The Effect of Alcohol on Pancreatic Blood Flow.

An Experimental Study.

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DECLARATION:

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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ABSTRACT:

The reference sample method using 15 micron diameter radionuclide labelled carbon microspheres was used to establish a rat model of pancreatic blood flow which was then used to follow up previously reported studies in dogs which showed that intravenously administered alcohol lead to a fall in pancreatic blood flow. In addition, the oral administration of alcohol alone and in combination with glucose was studied.

The literature regarding pancreatic blood flow was reviewed with special emphasis on techniques of measurement and the effect of intravenously administered alcohol.

While the rat model proved highly successful, it was not possible to use a dual injection technique as had been previously carried out in the larger experimental animal. This meant that instead of carrying out a control measurement on each animal it was necessary to have separate control and experiment groups.

The major findings of the study were of difference in pancreatic blood flow between fasted (105 ± 9, mean ± s.e.) and non-fasted animals (134 ± 11) which was significant at the 0.025 level using the unpaired t test ($t=2.14$, df 18, $p<0.025$). No significant changes in pancreatic blood flow were observed with alcohol administered via intravenous or via gastric infusion compared to control groups.

The major finding of the study was that a combination of alcohol and glucose administered via gastric infusion was found to produce a rise in pancreatic blood flow (161 ±19) which was greater than that seen with either intravenous alcohol alone

(111 ± 9) or glucose alone (90 ± 6). This change was highly significant (t=2.70, df 10, $p<0.0125$).

It is concluded that the rat is a suitable experimental model for studying pancreatic blood flow with the microsphere method, that fasting significantly lowers pancreatic blood flow, and that the combination of alcohol and glucose is a potent stimulator of pancreatic blood flow.

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PART 1: BACKGROUND AND LITERATURE REVIEW.

CHAPTER 1. INTRODUCTION, OBJECTIVES AND HYPOTHESES:

1.1 Introduction

a. Pancreatic blood flow:

Interest in pancreatic blood flow dates to 1865 when Claude Bernard observed the colour of the rabbit pancreas to change from pale white in the fasting state (figure 1.1) to a deep red colour following feeding, (figure 1.2) leading him to suggest that pancreatic blood flow increased with the ingestion of food.

Despite this early insight into the physiology of pancreatic blood flow, data was slow to accumulate. This was partly due to the time taken to develop sophisticated measuring techniques but also because of the complexity and relative inaccessibility of the pancreas and its blood supply. This has resulted in the use of a wide variety of experimental methods of varying accuracy which have been used over the years to measure pancreatic blood flow, culminating in the introduction of the radioactive microsphere method by Rudolph and Heymann in 1967. These methods are reviewed and their relative merits discussed in chapter 3.

Not unexpectedly, the major areas of interest in the field of pancreatic blood flow have been the effects of the gastro-intestinal hormones, (secretin and pancreozymin in particular) and the effect of the autonomic nervous system and its transmitters. The effect of glucose and the effect on pancreatic blood flow of a wide variety of vaso-active agents and drugs (including alcohol) have also been studied, as have the changes

observed in pancreatitis. The literature relating to these studies is reviewed and summarized in chapter 2.

b. Alcohol and Pancreatitis:

One of the major stimuli of research into pancreatic physiology has been the clinical condition of pancreatitis, or inflammation of the pancreas, a common disorder which results in considerable morbidity and mortality. Clinically, two major forms of the disease exist, an acute haemorrhagic form and a chronic form, the latter being the more common, but fortunately the less severe.

Although the cause is unknown, the consumption of alcohol was recognized as a major aetiological factor by Friedreich as early as 1878 when he first drew attention to what he called "drunkards pancreas" (cited by Gambill 1973). Since that time, a vast number of experimental and clinical studies (summarized in the detailed reviews of Creutzfeltd 1970 and Gambill 1980), have confirmed the following:

1) alcohol is the major aetiological factor in up to 20% of all cases of acute and up to 70% of cases of chronic pancreatitis
2) the pathological mechanism of acute pancreatitis, of whatever aetiology, is auto-digestion of the gland and its surrounds by its own activated enzymes (first suggested by Chiari in 1896.)
3) that surgically induced ischaemia is a reliable way of producing acute pancreatitis in the experimental animal (first demonstrated by Smyth in 1940.)

Considering that this information has been available for over 100 years, it is surprising that the exact steps involved in the pathogenesis of alcohol induced pancreatitis, i.e. how alcohol leads to the presence of activated enzymes in the interstitium of the gland,

remains unknown as does the relevance of ischaemia in the pathogenesis of pancreatitis in man.

These questions are not merely of academic physiologic interest, since alcohol induced pancreatitis is a common disorder estimated to affect hundreds of patients per year who have a mortality of up to 17% and an even greater morbidity (Hermon-Taylor 1977). Patients are often admitted in the early stages of the disease and while in many the disease does not progress beyond mild interstitial oedema which recovers after a few days, in others the clinician must watch helplessly as the acute haemorrhagic variety of the disease develops and leads to the all too well known attendant morbidity and mortality (Boyer 1960).

The use of prognostic indices (Ranson 1982), has led to the ability to select, early in the course of the disease, those patients who are at greater risk of developing complications, but, without knowledge of the pathologic steps involved, it is not possible at present to significantly modify the course of the disease.

To date, much attention has been paid to the effect of alcohol on pancreatic exocrine function and the subsequent development of chronic pancreatitis but little is known about the effect of alcohol on pancreatic blood flow.

Horwitz in 1982 demonstrated that intravenous alcohol produced a significant fall in pancreatic blood flow in dogs and postulated on the basis of ischaemia being a well recognized cause of experimental pancreatitis, that ischaemia was thus a possible pathogenic mechanism of alcohol induced acute pancreatitis.

These findings have been confirmed by Slavotinek (1983) and Friedman (1983) but there had been no further published work on the mechanism involved in the lowering of pancreatic blood flow by alcohol, nor any postulated explanation of just how this reduction in blood flow might lead to extravasation and subsequent activation of proteolytic enzymes in the interstitium of the gland. There were no published reports of the effect of alcohol on pancreatic blood flow in any other laboratory animals at the time the research was carried out.

Due to the long delay between the experimentation and presentation of this thesis, a detailed Medline search has been carried out in order to review pancreatic blood flow findings as they pertain to 2013.

This review has failed to find any experimental studies other than that of Foitzik (1998) in which microscopy of the exposed pancreas in cats was used to study the effect of alcohol on capillary blood flow. The main aim of the study was to test the hypothesis that endothelin-1 mediates an ethanol-induced pancreatic injury through impairment of perfusion.

No studies were found in which the microsphere method was used to measure blood flow in rats.

1.2 Objectives :

The primary objective of this study was to establish an experimental model for the measurement of pancreatic blood flow rats in order to test the hypothesis that alcohol will reduce pancreatic blood flow.

Experiments were carried out in which alcohol was administered,

1) intravenously

2) orally

3) orally in combination with glucose

1.3 Hypotheses :

The primary aim of this study was to use a rat model to test the hypothesis that alcohol administration, as had been demonstrated in dogs by previous studies (Horwitz 1982, Slavotinek 1983, Freidman, 1983) could significantly reduce pancreatic blood flow. Alcohol administration was to be carried out intravenously, as in the studies of Friedman and Slavotinek and Horwitz.

The secondary hypothesis was that simultaneous glucose administration would alter the effect alcohol had on pancreatic blood flow. This secondary hypothesis was made prior to any literature search. It is based on the fact that the pancreas contains both an exocrine portion responsible for the secretion of enzyme rich fluid and an endocrine portion which is responsible for the secretion of insulin and the clinical observation that alcohol ingestion by man is often in the form of solutions with a high alcohol content (spirits) combined with soft drinks which have a high glucose content.

CHAPTER 2. THE PANCREAS:

2.1 Morphology.

The pancreas is an elongated mixed exocrine and endocrine gland which extends from within the "C" shaped duodenum to the hilum of the spleen and is divided morphologically into a head, body and tail. In addition, there is an uncinate process which lies posterior to the head. The entire gland occupies a retroperitoneal position.

The exocrine tissue comprises the vast bulk of mass of the gland and is similar in morphology to the salivary glands. Its secretion of water, electrolytes and enzymes ("pancreatic juice") drains into a duct system which opens into the duodenum, either separately or more commonly (up to 85% of individuals according to Gambill 1973), in association with the common bile duct via a common ampullary channel. Outflow, and more importantly, reflux into the duct from the duodenum is controlled by the sphincter of Oddi.

The enzymes produced by the pancreas are responsible for the digestion of proteins, fats and carbohydrates and are secreted in an inactive or pro-enzyme form to prevent auto-digestion of the pancreas. In the duodenum, there is conversion of the pancreatic pro-enzyme trypsinogen to the active form of the enzyme, trypsin, which in turn activates the other pro-enzymes.

2.2 Hormonal control:

Pancreatic secretion is largely under the control of the gastrointestinal hormones secretin and cholecystokinin (CCK), sometimes referred to as pancreozymin. Secretin arises from the duodenal mucosa and enters the circulation in response to the entry of gastric contents (and in particular acid) to produce an increased volume of pancreatic juice which has a high bicarbonate but low protein content. Cholecystokinin (which is also secreted by the duodenal mucosal) does not produce as great an increase in volume, but does increase the protein content of pancreatic fluid. A similar effect is seen with stimulation of the vagus nerve i.e. an increase in pancreatic juice of high enzyme but low bicarbonate content, (Wormsley 1977).

The endocrine portion of the gland, which accounts for only 1% of the mass of the gland, is scattered throughout the gland in small spherical collections of cells referred to as the Islets of Langerhans. The secretory products of the Islets, insulin, glucagon and gastrin are released directly into the circulation.

2.3 Blood supply:

Embryologically, the pancreas is derived from the endoderm of the foregut. However, the ventral and dorsal diverticulae which eventually fuse to give rise to the gland are situated close to the junction of the fore and mid gut. It is not surprising therefore that blood supply of the pancreas is derived from both the artery of supply of the foregut (coeliac) and that of the midgut (superior mesenteric).

a. Arterial supply:

In the rat, the pancreas is softer and a little more diffuse than in man, but it has a similar blood supply. The coeliac axis (first major branch of the abdominal aorta) divides into a splenic artery which courses along the superior border of the pancreas towards the hilum of the spleen, supplying the pancreas via multiple small branches as it does so.

Another major branch of the coeliac axis, the hepatic artery gives a gastro-duodenal branch which passes posterior to the pylorus of the stomach giving off the superior pancreatico-duodenal artery which courses in the groove between the pancreas and duodenum, supplying both, again via multiple small branches. The superior mesenteric artery supplies an inferior pancreatico- duodenal artery which ascends to anastomose with its superior counterpart (fig. 2.1).

b. Venous drainage:

As in man, this parallels the arterial supply feeding into the splenic and superior mesenteric veins which unite to form the portal vein. The blood supply of the pancreas is thus not only diverse with many arteries of supply and many draining veins but these vessels are intimately connected with those of the duodenum and spleen. The problems this creates in attempting to measure blood flow will become apparent in the ensuing chapter.

c. Micro-circulation:

An interesting feature of the blood supply of the pancreas relates to the arterial supply and venous drainage of the Islets of Langerhans. Although morphologically and physiologically independent of the surrounding acinar tissue, the islets channel their venous outflow to the acinar circulation rather than directly into the veins draining the pancreas as a whole.

Their blood supply is thus in series rather than in parallel as was first alluded to by the morphologic studies of Wharton (1923). This has since been confirmed by light microscopy (Wharton 1932), and scanning electron microscopy (Fujita 1973).

These findings were also confirmed by Lifson (1980) who demonstrated that the islets receive 11-23% of the total blood supply of the pancreas in spite of the fact that they only comprise 1-2% of the total weight of the gland.

By using a technique of retrograde injection of microspheres into the pancreatic veins, Lifson was able to demonstrate that all of the microspheres were trapped in exocrine gland capillaries leading him to postulate that the venous outflow of the Islets is directed to the exocrine glands. This postulate was subsequently confirmed by scanning electron microscopy by Murakami 1997.

The existence of an “insulo-acinar” portal system was thus demonstrated but to date there has not been any suggestion regarding its possible physiological implications.

2.4 Pancreatic Blood Flow

a. Introduction:

An extensive search for papers reporting pancreatic blood flow was carried out using the Medline database and copies of these original articles were obtained where ever possible. The bibliographies of these papers were then examined in detail and further articles traced.

The papers were examined from the point of view of the results obtained as well as the technique employed. This chapter provides a chronological overview, with particular emphasis on technique and of course the effect of alcohol. Details of the various methods are discussed in chapter 3, with the most accurate and reliable methods considered first with appropriately less attention given to those techniques whose value is more historic than practical.

b. Historical Overview:

The study of the heart and the circulation of blood have been prominent in the history of Medicine since the days of the Ancient Greeks with Hippocrates having been credited with the concept that arteries were connected to veins and contain blood which circulates to distribute warmth and life to the whole body (Doby 1963).

Many others have been outstanding contributors to our understanding of the circulation. William Harvey (1578-1658), was the first to postulate the existence of capillaries, thus "completing the circuit" and explaining the nature of the circulation. The Reverend

Stephen Hales (1677-1761) was a prominent physiologist of his day who not only was the first to measure blood pressure, but who also made quantitative measurements of cardiac output by making wax casts of the ventricles of animals.

Steps toward the actual measurement of cardiac output came from Hering in 1829 who was the first to measure circulation time. In 1860, the German mathematician Fick postulated that cardiac output could be measured by knowing the concentration of oxygen or carbon dioxide entering and leaving the heart and in 1866 Grehant and Quinquard successfully applied Fick's principle to the measurement of cardiac output in the dog (see Woodcock 1975 for a detailed review).

The first recorded interest in pancreatic blood flow was by Claude Bernard in his classic treatise on the pancreas in 1856 (translated by Henderson 1985). This contained detailed observations and experiments on the physiology of pancreatic secretion and content of pancreatic fluid. Almost as an aside, the treatise contained a comment supported by a drawing (fig. 1 and 2), that the pancreas was redder and by inference, contained more blood in animals having just eaten compared to animals in the fasting state.

The first attempts to measure pancreatic blood flow were by Francois-Frank in 1896 (cited by Tankel), Edmunds (1909), Anrep (1916) and Mann (1917) and involved the technique of plethysmography. This technique is only marginally more objective than observation of the organ for vasodilatation and is based on the assumption that increased blood flow to an organ will lead to an increased volume of the gland. This increased volume is measured by encasing the organ in a rigid container which is connected to a pressure recording device. Extensive manipulation of the pancreas is

required with this technique and the results obtained can only be considered subjective at best.

Early interest in pancreatic blood flow concerned the adrenal hormones with Edmunds (1909) studying the effect of adrenaline and other agents on pancreatic secretion and on pancreatic blood flow using a plethysmograph. He demonstrated a decrease in secretion associated with a marked increase in volume followed by a rapid diminution in size with adrenaline infusion.

Similar changes were observed with compression of the thoracic aorta and Edmunds to conclude, as had Claude Bernard, that a close relationship existed between the blood supply and secretory activity of the organ.

Other techniques used in the early part of the 20th century to evaluate the effect of pancreatic hormones on blood flow and secretion involved the measurement of venous outflow either with simple drip chambers, Anrep (1916) or the more complex Stromuhr devices of Bennett (1933).

These methods all required extensive manipulation of the gland because of its diverse venous drainage and without exception, these papers from early in that century rarely describe their method in detail and make no attempt at validation.

Anrep (1916) studied the effect of vagal stimulation on pancreatic secretion and blood flow in decerebrate dogs. Using the technique of venous outflow measurement, he was unable to demonstrate any effect of vagal stimulation on pancreatic blood flow but he was able to demonstrate a steady flow of pancreatic juice with infusion of secretin into

the jugular vein. He made no comment about the state of pancreatic blood flow during this infusion.

Apart from being one of the first to report the effect of secretin on pancreatic secretion, the paper is of interest because it reviewed the then current literature on pancreatic blood flow. It quotes work by Francois-Frank (1897) and May (1904) and describes the work of Edmunds (1909) (see above).

Still (1933) and his co-workers studied pancreatic blood flow in dogs with a stromuhr device, essentially a sophisticated "bucket and stop-watch" method of measuring venous output which is described in greater detail in chapter 3. Their results were expressed as the percentage increase in total volume of blood passing through the gland during the period of stimulation compared to the control rate.

They found only modest increases in pancreatic blood flow with secretin infusion but made the interesting observation that this infusion markedly increased the rate of pancreatic oxygen and carbon dioxide metabolism.

In a similar study in which pancreatic duct pressure was experimentally increased in association with secretin infusion, (Bennett 1933) increases in venous outflow of 20% to over 200% were found, leading to the conclusion that raised intra-duct pressure is able to stimulate pancreatic blood flow.

Richins (1953) used the novel technique of freeze drying to test the effect of sympathetic nerve stimulation on the flow of blood through the capillary beds of the cat pancreas. Histological sections were produced and the size of the capillaries and arterioles used to qualitatively estimate pancreatic blood flow at the time of death.

Although the results can only be considered subjective at best, the paper concludes that stimulation of the sympathetic nerve supply of the pancreas produces an increased volume of blood flow through the capillary beds, and that secretory activity is increased by this increased blood flow.

Using an equally ingenious technique of trans-illumination of the pancreas to assess vascular changes, Holton and Jones (1960) attempted to study the relationship between blood flow and secretion.

They demonstrated an increase in pancreatic vasodilatation with secretin and histamine infusion which was refractory to atropine but were unable to show that this effect was in any way affected by increased intra duct pressure as had been suggested by the work of Bennett and Still (1933).

Despite their assertion that this technique caused very little interference with secretion and in spite of evidence from Jones (1960) that there was correlation between the colour changes observed with trans-illumination and venous outflow, it appears that they merely confirmed the observation of Claude Bernard in 1856 that the pancreas becomes red and congested during digestion.

Kuznetsova (1962) used a stromuhr technique to study the effect of feeding on pancreatic blood flow in dogs. A definite increase in blood flow following feeding was seen and moreover this increase was paralleled by an increase in pancreatic secretion. Although the technique of blood flow estimation was subjective only and not validated in any way, the paper is of interest because the authors used actual feeding of the animals rather than infusion of pancreatic hormones of questionable purity in their study of the commonly studied question of the relationship of pancreatic blood flow to secretion.

The 1960's saw the introduction of radioactive indicator transport techniques of blood flow measurement and for the first time, qualitative assessment was replaced with quantitative estimation of pancreatic blood flow, albeit of questionable accuracy.

Gilsdorf (1965) used radioactive potassium (K^{42}) to study the effect of hypothalamic stimulation in dogs, concluding (without a suggestion as to a possible cause) that this lead to an increase in both pancreatic secretion and blood flow.

Delaney and Grim (1966) used both radioactive potassium (K^{42}) and rubidium (Rb^{86}) in measuring the effect of secretin and other agents such as adrenaline and nor-adrenaline on pancreatic perfusion in anaesthetised dogs.
They found a control value of 60mls/min/100 grams which was increased by up to 150% following infusion with secretin, nor-adrenaline and cortisone and concluded that since there was no effect on arterial blood pressure, that the observed rise must have been due to a lowering of pancreatic resistance.

Using the same technique but with radioactive rubidium (Rb^{86}) rather than potassium, Papp et al. (1966 a) compared pancreatic blood flow in dogs in which pancreatitis had been induced by injection of bile acids into the pancreatic duct with controls and found a statistically significant fall of the order of 50%.
In another series of experiments Papp et al. (1966 b) used heated thermocouples to assess the change in pancreatic blood flow following administration of histamine, bile acids and adrenaline. They demonstrated vasodilatation with bile acids and histamine but no effect with nor-adrenaline.

Further work on pancreatitis and blood flow was carried out by Hermreck (1968) who studied the effect of acute oedematous pancreatitis on blood flow in dogs.

The pancreatitis was induced by injection of bile into the cannulated pancreatic duct while blood flow was assessed by the measurement of venous outflow following the surgical isolation of the gland from the duodenum. This study showed no decrease in pancreatic blood flow in the early oedematous phase of the development of pancreatitis. It also showed that even after the development of haemorrhage and necrosis, oxygen consumption (which was also measured) continued at normal rates or greater suggesting continued high metabolic activity.

The possible role of hypothalamic stimulation on the production or progression of pancreatitis was studied by Gilsdorf and co-workers (1965) on the basis that splanchnic nerve block had been proposed as a treatment of pancreatitis. Using radioactive potassium in non-anaesthetised cats, they demonstrated no significant increase in pancreatic blood flow with hypothalamic stimulation, concluding that any beneficial effect of splanchnic nerve blockage in pancreatitis must be due to division of afferent pain fibres.

Eichelter (1966) used an electro-magnetic flow probe around the pancreatic-duodenal artery of anaesthetised dogs to study the haemo-dynamics of pancreatic secretion. A rapid increase in both secretion and pancreatic blood flow (71%) following secretin administration was reported as was the fact that these changes were accompanied by an increase in pancreatic oxygen consumption.

Barlow et al. (1968) also studied the relationship of pancreatic blood flow to secretion. Using venous outflow in fasted anaesthetised cats after isolation of the pancreas from the splanchnic circulation, they found a slight increase in pancreatic blood flow following injection of secretin. A profuse flow of juice was obtained and maintained with subsequent injections of secretin but the blood flow returned to and remained at basal levels.

They also used the technique of measurement of electrical conductance across the gland despite admitting that that changes in conductance are brought about by alterations in the composition as well as volume of extra cellular fluid, both of which vary during the secretory cycle of the gland.

Aune and Semb (1969) described the technique of measurement of hydrogen gas clearance to determine pancreatic blood flow in conscious and anaesthetised dogs. They found basal values in the anaesthetised compared to the conscious animal to be reduced by almost 50% but found that regardless of conscious state, both secretin and pancreozymin were effective in increasing this value.

Using the same technique in anaesthetised pigs, the same authors reported a 300% increase in pancreatic blood flow after the infusion of 5% glucose intravenously, an effect which could be blocked by the concomitant injection of insulin (Semb 1971). This effect was not seen with the administration of similar volumes of mannitol nor saline.

The authors suggested that the results point to a close functional link between the exocrine and endocrine portions of the gland and they speculate on the possibility of secretin being the mediator of their observed increase in pancreatic blood flow.

Mandelbaum (1969) used venous outflow measurements to test the effect of hypertonic glucose on pancreatic blood flow in anaesthetised dogs during extra-corporeal bypass. Although an increase in insulin secretion was noted with the infusion of hypertonic glucose, there was no associated increase in pancreatic blood flow.

These experiments were conducted under conditions of total cardiopulmonary bypass with maintenance of cardiac output, a clearly artificial physiological situation. It was postulated that the findings of other authors who did demonstrate an increase in pancreatic blood flow following the infusion of glucose were not true changes in pancreatic blood flow but a consequence of an increase in cardiac output. There does not however seem to be any supportive evidence for this postulate.

Goodhead (1970) studied pancreatic blood flow in pentothal anaesthetised dogs using radioactive rubidium. A marked increase in secretion and a three-fold increase pancreatic blood flow following an infusion of secretin was seen with a similar but less pronounced increase with pancreozymin, urecholin and pentagastrin.

The increased blood flow following secretin infusion was associated with the output of a large volume of pancreatic juice of low viscosity and these authors were in no doubt of the existence of a relationship between increased secretion and blood flow.

Vance 1970 used microspheres to test the effect of glucose and various other agents on pancreatic blood flow, finding that an infusion of glucose resulted in a 37% increase in

flow. This is in keeping with the findings of Aune and Semb (see above) but unfortunately, although his method was ingenious, it was not validated.

Microspheres of 15 micron diameter were injected into the left ventricle before and after perfusion of the test substance into the superior pancreatico-duodenal artery. Microsphere content and therefore pancreatic blood flow were then compared for the parts of the gland which were and were not supplied by the artery and therefore influenced by the test substance.

No mention is made of how the part of the pancreas supplied by the superior pancreatico-duodenal artery was identified, nor are any control or preliminary studies reported.

Sasaki and Wagner (1971) used 50 micron diameter microspheres to measure the distribution of cardiac output in conscious and anaesthetised rats. Pancreatic blood flow was measured as 0.8% of cardiac output in the conscious animals but a significantly higher 1.3% of cardiac output in the pentobarbital anaesthetised animals. These authors also mention in passing that they observed the fraction of cardiac output distributed to the gastrointestinal tract was higher in the postprandial than in the fasting state.

Malik (1974) applied a similar method but with 15 micron diameter microspheres to determine regional blood flow in conscious rats. He found that the technique caused no alteration of cardiovascular function and therefore demonstrated the feasibility of using the microsphere method in rats. Although the distribution of cardiac output had previously been determined in rats, this was the first published report using the reference organ method. Their finding of pancreatic blood flow of 152 $\pm$ 70 mls/min/100 grams of tissue and renal blood flow of 525 $\pm$ 32 mls/min/100 gm correlates well with the findings of Benveniste (1985).

Papp (1973) in another series of experiments in dogs using an electro-magnetic (EM) flow meter and heated thermocouples studied the effect of secretin, histamine and bile acids on pancreatic blood flow. All of these agents increased flow with secretin producing the largest detected increase (50%). Their measured values for blood flow through the superior pancreatico-duodenal artery (which they commented on as supplying the majority of the gland) corresponded to the values obtained by other studies using EM flow meters (Eichelter 1966, Papp 1969) but were 30% more than values quoted by authors using the radioactive rubidium technique. Studley (1985) found a similar discrepancy and concluded that EM flow meters overestimated pancreatic blood flow because of connections to the duodenum.

Lenninger (1973) measured pancreatic blood flow in cats by using a photoelectric cell to

assess venous outflow. The effect of acetylcholine and papavarine were studied and found to increase both pancreatic blood flow and secretion in combination.

The significance of the results is unclear since by the author's own admission, the considerable disruption resulting from operative preparation of the gland lead to difficulties in interpreting the results.

Glazier and Needham (1974) used a variation of the Fick principle to measure pancreatic perfusion rate in dogs. Their method was to inject radioactive Xenon into the splenic vein. With the pancreas having been previously isolated from the stomach and duodenum, radioactivity in the gland was then monitored and clearance curves constructed. Intravenous injection of secretin resulted in a peak increase in perfusion

rate of 3 to 4 times control rates. This occurred at 1.5 minutes with restoration of control rates at 7.5 minutes.

Takeuchi (1974) used an isolated perfused canine pancreas model to test the effect of various biogenic substances. Results were expressed as a percentage change in the blood flow compared with the resting state. A marked rise in blood flow was seen with secretin infusion and a similar increase in blood glucose levels was seen with glucagon. Although a large number of biogenic substances were tested, surprisingly, alcohol was not included in this study.

Fischer et al. (1976) used implanted EM flow probes to measure superior pancreatico-duodenal blood flow in dogs. They found that pancreatic blood flow was rapidly raised by the intravenous administration of glucose and that this increase was seen in conscious as well as anaesthetised animals.

Beijer et al. (1977) also used EM flow probes in anaesthetised dogs and demonstrated an increase in both pancreatic blood flow and secretion following the administration of secretin.

