one major tributary of the portal trunk to another. The two large anastomoses which were associated with veins draining the pancreas consisted of 1) the 'gastroduodenal vein - splenic vein' loop; and 2) the 'gastroduodenal vein - caudal pancreaticoduodenal vein' loop (Figure 4 and Plates 2 & 3). Highly variable between specimens were the subsets of smaller anastomosing loops which were evident within the confines of the larger loops (Appendix 1).

The 'gastroduodenal vein - splenic vein' anastomosis was comprised of gastroduodenal vein, right gastroepiploic vein, left gastroepiploic vein and splenic vein (see Figure 4 - green route). After leaving the gastroduodenal vein, the right gastroepiploic vein coursed ventrally along the greater curvature of the stomach to anastomose with the left gastroepiploic vein prior to joining with the splenic vein, which also drained the spleen and left lobe of the pancreas. Veins draining the angle[8] and left lobe of the pancreas joined

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[7] In strictly anatomical terms, the portal vein extends from the liver to the point at which the cranial and caudal mesenteric veins branch. Unlike the caudal mesenteric vein, which is clearly a separate branch of the portal vein, the cranial mesenteric vein represents the portal vein's caudal extension and can therefore be considered together with the portal vein as a functional unit. In this thesis, the term 'main portal trunk' refers to that functional vessel which is composed of both the portal vein and the cranial mesenteric vein.

[8] The term 'angle' of the pancreas is not recognized by the Nomina Anatomica Veterinaria, however it is used in this thesis to describe that area of the canine pancreas which is formed by the junction of the right and left lobes.

with either the gastroduodenal vein, the right gastroepiploic vein, the splenic vein or one of its branches, or the portal trunk directly (Appendix 1).

The 'gastroduodenal vein - caudal pancreaticoduodenal vein' loop was comprised of gastroduodenal vein, cranial pancreaticoduodenal vein, pancreatic and duodenal branches of the cranial pancreaticoduodenal vein, and caudal pancreaticoduodenal vein (see Figure 4 - orange route). These vessels provided drainage to the duodenum, and the body and right lobe of the pancreas. One or two small tributaries were commonly observed to leave the duodenal branch of the cranial pancreaticoduodenal vein at various levels along the descending duodenum. These passed either through the pancreas, usually at the extremity of the right lobe, or coursed along its ventral or dorsal aspect, sometimes joining with the pancreatic branch, and then with the caudal pancreaticoduodenal vein, prior to merging with the portal trunk.. The variability observed between specimens in this region (see Appendix 1) appeared to account for the somewhat random number and distribution of small anastomoses in the area.

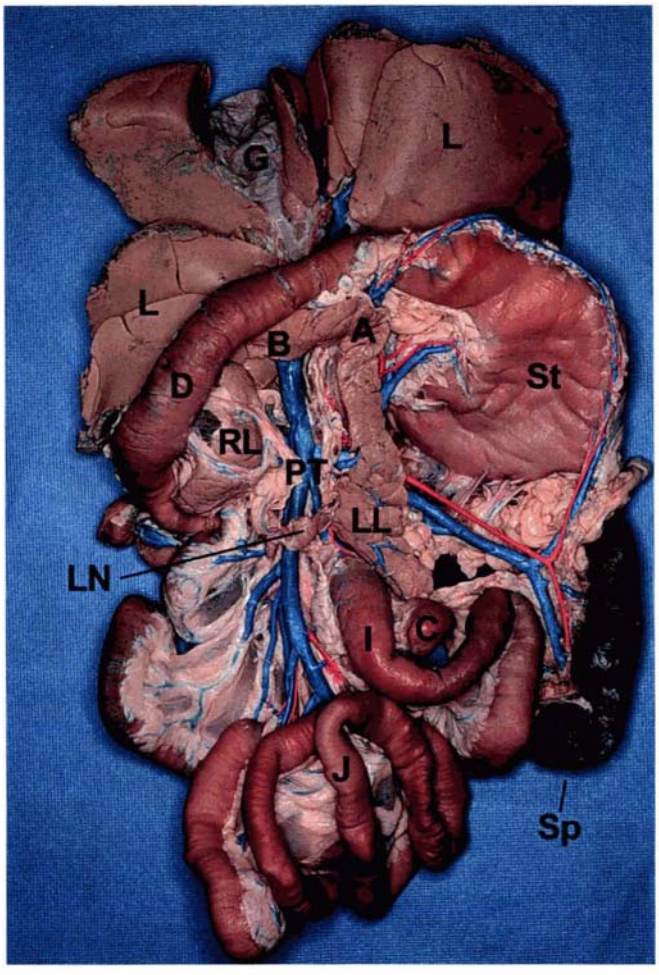

Plate 1. Intact, fully preserved arterial and venous latex cast showing the gastrointestinal tract and hepatic portal system of the dog (ventral view - specimen #1). The omental tissues, a large portion of the mesentery and the large colon have been removed. The greater curvature of the stomach has been reflected cranially to reveal the angle and left lobe of the pancreas. The jejunum has been pulled caudally to reveal the branching pattern of the jejunal vessels. The veins of the portal system in this and all subsequent plates are shown in blue. A angle of pancreas; B body of pancreas; C caecum; D descending duodenum; G gall bladder; I ileum; J jejunum; L liver; LL left lobe of pancreas; LN mesenteric lymph node; PT portal trunk; RL right lobe of pancreas; Sp spleen; St stomach. (Photograph by Angus Fordham)

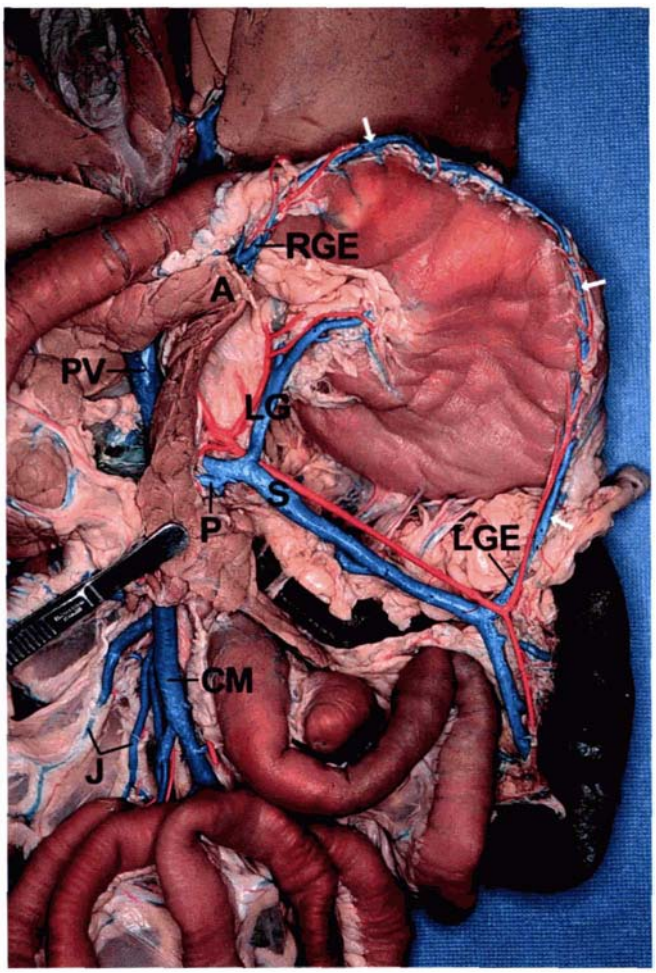

Plate 2. Ventral view of same specimen as depicted in Plate 1 showing the splenic vein and its branches. In this photograph, the left lobe of the pancreas has been reflected to the right to show the splenic vein along it length and its bifurcation with the left gastric vein. The splenic vein is one of three major branches of the portal vein which drain the pancreas. Note also the large anastomosing loop (the 'gastroduodenal vein-splenic vein' loop) which can be followed from the angle of the pancreas to the splenic vein, passing along the greater curvature of the stomach (arrows). A angle of pancreas; CM cranial mesenteric vein; J jejunal veins; LG left gastric vein; LGE left gastroepiploic vein; P pancreatic vein (joining splenic vein); PV portal vein; RGE right gastroepiploic vein; S splenic vein. (Photograph by Angus Fordham)

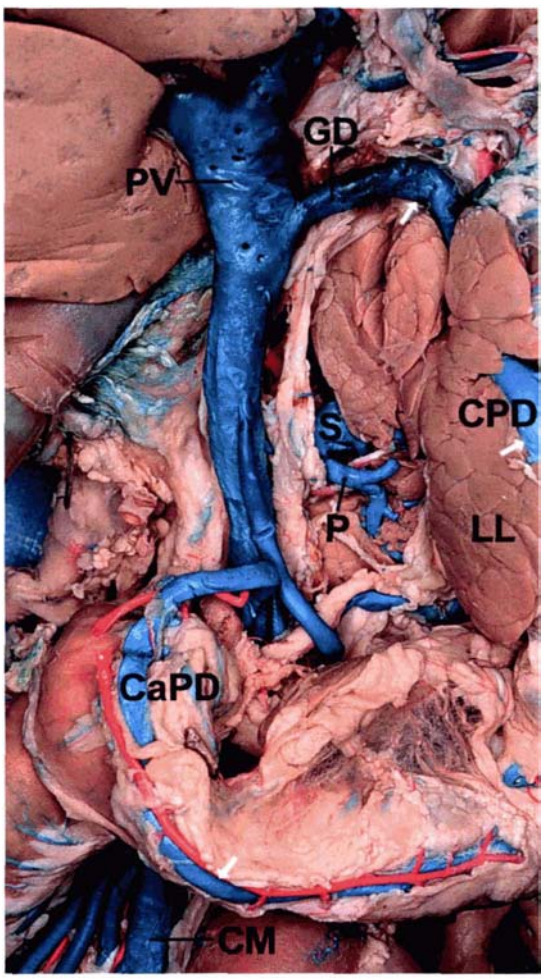

Plate 3. Ventral view of same specimen as depicted in Plates 1 and 2 showing the gastroduodenal and caudal pancreaticoduodenal veins. In this photograph, the descending duodenum has been reflected to the left revealing the dorsal aspect of the pancreas. The gastroduodenal vein and the caudal pancreaticoduodenal vein comprise two of the three major branches of the portal vein which drain the pancreas. The cranial pancreaticoduodenal vein, which is a branch of the gastroduodenal vein, anastomoses with the caudal pancreaticoduodenal vein, which is a branch of the cranial mesenteric vein, to form a 'gastroduodenal vein-caudal pancreaticoduodenal vein' loop (arrows). The splenic vein is also evident in this view. CaPD caudal pancreaticoduodenal vein; CM cranial mesenteric vein; CPD cranial pancreaticoduodenal vein; GD gastroduodenal vein; LL left lobe of pancreas; P pancreatic vein; PV portal vein; S splenic vein. (Photograph by Angus Fordham)

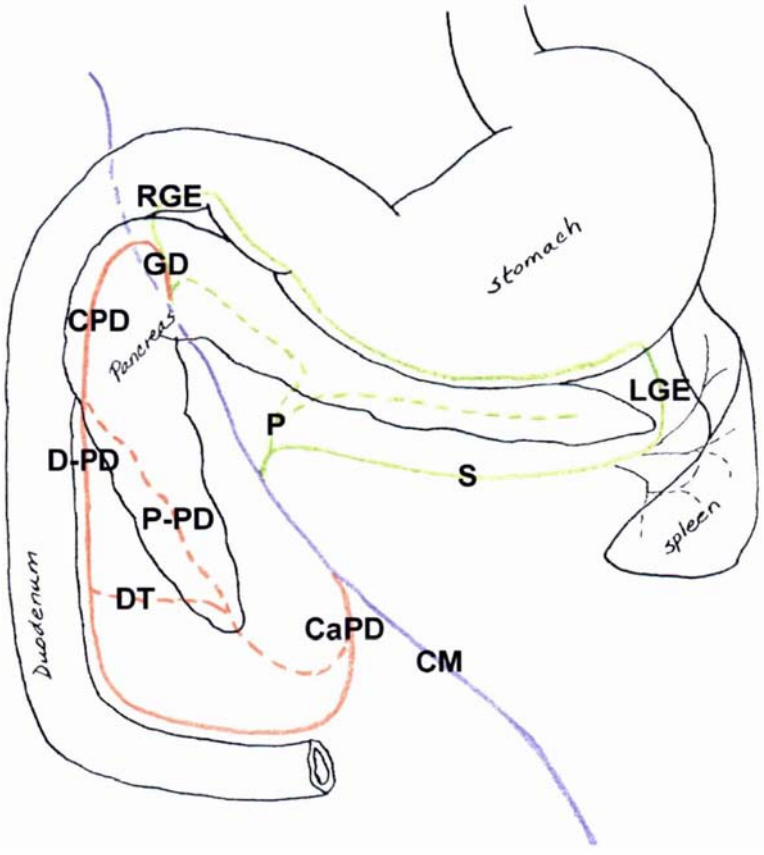

Figure 4. Schematic representation of the gastrointestinal tract and portal system of the dog showing typical anastomosing patterns of veins draining the pancreas (ventral view; composite diagram prepared from 6 latex specimens). The large 'gastroduodenal vein-splenic vein' loop is represented by a solid green line; the large 'gastroduodenal vein-caudal pancreaticoduodenal vein' loop is represented by a solid orange line; hashed lines represent typical drainage patterns and subsets of these loops; the main portal trunk is shown in purple. CaPD caudal pancreaticoduodenal vein; CM cranial mesenteric vein; CPD cranial pancreaticoduodenal vein; D-CPD duodenal branch of pancreaticoduodenal vein; DT duodenal branch tributary; GD gastroduodenal vein; LGE left gastroepiploic vein; P pancreatic vein; P-CPD pancreatic branch of pancreaticoduodenal vein; RGE right gastroepiploic vein; S splenic vein (Template shown was modified and reprinted from Miller's Guide to the Dissection of the Dog, Evans & de Lahunta, p 208, 1996, by permission of the publisher, WB Saunders Company)

VENOUS ANATOMY OF THE DESCENDING DUODENUM, AND THE BODY AND RIGHT LOBE OF THE PANCREAS

Plate 4 shows the venous anatomy of the descending duodenum, and the body and right lobe of the pancreas. Upon entering the pancreas, the gastroduodenal vein first receives a small tributary, the right gastric vein, before splitting into its larger right gastroepiploic and cranial pancreaticoduodenal branches. The right gastroepiploic vein emerges from the pancreas ventrally, distal to the pylorus before turning cranially to continue its course along the greater curvature of the stomach (Plate 5). This vein was also observed to divide soon after its emergence from the pancreas, sending off smaller, caudally-directed tributaries to the adjacent duodenum. In the area of close apposition, the single cranial pancreaticoduodenal vein is enclosed between the pancreas and duodenum, particularly along its ventral aspect. Here, small tributaries emanating from the pancreas and duodenum were observed to enter the cranial pancreaticoduodenal vein directly. Splitting of the cranial pancreaticoduodenal vein into its respective pancreatic and duodenal branches was observed to occur near the site at which the close apposition of the pancreas and duodenum terminated (Plate 6).

In the preserved specimens, numerous venules and small veins were seen superficially coursing through the wall of the duodenum beneath the serosa and perpendicular to the long axis of the bowel. In this area, the venous network was dense, and in two specimens (specimens #2 & #4), a distinct bluish haze could be appreciated along the surface of the bowel. This was particularly evident in the proximal duodenum of specimen #4 whereby the bluish tint imparted by the latex was more intense in the area of close apposition compared to the more distal aspect of the duodenum where pancreatic contact is normally lacking (Plate 7). These small duodenal vessels became larger as they approached the surface of the gut wall. Here they could be seen to either exit the duodenum in parallel fashion singly and enter the common tributary singly, or to first converge upon one another in 'tree-like' fashion prior to entering the common tributary which was shared by the body and right lobe of the pancreas.

With increasing maceration of specimens, smaller caliber vessels were visible. Small branches emanating from the pancreas were identified by the presence of fine tufts of

delicately woven capillaries which were often found to contain residual pancreatic tissue (Plate 8). These delicate tufts were sometimes seen to anastomose with one another, and when this occurred, they were connected by one or more venules (Plate 9) While many of the capillary tufts and the tributaries into which they drained resided as terminal units off the cranial pancreaticoduodenal vein, the right gastroepiploic vein, or one of their branches, in areas where the pancreas directly overlapped the duodenum, many such units were observed to first converge with a duodenal tributary en route to the parent vessel (Plate 10). In specimen #4, the capillary end of two separate pancreatic tuft units was adherent to vessels which appeared to emanate from the duodenum (Plate 11). The source of these adhesions however could not be determined from gross examination. Capillary tufts were not a feature of the duodenum, irrespective of the degree of maceration achieved. Rather, the capillary beds in this area formed an intricate latticework pattern which lay below the serosal surface of the bowel (Plate 12).

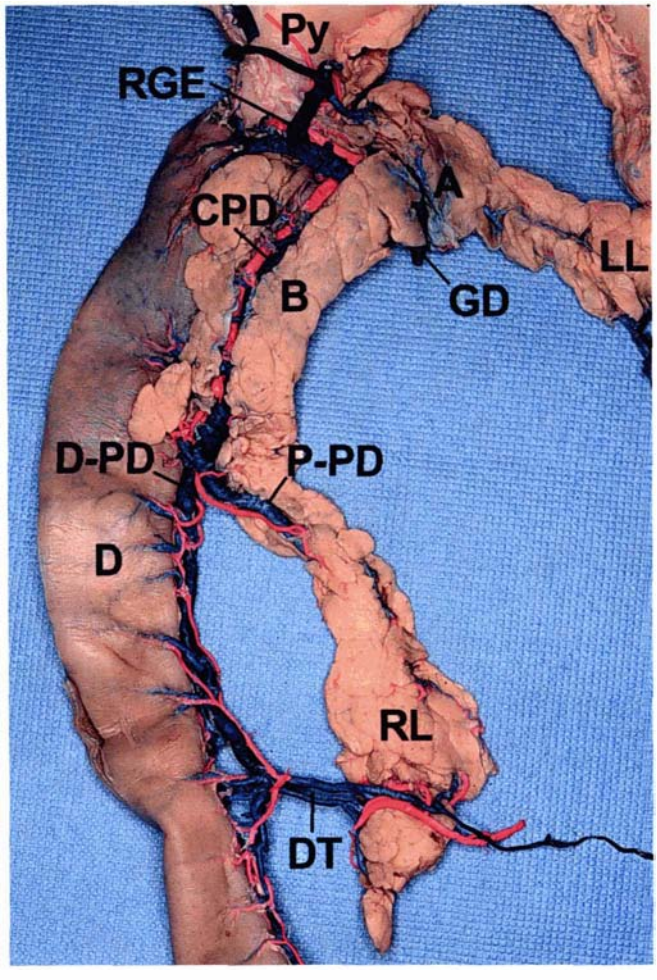

Plate 4. Fully preserved arterial and venous latex cast showing the venous anatomy of the descending duodenum, body and right lobe of the pancreas (ventral view - specimen #2). In this photograph, the mesentery has been removed and the body of the pancreas has been dissected along its length to reveal the cranial pancreaticoduodenal vein. A angle of pancreas; B body of pancreas; CPD cranial pancreaticoduodenal vein; D descending duodenum; D-PD duodenal branch of pancreaticoduodenal vein; DT duodenal branch tributary; GD gastroduodenal vein; LL left lobe of pancreas; P-PD pancreatic branch of pancreaticoduodenal vein; Py pylorus; RGE right gastroepiploic vein; RL right lobe of pancreas. (Photograph by Angus Fordham)

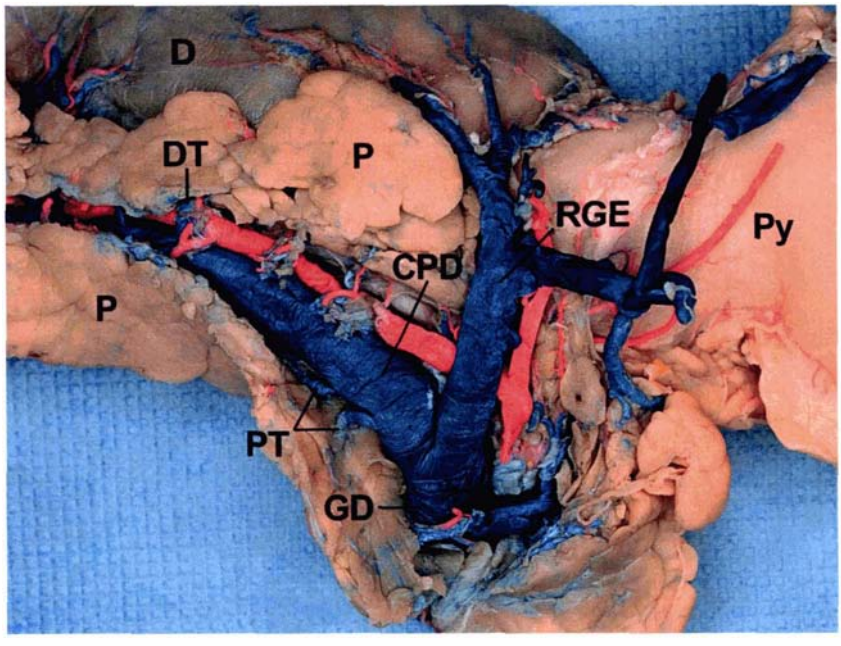

Plate 5. Ventral view of same specimen as depicted in Plate 4 showing the gastroduodenal vein and its branches. The specimen has been rotated 90° and the angle of the pancreas has been dissected along its ventral aspect to reveal the branching pattern of the gastroduodenal vein. CPD cranial pancreaticoduodenal vein; D descending duodenum; DT duodenal tributary; GD gastroduodenal vein; P pancreas; PT pancreatic tributaries; Py pylorus; RGE right gastroepiploic vein. (Photograph by Angus Fordham)

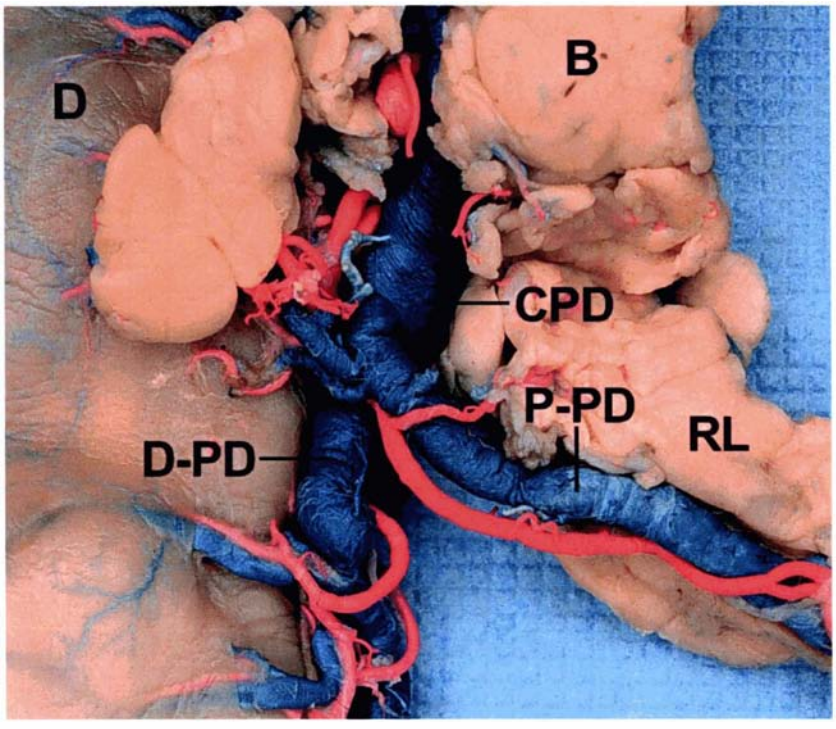

Plate 6. Ventral view of same specimen as depicted in Plates 4 and 5 showing the location at which the cranial pancreaticoduodenal vein splits. The cranial pancreaticoduodenal vein splits into its respective branches near the junction of the body with the free part of the right lobe of the pancreas. At this site, the close apposition between the pancreas and duodenum terminates. B body of pancreas; CPD cranial pancreaticoduodenal vein; D descending duodenum; D-PD duodenal branch of pancreaticoduodenal vein; P-PD pancreatic branch of pancreaticoduodenal vein; RL right lobe of pancreas. (Photograph by Angus Fordham)

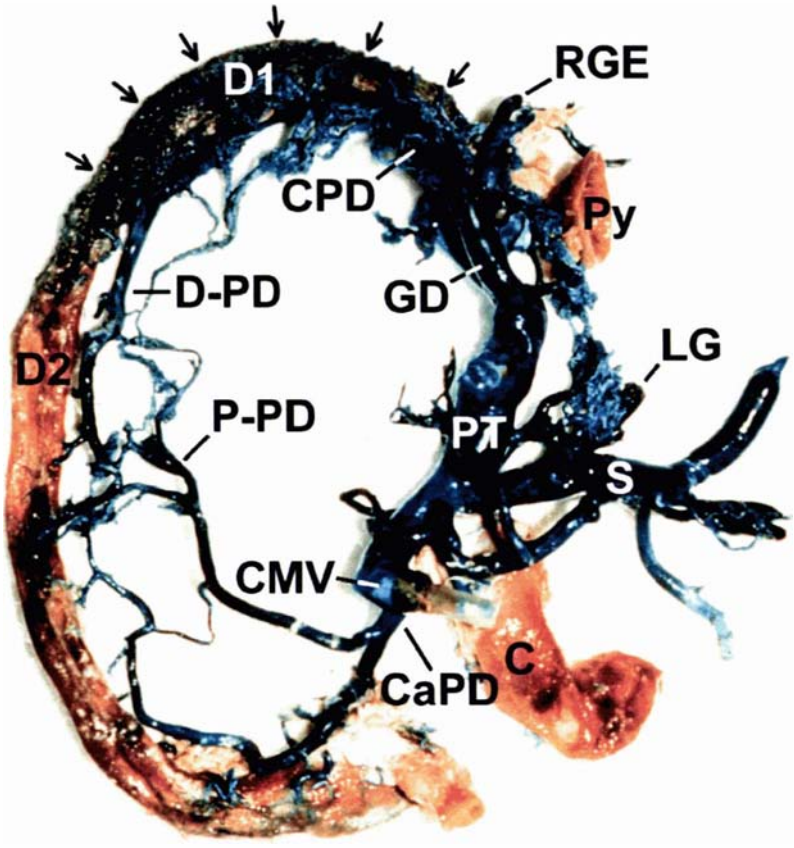

Plate 7. Incompletely macerated venous latex cast showing the deep venous anatomy in the area of close apposition between the duodenum and pancreas (ventral view - specimen #4). Note the high density of veins coursing along the proximal descending limb of the duodenum (arrows) compared to the more distal aspect of the duodenum where pancreatic 'contact' is normally lacking. C caecum; CaPD caudal pancreaticoduodenal vein; CM cranial mesenteric vein; CPD cranial pancreaticoduodenal vein; D1 proximal descending limb of duodenum; D2 distal descending limb of duodenum; D-PD duodenal branch of pancreaticoduodenal vein; GD gastroduodenal vein; LG left gastric vein; P-PD pancreatic branch of pancreaticoduodenal vein; PT portal trunk; Py pylorus; RGE right gastroepiploic vein; S splenic vein (Photograph by Massey University Photography Unit)

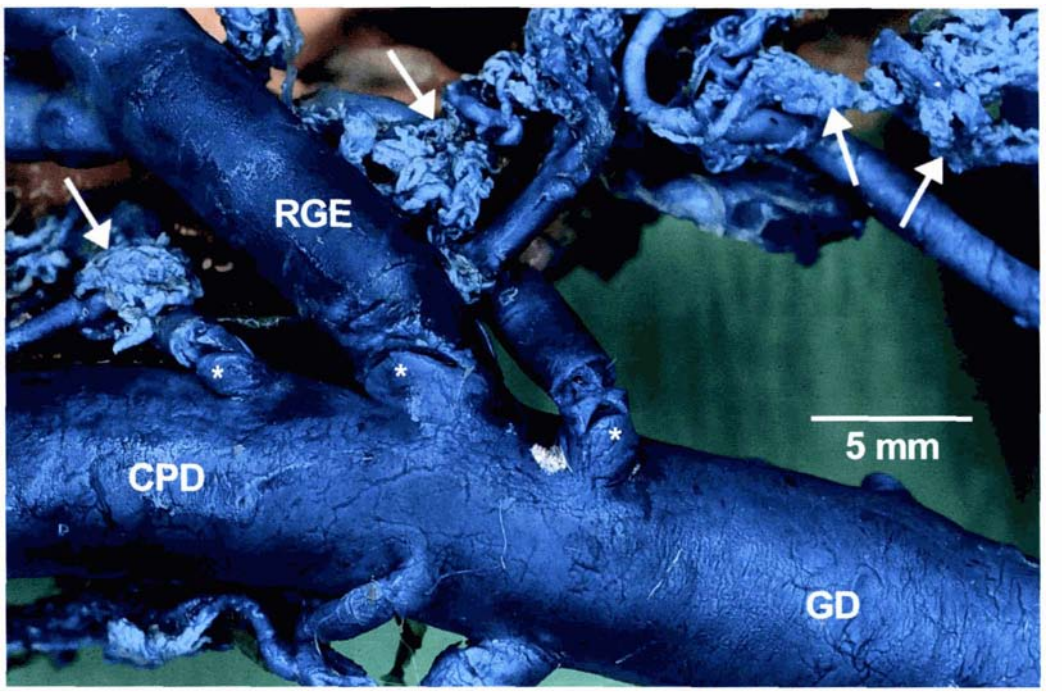

Plate 8. Ventral view of same specimen as depicted in Plate 7 showing capillary beds of the pancreas. This high magnification photograph was taken at the level of the bifurcation of the gastroduodenal vein. In this specimen, the pancreas has been almost completely digested by the beetles leaving behind fine 'tufts' of delicately woven capillaries (arrows). In this view, the tufts of capillaries and the venules into which they drain reside as terminal units ('capillary tuft units') off the cranial pancreaticoduodenal and right gastroepiploic veins. Capillary tufts were a distinctive feature of veins draining the pancreas. The capillary beds seen to the right of the right gastroepiploic vein drain the angle of the pancreas, while the capillary beds seen to the left drain the body of the pancreas. CPD cranial pancreaticoduodenal vein; GD gastroduodenal vein; RGE right gastroepiploic vein; * valve imprint. (Photograph by Shelley Ebbett)

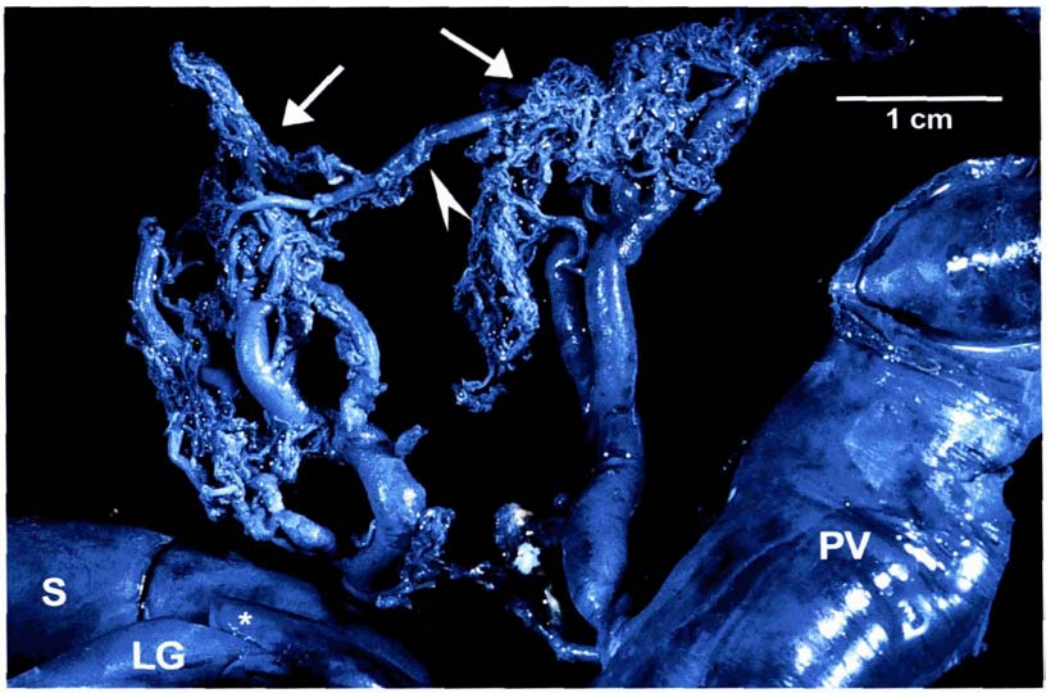

Plate 9. Dorsal view of same specimen as depicted in Plates 7 and 8 showing anastomosing venule between capillary beds of the pancreas. This high magnification photograph was taken near the junction of the splenic vein with the portal vein. The left gastric vein has been reflected ventrally. Two large tributaries, each containing several capillary tuft units, are shown (arrows). The capillary beds on the left tributary join the splenic vein, while the capillary beds on the right feed into the portal vein directly. Note the communicating venule which links the two (arrowhead). These capillary beds drain the left lobe of the pancreas. LG left gastric vein; PV portal vein; S splenic vein; * valve imprint. (Photograph by Shelley Ebbett)

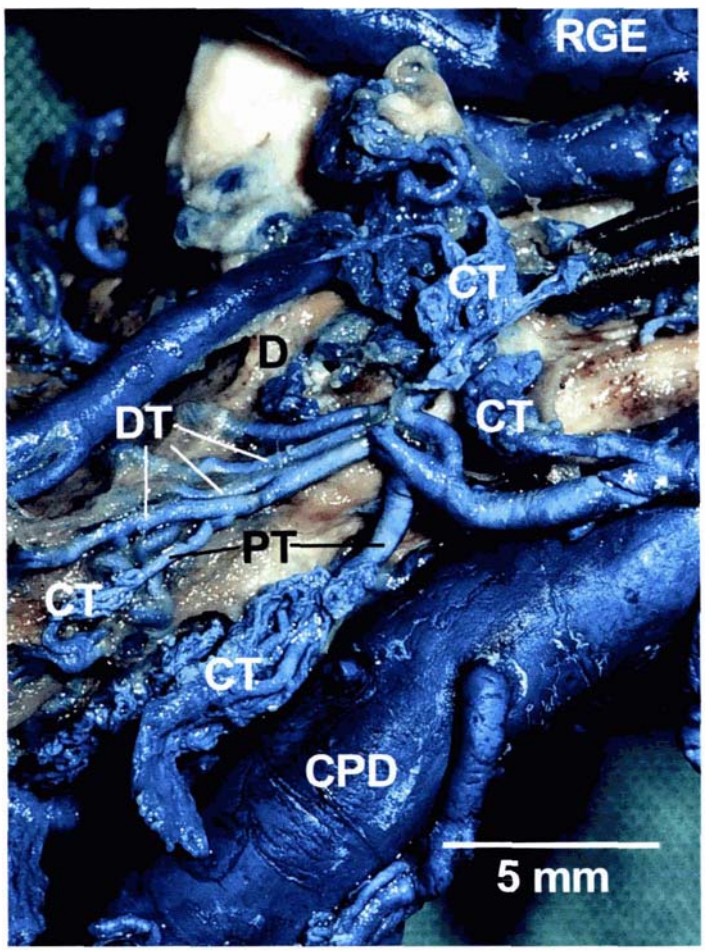

Plate 10. Ventral view of same specimen as depicted in Plates 7 to 9 showing common venous drainage patterns of the duodenum and pancreas. Unlike the pancreas which has been almost completely digested away, the descending duodenum remains to some extent intact. This high magnification photograph was taken in the area of close apposition between the duodenum and pancreas where numerous vessels can be seen emanating from both. Note that in this area, pancreatic tributaries are sometimes seen to first converge with duodenal tributaries prior to joining the cranial pancreaticoduodenal vein. In this view, cranial is to the upper right of the photograph. CPD cranial pancreaticoduodenal vein; CT capillary tuft; D descending duodenum; DT duodenal tributaries; PT pancreatic tributaries; RGE right gastroepiploic vein; * valve imprint. (Photograph by Shelley Ebbett)

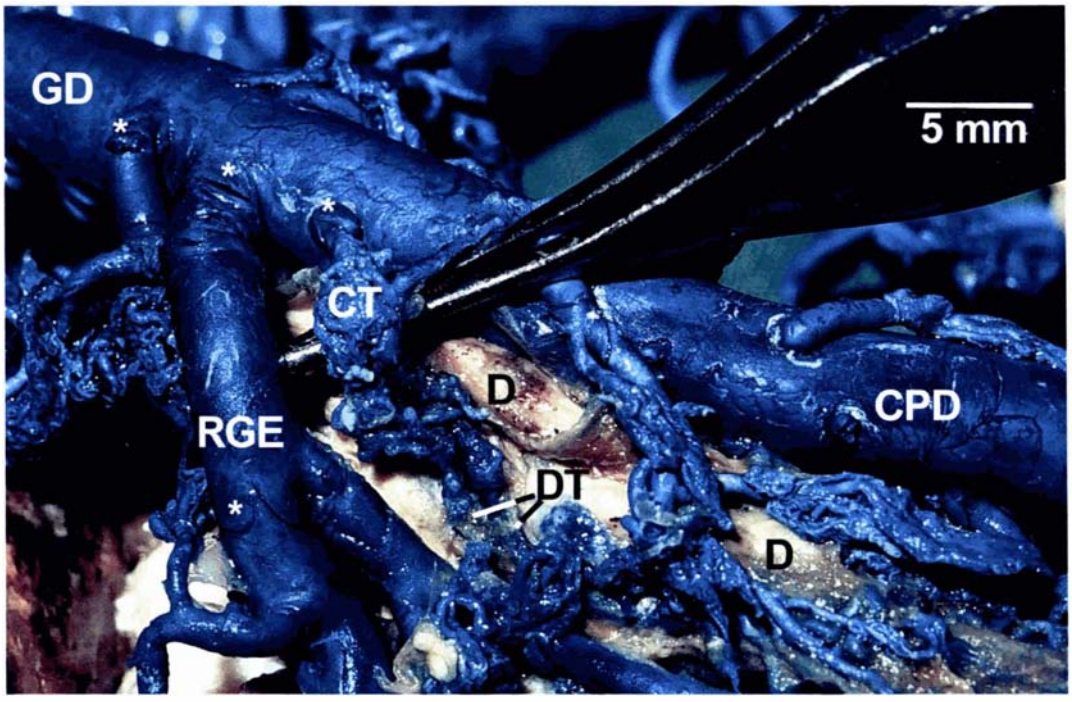

Plate 11. Ventral view of same specimen as depicted in Plates 7 to 10 showing one of two different sites along the duodenum in which a pancreatic capillary tuft was found adherent to vessels draining the duodenum. This high magnification photograph was taken at a level slightly more cranial to that shown in Plate 10. In this area, a tangled knot of capillaries and venules can be seen overlying the tip of a pair of forceps. It could not be determined from gross examination whether the capillary tuft shown was simply 'stuck' to the underlying duodenal tributary/tributaries, or whether a more complicated relationship of these vessels existed. In this view, cranial is to the upper left of the photograph. CPD cranial pancreaticoduodenal vein; CT capillary tuft; D descending duodenum; DT duodenal tributaries; GD gastroduodenal vein; RGE right gastroepiploic vein; * valve imprint. (Photograph by Shelley Ebbett)

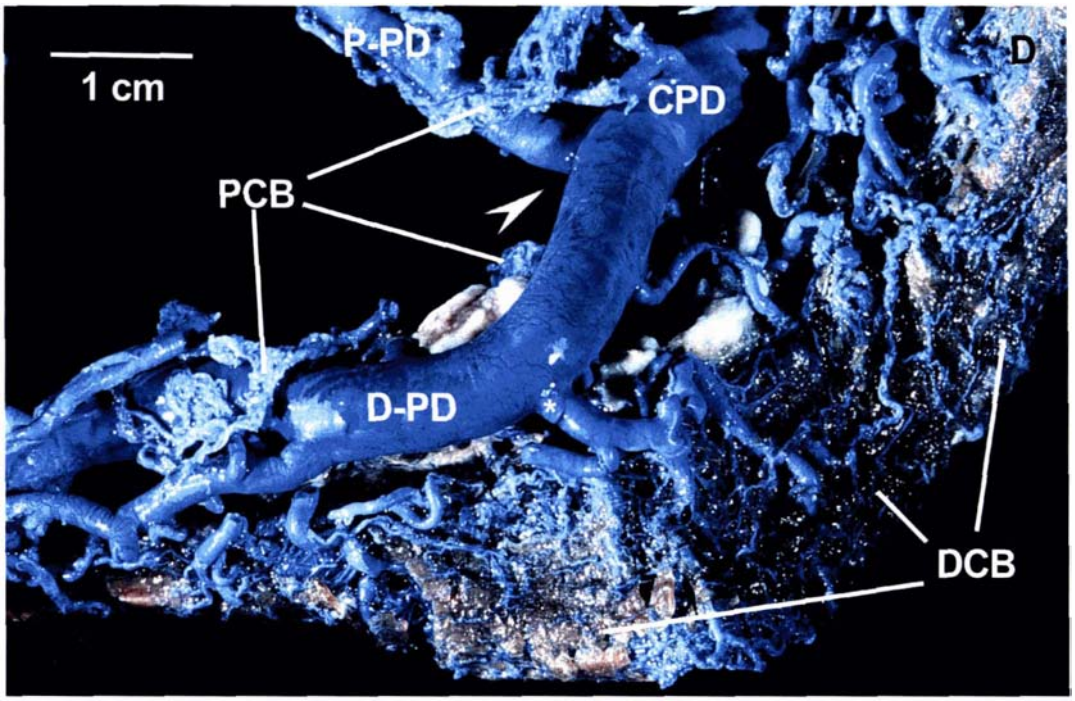

Plate 12. Dorsal view of same specimen as depicted in Plates 7 to 11 showing the organization of the capillary beds and receiving venules of the duodenum. This high magnification photograph was taken at a point distal to that which is shown in plates 10 and 11, just past the bifurcation of the cranial pancreaticoduodenal vein (arrowhead). The duodenum has been reflected to show its dorsal surface. Note the intricate latticework pattern of the duodenal vessels compared to that of the pancreas. Capillary tufts were not a feature of the duodenum irrespective of the degree of maceration. In this view, cranial is to the upper right of the photograph. CPD cranial pancreaticoduodenal vein; D descending duodenum; DCB duodenal capillary beds; D-PD duodenal branch of pancreaticoduodenal vein; PCB pancreatic capillary beds; P-PD pancreatic branch of pancreaticoduodenal vein; * valve imprint. (Photograph by Shelley Ebbett)

ANATOMICAL EVIDENCE FOR VALVE-DIRECTED BLOOD FLOW

Symmetrically bi-lobed, 'V'-shaped flap impressions resembling imprints of venous valves were observed adjacent to both major and minor branch points in all specimens which were closely examined (Plates 13 - 16 and previous Plates 8, 9, 10, 11 & 12). Imprints were identified on venous casts having diameters which ranged in size from 1-9 mm. Where present, the apex of the 'V' (the apposed free margins of the intra-luminal valve leaflets) consistently pointed in the direction of the larger tributary to which the vein joined, i.e., always in the direction of the anastomosing loop or centrally toward the portal trunk. Uni-lobed, and/or tri-lobed imprints were less frequently observed. In many instances, valvular imprints took on a blunted appearance, resembling that of a 'heart' more than that of a 'V'. When these were observed, they were consistently found to reside at the tips of smaller tributaries. Their presence suggested that latex filling had proceeded *against* the normal direction of blood flow, forcing the valves closed in the process.

Figure 5 is a composite drawing prepared from the six specimens examined for the presence of valves (specimens #1 to #6). This figure shows both the collective locations of imprints as well as the direction of blood flow. Imprints were not seen at *all* branch points and were not always observed in the same areas when individual specimens were compared. Notably however, the number of valvular impressions identified was found to increase with increasing maceration of specimens. Valves were identified adjacent to primary, secondary and tertiary branch points relative to the portal trunk. Large anastomosing circles were devoid of valvular imprints except at major branch points, and none of the specimens examined contained impressions along the portal vein or at the junction of the gastroduodenal vein with the portal vein (see Appendix 2 for locations of valves in individual specimens).

