THE NEURAL AND HORMONAL CONTROL OF SPLANCHNIC BLOOD FLOW IN NORMAL AND ABNORMAL MAN.

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The Neural and Hormonal Control of Splanchnic Blood Flow

In Normal and Abnormal Man

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In this thesis the merit of a non-invasive Doppler ultrasound method of measuring superior mesenteric artery (a major constituent of the splanchnic vascular bed) blood flow in man is evaluated. The reproducibility of this method is assessed and then applied to determine the neural and humoral control of the splanchnic vascular bed in normal subjects, patients with sympathetic denervation (primary autonomic failure) and essential hypertension.

Sympatho-neural activation by pressor tests and head-up tilt caused marked splanchnic vasoconstriction associated with a rise (pressor tests) or maintenance (tilt) of blood pressure in normal subjects but not in patients with sympathetic denervation in whom severe postural hypotension occurred.

Sympatho-inhibition by clonidine, a centrally acting α2 adrenoceptor agonist lowered blood pressure, caused a fall in cardiac output and actively dilated the mesenteric artery in normal subjects and in patients with central sympathetic denervation. In patients with peripheral sympathetic failure clonidine did not lower blood pressure or dilate the superior mesenteric artery.

Alcohol ingestion lowered supine blood pressure and dilated mesenteric vessels in sympathetic denervation but not in normals. These responses could be prevented by Octreotide, a somatostatin analogue which inhibits release of gut peptides.

In hypertensives the resting mesenteric vascular resistance was higher than in controls and sympatheo-inhibition by clonidine reversed these changes and lowered blood pressure.

In normal subjects, angiotensin converting enzyme inhibition by captopril caused active mesenteric vasodilatation but failed to lower blood pressure. Mesenteric vasoconstriction occurred during tilt indicating that captopril induced mesenteric vasodilatation was independent of sympathetic activity.

These results suggest that the sympatheo-neural and hormonal (renin-angiotensin and gut peptides) control of the mesenteric vascular bed is important for maintenance of blood pressure. Thus abnormal splanchnic vascular responses contributes to severe postural and post-alcohol hypotension in sympathetic denervation. Similarly a higher sympathetic activity and splanchnic vascular resistance may play a part in the pathogenesis of essential hypertension.
The splanchnic vascular bed is the largest vascular bed in the human body. Studies in animals have shown that the splanchnic circulation plays an important part in the reflex control of the cardiovascular system, in particular blood pressure regulation. However, in humans, the role of the splanchnic circulation in overall cardiovascular regulation is unclear as a major impediment to the study of the splanchnic vascular responses in humans has been the lack of a non-invasive method capable of reliably measuring splanchnic blood flow in unanaesthetised subjects. Recent developments in non-invasive Doppler ultrasound method for measuring blood flow within a major vessel in the splanchnic vascular bed has been a major advance in the study of the splanchnic vascular haemodynamics.

The initial studies presented in this thesis assesses the reliability and reproducibility of a Doppler ultrasound measurement of superior mesenteric artery blood flow in normal subjects. The haemodynamic changes within the superior mesenteric artery in response to various sympahto-neural stimuli in normal subjects and patients with sympathetic denervation due to primary autonomic failure was investigated in a further series of studies. The autonomic failure patients provide an important human model for studying the neural control of the splanchnic vascular bed which is entirely innervated by the sympathetic nervous system. Further studies explored the neural and hormonal control of the splanchnic vascular bed using neuro-pharmacological probes and peptides, with a particular emphasis on the mechanisms contributing to postural hypotension in autonomic failure, or hypertension in patients with essential hypertension.
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DECLARATION

I declare that the studies described in this thesis were carried out by myself at the Department of Medicine, St Mary's Hospital Medical School and at the Autonomic Unit, University Department of Clinical Neurology in the National Hospital for Neurology and Neurosurgery at Queen Square, London, over a period between January 1990 to June 1992. I was solely responsible for recruitment of patients, most of whom were under the care of Professor Mathias. I was responsible for setting up, validation and carrying out the measurements of superior mesenteric artery and portal venous blood flow described in the studies and subsequent analysis of all blood flow measurements. I collected and centrifuged the blood samples in the studies requiring blood collection and was also responsible for the care of the patients after each study. I carried out the entire word processing, statistical analysis (with advice from the statistician attached to the medical school) and graphic work used in the thesis.
DEDICATION

This thesis is dedicated to my father Professor A. K. Roy Chowdhury who taught me medicine and encouraged me to participate in research.
PUBLICATIONS AND PLATFORM PRESENTATIONS IN INTERNATIONAL MEETINGS BASED ON STUDIES DESCRIBED IN THIS THESIS:


Work contained in this paper presented to the Clinical Autonomic Research Society meeting (8th), London, November 1990 and the European Hypertension Society meeting, Milan, Italy, June 1991.


4. Thomaides T, Ray-Chaudhuri K, Mathias CJ. Superior mesenteric vascular resistance is higher in hypertensives and is lowered by clonidine, unlike in normal subjects. Journal of Hypertension 1991;9(sup 6):s82-s83.

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Presented to the European Neurological Society, Lausanne, Switzerland, July 1992.


ABBREVIATIONS:

SMA = Superior mesenteric artery
SMABF = Superior mesenteric artery blood flow
SMAVR = Superior mesenteric artery vascular resistance
MBP or MABP = Mean arterial blood pressure
CI = Cardiac index
FBF = Forearm blood flow
FVR = Forearm vascular resistance
DSBF = Digital skin blood flow
DSVR = Digital skin vascular resistance
FTT = Finger (index) temperature
FI = Falsatility index
AF = Autonomic failure
PAF = Pure autonomic failure
MSA = Multiple system atrophy
MA = Mental arithmetic
CC = Cutaneous cold
ISE = Isometric exercise
SEM = Standard error of mean
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CHAPTER 1

THE SPLANCHNIC CIRCULATION, BACKGROUND AND PHYSIOLOGY.
1.1 THE HISTORICAL PERSPECTIVE OF THE SPLANCHNIC CIRCULATION:

Current knowledge of the intestinal and in particular the splanchnic vascular circulation and physiology are substantially based on the important contributions of eminent scientists such as Aristotle, Erasistratos, Galen, Harvey, Polder, Starling and Morey to name only a few. Why the intestinal circulation became an object of interest for these scientists remains an enigma, although it may be that the mesenteric vessels were the most accessible and visible and were easy to dissect from an eviscerated animal.

In a cave at Lascaux, France, can be found paintings depicting the loops of intestine dangling from the belly of a bison, cut open by a spear (Bradley, 1982) which is probably the earliest known portrayal of intestinal vasculature, being about 30,000 years old. Similar paintings depicting deers have also been found in the Bhimbetka caves in India (Roy-Chowdhury, 1988), indicating the interest of Paleolithic man in the dissection of animals and viewing the viscera. Circa 1600 B.C., written descriptions of the splanchnic circulation appeared in the Egyptian literature and subsequently Diogenes and Hippocrates described connection between forearm veins and the splanchnic circulation in 5th century B.C. This led to the then common practice of phlebotomy for abdominal complaints (Bradley, 1982). In his treatise, De partibus animalium, Aristotle (322-384 B.C.) also described the connection between abdominal organs and the arm veins and believed that the viscera fastens the mesenteric blood vessels to the body. Later, the term "sanguification" was ascribed by Greek physiologists to the formation of blood from food by the splanchnic vascular bed.

About 300 B.C., Erasistratos claimed that blood from the intestine must flow through liver to the heart; he was also the first to propose that movements of the gastrointestinal system intermittently compressed the splanchnic vascular bed thereby helping blood flow to the liver.
Galen (ca. A.D. 129-199) then wrote extensively on various aspects of vascular anatomy and physiology. He described the splanchnic circulation in greater detail and also further refined the "sanguification" theory. About 1400 years later, in the 17th century, William Harvey proposed that the portal venous blood was the same blood that perfused the stomach, pancreas and intestine. Harvey was the first to move away from the concept of "sanguification". In 1830, Poiseuille measured mesenteric venous pressure and this led to the formulation of the currently held principles of vascular hydraulics. He invented the mercury manometer which was subsequently used by Baylis and Starling (1894) in the later part of the 19th century. First measurement of flow in the splanchnic vascular bed was accomplished by Burton-Opitz (1903) using the streamuhr.

In the 20th century, more interest focussed on the splanchnic circulation and included work by Brodie and Vogt (1910) who measured intestinal blood flow during absorption of salt and protein, and Lawson and Chumley (1940) who studied the influence of motility on intestinal blood flow. Florey in 1927 observed that splanchnic nerve stimulation increased lymphatic contractility while the reverse occurred with vagal stimulation. New techniques were developed for blood flow measurement in the 1950's and included the introduction of the microsphere technique for measuring distribution of blood flow in bowel wall by Grim and Lindseth (1958) and use of the dye dilution method utilising the Fick principle to measure splanchnic blood flow as described initially by Bradley et al (1960) and utilized by Rowell (1975).

Non-invasive methods for measurement of splanchnic blood flow were first described by Kaude & Wright (1981) who described ultrasonographic visualisation of major mesenteric arteries, Shepherd and Reidel (1982) who described a laser Doppler method for measuring intestinal mucosal blood flow and Nimura et al (1983) who measured the superior mesenteric artery blood flow using a Doppler ultrasound method. This method has since been used in the study of the splanchnic and various other regional circulations under physiological and pathophysiological conditions.
1.2 ANATOMY OF THE SPLENCHNIC CIRCULATION:

(A) Vascular Anatomy:

The splanchnic circulation is a complex circulation supplying a series of organs including the stomach, small and large intestines, spleen, pancreas and liver collectively referred to as the splanchnic organs. The blood supply to these organs is derived from three major blood vessels, the superior mesenteric, inferior mesenteric and coeliac artery, all of which originate from the abdominal aorta. The coeliac trunk emerges beneath the diaphragm and branches into the common hepatic artery which further divides into several branches. These are the right and left gastric, gastro-duodenal and splenic artery supplying parts of the stomach, the superior portion of the duodenum and spleen. As the studies described in the following chapters are mainly concerned with superior mesenteric artery blood flow, the anatomy of this artery will be discussed in detail. The superior mesenteric artery (SMA) is the second splanchnic branch of the aorta and originates from the aorta about a centimetre below the coeliac trunk, at the level of L1. (Fig.1.1) It passes between the head and the neck of the pancreas and enters the root of the mesentery at the point where the mesentery is applied to the inferior vena cava. The artery then runs in the mesentery in close association with the superior mesenteric vein which lies to its immediate right and travels to its termination as the ileocolic artery in the right iliac fossa. The SMA provides 12 or more mesenteric branches to jejunum and ileum and these divide and reunite to form multiple tiers of intercommunicating arcades. Within the intestinal serosa, the branches of the SMA arborize into further branches which form anastomoses with similar vessels arching in from the opposite direction, at the antimesenteric border of the cylindrical intestine (Noer, 1943, Reynolds and Swan, 1972). The jejunal and ileal arteries fan out from the left side of the SMA to form loops from which the vasa recta arise. The twigs of the vasa recta anastomose freely within the submucosa of the intestine.
Fig 1.1: Figure showing the origin of the superior mesenteric artery and the coeliac artery from the aorta in longitudinal section.
The other major branches of the SMA are the inferior pancreato-duodenal, jejunal and ileal supplying the small intestine, the ileo-colic, right and middle colic artery supplying the large intestine. In 17% of normal subjects the SMA may give rise to an accessory right hepatic artery. The branches of SMA supply the entire small intestine except the superior portion of the duodenum and right half of the transverse colon. The three colic branches of the SMA meet with the colic branches of the inferior mesenteric artery to form the "marginal artery of Drummond" which parallels the entire colon.

The inferior mesenteric artery supplies the large gut from the left end of the transverse colon to the lower end of the rectum. It arises from the aorta at the level of L3 vertebra and descends retroperitoneally and enters the pelvis as the superior rectal artery. The appendicular artery and several small cecal branches arise from the terminal ileal branches of the SMA.

The gastro-intestinal venous system effectively consists of the portal system and it also drains the abdominal viscera such as the spleen, pancreas, stomach, large and small intestines and the mesenteries. The volume of blood flowing into the portal vein and the pressure exerted upon the portal venous blood are largely determined by the resistances to perfusion by arterial blood within each of this portal units. The portal vein is formed at the junction of the splenic and superior mesenteric veins and lies anterior to the inferior vena cava and posterior to the neck of the pancreas. The superior mesenteric vein drains parts of the stomach, the pancreas, the small intestine and colon up to the transverse colon. This vein ascends in the mesentery anteriorly and to the right of the SMA and receives branches from the mesenteric venous arcades of the jejunum, ileum and proximal large intestine. The inferior mesenteric vein joins the splenic vein and drains the area supplied by the inferior mesenteric artery. The portal vein enters the hilum of the liver lying close to the hepatic artery and the common bile duct. The portal vein is structurally weakly muscled and this suggests limited distensibility and easy
collapsibility of this vein. The portal vein bifurcates into a right and left branch. The right branch enters the right hepatic lobe and the left branch subdivides to supply other lobes of the liver. The portal vein also receives contributions from the gastric, cystic and other veins and then further subdivide into smaller branches to form sinusoids. The vessels converge to the sinusoids to form the hepatic veins which join the inferior vena cava.

(B) INNERRATION:

1. SYMPATHETIC NERVES:

(a) Splanchnic Nerves:
The intestine and the intestinal vessels are richly supplied by sympathetic postganglionic fibers which accompany the superior mesenteric artery. These fibers originate principally from the splanchnic nerve (Greenway, 1984) and consist of the greater and lesser splanchnic nerves (Fig.1.2). The greater splanchnic nerve is formed by the preganglionic fibers from fifth to tenth thoracic ganglia and supply the coeliac plexus which surrounds the coeliac artery and the root of the superior mesenteric artery. The lesser splanchnic nerve is formed by the preganglionic fibers from ninth to the tenth and sometimes eleventh thoracic ganglia and supplies the aortico-renal ganglion. There are connections between the coeliac and aortico-renal ganglia and also to a number of secondary plexuses such as the superior mesenteric plexus containing the superior mesenteric ganglion and the abdominal aortic plexus.

(b) Lumbar Sympathetic Nerves:
The vascular bed of the superior mesenteric artery also receives a small contribution from sympathetic nerve fibers originating from first and second lumbar region of the spinal cord (Greenway, 1984). These nerve fibres do not run within the splanchnic nerves (Brooksby & Donald, 1970).
(c) Sympathetic Vasodilator Nerves:

There appears to be evidence of existence of presynaptic cholinergic receptors within the intestinal vascular bed though it is thought that they are not innervated (Greenaway, 1984). Sympathetic vasodilator nerves have been thought to occur in the skeletal muscles in humans, although Walling and Sundlof (1982) have disputed this finding. They recorded efferent sympathetic nerve activity in two humans during vasovagal fainting and recorded abrupt cessation of nervous activity with the onset of hypotension but no increase in other nervous activity as would be expected if the vasodilator nerves were activated.

Stimulation of the sympathetic nervous system, either directly, or in a reflex fashion by using the neurotransmitter noradrenaline, results in constriction of splanchnic, skeletal muscular and adipose tissue vessels in animal and human tissues. The vasoconstriction in splanchnic vessels however, may not persist, due to the phenomenon of "autoregulatory escape", first shown by Folkow et al (1964). It occurs mainly due to dilatation in the arterioles and not the precapillary sphincters or venous capacitance vessels (Folkow et al, 1964; Patel et al, 1981). It is unclear whether autoregulatory escape occurs in human splanchnic vascular bed in vivo. The neurochemical basis for sympathetic vasoconstriction is dependent on alpha adrenoceptor activation and both alpha 1 and alpha 2 adrenoceptors are present within the splanchnic vascular bed. Beta adrenoceptors also influence splanchnic circulation and stimulation of beta adrenoceptors may cause dilatation in the splanchnic vascular bed (Taira & Yabuuchi, 1977). Sympathetic stimulation may also release various vasoactive co-transmitters including neuropeptide Y and adenosine triphosphate. Renin and vasopressin, both vasoconstrictors, may also be released after sympatho-neural activation in vivo. During haemorrhage and hypovolemia, the splanchnic vascular changes may be due to rise in these hormones and the changes can be reversed by appropriate antagonists (Cardiner et al, 1989).
2. PARASYMPATHETIC NERVES:

The parasympathetic nerve supply comes mainly from the vagus nerve through its various branches which include the oesophageal, gastric, coeliac and hepatic branches. The coeliac plexus contain branches from the posterior vagal trunk and pelvic parasympathetic nerves supply the inferior mesenteric ganglion. Both sympathetic and pelvic parasympathetic fibres from the pelvic splanchnic nerves supply the superior gastric plexus.

The extrinsic cholinergic nerves do not appear to cause vascular responses (Greenway, 1984) within the intestinal vascular bed. Vagal stimulation causes gastric vasodilatation but it is unclear whether the vasodilatation is caused directly by vasodilator nerves or secondary to acid secretion or increased metabolism (Martinson, 1965). Vagotomy does not affect superior mesenteric artery blood flow (Tibblin et al, 1969) and vagal stimulation below the level of heart has been shown not to alter intestinal vascular resistance during various stimuli (Kewenter, 1965). This further indicates that vagal nerves probably do not convey specific vasodilator fibres to the intestinal vascular bed.

3. THE ENTERIC NERVOUS SYSTEM:

This consists of neurons and supporting cells within the walls of the gastro-intestinal tract and related viscera (Furness & Costa, 1987). Two major enteric plexuses are described within the intestinal wall which are capable of working independently of the sympathetic and parasympathetic nervous system, by a local reflex pathway. These plexuses are the myenteric (Auerbach's) plexus and the submucous Meissner's plexus. The enteric nervous system is linked closely to the endocrine cells within the gastro-intestinal tract (Fig.1.2).

These cells are actively concerned with the release of various hormones such as the vasoactive intestinal polypeptide (VIP) and serotonin. Many of the hormones released have vascular effects such as vasodilatation by VIP or may modulate the actions of the autonomic nerves.
4. NON-ADRENERGIC AND NON-CHOLINERGIC NERVES:
The existence of a non-adrenergic non-cholinergic neural system was established by Burnstock et al (1963), Bennett et al (1966) and Martinson & Muren (1963). These nerves were recognized in a wide variety of organs such as the urinary bladder, lung, oesophagus and parts of the vascular system (Burnstock, 1969). According to the putative transmitter in these nerves they could be subdivided into purinergic, peptidergic and aminergic nerves.

(a) Purinergic Nerves:
The non-adrenergic non-cholinergic nerves that supply the smooth muscle of the gastrointestinal tract, portal vein in rabbits and urinary bladder seem to utilize adenosine triphosphate (ATP) as the principal neurotransmitter and so are purinergic. Recent evidence suggests that the purinergic receptors could be further subdivided (Burnstock, 1986).

(b) Peptidergic and aminergic nerves:
Some non-adrenergic non-cholinergic nerves utilize neurotransmitters other than ATP. Up to nine morphologically distinguishable types of neurones have been shown in the enteric plexuses (Cook & Burnstock, 1976). These include VIP, neuropeptide, somatostatin, bombesin, calcitonin gene-related peptides among others (Fig.1.2).

(c) Non-adrenergic non-cholinergic innervation of blood vessels:
Non-adrenergic non-cholinergic fibres supplying the portal vein in the rabbit cause vasodilatation possibly by purinergic inhibitory nerves. In the cerebral artery of the rabbit, stimulation of the non-adrenergic non-cholinergic nerves cause vasoconstriction possibly due to serotonergic perivascular nerves (Burnstock, 1986). The effects of non-adrenergic non-cholinergic nerve stimulation on the splanchnic vessels apart from the portal vein in rabbits, is unclear.
Fig. 1.2: NS = Nervous system. Non A Non C = Non adrenergic non cholinergic.

INNERVATION OF THE SPLENCHNIC VASCULAR BED & INTESTINE

Sympathetic

Parasympathetic

Enteric

Non A Non C

Vagal

Auerbach’s plexus

Meissner’s plexus

Close link with endocrinial cells in GI tract

Greater Splanchnic

Lesser Splanchnic

(a) Splanchnic N (T5-T10)

(b) Lumbar (L1-L2)

(c) Vasodilator Receptors (? Innervation)

Purinergic N

Peptidergic & Aminergic N

P1

P2

(VIP, Sub.P, Enkephalin, GRP, CGRP, NPY, Neurotensin, Somatostatin & others)

(Neuromodulation)

(Adenosine & AMP)

(Transmitter)

(ATP & ADP)
1.3 THE IMPORTANCE OF THE SPALANCHNIC CIRCULATION IN PHYSIOLOGICAL AND DISEASE STATES:

(A) Physiology:

Background.

The gastro-intestinal circulation is the largest vascular bed in the body. In mammals, the splanchic organs receive about 25% of the cardiac output at rest and contain up to 30% of the total blood volume (Rowell & Johnson, 1984). The splanchic circulation plays a major role in digestive and absorptive processes which are important for energy production and maintenance of water and electrolyte balance. The splanchic circulation is also thought to play an important role in cardiovascular homeostasis, in particular blood pressure regulation as this region comprises one-fourth of total vascular resistance (Rowell & Johnson, 1984). The rich sympathetic innervation of the splanchic capacity vessels suggest that large quantities of blood may be rapidly mobilized from this vascular bed which has been called the "venesector and blood giver of the circulation" (Katz & Robbard, 1939). Thus alterations in the rate of blood flow to and the volume of blood contained in this vascular bed have been regarded as major factors in the cardiovascular responses to stress (Donald, 1981). The mechanisms governing alterations in splanchic blood flow and blood volume may be **Mechanical or passive** - dependant on the intrinsic myogenic tone of resistance vessels and the compliance of the capacitance vessels and **Active** - dependant on neural or hormonal contributions. This thesis primarily concerns the evaluation and assessment of the latter mechanism, in normal man and various disease states.

The systematic study of changes in splanchic circulation occurring either passively or due to direct and reflex activation of sympathetic nervous system, has largely been confined to studies in anaesthetized cat and dog and quantitative evaluation of the splanchic circulatory physiology have proved extremely difficult for the following reasons:
a. The relative inaccessibility and complex arrangement of the hepato-splanchnic vascular bed.

b. Inadequacies of the available methods.

c. Uncertainties arising from differences in various species of animals studied. This could be due to differences in effective drug doses used, variation in the intensity and manner of the various stimuli applied, dissimilar underlying fundamental physiological mechanisms in the different species or factors such as interaction of baroreflexes with other reflexes and local factors. Moreover, interpretation of the hepatic-splanchnic vascular changes in one species (e.g. man) on the basis of available data in another species (e.g. dog) must be made with caution and may not be completely relevant.

Before discussing human data, consideration will be given to the animal models that have been used and to the experimental conditions that may have influenced animal studies.

(a) Experimental animal models: The following animals have been studied:

1. Dogs: The dog has been used extensively possibly because of its easy availability, size and relative ease of handling. The splanchnic responses to shock have been studied extensively in dogs and Swan et al (1977) found that the splanchnic vascular responses to shock (Escherichia coli endotoxin) in dogs differed markedly from primates (rhesus monkeys).

2. Rats: Rats have been increasing in popularity as an animal model for studies of shock. The advantages are the low cost, ease of maintenance and the availability of controlled breeding which reduces individual variation.

3. Cats: Cats have been used extensively for metabolic studies and studies of splanchnic vascular responses to haemorrhage. Responses to splanchnic artery occlusion in cats differ from those of dogs (Andreen & Irestedt, 1979) and cats may exhibit idiosyncratic reactions to various anaesthetics.

4. Primates: Species used include monkey and baboons. These are expensive and some species are unavailable.
5. **Others:** These include *rabbits, sheep and pigs.* The gastrointestinal vascular arrangement of rabbits are similar to that of cats and sheep have been studied largely in surgical research. Pigs have been studied for research on gastric ulceration and in short term ischaemia studies involving measurement of hepatic blood flow (Booker & Burstad, 1974).

(b) **Effect of experimental conditions:** The following factors are considered briefly:

1. **Anaesthesia and Drugs:** Most studies in animals and in man involve anaesthesia. Anaesthesia, in general, reduces splanchnic blood flow (Kreitsa et al, 1981) and have varying effects in splanchnic vascular resistance. The splanchnic haemodynamic effects of anaesthesia are directly proportional to the depth of anaesthesia and also depend on the duration. It is unclear whether the vascular responses to different anaesthetic agents are species-dependent.

   Drugs used in various studies include *anticholinergics, sedatives and muscle relaxants.* All these have varying effects on the splanchnic vascular bed. Morphine, for instance, reduces splanchnic blood flow (Mailman, 1980) while succinylcholine increases splanchnic blood flow in cats (Savolainen, 1969).

2. Other factors which may alter splanchnic blood flow and vascular resistance are summarised in Table 1.1 and include, *blood volume, respiration, visceral manipulation, temperature, feeding, extracorporeal circulation and urinary bladder distension.*
References

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<th>SBF Model</th>
<th>SB</th>
<th>Model</th>
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Table 1.1

Experimental Conditions Affecting Measurement of Splanchnic Blood Flow

Bradley distribution
Food
Temperature (heal)
Laparotomy
Blood loss
Hypoglycemia or akathisia
3. Scopolamine
2. Morphine
1. Benzodiazepines
Sedatives & NM blockers:
3. Spinal
2. Cyclopropane
1. Halothane
Anesthetic agents:
In humans, studies involving measurement of splanchnic circulatory changes have been limited, owing to methodological difficulties and the invasive nature of the techniques commonly employed. Rowell (1975) has reviewed changes in splanchnic circulation in humans during exercise, lower body negative pressure simulating haemorrhage and thermal stress and these are discussed briefly in the following paragraphs. Recently, in animal studies, the humoral/autacoid and adrenoreceptor control of the splanchnic vascular bed has been elegantly delineated in experiments using gastrointestinal hormones such as secretin, gastrin, neurotensin and others (Chou et al, 1984), various peptides such as angiotensin II, arginine vasopressin (Bennett and Gardiner, 1986), atrial natriuretic peptide (Gardiner et al, 1988), calcitonin gene related peptide (Gardiner et al, 1989), acetylcholine, bradykinin and endothelin (Gardiner et al, 1990) and adrenoreceptor antagonists (Gardiner & Bennett, 1988). However, the role of the splanchnic vascular responses in overall cardiovascular homeostasis during various physiological and pharmacological stimuli, such as described above, in normal and abnormal man, remain largely undocumented.

**Reflex control of the hepato-splanchnic circulation:**

This has been reviewed by Rowell (1974) during stimuli which include haemorrhage, lower body negative pressure, orthostasis, thermoregulatory reflexes or heat stress and exercise.

**Haemorrhage** shows marked variability in vasomotor responses in the splanchnic vascular bed. In cats, during haemorrhage, there is constriction of the splanchnic vascular bed which releases up to 25%-65% of the total blood volume lost in haemorrhage (Greenway & Lister, 1974). In dogs, however, only about 14% of the volume lost in haemorrhage comes from the liver (Carneiro & Donald, 1977). Price et al (1966) removed 15%-20% of the estimated total blood volume from normal human subjects and showed that there was a 40% reduction in splanchnic blood volume though they did not observe a change in
splanchnic blood flow (SBF), cardiac output, heart rate and mean arterial blood pressure.

*Lower body negative pressure (LBNP)* can simulate haemorrhage in humans if LBNP is applied at levels sufficient to draw up to 750 mls of blood into the vessels in the leg. Rowell et al (1974) showed that by applying sustained LBNP (-50 mmHg) below the iliac crests, there was a marked reduction in cardiac output, stroke volume and central blood volume. The SBF fell by 34% and the splanchnic vascular resistance (SVR) rose by 34% and splanchnic vasoconstriction accounted for one-third of the decrease in total vascular conductance. The response was mediated by sympathetic vasoconstrictor nerves as in hypertensive subjects, surgical ablation of the splanchnic nerves prevents splanchnic vasoconstriction during head-up tilt (Wilkins et al, 1951) which also pools blood in legs. This may explain why splanchnic denervation was temporarily effective when used previously, in some patients with severe hypertension (Page & Heuer, 1937).

The *arterial baroreceptors* seems to exert a greater effect on the splanchnic vascular bed than the *cardiopulmonary baroreceptors*, in humans. This is because during LBNP, the increments in SVR correlate more closely with the fall in aortic pulse pressure than with the fall in right atrial pressure.

The *chemoreceptors* also play a part in control of blood flow in the splanchnic region and the reflex effect of hypoxic stimulation of chemoreceptors in the splanchnic region is vasoconstriction (Korner et al, 1967).

*Orthostatic adjustments* during upright posture also involve substantial changes in SBV. During assumption of upright posture, about 600-700 ml of blood move into the leg veins thus reducing the central venous pressure, stroke volume and cardiac output (Gauer & Thron, 1963). The SVR increases by 45% and the SBF falls by 40% during upright posture and makes a major contribution to total changes in systemic vascular conductance and towards maintenance of mean arterial blood pressure. This has clinical implications in patients with sympathetic denervation who experience severe postural
hypotension when tilted head-up, as discussed in the following chapters. Postural hypotension also occurred in hypertensive patients after splanchnic sympathectomy (Wilkins et al, 1951).

Exercise causes redistribution of blood particularly from the non-working regions such as splanchnic vascular bed and the kidneys (Rowell, 1974) to the muscles where blood flow increases markedly. The SBF is reduced while heart rate and plasma noradrenaline levels rise and blood pressure is maintained despite muscular vasodilatation. In dogs, however, the SBF or renal blood flow does not decrease during even severe exercise (Vatner, 1975) though the SVR and blood pressure increase. A combination of neural and hormonal factors are probably responsible for splanchnic vasoconstriction during exercise.

Thermoregulatory reflexes or heat stress is associated with splanchnic and renal vasoconstriction in many species and particularly in humans and other primates (Rowell & Johnson, 1984; Hales et al, 1979). Thermal stimulation of the receptors in the hypothalamus and spinal cord increases the vasoconstrictor activity and SVR rises in parallel with heart rate, cardiac output and plasma noradrenaline levels (Escourrou et al, 1983). As there is a marked rise in cardiac output during heat stress, the reduction in SBF makes a contribution of 10%-20% in the increase in skin blood flow. The splanchnic circulation, however, plays an important role in distribution of blood volume during heat stress. This is because heat stress response provides a good illustration to the concept of Krogh's model (Krogh, 1912) where the cardiovascular system is conceptually divided into a compliant and non-compliant section. Distribution of blood flow between the two circuits determines the volume of blood available for cardiac filling. During heat stress, the rise in blood flow in the skin is compensated by reduction in flow in the splanchnic region.

A number of circulating and locally produced hormones influence SBF and SVR. Food, in particular carbohydrates increase SBF possibly by release of vasodilatory gut peptides. In normal subjects the blood pressure is maintained by compensatory changes in cardiac
output and skeletal muscular vascular resistance (Mathias, 1990). In patients with sympathetic denervation such as those with chronic primary autonomic failure food ingestion causes marked fall in blood pressure, possibly due to splanchnic vasodilatation and lack of compensatory changes in other vascular beds (Mathias et al, 1989).

**(B) The involvement of the splanchnic circulation in disease states:**

1. **Neurological Disorders:**

   **(a) Chronic primary autonomic failure:** These patients have sympathetic denervation and include patients with Pure Autonomic Failure (PAF) or Multiple System Atrophy (MSA). Abnormality of splanchnic vascular responses during upright posture in these patients are probably responsible for the severe postural hypotension which usually occurs in these patients. This is described in detail later. These patients also have marked postprandial hypotension due to food induced vasodilatation in the splanchnic vascular bed and lack of compensatory changes in other vascular beds (Mathias, 1990). Octreotide, a somatostatin analogue which prevent release of vasodilatory gut hormones prevent splanchnic vasodilatation after food in these patients and reduce post-prandial hypotension (Kooner et al, 1990). Different components in food affect SBF differently and thus have a differing effect on blood pressure (Mathias et al, 1989).

   **(b) Syncope:** Syncope refers to fainting or a transient loss of consciousness resulting from an inadequate cerebral flow. During vasovagal syncope, hypotension and bradycardia occurs and the main cause of hypotension is usually skeletal muscle and other regional (possibly splanchnic) vasodilatation (Hainsworth, 1986). Fainting can also be precipitated by activation of cardiac receptors (*Bezold-Jarisch reflex*) which dilates resistance and capacitance vessels in the abdomen (Hainsworth, 1991). A similar response may occur in humans after injection of contrast medium into the coronary circulation or during myocardial ischaemia (Hainsworth & McGregor, 1987).
(c) Parkinson’s disease and Multiple Sclerosis: Patients with Parkinson’s disease (PD) may suffer from autonomic impairment as part of PD or due to drug therapy. Some PD patients also may have symptoms suggestive of post-prandial hypotension. Whether the abnormal splanchnic circulatory responses are involved in the pathogenesis of these symptoms are unclear and needs evaluation. In advanced multiple sclerosis patients there may be diffuse demyelination in the brain and spinal cord affecting the autonomic outflow to the vascular system. Abnormalities of splanchnic vascular responses during sympato-neural activation in these patients may unmask subtle autonomic deficits.

2. Vascular Disorders:

(a) Hypertension: There is increasing evidence as based on studies in animals and humans, that the splanchnic vascular bed possibly plays an important role in the pathogenesis and treatment of hypertension. There may be an increased sympathetic tone in hypertension and the SVR may be higher than in normal subjects. In Dahl salt sensitive rats there is evidence of specific supersensitivity of the mesenteric vascular bed to noradrenaline and periarterial nerve stimulation (Kong et al, 1990).

(b) Post-prandial angina: In subjects with coronary vascular disease, shunting of blood to the mesenteric circulation after a meal may cause symptoms of angina and may lead to myocardial infarction.

3. In elderly subjects: Elderly subjects often have postural hypotension and may suffer from post-prandial hypotension (Lipsitz et al, 1985; Potter et al, 1990). It is probable that splanchnic vasodilatation plays an important role in the pathogenesis of postural and post-prandial hypotension which may contribute to stroke or angina if cerebral or coronary diseases were coexistent.
4. Gastro-intestinal Disorders:

(a) Dumping Syndrome: The classical dumping syndrome often occurs after gastric drainage procedures when there is a rapid entry of hyperosmolar solution in the jejunum causing fluid absorption within the gut. This is usually associated with a reduction in effective plasma volume, a rise in haematocrit and symptoms such as weakness, palpitation and sometimes a modest fall in blood pressure (Roberts et al, 1954). In patients with the early dumping syndrome, food ingestion especially with high carbohydrate content, increases SMABF more than in normal subjects and may account for the symptoms (Aldoori et al, 1985).

(b) Mesenteric Ischaemia: In this condition patients often have postprandial pain, diarrhoea and weight loss. The SMA and coeliac artery velocity waveforms have been quantified after visualisation of these arteries using non-invasive ultrasonographic techniques, in patients with mesenteric insufficiencies (Nicholls et al, 1986). Failure of increase in diastolic flow in the SMA after a liquid meal challenge (the intestinal stress test), has been proposed as an indication for mesenteric angiography for confirmation of diagnosis of mesenteric ischaemia (Monea et al, 1991).

5. Diabetes Mellitus:
In streptozotocin induced diabetic rats there is mesenteric vasodilatation which is not suppressed by octreotide (Bennett & Gardiner, 1992). In patients with diabetic autonomic neuropathy, abnormal splanchnic vascular responses have been thought to play a part in the pathogenesis of postural hypotension (Stevens et al, 1991).
1.4 AIMS OF THE PRESENT STUDY:

The aims of the studies as described in the following chapters are as follows:

1. To set up and validate a reproducible non-invasive pulsed Doppler ultrasound method for measuring superior mesenteric artery blood flow and portal blood flow in man.

2. To identify various aberrations in the anatomy of the superior mesenteric artery and to relate this to measurement of blood flow and also to assess the validity of alternative (to that of the measurement of volumetric blood flow) Doppler indices in measurement of haemodynamic changes within the superior mesenteric artery.

3. To utilize the method to assess haemodynamic changes in the splanchnic vascular bed in normal man during various physiological stimuli which causes activation of the sympathetic nervous system.

4. To compare the splanchnic vascular and other regional haemodynamic changes during sympatho-neural activation in normal man and in patients with sympathetic failure who have severe postural hypotension (pure autonomic failure and multiple system atrophy) with particular emphasis on splanchnic haemodynamic changes during head-up tilt.

5. To use various pharmacological agents (clonidine, alcohol, captopril and octreotide) to investigate the neural and hormonal control of the splanchnic vascular bed in normal man and also in patients with primary autonomic failure.

6. To investigate the splanchnic and other regional vascular and neurohormonal responses in patients with essential hypertension using anti-hypertensive agents such as clonidine or captopril. These studies were aimed at underlining the possible role of the splanchnic vascular bed in the pathogenesis and treatment of hypertension.
CHAPTER 2.

THE MEASUREMENT OF HEPATIC AND SPLANCHNIC BLOOD FLOW WITH SPECIAL REFERENCE TO SUPERIOR MESENTERIC ARTERY BLOOD FLOW.
The gastrointestinal and splenic vascular beds drain into the portal vein which supplies the liver with up to 75% of its blood supply (Fig. 2.1). Blood flow from all the splanchnic organs drain into the portal vein and then from liver to the hepatic veins which drain into the inferior vena cava. Because of the common venous drainage all the splanchnic organs can be grouped together into a single hypothetical organ and changes in blood flow to one splanchnic organ will usually produce the same magnitude of change in total splanchnic blood flow unless it is compensated by an equivalent and opposite change in another parallel organ (Rowell, 1974). Measurements of blood flow in the splanchnic vascular bed have thus focussed on measurement of either total or regional blood flow, initial methods in intact animals and man reflecting changes in total blood flow only.

2.1 INTRODUCTION:

Measurement of hepatic/splanchnic blood flow has been difficult owing to problems discussed previously. Various methods have been applied for measurement of total and regional splanchnic blood flow (SBF) and these can be classified into invasive and the presently available non-invasive methods particularly in man. These methods usually determine blood flow in the major splanchnic vessels such as the superior mesenteric artery and the portal vein while some techniques also measure the total splanchnic blood flow. Initially measurement of SBF was restricted to direct invasive methods which could only be implemented in animals. Subsequently measurement of SBF became possible in humans, but could only be achieved by using techniques requiring multiple catheterisations which restricted its use. Recent technical advances have made it possible to measure and quantify regional blood flow within the splanchnic vascular bed in humans, using a non-invasive Doppler ultrasound technique.
fig 2.1: A simplified diagram of the splanchnic vascular bed. The figure illustrates the parallel configuration of the gastrointestinal organs and blood vessels, the dual blood supply of liver and the common venous drainage of all organs (taken from Howell LB, 1975).
2.2 DIFFERENT TYPES OF BLOOD FLOW MEASUREMENT:

Types of blood flow measurement could be classified according to the size of vessel/nature of tissue or the nature of the investigation (invasive and non-invasive). Some key points are considered below as the details of individual techniques are beyond the scope of this thesis.

**Large vessel technique:** This indicates measurement of blood flow through vessels larger than 0.5mm. The methods include use of flowmeters, indicator dilution, angiographic spillover, video-dilution, ultrasonic Doppler.

**Small vessel technique:** This indicates measurement of blood flow through smaller vessels (pre-capillaries to 0.5mm) and employ microsphere injections, optical image splitters, video playback, fluorescence microscopy and microscopic analysis (post-mortem) of tissue perfused with substances such as gelatin, starch etc.

**Capillary or exchange vessel technique:** This is based on the assumption that rates of movement across capillary walls are influenced by blood flow. The techniques employed include H₂O filtration, ^42^K and ^86^Rb clearance, clearance of radioactive markers from the peritoneal cavity, clearance by pH trapping of agents such as aniline, barbital and aminopyrine (Larsen & Moody, 1981).