Jarhult (1977) studied the effect of intra arterial injections of hypertonic solutions of xylose, glucose and sucrose on pancreatic blood flow in cats using a photo electric cell to measure venous outflow from the splenic vein following ligation of all vessels connecting the pancreas to the stomach and duodenum. The technique provided only "semi-quantitative" blood flow measurement, but the authors point out that the results obtained with various secretagogues and vasodilators were consistent with results

obtained by other techniques. They make the suggestion that the vasodilatation observed was due to the hyper-osmolar nature of the solutions rather than an islet response to glucose since xylose and sucrose do not stimulate the islets.

Vaysse (1977), used an isolated, perfused gland technique to study the effects of catecholamines on vascular resistance and blood flow on the pancreas of dogs. The pancreas was removed and separated from the duodenum and a perfusion circuit set up and maintained with heparinized, oxygenised blood infused into the superior mesenteric and coeliac trunk. Venous blood was then collected from the portal vein. Results were expressed as percentage changes in vascular resistance and by inference, blood flow.

Lifson (1980) used non-radioactive microspheres in a study of acinar versus Islet blood flow in rabbits. Spheres were injected into the left ventricle and the pancreas was then studied histologically. The Islets were found to have received 11-23% of the spheres while the remaining 77-89% were found in the acinar tissue. By using retrograde (i.e. venous) post-mortem injection of spheres, they were able to show that no microspheres reached the Islets while with orthograde (i.e. arterial) post mortem injection resulted in the same distribution as seen in the in-vivo studies, thus confirming the existence of an insulo-acinar portal system.

Studley et al..(1985), compared pancreatic blood flow in dogs measured by EM flow meter and Krypton^{85}. They considered normal canine pancreatic flow to be 60-65ml/min/100gm and found good correlation between the two methods provided that all vascular connections between the pancreas and the duodenum had been divided. If this division was not carried out then the EM flow meter over-estimated blood flow by

approximately 35% a figure remarkably similar to the estimate of Eichelter (1966), Papp (1969) and Studley (1985) (see above) .

In spite of this large volume of work and the variety of methods employed, it was not until the introduction of the microsphere method by Rudolph and Heyman in 1967 that any method of pancreatic blood flow measurement came anywhere near the ideals outlined by Jackobsen (1981) i.e. accurate, continuous, instantaneous, reproducible, quantitative recording of complete or partial organ blood flow which is non-invasive and not harmful. For the first time it was possible to make measurements of pancreatic blood flow with minimum interference to the intact animal and in particular without any manipulation of the duodenum or the pancreas.

Horwitz and Myers (1982) using the microsphere method, studied the effect of alcohol on pancreatic blood flow in conscious dogs and found a 37% fall in pancreatic blood flow. No significant change was found to the blood flow to the stomach, spleen, large or small intestine. They concluded that this fall was due to an increase in vascular resistance but subsequent experiments with sympathetic blocking agents, prostaglandin synthesis inhibitors and histamine antagonists failed to block the previously demonstrated increase in pancreatic vascular resistance and associated fall in blood flow. Pre administration of hypertonic mannitol did not prevent the fall in blood flow but with a further infusion of mannitol the flow was restored to control values.

Their conclusion was that mannitol reversed the elevation in pancreatic vascular resistance produced by alcohol by allowing cellular fluid to shift into the extracellular space.

Friedman (1983) studied the effect of intravenous alcohol on pancreatic and splanchnic blood flow in conscious dogs, finding a 25% fall in pancreatic flow with a concomitant but not significantly correlated rise in hepatic arterial blood flow. In pentobarbital anaesthetised dogs, a similar rise in hepatic blood flow was seen but the fall in pancreatic blood flow was not. Colonic blood flow was increased and was the only significant change noted. The increase in colonic blood flow was seen in both awake and anaesthetised animals.

Slavotinek et al. (1986) used the microsphere method in dogs to test the effect of intravenously administered alcohol on pancreatic blood flow and found a statistically significant decrease compared to controls as had Horwitz (1982) and Friedman (1983). They also studied pancreatic blood flow in rats with experimentally induced pancreatic fibrosis. They found a control blood flow of 134 mls/min/100g (s.d. 19) which was reduced by nearly 50% in the fibrotic part of the gland. As well as this lower basal blood flow, the fibrotic part of the pancreas showed a reduced response to secretin stimulation, with a two-fold (rather than the three-fold increase seen in the normal gland) .increase in blood flow

Kogire et al. (1988) studied the effect of intravenously administered alcohol on hepatic and pancreatic blood flow in anaesthetised dogs. Using an ultrasonic flow meter on the hepatic artery they showed a 50% increase in blood flow. No increase in portal blood flow was found nor was there any change in pancreatic blood flow as determined by a laser-Doppler flow meter positioned on the surface of the pancreas.

These results appear to conflict with the findings of Horwitz, Friedman and Slavotinek but the technique was not validated against other established methods. In addition the

stomach and bile duct were cannulated thus preventing the entry of gastric juice or bile into the duodenum and eliminating any possible indirect effects of alcohol on pancreatic blood flow such as release of secretin from the duodenum.

2.5 Pancreatitis.

a. Introduction

The acute haemorrhagic form of the disease is characterised by a fulminating course of abdominal pain, retro-peritoneal haemorrhage and cardiovascular collapse. It has long been associated with alcohol ingestion but is considered unlikely to occur following an occasional alcoholic bout in a person who is not a chronic user (Balart 1982).

The condition has a significant mortality rate and survival may be attended by recurrent bouts of abdominal pain if the patient continues to imbibe alcohol. Apart from general supportive measures relating to fluid replacement and control of infective complications, there is no known way of modifying the course of the disease or averting its final outcome.

The major distinguishing feature of alcohol induced chronic pancreatitis is episodic or continuous abdominal pain occurring in predominantly young men after many years of excessive alcohol intake (Mallison 1977, Sarles 1986).

Morphologic damage including calcification of the interstitium of the gland and pancreatic duct stones is a common finding (Balart 1982, Wilson 1983) and forms the basis of what has become known as the Marseilles classification (Sarles 1974). Another well recognized feature of alcoholic pancreatitis is that it rarely occurs in association with alcoholic cirrhosis despite the fact that both occur in patients with similar backgrounds. The consumption of different types of alcoholic beverage has been

suggested as a possible explanation (Mackay 1966) but is not supported by epidemiological studies, (Sarles 1985)

Although the suggestion that acute pancreatitis of whatever aetiology is the result of auto digestion of the gland by its own activated proteolytic enzymes is well accepted, how the normally inactive forms of the enzymes secreted by the pancreas come to be in the interstitium of the gland and moreover, how they come to be activated is unknown.

Various theories have been proposed to explain the pathogenesis of pancreatitis in general and these will now be reviewed as they have relevance in understanding the rationale behind many of the experimental studies which have been carried out, especially those relating to alcohol and pancreatic blood flow.

b. Pathogenesis of Pancreatitis:

The major theories are listed and summarized below and then considered in detail.

1. Secretion/hyper-secretion against obstruction
2. Biliary and/or duodeno-pancreatic reflux
3. Direct toxicity
4. Dietary factors
5. Vascular factors

1. Secretion / hyper-secretion against obstruction:

Secretion, or possibly hyper-secretion against pancreatic duct obstruction with subsequent small duct damage and extravasation of enzymes is the proposed explanation for gallstone pancreatitis and has been extended to alcohol induced pancreatitis by several authors (Dreiling 1960, Schapiro 1966, Sarles 1985). Sarles and his co-workers (1971), have demonstrated that chronic alcohol consumption results in the secretion of a protein rich fluid which in turn leads to the formation of protein plugs and stones within the ducts, chronic calcifying pancreatitis and damage to acinar cells. Against this background of partial duct obstruction, stimulation of pancreatic secretion by alcohol either directly or via the stimulation of gastrin, may lead to increased intra-duct pressure with rupture of small ductules.

This theory accounts for many of the well known clinical features of alcohol induced pancreatitis; occurrence only after a long period of alcohol ingestion, repeated acute attacks against a background of chronic pancreatitis and the finding of interstitial calcification and ductal stones. It however fails to explain how the pancreatic enzymes are activated. The theory is also in question because there is no convincing evidence of raised intra-duct pressures in patients with chronic pancreatitis and that pancreatic duct obstruction alone is not sufficient to produce pancreatitis in experimental animals (Kalant 1969). Moreover, it has been suggested that the protein plugs and pancreatic calcification commonly seen in the pancreas of chronic alcoholics, may be the effect, rather than the cause of the inflammation which is commonly observed (Howard 1971).

2. Biliary and/or duodeno-pancreatic reflux:

Reflux of duodenal or pancreatic juice into the biliary tract secondary to dysfunction of the Sphincter of Oddi forms the basis of several theories which have been proposed over the years to account for pancreatitis associated with gallstones as well as alcoholic pancreatitis. Injection of bile and duodenal contents into the pancreatic duct has been used to produce pancreatitis in experimental animals for many years (Smyth 1940), but this theory has largely been discarded despite evidence that alcohol increases tone in the Sphincter of Oddi, (Pirola 1970). It is not tenable because of evidence that even in the presence of a common channel, pancreatic duct pressure remains higher than common bile duct pressure, implying that reflux would not occur (White 1964, Gilsdorf 1965).

3. Direct toxicity*:*

The suggestion of a direct toxic action of alcohol on the pancreas has been made on the basis that other drugs such as azathioprine and the chlorothiazides, are associated with pancreatitis (Balart 1982), that methanol has a toxic effect on the pancreas (Gambill 1973), the fact that acetaldehyde, a breakdown product of alcohol leads to intracellular disruption of microtubules within hepatocytes (Dreiling 1979) and that similar intracellular changes occur in pancreatic exocrine cells after the long term ingestion of alcohol (Darle 1970). There is however, no evidence to suggest that ethanol has a similar action to methanol nor any other drugs associated with pancreatitis and the breakdown of alcohol (and thus the concentration of acetaldehyde) occurring in pancreatic cells is minimal (Estival 1961).

4. Dietary factors:

Evidence for the possible role of dietary factors in the pathogenesis of alcohol induced pancreatitis has been reviewed by Wilson and Pirola (1983) and is based on the fact that both malnutrition and hyper-triglyceridaemia are associated with pancreatitis in the absence of alcohol ingestion and that both are features of the chronic alcoholics who develop alcoholic pancreatitis (Hermon-Taylor 1977). In addition, there is experimental evidence that rats fed long term alcohol demonstrate changes in the zymogen concentration in acinar cells and moreover, that such changes are dependent upon the ratio of fat to protein in the diet (Sarles 1971). In the absence of controlled dietary studies of alcoholic pancreatitis, this experimental evidence is insufficient for diet to be considered a major aetiologic factor (Sarles 1986).

5. Vascular factors*:*

Ischaemia was first proposed as an aetiological factor in pancreatitis by Panum in 1862 (cited by Smyth 1940). Knape 1912 (cited by Gambill 1973), showed that pancreas extract or trypsin, applied to the surface of the organ slowed down pancreatic blood flow and led to capillary stasis and ecchymoses. More direct evidence of arterial occlusion being able to produce pancreatitis came from Smyth (1940), who produced pancreatitis in dogs by injecting mercury droplets into the arteries supplying the pancreas and Pfeffer (1962), who was able to obtain similar results with the injection of microspheres.

While any tissue will become necrotic if the degree of ischaemia is severe enough, the pancreas seems particularly sensitive as shown by experiments in which partial occlusion of the gastro-duodenal artery lead to acute pancreatitis in dogs (Block 1954).

A common finding in a number of different studies has been that the production of oedema, inflammation and necrosis (the experimental equivalent of pancreatitis) requires both duct obstruction and impairment of arterial supply (or venous drainage) to be present (Block 1954, Nestle 1957, Popper 1948).

Blood flow has been measured in animals in whom pancreatitis was experimentally induced by the injection of bile into the ducts. Some authors found a 50% reduction in blood flow compared to controls (Papp 1966, Goodhead 1968) while others failed to demonstrate any fall in pancreatic blood flow (Hermrech 1968).

It is difficult to interpret these somewhat conflicting results, especially as it is difficult to know whether any observed fall in blood flow is the ***cause*** of the pancreatitis or merely an ***effect*** of it. The importance of these reports is that they do demonstrate that an association may exist between pancreatitis and lowered pancreatic blood flow.

Chapter 3. Blood Flow Measurement Techniques

3.1 Introduction:

In selecting a blood flow measurement technique one should aim for a technique which is accurate, provides continuous, instantaneous, reproducible, quantitative recording of complete or partial organ blood flow and which is non-invasive and not harmful. It should be independent of unproven assumptions or other measurements and ideally it should be economic with regard to time, effort and expertise (Jackobsen 1981).

In search of this ideal, techniques of blood flow measurement employed over the years have been developed from many fields of physics and most of these techniques have been applied to pancreatic blood flow measurement, as can seen from the previous chapter.

Of critical importance is the need for the method to provide a result which is expressed in absolute units of blood flow – millilitres per minute per gram of tissue. Only then is it possible to compare organ blood flow under different physiological conditions.

The following chapter reviews in descending order of importance the methods used to measure pancreatic blood flow over the years.

Particular attention is paid to methods using indicator transport or dilution (Fick Principle) and the underlying mathematical basis of these methods is presented in detail.

3.2 Indicator transport methods (Fick Principle):

It is over one hundred years since the mathematician Adolf Fick suggested a method of measuring cardiac output. He postulated that by measuring the concentrations of oxygen and/or carbon dioxide in arterial and mixed venous blood and their uptake or release by the lungs in a specified period of time, the cardiac output could be calculated, thereby introducing the principle which now bears his name and which forms the basis of so many methods of blood flow measurement (Fick A. Uber die Messung des Blutquantums in den Herzventrikeln Sitz. Physik-Med. Wurzburg. 1870: 16, cited by Woodcock 1975). Because of its central importance to the method employed in this study, the Fick Principle is considered in detail.

a. Mathematical basis of the Fick Principle.

The Fick principle is based on the fact that the change in the concentration of a detectable indicator (e.g. oxygen or carbon dioxide) between the inflow point (**Ci**) and outflow point (**Co**) of an organ (e.g. the heart) is proportional to the blood flow through that organ (**F)**, (i.e. cardiac output) and the total amount of indicator (**Q**) passing through. This is expressed mathematically as**,**

$$F = \frac{Q}{Co - Ci}$$

This expression provides a relatively easy way to calculate cardiac output as in spite of slight variations over the cardiac cycle, the constant uptake of an indicator (oxygen) into

the circulation leads to a steady state, mean concentration. By measuring the oxygen concentration entering and leaving the heart and measuring the total amount of oxygen taken up, cardiac output can be measured accurately, provided there is no arterio-venous shunting of blood (Visscher 1953).

While the heart has oxygen and carbon dioxide as easily measured indicators which achieve a steady state concentration, this is more difficult to achieve in regards to other organs such as the pancreas. Most methods therefore involve the injection of a bolus of indicator into the circulation which rapidly achieves a maximum concentration and then falls away in proportion to the blood flow through the pancreas (the faster the flow, the more rapidly the indicator is transported or "washed" out.)

Mathematically, the flow can be expressed as;

$$F = \int_{\infty}^{0} \frac{Q}{C(t).dt}$$

where F = total organ blood flow

Q = total amount of indicator injected

C = concentration of the indicator at given time (t)

In practical terms, even if sufficient samples can be taken to accurately graph the rate of fall off of the indicator, the calculation of the integral of the amount of indicator passing through the organ in time dt, is a limiting factor.

An elegant solution is that of Sapirstein (1958) who measured cardiac output as well as the total amount of indicator injected. From (2) above we can derive,

$$\int_{\infty}^{0} C(t).dt = \frac{Q}{F}$$

If the indicator is injected into the left atrium of the heart and is evenly mixed with the blood leaving the heart, then,

$$\int_{\infty}^{0} C(t).dt$$

is constant.

For organs **1, 2,.....n;**

$$\int_{\infty}^{\circ} C(t)\,dt = \frac{Q}{F} = \frac{Q_1}{F_1} = \frac{Q_2}{F_2} = \ldots\ldots\ldots \frac{Q_n}{F_n}$$

where **Q** is the total indicator injected

$Q_1, Q_2,...Q_n$ is the indicator passing through organs **1,2,.n**

F is the cardiac output

$F_1,F_2,...F_n$ is the flow to organs **1,2, ...n**

If the cardiac output **C.O.** is known, then flow to individual organs can be calculated from,

$$F_{1,2,.....n} = \frac{Q_{1,2,.....n} \times C.O.}{Q}$$

thus avoiding the necessity of complex mathematics.

While an improvement on standard indicator dilution methods, the calculation of cardiac output by an independent method is not always easy and adds a further source of error.

This difficulty was overcome by the development of the reference organ method (Domenech 1969, Buckberg 1971).

Very simply, if the flow (in absolute terms) to one organ is known the others can be calculated without having to measure the cardiac output. From (5), if the flow to one organ (F_1) is known and the amount of indicator passing through it (Q_1), can be measured, then,

$$\frac{F}{Q}\frac{1}{1} = \frac{C.O.}{Q}$$

Substituting (6) into (5) gives ,

$$F_2, F_3, ...F_n = Q_2, Q_3, ... Q_n \times \frac{F1}{Q1}$$

so if $Q_{2,3....n}$ can be measured, then, $F_{2, 3, ...n}$ can be calculated.

The organ whose flow is known is the "reference" organ and can be an artificial one, consisting of a syringe mounted on a withdrawal pump set at a known rate and connected to the circulation via a catheter, usually in the femoral artery.

b. Indicators used to measure blood flow.

Various substances (including radioactive microspheres) have been used as indicators in measuring pancreatic blood flow according to the Fick Principle and will be considered as separate techniques as each has its own technical difficulties.

i. Radioactive potassium / rubidium.

Introduced by Sapirstein in 1958, the method involved the injection of radioactive potassium (K^{42}) and rubidium (Rb^{86}) compounds into the circulation via a catheter in the inferior vena cava, following which they were distributed throughout the body in proportion to the blood flow to the various organs. Blood was simultaneously withdrawn from the femoral artery through a counting chamber fixed in a gamma counter and the data used to draw an isotope dilution curve from which the cardiac output could be calculated. The animal was killed 30-40 seconds after injection of the isotope, prior to its recirculation and the amount of radioactive indicator in the pancreas measured.

Flow to the pancreas (F) was then calculated from (5), where Q_1 is the indicator measured in the pancreas and Q the total indicator injected

$$\mathbf{F = \frac{Q_1 \times C.O.}{Q}}$$

The major limitations of this method are variable organ distribution and further uptake of indicator on subsequent recirculation, which is only partly overcome by rapid arrest of the circulation following injection. Although quoted as providing reproducible results, (Sapirstein 1958), errors of up to 10% have been estimated (Delaney 1966). This method has never-the-less been used to provide valuable information on the effect of pancreatic hormones on blood flow (Delaney 1966, Gilsdorf 1965, Goodhead 1970) and was really the fore-runner of the microsphere method.

ii. Hydrogen gas:

A variation of the above method has been to measure the rate of clearance of an indicator from the pancreas. Inhaled hydrogen gas is allowed to reach a steady state maximum concentration in the pancreas. The rate of clearance is then measured using implanted platinum electrodes. These combine with the tissue hydrogen to produce an electrical current which is proportional to the hydrogen concentration (Aune and Semb 1968).

The major drawbacks with this method are that only *relative* blood flow values are obtained, there is considerable variation in blood flow from different parts of the gland and it is technically difficult. The method was not widely used.

iii. Xenon gas:

In a similar way, the clearance of injected radioactive Xenon gas into the pancreas (Bor 1974) or gastroduodenal artery (Glazier 1974) has been used to calculate pancreatic

blood flow. Again only a relative measure of blood flow is obtained and it is not possible to obtain a value for total organ blood flow.

iv. Microspheres:

Introduced by Rudolph and Heymann in 1967, insoluble, radioactively labelled carbonised microspheres of uniform size (3M Corp.) have been used to great advantage in the measurement of total and regional organ blood flow.

In order to calculate flow to individual organs using indicator transport methods, it is necessary to be able to measure the amount of indicator passing through that organ. By selecting microspheres of 15 micron diameter which are all trapped in the capillary circulation (Fan 1979), calculation of the indicator passing into each organ simply becomes a matter of counting the microspheres trapped within the organs. There is not a problem with recirculation nor any need to arrest the circulation in the sudden manner employed by Sapirstein (1958) and Delaney (1966), making the subsequent calculation of blood flow extremely accurate.

With the femoral artery cannulated and connected to a syringe mounted on a withdrawal pump, an artificial reference organ of known flow (**F_1**) is created. Counting the number of microspheres in the syringe (**Q_1**) allows total or regional pancreatic blood flow to be measured without the necessity to calculate cardiac output.

From the following expression ;

$$F_2, F_3, ...F_n = Q_2, Q_3, ... Q_n \times \frac{F1}{Q1}$$

can be derived the following,

$$F_{panc} = Q_{panc} \times \frac{F1}{Q1}$$

Moreover, by using two sets of microspheres with different radio-nuclide labels, Kaihara (1968) demonstrated that two separate blood flow experiments could be carried out in each animal. This means that a blood flow measurement (control) can be made prior to administration of a test substance (e.g. alcohol). As each animal acts as its own control, subsequent statistical analysis of the results is thus simplified.

Technical details of the microsphere method are described in greater detail in chapter 5.2

3.3 Venous outflow :

The collection and measurement of venous outflow of an organ appeals as a simple method of blood flow estimation and has commonly been applied to measurement of pancreatic blood flow.

Anrep (1916) and Barlow (1968), simply collected the venous outflow in a drip-chamber (the so called "bucket-and-stopwatch" method), while Bennett (1933) and Kuznetsova (1962) employed more complicated "stromuhr" recording devices and Lenninger used a photo-electrical cell connected to a drum recorder.

The major problem in the application of these techniques to the measurement of pancreatic blood flow is the diverse venous drainage of the gland which parallels its arterial supply. The approaches which have been used to overcome this problem involve extensive surgical manipulation to ligate the draining veins so as to channel all of the

venous drainage into a single vein. The methods include volumetric estimation of blood flow out of;

1) The superior pancreatico-duodenal vein (Mandelbaum 1969) or splenic vein (Lenninger 1973) following ligation of all other veins draining the pancreas and spleen
2) splenic vein following ligation of the vessels connecting the pancreas and duodenum plus splenectomy (Jarhult 1977)
3) splenic vein following the complete isolation of the pancreas with in-vitro perfusion of the gastro-duodenal and splenic arteries (Coddling 1977, Vaysse 1977, Saharia 1977).)

All of these techniques by necessity result in a major disturbance, (if not complete destruction) of the perivascular nervous supply of the gland. With isolated in situ perfusion of the gland, there is the added disadvantage that blood flow can only be expressed as a change in the peripheral resistance of the gland.

3.4. Electromagnetic (EM) flow-meters.

The description and use of these instruments is reviewed in detail by Schenk (1967).
In summary their use is based on the principle that movement of an electrical conductor through a magnetic field induces a voltage across the conductor which is proportional to the number of magnetic lines of force cut per unit time. A moving stream of blood is the conductor (by virtue of its ionic concentration) and the magnetic field is contained in an

external assembly which encircles the vessel under study. This also contains electrodes for detecting changes in the current generated and its circular shape is used to maintain a fixed vessel diameter. The probes are quite small and have the advantage that they can be implanted, thereby providing in vivo, continuous recording of blood flow. The disadvantages of the electromagnetic flow-meter technique are that a reference value is needed at regular intervals to calibrate the flow-meter and that the orientation of the probe in relation to the vessel is critical. These are easily overcome in experiments where the vessel being studied is exposed and can be occluded to provide reference zero values, but can lead to gross inaccuracies in experiments involving implanting of the electrodes in live experimental animals.

Once again there is the recurring problem of multiple arterial supply and venous drainage of the pancreas, with isolation of the blood supply from the duodenum and spleen involving extensive dissection and possible damage to the autonomic nerve supply of the gland. Studley (1985) has estimated that blood flow using these instruments may be overestimated by up to 35%.

While EM flow meters provide direct flow measurements, these values are expressed as mls./minute and while it is therefore possible to calculate percentage changes in blood flow, it is not possible to make comparisons with studies quoting blood flow as mls./min./100 grams of tissue.

Never-the-less, valuable information on the rapid effect of glucose (Fischer 1976) and secretin (Eichelter 1966, Beijer 1979) on pancreatic blood flow has been obtained by the use of electromagnetic flow meters and they remain the only effective method to date to provide continuous readings of blood flow.

3.5. Miscellaneous methods:

a. Plethysmography:

One of the earliest methods employed, the underlying principle of the technique is that an increase in blood flow to an organ leads to an increase in the blood volume of the organ and that this change can be detected by monitoring the changes in total organ volume. This is most commonly accomplished by enclosing the organ in a rigid fluid-filled, vessel or jacket to which is connected a device capable of recording volume changes (Brodie 1905). The method provides a means of indirectly assessing changes in blood flow and is well suited to the measurement of limb blood flow. Its application to the measurement of pancreatic blood flow requires extensive surgical mobilization as well as ligation of the pancreatic duct and in addition the consistency of the pancreas does not transmit of volume changes well (Tankel 1957).

Apart from the early reported measurements of pancreatic blood flow by Francois-Franck and Hallion in 1896 (cited by Tankel 1957), Edmunds (1909) and Anrep (1916), plethysmography has not featured prominently in the modern study of pancreatic physiology.

b. Light conduction:

Based on the relation between the quantity of blood in the tissue and the absorption of light by tissue, this method was used to estimate changes in pancreatic blood flow in

cats by mobilizing the pancreas and mounting it between a light source and a detector (Holton 1960). The pancreas is of course not completely translucent and diffraction of light passing through the organ complicates interpretation of the results which appear to be little more objective than the direct observation of the pancreas made by Claude Bernard.

c. Electrical conductance.

The extracellular fluid of the pancreas contains ions and therefore will conduct an electrical current between electrodes. Changes in blood flow will affect the volume and content of the extracellular fluid and the resultant change in conductance has been used as an indirect measure of blood flow changes (Barlow 1968).

d. Heated thermocouples.

Instead of electrical conductance, Papp (1966), used heated thermocouples to measure changes in extra cellular fluid ion concentration and indirectly, pancreatic blood flow. Once again, results are entirely relative and must be interpreted with great caution.

e. Histological studies.

A method of rapid freeze drying of the pancreas to provide sections for histological analysis has been described (Richins 1953). The vascular pattern and in particular the degree of capillary filling was used as a indication of blood flow but no correlation with other methods of blood flow is given and there have been no further reports of the method.

f. Ex-vivo perfusion.

In this method described by Vayesse (1977), the isolated pancreas is perfused with heparinised blood and changes in pancreatic resistance estimated. These changes are used as an indicator of pancreatic blood flow but extrapolation to the situation in the intact animal must be made with great caution. A similar method was used by Takeuchi (1974) who tested the effect of over 60 biogenic and pharmacologic agents (but curiously not alcohol).

PART 2. METHODS :

RADIOACTIVE MICROSPHERE MEASUREMENT OF BLOOD FLOW:

Introduction ;

Radio-active tracer microspheres of 15 micron diameter (3M Corp.) were used in this study to measure blood flow according to the method described by Hales (1974). Introduced by Rudolph and Heymann (1967), the method is a variation of the Fick principle, whose mathematical derivation has been outlined in the previous chapter.

The following chapters present in detail the experimental method used in this study to measure pancreatic and gastro-intestinal tract blood flow in rats.