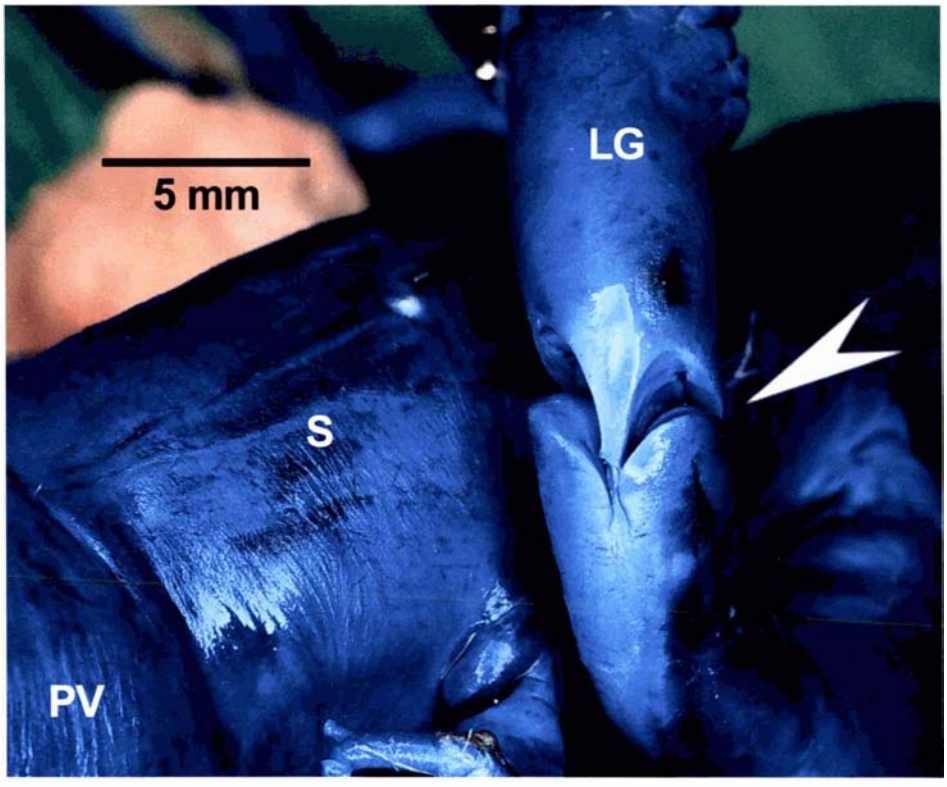

Plate 13. Incompletely macerated venous latex cast showing appearance of valve imprints (dorsal view - specimen #4). In this photograph, the left gastric vein has been stretched to demonstrate a latex cast of a valve imprint (arrowhead). Note the bi-lobed appearance of the imprint and its 'V' shape which was formed by the intra-luminal projection of two valve leaflets. The apex points in the direction of blood flow, which in this case is toward the splenic vein. This valve imprint is also visible in Plate 9. LG left gastric vein; PV portal vein; S splenic vein. (Photograph by Angus Fordham)

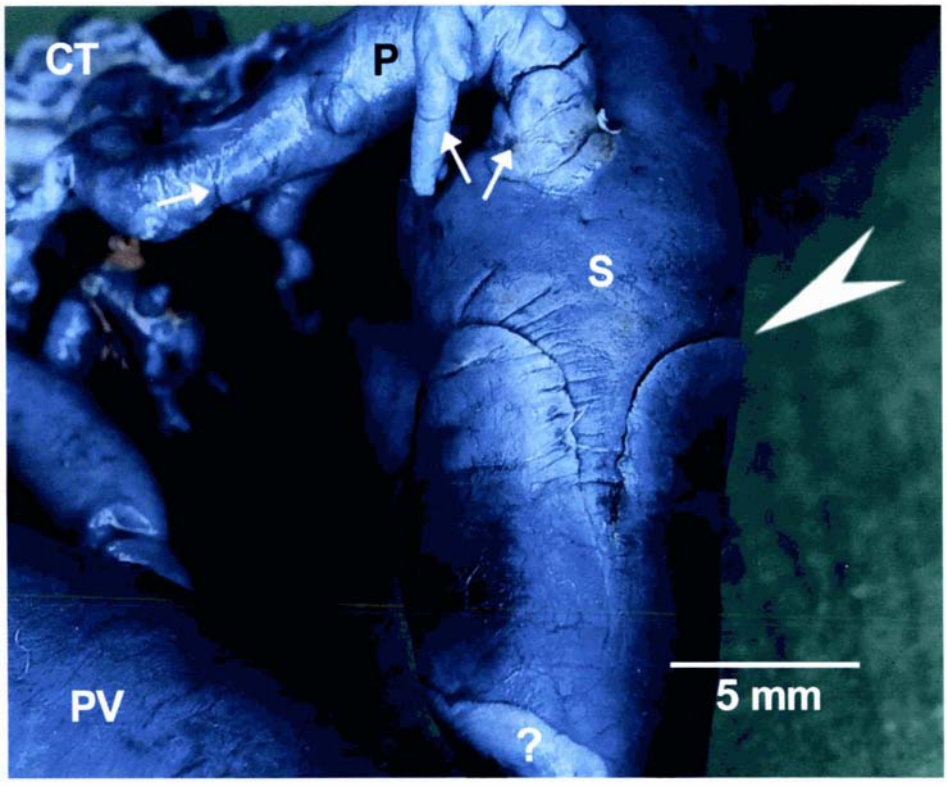

Plate 14. Ventral view of same specimen as depicted in Plate 13 showing valve imprints along the splenic vein and a pancreatic tributary. This photograph was taken near the junction of the splenic vein with the portal vein. The pancreatic tributary seen at the top of the photograph originated in the left lobe of the pancreas. Note the large valve imprint along the splenic vein (arrowhead) and the multiple small valve imprints along the pancreatic veins (arrows). These valves are directing blood toward the portal vein. Valves were found in predictable locations near incoming tributaries, were observed in parallel along adjacent tributaries, and in sequence along the same vein. CT capillary tuft; P pancreatic tributary; PV portal vein; S splenic vein; ? possible ostial valve imprint. (Photograph by Angus Fordham)

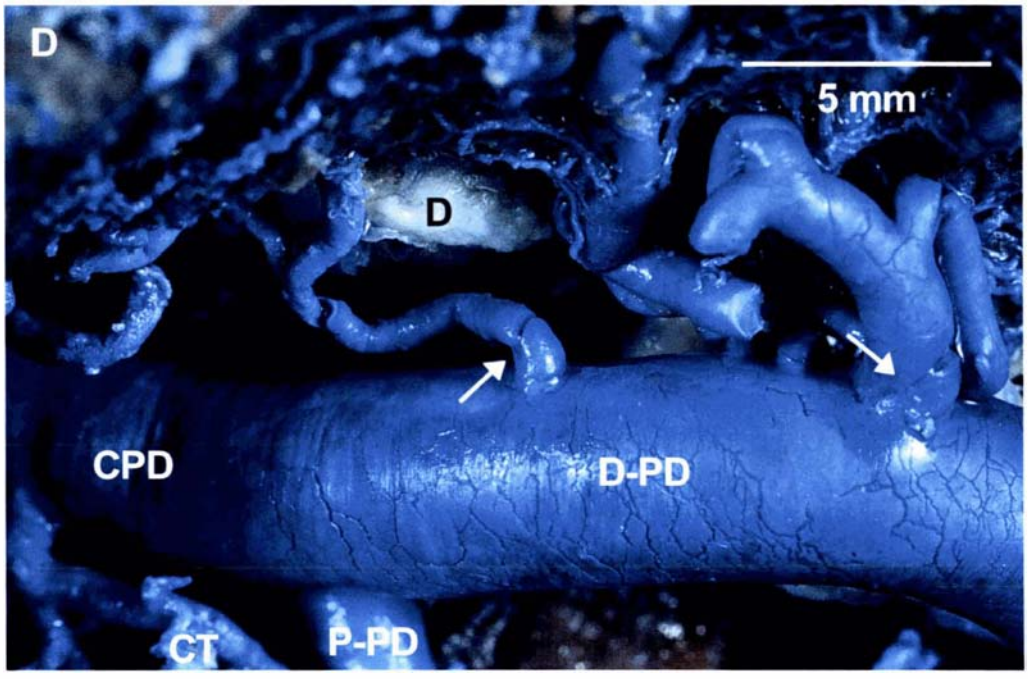

Plate 15. Dorsal view of same specimen as depicted in Plates 13 and 14 showing valve imprints along duodenal tributaries. This photograph, taken just caudal to the bifurcation of the cranial pancreaticoduodenal vein, shows two small valve imprints (arrows) which are directing blood from the duodenum into the duodenal branch of the parent vessel. CPD cranial pancreaticoduodenal vein; CT pancreatic capillary tuft; D duodenum; D-PD duodenal branch of pancreaticoduodenal vein; P-PD pancreatic branch of pancreaticoduodenal vein. (Photograph by Shelley Ebbett)

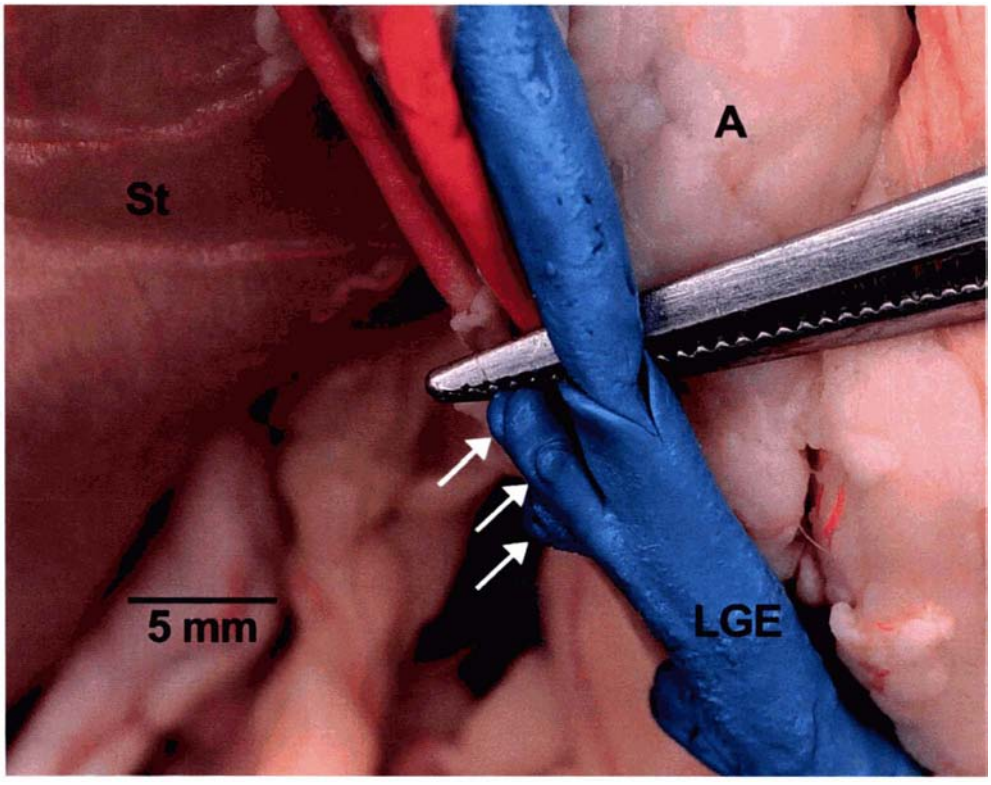

Plate 16. Intact, fully preserved arterial and venous latex cast showing 'dead-end' valve imprints (ventral view - specimen #1). This photograph of the left gastroepiploic vein was taken near its point of divergence from the greater curvature of the stomach. Note the typical valve imprint seen below the level of the forceps, the apex of which points in the direction of the splenic vein. Four smaller tributaries can also be seen merging with the left gastroepiploic vein, and upon close examination, valve imprints with a more blunted appearance are visible at their tips (arrows). Their presence suggested that latex filling had proceeded *up* the veins, snapping the valves closed and precluding filling distal to these sites. Valves became a more frequent finding at this level of the 'gastroduodenal vein - splenic vein' loop, as incoming tributaries approached the large splenic vein. A adipose; LGE left gastroepiploic vein; St stomach (Photograph by Angus Fordham)

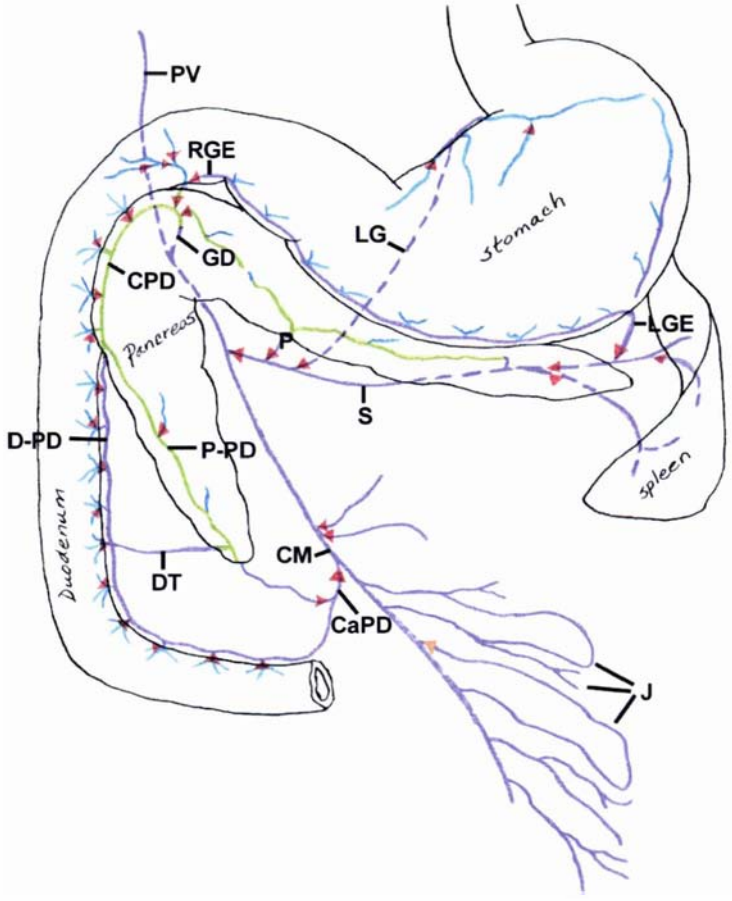

Figure 5. Schematic representation of the gastrointestinal tract and portal system of the dog showing common locations of valve imprints and direction of blood flow (ventral view; composite diagram prepared from 6 latex specimens). This diagram shows the typical locations in which valve imprints were identified in the specimens examined (red arrowheads). Imprints were observed at primary, secondary and tertiary branch points relative to the main portal trunk. The direction in which valves pointed was always toward a large anastomosing loop or centrally toward the portal vein. Imprints were not identified along the main portal trunk, the gastroduodenal vein, or the central-most areas of large anastomosing loops, suggesting that blood entering these vessels from adjoining tributaries follows the path of least resistance (either cranially or caudally) to the portal vein. Solid purple lines represent veins which are freely visible passing ventral to the viscera; hashed purple lines represent veins which are freely visible passing dorsal to the viscera; dark blue and light blue lines represent veins which are visible on the ventral and dorsal aspects of the viscera, respectively; green lines represent veins passing through the pancreas. CaPD caudal pancreaticoduodenal vein; CM cranial mesenteric vein; CPD cranial pancreaticoduodenal vein; D-PD duodenal branch of pancreaticoduodenal vein; DT duodenal branch tributary; GD gastroduodenal vein; J jejunal vein; LG left gastric vein; LGE left gastroepiploic vein; P-PD pancreatic branch of pancreaticoduodenal vein; PV portal vein; RGE right gastroepiploic vein; S splenic vein; P pancreatic tributary (Template shown was modified and reprinted from Miller's Guide to the Dissection of the Dog, Evans & de Lahunta, p 208, 1996, by permission of the publisher, WB Saunders Company)

DISCUSSION

The vascular casting technique employed in this study provided the opportunity to examine the veins of the hepatic portal system in progressively greater detail by subjecting nine canine, latex-injected preparations to differing degrees of dissection and maceration. Using this technique, along with sequential examination of specimens at various stages of maceration, the general arrangement of the portal system could be delineated and the intricate details of these vessels both within and around the pancreas and duodenum described. This technique also proved to be a valuable tool for acquiring information pertinent to the study of directed blood flow.

The general arrangement of the portal system, as described in this study, is consistent with current knowledge of the general anatomy of the portal system in the dog (Chapter 2). That is to say that the primary branches, the gastroduodenal, splenic and caudal pancreaticoduodenal veins, together with the ileocolic, cranial mesenteric, caudal mesenteric and jejunal veins, feed into the main portal trunk, albeit somewhat variably, en route from gut to liver. While a general description of the anatomy of the portal system of the dog was published by Vitums (1959), the present study provides the first *detailed* account of the intricate venous anatomy of the duodenum and pancreas in the area of their close apposition.

The primary objective of this study was to determine if there was evidence to support the existence of a duodenum-to-pancreas venous communication. Specifically, it was of interest to determine two things: first, whether any interconnection of veins between the duodenum and pancreas existed such that blood draining the duodenum would first perfuse pancreatic tissue, and second, whether there was evidence which would suggest a high likelihood that blood does indeed flow in such a manner[9]. Although there was no unequivocal support for such a hypothesis, a number of interesting observations were made

[9] While the focus of this thesis was to examine the possibility of local communication between the duodenum and the *exocrine* pancreas, the vascular casting technique employed did not allow one to distinguish whether particular vessels were associated with exocrine or endocrine tissues.

regarding the venous anatomy of the duodenum and pancreas, and blood flow throughout the hepatic portal system as a whole.

While intact, fully-preserved specimens and specimens which were completely macerated provided the most meaningful information regarding the topography and general arrangement of the portal system, the intricate details of the venous blood supply to the pancreas and proximal duodenum were best appreciated by examining specimens in which only the duodenum and its indirect venous attachments to the pancreas remained. Fortuitously in this study, digestion by beetles of pancreatic tissue proceeded at a rate which exceeded digestion of the bowel wall. The presence of an intact duodenum thereby served as a reference point. Further, the presence of different capillary beds having distinct appearances in the pancreas and duodenum allowed for a clear separation of the origin of veins in those areas where the duodenum and pancreas had been closely apposed and where both the pancreas and duodenum had been, for the most part, fully removed by digestion.

The appearance of the pancreatic capillary beds in this study is reminiscent of that which has been previously described. The microvasculature of the pancreas has been closely examined in a number of different species, including the dog (Beck and Berg, 1931; Bunnag *et al.*, 1963; Fujita and Murakami, 1973; Henderson and Daniel, 1979). Using ink-infused or dye-infused whole mount preparations or scanning electron microscopy of corrosion casts, these studies have revealed the three-dimensional nature of the interconnections between the pancreatic islets, acini and ducts. Afferent vessels entering the islet give rise to capillaries which, upon exiting the islet, follow one of two courses. In larger islets, efferent capillaries coalesce at the edge of the islet into collecting venules which then empty directly into a vein. In smaller (intralobular) islets, efferent capillaries first pass through pancreatic exocrine tissue before coalescing, and in this manner form an 'insulo-acinar' portal system. In dogs, as in rabbits, pigs, cats, cattle, monkeys (Murakami *et al.*, 1993) and man (Murakami *et al.*, 1992), essentially all the islets in the pancreas are intralobular in location and usually emit portal vessels only. The net result is a three-dimensional array of arterioles and venules with a dense and interconnecting lobular

capillary plexus, similar to the 'tuft-like' appearance of the capillary beds observed in the pancreas of this study (Plates 8 & 9).

While the majority of pancreatic capillary beds and the tributaries into which they drained could be seen to enter the cranial pancreaticoduodenal vein separately, many such units emanating from the pancreas in the area of close apposition coalesced first with smaller tributaries leaving the wall of the duodenum (Plate 10). Thus, the cranial pancreaticoduodenal vein was *not* the *sole* site of common drainage between the pancreas and the duodenum, as most general anatomy textbooks would lead one to believe. Rather, in this area, blood leaving the pancreas also joined tributaries of duodenal origin *before* entering the major down-stream vessel. Of interest in this study was the finding of two small capillary tuft units (one of which is shown in Plate 11) which could not be lifted free from the wall of the duodenum near the tip of their capillary beds. The number, small size and tortuosity of the vessels involved, along with the 'stickiness' of the very fine strands of latex, precluded possible identification of a duodenal tributary which might have entered these capillary beds directly. While small, underlying duodenal tributaries could be seen to closely approach these beds, there was no suggestion (such as the presence of valves) that blood was being shunted in their direction. It is interesting however, that in the pancreas, the extension of highly permeable efferent capillaries from the islet into the surrounding acinar tissue is a well recognized means by which hormonal communication between the endocrine and exocrine pancreas is thought to occur [for physiological evidence for an insulo-acinar portal system, see the reviews by Williams and Goldfine (1985) and Bonner-Weir (1993)]. That such a portal system could similarly exist between the endocrine system of the gut and exocrine and/or endocrine pancreatic tissue is intriguing and warrants further investigation.

Reports on the nature and occurrence of venous valves can be traced back to as early as the sixteenth century [see Franklin (1927) for a review]. Although valves or valve-like structures have been reported in most segments of the venous system, many textbooks in current usage focus attention on valves of the extremities and fail to acknowledge the significance of valves in the venous system as a whole. The presence of valves in the abdomen and thorax has in some cases been simply ignored (Fawcett, 1986; Weiss, 1988;

Burkitt *et al.*, 1993), while in others, their presence has been explicitly denied (Cormack, 1987), and this author was able to find only two textbooks which made clear reference to valves in veins other than those which exist in the extremities (Alexander, 1963; Ghoshal *et al.*, 1981). It is interesting how such information can fall from view, only to be rediscovered in the quest for new knowledge.

An extensive historical review of the literature of valves in veins was published by Franklin (1927). In this paper, Franklin summarized earlier detailed accounts of the nomenclature, structure and distribution of valves throughout the body, including those of the portal system. In this review, valves were divided into two classes: *ostial* and *parietal*. Ostial valves are said to occur less frequently than parietal valves, however when present, they are situated at the entrance of smaller veins with larger veins and consist usually of a single fold, the insertion of which occupies approximately two-thirds of the circumference of the entry. Although ostial valves were apparently uncommon in the veins of the present study, the presence of an ostial valve imprint was suspected in one specimen (Specimen #4) at the junction of the splenic vein with the portal vein (Plate 14). Parietal valves, on the other hand, are situated *adjacent* to the entrance of tributaries. They may have from one to five cusps. In man, as in the dogs studied here, bicuspid valves are the rule, however unicuspid and tricuspid valves have also been reported. In animals, tricuspid, quadricuspid and quincuspid valves have been described. Valves in the hepatic portal system have been identified in the 'small gastrosplenic' branches of the portal vein, the splenic vein, and the 'long and short intestinal veins' of dogs. No specific mention was made regarding the presence (or lack thereof) of valves along the main portal trunk of the dog, however, valves have never been identified in this location in man. No indication of the extent to which the portal system of the dog was examined or of the numbers of valves identified was provided. It was implied however, that the number of valves observed in the portal vessels was relatively sparse in comparison to the number of valves in the veins of the limbs. The results of the present investigation are complementary to the information provided in Franklin's 1927 report, and contribute new knowledge regarding the presence and distribution of valves in the veins of the hepatic portal system of the dog.

The supposition that blood draining the gut, liver and pancreas always flows in the direction of the portal vein is well supported by the results of the present study. Collectively speaking, valve-like impressions consistently pointed centrally (Figure 5), being present at the junctions of medium-sized veins (secondary and tertiary vessels) with the large anastomosing loops (primary vessels) into which they drained, at the peripheries of the gastroepiploic veins (where they joined the gastroduodenal vein and splenic vein), at all major branch points along the splenic vein, and where the splenic vein merged with the portal trunk. Of particular interest was the observation that valvular imprints were not found along the portal vein itself, the gastroduodenal vein (even at its junction with the portal vein), or along the cranial pancreaticoduodenal vein or either of its respective branches; the one exception being where the latter branches merged and/or where they entered the portal trunk. Assuming that their absences from these sites is a consistent (possibly) functional feature, then it would appear that there is no *mechanical* barrier to portal blood entering this loop at the level of the gastroduodenal vein. That is to say, that if valves were the *only* factors dictating directionality of blood flow, then it is conceivable that portal blood draining other areas of the gastrointestinal tract could enter (or re-enter) and proceed through the 'gastroduodenal vein - caudal pancreaticoduodenal vein' anastomosing loop. Similarly, it is also possible that blood travelling through the cranial pancreaticoduodenal vein could enter the pancreas via passive means, since valves were not consistently observed along *all* incoming pancreatic tributaries. Such a scenario seems unlikely however, given that blood flow through the venous system follows the path of least resistance, typically from smaller to larger caliber vessels. In addition, the inability to identify valve imprints along *all* incoming tributaries does not definitely imply that they are in fact absent from these vessels (see below).

Several factors could account for the absences of valvular imprints from some vessels in the specimens of this study. The most obvious reason would be that they simply do not exist in all veins. For example, it would seem logical that venous blood needs simply to be directed to a major anastomosing loop, from which it then follows the path of least resistance (either cranially or caudally) to the portal vein. This could explain the apparent deficiency of valves in the (central areas of the) large anastomosing loops. Alternatively, the absence of valve imprints could be a reflection of technique. The degree of vascular

filling with latex is a function of not only the total amount of latex infused, but also its viscosity, the rate and pressure at which filling occurs, and the maintenance of (somewhat higher) post-infusion pressures during the early stages of solidification. The observation however, that valve imprints were identified on casts measuring the same size as veins in which valves are known to occur (Fawcett, 1986; Weiss, 1988) suggests that the degree of vascular filling was adequate for the intended purpose.

It was of interest, having identified such large numbers of 'one-way' valves, that the degree of filling occurred to the extent that it did. The fact that small tributary filling occurred against the normal direction of blood flow suggests a relative incompetency of these valves. This assessment is in agreement with the results of earlier studies (see Alexander (1963) which showed that reversal of blood flow through a vein in a loop of dog intestine results in a substantial retrograde flow which lasts for several minutes prior to the onset of oedema. This situation contrasts with that observed in healthy veins in the extremities where valves have been shown to present complete resistance to retrograde flow until very high pressures are reached. Despite this knowledge, some of the veins in the present study failed to fill distal to the valve site. It would seem logical that in these and other areas where filling took place *against* the normal direction of blood flow, that optimal filling would have been achieved by administering the latex at a relatively slow rate and low pressure, and then maintaining similar or slightly higher pressures for a given period of time thereafter - as was done in the present study. For those vessels in which filling took place in the *normal* direction of blood flow, the absence of valve imprints in expected locations could have resulted from relatively rapid infusion rates and/or pressures which managed to force valves open during the filling process. Similarly, failure to maintain adequate post-infusion pressures could allow for displacement of the latex from around the valve leaflets prior to solidification. Despite these potential problems, the latex vascular casting technique employed in this study was sufficiently sensitive for outlining the structures of interest, and was therefore considered to be a useful tool for studying factors relating to the presence of directed blood flow.

In conclusion, this study used latex vascular casts to describe the macroscopic anatomy of the hepatic portal system of the dog and to reveal valve-like structures which support the

existence of directed blood flow. In addition, examination of incompletely macerated specimens revealed the detailed venous anatomy of the duodenum and pancreas in their area of close apposition. Although evidence to support a direct duodenum-to-pancreas venous communication is weak based upon the results of the present study, the possibility that the pancreas is in some way 'portal' to the duodenum cannot be entirely excluded. Further insight regarding the presence of communicating links between the duodenum and pancreas may be provided by *microanatomical* studies of this area in the dog. The microscopic features of the veins of the hepatic portal system, and the duodenum and pancreas in their area of close apposition, is the subject of Chapter 4.

Chapter Four

MICROSCOPIC INVESTIGATION OF THE DUODENO-PANCREATIC AREA AND HEPATIC PORTAL SYSTEM OF THE DOG

SUMMARY

In an attempt to further define the anatomical relationships of the pancreas with the duodenum and hepatic portal system of the dog, a microscopic survey was performed. The primary objective of this investigation was to find microscopic evidence that might support the idea of a direct duodenum-to-pancreas venous communication. One thousand eight hundred and forty three haematoxylin/eosin-stained tissue sections from the duodeno-pancreatic area and representative veins of the hepatic portal system were examined. The results showed that in the area of *closest* apposition, the pancreas is separated from the duodenum by a single thin sheet of fibrous connective tissue. Veins course from the more dorsal and ventral aspects of the duodenum to the pancreas, rather than directly from the mesenteric border of the intestine. Veins leaving the duodenum course *between* rather than *through* pancreatic lobules, and converge with progressively *larger* rather than *smaller* tributaries within interlobular septa. Taken together, these observations provided little histological support for the existence of a portal circulation between the two organs. An incidental finding in the veins was a small number of luminally-projecting 'folds' having histological features that were reminiscent of carotid sinus baroreceptors. These features included a dense collagen core which comprised the tunica adventitia, nerves within the collagen core or near the base of the fold, and/or, a suggestion of thinning of the tunica media along the luminal extremity of the fold. These structures have not previously been described and their appearance led the author to speculate that they may have a sensory function. The possibility that the folded areas of the veins received sensory innervation was investigated immunocytochemically by examining 417 sections of veins from the hepatic portal system for the presence and distribution of the sensory neuropeptides, substance P (Sub P), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP). Overall, the general pattern of immunoreactivity observed within and around the walls of the veins and arteries in the present study is consistent with known patterns of sensory innervation of blood vessels. Positive immunoreactivity was randomly distributed around the veins and was *not* observed with greater frequency in the dense collagen cores of the previously identified folds. An unexpected observation was the presence of positive immunoreactivity at or near the level of the endothelium in the walls of the veins, but not the arteries. Although this information provided no evidence to suggest that the folded areas of the veins represented novel 'sensory' structures, the significance of positive immunoreactivity itself at the level of the endothelium was considered. The possibility that the veins of the hepatic portal system may have a 'chemosensory' function and that they are involved in reflex regulation of the pancreas is hypothesized.

INTRODUCTION

In the previous investigation (Chapter 3), latex vascular casts were used to reveal the general layout and macroscopic anatomy of the hepatic portal system of the dog. Particular emphasis was placed on the deep venous anatomy of the duodenum and pancreas in their area of close apposition. The primary goal of that study was to determine if there was gross anatomical support for direct duodenum-to-pancreas venous communication since it had been postulated that such an arrangement may play a role in the control of exocrine pancreatic function. While no strong evidence of a venous connection between the duodenum and pancreas was found, the possibility that the pancreas was somehow 'portal' to the duodenum could not be entirely excluded.

In an attempt to further define the anatomical relationships of the pancreas with the duodenum and hepatic portal system of the dog, a microscopic survey of the duodeno-pancreatic area was performed. In the first part of this study, attention was directed at characterizing histologically, the nature of the attachments between the duodenum and pancreas, the presence and course of travel of veins, arteries and nerves in the area, and at confirming the presence of 'directed' blood flow. It was anticipated that this information would complement the results of the macroscopic investigation outlined in the previous chapter and that further insight might be gained regarding the possibility of direct duodenum-to-pancreas venous communication. Observations of distinctive, inward-projecting folds of the walls of the veins then prompted a second investigation which expanded the focus of attention to the hepatic portal system as a whole. The second part of this study used immunocytochemistry to assess the possibility that the above-mentioned structures contained sensory innervation, and that there may exist a 'sensory' role for the veins of the hepatic portal system.

MATERIALS AND METHODS

ANIMALS AND TISSUES COLLECTED

All tissue samples used in the histological and immunocytochemical studies were obtained from dead animals which originated from either private owners who signed consent forms for the use of their dogs after death, or from a public pound, at the request of, and under

the authorization of, certified Animal Control Officers. The use of all animals was given prior approval by the Massey University Animal Ethics Committee.

Tissue samples were collected from a total of eight dogs of mixed age, breed and sex. Tissues taken from each dog included the following:

dog #1	pancreas (middle third of right lobe) and associated veins
dog #2	pancreas (body or 'duodeno-pancreatic' area, distal right and left lobes) and associated veins
dog #3	veins (pancreatic branch of cranial pancreaticoduodenal vein, duodenal vein tributaries and caudal pancreaticoduodenal vein)
dog #4	pancreas (entire) and veins (portal trunk; splenic vein, cranial pancreaticoduodenal vein, right gastroepiploic vein, ileocolic vein and a duodenal vein tributary)
dog #5	peripheral veins (external jugular vein, femoral vein, uterine vein, renal vein and axillary vein)
dog #6	veins (portal trunk; splenic vein, left gastroepiploic vein, left gastric vein, gastroduodenal vein, cranial pancreaticoduodenal vein and a duodenal vein tributary)
dog #7	renal vein (distended with blood clot)
dog #8	portal vein (distended with blood clot)

ANIMAL PREPARATION, SAMPLE COLLECTION AND FIXATION

Dogs were sedated by intramuscular injection of ACP (0.03-0.125 mg/kg body weight; Acepromazine, 2 mg/ml, C-Vet Ltd, Suffolk) and anaesthetized by intravenous injection of Nembutal (28 mg/kg body weight, to effect; Pentobarbitone sodium, 60 mg/ml, Techvet Laboratories Ltd, Auckland). Dogs were later euthanased with an intravenous injection of magnesium sulfate (saturated solution; laboratory prepared). Dogs #1 to #6 received heparin intravenously (1000 IU/kg body weight; Heparin sodium, 25,000 IU/ml, Leo Pharmaceutical Products, Denmark) prior to euthanasia.

Following death, a ventral midline longitudinal incision was made to expose the abdominal viscera. With the exception of dogs #5 and #7 in which peripheral veins were isolated, the

gastrointestinal tract was dissected free from its attachments and removed *en bloc*. Tissues of interest were dissected, removed and partially fixed whole in order to preserve anatomical relationships. Modifications in preparation and technique were necessary for two dogs. In order to maintain distention and minimize contraction artifact during fixation, heparin administration was eliminated prior to euthanasia in dog #7 (renal vein) and in dog #8 (portal vein). These tissue specimens were collected with a firm blood clot in place and were fixed in extension attached to a wooden tongue depressor. All tissues were placed in fixative within 15-30 minutes following death.

Specimens destined for routine histology were placed in a freshly prepared 10% formalin solution; those being submitted for immunocytochemistry were placed in freshly prepared Bouin's fluid (laboratory prepared - see Appendix 3). Tissues were allowed to harden in formalin for 12 hours or in Bouin's fluid for approximately one hour prior to removal. Once hardened, the tissues were further dissected to provide a number of samples of varying sizes and dimensions suitable for histological examination. In one of the two tissue samples prepared from dog #7, the blood clot was removed after trimming. Upon completion of these procedures, all samples were returned to the fixative for optimal penetration. After 24 hours, samples were placed in 70% alcohol to await paraffin wax processing.

PROCESSING AND EMBEDDING

Tissue samples were paraffin wax (melting point 56° C; Paraplast, Oxford Labware, St. Louis, Mo, USA) processed using an automatic tissue processor (Leica TP1050, Global Science Ltd, Auckland, NZ) according to the following schedule:

70% ethanol	1 hour
95% ethanol	1 hour
absolute ethanol	2 changes, 1 hour each
absolute ethanol	2 changes, 2 hours each
chloroform	1 hour
xylene	2 changes, 1 hour each
paraffin wax	2 changes, 2 hours each

Processed tissues were embedded in paraffin wax using a tissue embedding centre (Tissue Tech, Miles Scientific, Sakura Finetechnical Co, Ltd, Tokyo, Japan).

SECTION CUTTING

Sections were sequentially cut every 100-200 μm at a thickness of 6 μm using a rotary microtome (Leitz, Wetzlar, Germany), were floated in a warm water bath (Electrothermal, England) at 45°C, attached to PVA-coated (polyvinylacetate, National Starch and Chemical Co, Christchurch, NZ) slides, and incubated in a hot air oven at 60°C for four or more hours.

STAINING

Routine staining

Slides were de-waxed in xylene, brought to water through graded concentrations of alcohol, stained with haematoxylin and eosin (see Appendix 3 for protocol), and coverslipped using DPX (Distrene, Plasticiser, Xylene or distrene-tricresylphosphate-xylene; BDH, England).

Immunostaining

Immunocytochemistry was performed using rabbit polyclonal anti-(synthetic) Sub P antibody (Amersham International, pic, Buckinghamshire, England), rabbit polyclonal anti-(NKA/SK, code 7359, Peninsula) NKA antibody (Euro-Diagnostica AB, Sweden), rabbit polyclonal anti-rat (Code 6006, Peninsula) CGRP antibody (Euro-Diagnostica AB, Sweden), and a biotin-streptavidin-peroxidase detection system (donkey anti-rabbit IgG, biotinylated, species-specific whole antibody, and streptavidin biotinylated horseradish peroxidase preformed complex; Amersham International, Buckinghamshire, England). The antibodies used were tested for antigen specificity by the supplying companies. Optimum binding of the three primary antibodies was found to occur at concentrations of 1:4000 for Sub P and at 1:6000 for NKA and CGRP. Immunocytochemistry was performed on tissue sections obtained from dogs #2 and #6 using the following protocol:

Protocol for Immunocytochemistry Using the Biotin-Streptavidin-Peroxidase Detection System

1) Deparaffinize sections and bring to water.
2) Equilibrate in phosphate-buffered saline (laboratory prepared PBS; 0.01 M, pH 7.2; see Appendix 3) for 1 minute.
3) Dry slide around section with paper tissue.
4) Encircle section with PAP pen (Daido Sangyo Co, Ltd, Japan) to create fluid barrier.
5) Block non-specific binding sites using 1% bovine serum albumin (BSA) in PBS for 5 minutes.
6) Drain BSA drop from all but negative control sections.
7) Apply primary antisera to test sections; incubate in humidity chamber at 4 °C overnight.
8) Drain sections and wash in 3 changes of PBS for 1 minute each.
9) Drain thoroughly.
10) Apply anti-rabbit biotinylated antibody (1:200) to sections and incubate in humidity chamber at room temperature for 30 minutes.
11) Drain sections and wash in 3 changes of PBS for 1 minute each.
12) Apply streptavidin biotinylated horseradish peroxidase preformed complex (1:200) and incubate in a humidity chamber at room temperature for 15 minutes.
13) Drain sections and wash in 3 changes of PBS for 1 minute each.
14) React sections in 3,3'-diaminobenzidine tetrahydrochloride (DAB) solution (4 mg of DAB in 10 ml of PBS activated immediately before use by adding 10 µl of hydrogen peroxide) at room temperature for approximately 3 minutes.
15) Halt reaction by immersing slides in PBS.
16) Rinse in tap water.
17) Counterstain for 20 seconds in Mayer's Haemalum.
18) Rinse in tap water.
19) Blue in Scott's tap water for 1 minute.
20) Rinse in tap water.

21) Dehydrate through graded alcohols, clear in xylene and mount in DPX.

MICROGRAPHY

Slides were viewed, analyzed and photomicrographed with a Zeiss ™ Axiophot Photomicroscope (Carl Zeiss, Germany). Colour photomicrographs were taken using Kodak™ Ektachrome 64T film.

RESULTS

HISTOLOGY

A total of 281 haematoxylin and eosin-stained tissue sections prepared from dogs #2, #4 and #6 were examined to determine the histological features of the duodeno-pancreatic area. Emphasis was placed on the nature of the attachments between the duodenum and the pancreas, the presence of blood vessels, particularly veins, and their course of travel in the area of close apposition. A total of 1,562 haematoxylin and eosin-stained tissue sections prepared from dogs #1, #2, #3, #4 and #6 consisted of veins from representative areas of the hepatic portal system. These sections were examined to confirm the presence of valves and to study the walls of the veins. Approximately 650 additional tissue sections were examined from the remaining dogs (dogs #5, #7 and #8) for purposes of comparing the structures of the walls of the veins. Figure 1 shows the locations from which haematoxylin and eosin-stained sections were obtained for purposes of producing photomicrographs.

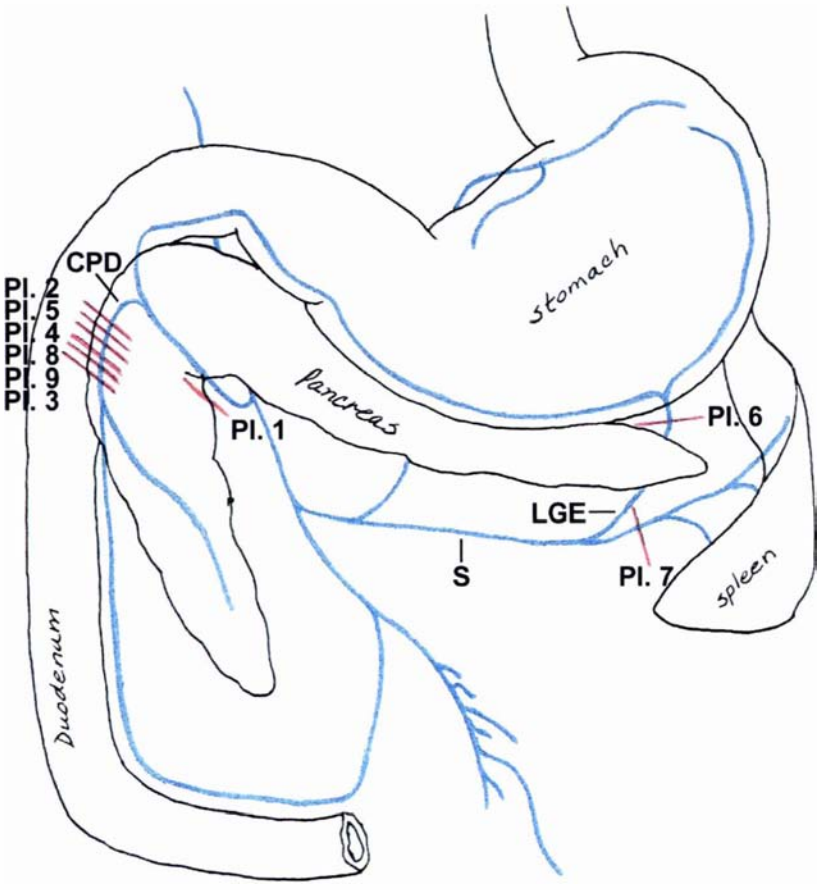

Figure 1. Locations from which haematoxylin and eosin-stained sections were obtained for purposes of producing photomicrographs (plates 1 to 9). CPD cranial pancreaticoduodenal vein; LGE left gastroepiploic vein; S splenic vein

The duodeno-pancreatic area and attachments

The pancreas was readily identified lying adjacent to the proximal descending limb of the duodenum. Surrounding the pancreas were one or more layers of fibrous connective tissue which formed the capsule, and from this capsule, septa extended inwards, dividing the pancreas into lobules (Plate 1). Between the lobules, dense sleeves of irregularly arranged fibrous connective tissue supported and surrounded the larger ducts, blood vessels, nerves and parasympathetic ganglia. Within lobules, a fine fibrous connective tissue stroma was observed surrounding the secretory end pieces. The substance of the gland was composed of secretory (acinar) tissue, as well as clusters of endocrine cells (islets of Langerhans) which were scattered randomly throughout the lobules.

Attaching the pancreas to the descending limb of the duodenum were irregularly arranged thin sheets of loose, fibrous connective tissue which, in most areas, were widely separated by adipose tissue (Plate 2). This delicate, adipose-laden appearance of the connective tissue was typical of the looser attachments between the pancreas and duodenum both proximal and distal to the area of closest apposition, and of the looser dorsal and ventral attachments of the duodeno-pancreatic area along its length. In the area of *closest* apposition however i.e., along the mesenteric border of the duodenum, the pancreas was separated from the duodenum by a single thin layer of fibrous connective tissue (Plate 3) which was continuous with the connective tissue encapsulating the gland. Nerves, lymphatics and blood vessels were commonly observed coursing through both the intervening connective tissues as well as through the surrounding capsule.