**Tissue flow technique:** This measures global blood flow through vessels of all sizes in a tissue of given size, and use techniques such as plethysmography, heat conductance, measurement of tissue radioactivity by injected markers.

**Functional flow technique:** The techniques employed include measurements of clearance of agents such as O₂, BSP, sulphur (colloidal), tritiated water and measurement of metabolic parameters (Larsen & Moody, 1981).

2.3 INVASIVE METHODS:

Invasive techniques available for measurement of SBF can be further subdivided into direct and indirect methods.
Direct methods: These are applicable usually to animals and involves placing a flow transducers either within a main or branch artery after cannulation of one of the major splanchnic vessels, usually the mesenteric artery. The latter has been particularly useful in chronic instrumentation for the study of conscious animals. Changes in total splanchnic blood flow are estimated from flow changes within a single vessel and assumes that the other arteries supplying different portions of the splanchnic regions respond in an identical manner. There is however a possibility of damage to the perivascular nerves supplying the downstream resistance vessels while placing the transducers within or around the vessels. Trauma, anaesthesia, manipulation, haemorrhage and loss through collateral channels may all contribute to the errors inherent in this procedure. Nevertheless, the great advantage of this method is that it is a direct measurement of blood flow.

Measurement of total splanchnic flow is possible if these transducers are placed on the portal vein or the hepatic artery. The levels of blood flow vary according to the circumstances of measurements and depends upon the species of animal, and whether the animals are conscious or anaesthetised.

The application of flowmeters to the circulation will alter the blood flow subsequent to insertion of the cannula and thus placing an obstacle in the path of the flowing blood and external constriction from surrounding the vessel with a rigid sleeve. Flowmeters used include:

(a) Flowmeters measuring pressure differences: These include the Venturi meters which is based on the generation of convective acceleration by variation in the cross-sectional area of a tube.

(b) The rotameter: This device measures mean blood flow in cannulated vessels and was originally intended for measuring gas flow.

(c) The electroturbinometer: This consists of a stainless-steel turbine driven by the blood stream and generates voltage which is proportional to the rotational speed.
(d) Bristle and Pendulum flowmeters: These are based on the principle that if the body is held in position by an elastic device, it will undergo displacement due to force and the degree of displacement can be taken as a measure of that force.

(e) Electromagnetic flowmeter: The principle of electromagnetic blood flow sensors is based on the fact that when blood flows through a magnetic field, it generates an electromotive force or voltage which can be assessed at signal electrodes. There are two types of flow sensors and these are perivascular and extracorporeal flow sensors. The perivascular flow sensor can be slipped around surgically exposed blood vessels and can be used for acute or chronic application. The extracorporeal flow sensor provides a continuous measurement of blood flow after insertion into an extracorporeal circulation by tubing connections. The electromagnetic flowmeters have several advantages which include direct transformation of mechanical impulses to electrical signals, negligible interference with blood flow, easily achieved instantaneous and continuous measurement of mean blood flow by integrating circuits (calibration being independent from velocity profile, viscosity and density of fluid) and applicability to all fluids with electrical conductivity equal to or higher than tap water. Furthermore no anticoagulation is needed and the perivascular sensors leave the vessels intact. The limitations of this technique include the frequent need for resetting the zero point, extreme sensitivity to minor changes in position and its invasive nature.

(f) Ultrasonic flowmeter: This measures blood velocity by recording ultrasound frequency shift upstream and downstream within a small length of vessel. This has many advantages including no interference with blood flow and the vessel remains intact and the signals can follow rapid changes in instantaneous blood velocity in the circulation. The Doppler technique has been compared to electromagnetic flowmeters by Gardiner et al (1990a) and a modified pulsed Doppler probe which was capable of resolving aliasing (discussed later), produced results in good agreement with the electromagnetic flowmeters.
Miscellaneous methods: These include use of markers such as dye, radio-opaque material or gas bubble, electrolytic polarisation and nuclear magnetic resonance.

Indirect methods: Various indirect invasive techniques for measuring SBF are available and are applicable to both man and animals. These involve three different methods: the clearance and extraction techniques which determines the total quantity of some substance removed from or added to the blood by the liver each minute, the single injection technique in which the percentile disappearance of some substance cleared from the blood perfusing the liver is measured and hepatic blood flow is calculated as that percentage of the blood volume and the dilution techniques which estimates flow from the extent of dilution of a known quantity of tracer by total outflow during a fixed time.

The dye extraction and clearance method utilizes the Fick principle, first used by Bradley et al (1945) and consists of infusion into the blood stream of non-toxic dyes such as indocyanine green, tetrachlor-tetraido-fluroscein (rose bengal), or sulphobromothalein sodium (BSP). These dyes are removed from the blood by the liver and splanchnic/hepatic blood flow can then be measured either by a constant infusion method or a plasma disappearance method.

Constant infusion method: In this method blood flow is measured by a constant infusion of the dye and catheterisation of the hepatic vein. The formula for calculating blood flow utilises the Fick principle as described by Adolf Fick (1870).

This method is usually applied under conditions of constant splanchnic blood flow and depends on the dye being removed only by the liver at a steady rate and on the absence of a significant entero-hepatic circulation.

BSP may be replaced by indocyanine green (ICG) which has the advantage of being removed only by the liver and has minimal entero-hepatic circulation. ICG is unconjugated and the plasma arterial levels are steadier in those with impaired hepatic function. The calculated HBF/SBF are lower than with BSP particularly in cirrhotic
patients. However, ICG is unstable on standing in aqueous solution and therefore not ideal for constant infusions.

2. Single injection technique: This involves the plasma disappearance method: In this method a single bolus intravenous injection is employed. HBFSBF is measured after injection of ICG followed by the analysis of the disappearance curve in a peripheral artery and hepatic vein. A heat-denatured albumin colloidal complex tagged with $^{131}$I (131 CAI) has been used in man to measure HBFSBF as it has minimal extra-hepatic removal and 94% extraction by the liver in man.

3. Dilution techniques: This depends upon the measurement of dilution of a known amount of tracer within the hepatic circulation over a measured time period. The measurements are adapted from the Hamilton-Stewart method for cardiac output measurement and the Kety-Schmidt method for measuring cerebral blood flow. $^{131}$I labelled human serum albumin is used which is injected into spleen and the concentration curve followed either by external counting over liver or by continuous sampling from hepatic venous outflow. The possibility of recirculation, nonuniform mixing, possible pooling of the tracer and the difficulties of intrasplenic injection limit the usefulness of this method.

Using the dye technique requires insertion of three intravascular catheters, one for dye infusion, one for measuring arterial dye concentration and one for measuring hepatic venous dye concentration. The latter is obtained by cannulating a large hepatic vein under fluoroscopic guidance.

These measurements yield values which are commonly known as estimated hepatic or splanchnic blood flow because the hepatic venous dye concentration, measured from one hepatic vein, is an estimate of average venous dye concentration for all hepatic veins (Rowell, 1974). As several veins drain the liver, the use of only one vein to measure venous dye concentration could lead to an atypical value and erroneous flow measurement. However this does not appear to be an important source of error in the
measurement of splanchnic blood flow (Rowell, 1974). Simultaneous comparison of flow measured by using dye and with flowmeters and using indicators which are almost completely extracted by the liver in one pass, yield similar values for splanchnic blood flow (Drapanas et al, 1960). There are potential sources of error using this method when applied to measurement of splanchnic blood flow. As the hepatic blood flow is a sum of hepatic arterial and portal venous blood flow, it provides an indirect estimate of splanchnic blood flow through changes in the portal blood flow, which is the common venous outflow of the splanchnic vessels. In disease states such as cirrhosis of liver and congestive cardiac failure the hepatic extraction of the dye may be reduced up to 20% because of significant shunts between portal vein and inferior vena cava. The total splanchnic blood flow in such circumstances is greater than the hepatic blood flow.

The dye dilution technique has also been utilized to selectively measure SMABF in subjects who have been or were to be operated upon for malignant diseases by Norryd et al (1975). Significant errors may occur with the use of ICG when measuring the intestinal blood flow. Comparison of ICG and electromagnetic flowmeter measurement of blood flow in isolated, autoperfused intestine of the dog showed that ICG overestimated intestinal blood flow by almost 40% (Donald & Yipintsoi, 1973). The authors also noted that errors were greater with pulsatile flow. Other limitations include the possibility of buildup of the indicator in blood if used repeatedly, sedimentation of ICG in saline, spectral shifts on dilution and incomplete recovery from some vascular beds. The technique of thermal dilution is less invasive because the indicator injection and detection of indicator curve can be accomplished by a single thermistor-tipped catheter.

(2) Angiographic techniques: (a) Spill-over angiographic reflux method: This procedure involves the interpretation of serial angiographic films (using a contrast medium such as 76% methylglucamine diatrizoate ) which are taken during intra-arterial injection of the medium delivered at a linearly accelerating rate. Injection of the medium into an artery at a linearly accelerating rate causes the rate of the contrast medium to ultimately
approach the rate of blood flow. At this point further increase in injection rate causes the dye to "spill" retrograde towards the heart and the rate of injection at the time of initial spillover equals the rate of blood flow. This technique was successfully used by Olin & Redman (1966) to measure renal blood flow in rabbits and since then has been used in human studies as part of angiography, by workers such as Clark et al (1980) who measured SMABF after dilatation of the SMA by prostaglandins. The limitations of this method include the subjective nature of data analysis, effect of size and shape of catheter on blood flow, the vasoactive properties of the contrast medium, the haemodynamic changes induced by lengthy, selective and rapid injections of medium (thus the duration of the injection has to be kept below 3 seconds or less), vascular trauma and the invasive nature of the procedure.

(b) Video dilution method: In this method, contrast medium is injected in an artery and is recorded on videotape during fluoroscopy. The blood flow in the artery can then be estimated as a fraction of the cardiac output. The results when correlated with electromagnetic flow reading show close correlation (Lantz et al, 1981). The advantage of this method is that it is more accurate than the "spillover" method, it can be conveniently applied during routine angiography in patients and it does not prolong catheterisation time. The limitations concern effect of contrast on circulation, mixing of contrast with flow, the technique of video recording, catheterisation and vascular trauma.

(3) Inert gas washout method: This method has been utilized to measure blood flow in the small intestine in cat and man (Hulten et al, 1976). In man studies have been performed in patients undergoing laparotomy for carcinoma of colon, ulcerative colitis or Crohn's disease (Hulten et al, 1976). Easily diffusible, lipid soluble inert gas such as $^{85}$Kr dissolved in 0.9% normal saline was injected intra-arterially as a single bolus via a cannula in a marginal intestinal artery. To measure total intestinal blood flow, the elimination rate of the $r$-activity of the isotope was recorded by a scintillation detector coupled to a spectrometer and a linear ratemeter.
2.4 NON-INVASIVE METHODS:
The difficulties of measurement of total and regional splanchnic blood flow in humans, due to the invasive nature of the procedures, led to the search of non-invasive techniques which can adequately and reproducibly measure haemodynamic changes within this large vascular bed. It is now possible to measure and quantify regional splanchnic blood flow, using a Doppler ultrasound method. A voluminous literature exists about the theory and mechanics of Doppler ultrasound machines and as the studies described in this thesis rely on the use of this technique, the practical principles will be considered in detail.

Background of Doppler Ultrasound Technique:
The physical principles which make Doppler blood flow measurement possible have been understood since World War II. In the past decade, development of instrumentation, mainly in the form of advances in miniaturisation of electronics has taken place rapidly. The equipments have evolved rapidly from continuous wave Doppler flow detectors that provided the user with a poorly localized audible Doppler signal for aural analysis to the presently available state-of-the-art colour flow mapping systems and duplex instruments. These combine real time ultrasound imaging with pulse Doppler techniques and can provide information of a physiologic as well as anatomic nature. Satomura (1959) developed the first Doppler flow detector and can be credited with opening the era of non-invasive diagnosis in vascular diseases. The clinical use of the Doppler flow detectors started in the 1960's in the area of peripheral vascular disease (Rushmer et al, 1966; Strandness et al, 1966). The initial instruments did not have direction sensitive circuitry. Pulsed Doppler system (discussed later) was first used for blood flow velocity measurement by Wells in 1969. Since then the Doppler ultrasound methods have been applied to various areas such as cardiology, obstetrics, general and regional circulation.

The advantages of measurement of SMABF include:

1. Easy visualisation and fairly constant anatomy of the SMA.
2. Plug flow velocity profile in proximal SMA which allows accurate measurement of volume blood flow.
3. SMA has a characteristic Doppler signal which can be easily distinguished from the neighbouring vessels (Fig 2.2).
4. It is entirely innervated by the sympathetic nerves thus allowing easier assessment of neural control of haemodynamic changes in the SMA.
5. There is good correlation of non-invasive SMABF values with invasive studies.
Fig 2.2: Real time two dimensional Doppler image showing the superior mesenteric artery (S) with a cursor placed within its lumen so as to obtain a characteristic Doppler frequency shift signal (lower panel).
Basic Physics and The Doppler Effect:

The Doppler effect was first described by Christian Johann Doppler (1803-1853) who postulated that the colour of a luminous body must change by relative motion of the body and the observer. When applied to sound waves, the Doppler principle states that if a sound source moves relative to an observer, the observer will detect a signal whose frequency is shifted from that source.

The frequencies of an ultrasound acoustic wave are, as the name implies, beyond the hearing threshold, i.e. > 20kHz. The ultrasound acoustic wave is a longitudinal compression wave and consists of a series of compressions and rarefactions where compressions and rarefactions are regions of increased and decreased pressures with respect to the ambient pressure. The pressure wave propagates through tissue at a velocity typical for each tissue and is dependant on the elastic modulus (M) and density (D) of the tissue medium. The elastic modulus of a tissue is determined by the strength of attachment of the molecules and cells in a small tissue volume and the density is a measure of the number of atoms in that tissue volume. As M and D are tissue characteristics, therefore it follows that each tissue has a characteristic velocity. The characteristic velocity of sound in tissue is calculated to be 1540 m/sec and this represents a satisfactory average value for each tissue (Nelson and Pretorius, 1988). The sound wave also has a characteristic frequency (number of pressure peaks per second) and wavelength (the distance between pressure peaks) and the relationship between wavelength (\( \lambda \)), frequency (f) and speed of propagation of sound wave in tissue (c) is given by:

\[ c = f \lambda \]

As the velocity is constant, the wavelength decreases as the frequency of the sound wave (determined by the transducer) increases. The wavelengths used in ultrasonic equipments
range from 0.08 to 0.016 mm which correspond to frequencies from 2-10 MHz respectively.

The ultrasonic waves will propagate through tissue until all its energy is dissipated and the amount of attenuation of ultrasonic vibration increases with distance from the transducer. The attenuation also depends on the frequency of the vibrations, the homogeneity of the ultrasonic beam and on the absorptive properties of the medium, and this relationship is expressed by:

\[ \alpha = a x f^b \]

where \( \alpha \) = coefficient of attenuation, \( a \) & \( b \) = constants characterised for a given medium and \( f \) = frequency of the ultrasonic source. Thus, the higher the frequencies, the higher the attenuation. For deep penetrations to image deep-seated vessels, lower frequencies such as 2-3.5 MHz is preferred while for superficial vascular examinations, a frequency range of 5-10 MHz is usually recommended. In certain topographical and pathological conditions difficulties may arise due to the high attenuation coefficients of calcium (5000 times that of water) and air (50 times higher than water).

**The Transducer:**

Various types of transducer systems, for use with the appropriate Doppler instrument, are now available. These include pencil probes, mechanical sector probes, intercavity probes and different types of array systems. The vibration of piezoelectric crystals are used as a source for ultrasonic energy. Originally Rochelle salt piezocrystal was used but this has now been replaced by polycrystalline ceramic containing artificial material such as titanate zirconate. These crystals vibrate when connected to a source of electrical energy and also are capable of producing electrical energy when exposed to mechanical vibrations. The transducer controls the frequency with which the Doppler instrument operates and is thus a critical component of the system. Doppler information may be obtained by either continuous or pulsed ultrasound waves (to be discussed later) and the transducer has to be designed specifically for either of these operations. A continuous
wave mode transducer needs a simple air cushion behind the crystal, as major portion of the energy is reflected into the insonating medium (Fronek, 1989). During pulsed applications, the short bursts of vibrations produce long "ringing" of the element after the electrical impulse has ceased, and this would interfere with detection of early reflected signals (Fronek, 1989). The piezoelectric element has therefore, to be backed by a cushioning material which would dampen the after-oscillations. The backing substance usually used consists of fine epoxy compressed powdered metallic material.

Another important criterion for design of transducer is the ultrasonic field it produces. The ultrasonic field consists of two components which are (a) the near field, where the ultrasonic field propagates as a cylindrical beam and (b) the far field, which commences at the end of the near field and starts to disperse and follows a conical confine (Fig.2.3, Kossoff, 1978).

The details of physical principles and application of various transducers has been reviewed by Kossoff (1978) and two important types of transducers will be considered here briefly.

**Mechanical sector scanners:** These are of various designs, but all involve the alternating between imaging and Doppler ultrasound. A single, mechanically focussed, transducer crystal is used which is swept through an angle of 15°-120° at a rate of five to 78 times a second. The sweep rate and the angle can be determined by the user depending upon the desired field of view. As the mechanical scanners use a single and relatively large (7.5-25 mm) transducer, the focal characteristics and thus the quality of the image are often good. However, because these are focussed mechanically, the focal length is fixed and different transducers are needed for scanning area in different depths in the tissue.
Fig 2.3: The ultrasonic field of a transducer (after Kossoff, 1978).
**Phased-array scanners:** This system allows the simultaneous display of images and Doppler spectra, unlike the mechanical sector scanners. There may be however, some degradation of the image and the Doppler signal to achieve this advantage. These transducers are composed of many transducer crystals (e.g. 128) which are mounted close to the scanhead in either a flat or a convex linear format. The focusing and sweeping of a phased array is done electronically by adjusting the phase or time delay of the electrical signal driving each element. This makes it possible to focus the array in a dynamic fashion and a single array can be used to give a large range of focal lengths, unlike the mechanically focussed transducers. These can be also used to simulate a sector scan and the choice between linear or sector array depends upon the intended application, sector being required for most abdominal applications while the linear arrays are ideal for obstetrics (Taylor and Holland, 1990).

**Blood Flow Measurement by Doppler Ultrasound:**

The ultrasonic beam generated by the transmitting crystal is reflected by the moving red blood cells which act as reflecting particles (Kato et al, 1962). The reflected energy is picked up by a second receiving crystal or the same crystal which has been switched from a transmitting mode to a receiving mode. The movement of the reflected energy results in a change in wavelength. As the velocity of sound is constant, the change in wavelength corresponds to a change in frequency. The magnitude of the change in frequency is dependent on (a) the velocity of wave in the medium, (b) the relative velocities of the reflector and the medium, (c) the frequency of the source. Many types of Doppler instruments provide Doppler information as frequency shift (in kilohertz) and Doppler shifts are only comparable when the frequency and the angle of insonation are given. The Doppler frequency shift \( f^D \) is given by the Doppler equation which states:

\[
2f_o \cdot v \cdot \cos\theta
\]

\[
f^D = \frac{\nu \cdot \cos\theta}{c}
\]
where \( f_0 \) = the incident frequency, \( v \) = flow velocity, \( c \) = speed of sound in tissue, \( \Theta \) = angle between the ultrasound beam and the flow direction.

It is possible therefore to measure the velocity of a moving object emitting or reflecting ultrasound waves from the measured Doppler shift by solving the above equation. There are however several factors which may limit the use of Doppler ultrasound in localizing and measuring blood flow. These need to be addressed before satisfactory measurement of blood flow and are discussed below along with possible ways of solving these problems. The factors which influence Doppler ultrasound recording and calculation are:

1. **Transducer performance.**
2. **Ultrasound attenuation in tissue.**
3. **Ultrasound scattering in blood.**
4. **Transmitting signal characteristics.**
   a. Choice of frequency.
   b. Aliasing.
   c. Angle of insonation.
5. **Received signal processing methods.**
   a. Spectral analysis and demodulation.
   b. Wall filter.
6. **Haemodynamics of blood flow and sample volume size:**

   1. **Transducer performance:** Details about transducers have already been described. All medical transducers have the disadvantage of finite aperture defects as the transducers are not point sources but have an active and finite surface area. This is governed by Huygen's principle where it is stated that wavelets generated by each minute element on the surface will interact with each other, producing a characteristic field of maximal and minimal vibration in the acoustic field generated by the transducer. To combat this, mechanical or electronic focusing of Doppler transducer systems is used to improve localisation of ultrasound echoes and yield uniform image intensity.
2. **Attenuation of ultrasound in tissues**: The ultrasound attenuation in many tissues increases almost linearly with frequency, causing high frequencies to be attenuated more than low frequencies (Goss et al., 1975; Nicholas, 1982). Thus for examination of abdominal vessels which are deep-seated, lower frequencies (2-3 MHz) are required as the distance from the transducer to the vessel could be several centimetres long. Attenuation of Doppler pulse in tissue could also change the displayed Doppler power spectrum, when very short pulses are used to achieve very small sample volume (Newhouse et al., 1977).

3. **Ultrasound scattering in blood**: The majority of ultrasound scattering from blood is thought to occur from the red blood cells (RBC, Shung et al., 1976). As the average diameter of a normal RBC (7 μm) is much smaller than a typically employed ultrasound wavelength (e.g. 300 μm at a 5 MHz frequency), a single ultrasound wavelength will meet nearly $10^5$ RBCs (Angelsen, 1980). The inhomogeneities of blood due to local variations in RBC concentration and diameter may give rise to many weakly scattered waves of random phase and amplitude that superimpose at the receiver to give the total received signal (Fig 2.4).

This type of scattering is called *Rayleigh scattering* and the amount of scattering depends upon the scatterers radius raised to the sixth power and the frequency raised to the fourth power. This indicates a strong size dependence and has the following implications. Firstly, the increase of backscatter with frequency means using high frequency would greatly increase the amplitude of ultrasound echoes from blood. However, attenuation of ultrasound in tissues increases with frequency and therefore depending on the thickness of the tissue path for ultrasound examination, a trade-off between frequency dependent backscatter from blood and ultrasound attenuation in tissue will determine the optimum frequency needed for ultrasound examination. Thus superficial (peripheral vascular) Doppler examinations are usually performed at frequencies of 5-7 MHz while imaging of deep-seated vessels are performed at 2-3.5 MHz. Secondly, ultrasonic backscatter from blood would cause spectral distortion if there is broadband ultrasonic pulse. Broadband
FIG. 2.4: Ultrasonic scattering in blood.
pulse would encompass high and low frequency components and high frequency components would produce stronger echoes than the low frequency ones causing distortion. However, the effects of attenuation and backscatter will nearly cancel each other on the spectrum of a 3.5 MHz ultrasonic pulse through a 6 cm tissue path (Taylor and Holland, 1990).

4a. Choice of frequency: This varies depending upon whether continuous-wave or pulsed-Doppler technique is being used. In the continuous-wave technique, the prime consideration is frequency dependent attenuation and so high frequencies (6-10 MHz) can only be used for superficial vessels and low frequencies reserved for deeper imaging. In the pulsed-Doppler technique, at higher frequencies there is better resolution and increased amplitude of returned Doppler signal but there is also increased attenuation which limits the depth of imaging. Also at higher frequencies there is increased chance of aliasing (discussed later) while lower frequencies enable detection of higher flow velocities without aliasing.

4b. Aliasing: Aliasing occurs when the velocity of blood exceeds an upper measurement limit resulting in display of erroneous velocity information. Aliasing occurs with pulsed sampling techniques and colour flow methods and is defined by the pulse repetition frequency, the vessel depth, transmitter frequency and angle of insonation (Nelson and Pretorius, 1988). The pulse repetition frequency (PRF or sampling frequency) is the frequency at which sequential tone bursts are transmitted by a pulsed Doppler equipment. When an ultrasonic pulse is transmitted into a tissue, the pulsed Doppler system must receive the last echo from this particular pulse before it transmits the next one. Current pulsed Doppler equipments measure Doppler shifts of echoes from the blood by extraction of the phase change in echoes from one PRF to the other. The mathematical details imply that the maximum frequency signal that may be detected is half that of the PRF. This maximum frequency is known as the Nyquist frequency and thus limits the maximum flow velocity that can be recorded with a Doppler instrument.
operating at a fixed PRF. The maximum velocity \(V_{\text{max}}\) can be calculated from the Doppler equation and the Nyquist frequency given by the PRF, as follows:

\[V_{\text{max}} = \frac{c \cdot \text{PRF}}{4 \cdot f_0 \cdot \cos\theta} \]

Thus the maximum measurable velocity can be increased by increasing the PRF, decreasing the \(f_0\) or increasing the angle of insonation (decreasing \(\cos\theta\)).

Aliasing will occur when sampled blood velocities produce Doppler shifts greater than the Nyquist frequency determined from the PRF. The aliased signal, when displayed, tend to wrap around the baseline and frequently appear below the baseline (Fig 2.5). The aliased frequencies thus misrepresent high-velocity forward flow as low-velocity reverse flow.

What are the ways the problem of aliasing can be resolved satisfactorily? One needs to consider the modified Doppler equation for calculation of maximum velocity as described earlier. Firstly, the sampling rate can be increased. But the rate of sampling is determined by the speed of sound in tissue and the depth of examination. For deep seated vessels with fast flow it may not be possible to increase PRF sufficiently to avoid aliasing. Secondly, one may convert to using continuous wave Doppler ultrasound technique where there is no Nyquist limit. But the disadvantage of continuous wave method is the loss of axial resolution though this has been satisfactorily used in carotid artery studies. Thirdly, as Doppler frequency shift is inversely related to the maximum velocity, aliasing can be prevented using a lower frequency. Though practicable for superficial vessel studies where higher frequencies are used, this is not of use for abdominal vascular studies as a low frequency is already in use. Fourthly, demodulation of Doppler signals can be used to prevent aliasing. During demodulation of the received Doppler signals, the baseline or the zero flow line can be adjusted by progressively lowering it till the entire range of velocity is displayed as fast forward flow. Most recent Doppler equipments incorporate demodulators to prevent aliasing. Finally, the aliased waveform may be utilised by adding the the reverse velocities to the cut-off peak above the baseline (forward flow) or by increasing the angle of insonation.
Fig 2.5: Upper panel showing Doppler shift signal displaying aliasing and poor signal to noise ratio. Lower panel shows the same signal after demodulation by adjusting the baseline of the Doppler spectrum.
4c. Angle of insonation: If we look at the Doppler equation, it is clear that one of the stronger variants affecting the Doppler frequency shift is the angle of insonation $\theta$ (angle between the ultrasound beam and axis of blood flow in the vessel examined). Blood flow velocities measured from the frequency shifts are inversely proportional to the cosine of the angle $\theta$. If the ultrasound beam is at right angles (90°) to the direction of flow then no signal will be obtained as cosine of 90° is 0. Typically at angles exceeding 70° Doppler shifts often appear above and below the baseline causing the so called "mirror-image" artifact. Experienced workers have suggested that angles between 30° to 60° are ideal for imaging (Taylor & Strandness, 1987) and Burns (1988) has noted that at 70° angle, an uncertainty of the measured angle of even 5° will cause a 25% error in calculated velocities. Rizzo et al (1990) has shown that in the SMA, changes in the measured peak systolic velocities (PSV) and mean velocities (MV) varied from -15% (PSV) and -23% (MV) at 30° to 120% (PSV) and 111% (MV) at 80°. The variation of velocities, which would determine measurement of volume blood flow, occurred markedly at angles above 70° thus further emphasising the need for strict control of this variable. It is therefore generally accepted that the upper limit of the angle of insonation should be 60° for abdominal vascular studies (Taylor & Holland, 1990) and Doppler studies involving angles greater than 60° should not be used for measurement of blood flow velocities or volume blood flow. In practice, angles between 30° and 60° are usually employed.

5a. Signal processing and demodulation of Doppler signals.

The function of a spectral analyser: The output of a Doppler ultrasound unit consists of time-varying signals containing frequency components which are in the audible range. The spectral analyser converts the time domain waveform to frequency domain whereby a complex time varying waveform is broken down into a spectrum of individual component frequencies. The spectrum consists of different frequencies due to the configuration of the velocity profile, the high frequencies at the far end of the power spectrum (representing peak velocities around the vessel axis) and the lower frequencies
reflecting slow velocity components close to vessel wall. The principle is analogous to refraction of light by a glass prism. The equipment described in this thesis uses a FFT analyzer which is capable of transforming incoming Doppler signals in the time domain to frequency domain in real time.

The FFT analyser initially samples and digitises the demodulated (discussed later), time-domain Doppler signal with a sampling rate usually sufficient to prevent aliasing. The output, after electronic computing, is displayed on a moving screen with time along the horizontal and frequency on the vertical axis. A grey scale intensity represents the power at each frequency. This is the Doppler spectral display and referred to as time velocity waveform. According to the Doppler equation, the frequency spectrum of the Doppler signal corresponds directly to the velocity spectrum in the blood flow. Thus the peak systolic, end diastolic, average velocity and other Doppler flow indices can be measured directly from the Doppler spectrum. Bandwidth of the Doppler spectrum also provides important information on the nature of blood flow.

**Demodulation:** The Doppler frequency shift (kHz) is carried on high frequency sound waves (MHz) and the process of separating the Doppler shift from the sound waves on which it is carried is called demodulation. The type of demodulation in modern equipment is the phase-quadrature heterodyne demodulation and this generates two channels of Doppler signals in the audible frequency range. One channel represents flow towards the transducer and the other represents flow away from the transducer. The principle of demodulation and spectral analysis has been discussed in detail by Atkinson and Woodcock (1982).

**5b. Wall filters:** Unwanted echoes particularly from the vessel wall often cause large amplitude ultrasound echo with low velocity Doppler frequency shifts which interfere with interpretation of spectral displays. Echoes from walls of blood vessels are large and often saturate the electrical circuitry which has been designed to amplify, demodulate and process low amplitude echoes from blood. This causes a loud thumping noise in the
audio frequency output and is known as the "wall thump" particularly in cardiology. The wall thump can be effectively eliminated by using a wall filter which is a variable cutoff, high pass filter, which electronically removes low frequency Doppler shifts at the demodulator output. The frequency cutoff point can be adjusted manually in most equipments and varies from 50 to 1600 Hz. However, while effective in removing unwanted echoes, the wall filter also can remove Doppler information from the incoming Doppler signals. This is particularly true in abdominal imaging where the wall filter should be set as low as possible (50-100 Hz) to avoid losing low frequency signals and maintain display of important diastolic flow which in particular can be abolished by a high filter.

6. Blood flow patterns and the effect of the size of sample volume: Information about the pattern of blood flow in a particular vessel can be obtained from the Doppler spectral display. Circulation is pulsatile and causes several types of blood flow and associated velocity distributions to occur. The three main types of blood flow are plug or flat flow, parabolic or laminar flow and turbulent flow. Blood flow can also assume intermediate forms between these three types. Plug flow consists of a flat and linear wave front and implies all the RBC's at the different flow laminae are moving at the same velocity and thus has a narrow velocity distribution (Fig 2.6).

This type of flow is seen in vessels such as the aorta and proximal SMA and produces a clean wave pattern with an absence of lower velocities (Taylor and Holland, 1990). The parabolic flow profile has a broader velocity distribution as the RBC's in contact with the vessel wall move slower than those in the centre of the blood stream thus producing a parabolic wavefront.
Fig 2.6: "Clean" Doppler shift signals from the superior mesenteric artery probably suggestive of plug flow.
Parabolic flow is seen in vessels usually smaller than 5 mm in diameter such as the proximal hepatic artery (Taylor et al, 1985). Turbulent flow has a broad velocity distribution and these range from very slow moving blood to very fast jets. Reverse flow commonly occurs in turbulent velocity patterns and is usually associated with narrowing of the vessel, stenosis or bifurcation of vessels (Nelson abd Pretorius, 1988). Relationship governing the type of flow is expressed by the Reynolds number which is given in the following equation:

\[ \text{Re} = \frac{\text{velocity} \times \text{radius} \times \text{density}}{\text{viscosity}} \]

\( \text{Re} \) = Reynolds number.

Turbulence will usually occur if:

\[ \text{Re} > 2000 \text{ for large vessels such as aorta.} \]
\[ \text{Re} > 1000 \text{ for straight arteries (SMA, Renal artery)} \]
\[ \text{Re} > 200 \text{ for smaller arterial branches.} \]

Thus the flow characteristics, in particular turbulence, depends largely on the geometry of the vessel and properties of blood.

Doppler spectrums are different for the different types of flow. For plug flow, the spectrum is "clean" and has a narrow bandwidth with minimal low velocity display (Fig 2.6). Calculation of velocity and thus volume blood flow has been shown to be most accurate and reliable from plug flow profile and sampling from any portion of the flow stream will produce uniform velocity distribution (Taylor et al, 1985). Turbulent flow will produce Doppler spectral displays which are much broader and with a relative increase in mid velocity signals as well as reverse flow due to eddy currents (Fig 2.5). It is important to recognise the geometry of the displayed waveform as turbulent flow may be indicative of stenosis of the vessel or diseased vessel due to atherosclerosis and plaque formation, although it may also occur under physiological conditions such as in the carotid bulb and origin of the SMA (Taylor et al, 1985). It is also important to remember that turbulent, non-laminar flow persists only for a limited distance before laminar flow is re-established. This distance is dependent on the size of the vessel and the velocities
generated within the blood vessel (Taylor & Holland, 1990). To diagnose a stenotic lesion within an artery, it should therefore, be preferably scanned for its entire length wherever possible.

**The measurement of volume blood flow:** Blood flow through a vessel is principally affected by two factors and the relationship is given by the equation: (Nelson & Pretorius, 1988)

\[
\text{blood flow} = \frac{\text{pressure differential between the end of the vessel}}{\text{resistance}}
\]

Blood flow indicates flow of the total amount of blood, at all velocities, past a certain point and thus if the entire velocity distribution is measured, then estimation of blood flow is possible through a blood vessel. The resistance to blood flow determines blood flow measurement and is given by the equation:

\[
\text{resistance} = \text{viscosity} \times \frac{\text{length}}{\text{radius}^4}
\]

and thus radius is the critical factor which governs the resistance to blood flow. This indicates small changes in radius will produce marked changes in the resistance which in turn will affect measurement of blood flow. In practice, blood flow measurement is made possible by ultrasonography by using the formula: \(\text{flow} = \text{mean velocity} \times \text{cross-sectional area}\). Careful measurement of the area after imaging, using a duplex scan and estimation of velocity distribution from the Doppler display allows measurement of blood flow (Gill, 1979; Qamar et al, 1986a). Using meticulous technique and pulsed Doppler machine, Gill (1979) showed errors in measurement of blood flow to be around 6%. Results of measurement of volume blood flow in normal man by Qamar et al (1986a) showed close correlation with invasive techniques and an acceptable coefficient of variation.
Instrumentation:

Three types of Doppler devices are generally available. These are as follows:

a. Continuous-Wave Doppler.

b. Pulsed-Wave Doppler and duplex Doppler imaging.


a. Continuous-Wave (CW) Doppler devices are widely used for detection and superficial evaluation of blood flow in peripheral vascular examinations and in obstetrics. The transmitter is on continuously and sends a continuous train of impulses reaching the insonated blood stream and a separate transducer receives a train of impulses from the blood stream. As the reflected particle is a moving red blood cell, the received frequency varies with the velocity of blood. The frequency - difference signal is then produced audibly as well as displayed graphically. The CW device has practical advantages which include facilitation of search for a vessel because of the continuous train of impulses, lack of aliasing and low cost. However, the CW system makes it difficult to select a desired signal when vessels lie close to one another and does not allow analysis of the velocity profile. There is also no information on the position of vessel and it is not possible to determine the exact source of the information as any moving object in the path of the beam will produce a signal.

b. Pulsed-Wave (PW) Doppler devices use an interrupted train of impulses (e.g. a transmitted frequency of 5 MHz is interrupted at the rate of 15 MHz). This was first described by Wells in 1969. Here a sound pulse is transmitted and the returning echo is detected by a single transmitter. The time lag between the transmitted pulse and the returning echo depends on the velocity of sound and is directly proportional to the depth of the reflector. The factors governing the PW system are the duration of the ultrasound pulse (pulse length) and the amount of time the beam is being transmitted (duty cycle)(Nelson and Pretorius, 1988). These two parameters impose an upper limit to the maximum velocity that can be measured beyond which aliasing may occur as discussed
previously. However, there are many benefits of this system. The small and controllable sample volume (by adjusting the gate opening time) selects well defined and characteristic signal sources thereby allowing identification of vessel as well as preventing interference from neighbouring vessels. Also a portion of the velocity profile can be selected if necessary. These features improve the accuracy of the measurements significantly.

**Duplex Doppler imaging:** This combines PW Doppler with two-dimensional real time imaging so as to allow precise localisation of the Doppler sample. The image and the velocity information are displayed on a real time display. The display provides information about the peak systolic velocity, end diastolic velocity and average velocity while the image with enlargement (incorporated in some machines) allows calculation of the cross sectional area of the sample volume from the diameter of the vessel. Moreover, computers incorporated in Doppler machines also allow calculation of other Doppler indices for haemodynamic information. This system has many advantages over a CW or PW system, the most important being the ability to quantify blood flow. The duplex system is now being increasingly used to study blood flow changes in the carotid vessels, renal vessels, foetal and uterine vessels and abdominal vessels.

c. **Colour flow devices:** In this system, the grey scale and the flow images are combined on a colour monitor.

**Artifacts:** Artifacts which may cause errors in calculation of velocities from the Doppler display include:

1. **Aliasing.**

2. **Mirror-Image artifact:** Here a similar time-velocity spectrum appears above and below the baseline when imaging is done with a high angle of insonation, a high signal to noise ratio and when weak Doppler signals are recorded.
Both invasive and non-invasive methods are available to measure splanchnic blood flow in man and animals. The direct measurement splanchnic and superior mesenteric artery blood flow in man have been limited because of the invasive and complicated nature of the methods. Recently non-invasive Doppler ultrasound method has been available to allow direct and real-time measurement of blood flow within the splanchnic vessels. With proper consideration of the underlying principles and meticulous attention to technique, the Doppler ultrasonography provides a powerful and valuable tool for understanding haemodynamic mechanisms within the splanchnic vascular bed under physiological and pathophysiological conditions in a wide range of subjects (normal man and disease states). The duplex Doppler system has the capacity for depth resolution and a variable sample volume while synchronising the two-dimensional and Doppler modes. The Doppler measurements can be used to determine presence and direction of flow, velocity characteristics and estimation of volume blood flow and with careful attention to the technique should provide important information regarding the changes within the splanchnic vascular bed in normal man during neural and hormonal stimuli and in various disease states.
CHAPTER 3

MEASUREMENT OF SUPERIOR MESENTERIC ARTERY BLOOD FLOW AND PULSATILITY INDEX IN NORMAL MAN: EFFECT OF ANATOMICAL VARIATIONS AND DIFFERENT AGE GROUPS AND ASSESSMENT OF REPRODUCIBILITY.
INTRODUCTION:

Non-invasive assessment of blood flow within the splanchnic vascular bed has recently been successfully accomplished using the duplex pulsed Doppler scanning as detailed in the previous chapter. Most of the studies have concentrated on measurement of superior mesenteric artery blood flow (SMABF) although studies measuring portal venous blood flow (Brown et al, 1989; Sabba et al, 1990; Kawasaki et al, 1991), splenic and common hepatic artery blood flow (Nakamura et al, 1989) have also been described. The superior mesenteric artery has been chosen for non-invasive assessment of splanchnic vascular changes as it has many theoretical advantages. These can be subdivided into physiological and methodological advantages. The physiological advantages are:

1. The SMA is a major constituent of the splanchnic vascular bed supplying the duodenum (except the superior part), small intestine and the large intestine (except the left half of the transverse colon). Changes in SMABF should therefore reflect changes in total splanchnic blood flow (as all the splanchnic organs have a common venous drainage) unless it is compensated by an equal and opposite change in a parallel organ (Rowell, 1974).