The methodology is covered in three parts ;

- Chapter 4 - the equipment
- Chapter 5 - preliminary steps
- Chapter 6 - details of experimental method

Chapter 4. The Equipment:

4.1 Introduction;

While the microspheres themselves were the key component of the method, a range of other equipment was used in the course of this study and is described in detail below. These include the polygraph machine used to monitor blood pressure and pulse rate, the withdrawal pump and agitator which were used during each blood flow experiment, the polythene scintillation tubes used to store organ samples and the analytical balance and gamma counter which were used to measure the weight and radioactive content respectively of the tissues under study.

Finally the personal microcomputers which were used for the calculation and storage of blood flow data and preparation of this thesis are described.

4.2. The Microspheres:

These are insoluble "carbonized tracer microspheres" and are available from the Nuclear Products Division of the 3-M Company, St. Paul Minn. USA. Their exact chemical composition has not been revealed, but they are known to consist of 75% carbon and 25% oxygen. Their specific gravity is 1.3 (c.f. 1.05 for whole blood) and they are available in sizes ranging from 3 to 50 microns with a variety of radionuclide labels. This radioactive label is incorporated into the plastic and therefore the radioactivity or to be more precise, the number of counts per minute (c.p.m.) of a given sample of microspheres will be related to sphere volume. The microspheres are uniform in size, thus the total radioactivity of a sample of spheres is directly proportional to the

number of spheres in the sample. This property of the microspheres is crucial to their use in the measurement of blood flow.

The microspheres used in this study were of 15 microns diameter and labelled with either Cerium (Ce^{141}) or Strontium (St^{85}), as follows:

Table 1. Radionuclide Properties

PROPERTY	**RADIONUCLIDE**	
	Ce^{141}	St^{85}
Half-life (days)	32.5	64.7
Principle radiation keV	145	513
Radiotoxicity group	Class 3	Class 3
Activity of bulk supply (mCi)	1	1

They are delivered in multiple injection vials suspended in a 10% Dextran solution with 0.19% by volume polyoxyethylene 80 sorbitan mono-oleate (Tween 80), a detergent added to the solution to prevent aggregation of the microspheres.

4.3 Arterial and Venous Cannulae:

In order to carry out the blood flow measurements, it was necessary to establish the following arterial and venous access ;

- Femoral artery for blood pressure monitoring
- Femoral artery for “reference organ”
- Femoral vein for infusion of alcohol etc
- Left ventricle via carotid artery for injection of microspheres

Vessels were cannulated with single lumen polythene tubing with an outer diameter of 0.96 mm. (Dural Plastics and Engineering, Dural N.S.W. cat. no. SP 45). One end of the cannula was stretched to provide a gentle bevel to assist with cannulation of the vessel, while a 23 gauge (0.63 x 32 mm.) luer lock needle was inserted into the other. To this was attached a three-way stopcock (figure 4.1). The cannula, needle and stopcock were all flushed with heparinised saline (1,000 units in 10 mls.) to expel air and to prevent coagulation of blood. The instruments used to expose the femoral vessels are displayed in figure 4.2. A stopcock, needle and cannula assembly is seen at the left of the figure.

4.4 Monitoring equipment:

Arterial blood pressure was monitored by cannulation of a femoral artery which was connected to a Grass Model 5D Polygraph recorder via a Statham transducer, (0-75 cm. Hg.)

This was calibrated against a mercury sphygmomanometer at the start of each blood flow experiment and was then used to ensure that each of the arterial cannulae were in suitable position and that the lines did not contain air (which resulted in damped arterial traces). It was also used to detect passage of the carotid cannula through the aortic valve into the left ventricle.

In figure 6, the normal arterial trace, fluctuating from a systolic pressure of approximately 150 mm Hg to a diastolic of 90 mm Hg is seen to change to a left ventricular trace characterised by a diastolic pressure of close to zero as the cannula passes into the left ventricle.

4.5 Injection / Withdrawal pump

A Braun corporation pump was used for infusion of test substances.

By mounting a syringe on the pump, it was possible to infuse the alcohol and glucose solutions intravenously.

Once the infusion was completed a further syringe was mounted and the pump used to withdraw blood at a given rate, thus establishing the so called reference organ.

4.6 Scintillation counting tubes:

Polythene vials (Beckman Scientific Insert Vial BGC5C) were used These were of 5ml. capacity, 55mm in length with a diameter of 12mm and had screw top lids. Prior to each experiment, approximately 40 tubes (with their lids in place) were numbered and then weighed on a Mettler H10T analytical balance to an accuracy of 0.0001 grams. After each blood flow experiment, tissue samples were collected and loaded into appropriately labelled tubes. These were then centrifuged at high speed for 2 minutes to ensure that all tissue was compacted into the bottom of the tube, thus leading to more accurate measurement of the radioactivity level.

As a final check, the tubes were re-weighed prior to loading into the gamma counter.

4.7 Gamma counter:

A Searle Nucleonics gamma counter was used which allowed for automated counting of large numbers of tubes. Counting was more accurate in the depth of the well, hence the selection of short polythene tubes and the need for centrifugation of the contents.

4.8 Microcomputer:

A Wang personal computer was initially used during the course of experiments in 1984 to store experimental data (Microsoft Multiplan) and to calculate blood flows using a programme written in Basic. This pc also had a word processing facility which was used for initial preparation of this thesis.

The final draft was edited using the latest version of Microsoft Office Word.

Chapter 5. Preliminary Steps:

5.1 Experimental animals:

A small colony of Portland rats was purchased and a breeding programme established. The animals were kept in cages containing up to 15 rats and had continuous access to dry pellets and water. The building was air-conditioned to provide a stable temperature of 20 degrees C and lighting was controlled to provide alternate 12 hour periods of light and dark.

5.2 Quality control of the microspheres:

The manufacturer (3M Corporation) guarantees that the variation in size of the microspheres will be less than ± 5 microns and that there will be a minimum of irregular or damaged spheres. This was verified in the preliminary experiments (vide infra).

5.3 Microsphere standard:

The application of microspheres to blood flow measurement is based on the fact that the radionuclide label is incorporated into the spheres and because of their uniform size, each sphere has the same radioactive content. To calculate the number of spheres present in each organ of study, it is simply necessary to measure the total radioactivity of the organ, and then divide by the count per unit sphere (c.p.s.). While the c.p.s. at the time of manufacture may be known, there will be continual loss of radioactivity with time and the c.p.s. must therefore be calculated each and every time a blood flow measurement is made. To do this, a sample containing an exact number of spheres is

required. The radioactivity of this "Standard" sample can then be measured and division by the number of spheres provides the current c.p.s.

The preparation of described in the following chapter.

5.4 Calibration of gamma counter:

Prior to carrying out the double sphere experiments, the radioactivity profiles were graphed for each of the two radionuclide agents (Cerium and Strontium) and settings selected to provide minimum overlap. For the single sphere experiments, a setting providing maximum sensitivity was used.

5.5 Laboratory :

Partly for radiation safety purposes and partly for convenience, a dedicated laboratory was used for the duration of the project. An appropriately labelled cupboard was used to store the microspheres and a separate set of instruments which were used to dissect radioactively contaminated animals. The laboratory contained a table covered with disposable plastic sheeting, and had facilities for washing instruments as well as for laboratory personnel. Other equipment kept in the laboratory throughout the duration of the project included the Polygraph recorder and withdrawal pump.

5.6 Radiation safety:

The laboratory used for storage of the microspheres and the preliminary experimental procedures such as weighing of the plastic tubes as well as the animal theatre where the experiments were carried out, were under the control of the Radiation Safety Officer of

the University of Adelaide. Strict safety precautions were employed including the use of surgical gowns and plastic gloves when handling the microspheres and the animals. Throughout the course of the study, all staff wore radiation surveillance badges.

Chapter 6. Details of Experimental Method:

The major steps involved in each blood flow measurement experiment were the preparation and anaesthesia of the animals, preparation and injection of the microspheres followed by removal and study of organs of interest. Calculation of blood flow was carried out and the radio-active tissues then disposed of.

Details are as below.

6.1 Preparation of the animals:

Adult rats of either sex were used. Animals smaller than 250 gms. were avoided because in these, cannulation of the femoral arteries and veins proved too difficult. The animals for an experiment were selected and the weight written on their tails.

Anaesthesia:

After being weighed, the animals were transferred to the laboratory, where anaesthesia was induced by intra peritoneal injection of 0.12 ml / 100 gm. body weight of a 60 mg% solution of pentobarbitone. Disposable, sterile 1ml. tuberculin syringes and 26 gauge needles were used to prevent any possible contamination of the stock solution. The injection was made into the left iliac fossa in order to avoid the caecum and was followed by complete anaesthesia within 5 minutes. In most cases anaesthesia lasted for 60 minutes.

Respiration was maintained by spontaneous ventilation and apart from initial retraction of the tongue to prevent gagging, no special measures were necessary.

Over the period of study, approximately 5% of the rats died from an apparent sensitivity to the anaesthetic and a similar number failed to reach a satisfactory level of anaesthesia, the majority being due to anatomical causes such as injection into the bowel, abdominal wall or into a retracted testicle.

Once anaesthetised, the groins, abdominal wall and neck were shaved and the animal positioned supine on a plastic covered cork board. The legs were taped down to provide access to the groins and neck.

Vascular access:

Exposure of the femoral vessels in the groins and the carotid artery in the neck was carried out with a combination of sharp and blunt dissection using fine ophthalmic forceps and scissors shown in figure 4.2.

Approximately 5mm of the femoral artery just distal to the origin of the inferior epigastric artery vessel was exposed and two lengths of fine 3 0 black silk surgical threads passed under as slings. Traction on the slings proximally and distally allowed the exposed section to be effectively “clamped” while a small transverse incision was made with a scalpel to facilitate cannulation (figure 4.3).

The tapered end of a length of polythene tubing was then introduced and gently advanced as the pressure on the proximal sling was released.

Once sufficiently far advanced, the sling was tied, thus anchoring the cannula in place. Figure 4.4 shows the tie being secured. The tip of the index finger is seen securing the silk tie and reinforces the small size of the vessels.

The distal sling was tied, the cannula taped to the cork board and cannulation was complete (figure 4.5).

This process was repeated until both femoral arteries and one femoral vein was cannulated. In figure 4.6 all vessels are cannulated. The right femoral artery cannula is connected to the Statham transducer, the left femoral vein cannula to the syringe mounted on the infusion pump (the artificial or "reference " organ) while the right carotid cannula remains clamped.

The right femoral arterial cannula was connected via the transducer to the Grass machine for cardiovascular monitoring throughout the experiment. Figure 4.7 shows calibration of the femoral artery trace.

In the neck, an incision was used to expose the common carotid artery. The whole carotid sheath was exposed and then the artery carefully isolated from the vagus nerve. Cannulation proceeded as for the femoral arteries except that the cannula was connected to the polygraph machine and then carefully advanced until the resistance of the aortic valve was felt. With gentle rotation, the cannula could be made to pass through the valve into the left ventricle. In figure 4.8, the normal arterial trace, fluctuating from a systolic pressure of approximately 150 mm Hg to a diastolic of 90 mm Hg is seen to change to a left ventricular trace characterised by a diastolic pressure of close to zero as the cannula passes into the left ventricle.

Monitoring of vital signs

Once the right femoral cannula was connected via the transducer to the polygraph reorder and calibration complete, the rate of the recorder was set to 10mm per second, resulting in sufficient expansion of the blood pressure trace to allow the accurate measurement of pulse and respiratory rate (figure 4.9).

Continuous blood pressure monitoring during the blood flow experiments was used to ensure that injection of the spheres caused no major physiological disturbance.

In figures 4.10 and 4.11, “W on” and “W off” indicates the points at which the withdrawal pump was switched on and off and “Inj. on” and “Inj. off” the duration of alcohol and/or glucose injection.

It can be seen that during the experiment from which figure 4.10 originates, the blood pressure and pulse rate remained steady throughout, while during the experiment depicted in figure 4.11, a major drop in blood pressure occurred at the time of injection leading to its exclusion.

This occurrence was found to be particularly common in rats weighing less than 250 gms. presumably because of the small diameter of their femoral arteries.

Gastric cannulation:

It was found that passage of an oral gastric cannula in the anaesthetised animals resulted in great respiratory distress to the animals and the injection of even small volumes of fluid into these tubes was accompanied by reflux up the oesophagus and into the lungs with dire consequences. A technique was thus devised in which the cervical oesophagus was isolated on the left side of the neck (through the same incision used to isolate the carotid artery) and the upper end ligated with fine silk. A small transverse opening was made with iris scissors and the cannula threaded down into the stomach and then secured with a further silk tie. This procedure was easily performed and with care to avoid damage to the vagus nerve, resulted in no changes in the animal's vital signs.

Intravenous injection:

The alcohol was injected intravenously by infusion, using a 1.2 ml. tuberculin syringe mounted on the Braun perfusion pump. The usual rate employed was 0.1 ml. per minute.

6.2 Preparation and injection of microspheres:

Microspheres:

After preparation of the animal, the vial containing the microspheres was removed from its protective lead container and thoroughly agitated using a mechanical agitator. One drop was then withdrawn and injected into a small petri dish. One ml. of saline followed by 1 ml. of microsphere solution was then drawn into the injection apparatus, consisting of a glass mixing chamber with silastic tubing on each side.
To one length of tubing was attached a drawing up needle (19 gauge) and to the other a 2ml. syringe. Figure 4.12 shows the vial containing the microspheres, the petri dish in which the spheres and saline were mixed and the mixing chamber with its attached syringe. Figure 16 shows a close up view of the mixing chamber.

This technique of drawing up saline followed by the microspheres resulted in the microspheres being in the glass chamber with a "plunger" of saline in the syringe and tubing (figure 4.13).
Figure 4.14 shows the process of preparing the glass chamber injection apparatus. Once so prepared, it was carried over to the operating table, positioned at the head of the board and the needle inserted into the carotid artery cannula. The rubber shod

haemostat used to clamp the cannula was then removed and the glass mixing chamber applied to the agitator until the time of injection.

The injection of microspheres was carried out by hand over approximately 30 seconds, following which the carotid cannula was re-clamped.

Reference organ:

The Braun perfusion pump which was used to infuse the alcohol solution was then used to apply suction to a 2ml. syringe connected to one femoral artery cannula (figure 4.15). Again, the three-way stopcock was removed and replaced with a No. 23 gauge needle and a haemostat was used to provide occlusion until commencement of the experiment.

Injection and withdrawal:

Once the injection apparatus and withdrawal pumps were connected, the microspheres were thoroughly mixed, and the Polygraph machine was switched to a recording rate of 5cms/second to allow easy detection of fluctuations in blood pressure. The clamp on the femoral cannula was removed and the withdrawal pump switched on. The time was marked on the pressure tracing ("W on", figure 4.10) and a period of 15 seconds allowed following which the microspheres were injected (“Inj. on”). Once the injection was completed (“Inj. off”) the pump was run for a further 15 seconds before being switched off (“W off”).

As previously indicted, experiments in which a major drop in blood pressure occurred immediately after the injection of microspheres (such as seen in figure 4.11) were rejected.

6.3 Preparation of samples for counting*:*

At the completion of the experiment, the animal was killed with an over dose of Pentothal given intravenously and the cannulae disconnected from the Polygraph recorder and withdrawal pump and tied. The injection apparatus was flushed with saline and returned to the radioactive cupboard and the withdrawal pump removed from the operating table. Samples were then collected as follows:

a. Pancreas:

Using a special set of instruments (which were kept in the "radioactive" cupboard), a midline incision was made and the pancreas the spleen were delivered from the abdominal cavity (figure 4.16). The spleen was removed by dividing its connections to the pancreas and stomach. The pancreas was then grasped at its distal end and its superior and inferior borders freed. Finally its connections to the duodenum were divided, any fat was trimmed and then the whole organ was then placed in a counting tube (figure 4.17).

b. Blood:

The reference syringe was removed from the pump along with its needle and cannula and the blood injected into a counting tube. Two millilitres of saline were then drawn into the syringe and injected into a second tube to flush out any remaining microspheres. This process was repeated for a second time to be absolutely sure that no

spheres were left behind . The four counting tubes were appropriately labelled (figure 4.18).

c. Other organs:

The whole stomach was removed and divided along the macroscopic junction between the fundus and antrum which were then placed in separate tubes after the contents were expelled. A 3-4 cm. length of duodenum, mid small bowel and distal colon were also removed for counting after removing the contents and mesentery. A portion of the liver and both kidneys were removed and placed in counting tubes. Other organs sampled were the testes and brain and samples were also collected of skin and muscle.

6.4 Calculation of blood flow:

Individual tubes were weighed, centrifuged and then placed in the gamma counter along with 3 empty tubes (background radiation) and the microsphere "standards". Pre and post experiment tube weights and radioactivity levels were then entered on computer using a programme written in Basic. (Background radiation was taken as the mean of the 3 empty tubes and was subtracted from all other readings).

Reference organ flow (mls./minute) was calculated from the volume of blood in the syringe (or weight, since specific gravity of blood is close to 1.0), divided by 1.25 (length of withdrawal in minutes). The number of spheres in the syringe was calculated from total syringe count of tubes 1, 2 and 3 (the blood plus washings) divided by the microsphere standard.

The total number of microspheres in each organ sample was then calculated in similar fashion, i.e. total organ radioactivity divided by microsphere standard.

Thus from the expression in chapter 3.2, if F1 and Q1 (reference organ flow and indicator content) are known and Q2,3....n have been measured flow was then calculated for organs F2, F3,...Fn from;

$$F2,3...n = \frac{Q2,3...n \times F1}{Q1}$$

and expressed as mls. per minute per 100 grams of tissue.

Left and right renal blood flows were compared and if they varied by more than 10%, uneven distribution was assumed to have occurred and the blood flow experiment was excluded. All data and results were then printed out after blood flow data was stored using Multiplan (a preliminary computerised spreadsheet of the Microsoft corporation), which was used to provide automatic updating of mean, standard deviation and standard error.

6.5 Disposal of radioactive tissue:

At the completion of the blood flow experiment, the remains of the animal, cannulae, plastic sheeting and scintillation tubes were sealed in large plastic bags which then stored in an appropriately labelled freezer for 3 months (slightly greater than 2 half lives) before being incinerated.