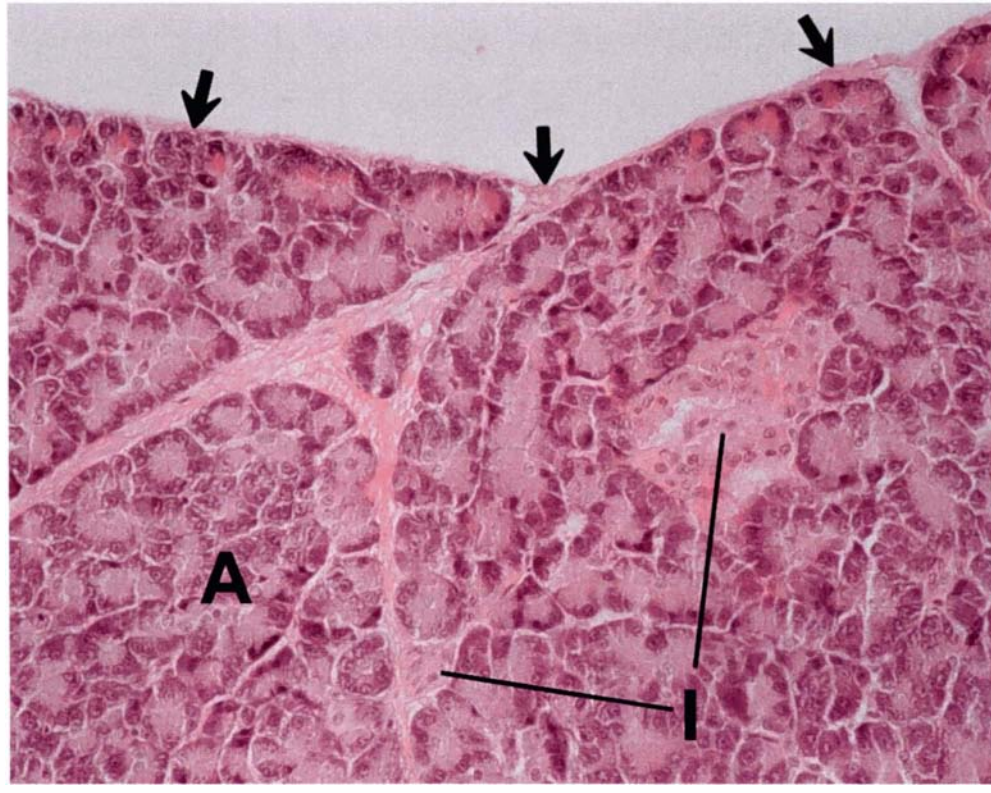

Plate 1. Section of canine pancreas. A fibrous connective tissue capsule surrounds the gland (arrows). From the capsule, septa extend inwards dividing the pancreas into lobules. Within lobules, a fine fibrous connective tissue stroma is observed surrounding the secretory endpieces. The substance of the gland is composed of acinar tissue (A) as well as clusters of islet cells (I) scattered throughout the lobules. (200x)

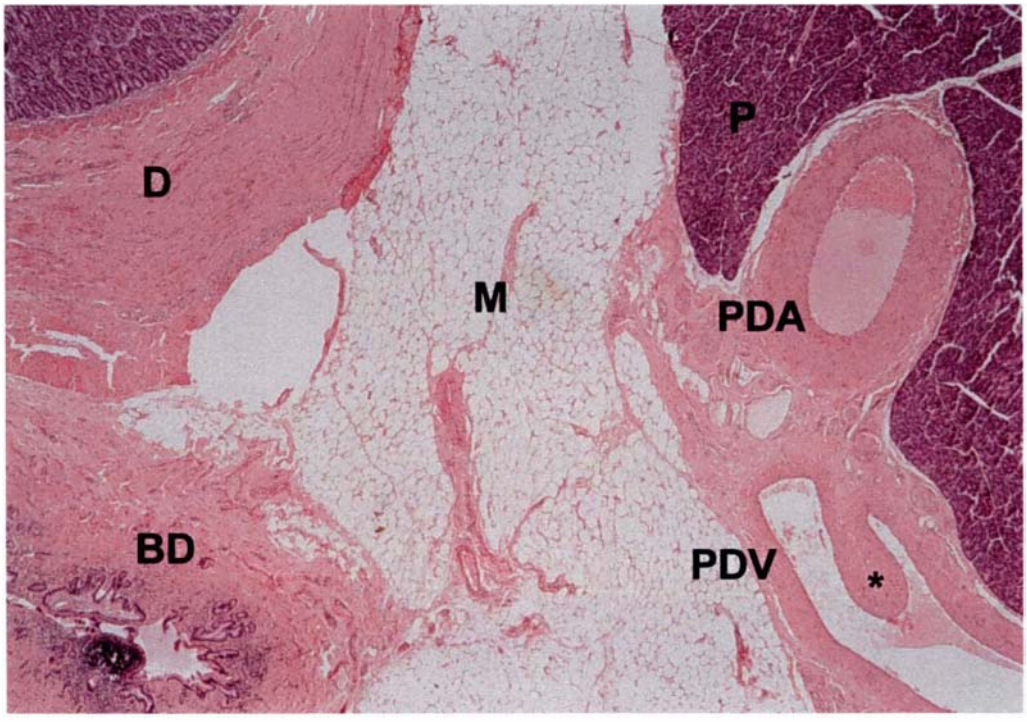

Plate 2. Dorsal aspect of section of the canine duodeno-pancreatic area just cranial to the area of closest apposition. The mesoduodenum (M) is composed of irregularly arranged thin sheets of fibrous connective tissue which is widely separated by adipose tissue. BD bile duct; D duodenum; P pancreas; PDA cranial pancreaticoduodenal artery; PDV cranial pancreaticoduodenal vein; * 'infolding' of vein wall. (25x)

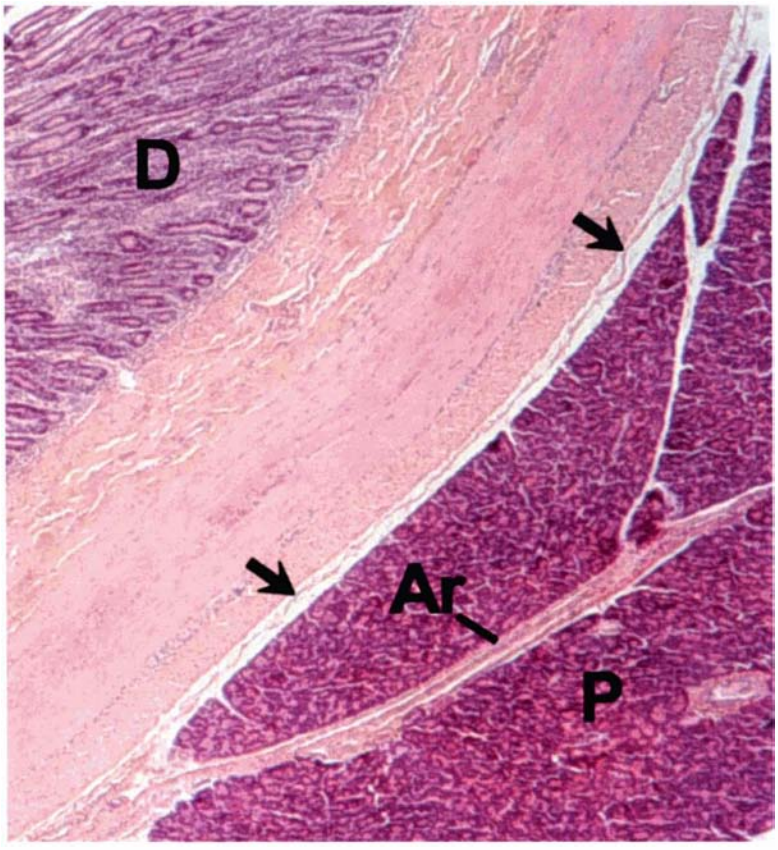

Plate 3. Area of closest apposition between duodenum and pancreas. A single thin layer of fibrous connective tissue (arrows) separates the pancreas from the duodenum. Ar arteriole coursing obliquely through pancreas to enter duodenum along its ventral aspect; D duodenum (mesenteric aspect); P pancreas. (55x)

Blood vessels and nerves

Arterioles, venules and nerves entered (or emanated from) the duodenum through smooth discontinuities in the tunica muscularis externa (Plate 4). These discontinuities took the form of connective tissue-filled vascular channels or neural channels. Of the prepared slides in which the tissues were judged to be of satisfactory quality, such structures were observed to enter (or arise from) primarily the dorsal and ventral aspects of the duodenum, rather than to directly enter (or exit) the mesenteric border where the pancreas most closely approached the duodenum (see Plate 3). Arterioles and venules were typically observed to traverse the tunica adventitia of the duodenum in oblique fashion, and in some instances, to course through the pancreas via interlobular septa. In serial sections, vessels were frequently seen to diverge from (or converge with) larger tributaries, after (or before) coursing from (or toward) their parent vessels, which often resided in large interlobular septa deep within the pancreas. There was no microscopic evidence, such as the presence of highly tortuous veins closely apposed to arteries, to suggest that a local countercurrent exchange mechanism exists. Nerve bundles were common around blood vessels, with the greatest density typically observed around arteries.

Valves

Valves were commonly observed at points of convergence with larger venous tributaries throughout the veins of the duodeno-pancreas. Valve leaflets were recognized histologically as luminal-projecting folds of tunica intima which contained a thin connective tissue core and which were surrounded by an endothelial lining exposed to the blood. At the base of each valve cusp was a thickened band of supporting collagenous tissue (Plate 5). Sections of valves or parts of valve leaflets were observed in venules in all areas of the pancreas including the capsule and surrounding adventitia as well as throughout the veins of the hepatic portal system as a whole. Valves were not observed in either the cranial pancreaticoduodenal vein or along the main portal trunk.

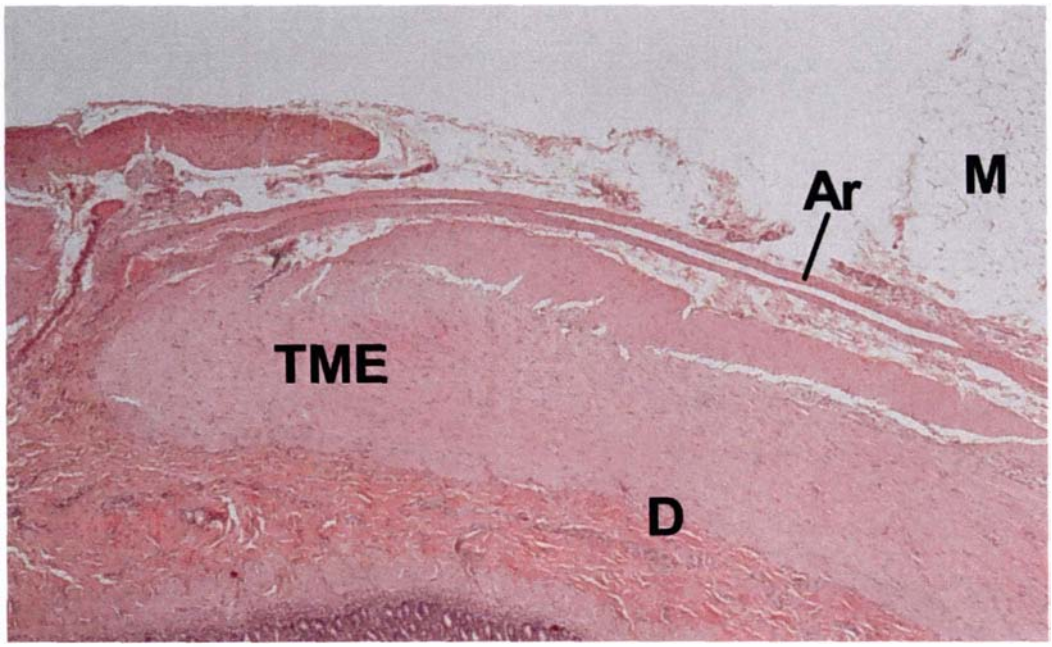

Plate 4. Arteriole entering dorsal aspect of duodenum. Blood vessels and nerves entered (or emanated from) the duodenum through smooth discontinuities in the tunica muscularis externa (TME). These structures were observed to enter (or arise from) primarily the more dorsal and ventral aspects of the duodenum rather than the mesenteric border where the pancreas most closely approached the duodenum. Ar arteriole; D duodenum; M mesoduodenum. (25x)

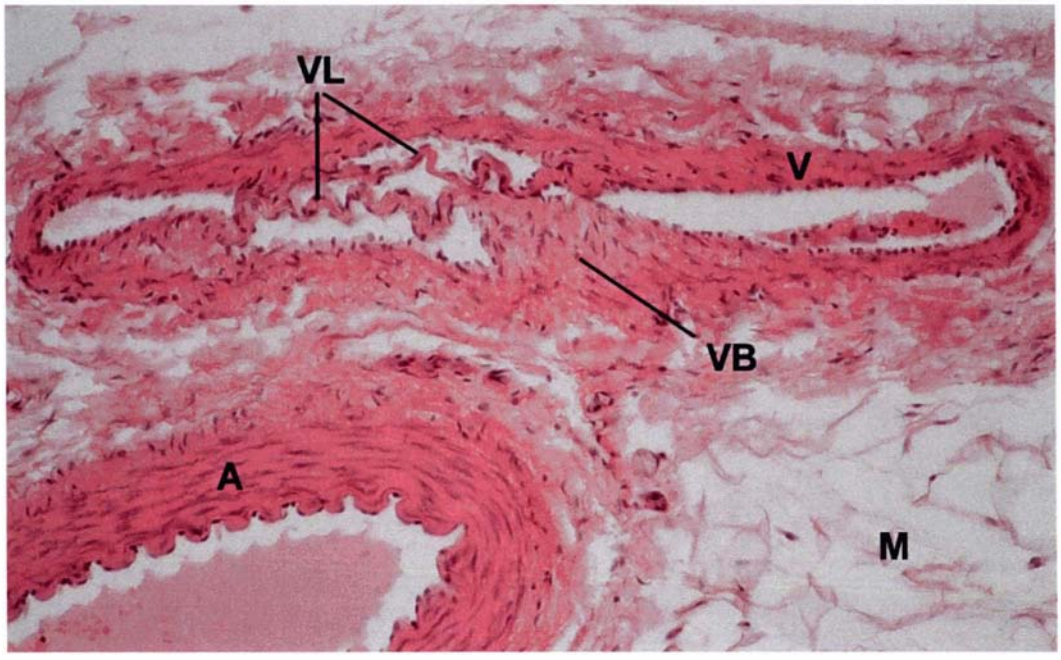

Plate 5. Cross section of vein with valve. Valve leaflets were recognized histologically as luminal-projecting folds of tunica intima which contained a thin connective tissue core and an endothelial lining exposed to the blood. A thickened band of supporting collagenous tissue marked the base of each valve cusp. A artery; M mesoduodenum; V vein; VB valve base; VL valve leaflets. (210x)

Configuration of veins

Whereas the arteries coursing within and around the duodeno-pancreatic area assumed a relatively consistent round to oval shape and inner contour, the veins were highly variable in these respects. In most of the medium to large veins examined in this, as well as other areas of the hepatic portal and systemic venous systems, one or more 'infoldings' protruded into the lumen of the vein (Plate 6; also see Plate 2). These inward-directed 'folds', which consisted of tunica intima, tunica media and tunica adventitia, were often observed to extend for several hundred μm along the length of the vein before gradually disappearing and giving way to a new generation of folds. In most instances where the veins had not been distended prior to fixing (dogs #1 to #6), the folds were artefactual, resulting from collapse and/or contraction of the muscular layer of the wall of the vein. This was confirmed by examination of serial sections of the hepatic portal vein (dog #8) and a renal vein (dog #7) after having been fixed in a distended state. In all slides fixed in such a manner, the morphological appearance of the walls of the veins was uniform and circular (not shown).

Unique to a few folds however, was the presence of one or more of the following features: a dense collagen core which comprised the tunica adventitia (Plates 7, 8 & 9); nerves within the collagen core (Plate 7) or near the base of the fold (Plates 7, 8 & 9); and/or, a suggestion of thinning of the tunica media along the luminal extremity of the fold (Plates 8 & 9). The presence of nervous elements seen in association with some of these folds prompted further investigation into the nature of their innervation. The supposition was that nerves from sensory endings located in the more superficial regions of the vessel wall extended through the tunica adventitia to unite with the relatively large nerve bundles deep to the folds.

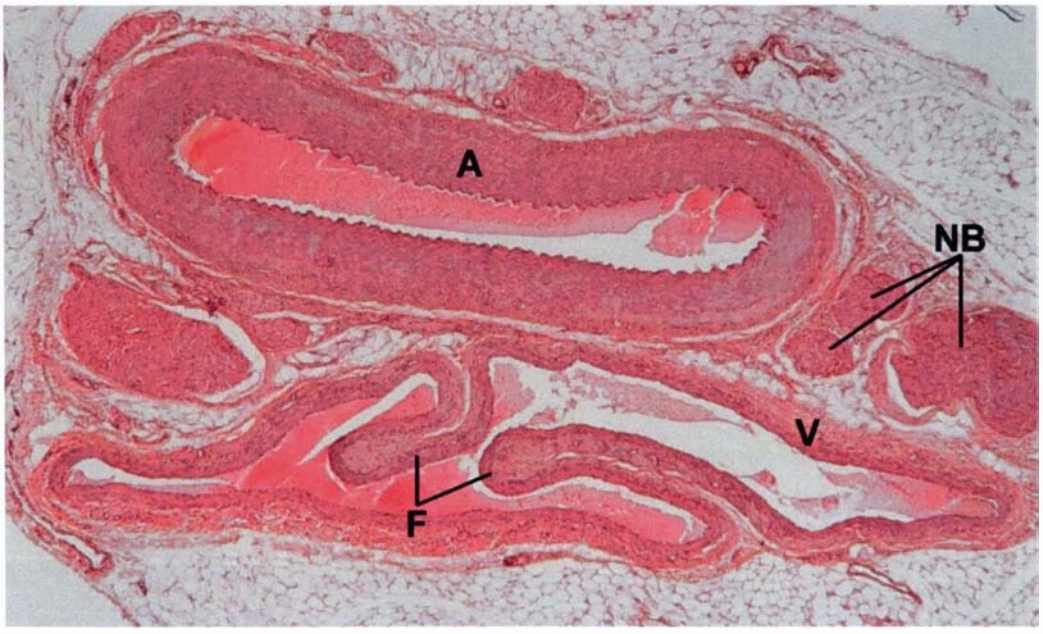

Plate 6. Folds. This photomicrograph shows the typical appearance of the inward-projecting 'folds' of vein wall which were commonly observed throughout both the hepatic portal system and systemic venous circulation. These folds were artifactual. They resulted from collapse of the vein along its length during fixation. A artery; F folds; NB nerve bundle; V vein. (35x)

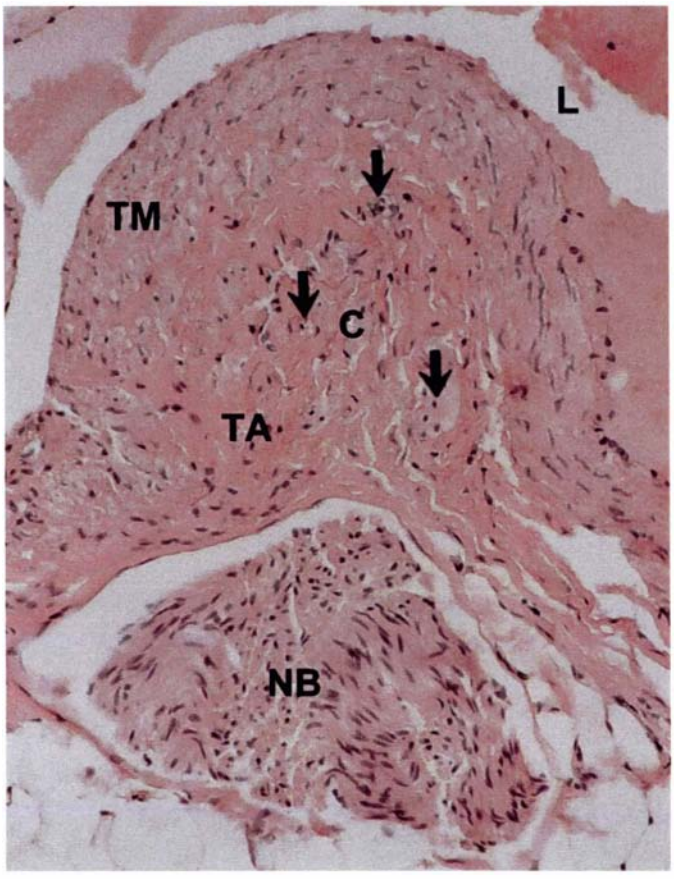

Plate 7. 'Atypical' fold. This plate shows some of the histological features of the less commonly observed 'atypical' folds. Note the dense collagen core (C) which comprises the tunica adventitia (TA), nerves coursing through the core (arrows), and the large nerve bundle (NB) near the base of the fold. Compare this fold to the folds in Plate 6. L lumen; TM tunica media. (160x)

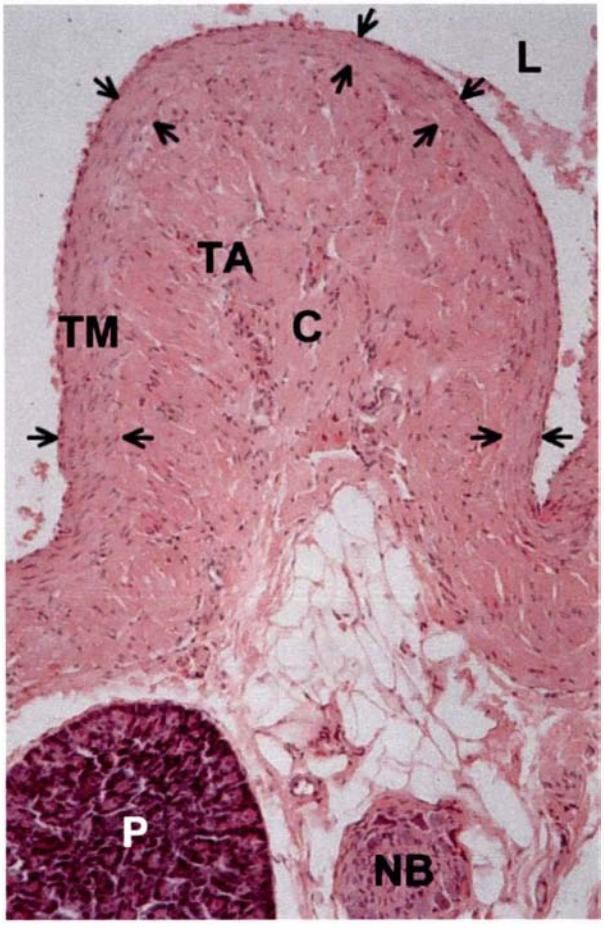

Plate 8. 'Atypical' fold. Note the dense collagen core, large nerve bundle and thinning of the tunica media in the upper half of this fold (compare distances between arrows). C collagen core; L lumen; NB nerve bundle; P pancreas; TA tunica adventitia; TM tunica media. (75x)

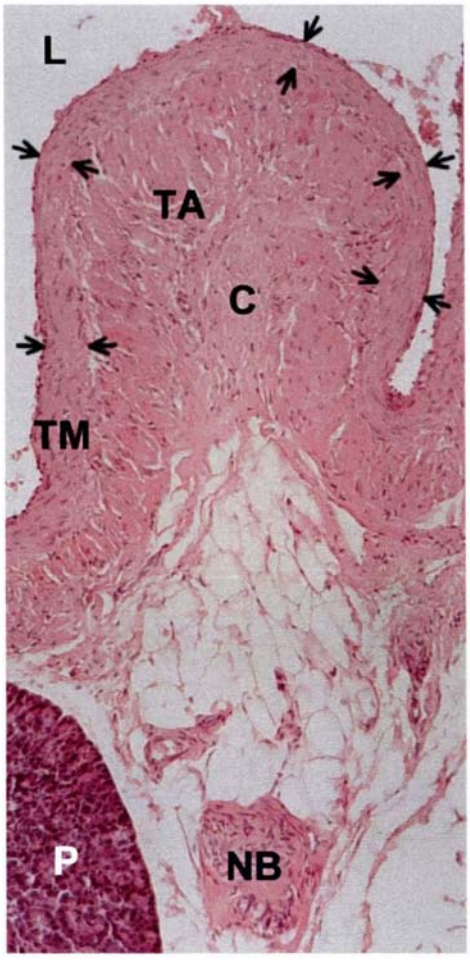

Plate 9. 'Atypical' fold. Note the variability in thickness of the tunica media in different areas of this fold (compare distances between arrows). C collagen core; L lumen; NB nerve bundle; P pancreas; TA tunica adventitia; TM tunica media. (80x)

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To explore the possibility that the 'atypical' folds described above might receive sensory innervation, the distribution of Sub P-, NKA- and CGRP-like immunoreactivity (Sub P-LI, NKA-LI and CGRP-LI, respectively) within and around the walls of the veins was studied. Figure 2 shows the locations from which the immunocytochemistry photomicrographs were obtained.

A total of 417 sections from seven different veins in the hepatic portal system were examined for the presence and distribution of the sensory neuropeptides, Sub P, NKA and CGRP. The proportion of sections examined in which positive Sub P-, NKA- and CGRP-LI was identified in or around the walls of the veins and their companion arteries[10] is shown in Table 1. Positive immunoreactivity to all three antibodies was sporadically and infrequently observed within and around the walls of the veins in all areas of the hepatic portal system examined. This was in contrast to the relatively high frequency of positive immunoreactivity observed within or around the walls of the corresponding arteries which were usually present on the same section. Immunoreactivity to each of the three antibodies in veins and arteries was most commonly observed in the middle to outer third of the tunica media, at the junction of the tunica media with the tunica adventitia, within the tunica adventitia and in surrounding nerve bundles (Plates 10 & 11). Less frequently, areas of intense immunoreactivity were observed to approach the level of the endothelium in the walls of the veins, but *not* of the arteries (Plates 12, 13, 14 & 15). Positive immunoreactivity was *not* observed within the dense collagen cores of the previously identified atypical folds. Negative controls are shown in Plates 16 and 17.

[10]Companion artery was defined as the artery on the same section which was nearest in size to the primary vein being examined.

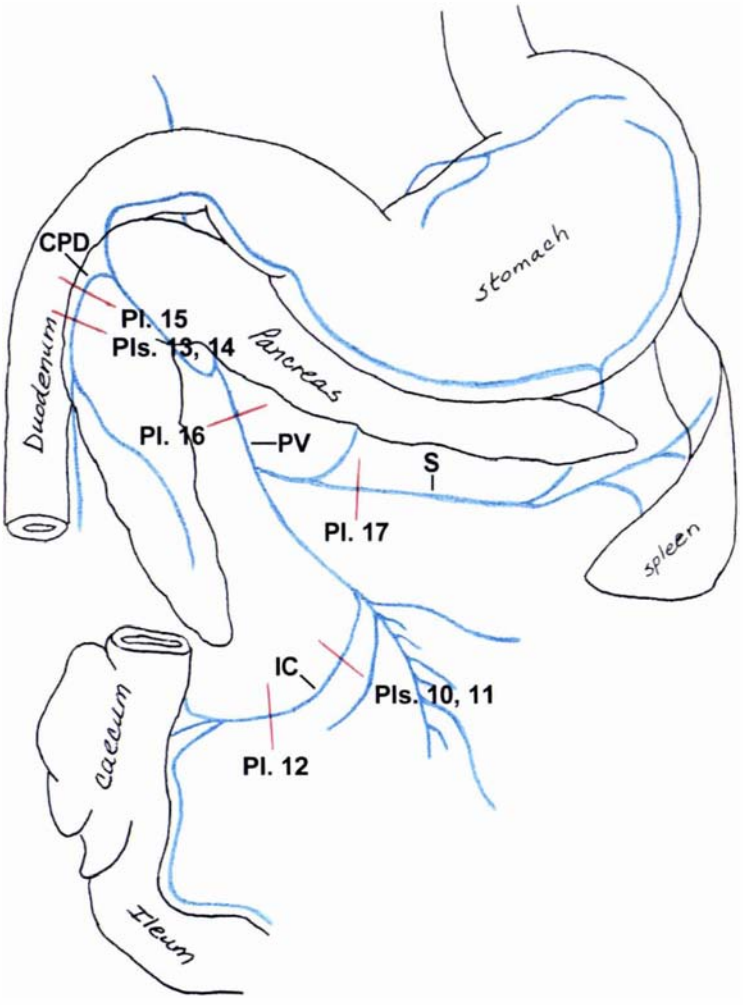

Figure 2. Locations from which immunocytochemistry sections were obtained for purposes of producing photomicrographs (plates 11 to 17); CPD cranial pancreaticoduodenal vein; IC ileocolic vein; PV portal vein; S splenic vein.

Table 1. Proportion of sections examined in which were observed one or more areas of positive immunoreactivity to each antibody in or around the walls of the veins and their companion arteries

	Vein			Companion Artery		
	SUB P	**NKA**	**CGRP**	**SUB P**	**NKA**	**CGRP**
Portal trunk	20/34 (58.8%)	19/31 (61.3%)	23/34 (67.6%)	32/34 (94.1%)	28/31 (90.3%)	30/34 (88.2%)
Gastroduodenal	1/12 (8.3%)	1/13 (7.7%)	1/12 (8.3%)	11/12 (91.7%)	10/13 (76.9%)	10/12 (83.3%)
Cr pancreatico-duodenal	12/29 (41.4%)	6/30 (20.0%)	13/29 (44.8%)	29/29 (100%)	28/30 (93.3%)	28/29 (96.6%)
Duodenal tributary	3/12 (25.0%)	4/10 (40.0%)	1/1 (100%)	5/12 (41.7%)	5/10 (50.0%)	1/1 (100%)
Splenic	8/33 (24.2%)	5/33 (15.2%)	15/36 (41.7%)	25/33 (75.8%)	21/33 (63.6%)	30/36 (83.3%)
Left gastric	2/12 (16.7%)	0/12 (0%)	2/12 (16.7%)	10/12 (83.3%)	6/12 (50.0%)	10/12 (83.3%)
Left gastro-epiploic	1/12 (8.3%)	1/8 (12.5%)	2/12 (16.7%)	9/12 (75.0%)	3/8 (37.5%)	2/12 (16.7%)
OVERALL	**47/144 (32.6%)**	**36/137 (26.3%)**	**57/136 (41.9%)**	**121/144 (84.0%)**	**101/137 (73.7%)**	**111/136 (81.6%)**

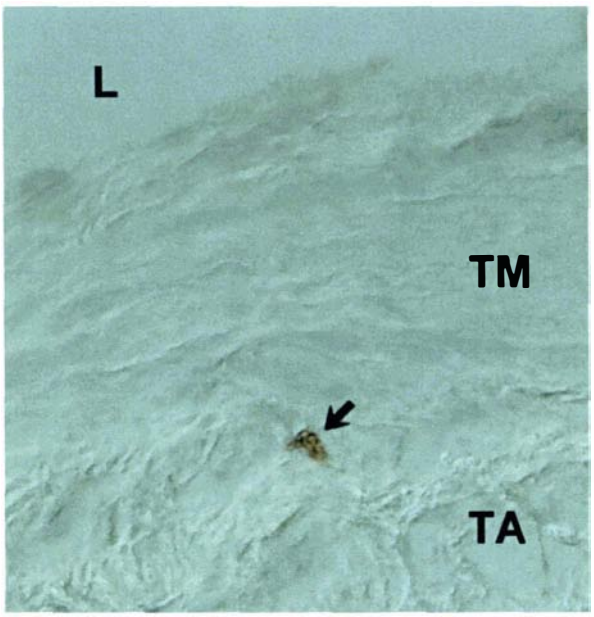

Plate 10. Typical location in which immunoreactivity was observed in the walls of arteries and veins. Immunoreactivity to each of the three antibodies was most commonly observed in the middle to outer third of the tunica media, at the junction of the tunica media with the tunica adventitia, within the tunica adventitia, and in surrounding nerve bundles. Arrow points to Sub P-LI found near the junction of the tunica media with the tunica adventitia in the portal vein. L lumen; TA tunica adventia; TM tunica media. (1000x)

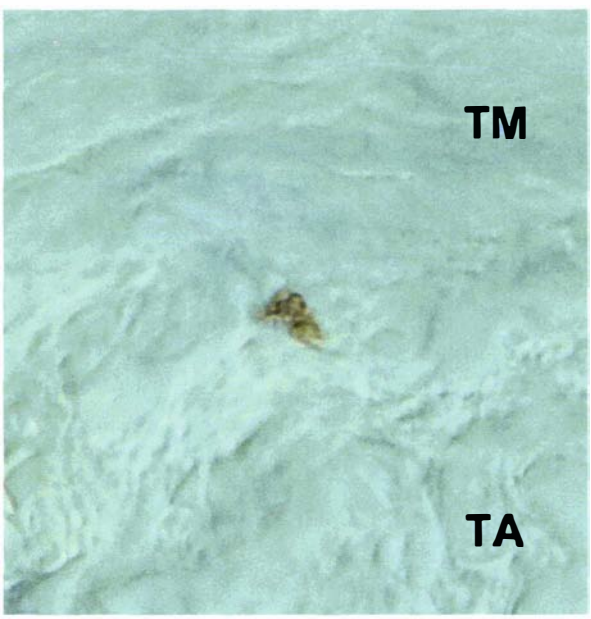

Plate 11. Slightly higher magnification of immunoreactivity shown in Plate 10. (1500x)

Plates 12., 13., 14. & 15. Sub P-, NKA- and CGRP-LI at or near the endothelium. Positive immunoreactivity (arrow) was sometimes observed to approach the level of the endothelium in the walls of the veins, but not the arteries. E endothelium; L lumen; TM tunica media.

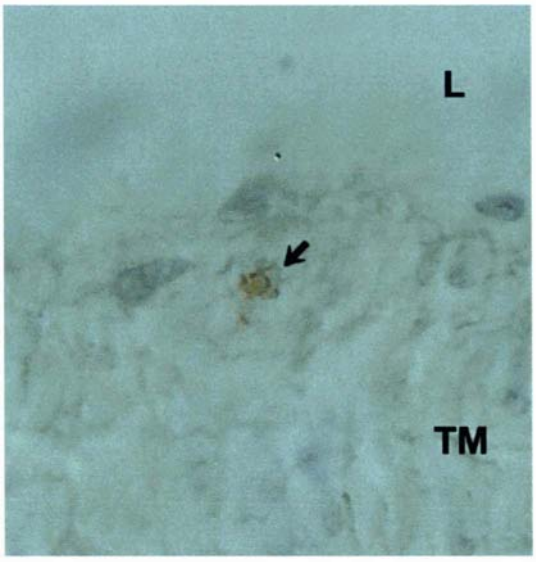

Plate 12. NKA-LI - portal trunk (1500x)

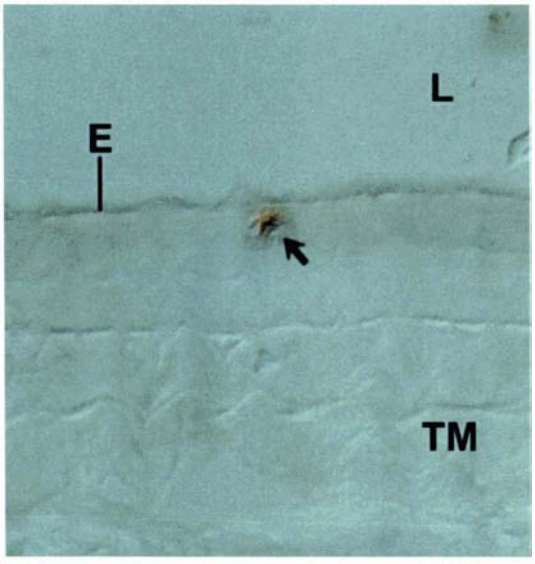

Plate 13. CGRP-LI - cranial pancreaticoduodenal vein (1500x)

Plate 14. Sub P-LI - cranial pancreaticoduodenal vein (1500x)

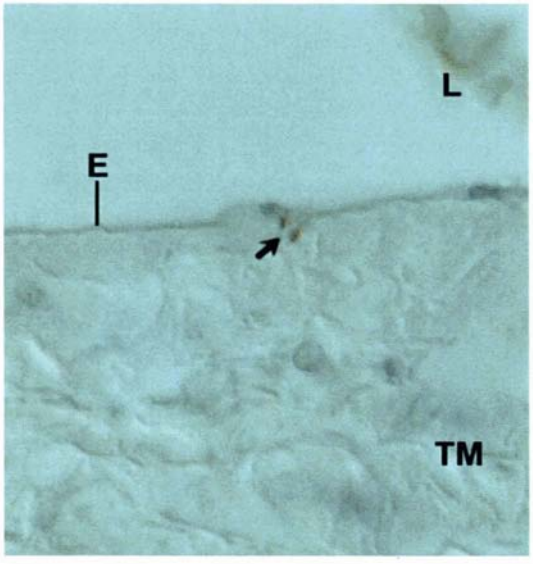

Plate 15. Sub P-LI - cranial pancreaticoduodenal vein (1500x)

Plates 16. & 17. Negative controls. Immunoreactivity was not observed in the walls of the veins or arteries of negative control slides. E endothelium; L lumen; TA tunica adventitia; TM tunica media

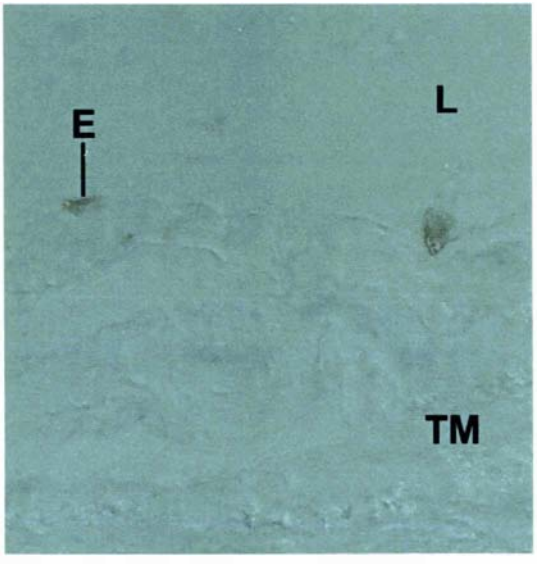

Plate 16. Portal trunk (1000x)

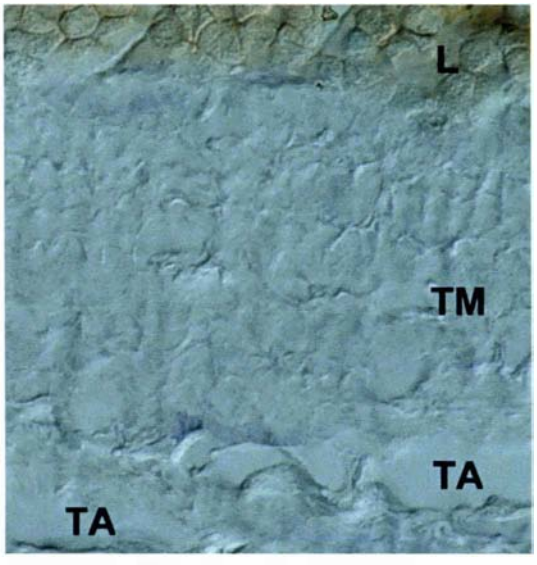

Plate 17. Splenic vein (1000x)

DISCUSSION

This investigation has explored the microscopic anatomy of the duodeno-pancreatic area and hepatic portal system of the dog. Relevant to the primary goal of this study was a descriptive account of the anatomical and histological features of the pancreas in its area of close apposition with the duodenum. While the histological observations are consistent with current knowledge regarding the microscopic appearance of the pancreas, duodenum and veins on an individual basis, a descriptive account of the microscopic *relationships* of these structures does not appear in the literature. In this respect, the results provide new information which is both relevant and complementary to the macroscopic anatomy previously described (Chapters 2 and 3).

Histologically, the canine pancreas is 'attached' to the proximal descending limb of the duodenum by thin sheets of fibrous connective tissue which, in some areas, are widely interspersed with fat. These histological features typify mesenteries as a whole, and in this case, they represent the mesoduodenum, the mesentery into which the right lobe of the pancreas expands during early development. In the area of *closest* apposition, only a single thin sheet of connective tissue remains. This layer originates from the mesoduodenum and forms a continuous, interposing capsule between the pancreas and duodenum. The 'area of close apposition' can therefore now be more precisely defined as that portion of the pancreas which closely approximates, yet remains anatomically distinct from, the duodenum.

The relative paucity of blood vessels and nerves coursing directly between the mesenteric border of the duodenum and the pancreas is consistent with the macroscopic anatomy previously described (Chapter 3). Latex-cast specimens of the canine hepatic portal system also revealed that venules emanated from the more dorsal and ventral aspects of the duodenal wall and coursed obliquely through the mesoduodenum, rather than emanating from the mesenteric border of the duodenum and crossing directly into the pancreas. In that, as well as the present study, smaller tributaries were observed to converge with progressively larger tributaries as they continued their course toward the cranial pancreaticoduodenal vein. Histologically however, veins and arteries were rarely able to be followed directly to their parent vessels which were not located immediately

adjacent to the duodenum, but distantly, within deep folds of the pancreas. Similarly, despite serial sectioning, the specific course of travel of nerves could not be traced, although this may have been due, in part, to the inability to readily distinguish nerves without the use of specific (silver) staining methods. Demonstration of nerves leaving the duodenum and entering the pancreas would have provided histological evidence that the enteric nervous system indeed innervates the pancreas in the dog, as has been shown in the rat (Anglade *et al.*, 1987).

The histological methods employed were also found to be an insensitive method for differentiating between venules of pancreatic and duodenal origin when compared to the latex casting and maceration technique used in Chapter 3. It was therefore not possible to recognize pancreatic tributaries which joined first with duodenal tributaries before entering the cranial pancreaticoduodenal vein, or to demonstrate or refute the presence of veins which may have formed communicating channels between either the duodenum, or the cranial pancreaticoduodenal vein, and the pancreas. The observation however, that veins leaving the duodenum coursed *between* rather than *through* pancreatic lobules, and that they converged with progressively *larger* rather than *smaller* tributaries within interlobular septa, provided little histological support for the existence of a portal circulation between the two organs. Similarly, the absence of closely apposed veins/lymphatics with arterioles in or around the duodeno-pancreatic area also failed to provide histological support for the existence of a local countercurrent exchange mechanism in this area.

Also consistent with the macroscopic anatomy was the presence of valves throughout most areas of the hepatic portal system. Although direction of blood flow could not always be determined, when valves were present, they were commonly observed at expected locations, i.e., near the bifurcations of smaller tributaries with larger tributaries, when serial sections were considered as a whole. The inability to consistently determine direction of blood flow on any given tissue section, as well as the inability to distinguish between veins of pancreatic and duodenal origin, precluded investigation of the possibility that blood (as a result of strategic placement of valves) may somehow be diverted through the pancreas before entering, or re-entering, the parent vessel. Overall, the results provide no new or additional information which supports the existence of a venous communication

between the duodenum and pancreas. The results however, *do* profess to the widespread nature of venous valves in these blood vessels in the dog.

An intriguing component of the present investigation was the relatively common occurrence of one or more luminal-projecting 'folds' of the vein wall. These folds, which extended for several hundred μm along the length of the vein, were found throughout both the hepatic portal and systemic venous systems. The large majority of these structures were clearly artefactual as was demonstrated in the present investigation, and as has been shown by others (Bacon and Niles, 1983; Geneser, 1986). Unexpected however, were the observations that in a small number of folds, a dense collagen core was comprised of tunica adventitia. Within this core, nerves were sometimes observed, and in some cases, there was thinning of the tunica media in the area of the fold. These unusual microscopic features were reminiscent of the carotid sinus baroreceptors which are characterized histologically by a thin tunica media and a thick, collagen-dense tunica adventitia. The latter houses the fine afferent nerve fibres of the pressoreceptors [for references, see Bader (1963)]. Although this author could find no reference to the presence of inward-projecting 'folds' along the vessel wall, the histological descriptions of the wall of the artery in the carotid sinus were not entirely dissimilar to the findings of the present study. It was these observations which prompted this investigator to speculate that the 'folds' may have a sensory function.

Despite the above mentioned histological similarities, the function of pressoreceptors has little relevance to our investigation of a possible role for the portal system in the control of pancreatic function. Of greater interest would be the identification of structures within the portal system which 'sensed' nutrients, hormones or other products of digestion, i.e., portal 'chemoreceptors', and which then (via local or long pathways involving the central nervous system) exerted some direct regulatory influence on the pancreas. Such a chemosensory role for the portal system is not unprecedented, at least as far as reflex regulation of the *endocrine* pancreas is concerned. The hepatico-portal area has been shown to be sensitive to glucose (Niijima, 1969; Russek, 1970; Schmitt, 1973), the incretin hormone, glucagon-like peptide-1 (Nishizawa *et al.*, 1996), and to a number of amino acids (Tanaka *et al.*, 1986; Tanaka *et al.*, 1990; Saitou *et al.*, 1993; Niijima and Meguid, 1995).

Vagal arginine (Tanaka *et al.*, 1986), alanine and leucine (Tanaka *et al.*, 1990) sensors in the liver have been shown to modulate amino acid-induced pancreatic hormone secretion, and glycine sensors (Saitou *et al.*, 1993) in the hepatico-portal system have been shown to exert reflex regulation on pancreatic vagal nerve activity. There is also strong evidence for the existence of receptors in the hepatico-portal system which are sensitive to changes in osmolality, and which play an important *early* role in systemic osmoregulation following ingestion of a meal (Haberich, 1968). Although little is known about the structure(s) of the above-mentioned 'receptors', such diverse responses by the liver and/or the portal system to absorbed nutrients and hormones illustrate the complex nature of the sensory mechanisms controlling homeostasis. They further show that the veins of the hepatic portal system have far more complex functions than acting as simple conduits for blood.

The possibility that the 'folds' of the present study might represent specialized 'sensory' structures was investigated immunocytochemically by examining the veins for the presence and distribution of the sensory neuropeptides, Sub P, NKA and CGRP. The method employed involved application of specific antisera (anti-Sub P antibody, anti-NKA antibody and anti-CGRP antibody) to the tissues of interest, labelling the reacted antibody with a second, biotinylated antibody, and then reacting *this* product with a preformed biotin-streptavidin-enzyme complex. Final addition of the chromogen, DAB, produced a brown end-product at positive sites of immunoreactivity. The biotin-streptavidin staining method was selected because of its greater sensitivity compared to other direct and indirect methods, and the excellent results which can be obtained on fixed, paraffin-embedded specimens (Boenisch, 1989).

The tachykinins (Sub P and NKA) and CGRP are neuropeptides contained within a distinct subpopulation of primary afferent neurons. The criterion for classification of these neurons is pharmacological, based on their sensitivity to the stimulant and desensitizing actions of capsaicin. The peptides are synthesized in the cell bodies of dorsal root ganglia and are transported to both peripheral and central terminals where, upon release, they function as neurotransmitters in both the peripheral and central nervous systems. With the exception of their presence in a small number of non-neuronal cell types in the peripheral

nervous system, the tachykinins and CGRP are primarily of neuronal origin and the most widespread sources are the peripheral endings of capsaicin sensitive nerves. Receptor endings of visceral primary afferent neurons are located in the walls (or in the parenchyma) of internal organs, in the vessels that supply the viscera, and in the serosal membranes that cover them. Visceral primary afferent fibres travel to the central nervous system via the sympathetic and parasympathetic nerves [for references and extensive reviews on the physiology and pharmacology of primary afferent neurons, see Cervero & Foreman (1990), Otsuka & Yoshioka (1993) and Maggi (1995)].