2. The SMA is entirely innervated by the sympathetic nervous system and blood flow responses in the SMA, therefore, form a useful way of studying the neural control of the splanchnic vascular bed by using sympatho-neural stimulus in normal man and in disease conditions where abnormality of sympathetic nervous system occurs.

The methodological advantages are:

1. Relative ease in identification of the artery on abdominal scanning.
2. Fairly constant anatomy.
3. The proximal portion of the SMA has a linear course before it undergoes curvature and thus sampling from the proximal SMA allows a lower angle of...
insonation, a high angle of insonation being an important source of error in volume blood flow calculation (Rizzo et al, 1990).

4. The blood flow velocity profile in the proximal SMA is usually of the plug flow type and as discussed previously measurement from plug flow profile yields the most ideal measurement of volume blood flow (Taylor et al, 1985).

In spite of these advantages, measurement of SMABF can be difficult and may produce variable results particularly as the artery lies deep within abdomen and close to other large arteries such as the aorta. Other sources of errors have already been alluded to (Chapter 2). The reliability of the results of SMABF measurement depends on the skill of the operator as well as quality of the hardware of the Doppler machine used. Alternative Doppler indices have been devised to overcome inaccuracies in measurement of volume blood flow by alternative quantification of the shape of blood velocity waveforms. These indices include the $A/B$ ratio (Holland et al, 1984), the resistive or Pourcelot index (Taylor et al, 1985) and the pulsatility index (Gosling and King, 1974). The latter is defined as peak to peak amplitude of a waveform divided by the mean amplitude over the cardiac cycle and has been shown to indicate downstream resistance to blood flow (McCallum et al, 1978). PI is also sensitive in differentiating abnormal waveforms, as it takes into account the mean velocity (Wladimiroff et al, 1987) and has been successfully used in relation to carotid (Gangar et al, 1991), renal (Rigsby et al, 1987), uterine (Vyas et al, 1989) and superior mesenteric artery (Qamar et al, 1986).

This study was undertaken to assess the reproducibility of measurement of resting SMABF and PI in healthy subjects both in the short term (same day) and long term (studies separated by two months) and the inter observer variation of SMABF and PI. The effect of variable age group and different types of origins of SMA on the measurement of SMABF and PI were also studied.
EQUIPMENT:

All measurements of SMABF and PI described in this study and the following studies were performed using an Acuson 128 ultrasound system (Acuson Inc., Mountain View, California, USA) equipped with a 28 mm (aperture size), 3.5 MHz (penetration frequency), phased-array sector scanner (S328) (Fig 3.1). This is a duplex scanner which combines B-mode (2-dimensional) and pulsed Doppler ultrasound in a single machine along with a microcomputer and a video recorder with freezing and recording facilities. Demodulated, audio frequency time domain signals from the insonated blood vessel are converted by a fast Fourier transform analyser in real time to frequency domain and displayed as amplitude weighted Doppler frequencies. The images are stored in an attached videotape for later analysis. In the pulsed Doppler mode, velocities are displayed bounded by the gate (ranging from 1.5 mm to 30 mm, adjusted manually) on the cursor. The 2-D image and the Doppler spectral display can be displayed at the same time or individually. The equipment also incorporates variable cut off wall filters (100-1000 Hz) to reduce interference from "wall thump" and a regional expansion selection programme to enlarge the image in real time so as to facilitate measurement of vessel diameter. In addition there is also a range of functions designed to improve quality of image and vascular calculations. These are, adjustment of baseline and facility for changing velocity, log compression, pre and post processing and are discussed later.
Fig 3.1 Acuson 128 computed sonography system with its component parts.
**METHODS AND MATERIAL: Measurement of SMABF:** SMABF was measured using a real-time, transcutaneous pulsed Doppler ultrasound method using the machine Acuson 128 with the subjects being in the supine position. Satisfactory visualisation of the superior mesenteric artery (SMA) on its long axis, arising from the aorta was obtained by a longitudinal epigastric scan using a 3.5 MHz transducer and the B-mode images were stored in a video recorder attached to the machine. After identification of the SMA, the Doppler mode was switched on manually and the pulsed Doppler sample volume cursor was then placed in the lumen of the SMA, usually within 1cm from its origin from the SMA. By adjustment of the probe position, and altering the size of the Doppler gate (usually within 2-4mm), characteristic SMA signals (Fig 3.2a), easily distinguishable from the coeliac artery and the aorta, were obtained which is displayed as Doppler frequency shift on the screen. The angle of insonation is automatically calculated by the software of Acuson and displayed on the screen and was kept as constant as possible. Peak systolic velocity and end diastolic velocity of the SMA blood velocity waveforms were obtained in real time by freezing the image on the screen and placing Doppler caliper markers (appearing as a vertical line with a crosshair) at the peak of the waveform (systolic) and at the end of the waveform (end diastole)(Fig 3.2b). The crosshair marks the point at which velocity is measured and the vertical line marks the point at which to measure the mean of the Doppler signal. Time average velocity (TAV) (integration of the area under each individual velocity waveform) was then calculated in real time by the incorporated Acuson spectrum analyzer which measures:

a. The difference in velocity between the two caliper markers ($\Delta V$).
b. The difference in time between the caliper markers ($\Delta T$)
c. The rate of acceleration between the two points (Accl).
d. Time average velocity (TAV) between the two points from peak systolic and end diastolic velocities, by using the Doppler formula:
Fig 3.2 (a): (Upper panel) Figure showing characteristic Doppler frequency shift signal obtained by placing the sample volume cursor within the lumen of the superior mesenteric artery (S). C = coeliac artery, A = aorta.

Fig 3.2 (b): (Lower panel) Caliper markers placed at peak systole and end diastole of frozen Doppler frequency shift signals (encompassing three cardiac cycles) obtained from the superior mesenteric artery.
2 \frac{F_o v \cos \theta}{c} = \frac{F_d}{c}

where $F_d =$ Doppler frequency shift, $F_o =$ incident frequency, $v =$ flow velocity, $c =$ speed of sound in tissue, $\theta =$ angle of insonation (usually kept between $30^\circ$-$60^\circ$). At least three cardiac cycles (three individual waveforms including all individual variations in the shape of displayed waveform) were included for each measurement of TAV. Waveforms suggestive of plug flow were preferably selected for measurement of volume blood flow and this was obtained by adjusting the position of the sample volume cursor till typical waveforms (suggestive of plug flow) were obtained (Fig 3.2a). Waveforms were rejected when the spectral display and the audible signal obtained in the Doppler mode suggested turbulence or when aliasing occurred which could not be adequately dealt with by heterodyne demodulation and lowering the baseline.

**Measurement of volume blood flow:** The diameter of the SMA at the point of spectral sampling was calculated from the high resolution picture recorded in the videotape using a built in regional expansion system which enlarged the image to facilitate the measurement. The enlargement of the image occurs without any loss of resolution while in most cases the resolution actually increases. After freezing the high resolution, expanded image of the SMA, imaging dimension markers were placed manually to measure the diameter which was automatically calculated by the computer software (Fig 3.3). Diameter readings were only recorded when there was clear visualisation of the lumen of the artery, the picture of which could be enhanced using a series of image processing functions also incorporated within the machine. These include:
Fig 3.3: Calculation of diameter of the proximal SMA (using manual dimension markers) from where sampling is obtained after expansion of the image by Regional Expansion Selection programme.
1. **Adjusting the dynamic range of the image (Log compression).** The Acuson system assigns different shades of black and white to different levels of sound intensities. Adjusting the log compression changes the dynamic range of the image gray scale.

2. **Preprocessing the image:** This accentuates the edges of the images and there are three preprocessing levels.
   - Level 0 = Smooth border
   - Level 1 = Moderately sharp border
   - Level 2 = Crisp border

3. **Adjusting persistence:** Persistence accentuates subtle differences in tissue texture and six levels (0-5) are available. For rapidly changing anatomical structures such as the SMA diameter level 0 to 2 was used.

4. **Postprocessing the image:** This helps in providing high contrast (post 5 - post 9) to low contrast (post 1 - post 4) as necessary.

Blood flow was then calculated using the formula:

\[ F = TAV \times \pi r^2 \times 60 \]

where \( F \) = flow in ml/min, \( r \) = radius of SMA in mm at the point of sampling and \( TAV \) = time average velocity in cm/sec.

**Measurement of pulsatility index (PI):** PI was calculated retrospectively from videotaped images by selecting a waveform and using the Doppler caliper markers to select the maximum (Max) and minimum (Min) peak velocity and the time average maximum velocity (Tamx) (done by tracing the envelope of the spectral display over a single cardiac cycle after going into the trace mode, Fig 3.4) and the PI value is then automatically calculated by the software from the formula:
Fig 3.4: The pulsatility index of a Doppler spectral waveform obtained from the superior mesenteric artery being calculated after tracing the contour (dotted line) of a selected waveform.
Measurement of blood pressure: Blood pressure (BP) was recorded non-invasively using an automated sphygmomanometer (Sentron, Bard Biomedical, USA) which has been calibrated against a mercury sphygmomanometer. Mean blood pressure was calculated from the formula systolic blood pressure plus twice diastolic blood pressure divided by three.

Study 1: Reproducibility of PI and volume flow estimates. Variations in the origin of SMA.

Subjects: 30 subjects were initially studied. In 5 the SMA could not be adequately visualised and the study was completed in 25 (mean age 37 years, range 22-66 years, 10 males). None was on medication and all were normotensive. They fasted overnight (to reduce the established effect of food on SMABF, Moneta et al, 1988) and were studied at 0900 h in a temperature controlled room (24°C) after a 30 min supine rest to allow stabilisation and allow familiarisation with equipment. Their body weight ranged from 42 kg to 80 kg (mean: 64 kg). All gave informed consent and the study was approved by the Ethical Committee of St. Mary's Hospital.

All real time scanning was performed by one investigator (KRC). Calculation of volume blood flow within the superior mesenteric artery (SMABF) and PI was accomplished retrospectively by 2 observers (KRC, observer 1 and SR, observer 2) independently on separate occasions. Coefficient of variation of SMABF and PI was calculated based on four separate measurements at 30 min intervals (short term) in 18 subjects and resting measurements (long term) on two occasions at 2 months interval in 8 subjects.

For each measurement of blood flow, a mean of at least 6 TAVs over 3-4 cardiac cycles and 6 separate diameter measurements were used. To reduce errors in blood flow measurement, waveforms with strong signals were selected for flow velocity measurement. When variable Doppler frequency shift occurred from the sampling region, the study was repeated on another day or...
was excluded from the study. When aliasing occurred, it was corrected by 
rejecting aliased waveforms and by using higher velocity scales. Recordings 
involved angle of insonation exceeding 60° or unsatisfactory diameter 
estimation due to poor picture quality were excluded from the study. In all, the 
angle of insonation was kept as constant as possible so as to reduce angle-
induced errors in frequency shift as a source of variability in the calculation of 
Duplex velocity parameters. The gate size of the sample volume was also kept 
constant between 2-4 mm. When interference of Doppler signals occurred due to 
excessive pulsatility of vessel wall, a variable cut-off wall filter was used. 
However, a filter in excess of 100Hz was not used so as to avoid losing low 
frequency signals.

For each measurement of PI, a mean value of at least six PI measurements 
selected from uniform intensity waveforms from six displays were used. PI 
measurements were made even when flow measurements were not possible due 
to either a high angle of insonation or difficulty in estimation of the diameter 
of SMA, as long as satisfactory SMA signals were obtained.

Prior to measurement of flow, the type of origin of the SMA was recorded and 
classified as:
Parallel (Type 1): a parallel origin of the SMA and the coeliac artery (CA) 
from the aorta. (Fig 3.5a).
Divergent (Type 2): a divergent and often common origin of the SMA and CA. 
(Fig 3.5b).
Others (Type 3): SMA coiling behind the coeliac artery or SMA arising in the 
reverse direction (Fig 3.6).
Fig 3.5a: (upper panel) A parallel (type 1) origin of the superior mesenteric artery.

Fig 3.5b: (lower panel) A divergent (type 2) origin of the superior mesenteric artery. C = Coeliac artery, S = Superior mesenteric artery, A = Aorta.
Fig 3.6: An aberrant origin of the superior mesenteric artery (arising in reverse direction), type 3 origin.
Protocol:

After 30 min supine rest BP was recorded in each subject at 4 min interval. Recording of SMABF was made at 0 min and then at 30 min interval for two hours. Six real time measurement of TAV were recorded on the videotape for each measurement for retrospective analysis and measurement of blood flow. In 6 subjects basal measurement of SMABF were repeated under identical conditions at two months interval on two occasions.

Study 2: Estimates of SMABF and PI in younger and older subjects.

On a separate occasion basal SMABF was recorded in normal subjects of two different age groups. These consisted of 14 younger subjects (mean age=24 yrs, range 19-33 yrs, nine females and five males) and 12 older subjects (mean age=63 yrs, range 58-80 yrs, six females and six males). All were normotensive although resting BP tended to be higher in the older group and none was on medication at the time of study. All were studied as described in study 1 under identical conditions.

Statistical analysis:

Statistical analysis was performed using the Minitab data analysis software (1988, Inc.). Correlation of various measurements at rest between the two observers were examined using linear regression analysis and expressed as Pearson's correlation coefficient.

Coefficients of variability were obtained by dividing the standard deviation by the mean. Variability was then expressed as the mean of coefficients of variability.

In study two, basal BP, SMABF and SMA diameter values between the two groups (younger and older) were compared by means of Mann-Whitney test. Data are expressed as means ± s.e.m. P < 0.05 was considered significant.
RESULTS: Study 1:

In 25 subjects resting superior mesenteric artery blood flow or pulsatility index could be measured satisfactorily. 5 were excluded from the study because of poor visualisation of the superior mesenteric artery in 3, an excessively pulsatile superior mesenteric artery in 1 and due to an aberrant origin of superior mesenteric artery in 1.

Resting superior mesenteric artery blood flow measurements could be made in 18 out of the 25 subjects. 7 were excluded because of an angle of insonation greater than 60° in 5 and noisy signal due to wall thump and turbulence in 2 subjects. However, pulsatility index could be measured satisfactorily in these 7 subjects.

The mean angle of insonation in 18 subjects was 41±2° (range 18-60°).

Mean diameter (mean of 6 measurements of both observers, KRC and SR) of the superior mesenteric artery was 7±0.1 mm and there was a close correlation between the 2 observers (r=0.72, P<0.05). (Fig 3.7)

Mean resting superior mesenteric artery blood flow in 18 subjects and mean resting pulsatility index in 25 subjects were 508±35 ml/min and 283±10 respectively. There was a highly significant correlation between the 2 observers in the measurement of superior mesenteric artery blood flow (r=0.89, P<0.01) and pulsatility index (r=0.97, P<0.001). (Fig 3.7)

Within subject coefficients of variability in the short term in 18 subjects was 7.7% for superior mesenteric artery blood flow and 5.6% for pulsatility index. Long term coefficients of variability in 8 subjects were 8% for superior mesenteric artery blood flow and 6.2% for pulsatility index.

8 subjects had a parallel origin of the superior mesenteric artery while 17 had a divergent origin. Of the 17 with a divergent origin, 5 had a common origin of SMA with coeliac artery. 4 out of the 5 subjects in whom the angle of
insonation was greater than 60° and were therefore excluded from the study, had a divergent origin of the SMA.

**Study 2:**

Resting mean arterial blood pressure (MABP) was higher in the older subjects (97.3±3 mmHg) compared to the younger subjects (82.6±3, P<0.05, Table 3.1) although both groups were normotensive. The basal mean diameter of the SMA was 7.6±0.1 in the younger group and 7.1±0.1 in the older group (P=0.09, Table 3.1). Basal mean values of SMABF were 490±30 ml/min in the younger group and 412±31 in the older group (P=0.08, Table 3.1). Basal mean PI values were 280±11 in the younger subjects and 296±16 in the older subjects (P=0.09, Table 3.1).
Fig. 3.7: Correlation of measurements of diameter of the superior mesenteric artery (top panel), blood flow (SMABF, middle panel) and pulsatility index (PI, lower panel) between two observers (1 & 2) in 25 healthy subjects.
### Table 3.1: Table showing resting mean arterial blood pressure (MABP), diameter of the superior mesenteric artery (SMA), superior mesenteric artery blood flow (SMABF) and pulsatility Index (PI) values in younger and older subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>young (24 yrs)</th>
<th>older (63 yrs)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=14)</td>
<td>(n=12)</td>
<td></td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>82.6±3</td>
<td>97.3±3</td>
<td>P=0.002</td>
</tr>
<tr>
<td>Diameter of SMA (mm)</td>
<td>7.8±0.1</td>
<td>7.1±0.1</td>
<td>P=0.09</td>
</tr>
<tr>
<td>SMABF (ml/min)</td>
<td>490±30</td>
<td>412±31</td>
<td>P=0.08</td>
</tr>
<tr>
<td>PI</td>
<td>280±11</td>
<td>290±16</td>
<td>P=0.1</td>
</tr>
</tbody>
</table>
DISCUSSION:
In this study, the reproducibility of measurement of SMA blood flow and PI have been studied and attempt has been made to correlate measurements of PI, SMABF and other parameters such as diameter of SMA in normal subjects between two observers. The relationship of SMABF and PI to the various origins of SMA and different age groups also have been studied.

Two main types (parallel and divergent) of origin of the SMA were identified, a divergent form being more common. A divergent origin may include a common origin of the SMA and CA and might necessitate placement of the sample volume at a more distal portion of the SMA so as to avoid turbulence, which may occur in the SMA at its origin from the aorta (Taylor et al, 1985). This could lead to inaccuracies in the measurement of SMABF for the following reasons. Firstly, the angle of insonation may be higher in the distal portion of the SMA; 4 out of the 5 subjects, in whom flow could not be measured in study 1 had a divergent origin. Secondly, the diameter of the SMA is reduced more distally and this could lead to difficulties in estimation of changes in diameter and thus SMABF responses. Finally, a sample volume from the distal portion of the SMA (beyond the first 2 cm from its origin) may have a different Doppler spectral distribution as compared to the proximal SMA. This is because of differences in flow velocity profiles in proximal and distal SMA. Due to the pulsatile nature of arteries, different vessels show varying flow velocity profiles. These include flat or plug flow (flow laminae moving at the same velocity) profile in the aorta, parabolic flow (centre of the flow laminae moving at a higher velocity than the periphery) profile in small vessels (proximal hepatic artery) or an intermediate flow (between plug and parabolic) profile in the coeliac trunk (Taylor et al, 1985). In the SMA, flow tends to become parabolic distally due to narrowing of the vessel, and ideally sampling should be made from an area just distal to its origin from the aorta (within 1 cm) where plug or intermediate flow is present. Vessels with plug
flow have been shown to yield the most reliable measurement of volume blood flow, as during plug flow, any sample from the lumen of the vessel gives average velocity from which flow can be calculated (Taylor et al, 1985). The less common parallel origin of the SMA usually implies a satisfactory sample volume placement within the SMA but a greater chance of noise interference of the spectral display if there is a highly pulsatile coeliac artery which is adjacent.

Using all the precautions described in the method section, measurements of SMABF and PI were found to be highly reproducible with an acceptable coefficient of variability when reassessed over the short and long term. The advantages of measurement of SMABF using the non-invasive pulsed Doppler technique include its real time and continuous measurements and non-invasive nature avoiding injection of dyes, catheterisations and trauma to vessels and nerves. However, there are several potential sources of error in measurement of SMABF using the pulsed Doppler ultrasound method. The main sources of error are related to (a) location of vessel, (b) the angle of insonation, (c) the measurement of diameter of SMA, (d) the prevention of aliasing, (e) avoiding losing maximal frequency shifts, (f) the effect of attenuation of the ultrasound beam in tissue and (g) background noise and interference. These have already been discussed in chapter two and consideration will be given to steps aimed to reduce these problems in this study.

The location of SMA is relatively simple owing to its fairly constant anatomy although it is important to identify the type of origin of SMA as this may influence measurement of SMA. The identification of the vessel is made easier by proper selection of volunteers (usually slim subjects) and overnight fasting which allows optimum 2-D visualisation of the SMA.

The angle of insonation is an important potential source of error particularly as the blood flow velocities calculated from the Doppler shifts are proportional
to the inverse of the cosine of the angle $\theta$ and at 90° the shift frequency is theoretically nil as cosine 90° = 0 (Taylor & Holland, 1990). However, at too low an angle there may be difficulty in obtaining signals because of total reflection of sound waves at the vessel walls and resultant interference with background noise. In this study, all examinations involving an angle of insonation above 60° were rejected. Angle of insonation was kept constant as far as possible within 35°-55° and this could be achieved by insonating the proximal portion of the SMA where it has a linear course allowing a lower angle of insonation and also by adjusting the position of the probe and the transducer.

The measurement of the diameter of the SMA is another important potential source of error in measurement of volume blood flow. The SMA is a medium sized artery (resting diameter <0.3 cm) and the manual placement of the dimension cursors and limited resolution of B-mode imaging in some equipments possibly increase the chances of error in measurement of diameter. Qamar et al (1986a) found that their measurement of diameter was slightly lower than measured in in-vitro models and this could lead to an error of upto 20% in a vessel of 7mm diameter. This error however, they postulated, would be neutralized by an overestimation of velocity due to non-uniform insonation of the vessel. In this study, a high resolution scanner was used whose resolution was enhanced further when the regional expansion selection mode was operated which enlarged the image of the SMA. This enabled clear, high contrast picture of the lumen of the SMA where the diameter measurement markers could be easily placed. Using selected, expanded and high resolution images, there was close correlation in measurement of diameter between the two observers. Furthermore, the accuracy of the on-screen calipers was estimated to be better than 0.5mm and for each measurement, an average from at least six separate diameter measurements were used. Using averaged diameter measurements Nakamura et al (1989) have reduced the error in diameter
measurement to less than 3.5% in a vessel with a diameter of 7 mm. The results of the mean basal diameter measurements in the present studies (1 and 2) agree well with values reported by other workers (Table 3.2).

Table 3.2 Measurements of diameter of the SMA using the duplex pulsed Doppler ultrasound technique in man.

<table>
<thead>
<tr>
<th>Diameter of SMA</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>7mm</td>
<td>Qamar et al</td>
<td>1986</td>
</tr>
<tr>
<td>7mm</td>
<td>Nakamura et al</td>
<td>1989</td>
</tr>
<tr>
<td>6mm-7mm</td>
<td>Rizzo et al</td>
<td>1990</td>
</tr>
<tr>
<td>7.3±0.2 mm</td>
<td>Braatvedt et al</td>
<td>1991</td>
</tr>
<tr>
<td>7±0.07 mm</td>
<td>Best et al</td>
<td>1991</td>
</tr>
</tbody>
</table>

Aliasing occurred infrequently in this study as the sampling rate (pulse repetition frequency) was adequate in most scans to prevent aliasing. However, when aliasing occurred this was dealt with by using higher velocity scales and shifting the baseline using the demodulation facility and also by rejecting aliased waveforms.

Attenuation of ultrasound in tissue is a problem particularly when imaging the SMA which is a deep seated abdominal vessel. However, as discussed previously, at the transducer frequency of 3.5 MHz (used in this study) and over a distance of 6 cm (approximately the distance from surface to SMA) the attenuating effect on the spectrum is cancelled by a reverse effect of scattering of ultrasound from blood (Taylor & Holland, 1990).

Finally, contamination of signals by background noise causing a poor signal to noise ratio can cause error in the calculation of velocities. When interference due to excessively pulsatile artery or wall motion (wall thump) occurred, a variable cut off wall filter (100 Hz) was used to reduce the background noise.
However, filters in excess of 100 Hz were not used so as not to lose low frequency signals. When background noise persisted the study was repeated on another day.

Measurement of volume blood flow in the SMA was thus made based on multiple measurements of time averaged velocity and diameter of SMA which incorporated maximal values obtained during scanning. Nakamura et al (1989) suggested that time averaged measurement of diameter substantially reduces errors in measurement of diameter and achieves accurate measurement of blood flow. In another study Ranke et al (1992) reported on the accuracy and sources of error in conventional and colour Doppler ultrasonography and concluded that multiple and averaged measurement yields the most accurate blood flow measurements. In our laboratory, previous studies have indicated good correlation of estimated and actual blood flow rates using the pulsed Doppler technique with in vitro models (Kooner et al, 1989b; Ranke et al, 1992). Similar results have been reported by Scheurlen et al (1992) in another in-vivo validation study, but only after meticulous attention to all the variables. Using all the precautions for controlling the variables, the measurement of SMABF was found to be highly reproducible in our hands with a close agreement between two observers and the basal SMABF values are in general agreement with most of the other workers. However, a substantial variability in measurement of SMABF still exists in some reported studies utilizing non-invasive measurement of SMABF. This is illustrated in Table 3.3 where values of basal SMABF in man, by different workers are summarised.
Table 3.3 Values of basal superior mesenteric artery blood flow (SMABF) in man using a duplex pulsed Doppler ultrasound method.

<table>
<thead>
<tr>
<th>SMABF (ml/min)</th>
<th>Age(yrs)</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>377±166</td>
<td>54 (I pt)</td>
<td>Jager et al,</td>
<td>1984</td>
</tr>
<tr>
<td>517±19</td>
<td>37</td>
<td>Qamar et al,</td>
<td>1986</td>
</tr>
<tr>
<td>538±37</td>
<td>32</td>
<td>Moneta et al,</td>
<td>1988</td>
</tr>
<tr>
<td>478±166</td>
<td>37</td>
<td>Nakamura et al,</td>
<td>1989</td>
</tr>
<tr>
<td>532±38</td>
<td>23</td>
<td>Braatvedt et al.</td>
<td>1991</td>
</tr>
<tr>
<td>513±29</td>
<td>26</td>
<td>Cooper et al,</td>
<td>1991</td>
</tr>
<tr>
<td>377±166</td>
<td>26</td>
<td>Scheuren et al,</td>
<td>1992</td>
</tr>
</tbody>
</table>

The variability in measurement of SMABF in some of the above studies could have resulted from a number of factors. These include using different ultrasound systems and transducer frequencies, use of high pass filter (Scheuren et al, 1992), older age group of subjects (Jager et al, 1984), semi-supine posture (Moneta et al, 1988) and smaller body mass of subjects (Nakamura et al, 1989).

The results of this study also agree with results of invasive measurements of SMABF as carried out by Clark et al (1980) using spillover angiographic reflex technique (mean SMABF = 456 ml/min) and Hulten et al (1976) using the inert gas washout technique (total small intestinal blood flow of 500-600 ml/min). Measurement of SMABF by the dye dilution method (Norroyd et al, 1974) however, yielded higher SMABF values (706±108 ml/min). This could be due to the invasiveness of the technique and that venous outflow, which is measured in this technique is higher than arterial inflow. Furthermore, vasoactive properties of the contrast medium and haemodynamic changes induced by the initial injection of contrast medium, as discussed in chapter 2,
could have also contributed to the overestimation of flow. In another study, the dye dilution method was found to overestimate measurement of blood flow by 18% when compared to direct methods in the laboratory (Lanciant & Jacobson, 1976).

Due to ethical reasons there have been no studies comparing non-invasive measurement of SMABF with invasive measurements. However in man, Payen et al (1982) found good correlation between intra-arterial and Doppler ultrasound measurement of carotid blood flow. In animal studies, Eik-Nes et al (1984) found a close correlation between electromagnetic and Doppler ultrasound measurement of aortic blood flow in pigs. Nakamura et al (1989) reported a close correlation between SMABF measured by Doppler ultrasonography and electromagnetic flowmeter in dogs. The agreement of basal SMABF values obtained in this study with majority of other workers using a similar technique and indeed, with some invasive studies, indicate that with properly controlled technique, measurement of SMABF is reliable and reproducible and has the potential to provide valuable insight into gastrointestinal physiology.

As errors may be associated with measurement of volume blood flow measurement, the reproducibility of PI, an alternative Doppler index, was also assessed. In our hands measurement of PI was found to have a greater reproducibility than volume blood flow measurement and PI could be measured satisfactorily in all 25 subjects while flow could be measured in 18 subjects. This indicates a greater practical advantage of measurement of PI over SMABF as PI can be measured retrospectively. This is of importance, particularly during short lived stimulus, as measurement of volume blood flow (involving freezing B-mode image in real time to compute the time average velocity) in such occasions, may miss short lived haemodynamic changes and also limits the time available for making other observations. Furthermore, measurement of PI is independent of angle of insonation and diameter of the
SMA, two major variables which determine the accuracy of blood flow measurement. Thus PI measurement is possible as long as satisfactory Doppler frequency shifts can be obtained after imaging although flow measurements may not be possible. This would increase the number of subjects in whom haemodynamic studies may be performed as shown in this study where PI could be measured in greater number of subjects. However unlike measurement of volume blood flow, PI is semiquantitative and a relatively crude index of haemodynamic changes. Skidmore et al (1980) have shown that volume blood flow measurement is a more sensitive indicator of changes in vascular resistance than PI which seems to be less sensitive to changes in mean blood pressure. Studies attempting to correlate PI with regional blood flow have been few and almost exclusively limited to cerebral and uteroplacental circulations with varying results. Hansen et al (1983) described measurement of PI and brain blood flow using a microsphere and Doppler ultrasound method in piglets after administration of pancuronium and artificial alteration of the PaCO2. They found a poor correlation between brain blood flow and changes in PI. In another study, however, Greisen et al (1984) compared the Doppler ultrasound and 133Xenon clearance method for measuring cerebral blood flow in newborn infants and found a significant correlation between blood flow and PI. Of the two studies where PI of the SMA has been calculated (Qamar et al, 1986b; Nakamura et al, 1989) there has been no correlation with SMABF. Ranke et al (1992) measured PI values with conventional and colour Doppler ultrasonography and found a significant difference in PI values within the two systems under similar experimental conditions. Thus ideally, measurement of PI should be coupled with measurement of volume blood flow to assess haemodynamic changes within a vessel.

In study 2, the basal measurements of SMABF in the younger and the older groups were not significantly different although values in older subjects were lower (Table 3.1). Both groups were normotensive although there was a
significant difference in mean blood pressure between the two groups, blood pressure being higher in the older group. Qamar et al (1986) found no correlation of SMABF and age group of subjects although they did not study two age groups separately. The lower SMABF values in the older subjects in this study could be partly explained by a lower (although non-significant) diameter of the SMA when compared to the younger subjects. This may be due to age related narrowing of the vessel or intimal calcification which reduced the lumen size of the vessel. The latter however, is unlikely as enlargement of the image allowed closer examination of the lumen of the artery. Another explanation for the lower SMABF in the older subjects may be the higher blood pressure in these subjects. There is evidence that sympathetic nerve activity increases with age and is reflected by increasing plasma catecholamine concentrations and nerve traffic impulses from cutaneous and muscle sympathetic nerves (Davies & Sever, 1988). The precise mechanisms are unclear although it appears circulatory clearance of catecholamines and neural secretion rate of endogenous catecholamines is increased in the elderly subjects (Christensen, 1982). Although the older subjects in this study are not "elderly" yet it is possible a greater sympathetic activity in these subjects was responsible for the higher blood pressure and an increased tone in the SMA, resulting in a lower diameter and volume blood flow value. The PI measurements in the two groups did not differ much and this observation agrees with that of Skidmore et al (1980) who suggested PI is less sensitive to changes in mean blood pressure when compared to volume blood flow.
CONCLUSIONS:

Measurement of volume blood flow and pulsatility index of the SMA blood velocity waveform is reliable and reproducible with a low inter-observer variability, provided all the variables affecting blood flow measurement are controlled carefully. Measurement of SMABF should preferably be carried out using the same type of pulsed Doppler equipment particularly when results of different studies are compared, as results from different types of pulsed Doppler equipment may vary. Furthermore, a range of normal values needs to be defined for each laboratory. There also appears to be lower basal SMABF values in older subjects which could be related to their higher blood pressure compared to younger subjects. This emphasises the need for close age matching when comparing changes in SMABF in normal and disease conditions. PI is also highly reproducible and can be used in situations where SMABF measurements are not possible. It is however semiquantitative and less sensitive to changes in mean blood pressure. Measurement of SMABF and PI therefore could be used to study the haemodynamic changes within the superior mesenteric artery during various neural and pharmacological stimuli which would provide important information about the role of the splanchnic vascular bed in cardiovascular homeostasis in man.
CHAPTER 4

CHANGES IN PULSATILITY INDEX AND SUPERIOR MESENTERIC ARTERY BLOOD FLOW DURING SYMPATHO-NEURAL ACTIVATION IN NORMAL SUBJECTS.
INTRODUCTION:
In man, sympatho-neural activation can be achieved by a range of physiological tests which activate the sympathetic nervous system and either raise or maintain blood pressure (Fig. 4.1). Stimuli which activate the sympathetic efferent pathways and raise blood pressure are often grouped together as pressor tests and can be subdivided into centrally and peripherally acting pressor tests.

Fig 4.1: Effect of pressor tests and head-up tilt on blood pressure in normal subjects.
The centrally acting pressor tests include cortical arousal by stimuli such as mental arithmetic (MA) or sudden noise; the peripherally acting pressor stimuli include isometric exercise (ISE) and cold pressor test or cutaneous cold (CC). Another stimulus which activates the sympathetic nervous system but maintains blood pressure is postural challenge or head-up tilt at 45° or 60°. These simple bedside tests of sympathetic function are well validated, reproducible and routinely used in the autonomic laboratory in the investigation of autonomic disorders (Mathias & Bannister, 1992). In man, the overall systemic and hormonal responses to these stimuli have been documented but the splanchnic vascular responses, are unknown. In the following study, changes in superior mesenteric artery blood flow (SMABF, as an index of splanchnic vascular changes) have been studied during sympatho-neural activation induced by stimuli which raise blood pressure (mental arithmetic, cutaneous cold and isometric exercise) or maintain blood pressure (head-up tilt) in 18 healthy subjects. Systemic and other regional (forearm blood flow, cardiac output) vascular responses were also studied.

SUBJECTS AND METHODS:

Subjects:
24 normal subjects were studied initially. In 16 (mean age of 26 years, range 24 to 35 years, seven male and nine female,) there was satisfactory ultrasound visualisation of the SMA and the entire study was completed. All were healthy with no previous disorder. None was on medication and their mean body weight was 64 kg. They fasted overnight and were studied at 0900 hours in a temperature controlled (24°C) room. All gave informed consent and the study was approved by the Ethical Committee of St. Mary's Hospital.
HAEMODYNAMIC MEASUREMENTS:

Blood pressure (BP) and heart rate (HR) were measured noninvasively using an automated sphygmomanometer (Sentron, Bard Biomedical, USA) every three minutes and during each stimulus. Automated sphygmomanometry uses the auscultation of Korotkoff sounds using a microphone incorporated in the cuff. Using a transducer and a microprocessor, the machine records oscillations in the air contained in the bladder of the cuff and uses the appearance of oscillations above the basal oscillations to calculate systolic blood pressure. The point of disappearance of the oscillations is taken as diastolic blood pressure. This method is now being widely used as it is non-invasive unlike direct measurement of blood pressure. White et al (1990) found supine blood pressure measurement by this device is of the same accuracy as measurements made by experienced clinicians. Sidery & Macdonald (1991) showed that in normotensive subjects, estimation of blood pressure by automated sphygmomanometry was reproducible, particularly during head-up tilt, when this device appeared to be more sensitive than conventional or random-zero sphygmomanometer. Comparison of automated blood pressure recording with direct intra-arterial measurement of blood pressure showed good correlation (Woittiez et al, 1986).

Using this method, mean blood pressure was calculated from the formula systolic + twice diastolic blood pressure divided by three.

SMABF and PI was measured using a pulsed Doppler method with a real time, 2 D ultrasonic scanner (Acuson 128 computed sonographic system, 3.5 MHz sector transducer) as described previously. Superior mesenteric artery vascular resistance (SMAVR) was calculated from the ratio of mean arterial blood pressure and SMABF, assuming zero venous pressure. An inverse relationship of vascular resistance with blood flow suggested active or passive vascular changes within the splanchnic vascular bed.
Aortic Blood Velocity: Ascending aortic blood velocity was measured using a continuous-wave Doppler transmitter and receiver operating at 3 MHz (Exerdop, Quinton Instrument Co., a division of A.H.Robins Inc., Seattle, Washington). This incorporates a bidirectional, continuous wave Doppler unit with an ultrasonic beam designed to receive scattered signals from a distance of 6-14 cm from the transducer. The transducer consists of a handle which incorporates piezoelectric crystals for transmitting ultrasound and receiving echoes. The recordings are processed off-line using spectral frequency analysis and the results are used to determine the highest frequency present during each analysis interval. The transducer was placed in the suprasternal notch with adequate coupling fluid and then positioned so as to aim the beam towards the expected location of the aortic root. Initially a slow search for the region was made while listening to the audio output of the Doppler shift frequencies. Signals were considered to be adequate when it yields the highest systolic velocity integral. This is concomitant with
a) characteristic high pitched crisp and clear sounds during systole with a rapid onset and falling to a minimum during cessation of flow in diastole.
b) inspection of visual signals displayed and analyzed on an oscilloscope (model D61, telequipment), rising sharply to a maximum velocity during early phase of systole and falling to minimum levels before diastole and remaining in that position until the beginning of the next systole.

The analogue signals were processed by the system which allowed detection of signals representing flow towards the transducer only. The maximum Doppler frequency shift in the signal corresponding to the maximum velocity of the blood from the ascending aorta occurring at any moment was recorded during 20 complete and consecutive cardiac cycles.

Stroke distance (SD), a measure of stroke volume, was calculated from continuous integration of each systolic velocity signal. Relative cardiac output (cardiac index) could then be calculated from the product of stroke distance
and heart rate. A mean value of 20 complete and consecutive cardiac cycle was used for each observation. This method for measuring cardiac output has been validated against invasive techniques by other workers (Huntsman et al, 1983; Sabbah et al, 1986).

Forearm Blood Flow (FBF): Forearm blood flow was measured by venous occlusion plethysmography using the method described by Whitney (1953). Initially water-filled plethysmography was used for measuring volume changes in the forearm or calf (Barcroft & Edholm, 1943). The major disadvantages of the water-filled plethysmograph was the cumbersome application to the limb in order to prevent leaks and limitation of movement of the subjects. Substantial advance to the limitations of the water-filled plethysmography was introduced by Whitney (1949, 1953) who used a mercury-filled silastic tubing wrapped around the extremity examined. The resistance of the mercury gauge R is determined by its length l, cross-sectional area A and the specific resistance of mercury $\rho$ as shown in the following equation:

$$ R = \frac{\rho}{\frac{A}{l}} $$

In a limb with cross-sectional area of Q and a circumference of l, l becomes $\pi Q$, where $\pi = \frac{V}{Q}$, where $V$ = constant depending upon the form of the cylindrical cross section. R is then given by the following equation:

$$ R = \frac{a^2 \rho}{\frac{V}{Q}} $$

where $V$ = volume of mercury. Q is however given by $\frac{V}{L}$ where $V$ = volume of the cylinder and $L$ = length of cylinder. The above equation thus becomes:

$$ R = \frac{a^2 \rho}{\frac{V}{L}} $$
If \( L \) is constant and cross-sectional configuration is not affected (a remains constant) during minute changes in volume then the equation can be rewritten as:

\[
dR/R = dV/V
\]

which confirms that relative change in electrical resistance is equal to the relative volume change.

Originally the system needed to be calibrated using a micrometer but Whitney modified the system and simplified calibration using a calibration screw inserted in the mounting itself.