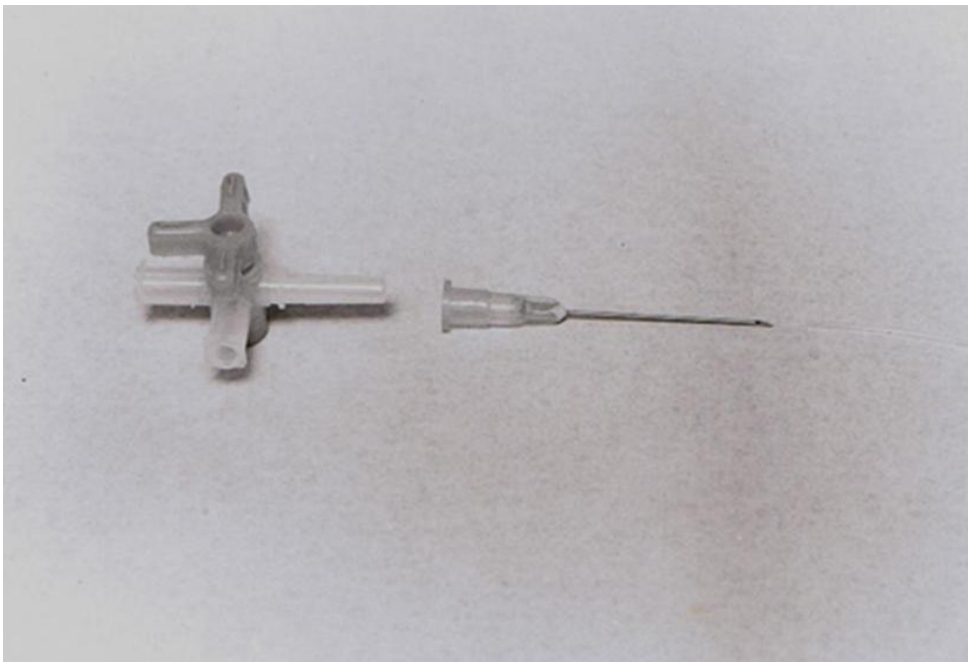


Fig. 4.1

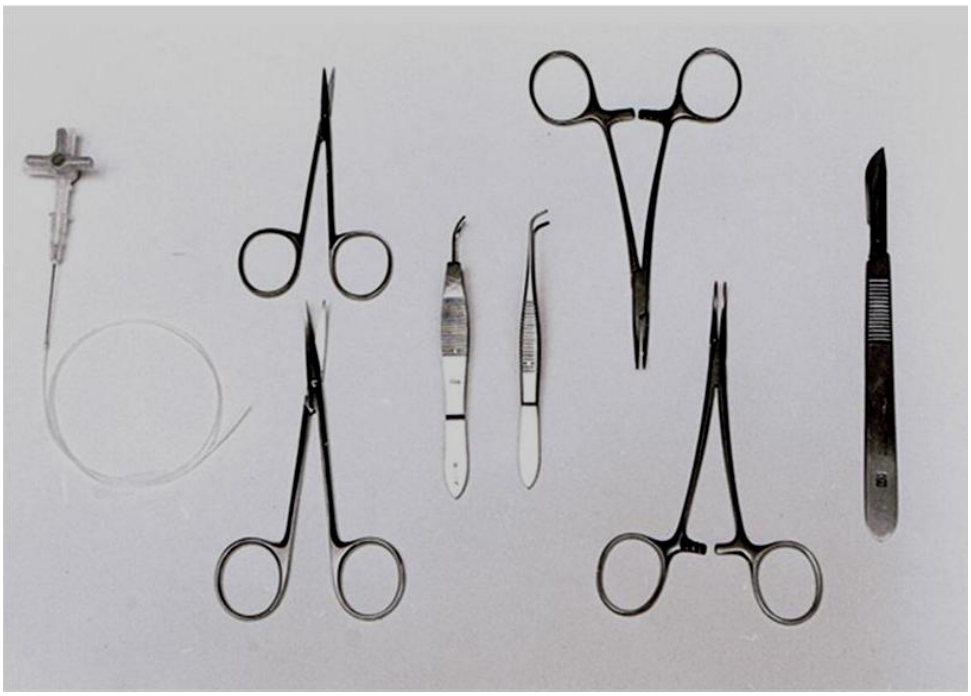


Fig. 4.2

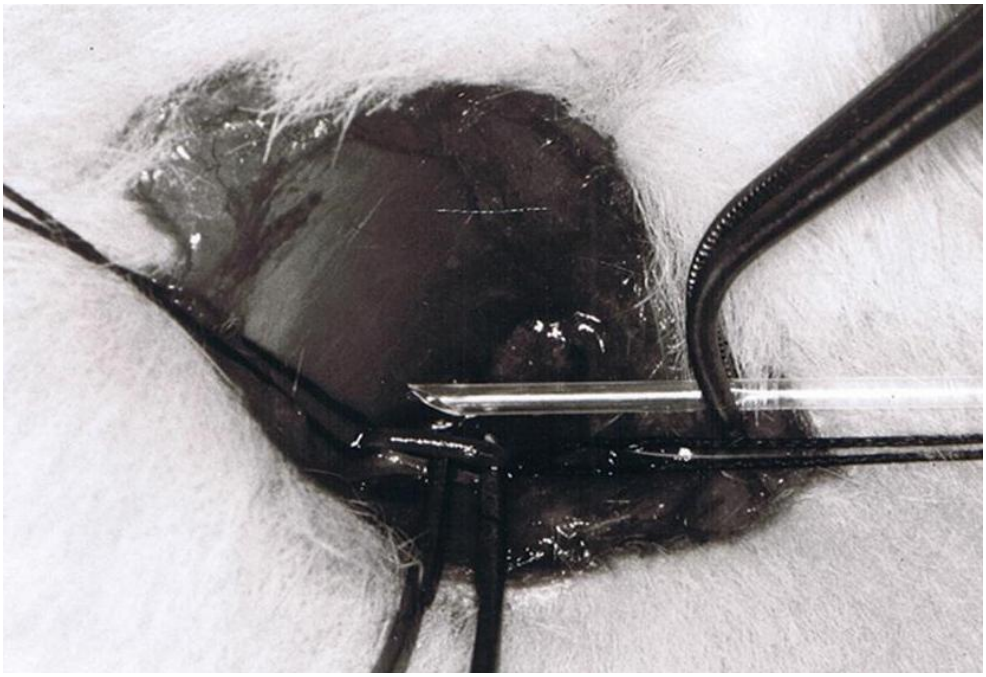


Fig. 4.3

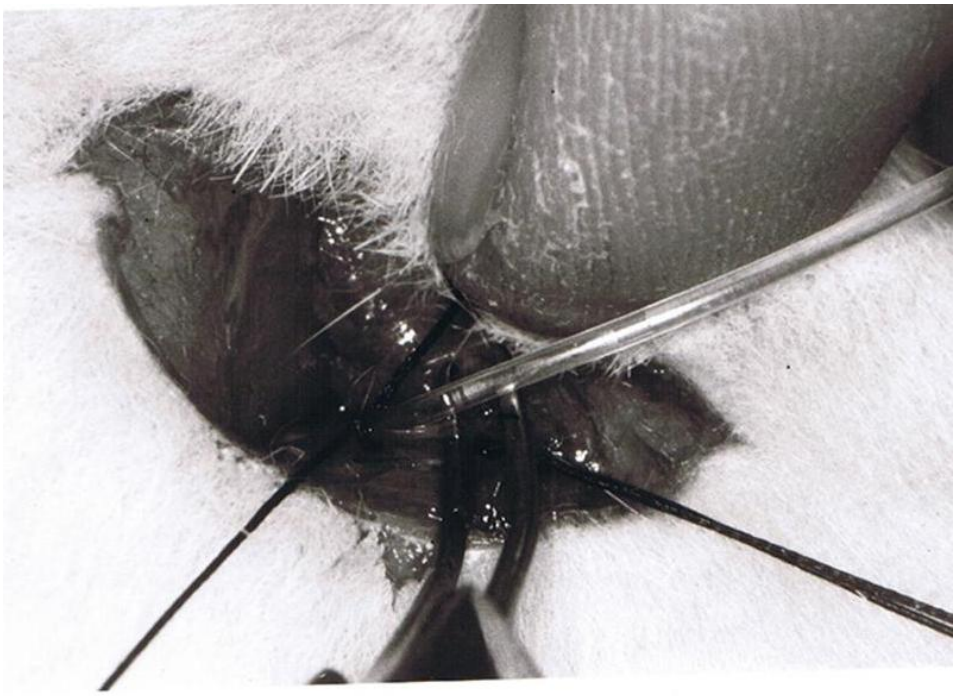


Fig. 4.4

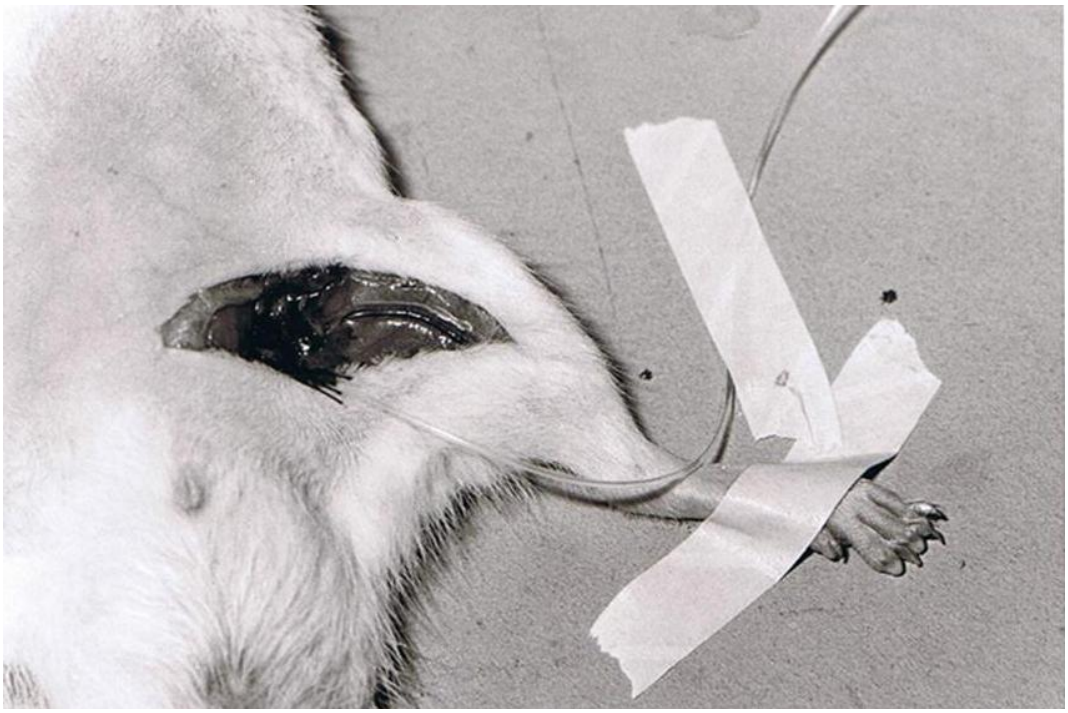


Fig. 4.5

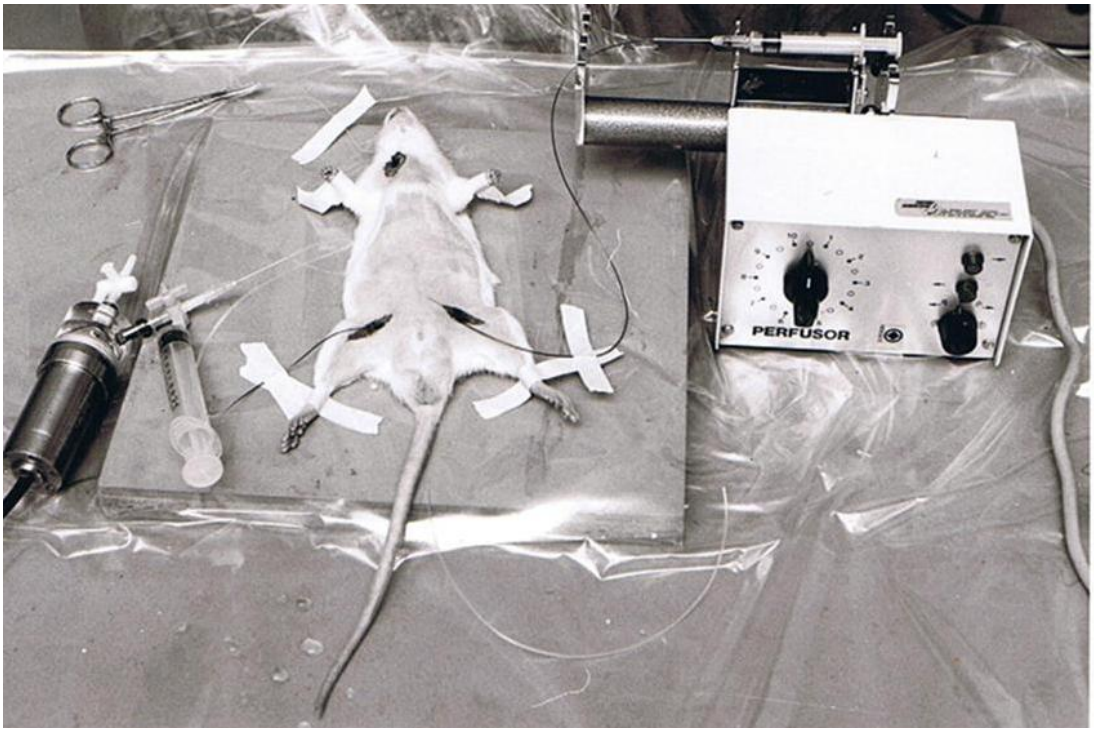


Fig. 4.6

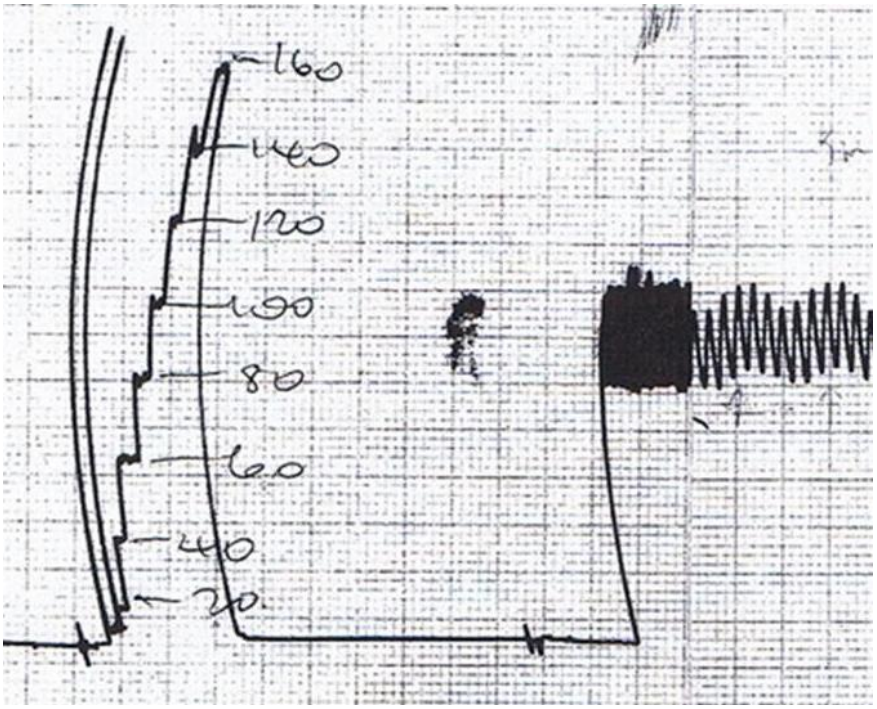


Fig. 4.7

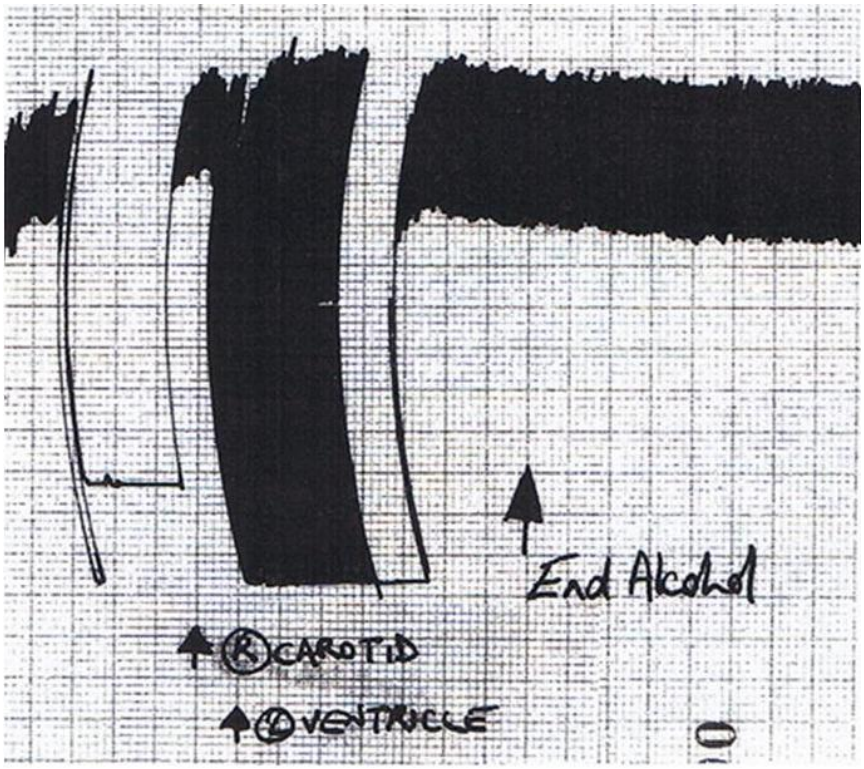


Fig. 4.8

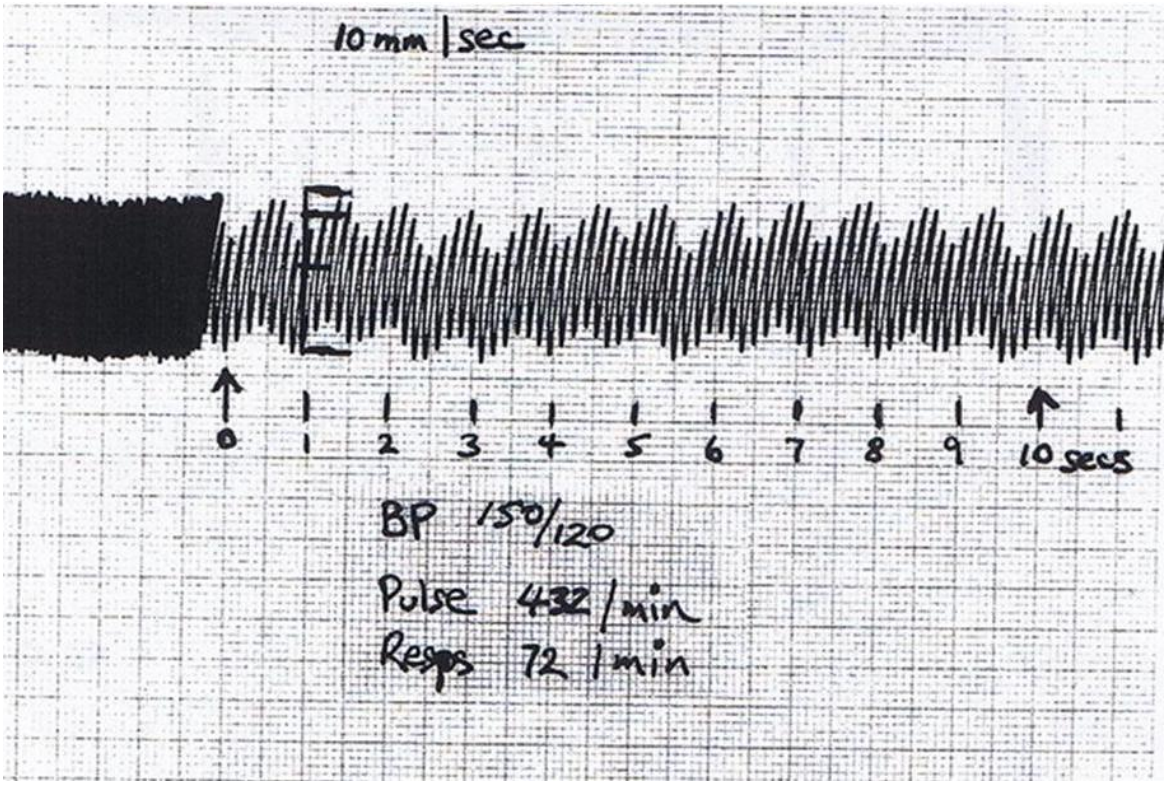

10 mm | sec
0
1
2
3
4
5
6
7
8
9
10 secs
BP 150/120
Pulse 432/min
Resps 72/min

Fig. 4.9

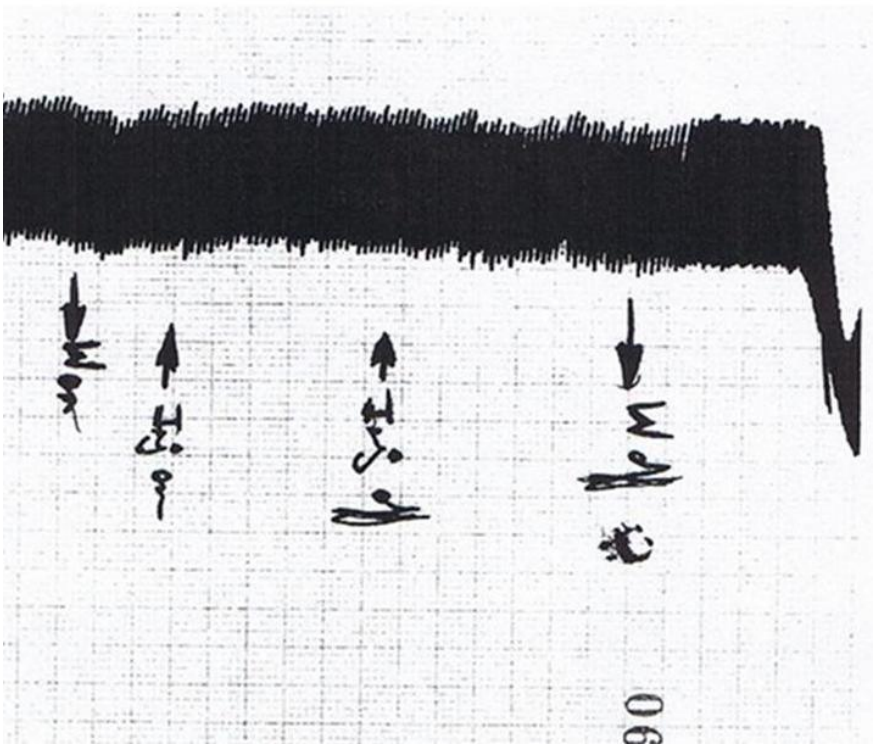


Fig. 4.10

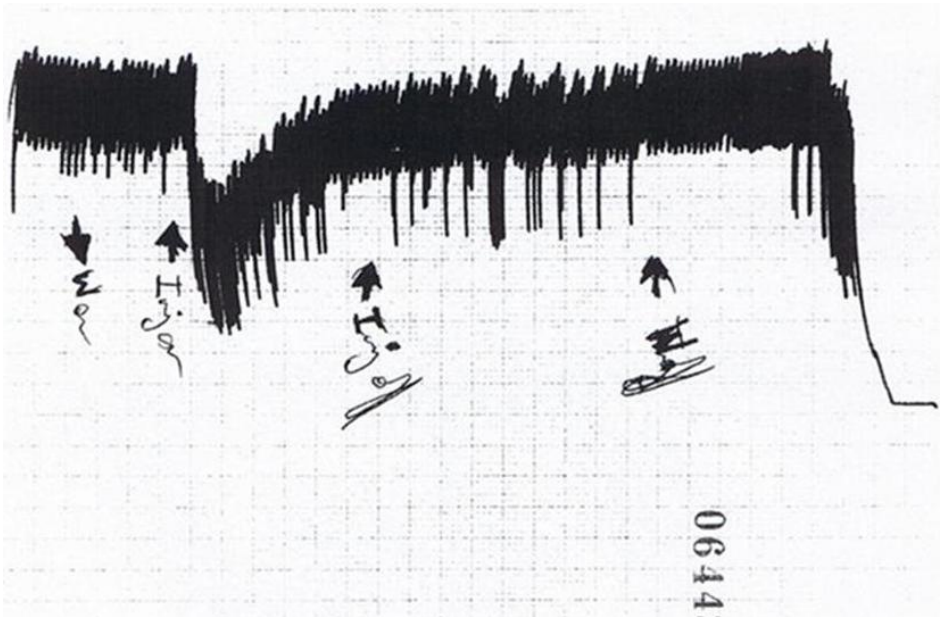


Fig. 4.11

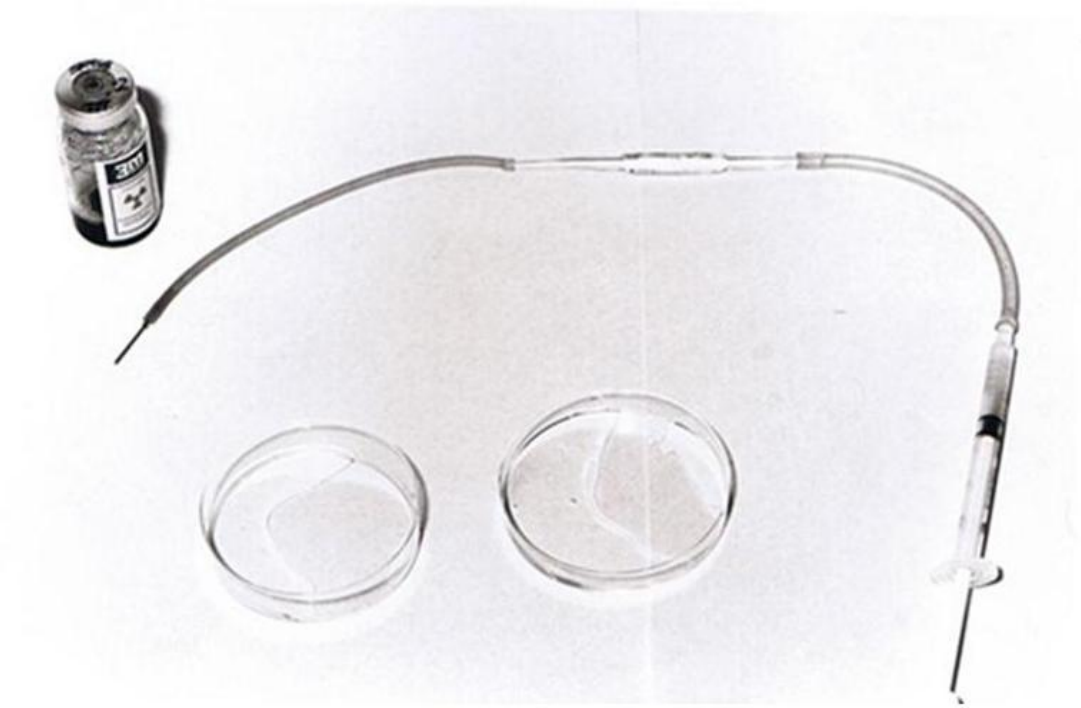


Fig. 4.12

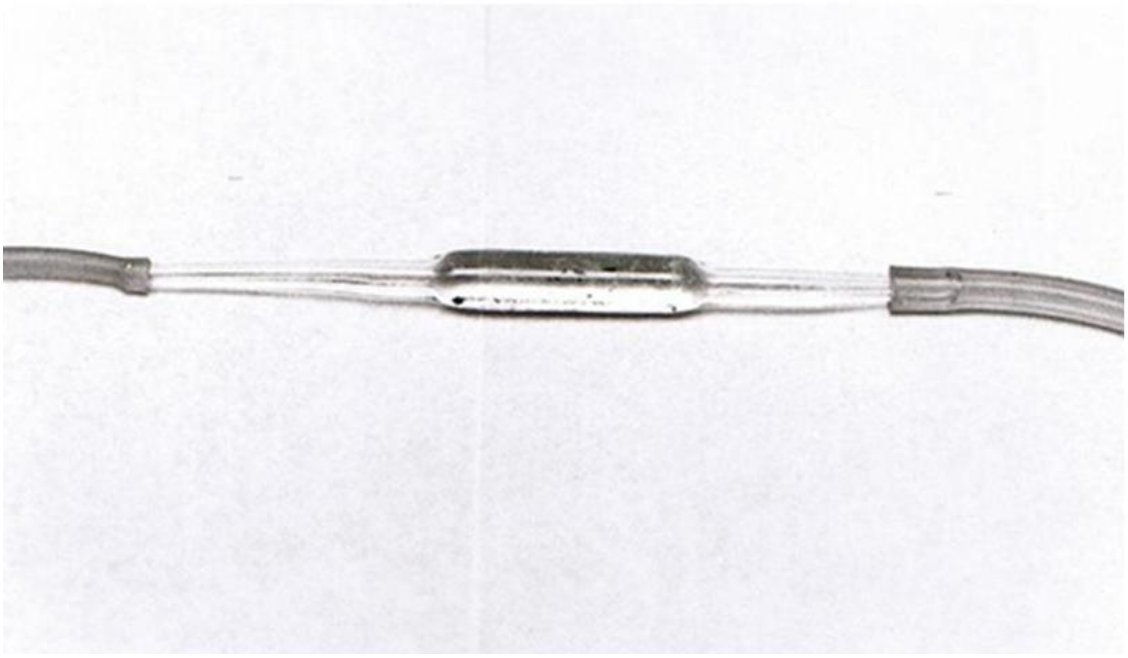


Fig. 4.13

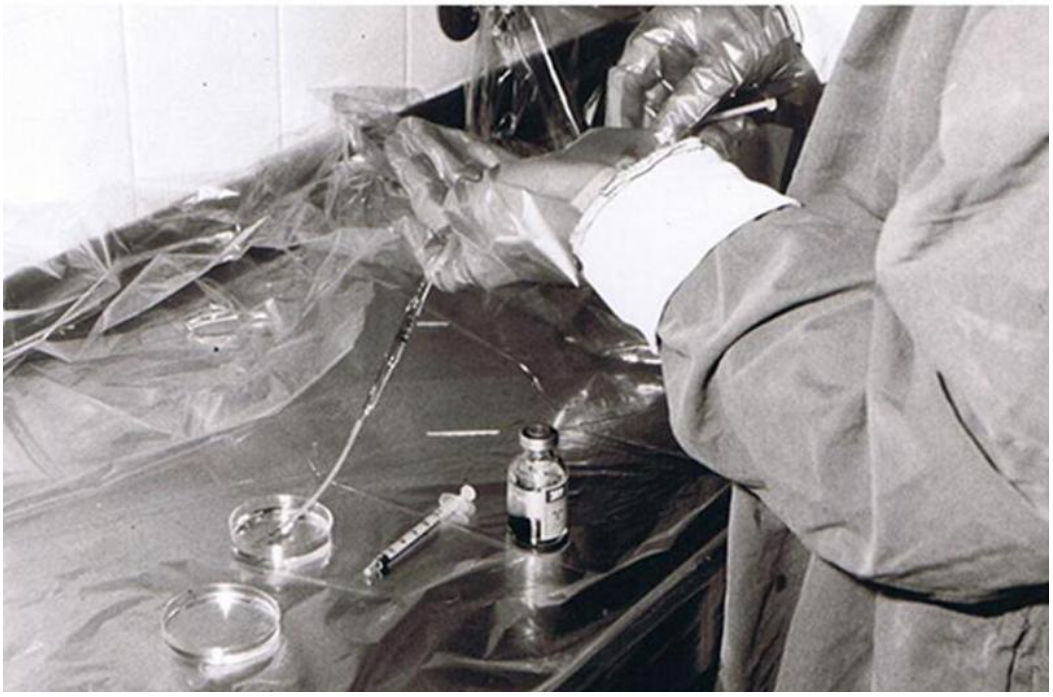


Fig. 4.14

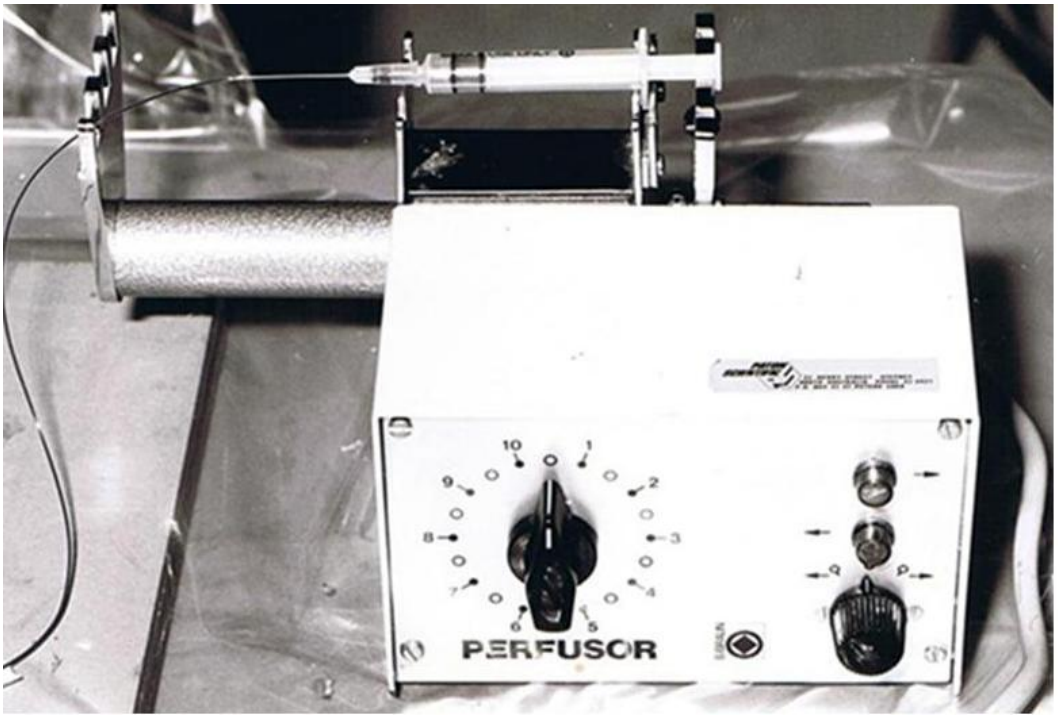


Fig. 4.15

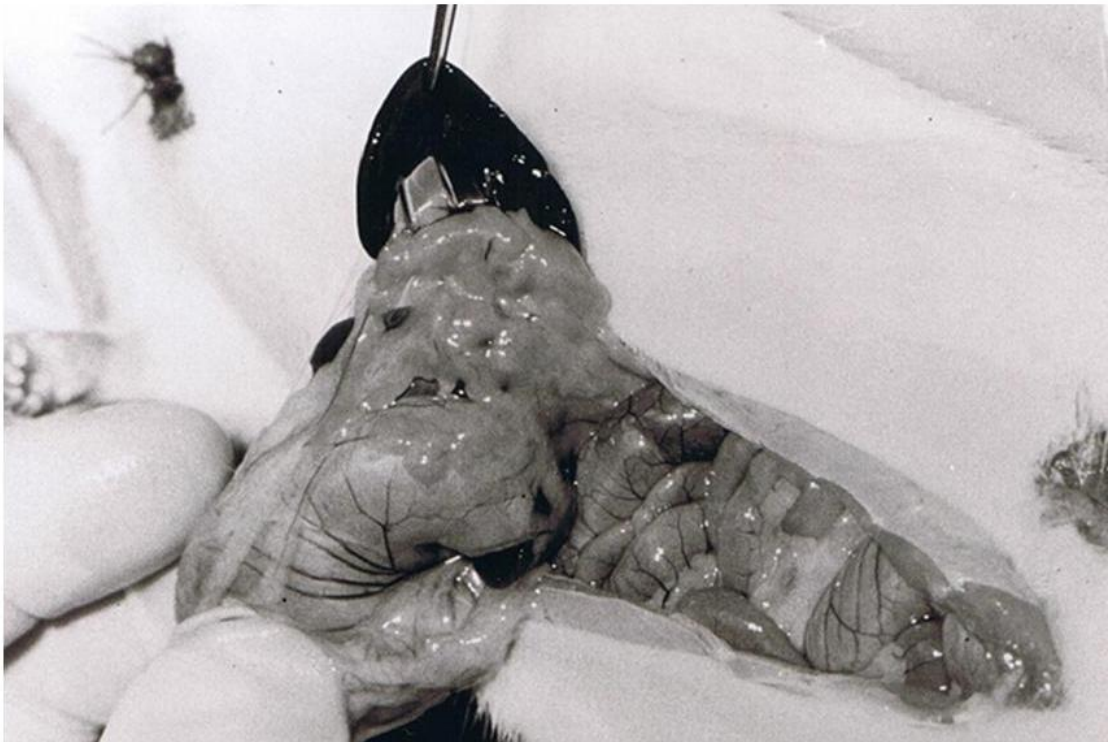


Fig. 4.16

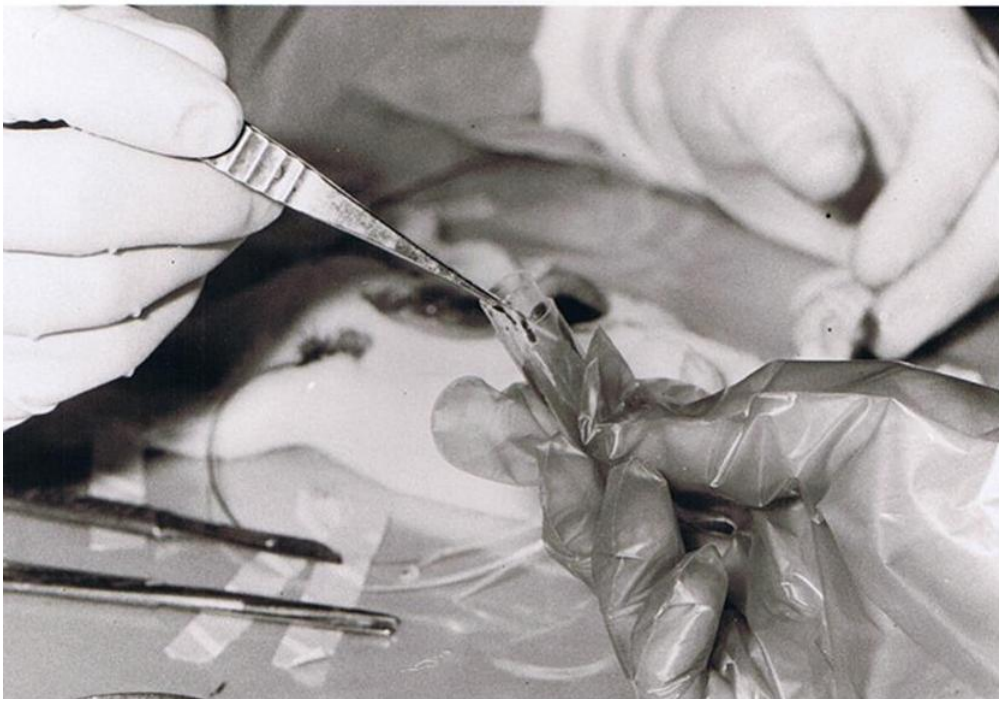


Fig. 4. 17

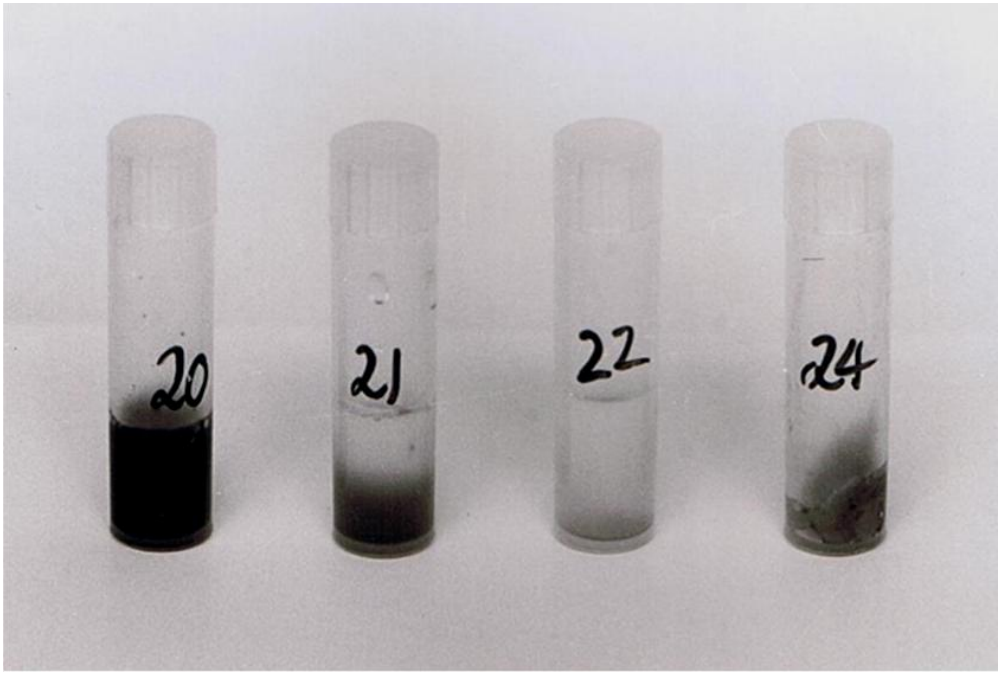


Fig. 4.18

PART 3. RESULTS:

INTRODUCTION ;

Results are grouped into four main chapters;

Chapter 7. Validation experiments

Chapter 8. Pancreatic blood flow

Chapter 9. Gastro-intestinal blood flow

The first, chapter 7, presents a set of experiments aimed at validating the microsphere method of blood flow measurement and includes a series of experiments related to blood alcohol and blood glucose levels which were carried out prior to the blood flow experiments.

Chapter 8 contains the results of pancreatic blood flow with and without alcohol infusions and is the main body of experimental work carried out in this project. Although not a specific aim of the study, the microsphere technique allowed simultaneous measurement of blood flow to the whole of the gastrointestinal tract and these are presented in Chapter 9.

The literature review did not specifically encompass visceral blood flow and detailed statistical analysis of these results was not undertaken.

This chapter could be regarded as a suggestion for further research.

CHAPTER 7. VALIDATION EXPERIMENTS.

Prior to carrying out blood flow experiments, a number of experiments relating to the microspheres themselves as well as the validity of the microsphere technique in our laboratory, were carried out. These are divided into those relating to the microspheres themselves and those relating to the blood flow measurement technique.

7.1 Microspheres

Experiments were carried out as follows;

a. Size and shape
b. Microsphere standard
c. Mixing
d. Recirculation
e. Effect on local tissues

a. Assessment of size and shape:

The aim was to test the manufacturer's claim regarding the size and shape of the microsphere supplied and to test for the presence of aggregation or clumping which would lead to inaccuracies in blood flow measurement by producing slides with sufficient numbers to examine (300 - 400) and with a distribution of spheres such that each slide did not have so much overlap as to make identification of individual spheres impossible.

Streaks of microspheres were made onto graduated (50 micron squares) ground glass slides and examination was then carried out by using high powered microscopic examination (x 400).

Six satisfactory separate satisfactory slides were prepared and examined and in none of these six separate slides was any clumping of the spheres seen. Only 4 - 5 small or irregular shaped spheres were found in the total of almost 2000 microspheres examined. Several micro-spheres were identified which were marginally smaller or

larger than 15 microns (figure 7.1) and in addition a similar number of irregularly shaped microspheres were found (figure 7.2). In all cases, the irregularities were of minor degree only.

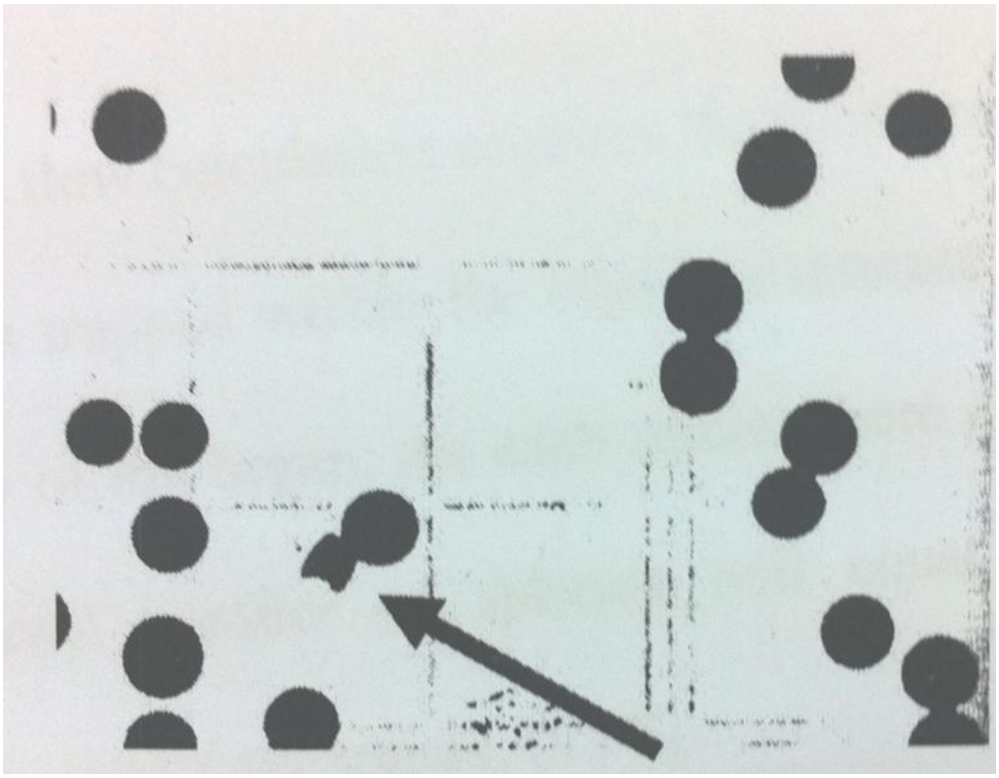


Fig. 7.1

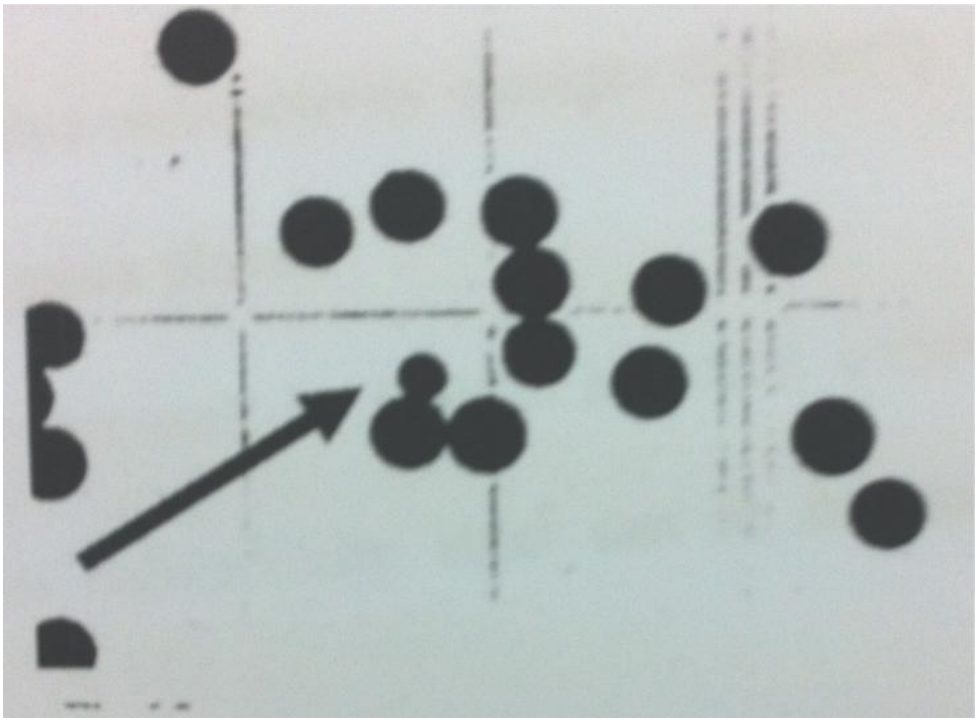


Fig. 7.2

b. Microsphere standard:

As described in chapter 2, blood flow calculation requires the accurate measurement of the total number of microspheres trapped within the organ of interest. This is done by measuring the total radioactivity of the organ. As each microsphere contains the same amount of radionuclide the total number of spheres will equal the total organ radioactivity count divided by the count per sphere, (CPS).

Two problems need to be overcome in measuring the CPS.

Firstly, it would be impossible to accurately and reproducibly measure the radioactivity of a single sphere and secondly, this radioactivity changes constantly due to the natural process of radioactive decay that takes place.

In practical terms it therefore necessary to have a standard collection of a known number of microspheres whose radioactivity level can be measured prior to each experiment being carried out.

A microsphere "standard" was prepared by placing a streak of microspheres onto a small piece of filter paper. This “standard” then contained a sufficiently large number of spheres to allow accurate measurement of its radioactivity, yet few enough to allow accurate counting. The exact number of microspheres on the filter paper “standard” was determined by counting under the microscope by three independent observers who each carried out the measurement on three separate occasions.

The strontium standard thus prepared had 824 microspheres whilst the cerium standard had 412 individual microspheres.

The filter papers containing the microspheres were then sealed in scintillation counter tubes. Calculation of the CPS was made by simply measuring the radiation count of the scintillation tube containing the spheres (standard), subtracting the radiation count of a blank scintillation tube (background radiation) and dividing by the number of spheres contained in the standard ;