This study revealed the presence and distribution of Sub P-, NKA- and CGRP-LI in the veins of the hepatic portal system. Consistent with previous reports [for references, see Maggi (1995)], the neuropeptides were well-represented around blood vessels, and, positive immunoreactivity was observed with greater frequency around arteries compared to veins. With regard to the primary objective of this study however, positive immunoreactivity was *not* observed with greater frequency in the dense collagen cores of the previously identified 'atypical' folds, i.e., the three peptides, when present, appeared to be randomly distributed around the circumference of the vein. Although it is conceivable that the presence of fine immunoreactive neurofilaments which were concentrated in the tunica adventitia of the 'folds' may have gone unnoticed under the light microscope, the results of this study provided little evidence to suggest that the folded areas of the veins represent novel 'sensory' structures.

The general pattern of immunoreactivity observed around the veins and arteries in the present study was consistent with known patterns of innervation of blood vessels (Woollard, 1926; Hinsey, 1928), and both Sub P-LI (Barja and Mathison, 1982; Furness *et al.*, 1982; Barja and Mathison, 1984) and CGRP-LI (Sasaki *et al.*, 1986; Carrier and Connat, 1996) have been localized to the outer muscle layers and/or the tunica adventitia in the hepatic portal vein of rats and guinea pigs. Unexpected, however, were the areas of positive immunoreactivity which were observed at or near (within the luminal third of the tunica media) the level of the endothelium in the walls of the veins, but not the arteries. The possibility that these immunoreactions represented 'non-specific' binding was initially considered, however this seemed unlikely since no similar reactions were observed in

controls and antibodies were known to have been tested for antigen specificity. If one is to therefore assume that binding in these sites is specific, one is still left with the question of the *origin* of the peptides, i.e., are they of neuronal or non-neuronal origin? Immunoelectronmicroscopy has localized Sub P to the endothelium of mesenteric arteries (Loesch and Burnstock, 1988) and electron microscopic immunoreactions to CGRP have recently been identified in endothelial cells of the carotid body artery and its branches (Ozaka *et al.*, 1997). In the latter study, Ozaka, *et al.* suggested that CGRP was synthesized, stored, and presumably released from these cells. Whether a similar non-neuronal origin of the peptides exists for the immunoreactivity observed in the present report is unknown. Immunoelectronmicroscopy would have been an invaluable tool for clarifying this issue.

Overall, while it would appear from the available information that the 'folds' of the present study do *not* represent specialized 'sensory' structures, the interesting observation of positive immunoreactivity to the tachykinins and CGRP at (or near) the endothelium raises questions regarding the function of the neuropeptides at this level. It would seem that sensory innervation reaching the level of the endothelium would be ideal for 'sampling' the contents of venous blood, and indeed, such a theory has some basis in that one important function of (visceral) primary afferent neurons is the sensing of changes in the internal environment - changes which then trigger or modulate reflex control of visceral function [for references, see Cervero and Foreman (1990) and O'Donohue, *et al.*, (1990)]. Sensory nerve fibres reaching the level of the endothelium, or neuropeptides released from endothelial cells, may be involved in the conduction of impulses to the central nervous system. It would be of particular relevance to this thesis if it could be demonstrated that sensory nerve endings residing near the luminal side of the portal vasculature were sensitive to the chemical components of portal blood and that either local or even central processing of this information resulted in reflex regulation of pancreatic function. Although evidence to support such a hypothesis is tenuous, the possibility remains that the hepatic portal system may play a supplementary role in the control of exocrine pancreatic function. That such a role may exist for the veins of the hepatic portal system is the subject of Chapter 5.

Chapter Five

LATENCY OF PANCREATIC FLUID SECRETORY RESPONSE TO SECRETIN INJECTED ARTERIALLY AND INTO SELECTED VEINS OF THE HEPATIC PORTAL SYSTEM

SUMMARY

The purpose of this investigation was to explore the possibility that a local sensory mechanism for secretin originates within the walls of the veins of the portal system and that such a mechanism is involved in the reflex regulation of exocrine pancreatic function. To test this hypothesis, the latency of the pancreatic fluid secretory response to a bolus of secretin injected into the aorta (A site) was compared to that of secretin injected into a peripheral vein (superficial dorsal metatarsal vein = SDMV site) and into selected veins of the hepatic portal system (cranial pancreaticoduodenal vein = PDV site; portal vein = PV site; mesenteric vein = MV site). Overall, the latency of the pancreatic fluid secretory response to secretin injected into the portal system was longer than that following injection into the aorta, suggesting that a local sensory mechanism for secretin does not originate within the walls of the portal vasculature. However, pancreatic cannulae of two diameters were used in this study. The mean latencies were shorter in the dogs in which the larger pancreatic duct cannula was used, suggesting a 'delaying' effect due to greater resistance to flow through the smaller cannula. In addition, the latency after injection into the PV site was generally shorter than that after injections into the other venous sites, and response times after injection into the PV site approached that at the A site of injection. This is in marked contrast to the results of the dogs in which the small cannula was used, where there were no differences in mean latency between the venous sites of injection, but a clear difference between latencies at the A and PV sites of injection. These differences in latency data when broken down by pancreatic duct cannula size raised questions regarding the validity of this model, which should be examined further.

INTRODUCTION

Previous studies by this author in which the macroscopic and microscopic anatomy of the duodeno-pancreatic area in the dog were examined (Chapters 3 and 4), failed to provide strong evidence for the existence of a direct duodenum-to-pancreas venous communication. Such a 'short-cut' mechanism was postulated to be a potential means by which the gut exerts some degree of local regulatory control over the exocrine pancreas without the absolute requirement for absorbed nutrients, hormones and metabolites to first enter the systemic circulation. Because these studies failed to identify either a direct interconnection of veins between the duodenum and pancreas, or evidence which would

suggest that portal blood flow is *directed* towards the pancreas, it was concluded that the possibility of a vascular shunt between the two organs was remote.

An alternative means by which information regarding the digestive status of the gut could be directly relayed to the pancreas is through a local mechanism, essential sensory components of which exist within the walls of the veins. This idea was originally prompted by the results of an earlier microscopic survey of the hepatic portal system (Chapter 4) which revealed, in the walls of at least some veins, histological features which resembled in some ways those described for pressoreceptors of the carotid sinus and aortic arch (for references, see Bader, 1963). The feasibility of such a mechanism is also supported by physiological evidence for *other* sensory modalities which appear to reside within the portal system or liver (Haberich, 1968; Sakaguchi and Yamaguchi, 1979; Stoppini *et al.*, 1984; Saitou *et al.*, 1993). Osmosensitive mechanisms for example, have been shown to facilitate rapid and early homeostatic adjustments in water balance (Haberich, 1968). These features, which act *before* cerebral osmoreceptors become involved, would appear to be adaptive for preventing wide surges in systemic osmolality following ingestion of a meal. In addition, this author has recently demonstrated within the hepatic portal system, endothelium-associated immunoreactivity to substance P (Sub P), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP), neuropeptides which are associated with primary sensory afferent nerve fibres (Chapter 4). While neither the specificity nor the biological significance of the latter findings is known, it is possible that an additional, as yet unrecognized regulatory role can be further assigned to the veins of the hepatic portal system.

In order to test the hypothesis that a sensory mechanism exists within the hepatic portal system of the dog, and that such a sensory mechanism plays a role in the control of exocrine pancreatic function, a study was designed to examine and compare between sites, the latencies of the pancreatic fluid secretory response to a known, exogenously administered pancreatic secretagogue. Secretin is a naturally-occurring polypeptide hormone which is synthesized by specialized endocrine cells (S cells) in the small intestinal mucosa (Bussolati *et al.*, 1971; Polak *et al.*, 1971). This hormone, normally released in response to acid and fat digests in the upper small intestine (Faichney *et al.*,

1981; Schaffalitzky de Muckadell *et al.*, 1981), is a potent and highly efficacious stimulant of pancreatic fluid and bicarbonate secretion. Secretin has been shown to inhibit gastric emptying (Raybould and Holzer, 1993) and gastric acid secretion (Li *et al.*, 1998) in rats via a capsaicin-sensitive vagal afferent pathway, and while a great deal is known regarding pancreatic enzyme secretion following stimulation of vagal afferent pathways by cholecystokinin (Li and Owyang, 1993; Li and Owyang, 1994), virtually nothing is known about the ability of secretin to stimulate pancreatic secretion via sensory neural pathways. These properties, along with the ability of secretin to produce immediately measurable increases in output of pancreatic fluid, are why this hormone was selected for preliminary testing.

The purpose of this investigation was to compare the latencies of the pancreatic fluid secretory response to secretin injected arterially and into selected veins of the hepatic portal system of the dog. The rationale was that if the pancreatic fluid secretory response to secretin injected into the portal system was in part mediated by a local sensory mechanism, essential components of which exist within the walls of the veins, then this response would have a shorter latency than the response to secretin injected into the aorta. If such a mechanism does not exist within the walls of the veins for secretin, then it was assumed that differences in the measured latencies would simply reflect the time necessary for systemic transport of secretin from the site of injection to the pancreas.

MATERIALS AND METHODS

ANIMALS AND ANIMAL PREPARATION

All dogs used for this experiment were sourced from the Animal Health Services Centre (Massey University, Palmerston North, NZ) for purposes of acute, terminal experimentation. As part of the normal turnover of animals bred for research purposes, the dogs employed for this study were destined for euthanasia. The dogs were under anaesthesia throughout the entire study, never regained consciousness and were overdosed with the anaesthetic upon completion of the experiment. Massey University Animal Ethics approval was obtained for all procedures prior to the start of the experiments.

Sixteen lean dogs, approximately eight months to three years of age and weighing 13 to 25 kg, were used for this experiment. The dogs were of mixed breed and consisted of eight males and eight females. Four of the 16 dogs were randomly assigned as control dogs prior to beginning the experiment. All dogs were determined to be healthy at the time of admission based upon standard clinical examination and routine pre-operative blood analyses (packed cell volume and total protein concentration). All dogs were fasted for 16 to 18 hours prior to anaesthesia but had free access to water. One dog was used on each experimental day and usually two experiments were performed each week.

ANAESTHESIA AND PRE-SURGICAL SET-UP

On the day of the experiment, an 18 gauge, 5.1 cm indwelling teflon catheter (Quik-Cath Dupont catheter, Travenol Laboratories Inc, Deerfield, Ill., USA) was placed in a foreleg vein to facilitate administration of induction agents, maintenance fluids and secretin infusion. Anaesthesia commenced between 08:30 and 09:00 hours on experimental days. Anaesthesia was induced via cephalic vein injection of diazepam (0.5 mg/kg as a rapid bolus; Pamlin Injection, Parnell Labs NZ Ltd, Auckland, NZ) followed immediately by thiopental (10 mg/kg to effect; Intraval Sodium, May & Baker, Dagenham, England). Dogs were intubated and maintained on a mixture of halothane (Fluothane, ICI NZ Ltd, Auckland, NZ) and oxygen.

Following induction, dogs were positioned in dorsal recumbency on an electric heating pad below which lay a sheet of bubble wrap which was of suitable size for securing around the dog (after surgery) to prevent hypothermia. A catheter was passed through the urethra into the bladder of male dogs to prevent urine contamination of the abdomen. In order to facilitate continuous removal of gastric juices and thereby minimize entry of gastric contents into the duodenum, a fenestrated feeding tube (Levin tube, Jackson Allison Medical and Surgical Ltd, Auckland, NZ) was passed through the oral cavity into the stomach and connected to a suction pump (A & H Surgical Suction Apparatus, Model T.J.20, Allen & Hanburys Ltd Surgical Engineering Division, London, England). Placement of this tube in the stomach was verified after opening the abdomen. Because endogenous release of secretin (and subsequent release of pancreatic fluid from the pancreas) may result from gastric acid entering the duodenum, random samples of gastric

fluid were collected via the feeding tube from seven dogs for pH determination (PHM220 Lab pH Meter, Radiometer/Copenhagan, Radiometer Analytical S.A., Lyon, France).

The abdomen was clipped and prepared for surgery. Maintenance fluids (0.15 M NaCl solution; Baxter Viaflex, Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia) were administered into the cephalic vein catheter via peristaltic pump (10 ml/kg/hr; Gilson Minipuls 3, Le Bel, France). Direct blood pressure readings were made following cannulation of either a pedal artery (20 g, 5.1 cm teflon catheter; Quik-Cath Dupont catheter, Travenol Laboratories Inc, Deerfield, Ill., USA) or a femoral artery (single lumen polyethylene tube, ID 2.00 x OD 3.00 mm; Dural Plastics and Engineering, Auburn, NSW, Australia). Anaesthetic monitoring consisted of heart rate, respiratory rate, systolic and diastolic blood pressure recordings every ten minutes. Assessments of anaesthetic depth were made but not recorded using reflex responses, jaw tone, eye position, mucus membrane colour and capillary refill time. Body temperature was recorded usually once per hour. Appendix 4 shows the anaesthetic form used for each dog as well as the results of anaesthetic monitoring for dog #15.

SURGICAL PROCEDURES AND EXPERIMENTAL SET-UP

Each experiment required eight surgical procedures. These included: ligation of the main pancreatic duct; cannulation of the accessory pancreatic duct; cannulation of the aorta, portal vein, cranial pancreaticoduodenal vein and a mesenteric vein. For purposes of comparing the latency of response to secretin injected into a peripheral systemic vein, the superficial dorsal metatarsal vein was also cannulated. Following completion of these procedures, EMG recording electrodes were placed in the gastric antrum and duodenum to detect changes in gastrointestinal contractile activity that might influence pancreatic fluid output.

Cannulation of the aorta and superficial dorsal metatarsal vein was performed prior to opening the abdomen. All vascular cannulae consisted of single lumen PVC tubing (Dural Plastics and Engineering, Auburn, NSW, Australia), were of the same length and had the same internal diameter (length: 0.50 cm; ID: 0.50 mm x OD: 0.80 mm; dead space: 0.11 ml). The aorta was cannulated via a femoral artery using a cannula of the same length and

internal dimensions, but which had been inserted and glued into an outer PVC sleeve (ID: 1.20 mm x OD: 1.70 mm) to provide greater rigidity when inserting against the flow of arterial blood. This modified cannula was advanced through the femoral artery into the aorta and positioned so that the tip of the cannula was at or just cranial to the level of the diaphragm. All vascular cannulae, except the dorsal metatarsal vein cannula, were secured to the vessel into which they were introduced by double ligation. The dorsal metatarsal vein was cannulated through a previously introduced 18 gauge, 5.1 cm indwelling teflon catheter which was glued in place. The tip of this cannula was positioned at or near the level of the hock. All vascular cannulae were capped with labelled injection ports. All vascular cannulae were filled with heparinized saline (5 IU heparin/ml saline; Multiparin Heparin Injection, Fisons Pharmaceuticals, Leicestershire, UK) prior to introduction into the vessel, and were usually flushed on an hourly basis thereafter.

Following the above cannulation procedures, a ventral midline incision was made to expose the abdominal viscera. The duodenum and pancreas were isolated and reflected ventrally to identify and ligate the main pancreatic duct. The accessory pancreatic duct, which was most accessible from a ventral approach, was isolated by gently dissecting between the pancreas and duodenum. In most dogs, the accessory pancreatic duct was found to bifurcate just inside the pancreas, necessitating ligation of usually the caudally-directed branch. Into the cranially-directed branch, one of two different-sized PVC cannulae (Dural Plastics and Engineering, Auburn, NSW, Australia) was placed. In dogs #1 to #8, a 50 cm long cannula with known dead space (0.30 ml) and internal diameter (ID: 0.86 mm x OD: 1.27 mm) was used. Because of difficulties encountered obtaining pancreatic fluid consistently through this small diameter, a cannula of the same length but with larger internal dimensions (ID: 0.97 mm x OD: 1.27 mm; dead space: 0.40 ml) was placed in the accessory pancreatic ducts of dogs #9 to #16. All cannulae were filled with 0.15 M saline prior to placement.

Cannulation of the cranial pancreaticoduodenal vein was accomplished by introducing a cannula into the duodenal branch of the cranial pancreaticoduodenal vein and advancing it cranially until its tip lay immediately caudal to the site of termination of close contact of the pancreas with the duodenum. This was usually caudal to the level of convergence

of the duodenal branch with the pancreatic branch. In order to ensure that the secretin bolus was directed through the cranial pancreaticoduodenal vein alone, the pancreatic branch was ligated. The portal vein was cannulated via a conveniently located jejunal vein tributary, and an attempt was made to position the tip of this cannula just cranial to the bifurcation of the gastroduodenal vein. The mesenteric vein cannula was introduced in similar fashion, however its tip was positioned 2.5 cm cranial to its site of introduction into the jejunal vessel.

For measurement of electrical activity along the bowel wall, teflon-coated, multi-strand biomed electrodes (#AS633, Cooner Wire Company, Chatsworth, Ca., USA) were positioned intramurally in the gastric antrum and in the distal half of the descending duodenum. Antro-duodenal myoelectrical activity, blood pressure and pancreatic fluid output were simultaneously recorded using a JRAK module system (JRAK Biosignals Pty Ltd, Australia) and a four channel chart recorder (Gould Inc Instrument Division, Cleveland, Ohio, USA). Pancreatic fluid output (drop rate) was measured using an optical transducer and drop counter designed to integrate with the JRAK system.

Figure 1 shows the final set-up for the experiment after all surgical procedures were completed.

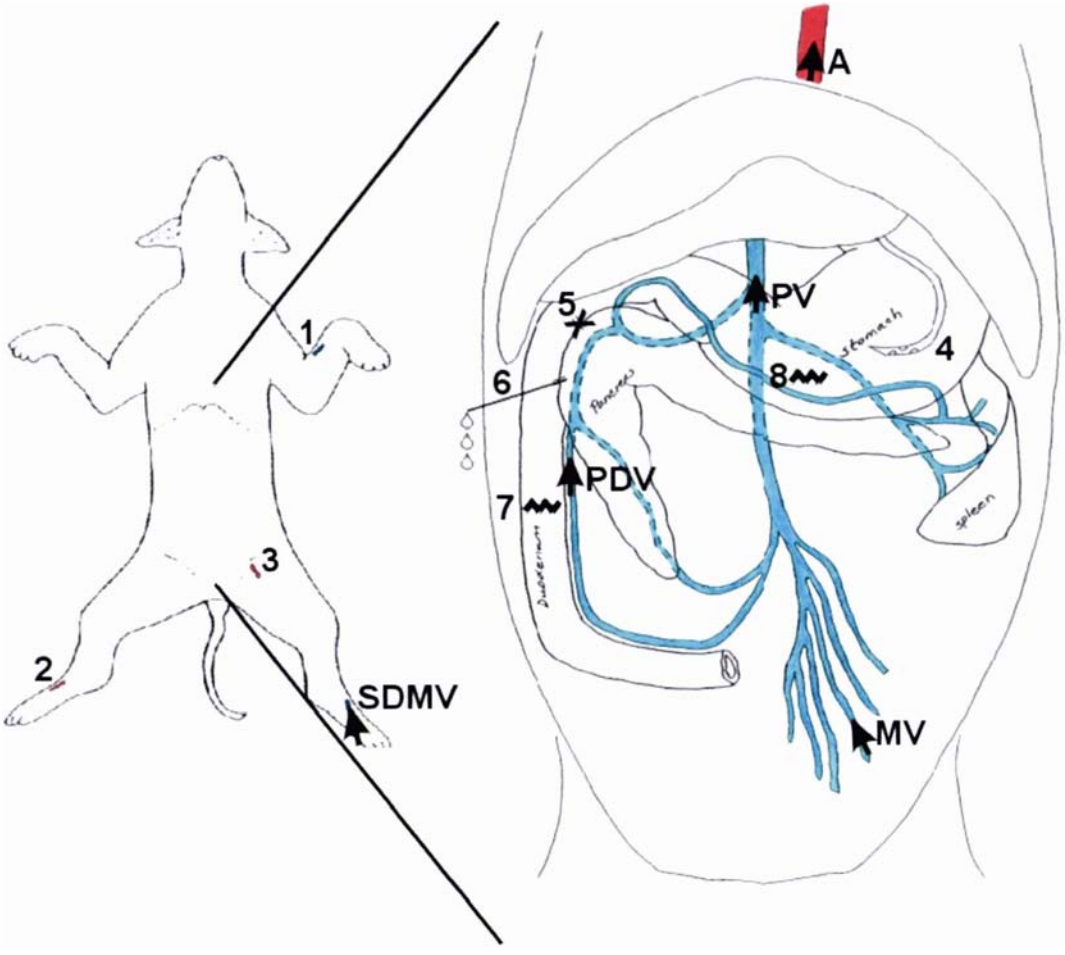

Figure 1. Final set-up of experiment. Arrows mark the locations at which the tips of cannulae were positioned for administration of secretin boluses. Each arrow points in the direction that the bolus was administered. A aortic cannula; MV mesenteric vein cannula; PDV cranial pancreaticoduodenal vein cannula; PV portal vein cannula; SDMV superficial dorsal metatarsal vein cannula. 1 cephalic vein catheter for administration of induction agents, fluids and secretin infusion; 2 pedal artery catheter for measurement of blood pressure; 3 femoral artery site of introduction of aortic cannula; 4 oro-gastric tube for continuous suction of gastric juices; 5 main pancreatic duct ligated; 6 accessory pancreatic duct cannulated; 7 duodenal EMG recording electrode; 8 antral EMG recording electrode

Following completion of the above procedures, the viscera were carefully replaced in the abdomen, and in order to prevent the tissues from drying out, the abdomen was gently packed with saline-moistened laparotomy towels. The ends of all cannulae were exteriorized and their patency checked prior to closing the abdominal wall with towel clamps. The underlying layer of bubble wrap was fastened snugly around the body of the dog and the system was allowed to stabilize for a minimum of one hour.

EXPERIMENTAL DESIGN

The reader is referred to Appendix 5 for details of the checklist and protocol followed for each experiment.

Preparation of secretin solutions

All secretin-containing solutions consisted of synthetic secretin (Sigma, Biolab Scientific Ltd, Auckland, NZ) dissolved in 0.15 M NaCl with 0.5 % bovine serum albumin (BSA; Fraction V Powder, Sigma, Biolab Scientific Ltd, NZ). Bovine serum albumin (2.5 gms) was added to a 500 ml bag of isotonic saline solution for infusion and to a smaller stock volume of saline for preparation of boluses (50 mg BSA in 10 ml). From the stock volume, 1 ml of the BSA/saline solution was drawn-up into each of five labelled, 1-ml syringes. These solutions were prepared the day before the experiment and were refrigerated overnight. On the morning of the experiment, 10 μg secretin was dissolved in the 500 ml bag of saline containing BSA to make a final concentration of 20 ng secretin/ml saline. To each 1-ml syringe containing saline/BSA was added 35 ng secretin/kg body weight. This dose was selected from a range of doses used in an earlier pilot study where the 35 ng/kg dose was judged to be optimal in terms of both the magnitude of the pancreatic fluid secretory response as well as the time necessary to return to baseline rate of secretion.

Intravenous infusion of secretin

To stimulate the flow of pancreatic fluid, the secretin infusion described above was begun following cannulation of the accessory pancreatic duct, and was continued for the remainder of the experiment. The secretin infusion was delivered via a second peristaltic pump (Gilson Minipuls 3, Le Bel, France) into the cephalic vein catheter at a concentration

of 20 ng/ml and an initial flow rate of 5-10 ml/kg/hr. This dose was then adjusted to obtain a baseline output of pancreatic fluid of approximately 3-6 drops per minute. Delivery rates of 0.15 M NaCl solution were adjusted as necessary so as not to exceed a total fluid (NaCl infusion plus secretin infusion) delivery of 10 ml/kg/hr.

Bolus administrations of secretin

Test boluses of secretin were given no sooner than one hour after completion of all surgical procedures and no sooner than 15 minutes after having achieved a stable pancreatic fluid output following administration of the previous bolus. The order of injection of the boluses was randomized using a computer-generated spreadsheet for each dog. Injection sites were identified as follows: A (aorta), PV (portal vein), PDV (cranial pancreaticoduodenal vein), MV (mesenteric vein = jejunal vein) and SDMV (superficial dorsal metatarsal vein). Prior to the first bolus of secretin and immediately following administration of each bolus, approximately 1 ml of pancreatic fluid was collected, weighed (Sartorius handy H51, Sartorius-Instruments Ltd, England) and the total protein concentration measured. Pancreatic fluid protein concentration was measured using an automated clinical chemistry analyzer (Boehringer Mannheim Urinary/CSF Protein assay, Roche Diagnostics NZ Ltd, Auckland, NZ). The method employed was based on a turbidometric endpoint which resulted from the reaction of benzethonium chloride and protein reacting in a basic medium.

Each secretin bolus was administered directly into the cannula over a 5 second period. Cannulae were immediately flushed over a similar time course with an equal volume of saline containing BSA, were then capped and flushed with a small volume of heparinized saline. The pancreatic response was recorded and allowed to return to steady state or previous baseline. Control dogs (#3, #8, #13 & #16) were treated identically to test dogs except they received boluses containing vehicle only (1 ml of 0.15 M NaCl with 0.5 % BSA).

Validation of procedures

Upon completion of the experiment, dogs were euthanased with an overdose of sodium pentobarbitone solution (Pentobarb 500, Chemstock Animal Health Ltd, NZ). In order to show that the PDV secretin bolus passed cranially through the vein without entering and stimulating the pancreas directly, 1 ml of indian ink (Quink Solv-x, Parker, England) was injected through the PDV cannula prior to euthanasia and again immediately following death. Serial sectioning through the pancreas after death failed to reveal ink in the pancreas of any dog.

Calculation of latency

The latency of the pancreatic fluid secretory response was measured as the time elapsing between the midpoint of the secretin bolus injection and a sustained decrease in the drop interval (time elapsed between drops) of more than three standard deviations, compared to the mean drop interval recorded during the immediate five minute pre-injection period. Drop intervals were calculated from the distance between consecutive drops (hand measured to the nearest 0.25 mm) from the recorded data at known paperspeed (see Appendix 6 for example of recorded data).

Calculation of drop size

To test whether variation in drop size could influence measurement of latency, the weight of each drop of pancreatic fluid (drop size) was determined by counting the number of drops in approximately 1 ml of fluid collected immediately post-injection following each secretin bolus. The number of drops in this volume of pancreatic fluid was then divided by the weight to give number of drops per gram of pancreatic fluid.

Duration of pancreatic fluid response and area under the curve

The duration of the pancreatic fluid response and the area under the curve (AUC) were determined following each secretin bolus injection. These data were used to describe the typical secretory response pattern and to assess whether pancreatic function significantly changed during the course of the experiment. Duration of the pancreatic response was defined as the time elapsing from injection of the secretin bolus until the number of drops of pancreatic fluid secreted per minute returned to within three standard deviations of the

mean number of drops secreted per minute during the immediate five minute pre-injection period (baseline drops per minute). The AUC was calculated by arithmetically summing the total secretory response, minus the baseline drops per minute, for the entire duration of the response.

Statistical analysis

All statistical analyses were performed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc, Cary, NC). Latency data for the 12 experimental dogs were analyzed by analysis of variance utilising a partially nested design. Factors included in the full model were cannula size, dog nested within cannula, site of injection and injection order. Injection order and interaction between cannula and site were non-significant and were deleted from the final model. The appropriateness of the model was confirmed using residual analysis (Appendix 7). Means reported are least squares means with standard errors. Differences between means were calculated using the Student's *t*-test.

Statistical analysis of drop size was performed using analysis of variance and covariance, with dog, cannula size, site, injection order, secretory rate and pancreatic fluid protein concentration included in the full model. Dog, cannula size, injection order and protein concentration had significant effects and were retained in the final model. Secretory rate was calculated as milligrams of fluid per minute post-injection and was log-transformed before analysis because of its left-skewed distribution.

Analysis of duration of response and AUC was performed for the small and large cannula groups of dogs, using analysis of variance with dog, site and injection order included in the model. Neither injection site nor injection order remained in the final model.

RESULTS

SECRETORY RESPONSE

An abrupt pancreatic secretory response was evident usually within the first minute following injection of secretin which peaked quickly and then declined to baseline over the following 10 to 15 minutes (Table 1 & Figure 2). No response was noted in control dogs where vehicle alone was injected. Both the durations and overall magnitudes of response as measured by the AUC were clearly dependent on the size of the cannula used in the experiment. Neither duration nor AUC however, were affected by injection site or order of injection, suggesting that exocrine pancreatic function remained constant throughout the course of the experiments.

Table 1. The pancreatic secretory response following administration of secretin into selected vessels, using either small or large pancreatic duct cannulae. Neither duration of response nor AUC was affected by site of injection or order of injection. Typical response curves are presented graphically in Figure 2. Min = minimum; Max = maximum

Cannula	Variable	Mean	SD	Min	Max
Small	Latency (sec)	36	11	17	60
	Duration (min)	9	7	3	25
	Area under response curve (AUC)	20	27	3	87
Large	Latency (sec)	33	14	13	75
	Duration (min)	16	5	6	26
	Area under response curve (AUC)	156	67	31	287

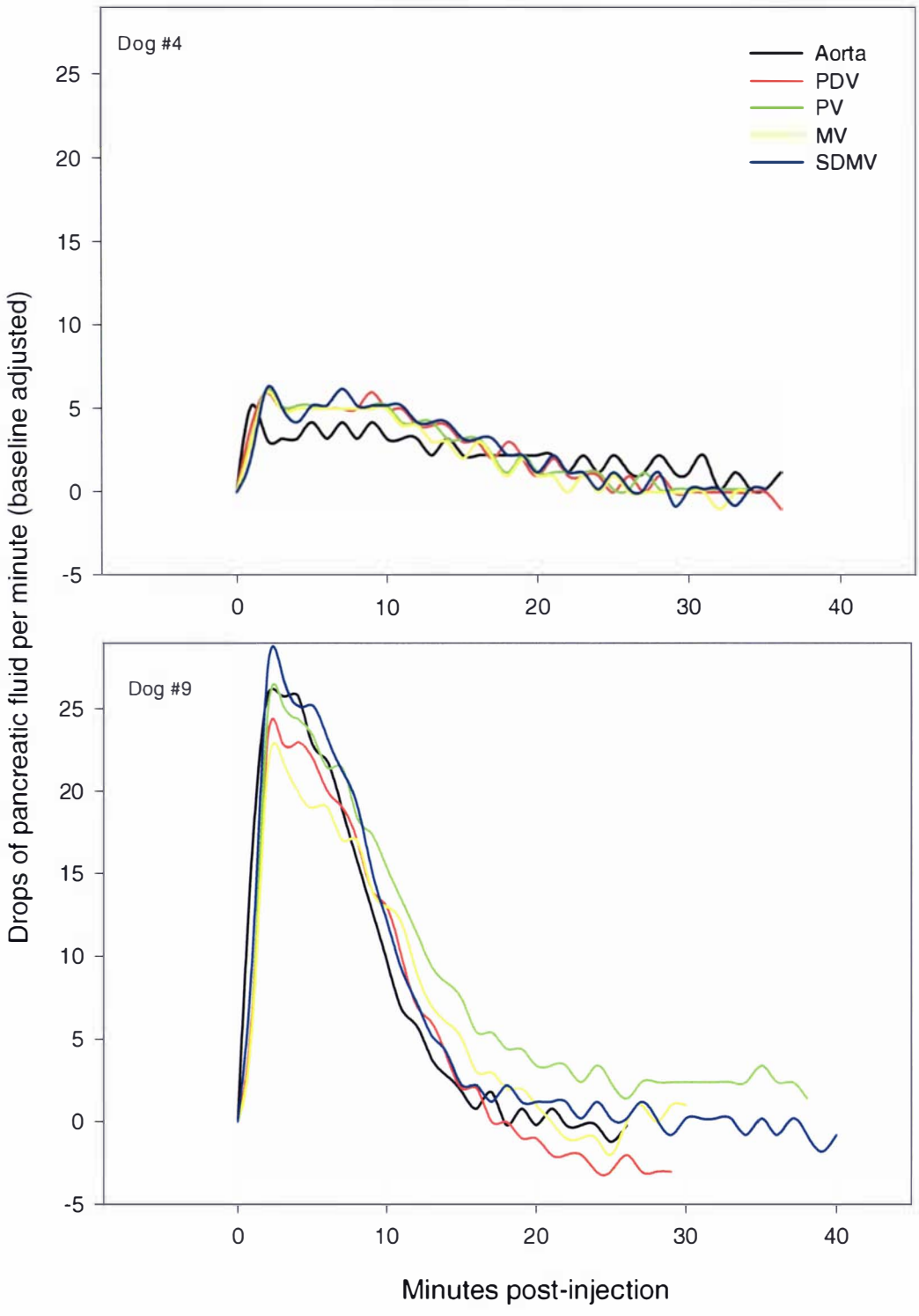

Figure 2. Typical pancreatic secretory response curves following administration of secretin into selected vessels, using either small (Dog #4) or large (Dog #9) pancreatic duct cannulae.

LATENCY OF RESPONSE

There was a delay between the time of secretin injection and the onset of the associated increase in exocrine pancreatic secretions. The duration of this delay, here called the latency of response, was significantly related to the secretin injection site ($p < 0.001$; Table 2) where generally, injection into the A site elicited a faster response than injection into any of the veins of the hepatic portal system or SDMV site (Figure 3). Differences in response times between the A site and all other sites of injection were significant ($p < 0.001$; refer to Appendix 8 for tabulation of least squares means). The latency of response to injections into the PV site were intermediate, being significantly faster than the latency of response to injections into the MV site ($p<0.05$), but not significantly different from the responses to injections into the PDV site or SDMV site.

The mean latencies were shorter in the group of dogs in which the larger pancreatic duct cannula was used ($p<0.05$; Table 2), suggesting a 'delaying' effect of greater resistance to flow in the smaller cannulae. In addition to the trend to shorter latencies, differences in latency between injection sites became more evident when the larger cannulae were

Table 2. ANOVA table from the analysis of latency of response (RTIME3): main effects model. Factors included in the full model were cannula size, dog nested within cannula, site of injection and injection order. Injection order and interaction between cannula and site were non-significant and were deleted from the final model.

Dependent Variable: RTIME3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	6387.62529552	425.84168637	7.68	0.0001
Error	39	2161.75380266	55.42958468		
Corrected Total	54	8549.37909818			

R-Square	C.V.	Root MSE	RTIME3 Mean
0.747145	21.58388	7.44510475	34.49381818

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CANNULA	1	320.41649306	320.41649306	5.78	0.0211
DOGID(CANNULA)	10	2451.75982372	245.17598237	4.42	0.0004
SITE	4	3212.29212734	803.07303183	14.49	0.0001

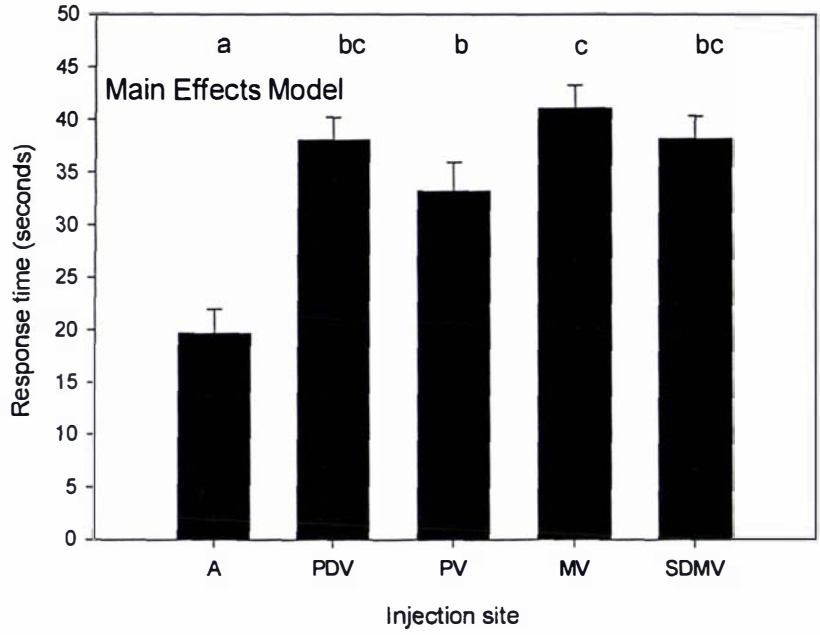

Figure 3. Main effects model for the effect of site of injection on the latency of response to secretin. Each bar represents the least squares mean and standard error for response time adjusted for variation arising from differing cannula size and differences between dogs. A aorta; MV mesenteric vein; PDV pancreaticoduodenal vein; PV portal vein; SDMV superficial dorsal metatarsal vein; a, b, c mean response times having superscripts which differ, are significantly different ($p < 0.05$)

used (Figure 4). Interestingly, the latency after injection into the PV site was generally shorter than after injections into the other venous sites ($p<0.05$ for MV and SDMV and $p=0.05$ for PDV) and response times after injection into the PV site approached that of the A site of injection ($p = 0.073$). This is in marked contrast to the results from the dogs in which the small cannulae were used, where there were no differences in mean latency between the venous sites but a clear difference between latencies for the A and PV sites ($p<0.01$; Figure 4).

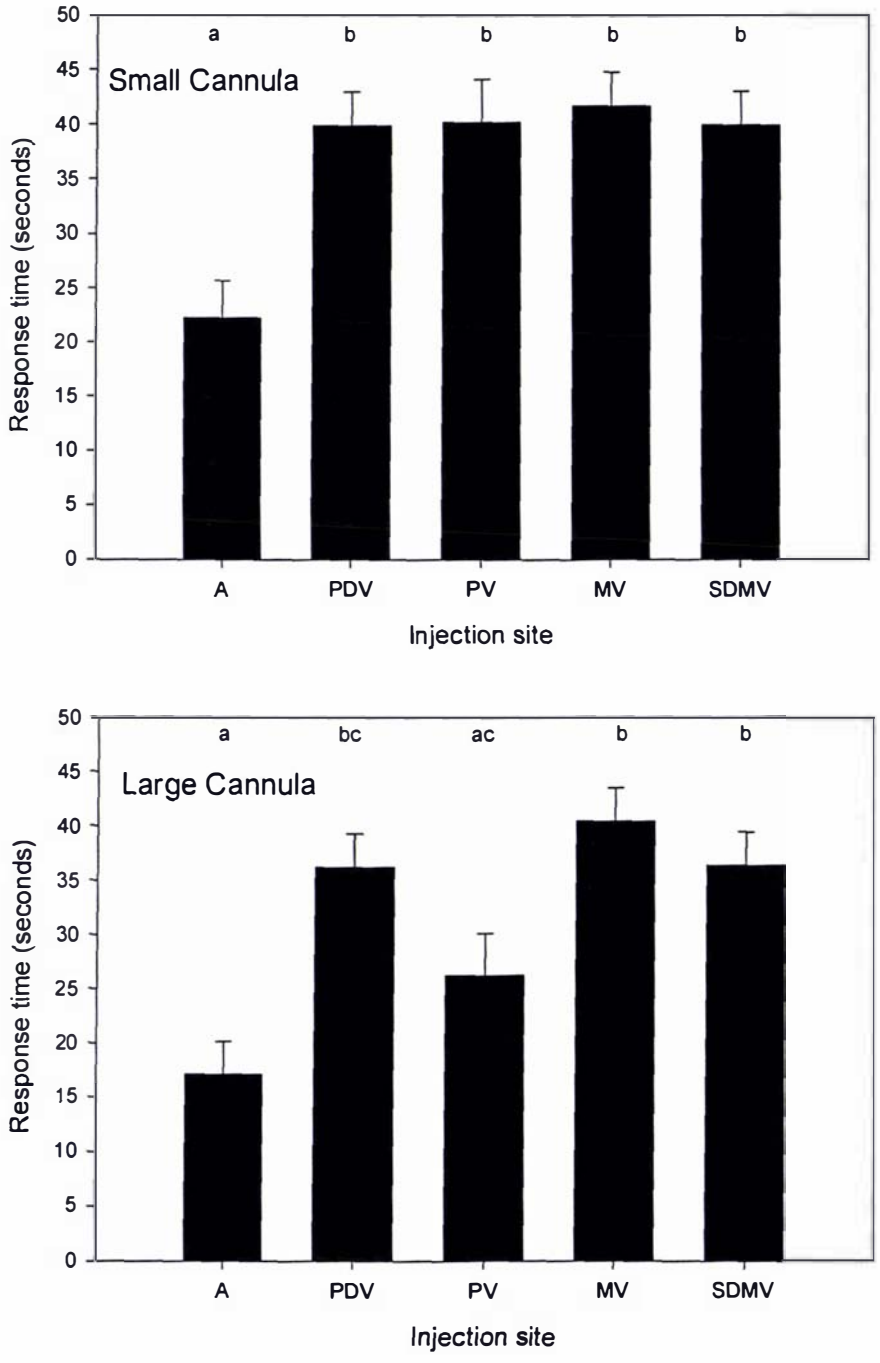

Figure 4. The effect of site of injection on latency of response to secretin, broken down by cannula size. Each bar represents the least squares mean and standard error for response time, adjusted for variation arising from differences between dogs. A aorta; MV mesenteric vein; PDV cranial pancreaticoduodenal vein; PV portal vein; SDMV superficial dorsal metatarsal vein; a, b, c mean response times having superscripts which differ, are significantly different ($p < 0.05$)

FACTORS POTENTIALLY AFFECTING THE SECRETORY RESPONSE

Antro-duodenal electrical activity

Electrical activity was sporadic at both antral and duodenal sites and quiescent periods were commonly observed. When present, spiking activity took the form of intermittent action potentials or intense bursts of electrical activity. There was no evidence of a regularly repeating cycle of myoelectrical activity at either site or between sites in any one dog throughout the period of anaesthesia.

While neither the amplitude nor frequency of action potentials was quantified, intense bursts of spiking activity appeared to occur at random intervals. These intermittent bursts of activity resembled phase III of the migrating myoelectric complex (MMC) and were sometimes observed to coincide with the immediate pre-secretin and post-secretin bolus administration period. That such complexes did not have a major affect on the latency of stimulated pancreatic secretion is suggested by the presence of similar complexes in both control dogs and test dogs during stable periods of pancreatic fluid output (Figure 5).

pH of gastric fluids

Secretion of pancreatic fluid may have resulted from the endogenous release of secretin if a sufficient volume of gastric acid had entered the duodenum. It was therefore of interest to determine if the pH of the gastric contents achieved a pH value low enough to stimulate the release of secretin from the duodenal mucosa. Although not quantified, small volumes (always less than 10 ml) of gastric juice were obtained by continuous suction over the course of each experiment. The contents of the stomach varied from clear with mucous, to particulate (resembling food), to bile-coloured, and on several occasions, the contents contained blood. Measurements obtained from a total of seven random samples of gastric juice collected from seven different dogs revealed the pH to range between 4.16 and 7.73 at ambient temperatures. A pH of less than or equal to 4.5 is required to stimulate secretin release (Meyer *et al.*, 1970); only one sample fell within this range.

Physiological state under anaesthesia

Average anaesthesia time for all dogs was 8 hours (range: 6 - 10 hrs). Patient stability was

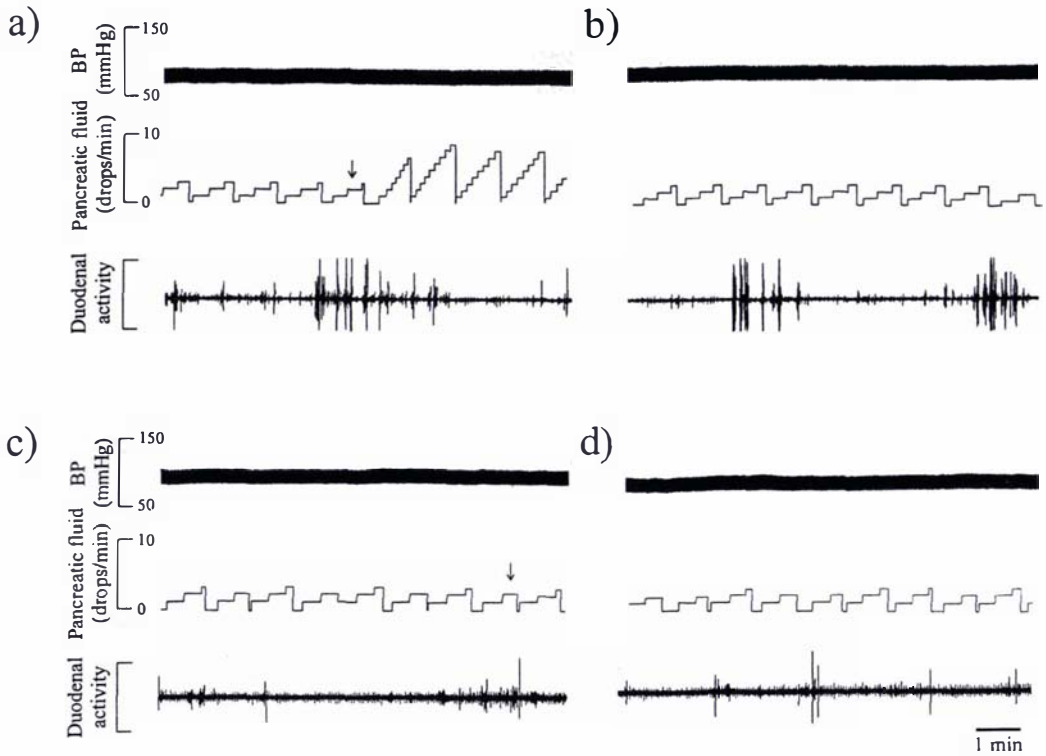

Figure 5. Duodenal electrical activity relative to blood pressure and pancreatic fluid output. Intermittent bursts of electrical activity resembling phase III of the MMC were sometimes observed to coincide with the immediate pre-secretin and post-secretin bolus administration period (a). That such complexes did not have a major affect on the latency of stimulated pancreatic secretion is suggested by the presence of similar complexes in both control dogs (c, d) and test dogs (b) during stable periods of pancreatic fluid output. Arrows mark the time of administration of either a secretin bolus (a) or vehicle alone (c). BP blood pressure

monitored by recording systolic and diastolic blood pressure, heart rate and respiratory rate every ten minutes. Body temperature was recorded usually once per hour. Of concern was the possibility that changes in the physiological parameters, due to the length of the anaesthetic period, might confound the experimental results. The measured parameters showed little variation over the course of the experiment (Tables 3 & 4) and were not suggestive of a declining physiological state, despite the fact that the data collected after the fifth injection were typically recorded 6-10 hours after induction of anaesthesia (see Appendix 4 for a completed anaesthetic monitoring record).