In this study and the following studies utilising this method the forearm blood flow was measured with the arm supported on an arm stand at the level of the heart. The wrist cuff was inflated above the arterial pressure and the arm cuff above venous pressure. Changes in the circumference of the arm were measured by using a double-stranded mercury in silastic tube gauge (model 2582, Ormed Ltd., Welwyn Garden City, Herts, UK). Signals from the gauge were transferred to a coupling unit (model 2583, Lectromed) for temperature compensation and then fed to a preamplifier (type MX2P, Lectromed). The signals were recorded on a chart recorder (type MX216, Lectromed). Reading were made on a 15 second cycle (inflation for 12 second and deflation for 3 second) and 12 successive readings were averaged for each measurement.

**PROTOCOL:**

Measurements were made initially after 30 mins supine rest, after the subject was familiarised with the equipment and there was equilibration and stabilisation. Interventions were non-randomly allocated and 15 min supine rest was allowed before each intervention to allow measurements to return to normal. Measurements were carried out before and during (120sec) mental arithmetic, cutaneous cold, isometric exercise and head up tilt (45 degrees on a tilt bed for 10 mins with measurements taken at 2 and 10 mins).
Mental arithmetic (MA): The subjects were asked to do rapid serial subtraction of 17 from 700 for 120 sec. All subjects were literate and the same person (KRC) supervised the test on each occasion.

Cutaneous cold (CC): One hand was immersed in ice slush in a bucket at 4°C for 120 sec.

Isometric exercise (ISE): One hand (the dominant hand) grips a partially inflated sphygmomanometer cuff (usually at 20 mmHg) at submaximal pressure (one third the maximal voluntary contraction pressure) for 120 sec.

Head-up tilt: Postural change was induced by using a tilt table and tilting the bed to 45° which was maintained for 10 min.

These tests activate the sympathetic efferent constrictor fibres to capacity and resistance vessels (Mathias & Bannister, 1992).

A 15 min rest was allowed between individual stimulus and readings were taken in the supine position, 10 mins prior to each test to ensure return to the baseline state.

The study was repeated in an identical manner in 6 subjects after 2 months to assess the reproducibility of the observations.

**STATISTICAL ANALYSIS:**
Comparison of mean values of BP, HR, CI, FBF, SMABF and PI before and during each stimulus was carried out by means of paired t test using the Minitab data analysis software. Mean baseline BP and SMA blood flow measurements, preceding each test were compared by one-way analysis of variance (ANOVA, Minitab). Correlation of measurements during initial and repeat studies were examined with linear regression analysis.

Data are presented as means ± s.e.m and statistical significance was accepted at \( P < 0.05 \).

Variability of FBF, CI, SMABF and PI measurements were obtained based on four observations at 30 min interval in 10 subjects. Coefficient of variability was obtained by dividing the standard deviation by the mean.
RESULTS

MBP: There was no significant differences in mean systolic and diastolic blood pressure preceding each stimulus (ANOVA, Table 4.1) indicating satisfactory return to baseline. MBP (in mmHg) rose significantly during the pressor tests, MA (85.6±2 to 93±3), CC (86±2 to 96±2) and ISE (89±2 to 98±2)(each P<0.05) and was maintained during head-up tilt (87±2 to 90±2 at 2 min and 87±2 to 89±1.6 at 10 min)(Fig 4.1).

HR: Changes in HR during the pressor tests and head-up tilt were not significant although HR tended to be higher during the pressor tests and head-up tilt (Table 4.1). In the three subjects who felt faint during head-up tilt, heart rate fell after an initial rise.

DIAMETER OF SMA: Mean diameter of SMA, preceding each test varied between 7.06±0.1 mm to 7.3±0.1 (P=NS, ANOVA, Table 4.1). Diameter of the SMA (in mm) fell during MA (7.3±0.1 to 6.5±0.1), CC (7.06±0.1 to 6.08±0.1), ISE (7.06±0.1 to 5.9±0.1) and head-up tilt at 2 min (7.1±0.1 to 6.2±0.2) and at 10 min (7.1±0.1 to 6.3±0.1)(each P<0.05, Table 4.2).

SMABF: The mean value of resting SMABF before each stimuli varied from 484±27 to 538±37 ml/min. Analysis of these resting SMABF values preceding each test, by ANOVA, showed no significant differences, indicating satisfactory return to the baseline state. In six out of the 24 subjects studied initially, there was poor visualisation of the SMA due mainly to bowel gas. An anatomical variation (type three origin) and an excessively pulsatile SMA (wall thump) prevented accurate measurement of SMABF in two subjects. There was a significant fall in SMABF (in ml/min) during MA (500±48 to 386±30), CC (538±37 to 354±36), ISE (523±31 to 368±34) and head-up tilt (484±27 to 240±26 at 2 min and 484±27 to 270±25 at 10 min)(each P<0.05, Table 4.2, Fig 4.2).
SMAVR: Calculated superior mesenteric artery vascular resistance (SMAVR) rose correspondingly (each \(P<0.05\)) during each pressor stimuli and head-up tilt indicating active constriction of SMA (Table 4.2, Fig 4.2).

PI: Baseline PI of the SMA blood velocity waveforms preceding each test varied from \(271\pm10\) to \(282\pm10\) (\(P=NS\), ANOVA, Table 4.2). PI rose during MA (\(282\pm10\) to \(312\pm13\)), CC (\(273\pm10\) to \(322\pm13\)) and ISE (\(271\pm10\) to \(307\pm12\))(Each \(P<0.05\), Table 4.2, Fig 4.3). PI however, was unchanged during head-up tilt (\(275\pm12\) to \(280\pm12\) at 2 min and \(275\pm12\) to \(283\pm13\) at 10 min, each \(P=NS\)).

Cl: Baseline resting CI (in units) varied from \(500\pm40\) to \(550\pm31\) before each stimuli and did not differ significantly. CI rose during MA (\(507\pm32\) to \(650\pm45\)) and ISE (\(500\pm44\) to \(626\pm42\)) and fell during head-up tilt (\(550\pm31\) to \(410\pm40\) at 2 min and \(400\pm30\) at 10 min). CI was higher during CC although this did not reach statistical significance (Fig 4.1).

FBF: Baseline FBF (in ml/100ml/min), preceding each stimulus, varied between \(2.32\pm0.3\) to \(2.9\pm0.4\) and was not significantly different. FBF rose (\(P<0.05\)) during MA with an accompanying fall in forearm vascular resistance (FVR, Fig 4.6). FBF was unchanged during CC and ISE although values tended to be higher during ISE with a lower FVR (\(P=NS\), Fig 4.6). FBF fell during head-up tilt with an accompanying rise in forearm vascular resistance (\(P<0.05\))(Fig 4.4).

Repeat measurements of SMABF during rest and during stimulation (MA, CC, ISE and head-up tilt) in 6 subjects studied 2 months later showed a close correlation (\(r=+0.71\), \(P<0.05\)) with previous measurements (Fig 4.5)

Symptoms: Three subjects felt faint and light-headed during head-up tilt. These symptoms were rapidly reversed on return to the horizontal position.

Coefficients of variation: Coefficients of variation for various measurements were 11% for SMABF, 8% for CI and 12% for FBF.
Fig 4.1: Changes in mean arterial blood pressure (MABP, upper panel) and cardiac index (CI, lower panel) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt at 2 min (T2) and 10 min (T10) in normal subjects.
Fig 4.2: Changes in superior mesenteric artery blood flow (SMABF, upper panel) and superior mesenteric artery vascular resistance (SMAVR, lower panel) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt at 2 min (T2) and 10 min (T10) in normal subjects.
Fig 4.3: Changes in pulsatility index of superior mesenteric artery blood velocity waveforms before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt at 2 min (T2) and 10 min (T10).
Fig 4.4: Changes in forearm blood flow (FBF, upper panel) and forearm vascular resistance (FVR, lower panel) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt at 2 min (T2) and 10 min (T10) in normal subjects.
Fig 4.5: Correlation between superior mesenteric artery blood flow values (baseline and during stimulation) during initial (1st recording) and repeat (2nd recording) study at 2 months interval. ($r=0.71$, $P<0.05$)
Table 4.1 Changes in mean systolic, diastolic blood pressure and heart rate before and during each stimulus in normal subjects.

<table>
<thead>
<tr>
<th></th>
<th>MA</th>
<th>CC</th>
<th>ISE</th>
<th>T2</th>
<th>T10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean systolic</strong></td>
<td>Pre</td>
<td>During</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>blood pressure</strong></td>
<td>(mmHg)</td>
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<td></td>
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<tr>
<td>Pre</td>
<td>120±10</td>
<td>120±12</td>
<td>122±10</td>
<td>121±11</td>
<td>121±11</td>
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<tr>
<td>During</td>
<td>131±13</td>
<td>130±10</td>
<td>130±12</td>
<td>120±9</td>
<td>121±9</td>
</tr>
<tr>
<td><strong>Mean diastolic</strong></td>
<td>Pre</td>
<td>During</td>
<td></td>
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<td><strong>blood pressure</strong></td>
<td>(mmHg)</td>
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<tr>
<td>Pre</td>
<td>69±7</td>
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<td>71±8</td>
<td>70±7</td>
<td>70±7</td>
</tr>
<tr>
<td>During</td>
<td>74±10</td>
<td>79±9</td>
<td>79±11</td>
<td>75±8</td>
<td>73±7</td>
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<tr>
<td><strong>Mean heart rate</strong></td>
<td>Pre</td>
<td>During</td>
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<td>(beats/min)</td>
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</tr>
<tr>
<td>Pre</td>
<td>66±2</td>
<td>64±2</td>
<td>62±2</td>
<td>66±3</td>
<td>66±3</td>
</tr>
<tr>
<td>During</td>
<td>75±3</td>
<td>69±3</td>
<td>75±3</td>
<td>74±2</td>
<td>76±3</td>
</tr>
</tbody>
</table>

MA=mental arithmetic, CC=cutaneous cold, ISE=isometric exercise, T2 and T10 are 2 and 10 min during head-up tilt.
Table 6: Changes in diameter of the superior mesenteric artery, superior mesenteric artery velocity, and superior mesenteric artery flow before and during exercise (ISE) and head-up tilt (TUT) and to min (T2) and 10 min (T10).

<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>ISE</th>
<th>CC</th>
<th>MA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
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<tr>
<td>T2</td>
<td></td>
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</tbody>
</table>

p = p > 0.05
In this study SMA blood flow and pulsatility index has been measured during stimuli which cause sympato-neural activation. In normal man, vasoconstriction in the splanchnic region has been described during powerful stimuli, such as lower body negative pressure (Rowell, 1974), exercise (Grayson & Mendel, 1965), haemorrhage (Price et al, 1966) or thermoregulatory stress (Rowell & Johnson, 1984; Qamar & Read, 1987). The responses observed were considered important in blood pressure regulation (Rowell & Johnson, 1984). However changes in SMA blood flow during sympato-neural activation by pressor tests or head-up tilt, which are routinely used in autonomic assessment, have not been previously described.

Consideration will be given initially to the various sympato-neural stimuli used in this study. Pressor tests such as MA, CC and ISE activate the sympathetic efferent constrictor fibres which supply capacitance and resistance vessels and raise blood pressure (Mathias & Bannister, 1992). However, various receptors such as the arterial baroreceptors and chemoreceptors, cardiopulmonary mechanoreceptors and skeletal muscular receptors govern changes in sympathetic outflow. MA is a centrally acting pressor test with the afferent input activating the cortical and hypothalamic centres while afferent impulses from CC and ISE travel from the periphery to the cerebral cortex and descend from the major nuclei via the preganglionic sympathetic neurons in hypothalamus, midbrain and brain stem. These nuclei include locus coerulius, nucleus ambiguus and the nucleus tractus solitarius. The pathways continue in the cervical spinal cord where the axons synapse with the sympathetic neurons of the intermediolateral cell columns (Spyer, 1992). Myelinated axons then emerge from the thoracic and upper lumbar spinal segments, within the white rami and synapse within the paravertebral ganglia. Postganglionic unmyelinated fibres then run in the grey rami and innervate target organs except the adrenal medulla which is solely innervated by preganglionic fibres. Owing to the highly complex functioning of the central and peripheral sympathetic sensors, the sympathetic nerve discharges occur in a markedly differential pattern (Shepherd & Shepherd, 1992). During peripheral or
central reflex stimulus, effects of sympathetic efferent activation may be variable
between different organs, vascular beds and in some organs between resistance and
capacitance vessels (Shepherd & Shepherd, 1992; Bennett & Gardiner, 1992). Therefore
although sympathetic activation by the pressor stimuli used in this study is
expected to cause splanchnic vasoconstriction as with lower body negative pressure
and exercise, it is by no means certain and needs to be documented in man. This is
important as some pressor tests such as isometric exercise may increase blood flow in
certain vascular beds (Shepherd & Shepherd, 1992) and plasma noradrenaline level,
which is used as an index of sympathetic function, may not be a reliable indicator of
splanchnic vascular changes. This is because the skeletal muscle contributes towards
one fifth of the plasma noradrenaline level in comparison to a minimal contribution
from the splanchnic vascular bed due to clearance of catecholamines by the liver
(Shepherd & Mancia, 1986).

During head-up tilt there is gravitational shift of blood from the cardiopulmonary
region to the dependent parts. A series of neurohormonal responses follow which
includes reflex activation of the baroreflex pathways, decreased activity of the
cardiopulmonary and arterial mechanoreceptors, vasoconstriction in vascular beds
such as the muscle and renal, increase in plasma renin activity and noradrenaline
(Shepherd & Mancia, 1986) and reflex constriction of the precapillary vessels
(Hainsworth, 1990). Consequent to these neural and hormonal responses, BP is
maintained. There is however, a difference in responses after head-up tilt and
standing (Welling, 1992). During head-up tilt there is a gradual rise in diastolic blood
pressure unlike during standing where diastolic BP increases quickly by about 10
mmHg, often with an overshoot in rise of heart rate rise as well (Welling, 1992). The
differences in responses during head-up tilt and standing could be attributed to
muscular contraction in the abdominal muscles and the leg during standing
(Sprangers et al, 1991) which does not occur during head-up tilt as the subject is
supported. Thus head-up tilt may provide a more accurate estimation of baroreflex
pathway activation. In this study, measurements were commenced after 2 min of
head-up tilt as arterial recordings have shown that changes in blood pressure (if any) will usually be apparent by this time (Bannister & Mathias, 1992).

In our study adequate sympatho-neural activation was confirmed by a significant rise in BP during each of the pressor tests and maintenance of BP during head-up tilt. Heart rate tended to rise although not significantly. The rise in BP during the pressor stimuli and maintenance of BP during head-up tilt was associated with a significant reduction in SMABF. A corresponding rise in calculated mesenteric vascular resistance (SMAVR) indicated that the reduction in SMABF was due to active constriction of the SMA. Furthermore, a significant rise in PI during the pressor tests confirmed vasoconstriction of the SMA, as a rise in PI is indicative of rise in downstream vascular resistance. PI however, remained unchanged during head-up tilt when it was expected to rise. The possible explanation for this is discussed later.

CI, a measure of relative cardiac output rose during MA and ISE and remained unchanged during CC. The rise in cardiac output during MA and ISE possibly contributed to the rise in BP along with splanchnic vasoconstriction; during CC, changes in splanchnic and possibly other vascular beds played a part in the BP response. FBF rose during MA and tended to be higher during ISE and remained unchanged during CC. The mechanism of rise in FBF during MA is unknown and could be analogous to the rise in FBF during ISE, which has also been reported by Cotzias and Marshall (1991). Several explanations for the rise in FBF during ISE have been offered. These include accumulation of products of muscle metabolism which activate chemosensitive endings in the muscle causing vasodilatation, a beta-adrenoreceptor mediated vasodilatation and a neurally induced reflex (Kaijser, 1991; Shepherd & Shepherd, 1992). It is possible a combination of these factors played a part in the responses observed in the present study.

During head-up tilt, CI and FBF fell as has been reported in other studies (Shepherd & Mancia, 1986) and there was a corresponding rise in calculated FVR. However changes in FBF may not be representative of changes in the entire skeletal muscular bed (Bennett & Gardiner, 1992). In normal man, for instance, vascular responses
within the forearm and calf vascular beds may differ. In this study, measurements of calf blood flow were however, not made.

During head-up tilt, SMABF fell markedly with a corresponding rise in SMAVR but the PI remained unchanged. The precise reasons for this discrepancy is unknown. Although measurement of PI is independent of the estimation of vessel diameter and angle of insonation, it is governed by several factors which can alter the shape of the arterial flow velocity waveform. These include, cardiac contractility and output, vessel wall compliance, blood velocity, turbulence and distance of the point of recording from the heart (McDonald, 1974). Therefore, the fall in cardiac output during head-up tilt may have led to a reduction in both the systolic and diastolic components of flow volume and thus the ratio, which is PI, remained unchanged. Lack of a consistent rise of forearm vascular resistance and cardiac output (during CC) during the pressor tests indicated that the documented constriction of the SMA (suggestive of splanchnic vasoconstriction) played a major role in the pressor response. Similarly, during head-up tilt, maintenance of BP while cardiac output fell, was achieved by a marked splanchnic and possibly forearm vasoconstriction. Though there was forearm vasoconstriction, it is not clear whether there was generalised skeletal muscular vasoconstriction during head-up tilt as calf blood flow measurements were not made (as discussed previously). This is may be important as Duprez et al (1987) have shown that in normal subjects, postural cardiovascular reflexes involve forearm, but not calf resistance vessels. Measurements in some of the other vascular beds, which could have played a part in these vascular responses were not made in this study. These include the renal and cerebral vascular beds. However, a strong autoregulatory mechanism exists in both these vascular beds (Owman, 1986; Hainsworth, 1992) and it is therefore unlikely that the renal and cerebral vascular beds played a major role in the observed vascular responses.

In the three subjects who felt faint during head-up tilt, the heart rate fell and there was hypotension. This was possibly due to vasovagal syncope as described originally by Lewis (1932). Short lasting orthostatic dizziness is now recognised, after prolonged
Supine rest, particularly in young subjects (Weiling, 1992). All three subjects who felt faint were aged between 24 to 26 years, and thus may belong to the above category. The mechanism of constriction of the SMA during pressor stimuli and head-up tilt is most likely to be neurally induced because of its early onset although a humoral contribution cannot be excluded, particularly as hormonal measurements were not made. The possible humoral agents mediating vasoconstriction of the SMA in response to sympathetic activation, apart from noradrenaline, could be angiotensin II, vasopressin and endothelin among others. It is unlikely however, the renin-angiotensin system and vasopressin release played a part in the pressor responses during the short lasting pressor stimuli as these agents are usually released after continued sympathetic activation, such as with prolonged standing. The role of endothelin is yet to be fully established although recent evidence suggest that plasma endothelin increases with upright posture (Kaufmann et al, 1991) and is influenced by baroreflex activation. It is possible therefore that vasoconstriction of the SMA during head-up tilt could have partly been induced by humoral factors.
CONCLUSIONS:

Sympatho-neural activation by pressor tests (raising blood pressure) and head-up tilt (maintaining blood pressure) causes active splanchnic vasoconstriction in normal subjects. The non-invasive pulsed Doppler ultrasound technique is capable of demonstrating constriction within the SMA, even during short-lived stimuli. Measurement of the pulsatility index of the superior mesenteric artery blood velocity waveforms is an useful adjunct to measurement of volume blood flow although PI measurements appear to be inaccurate during head-up tilt and therefore should not be used during postural change.
CHAPTER 8:
ASSESSMENT OF SYSTEMIC, MESENTERIC AND OTHER REGIONAL VASCULAR RESPONSES TO PRESSOR STIMULI AND HEAD-UP TILT IN NORMAL SUBJECTS OF TWO DIFFERING AGE GROUPS.
INTRODUCTION:

Studies described in chapter 3 indicated that there may be a difference in SMABF in young and older normal subjects, although this was not significant. There was a difference in resting mean arterial blood pressure in the two groups as well, BP in older subjects being higher than in younger subjects. Whether the differences would apply to splanchnic vascular responses to sympato-neural stimuli in older subjects, as described in younger subjects earlier (chapter 4), is not known.

The autonomic nervous system is affected by ageing and in general, as age advances the overall homeostasis remains active although the efficiency of the autonomic responses may be gradually reduced (Johnson, 1992). Elderly healthy subjects, despite being off drug therapy, are known to respond differently to certain stimuli in comparison to younger normal subjects. Examples are the blood pressure (BP) responses to head-up tilt and food ingestion, either of which can lower BP (Caird et al, 1973; Lipsitz et al, 1986; Potter et al, 1989) and the effect of ageing on thermoregulation (Johnson et al, 1990). The differences in responses to various stimuli in the young and the elderly have mainly focussed on orthostasis and mental stress tests such as mental arithmetic. Recent studies indicate that there may be a differential response to mental stress in young and elderly healthy subjects, as based on power spectral analysis of heart rate variability (Moriguchi et al, 1991) or the P-300 component of event related long latency potential which is intimately related to information processing in brain (Toledo-Morell et al, 1991). A further study has shown an exaggerated pressor response to isotonic exercise in elderly hypertensive subjects (Alguacil et al, 1991).

In this study, a range of responses were noted in normal subjects below the age of 65, who were therefore not classed as elderly, during sympato-neural stimuli including mental arithmetic. As this stimulus is often used in clinical and research studies, the systemic and regional, in particular superior...
mesenteric artery blood flow responses, were studied in two age groups, before and after a range of stimuli commonly used in cardiovascular and autonomic laboratories. The normal subjects included those with a mean age of 21 years (younger) or mean age of 62 years (older), the two groups being separated by at least 30 years. The similarities and differences in responses between these two groups with differing ages are reported.

MATERIAL AND METHODS:

8 younger healthy subjects (mean age 21 years, range 19-27 years) and 8 older healthy subjects (mean age 62 years, range 58-72 years) were studied in an identical manner. The age gap between the oldest member of the younger group and the youngest member of the older group was 30 years. All were normotensive, literate and none were on medication at the time of the study. They were studied after an overnight fast in a temperature controlled room (24°C) at 0900 hrs. All subjects were rested for 30 min in the supine position to allow familiarisation with equipment and equilibration before baseline measurements begun.

Measurements consisted of blood pressure and heart rate by automated sphygomanometry (BP, HR, Sentron Bard Biomedical), forearm blood flow by venous occlusion plethysmography (FBF, mercury in silastic strain gauge plethysmography), cardiac index by continuous wave Doppler ultrasound (CI, Exerdop, Quinton Instruments) and superior mesenteric artery blood flow by Doppler ultrasound flowmetry (SMABF, Acuson 128, 3.5 MHz sector transducer). All measurements were performed as previously described.

After baseline measurements, the subjects underwent mental arithmetic (MA, forced serial 17 subtraction from 700 for 120 sec), cutaneous cold (CC, one hand immersed in ice slush at 4°C for 120 sec), isometric exercise (ISE, dominant hand gripping rolled BP cuff at one third the maximal voluntary pressure for 120 sec) and 45° head-up tilt for 10 min. The same investigator supervised the mental arithmetic and other pressor tests in each subject.
Measurements were made before (10 min) and at the end (120 sec) of each stimulus, with measurement during head-up tilt at 2 and 10 min. There was a rest period of 15 min between each test during which values returned to the baseline. The stimuli were applied in a strict within subject standardized procedure and every effort was made to keep resting levels and other confounding factors as constant as possible.

Calculations of SMABF, CI, FBF, mean arterial blood pressure (MABP), superior mesenteric artery and forearm vascular resistances were made as previously described.

MABP, CI, FBF and SMABF measurements at two and 10 min of head-up tilt are expressed as average values during head-up tilt.

STATISTICS:
Resting SMABF values were higher in younger subjects compared to older subjects. Changes in BP and SMABF are therefore, expressed as percent changes from baseline values. Absolute values before and during each stimulus were compared by paired t test. Mann Whitney test was used to compare changes in measured responses between younger and older subjects. P < 0.05 was considered significant and data are presented as means ± s.e.m.

Coefficient of variations of SMABF, CI and FBF were determined as described in chapter 3.

RESULTS:

MBP: In the younger subjects resting MBP between stimuli varied between 82±2 to 86±3 mmHg and it rose during MA by 13±2% (82±2 to 93±3 mmHg), during CC by 12±3% (83±2.5 to 93±3) and during ISE by 15±3% (86±3 to 99±3) (each P<0.05, Figs. 5.1 & 5.2). MBP was maintained during head-up tilt (85±2 to 88±2 mmHg, 4±1.7%, P=NS).

In the older subjects resting MBP between stimuli varied between 92±5 and 95±4 mmHg. Resting MBP tended to be higher in older controls though this was not statistically significant. There was no significant change in MBP
during MA (95±4 to 103±5, 9±3%, P=NS). In 4 out of the 8 subjects MBP rose (97 to 110, 80 to 90, 103 to 120 and 103 to 111 mmHg); individual changes in MBP during MA in the two groups are shown in Fig 5.3. MBP rose during CC by 14±2% (94±2 to 107±4), and during ISE by 25±5% (92±5 to 117±9)(each P<0.05) and was maintained during head-up tilt (93±3 to 95±3, 2.1±2%, P=NS) similar to the younger subjects. The percentage rise in MBP was higher in the older subjects during ISE (25±5%) than in the younger subjects (14±3%) though this did not achieve statistical significance when compared by Mann Whitney test.(Fig 5.2)

SMABF: In younger subjects resting SMABF tended to be higher than the older subjects although the difference was not significant. In younger subjects SMABF fell during MA by 37±6% (670±63 to 444±47 ml/min), during CC by 37±6% (670±63 to 444±47), during ISE by 39±4% (629±46 to 378±30) and during head-up tilt by 45±9% (574±31 to 314±65)(each P<0.05, Fig 5.4). There was a corresponding rise in calculated SMAVR during MA by 82±17% (0.13±0.01 to 0.24±0.02 units), during CC by 91±18% (0.11±0.01 to 0.23±0.02), during ISE by 91±19% (0.14±0.01 to 0.26±0.02) and during head-up tilt by 126±32% (0.15 to 0.35±0.05)(each P<0.05, Fig 5.5).

In the older subjects there was minimal change in SMABF during MA (14±6%, P=NS) though in the 4 subjects in whom MBP increased during MA, SMABF fell (Fig 5.3). Comparison of the percentage change in SMABF during MA showed a significant difference from the younger subjects.(Fig 5.6). There was no significant change in the calculated SMAVR during MA in the old (35.5±13%, P=NS) and comparison of percentage change in SMAVR in the younger (82±17%) and older (35.5±13%) subjects during MA showed a significant difference.(Fig 5.6) SMABF however fell similar to the younger subjects during CC by 44±4% (452±62 to 262±50), during ISE by 43±5% (471±53 to 276±41) and during head-up tilt by 54±3% (471±71 to 222±43)(each P<0.05, Fig 5.4). There was also a corresponding rise in the
SMAVR during CC by 94±16% (0.23±0.03 to 0.46±0.08 units), during ISE by 174±34% (0.18±0.02 to 0.52±0.14) and during head-up tilt by 134±12% (0.21±0.02 to 0.52±0.07) (each P<0.05). There was a significantly higher rise in SMAVR (174±34%) in the older subjects during ISE compared to the younger subjects (91±14%) suggesting a greater constriction of the SMA. (Fig 5.6) Individual changes in SMABF during MA in the younger and older subjects are shown in Fig 5.3.

FBF: In younger subjects, FBF tended to rise during MA (2.25±0.41 to 3.55±0.56 ml/100ml/min, 66±19%), and during ISE (2.07±0.34 to 2.66±0.41, 32±6%), with a corresponding trend towards lower FVR but this was not statistically significant (Fig 5.7, Table 5.1). During head-up tilt FBF fell (P<0.05) with a rise in the forearm vascular resistance (Fig 5.7, Table 5.1).

In the older subjects, FBF tended to rise to a lesser degree (29±6%) during MA (3.13±0.2 to 4.13±0.47, P=NS) with a corresponding lowering of FVR (Fig 5.7, Table 5.1). FBF however rose significantly in the older subjects (3.85±0.2 to 4.22±0.4, 36±6%, P<0.05) during ISE with a lowering of FVR. FBF fell during head-up tilt (3.15±0.25 to 1.62±0.17, P<0.01) with a rise in forearm vascular resistance (31.04±2.3 to 62.6±6.4 units, P<0.01) in older subjects. (Table 5.1) Changes in FBF during CC were not significant.

CI: Resting CI was significantly higher in the older subjects (702±77 units) than the younger subjects (472±45). Changes in CI were not significant during the stimuli except during head-up tilt when it fell (P<0.05) in older subjects. There was a trend towards a lower CI in the younger subjects during head-up tilt (Fig 5.8).
Fig 5.1: Changes in mean arterial blood pressure (MBP) in younger (upper panel) and older subjects (lower panel) during sympa-tho-neural activation.
Fig 5.2: Percentage rise in mean blood pressure (MBP) in younger (blank bars) and older subjects (filled bars) during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt (T).
Fig 5.3: Individual changes in mean blood pressure (\( \Delta \) MABP) and superior mesenteric artery blood flow (\( \Delta \) SMABF) in 8 younger subjects (upper panel) and 8 older subjects (lower panel) during mental arithmetic. (Each bar represents a subject.)
Fig 5.4: Changes in superior mesenteric artery blood flow (SMABF) in younger subjects (upper panel) and older subjects (lower panel) during sympatheo-neural activation.
Fig 5.5: Changes in superior mesenteric artery vascular resistance (SMAVR) in younger subjects (upper panel) and older subjects (lower panel) before and during sympa-tho-neural activation.

* = $P < 0.05$
Fig 5.6: Percentage rise in superior mesenteric artery vascular resistance (SMAVR, upper panel) and percentage fall in superior mesenteric artery blood flow (SMABF, lower panel) in younger (blank bars) and older (filled bars) subjects during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt (T).
Fig 5.7: Changes in forearm blood flow (FBF) in younger (upper panel) and older (lower panel) subjects before (P, blank bars) and during (filled bars) mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt (T).
Fig 5.8: Changes in cardiac index (CI) in younger (upper panel) and older (lower panel) subjects before (P, blank bars) and during (filled bars) mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt (T).

*=P<0.05
Table 5.1: Changes in forearm vascular resistance (FVR) in younger and older subjects before and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and head-up tilt (T).

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Younger Subjects (N=8)</th>
<th>Older Subjects (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>During</td>
</tr>
<tr>
<td>MA</td>
<td>48.9±6.5</td>
<td>33.4±6.6</td>
</tr>
<tr>
<td>CC</td>
<td>48.9±6</td>
<td>63.3±8.8</td>
</tr>
<tr>
<td>ISE</td>
<td>52.8±6</td>
<td>47.9±5.1</td>
</tr>
<tr>
<td>T</td>
<td>49.1±6.2</td>
<td>99.5±13</td>
</tr>
</tbody>
</table>

*=P<0.05
Our results indicate that although the majority of haemodynamic responses to stimuli causing sympatho-neural activation were similar in younger and older healthy subjects, there were certain differences. There was a selective impairment of the pressor response to MA in some of the older subjects associated with a lack of active constriction of the superior mesenteric artery, unlike younger subjects. During CC and ISE, BP rose in both younger and older subjects while BP was maintained during head-up tilt. In both groups sympatho-neural activation induced by these stimuli was associated with an active constriction of the SMA and the findings during these stimuli were similar to those reported in young healthy subjects (Chapter, 4).

The responses in the two groups during the stimuli, apart from MA, suggest that overall afferent, central and efferent reflex autonomic function was no different, despite the age gap. These tests, however, do not separate the different components of the autonomic pathways concerned with maintaining cardiovascular homeostasis. The reasons for the selective impairment of the pressor response to MA in half the older healthy subjects were not clear, but there were several possibilities. A limitation of this study was the small number of subjects in each group. Abnormal responses to MA in four out of the eight older subjects may have been due to poor mental performance and/or poor compliance in the older subjects and were thus open to chance. However, this is unlikely as these subjects were cooperative and performed MA, like the others within a strict time span. The performance, though not quantified objectively, did not seem to differ between the groups and was reproducible as has also been reported by Jern et al (1991). Whether these variant responses would apply to other tests of mental function remains to be tested. It is possible that MA unmasked a differential vascular response in the older subjects; forearm blood flow (as a measure of skeletal muscle blood flow) tended to rise during MA in the older subjects but this was no greater than in the younger

DISCUSSION:

Our results indicate that although the majority of haemodynamic responses to stimuli causing sympatho-neural activation were similar in younger and older healthy subjects, there were certain differences. There was a selective impairment of the pressor response to MA in some of the older subjects associated with a lack of active constriction of the superior mesenteric artery, unlike younger subjects. During CC and ISE, BP rose in both younger and older subjects while BP was maintained during head-up tilt. In both groups sympatho-neural activation induced by these stimuli was associated with an active constriction of the SMA and the findings during these stimuli were similar to those reported in young healthy subjects (Chapter, 4).

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subjects, and in some was not accompanied by a similar or greater degree of constriction of the SMA. As reported in the previous chapter, the splanchnic vascular bed plays an important role in BP regulation during sympatho-neural activation in normal subjects. Whether the lack of constriction of the SMA during MA in some elderly subjects was the cause of the abnormal pressor response is not clear from this study. The SMA responses to CC, ISE and tilt were however preserved indicating the ability of this vascular bed to respond to other sympatho-neural stimuli. In experimental animals and in some in-vitro human studies, prolonged stimulation of the splanchnic sympathetic nerves causes blood flow in intestinal arteries to return to normal after initial vasoconstriction, which has been described as the phenomenon of autoregulatory escape (Folkow et al, 1964; Greenway, 1984). It is possible therefore, that the lack of constriction of the SMA during MA could have been due to autoregulatory escape during prolonged sympathetic activation due to mental stress. This is unlikely however, as the stimulus was similar to other short lived stimuli (CC, ISE, tilt) where the response was similar to younger subjects. Furthermore, in this and the previous study vasoconstriction of SMA persisted even after 10 min of head-up tilt and intense splanchnic vasoconstriction has been reported for up to 1 hour during severe stress in man without autoregulatory escape, making this a less likely explanation (Rowell, 1971).

The abnormal responses to MA in some of the older subjects may be due to a defect in the central neuronal pathways responsible for the processing of higher functions, which modulate and then influence the sympathetic outflow. There are other examples where central neuronal dysfunction associated with aging has been described in healthy older subjects. Moriguchi et al (1991) used power spectral analysis of heart rate variability and showed that while head-up tilt increased sympathetic activity in older subjects (mean age 66 yrs), MA failed to stimulate sympathetic activity, unlike young subjects. In another study Toledo-
Morrell et al (1991) found a delayed P-300 component of long latency event related potentials, recorded from posterior regions of the scalp, during mental stress in healthy elderly subjects (mean age 71 years), but not in young healthy subjects. Event related potentials have been shown to depend upon the amount of information processing activity evoked by a stimulus and have been used to study age related alterations in cognitive function (Ford et al, 1985). Phillips et al (1991) found that healthy elderly men (mean age 70 years) have reduced osmotic thirst which is thought to be due to dysfunction of central connections of thirst pathways. Baroreceptor-heart rate reflex sensitivity is reduced with ageing (Gribbin et al, 1971) and Tonkin et al (1991) suggested that dysfunction of the afferent limb of the baroreflex arc occurs in the healthy elderly subjects off medication (mean age 82 years). These studies were carried out in subjects over 65 years and defined as elderly, while our subjects were slightly younger. We however feel that an arbitrary cut off point of 65 years may not be entirely relevant to a continuing process. The probable central abnormalities in our healthy older subjects, although specifically in relation to MA, may reflect the normal aging process as noted in other studies.

In our study we also compared systemic and regional haemodynamic responses to other stimuli in the younger and older subjects and overall these showed no difference. Isometric exercise tended to increase BP to a greater extent in the older subjects, but this was not statistically significant, and it was accompanied by a greater increase in forearm blood flow than in the younger subjects. This was also accompanied by a greater rise in superior mesenteric artery vascular resistance in the older subjects suggesting a more marked constriction of the SMA during ISE. This could be one explanation for the higher rise of BP in the older subjects during ISE. This is consistent with previous observations showing an increase in forearm blood flow in normal subjects during ISE and an exaggerated pressor response to isotonic exercise in older hypertensive
subjects (Cotzias & Marshall, 1991; Alguacil et al, 1991). The regional circulatory changes were however not previously reported.

CONCLUSIONS:
The pressor and splanchnic responses to mental stress may be different in younger and older healthy normal subjects. The precise reasons for these differences are not clear. In this study, owing to the small number of subjects included, the differential response to MA could have resulted from a chance finding. Systemic and regional haemodynamic responses to CC and head-up tilt are similar in both groups although the responses to ISE appear to be more pronounced in the older subjects. This study emphasises, despite the small number of subjects studied, the need for further evaluation of the systemic and regional haemodynamic responses to different forms of mental stress in a larger group of normal subjects over a wide age range. Furthermore, this study underlines the necessity for strict age matching and for using a range of tests in determining the responses to activation of the sympatho-neural pathways.
CHAPTER 6:

ABNORMALITY OF SUPERIOR MESENTERIC ARTERY BLOOD FLOW RESPONSES IN HUMAN SYMPATHETIC DENERVATION.
The importance of the splanchnic vascular bed which contains up to 30% of total blood volume and receives up to 25% of the resting cardiac output (Rowell, 1975) has already been indicated in the preceding studies in normal subjects. Studies in the previous chapters (4 & 5) showed that short lived pressor stimuli and head-up tilt caused active constriction of the SMA and raised (pressor tests) or maintained (head-up tilt) blood pressure. With some of these stimuli, such as head-up tilt, it was not clear if humoral changes may have contributed to the observed fall in SMABF and rise in superior mesenteric artery vascular resistance (SMAVR). This study was undertaken to further evaluate the role of neural factors (in particular the sympathetic nervous system) in SMA vascular responses, by observations in two groups of patients with primary autonomic failure, who on the basis of a range of physiological and biochemical tests had widespread sympathetic denervation.

As previous studies indicated that the SMA vascular and pressor responses to certain stimulus may be different in young and older healthy subjects, comparisons were made in age matched healthy subjects (controls).

Study 1: Studies in patients with chronic primary autonomic failure:

Much of the work described in this thesis has been carried out in patients with confirmed chronic primary autonomic failure (AF). Details of clinical presentation and pathology of AF patients have been described recently by Mathias (1987, 1991). A brief description of chronic primary autonomic failure will be considered here.
Primary Autonomic Failure (AF):

Autonomic disorders can be broadly classified into a primary autonomic failure where there is no identifiable cause and secondary autonomic failure where autonomic dysfunction is either due to a specific disease condition such as diabetes mellitus or is strongly associated with a condition such as Holmes-Adie syndrome or ageing. Autonomic dysfunctions can be further subdivided into a localized form where a specific organ is affected (Horner's syndrome, Chaga's disease) or is induced by drugs acting by modulating sympathetic or parasympathetic activity (Mathias, 1991).

Patients with primary autonomic failure are subdivided as those with pure autonomic failure (PAF) and those with probable multiple system atrophy (MSA, probable because the diagnosis can only be confirmed after neuropathological examination of the brain).

Pure Autonomic Failure: This included patients with autonomic failure who do not have any other neurological deficit. This probably includes the syndrome of "idiopathic orthostatic hypotension" which was first described by Bradbury and Eggleston (1925) although the description does not include the often associated involvement of sweat glands, urinary bladder, bowel, genitalia and pupil. Post mortem studies show that most cases of PAF are associated with loss of intermediolateral cell columns which is the final common pathway for the sympathetic outflow (Oppenheimer, 1980). Additional loss of ganglionic neurons have also been reported in PAF (Matthews, 1992). Neuropathological data is otherwise limited in PAF. Polinsky (1992) has reported on biochemical and pharmacological differences between PAF and MSA which favours a more peripheral lesion in PAF. The major differences are as follows:
**BIOCHEMICAL:**

1. Low supine plasma noradrenaline (NA) and decreased neuronal uptake of NA in PAF.

2. Decreased urinary NA metabolites.

3. Absent adrenaline response to arecoline, a cholinergic agonist which stimulates muscarinic and nicotinic receptors (Polinsky et al., 1991).