$$CPS = \frac{(Count - Background)}{No.\ of\ Spheres}$$

The microsphere standards were used throughout the period of experimentation.

c. Adequate mixing

The aim of this experiment was to validate the first blood flow measurement assumption i.e. that injection of the microspheres into the left ventricle of the heart leads to sufficient mixing that the spheres are then distributed in proportion to the cardiac output. It was also used to test the validity of using comparison of right and left kidney blood flow as an indicator of satisfactory mixing.

A study similar to that reported by Buckberg (1971) was carried out in which the right and left femoral arteries were cannulated and connected to syringes mounted on withdrawal pumps. A blood flow study was then carried out in the standard way and the number of spheres in each syringe compared. Provided adequate mixing of spheres takes place in the left ventricle, distribution to each of the femoral arteries should be equal, with a difference of less than 5% .

Ideally, comparison of femoral artery flow should have been carried out in each experiment but in practical terms it was easier to use comparison of kidney blood flow to test for errors in distribution, with a difference of less than10% considered by Buckberg (1971) to exclude mixing errors.

Blood flow measurement experiments were carried out in 6 animals using bilateral femoral artery cannulation and results are presented as number of microspheres per ml./min. and the percentage difference between left and right. The number of microspheres per gram weight of each kidney is also presented along with the percentage difference in bold.

Femoral Artery Withdrawal Series.

Expt.	**Withdrawal rate** (mls./min.)		**Femoral arteries** No. of spheres per. ml/min			**Kidneys** No. of spheres per. ml/min		
	Left	Right	Left	Right	% diff	Left	Right	% diff
3	1.00	1.08	6,674	6,633	**0.6**	26,052	26,870	**3.1**
1	1.08	1.09	9,119	9,250	**1.0**	32,145	29,793	**7.6**
5	1.05	1.02	5,221	5,370	**2.8**	21,936	23,493	**6.9**
6	1.05	1.03	5,929	5,578	**6.1**	24,664	24,012	**2.7**
4	1.07	1.04	7,711	8,278	**7.1**	18,823	21,860	**15.0**
2	1.08	1.09	8,355	7,764	**7.3**	37,721	41,616	**9.8**

Table 7.1

d. Recirculation:

The aim was to test for lack of recirculation of microspheres, i.e. that there is complete trapping of microspheres by capillary networks, in particular those of the pulmonary circulation.

It was assumed in choosing 15micron diameter microspheres that none would pass through the capillary beds of the lungs.

A bolus of microspheres was injected into the femoral vein. The kidneys and viscera were then removed the radioactivity levels measured and the number of trapped microspheres thus calculated.

Three experiments were carried out. The first with a bolus of microspheres (1.0 - 1.5 x 10^{6}) comparable to that used in a blood flow experiment and the others with double and triple this amount.

On each occasion, only background radiation was measured in the kidneys and viscera, indicating that all the spheres had been trapped in the pulmonary capillaries.

e. Effect on local tissues:

To test the assumption that the microspheres are inert in vivo, histology of the viscera was carried out after injection with microspheres in the usual way. For purposes of radiation safety, specimens were refrigerated for 2 half-lives, prepared and stained with haematoxylin and eosin and then examined under the microscope for signs of acute inflammation.

In none of the prepared tissue were any acute inflammatory cells seen surrounding the microspheres.

Figures 7.3 shows a section of pancreas at a magnification of x200.

An Islet can be seen to the right of the slide with a single microsphere in the centre. There is no apparent disruption of the tissue and no evidence of any inflammatory cells. A similar micrograph at the higher magnification of x400 is seen in 7.4

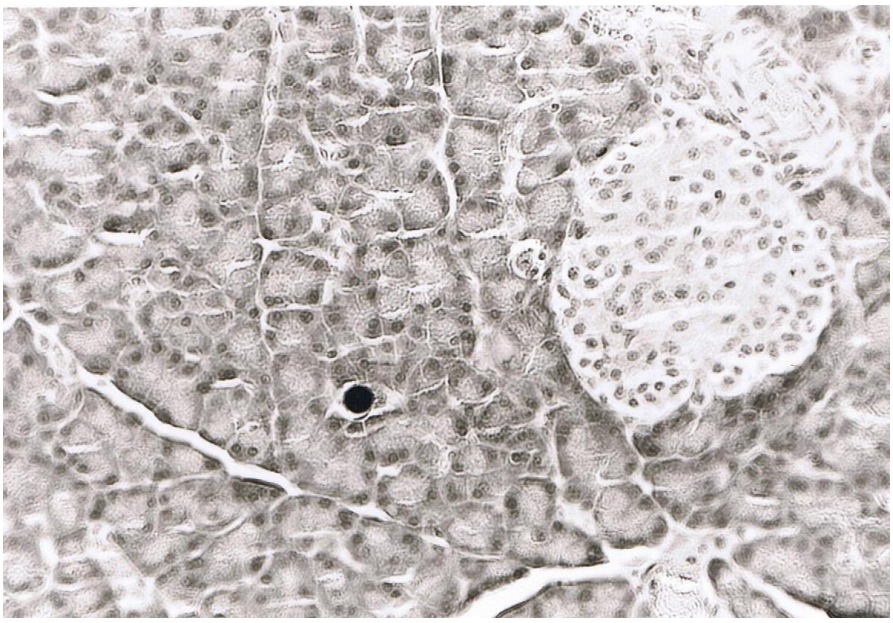


Fig. 7.3

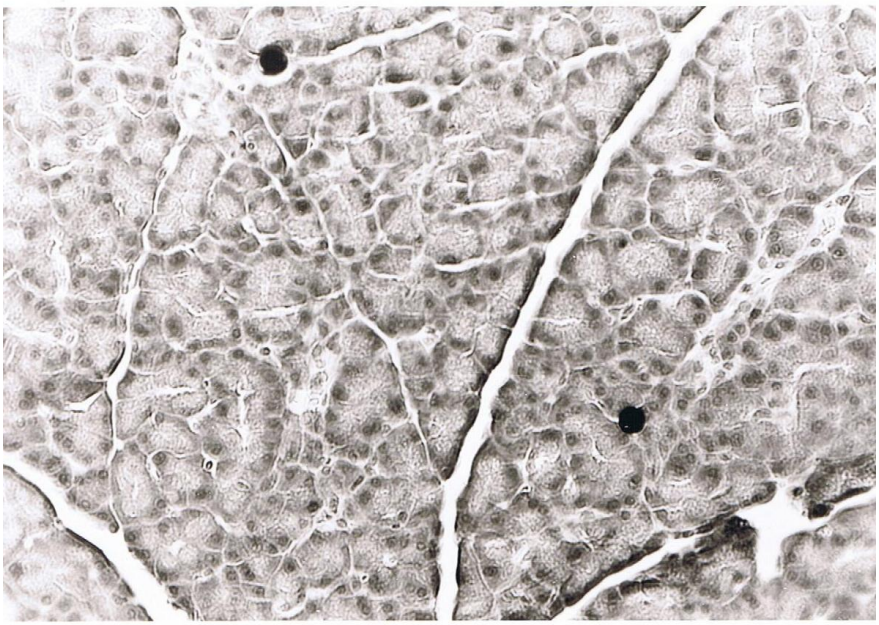


Fig. 7.4

7.2 Effect on cardiovascular function:

One of the major criteria for acceptance of a blood flow experiment is that no major change in cardiovascular function occurs due to the measurement technique used i.e. that the experimental technique itself does not bring about significant changes in organ blood flow.

A series of experiments were carried out testing the effect of microsphere injection on cardiovascular function.

In a series of 7 experiments, the polygraph recorder was run at a rate of 5cm per second before and after injection of the microspheres so as to allow accurate measure of the blood pressure, pulse rate and respiratory rate.

Pre and post blood flow experiment measurements of mean arterial blood pressure (mm Hg), pulse rate per minute and respiratory rate per minute are displayed in Table 6.2 and in graphs 6.1, 6.2 and 6.3.

Statistical comparison was made using the paired Student t test with the fall in blood pressure of approximately 10% being significant ($p<0.0025$). No statistically significant change was seen in the pulse or respiratory rate.

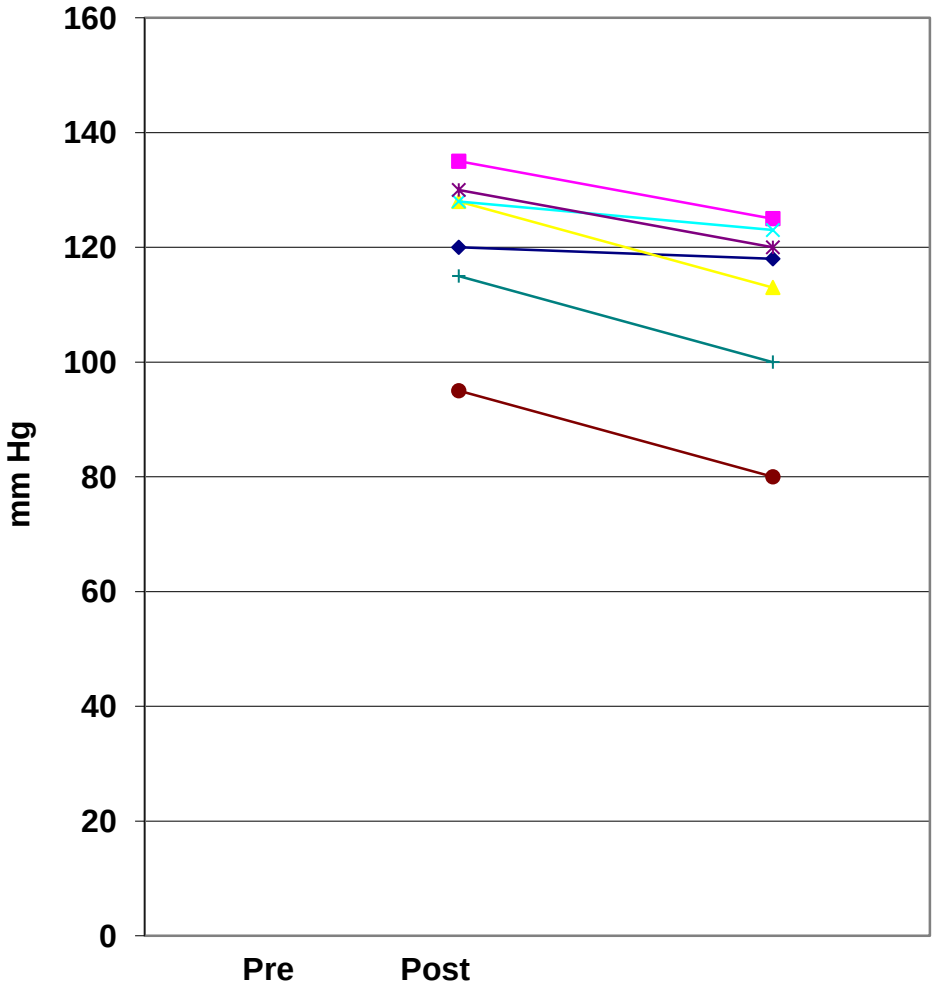
Physiological Changes Due to Blood Flow Measurement

Expt. No.	Mean BP (mmHg)		Pulse rate (per min)		Resp. rate (per min)	
	Pre	Post	Pre	Post	Pre	Post
1	120	118	436	444	60	60
2	135	125	396	378	72	66
3	128	113	428	424	76	68
4	128	123	435	448	64	60
5	130	120	432	430	66	75
6	95	80	352	336	52	48
7	115	100	468	456	84	84
Mean	122	111	421	417	68	66
S.D.	13	16	37	44	11	12
S.E.	5	6	14	17	4	4
% **diff.**	-8		-1		-3	
t	5.12		0.99		0.88	
df	6		6		6	
p	<0.0025		NS		NS	

Table 7.2

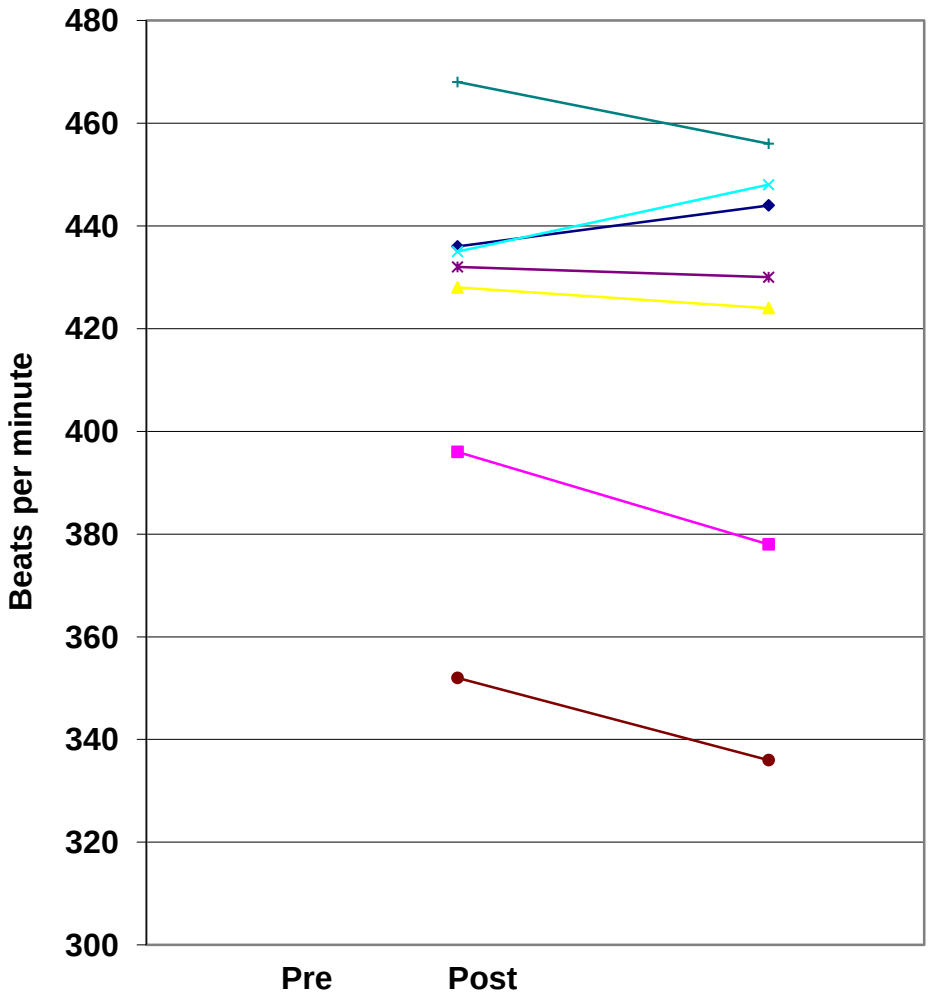
The statistically significant change in blood pressure can more easily be appreciated graphically.