Table 3. Summary statistics for mean arterial blood pressure (MABP), heart rate (HR), respiratory rate (RR) and body temperature (T) measured every 10 minutes after induction of anaesthesia in control dogs. Means reported are those for the period beginning approximately 1 hour before the first bolus injection of vehicle (the pre-injection period) and the periods following each bolus injection of vehicle (post-injection periods 1-5). Time elapsing from the start of the pre-injection period to completion of the experiment ranged between 3 and 4 hours.

		Pre-injection	Post-injection periods				
		period	1	2	3	4	5
MABP[1]	$\bar{x}$	83.5	86.6	86.6	83.1	77.6	81.3
(mm Hg)	sd	10	9.2	10.6	6.4	0.5	7.8
	n[2]	4	4	4	4	3	4
HR	$\bar{x}$	117.5	117	121.2	114.4	127.3	126.3
(beats/min)	sd	9.4	11.1	12.8	6.5	7.7	7.5
	n	4	4	4	4	3	4
RR	$\bar{x}$	35.4	31	27.2	21.3	25.2	25.3
(breaths/min)	sd	27.1	17.5	18.1	15.4	10.1	10
	n	4	4	4	4	3	4
T	$\bar{x}$	38.6	NA	37	37.5	39.3	38.2
(^{o}C)	sd	1.2	NA				1.3
	n	3	NA	1	1	1	4

[1] Mean arterial blood pressure was estimated from the systolic and diastolic pressures using the formula MABP = diastolic pressure + 0.33(systolic pressure-diastolic pressure) (Smith and Kampine, 1984)
[2] Number of dogs from which one or more observations of each parameter were averaged

FACTORS POTENTIALLY AFFECTING THE MEASUREMENT OF LATENCY

Order of injection

The order of injection was randomized to prevent bias due to changes in exocrine pancreatic function over the course of the experiment. Such changes could be due to residual effects of previous injections or to deterioration of the experimental preparation over time. Latency of response was not dependent on the order of injection. The fact that latency, duration and overall magnitude of response (see above) were independent of injection site order provides strong evidence that pancreatic function was unaffected by the lengthy experimental periods.

Table 4. Summary statistics for mean arterial blood pressure (MABP), heart rate (HR), respiratory rate (RR) and body temperature (T) measured before and after each bolus injection of secretin to test dogs. Parameters were recorded every 10 minutes after induction of anaesthesia. Means reported are those for the period beginning approximately 1 hour before the first bolus injection of secretin (the pre-injection period) and the periods following each bolus injection of secretin (post-injection periods 1-5). Time elapsing from the start of the pre-injection period to completion of the experiment ranged between 3 and 5.5 hours.

		Pre-injection	Post-injection periods				
		period	1	2	3	4	5
MABP[1]	$\bar{x}$	80.6	80.2	79.2	79.2	77	74.6
(mm Hg)	sd	10.2	9	7.4	8.2	7.9	7.6
	n[2]	12	12	12	12	12	12
HR	$\bar{x}$	126.6	126.8	126.9	127.8	125	128.4
(beats/min)	sd	10.6	9	9.7	9.4	11.1	11.1
	n	12	12	12	12	12	12
RR	$\bar{x}$	24.9	26.7	24.5	24.7	23.9	24.6
(breaths/min)	sd	7.5	11.4	11.5	9.2	8.7	6.5
	n	12	12	12	12	12	12
T	$\bar{x}$	39.1	38.4	38.1	38.6	39.2	38.3
(^{o}C)	sd	0.7	1.5	1.5	0.8	0.8	1.6
	n	4	6	6	5	4	9

[1] Mean arterial blood pressure was estimated from the systolic and diastolic pressures using the formula MABP = diastolic pressure + 0.33(systolic pressure-diastolic pressure) (Smith and Kampine, 1984)
[2] Number of dogs from which one or more observations of each parameter were averaged

Drop size

In this study, a decrease in the time elapsing between drops was used as an indicator of changes (increases) in secretory rate following injection of secretin. A change in drop size could confound the results by causing an over-estimation or an under-estimation of the true latency. Drop size was unaffected by site of injection or secretory rate before or after injection. Drop size was affected by dog, size of the pancreatic duct cannula, order of injection and protein concentration of the pancreatic fluid ($p < 0.01$). As would be expected, drop size was less with the small cannula (41.3 ± 0.30 drops/g) when compared to the large cannula (38.3 ± 0.23 drops/g; $p < 0.001$). The first site to be injected tended to have a smaller drop size, however order of injection was randomized so bias due to this effect was unlikely. The drop size decreased as protein concentration increased in

pancreatic fluid ($p < 0.01$) but protein concentration did not differ between sites of injection ($p = 0.8$).

Accuracy of cannula placement

The accuracy of cannulae placement was determined following death. Most problems involved placement of the PV cannula (Table 5). Of the 12 experimental dogs, the tip of the PV cannula was found to have been advanced cranial to the margin (hilus) of the liver in six (dogs #2, #4, #5, #9, #10, #14). Of the six remaining dogs, the tip of the PV cannula was found in a mesenteric vein (dogs # 6, #12 & #15), in the splenic vein (dog #1), in the portal vein (dog #7), or slightly more caudally in the cranial mesenteric vein (dog #11). In two dogs (dogs #5 & #15), the tip of the A cannula was closer to the heart than to the diaphragm. All other cannulae were properly positioned. Data for the PV site in dogs #1, #6, #12 and #15 were not included in the final analysis.

Table 5. Summary of locations of tips of cannulae in test dogs (as determined at post mortem examination) and the usefulness of each preparation for latency of response testing.

Dog ID	Minor Problems	Major Problems	Usefulness of Preparation
# 1		PV cannula in splenic vein	PV site not used
# 2	PV cannula cranial to liver hilus		All sites used
# 4	PV cannula cranial to liver hilus		All sites used
# 5	PV cannula cranial to liver hilus; A cannula close to heart		All sites used
# 6		PV cannula in mesenteric vein	PV site not used
# 7			All sites used
# 9	PV cannula cranial to liver hilus		All sites used
# 10	PV cannula cranial to liver hilus		All sites used
# 11	PV cannula in cranial mesenteric vein		All sites used
# 12		PV cannula in mesenteric vein	PV site not used
# 14	PV cannula cranial to liver hilus		All sites used
# 15	A cannula close to heart	PV cannula in mesenteric vein	PV site not used

DISCUSSION

It is evident from the two data sets for secretory response latency, one relating to smaller diameter and the other to larger diameter pancreatic duct cannulae (Figure 4), that the nature of the measured secretory response is sensitive to quite minor changes in the animal model employed. This highlights the need to consider the features of the animal model used, how they may have affected the results and thus the suitability of this animal model for the purposes to which it was put. Accordingly, the strengths and weaknesses of the model have been considered below.

Also, taken overall, and analyzed statistically using the main effects model, the results of the present experiment suggested that the latencies of the pancreatic fluid secretory response to secretin injected into the portal system and a peripheral vein were similar and were significantly longer than the latency of response to secretin injected into the aorta (Figure 3). These observations are consistent with the outcome expected if no sensory mechanism for secretin originates within the walls of the veins of the hepatic portal system, i.e., the shorter response time after arterial injection when compared to that for injections into all other sites reflect mainly differences in circulation time. However, when the data from subgroups with smaller diameter and larger diameter pancreatic duct cannulae were analyzed separately, a shorter latency for the portal vein site compared to the other sites in the portal system was evident in the large cannula group of dogs (Figure 4). This finding merits detailed consideration, and is also examined below.

Suitability of the animal model

The validity of the above data relies upon having examined, and minimized where possible, the potential influence of factors which may have either positively or negatively biased the results. These factors include potential complications associated with antro-duodenal myoelectrical activity, gastric acid entering the duodenum, stimulation of the pancreas by injected secretin via a pathway other than through the systemic circulation or the putative neural mechanism, and the effects of anaesthesia on the stability of the preparation and on the response of the pancreas.

Pancreatic fluid output may have been affected by changes in myoelectrical activity. Dogs and other species which eat at intervals of several hours or more, develop during the interdigestive period, cyclically recurring fronts of intense motor activity which originate in the stomach and slowly migrate throughout the length of the small bowel (Szurszewski, 1969; Code and Marlett, 1975). Associated with these intense bursts of myoelectrical activity (which characterize phase III of the MMC), are bursts of secretory activities by the stomach, liver and pancreas (DiMagno *et al.*, 1979; Vantrappen *et al.*, 1979; Konturek *et al.*, 1986). While no such coordinated progressive activity between the antral and duodenal electrodes was apparent in the present study, intense bursts of electrical activity did sometimes appear to coincide with secretin administration. The inability to have demonstrated the typical pattern of electrical activity between the antrum and duodenum is consistent with the results of an earlier study which found that "any unusual manipulation of the dog or the administration of an anaesthetic disturbs the sequence of the interdigestive pattern" (Code and Marlett, 1975). Although it could not be definitively proven that the apparently disrupted pattern of the MMC served to also 'uncouple' the secretory activities of the pancreas, changes in output of pancreatic fluid did not appear to coincide with periods of increased spiking activity (Figure 5).

The likelihood that entry of gastric juice into the duodenum had a major effect on the latency of stimulated pancreatic secretion was considered low for several reasons. First, spontaneous flow of pancreatic fluid was never observed in any dog under anaesthesia. Rather, the baseline flows were achieved by continuous low dose secretin infusion once the dogs were anaesthetized. Second, the pH threshold for secretin release from the intestinal mucosa is approximately 4.5 in dogs (Thomas and Crider, 1940), and a pH value below this was obtained in only one of seven samples of gastric contents. Although the pH measurements were made at ambient temperatures rather than at body temperature, this would be expected to have had a very small effect on the measured pH values, as would also be the case for carbon dioxide degassing during the aspiration process (B.D. Wagner, personal communication*). Finally, the pancreatic bicarbonate response to introduction of hydrochloric acid into the duodenum is proportional to the *amount of acid infused per unit time* (Preshaw *et al.*, 1966b). In view of the latter, even if the measured pH values were not representative, i.e., they were actually lower, it was considered unlikely in the

presence of continuous aspiration, that the amount of acid entering the duodenum in the fasted, anaesthetized state would have been sufficient to elicit a pancreatic response.

This experiment was not designed to test for the presence of a vascular connection between the duodenum and pancreas because the possibility of a *duodenal vein* tributary entering the pancreas was not controlled for. Nonetheless, cannula placement in this preparation was such that, if a vascular communication existed between the *pancreaticoduodenal vein* and the pancreas, secretin injected into the PDV site might have entered the pancreas. In order to show that the PDV secretin bolus passed cranially through the vein without entering and stimulating the pancreas directly, Indian ink was injected through the PDV cannula prior to euthanasia and again immediately following death. Serial sectioning through the pancreas after death failed to reveal ink in the pancreas of any dog. Although the existence of a local vascular pathway between the duodenum and pancreas could not be entirely ruled-out, it would appear from the results of this validation procedure that passage of blood from the pancreaticoduodenal vein through the pancreas does not occur. This study, like the latex vascular cast (Chapter 3) and microanatomical (Chapter 4) studies, provided little evidence to support the presence of a local vascular pathway of communication between the duodenum and pancreas.

In acute experiments designed to test pancreatic function, anaesthesia might be expected to obscure the effects of neural reflexes, the release of hormones or the response of the pancreas itself. When the conditions of anaesthesia are consistent both within and between preparations, as was the case in this experiment, it is assumed that the results are valid, at least under the conditions of this study. However, added steps were employed to minimize the effects of anaesthesia. These included the use of combination drug therapy for induction (small doses of diazepam reduce the requirement for thiopental), selection of a short-acting pre-medication and induction agent (diazepam and thiopental, respectively), use of the lowest plane of anaesthesia necessary for the procedures being performed, provision of circulatory support and maintenance of normothermia. Monitoring the patient throughout the anaesthetic period was critical to minimizing the effects of anaesthesia; it allowed circumvention of anaesthetic complications which *might* have invalidated experimental results and also provided evidence that the experimental preparation did not

deteriorate over time. For a more complete discussion on choice of animal model (acute versus chronic preparation) see Chapter 7.

Latency of response and the effect of cannula size

Interpretation of the results of the present investigation is complicated by the differences observed in response times between the small cannula group of dogs and the large cannula group of dogs (Figure 4). The most likely explanation for the observed differences in latencies is that the smaller diameter cannula impeded flow of pancreatic fluid. The observation that all response times were somewhat shorter in the group of dogs in which the larger cannula was used was therefore not unexpected, however if the differences in latencies between the two groups of dogs were solely attributable to decreased resistance to flow of pancreatic fluid through the larger cannula, then one would have expected to see proportional decreases at all sites. While differences were not significant between groups at the A, PDV, MV and SDMV sites, this was not the case at the PV site, where the latency was significantly shorter for the large cannula group of dogs ($p < 0.05$).

The reason(s) for the disproportionately shorter response time at the PV site in the large cannula group of dogs is (are) unknown. One possible explanation is the small sample size employed in this experiment. Four dogs (two from each cannula group) were not included in the final analysis of PV latency due to improper placement of the cannula, leaving data for only four dogs in each group available for final analysis. With such a small sample size, there would have existed an opportunity for individual dogs to have unduly influenced the results at this site. However, based on the standard errors calculated, the variability in latency of response at the PV site was similar to that at the other sites (Figure 4), and there were no apparent outliers in the data to explain such a discrepancy. An alternative explanation may be that the increased resistance to flow of pancreatic fluid through the small cannula was partially masking differences between response times, and that reducing the resistance due to the cannula provided data more representative of the true latency following injection at the PV site. Consistent with this was a *trend* toward differences in latencies between each of the other respective sites. It is this author's opinion that an 'unmasking' effect due to the larger cannula size best explains the observed differences in response times between the small and large cannula groups of dogs.

If the results of the large cannula group of dogs are taken to be more reflective of the true latencies, then it would seem unlikely that circulation time alone could explain why the mean latency value for the PV site was closer to that at the A site than it was to that at the MV site (Figure 4 and Appendix 8). It is possible that 'over-insertion' of the PV cannula in six cases and/or the A cannula in two cases (see Table 5) could account for such a discrepancy, however, the number of dogs in which cannulae were improperly positioned was equally represented in both groups of dogs. In addition, it would seem that the consequent decrease in circulation time would be insufficient to account for the observed drop in latency. One could also postulate that the portal vein and/or liver may be playing a regulatory role in the pancreatic response to secretin, however the fact that a similar effect on latency was not observed at the PDV and MV sites argues against this. On the other hand, it is conceivable that the inability to have been able to demonstrate a similar effect at these sites was due to changes in regional blood flow within the portal system, as has been well documented for dogs under halothane anaesthesia (Andreen *et al.*, 1977; Gelman *et al.*, 1984a), *or* to a dilutional effect of portal venous blood. In the latter case, a threshold concentration at the PV site, which could be achieved by close injection of secretin into the PV site but which could not be achieved with the same dose injected into the PDV or MV sites, may be necessary to initiate a pancreatic response. In view of the fact that the response sought would be supplementary to a primary hormonal effect by secretin, demonstration of the presence of an early bi-phasic response coupled with a greater total response at the portal sites would have provided stronger physiological support for an effect of the portal vein or liver on exocrine pancreatic function. Such an effect was not evident in these data (Figure 2). Taken together, no strong conclusions can be drawn regarding the significance of the differences in latencies at the PV site between the two different cannula sizes, nor between the latencies at the PV site compared to the A and MV sites in the large cannula group of dogs.

A sensory role for the hepatic portal system?

The hypothesis that a 'sensory' mechanism exists within the hepatic portal system for secretin implies the existence of an underlying nervous mechanism which relays information regarding the contents of venous blood to the pancreas, either directly via

pancreatic ganglia, indirectly via the sympathetic or parasympathetic nervous systems or via other non-adrenergic, non-cholinergic pathways. Latency studies, in response to intestinal and parenteral stimulants, in the absence and in the presence of antagonists of the autonomic nervous system, and before and after cutting the extrinsic nerves to the intestine and pancreas, have been used by other investigators as an indirect means of examining the involvement of nervous mechanisms in the total pancreatic response (Hickson, 1970; Greenwell and Scratcherd, 1974; Singer *et al.*, 1980a; Singer, 1983; Zabielski *et al.*, 1994). Using this methodology, Singer (1983) examined the possibility that an enteropancreatic reflex mediates the pancreatic fluid secretory response to intestinal stimulants in conscious dogs, as had previously been shown for pancreatic *enzyme* release in response to intraduodenal administration of tryptophan and oleate (Singer *et al.*, 1980a). In Singer's 1983 study, the mean latency of the pancreatic fluid secretory response to intraduodenal HCl was 91 seconds, compared to 62 seconds for tryptophan, 64 seconds for oleate, and 28 seconds for rapid intraportal or intravenous administration of secretin. Unlike the case for intraduodenal tryptophan and oleate, in which atropine and truncal vagotomy significantly increased the latency of the pancreatic fluid response, atropine and vagotomy had no effect on the latency of the response to intraduodenal HCl *or* to the intraportal injection of secretin. The results of Singer's 1983 study provided little support for the conclusion that an enteropancreatic reflex involving a vago-vagal cholinergic mechanism was involved in the early pancreatic response to intraduodenal HCl, and provide little further support for the conclusion that a 'portal vein-pancreatic' reflex involving a similar mechanism mediates an early pancreatic response to secretin. Although it was not the intent of this author to determine the extent of involvement of a particular neural pathway in our hypothetical reflex, the results of Singer's study would suggest that if a sensory role for the portal system or liver indeed exists for secretin, then a vago-vagal cholinergic mechanism probably is not involved.

Superimposition of neural and humoral reflexes

Finally, the assumption that a neural mechanism can be involved in the pancreatic response to a stimulant only if the latency of that response is shown to be shorter than the apparent circulation time, warrants some discussion. A postulated nervous mechanism may not necessarily result in a response which is *faster* than the minimum circulation time; in some

situations, the neurally-mediated component of that response may be superimposed on the non-neural component even though the response time is longer than the apparent circulation time. For example, Singer, *et al.* (1980a, 1983) demonstrated that the latency of the pancreatic *enzyme* response to intraduodenal tryptophan and oleate was significantly *shorter* than the apparent circulation time, whereas the latency of the pancreatic *fluid* response to the same intestinal stimulants was significantly *longer* than the apparent circulation time. In both instances, atropine and truncal vagotomy significantly increased the respective latencies. Singer's conclusions regarding the relative importance of the innervation as opposed to hormonal mechanisms in mediating the pancreatic response were based solely on whether or not the responses were delayed both by vagotomy and by the administration of atropine, rather than whether or not the responses were shorter than the minimum circulation time. It is important to recognize however that in Singer's studies, the latency of response to an unknown quantity of *endogenously released* hormone was compared to the latency of response to a known quantity of *exogenously administered* hormone, unlike the present investigation, in which the response to known, equivalent doses of exogenously administered hormone were compared between sites. Overall, these observations suggest that cautious interpretation of latency data (particularly that which compares the effects of endogenously released hormone with exogenously administered hormone), may in some cases be necessary before one accepts or rejects the presence of a superimposed nervous reflex. They also demonstrate the importance of examining the response times both in the absence and in the presence of antagonists of the autonomic nervous system, and before and after cutting the extrinsic nerves to the area(s) of interest.

In conclusion, the main effects model shows that the latency of the pancreatic fluid secretory response to secretin injected into the aorta is significantly shorter than the latency of the pancreatic response to secretin injected into the portal system. Examination of the latency data when broken down by pancreatic duct cannula size however, raises questions regarding the validity of this model. In order to determine the significance of the observed differences in latencies at the PV site between the two different cannula sizes, a second experiment was designed. That experiment, which is the subject of Chapter 6, was performed in an attempt to reproduce the results obtained at the PV site from the large cannula group of dogs, as well as to examine the relative importance of circulation time

versus a postulated portal vein or liver effect on the pancreatic fluid secretory response to secretin.

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Chapter Six

FURTHER INVESTIGATIONS INTO A POTENTIAL ROLE FOR THE PORTAL SYSTEM IN THE CONTROL OF EXOCRINE PANCREATIC FUNCTION

SUMMARY

The purpose of this investigation was to further explore the possibility of a local sensory mechanism for secretin, components of which exist within the walls of the veins of the hepatic portal system. This hypothesis was tested in two ways. First, the latency of the pancreatic fluid secretory response to a bolus of secretin injected into the aorta was compared to that of secretin injected into selected veins of the portal system and systemic circulation in 10 dogs. The injection of secretin into the aorta (A site) elicited a faster response when compared to injection into the caudal vena cava (CaVC site), portal vein (PV site), cranial mesenteric vein (CMV site) or superficial dorsal metatarsal vein (SDMV site), in agreement with the results presented in Chapter 5 for the main effects model. Differences in latency between sites generally reflected expected differences in circulation time. Second, the PV threshold dose was determined in each of 4 dogs. The PV threshold dose was the smallest dose of secretin which elicited a detectable pancreatic response following injection into the PV site. This PV threshold dose was then administered into each of the remaining non-portal vein sites (A, CaVC, CMV and SDMV). Responses to the PV threshold dose were obtained at each of the non-portal vein sites, providing further evidence against a specific role for the portal vein or liver in the mediation of secretin-induced pancreatic secretion. Collectively, the results of these two studies provide no support for a sensory role for the portal vein or liver in the pancreatic response to secretin.

INTRODUCTION

Previous studies by this author examined macroscopic and microscopic evidence for the presence of a direct duodenum-to-pancreas venous communication (Chapters 3 and 4). These studies failed to identify conclusively, interconnections of veins between the duodenum and pancreas. However, positive immunoreactivity to three sensory neuropeptides was observed at or near the level of the endothelium in the veins of the hepatic portal system (Chapter 4), giving rise to the hypothesis that this may represent a 'sensory' mechanism which plays a role in the control of exocrine pancreatic function. The study described in Chapter 5 was designed to test this hypothesis. It compared the latency of the pancreatic fluid secretory response to secretin injected into the aorta with the latency of the same dose of secretin injected into selected veins of the hepatic portal

system. It was postulated that if such a 'sensory' mechanism exists, then the latency of the pancreatic response to secretin injected into the veins of the portal system would be shorter than the latency of the same response to secretin injected into the aorta; otherwise, any differences in latency would be explained mainly by differences in circulation time.

The main effects statistical model used in the study described in Chapter 5 clearly demonstrated that the response times following injection of secretin into the portal system were significantly longer than that following injection into the aorta. Closer examination of the data however, raised questions regarding the validity of one aspect of the experimental design.. When broken down by size of the pancreatic duct cannula, there was a significantly shorter response time at the portal vein site in the group of dogs in which a larger pancreatic duct cannula was used, an effect which was believed to result from decreased resistance to flow of pancreatic fluid. This shorter latency of response at the portal vein site was not significantly different from the response time at the aortic site of injection in the large cannula group of dogs, but did differ significantly from that for the aortic site of injection in both the main effects model and small cannula group of dogs. The reason(s) for these apparent discrepancies are presently unclear.

It is possible that the inability to have demonstrated the expected longer response time at the portal vein site was due simply to the small number of animals employed in the experiment. It is also conceivable however, that the portal vein or liver somehow participated in the pancreatic response to secretin. While the latter explanation seems unlikely on the basis of the available data, other studies have demonstrated a sensory role for the liver in the reflex regulation of *endocrine* pancreatic function (Lee and Miller, 1985; Kabadi, 1993; Saitou *et al.*, 1993). Therefore, the possibility that controlling mechanisms for the *exocrine* pancreas also reside within the hepatico-portal area cannot be entirely excluded.

In order to test whether factors within the portal vein or liver might act in an additive or potentiating manner with secretin to elicit a more rapid pancreatic response, the following investigation was designed. In the first part of this experiment, an additional cannula was placed in the caudal vena cava and positioned such that its tip lay at the same level as that

of the portal vein cannula near the hilus of the liver. It was anticipated that if differences in response times between the portal vein and aorta truly existed and were attributable to circulation time alone, then the response times at the caudal vena cava and portal vein sites would be similar, and that these response times would be significantly *longer* than that at the aortic site. If other factor(s) were involved, such as an effect of the portal vein or liver on the pancreatic response, then it was anticipated that the results of the previous study would be reproduced i.e., that the response times at the portal vein site would be similar (or shorter) than those at the aortic site, and that both would be significantly *shorter* than that at the caudal vena cava site.

The second part of this experiment was designed to further evaluate a potential role for the portal vein or liver on the pancreatic response to secretin. An attempt was made to determine the threshold dose for secretin following injection into the portal vein. It was believed that if administration of this pre-determined dose of secretin into the remaining non-portal sites *failed* to elicit a pancreatic response, then this would provide stronger physiological support for a role for the hepatico-portal area in the control of exocrine pancreatic function. The relative contribution of circulation time versus that of a possible portal vein or liver effect on the pancreatic response to secretin is the subject of the remainder of this chapter.

MATERIALS AND METHODS

ANIMALS AND ANIMAL PREPARATION

All dogs used for this experiment were sourced directly from or through the Animal Health Services Centre (Massey University, Palmerston North, NZ) for purposes of acute, terminal experimentation. Some of the dogs used were part of the breeding colony at the facility, while others were obtained from farmers in the district who had sheep dogs surplus to their needs on the farm. These dogs were provided for the study as a humane alternative to shooting them. It was explained to the owners that the dogs would be used in a study of how the gut and related organs work, and that the dogs would be under anaesthesia throughout the study, would never regain consciousness and would be overdosed with an anaesthetic to kill them at the end of the procedures. The study and their death would therefore both be painless. The owners signed a consent form and were

paid for the dogs. Massey University Animal Ethics approval was obtained for all procedures prior to their being carried out.

Ten lean dogs, approximately six months to two years of age and weighing 13 to 25 kg, were used for this experiment. The dogs were typically of mixed breeding and consisted of six males and four females. Because none of the control dogs in the previous study (Chapter 5) showed any response to administration of vehicle alone, none of the dogs used in this experiment were assigned to serve as controls. All dogs were determined to be healthy at the time of admission based upon standard clinical examination and routine pre-operative blood analyses (packed cell volume and total protein concentration). All dogs were fasted for 16 to 18 hours prior to anaesthesia but had free access to water. One dog was used on each experimental day and usually two experiments were performed each week. All chemicals, materials and equipment used for this experiment were the same as those used in the previous experiment (Chapter 5).

ANAESTHESIA AND PRE-SURGICAL SET-UP

Dogs were anaesthetized, maintained under anaesthesia and prepared for surgery using the same protocol as that used in experiment 1 (see Chapter 5 for details). Briefly, on the day of the experiment, anaesthesia was induced with diazepam (0.5 mg/kg as a rapid bolus) immediately followed by thiopental (10 mg/kg to effect) via a previously introduced cephalic vein catheter. Dogs were subsequently maintained on a mixture of halothane and oxygen. Following induction, dogs were positioned in dorsal recumbency on an electric heating pad below which lay a large sheet of bubble wrap. A fenestrated oro-gastric feeding tube was passed into the stomach and connected to a suction pump in order to facilitate continuous removal of gastric contents and minimize entry of gastric juice into the duodenum. A urinary catheter was also passed via the urethra into the bladder of male dogs to prevent urine contamination of the abdomen. The abdomen was clipped and prepared for surgery. Maintenance fluids consisted of 0.15 M NaCl solution and were administered via peristaltic pump at a flow rate of 10 ml/kg/hr. Direct blood pressure readings were made from either a pedal artery or a femoral artery. Anaesthetic monitoring consisted of heart rate, respiratory rate, systolic and diastolic blood pressure recordings (see Appendix 9) as well as assessments of anaesthetic depth (reflex responses, jaw tone,

eye position, mucus membrane colour and capillary refill time; not recorded) every 10 minutes. Body temperature was recorded usually once per hour. Anaesthesia commenced between 08:30 and 09:00 on experimental days.

SURGICAL PROCEDURES AND EXPERIMENTAL SET-UP

For this experiment, the necessary surgical procedures included the following: ligation of the main pancreatic duct; cannulation of the accessory pancreatic duct; cannulation of the aorta, caudal vena cava, portal vein, cranial mesenteric vein and superficial dorsal metatarsal vein. Because in the previous investigation there appeared to be no correlation between gut electrical activity relative to pancreatic fluid output, in this experiment, electrodes to record antro-duodenal myoelectrical activity were not inserted.

Cannulation of the aorta, caudal vena cava and superficial dorsal metatarsal vein was performed prior to opening the abdomen. All vascular cannulae consisted of single lumen PVC tubing, were of the same length and had the same internal diameters (length: 0.50 cm; ID: 0.50 mm x OD: 0.80 mm; deadspace: 0.11 ml). The aorta and caudal vena cava were cannulated via a femoral artery and a femoral vein respectively, using a modified cannula which had the same dimensions, but which had been inserted and glued into an outer PVC sleeve to provide greater rigidity (ID: 1.20 mm x OD: 1.70 mm). As in the previous experiment, the tip of the aortic cannula was positioned slightly cranial to the level of the diaphragm. The caudal vena cava cannula was advanced so that its tip was palpable just caudal to the hilus of the liver (the position of this cannula could usually be verified during the operation). The dorsal metatarsal vein was cannulated through a previously introduced indwelling catheter. All vascular cannulae were secured to the vessel into which they were introduced by double ligation with or without glue. All vascular cannulae were capped with labelled injection ports, and were flushed with heparinized saline (5 IU heparin/ml saline) prior to introduction into the vessel, and then usually on an hourly basis thereafter.

Following the above cannulation procedures, a ventral midline incision was made to expose the abdominal viscera. The main pancreatic duct was ligated, and the accessory pancreatic duct was cannulated, this time using the larger of the two PVC cannulae used in the previous experiment (ID: 0.97 mm x OD: 1.27 mm; deadspace: 0.40 ml *versus* ID:

0.86 mm x OD: 1.27 mm; deadspace: 0.30 ml). All cannulae were filled with saline prior to placement. In this experiment, the portal vein was cannulated using the *modified* cannula (see above) since it was easier to advance and to confirm the location of this cannula during surgery. An attempt was made to position its tip within the portal vein at approximately the same level as the tip of the caudal vena cava cannula, usually just caudal to the liver. The cranial mesenteric vein cannula was introduced in a similar fashion and advanced to reach the cranial mesenteric vein rather than being left to reside within the jejunal vessel through which it was introduced. Pancreatic fluid output (see below) and blood pressure were simultaneously recorded using a JRAK module system and a two channel chart recorder. Pancreatic fluid output (drop rate) was measured using an optical transducer and drop counter designed to integrate with the JRAK system.

Figure 1 shows the final set-up for this experiment after all surgical procedures were completed.

Following completion of the above procedures, the viscera were carefully replaced in the abdomen and the abdomen was gently packed with saline-moistened laparotomy towels. The ends of all cannulae were exteriorized and their patency checked prior to closing the abdominal wall with towel clamps. The underlying layer of bubble wrap was fastened snugly around the body of the dog and the system was allowed to stabilize for a minimum of one hour.

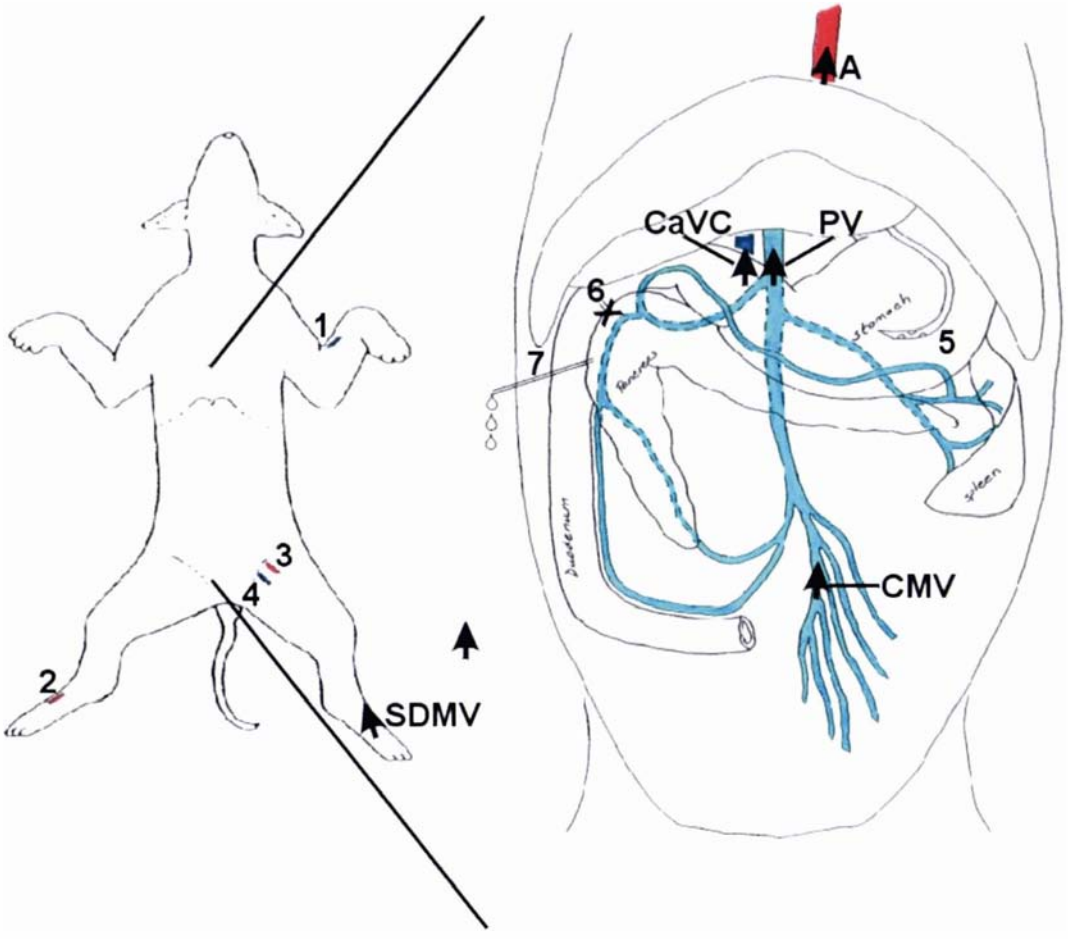


Figure 1. Final set-up of experiment. Arrows mark the locations at which the tips of cannulae were positioned for administration of secretin boluses. Each arrow points in the direction that the bolus was administered. Note the modifications in placement of cannulae for this experiment compared to experiment 1 (Figure 1, Chapter 5). A aortic cannula; CaVC caudal vena cava cannula; CMV cranial mesenteric vein cannula; PV portal vein cannula; SDMV superficial dorsal metatarsal vein cannula; 1 cephalic vein catheter for administration of induction agents, fluids and secretin infusion; 2 pedal artery catheter for measurement of blood pressure; 3 femoral artery site of introduction of aortic cannula; 4 femoral vein site of introduction of caudal vena cava cannula; 5 oro-gastric tube for continuous suction of gastric juices; 6 main pancreatic duct ligated; 7 accessory pancreatic duct cannulated

EXPERIMENTAL DESIGN

The reader is referred to Appendix 10 for details of the checklist and protocol followed for each dog.

Preparation of secretin solutions

All secretin-containing solutions consisted of synthetic secretin dissolved in 0.15 M NaCl with 0.5 % bovine serum albumin (BSA). As in experiment 1, BSA (2.5 gms) was added to a 500 ml bag of isotonic saline solution for infusion. For this experiment, a larger stock volume of the BSA/saline solution (60 ml) was prepared in order to accommodate the additional volumes necessary for threshold testing in part 2 of the experiment. From the stock volume, 1 ml of the BSA/saline solution was drawn-up into each of fifteen labelled, 1-ml syringes. Five of these syringes were used for initial bolus testing in part 1 of the experiment and the remaining syringes were used for threshold testing in part 2 of the experiment. In preparation for diluting the secretin boluses in the second part of the experiment, various sized aliquots of stock solution (0.5-10 ml) were dispensed into separate labelled glass vials. The above solutions were prepared the day before the experiment and were refrigerated overnight.

On the morning of the experiment, 10 μg secretin were dissolved in the 500 ml bag of saline/BSA for infusion (final concentration: 20 ng secretin/ml saline). Into five of the 1-ml syringes containing the BSA/saline solution was initially added 35 ng secretin/kg body weight, the same dose as that which had been used for bolus administration in the previous experiment. Due to the unacceptably long overall response times following administration of this dose to dog #1 in part 1 of the study (approximately 45 minutes per bolus), and because a lengthier procedure was anticipated in lieu of a 'two part' experiment, this dose was decreased to 5 ng secretin/kg body weight in all remaining dogs. For part 2 of the experiment, the initial secretin boluses and all subsequent dilutions were prepared as needed during the experiment (see below).

Intravenous infusion of secretin

The secretin infusion was begun following cannulation of the accessory pancreatic duct and was continued for the remainder of the experiment. The infusion was delivered via a second peristaltic pump into the cephalic vein catheter at an initial flow rate of 5-10 ml/kg/hr. This dose rate was then adjusted to obtain a baseline output of pancreatic fluid of approximately 3-6 drops per minute. Fluid delivery rates of 0.15 M NaCl solution were adjusted as necessary so as not to exceed a total fluid (NaCl infusion plus secretin infusion) delivery of 10 ml/kg/hr.

Secretin bolus administration - Part 1 (latency of response)

Secretin bolus administration began no sooner than one hour after completion of all surgical procedures and no sooner than 15 minutes after having achieved a stable pancreatic fluid output following administration of the previous bolus. The order of injection of the boluses was randomized using a computer-generated spreadsheet for each dog. Injection sites were identified as follows: A (aorta), CaVC (caudal vena cava), PV (portal vein), CMV (cranial mesenteric vein) and SDMV (superficial dorsal metatarsal vein). Because in the previous study (Chapter 5), drop size was unaffected by secretory rate before or after injection, and because the relationship between drop size and protein concentration was not strong, collection of pancreatic fluid for measurement of these parameters was not performed in this experiment.

Secretin boluses were administered directly into each cannula over a 5 second period. The cannula was immediately flushed over a similar time course with an equal volume of BSA/saline and was then capped and flushed with a small volume of heparinized saline. The pancreatic response was recorded and allowed to return to steady state or previous baseline prior to administration of the next bolus.

Secretin bolus administration - Part 2 (threshold determination and testing)

Determination of the threshold dose of secretin at the portal vein site commenced following completion of part 1 of the experiment. Administration of each dilution commenced no sooner than 10 minutes after having achieved a stable pancreatic fluid output following administration of the previous bolus. Dilutions were prepared as needed

by adding 1 μg secretin to a known volume of BSA in saline. From this stock solution, the calculated dose was removed and added to one of the ten remaining 1-ml syringes containing BSA in saline. All doses were administered in a total volume of 1 ml and in the same manner as that described for part 1 of the experiment. The first dose to be injected was 1/100th of the original 35 ng/kg dose. Subsequent boluses were usually double dilutions of this dose. Administration of serial dilutions was continued until a 'no response' was obtained at the PV site. After determining the threshold dose (see below for definition), threshold testing was performed by administering this dose into the A, CaVC, CMV and SDMV injection sites. As in part 1 of the study, the order of injections into the latter four sites was randomized using a computer-generated spreadsheet. Appendix 11 shows an example of the form used for each dog as well as the results of secretin threshold testing for dog #8.

Definitions of 'threshold dose' and 'positive response'

Threshold dose was defined as the *lowest* dose of secretin which would result in an increase in pancreatic fluid output at the PV site during at least one minute of the first five minutes post-injection. A detectable increase in pancreatic fluid output was subjectively assessed in dogs #1 to #4 by visually identifying any increase in drops per minute (as manifested by an obvious reduction in drop interval) as the chart recorder was running. Because this approach did not yield consistent and repeatable results, the threshold dose was later more specifically defined as the lowest dose of secretin which would result in an increase in pancreatic fluid output by the mean plus two standard deviations ($\bar{x}$ + 2 SD) of the number of drops per minute during a five minute pre-injection (baseline) period.

Baseline drops per minute were measured prior to administering each dilution of secretin. From this, $\bar{x}$ + 2 SD was calculated. Following administration of the secretin, drops per minute were again calculated for the first five minutes. An increase by greater than or equal to $\bar{x}$ + 2 SD of baseline output, during any minute of the first five minutes post-injection of secretin, constituted a positive response. Appendix 12 shows a tracing of a five minute baseline, illustrates the manner in which $\bar{x}$ + 2 SD was calculated from the raw data, and shows the minutes during which a response was obtained following administration of a dose of secretin into the PV site in dog #5.

For threshold *testing* at the A, CaVC, CMV and SDMV sites, the presence or absence of a response was determined in the same manner, but retrospectively, following completion of the experiment.

Calculation of latency

The latency of the pancreatic fluid secretory response was measured as the time between the midpoint of the secretin bolus injection and a sustained decrease in the drop interval (time elapsed between drops) of more than three standard deviations compared to the mean drop interval recorded during the immediate five minute pre-injection period. Drop intervals were calculated from the distance between consecutive drops (hand measured to the nearest 0.25 mm) from the recorded data at known paper speed.

Validation of the model

The model used in this experiment was validated in the previous chapter. The typical secretory response pattern to a secretin bolus is illustrated in Figure 2 and Table 2 of Chapter 5. Factors potentially affecting the secretory response or the measurement of latency were examined in some detail. The experimental preparation was found to respond consistently to an injected secretin bolus over the entire duration of the experiment and was not subject to variation due to gastric pH or antro-duodenal electrical activity. Measurement of the pancreatic secretory response was found to be unaffected by variation in drop size or order of injection.

Statistical analysis

Statistical analysis for part 1 of the experiment was performed using the GLM procedure of SAS (SAS Institute Inc, Cary, NC). Dog #1 was excluded from the analysis because a different dose of secretin was used. Latency data for the nine dogs was analyzed by analysis of variance. Factors included in the full model were dog, site of injection and injection order. Injection order had no significant effect and was dropped from the model. The appropriateness of the model was confirmed using residual analysis (Appendix 13). Means reported are least squares means with standard errors. Differences between means were calculated using the Student's *t*-test.

For purposes of both establishing *and* testing the threshold dose in part 2 of the experiment, calculations of pre-injection and post-injection $\bar{x}$ + 2 SD were performed using a portable calculator.

RESULTS

Physiological state under anaesthesia

Average anaesthesia time for all dogs was 9.7 hours (range: 8.5 - 12.6 hrs). The physiological stability of the dogs under anaesthesia was monitored throughout both parts of this experiment. Of concern was the possibility that deterioration of the physiological preparation might occur due to the length of the anaesthetic period. The measured parameters showed little variation over the course of each experiment (Table 1 and Table 2) and there were no meaningful trends identified that might suggest a declining physiological state, despite the length of anaesthesia.

Table 1. Part 1 summary statistics for mean arterial blood pressure (MABP), heart rate (HR), respiratory rate (RR) and body temperature (T) measured before and after each bolus injection of secretin. Parameters were measured every 10 minutes after induction of anaesthesia. Means reported are those beginning approximately 1 hour before the first bolus injection (pre-injection period) and ending after the last response (post-injection 5) had returned to baseline. Time elapsing from the start of the pre-injection period to completion of the experiment ranged between 2.5 and 5 hours.

		Pre-injection period	Post-injection periods				
			1	2	3	4	5
ALL DOGS							
MABP[1]	$\bar{x}$	75	77.1	77.7	77.7	78.8	80.2
(mm Hg)	sd	9.2	8.2	7	8.1	7.1	6.9
	n[2]	10	10	10	10	10	10
HR	$\bar{x}$	114.4	119.5	119.3	123.9	125.9	124.6
(beats/min)	sd	8.1	12.1	11	12.5	12.2	12.7
	n	10	10	10	10	10	10
RR	$\bar{x}$	30	31.9	29	30.3	31.2	30.4
(breaths/min)	sd	11.8	13.7	12.2	13.5	14.5	13.8
	n	10	10	10	10	10	10
T	$\bar{x}$	37	37.7	38	38	38.9	38
(^{o}C)	sd	0.8	1.6	0.6	1.4	0.7	1.2
	n	4	3	3	3	3	4

[1] Mean arterial blood pressure was estimated from the systolic and diastolic pressures using the formula MABP = diastolic pressure + 0.33(systolic pressure-diastolic pressure) (Smith and Kampine, 1984)
[2] Number of dogs from which one or more observations of each parameter were averaged

Table 2. Part 2 summary statistics for mean arterial blood pressure (MABP), heart rate (HR), respiratory rate (RR) and body temperature (T) measured over the course of the secretin threshold testing procedures. Parameters are recorded as averages of the 4 dogs for which threshold testing results were reported. Part 2 of the experiment began approximately 8 hours after induction of anaesthesia. Time elapsing from the beginning of Part 2 until completion of the experiment ranged between 1.3 and 5.3 hours.