4. Normal levels of dihydroxyphenylglycol, homovanillic acid, 5-hydroxyindole acetic acid and somatostatin levels in the cerebrospinal fluid.

**NEUROIMAGING:**

1. Normal glucose metabolism and dopamine uptake on neuroimaging (Positron Emission Tomography) (Fulham et al., 1991; Brooks et al., 1990).

Further subclassification of PAF is possible based on identification of precise enzyme deficiencies in the bio-synthetic pathway of noradrenaline and adrenaline. An example is the dopamine-beta-hydroxylase deficiency syndrome (Roberson et al., 1986).

**Multiple System Atrophy:** Here autonomic failure is associated with other neurological abnormalities. This is otherwise referred to as the Shy-Drager syndrome after the first neuro-pathological description of this disorder by Shy and Drager, (1960). Three clinical subtypes are now recognised (Mathias, 1991). These are autonomic failure with:

(a) **Striatonigral degeneration** presenting with mainly Parkinsonian features (extrapyramidal) and associated with loss of pigmented cells in the substantia nigra and locus ceruleus. Unlike idiopathic Parkinson's disease, patients with striatonigral degeneration present with autonomic failure and usually have a poor response to L-dopa therapy.
(b) **Olivopontocerebellar atrophy** usually presenting with cerebellar and pyramidal manifestations associated with neuronal atrophy in the olive, pons and cerebellum.

(c) **Multiple neuronal system atrophy** presenting with extrapyramidal, cerebellar and/or pyramidal manifestations and widespread neuropathological findings. The autonomic lesions in MSA lie principally within the brain and spinal cord. Lesions may also involve the respiratory centers resulting in the sleep apnoea syndrome and the Onuf's nuclei in the sacral portion of the spinal cord possibly resulting in bladder dysfunction. In MSA, there is also loss of intermediolateral cell columns as in PAF but the ganglionic neurons are usually preserved. As discussed previously, there are also differences in biochemical and pharmacological parameters (Polinsky, 1992) which favours the hypothesis that the lesions in MSA are central compared to peripheral lesions in PAF. The principal evidence for this is that plasma noradrenaline levels are lower in PAF and neuroimaging shows evidence of central lesions in MSA and not in PAF on the basis of regional cerebral metabolism. (Fulham et al, 1991; Brooks, 1992).

**Clinical manifestations of primary autonomic failure:**

1. **Postural hypotension**, which is often the presenting feature resulting from symptoms of cerebral ischaemia due to a fall in blood pressure. Postural hypotension may be aggravated by exercise, food and raised ambient temperature and lead to syncope.

2. **Supine hypertension** may occur due to reasons which are unclear and include adrenoreceptor supersensitivity, impaired baroreflex activity, increase in central blood volume because of translocation of blood from the periphery and iatrogenic effect.

3. **Anhidrosis or hypohydrosis** which is often noticed in warm weather. There is a preferential involvement of the eccrine glands which are concerned with thermoregulation whereas the apocrine glands may continue to function.
4. Abnormalities of thermoregulation in the form of hypo or hyperthermia may occur the latter often in association with anhydrosis.

5. Gastrointestinal symptoms such as constipation and rarely gastroparesis may occur.

6. Nocturia, probably resulting from recumbency is common and may lead to loss of weight and extracellular fluid volume and increased tendency for postural hypotension (Mathias et al, 1986).

7. Frequency, urgency and incontinence of urine may occur from bladder involvement.

8. Impotence also may be a feature.

9. Inspiratory stridor and sleep apnoea may occur in the later stages of the disease.

**MATERIAL AND METHODS:**

**Subjects & Patients:**

13 subjects with chronic primary autonomic failure (AF, mean age=56 yrs, range 46-72) and 10 age matched controls (mean age=52 yrs, range 36-68) were studied. All AF patients had severe postural hypotension (systolic fall of BP > 30 mmHg.) due to sympathetic failure and the majority had cardiac parasympathetic involvement. The diagnosis was confirmed by a series of autonomic function tests as described by Mathias and Bannister, 1992 (Table 6.1). Of the 13 patients with AF, 8 had pure autonomic failure (PAF) and 5 had multiple system atrophy (MSA). Despite a similar degree of sympathetic failure, there were differences in plasma noradrenaline levels, as discussed previously. Results in these two groups will therefore be considered separately. 10 healthy controls were studied in an identical manner. The study was approved by the ethics committee of St.Mary's Hospital and National Hospital for Neurology and Neurosurgery.
| BA/P | SP/V | RV/P | SP/H | RV/H | BA/P | SP/V | RV/P | SP/H | RV/H | BA/P | SP/V | RV/P | SP/H | RV/H |
|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 2    | A    | A    | A    | A    | A    | A    | A    | A    | A    | A    | A    | A    | A    | A    | A    | A    |
None of the controls were on drugs. In the patients medication consisted of fludrocortisone which was withdrawn 4 days prior to the study. They were studied after an overnight fast to exclude the established effect of food on superior mesenteric artery blood flow (SMABF) and ensure optimum ultrasound visualisation of the SMA (Qamar et al, 1986b; Moneta et al, 1988).

The study began at 0900 hours in a temperature controlled room (24°C). After an initial supine rest for 30 min to allow for familiarisation with equipment and stabilisation, measurements were made of SMABF (Acuson 128 Computed Sonography System, Acuson Corporation, California; 3.5 MHz. Sector Transducer), blood pressure and heart rate (BP, HR, Automated Sphygmomanometer, Sentron, Bard Biomedical, USA), forearm blood flow (FBF, mercury in silastic strain gauge plethysmography), and cardiac index (CI, continuous wave Doppler ultrasound, Exerdop, Quinton Instruments, USA). Subjects then non-randomly underwent a series of pressor tests which included mental arithmetic (MA, serial 17 subtraction from 700 for 120 sec), cutaneous cold (CC, free hand immersed in ice slush at 4°C for 120 sec), isometric exercise (ISE, gripping a rolled blood pressure cuff for 120 sec at one third maximal pressure using the dominant hand) as described in the previous chapter. They were then subjected to 45° head-up tilt for 10 min with measurements at 2 and 10 min. A 15 min period of equilibration was allowed between each stimulus and measurements were made before (10 min) and during (at 120 sec) each stimulus.

**Calculations:**

SMABF was measured by using a real time pulsed Doppler ultrasound method as previously described (Qamar et al, 1986a; Ray-Chaudhuri et al, 1991a). For each measurement, average values of three TAV's (each containing at least three cardiac cycles) were included. Precautions regarding angle of insonation, aliasing and background noise were taken as described previously. Cardiac index was calculated by multiplying stroke distance (a measure of stroke
volume) with heart rate and FBF was measured using strain gauge plethysmography as described previously. Mean arterial pressure (MAP) was calculated from the formula systolic BP + twice diastolic BP divided by three. Vascular resistance was calculated by dividing MAP by blood flow assuming zero venous pressure.

The measurement of basal SMABF was repeated under identical conditions in six controls and five patients with autonomic failure at an interval of three months to compare the long term reproducibility of SMABF measurements in controls and patients with autonomic failure.

**STATISTICS:**
Statistical analysis were carried out using a standard version Minitab data analysis software (Minitab, Inc, 1989). Blood flow (SMABF, FBF), BP, HR and CI values before and during each stimulus were compared by paired t test and \( P < 0.05 \) was considered significant. Changes in BP, SMABF and SMAVR between controls and patients and mean baseline SMABF values preceeding each test, were compared by analysis of variance (ANOVA). Within subject coefficients of variation of SMABF measurement were obtained by dividing the standard deviation by the mean and was then expressed as the mean of coefficients of variation.

Correlation of measurements during initial and repeat studies were examined using linear regression analysis.

Data are presented as means ± s.e.m.

**RESULTS:**

**MBP:** In the controls, during MA, BP rose in 6 subjects and was unchanged in 4, with no significant change in mean BP during MA (94.6±4.4 to 101.1±4.3, \( P = 0.11 \)). (Fig 6.1) BP rose during CC (91±3 to 102±3 mmHg) and ISE (89±4 to 109±8)(each \( P < 0.05 \)). BP was maintained during tilt at 2 min (89±2 to 93±3) and 10 min (89±2 to 93±3)(Fig 6.1).
In the PAF patients, BP was unchanged during MA (112.1±6.2 to 117.1±6.1), CC (113.8±6.3 to 120.7±4.8) and ISE (112.2±5.7 to 114.7±4.3)(each P=NS). BP however fell markedly during tilt at 2 min (111.2±5.3 to 68.6±7.4) and 10 min (111.2±5.3 to 63.8±4.5)(each P<0.01).(Fig 6.1)

In the MSA patients, BP was also unchanged during MA (107.8±7.9 to 104.8±7.1), CC (104±7.1 to 113.7±8.9) and ISE (109.4±9.5 to 110.8±8.4)(each P=NS). BP fell during tilt at 2min (110±9.9 to 77.7±3.1) and 10 min (110±9.9 to 79.1±3.8)(each P<0.01).

When compared by ANOVA, in controls, the rise in BP during CC (14±1%) and ISE (21±3%) but not during MA (6.9±1.6%), was significantly higher (each P<0.01) compared to PAF or MSA. The fall in BP during head-up tilt in PAF (38.5±5.5%) and MSA (27.3±5.8%) was however, markedly higher compared to controls (each P<0.001, Fig. 6.1)

**SMABF:** In the controls, mean resting SMABF was 439±61 ml/min (range 370-769). There was a non-significant reduction in SMABF during MA (439±61 to 318±54, P=NS, paired t test) though when compared by ANOVA, percentage change in SMABF was significantly higher than in the patients. The SMABF however fell during CC (462±50 to 289±40 ml/min), ISE (511±49 to 295±36) and tilt at 2 min (483±57 to 271±40) and 10 min (483±57 to 266±41)(each P<0.005).(Fig 6.2)

In the patients with PAF, the mean resting SMABF was not significantly higher (546±42 ml/min, range 330-725) than controls or MSA. There were no significant changes in SMABF during MA (546±42 to 444±36), CC (592±41 to 529±56) and ISE (606±43 to 492±52). During tilt there were no significant changes in SMABF either at 2 min (585±47 to 491±57) or 10 min (585±47 to 476±57)(each P=NS) though there was a marked fall in BP.(Fig 6.2)

In the MSA patients, resting SMABF was 456±73 ml/min (range 260-696). There were no significant changes in SMABF during MA (456±73 to 461±89),
CC (447±68 to 455±82), ISE (454±64 to 380±53), tilt at 2 min (375±59 to 300±45) and 10 min (375±59 to 308±58) (each P=NS). (Fig 6.2)

In the controls, rise in the calculated SMAVR was non-significant during MA (0.23±0.03 to 0.39±0.09 units, P=NS, paired t test). The SMAVR rose during CC (0.21±0.02 to 0.42±0.07 units), ISE (0.19±0.03 to 0.47±0.12) and tilt at 2 min (0.2±0.02 to 0.4±0.06) and 10 min (0.2±0.02 to 0.4±0.07) (each P<0.05) indicating active constriction of the SMA during these stimuli. (Fig 6.3)

In the PAF and MSA patients, there were no changes in calculated SMAVR during MA, CC, ISE or tilt. (Fig 6.3)

Analysis of mean baseline SMABF values preceding each stimulus in controls, PAF and MSA patients using ANOVA test showed no significant difference, though in MSA patients pre tilt SMABF values tended to be lower.

Changes in SMABF and SMAVR during each stimulus in controls were significantly (P<0.01, ANOVA) higher when compared to the patients (Figs 6.2 & 6.3).

Within subject mean coefficients of variation of resting SMABF values were 8.3% in the controls and 13% in AF patients.

CI: In the controls, there were no significant changes in the CI during the pressor tests though CI was higher during ISE (617±48 to 771±60, P=NS). CI fell during tilt at 2 min (687±43 to 489±68) and 10 min (687±43 to 502±65) (each P<0.05). (Fig 6.4)

In the PAF and MSA patients, the CI remained unchanged during the pressor tests and head-up tilt, though values tended to be lower during tilt. (Fig 6.4)

HR: Changes in HR were not significant in the controls, the PAF or MSA patients during the various stimuli. HR tended to rise in controls during head-up tilt but this failed to reach statistical significance. (Table 6.2)

FBF: In the controls, changes in FBF were not significant during the pressor tests though there was a trend towards a higher FBF and lower FVR during
MA and ISE (Figs. 6.5 & 6.6). In the controls, the FBF fell during tilt at 2 min
(2.7±0.3 to 1.4±0.3 ml/100ml/min, P<0.05)(Fig 6.5) with a corresponding
rise in FVR (41.8±8 to 83.3±17 units, P<0.05)(Fig 6.6) indicating an active
forearm vasoconstrictor response.

In the PAF and MSA patients, changes in FBF and FVR during the pressor
tests were not significant. FBF fell during tilt (2.13±0.34 to 1.11±0.1
ml/100ml/min in PAF and 2.7±0.3 to 1.3±0.19 in MSA, each P<0.05)(Fig
6.5) but without a corresponding significant rise in the calculated FVR
(55.1±4.5 to 64±7.6 units in PAF and 42±4.9 to 58±9 in MSA, each
P=NS)(Fig 6.6).

Measurements of baseline SMABF made on two occasions at an interval of
three months in six controls and five AF patients showed a close correlation
(r=+0.85, Fig 6.7).
Fig 6.1. Changes in mean arterial blood pressure (MABP) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and tilt at 2 min (T2) and 10 min (T10) in controls, pure autonomic failure (PAF) and multiple system atrophy (MSA) patients.

■ = P<0.05 vs Pre
* = P<0.01 vs PAF and MSA
** = P<0.001 vs controls
Fig 6.2. Changes in superior mesenteric artery blood flow (SMABF) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and tilt at 2 min (T2) and 10 min (T10) in controls, pure autonomic failure (PAF) and multiple system atrophy (MSA) patients.

■ = P<0.05 vs Pre
* = P<0.01 vs PAF and MSA
Fig 6.3. Changes in superior mesenteric artery vascular resistance (SMAVR) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and tilt at 2 min (T2) and 10 min (T10) in controls, pure autonomic failure (PAF) and multiple system atrophy (MSA) patients.

- ■ = P<0.05 vs Pre
- * = P<0.01 vs PAF and MSA
Fig 6.4. Changes in cardiac index (CI) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and tilt at 2 min (T2) and 10 min (T10) in controls, pure autonomic failure (PAF) and multiple system atrophy (MSA) patients.

■ = P<0.05 vs Pre
Fig 6.5. Changes in forearm blood flow (FBF) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and tilt at 2 min (T2) in controls, pure autonomic failure (PAF) and multiple system atrophy (MSA) patients.

■ = P<0.05 vs Pre
Fig 6.6. Changes in forearm vascular resistance (FVR) before (P) and during mental arithmetic (MA), cutaneous cold (CC), isometric exercise (ISE) and tilt at 2 min (T2) in controls, pure autonomic failure (PAF) and multiple system atrophy (MSA) patients.

■ = P<0.05 vs Pre
Fig 6.7. Correlation of Residual Superior Mesenteric Artery Blood Flow on 2 occasions (1st and 2nd recording).

Controls = ▽
Autonomic Failure = ■

(20 subjects: 6 controls and 5 autonomic failure patients)
Table 6.2: Changes in heart rate (HR) before and during mental arithmetic, cutaneous cold (CC), isometric exercise (ISE) and tilt at 2 min (T2) and 10 min (T10) in pure autonomic failure (PAF) and multiple system atrophy (MSA) patients

<table>
<thead>
<tr>
<th></th>
<th>CONTROLS</th>
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<th>MSA</th>
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<td>BEFORE</td>
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<tr>
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DISCUSSION:

Consideration has already been given to the technique used to measure superior mesenteric artery blood flow in this study and therefore will not be discussed here. The pulsed Doppler ultrasound method has also been utilized to measure SMABF in disease states such as diabetes mellitus (Best et al, 1991); intestinal ischaemia (Nicholls et al, 1986) and cirrhosis of liver (Sato et al, 1987), but this is the first study using this method to study splanchnic vascular responses in patients with autonomic failure.

The superior mesenteric artery, as described previously, is one of 3 vessels contributing to the splanchnic arterial supply. It is however, a major vessel supplying the duodenum (except the superior part), the entire small intestine and the ascending colon (except the distal part). It is richly innervated by sympathetic vasoconstrictor nerves and also has an anatomical advantage for ultrasonic visualisation, as it is anteriorly placed and allows a lower angle of insonation when the sample volume is placed within the proximal portion of the SMA. Measurement of SMABF therefore provides a reasonable indication of haemodynamic change in this large vascular bed.

In this study, in controls (who were age matched and thus older than subjects studied in chapter 4), the resting SMABF values were slightly lower than values obtained in the younger subjects in the previous study and agrees with the observation that basal SMABF values are lower in older subjects. In PAF, the basal SMABF values were higher although not significant. The precise reason for this is unclear but it is possible that post-ganglionic sympathetic denervation, as occurs in PAF, could have resulted in reduced sympathetic vascular tone in the SMA and thus an increased SMABF. In MSA, the SMABF values were similar to controls although there was greater variability in the short term.

In our normal subjects, stimuli known to cause sympatho-neural activation, such as cutaneous cold, isometric exercise and head-up tilt, actively constricted
the superior mesenteric artery. This occurred to a lesser extent during mental arithmetic in controls, when there was an impaired elevation of BP during this particular stimulus, unlike during cutaneous cold and isometric exercise. This may have been due to reduced responses in some of our older controls and is consistent with previous data indicating a selective impairment of the pressor response to MA in older but not young healthy subjects; the reasons for this difference remain unclear (Ray-Chaudhuri et al, 1992 & Chapter 5). However, changes in mesenteric responses were still significantly greater in controls during MA in comparison to the patients. Constriction of the superior mesenteric artery occurred during the other pressor stimuli (CC and ISE) which also induced pressor effects rapidly; the constriction therefore was likely to have been mediated neurally. We could not however, definitely exclude humoral factors contributing to the constriction of the superior mesenteric artery, especially during head-up tilt in the controls. This is because sympathetic stimulation during head-up tilt releases various hormones and neuropeptides with vasoactive effects, the best examples being the associated elevation of angiotensin II and vasopressin (Granger et al, 1980), and probably endothelin (Kauffman et al, 1991) all of which are potent constrictors of the splanchnic vascular bed.

In both groups of AF patients (PAF and MSA), there were no changes in BP or SMABF during the pressor tests. Furthermore, during head-up tilt, BP fell markedly although there were no significant changes in SMABF or SMAVR. The abnormal splanchnic haemodynamic responses in these patients were most likely due to lack of vasoconstrictor nerve activity, though other possibilities may exist. In our older patients, failure of constriction of the SMA may have been due to more rigid vessels, as may occur with aging. This is unlikely, however, as there was adequate constriction in age matched controls and previous studies in such patients have excluded stiff "fixed" vessels, as they are capable of the reverse, dilatation, after food ingestion (Kooner et al, 1989).
Abnormal humoral responses (renin-angiotensin, vasopressin and endothelin) to sympathetic stimulation during head-up tilt remain as other possibilities. Renin levels may rise in some patients with AF during head-up tilt (Mathias et al, 1977) but not in other patients (Bannister et al, 1977). Vasopressin levels also rise during head-up tilt in PAF patients (Zerbe et al, 1983) but this does not occur in MSA patients (Puritz et al, 1983). Constriction of the superior mesenteric artery during head-up tilt did not occur in either PAF or MSA, making it unlikely that lack of vasopressin alone played a singular role in the abnormal splanchnic responses.

Kauffmann et al (1991) showed that plasma endothelin rose in normal subjects during tilt but not in primary autonomic failure. They postulated that impaired baroreceptor reflex activation results in an absence of rise in endothelin levels in primary autonomic failure and may contribute to orthostatic hypotension. However, diabetics who may develop postural hypotension due to secondary autonomic failure, have been shown to have increased plasma levels of endothelin-1 (Takahashi et al, 1990). Thus, the role of endothelin in causing systemic vasoconstriction to maintain blood pressure is yet uncertain and therefore, the contribution of lack of endothelin in the pathogenesis of postural hypotension in primary autonomic failure remains to be established. The haemodynamic abnormalities, especially in relation to superior mesenteric artery constriction in these patients, therefore, were probably and predominantly the result of sympatho-neural failure.

In this study, there were no changes in FBF in patients with AF except during head-up tilt when there was vasoconstriction in the forearm vascular bed similar to controls. However, as explained previously this may not be an indicator of generalised vasoconstriction within the skeletal muscles particularly during head-up tilt when differential responses between forearm and calf vascular beds have been reported (Duprez et al, 1987). The results of this study thus suggest that during the stimuli we used, responses in the
splanchnic region may play a greater role in overall BP regulation than those in other regional vascular beds, such as in skeletal muscle.

However, apart from forearm blood flow, only certain aspects of cardiac function was measured, and it is not known what happened in other major vascular beds such as the renal and cerebral. It was unlikely however, that they could account for the overall BP responses during the stimuli used in normal man or the lack of response in autonomic failure. The renal vascular bed has a strong autoregulatory mechanism as demonstrated in conscious animals during low cardiac output states and hypotension (Vatner & Braunwald, 1975). In the conscious state, surgical or pharmacological renal denervation does not affect renal blood flow or renal vascular resistance (DiBona & Wilcox, 1992). In autonomic failure patients, renal blood flow and the glomerular filtration rate are maintained in spite of reduction of blood pressure by step-wise head-up tilt till the lower limits of autoregulation (60-70 mmHg) are reached (DiBona & Wilcox, 1992). Cerebral autoregulation (over a range of perfusion pressures from 70-140 mmHg) similarly normally ensures a preservation of oxygenation despite changing perfusion pressure during controlled haemorrhage (Lassen, 1974; Owman, 1986). Brooks et al (1989) studied ten patients with primary autonomic failure and demonstrated a preserved cerebral autoregulation during head-up tilt. Similar findings have also been reported by Thomas and Bannister (1980) previously.

Although our study was not directed towards determining the role of individual vascular beds in BP control, it suggests that during postural change, for instance, the level of constriction of the superior mesenteric artery is a major contributor to maintenance or fall of BP. Thus during head-up tilt, lack of constriction of the SMA as observed in the AF patients possibly played a major part in the pathogenesis of postural hypotension. This may have therapeutic implications, as drugs which have selective constrictor effects on this vascular bed may have a greater therapeutic value in preventing postural hypotension in
autonomic failure patients. This major neural defect in splanchnic control may explain why postural change after food ingestion considerably enhances symptoms of postural hypotension in some patients with autonomic failure (Mathias et al, 1991).

CONCLUSIONS:
It is concluded from this study that in normal man sympatho-neural activation by pressor stimuli which raise blood pressure, or head-up tilt which maintains blood pressure, causes active constriction of the superior mesenteric artery. This does not occur in patients with sympathetic failure who are not capable of reflex sympathetic vasoconstriction. The lack of constriction of the superior mesenteric artery probably contributes to the severe postural hypotension seen in these patients. This study indicates the importance of the integrity of sympathetic pathways in the neural control of the splanchnic vascular bed and its role in overall blood pressure regulation, especially during postural change.
CHAPTER 7:
SUPERIOR MESENTERIC ARTERY BLOOD FLOW AND NEUROHORMONAL RESPONSES TO THE CENTRALLY ACTING SYMPATHOLYTIC DRUG CLONIDINE. STUDIES IN NORMAL SUBJECTS AND PATIENTS WITH PRIMARY AUTONOMIC FAILURE.
INTRODUCTION:

CLONIDINE:

Structure: Clonidine is an imidazoline compound which is structurally related to tolazoline (an α adrenoline receptor blocker), naphazoline (a sympathomimetic agent) and antazoline (H₁ receptor blocker).

Mode of action: Clonidine is an α₂-adrenoreceptor agonist which was first marketed in 1966, in Germany, as an antihypertensive drug and its potent hypotensive action was documented by Knobloch & Morr (1966). Studies in animals and man have indicated that the hypotensive action of clonidine is mediated principally by a central reduction in sympathetic tone to the blood vessels and the heart via stimulation of α₂ adrenoreceptors in the pontomedullary region (Schmitt, 1977; Reid et al, 1977) and a facilitation of the vagal baroreceptor reflex (Gills et al, 1985). Besides the central α₂ adrenoreceptor agonist action, the antihypertensive action of clonidine has also been implicated in the activation of central imidazoline receptors (van Zweiten, 1991). There is also a peripheral component to the antihypertensive action of clonidine, this being the stimulation of presynaptic α₂ adrenoreceptors on the sympathetic nerve terminals (Brown & Harland, 1984). The central sympatholytic action of clonidine has been successfully used as a neuropharmacological probe to evaluate the sympathetic neural control of circulation in patients with tetraplegia (Reid et al, 1977; Mathias et al, 1979),
autonomic failure (Kooner et al, 1989) and renovascular hypertension (Mathias et al, 1983).

Besides its antihypertensive action, clonidine also has neurohormonal effects mediated by its central and peripheral sites of action. The central action principally involves the release of growth hormone and has been shown to occur in animals (Chambers & Brown, 1976) and in normal man (Martin, 1979; Mathias & Bannister, 1992). The site of action is thought to be the hypothalamic α2 adrenoreceptors, as the release of growth hormone by clonidine can be blocked by the α2 adrenoreceptor antagonist, yohimbine (Mazza et al, 1990). Clonidine also causes a reduction in stimulation evoked release of noradrenaline acting on the α2 adrenoreceptors in tissues (Langer, 1981) and inhibits release of noradrenaline induced by sympathetic activation, in pithed rats (Katalin et al, 1983). In humans, clonidine has been shown to lower plasma noradrenaline levels in normal subjects (Reid, 1981) and in hypertensive subjects (Kooner et al, 1988). Other hormonal effects of clonidine include lowering of plasma insulin levels (Barbieri et al, 1980) and increased levels of plasma glucose which may be dependent on a combination of central and peripheral action of clonidine (May et al, 1990).

The main side-effects of clonidine include dryness of mouth and sedation.

A previous study, infusing clonidine in patients with primary autonomic failure indicated that clonidine lowered blood pressure in patients with multiple system atrophy (MSA) but not in pure autonomic failure (PAF), despite a similar degree of cardiovascular reflex impairment in each group (Kooner et al, 1989). The overall haemodynamic and hormonal changes in MSA and PAF after clonidine were briefly described in this study, but the changes in the large splanchnic circulation were not described. In the present study, the haemodynamic mechanisms responsible for the differential responses to
clonidine in two groups of patients with primary autonomic failure, MSA and PAF have been investigated, with a particular emphasis on superior mesenteric artery blood flow. The aim of this study was to evaluate the splanchnic vascular responses after sympato-inhibition by clonidine in primary autonomic failure. Comparisons were made in age matched healthy controls. This study is divided in two sections. In the first, the systemic, splanchnic and other regional haemodynamic responses to clonidine will be described. In the second, the neurohormonal responses with an emphasis on growth hormone responses to clonidine, will be discussed.

**STUDY 1: THE SYSTEMIC AND REGIONAL HAEMODYNAMIC RESPONSES TO CLONIDINE.**

**MATERIAL AND METHODS:**

Ten patients with pure autonomic failure (PAF, 4 males, 6 females) aged between 45 and 72 years (mean 53 years) and ten patients with multiple system atrophy (MSA, 4 males, 6 females) aged between 46 and 69 years (mean 51 years) were studied in an identical manner. Known causes of secondary autonomic dysfunction such as diabetes mellitus and amyloidosis were excluded. All had severe postural hypotension and the diagnosis was confirmed on the basis of physiological, biochemical and pharmacological tests as described previously (Table 7.1). Drugs, which mainly consisted of fludrocortisone 0.1 mg only (none were on L-dopa) were stopped 4 days prior to the study. Fifteen healthy controls (7 males and 8 females) aged between 46 and 70 years (mean 52 years), none of whom were on any medication, were also studied in an identical manner. The study had ethical approval from St. Mary's Hospital and the National Hospital for Neurology and Neurosurgery, Queen Square London.

All subjects were studied after an overnight fast at 09:00 hours in a temperature controlled room (24°C). A 30 min rest period in the supine position was allowed initially for familiarisation with equipment and
techniques. A suitable forearm vein (usually in the antecubital fossa) was selected and a canula (Abbocath, 18G) was inserted under local anaesthesia (2% lignocaine) for infusion of clonidine.

The following measurements were made:

1. **Blood Pressure and Heart Rate (BP & HR):** by automated sphygmomanometry (Sentron), before and every five min after clonidine infusion.

2. **Stroke Distance and Cardiac Index (SD & CI):** by continuous wave Doppler ultrasound (Exerdop), before and every 15 min after clonidine infusion.

3. **Forearm Blood Flow (FBF):** by venous occlusion strain gauge plethysmography, before and every 15 min after clonidine infusion.

4. **Superior Mesenteric Artery Blood Flow (SMABF):** by pulsed Doppler ultrasound flowmetry (Acuson, 128), before and every 15 min after clonidine infusion.

5. **Digital skin blood flow (DSBF):** by laser Doppler flowmetry (Periflux PF2B, Perimed, Sweden), before and monitored continuously throughout the study period.

All measurements have been previously described except measurement of DSBF which will be considered here.

Digital skin blood flow was measured using a narrow, monochromatic (red) laser light source which is led to the skin surface by an optical fibre to the probe held in place by a plastic probe holder. The incident light source reaches the skin capillaries and red blood cells at a variety of different angles because of the random arrangement of the capillary loops. The light is thus repeatedly reflected and refracted and gradually absorbed. The multiple scattering of light produces a volume of isotopic illumination in front of the probe head which is a hemisphere with a radius of about 1 mm. The red blood cells moving in this illuminated volume are struck by the light and reflect it with alteration in frequency in the light (Doppler shift). The reflected frequency
shifted light is returned from the moving red blood cells in the microvasculature to the instrument by efferent optical fibres arranged in a parallel fashion to the afferent fibres which carry the light of the instrument. The noise resulting from variations in the light signal is reduced by dual efferent light guides (Nilsson et al, 1980). This modification serves to reduce the signals originating from the stationary reflection sites, while the Doppler shifted signals are amplified. The frequency shift of the light is detected by photodetectors, which produce a voltage signal directly proportional to the quantity of blood flow (velocity and number of red blood cells) in the superficial skin microvascular network. The Doppler shifted signals correspond to an average velocity obtained under an average angle. As the signal is a product of the number of red blood cells moving in the sample volume and the mean velocity of the moving particles and thus is neither velocity nor flow, the term blood cell flux has been suggested (Stern et al, 1977). Thus,

\[ \text{Flux} = \text{Red cell volume fraction} \times \text{velocity} \]

A good correlation of this form of measurement of blood flow from the finger tip with \(^{133}\text{Xenon washout studies in normal subjects exposed to ultraviolet induced local hyperaemia was found by Stern et al (1977).}\\

This method has been clinically applied to measure laser Doppler flux in plastic surgery, to assess vascular changes in the skin after needle trauma (Holloway, 1980), during tests of autonomic function (Low et al, 1983) and in the investigation of cutaneous vascular responses to warming in the diabetic foot (Stevens et al, 1991).

Measurements were then made before and 15, 30, 45 and 60 min after clonidine hydrochloride (Catapres, Boehringer-Ingelheim, 2 \( \mu \text{g/kg body weight} \) diluted to 20 mls with normal saline, infused intravenously over 10 min).

Superior mesenteric artery vascular resistance (SMAVR), forearm vascular resistance (FVR), digital skin vascular resistance (DSVR) and mean arterial blood pressure (MABP) were calculated as described previously.
STATISTICS:
Changes in SMABF are expressed as percent changes from baseline values. Statistical analysis was performed using students paired and unpaired t tests for absolute values and analysis of variance (ANOVA). P<0.05 was considered significant and data are presented as means ± s.e.m.

RESULTS:
MBP and HR: In the controls, supine MABP was 91±2.1 mmHg and fell to 80±2.5, 77±2.1, 76±2.5 and 76±2.3 at 15, 30, 45 and 60 min after clonidine respectively (each P<0.05, Fig 7.1). In the MSA patients, supine MABP was 111±4.8 mmHg and after clonidine fell to 92±5.1, 84±4.8, 86±4.6 and 86±5.4 at 15, 30, 45 and 60 min respectively (each P<0.05, Fig 7.1).
In the PAF patients, supine MABP was higher (113±6.5mmHg) than in the normal subjects (91±2.1mmHg) and remained unchanged after clonidine (Fig 7.1). Changes in HR after clonidine in controls, MSA and PAF patients were not significant (Table 7.2).
SMABF and SMAVR: In the controls, resting SMABF was 445±35 ml/min and rose by a maximum of 22.5±5% (445±35 to 538±41 at 30 min) after clonidine. In MSA, resting SMABF was 433±52 ml/min (similar to controls) and rose by a maximum of 38.8±14% (433±52 to 574±68 at 30 min) after clonidine (Figs 7.2 & 7.3). Resting SMABF was higher in PAF patients (601±62 ml/min) compared to MSA and controls (P<0.05) and was unchanged after clonidine (601±62 to 630±60, 4.5%; P=NS). Changes in SMABF in controls and MSA were significantly different from those in PAF (P<0.05)(Fig 7.3).
There was a corresponding fall in SMAVR after clonidine by 30.6±3.3% (0.22±0.02 to 0.15±0.02 units at 45 min) in controls and by 43±6.3% (3±0.05
to 0.17±0.03 at 45 min) in MSA (each P<0.05, Figs 7.2 & 7.3). SMAVR remained unchanged in PAF after clonidine (10.6±5.3%, 0.21±0.03 to 0.18±0.03, P=NS, Figs 7.2 & 7.3). There was a significant difference between the percentage change of SMAVR after clonidine at 30 min when controls and MSA patients were compared with PAF patients (each P < 0.05) (Fig 7.3).

**FBF and FVR:** In controls and MSA after clonidine, FBF fell, but without a rise in calculated FVR (Table 7.3). In PAF there was no change in FBF or FVR after clonidine (Table 7.3).

**DSBF and DSVR:** There was a trend towards a higher skin digital blood flow after clonidine at 30 min in controls (44±4 to 56±4 arbitrary units) and at 45 min in MSA (43±9 to 54±11 arbitrary units) (each P=NS) with a corresponding fall in skin digital vascular resistance (Table 7.3). Skin digital blood flow and skin digital vascular resistance were unchanged after clonidine in PAF (Table 7.3).

**CI:** In controls after clonidine, cardiac index did not fall significantly (775±81 to 645±66 units at 30 min, P=NS), but it fell in MSA patients at 15 min (709±47 to 577±43 units), 30 min (709±47 to 560±47) and 45 min (709±47 to 543±45) (each P < 0.05, Table 7.2). CI remained unchanged in PAF patients after clonidine (Table 7.2).
Table 7.1 Results of a series of autonomic function tests and plasma noradrenaline (NA) levels in 10 patients with pure autonomic failure (PAF) and 10 patients with multiple system atrophy (MSA). SA = Sinus arrhythmia, HV = Hyperventilation, Valsalva = Valsalva manoeuvre, BP = Blood pressure, HR = Heart rate, A=Abnormal and N=Normal.

<table>
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<th>Tilt BP/HR</th>
<th>Valsalva BP/HR</th>
<th>Pressor Tests BP</th>
<th>SA HR</th>
<th>HV HR</th>
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* In normal subjects, plasma noradrenaline levels under similar circumstances are 300±40 pg/ml whilst supine, and 500±65 pg/ml during head up tilt.
FIG 7.1: Changes in mean arterial blood pressure (MABP) before (0) and after clonidine in controls (C), multiple system atrophy (MSA) and pure autonomic failure (PAF) patients.
FIG 7.2: Changes in superior mesenteric artery blood flow (SMABF) and superior mesenteric artery vascular resistance (SMAVR) before (0) and after clonidine in controls (C), multiple system atrophy (MSA) and pure autonomic failure (PAF) patients. The fall in SMAVR was significant (P<0.05) in C and MSA at 30 and 45 min, with no change in PAF. *= P <0.05
FIG 7.3: Percentage change in superior mesenteric artery blood flow (SMABF) and superior mesenteric artery vascular resistance (SMAVR) before (0) and after clonidine in controls (C), multiple system atrophy (MSA) and pure autonomic failure (PAF) patients.

* = P<0.05, MSA vs PAF and C vs PAF
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<td>69.5 ± 2.6</td>
<td>66.1 ± 2.4</td>
<td>64.8 ± 2.8</td>
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<td>66.6 ± 2.6</td>
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<td><strong>DBP</strong></td>
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<tr>
<td>65.9 ± 3.5</td>
<td>73.5 ± 4.4</td>
<td>74.8 ± 4.4</td>
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<tr>
<td>66.3 ± 2.1</td>
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<tr>
<td><strong>SBP</strong></td>
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<td>183.4 ± 5.6</td>
<td>172.5 ± 12</td>
<td>126.5 ± 12</td>
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<tr>
<td>161.8 ± 11.1</td>
<td>115.9 ± 7.8</td>
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<td>120.1 ± 11.0</td>
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Table 7.2: Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), and arterial index (AI) before (0) and after (60 min) CCB.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>PAF</th>
<th>MSA</th>
<th>(unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.55 ± 0.32</td>
<td>3.08 ± 0.33</td>
<td>(mmHg)</td>
</tr>
<tr>
<td>15</td>
<td>2.09 ± 0.28</td>
<td>2.42 ± 0.29</td>
<td>(mmHg)</td>
</tr>
<tr>
<td>30</td>
<td>2.25 ± 0.26</td>
<td>2.23 ± 0.29</td>
<td>(mmHg)</td>
</tr>
<tr>
<td>45</td>
<td>2.25 ± 0.26</td>
<td>2.23 ± 0.29</td>
<td>(mmHg)</td>
</tr>
<tr>
<td>60</td>
<td>2.25 ± 0.26</td>
<td>2.23 ± 0.29</td>
<td>(mmHg)</td>
</tr>
</tbody>
</table>

*Table 7.3: Changes in forearm blood flow (FBF) forearm vasoconstriction and duplicate systolic blood pressure (PSA) and pure sphygmomanometric fallure (PAF) fallure.

Reference: Reference (PS/A) before (g) and after administration in control.

Reference: Resistance (PAF) before (g) and after administration in control.

Reference: Reference (PAF), digital skin blood flow (PSA) and digital skin
DISCUSSION:

These studies confirm that in patients with chronic primary autonomic failure, clonidine causes differential responses in blood pressure. Like controls, there is a reduction in blood pressure in MSA after clonidine, which differs from the lack of fall in blood pressure in PAF. Both the MSA and the PAF patients had clear evidence of sympathetic denervation, as based on a combination of physiological tests and on the impaired response in plasma noradrenaline levels to head up tilt, when plasma noradrenaline failed to rise although their blood pressure fell. There were however differences in the basal levels of noradrenaline between the groups, these being within the normal range in the majority with MSA, while this was the reverse in PAF, in whom the levels were subnormal. As discussed previously, this favours sympathetic failure caused by a central lesion in MSA, and a more peripheral lesion in PAF.