Changes in Mean Arterial Blood Pressure



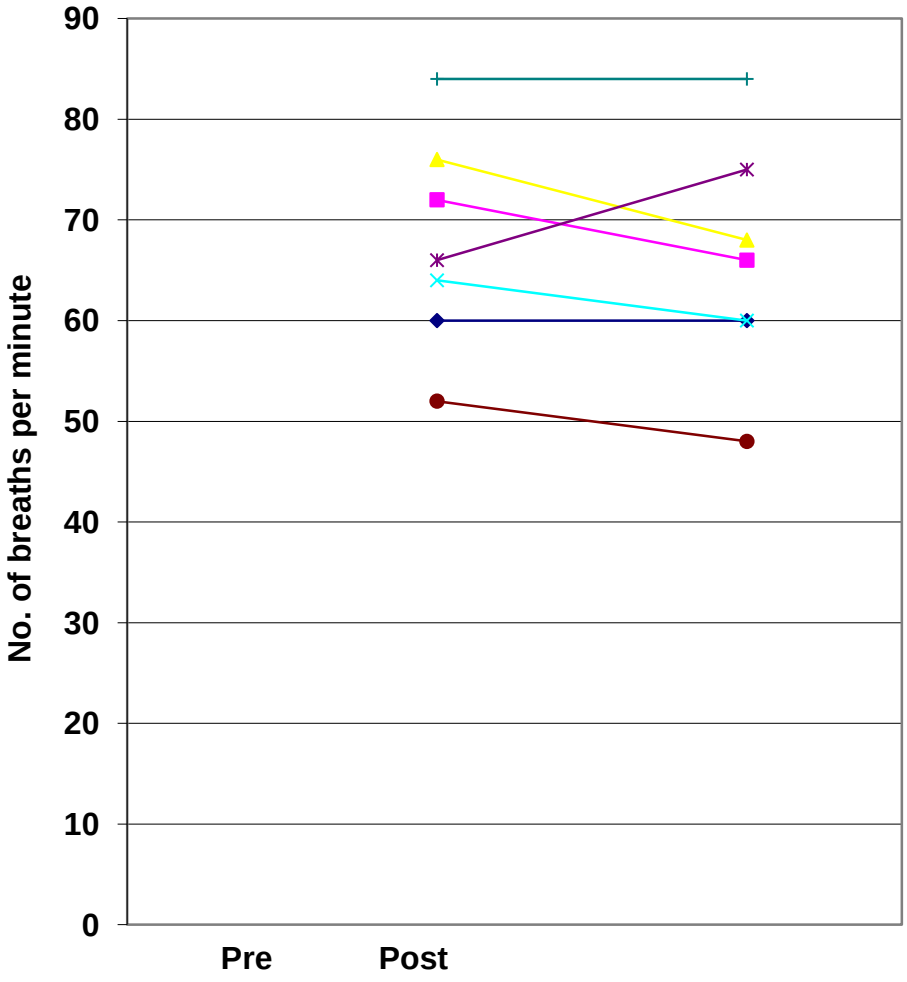
Graph 6.1

Changes in Pulse Rate



Graph 6.2

Changes in Respiratory Rate



Graph 6.3

7.3 Double injection series:

The aim of this experiment was to test the effectiveness of using 2 differently labelled microspheres in one blood flow experiment.

One of the major advantages of the microsphere method is that the capillary trapping of the microspheres in a sense "stores" the blood flow measurement until the organs are harvested and the number of microspheres measured. If a second bolus of microspheres with a different radio-nucleide label is then injected, a further blood flow measurement is able to be made in the same animal. This provides an ideal experimental situation in which each experimental animal can act as its own control allowing paired comparisons as in the study of Slavotinek (1982).

In a series of 12 experiments, dual microsphere injections were carried out.

Organs were harvested in the usual way and dual blood flow calculations were made by measuring the presence of both isotopes (and thus the number of different microspheres) in each organ. As previously described, a percentage difference of less than 10% was used as an assessment of adequate mixing of microspheres prior to their being ejected from the left ventricle. The following table displays the percentage difference between the kidneys in each of the twelve experiments.

Double Injection Series.
102

% Difference Left/Right kidneys		
Expt.	Flow 1	Flow 2
1	14	90
2	13	7
3	37	41
4	5	32
5	67	6
6 *	5	5
7	18	18
8 *	7	5
9 *	3	6
10	5	13
11	25	67
12 *	2	8

Table 7.3.

* these studies had acceptable differences in kidney blood flow.

7.4 Blood alcohol / glucose levels :

The effect of alcohol on pancreatic blood flow was studied by the infusion of a solution of 50% alcohol intravenously as in the previous studies of Slavotinek and Horwitz and by the instillation of alcohol with and without glucose into the stomach of the anaesthetised animal.

Ideally, the blood alcohol and blood glucose levels should have been measured after each experiment. However because of the possibility of radioactive contamination of the equipment used for blood alcohol and blood glucose analysis (high pressure liquid chromatography, HPLC), a series of preliminary experiments were carried out to assess the blood levels achieved by standard doses of alcohol and glucose.

Blood Alcohol Levels

Expt. No.	Blood alcohol (mg/100ml)
1	0.12
2	0.13
3	0.07
4	0.17
5	0.05
6	0.09
7	0.09
8	0.11
Mean	0.10

Table 7.4

The intra-gastric infusion group were all fasted and received 2gm/kg alcohol mixed with either normal saline or glucose 1gm/kg. Two series of experiments were carried out in which blood alcohol levels and blood glucose levels were determined after administration of either alcohol and saline or alcohol and glucose.

IG Alcohol 2gms/kg : Saline 1ml

Expt. No.	Bl. Alc. (mg/100ml)	BSL (mmol/l)
1	0.11	4.40
2	0.13	2.90
3	0.11	3.5
4	0.10	3.7
5	0.08	3.5
Mean	0.11	3.6

Table 7.5

IG Alcohol 2gms/kg : Glucose 1gm/kg

Expt. No.	Bl. Alc. (mg/100ml)	BSL (mmol/l)
1	0.16	6.00
2	0.09	3.80
3	0.06	4.20
4	0.09	4.1
5	0.11	6.0
6	0.09	5.5
Mean	0.10	4.9

Table 7.6

CHAPTER 8. PANCREATIC BLOOD FLOW.

Seven series of pancreatic blood flow experiments were carried out and these can be conveniently divided into 3 major groups, controls, intravenous alcohol and intra-gastric alcohol. The control and the intravenous alcohol groups were subdivided into a fasted and a non-fasted group, while the intra-gastric alcohol group was separated according to whether the alcohol was diluted with saline or glucose.

The experiments are presented as follows;

Group 1. Controls

Expt. 1.1 Fasted

1.2 Non-fasted

1.3 Intra-gastric glucose

Group 2. Intra venous alcohol

Expt. 2.1. Fasted

2.2. Non-fasted

Group 3. Intra-gastric alcohol

Expt. 3.1. Alcohol /saline

3.2. Alcohol/ glucose

CHAPTER 8.1 CONTROLS.

The initial study group of animals was fasted from food but not fluids for a period greater than 12 hours. This was found to result in an empty stomach (which under normal circumstances was distended with foodstuff). The blood flow study was rejected if the stomach was not completely empty.

Satisfactory blood flow studies were carried out on 11 animals with a mean weight of 310 gms. The mean pancreatic blood flow for the group was 105 mls/min/100gms with a standard deviation (S.D.) of 29 and standard error (S.E.) of 9. (See table 9.1 below.)

The second group of animals were allowed free access to food and fluids prior to being studied. These are referred to as non-fasted and abbreviated to 'NF'. Invariably, their stomachs were found to be distended with foodstuff. Satisfactory blood flow studies were carried out on 9 animals with a mean weight of 335 gms. The mean pancreatic blood flow for the group was 134 mls/min/100gms with a standard deviation (S.D.) of 32 and standard error (S.E.) of 11. (See table 8.2. below.)

The third group of animals was fasted as per group 1.2 and in addition received an intra-gastric infusion of glucose prior to the blood flow measurement experiment.

The three groups are compared and their differences analysed (table 8.3).

The results are also displayed graphically (graph 8.1)

CONTROLS : FASTED

Expt. No.	Wt. (gms)	P.B.F. (mls/min/100gms.)
1	280	106
2	300	96
3	335	99
4	370	168
5	220	116
6	255	92
7	320	138
8	350	90
9	310	110
10	315	57
11	360	80
Mean	310	105
S.D.		29
S.E.M.		9

Table 8.1

CONTROLS : NON-FASTED

Expt. No.	Wt. (gms)	P.B.F. (mls/min/100gms.)
1	335	155
2	300	108
3	270	150
4	340	156
5	295	71
6	340	137
7	310	139
8	360	175
9	260	114
Mean	335	134
S.D		32
S.E.M.		11

Table 8.2

CONTROLS : INTRA GASTRIC GLUCOSE

Expt. No.	Wt. (gms)	P.B.F. (mls/min/100gms.)
1	285	99
2	260	104
3	240	72
4	285	92
5	270	85
Mean	268	90
S.D		13
S.E.M.		3

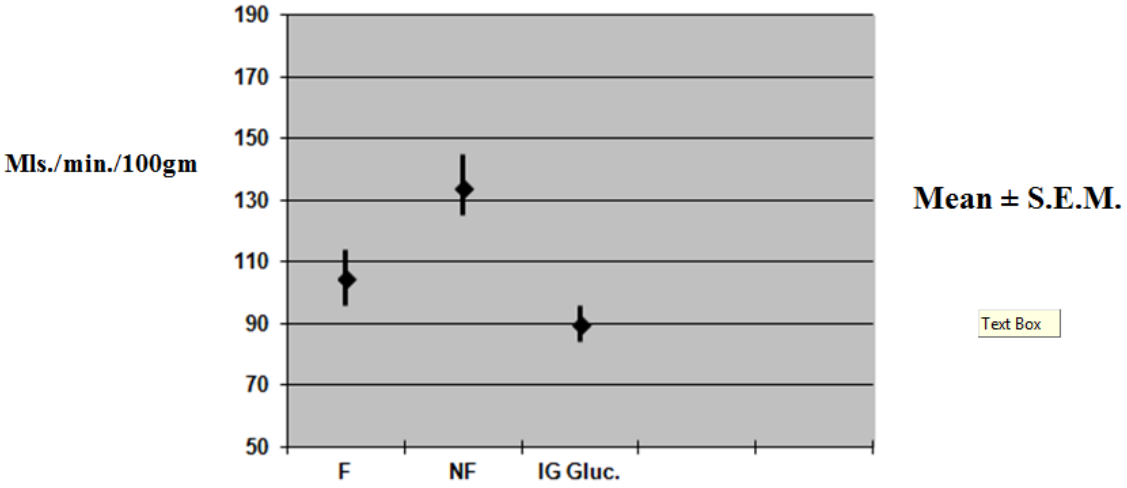
Table 8.3

Comparison of fasted, non-fasted and IG glucose groups

	Fasted (Group 1.1) n=11	Non-Fasted (Group 1.2) n=9	Fasted IG glucose (Group 1.3) n=5
Mean	105	134	90
S.D.	29	32	13
S.E.	9	11	6
% change		+ 28	-14%
df.		18	14
t		2.14	1.09
p		<0.025	N.S.

Table 8.4

Controls ; Fasted / Non-fasted / IG Glucose



Graph 8.1

F Fasted
NF Non fasted
IG Intra gastric glucose

CHAPTER 8.2 INTRAVENOUS ALCOHOL.

The second group studied were animals given an intravenous infusion of alcohol, both fasted (from food but not fluids for a period greater than 12 hours) and non-fasted. Satisfactory blood flow studies were carried out on 7 fasted animals with a mean weight of 289 gms. The mean pancreatic blood flow for the group was 110 mls/min/100gms with a standard deviation (S.D.) of 38 and standard error (S.E.) of 14. (See table 8.5 below.)

This group was compared with the fasted controls (table 8.6). No statistically significant difference was seen. The results are displayed in graph 8.2

A further group of animals was allowed free access to food and fluids prior to being studied and form the 'non-fasted' group. Satisfactory blood flow studies were carried out on 5 animals with a mean weight of 340 gms. The mean pancreatic blood flow for the group was 129 mls/min/100gms with a standard deviation (S.D.) of 9 and standard error (S.E.) of 4. (See table 8.7. below.)

Again this group was compared to the non-fasted controls (table 8.8) and again no statistically significant difference was seen. The results are displayed in graph 8.3.

The two groups receiving intra-venous alcohol were compared (table 8.8) and displayed in graph 8.4.

The difference between these groups was not statistically significant but as can be seen from graph 8.5 the difference was similar to that seen between the fasted and non-fasted controls.

IV ALCOHOL: FASTED

Expt. No.	Weight (gms)	P.B.F. (mls/min/100gms.)
1	320	148
2	215	109
3	340	81
4	370	106
5	225	165
6	230	51
7	320	113
Mean	289	110
S.D.		38
S.E.		14

Table 8.5

IV ALCOHOL: NON FASTED

Expt. No.	Wt. (gms)	P.B.F. (mls/min/100gms.)
1	350	120
2	300	140
3	270	120
4	400	135
5	380	129
Mean	340	129
S.D.		9
S.E.		4

Table 8.6

Comparison; Fasted Controls / Fasted - IV alcohol

	Group 1.1 Fasted n=11	Group 2.1 IV alcohol n= 7
Mean	**105**	**110**
S.D.	29	38
S.E.M.	**9**	**14**
% change	**+5**	
t	**0.36**	
p	**NS**	
(unpaired t test)		

Table 8.7

Comparison; Non-Fasted Controls / IV alcohol -Non-fasted

	Group 1.2 Non-fasted n= 9	Group 2.2 IV Alcohol n=5
Mean	**134**	**129**
S.D.	**32**	**9**
S.E.M.	**11**	**4**
% change	**-4**	
t	**0.35**	
p	**N.S.**	
(unpaired t test)		

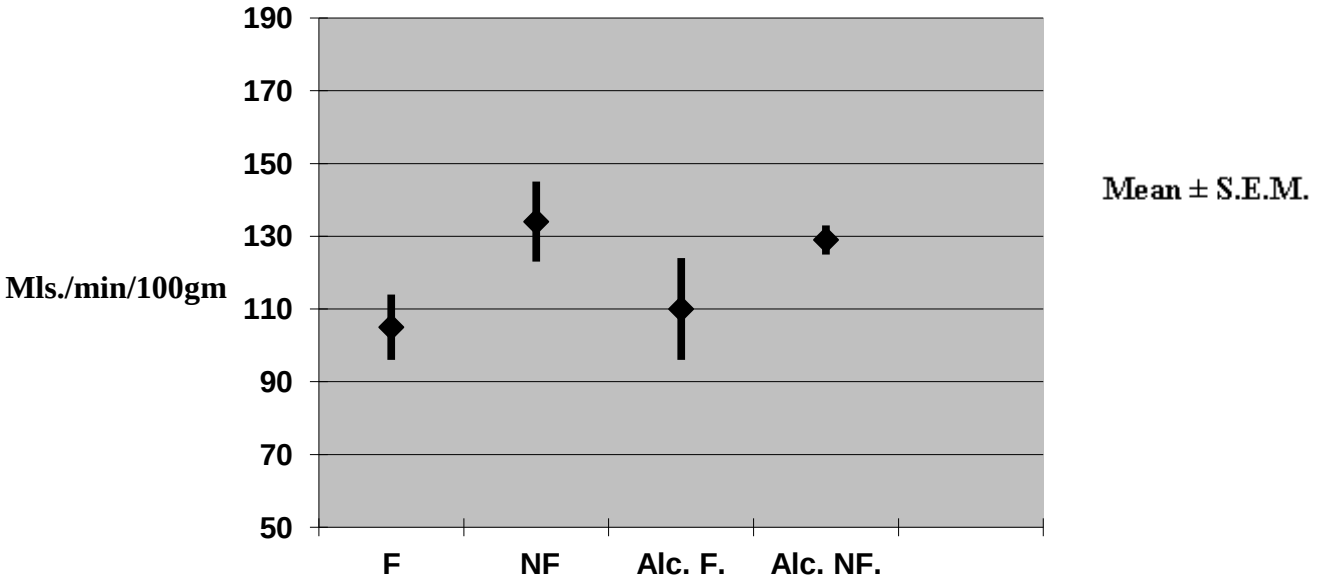
Table 8.8

Comparison; IV alcohol – Fasted / Non-fasted

Group	**2.1 IV Alcohol Fasted**	**2.2 IV Alcohol Non-fasted**
Number	7	5
Mean	110	129
S.E.	14	4
% change	+17	
t	1.04	
p	**N.S.**	
(unpaired t test)		

Table 8.9

Comparison; Controls / IV alcohol – Fasted / Non-fasted



Graph 8.2

CHAPTER 8.3. INTRAGASTRIC INFUSION.

1. Alcohol in saline

This group received a 2 ml. intra-gastric infusion of alcohol (2gm/kg) in saline through a cannula inserted into the stomach via the cervical portion of the oesophagus as described in chapter 6.1 e.

The infusion took place over a period of 5 minutes and blood flow measurements were made after a further delay of 5 minutes.

A second group received a similar amount of alcohol (2 gm/kg) combined with glucose, 1gm/kg.

The control group receiving intra gastric glucose (Table 8.3) is reproduced and used for comparison.

INTRA-GASTRIC ALCOHOL

No.	Wt. (gms.)	P.B.F. (mls/min /100gms)
1	245	72
2	400	144
3	230	123
4	265	119
5	210	100
6	250	95
7	280	121
Mean	269	111
S.D.		23
S.E.M..		9

Table 8.10

INTRA GASTRIC GLUCOSE

No.	Wt. (gms.)	P.B.F. (mls/min/100gms.)
1	285	99
2	260	104
3	240	72
4	285	92
5	270	85
Mean	268	90
S.D		13
S.E.M.		3

Table 8.3

INTRA-GASTRIC ALCOHOL + GLUCOSE

No.	Wt. gms.	P.B.F. mls./min/100gms
1	250	162
2	330	117
3	345	124
4	310	189
5	305	215
Mean	308	161
S.D.		42
S.E.		19

Table 8.11

Comparison IG glucose / IG alcohol

	IG Gl,	IG Alc
number	5	7
mean	90	111
s.d.	13	23
s.e.	6	9
% change	+23	
t	1.74	
df	10	
p	N.S.	

Table 8.12

Comparison IG glucose / IG alcohol + glucose

	IG Gl	IG Alc+Gl
number	5	5
mean	90	161
s.d.	13	42
s.e.	6	19
% change	+79%	
t	1.74	
df	8	
p	<0.003	

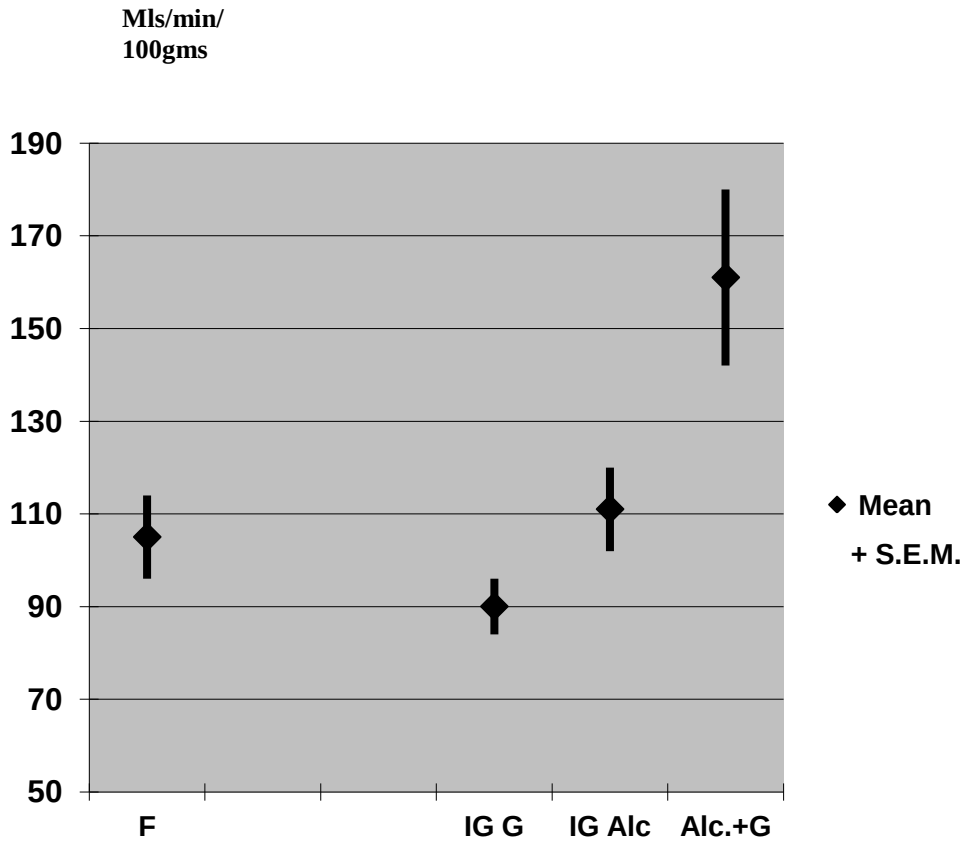
Table 8.13

Comparison IG Alcohol / IG Alcohol + Glucose

	IG Alc	IG Al+G
number	7	5
mean	111	161
s.d.	23	42
s.e.	9	19
% change	+45	
t	2.70	
df	10	
p	< 0.0125	.

Table 8.14

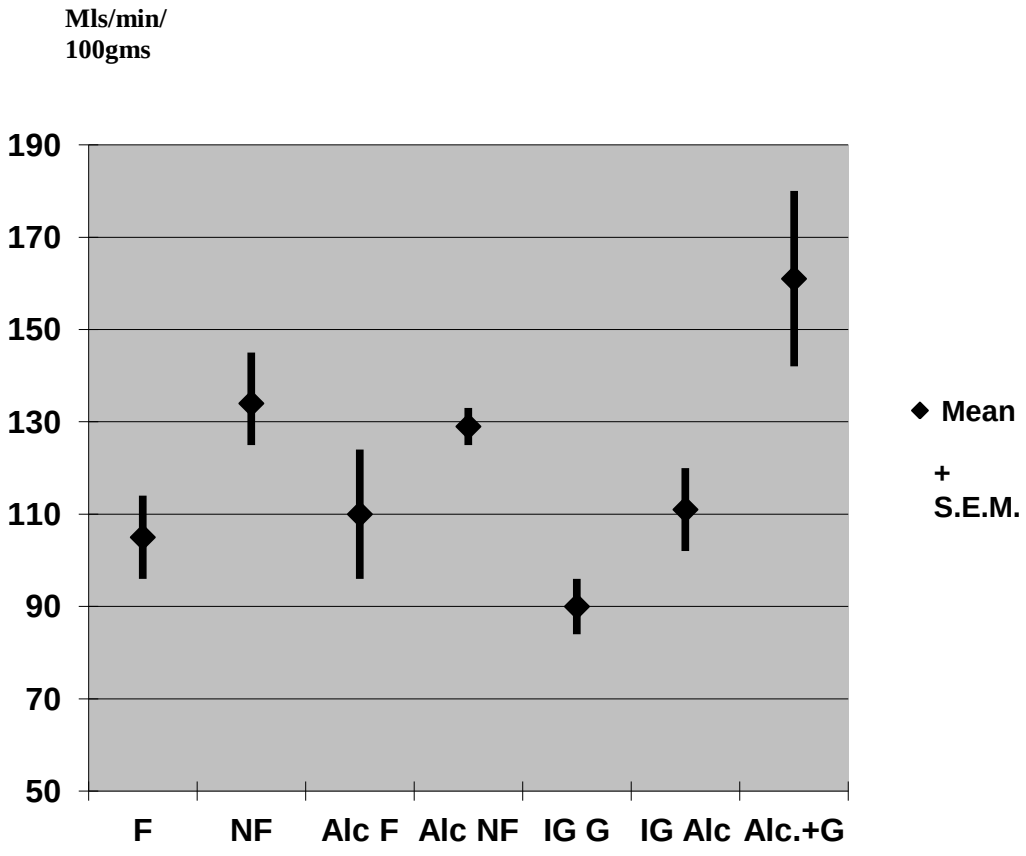
INTRA-GASTRIC INFUSION SERIES



Graph 8.3

F Fasted Control
IG Gl Intra gastric glucose
IG Alc Intra gastric alcohol
IG Alc+Gl Intra gastric Alcohol + Glucose

Comparison Controls / IV Alcohol / IG Glucose and Alcohol



Graph 8.4

F	Fasted control
NF	Non-fasted controls
IG Gl	Intra gastric Glucose
IG Alc	Intra gastric alcohol
IG Alc+G	Intra gastric Alcohol+Glucose

CHAPTER 9. GASTRO-INTESTINAL TRACT (GIT) BLOOD FLOW

Introduction ;

The microsphere method of blood flow measurement is based on injection of the spheres into the left ventricle of the heart which are then distributed throughout the body. This means that the blood flow to each and every organ and tissue of the body is able to be measured each time a blood flow experiment is carried out.

While the focus of this study was pancreatic blood flow and therefore only calculation of blood flow to the pancreas and kidneys (in order to test for even distribution) was necessary, a range of other tissues were sampled.

These included the kidneys, liver, spleen, testes and brain as well as different levels of the gastro-intestinal tract ,including the antrum and fundus of the stomach, the duodenum, the ileum and the colon. In addition samples of skeletal muscle and skin were also taken and tested.

Blood flow to the liver was extremely low (as only hepatic arterial rather than portal venous flow was able to be measured) and blood flow to the spleen so variable that no meaningful interpretation of the data was possible.

The brain, testes, skin and skeletal muscle had blood flows which were so low that no meaning-full conclusions could be made.

Significant blood flow changes through the gastrointestinal tract were observed and although not part of the original study plan, the results are presented in the following chapter.