		Post-injection periods				
		1	2	3	4	5
DOGS #1, #5, #8 & #9						
MABP[1]	$\bar{x}$	75.7	75.8	75	75.2	71.8
(mm Hg)	sd	5.4	8.7	7.5	7.9	8.8
	n[2]	4	4	4	4	4
HR	$\bar{x}$	118.6	121.2	116.5	118.2	124.9
(beats/min)	sd	7.7	1.2	7.7	9.9	10.4
	n	4	4	4	4	4
RR	$\bar{x}$	30.3	29.8	28.9	32.8	21.5
(breaths/min)	sd	17.9	22.4	19.1	21.4	22.8
	n	4	3	4	4	3
T	$\bar{x}$	36.9	NA	39.1	38.4	39.3
(^{o}C)	sd	0		0	1.1	0
	n	1		1	2	1

[1] Mean arterial blood pressure was estimated from the systolic and diastolic pressures using the formula MABP = diastolic pressure + 0.33(systolic pressure-diastolic pressure) (Smith and Kampine, 1984)
[2] Number of dogs from which one or more observations of each parameter were averaged

Part 1 - Latency of response

The latency of the pancreatic fluid secretory response to secretin differed significantly between injection sites ($p < 0.001$; Table 3). Injection into the A site elicited a faster response when compared to injection into the CaVC, PV, CMV and SDMV sites (Figure 2). Differences in response times between A and all other sites were significant ($p < 0.05$; Appendix 14). The latency of response at the CaVC site was intermediate, being significantly faster than the PV and CMV sites ($p < 0.05$), but not different from the SDMV site. As in the Chapter 5, latency of response was found to be unrelated to the order of injection.

Table 3. ANOVA table from the analysis of latency of response (RTIME3). Factors included in the full model were dog, site of injection and injection order. Injection order was non-significant and was deleted from the final model.

Dependent Variable: RTIME3

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	8219.31961478	684.94330123	7.27	0.0001
Error	30	2824.52648289	94.15088276		
Corrected Total	42	11043.84609767			

R-Square	C.V.	Root MSE	RTIME3 Mean
0.744244	20.86686	9.70313778	46.50023256

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOGID	8	4584.11601711	573.01450214	6.09	0.0001
SITE	4	3480.55810711	870.13952678	9.24	0.0001

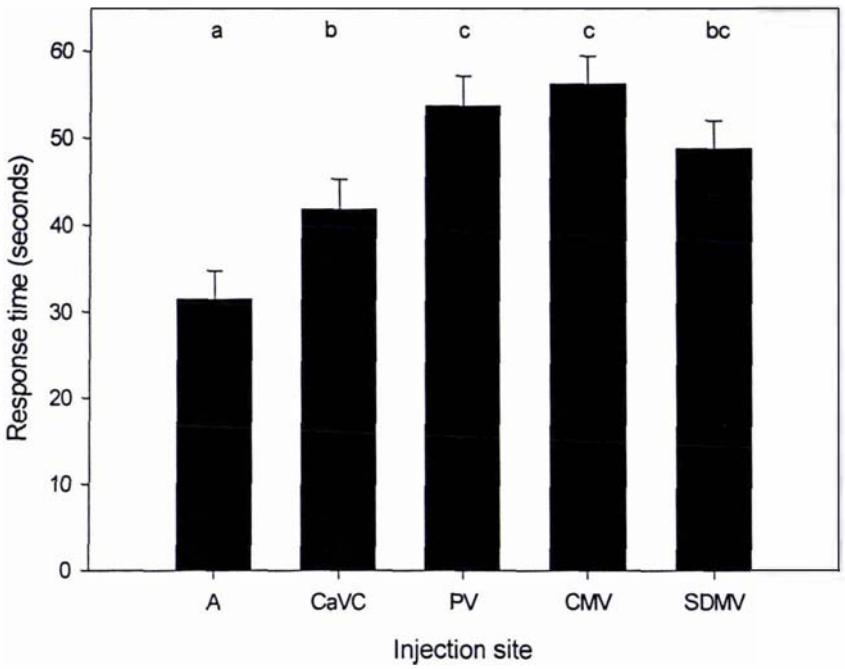


Figure 2. The effect of site of injection on latency of response to secretin. Each bar represents the least squares mean and standard error for response time adjusted for variation arising from differences between dogs. A aorta; CaVC caudal vena cava; CMV caudal mesenteric vein; PV portal vein; SDMV superficial dorsal metatarsal vein; a, b, c mean response times having superscripts which differ, are significantly different ($p < 0.05$).

Part 2 - Threshold testing

Threshold testing was performed in 10 dogs, however the threshold dose for secretin following injection into the PV site was determined properly in only four (dogs #1, #5, #8 & #9). Application of the $\bar{x} + 2$ SD definition to the data collected from the dogs which were initially subjectively assessed (as described for dogs #1 to #4 in Materials and Methods) revealed that the threshold dose was correctly identified in only one of these dogs (dog #1). In two of three remaining dogs in which data were collected (dogs #6 & #7), a 'supra-threshold' dose was inadvertently administered into the remaining injection sites. Anaesthesia-related complications precluded completion of threshold testing in dog #10.

Table 4 shows the results obtained from four dogs following administration of the PV threshold dose into the A, CaVC, CMV and SDMV injection sites. The range of dilutions for the threshold dose at the PV site was 1/2500th to 1/100th of the 35 ng/kg dose. The PV threshold dose generated a response following seven of 16 injections at other sites. The only dog for which no response was observed at the remaining sites to the threshold dose was dog #9. In this dog, the threshold dilution was 1/2500, and this dose generated a response at the PV site during minute five only. Although small numbers precluded statistical analysis, a response was generated at each injection site on at least one occasion, and there appeared to be no clear pattern to the *occurrence* of the responses, nor to their timing.

Table 4. Portal vein threshold dose and results of threshold testing following administration of secretin into the aorta (A), caudal vena cava (CaVC), cranial mesenteric vein (CMV) and superficial dorsal metatarsal vein (SDMV) in four dogs; minutes during which a response was observed are shown in parentheses for each site; NR = no response

Dog ID	PV Threshold Dose (35 ng/kg /X)	Order of injection	A	CaVC	CMV	SDMV
#1	100 (minutes 2, 3)	SDMV A CMV CaVC	Response (minutes 1, 2, 3, 4, 5)	Response (minutes 1, 2, 3, 4, 5)	Response (minutes 2, 3, 4, 5)	Response (minutes 2, 3, 4)
#5	100 (minutes 2, 3, 4, 5)	CMV SDMV A CaVC	NR	NR	Response (minute 4)	Response (minutes 3, 4)
#8	500 (minutes 3, 4, 5)	CMV A CaVC SDMV	Response (minutes 4, 5)	NR*	NR	NR
#9	2500 (minute 5)	CaVC A SDMV CMV	NR	NR	NR	NR

* baseline period overlapped with previous injection at A site

Verification of placement of cannulae

Upon completion of the experiment, dogs were euthanased with an overdose of sodium pentobarbitone. Cannulae placement was determined following death (Table 5). Of the 10 experimental dogs, the tip of the CaVC cannula was located 2.5 cm into the chest of one dog when the PV cannula was in proper position (dog #2). In one dog (dog #4), the PV cannula had become looped and was pointing caudally. In all other dogs, the tip of the CaVC cannula was roughly parallel to the tip of the PV cannula. The location of the tip of the CMV cannula was close to the liver in two dogs (dogs #4 & #6) and in the jejunal vein *close* to its point of entry into the cranial mesenteric vein in another (dog #9). In three dogs (dogs #6, #7 & #8), the SDMV cannula would not thread through the indwelling catheter to reach the level of the hock. Data for the CaVC cannula in dog #2 and for the PV cannula in dog #4 were not included in the analysis for part 1 of the experiment.

Table 5. Summary of locations of tips of cannulae in all dogs (as determined at post mortem examination) and the usefulness of each preparation for latency of response and threshold testing.

Dog ID	Minor Problems	Major Problems	Usefulness of Preparation for Latency Tests	Usefulness of Preparation for Threshold Tests
# 1			All sites used	All sites used
# 2		CaVC cannula in chest	CaVC site not used	NA[1]
# 3			All sites used	NA
# 4	CMV cannula in portal vein	PV cannula looped - pointing caudally	PV site not used	NA
# 5			All sites used	All sites used
# 6	CMV cannula in portal vein; SDMV cannula did not reach hock		All sites used	NA
# 7	SDMV cannula did not reach hock		All sites used	NA
# 8	SDMV cannula did not reach hock		All sites used	All sites used
# 9	CMV cannula in jejunal vein		All sites used	All sites used
# 10			All sites used	NA

[1] Not applicable: data from these dogs were not used.

DISCUSSION

The results of part 1 of this investigation clearly demonstrate that the latency of the pancreatic fluid secretory response to secretin injected into the aorta is significantly shorter than the latency of response to secretin injected into any of the veins of the portal system or systemic circulation. Although the response time at the CaVC site was shorter than the response time at the PV site ($p < 0.05$), both were significantly longer than the response time following administration of secretin into the aorta ($p < 0.05$). The results suggest that the differences observed in response times between injection sites largely reflect differences in circulation time, and provide little support for a role for the portal vein or liver in the pancreatic response to secretin.

Despite the use of the larger pancreatic duct cannula, the results of the present investigation are in agreement with the results obtained from the main effects model and small cannula group of dogs in the first experiment (Chapter 5). While the similarities in response times observed between the PV site and the A site in the large cannula group of dogs in the previous experiment remain unexplained, the results of the present investigation are believed to carry considerably more weight due to the larger sample size and refined technique used in the present study. It is possible that the lower dose of secretin used in this experiment may have somehow accounted for the differences observed between the two experiments, however this explanation seems unlikely. In preliminary studies by Singer (1983) who examined the effect of dose on the latency of response to secretin, intravenous bolus injections of secretin were found to produce a dose-dependent decrease in the latency of the pancreatic fluid response. The shortest latency was seen after injection of secretin at a dose of 250 ng/kg (a dose 50 times that used in the present study). Further doubling the dose did not significantly shorten the latency. On the basis of this information, it is difficult to reconcile how a different dose of secretin would preferentially affect the response time at the PV site while having no detectable effect at the other sites. What seems more likely is that the net effect of using a lower dose of secretin would be a proportional increase in the latencies at all sites, as was observed for the A, PV and SDMV sites in the present investigation (compare Figure 2, Chapter 6 with Figure 4, Chapter 5).

An interesting and rather unexpected outcome of the present investigation was the significantly shorter response time at the secretin-injected CaVC site compared to the PV site when the tips of both cannulae were placed at roughly the same level (near the hilus of the liver) in their respective veins. There are several possible explanations for this. First, it is conceivable that some degree of metabolism of secretin occurred within the liver following injection into both the PV and CMV sites, and that a "first-pass effect" resulted in partial inactivation of the injected hormone. Partial inactivation of the hormone would result in exposure of the pancreas to a lower total concentration of drug which, for reasons noted above (Singer, 1983), would be expected to somewhat prolong the latency. In the dog however, the kidneys are known to eliminate a large proportion of the hormone (up to 60%) as has been shown by experiments with ligation of the renal artery supply (Lehnert *et al.*, 1974), and Strunz *et al.* (1978) proposed that degradation of secretin may take place in the whole of the vascular bed, suggesting that the liver may not have a major effect. In fact, a role of the liver in the catabolism of secretin has been questioned. Data compiled in the late 1960's and early 1970's suggested that hepatic elimination was minimal. Bioassay studies in dogs have demonstrated a small loss of pancreatic response when secretin was infused into the portal vein as compared to infusion into a femoral artery, however this loss was only observed at doses of 0.25 clinical units per kilogram per hour or less ($\leq$ 0.25 CU/kg/hr) (Lehnert *et al.*, 1974). Because weight-for-weight comparisons of potency cannot be made when the weight of pure secretin contained within a secretin preparation is unknown, it was not possible in the present investigation to determine whether this dose was exceeded. The observation however, that the latency of response to secretin at the SDMV site did not differ significantly from that at the PV and CMV sites argues against a major role for the liver in the catabolism of secretin under the conditions of this study.

Alternative explanations for the observed differences in latency between the CaVC and PV sites include the distance (or course) that the secretin bolus must travel to reach the pancreas, and blood flow through each of the respective veins. Secretin, following injection into the portal vein, must first traverse the liver, caudal vena cava, heart, lungs and systemic arteries before finally reaching the pancreas. This is in contrast to the course of travel of secretin following injection into the caudal vena cava which bypasses the

hepatic sinusoids. While blood flow through the portal vein has been well documented in both conscious and anaesthetized dogs (Andreen *et al.*, 1977; Gelman *et al.*, 1984a; Gelman *et al.*, 1984b; Nyland and Fisher, 1990; Lamb and Mahoney, 1994), there are apparently no similar reports of blood flow through the caudal vena cava. It is likely however, that blood flow through the caudal vena cava is more rapid than that through the hepatic portal system. A combination of a shorter distance travelled by the CaVC bolus, and a greater blood flow at the CaVC injection site, therefore probably accounts for the significantly shorter response time observed at that site.

The second part of this experiment was originally designed to test the hypothesis that a role for the portal system exists in the pancreatic response to secretin, and to provide additional physiological support for such a role *if* the results of the PV response times obtained with the large cannula group of dogs (Chapter 5) could be successfully reproduced. The methods employed were based on the supposition that a neural reflex exists which could be initiated by a portal or hepatic action of the hormone. If this was the case, then it was believed that the lowest dose of secretin which was capable of initiating a pancreatic response at the PV site, would be incapable of initiating the same response at the remaining non-portal sites of administration.

Of the 16 injections administered into the non-portal vein sites, a response was elicited on seven occasions. Furthermore, the PV threshold dose was found to generate a response at *each* site of injection on at least one occasion. The implications of these observations can be explained thus: if the *estimated* threshold dose is close to the *true* physiological threshold dose (wherever the injected hormone acts), then repeated administration of this dose at the PV site would, through the laws of probability, be expected to result in a barely detectable response about half of the time and no response about half the time. If there were no modifying effect of the liver or portal vein on the response to secretin, then administration of the threshold dose at the other sites should elicit a similar response. The observation therefore, that a detectable response was recorded in 7 of 16 injections, and at all sites at least once, argues against a specific role for the portal vein or liver in the mediation of a secretin-induced pancreatic response.

Regarding the methodology however, there were a number of concerns which raised questions regarding the validity of the results. Many of these concerns, which were not anticipated at the time the study was designed, stemmed from the physical and time constraints imposed by attempting to perform two experiments with the same dog on the same day, an approach which was intended to make optimal use of each animal. The major concerns related to: the definition of the term "response"; the means by which the threshold dose was estimated; the possibility that there may have been a residual effect from the previous injection during either threshold determination or threshold testing, and the potential effect of decreasing sensitivity of the pancreas following continuous infusion and/or multiple injections of secretin. These problems, which may have biased the results in either direction, are discussed separately below.

In this study, the term "response" was defined as an increase in pancreatic fluid output following injection of secretin which was equal to or greater than $\bar{x}$ + 2 SD of the number of drops per minute measured during a five minute pre-injection period. The time frame over which such a response was considered to be acceptable was rather arbitrarily defined as the first five minutes after injection, a number which was well within the time frame in which responses were observed in earlier latency studies performed by this author (Chapters 5 and 6). From Table 4 it can be seen that there was great variability in the timing of responses, leading one to question whether a response during minute 1 was equivalent to a response during minutes 4 or 5. Also of concern was the one dog for which the threshold dose was 1/2500, a dilution far greater than that determined for the other three dogs. It was interesting to note that this was the only dog in which the threshold dose at the PV site generated a response during a *single* minute of the 5 minute window, and that this dose failed to generate a response in any of the remaining injection sites. Whether the PV response observed during minute 5 occurred by chance alone and actually represented a sub-threshold dose, or whether this dilution was actually closer to true threshold, is unknown. Knowledge of the relative changes in latency and magnitude of response at the lower end of the dose range would have helped to clarify these issues, however this information could have only been obtained by performing a full dose-response curve at each injection site.

The endpoint for threshold determination in this study relied upon having determined two things: the lowest dose which would *elicit* a response at the PV site, and a *lower* dose which would *fail to elicit* a response at the PV site. Unfortunately, time constraints meant that a limited number of dilutions could be tested. As a result, it is not known how closely the *estimated* threshold dose approximated the *true* threshold dose. This, in combination with the effect of probability (i.e., as one nears threshold, a response may be generated at one time, but not at others), likely explains the random distribution of responses in Table 4.

A third factor which may have led to misleading results relates to the possibility that the pancreas was still in the process of recovering from the previous injection when the baseline $\bar{x}$ + 2 SD was calculated. This was known to have occurred in at least one case (Table 4). A residual effect of the previous injection during threshold *determination* may have resulted in an overestimation of the true threshold, whereas a similar error during threshold *testing* may have resulted in an inability to demonstrate a response when a response had actually occurred. The fact that the biological half-life of secretin in dogs is in the range of 2-3 minutes (Lehnert *et al.*, 1972; Rayford *et al.*, 1975) would suggest that during threshold testing, even if a significant response had not occurred until minute 5 (as was the case with dog #9), the biological activity of the drug would have been exceptionally low by minute 10 (the earliest time at which the next dose would have been administered). The possibility remains however, for sites which were injected as soon as 10 minutes after the previous bolus, that the five minute baseline *preceding* the injection may have overlapped with a period during which the pancreas was still in a phase of recovery.

Finally, it is conceivable that the pancreas became desensitized to some degree in response to repeated hormonal stimulation. This would have been expected to decrease the chances of obtaining a response to secretin over time. Declining responses of the pancreas to either continuous pancreatic stimulation by secretin or to repeated instillations of HCl into the duodenum have been noted by others (MacKay and Baxter, 1931; Christodoulopoulos *et al.*, 1961; Baron *et al.*, 1963; Henriksen, 1966). Henriksen (1966) showed that in anaesthetized dogs, the pancreatic response to a sub-maximal dose of secretin decreased

when the interval between injections was 60 minutes, but that there was no decrease in response when the interval between injections was extended to 120 minutes. In the present study however, there was no consistent pattern to the responses when order of injection was taken into account, despite injection intervals of less than one hour. It is possible that a refractory period to secretin may be shorter or may not exist at doses which approach threshold. In addition, although the infusion rate of secretin necessary to achieve a baseline pancreatic fluid output of approximately 3-6 drops per minute varied, there was no trend to an increasing requirement over time. Pancreatic exhaustion may also have been minimized in the present study by administering a background secretin infusion which achieved a lower baseline output (3-6 drops/minute) than that desired during other studies (Singer *et al.*, 1980a; Singer, 1983). Finally, the consistent responses noted in the present study may have in part resulted from the careful monitoring of the patient throughout the anaesthetic period and the many steps taken to minimize the effects of anaesthesia.

Overall, the technical difficulties discussed above diminish the value of this component of the investigation, and perhaps preclude the ability to draw conclusions of any kind. Some of the concerns outlined above would be expected to bias *towards* generating a response, while others would bias *against* generating a response. If any conclusion is to be drawn however, it is that this part of the experiment provides no new or additional evidence for a sensory role for the portal system or liver in response to secretin administered intra-portally. This conclusion is fully compatible with the results of part 1 of the experiment, which demonstrated that the latency of the pancreatic response to secretin injected into the aorta was significantly shorter than the latency of response to secretin injected into any of the veins of the hepatic portal system or systemic circulation. Collectively, the results of these two studies provide no support for a role for the portal system or liver in the pancreatic response to secretin.

Chapter Seven

GENERAL DISCUSSION

INTRODUCTION

One cannot help but be impressed by the complexity, yet coherence of design, of the mammalian digestive system. This intricate system for nutrient processing consists anatomically, of an ordered series of highly specialized organs that are remarkably well-adapted and positioned with respect to one another for performing their specific functions in the digestive process. Perhaps even more impressive are the complex *interactions* (physical, humoral, neural) that take place between the organs of digestion - interactions that exert both positive and negative feedback control on one another. The net result is a response by each organ 'participant' that is well harmonized, both in timing and in magnitude, with the specific needs of the digestive process at any point in time.

However logical the layout of the digestive system may at first appear, there are a number of aspects of its design that are more difficult to explain given our current understanding of the physiology. For example, although the location of the pancreas relative to the duodenum seems logical considering the need for delivery of water, bicarbonate and enzymes into the upper small intestine, it is surprising that the response by the exocrine pancreas to sensory information arising in the gut should, out of necessity, travel by pathways involving the CNS and/or the systemic arterial blood supply. Neither pathway apparently takes advantage of the proximity of the pancreas to the gut and its associated portal vasculature.

Despite the close apposition of the pancreas with the proximal duodenum, it has generally been held that the gut communicates with the pancreas only via 'long' pathways involving the systemic arterial blood supply, the CNS, or a combination of both. The possibility that a *local* form of communication could exist has been recognized for almost a century but such 'unconventional' forms of control have escaped close scrutiny by modern researchers, perhaps due to rapid advances in our understanding of the more *conventional* forms of control. Cholecystokinin (CCK), for example, has been shown to play a complex role in regulating the secretion of pancreatic enzymes. Although its action as a classical endocrine

hormone is well-accepted, there is now considerable evidence that shows that long vago-vagal reflexes are the predominant mechanism whereby CCK exerts its effects (Chapter 1). The study of the role gastrointestinal hormones as neurotransmitters and/or neuromodulators has become a rapidly growing field – one that will no doubt shed new light on the mechanisms of control of pancreatic function.

The hypothesis proposed in this thesis was that local avenues of communication exist that take advantage of the developmental proximity of the pancreas to the gut and hepatic portal system. As discussed in Chapters 1 and 2, the biological significance of these relationships was questioned when this author observed in a number of different species, varying degrees of intimacy of the pancreas with respect to the duodenum and veins of the hepatic portal system. Further support for the hypothesis was provided by a number of other researchers who *also* speculated, but did not specifically test, that the position of the pancreas relative to the proximal small intestine may have functional significance (Pavlov, 1910; Thomas, 1948; Thambugala and Baron, 1971; Bodanszky *et al.*, 1973; Tiscornia *et al.*, 1976a; Tiscornia, 1977; Kirchgessner and Gershon, 1990). At the outset of the work described here, it was believed that the most likely means by which communication could occur between the small intestine and the pancreas was through some form of local vascular transfer, either directly or via a counter-current exchange mechanism, or by a local neural relay of information via the enteric nervous system. During the course of these studies, yet another potential mechanism became apparent: a communication pathway between the *hepatic portal system* and the pancreas. In this scenario, specialized sensory structures in the walls of the portal vasculature would transmit information to the pancreas regarding the digestive status of the animal. These specific mechanisms, which have not been examined to any significant extent, were considered worthy of further study.

The technical difficulties involved with studying regulation of the exocrine pancreas (difficulties with which this author has become altogether too familiar) may in part be responsible for the paucity of research in this area. As described in Chapter 1, any intervention, whether it be physical (i.e., transplantation of the pancreas) or pharmacological (i.e., pre-treatment with agonists or antagonists of the autonomic nervous system), will potentially modify pancreatic function through more than one pathway. This

circumstance is problematical because it then becomes difficult to draw accurate conclusions regarding the role(s) of conventional controlling mechanisms when the contribution(s) of other potential, but less well-understood mechanisms of control are unknown. *Pharmacological* intervention may *also* modify pathways – pathways that normally do not operate under physiological conditions. The issue of supraphysiological doses of secretagogues used in many earlier studies of pancreatic function was addressed by Li and Owyang (1993, 1994). They showed that CCK, at "physiological" concentrations, stimulates pancreatic enzyme secretion in anaesthetized rats via a capsaicin-sensitive afferent vagal pathway, a pathway that originates in the gastroduodenal mucosa. Whereas these results were in disagreement with those of previous researchers (Henriksen, 1969; Konturek *et al.*, 1972; Konturek *et al.*, 1974a; Debas *et al.*, 1975a; Solomon and Grossman, 1979), Li and Owyang attributed these contradictory findings, in part, to the use of "supraphysiological" doses of CCK. These observations regarding the definition of the descriptor 'pharmacological' not only revolutionized our understanding of the role of gastrointestinal peptides as both hormone and neuromodulator, but also helped to explain much of the confusion that arose from the results of earlier studies in which supraphysiological doses of pharmacological agents were used.

The potential implications of the research detailed in this thesis are significant. Should the existence of a local pathway of communication exist, it would cast considerable doubt on a large body of research that was designed to evaluate only the involvement of *conventionally accepted* forms of control. For example, the conclusions drawn from the landmark latency studies of Singer's group (Singer *et al.*, 1980a and Singer, 1983) might not be valid if a local vascular or neural pathway was shown to exist between the duodenum and pancreas. While their work has helped to shape our understanding of the involvement of nervous and hormonal mechanisms in the control of exocrine pancreatic function, their experiments were not specifically designed to test for the existence of short vascular or neural reflex pathways. Consequently, and at their own admission, the presence of local forms of control could not be disproved.

RESEARCH OUTCOMES

OVERVIEW

As a preliminary step in investigating whether local avenues of communication exist between the duodenum and pancreas, a selective venous casting technique was employed. In this study, which provided the opportunity to examine the complex venous drainage patterns of the duodeno-pancreas, little evidence was found that was in direct support of a local vascular pathway of communication between the two organs. Latex-injected casts of the gut and hepatic portal system showed that venous tributaries of duodenal origin were sometimes observed to coalesce first with veins draining the pancreas prior to entering the cranial pancreaticoduodenal vein. Common drainage of venous blood at this level has not previously been described. In the one specimen macerated to the extent that only attachments to the proximal duodenum remained, duodenal tributaries were also observed to closely approach capillary beds of pancreatic origin. Unfortunately, the relationships of these vessels with one another could not be precisely determined and it is therefore not known whether these tiny networks represented a local portal circuitry between the duodenum and pancreas. Imprints of venous valves were also identified in this study contrary to what many anatomy textbooks would lead one to expect. They were found adjacent to primary, secondary and tertiary branch points relative to the main portal trunk. In all cases, the valve imprints directed blood towards the portal vein, and there was no evidence of strategic placement of valves that would suggest that blood draining the duodenum was being shunted through the pancreas.

The latex vascular casting technique employed in this study was a very sensitive technique for outlining the structures of interest, in particular the small vessels of the duodeno-pancreas and the valves of the hepatic portal system. As mentioned earlier however, the latex had a tendency to become 'sticky' during prolonged periods of examination out of water. As a general rule, the finer strands of a latex cast do *not* adhere to one another unless the medium within which the cast is stored dries out. When this happens, the elements of the cast *will* tend to stick together, especially after repeated handling (Allan Nutman, personal communication*). It is possible (and in this author's mind, probable) that this effect was most likely to have accounted for the author's observation (Chapter 3) of duodenal tributaries closely approaching and disappearing into pancreatic capillary

beds. Other potential methods used in vascular research, such as injection of dyes or radiographic contrast media, were considered to be of little value for testing the hypothesis presented in Chapter 3. If a vascular connection indeed exists, it is possible that only a small portion of the duodenal venous drainage passes through the pancreas. To adequately test this hypothesis, these materials would have to have been injected into *every* vein draining the duodenum to ensure that no vascular connections were missed.

As with the latex casting study discussed above, little *histological* support for the existence of a portal circulation between the duodenum and pancreas was found in the microanatomical study that followed (Chapter 4). Nor was there evidence of any sort (i.e., highly tortuous veins closely apposed to arteries) to suggest that a local countercurrent exchange mechanism exists between veins draining the duodenum and arteries supplying the pancreas. The histological techniques employed however, were not a particularly sensitive technique overall, despite the large number of sections examined. Due to widely differing tissue densities, significant problems were encountered achieving optimal fixation for both the duodenum and pancreas simultaneously, so many sections of pancreas in the area of close apposition were torn and unusable. It was therefore difficult, despite *attempts* at serial sectioning, to follow particular veins from their points of origin in the duodenum to their final destination at the cranial pancreaticoduodenal vein. With respect to nerves, although a nervous pathway was initially hypothesized, little specific effort was put towards defining potential pathways of communication via the enteric nervous system other than a general survey of the area. This was primarily because, at this early stage, the main focus was on a vascular communication. In retrospect, it would have been valuable to have *also* focussed on following the pathways of specific nerves, recognizing of course, that specialized staining procedures (such as silver-based impregnation methods or immunocytochemistry using antibodies to neurofilament proteins) would have been required. This information would have at least allowed for a preliminary conclusion regarding the possibility of a direct *neural* pathway of communication between the duodenum and pancreas in the dog, as has been previously demonstrated histologically in rats (Anglade *et al.*, 1987).

Overall, it was strongly believed by this stage of the thesis that, although the possibility could not be entirely ruled-out, there was no firm evidence to suggest that a direct functional interconnection of veins exists between the duodenum and pancreas. Additional evidence against the presence of a vascular route of communication was obtained later in the thesis. As a means of validating the results of the experiment in Chapter 5, Indian ink was injected into the cranial pancreaticoduodenal vein cannula prior to euthanasia and again immediately following death in order to show that the secretin bolus passed cranially through the vein *without* entering the pancreas. Serial sectioning through the pancreas after death failed to reveal ink in the pancreas of any of the dogs. This combination of results from the three studies suggested to this author that a direct duodenum-to-pancreas venous communication was unlikely.

An incidental finding during the microanatomical study was the large number of luminally-projecting 'folds' of vein wall that could be demonstrated throughout both the hepatic portal system and veins of the systemic circulation (Chapter 4). Most of these folds were *clearly* artifactual, however a small number of the folds observed had histological features that were somewhat reminiscent of sensory organelles. This inability to assign "artifact" to *all* such structures prompted the use of immunocytochemistry to determine if these structures served a 'sensory' role. Although this could not be demonstrated, identification of positive immunoreactivity to each of three sensory neuropeptides at or near the level of the endothelium suggested to *this* author, that perhaps a 'sensory' role exists for the veins of the hepatic portal system. The possibility that the portal vein or its tributaries may have a 'chemosensory' function was intriguing, and it was hypothesized that these or other heretofore unrecognized sensory structures in the portal vasculature might be involved in reflex regulation of the pancreas.

In order to test the hypothesis that components of a sensory mechanism exist within the hepatic portal system of the dog, and that such a sensory mechanism plays a role in the control of exocrine pancreatic function, a physiological experiment was designed to examine and compare between sites, the latency of the pancreatic fluid secretory response to a known, exogenously administered pancreatic secretagogue (Chapter 5). Secretin was selected over CCK for preliminary testing because secretin has been shown to inhibit

gastric emptying (Raybould and Holzer, 1993) and gastric acid secretion (Li *et al.*, 1998) via a capsaicin-sensitive vagal afferent pathway in rats, and while a great deal is known regarding pancreatic enzyme secretion following stimulation of vagal afferent pathways by CCK (Li and Owyang, 1993 and 1994), virtually nothing is known about the ability of secretin to stimulate pancreatic secretion via similar (or other) sensory neural pathways. Secondly, secretin, unlike CCK which requires laboratory evaluation to determine changes in (enzyme) output, produces immediately measurable increases in output of pancreatic fluid.

Before discussing the results of the first physiological experiment, it would seem wise to examine the choice of animal model, both with respect to the species chosen and the experimental set-up. First, because of its size, compliance and pancreatic anatomy, the dog is the species of choice for pancreatic secretory studies (Niebergall-Roth *et al.*, 1997), and indeed, an enormous amount of literature has been generated from research performed in this species. Dogs were chosen for the present physiological experiments because they are one of the species in which the body of the pancreas is closely associated with the proximal descending limb of the duodenum. Dogs were used as acute rather than chronic preparations because it was the desire of both the author and her supervisors that the impact of the experimental procedures on the animals be minimal – it was considered better to first test the experimental hypotheses in anaesthetized animals so the animals would not need to be put through recovery surgery needlessly. The possible effects of anaesthesia on the reflexes of interest are discussed below.

Second, when proposing these studies it was required that a choice be made between experimental models. Historically, three animal models have been developed for collecting pancreatic secretions in dogs (recently reviewed by Niebergall-Roth *et al.*, 1997). They include pancreatic fistulas, duodenal pouches (that collect the pancreatic secretion), and duodenal fistulas (through which a thin cannula is inserted into the pancreatic duct). The animal model used for collecting pancreatic secretions in the dogs of the present studies was a temporary pancreatic fistula. This model, which is most commonly used in acute studies (Niebergall-Roth *et al.*, 1997), clearly has the distinct

advantages of fewer technical difficulties and, because of anaesthesia, minimal impact on the animals.

In retrospect, however, the use of this model may not have been optimal for testing the putative nervous reflexes in question. Anaesthesia can be expected to reduce the response from the pancreas to stimuli that act through the CNS (Niebergall-Roth *et al.*, 1997). Whether this is true of nervous reflexes acting locally is uncertain; that the normal migrating myoelectric complex in the canine gut is disrupted during anaesthesia in the fasted dog would suggest that local reflexes mediated locally by the enteric nervous system may also be inhibited. This criticism may not be as serious as it at first seems – clearly a multitude of neural reflexes acting through both central and peripheral nerve terminals are functional, albeit somewhat modified, in the anaesthetized dog. In the current studies, it was not the absolute magnitude of response that was of interest, but the relative latency of response at various sites of injection. It was also shown that anaesthesia was very stable during these experiments (see discussion below) and that pancreatic secretion did not deteriorate during the course of the experiment. Nonetheless, it seems that more definitive conclusions could be drawn in future studies if they were performed using chronic rather than acute, animal models.

The results of the first experiment, contained in Chapter 5, were somewhat equivocal. Overall, the results indicated that the latency of the pancreatic fluid secretory response to secretin injected into the portal system was longer than that following injection into the aorta, suggesting that a local sensory mechanism for secretin *does not* exist within the walls of the portal vasculature. However, as a complicating factor, pancreatic cannulae of two diameters were used in this study. The mean latencies were shorter in the dogs in which the larger pancreatic duct cannula was used, suggesting a greater resistance to flow through the smaller cannula. More importantly, choice of cannula appeared to affect the relative latencies for different venous sites so that, in dogs with the large cannula, the latency after injection into the portal vein was significantly shorter than that after injection at the mesenteric vein, and was more similar to that of injection into the aorta. These differences in latency data when broken down by pancreatic duct cannula size clearly complicated interpretation of the results. In an earlier pilot study performed on 10 dogs,

various sizes of pancreatic duct cannulae were used along with various doses of secretin in an attempt to determine the optimal dose for later use in the latency studies. Unfortunately, the magnitude and duration of the pancreatic response to secretin was not considered in relation to the size of the pancreatic duct cannula. Certainly it was easier to introduce and thread a cannula of smaller external dimensions, and perhaps it was also less traumatic to the pancreatic duct. Identification of this problem half-way through the experiment led ultimately to results that raised questions regarding the validity of both models. A decision was therefore made to design a second experiment in an attempt to reproduce the results obtained at the portal vein site in the large cannula group of dogs (second latency experiment), as well as to examine the relative importance of circulation time versus a postulated portal vein or liver effect on the pancreatic fluid secretory response to secretin (threshold testing).

Results from the studies in Chapter 6 primarily served to clarify some of the ambiguous findings in Chapter 5. Using a slightly modified set-up of cannulae, the latency of the pancreatic fluid secretory response to a bolus of secretin injected into the aorta was compared to that of secretin injected into selected veins of the portal system and a peripheral vein. Overall, the results were in agreement with the results presented in Chapter 5 for the main effects model, i.e., the differences in latency between sites generally reflected expected differences due to circulation time. Using the same preparations, the threshold dose of secretin at the PV site was determined in each of 4 dogs and this dose was then administered into the remaining sites in order to (further) test the hypothesis that the portal vein or liver may somehow potentiate the effect of secretin. Secretory responses were observed to the threshold dose following seven of 16 injections, which is about the proportion one would expect in the absence of a modifying effect of the liver or portal vein, providing further evidence *against* a specific role for the portal vein or liver in the mediation of a secretin-induced pancreatic response. Collectively, the results of these two studies provided *no* support for a sensory role for the portal vein or liver in the pancreatic response to secretin.

THE ROLE OF ANAESTHETIC MONITORING IN PHYSIOLOGICAL STUDIES

During the course of the experiments, a substantial amount of time was devoted to ensuring the stability of the anaesthetized patient. This was considered to be a vital component of the experiment, since deterioration of patient status over time was likely to have adversely affected the results. As indicated in Chapter 5, there were a number of measures taken to minimize the effects of anaesthesia. These included the use of lower doses of more than one drug for induction of anaesthesia, the use of short-acting pre-medications and induction agents, the use of the lowest plane of anaesthesia necessary for the procedures being performed, the provision of circulatory support, and maintenance of normothermia. In addition, stringent monitoring of the anaesthetized animal was critical for circumventing potential complications that may have arisen as a result of gradual deterioration over time. Careful monitoring usually provided clues that the status of the animal was changing. Early identification of such trends allowed for early intervention, and a return of the patient to a more physiological state.

Perhaps the most difficult system to regulate during anaesthesia was body temperature. Short of ambient temperature control (which is usually not achievable), great measures must be taken to prevent hypothermia, especially early during the surgical set-up when the abdomen is open and evaporative heat losses are at a maximum. The bubble wrap used in the present studies was an extremely efficient means of heat conservation in the anaesthetized dogs once all surgical procedures were complete, however, *hyper*thermia was also encountered as a result of its use. Early identification and attention to trends toward either hypo- or hyperthermia were especially important in these experiments due to the long lag time seen between intervention and response.

Overall, although the effects of changes in physiological parameters on the results of the experiments were not specifically examined in these studies, it is the opinion of this author that ensuring the stability of the anaesthetized animal throughout the course of an acute physiological experiment greatly enhances the chance of obtaining valid physiological data.

OVERALL RESEARCH CONCLUSIONS

In reviewing the research outcomes for this series of studies (Chapters 2 to 6), it appears that little evidence has been provided that supports the existence of a local means of communication between either the duodenum or the hepatic portal system and the pancreas. This of course does not mean that one does not exist. Certainly the macroscopic and microscopic surveys of the area of close apposition did not reveal clear evidence of any vascular configurations which suggest venous shunting of blood through the pancreas. This potential method of communication therefore seems unlikely. Similarly, there was no evidence to suggest that a nervous pathway of communication exists between the veins of the portal vasculature and the pancreas, although these studies tested only one of many possible initiators of such a reflex, that being a mechanism sensitive to secretin. The possibility that other local forms of communication exist that exert control over the exocrine or endocrine pancreas await further investigation. Potential directions for future research in this area are discussed below.

FUTURE DIRECTIONS

This thesis has presented the results of a series of studies that have attempted, for the first time, to demonstrate the existence of a local form of communication between the duodenum and/or the hepatic portal system and the pancreas, and to show that this form of communication plays a role in the control of exocrine pancreatic function. The form of communication sought was one that does not rely on the CNS or delivery of humoral agents via the systemic arteries, but rather takes advantage of the developmental proximity of the pancreas to the gut and its associated portal vasculature.

Although there was little evidence found that would support the existence of any such form of communication in the present studies, there is a great deal more that can be learned from research efforts focussed in this direction. For example, it is conceivable that other nutrients, hormones or perhaps metabolites in portal blood may participate in initiating reflex regulation of the exocrine pancreas. Testing such pathways in conscious animals would have its obvious advantages. It is also entirely possible that *other* nervous pathways

exist, for instance, one that is mediated by the enteric nervous system; such a mechanism could play an important supplementary role in (reflex) regulation of the pancreas.

Investigating the role of the enteric nervous system in the control of exocrine pancreatic function is likely to be a most fruitful mechanism to next explore. Building upon the work of other researchers in this area, it would seem that the logical first step would be histological confirmation of the existence of nerves running directly from duodenum to pancreas in the dog, evidence to show that these nerves indeed represent projections of the enteric nervous system, and finally evidence that would suggest that such projections may have functional significance, as has been shown in the rat (Kirchgessner and Gershon, 1990). The projections of the enteric nervous system have been extensively studied using immunocytochemistry, retrograde tracers and electrophysiology (Dalsgaard *et al.*, 1983; Lee *et al.*, 1986; Doerffler-Melly and Neuhuber, 1988), and the ability of nerves from the gut to alter the activity of pancreatic neurons has been assessed *in vitro* by activating enteric neural reflex pathways by luminal application of veratridine. Cytochrome oxidase activity, an endogenous metabolic marker for neuronal activity, increases rapidly in stimulated neurons when the rate of discharge of action potentials is raised (Mawe and Gershon, 1986). The cytochrome oxidase activity of neurons and acinar cells can be demonstrated histochemically in attached segments of pancreas and can be measured by computer-assisted microdensitometry (Kirchgessner and Gershon, 1990). Before further experimentation on live dogs can be justified however, it is the opinion of this author that it would be imperative to first confirm that these anatomical and physiological relationships found in the rat similarly exist in the dog.

Having demonstrated that enteric neurons project to the pancreas in the dog, and that the enteric nervous system may in some way influence the activities of the exocrine pancreas, one could then move into a physiological experiment that uses and builds upon some of the concepts and techniques developed by Singer *et al.* (1980a, 1983). As pointed out by Singer, it is not easy to make a preparation in which hormonal mechanisms are eliminated while nervous pathways are left intact. Thus it has not been possible to exactly quantify the role of the hormonal versus neural components of the pancreatic exocrine response. Singer found strong evidence for a vago-vagal component to the early pancreatic response

to intraduodenal tryptophan and oleate, but surmised that the response remaining after administration of atropine or vagotomy must be entirely hormonal. This conclusion does not draw on the possibility that *some* of the residual response after atropine or vagotomy may be mediated by local (non-cholinergic) nervous pathways.

The objective of the proposed experiment would be to examine the residual response by comparing the pancreatic response following rapid intraduodenal injection of pancreatic stimulants (i.e., tryptophan, oleate and HCl) in dogs, before and after vagotomy, and before and after severing the connections of the enteric nerves. The experimental model used in this experiment would be the conscious dog fitted with a chronic pancreatic fistula and a duodenal cannula placed opposite the accessory pancreatic duct. Both magnitude and latency of secretory and enzyme responses would be measured because it is conceivable that the putative local neural reflex could have a permissive or supplementary role on pancreatic secretion that may affect magnitude without altering latency. An experiment such as this, although technically demanding, would provide a relative measure of the contribution of both 'long' and 'short' entero-pancreatic neural reflexes in the control of the exocrine pancreatic secretion, and may also have relevance to the study of reflexes that influence pancreatic *endocrine* function

CONCLUDING REMARKS

Exocrine pancreatic function and its control remains an important area of research as evidenced by the large number of research activities ongoing in the field. Despite the inability to have demonstrated a specific local pathway of communication between the duodenum and pancreas in *this* series of studies, it seems likely, based on accumulated evidence from the literature, that the activities of the exocrine pancreas will eventually be shown to be influenced by the bowel through at least three different mechanisms: first, by an endocrine mechanism that is dependent on the release of hormones from the gastrointestinal mucosa; second, by a neural mechanism, which involves centrally processed reflexes; and finally, by a local mechanism, which operates independently of these pathways. It remains for future experiments to determine the physiological role(s) played by each.

It is humbling to note that many of the frustrations and difficulties encountered during the course of these studies were evident to researchers over 100 years ago. Pavlov (1910) quotes Heidenhain thus:

> "Indeed, every observer who has been occupied for any length of time investigating the functions of the pancreas will leave this field with a feeling of dissatisfaction in consequence of the extremely large number of fruitless experiments he is obliged to subtract from the total number of his investigations; for not even the greatest care, nor the ripest experience in making of pancreatic fistulae, will overcome the incomprehensible sensitiveness of the organ, which only too often annuls its function for a length of time after the operation, a function which does not resume even under the influence of the most favourable secretory conditions. A degree of uncertainty, therefore, always clings to the results of such observations, which is not set aside even by frequent repetition of the experiments. I must openly confess that I have never undertaken a series of experiments which entailed the sacrifice of so many dogs and with such poor results."

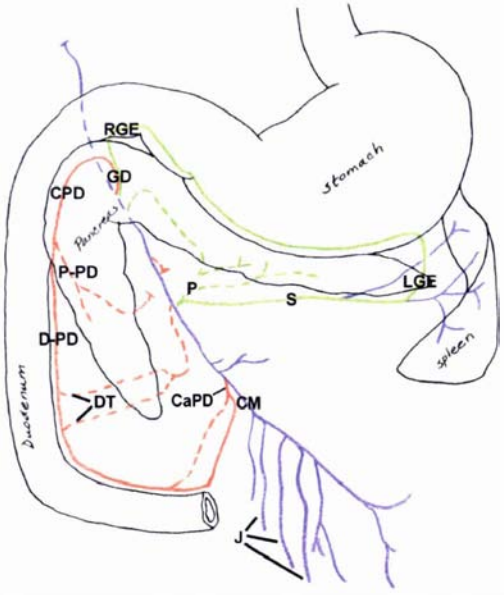
Through the efforts of these ingenious early researchers, and those that came after them, our understanding of the complexities of pancreatic control have slowly progressed; although there is much yet to be learned, it is the fervent hope of this researcher that the findings contained herein will be of value to those who find themselves in the future "occupied for any length of time investigating the functions of the pancreas".

* Personal Communication with Allan Nutman, Institute of Veterinary, Animal and Biomedical Sciences.