The hypotensive action of clonidine, as based on studies in animals (Kobinger, 1977) and in tetraplegic and normal man (Mathias et al, 1977; Reid et al, 1977), is due mainly to a central action. The proposed central nervous system sites for antihypertensive action of clonidine include the hypothalamus (anterior preoptic region, posterior hypothalamus), locus coeruleus, and the brainstem (paramedial depressor region, nucleus tractus solitarius, nucleus reticularis lateralis, gigantocellularis and rostroventrolateralis (Gillis et al, 1985; Jarrott et al, 1987). It is possible that the brainstem area was spared in MSA patients allowing clonidine to act on the vasomotor centres causing a fall in blood pressure. In PAF, however, there was peripheral post-ganglionic sympathetic denervation with a lower sympathetic tone as indicated by the low plasma noradrenaline levels. It is likely therefore, that clonidine failed to lower sympathetic tone and thus blood pressure further in PAF, owing to peripheral sympathetic denervation and a prevailing low sympathetic tone. Indeed, Robertson et al (1983) described a pressor effect of clonidine (given orally) in four patients with presumed PAF and thought that this could be related to
postjunctional $\alpha_2$ receptor stimulation by oral clonidine. In this study, a rise in blood pressure after clonidine in PAF patients was not seen. The differences may be attributed to the differences in the mode of administration and dosage of clonidine and the number of patients in the present study and that carried out by Robertson et al (1983).

Blood pressure falls during sleep and it may be argued that the fall in blood pressure in the controls and the MSA patients was partly mediated by clonidine induced sedation. Hossmann et al (1980) reported a similar degree of sedation and hypotension after oral nitrazepam and clonidine in five healthy subjects. However, Kooner et al (1989d) showed that in hypertensive subjects, 10 mg of nitrazepam which caused a similar degree of sedation to clonidine, confirmed by clinical observation and a visual analogue scale, did not lower blood pressure or plasma noradrenaline levels. This suggested that a substantial and prolonged hypotension after clonidine was unlikely to be due to its sedative effects. In this study, a similar degree of sedation was observed in all subjects and was restricted to short lasting drowsiness. Moreover, in PAF patients, despite of a similar degree of drowsiness after clonidine, blood pressure did not fall, making it unlikely that the hypotension after clonidine was secondary to its sedative action.

Kooner et al (1989c) showed that in MSA and PAF patients after clonidine, despite measurements of cardiac function and flow in a number of peripheral vascular regions such as the skin and the forearm muscle, there were no clear haemodynamic explanations for the differential pressor response. This raised the possibility of differential effects of clonidine upon a major vascular bed, such as the splanchnic region. This has been difficult to evaluate in the past owing to the need for invasive techniques available for measurement of splanchnic blood flow. The utilisation of Doppler techniques for the measurement of SMABF, enabled us to make rapid and repeated
measurements in the SMA, richly innervated by the sympathetic nervous system.

Using this technique, the basal SMABF levels in controls were comparable to basal levels in the studies described in the previous chapters although the values were slightly lower than those in younger subjects as described in chapter 4. This could be due to the relatively older age of the controls in this study. The basal levels of SMABF in MSA were similar to those in controls; levels in PAF, however were higher. The precise reasons for this are unclear. A greater degree of peripheral denervation in PAF may have contributed. Circulating levels of various vasoactive peptides, neuropeptides and neurohormones, are known to be different in PAF as compared with MSA (Polinsky, 1992), and it is possible this also may have contributed to the higher basal SMABF in PAF patients. The calculated SMAVR however was similar in PAF and controls, but considerably higher in MSA. The precise reason for this is unclear and it may reflect a tendency in MSA patients to greater constriction within this vascular bed.

After clonidine, SMABF rose in the controls and in MSA, but not in PAF. Reciprocal changes were reflected in calculated SMA vascular resistance. In controls this suggests reduction of neural sympathetic tone to this vascular bed and a resultant increase in splanchnic blood flow. The response to clonidine in MSA suggested that their basal sympathetic outflow was within normal limits. However, they had impaired autonomic responses to a range of stimuli which are dependent on the integrity of sympathetic pathways, and the apparently "normal" basal tone may indicate partial preservation of sympathetic pathways, particularly in the presence of pressor supersensitivity (Mathias et al, 1985). It is unlikely that these effects of clonidine were secondary to changes in vasoactive peptides. In controls, iv clonidine has no effect on vasopressin and either elevates (Mathias et al, 1985) or causes no change in renin and thus angiotensin II levels (Kooner et al, 1991). The latter, a rise in plasma
angiotensin II levels, should constrict the SMA, rather than dilate it. After clonidine, the dilatation in the SMA coincided with the substantial fall in blood pressure in both MSA and controls.

In PAF, however, after clonidine, there were no changes in SMABF or in SMAVR. This is consistent with peripheral sympathetic denervation and the inability of clonidine to further reduce low sympathoneural tone as discussed previously. In PAF, the SMA may have been dilated maximally before clonidine, and further dilatation therefore was not possible. It was unlikely that an inter-relationship with humoral factors, such as an abnormal level of vasoactive substances, was responsible, although this cannot be entirely excluded. The lack of change in this major vascular bed in PAF was associated with an absent depressor response (lack of fall of blood pressure) to clonidine, as distinct from MSA.

Although previously described, the changes in cardiac function and in other regional beds bear mention, particularly in relation to the differences in the two subgroups. In neither group, as in the controls, was there a significant change in heart rate. Previous studies, in normal subjects, have indicated that changes in heart rate after clonidine may be inconsistent, with a fall in heart rate in some (Hossman et al, 1980) and remaining unchanged in others (Kooner et al, 1991; Macphee et al, 1992). Cardiac index however, fell in MSA and to a lesser extent in the controls; in MSA patients this suggests a contribution of residual sympathoneural activity to cardiac function. This did not occur in PAF. In controls, and neither MSA or PAF, there were changes indicating active responses in the forearm muscle vasculature, excluding significant effects upon the forearm and possibly skeletal muscle bed. The forearm blood flow is a resultant of muscle and skin blood flow, and the lack of change in forearm vascular resistance after clonidine in normal subjects has been previously recorded (Kooner et al, 1991). This may also reflect the fact that forearm
blood flow is not predominantly controlled by sympathetic vasoconstrictor
nerves.

DSBF tended to rise with a trend towards a lower vascular resistance (although
not significant) in MSA and controls; DSBF was unchanged in PAF. Unlike
the forearm blood flow, digital skin blood flow is mainly controlled by
sympathetic vasoconstrictor nerves and the trend towards a rise in DSBF in
controls thus reflects a reduction in sympathetic vasoconstrictor tone which has
also been noted before (Kooner et al, 1991). In MSA, a similar trend towards a
rise in DSBF and fall in DSVR possibly reflects a partially preserved
postganglionic sympathetic pathway, as also indicated by a fall in blood
pressure after clonidine in these patients. In PAF, however, a lack of change in
DSBF or DSVR is in keeping with the peripheral sympathetic denervation and
the inability to lower blood pressure after clonidine in these patients.

The implications of this study in relation to the mode of action of clonidine and
its relevance to the understanding of the abnormalities in the two groups with
chronic primary autonomic failure will now be discussed. Firstly they suggest
that a major component of the haemodynamic effects of clonidine contributing
to systemic blood pressure changes may be through the splanchnic circulation.
This may explain why there have been few changes observed in other vascular
beds, particularly those in the periphery and in skeletal muscle. Secondly, they
indicate that pressor responses may be dependent on the splanchnic vascular
bed and support an important role in the reverse situation, postural
hypotension, as suggested by previous studies described in chapter 6 (Ray-
Chaudhuri et al, 1992). Finally they emphasize the differences between the two
groups with autonomic failure, despite the similarity in postural hypotension
and response to physiological tests designed to determine the degree of
sympathetic failure. Distinguishing these groups may be of relevance to clinical
management. It has been previously suggested that clonidine may raise blood
pressure and reduce postural hypotension in autonomic failure (Robertson et
al, 1983) but these benefits could not be confirmed in this study. These observations were however, made in PAF, who may have exhibited a supersensitive pressor response to clonidine.

CONCLUSION:
This study confirms the differential pressor responses to clonidine in the two major groups with primary autonomic failure, MSA and PAF. These observations are not satisfactorily explained by changes in cardiac function and in peripheral vascular changes, and are likely to be largely dependent upon differential responses within the splanchnic vascular bed.
STUDY 2: THE GROWTH HORMONE RESPONSES TO CLONIDINE.

INTRODUCTION:
In this section the neurohormonal responses to clonidine in MSA, PAF and normal subjects will be discussed. It has been previously reported that clonidine failed to elevate growth hormone levels in primary autonomic failure (da Costa et al, 1984). However, this early study was conducted on a small number of patients, and it was not possible to differentiate between the two major groups (PAF and MSA) with primary autonomic failure. Distinction between the two groups (PAF and MSA) may be difficult, especially in the early stages, when patients with MSA may present with autonomic failure only or may need to be separated also from idiopathic Parkinson's disease. The effect of clonidine on growth hormone release in patients with well defined MSA and PAF was therefore studied to ascertain if the growth hormone response may serve as a neuroendocrine marker to distinguish between these two varieties of primary autonomic failure.

SUBJECTS AND METHODS:
Subjects were studied in an identical manner as in study 1. The indwelling venous cannula inserted into a forearm vein was used for infusing clonidine and for collecting blood samples for analysis. Blood collections were made 15 min before clonidine and at 15 min intervals thereafter for 60 min after 2 μg/Kg of clonidine (Catapres, Boehringer Ingelheim) in 20 ml N-saline was infused i.v. over 10 min. Measurements of plasma levels of catecholamines (noradrenaline, adrenaline, dopamine), glucose, insulin and growth hormone were made at 10, 15, 30, 45 and 60 min.

The i.v. cannula was kept patent with heparinized saline solution. Blood was collected in heparinized tubes, immediately centrifuged and plasma was stored at -20°C until assayed. Samples from each patient were assayed in the same run. Plasma noradrenaline, adrenaline and dopamine were measured by high performance liquid chromatography with an electrochemical detector, plasma
glucose levels were measured by glucose oxidase method using a chem Lab continuous flow autoanalyzer, plasma insulin by radioimmunoassay using RSL 125I Insulin kit (ICN Biomedicals, Inc.) and growth hormone by I-labelled growth hormone immunoradiometric assay (IRMA assay) by Kit (NETRIA; North East Thames Radioimmunoassay). The intra- and inter-assay variabilities were 4.3% and 4.7% for catecholamines, 3.2% and 4.8% for glucose, 2.7% and 5.4% for insulin and 3.1% and 7.4% for growth hormone, respectively.

The data are presented as means ± SEM. Statistical analysis was performed using Student's paired t test. Changes in growth hormone between controls, MSA and PAF patients were compared by analysis of variance (ANOVA, Minitab data analysis software, Inc. 1989). The level of significance was taken as P < 0.05.

RESULTS:
In controls and MSA, the basal plasma noradrenaline levels were not statistically different (1454±189 pmol/l and 2146±372 pmol/l, respectively, NS), and fell in each group after clonidine (to 922±118 pmol/l and 1005±331 pmol/l, P<0.05 each) (Table 7.4).

In PAF, plasma noradrenaline levels were substantially lower at rest (P<0.05), with a further fall after clonidine (Table 7.4).

Basal plasma levels of GH were similar in controls and and PAF and tended to be higher in MSA although non-significant (Fig 7.4). After clonidine, GH levels rose in controls, with peak levels at 60 min (14.4±5.7 mU/l; P<0.05). In PAF, peak GH levels also were reached at 60 min (15.4±5.7 mU/l; P<0.05). In MSA there was no rise in GH (Fig 7.4). Comparison of changes in GH levels between groups showed no difference between controls and PAF, but both differed from MSA (P<0.05 at 60 min).

Basal plasma glucose levels were similar in controls and patients, with a rise in each group after clonidine (Table 7.4).
There were no significant differences in insulin levels in the controls and patients, either before or after clonidine (Table 7.4).
Fig 7.4: Changes in plasma growth hormone levels before (0) and 15, 30, 45 and 60 min after clonidine in controls (C), multiple system atrophy (MSA) and pure autonomic failure patients
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
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<td>Glucose</td>
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<td>Dopamine</td>
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<td>Adrenaline</td>
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<td>Noradrenaline</td>
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<td>140</td>
<td>114</td>
<td>94</td>
<td>77</td>
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<td>PaP</td>
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<td>Glucose</td>
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<td>Noradrenaline</td>
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DISCUSSION.

This study indicates that after clonidine there is a rise in plasma GH levels in normal subjects and in PAF patients, but not in MSA patients. Plasma noradrenaline, adrenaline and dopamine responses to clonidine were similar in each group, there being a significant change in plasma noradrenaline levels only. Plasma noradrenaline levels fell mainly in controls and MSA as previously reported due to the sympatholytic action of clonidine. In PAF, the fall in plasma noradrenaline level was much smaller because of a preexisting low noradrenaline level. Plasma glucose and insulin responses were similar in both groups, glucose levels rising after clonidine in each group as has been previously reported (May et al, 1990). The main difference in hormonal responses to clonidine in PAF and MSA pointed towards a differential control of growth hormone release in these two groups and suggests a major difference in the central neural mechanisms responsible for GH release in the two groups with primary autonomic failure. This was not evident from the previous study (da Costa et al, 1984) where GH did not rise, favouring a central sympathetic degeneration as the reason for the impaired response. The majority of those patients, on retrospective analysis, fell into the MSA category.

In normal man, GH secretion in response to clonidine is dependent on its α2 adrenoreceptor action on the hypothalamus and is prevented by the alpha 2 adrenoceptor antagonist, Yohimbine (Mazza et al, 1990). Clonidine is presumed to release GH release hormone (GHRH), which then acts directly, or through other pathways, to stimulate GH release from the anterior pituitary (Mazza et al, 1990). There may be additional effects through suppression of somatostatin, which otherwise inhibits GH release (Alba Roth et al, 1989). Clonidine does not appear to directly stimulate somatotrophs in the anterior pituitary, as based on experimental and in vitro evidence (Alba Roth et al, 1989; Müller et al, 1988).
The major differences in GH responses to clonidine in the two groups with autonomic failure warrant consideration of various possibilities. Penetrability of clonidine into the central nervous system may have been lower in MSA, but seems unlikely, as it is a highly lipophilic substance which readily crosses the blood brain barrier (Reid, 1981). It also causes sedation which, although not quantified, was similar in all subjects. Furthermore, both blood pressure and plasma noradrenaline levels fell in MSA, as in the controls. These responses are recognised as being predominantly due to the central sympatholytic effects of clonidine (Reid et al, 1977; Kooner et al, 1991).

Older subjects may have impaired GH secretion (Rudman et al, 1981) with blunting of the response to clonidine, hence the close age matching of controls in this study. Furthermore, the PAF patients, in whom GH rose, were of a similar age and body weight.

GH responses are also impaired in endogenous depression; this is postulated to be the result of reduced central monoamine levels and is consistent with the ability of desmethylimipramine to reverse both depression and GH responses (Checkley et al, 1981). No formal depression score rating was performed in this study, but none of our patients was clinically depressed or was on antidepressant therapy.

It could be argued that sedation caused by clonidine may have confounded the rise in growth hormone level in controls and PAF. However, sedation was mild and short lasting in all subjects, most subjects being drowsy after clonidine and able to respond to commands. None had stage III, IV or REM sleep. Endogenous modifications of growth hormone release is known to occur after at least an hour of sleep (Reichlin, 1985). Furthermore, a similar degree of drowsiness was also observed in the MSA patients in whom growth hormone levels did not rise.

It seems likely that the impaired GH response to clonidine in MSA resulted from abnormalities in those neuronal systems and/or pathways from the
hypothalamus, concerned with secretion of GH. As discussed previously, in normal man, alpha adrenoceptor activation stimulates growth hormone releasing hormone, or suppresses somatostatin. The opiate and cholinergic systems, which are known to be linked with the noradrenergic system (May et al, 1990), may also influence GH release (Delitala et al, 1983). This study suggests that in PAF there is sparing of these central systems and pathways, unlike in MSA. This is consistent with previous data favouring lesions in the spinal cord and/or periphery in PAF, with lesions within the hypothalamus and brain stem in MSA (Polinsky, 1992). The present observations in these patients may provide a unique means of determining further which systems control GH release in man.

The GH response to clonidine, therefore, enables an unmasking of central neurological abnormalities in MSA and helps separate it from PAF. This could be of importance in the early stages of the disorders, when MSA patients may present with only autonomic failure. Furthermore, it may keep separate MSA patients from idiopathic Parkinson's disease (without autonomic failure), as both present with Parkinsonian features. Recent post-mortem evidence indicates that between 7 to 22% of patients considered to have Parkinson's disease may have MSA (Hughes et al, 1992). Distinction at an early stage is important, as in MSA there is a poor response to drug treatment, a wide range of complications may occur which increases their morbidity and contributes to mortality, and offers a less favourable prognosis.
CONCLUSIONS:
There are marked differences in the GH response to clonidine in PAF and MSA. Clonidine stimulates growth hormone release in PAF but not MSA. This test has advantages over others as it results from central adrenergic stimulation with end points independent of peripheral effects, such as blood pressure or plasma catecholamine levels (Gemmil et al, 1993). Clonidine is a safe drug and the test can be performed on an outpatient basis. The differences indicate that the GH response to clonidine may therefore serve as an objective neuroendocrine marker to separate patients with pure autonomic failure from those with parkinsonian and/or cerebellar features, as part of multiple system atrophy (Shy-Drager syndrome).
CHAPTER 8:
SYSTEMIC, SUPERIOR MESENTERIC ARTERY BLOOD FLOW AND
NEUROHORMONAL RESPONSES AFTER CLONIDINE IN
HYPERTENSIVE SUBJECTS
INTRODUCTION:

In this study, the splanchnic vascular responses during clonidine infusion are described in patients with essential hypertension.

Previous studies have indicated that a higher sympathetic nervous system activity may play a part in the pathogenesis of essential hypertension (Vlachakis et al, 1980; Floras, 1992; Egan et al, 1987). Increased sympathetic activity elevates cardiac output and total peripheral resistance (TPR) both of which can raise arterial blood pressure and previous studies have shown that a raised cardiac output and TPR occurs in hypertension (Overbeck et al, 1980). Regional vasoconstriction has been thought to be associated with systemic vasoconstriction to account for the rise in TPR in hypertensives. In hypertensive humans and animal models, vasoconstriction and a raised sympathetic nerve activity have been documented in the skeletal muscle (Anderson et al, 1989, Egan et al, 1987) and the renal vascular bed (Oparil, 1986).

The role of the neural control of the splanchnic vascular bed in the pathogenesis of hypertension was suspected as early as the 1930s. In 1937, Page and Heuer reported on splanchnic nerve section in patients with severe hypertension. In some patients this operation was effective presumably because of vasodilatation in this large vascular bed with a fall in peripheral vascular resistance. In patients with alcoholic cirrhosis clonidine reduces portal venous pressure and measurements of noradrenaline spillover indicate that this is due to a reduction in the sympathetic tone in this part of the vasculature (Willett et al, 1986). However, a limited number of studies have been carried out to document the changes in the splanchnic vascular bed in hypertension. Most studies have been carried out in animal models owing to the methodological problems of studying the splanchnic vascular changes in hypertensive humans. The animal model used most extensively, as a model for essential hypertension, has been the spontaneous hypertensive rat (SHR) of the Aoki-Okamoto strain.
Several studies in SHR have shown that there is reduced splanchnic blood flow and elevated splanchnic vascular resistance in the resting state (Ferrone et al, 1979; Tobia et al, 1974). Kong et al (1991) have recently showed supersensitivity of the mesenteric vascular bed of hypertensive Dahl salt sensitive rats to noradrenaline and periarterial nerve stimulation suggesting that vasoconstriction in this vascular bed may play an important part in the pathogenesis of hypertension.

Splanchnic haemodynamic changes in hypertensive humans remain poorly documented. Using invasive and indirect methods McGiff & Quilley (1981) showed a decreased splanchnic blood flow with a markedly elevated splanchnic vascular resistance in patients with renovascular hypertension. However, other studies using similar methods, indicated mild elevation of splanchnic vascular resistance and unchanged splanchnic blood flow in patients with essential hypertension (Messerli et al, 1975; Wilkins et al, 1952). Mathias et al (1983) used clonidine as a neuropharmacological probe to further evaluate the role of the sympathetic nervous system in patients with hypertension, particularly of renovascular origin. The overall, regional (renal, cardiac, skeletal muscular and cutaneous) and hormonal responses to clonidine in hypertensive subjects have been described (Reid et al 1981, Kooner et al, 1988). However, in hypertensive subjects, changes in the splanchnic vascular bed after sympathoinhibition by clonidine, are unknown.

In the present study, the changes in systemic, regional, in particular superior mesenteric artery blood flow and neurohormonal responses to clonidine infusion in 14 patients with essential hypertension are reported.

Comparisons were also made in age and sex matched healthy subjects (controls) in this placebo controlled study.
METHODS:
14 patients with essential hypertension (mean age 54 years, range 36-72, 10 male and four females) were studied. A primary cause for hypertension were excluded in all patients by a series of investigations. Investigations included serum electrolyte, glucose, renal and hepatic function profile, lipid profile, routine urinary examination and urinary VMA estimation, ECG, chest Xray, renal ultrasound scan, renal artery digital subtraction angiography and plasma renin activity when indicated. None was on medication at the time of the study. Other than left ventricular hypertrophy in some patients, there was no evidence of target organ damage. 14 healthy age and sex matched subjects (mean age 51, range 36-72 yrs) were also studied in an identical manner. All were studied after an overnight fast before each study day to exclude the established effect of food on superior mesenteric artery blood flow and to allow optimum ultrasound visualisation of the SMA (Moneta, et al, 1988). All were studied in a temperature controlled room (24°C) at 0900 hrs after a supine rest for 30 min to allow familiarisation with equipment and stabilisation. An indwelling venous cannula (Abbocath, 18G) was inserted into a forearm vein under local anaesthesia (2% lignocaine) for blood collection and infusion of clonidine. The intravenous cannula was kept patent with heparinized saline solution. Haemodynamic measurements were repeated in seven hypertensive patients and seven normal subjects before and after infusion of normal saline (placebo) under identical conditions on a separate occasion. The study was approved by the Ethical Committee of St. Mary's Hospital.

Measurements:
Measurements of blood pressure and heart rate (BP, HR, before and every five min following clonidine/placebo infusion), stroke distance (SD, before and every 15 min following clonidine/placebo infusion), forearm blood flow (FFB, before and every 15 min following clonidine/placebo infusion), digital (thumb) skin blood flow (DSBF, before and continuously during clonidine/placebo
infusion), index finger and body skin temperature by multiple site thermistors (Pan Labs Inc., Bothell, Washington placed on forehead, cheek, chest and index finger) and superior mesenteric artery blood flow (SMABF, before and every 15 min following clonidine/placebo infusion) were made during the study.

All methods are non-invasive and have been described in detail in the previous chapters.

Calculations:

Mean arterial blood pressure (MABP), Ascending aortic blood velocity and Stroke distance (SD), CI (relative cardiac output), FBF, DSBF and SMABF were calculated as described previously. Precautions regarding measurement of SMABF were taken as described previously.

Forearm vascular resistance (FVR), digital skin vascular resistance (DSVR) and the SMA vascular resistance were calculated from the ratio of the MAP and respective blood flow values, assuming zero venous pressure.

Blood collection and analysis:

Blood samples (20 mls on each occasion) collected at 0 min and at 15, 30, 45 and 60 min after clonidine/placebo were collected in tubes stored in ice until centrifugation at 4°C for separation of plasma.

Plasma noradrenaline and adrenaline were measured after collecting 5 mls of blood in tubes containing 20 ul EGTA (0.095% wt/vol) and storing the plasma at -70°C till the assay which was done by high performance liquid chromatography with an electrochemical detector (Smeddes et al, 1982). The intra and interassay coefficients of variation for noradrenaline were 7.8% and 6.6% and adrenaline was 9.2% and 11%.

PRA was measured after collection of 5 mls of blood in pre-chilled tubes containing EDTA (potassium salt) subsequent radioimmunoassay of angiotensin I generated from its endogenous substrate in the presence of angiotensinase and
converting enzyme inhibitors (Boyd et al, 1969). The intra-assay and inter-assay coefficients of variation were 4% and 7% respectively.

Glucose levels were analyzed by a glucose oxidase method using a chem Lab autoanalyzer (Trinder, 1969). Insulin was assayed by radioimmunoassay using RSL, $^{125}$I Insulin kit (ICN Biomedicals, Inc.) and growth hormone by $I$-labelled growth hormone immunoradiometric assay (IRMA assay kit, NETRIA, North East Thames Radioimmunoassay). The intra-assay and inter-assay variabilities were 3.2% and 4.8% for glucose, 2.7% and 5.4% for insulin, 3.1% and 7.4% for growth hormone respectively.

PROTOCOL:
After 30 min rest in the supine position, collection of blood and basal haemodynamic measurements were commenced. Clonidine (Boehringer Ingelheim, 2 $\mu$g/kg body weight i.v. infused slowly over 10 min by an infusion pump) or placebo (20 ml 0.9% normal saline i.v.) was then infused on separate days, at least one week apart. Measurements (except BP and HR) and blood collection were made at 15 min interval for 60 min.

STATISTICS:
Data are presented as means ± SEM. Analysis of variance (Minitab data analysis software, Minitab Inc, 1989) was used for data analysis. Multiple t tests were performed on means at 0, 15, 30, 45 and 60 min to further characterize significant differences. P<0.05 was considered significant.

Variability of the various measurements were obtained, based on basal readings on the two study days, by dividing the standard deviation by the mean. Variability is then expressed as the mean coefficients of variability.

The coefficients of variation were 7.4% for CI, 7.2% for FBF, 11.6% for DSBF and 7.3% for SMABF.
RESULTS: A. Haemodynamic measurements:

Systolic, Diastolic and MABP: Basal BP (in mmHg) was higher in hypertensives (168±6/100±2) than in the normotensives (125±4/73±2). After clonidine, systolic, diastolic and MABP fell in hypertensives (168±6/100±2 to 118±5/78±3, 121±2 to 96±3 MABP at 60 min, P<0.05). Blood pressure also fell in controls, but to a lesser extent after clonidine (125±4/73±2 to 104±3/62±2, 91±2 to 76±3 MABP at 60 min, P<0.05, Fig 8.1).

Basal blood pressure readings in the placebo phase were similar to the clonidine phase in hypertensives (156±10/94±3, 116±3 MABP) and controls (120±11/74±4, 90±5 MABP). After placebo, there were no changes in systolic, diastolic and MABP in hypertensives or controls (Fig 8.1).

Diameter of the SMA: In hypertensives the mean resting diameter (in mm) of the SMA was lower (6.9±0.06 in clonidine phase and 6.8±0.06 in placebo phase) than controls (7.2±0.06 in clonidine phase and 7.3±0.06 in placebo phase, P=NS).

SMABF and SMAVR: In hypertensives, resting SMABF was 355±22 ml/min during the clonidine phase and 390±45 during placebo phase. Resting SMABF was higher in controls during the clonidine (445±35) and placebo phase (456±19). In hypertensives, after clonidine, SMABF rose at 15 min (490±30), at 30 min (561±35), 45 min (518±35) and 60 min (492±38)(each P < 0.005, Fig 2). In controls, after clonidine, SMABF fell to a lesser extent at 45 min (523±34, P<0.05, Fig 8.2). After placebo, SMABF was unchanged in hypertensives and controls (Fig 8.2).

The resting calculated SMAVR (in units) was higher (P<0.05) in hypertensives during the clonidine (0.34±0.01) and placebo phase (0.31±0.02) compared to the controls (0.22±0.01 during clonidine and 0.2±0.01 during placebo). SMAVR fell after clonidine, in hypertensives, at 15 min (0.21±0.01), at 30 min (0.17±0.01), 45 min (0.19±0.06) and 60 min (0.2±0.01)(each P<0.01, Fig 8.3). In controls, after clonidine, SMAVR fell at 30 min (0.15±0.01), at 45 min
After placebo, SMAVR remained unchanged in hypertensives and controls (Fig 8.2).

HR: After clonidine and placebo, HR was unchanged in controls and hypertensives (Table 8.1).

CI: Resting CI was 789±36 units in hypertensives and 623±67 in controls. After clonidine, in hypertensives, CI fell at 30 min (444±56) and 45 min (445±55) (each P<0.05, Fig 8.3). In controls, CI remained unchanged after clonidine. After placebo, CI was unchanged in hypertensives and controls.

FBF and FVR: After clonidine, in hypertensives, FBF (in ml/100ml/min) fell at 15 min (4.2±0.4 to 3.6±0.4), 30 min (3±0.3) and 45 min (3±0.3) (each P<0.05, Table 8.2). In controls, after clonidine, FBF fell as well (3.2±0.3 to 2.3±0.3 at 30 min and 2.3±0.3 at 45 min, each P<0.05, Table 8.2). However, after clonidine, changes in FVR were not significant in hypertensives or controls. FBF and FVR were unchanged after placebo in hypertensives and controls.

DSBF and DSVR: Changes in DSBF and DSVR were not significant after clonidine or placebo, in hypertensives or controls although there was a trend towards increased DSBF and a lower DSVR after clonidine in both groups (Table 8.2).

Finger (index) temperature (FTT) rose after clonidine in hypertensives (31±0.9 to 33±0.8°C at 45 min) and controls (32±0.8 to 34±0.4 at 45 min) (each P<0.05, Fig 8.4). Changes in skin temperature, at other sites after clonidine and placebo, were not significant in hypertensives or controls. FTT was unchanged in the placebo phase.

B. Biochemical measurements:

Noradrenaline (NA) and Adrenaline: Basal plasma NA levels (in pg/ml) were similar (486±136 in controls and 499±100 in hypertensives) in both groups while adrenaline levels tended to be lower in hypertensives (46±7 in controls...
and 33±5 in hypertensives, P=NS). Plasma NA levels showed a moderate but non-significant fall after clonidine in hypertensives and controls while adrenaline levels fell significantly in both groups after clonidine (Table 8.3).

Glucose and Insulin: Basal plasma glucose and insulin levels were similar in both groups (Table 8.3). Plasma glucose levels rose after clonidine in both groups. Plasma insulin levels showed a moderate but non-significant fall in both groups at 45 min after clonidine (Table 8.3).

Plasma Renin Activity (PRA): Basal PRA levels were similar in both groups and remained unchanged after clonidine (Table 8.3).

Growth Hormone (GH): Plasma GH levels (in mU/l) rose after clonidine in controls (5.7±2.7 to 18±4 at 60 min) and hypertensives (5.7±2.7 to 18±4 at 60 min)(Each P<0.05, Table 8.3).

Symptoms: All subjects were asymptomatic after clonidine or placebo. After clonidine most subjects had dry mouth and felt drowsy but none fell asleep. Sedation did not occur after placebo.
Fig 8.1: Changes in mean arterial blood pressure (MABP) in controls (C) and hypertensives (H) before (0) and 15, 30, 45 and 60 min after clonidine or placebo infusion.

Clon = Clonidine, Pl = Placebo
Fig 8.2: Changes in superior mesenteric artery blood flow (SMABF, upper panel) and vascular resistance (SMAVR, lower panel) in controls and hypertensives (H) before (0) and 15, 30, 45 and 60 min after clonidine or placebo infusion.

Clon = Clonidine, Pl = Placebo
Fig 8.3: Changes in cardiac index (CI) in controls (C) and hypertensives (H) before (0) and 15, 30, 45 and 60 min after clonidine or placebo infusion.

Clon = Clonidine, Pl = Placebo
Fig 8.4: Changes in index finger temperature (FIT) in controls (C) and hypertensives (H) before (0) and 15, 30, 45 and 60 min after clonidine or placebo infusion.

Clon = Clonidine, PI = Placebo
Table 8.1: Changes in heart rate in controls (C) and hypertensive patients (H) before (0) and at 15, 30, 45 and 60 min after clonidine and placebo.

<table>
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<th>TIME (min)</th>
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<th>30</th>
<th>45</th>
<th>60</th>
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<td>C (Clonidine)</td>
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<td>65±2</td>
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<tr>
<td>H (Clonidine)</td>
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<td>63±2</td>
<td>63±2</td>
<td>64±2</td>
</tr>
<tr>
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<td>64±3</td>
<td>63±2</td>
<td>64±3</td>
<td>66±2</td>
</tr>
<tr>
<td>H (Placebo)</td>
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</tr>
<tr>
<td>Time (min)</td>
<td>FBF ml/100ml/min</td>
<td>FVR Units</td>
<td>DSBF (%)</td>
<td>DSVR Units</td>
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<tr>
<td></td>
<td>Clon C</td>
<td>Clon H</td>
<td>Plac C</td>
<td>Plac H</td>
<td></td>
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<tr>
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<tr>
<td></td>
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<td>Clon H</td>
<td>Plac C</td>
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<tr>
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<td>39±3</td>
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<td>Clon C</td>
<td>Clon H</td>
<td>Plac C</td>
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<td>Plac C</td>
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<tr>
<td>45</td>
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<tr>
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Table 8.2: Changes in forearm blood flow (FBF), forearm vascular resistance (FVR), digital skin blood flow (DSBF) and digital skin vascular resistance (DSVR) in controls (C) and hypertensive patients (H) after clonidine or placebo infusion. * = P<0.05
Table 8.3: Changes in noradrenaline (NA), adrenaline (A), glucose (GL), insulin (INS), growth hormone (GH) and plasma renin activity (PRA) in controls (C) and hypertensive patients (H), before (0) and at 15, 30, 45 and 60 min after clonidine.

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<th>45</th>
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<tr>
<td>NA (g/ml)</td>
<td>C 486 ± 136</td>
<td>365 ± 118</td>
<td>339 ± 108</td>
<td>298 ± 89</td>
<td>382 ± 118</td>
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<tr>
<td></td>
<td>H 499 ± 100</td>
<td>387 ± 96</td>
<td>348 ± 87</td>
<td>361 ± 90</td>
<td>366 ± 92</td>
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<tr>
<td>A (g/ml)</td>
<td>C 46.7 ± 7.2</td>
<td>38.1 ± 7.7</td>
<td>27.8 ± 4.2*</td>
<td>27.5 ± 4.1*</td>
<td>30.4 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>H 32.6 ± 4.9</td>
<td>28.3 ± 5.8</td>
<td>22.6 ± 3.6</td>
<td>18.9 ± 3.2*</td>
<td>22.5 ± 5.7</td>
</tr>
<tr>
<td>GL (mmol/l)</td>
<td>C 4.1 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.6 ± 0.1*</td>
<td>4.5 ± 0.1*</td>
<td>4.5 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>H 4.3 ± 0.2</td>
<td>4.7 ± 0.2</td>
<td>5.0 ± 0.3*</td>
<td>4.8 ± 0.2*</td>
<td>4.7 ± 0.22</td>
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<td>3.9 ± 0.6</td>
<td>4.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>H 4.2 ± 0.7</td>
<td>3.6 ± 0.6</td>
<td>3.7 ± 0.6</td>
<td>3.3 ± 0.6</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>GH (ug/l)</td>
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<td>4.6 ± 1.7</td>
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<td>14.7 ± 3.4*</td>
<td>15.9 ± 4*</td>
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<td></td>
<td>H 5.7 ± 2.7</td>
<td>6.1 ± 1.8</td>
<td>13.0 ± 3.5</td>
<td>16.8 ± 3.9*</td>
<td>17.9 ± 4.1*</td>
</tr>
<tr>
<td>PRA (y/ml/hr)</td>
<td>C 2744 ± 698</td>
<td>2524 ± 720</td>
<td>2434 ± 672</td>
<td>2595 ± 810</td>
<td>2802 ± 920</td>
</tr>
<tr>
<td></td>
<td>H 2818 ± 542</td>
<td>2715 ± 556</td>
<td>2131 ± 515</td>
<td>2365 ± 551</td>
<td>2803 ± 669</td>
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* = p<0.05 vs 0 min
THE AIM OF THE PRESENT STUDY WAS TO ESTABLISH THE ROLE OF THE SPLENCHNIC VASCULAR BED IN THE CONTROL OF BLOOD PRESSURE IN HYPERTENSIVE SUBJECTS. IN THIS STUDY, THE RESTING SUPERIOR MESENTERIC ARTERY BLOOD FLOW WAS LOWER AND VASCULAR RESISTANCE WAS HIGHER IN HYPERTENSIVE SUBJECTS. AFTER CLONIDINE, BLOOD PRESSURE FELL IN HYPERTENSIVES AND NORMAL SUBJECTS AND WAS ASSOCIATED WITH AN ACTIVE DILATATION OF THE SUPERIOR MESENTERIC ARTERY. CARDIAC INDEX FELL IN HYPERTENSIVES WHILE SKELETAL AND DIGITAL SKIN VASCULAR RESISTANCE WERE UNCHANGED IN BOTH GROUPS. ALTHOUGH NON-SIGNIFICANT, PLASMA CATECHOLAMINE AND INSULIN LEVELS SHOWED A TREND TOWARDS A LOWER VALUE AFTER CLONIDINE IN BOTH GROUPS. PLASMA GLUCOSE AND GROWTH HORMONE ROSE AFTER CLONIDINE IN HYPERTENSIVES AND CONTROLS.

THE FINDING THAT THE RESTING SUPERIOR MESENTERIC ARTERY BLOOD FLOW WAS LOWER AND THE CALCULATED MESENTERIC VASCULAR RESISTANCE WAS HIGHER IN HYPERTENSIVES, MERIT DISCUSSION. IT MAY BE ARGUED THAT TECHNICAL ERRORS ASSOCIATED WITH DOPPLER MEASUREMENT OF SMABF MAY HAVE RESULTED IN A LOWER MESENTERIC FLOW IN HYPERTENSIVES. HOWEVER, ADEQUATE PRECAUTIONS (AS DESCRIBED PREVIOUSLY) WERE TAKEN DURING MEASUREMENT OF SMABF AND SMAVR TO REDUCE THE TECHNICAL ERRORS. FURTHERMORE, ALL STUDIES WERE PERFORMED UNDER IDENTICAL CONDITIONS AND IN AGE AND SEX MATCHED SUBJECTS SO AS TO REDUCE THE EFFECT OF THESE VARIABLES.

SECONDLY, IN HYPERTENSIVE SUBJECTS, THE VESSEL GEOMETRY MAY BE ALTERED EITHER DUE TO ATHEROMA AND A CALCIFICATION OF THE VESSEL WALL OR TO VASCULAR HYPERTROPHY LEADING TO AN UNDERESTIMATION OF BLOOD FLOW MEASUREMENT. HYPERTROPHIED VESSELS ARE PROBABLY MORE SENSITIVE TO VASOCONSTRICTOR STIMULI AND EVEN A "NORMAL" LEVEL OF SYMPATHETIC TONE MAY CAUSE A GREATER DEGREE OF VASOCONSTRICTION ENHANCING PERIPHERAL VASCULAR RESISTANCE AND INCREASING BLOOD PRESSURE (FOLKOW, 1978, 1982). HOWEVER, IN THIS STUDY THE DIAMETER OF THE SMA IN HYPERTENSIVES AND CONTROLS WERE SIMILAR. THE HIGH RESOLUTION ENLARGEMENT OF THE IMAGES ALLOWED VISUALISATION OF THE VESSEL WALL WHICH APPEARED TO BE OF UNIFORM THICKNESS IN ALL SUBJECTS. HOWEVER, AS FORMAL MEASUREMENTS OF VESSEL
wall thickness was not made, vascular hypertrophy contributing to differences in blood flow between controls and hypertensives cannot be confidently excluded.

Several animal studies have indicated that the splanchnic vascular resistance is higher in the SHR (Tobia et al, 1974; Nishiyama et al, 1976) while Kong et al (1991) showed that in Dahl salt sensitive hypertensive rats, the mesenteric vascular bed is supersensitive to noradrenaline and periarterial nerve stimulation. McGiff and Quilley (1981) reported a disproportionately high splanchnic vascular resistance in patients with renovascular hypertension. Messerli et al (1975) reported no change in estimated hepatic blood flow and a mild increase in splanchnic vascular resistance in essential hypertension. However, these studies were conducted using an indirect and invasive measurement of hepatic blood flow unlike the present study where direct and continuous measurement of blood flow, within a major constituent artery of the splanchnic vascular bed, could be made.