GIT Blood Flow ; Controls / IV Alcohol

	Controls		IV Alcohol	
	Fasted	Non- Fasted	Fasted	Non-fasted
Stomach;				
Fundus	**23±2**	**24±3**	**24±2**	**27±3**
Antrum	**60±5**	**60±5**	**87±14** [1]	**130±27** [5]
Duodenum	**104±9**	**117±1**	**184±21** [2]	**147±12** [ns]
Ilium	**119±11**	**124±12**	**194±23** [3]	**153±18** [ns]
Colon	**44±7**	**45 ±5**	**112±26** [4]	**129±28** [6]

Table 9.1

Differences between fasted and non fasted controls were not statistically significant. Difference between control and IV alcohol groups were tested using paired student t test. Statistically significant differences were as follows;

1 p= 0.04, 2 p= 0.001, 3 p= 0.004, 4 p= 0.008, 5 p= 0.006, 6 p= 0.002

GIT blood flow; Intra gastric Infusion

	Fasted Controls	Glucose	Alcohol	Alcohol +Glucose
Stomach:				
- **Fundus**	**23±2**	**80±20** [1]	**35±5**	**30±4**
- **Antrum**	**60±5**	**100±18** [2]	**119±9** [4]	**89±7**
Duodenum	**104±9**	**170±20** [3]	**241±46** [5]	**174±18** [7]
Ilium	**119±11**	**112±17**	**200±32** [6]	**195±27** [8]
Colon	**44±7**	**30±3**	**57±6**	**123±+8** [9]

Table 9.2

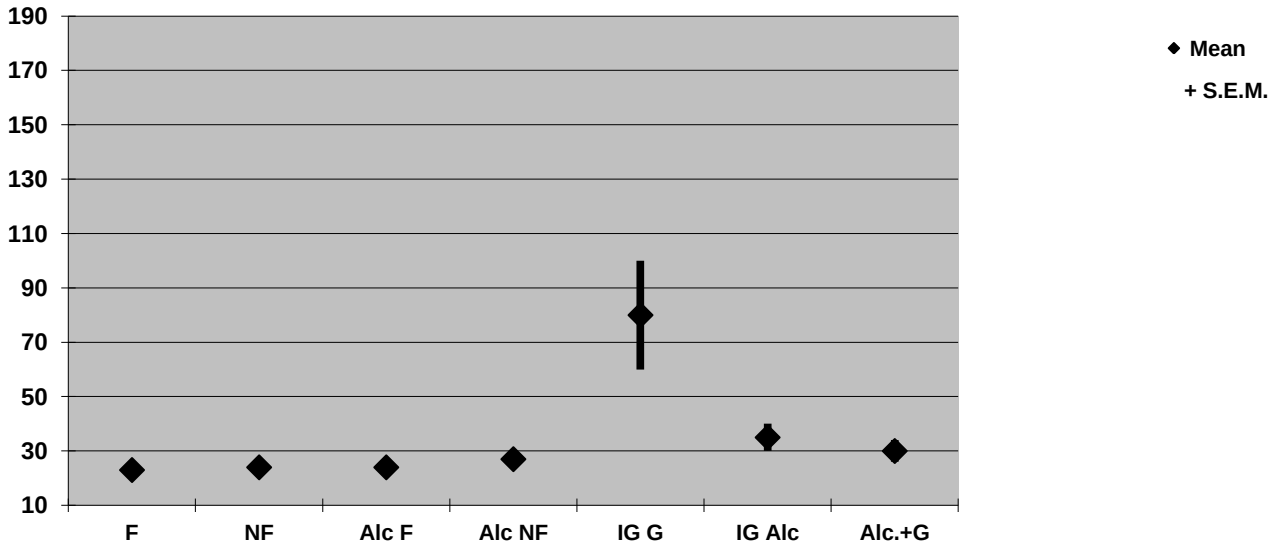
Statistically significant differences as follows ;

1 p= 0.006, 2 p= 0.01, 3 p= 0.006, 4 p= 0.002, 5 p= 0.002, 6 p= 0.01

7 p= 0.001, 8 p= 0.006, 9 p= 0.002

Stomach – Fundus

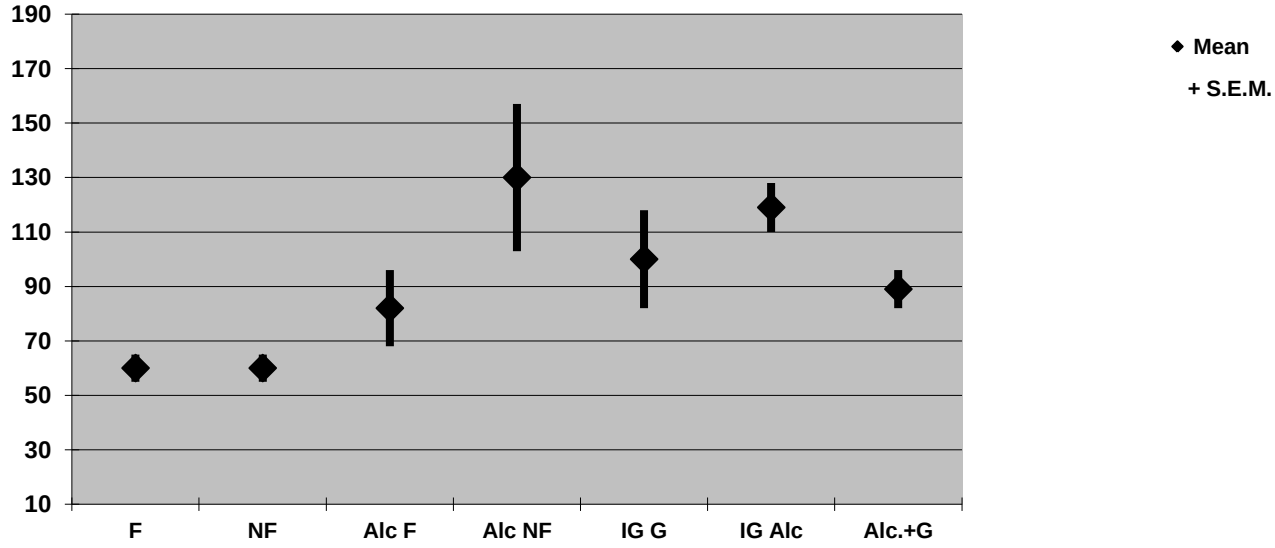
Mls./min./
100 gms.



Graph 9.1

Stomach - Antrum

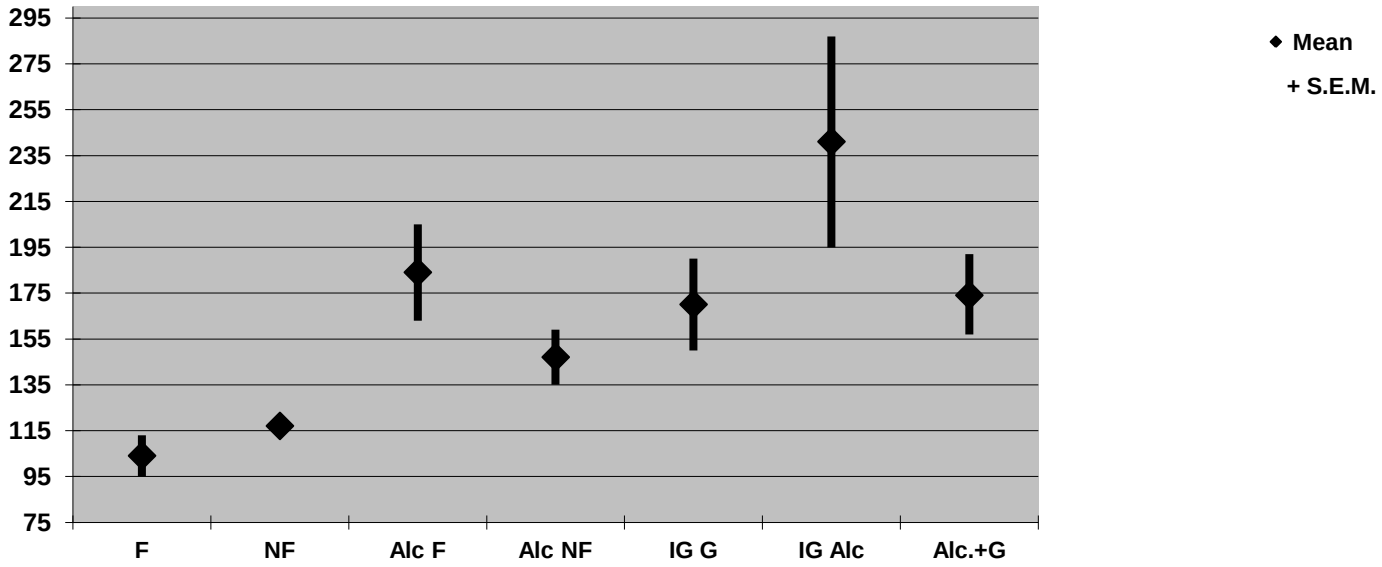
Mls./min./
100 gms.



Graph 9.2

Duodenum

Mls./min.
100 gms.

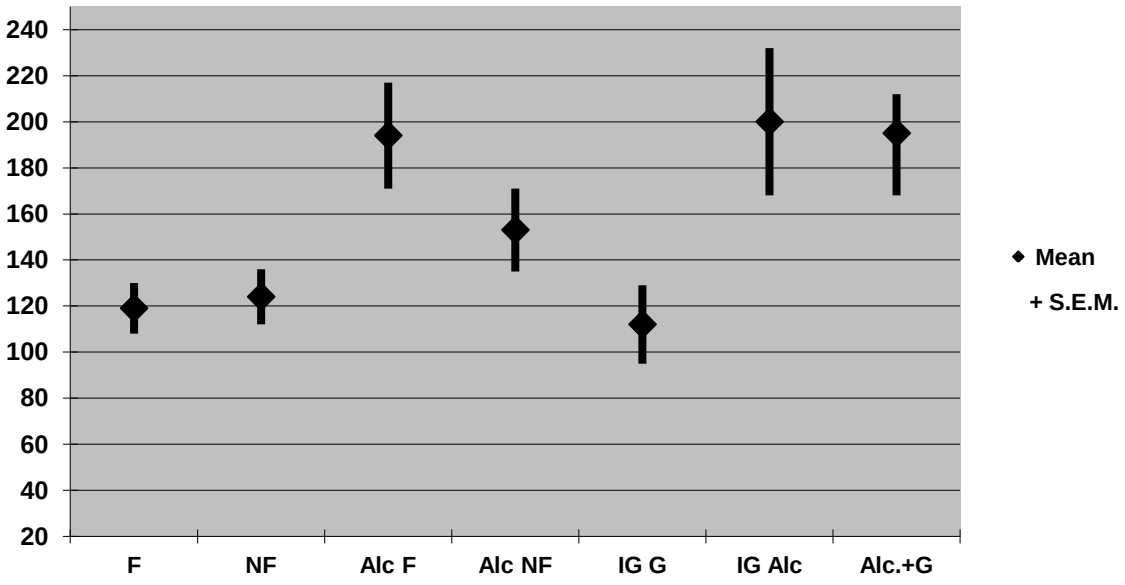


/min./

Graph 9.3

Ileum

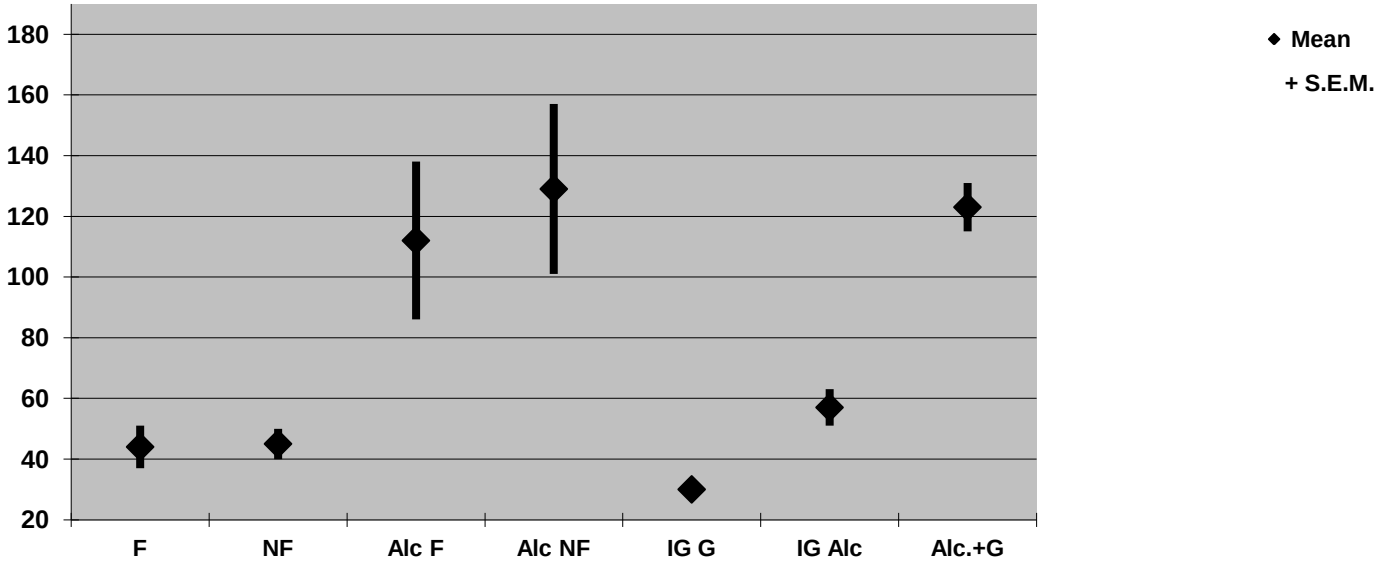
Mls./min./
100 gms.



Graph 9.4

Colon

Mls./min./
100 Gms.



Graph 9.5

Chapter 10. DISCUSSION:

Introduction;

The research carried out resulting in this thesis was undertaken during a post surgical fellowship appointment of one year as a temporary lecturer to the University of Adelaide, Department of Surgery at the Queen Elizabeth Hospital.

This project was chosen because the primary focus of the research being carried out by the department at the time related to pancreatitis. The microsphere method of blood flow measurement had been established in a dog model and a relationship between alcohol administration and lowering of blood flow was demonstrated and reported by Slavotinek (1982).

There were however, increasing ethical and economic issues associated with the use of the dog as an experimental animal. The use of stray dogs which had been collected by the local council and which were due to be destroyed was ultimately banned by the University ethics committee. While it was deemed acceptable to use animals specifically bred for research projects and while the University had such a breeding program in place, the cost of breeding and sustaining the animals until they were old enough to be used was prohibitive.

The microsphere method was thus established in rats and a series of experiments were carried out investigating the effect of secretin on blood pancreatic flow. A further series of blood flow experiments were carried in rats in which pancreatic fibrosis had been induced by surgical ligation.

The latter experiments were reported to the Surgical Research Society of Australia and the abstract published (Slavotinek 1986) but the experiments relating to secretin infusion were neither formally presented nor published.

These blood flow experiments were all carried by the Department's research assistants, Miss Elaine Deakin, Mrs. Sue Nance and Mr. Ken Porter (who appear in the acknowledgements) and supervised by Mr A.H. Slavotinek, Senior lecturer in Surgery. Mr Slavotinek also supervised this project.

Apart from routine care of the animals which was carried by the animal house technicians, all aspects of the experiments including preparation for and delivery of anaesthesia, establishment of all arterial and venous cannulation and connection to various recording devices were carried out by the primary investigator. All steps of the blood flow experiment apart from the injection of microspheres into the left atrial cannula and all post experiment activities such as harvesting of tissues, their preparation and insertion into the radiation counter and finally calculation of individual blood flows were also carried by the primary investigator.

Hypotheses ;

As stated in the introduction, the primary aim of this study was to use the rat model to test the hypothesis that alcohol administration, as had been demonstrated in dogs by previous studies (Horwitz 1982, Slavotinek 1983, Freidman, 1983) could significantly reduce pancreatic blood flow.

The secondary hypothesis regarding the effect on pancreatic blood flow of a combination of glucose and alcohol was made prior to any literature search. It was based on the fact that the pancreas contains both an exocrine portion responsible for the secretion of enzyme rich fluid and an endocrine portion which is responsible for the secretion of insulin and the clinical observation that alcohol ingestion in man is often in the form of solutions with a high alcohol content (spirits) combined with soft drinks which have a high glucose content. The Islets of Langerhans (the endocrine portion of the pancreas are scattered throughout the parenchyma of the exocrine pancreas and changes in their blood flow are reflected in total pancreatic blood flow as measured by the microsphere technique. The literature review revealed that although the Islets occupy a small volume of the gland, they account for up to 20% of its total blood supply (Lifson 1980) and that therefore any solution affecting Islet blood flow (such as a one containing glucose) could be expected to exert a significant effect on total pancreatic blood flow. Moreover, alcohol itself can affect Islet blood flow with evidence that alcohol can alter the glucose tolerance curve in man (Nikkila 1975) and that a more profound hypoglycaemia can be produced in patients when alcohol is administered with a glucose load (gin and tonic) than when administered alone (O'Keefe 1977) .

Apart from the papers cited above, the literature review carried out failed to identify any experimental studies in which a combination of alcohol and glucose was studied. In fact no other papers even acknowledge the presence of the Islets, let alone take into account the effect that Islet blood flow would have on total pancreatic flow.

All of the papers reviewed used intravenous infusion of alcohol in their studies thereby avoiding any possible confounding factors such as the influence of alcohol on gastric and duodenal mucosa.

Extrapolation of these results to the clinical situation in man however must be questioned. Coming from a background of clinical surgery, it therefore seemed logical to also study the effect of orally administered alcohol both alone and in combination with glucose.

As will be seen, the decision to test alcohol in combination with glucose delivered parenterally resulted in the most significant findings of this study, namely that a combination of alcohol and glucose delivered orally lead to a statistically significant increase in pancreatic blood flow compared to alcohol or glucose alone.

Literature Review ;

The preliminary literature review was divided into two broad aspects, the pancreas itself, including anatomy, physiology and pathology. Particular attention was paid to the condition of acute pancreatitis, the aetiology of alcohol related pancreatitis and the possible role of blood flow changes. The second major aspect of the literature review was that of pancreatic blood flow with the emphasis on blood flow measurement techniques, in particular the microsphere method.

a. Anatomy and Physiology

Review of the basic anatomy of the pancreas was a useful exercise as it highlighted the problems associated with the accurate measurement of blood flow. The gland is closely applied to the duodenum and spleen with which it shares a common arterial supply and venous drainage. Moreover both the arterial supply and venous drainage consist of

numerous vessels, these being derived from both the artery of the foregut (coeliac axis) and the artery of the mid gut (superior mesenteric). Simple measurement of arterial inflow or venous drainage (as applied for example to assess renal blood flow) is simply not possible. Methods which employ surgical intervention to isolate either the arterial or the venous drainage of the pancreas involve major dissection and results thus obtained must be considered with great caution. In none of the papers reviewed in which surgery was used to isolate pancreatic arterial or venous blood flow in order to simplify blood flow measurement, was any attempt made to assess the effect of such surgical manipulations on normal pancreatic blood flow.

Another anatomical problem not addressed when these methods were employed is possible interference with the peri-vascular nerve supply to the gland.

Physiologists had long recognised the importance of the nervous system in maintenance and control of splanchnic blood flow and indeed one of the earliest published papers on pancreatic blood flow was that of Anrep in 1916 in which the effect of vagal stimulation on pancreatic blood flow was studied.

Studies in which the pancreas is isolated from the duodenum or removed altogether, must disrupt its splanchnic nerve supply and therefore any blood flow results obtained should take into account changes resulting from this surgical manipulation. In none of the papers reviewed, was any account taken of this factor.

Another important relationship between the pancreas and duodenum highlighted during this part of the literature review related to the hormones secretin and cholycytokinin, both of which are secreted by the duodenal mucosa. These hormones have profound effects on pancreatic secretion and blood flow. Thus if surgical manipulation of the duodenal blood supply affects mucosal secretion of these hormones, it will also affect

pancreatic blood flow. Again none of the studies in which the blood supply to the pancreas and duodenum are operatively separated make any mention of, let alone make any assessment of the effect of such surgery on the secretion of these hormones.

Perhaps the most significant factor to emerge from the literature review of the anatomy and physiology of the pancreas is the relationship of the exocrine and endocrine portions of the gland. The Islets of Langerhans are collectively an endocrine "organ" but unlike others such as the thyroid, parathyroid and pituitary which exist as distinct anatomic entities, they are scattered throughout the pancreas, an exocrine gland.

Without exception, the papers in which the effect of alcohol on pancreatic blood flow was studied ignored the contribution of the endocrine portion of gland. This is in spite of evidence that alcohol has been shown to influence Islet blood flow and that the endocrine portion of the gland has been shown to account for up to 20% of total organ blood flow (Lifson 1980).

If a physiologic or pharmacologic agent was to produce a doubling of the blood flow of just the endocrine portion of the pancreas, the total organ blood flow would increase by 40%. A similar isolated doubling of the exocrine portion would show an 80% increase in total flow

It was not until the project was well underway that the issue of separate endocrine and exocrine flow became apparent. Once the importance of differential blood flow was appreciated, further reading revealed a technique allowing for the enzymatic separation of the Islets from the exocrine tissue of the pancreas (Lacy 1967). The microsphere method is such that once the microspheres lodge in tissues, they remain there and in effect become a permanent record of the blood flow at the time of the experiment. Enzymatic separation of the Islets from the exocrine tissue would therefore allow a

breakdown of total blood flow measurement into individual exocrine and endocrine portions of the pancreas. Had time and budgets allowed, the blood flow experiments in which alcohol and glucose were used in combination, would have been repeated with a view to gauging the extent of endocrine versus exocrine blood flow increase.

The existence of the "insulo-acinar portal system" was another fascinating feature of the review. Lifson (1980) found that following retrograde venous injection of microspheres into the draining veins of the pancreas, no microspheres were found within the Islets, the inference being that the venous drainage of the Isles in to the exocrine circulation, a so called ‘insulo-acinar portal’ system. Although its existence is unexplained, it does imply a functional reason for the Islets to be scattered throughout the gland, rather than grouped together like all of the other endocrine glands in the body. Further investigation of this phenomenon was beyond the scope of this study but it does validate the choice of the secondary hypothesis.

b. Pancreatitis:

Pancreatitis still presents a major clinical problem and has undoubtedly been a major stimulus behind much of the basic physiologic research into the pancreas and the effect that alcohol has on it. Alcohol has a long clinical association with pancreatic disease and a large body of experimental work exists concerning its effect on pancreatic physiology. The exact pathogenesis of alcoholic pancreatitis, acute or chronic, remains unknown. No currently held theory is able to account for all the known clinical and experimental facts, nor why it has not been possible to produce pancreatitis in experimental animals using alcohol alone. No theory is able to explain why far fewer alcoholics develop pancreatitis compared with those who develop cirrhosis of the liver. The protein plug theory of Sarles (1971) seems to adequately explain the development

of chronic pancreatitis but fails to explain the acute haemorrhagic variety of the disease. Horwitz (1983) and Friedman (1983) both rightly point to experimental work linking pancreatitis with ischaemia, but the 20-30% reduction in blood flow demonstrated by these authors seems insufficient to trigger the changes of acute pancreatitis. While this evidence lends credence to the possible importance of changes in blood flow, it would seem that major vessel obstruction in addition to pancreatic duct occlusion, (or some other as yet unidentified co-factor) is required, thus pointing to a multi-factorial aetiology.

c. Pancreatic Blood Flow :

The search for papers reporting pancreatic blood flow carried out using the Medline database and the papers listed in their respective bibliographies resulted in a fascinating condensed history of blood flow measurement. It was instructive to follow the developments in physiologic blood flow measurement from plethysmography and venous outflow through to indicator dilution techniques and the technologically sophisticated electromagnetic flow probes. It was also instructive to follow the development of what could be called 'scientific method' as without exception papers from early in the century rarely describe the method used in detail and make no attempt at validation. Control groups were not used and statistical analysis was non-existent !

There is no doubt that if submitted today, the majority of these early papers would never have been seen in print.

It was not surprising then, that even late in the 20th century, although assumed to be true, it was by no means proven conclusively that an increase in pancreatic blood flow was related to an increase in secretion nor that infusion of the pancreatic hormone secretin could produce an increase in blood flow. The observation by Bernard that the

pancreas was red and engorged in animals having been fed compared to those fasted was not alluded to in any of the reviewed papers except that of Kuznetsova (1962).

The most interesting and perhaps most significant feature of the literature review was the existence of an insulo-acinar portal system whereby the venous outflow of the Islets is directed to the exocrine glands. This was first alluded to by Lifson (1980) who also studied differential blood flow and found that up to 20% of total pancreatic blood flow was directed to the Islets.

In none of the papers reviewed was the potential of differential flow considered, with the pancreas considered to be homogenous in relation to blood flow.

b. Pancreatic Blood Flow Techniques:

The methods identified in the literature review were critically compared to the theoretically ideal blood flow technique described by Jackobsen (1981). The criteria of accurate, continuous, instantaneous, reproducible, quantitative recording of complete or partial organ blood flow which is non-invasive and not harmful were in most part missing from nearly all of the techniques reviewed and many other studies employing methods such as light transmission (Jones 1960) and histologic assessment (Richins 1953), devote little or no discussion of any possible inaccuracies in blood flow estimation nor cite any references validating the techniques.!

Many of the techniques reviewed (including the microsphere method) were based on indicator transport or dilution techniques.

In its simplest form, an indicator injected into the blood stream at one point will arrive at a distal point in a concentration proportional to rate of flow. This principle was

elegantly described in 1870 by (and subsequently named for) the German mathematician and physiologist Adolf Fick (Acierno 2000).

Early use of indicator transport was made with the use of radioactive rubidium (Delaney 1966, Gilsdorf 1967), Xenon washout (Glazier 1974) and of course this is the basis of the radioactive microsphere method (Sasaki 1971, Malik 1976, Fara 1975) Slavotinek (1982), Horwitz (1982) and Friedman (1983).

Because of its key importance in understanding the basis of the microspheres method, and because the terms 'indicator dilution' and 'indicator transport' tended to be used in such a way as to imply a fundamental difference (Woodcock 1975), the mathematics was studied in detail (chapter 3.3.1).

In essence, total organ blood flow (F) can be expressed as the integral of the total amount of indicator injected (Q) divided by the concentration (C) of the indicator at a given time and expressed by the following formula.

$$F = \int_{\infty}^{0} \frac{Q}{C(t).dt}$$

Use of this simple formula involves not only measurement of the cardiac output (Q) but calculation of the integral of the concentration over a time period. This expression can be expressed graphically with the integral being the area under the curve but measurement of this area is by no means easy.

In chapter 3.3 there is a detailed explanation of the use of a reference organ whose blood flow is known and how this enables the simplified calculation of blood flow of any organ. The elegance and simplicity of this technique with its avoidance of complicated mathematics cannot be stressed enough.

The literature review very much vindicated the selection of the microsphere method for this study as it meets all of Jackobsen's criteria apart from being able to provide continuous blood flow recording and the fact that the experimental animal needs to be killed in order to measure blood flow.

Method ;

The technical details of the method are described in detail in chapter 6 and in practice blood flow measurement proved to be very straight forward.

Experimental Animal

The dog has been the experimental animal used by the majority of workers and while cats were used by Holton (1960), Jones (1960) and Richins (1953) and pigs by Semb (1971), not unexpectedly, the rat is the second most commonly used animal in pancreatic blood flow experiments. With increasing public awareness and opposition to the use of domestic animals such as dogs and cats, it is likely that rats will continue to be the dominant experimental animal used in pancreatic blood flow experiments.