Appendix 1

DEPICTION OF ANASTOMOSES AND VENOUS DRAINAGE PATTERNS IN INDIVIDUAL SPECIMENS USED IN THE DERIVATION OF THE COMPOSITE DIAGRAM (CHAPTER 3, FIGURE 4)

The 'gastroduodenal vein-splenic vein' loop is represented by a solid green line; the 'gastroduodenal vein-caudal pancreaticoduodenal vein' loop is represented by a solid orange line; hashed lines represent venous drainage patterns and subsets of these loops; the main portal trunk and all unrelated branches are shown in purple; ⊣ end of vein segment. The splenic vein (and its branches) of specimen #4, and all veins of specimens #5 and #6 were superimposed on the template in their expected locations. **CaPD** caudal pancreaticoduodenal vein; **CM** cranial mesenteric vein; **CPD** cranial pancreaticoduodenal vein; **D-PD** duodenal branch of pancreaticoduodenal vein; **DT** duodenal branch tributary; **GD** gastroduodenal vein; **J** jejunal vein; **LGE** left gastroepiploic vein; **P** pancreatic vein; **P-PD** pancreatic branch of pancreaticoduodenal vein; **RGE** right gastroepiploic vein; **S** splenic vein **(Template shown was modified and reprinted from Miller's Guide to the Dissection of the Dog, Evans & de Lahunta, p 208, 1996, by permission of the publisher, WB Saunders Company)**



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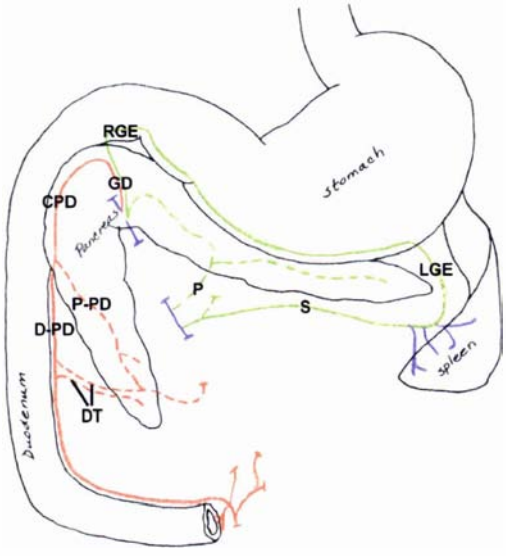

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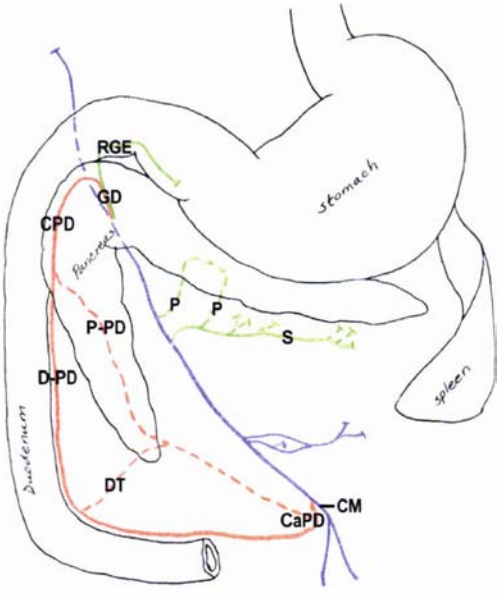

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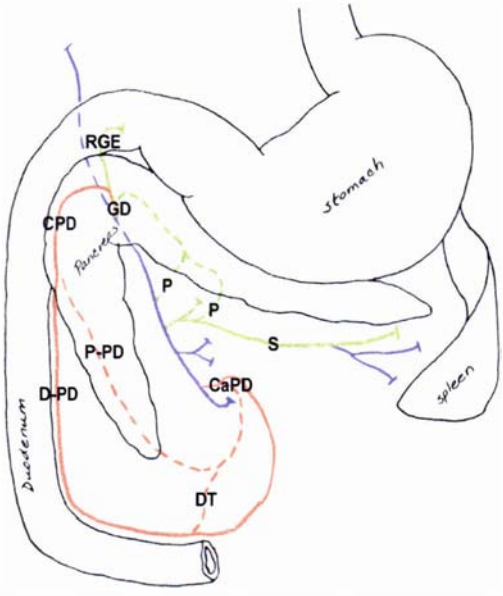
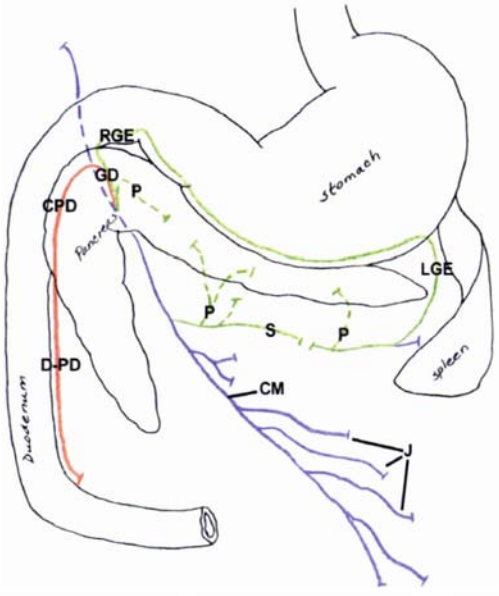
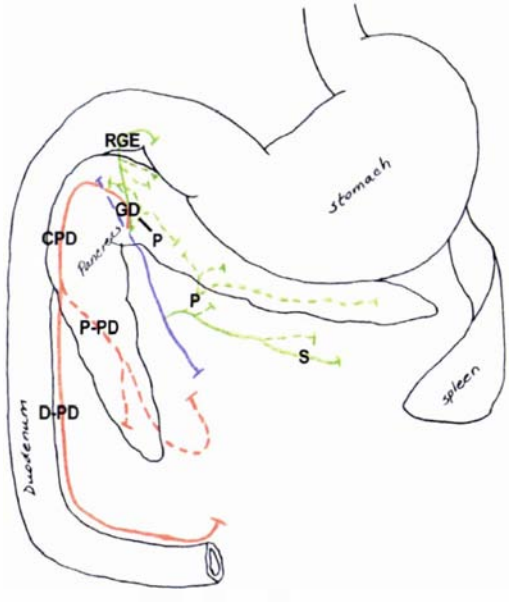


Specimen #4: Incompletely macerated venous latex cast



Specimen #5: Completely macerated venous latex cast

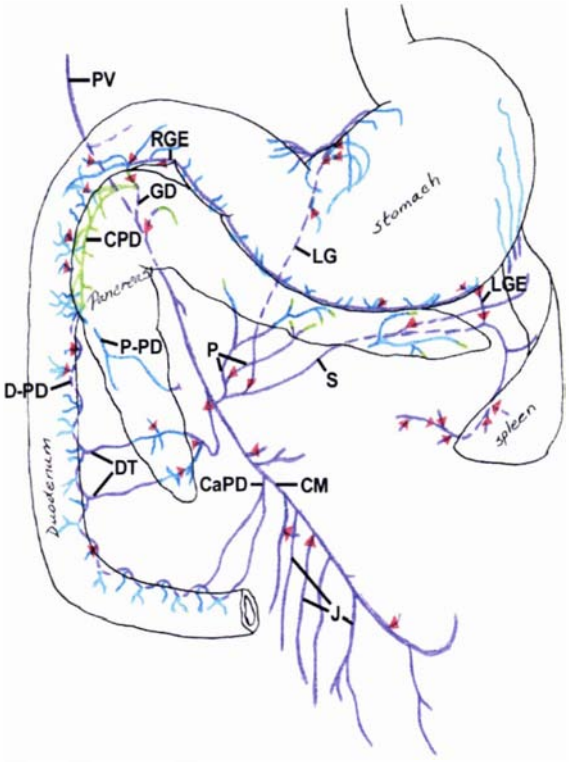


Specimen #6: Completely macerated venous latex cast

Appendix 2

DEPICTION OF LOCATIONS OF VALVE IMPRINTS IN INDIVIDUAL SPECIMENS USED IN THE DERIVATION OF THE COMPOSITE DIAGRAM (CHAPTER 3, FIGURE 5)

Red arrowheads show the specific locations in which valve imprints were identified and point in the direction of blood flow. Only bi-lobed valves were recorded. Imprints were not seen at *all* branch points and were not always observed in the same areas when individual specimens were compared. Note that the number of valve imprints observed increased with increasing maceration of specimens. In specimen #5, the pancreatic branch of the cranial pancreaticoduodenal vein could not be identified. **Solid purple** lines represent veins which are freely visible passing ventral to the viscera; **hashed purple** lines represent veins which are freely visible passing dorsal to the viscera; **dark blue** and **light blue** lines represent veins which are visible on the ventral and dorsal aspects of the viscera, respectively; green lines represent veins passing through the pancreas. **CaM** caudal mesenteric vein; **CaPD** caudal pancreaticoduodenal vein; **CM** cranial mesenteric vein; **CPD** cranial pancreaticoduodenal vein; **CT** capillary tufts; **D-PD** duodenal branch of pancreaticoduodenal vein; **DT** duodenal branch tributary; **GD** gastroduodenal vein; **IC** ileocolic vein; **J** jejunal vein; **LG** left gastric vein; **LGE** left gastroepiploic vein; **P** pancreatic tributary; **P-PD** pancreatic branch of pancreaticoduodenal vein; **PV** portal vein; **RGE** right gastroepiploic vein; **S** splenic vein



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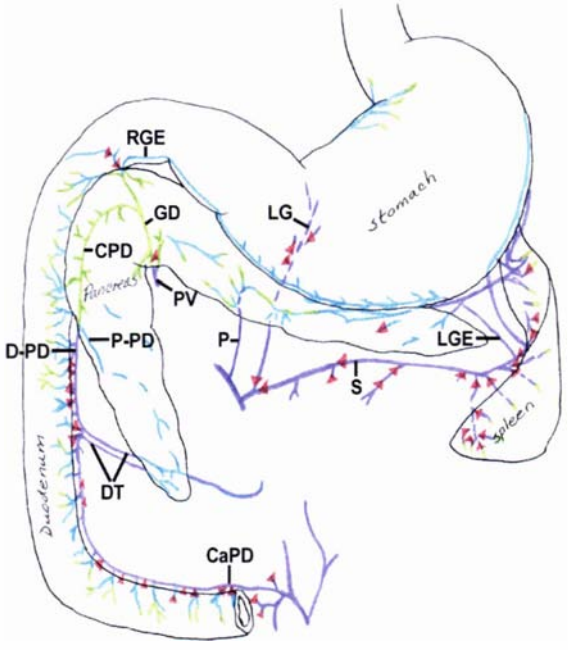
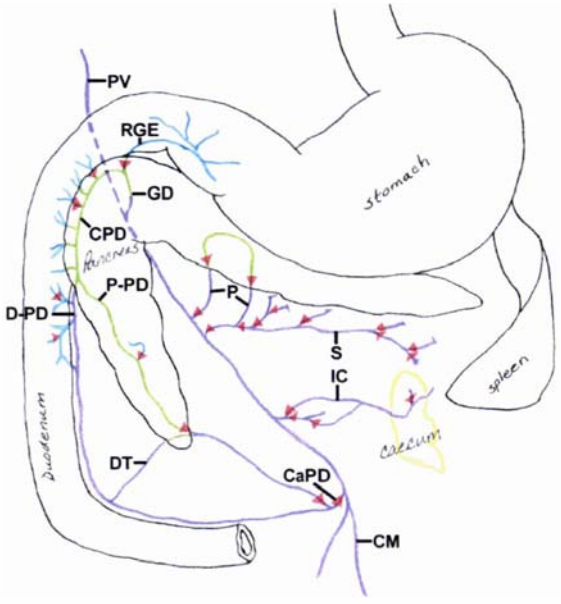
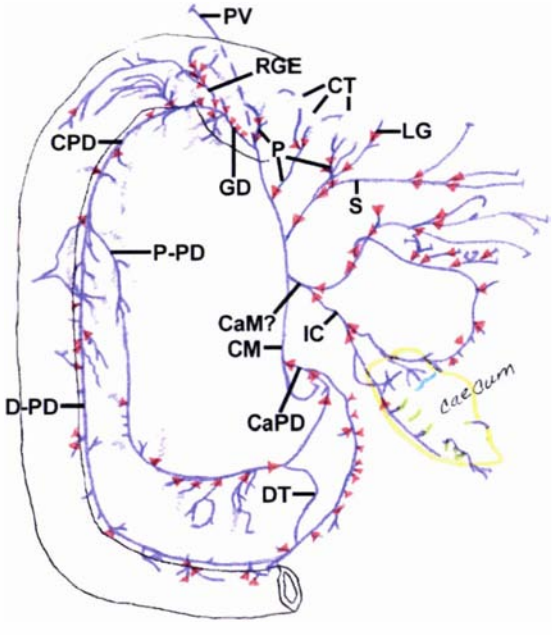


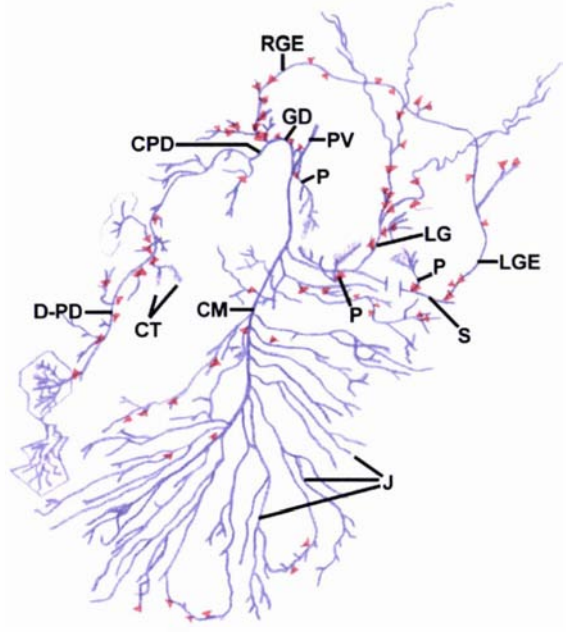
Specimen #2: Preserved arterial and venous latex cast



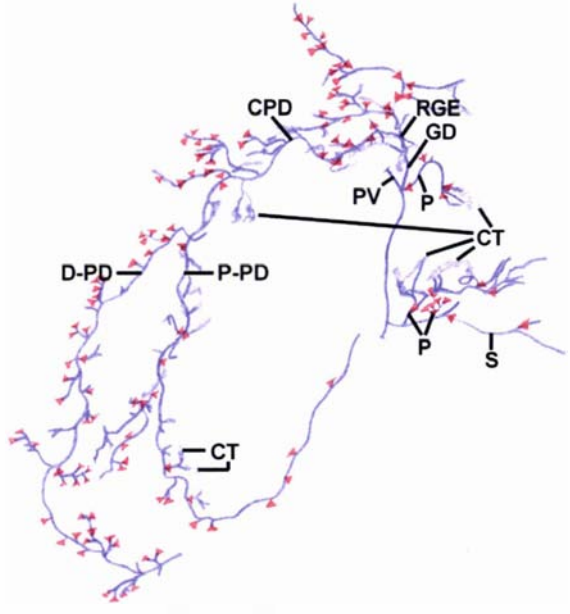
Specimen #3: Preserved venous latex cast



Specimen #4: Incompletely macerated venous latex cast



Specimen #5: Completely macerated venous latex cast



Specimen #6: Completely macerated venous latex cast

Appendix 3

LABORATORY METHODS – CHAPTER 4

PREPARATION OF BOUIN'S FLUID SOLUTION

Bouin's fluid solution was prepared by adding 250 ml of formalin (40% w/v formaldehyde) and 50 ml of glacial acetic acid to 750 ml of saturated (1.2% w/v) aqueous picric acid.

HAEMATOXYLIN/EOSIN STAINING PROTOCOL

This protocol was adapted from that of Kiernan, 1990.

23) Dewax and bring to water
    - A Two changes of xylene, 5 minutes each
        - a Absolute alcohol, until slides drain clear
        - b 70% alcohol, until slides drain clear
        - c Wash in tapwater
24) Stain in Mayer's Haemalum for 10 minutes
25) Wash in tapwater
26) Blue in Scott's tapwater for 2 minutes
27) Rinse in tapwater
28) Stain in 1% aqueous eosin Y for 2 minutes
29) Rinse in tapwater
30) Differentiate and dehydrate in 70% alcohol followed by two changes of absolute alcohol
31) Clear in two changes of xylene
32) Mount (coverslip) with DPX

This procedure stains nuclei and other basophilic structures blue/black, other tissue components shades of pink to red, and strongly acidophilic structures bright red.

PHOSPHATE BUFFERED SALINE SOLUTION

Phosphate buffered saline for immunocytochemistry was prepared by combining 4.54 grams anhydrous disodium hydrogen phosphate, 1.09 grams potassium dihydrogen phosphate and 36.0 grams sodium chloride with 4 litres of glass distilled water.

COMPLETE ANAESTHETIC RECORD: DOG #15 - CHAPTER 5

MASSEY UNIVERSITY ANAESTHETIC RECORD

PATIENT DETAILS

DATE 19/5/97 OWNER'S NAME .....

CASE NUMBER Expt 1, Dog 15 ANIMAL'S NAME Rob

WARD/CAGE/BOX ..... ANAESTHETIST ..... SUPERVISOR .....

SPECIES canine BREED working dog COLOUR brown/white

AGE mature SEX M WEIGHT (kg) 17.0 CONDITION OK

TEMPERAMENT timid FASTED (YES) NO

PHYSICAL EXAM

HR (bpm) 100 RR 32 TEMPERATURE 38

MM pink C T <1st HYDRATION normal ?

PULSE RATE / QUALITY 100/strong

HEART SOUNDS / RHYTHYM normal / RSA

LUNG SOUNDS normal UPPER AIRWAY normal

BLOOD WORK

PCV (%) 49 TP (g/dl) 72 BUN ..... C EAT .....

OTHER ....

....

HISTORY

PRESENT COMPLAINT .....

.....

.....

PRESENT MEDICATION/S

DRUG	DOSE RATE	DURATION

PREVIOUS ANAESTHESTICS

DATE COMMENTS (ie Drugs used, complications encountered etc)

....

....

....

Anaesthetic assessment | (Good) | Fair | Poor | Grave | Emergency |

PROCEDURE/S

1 accessory pancreatic duct ligation
2 main pancreatic duct cannulation
3 gastric aspiration
4 electrophysiology (bowel)
5 vascular cannulations
6 Secretin infusion
7 secretin bolusing

ANTICIPATED PROBLEMS / CONSIDERATIONS

1 hypo/hyperkensions
2 hypo/hyperthermia
3 small vessel bleeding
4 .....
5 .....

	DRUG	DOSE RATE (mg/kg)	DOSE (mg)	CONC. (mg/ml)	VOL (ml)	ROUTE	TIME	used (Calculated) DOSE	VOL
Pre-medication									
	diazepam	0.5	8.5	5	1.7	IV	8.34 am		
Induction	Thio	10	170	25	6.8	IV	8.34 am	125mg	5ml
	(drawn up: 8ml; give 5)								

	SIZE	SITE
IV CATHETER	18g 2"	cephalic vein

	ET. TUBE (size)	AGENT	CARRIER GAS	APPARATUS	
Maintenance	9mm (?)	halothane	Oxygen	circle	Humidifier ☐

	DRUG	DOSE RATE (mg/kg)	DOSE (mg)	CONC. (mg/ml)	VOL. (ml)	ROUTE	TIME	
	secretin	≈0.60μg in 0.9% saline			1	MV	12.20 pm	
Intra-op	"	with 0.5% BSA	"		"	PV	12.53 pm	T.A.T ☐
Medication	"	"	"	"	"	A	1.27 pm	AB. ☐
	"	"	"	"	"	3DMV	~2.07 pm	NSAID ☐
	"	"	"	"	"	PDV	2.29 pm	
	Pentobarb	125	2.1g in 3 500		4.2	IV	3.29 pm	

	TYPE	RATE (ml/kg/hr)	Route	VOL/ min (ml/min)	INFUSION (drips/sec)	TOTAL (ml)	
Fluids	0.9% saline	10	IV	2.8	≈ 7dr/15sec	MP3= 6.6	(MP3 = 5 with MP2 = 6)
0.02μg/ml	secretin	0.05μg/kg/hr	IV	0.71		MP2= 6	

LOCAL BLOCK Epidural ☐ Brachial Plex. ☐ Other ☐

MONITORING Blood Pressure Doppler ☐ Direct ☒ Oesoph. ☑
Pulse Ox. ☐ Capnograph ☐ Stethoscope
E.C.G ☐ Thermometer ☑

COMMENTS .....

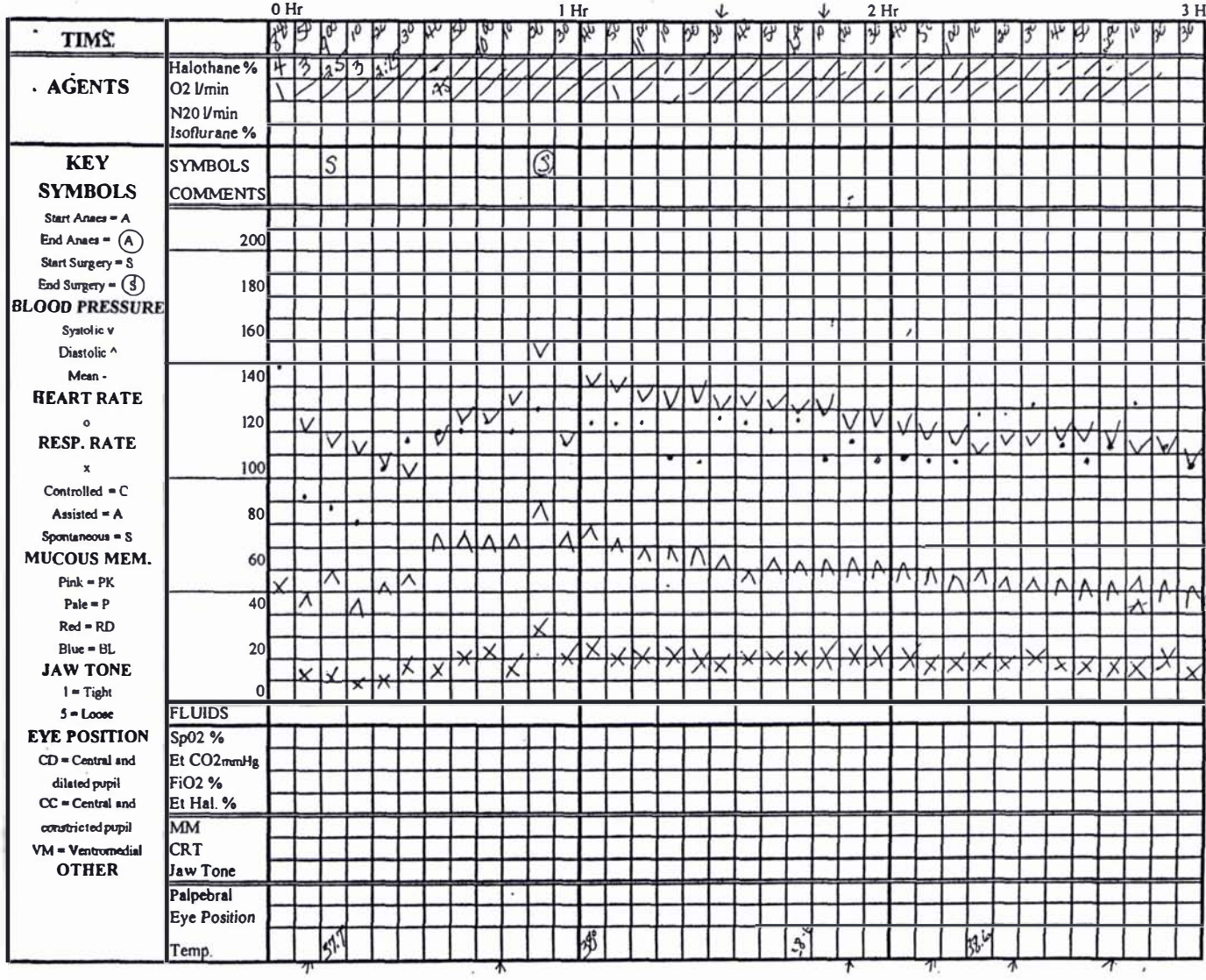
.....

COMPLICATIONS P.V. cannula not properly placed

.....

.....

.....



NORMAL PARAMETERS

HEART RATE

CATS

120 - 200 Bpm

DOGS

Sm. 100 - 180 Bpm

Lge. 60 - 120 Bpm

BLOOD PRESSURE

Systolic > 90

Mean > 70

RESP. RATE

CATS

12 - 20 Br/min

DOGS

Sm. 6 - 16 Br/min

Lge. 4 - 8 Br/min

SPO2

On 100% Oxygen

>95%

COMMENTS

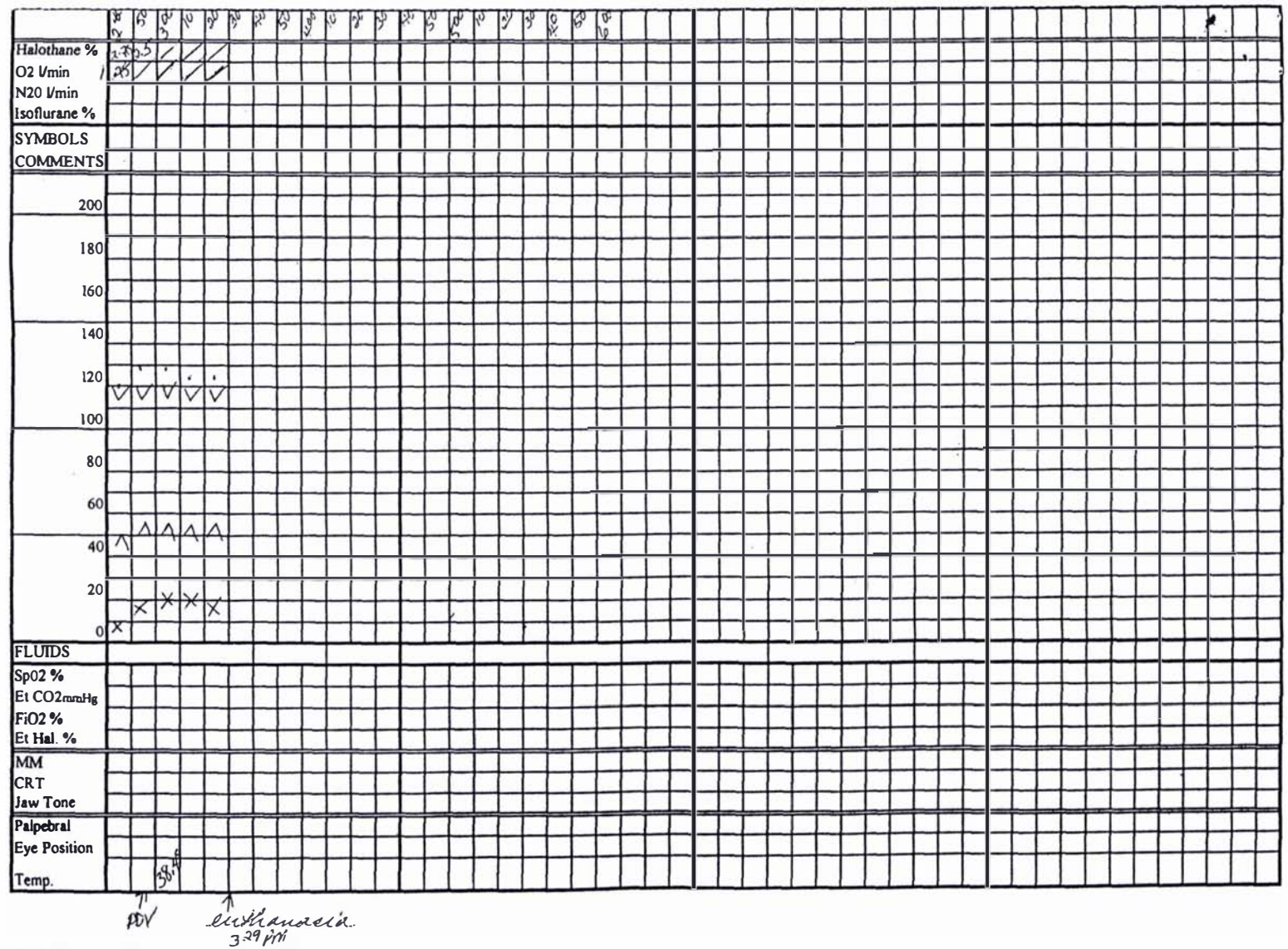
A 8:37am > 6hrs

(A) 3:29 > 55min

S 9:42am > ~1hr

(S) 10:42am > 20min

begin secretion infusion 10:09am


Halothane %
O2 l/min
N20 l/min
Isoflurane %
SYMBOLS
COMMENTS
200
180
160
140
120
100
80
60
40
20
0
FLUIDS
Sp02 %
Et CO2mmHg
FiO2 %
Et Hal. %
MM
CRT
Jaw Tone
Palpebral
Eye Position
Temp.
38.4
PDV
euthanasia
329 pm

CHECKLIST AND PROTOCOL: DOG #15 – CHAPTER 5

CHECKLIST AND PROTOCOL FOR EXPERIMENT I, DOG #15
(To be performed August 19, 1997)

Pre-experiment set-up:

1
- Obtain dog.
- Perform PE, PCV/TP, weigh (re-weigh day before if necessary).
- Inform Pauline/Jill about date/time of expt.
- Leave orders for feeding and need for dog to be walked on morning prior to expt.
- Fill out anaesthesia form. Calculate anaesthetic requirements, fluid and secretin infusion rates.

2
- Advise Jodi/Christine about date/time of expt.
- Leave list specifying equipment needed.
- Advise regarding when equipment is to be picked up.

3
- Decide on anaesthetic machine.
- Hook-up P&A oxygen cylinder.

4
- Make up secretin in 500 ml 0.9% saline with 0.5% BSA for infusion.
- Make up secretin boluses (0.035 µg/ g X 5).

5 Obtain equipment:
- Scrub-top, hair cover, coveralls, tennis shoes, socks, gum boots, keys
- Clip board with anaesthesia form, pen, watch, calculator
- Extension cord
- Heater(s) broken
- Thermometer
- Buckets for trash/cleaning equipment/large trash bag
- Hot water source
- Clippers/brush/cooling & cleaning spray
- Vacuum cleaner
- Prep (swabs, solutions)
- Sharps container
- 18 g 2-inch Teflon catheters
- 0.9% saline (1 L)
- Fluid administration set
- Tape (white)
- Drug labels
- Thio/diazepam (3 ml and 10-12 ml syringes)
- Endotracheal tube, lube, laryngoscope, tie, cuff inflator
- Anaesthetic machine
- Oxygen/halothane
- Oesophageal stethoscope
- Urinary catheter and collection bag (if male)
- Stomach tube (small fenestrated feeding tube, adaptor bubble tubing) and suction equipment
- Pads for prepping table, +/- small air bag(s)
- Kidney bowl with swabs and prep
- Heparinized saline
- 18 g needle
- Swabs
- 20 g 2-inch teflon catheters (BP)
- larger gauge tubing to fit femoral artery if n essary (BP)
- 3 ml syringe containing heparinized saline
- 3-way stopcock
- 'Super-glue'
- Tape (brown)
- Dissection equipment for femoral artery cannulation

- Arterial cannula
- Ties for cannulation (black)
- 3 ml syring containing heparinized saline
- 3-way stopcock(s)
- Pad, air bag(s), towel, bubble wrap, heating pad, towel
- Overhead light
- Infusion pumps and calibration graphs
- Stand for fluids
- Secretin infusate
- Fluid administration set
- Additional 18 g Teflon catheters
- Injection ports
- Blood pressure hook-up lines, transducer and stand, recording instrumentation
- 20 ml syringe
- Heparinized saline
- Exam gloves (medium)
- Surgical instrumentation
- Small forceps, etc
- Ties for cannulations (black)
- Small container or kidney bowl with sterile saline for soaking cannulae
- Cannulae (10 or more *vascular* cannulae), all same size, internal diameter and length with known volume (ID 0.50, OD 0.80; 50 cm contains 0.11 ml deadspace), and 25 g needles for vessel cannulations; main pancreatic duct cannula should be larger and longer (ID 0.97, OD 1.27; 48.4 cm cannula contains 0.63 ml deadspace), and requires 20 g needle
- Heparinized saline for flush
- Syringes (1 ml, 3 ml, 6 ml, 12 ml, 20 ml)
- Needles (variety of sizes)
- Injection ports
- Permanent black marker
- Drop counter equipment and recording instrumentation and stand
- Scale (or access to)
- Graduated cylinders (weighed) for measuring drop size; small aker or cup for collection
- 1 ml cups (labelled #1-#6) with lids for submitting pancreatic fluid samples to clin path for TP and amylase measurements
- Aluminium foil
- Ele trodes for measuring MMC and recording instrumentation
- Cello tape
- Secretin boluses (refrigerated as 1 ml boluses)
- Indian ink
- Pentobarbital, syringe and n edle
- Gurney
- Broom and mop
- T-pieces
- Small container for collecting small sample of stomach contents
- Radio

6 Set up equipment:

Day before:

- Make-up 0.5% BSA in 500 ml saline, label and refrigerate; make up additional 10 ml 0.5% BSA in saline for flushing boluses; cover, label and refrigerate.
- Draw up 0.5% BSA in 0.9% saline in 5 separate 1 ml syringes, label and refrigerate; check and organize for diluting secretin aliquots on morning of experiment.
- Obtain 0.9% saline (1L) for maintenance fluids; hook-up administration set, label and place in bucket or refrigerator.
- Che k heparinized saline stock; make-up additional (5 IU/ml) if necessary; label and refrigerate.
- Know weights of graduated cylinders.
- Collect and set-up instrumentation for monitoring (BP, pancreatic juice, MMC) - BG. enough paper for chart r order?

Calibrate infusion pumps; set-up in surgery room; bring graphs - BG.
Arrange tables for prepping and surgery.
Hook-up clippers and vacuum cleaner; check their operation.
Check oxygen and halothane levels.
Set-up anaesthetic drugs, catheters, tape, machine (O_2), endotracheal tube & accessory equipment, etc.
Set up tables, bubble wrap, towels, heating pads, overhead light, heater, suction equipment.
Set-up kidney bowl with swabs and prep near-by.
Place cannulae in dish.
Ensure that injection ports are labelled.
Check surgery equipment.

Morning of:

Turn on scavenge.
Turn on heating pad, overhead light and heater.
Turn on oxygen; pressure check anaesthetic machine.
Place maintenance fluids in hot water bath.
Half fill a bucket with cold water for soaking equipment after use.
Check operation of laryngoscope and ET tube cuff.
Place jelly on ET tube.
Prepare prep (place solutions on swabs in kidney bowl).
Go to 5th floor: thaw one 10 µg aliquot of secretin; dispense 0.035 µg secretin/kg (0.60 µg) into each of the 5 1-ml syringes containing 0.5% BSA in 0.9% saline (made-up day before); re-label and re-freeze remaining secretin (~7.0 µg). Thaw a second 10 µg aliquot and add all 10 µg to the 500 ml bag 0.9% saline with 0.5% BSA for infusion; take labelled secretin boluses, secretin infusate and extra 0.5% BSA solution downstairs. (slightly modified (4221 calibrate sheet)
Attach fluid administration set to secretin infusate and place this as well as the secretin boluses and additional BSA in refrigerator.
Take thio (from refrigerator) to surgery; draw up calculated amount into labelled syringe.
Take heparinized saline (from refrigerator) to surgery.
Fill up 3 ml syringe with heparinized saline for arterial line; attach stopcock.
Fill up 20 ml syringe with heparinized saline for attachment to transducer.
Soak cannulae and ties in sterile saline.

Experimental Procedures:

1 Collect dog.

2 <u>Anaesthesia</u>
Remove IV fluids from hot water bath and hang.
Clip, swab and place IV catheter in cephalic vein.
Attach fluid line (gravity flow - 10 ml/kg/hr).
Tape catheter and IV line in place.
Administer diazepam followed by thio into catheter.
Intubate.
Hook up to anaesthetic machine (O_2 flow rate 30 ml/kg to start; decrease to 10 ml/kg)
Monitor and record vitals on anaesthetic chart q 10 minutes.
Place dog in dorsal recumbency on pads.
Place oesophageal stethoscope.
Pass oro-gastric feeding tube for suction.
Clip abdomen and over both superficial dorsal metatarsal veins and over both pedal and femoral arteries.
Vacuum hair.
Begin prepping all areas (care with alcohol).
Cannulate pedal artery or femoral artery if necessary (BP monitoring).
Move to surgery table.

3 Surgery
Position.
Take body temperature.
Extend leg/legs and tie down; hook-up maintenance fluids to infusion pump and set desired flow rate.
Wrap snuggly and tape bubble wrap around head and torso.
Hook-up BP.
Place 18 g IV catheter in a superficial dorsal metatarsal vein; flush. Cap with labelled injection port (SDMV).
Hook-up suction to stomach tube.
Measure length of cannula needed for aortic cannulation (mark); flush with heparinized saline.
Cut down and place arterial line in femoral artery (A line); thread cannula to level of diaphragm; flush and place labelled injection port (A) or stopcock on end.
Make ventral midline incision.
Verify placement of stomach tube.
Identify and ligate accessory pancreatic duct.
Identify and cannulate main pancreatic duct (with larger cannula-see eqpt list); place clear injection port on end or hook up to drop counter.
Identify pancreaticoduodenal vein; cannulate, flush and place labelled injection port (PDV) on end.
Hook-up drop counter; standardize level to BP transducer.
Turn off saline; set secretin infusion flow rate to 5-10 ml/kg/hr until a pancreatic response is obtained; then decrease flow rate to 1/4 of this and adjust as necessary to obtain and maintain a baseline of 3-4 drops per minute of pancreatic juice.
Identify mesenteric vein (jejunal vein); cannulate and advance to portal vein, flush and place labelled injection port (PV) on end.
Identify second mesenteric vein (jejunal vein), cannulate, flush and place labelled injection port (MV) on end.
Place electrodes in gastric antrum and duodenum. Hook up to physiograph. Turn off heating pad if necessary to obtain adequate tracing (turn heating pad on again if dog becomes hypothermic).
Carefully return viscera to abdomen, pack gently being sure to leave cannulae exteriorized.
Wrap underlying bubble wrap around dog and tape closed (monitor body temperature); make holes if necessary to exteriorize cannulae.
Flush all cannulae with heparinized saline frequently.
Place 16 g needle into injection port of SDMV and thread cannula through needle; flush and cap.
Flush all vascular cannulae with heparinized saline.
Leave system to stabilize for one hour.

3 Experiment:
Randomize order of secretin bolusing into various sites; for dog #15:
MV
PV
A
SDMV
PDV
Determine number of drops/ml pancreatic fluid during baseline. Weigh and record. Repeat for each test. Place sample into labelled 1-ml lidded cups.
Flush each cannulae with heparinized saline regularly throughout experimental period.
Set time scale and chart recorder to desired settings (increase paper speed to 50 mm/sec for pre-injection baseline 5 minute period and bolus testing). LABEL all settings on paper chart.
Administer secretin boluses into vascular cannulae over 5 second period; START time is when ½ of bolus has been administered (mark on chart recorder and record time of injection).
Flush immediately with 0.2 ml 0.5% BSA in 0.9% saline.
Flush with small volume of heparinized saline.
Observe pancreatic effect on chart recorder; allow to return to baseline for 10-15 minutes.
Take freshly collected sample of gastric contents to 5th floor for pH measurement.
Inject small quantity of indian ink into PDV while observing pancreas for discoloration.

4 Euthanase dog with pentobarbital.
Remove instruments.

Disconnect equipment.
Remove arterial line and tape.
Remove endotracheal tube.
Remove stomach tube.
Place dog on gurney and cover.
Take to post mortem room.

5 Inject indian ink into PDV; observe pancreas for discoloration. Cut into pancreas and observe further.
Verify placement and remove all cannulae.
Dispose of dog.

6 Soak, then flush cannulae.
Wash syringes, small instruments, etc. Place on paper towels to drain.
Consolidate all equipment onto carts and remove from surgery theatre.
Clean up surgery theatre.
Sweep and mop floor in surgery theatre if necessary.
Dispose of trash.
Return gurney, laundry items and instruments to surgery. Place instruments in warm water to soak.
Turn off scavenge.
Submit pancreatic juice samples to clin path (6 samples; place in refrigerator if late in day).

7 Collect recorded information and anaesthesia chart.
Complete anaesthesia record.
Fold up chart and attach to anaesthesia record.
Record equipment used (anaesthesia and surgery).
Fill in diary.

18/8/97

. Secretin Infusion and Bolus Calculations wt = 17 kg

1. BSH
   - Add 2.5gms BSH to 500 ml 0.9% saline; label and refrigerate.
   - Make up an additional 10 ml by adding 50 mg (0.05gm) BSH to 10 ml saline; from this, draw up 9 x 0.2 ml of the BSH solution into each of 5 1-ml syringes; make up labels; cap syringes, cover remaining BSH and refrigerate all.

2. Secretin
   - Aliquots of 100µg vial secretin in following manner:
     1. Add 1000 µl distilled water to vial; vortex (100µg in 1000µl = 0.1µg/µl)
     2. Obtain 12 1-ml cups with lids; Place "510" on 6 of the cups plus the reconstituted vial; write "55" on the remaining 6 vials.
     3. Place 100µl into each of the 6 cups marked "510" (10µg in 100µl) Place 50µl into each of the 6 cups marked "55" (5µg in 50µl)
   - Boluses

     To one of the "55" aliquots, add 150µl BSH solution to make final concentration of 5µg in 200µl or 0.025µg/µl.
     Add 0.60µg (23.8µl) to each of the 1ml syringes containing BSH in saline; cap, label and refrigerate

     vortex

     Make up label for remaining secretin:

     Secretin
     "2µg in" 8µl
     18/8/97
     1 thaw

   - Infusion

     Add the remaining 10µg in the original reconstituted vial to the 500ml bag 0.9% saline with 0.5% BSH to make final concentration of 10µg in 500ml or 0.02µg/ml. Label, attach fluid administration set and refrigerate.

CLINICAL PATHOLOGY REPORT
DEPARTMENT OF VETERINARY PATHOLOGY
AND PUBLIC HEALTH
MASSEY UNIVERSITY
Phone (06) 3569099 Extn 7409 Fax (06) 3502270

Date: 20/8/97
Case No:
Lab No: R566

Owner's Name: Maureen Wichtel		**Animal's ID:**	
Species: Canine	**Breed:**	**Age:**	**Sex:**
Specimens: Pancreatic Juice		**Veterinarian:** M Wichtel	

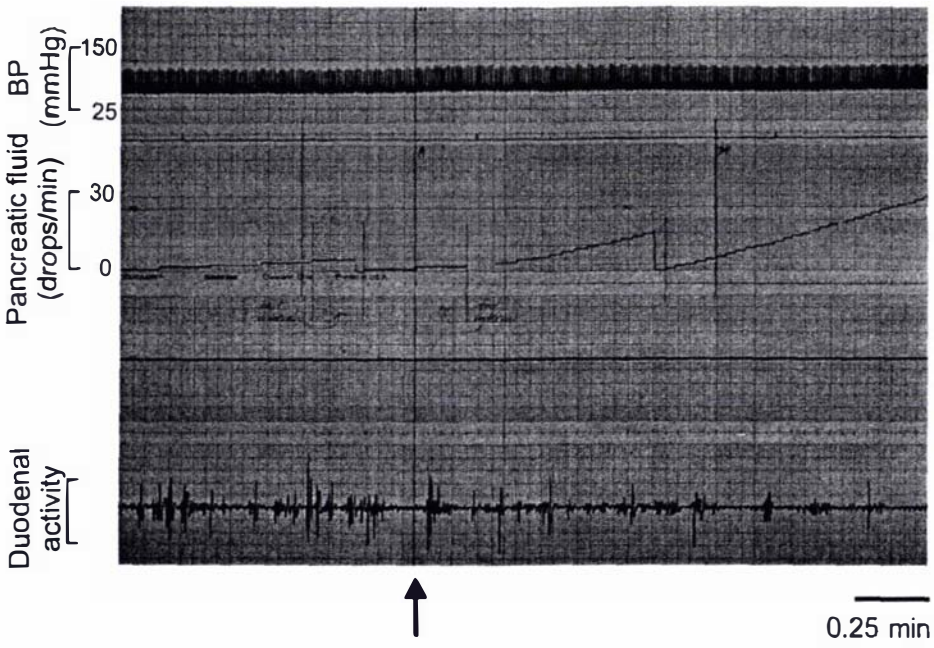
	Total Protein g/L
#1	1.1
#2	2.0
#3	2.2
#4	2.0
#5	1.6
#6	2.2

Comments:

Signed: ..... Date: 20/8/97

PHYSIOLOGICAL DATA OUTPUT – CHAPTER 5

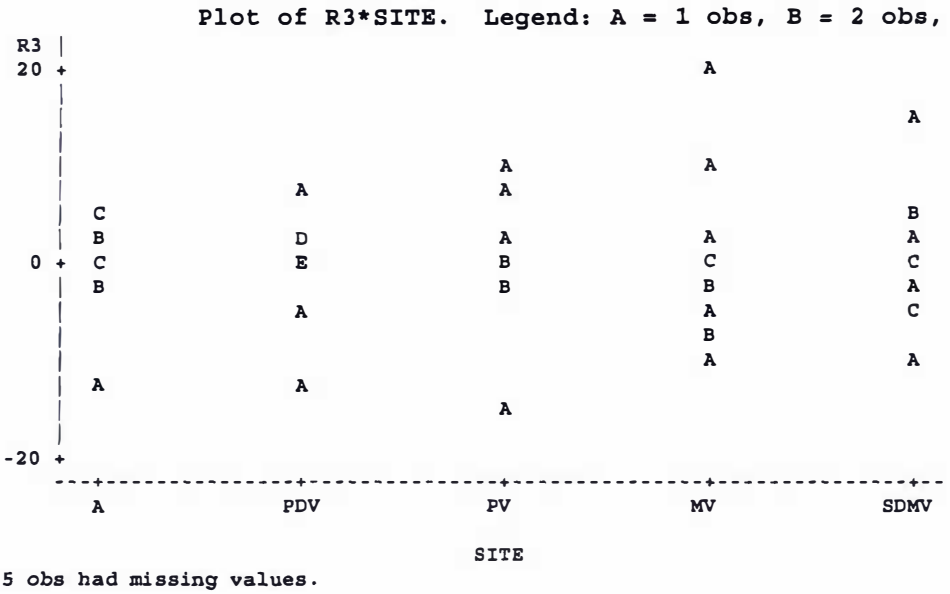
This is an example of physiological data output recorded using a JRAK module system and a four channel chart recorder. **BP** blood pressure; **Arrow** time at which secretin bolus was administered. Myoelectrical activity is shown for the duodenum site only.