The blood pressure fell in both hypertensive subjects and controls after clonidine. As based on studies in tetraplegic and normal subjects, the hypotensive action of clonidine has been shown to be mainly centrally mediated (Reid et al, 1977; Mathias et al, 1979). In this study evidence of the central action of clonidine is provided by the fact that plasma GH levels rose in hypertensives and controls, after clonidine. The fall in blood pressure in both groups was associated with an active dilatation of the SMA. There were no changes in forearm vascular resistance although forearm blood flow rose after clonidine. However, digital vascular resistance fell while finger temperature rose after clonidine, indicating the withdrawal of the sympathetic tone from the digital vessels, which is mainly controlled by the sympathetic vasoconstrictor fibers. Similar findings have been reported from studies in patients with pre and post-ganglionic lesions previously (Kooner et al, 1991). The inability of clonidine to reduce vascular resistance in forearm and skin
vascular beds (except the digital vessels) suggested that the observed vasodilatation within the splanchnic vascular region played a major role in the hypotensive response after clonidine.

The CI fell in hypertensives after clonidine and it may be argued that this contributed to the hypotensive effect of clonidine. While this may be true, it is unlikely that changes in CI alone played a major part in the hypotensive responses to clonidine as the blood pressure also fell in controls, in whom CI did not fall. Measurements in other major vascular beds such as the renal and cerebral were not made. However, both the renal and the cerebral vascular beds exhibit strong autoregulatory tendencies until the blood pressure falls to a critically low level. Such a severe hypotension did not occur in this study in any subjects. Furthermore, previous studies in patients with unilateral renal artery stenosis have shown that the hypotensive action of clonidine is independent of the suppression of renin-angiotensin-aldosterone system (Mathias, 1991b). A major contribution of the renal and cerebral vascular beds to the hypotensive responses to clonidine, in this study cannot be ruled out but is unlikely.

The mechanism of dilatation of the SMA after clonidine is likely to be neurally mediated although a hormonal contribution cannot be excluded. Plasma catecholamines showed a moderate but non-significant fall after clonidine indicating the central sympatholytic action of clonidine. Plasma renin activity was unchanged, insulin levels showed a trend towards a lower value while glucose and growth hormone levels rose. Hyperglycaemia may cause hypotension (Mathias, 1989) as may a rise in insulin level (Miles & Hayter, 1963) in patients with autonomic failure. However, none of our patients had autonomic dysfunction and thus this possibility is unlikely. Measurements of plasma vasopressin were not made but previous studies have showed that plasma vasopressin levels are not suppressed after clonidine (Mathias, 1963). Release of vasodilatory peptides such as insulin, VIP or neurotensin also may have influenced the changes in SMABF. However, in this study, after
Clonidine, insulin levels tended to fall. Measurements of VIP and neurotensin were not made. Previous studies using clonidine in patients with peripheral sympathetic denervation have shown no change in SMABF after clonidine (Thomaides et al, 1992). This suggested that clonidine-induced splanchnic vascular changes in PAF patients were probably neurally induced. A similar mechanism is therefore the most likely explanation for SMA vasodilatation in hypertensive patients, as seen in this study.

The clinical implications of this study merit discussion. The selective increase in superior mesenteric artery vascular resistance in hypertensive subjects, as shown in this study, indicates that reversal of this change may be a major haemodynamic mechanism to lower blood pressure by antihypertensive drugs. Drugs acting preferentially on the splanchnic circulation may provide an important therapeutic option in hypertension. These observations may also apply to patients with heart failure in whom there is often a higher sympathetic tone and possibly a higher splanchnic vascular resistance. Drugs causing vasodilatation in the splanchnic vascular bed, containing up to 30% of the total blood volume, could markedly reduce afterload and improve heart failure.

CONCLUSIONS:

Superior mesenteric artery blood flow is lower and calculated vascular resistance is higher in hypertensives. The resting superior mesenteric artery vascular resistance is lowered by clonidine suggesting a role for the sympathetic nervous system in the pathogenesis of hypertension. As there were fewer changes in the other vascular beds, the vasodilatation of the superior mesenteric artery may contribute to the fall in blood pressure after clonidine. The hypotensive action of clonidine thus appears to be dependent on the dilatation of the splanchnic vascular bed and this study favours a major role of the splanchnic vascular bed in the maintenance of blood pressure in patients with essential hypertension.
CHAPTER 9:
STUDIES WITH ALCOHOL INGESTION:

EFFECTS ON SUPERIOR MESENTERIC ARTERY BLOOD FLOW, OTHER REGIONAL CIRCULATIONS AND NEUROHORMONAL RESPONSES IN NORMAL SUBJECTS AND PRIMARY AUTONOMIC FAILURE:
BACKGROUND AND INTRODUCTION:

Studies described in the previous chapters (4-7) indicated that the sympatho-neural control of the splanchnic vascular bed plays an important role in blood pressure regulation and cardiovascular homeostasis. This was particularly evident in patients with primary autonomic failure where abnormalities of splanchnic vascular responses due to sympathetic denervation possibly resulted in abnormal blood pressure control and postural hypotension (Ray-Chaudhuri et al, 1992; Thomaides et al, 1992).

Several epidemiological studies indicate an association between chronic alcohol consumption and an increase in blood pressure (Howes & Reid, 1986a), though reports on effects of acute alcohol ingestion on systemic and regional haemodynamic parameters in man, are often diverse and contradictory (Howes & Reid, 1986b; Stott et al, 1987). Alcohol has been shown to cause splanchnic vasodilatation in experimental animals (Altura et al, 1983a; Carmichael et al, 1988), but the effects on the splanchnic circulation in man are unclear. A limited number of studies has been performed to assess splanchnic haemodynamic changes after alcohol ingestion in humans utilising invasive and indirect estimation of hepatic blood flow with contradictory results (Mendeloff 1954; Castenfors et al 1960; Ready et al 1989). Changes in superior mesenteric artery blood flow after alcohol in normal humans and AF patients have not been studied before.

A large proportion of patients with primary autonomic failure are intolerant to alcohol ingestion. The symptoms experienced after drinking even small quantities of alcohol include feeling lightheaded and dizzy, particularly on assumption of upright posture. These symptoms indicate cerebral ischaemia and the underlying mechanisms are unknown. These symptoms are, however, similar to those experienced by these patients after food ingestion.

The haemodynamic and neurohormonal alterations after food ingestion have been previously described in normal subjects (Potter et al, 1989), elderly
subjects (Lipsitz et al, 1986) and in patients with primary autonomic failure (Mathias et al, 1991). In AF patients, a standard liquid meal ingestion causes a pronounced fall in supine blood pressure and worsens the postural fall in blood pressure further (Mathias et al, 1991). This is thought to be due to food induced vasodilatation of the superior mesenteric artery, possibly mediated by the release of vasodilatory gut peptides (Mathias, 1990) and can be prevented by the peptide release inhibitor Octreotide, a somatostatin analogue (Kooner et al, 1989a). The hypotensive action of food ingestion is probably the reason why AF patients feel lightheaded after a meal.

In this study, the effects of acute ingestion of alcohol or placebo on several haemodynamic parameters, with a particular regard to the superior mesenteric artery blood flow have been studied in normal subjects and patients with primary autonomic failure. The results in normal subjects and autonomic failure patients are described separately as studies 1 and 2.

STUDY 1: STUDIES IN NORMAL SUBJECTS

MATERIAL AND METHODS:

SUBJECTS:

Ten healthy subjects (6 males, 4 females) aged 22-51 years (mean 31) were studied. In all subjects alcohol consumption was low (10-40g per week). All subjects were off medication and had normal liver function tests. The mean weight was 68 Kg (range 50-81). The study was approved by the St. Mary's Hospital Ethical Committee.

PROTOCOL:

All subjects were studied on two separate occasions at 0900 hrs, in a temperature controlled room (24±2°C), after an overnight fast. Alcoholic drinks were not allowed for three days prior to the study. Smoking and caffeinated beverages were stopped at midnight before the study. An indwelling venous cannula was inserted into a forearm vein. After a 30min
period of supine rest, basal measurements were taken and blood samples collected. Subjects were then randomly given a drink of either alcohol (0.5g/Kg body weight, in the form of 40% vodka diluted in sugar free orange juice, aspartame 0.8g/100ml) or placebo (sugar free orange juice, aspartame 0.8g/100ml), ingested over 10 min in the supine position. Measurements and blood samples were repeated at 15, 30 and 45 min after each drink; subjects were then tilted 45° head-up for 5min, during which measurements and blood samples were taken.

Vodka was used as the alcoholic drink as vodka is a pure spirit free of congeners such as fusel oil. Furthermore, vodka is tasteless. The composition of the ingestant (alcohol and orange juice), used in this study, was modified from the original protocol of Cook and Brown (1932) used for studying alcohol induced skin vasodilation.

MEASUREMENTS:
Blood pressure and heart rate, cardiac index, forearm blood flow, digital (thumb) skin blood flow and superior mesenteric artery blood flow were measured non-invasively as described previously. Coefficients of variability were 10.2% for the SMABF, 11.7% for the CI, 21.8% for the FBF and 15.8% for the DSBF.

Blood samples were collected for the estimation of plasma noradrenaline and adrenaline, glucose, insulin and alcohol (ethanol) levels. Blood alcohol levels were determined by an alcohol colour mono-assay (Trinder, 1967). The intra and interassay variability of alcohol assay was 2.8% and 3.5%.

STATISTICS:
Student's t test was used to compare results. Statistical significance was accepted at p<0.05. Data are presented as mean values ± SEM at each time point.

RESULTS (STUDY 1):
Alcohol levels.
After alcohol ingestion, plasma alcohol concentrations increased from basal values of 1.9±1.3mg/100ml to 35.8±6.3 at 15min, 61.6±6.5 at 30min, 53.6±5.4 at 45min (each p < 0.001). There were no changes after placebo (Fig 9.1).

BP, HR, CI: Changes in BP, HR and CI following alcohol and placebo ingestion are shown in Fig 9.2 and Table 9.1. The mean arterial blood pressure (MAP) was unchanged after alcohol or placebo, though immediately after alcohol ingestion MAP tended to increase transiently (87.4±3.4 to 92.7±4.0mmHg; NS). Systolic and diastolic BP were unchanged after either drink, though during tilt there was a trend towards a lower systolic blood pressure after alcohol (126.0±4.6 at 45min to 115.2±5.3mmHg; NS). HR was unchanged after placebo, but increased after alcohol during tilt (67.4±2.1 at 45min to 75.5±2.5 b/min; p<0.05). CI was unchanged after both alcohol and placebo, but fell during tilt after alcohol.

SMABF, SMAVR, PI: The results of SMABF and SMAVR are shown in Fig 9.3. After alcohol, SMABF rose at 15min (319±16.6 to 493.3±42.2ml/min) and was elevated at 30min (486.3±37.2) and 45min (450.1±27.0) (each p<0.005), with a corresponding fall in SMAVR (0.28±0.02 units to 0.19±0.02 at 15min, 0.20±0.02 at 30min, 0.20±0.01 at 45min) (each p<0.05). After placebo SMABF and SMAVR were unchanged. During tilt, SMABF fell and SMAVR rose significantly in both the alcohol and placebo phases. The pulsatility index (PI) (Fig 9.4) fell after alcohol (309.0±15.8% to 221.9±19 at 15min, 225.7±13.9 at 30min, 239.6±13.9 at 45min) (each p<0.005). After placebo, the PI did not change.

DSBF, DSVR: The DSBF (Fig 9.5) rose after alcohol (21.1±1.9 flow units to 28.6±1.6 at 30min, 30.8±1.5 at 45min) (each p<0.01), with a corresponding fall in DSVR (4.5±0.5 units at 0min, to 3.3±0.2 at 30min, 3.0±0.2 at 45min) (each p<0.05). During tilt after alcohol, DSBF fell significantly (20.1±2.6;
p < 0.005) with an increase in DSVR (5.3 ± 0.9; p < 0.05). Neither DSBF nor DSVR changed after placebo.

**FBF, FVR:** The results of forearm blood flow (FBF) and vascular resistance (FVR) are shown in Table 9.1. After alcohol, FBF tended to rise (4.7 ± 0.7 ml/min/100 ml to 6.3 ± 1.2 at 45 min; NS) without a change in FVR. During tilt, FBF fell after placebo (5.2 ± 0.7 at 45 min to 2.7 ± 0.6; p < 0.03), with a corresponding increase in FVR (20.5 ± 2.9 units at 45 min to 44.4 ± 8.6; p < 0.05).

**STUDY 2: STUDIES IN PRIMARY AUTONOMIC FAILURE PATIENTS**

**MATERIAL AND METHODS:**

**PATIENTS:** 15 patients with chronic primary autonomic failure were studied. Nine patients had PAF (mean age 54 years, range 42-66, four females and five males) and six patients had MSA (mean age 58 years, range 44-66, three females and three males). As discussed previously, these two groups will be considered separately as, the functional site of lesion is mainly peripheral in PAF but central in MSA. All patients were studied while off drugs. These mainly included fludrocortisone (0.1 mg). All drugs were discontinued four days before each study. The MSA patients were not on L-dopa or other anti-Parkinsonian medication. Most were low intake alcohol drinkers (alcohol consumption 10-40 g weekly) while some did not drink alcohol.

All had normal liver function tests. They were asked to abstain from alcohol for five days and caffeinated beverages and tobacco for 24 hours prior to the study as tobacco and caffeine may interfere with alcohol absorption (Johnson et al, 1991). The study was approved by the Ethical Committee of St. Mary's Hospital.

**MEASUREMENTS:**

All were studied in a temperature controlled room (24°C) at 0930 hrs after an overnight fast and measurements and blood collection as described in study 1 were made.
PROTOCOL:
Protocol identical to study 1 was followed. After collection of blood for measurement of plasma noradrenaline, adrenaline, insulin, glucose and alcohol levels, patients were given either alcohol (0.5g/kg, 40% Vodka diluted to 300 ml in chilled sugar free orange juice) or placebo (300 ml sugar free orange juice) on separate days. Patients drank alcohol/placebo in the supine position over 10 min. Sugar free orange juice was used to avoid the effect of glucose on SMABF and especially as glucose lowers BP in patients with autonomic failure. (Qamar et al, 1986; Mathias et al, 1989). Measurements and blood collections (15 ml on each occasion) were made at 15 min interval after the drink for 45 min. Patients were then tilted head-up for 10 min and measurements and blood collection were repeated. In some patients head-up tilt was terminated early because of postural symptoms such as dizziness and feeling faint.

RESULTS (STUDY 2):
BP: In PAF, after alcohol, supine MABP fell from 118±7 mmHg to 112±6 at 15 min, 102±7 at 30 min and 93±7 at 45 min (P<0.05). MABP fell further during head-up tilt (93±7 to 57±6, P=0.002, Fig 9.6). After placebo, there were no changes in supine MABP at 15 min (118±6 to 119±7 mmHg), 30 min (116±6) and 45 min (114±6)(Fig 9.6). MABP however fell during head-up tilt (114±8 to 74±8, P=0.004, Fig 9.6) but the level was lower in the alcohol phase (93±7 to 57±6).

In MSA, after alcohol, supine MABP fell to a lesser degree at 30 min (112±9 to 106±10) and 45 min (93±10) though this did not achieve statistical significance. MABP fell further during head-up tilt after alcohol (93±10 to 68±16, P<0.05)(Fig 9.7). In MSA, after placebo, MABP was unchanged while supine but fell during head-up tilt (113±12 to 80±15, P<0.05) the levels being lower in the alcohol phase (93±10 to 68±16)(Fig 9.6).
SMABF, SMAVR, PI: In PAF, after alcohol, SMABF rose markedly from 369±20 to 613±34 at 15 min, to 669±29 at 30 min and to 654±50 at 45 min (each P<0.001, Fig 9.7). SMABF was unchanged during head-up tilt (654±50 to 480±44, P=NS). In MSA, after alcohol, SMABF also rose from 382±43 to 514±78 at 30 min, and 577±59 at 45 min (P<0.05, Fig 9.7). SMABF remained unchanged during head-up tilt (577±59 to 400±47, NS) although values tended to be lower.

In PAF and MSA, after placebo, there were minimal changes in SMABF at 15 min (397±33 to 419±53 in PAF, 385±54 to 432±46 in MSA), at 30 min (438±53 in PAF, 434±71 in MSA), at 45 min (448 ±53 in PAF, 411±62 in MSA) and during head-up tilt (448±77 to 388±50 in PAF, 411±62 to 363±42 in MSA)(each P=NS, Fig 9.7).

In PAF, after alcohol, the corresponding calculated SMAVR fell from 0.32±0.02 units to 0.18±0.01 at 15 min, 0.14±0.008 at 30 min and 0.14±0.02 at 45 min (each P<0.001, Fig 9.7). SMAVR was unchanged during head-up tilt (0.14±0.02 to 0.11±0.01, P=NS). In MSA, after alcohol, calculated SMAVR fell to a lesser extent at 15 min (0.3±0.03 to 0.21 ±0.02, NS), at 30 min (0.22±0.02, NS) and at 45 min (0.16±0.01, P<0.05)(Fig 9.7). SMAVR was unchanged during head-up tilt (0.16±0.01 to 0.18±0.03). There were no changes in calculated SMAVR after placebo in PAF and MSA while supine and during head-up tilt. (Fig 9.7).

In PAF, after alcohol PI (in units) fell from 309±15 to 235±28 at 15 min, 241±21 at 30 min and 240±20 at 45 min. (each P<0.05, Table 9.2). In MSA, after alcohol, PI fell at 30 min (300±10 to 249±13) and 45 min (239±10)(each P<0.05, Table 9.3). Changes in PI, in PAF and MSA, after placebo were not significant. Measurement of PI was not made during head-up tilt for reasons mentioned in chapter 4.

CI, HR, DBF, FBF: In PAF and MSA, CI (in units) was unchanged after alcohol and placebo except during head-up tilt when it fell (P<0.05) in PAF.
and MSA (Table 9.3). In PAF and MSA, changes in the HR were not significant after alcohol or placebo (Table 9.3).

In PAF and MSA, changes in DBF or FBF were not significant after alcohol or placebo though values tended to be lower during head-up tilt (Table 9.3).

**Plasma alcohol, noradrenaline and adrenaline levels:**

In PAF and MSA, plasma alcohol levels (in mg/dl) rose after alcohol at 15 min (1.5±0.6 to 35.2±5.8 (PAF), and 2.2±0.9 to 33±8 (MSA)), at 30 min (51±6.1 (PAF) and 47±9 (MSA)) and at 45 min (54.2±6 (PAF) and 56±8 (MSA)) (each P<0.001, Fig 9.8). Basal plasma noradrenaline levels tended to be higher in MSA compared to PAF although this did not achieve statistical significance (Table 9.4). In PAF and MSA, plasma noradrenaline and adrenaline levels were unchanged after alcohol and placebo (Table 9.4).

**Plasma insulin and glucose levels:**

In PAF and MSA, plasma glucose levels were unchanged after alcohol and placebo (Table 9.4). In PAF, after alcohol, insulin levels tended to rise though not significant (Table 9.4). In MSA, after alcohol and placebo, there were no changes in plasma insulin levels (Table 9.4).

**Symptoms:** Six out of the nine PAF patients and three out of six MSA patients complained of feeling dizzy and faint when tilted after alcohol. The symptoms were rapidly reversed on return to the horizontal position. After placebo, none of the patients were symptomatic when tilted.
Fig 9.1: Figure showing blood alcohol levels in normal subjects after alcohol ingestion.
Fig 9.2: Figure showing changes in mean arterial pressure (MAP), systolic blood pressure (SBP) and diastolic blood pressure (DBP) after alcohol and placebo ingestion in normal subjects.
Fig 9.3: Changes in superior mesenteric artery blood flow (SMABF) and superior mesenteric artery vascular resistance (SMAVR) in normal subjects after alcohol and placebo.
Fig 9.4: Changes in pulsatility index of the superior mesenteric artery blood velocity waveform (PI) after alcohol and placebo ingestion, while supine, in normal subjects.
Fig 9.5: Changes in digital skin blood flow (DSBF) and vascular resistance (DSVR) after alcohol and placebo, while supine and during tilt (shaded area) in normal subjects.
Table 9.1: Values before (0), at 15, 30, 45min and during 10mln till after alcohol and placebo ingestion in normal subjects.

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>TILL</th>
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<tbody>
<tr>
<td>Heart rate (b/min)</td>
<td>63.0±2.0</td>
<td>66.2±2.6</td>
<td>66.0±2.3</td>
<td>67.4±2.1</td>
<td>75.5±2.5 *</td>
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<tr>
<td>Cardiac index (units)</td>
<td>650.1±58.0</td>
<td>709.4±78.3</td>
<td>685.8±50.8</td>
<td>714.1±52.4</td>
<td>543.3±27.6 *</td>
</tr>
<tr>
<td>Forearm blood flow (ml/min/100m)</td>
<td>4.7±0.7</td>
<td>5.0±0.9</td>
<td>5.8±1.2</td>
<td>6.3±1.2</td>
<td>3.3±0.7</td>
</tr>
<tr>
<td>Forearm vascular resistance (units)</td>
<td>21.4±2.4</td>
<td>22.7±3.5</td>
<td>22.1±3.9</td>
<td>20.5±3.9</td>
<td>34.8±7.0</td>
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</table>

* P = alcohol, p = placebo.  *= p<0.05.
Fig 9.6: Changes in mean arterial blood pressure (MABP) after alcohol and placebo ingestion while supine and during tilt in 9 pure autonomic failure (PAF) and 6 multiple system atrophy (MSA) patients.
Fig 9.7: Changes in superior mesenteric artery blood flow (SMABF) and vascular resistance (SMAVR) after alcohol (continuous line) and placebo (interrupted line) in PAF and MSA patients.
Fig 9.8: Changes in plasma alcohol level after alcohol ingestion in PAF and MSA patients. Dotted lines represent values after placebo ingestion.
Table 9.2: Changes in pulsatility index (PI) of superior mesenteric artery blood flow after alcohol and placebo ingestion in PAF and MSA patients.

* = P < 0.05

<table>
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<tr>
<th>Time (min)</th>
<th>Alcohol</th>
<th>Placebo</th>
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<tbody>
<tr>
<td>0</td>
<td>309±15</td>
<td>239±30</td>
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<tr>
<td>15</td>
<td>235±28</td>
<td>241±21</td>
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<tr>
<td>30</td>
<td>240±20</td>
<td>240±20</td>
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<tr>
<td>45</td>
<td>280±24</td>
<td>295±26</td>
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<tr>
<td>300±18</td>
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<td>308±26</td>
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<table>
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<th>PI</th>
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<td>MSA</td>
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* = P < 0.05
Before (0 min) and 16.30/16.36 min during head-up (U) after alcohol and
femoral vascular resistance (FVR) in the para-portal and peri-muscular arteries.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MSA (unita)</th>
<th>DFR (%)</th>
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<tr>
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<th>Time (min)</th>
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<td>45</td>
<td>22</td>
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</tbody>
</table>

Table 2: Changes in femoral resistance (FR), diaphragm blood flow (DFR) and
diaphragm skin vascular resistance (DVR), diaphragm blood flow (DFR) and
femoral vascular resistance (FVR) in the para-portal and peri-muscular arteries.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MSA (unita)</th>
<th>DFR (%)</th>
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<tbody>
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<td>0</td>
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<td>45</td>
<td>22</td>
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Table 3: Changes in femoral resistance (FR), diaphragm blood flow (DFR) and
femoral vascular resistance (FVR) in the para-portal and peri-muscular arteries.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MSA (unita)</th>
<th>DFR (%)</th>
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<td>Time (min)</td>
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<tr>
<td>Noradrenaline (pg/ml)</td>
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<tr>
<td>PAF</td>
<td>a</td>
<td>94±30</td>
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<tr>
<td></td>
<td>p</td>
<td>108±30</td>
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<tr>
<td>MSA</td>
<td>a</td>
<td>280±90</td>
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<tr>
<td></td>
<td>p</td>
<td>270±96</td>
</tr>
<tr>
<td>Adrenaline (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>a</td>
<td>60±9</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>68±10</td>
</tr>
<tr>
<td>MSA</td>
<td>a</td>
<td>70±20</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>57±29</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>a</td>
<td>4.9±0.1</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>4.9±0.1</td>
</tr>
<tr>
<td>MSA</td>
<td>a</td>
<td>4.1±0.2</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>4.4±0.2</td>
</tr>
<tr>
<td>Insulin (mIU/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>a</td>
<td>7.2±2</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>6±1</td>
</tr>
<tr>
<td>MSA</td>
<td>a</td>
<td>6.9±1</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>7±1</td>
</tr>
</tbody>
</table>

Table 9.4: Changes in plasma noradrenaline, adrenaline, glucose, insulin levels before (0 min) and at 15, 30, 45 min and during head-up tilt (T) after alcohol (a) and placebo (p) ingestion in nine PAF and six MSA patients. Normal supine levels of plasma noradrenaline = 300±40 and during head-up tilt = 490±65 pg/ml.
DISCUSSION:

In this study, in normal subjects, blood pressure was unaltered after alcohol ingestion except during head-up tilt when systolic blood pressure was lowered. In PAF and MSA patients, alcohol ingestion lowered supine BP significantly and caused a further reduction in BP during head-up tilt. None of the normal subjects were symptomatic after alcohol ingestion. However, most patients complained of dizziness when tilted after alcohol, but not after placebo. In normal subjects, during head-up tilt, heart rate, FVR, DSVR rose and CI fell. In AF patients, the fall in supine BP was not associated with a compensatory rise in heart rate, cardiac output, skeletal muscular or cutaneous vascular resistance, and plasma catecholamine levels, thus confirming the inability of these patients to activate baroreflex and sympatho-adrenal pathways. After alcohol, in normal subjects, PAF and MSA, there was, however, an increase in the SMABF and a corresponding fall in the calculated SMAVR, indicating active vasodilatation in this artery, a major constituent of the splanchnic vascular bed.

The importance of the splanchnic vascular bed in overall BP regulation in normal man and AF patients has been previously discussed. Vasodilatation of the SMA, as occurs after food ingestion in patients with primary autonomic failure, is associated with a fall in BP in the absence of other regional compensatory responses (Mathias, 1990). In this study, after alcohol, there was a similar dilatation of the SMA, and a lack of regional and sympatho-adrenal compensatory responses which also appeared to contribute to the hypotensive effect of alcohol in AF patients. In normal subjects, blood pressure appeared to be maintained in spite of SMA vasodilatation.

The effects of alcohol on the splanchnic vascular bed are varied and results are often conflicting, especially as acetaldehyde, a major metabolite of alcohol, may cause either vasodilatation and vasoconstriction (Altura et al, 1978; Altura & Gebrewold, 1981). In rats, acute oral alcohol inhibits spontaneous
contractile activity in the mesenteric vessels and increases portal blood flow, possibly through release of the vasodilator adenosine, a metabolite formed from acetate metabolism (Carmichael et al, 1987; Carmichael et al, 1988). In humans, there have been no studies utilising a direct method for measuring splanchnic blood flow after acute alcohol ingestion. A limited number of studies have been performed assessing estimated hepatic blood flow after alcohol infusion by indicator dilution method. Mendeloff (1954) reported an increase in estimated hepatic blood flow after alcohol infusion in normal subjects. Castenfors et al (1960) however, reported no change in hepatic blood flow after alcohol infusion in normal subjects. In patients with alcoholic cirrhosis, Ready et al (1989) found no change in portal pressure after alcohol infusion. However, the effect of alcohol on superior mesenteric artery blood flow in normal subjects and AF patients is unknown.

The precise mechanism for the mode of action of alcohol causing splanchnic vasodilatation, as observed in this study, is not clear. Alcohol may selectively cause vasoconstriction in certain vascular beds, such as the coronary or cerebral circulation (Altura et al, 1983b; Altura et al, 1983c) but is regarded mainly as a vasodilator (Gillespie, 1967; Kupari, 1983). This may occur either due to a direct vasodepressant action of alcohol on smooth muscle, or through neurogenic and hormonal/autacoid mechanisms. Alcohol has been shown to impair the vascular responses to noradrenaline, adrenaline, vasopressin and prostaglandin F2 and interfere with the movement of calcium ions (Altura et al, 1978). Cutaneous vasodilatation in the affected limb of a patient with a unilateral sympathectomy suggested a direct and/or peripheral vasodilator action of alcohol requiring intact postganglionic innervation of the blood vessels (Johnson & Robinson, 1987). In this study, in normal subjects the digital skin blood flow rose after alcohol but not in AF. The inability of AF patients to activate the postganglionic sympathetic nerves was confirmed in both groups by a lack of rise in plasma noradrenaline or adrenaline after alcohol.
alcohol, in AF, there was no vasodilatation in the periphery in skeletal muscle or the cutaneous vascular bed. This excludes a direct effect of alcohol on these vascular beds; vasodilatation here may be dependant on central vasomotor effects, as postulated in tetraplegics (Malpas et al, 1990). In AF, there was vasodilatation of the SMA after alcohol however, which was independent of the central and peripheral nervous system. This study therefore indicates a selective vasodilatory action of alcohol on the splanchnic vascular bed either directly or through factors which include the release of vasodilatory gut peptides.

BP while supine fell after alcohol in AF patients but not in normal subjects, although SMA vasodilatation was observed in all. In normal subjects, the reason for the maintenance of BP in spite of SMA vasodilatation is unclear particularly as there was no compensatory rise in heart rate or cardiac output. Reports of changes in blood pressure after acute alcohol administration in normal subjects have been variable and while some have found a rise in systolic blood pressure (Grassi et al, 1989) others have reported no change (Howes & Reid, 1985) or a fall in BP (Kupari, 1983). It is possible, in normal subjects, that vasoconstriction may have occurred in certain regions (such as the renal or pulmonary) where measurements were not made, particularly as alcohol is known to have a direct vasoconstrictor effect on certain vascular beds.

In both PAF and MSA patients supine BP fell after alcohol, although in the MSA patients there was a smaller fall in supine BP. In MSA, BP however fell to a similar extent as in PAF during head-up tilt. Plasma insulin levels appeared higher in PAF after alcohol. Insulin has been shown to lower blood pressure in patients with primary autonomic failure (Mathias et al, 1987) or autonomic impairment due to either tetraplegia or diabetes mellitus (Mathias, 1990; Miles & Hayter, 1968). A greater rise of plasma insulin (and possibly other vasodilatory gut peptides such as neurotensin) in PAF, as may occur in an analogous situation after a meal (Mathias et al, 1989), could have therefore
contributed to a greater degree of SMA vasodilatation and thus a further lowering of supine blood pressure in PAF patients. Alcohol ingestion was associated with a significant fall in BP to lower levels on postural change in both PAF and MSA patients. After alcohol, patients felt dizzy and lightheaded while tilted, unlike after placebo when BP fell to a lesser degree. Previous studies in normal subjects have shown that alcohol causes tachycardia and lowers BP in the erect position (Howes & Reid, 1985). This also occurred in the present study although the fall in blood pressure in normal subjects was not significant. Alcohol is also known to unmask a negative inotropic effect in normal subjects after autonomic blockade with propranolol and atropine (Chiid et al, 1979). In this study, however, during head-up tilt, there were similar splanchnic and other regional responses after alcohol and placebo, though BP fell more after alcohol in AF patients. In AF, the fall in BP to lower levels during tilt after alcohol could therefore be due to a lower supine BP before tilt, especially in the presence of splanchnic vasodilatation, a major contributor to systemic BP, as based on previous studies in similar patients.

The relationship between alcohol and postural hypotension in PAF and MSA patients raises a number of clinical issues. The hypotensive effect of food may be compounded by alcohol particularly during assumption of the upright posture. Vodka, a pure spirit, was used in our study. It is possible that different varieties of alcohol with varying compositions and congeners have a variable effect on BP. Alcohol rich in carbohydrate, such as beer, however, may lower BP further as carbohydrate is a potent hypotensive component of food in AF (Mathias et al, 1989). Alternatively, the pressor agent tyramine, known to be present in some red wines may prevent a fall in BP. Furthermore, carbonated forms of alcohol such as sparkling wine may be absorbed faster and thus have a greater hypotensive effect. An additional aspect may be that in women, blood alcohol levels rise to a greater degree than men, because they have a decreased activity of gastric alcohol dehydrogenase (Frezza et al, 1990).
Finally substances such as caffeine, often ingested after food and alcohol may reduce the fall of BP (Onrot et al, 1986). A number of factors may thus obscure the relationship between alcohol ingestion and postural intolerance, and this may not be clear without questioning and objective assessment. There are also therapeutic implications, as drugs used to reduce postural hypotension in PAF and MSA may be less effective if alcohol is consumed. This could be analogous to the negation of the benefit derived from the pressor agent dihydroergotamine as a result of post-prandial hypotension (Hoeldtke et al, 1986).

The observation that alcohol ingestion causes hypotension in PAF and MSA may apply to other groups with autonomic failure. Whether patients with secondary autonomic failure, which include diabetes mellitus and amyloidosis, have the same problem is not known, as local factors including vasodilatatory gut peptide release may vary. Elderly subjects are known to have both postural and post-prandial hypotension, and alcohol may lower their BP (Lipsitz, 1986; Potter et al, 1989). This may be of clinical importance as hypotension may contribute to syncope, myocardial ischaemia and strokes, especially if coronary and cerebrovascular disease were coexistent.

CONCLUSIONS:
Alcohol ingestion lowers supine BP and enhances the fall in BP further during postural change in PAF and MSA patients but not in normal subjects. The hypotensive action of alcohol in these patients appears to be due to vasodilatation of the SMA, possibly induced by a local and it appears (on the basis of lack of changes in skeletal muscular and cutaneous vascular beds), a selective vascular action of alcohol mediated directly or through release of vasodilator substances. The fall in BP and the enhancement in postural hypotension induced by alcohol is probably responsible for the symptoms observed after alcohol in PAF and MSA.
CHAPTER 10:
SYSTEMIC, SUPERIOR MESENTERIC ARTERY BLOOD FLOW AND
NEUROHORMONAL CHANGES WITH OCTREOTIDE, A
SOMATOSTATIN ANALOGUE, AFTER ALCOHOL INGESTION IN
PRIMARY AUTONOMIC FAILURE:
INTRODUCTION:
The previous study suggested that alcohol ingestion causes supine hypotension, worsens postural hypotension and dilates the SMA in patients with primary autonomic failure. Food ingestion, in patients with AF, is also associated with a marked fall in blood pressure and SMA vasodilatation (Mathias et al, 1986). Release of vasodilatory gut peptides/autacoids have been implicated in the hypotensive response and SMA vasodilatation after food ingestion (Raimbach et al, 1989) and it is possible, a similar response also played a part in alcohol induced vasodilatation of the SMA, in AF patients.

Somatostatin, a hypothalamic tetradecapeptide, was isolated in 1972 and was shown to exert numerous extrahypophyseal effects including secretory inhibition of various gut peptides and hormones (Blei & Groszmann, 1984; Konturek et al, 1981). In man, somatostatin infusion reduces total splanchnic blood flow (Wahren & Felig, 1976) and also causes a differential response in various circulatory beds. For example, in human external iliac artery, blood flow increases by 43% during a 1 min infusion of somatostatin (1μg/kg/min) while splanchnic arterial inflow decreases (Tyden et al, 1979). Furthermore, systemic vascular resistance remains unaltered after somatostatin while splanchnic blood flow is reduced (Bosch et al, 1982). This suggests that somatostatin may exert a selective vasoconstrictory effect on the mesenteric vascular bed in humans.

Octreotide, a somatostatin analogue, in a dose of 50 μg subcutaneously, effectively inhibits the release of a wide range of peptides and autacoids which are normally released in response to food ingestion (Blei & Groszmann, 1984). In primary autonomic failure, Octreotide has been shown to prevent glucose induced hypotension (Raimbach et al, 1989) and post-prandial SMA vasodilatation (Kooner et al, 1989a). Therefore, to test the hypothesis that alcohol induced vasodilatation of the SMA in AF could be due to release of
vasodilatatory peptides, studies were carried out with alcohol ingestion after pretreatment with Octreotide in six patients with primary AF.

**MATERIAL AND METHODS:**

Six patients with primary autonomic failure were studied on two separate occasions. Patients were aged between 50 and 67 years and five had PAF while one had MSA. All had severe postural hypotension and their diagnosis was proven by a series of physiological and biochemical autonomic function tests as discussed earlier.

**PROTOCOL:**

Protocol similar to that in chapter 9 was followed. On the first occasion haemodynamic and biochemical measurements were made 30 min before and at 15 min intervals for 45 min after ingestion of alcohol (40% vodka diluted in 300 ml of sugar free orange juice). Subjects were then tilted head-up for 5 min and measurements and blood samples repeated. On the second occasion the same protocol was repeated 30 min after pretreatment with 50μg of Octreotide (Sandostatin, Sandoz) administered subcutaneously.

Measurements included BP, HR, CI, FBF and SMABF. Blood was collected for plasma alcohol levels, catecholamine (adrenaline and noradrenaline), insulin and glucose levels.

**RESULTS:**

**MAP:** MAP fell after alcohol, while supine, (125±6 to 95±9 mmHg) at 45 min and fell further during tilt (96±9 to 58±8)(each P<0.05, Fig 10.1). All patients felt faint while tilted and symptoms were rapidly reversed on return to the horizontal position.

After Octreotide and alcohol, MAP remained unchanged while supine, (122±5 to 124±6 at 45 min) and fell during tilt only (124±6 to 30±5, P<0.01, Fig 10.1). None of the patients were symptomatic during tilt.

SMABF and SMAVR:
After alcohol, SMABF rose (393±26 to 716±17 ml/min at 45 min) with a corresponding fall in vascular resistance (SMAVR, 0.32±0.03 to 0.14±0.01 units)(each P<0.05, Fig 10.2). After Octreotide and alcohol, SMABF (465±45 to 356±25) and SMAVR (0.27±0.02 to 0.34±0.03) did not change significantly (Fig 10.2). After alcohol, during head-up tilt, SMABF fell (680±60 to 415±63, P<0.05) without any change in calculated SMAVR (0.15±0.01 to 0.17±0.03). After Octreotide and alcohol, changes in SMABF or SMAVR were not significant.

HR, CI, and FBF:
Changes in HR, and FBF were not significant Table 10.1). CI fell during tilt only in both phases (Table 10.1).

ALCOHOL, CATECHOLAMINE, INSULIN AND GLUCOSE LEVELS:
Plasma alcohol levels rose (P<0.05) after alcohol ingestion in both phases (Fig 10.3) while insulin levels fell (P<0.05) after Octreotide and alcohol (Fig 10.6). Changes in plasma catecholamines (noradrenaline and adrenaline) and glucose levels were not significant (Table 10.2).
Fig 10.1: Changes in mean arterial blood pressure (MAP) before (0 min) and after Octreotide followed 30 min later by alcohol ingestion (interrupted line) and alcohol ingestion only (continuous line). a15, a30, a45 indicates measurements at 15 min intervals after alcohol ingestion and shaded area represents measurements during head-up tilt.
Fig 10.2: Changes in SMABF (upper panel) and SMAVR (lower panel) before (0 min) and after Octreotide followed 30 min later by alcohol ingestion (interrupted line) and alcohol ingestion only (continuous line). a15, a30, a45 indicates measurements at 15 min intervals after alcohol ingestion and shaded area represents measurements during head-up tilt.
Fig 10.3: Changes in blood alcohol level after Octreotide injection and alcohol ingestion (continuous line) and alcohol ingestion only (interrupted line). Measurements are made at 15 min intervals in both phases after alcohol ingestion.
Fig 10.4: Changes in plasma insulin level after Octreotide injection and alcohol ingestion (interrupted line) and alcohol ingestion only (continuous line). Measurements are made at 15 min intervals in both phases after alcohol ingestion.
### Table 1.1: Changes in Heart Rate (HR), Cardiac Index (CI), forearm blood flow

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>45</th>
<th>90 (post occlusion), 15 (post occlusion)</th>
<th>30</th>
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<tbody>
<tr>
<td>HR (bpm)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI (unit)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (min)</td>
<td>0</td>
<td>30 (post Oxpoxide)</td>
<td>15 (post Alcohol)</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---</td>
<td>-------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>4.8 ± 0.4</td>
<td>4.8 ± 0.4</td>
<td>4.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>(mg/dl)</td>
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The table above illustrates the changes in alcohol and glucose levels after 30 minutes and 15 minutes from the consumption of Oxpoxide and Alcohol.