In this study, the rat proved to be a highly successful choice of experimental animal. The University of Adelaide Department of Surgery breeding programme was able to supply and house large numbers of suitable animals which were reasonably easy to handle.

Cannulation of the femoral arteries and veins demanded a high degree of manual dexterity and was tedious but achievable in most animals weighing over 250gms.

Intra gastric infusion of solutions proved to be a problem as it was not possible to reliably insert a naso- gastric cannula. Passage via the mouth invariably led to tracheal rather than oesophageal cannulation and even if successful, in the majority of cases gastro-oesophageal reflux occurred leading to tracheal aspiration when installation of alcohol or glucose was attempted.

A technique was therefore devised for the isolation of the oesophagus via an incision in the neck. A cannula was then inserted into the stomach via a small incision in the oesophagus. This technique produced no physiological disturbance and proved to be a simple and reliable method of introducing intragastric solutions. It was not described in any of the papers reviewed.

In spite of being time consuming, taking into account all of the preliminary weighing and the post experiment counting, calculating and cleaning up it was possible to carry out 5-6 experiments per day. In all, taking into account preliminary and discarded experiments, nearly 120 individual blood flow experiments were carried out during the 12 month research period.

The major disadvantage of the rat proved the inability to carry out dual isotope studies as had been carried in the previously reported studies using the dog as the experimental animal.

By using microspheres labelled with two different isotopes, the microsphere method can be used to carry out two independent blood flow measurements in a single animal. The isotopes caesium141 and strontium85 are commonly used as their radioactive profile is sufficiently different to enable accurate measurement of both once tissue harvest has occurred.

Typically measurements are made before and after administration of a test substance such as alcohol. In this setting each animal provides its own control blood flow measurement and an experimental blood flow measurement. This simplifies the statistical analysis and enables fewer experiments to be carried out.

The reference organ technique requires withdrawal of a volume of blood at a constant rate from the experimental animal during the circulation of the microspheres (in these experiments a volume of 2ml was withdrawn.)

While the blood loss associated with one blood flow experiment caused no physiological disturbance to the mean blood pressure, pulse or respiratory rate, as seen in table 4, chapter 7.2, there was a statistically significant decrease in the mean blood pressure of 10% following the second blood flow experiment.

While such a reduction in blood pressure was statistically significant it may not have proved a deterrent had it not been for the discrepancy seen in the distribution of flow to the kidneys. As suggested by Buckberg (1971), a difference of less than 10% between the left and right kidneys had been set as the benchmark for adequate mixing of the microspheres prior to distribution throughout the body. In table 6.3 it can be seen that only four of the 12 double injections series experiments would have been accepted according to this criteria. This was considered to be much too wasteful of time, effort and experimental animals and so the dual isotope or double injection experiments were abandoned. Although this meant that it was not possible use each animal as its own control as was done in the studies of Slavotinek (1982) where the dog was the experimental animal, this deficiency was more than compensated for by the ability to study larger numbers.

Anaesthesia.

Unlike the experiments reported by Slavotinek, Horwitz and Friedman in which the animals were awake, it was a necessary practical condition of using rats that they be anaesthetised.

Bond (1980) reported a generalised lowering of visceral blood flow in the anaesthetised compared to the awake dog and while the actual value of pancreatic blood flow may be expected to vary between the awake and anaesthetised animal, there was nothing to suggest that the response to alcohol would be any different in the two groups.

The anaesthetic technique itself proved reliable and provided cardiovascular stability in the animals with weights of 250gms or more.

The most difficult aspect of the blood flow technique was ensuring adequate mixing of the spheres in the left atrium of the heart prior their ejection into the general circulation. This would be best assessed by comparing flow through both femoral arteries into withdrawal syringes (i.e. reference organs). A difference in flow of less than or equal to 5% would be expected with adequate mixing. This was not a practical exercise in the rat model and the difference in renal blood flow of less than 10% was used as per Buckberg (1971).

Results ;

Validation experiments;

From the extensive literature review it was apparent that the microsphere method was not only the most suitable method available but also the most accurate. Moreover there was a substantial body of evidence attesting to its accuracy and validating the assumptions upon which the method is based Rudolf (1967), Hales (1974, 1977), Sapirstein (1958). While the evidence of the accuracy of the method from these papers was compelling, it was felt important to reproduce some of the validation experiments as this would lead to a better overall understanding of the fundamental principles of the method, as indeed it did.

The first of these was an assessment of the microspheres themselves. The manufacturer's claims regarding uniformity of size and shape were confirmed with a histological study. The small and the irregular microspheres seen in figures 7.1 and 7.2 respectively were rarities.

Establishing a microsphere standard i.e. a reusable container with an exact number of spheres was relatively straightforward. Once established they were used throughout the period of experimentation. As can be seen from figure 7.1 and 7.2, it is possible to produce a smear of microspheres such that each individual can be identified and counted.

Assumptions of microsphere technique.

The next set of preliminary experiments were designed to validate the underlying assumptions of the microsphere technique but were also invaluable in developing

expertise in basic techniques of the blood flow measurement experiments. All aspects of the technique were carried out by the principle investigator with only the mixing and injection of the microspheres being carried out by laboratory staff.

Confirmation was made of the assumptions on which the method is based i.e. that the microspheres are ;

1) thoroughly mixed with blood at the site of injection
2) distributed in direct proportion to the cardiac output
3) completely trapped on the first pass through the organ of study
4) inert with respect to the physiology of the circulation

Assumption 1 was tested by simultaneous withdrawal from both femoral arteries according to the method described by Buckberg (1971). Given that the microspheres are thoroughly mixed with blood within the left ventricle prior to distribution, then assumption 2, that they are distributed in direct proportion to the cardiac output, also holds true.

Complete trapping of microspheres was demonstrated by injection of large boluses of spheres into the femoral vein. None were found to pass through the capillaries of the lung, implying that all microsphere detected in the viscera in subsequent experiments where the microspheres were injected into the left ventricle, must have arrived there on a ‘first pass ‘ basis and not through recirculation.

With respect to assumption 4, no acute inflammation was seen in any of the histological sections prepared, however a long term study i.e. leaving the microspheres in situ for a prolonged period before looking for tissue damage was not carried out. Such long-term

studies confirming the inert nature of the microspheres have been carried out and reported by Hales (1974).

With respect to the effect of the technique on the physiology of the circulation, this was tested and the results presented in chapter 7.2

The removal of blood (the reference organ) did result in a statistically but not clinically significant fall in blood pressure. No statistically significant change was seen in pulse rate or respiratory rate.

The effect on blood pressure was compounded in the double injection series and was almost certainly the reason for the wide ranging difference in kidney blood flow seen. From table 7.3 it is apparent that in only 4 of the 12 experimental studies was the percentage difference in kidney blood flow less than 10% in both experiments. Thus only four experiments would have been accepted.

Because of this poor return of acceptable experiment numbers, the double injection series was abandoned.

Chapter 7.4 reports results of blood alcohol and blood sugar levels achieved following intravenous and intra gastric infusion. The mean level of 0.11 mg/100 mls of blood corresponds to moderate intoxication in man. Similar blood alcohol levels were obtained from both intravenous and intragastric infusion.

The aim of the validation experiments 6.1 c was to show that the difference in kidney blood flow was a valid measure of adequate mixing. Of the six experiments performed, only one would have been accepted based on kidney flow but rejected on femoral artery flow.

Preliminary Experiments

The results of previously unreported and unpublished data on pancreatic and gastrointestinal tract blood flow in rats including the effect of intravenous secretin infusion (Slavotinek et al, addendum 2) were compared with the initial results of this study.

Given that these blood flow measurement experiments of were carried out by full time research staff of the department of Surgery, the close correlation of the results with those of this study (table 6.2 and graph 6.2) was really a confirmation that a sufficient level of expertise had been achieved to commence the experiments proper.

The value of pancreatic blood flow measured in both series also closely correlates with the results of Malik (1974) who used the microsphere technique in rats. The reported pancreatic blood flow of 152 $\pm$ 70 mls/min/100 grams of tissue and renal blood flow of 525 $\pm$ 32 mls/min/100 gm correlates well with the findings of this study (pancreas 134 $\pm$ 11, kidney 411 $\pm$ 18) and that of Slavotinek (pancreas 124 $\pm$ 17, kidney 351 $\pm$ 18).

Pancreatic blood flow experiments;

Controls :

Table 8.1 and 8.2 display the results of blood flow experiments carried out in fasted and non fasted animals in whom no alcohol or glucose was infused. These acted as the control group for subsequent experiments.

In the fasted group of 11 animals, the mean pancreatic blood flow was found to be 105 mls./min./100gms. The standard deviation of the mean was 29 and the standard error of the mean 9.

By contrast in the non-fasted group, the mean pancreatic blood flow was found to be 134 mls./min./100gms. with a standard deviation of the mean of 32 and a standard error of the mean of 11.

The third group of controls (table 8.3) were fasted animals who received an intragastric infusion of glucose. The mean pancreatic blood was found to be 90 mls./min./100gms.with a standard deviation of 13 and standard error of the mean of 3.

The fasted and non fasted groups were compared using the unpaired t test (table 8.4) and the 28% increase in the measured pancreatic blood flow of the non-fasted group compared to the fasted group and this change was found to be significant at the 0.025 level.

This statistically significant difference between the two groups is well demonstrated in graph 8.1 which displays mean pancreatic blood flow (P.B.F.) and standard error of the mean (S.E.M.) and is consistent with the observation made by Claude Bernard in 1856

that the pancreas of the rabbit was engorged with blood when the stomach of the animal was full.

That a difference in pancreatic blood between the fasting and non-fasting state would exist, given the main function of the pancreas is to produce digestive enzymes, seems intuitive but what is somewhat surprising is that it was not addressed in the very many studies which took place over the course of nearly a century. Although alluded to by Kuznetsova (1962) who reported an increase in both pancreatic blood flow and secretion when dogs were fed, the difference in blood flow between the fasting and non fasting state has not previously been reported. The studies of Friedman, Horwitz and Slavotinek were all carried out on fasted dogs with no mention of any possible difference to the non fasted state.

Somewhat surprisingly, no statistically significant difference between the fasted group and the intra-gastric glucose group was seen. While a glucose infusion could be expected to increase Islet blood flow, the period between infusion and blood flow measurement may not have been long enough for a change to have occurred. A significant increase in blood flow was however seen in the stomach and duodenum (table 9.2).

Intravenous alcohol infusion

Having established that a statistically significant difference existed in the measured pancreatic blood flow between the fasted and non-fasted control groups, the effect of alcohol was also studied in both fasted and non-fasted groups.

A group of 7 fasted animals received an intravenous infusion of alcohol prior to blood flow measurement. A solution of 50 % alcohol in saline was used, the volume varying to provide a dose of 1gm/kg.

The mean pancreatic blood flow was found to be 110 mls./min/100gms.with a standard deviation of 38 and standard error of the mean of 14 (table 8.5)

These results were compared with fasted controls (table 8.6) and the difference found to be non-significant. This is well demonstrated in graph 8.2.

A group of 5 non fasted animals were studied after an identical intravenous infusion of alcohol. The mean pancreatic blood flow was 129 mls./min./100gms.with a standard deviation of 38 and standard error of the mean of 4 (table 8.6.)

Similar results were found when this group was compared to their non-fasted controls (table 8.7) i.e. no statistically significant difference which is well demonstrated in graph 8.3.

Comparison of the fasted versus non-fasted intravenous alcohol groups was made (table 8.8, graph 8.4).

The difference seen did not achieve statistical significance, but was similar to that seen in the fasted group as demonstrated in graph 8.5 and almost certainly represents a type 2 statistical error (i.e. a significant difference exists but has not been demonstrated because of small numbers).

Although this uncertainty may have been resolved with increased numbers, the aim of the study was to test the hypothesis that alcohol would lead to a lowering of pancreatic flow in the rat model as had been reported in dogs by Slavotinek, Horwitz and Meyer. Having demonstrated that no difference existed, no further experiments in which

intravenous alcohol was infused were carried out and attention turned to the effect of alcohol introduced via gastric infusion.

Intragastric alcohol

Table 8.11 displays the results of 7 experiments in which alcohol alone was infused into the stomach of fasted animals.

The mean value of 111 mls./min./100gms. (S.D. 23, S.E. 9) is similar to the results of the fasted control group, (table 8.1) where the mean blood flow was 105 mls./min./100gms. (S.D. 29, S.E. 9) and virtually identical to the fasted intravenous alcohol group, (table 8.5) which had a mean of 110 mls./min./100gms. (S.D. 38, S.E. 14).

In designing physiological experiments, especially those involving intact animals, variables are minimised so as to exclude confounding factors.

In the papers of Slavotinek (1983) and Friedman (1983), the only variable affecting the value of pancreatic blood flow between the control and experimental groups was the blood alcohol level which was the result of intravenous administration while the study of Horwitz (1982) reported the effect on pancreatic blood flow of parenterally infused alcohol. While a similar lowering of pancreatic blood flow was seen, potential secondary effects of the alcohol on the gastric mucosa could not be excluded.

For example, it was shown by Llanos (1977) that oral ingestion of alcohol in man and dogs leads to an increase in plasma levels of secretin and suggested that this may not be a direct effect but a secondary one as result of an increase of gastrin released from the stomach.

In this study, orally administered alcohol did not lead to a significant change in pancreatic blood flow. The introduction orally of a combination of alcohol and glucose did however produce a statistically significant increase in pancreatic blood flow 80% greater than glucose alone and 45% greater than alcohol alone.

These differences are well demonstrated in graph 8.6 which displays the intra-gastric results and graph 8.7 in which all 7 groups are displayed.

Statistical analysis showed both of these results to be highly significant, $p<0.003$ for glucose alone and $p<0.0125$ for alcohol alone and vindicate the hypothesis that ingestion of combinations of alcohol and glucose, as occurs in real life, may affect pancreatic blood flow differently to alcohol alone. The paper of O'Keefe (1977) which reported hypoglycaemia in human subjects ingesting a combination of alcohol and glucose (gin and tonic) was the only paper from the literature review which considered this combination.

Determination of how the increase in pancreatic flow observed came about, in particular whether it was due to gastrin release from the stomach as had been demonstrated by Llanos (1977) was beyond the scope of this study as was separation of blood flow into that derived from the exocrine and endocrine portions of the gland. .

Gastro-intestinal blood flow

As outlined in the methodology, the injected microspheres are distributed throughout the body of the experimental animal and it is therefore possible to obtain blood flow measurements from every organ and tissue.

Chapter 9 presents a summary of gastro-intestinal tract blood flow from the stomach to the colon.

Limited statistical analysis was carried out and the findings displayed in tables 9.1 and 9.2. Graphs are provided for the stomach (the thin walled fundus and the muscular antrum), the duodenum, mid portion of the ilium and the colon.

The finding of interest are that no significant difference was measured at any level of the GIT between the fasted versus non-fasted controls as was seen in the pancreas.

An increase in blood flow at all levels was seen with IV alcohol in both fasted and non-fasted animals ranging from 45% in the stomach through to 300% in the colon.

Intragastric infusion of alcohol showed a marked increase in blood flow in the antrum of the stomach, duodenum and ilium but no effect on the colon. Similar changes were seen with intra gastric glucose infusion.

The combination of intra gastric alcohol and glucose had similar effects on the stomach, duodenum and ilium to alcohol and glucose infused separately but a three-fold increase was seen in colonic blood flow.

While interesting, it is not possible to comment further on possible mechanisms.

Conclusions ;

The microsphere method of blood flow measurement is to date the method which comes closest to the ideals outlined by Jackobsen (chapter 2.4) namely providing accurate, reproducible results while producing minimal physiologic interference to the organs of study.

The microsphere method of blood flow measurement is easily carried out in the rat except that the blood flow measurements need to be carried out under general anaesthesia.

It is not possible to carry dual isotope experiments in the rat because withdrawal of the second volume of blood (reference organ) leads to unacceptable changes in cardiovascular function.

Isolation and cannulation the oesophagus in the neck provides a safe and reliable way of infusing solutions into the stomach of the anaesthetised rat. This technique was not described in any of the papers reviewed.

A difference in pancreatic blood flow was demonstrated between fasted and non fasted animal which was statistically significant. This difference had been alluded to but not formally confirmed in any of the papers reviewed.

The intravenous infusion of alcohol produced no significant change in pancreatic blood flow in either fasted or non-fasted animals thus negating the primary hypothesis.

The combination of alcohol and glucose infused into the stomach produced a highly significant increase in pancreatic blood flow thus validating the secondary hypothesis.

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APPENDIX 1.

CONTROL BLOOD FLOW: FASTED.												MEAN	S.D.	S.E.
NUMBER *	67	5	48	84	66	52	50	85	6	83	24			
% DIFF. **	-3	-2	0	0	1	1	1	2	3	8	8			
WEIGHT (GMS)	280	300	335	370	220	255	320	350	310	315	360	310	45	
PANCREAS	106	96	99	168	116	92	138	90	110	57	80	105	29	9
LIVER	9	8	7	13	13	3	8	7	15	6	13	9	4	1
SPLEEN	18	41	37	134	93	24	115	26	43	14	58	55	41	12
STOMACH:														
FUNDUS	30	14	25	19	27	33	30	21	15	12	24	23	7	2
ANTRUM	61	64	62	94	63	50	58	42	73	37	55	60	15	5
DUODENUM	82	104	82	187	97	111	91	79	127	92	93	104	31	9
ILEUM	95	79	109	138	74	169	143	112	175	134	82	119	35	11
COLON	77	40	31	39	48	46	27	38	98	20	25	44	23	7
KIDNEY														
LEFT	419	248	455	512	365	380	489	295	320	331	316	375	84	25
RIGHT	434	254	457	514	363	375	482	291	309	307	291	371	89	27

APPENDIX 2.

CONTROL BLOOD FLOW: NON-FASTED										MEAN	S.D.	S.E.
NUMBER	87	20	63	1	23	3	82	4	22			
% DIFF.	-8	-6	-4	-3	-1	-1	3	3	7	-8	5	2
WEIGHT	335	300	270	340	295	340	310	360	260	335	34	11
PANCREAS	155	108	150	156	71	137	139	175	114	134	32	11
LIVER	5	6	3	9	3	12	6	8	6	6	3	1
SPLEEN	60	40	39	112	85	133	23	156	102	83	46	15
STOMACH:												
FUNDUS	21	21	22	15	16	28	19	24	46	24	9	3
ANTRUM	54	71	54	60	36	64	46	71	88	60	15	5
DUODENUM	150	120	81	137	72	142	84	139	126	117	30	10
ILEUM	187	70	152	140	92	119	87	130	141	124	37	12
COLON	46	47	46	31	30	32	56	54	65	45	12	4
KIDNEY												
LEFT	438	439	345	453	351	389	350	498	438	411	54	18
RIGHT	475	466	360	467	354	391	340	482	409	416	57	19

APPENDIX 3.

FASTED:	INTRA-VENOUS ALCOHOL 1 GM./KG.							MEAN	S.D.	S.E.
NUMBER	12	14	25	13	16	17	9			
WEIGHT (GMS.)	320	215	340	370	225	230	320	289	63	24
% DIFF. *	-8	-5	-5	0	1	2	2	3	3	1
PANCREAS	148	109	81	106	165	51	113	110	38	14
LIVER	5	17	9	7	10	4	13	9	5	2
SPLEEN	179	109	40	123	230	39	164	126	71	27
STOMACH:										
FUNDUS	26	21	16	17	35	23	27	24	7	2
ANTRUM	88	67	49	117	156	60	70	87	38	14
DUODENUM	225	159	176	160	287	113	169	184	56	21
ILEUM	134	162	251	149	210	293	157	194	60	23
COLON	90	158	33	71	220	164	50	112	69	26
KIDNEY										
LEFT	390	443	457	391	392	376	284	390	56	21
RIGHT	423	465	479	391	389	367	277	399	68	26

APPENDIX 4.

NON-FASTED: INTRA-VENOUS ALCOHOL 1GM/KG.						MEAN	S.D.	S.E.
NUMBER								
% DIFF.	-3	-2	-1	-0	0			
WEIGHT	350	300	270	400	380	340	54	24
PANCREAS	120	140	120	135	129	129	9	4
LIVER	18	19	3	6	2	10	8	4
SPLEEN	88	143	43	54	54	76	41	18
STOMACH:								
FUNDUS	21	35	24	33	23	27	6	3
ANTRUM	237	107	85	113	106	130	61	27
DUODENUM	125	142	190	122	156	147	28	12
ILEUM	131	193	184	96	161	153	40	18
COLON	155	183	184	68	56	129	63	28
KIDNEY								
LEFT	385	684	417	522	254	452	161	72
RIGHT	396	698	423	523	253	459	165	74

APPENDIX 5.

INTRA GASTRIC GLUCOSE: FASTED

						MEAN	S.D.	S.E.
NUMBER	73	75	79	78	76			
% DIFF.	7	6	4	0	9	5	3	2
WEIGHT	285	260	240	285	270	268	19	8
PANCREAS	99	104	72	92	85	90	13	6
LIVER	13	30	5	27	28	21	11	5
SPLEEN	76	74	32	144	49	75	43	19
STOMACH:								
FUNDUS	75	151	33	59	81	80	44	20
ANTRUM	90	173	82	79	78	100	41	18
DUODENUM	213	219	151	152	117	170	44	20
ILEUM	171	72	116	115	87	112	38	17
COLON	41	23	34	26	25	30	8	3
KIDNEY								
LEFT	288	239	281	230	304	268	32	14
RIGHT	310	253	292	230	279	273	32	14

APPENDIX 6.

INTRA-GASTRIC ALCOHOL: FASTED.

								MEAN	S.D.	S.E.
NUMBER	71	72	69	70	68	43	45			
% DIFF.	9	6	0	3	4	4	6	5	3	1
WEIGHT	245	400	230	265	210	250	280	269	62	24
PANCREAS	72	144	123	119	100	95	121	111	23	9
LIVER	20	11	6	15	7	11	25	14	7	3
SPLEEN	66	60	83	67	54	63	61	65	9	3
STOMACH:										
FUNDUS	35	40	17	43	57	25	28	35	13	5
ANTRUM	128	154	102	108	146	95	103	119	23	9
DUODENUM	134	444	368	181	193	126	244	241	121	46
ILEUM	106	316	181	187	311	114	183	200	84	32
COLON	47	70	34	44	83	54	65	57	17	6
KIDNEY										
LEFT	287	294	384	347	432	333	433	359	60	23
RIGHT	314	312	385	335	416	320	409	356	46	17

APPENDIX 7.

INTRA-GASTRIC ALCOHOL + GLUCOSE: FASTED.

						MEAN	S.D.	S.E.
NUMBER	44	42	40	38	41			
% DIFF.	6	3	1	6	8	5	3	1
WEIGHT	250	330	345	310	305	308	36	16
PANCREAS	162	117	124	189	215	161	42	19
LIVER	9	6	3	5	13	7	4	2
SPLEEN	23	125	52	89	155	89	53	24
STOMACH:								
FUNDUS	26	27	22	36	41	30	8	4
ANTRUM	85	93	62	101	102	89	16	7
DUODENUM	216	182	108	182	182	174	40	18
ILEUM	246	264	120	174	170	195	59	27
COLON	72	175	70	94	205	123	63	28
KIDNEY								
LEFT	440	486	509	521	601	511	59	26
RIGHT	469	500	504	493	553	504	31	14

APPENDIX 8.

IV ALCOHOL - COMPARISON OF FASTED AND NON-FASTED GROUPS.

	FASTED MEAN (n=7) + S.E.	NON-FASTED MEAN (n=5) + S.E.	% CHANGE	t	p (df=10)
WEIGHT (GMS.)	289 + 24	340 + 24			
PANCREAS	110 + 14	129 + 4	17	1.04	NS
LIVER	9 + 2	10 + 4	11	0.09	NS
SPLEEN	126 + 27	76 + 18	-40	1.4	NS
STOMACH:					
FUNDUS	24 + 2	27 + 3	13	0.96	NS
ANTRUM	87 + 14	130 + 27	49	1.51	NS
DUODENUM	184 + 21	147 + 12	-20	1.36	NS
ILEUM	194 + 23	153 + 18	-21	1.32	NS
COLON	112 + 26	129 + 28	15	0.43	NS
KIDNEY					
LEFT	390 + 21	452 + 72	16	0.96	NS
RIGHT	399 + 26	459 + 74	15	0.88	NS

APPENDIX 9.

COMPARISON OF FASTED CONTROLS WITH IV ALCOHOL GROUP.

FASTED	CONTROL MEAN + S.E. (n=11)	IV-ALCOHOL MEAN + S.E. (n=7)	% CHANGE	t	p (df=16)
WEIGHT (GMS.)	310 + 14	289 + 24			
PANCREAS	105 + 9	110 + 14	+ 5	0.36	NS
LIVER	9 + 1	9 + 2	0	0.01	NS
SPLEEN	55 + 12	126 + 27	+ 129	2.72	<0.01
STOMACH:					
FUNDUS	23 + 2	24 + 2	+ 4	0.25	NS
ANTRUM	60 + 5	87 + 14	+ 45	2.13	<0.025
DUODENUM	104 + 9	184 + 21	+ 77	3.93	<0.0025
ILEUM	119 + 11	194 + 23	+ 63	3.36	<0.0025
COLON	44 + 7	112 + 26	+ 155	3.03	<0.005
KIDNEY					
LEFT	375 + 25	390 + 21	+ 4	0.41	NS
RIGHT	371 + 27	399 + 26	+ 8	0.71	NS

APPENDIX 10.

COMPARISON OF NON-FASTED CONTROLS AND IV ALCOHOL GROUP.

	CONTROL MEAN (n=9) ± S.E.	IV-ALCOHOL MEAN (n=5) + S.E.	% CHANGE	t	p (df=12)
WEIGHT (GMS.)	335 ± 11	340 + 24			
PANCREAS	134 ± 11	129 + 4	- 4	0.35	NS
LIVER	6 ± 1	10 + 4	+ 67	1.06	NS
SPLEEN	83 ± 15	76 + 18	- 8	0.28	NS
STOMACH:					
FUNDUS	24 ± 3	27 + 3	+ 13	0.78	NS
ANTRUM	60 ± 5	130 + 27	+ 117	3.32	<0.005
DUODENUM	117 ± 10	147 + 12	+ 26	1.86	<0.05
ILEUM	124 ± 12	153 + 18	+ 23	1.37	NS
COLON	45 ± 4	129 + 28	+ 187	4.02	<0.0025
KIDNEY					
LEFT	411 ± 18	452 + 72	+ 10	0.72	NS
RIGHT	416 ± 19	459 + 74	+ 10	0.7	NS

APPENDIX 11.

Pancreatic and gastro-intestinal blood flow :
Control vs Secretin (Slavotinek)

Organ	Control - non fasted	Secretin - IV infusion
Pancreas	124 ± 17	468 ± 118
Liver	5 ± 3	3 ± 2
Stomach : - Fundus	21 ± 16	210 ± 86
Stomach : - Antrum	75 ± 29	276 ± 86
Duodenum	137 ± 58	460 ± 110
Ileum	127 ± 44	203 ± 42
Colon	46 ± 44	49 ± 18
Kidney	351 ± 106	547 ± 106