RESIDUAL ANALYSES - CHAPTER 5

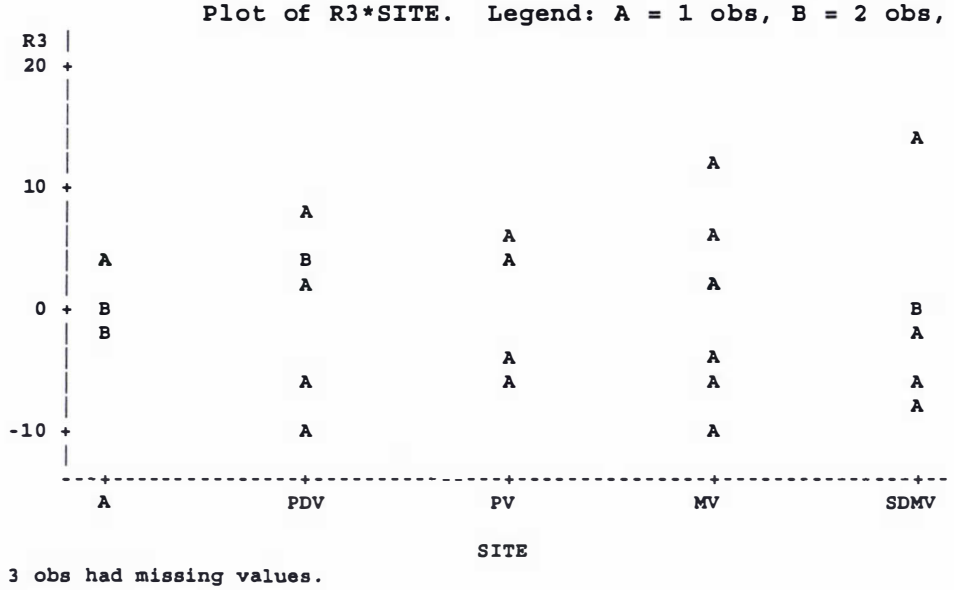
Residual analyses are used to confirm the appropriateness of the statistical model. If there was a systematic bias towards variability at any given site, this would be reflected by a distribution of residuals offset from zero. **Final Model** main effects model; **Cannula 1** small cannula; **Cannula 2** large cannula **A aorta; MV mesenteric vein; PDV cranial pancreaticoduodenal vein; PV portal vein; R3** residual; **SDMV superficial dorsal metatarsal vein**

Residual Analysis: Final Model Partially Nested Design



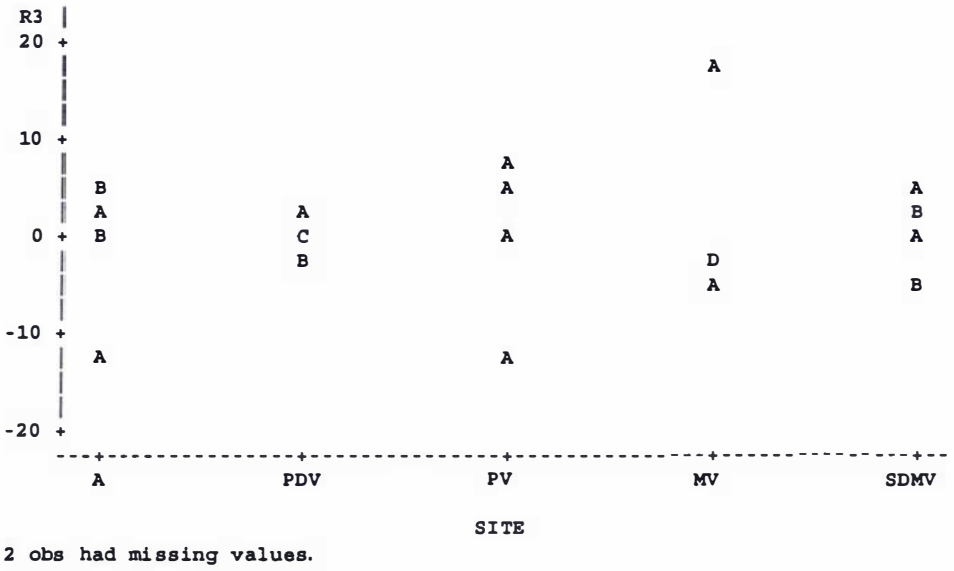
NOTE: 5 obs had missing values.

Residual Analysis: Cannula 1 Only



NOTE: 3 obs had missing values.

Residual Analysis: Cannula 2 Only



NOTE: 2 obs had missing values.

Appendix 8

MEANS FOR LATENCY OF RESPONSE – CHAPTER 5

Output from SAS showing means for latency of response at each injection site are presented below, first for the effect of site alone (main effects model), and second for the effect of site blocked by cannula size (interaction model). For each site, the least squares mean is provided along with its standard error. Also tabulated are the p values for the differences between any two means. **RTIME3** response time in seconds; **Block 1** small cannula; **Block 2** large cannula. **A aorta; MV mesenteric vein; PDV cranial pancreaticoduodenal vein; PV portal vein; SDMV superficial dorsal metatarsal vein**

Main Effects Model

SITE	RTIME3 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
A	19.6944658	2.2683758	0.0001	1
PDV	38.0616667	2.1492166	0.0001	2
PV	33.2085043	2.7412250	0.0001	3
MV	41.0883333	2.1492166	0.0001	4
SDMV	38.1750000	2.1492166	0.0001	5

i/j	1	2	3	4	5
1	.	0.0001	0.0005	0.0001	0.0001
2	0.0001	.	0.1714	0.3255	0.9704
3	0.0005	0.1714	.	0.0293	0.1619
4	0.0001	0.3255	0.0293	.	0.3437
5	0.0001	0.9704	0.1619	0.3437	.

Interaction Model

BLOCK	SITE	RTIME3 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
1	A	22.2712264	3.4190539	0.0001	1
1	PDV	39.9166667	3.0561732	0.0001	2
1	PV	40.2004717	3.9005809	0.0001	3
1	MV	41.7500000	3.0561732	0.0001	4
1	SDMV	39.9583333	3.0561732	0.0001	5
2	A	17.0766667	3.0561732	0.0001	6
2	PDV	36.2066667	3.0561732	0.0001	7
2	PV	26.2216667	3.8958717	0.0001	8
2	MV	40.4266667	3.0561732	0.0001	9
2	SDMV	36.3916667	3.0561732	0.0001	10

i/j	1	2	3	4	5	6	7	8	9	10
1	.	0.0005	0.0016	0.0002	0.0005	0.2650	0.0045	0.4511	0.0004	0.0040
2	0.0005	.	0.9547	0.6740	0.9924	0.0001	0.3965	0.0090	0.9067	0.4203
3	0.0016	0.9547	.	0.7564	0.9613	0.0001	0.4257	0.0158	0.9639	0.4473
4	0.0002	0.6740	0.7564	.	0.6810	0.0001	0.2081	0.0035	0.7613	0.2233
5	0.0005	0.9924	0.9613	0.6810	.	0.0001	0.3913	0.0088	0.9143	0.4148
6	0.2650	0.0001	0.0001	0.0001	0.0001	.	0.0001	0.0732	0.0001	0.0001
7	0.0045	0.3965	0.4257	0.2081	0.3913	0.0001	.	0.0515	0.3356	0.9661
8	0.4511	0.0090	0.0158	0.0035	0.0088	0.0732	0.0515	.	0.0069	0.0475
9	0.0004	0.9067	0.9639	0.7613	0.9143	0.0001	0.3356	0.0069	.	0.3569
10	0.0040	0.4203	0.4473	0.2233	0.4148	0.0001	0.9661	0.0475	0.3569	.

COMPLETE ANAESTHETIC RECORD: DOG #8 – CHAPTER 6

MASSEY UNIVERSITY ANAESTHETIC RECORD

PATIENT DETAILS

DATE (of procedure) 3/11/97 OWNER'S NAME .....

CASE NUMBER Dog #8 ANIMAL'S NAME Bruce

WARD/CAGE/BOX ..... ANAESTHETIST ..... SUPERVISOR .....

SPECIES canine BREED pig dog COLOUR brown/black

AGE yrs adult SEX M WEIGHT kg 20.5 kg CONDITION very good

TEMPERAMENT bouncy dog FASTED (YES) NO

ATTITUDE very good

PHYSICAL EXAM

HR (bpm) 120 RR 20 TEMPERATURE 38.9°C

MM pink CRT fast HYDRATION normal

PULSE RATE/QUALITY 120/strong

HEART SOUNDS / RHYTHYM normal/normal

LUNG SOUNDS normal UPPER AIRWAY normal

BLOOD WORK

PCV (%) 51 TP (g/dl) 73 BUN ..... CREAT .....

OTHER ....

....

HISTORY

PRESENT COMPLAINT .....

.....

.....

PRESENT MEDICATION/S

DRUG	DOSE RATE	DURATION

PREVIOUS ANAESTHESTICS

DATE COMMENTS (ie Drugs used, complications encountered etc)

.....

.....

.....

Anaesthetic assessment | (Good) | Fair | Poor | Grave | Emergency

PROCEDURE/S SURGEON ANTICIPATED PROBLEMS / CONSIDERATIONS

1 accessory pancreatic duct ligation ..... hypo / hyperthermia .....

2 main pancreatic duct cannulation ..... hypo / hypertension .....

3 multiple vessel cannulation ..... small vessel oozing / bleeding

4 gastric aspiration ..... 4 .....

5 secretin infusion ..... 5 .....

6 secretin bolusing

7 PV threshold testing

wt = 20.5 kg

	DRUG	DOSE RATE (mg/kg)	DOSE (mg)	CONC. (mg/ml)	VOL (ml)	ROUTE	TIME	(Actual Given) DOSE	VOL.
Pre-medication									
	diazepam	0.5	10.25	5	2.05	IV		all	
Induction	thio	10	205	25	8.2	IV		198 mg / 200	8 ml

	SIZE	SITE
IV CATHETER	18g 2"	cephalic vein

ET. TUBE (size)	AGENT	CARRIER GAS	APPARATUS	
11 mm	halothane	oxygen	circle	
Maintenance				Humidifier ☐

	DRUG	DOSE RATE ~~(mg/kg)~~ µg	DOSE (mg)	CONC. (mg/ml)	VOL. (ml)	ROUTE	TIME	
	secretin	0.005	0.025	µg in	1 ml	A	2:00 pm	(low dose) CMV
Intra-op	0.9% saline with 0.5% BSA					CMV	2:21 pm	T.A.T ☐ A
Medication	"	"	"	"	"	SDMV	2:52 pm	AB. ☐ CaVC
	"	"	"	"	"	CaVC	3:12 pm	NSAID ☐ SDMV
	"	"	"	"	"	IV	3:37 pm	Lacrilube ☐
	pentobarb	125mg/kg	2.6gms	500mg/ml	5.1	IV	7:53 pm	

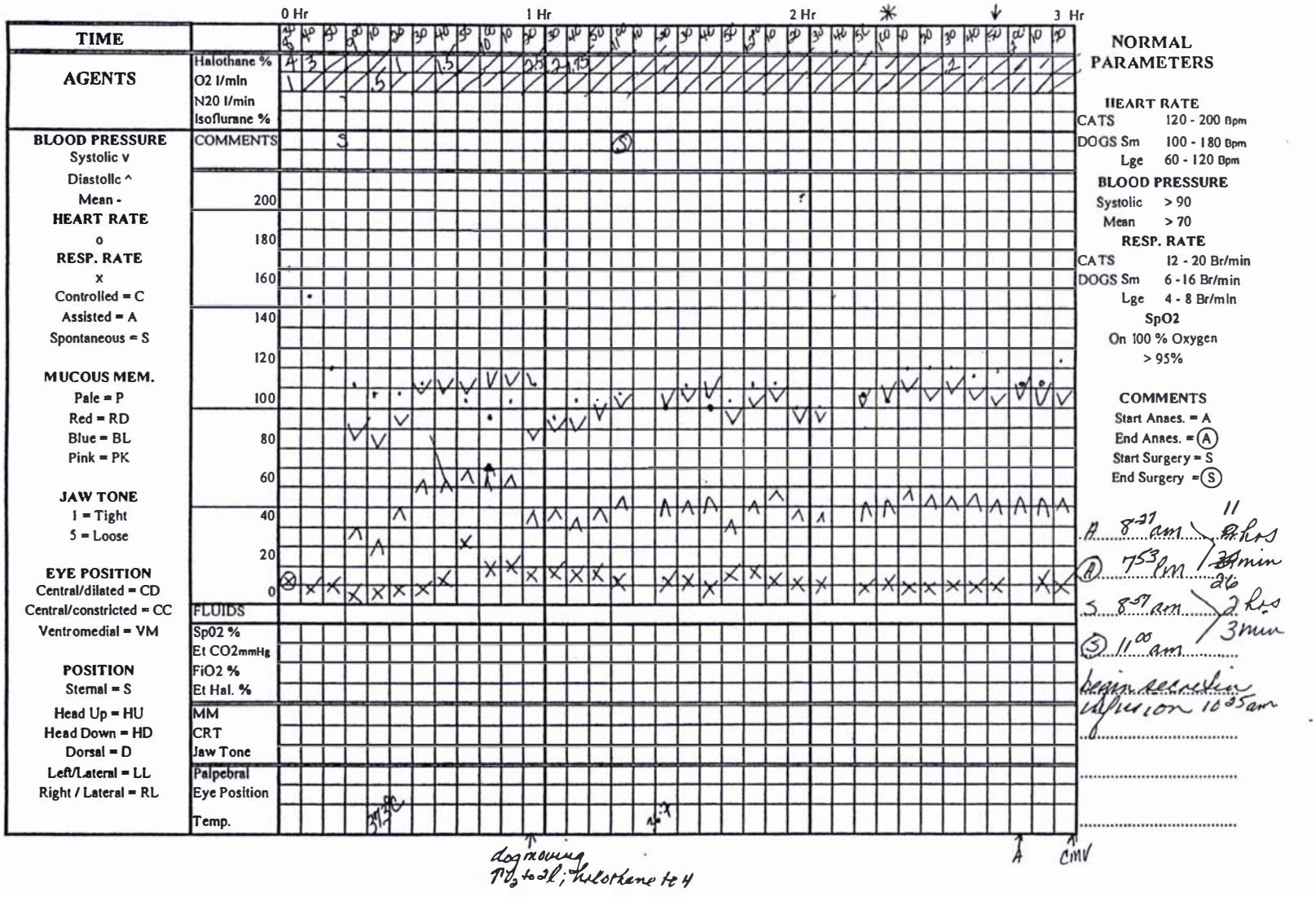
	TYPE	RATE (ml/kg/hr)	Route	VOL./ min (ml/min)	INFUSION (drips/sec)	TOTAL (ml)	
	0.9% saline	205	IV	3.4	8.5 d/15/sec	MP3 = 8.1	Syringe ☐
0.02µg/ml	secretin	0.05 µg/kg/hr	IV	0.85		MP2 = 7.3	Drip Sets ☑

MP3 = 6 when MP2 = 7.3
MP3 = ~~7~~ 3.9 when MP2 = 14.6

LOCAL BLOCK		Epidural	☐	Brachial Plex.	☐	Other	☐
MONITORING	Blood Pressure	Doppler	☑	Direct	☐	Oesoph.	☑
		Pulse Ox.	☐	Capnograph	☐	Stethoscope	
		E.C.G	☐	Thermometer	☑	Fluid Pumps	☑

COMMENTS .....

COMPLICATIONS difficulty cannulating cephalic vein; multiple attempts necessary to get pancreatic juice to flow properly (much surgical manipulations & re-cannulation); very long anaesthesia time; early hypotension + hypothermia (both recovered); pale xerosanguinous pancreatic fluid; small vessel bleeding/oozing, especially from skin and around pancreatic duct.



NORMAL PARAMETERS

HEART RATE

CATS	120 - 200 Bpm
DOGS Sm	100 - 180 Bpm
Lge	60 - 120 Bpm

BLOOD PRESSURE

Systolic	> 90
Mean	> 70

RESP. RATE

CATS	12 - 20 Br/min
DOGS Sm	6 - 16 Br/min
Lge	4 - 8 Br/min

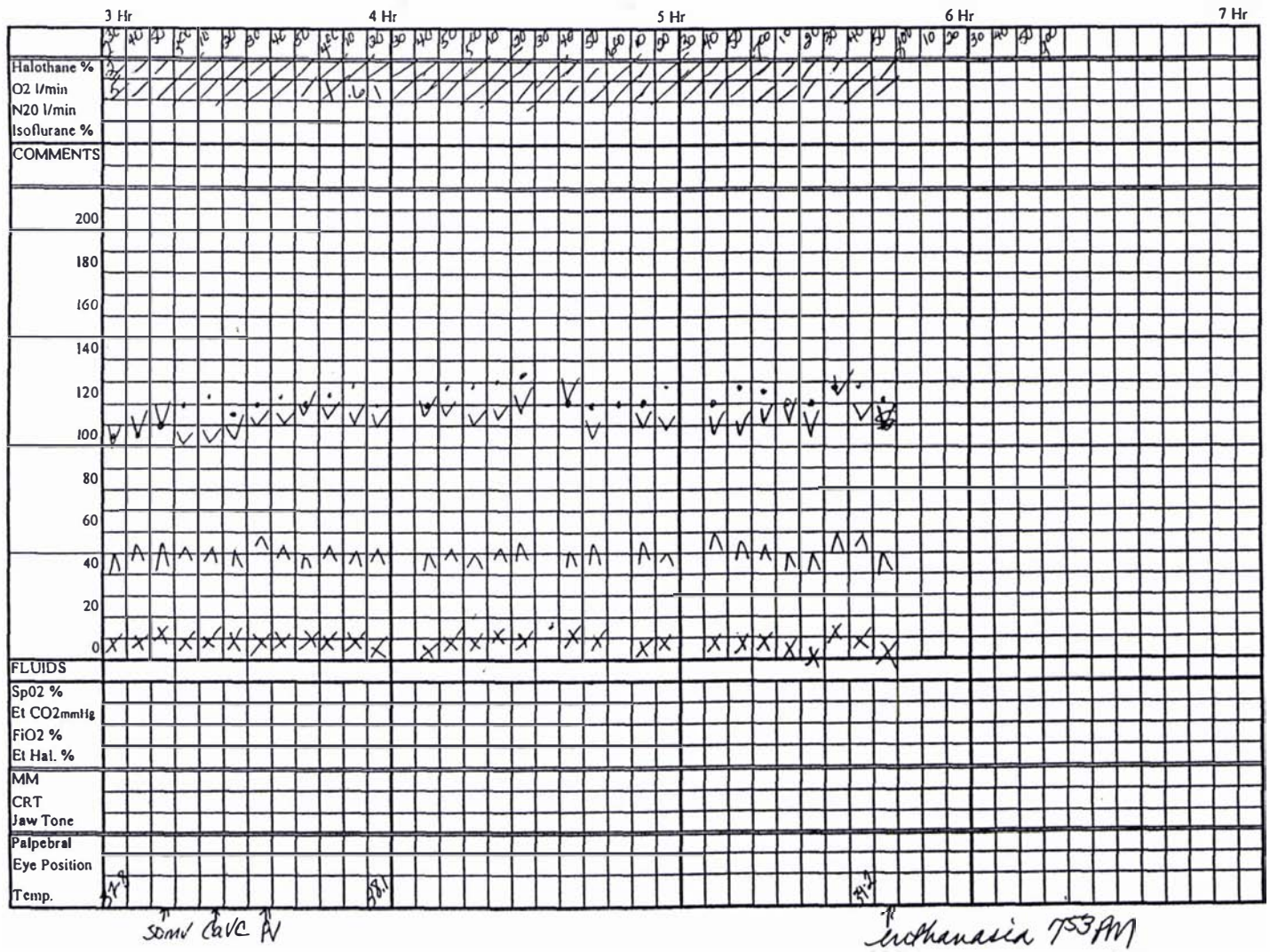
SpO2

On 100 % Oxygen
> 95%

COMMENTS

Start Anaes. = A
End Anaes. = (A)
Start Surgery = S
End Surgery = (S)

A 8.27 am
(A) 7.53 pm
S 8.57 am
(S) 11.00 am
begin resection
infusion 10.25 am


3 Hr
4 Hr
5 Hr
6 Hr
7 Hr
Halothane %
O2 l/min
N20 l/min
Isoflurane %
COMMENTS
200
180
160
140
120
100
80
60
40
20
0
FLUIDS
Sp02 %
Et CO2mmHg
FiO2 %
Et Hal. %
MM
CRT
Jaw Tone
Palpebral
Eye Position
Temp.
Euthanasia 753 AM

Appendix 10

CHECKLIST AND PROTOCOL: DOG #8 – CHAPTER 6

CHECKLIST AND PROTOCOL FOR EXPERIMENT 2, DOG #8
(To be performed November 3, 1997)

Pre-experiment set-up:

1
- Obtain dog.
- Perform PE, PCV/TP, weigh (re-weigh day before if necessary).
- Inform Pauline/Jill about date/time of expt.
- Leave orders for feeding and need for dog to be walked on morning prior to expt.
- Fill out anaesthesia form. Calculate anaesthetic requirements, fluid and secretin infusion rates and bolus requirements.

2
- Advise Jodi/Christine about date/time of expt.
- Leave list specifying equipment needed.
- Advise regarding when equipment is to be picked up.

3
- De ide on anaesthetic machine.
- Hook-up P&A oxygen cylinder.

5 Obtain equipment:
- Scrub-top, hair cover, coveralls, tennis shoes, socks, gum boots, keys
- Clip board with anaesthesia form, pen, watch, calculator, ruler
- Extension cord
- Thermometer
- Buckets for trash/cleaning equipment/large trash bag
- Hot water source
- Clippers/brush/cooling & cleaning spray
- Vacuum cleaner
- Prep (swabs, solutions)
- Sharps container
- 18 g 2-inch Teflon catheters
- 0.9% saline (1 L and 0.5 L)
- Fluid administrat on set
- Tape (white)
- Drug labels
- Thio/diazepam (3 ml and 10-12 ml syringes)
- Endotracheal tube, laryngoscope, tie, cuff inflator
- Anaesthetic machine
- Oxygen/halothane
- Oesophageal stethoscope
- Urinary catheter and collection bag (if male)
- Stomach tube (small fenestrated feeding tube, adaptor bubble tubing) and suction equipment
- Pads for prepping table, +/- small air bag(s)
- Kidney bowl wit swabs and prep
- Heparinized saline
- 18 g needle
- Swabs
- 20 g 2-inch teflon catheters (BP)
- larger gauge tubing to fit femo al artery if necessary (BP)
- 3 ml syringe containing heparinized saline
- 3-way sto ock
- 'Super-glue'
- Ta e (brown)
- Permanent black marking pen
- Arterial cannula
- Caudal vena cava (CaVC) cannula
- Ties for cannulation (black)

- 3 ml syring containing heparinized saline
- 3-way stopcock(s)
- Pad, air bag(s), towel, bubble wrap, heating pad, towel
- Overhead light
- Infusion pumps and calibration graphs
- Stand for fluids
- Secretin infusate
- Fluid administration set
- Additional 18 g Teflon catheters
- Injection ports
- Blood pressure hook-up lines, transducer and stand, recording instrumentation
- 20 ml syringe
- Heparinized saline
- Exam gloves (medium)
- Surgical instrumentation
- Small forceps, clamps, etc
- Small container or kidney bowl with sterile saline for soaking cannulae
- Cannulae (10 or more *vascular* cannulae), all same size, internal diameter and length with known volume (ID 0.58, OD 0.80; 50 cm contains 0.11 ml deadspace), and 25 g needles for vessel cannulations; main pancreatic duct cannula should be larger and longer (ID 0.97, OD 1.27; 48.4 cm cannula contains 0.63 ml deadspace), and requires 20 g needle
- Heparinized saline for flush
- Syringes (1 ml, 3 ml, 6 ml, 12 ml, 20 ml)
- Needles (variety of sizes)
- Drop counter equipment and recording instrumentation and stand
- Small beaker or cup for collection of pancreatic fluid
- Cello tape
- Chilly bin and access to ice
- Secretin boluses and additional 0.5% BSA
- 10 and 20 ml lidded cups/jars, labelled; each containing (pre-measured) 10 and 20 ml 0.5% BSA
- Hamilton syringe (100 µl)
- Distilled water for rinsing Hamilton syringes
- Several 1 ml syringes and needles to cap boluses (15 minimum)
- Labels
- Pentobarbital, syringe and needle
- Gurney
- Broom and mop
- Small container for collecting small sample of stomach contents
- Ruler
- Radio

6 Set up equipment:

Day before:

- Make-up 0.5% BSA in 500 ml saline, label and refrigerate; make up additional 55 ml 0.5% BSA in saline for additional boluses and for flushing.
- Draw up 0.5% BSA in 0.9% saline in 15 separate 1-ml syringes, label and refrigerate.
- Place 10,000 and 20,000 µl 0.5% BSA into each of 2 labelled lidded cups/jars; cover, label and refrigerate all.
- Check and organize for diluting secretin aliquots on morning of experiment.
- Obtain 0.9% saline (1L) for maintenance fluids; hook-up administration set (use secretin infusion administration set for maintenance fluids on second experimental day; discard maintenance fluid administration set from previous experiment), label and place in bucket or refrigerator.
- Check heparinized saline stock; make-up additional (5 IU/ml) if necessary; label and refrigerate.
- Collect and set-up instrumentation for monitoring (BP, pancreatic juice, MMC) - BG. enough paper for chart recorder?
- Calibrate infusion pumps; set-up in surgery room; firing graphs - BG.
- Arrange tables for prepping and surgery.

- Hook-up clippers and vacuum cleaner; check their operation.
- Check oxygen and halothane levels.
- Set-up anaesthetic drugs, catheters, tape, machine (O_2), endotracheal tube & accessory equipment, etc.
- Set up tables, bubble wrap, towels, heating pads, overhead light, heater, suction equipment.
- Set-up kidney bowl with swabs and prep near-by.
- Place cannulae in dish.
- Ensure that injection ports are labelled.
- Check surgery equipment.

Morning of:

- Turn on scavenge.
- Turn on heating pad
- Turn on oxygen; pressure check anaesthetic machine.
- Place maintenance fluids in hot water bath.
- Half fill a bucket with cold water for soaking equipment after use.
- Check operation of laryngoscope and ET tube cuff.
- Prepare prep (place solutions on swabs in kidney bowl).
- Take chilly bin to 7th floor; fill with ice.
- Go to 5th floor: Thaw one 10 µg aliquot secretin and add all 10 µg to the 500 ml bag 0.9% saline with 0.5% BSA for infusion; attach administration set and place in chilly bin or refrigerator.
- Thaw one 5 µg aliquot of secretin; dispense 0.005 µg secretin/kg (0.10 µg) into each of the 5 1-ml syringes containing 0.5% BSA in 0.9% saline (made-up day before); note remaining contents; place remainder in chilly bin on ice.
- Place labelled secretin boluses, additional 1-ml syringes containing BSA, lidded cups/jars containing BSA (2), extra 0.5% BSA and distilled water (+/- secretin infusate) in chilly bin with remaining secretin (aliquot).
- Collect 100 µl Hamilton syringe.
- Take chilly bin and Hamilton syringe downstairs to surgery.
- Take thio (from refrigerator) to surgery; draw up calculat d amount into labelled syringe.
- Take heparinized saline (from refrigerator) to surgery.
- Fill up 1 ml syringe with heparinized saline for flushing cannulae.
- Fill up 3 ml syringe with heparinized saline for arterial line; attach stopcock.
- Fill up 20 ml syringe with heparinized saline for attachment to transducer.
- Soak cannulae and ties in sterile saline.

Experimental Procedures:

1 Collect dog.

2 <u>Anaesthesia</u>
Remove IV fluids from hot water bath and hang.
Clip, swab and place IV catheter in cephalic vein.
Attach fluid line (gravity flow - 10 ml/kg/hr).
Tape catheter and IV line in place.
Administer diazepam followed by thio into catheter.
Intubate.
Hook up to anaesthetic machine (O_2 flow rate 30 ml/kg to start; decrease to 10 ml/kg).
Monitor and record vitals on anaesthetic chart q 10 minutes.
Place dog in dorsal recumbency on pads.
Place oesophageal stethoscope.
Pass oro-gastric feeding tube for suction.
Clip abdomen and over both superficial dorsal metatarsal veins and over both pedal and femoral arteries.
Vacuum hair.
Begin prepping all areas (care with alcohol).
Cannulate pedal artery or femoral artery if necessary (BP monitoring).

Move to surgery table.

3 Surgery

Position.
Take body temperature.
Extend leg/legs and tie down; hook-up maintenance fluids to infusion pump and set desired flow rate.
Wrap snuggly and tape bubble wrap around head and torso.
Hook-up BP.
Hook-up suction to stomach tube.
Measure length of cannula needed for aortic and caudal vena cava cannulations (mark); flush with heparinized saline.
Flush arterial cannula with heparinized saline. Cut down and place arterial line in femoral artery (A line); thread cannula to level of xyphoid (should place tip in aorta just cranial to diaphragm); flush to verify patency and place labelled injection port (A) or stopcock on end.
Flush venous line with heparinized saline. Place cannula in femoral vein; thread to level of xiphoid (should place tip in caudal vena cava at level of liver); flush again to verify patency and place labelled injection port (CaVC) on end.
Make ventral midline incision.
Verify placement of stomach tube.
Try to palpate/visualize portal vein and caudal vena cava.
Identify and ligate accessory pancreatic duct.
Flush pancreatic duct cannula with saline. Identify and cannulate main pancreatic duct (with larger cannula - see eqpt list).
Set-up drop counter; standardize level to BP transducer; hook-up pancreatic duct cannula once pancreatic juice is flowing freely.
Turn off saline; set secretin infusion flow rate to 5-10 ml/kg/hr until a pancreatic response is obtained; then decrease flow rate as necessary to obtain and maintain a baseline pancreatic juice flow of 2-4 drops per minute.
Flush PV cannula with heparinized saline. Identify mesenteric vein (jejunal vein); cannulate and advance cannula to xiphoid (should place tip in the portal vein inside of theliver); flush and place labelled injection port (PV) on end; palpate PV and CaVC to verify position .
Flush cranial mesenteric vein (CMV) cannula. Identify second mesenteric vein (jejunal vein); cannulate and advance to a point midway between the site of entry and the xiphoid process (should place tip in CMV); flush and place labelled injection port (CMV) on end.
Check patency of abdominal cannulae before closing abdomen.
Carefully return viscera to abdomen, pack gently being sure to leave cannulae exteriorized.
Place 18 g IV catheter in a superficial dorsal metatarsal vein; flush; secure in place. Cap with labelled injection port (SDMV).
Wrap underlying bubble wrap around dog and tape closed (monitor body temperature); make holes if necessary to exteriorize cannulae.
Flush all vascular cannulae with heparinized saline frequently.
When ready to inject into the SDMV site, place 16 g needle into injection port of SDMV and thread heparinized cannula through needle; flush.
Leave system to stabilize for one hour.

3 Experiment :

Randomize order of secretin bolusing into various sites; for dog #8:

(0.005 µg/kg boluses)	(low-dose boluses)
A	CMV
CMV	A
SDMV	CaVC
CaVC	SDMV
PV	

Flush each cannulae with heparinized saline regularly throughout experimental period.
Set time scale and chart recorder to desired settings. LABEL all settings on paper chart.
Administer secretin bolus into first injection site (see above). Administration time is 5 seconds; START time is when ½ of bolus has been administered (mark on chart recorder and record time of injection).
Flush immediately with 0.2 ml 0.5% BSA in 0.9% saline.

Flush with small volume of heparinized saline.
Observe pancreatic effect on chart recorder; allow to return to baseline for 5-10 minutes before *injecting* at next site.
Repeat all procedures until each site has received a 0.005 µg/kg secretin bolus.
Into the PV cannula, administer 1/100th of a 0.035 µg/kg secretin bolus, followed by 1/10th of a 0.035 µg/kg secretin bolus if no response is observed to the former. Continue diluting secretin boluses until a 'just noticeable' response is observed ("threshold dose") at this site. Once a "threshold dose" for the PV site is determined, administer the same dose into each of the other four vascular cannulae in the order shown above for 'low-dose boluses'.

****For *identification* of "threshold" dose, baseline drops/minute over a five minute period will be determined from the paper tracing. From this, the mean and standard deviation will be calculated. "Threshold dose" *will* be defined as that dose of secretin which results in an increase in pancreatic fluid output by the mean plus 2SD during any minute of the first five minute post-bolus period****.

4 Euthanase dog with pentobarbital.
Remove instruments.
Disconnect equipment.
Remove arterial line and tape.
Remove endotracheal tube.
Remove stomach tube.
Place dog on gurney and cover.
Take to post mortem room.

5 Verify placement and remove all cannulae.
Dispose of dog.

6 Soak, then flush cannulae.
Wash syringes, small instruments, etc. Place on paper towels to drain.
Consolidate all equipment onto carts and remove from surgery theatre (if necessary).
Clean up surgery theatre.
Sweep and mop floor in surgery theatre if necessary.
Dispose of trash.
Return gurney, laundry items and instruments to surgery. Place instruments in warm water to soak.
Turn off scavenge.

7 Collect recorded information and anaesthesia chart.
Complete anaesthesia record.
Fold up chart and attach to anaesthesia record.
Record equipment used (anaesthesia and surgery).
Fill in diary.

2/11/97

Secretin Infusion and Bolus Calculations for Experiment 2, Dog #8

Wt = 20.5 kg

1. BSA solutions (0.5%) - day before expt
   A. Add 2.5 gms BSA to 500 ml 0.9% saline for infusion; label and refrigerate.
   B. Make up additional BSA by adding 275 mg (0.275 gms) BSA to 55 ml 0.9% saline; label.
      I From this, place 20 ml and 10 ml into each of 2 labelled, lidded cups and jars.
      II Draw up 1000 µl into each of 15 1-ml syringes, cap; label in the following manner:

syringe #	Secretin content
1-5	0.10 µg

      * leave the 6 rest unlabelled
      III Cover and refrigerate all.
2. Secretin - morning of experiment
   A. Infusion
      Thaw 1 10-µg aliquot secretin and place all into the 500 ml bag 0.9% saline with 0.5% BSA; attach administration set and refrigerate.
   B. Boluses
      Thaw 1 5-µg aliquot secretin (5µg in 50µl); add to this 950µl 0.5% BSA solution to give a final concentration of 5µg in 1000µl or 0.005µg/µl, vortex. Into each of the 0.10µg labelled syringes (5), add 0.10µg (0.10µg = 20µl; remove 20µl first from each syringe!), cap; note remaining contents:

      ≈ 4.5µg in 897.5µl
      (1µg in 200µl)

      Place this and the 5 1-ml syringes in the chilly bin.
3. Distilled water - day before experiment
   A. Place ≈ 50 ml distilled water into a small jar or cup; label and cover; place in chilly bin

EXAMPLE OF COMPLETED SECRETIN THRESHOLD TESTING FORM: DOG #8 – CHAPTER 6

EXPERIMENT 2

SECRETIN "THRESHOLD" TEST
(PV vs CaVC, SDMV, CMV, A)

Date of experiment: Monday, 3 November, 1997
Dog id: Dog #8; Bounce
Wt: 20.5 kg
0.035 µg/kg = 0.72 µg
TD = Threshold dose = 0.72/500

	Dose Rate (µg/kg)	Dose (µg)	Concentration (µg/µl)	Volume (µl)*	Route (Site of injection)	Time of Injection	Response Observed ?
	0.72/100	0.0072	1/10,000	72	PV	5^{18} pm	yes
	0.72/250	0.0029	1/10,000	29	PV	5^{49} pm	yes
TD →	0.72/500	0.0014	1/20,000	28	PV	6^{13} pm	yes
	0.72/750	0.0010	1/20,000	20	PV	6^{36} pm	no
	0.72/500	0.0014	1/20,000	28	CMV	7^{05} pm	no
	"	"	"	"	A	7^{20} pm	yes
	"	"	"	"	CaVC	7^{31} pm	no*
	"	"	"	"	SDMV	7^{42} pm	no

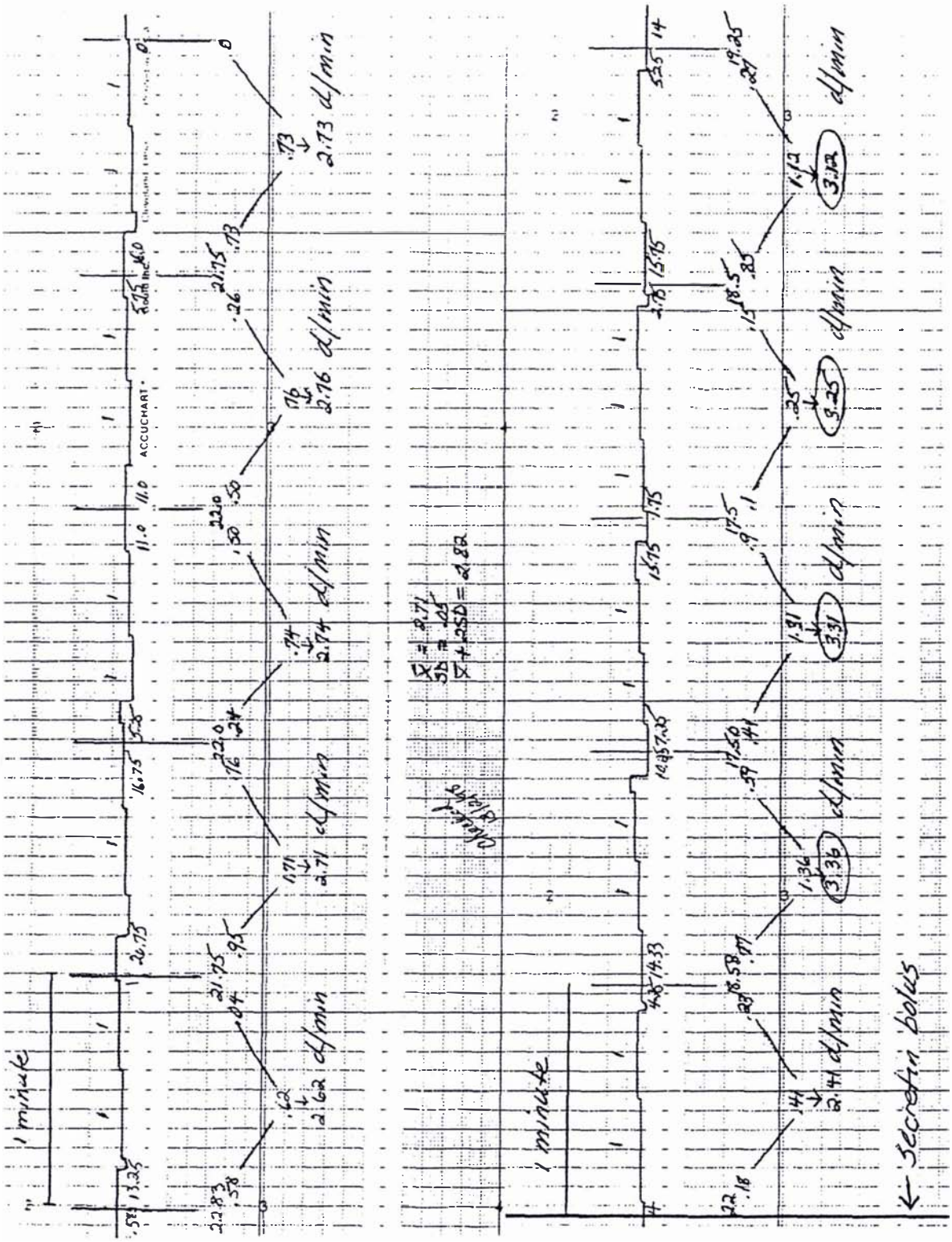
* Volume to be added to 1-ml syringe containing 0.5% BSA in 0.9% saline; total volume equal 1000 µl

* part of baseline overlapped with previous response to aortic injection

Appendix 12

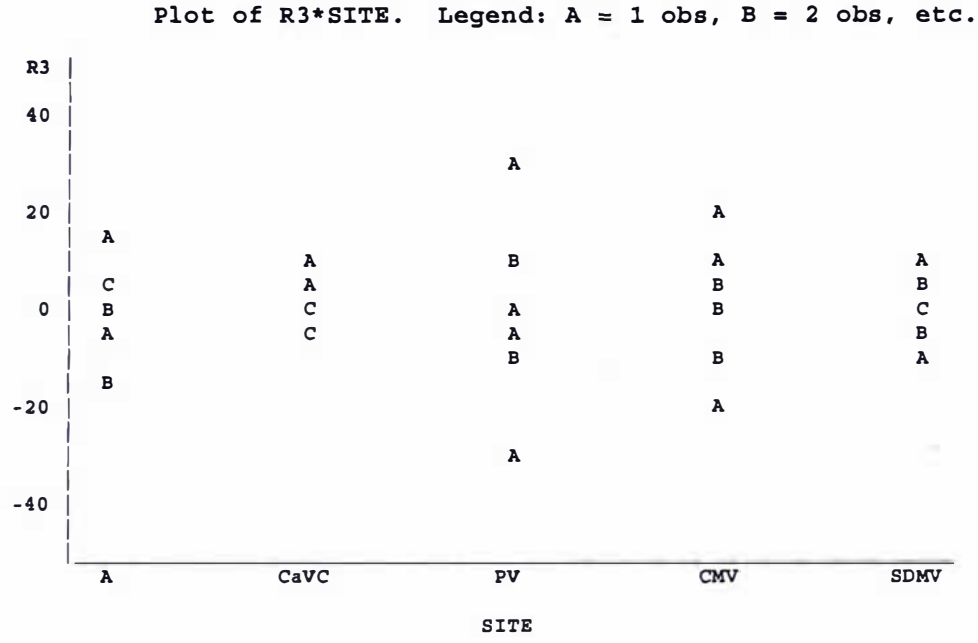
METHOD FOR THRESHOLD DETERMINATION – CHAPTER 6

The method used for threshold determination at the PV site is shown on the following page. The example provided is from dog #5. Baseline drops per minute were measured during the course of the experiment from the raw data prior to administering each dilution of secretin. From this, $\bar{x}$ + 2 SD was calculated. Following administration of each dilution of secretin, drops per minute were again calculated for the first five minutes. An increase by greater than or equal to $\bar{x}$ + 2 SD of baseline output, during any minute of the first five minutes post-injection of secretin, constituted a positive response. The top tracing illustrates the manner in which $\bar{x}$ + 2 SD was calculated from the raw data during the five minute *pre*-injection period, while the bottom tracing shows the minutes during which a response was obtained (circled numbers) following administration of a pre-determined dose of secretin into the PV site. The same method was used for determining whether a response had occurred during the threshold *testing* part of the experiment.


1 minute
2.62 cl/min
2.71 cl/min
2.94 cl/min
2.76 cl/min
2.73 cl/min
ACCUCHART
1 minute
2.44 cl/min
3.36 cl/min
3.31 cl/min
3.25 cl/min
3.12 cl/min
← seconds boluses

RESIDUAL ANALYSES – CHAPTER 6

Residual analyses are used to confirm the appropriateness of the statistical model. If there was a systematic bias towards variability at any given site, this would be reflected by a distribution of residuals offset from zero. **A aorta; CaVC caudal vena cava; CMV mesenteric vein; PDV cranial pancreaticoduodenal vein; PV portal vein; R3** residual; **SDMV superficial dorsal metatarsal vein**



NOTE: 2 obs had missing values.

MEANS FOR LATENCY OF RESPONSE – CHAPTER 6

Output from SAS showing means for latency of response at each injection site are presented below. For each site, the least squares mean is provided along with its standard error. Also tabulated are the p values for the differences between any two means. **RTIME3** response time in seconds; **A aorta; MV mesenteric vein; PDV cranial pancreaticoduodenal vein; PV portal vein; SDMV superficial dorsal metatarsal vein.**

SITE	RTIME3 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
A	31.5100000	3.2343793	0.0001	1
CaVC	41.7896644	3.4781275	0.0001	2
PV	53.6374063	3.4781275	0.0001	3
CMV	56.1966667	3.2343793	0.0001	4
SDMV	48.7766667	3.2343793	0.0001	5

Pr > |T| H0: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4	5
1	.	0.0385	0.0001	0.0001	0.0007
2	0.0385	.	0.0226	0.0050	0.1517
3	0.0001	0.0226	.	0.5940	0.3143
4	0.0001	0.0050	0.5940	.	0.1152
5	0.0007	0.1517	0.3143	0.1152	.

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