Note: D = Day
G = Glucose
This study shows that alcohol induced hypotension and vasodilatation of the SMA, in patients with AF, can be prevented by pre-treatment with Octreotide. After alcohol, as observed in the previous studies (chapter 9), there was a fall in supine BP with a further lowering of BP during head-up tilt causing all patients to be symptomatic. There was active vasodilatation of the SMA while supine. Although SMABF fell during head-up tilt, after alcohol, this was not associated with a rise in calculated SMAVR indicating that there was no active vasoconstriction within the SMA during tilt. After Octreotide injection, alcohol ingestion failed to lower BP and there was no vasodilatation of the SMA. Although BP fell during tilt, the values were higher than after alcohol only and none of the patients complained of feeling dizzy while tilted. After Octreotide, there was also a rapid and short lasting pressor effect associated with a rise in MAP and SMAVR and a moderate fall in SMABF which persisted even after alcohol ingestion. This was unlikely to be due to a stress response as after Octreotide there was no discomfort and none of the patients had positive pressor responses to other pressor tests. After Octreotide, basal levels of insulin (a gut peptide) were reduced unlike after alcohol only, when it tended to rise. In man, insulin may cause hypotension in primary and secondary autonomic failure (Miles & Hayter, 1968; Mathias et al, 1987). In elderly subjects, postprandial hypotension is at least partly thought to be mediated by a rise in insulin levels (Potter et al, 1989). Thus, in this study, the trend towards a higher insulin after alcohol ingestion may have played a part in causing splanchnic vasodilatation after alcohol ingestion. Other vasodilatory peptides known to cause vasodilatation in the gut include vasoactive intestinal polypeptide (VIP), neurotensin, substance P and possibly glucagon and gastric inhibitory polypeptide (GIP) (Chou et al, 1984). Although in this study, measurements of VIP, neurotensin, substance P and glucagon...
were not made it is likely that these were released after alcohol ingestion and contributed to splanchnic vasodilatation. Inhibiting the release of insulin and possibly other gut peptides such as neurotensin and vasoactive intestinal polypeptides (VIP) by Octreotide, may have prevented the alcohol induced vasodilatation of the SMA and thus the hypotension. Furthermore, in AF patients there may be relative deficiency of somatostatin similar to animals after sympathectomy (Schusdziarra et al, 1980). It may be speculated that exogenous somatostatin in the form of Octreotide prevented the abnormal splanchnic vasodilatatory response (induced by unopposed action of gut peptides) to alcohol ingestion in AF patients.

There are clinical implications of this study. Octreotide has been used in the treatment of autonomic neuropathy (Hoeldtke et al, 1986) and has been shown to prevent post-prandial hypotension in AF patients (Raimbach et al, 1989). The prevention of alcohol induced hypotension by Octreotide confirms the role of Octreotide in post-cibal disorders. Octreotide also reduced the signs and symptoms of postural hypotension in AF patients after alcohol ingestion. Although BP was lowered, none of the patients were symptomatic unlike after alcohol. This also suggests that in patients susceptible to post-prandial hypotension, Octreotide may help in the management of postural hypotension the symptoms of which may be aggravated after a meal or alcohol ingestion.

**CONCLUSIONS:**

In conclusion, in patients with primary AF, Octreotide prevented alcohol induced supine hypotension and vasodilatation of the SMA. Octreotide also reduced the signs and symptoms of postural hypotension. The mechanism of alcohol induced vasodilatation of the SMA and the resultant hypotension is therefore at least partly mediated by the release of vasodilatatory gut/pancreatic peptides.
CHAPTER 11:
HAEMODYNAMIC CHANGES IN THE SUPERIOR MESENTERIC ARTERY DURING ANGIOTENSIN CONVERTING ENZYME INHIBITION. STUDIES WITH CAPTOPRIL.
INTRODUCTION:
Captopril is an angiotensin converting enzyme (ACE) inhibitor which causes arteriolar vasodilatation, and lowers blood pressure (BP) and systemic vascular resistance in normal subjects and patients with hypertension and heart failure (Williams, 1988; Faxon et al, 1984; Ventura et al, 1985; Cody & Laragh, 1982). It acts by preventing the formation of angiotensin II (AII) both in the circulation, and probably in cardiac and vascular tissue, although other actions through release of bradykinin, prostacyclin and endothelium-derived relaxing factor, sympatho-neural inhibition, free radical scavenging and calcium channel blockade may contribute (Swales & Heagerty, 1987; Thurston & Swales, 1978; Lees et al, 1990; Westlin & Mullane, 1988; Cardiner et al, 1990; Vanhoutte et al, 1989; Jeremy et al, 1990). In normal man, captopril causes renal and skeletal muscular vasodilatation but its effect on the large splanchnic vascular bed, which plays an important part in BP regulation in man, is unclear. (Muller et al, 1990; Mabie et al, 1990; Banas, 1992; Crossley et al, 1984; Tesar et al, 1988). There have been previously no studies using direct measurements of superior mesenteric artery blood flow after captopril in normal man. In this study, results of non-invasive assessment of superior mesenteric artery and portal blood flow responses to a single dose of oral captopril in twelve healthy subjects, in a single blind placebo controlled study is reported. Systemic, other regional (cardiac index, forearm and cutaneous blood flow), and neurohormonal responses to captopril were also studied. Measurements were also made during head-up tilt so as to assess the effect of captopril on the vascular responses influenced by the sympathetic nervous system.

MATERIAL AND METHODS:
12 healthy subjects (mean age 31 years, range 20-64 years, male=4, female=8) were studied on two separate occasions, at least one week apart. All were on an unrestricted salt diet and fasted overnight before each study day to exclude the
established effect of food on superior mesenteric artery blood flow (SMABF). None was on medication and all were studied in a temperature controlled room (24°C) at 0930 hrs after supine rest for 30 min to allow familiarisation with equipment. An indwelling venous cannula (Abbocath, 18G) was inserted into a forearm vein under local anaesthesia (2% lignocaine) for blood collection. The intravenous cannula was kept patent with heparinized saline solution. The study was approved by the Ethical Committee of St. Mary's Hospital.

MEASUREMENTS:
The following measurements were made during the captopril and placebo phases:
Blood pressure and heart rate (BP, HR), stroke distance (SD, forearm blood flow (FBF), digital (thumb) skin blood flow (DSBF), superior mesenteric artery blood flow (SMABF) and portal venous blood flow (PBF).
All methods were non-invasive and have been previously described except portal venous blood flow the key details of which are provided below.
PBF was measured using the Acuson 128 machine and using a Doppler principle similar to measurement of SMABF. Measurements were made after visualisation of the portal vein on its long axis using an intercostal approach, as has been described previously (Brown et al, 1989). The sample volume cursor was positioned at the centre of the lumen of the vein midway between the confluence of the splenic and superior mesenteric veins and the division of the portal vein into left and right hepatic branches. PBF was calculated using the same formula as described before.

Blood was collected for basal levels of plasma noradrenaline, adrenaline, renin activity (PRA), angiotensin II (All), insulin, electrolytes (sodium and potassium) and glucose. Blood samples (20 mls on each occasion) after captopril or placebo were collected in tubes stored in ice until centrifugation at 4°C for separation of plasma.
All assays were carried out as previously described.
AII was measured by solid phase radioimmunoassay (Eurodiagnostics Angiotensin II kit) following ethanol extraction. The intra-assay and inter-assay coefficients of variation were 5% and 8% respectively.

**PROTOCOL:**

After baseline measurements and blood collection, subjects were given either oral captopril (50 mg, Capoten, Squibb) or placebo (50 mg, vitamin C, Boots) randomly on separate days, at least one week apart in a single blind fashion. The tablets were swallowed in the supine position with 50 ml of water. Measurements (except BP and HR) and blood collection (except AII and electrolytes) were made at 30 min intervals for 120 min. Blood samples for plasma AII and electrolyte measurements were taken at 60 min intervals. Subjects were then tilted head-up at 45° for 10 min and measurements and blood collection were repeated during tilt. Head-up tilt was reversed in some subjects who felt dizzy and lightheaded and had to be returned to the horizontal.

**STATISTICAL ANALYSIS:**

Data are presented as means ± SEM. Analysis of variance (Minitab data analysis software, Minitab Inc, 1989) and area under the curve using the method of summary measures were used for data analysis (Matthews et al, 1990). Multiple t tests and Mann-Whitney U test were performed on means at 0, 30, 60, 90, 120 min and during head-up tilt to further characterize significant differences. P<0.05 was considered significant.

Variability of the various measurements were obtained, based on basal readings on the two study days, by dividing the standard deviation by the mean. Variability is then expressed as the mean coefficients of variability. The coefficients of variation were 7.4% for CI, 7.2% for FBF, 11.4% for DSBF, 7.3% for SMABF and 12.2% for PBF.
RESULTS: A. Haemodynamic measurements:

Systolic, Diastolic and MABP: After captopril, there were no significant changes in systolic BP though there was a small but insignificant fall in diastolic and MABP (Fig 11.1). During head-up tilt, systolic and diastolic BP tended to fall, though not significantly (115±3 to 105±6 systolic and 65±2 to 60±2 diastolic at 120 min). MABP also was lower during head-up tilt (82±2 to 75±3, P=NS, Fig 11.1). There was a fall in MABP in three out of the four subjects who felt faint when tilted after captopril. In two of these subjects MABP was lower while supine at 120 min, after captopril.

After placebo, there were no changes in systolic, diastolic and MABP while supine and during head-up tilt (Fig 11.1).

Diameter of the SMA: Resting mean diameter of the SMA was 7.4±0.09 mm during captopril and 7.3±0.07 during placebo. After captopril, diameter of the SMA increased at 60 min (7.7±0.1), 90 min (7.8±0.09) and 120 min (7.8±0.09) (each P < 0.05, Fig 11.2). After placebo, SMA diameter remained unchanged. During head-up tilt, diameter of the SMA fell after captopril (7.8±0.1 to 7.1±0.1) and after placebo (7.3±0.1 to 6.5±0.1)(each P < 0.05, Fig 11.2).

SMABF and SMAVR: Resting SMABF was 430±22 ml/min on the captopril phase and 432±21 on the placebo phase. After captopril, SMABF rose at 30 min (430±22 to 554±45, P < 0.05), at 60 min (609±33), 90 min (700±40,) and 120 min (715±60, each P < 0.001, Fig 11.3). SMABF fell during head-up tilt (715±60 to 353±25, P < 0.001) as has been shown to occur in previous studies, in normal man (Ray-Chaudhuri et al, 1991). In two out of the four subjects who felt faint after captopril, and in whom MABP was lower while supine, the diameter of SMA and SMABF increased at 90 and 120 min.

There was a corresponding fall in calculated SMAVR after captopril, at 30 min (0.19±0.01 to 0.15±0.01 units), 60 min (0.13±0.006), 90 min (0.12±0.009)
and 120 min (0.12±0.01) (each P < 0.001, Fig 11.3). During head-up tilt, SMAVR rose (0.12±0.01 to 0.22±0.01, P < 0.001).

After placebo, SMABF and SMAVR were unchanged except during head-up tilt, when SMABF fell (460±26 to 273±15 ml/min, P < 0.001) and SMAVR rose correspondingly (0.19±0.01 to 0.32±0.02 units, P < 0.001, Fig 11.3).

**PI:** Resting PI was 322±13% during captopril and 314±13 during the placebo phase. After captopril, PI of the SMA velocity waveform fell due to increased diastolic flow in the SMA, confirming vasodilatation. PI fell at 30 min (322±13 to 267±17%), 60 min (265±19), 90 min (246±18) and 120 min (250±20) (each P < 0.01, Fig 11.4) after captopril. After placebo, PI of the SMA velocity waveform was unchanged (Fig 11.4). During head-up tilt, PI remained unchanged after captopril (250±20 to 220±17) and placebo (330±20 to 300±25) (each P=NS).

**PBF:** Resting PBF was 536±26 ml/min during captopril, and 586±42 during the placebo phase. After captopril, PBF rose at 30 minutes (536±36 to 743±78), 60 min (727±77), 90 min (742±65) and 120 min (719±75) (each P < 0.05, Fig 11.5). After placebo, changes in PBF were not significant (Fig 11.5). Measurement of PBF was not made during head-up tilt owing to difficulty in visualisation of the portal vein.

**HR:** After captopril and placebo, HR was unchanged except during head-up tilt when it rose after captopril (69±3 to 84±5 b/min) and after placebo (71±4 to 86±3) (each P < 0.05, Fig 11.6). Heart rate did not rise during head-up tilt in three out of four subjects who felt faint while tilted after captopril.

**CI:** Resting CI was 747±67 units during captopril and 885±82 during placebo. After captopril CI rose at 30 min (885±71), 60 min (875±71), 90 min (925±76) and 120 min (965±72) (P < 0.05 at 120 min, Fig 11.6). CI fell during head-up tilt (965±72 to 631±89, P < 0.01, Fig 11.6). After placebo, changes in CI were not significant except during head-up tilt when CI values were lower (843±86 to 644±65, P=0.06).
**FBF and FVR:** After captopril, FBF rose at 30 min (2.9±0.2 to 3.1±0.2 ml/100ml/min), 60 min (3.3±0.3), 90 min (3.4±0.3) and 120 min (3.8±0.2)(P < 0.01 at 120 min, Table 11.1). FBF fell during head-up tilt (3.8±0.2 to 2.1±0.3, P < 0.001). There was a corresponding fall in FVR after captopril which was significant at 120 min (28.6±2.2 to 22±1.1 units, P < 0.05, Table 11.1). FVR rose during head-up tilt (22±1.1 to 42.5±6.7, P < 0.05).

After placebo, changes in FBF and FVR were not significant except during head-up tilt when FBF fell with a corresponding rise in FVR (Table 11.1).

**DSBF and DSVR:** Changes in DSBF and DSVR were not significant after captopril or placebo though there was a trend towards a higher DSBF and lower DSVR after captopril (Table 11.1). DSBF fell during head-up tilt after captopril and placebo with a corresponding rise in DSVR (Table 11.1).

### B. Biochemical measurements:

**PRA:** Basal values of PRA were 2.6±0.3 ng/ml/hr during captopril and 3.0±0.5 during placebo. After captopril, PRA rose at 30 min (2.6±0.3 to 19.6±8.4), 60 min (23.8±6.7), 90 min (25.6±9.2) and 120 min (18.2±7)(each P < 0.05, Fig 11.7). During head-up tilt, changes in PRA were not significant (Fig 11.7). After placebo, there were no changes in PRA while supine and during head-up tilt (Fig 11.7).

**All:** Basal All levels were 95±11 pg/dl during captopril and 80±15 during placebo. After captopril, All fell at 60 min (96±11 to 64±10) and at 120 min (63±10, each P < 0.05, Fig 11.7). During head-up tilt changes in All were not significant. After placebo, All levels were unchanged while supine and during head-up tilt.

**Noradrenaline (NA) and Adrenaline:** Plasma NA levels were unchanged after captopril and placebo, while subjects were supine (Table 11.2). During head-up tilt, plasma NA tended to rise after captopril (483±116 to 755±151 pg/ml) though this was not significant (P=0.1). However, in three out of four subjects who felt faint while tilted after captopril, plasma NA did not rise appreciably.
After placebo, during head-up tilt, NA rose (323±32 to 548±66, \( P < 0.05 \), Table 11.2).

**Insulin, Glucose and Serum Electrolytes:** Changes in plasma insulin, glucose and electrolyte levels were not significant after captopril or placebo (Table 11.2).

**Symptoms:** All subjects were asymptomatic while supine, after captopril or placebo. During head-up tilt, four subjects felt faint after captopril. These symptoms were rapidly reversed on return to the horizontal position.
Fig 11.1: Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) in normal subjects before (0) min and 30, 60, 90, 120 min and during head-up tilt after captopril or placebo ingestion.
Fig 11.2: Changes in the diameter of superior mesenteric artery (SMA) before (0) and 30, 60, 90, 120 min and during head-up tilt after captopril and placebo ingestion in normal subjects.
Table 11.3: Changes in superior mesenteric artery blood flow (SMABF, upper panel) and vascular resistance (SMAVR, lower panel) in normal subjects before (0) min and 30, 60, 90, 120 min and during head-up tilt after captopril (continuous line) and placebo (interrupted line) ingestion.
Table 11.4: Changes in pulsatility index (PI) of superior mesenteric artery blood velocity waveform before (0) min and 30, 60, 90 and 120 min after captopril and placebo ingestion in normal subjects.
Fig 11.5: Changes in portal venous blood flow (PBF) before (0) min and 30, 60, 90 and 120 min after captopril or placebo ingestion in normal subjects.
Fig 11.6: Changes in heart rate (HR) and cardiac index (CI) before (0) min and 30, 60, 90, 120 min and during head-up tilt after captopril or placebo ingestion in normal subjects.

\* = p<0.05
Fig 11.7: Changes in plasma renin activity and angiotensin II levels before (0) and at 30, 60, 90, 120 min and during head-up tilt after captopril or placebo ingestion in normal subjects.

* = p<0.05
<table>
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<tr>
<th></th>
<th>0</th>
<th>30</th>
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<tr>
<td>c</td>
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<td>3.3±0.2</td>
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<tr>
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<td>62±12°</td>
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<td>18±2</td>
<td>18±2</td>
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<tr>
<td><strong>DSVR</strong> (units)</td>
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<tr>
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Table 11.1. Changes in forearm blood flow (FBF), forearm vascular resistance (FVR), digital skin blood flow (DSBF) and digital skin vascular resistance (DSVR) before (0), at 30, 60, 90, 120 min and during head-up tilt (T) after captopril (c) or placebo (p) ingestion in 12 healthy subjects. ° = P<0.05.
<table>
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<td></td>
<td>p</td>
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<tr>
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<td></td>
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<tr>
<td>(mUnits/l)</td>
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<tr>
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<td>5.3±0.9</td>
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Table 11.2: Plasma electrolyte and hormonal responses before (0) and 30, 60, 90, 120 min and during head-up tilt (T) after captopril (c) or placebo (p) ingestion in 12 healthy subjects. ⊹ = P<0.05.
In this study, a marked increase in SMABF and PBF in normal subjects has been demonstrated after ACE inhibition by captopril, which caused a significant rise in PRA levels and a fall in plasma AII levels. There were no other significant biochemical changes, particularly in plasma catecholamine levels, after captopril. After captopril there was also a rise in FBF and cardiac output with a trend towards a lower BP, particularly during head-up tilt. The results of direct non-invasive measurement of SMABF and PBF after captopril in this study differ from previous reports of invasive and indirect measurement of splanchnic vascular responses to captopril in man. Studies in heart failure, hypertensive or cirrhotic patients after captopril have indicated either no change or a decrease in hepatic blood flow measured by the indocyanine green clearance method, although some observed a reduction in splanchnic vascular resistance and hepatic venous wedge pressure (Faxon et al, 1984; Ventura et al, 1985; Crossley et al, 1984; Eriksson et al, 1984). The indocyanine green clearance method, however is dependent on hepatic metabolism and clearance and is an indirect measure of hepatic blood flow, which is the sum of hepatic arterial and portal venous flow. Hepatic extraction of indocyanine green can be reduced by 20% in disease states such as cirrhosis and congestive cardiac failure, thus resulting in errors in measurement (Caesar et al, 1961). This may explain the variable and contradictory results in such studies, where direct measurement of splanchnic blood flow were not made. As discussed in chapters 2 and 3, potential errors in measurement of SMABF may occur due to a high angle of insonation or error in the measurement of diameter of the SMA. Measures taken to account for these inaccuracies included keeping the angle of insonation between 20°-45° and using a regional expansion system to facilitate measurement of the diameter of the SMA. Additionally, the PI of the SMA blood velocity waveforms, a reliable indicator of changes in downstream resistance in the SMA, was also measured. The fall
in PI after captopril was probably due to vasodilatation causing an increase in diastolic flow in the SMA as has also been observed after food ingestion (Qamar et al, 1986b). The reason for the lack of change in PI during head-up tilt is unclear and may be due to underestimation of PI values during tilt due to a fall in cardiac output, as cardiac output influences the shape of blood velocity waveforms and thus PI. This is consistent with results of studies in chapter 4.

Measurements of gastro-intestinal venous outflow were made in the portal vein. There was a rise in PBF after captopril further indicating vasodilatation in the splanchnic vascular bed. This is similar to the 32.2% rise in portal blood flow observed in a study using a Dopplerian flowmeter, after a single 25 mg dose of captopril, in normal subjects (Tesar et al, 1988).

The mechanisms responsible for splanchnic vasodilatation after captopril need discussion. A major factor is likely to result from inhibition of ACE by captopril as indicated by a rise in circulating renin activity and a fall in plasma AII. In animal studies, infusion of exogenous AII causes marked mesenteric vasoconstriction which can be abolished by captopril (Gardiner et al, 1988; Bulkley et al, 1985). Recent evidence also indicates that locally generated AII from the vascular wall of perfused rat mesenteric arteries is interlinked with vascular production of endothelin, a potent vasoconstrictor (Goldschmidt & Tallarida, 1991). Thus, local vascular inhibition of AII by captopril may have also contributed to splanchnic vasodilatation. Captopril however also has other actions and further studies using specific AII antagonists will be needed to further define the role of angiotensin II on the splanchnic vascular bed in man.

Captopril however, has effects on other vasoactive substances and autacoids. In animal studies, captopril and its isomer causes a selective superior mesenteric vasodilatation (Muller et al, 1990). This may be related to its thiol group, which may augment endothelial cell-derived nitric-oxide, either by scavenging superoxide radicals or by interacting with the dynamics of the L-arginine-nitric oxide system (Westlin & Mullanc, 1998; Gardiner et al, 1990; Muller et al,
Captopril is also known to augment the release, and prevent breakdown, of the vasodilator bradykinin, which may release prostacyclin or endothelium-derived relaxing factor (EDRF), which are potent vasodilators (Gardiner et al, 1990; Vanhoutte et al, 1989). In rats, infusion of bradykinin has been shown to increase mesenteric blood flow (Gardiner et al, 1992). In addition, a calcium channel blocking action and an endothelium dependent relaxation of blood vessels due to release of EDRF induced by shear stress also has been proposed (Vanhoutte et al, 1989; Jeremy et al, 1990). In this study, there was a significant increase in the diameter of the SMA after captopril and it is possible that the latter mechanism played a part.

Circulating or locally formed AII inhibits the neuronal uptake, and facilitates the exocytotic release of noradrenaline which leads to augmented adrenergic neurotransmission and vasoconstriction (Clough et al, 1982). This response of blood vessels to sympathetic nerve stimulation is depressed by captopril (and its direct inhibitory effect on the alpha adrenoceptor activation), both in vitro and in vivo Vanhoutte et al, 1989; Clough et al, 1982; De Jonge et al, 1981). It may be argued, that in this study, the splanchnic vasodilation after captopril was due to such effects, especially as the sympathetic nervous system is known to influence this vascular bed. In the present study however, there were no changes in plasma catecholamine levels after captopril. Furthermore, during head-up tilt the SMA constricted and plasma NA rose in the majority of subjects. This indicated preservation of these sympatho-neural responses in this vascular bed. It is therefore unlikely that sympatho-inhibition played a part in the splanchnic vasodilation.

In this study, forearm blood flow and cardiac output rose after captopril as has been previously reported (Ventura et al, 1985; Banas 1992). There was a small but insignificant rise in cutaneous blood flow. It is presumed that captopril caused renal vasodilatation as previously described, and maintained cerebral blood flow, as has been reported even with a lower BP (Banas 1992; Paulson et
In this study systemic BP was maintained in the majority, despite vasodilatation in skeletal muscle, splanchnic, cutaneous and, we assume, the renal vascular beds. This maintenance of BP may have been due to a compensatory rise in cardiac output, though this was probably inadequate in some subjects, in whom BP tended to be lower after captopril particularly during head-up tilt, during which there could have been a further translocation of blood into dependent veins. The marked fall in BP in three out of the four subjects who felt faint when tilted after captopril, could thus be due to a combination of vasodilatation in splanchnic and other region and through the activation of the Bezold-Jarisch reflex (Mark, 1983). This reflex results in hypotension and bradycardia and has been proposed as a mechanism for the fall in BP in heart failure patients after the first dose of captopril (MacFayden et al, 1991). In the three subjects who fainted, heart rate did not rise and BP fell, suggesting that the Bezold-Jarisch reflex could have played a part in this response.

The possible clinical implications of these findings will now be considered. Studies in chapter 8 indicated a selective increase in SMAVR in patients with essential hypertension. Reversal of these changes by captopril may be a major haemodynamic mechanism to lower BP although this has not yet been studied. Furthermore, after captopril, there is no interference with the sympathoneural control of this large vascular bed, where lack of control may contribute to postural hypotension, as demonstrated in patients with sympathetic failure (Ray-Chaudhuri et al, 1992). This may explain the lack of postural hypotension by captopril and other ACE-inhibitors, when given chronically. Acute ingestion of the drug however may cause hypotension, especially in elderly subjects (Reid, 1987), and the mechanisms include activation of the Bezold-Jarisch reflex (MacFayden et al, 1991), although vasodilatation in splanchnic and other regional vascular beds may be contributory. Captopril is also used in patients with heart failure who often have a higher sympathetic tone and levels of
renin/AII which could increase splanchnic vascular resistance. Vasodilatation in the splanchnic region (which normally contains up to 30% of total blood volume) could contribute to reducing after-load and may be a further mechanism to explain the beneficial action of captopril in heart failure.

CONCLUSIONS:
It is concluded that acute ingestion of the ACE-inhibitor captopril causes a marked increase in SMABF and PBF along with a reduction in SMAVR, indicating splanchnic vasodilatation. This may be due to inhibition of AII formation by captopril, although other factors may be contributory. Vasodilatation in the large splanchnic region may contribute to the beneficial effect of captopril in hypertension and congestive cardiac failure. This study also indicates the possible role played by angiotensin II in the control of this vascular bed.
CHAPTER 12;
SUMMARY AND OVERALL CONCLUSIONS:

(A) SUMMARY:

The importance of the splanchnic circulation in the reflex control of the cardiovascular system and in particular blood pressure control has been documented in animal studies. As indicated previously, the invasive nature of the methods needed to measure splanchnic blood flow in humans and associated methodological problems have eluded documentation of the neural and hormonal control of this major vascular bed under physiological and pathophysiological states. The emergence of the non-invasive Doppler ultrasound method of measuring haemodynamic changes within the major splanchnic vessels such as the superior mesenteric artery has added a new dimension to the understanding of the role of the splanchnic circulation in human gastro-intestinal and cardiovascular physiology.

In the initial study (chapter 3), the use of a Doppler ultrasound method for measuring SMABF in normal healthy volunteers of differing age groups are described. With proper precautions and attention to detail this method was shown to provide a reliable, reproducible and direct measurement of SMABF, an important constituent of the splanchnic vascular bed.

In the following study (chapter 4), using this method, short term dynamic changes in SMABF during sympathetic activation could be measured for the first time, in normal subjects. This study indicated that the sympatho-neural control of the splanchnic circulation probably plays a major role in blood pressure control in normal subjects. Sympathetic activation by pressor stimuli raised blood pressure and caused active vasoconstriction of the SMA while during head-up tilt blood pressure was maintained by vasoconstriction in the SMA. Furthermore, this study indicated that the Doppler ultrasound method
was sensitive enough to measure shortlasting changes within the splanchnic vascular bed during various physiological stimuli.

The neural control of the SMA was investigated further by studies in patients with confirmed primary autonomic failure who form an unique human model of sympathetic denervation. Studies described in chapter 6 indicated that in primary AF, sympa-tho-neural stimuli in the form of pressor tests failed to vasoconstrict the SMA and raise blood pressure unlike in normal subjects. During head-up tilt, absence of vasoconstriction of the SMA was probably responsible for the disabling postural hypotension seen in these patients. Measurements in other vascular beds were also made and did not show marked differences in normal subjects and patients with AF. This further outlined the importance of the splanchnic vascular bed and the integrity of the sympathetic nervous system in overall blood pressure control in humans.

A series of studies were then undertaken, described in chapters 7 and 8, to evaluate SMA vascular responses during sympatho-inhibition by infusion of the \( \alpha_2 \) adrenoceptor agonist, clonidine. In previous studies, clonidine has been shown to cause hypotension through its central sympatholytic action. Its effects on the entirely sympathetically innervated SMA, in humans, were however unknown. In normal subjects clonidine caused hypotension and vasodilatation of the SMA along with a fall in cardiac output. In patients with AF, however, clonidine caused a differential response. In those with a predominantly peripheral sympathetic failure (PAF), clonidine failed to lower blood pressure or dilate the SMA. In patients with a predominantly central sympathetic failure (MSA), clonidine lowered blood pressure and actively dilated the SMA. This study, for the first time in humans, indicated that clonidine induced splanchnic vascular responses may distinguish between the two forms of primary AF which may be difficult clinically. In the same study, the neurohormonal effects of clonidine in normals and AF patients were also studied. While clonidine increased growth hormone levels in PAF, it failed to
do so in MSA suggesting a defect in the central neuronal release mechanism of growth hormone by clonidine in MSA. The growth hormone response to clonidine thus emerged as a possible neuroendocrine marker to help differentiate between the two forms of primary AF.

Although the role of splanchnic circulation in autonomic failure was investigated in these studies, the haemodynamic changes in the splanchnic vascular bed in the reverse situation, that is hypertension, is unknown. In essential hypertension, evidence is growing which implicate sympathetic nervous system hyperactivity. As the splanchnic vascular bed is entirely and functionally innervated by the sympathetic nervous system it is conceivable that the splanchnic circulation contributes substantially to the pathogenesis of hypertension. Studies in chapter 8 were therefore undertaken to evaluate the SMA vascular responses during sympatho-inhibition by clonidine in humans. Basal measurement of SMAVR was significantly higher in hypertensives along with a higher cardiac output. Clonidine lowered blood pressure and dilated the superior mesenteric artery in hypertensives along with a fall in cardiac output. This was probably due to the central sympatholytic action of clonidine as mild sedation and rise in growth hormone levels occurred in all subjects. Changes in muscle and cutaneous blood flow were not significant after clonidine and the results suggested that, in essential hypertension, a higher sympathetic tone possibly contributes to hypertension by causing splanchnic vasoconstriction.

In animal studies, the splanchnic circulation has been shown to be affected by various hormones and peptides. In man there is sparse data although agents such as vasopressin are known to have powerful effects on the splanchnic circulation and this forms the basis of the use of vasopressin in the treatment of bleeding oesophageal varices in portal hypertension. In the next series of studies the effect of various pharmacological agents on the splanchnic vasculature in humans was investigated in a clinical context. Acute alcohol ingestion is associated with signs suggestive of cerebral ischaemia in patients
with AF. Studies in chapter 9 compared the effect of alcohol and placebo ingestion on SMABF and other regional circulation in normal subjects and AF patients. Alcohol dilated the SMA in both groups but while blood pressure was maintained in normals there was supine hypotension and worsening of postural hypotension in AF. As changes in the other vascular beds were not significant, the hypotensive effect of alcohol in AF was likely to be due to a direct splanchnic vasodilatory effect. Studies described in chapter 10 showed that using Octreotide, a somatostatin analogue which inhibits the release of various gut peptides and hormones, prevented the hypotension induced by alcohol in AF. This indicated, that, in humans alcohol induced splanchnic vasodilatation was probably also mediated by the release of various gut hormones and peptides. These studies indicated the importance of humoral control of the splanchnic circulation in overall blood pressure control in humans.

The concluding study in this thesis investigates the role of angiotensin converting enzyme inhibition in the control of splanchnic circulation. Studies in normal subjects were undertaken with haemodynamic and biochemical measurements before and after an oral dose of captopril (an angiotensin converting enzyme inhibitor) or placebo. After captopril, SMABF and FBF rose markedly and the blood pressure was maintained while supine, although, during head-up tilt, blood pressure tended to be lower leading to pre-syncopal symptoms in some volunteers. FBF also rose but to a lesser extent. There was a rise in PRA levels along with a fall in plasma AII levels suggesting that adequate suppression of the renin-angiotensin system occurred after captopril. The maintenance of blood pressure after captopril despite of splanchnic vasodilatation could be partially explained by a raised cardiac output induced by captopril. This study, for the first time, showed that ACE inhibition by captopril has major effects on the splanchnic vascular bed and suggested an important role of angiotensin II in the haemodynamic control of the splanchnic vascular bed which has been
previously suspected but not documented. Studies are now under way to investigate the splanchnic vascular responses after captopril in hypertensives.

(B) RESEARCH AND CLINICAL IMPLICATIONS:

1. The Technique:

Studies described in the initial chapters of this thesis indicate that the non-invasive measurement of superior mesenteric artery blood flow by Doppler ultrasound is reliable, reproducible and easily applicable to a wide range of physiological and patho-physiological conditions. With proper attention to detail and appropriate precautions to avoid technical errors (discussed in chapter 2) this method will become invaluable in the understanding of gastrointestinal and splanchnic vascular physiology in normal subjects and various disease conditions. The Doppler ultrasound method of measuring blood flow within the major splanchnic vessels has already been used as part of an intestinal stress test to aid diagnosis of mesenteric ischaemia. Studies in this thesis indicate that this method may also help to differentiate between types of primary autonomic failure and monitor the responses to treatment of postural and post-prandial hypotension which occurs commonly in primary AF as well as in Parkinson's disease.

2. Clinical Implications:

A. Autonomic Failure:

The clinical implications of these studies are multiple. The initial studies, described in this thesis, indicate the major role played by the sympathetic nervous system and splanchnic vascular bed in the overall blood pressure
control and cardiovascular homeostasis in normal subjects. In patients with sympathetic denervation such as primary AF, abnormalities of splanchnic in particular SMABF responses are probably primarily responsible for severe postural hypotension. Therapies aimed towards vasoconstriction of the splanchnic vascular bed could therefore be useful in treatment of postural hypotension. Indeed, vasopressin and somatostatin, used successfully to treat disabling postural hypotension in these patients are potent splanchnic vasoconstrictors. Preliminary studies (Stevens et al, 1991) suggest that a similar abnormality of splanchnic vascular responses may also occur in other causes of AF such as diabetes mellitus and possibly amyloidosis.

The differentiation of various forms of primary AF in the early stages is often difficult as MSA patients may initially present with features of PAF. Patients with early MSA may also be difficult to differentiate from Parkinson’s disease. Results of studies described in chapter 7 indicate that the growth response to clonidine may serve as an important neuro-endocrine marker to separate the two groups of AF in the early stages. The test is simple, may be performed on an outpatient basis and may also be applicable in differentiating patients with PD from early MSA. Similarly the hypotensive and splanchnic vascular responses to clonidine may also serve in differentiation of PAF and MSA patients, there being no fall in BP or SMA vasodilatation after clonidine in PAF. Early differentiation is important as the prognosis, management and complications are markedly different in the two groups.

Food is a potent hypotensive agent in patients with primary AF and studies in chapter 9 shows that even mild quantities of alcohol causes supine hypotension and worsens postural hypotension in these patients. As food and alcohol are often consumed together the hypotensive action of alcohol may be compounded leading to cerebral or coronary ischaemia if cerebrovascular or coronary disease is coexistent. Furthermore, alcohol or food induced syncope may be the
first presenting feature of patients with PAF or MSA, and the diagnosis may be
delayed if a thorough history in relation to alcohol/food ingestion is not
elicited. If alcohol induced hypotension is documented in these patients advice
regarding postural change after alcohol may be beneficial. Thus type of alcohol
to drink (beers with high carbohydrate content preferably avoided while red
wine containing tyramine, a vasoconstrictor, ) and timing of drugs used to
treat postural hypotension is important. Drug treatment is particularly
valuable to the management of postural hypotension as unrecognized post­
prandial or post-alcohol hypotension may negate the benefit from therapy
aimed towards treating postural hypotension. Studies in chapter 10 and
previous work from the same laboratory (Kooner et al, 1989) indicate that
Octreotide, by causing splanchnic vasoconstriction, could be an effective
measure to counteract post-prandial and post alcohol hypotension in AF.

B. Essential Hypertension:

The importance of the splanchnic vascular bed in essential hypertension is also
discussed in the present studies. Sympathetic overactivity is probably
responsible for a raised splanchnic vascular resistance in hypertensives and use
of sympatho-inhibitory drugs such as clonidine may reduce blood pressure by
causing dilatation within the splanchnic vascular bed. However, other central
side effects of clonidine such as drowsiness and dry mouth limits the usefulness
of this approach. The entirely sympathetic innervation of the splanchnic
vascular bed also implies that antihypertensive drugs acting principally
through sympathto-inhibition may interfere with the normal sympathto-neural
activation during postural change and unmask unacceptable postural
hypotension.
The role of ACE inhibition, a widely used antihypertensive approach, is also important in splanchnic vascular control. Captopril, the most widely used and studied ACE inhibitor causes marked splanchnic vasodilatation in normal subjects (chapter 11) and this, presumably, is a major mechanism of its antihypertensive action. During head-up tilt, which causes sympathetic activation, captopril did not prevent splanchnic vasoconstriction in these subjects, suggesting that it exerts its effect principally through the renin-angiotensin system.

Thus antihypertensives, which cause splanchnic vasodilatation independently of sympatho-inhibition may be particularly useful as they do not cause marked postural hypotension.

(C) FUTURE PROSPECTS:

The studies described in this thesis open up a number of areas for further research. The importance of the splanchnic vascular bed in overall cardiovascular control in normal subjects and primary AF has been established. However, in humans, the possible importance of the splanchnic vascular bed in other causes of autonomic failure such as diabetes mellitus and chronic alcoholism remains largely unknown. This needs to be pursued as a non-invasive method for assessing splanchnic circulatory changes is now available and may substantially help the management of autonomic failure in these patients. For example, studies evaluating postural and post-prandial changes in diabetics and effects of antihypertensive therapy on splanchnic blood flow in those with coexistent hypertension should be performed.

Similarly studies in other disorders such as amyloidosis and connective tissue disease where autonomic dysfunction occurs are necessary to define whether
abnormal splanchnic circulatory responses lead to impaired cardiovascular homeostasis.

As patients with MSA are often mistaken for Parkinson's disease, similar studies in PD are already being undertaken. This may yield valuable information which will help the management of PD patients who may suffer from autonomic dysfunction either due to the drugs or disease process. The growth hormone response to clonidine and its ability to differentiate between MSA and PD should also be investigated.

In patients with multiple sclerosis, demyelination occurs in a scattered fashion throughout the nervous system. Autonomic dysfunction has been recorded in MS previously and preliminary studies from our laboratory (Thomaides et al, 1992) suggest that abnormality of SMABF blood flow and growth hormone responses to clonidine occur in secondary progressive MS. Further studies are required to establish the exact nature of these abnormalities and their influence on the symptoms of MS.

Finally, in essential hypertension, the effect of various drugs on the splanchnic vascular bed can now be studied noninvasively. Selective splanchnic vasodilators may play an important role in hypertension management and should be investigated. Central α² adrenoreceptor agonists such as rilmenidine have been introduced and may have the beneficial effects of clonidine without the central side effects. Similarly the effect of newer ACE inhibitors such as quinapril or fosinopril on the splanchnic vascular bed need to be documented. The role of the splanchnic circulation in heart failure remains largely unknown and is an area where there is scope for extensive research, particularly in relation to the effect of vasodilators and drugs causing selective splanchnic vasodilatation.
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