

AN EXAMINATION OF THE MEASUREMENT OF MEAN
CIRCULATORY FILLING PRESSURE IN
THE ANAESTHETIZED RAT

CENTRE FOR NEWFOUNDLAND STUDIES

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MEASUREMENT OF MEAN
CIRCULATORY FILLING PRESSURE
IN THE ANAESTHETIZED RAT

By

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in partial fulfilment of the requirements for the degree of
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ABSTRACT

The measurement of mean circulatory filling pressure (MCFP) is widely used in research, yet the basic assumption of equal pressures throughout the body during MCFP measurement has not been adequately tested. Therefore, this study examined whether there is pressure equilibrium between tributary veins and the central vena cava during the MCFP manoeuvre. The time course needed for pressure equilibrium was also examined. Pressures in the iliac artery, the hepatic portal vein (HPVP), the renal vein (RVP) and the inferior vena cava (IVCP) were determined at 4-second intervals over a 20-second period of circulatory arrest, produced by inflating a right atrial balloon, under the conditions of normal blood volume (NBV), 10% blood volume depletion (-10% BV) and 10% blood volume expansion (+10% BV) in urethane-anaesthetized rats. HPVP was found to be higher than IVCP during the full period of arrest at -10% BV and during the first 16 seconds of arrest at +10% BV. At NBV, HPVP was significantly higher than IVCP only at the 4th second of the arrest. RVP was virtually equal to IVCP in the three volume states. At the 8th second of the arrest in -10% BV, HPVP was 6.2 ± 0.8 mmHg and IVCP was 3.4 ± 0.2 mmHg; in +10% BV, HPVP was 7.7 ± 0.5 mmHg and IVCP was 6.2 ± 0.4 mmHg, differences between HPVP and IVCP being significant ($p < 0.01$ for each of the comparisons). To test if this pressure

disequilibrium resulted from reflex vasoconstriction, the MCFP manoeuvre was carried out following ganglionic blockade with hexamethonium and atropine. HPVP was found still to be significantly higher than IVCP for 12 seconds in both -10% and +10% BV conditions. It is concluded that during conditions of rapid blood volume alterations, HPVP is significantly higher than IVCP during the MCFP manoeuvre; physical transhepatic resistance at zero inflow appears to play a role in the constitution of this pressure disequilibrium.

Keywords: mean circulatory filling pressure (MCFP)
hepatic portal venous pressure
blood volume alteration
ganglionic blockade
anaesthetized rats

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Chapter 1

INTRODUCTION

1.1 Definition

Adequate circulation is a primary requirement for survival, and proper filling of the vascular beds with blood is a prerequisite for adequate circulation. The degree of filling of the total cardiovascular system with blood can be determined through measuring mean circulatory filling pressure (MCFP). MCFP has been commonly defined as the pressure that one would be able to measure at every point throughout the entire cardiovascular system if the heart was suddenly stopped, and the blood was immediately redistributed in such a way that all pressures in the cardiovascular system were equal (Guyton et al, 1973; Rothe, 1983a, 1983b).

1.2 Chronology of MCFP determination

The concept of MCFP originated about 100 years ago. Its conceptual evolution and technical development can be divided into three periods. Initially, MCFP studies explored the possible mechanisms responsible for the development of cardiac failure. The second period was occupied by studies where MCFP measurement was utilized to explain the regulation of cardiac output in various physiological and pathophysiological situations. Currently MCFP measurement is

used pragmatically to investigate certain changes in circulatory status.

1.2.1 The early development of MCFP measurement

While examining the haemodynamic features of various heart diseases and the cause of cardiac failure, Starling (1896,1897) hypothesized that there was a mean systemic pressure at the neutral pressure points on the venous side. The establishment of the mean systemic pressure theory had considerable influence on understanding heart failure and the development of relevant symptoms and signs. He reasoned that, if the circulation had been stopped and the blood had been at rest in the entire system, and if all sections of the circulatory system had been arranged in one plane, the pressures in all parts of the system must be equal. He called this equalised pressure mean systemic pressure and claimed it was about 10 mmHg in a large dog. He further speculated that, as the circulatory system was a continuous, closed circuit and each of its parts had a limited range of pressure alterations, there must be a neutral point somewhere in the circulatory circuit at which the pressure was neither raised, nor lowered during rhythmic contractions of the heart. In other words, the pressure at the neutral point was independent of cardiac activity and was equal to the mean systemic pressure.

Clearly, these statements were useful to explain the

clinical pictures of heart failure, especially the development of cardiac oedema. Comparing Starling's mean systemic pressure with MCFP defined in the early part of this thesis, one immediately notices that the two pressures are conceptually similar. Later, studies assigned the names of hydrostatic mean pressure (Bolton, 1903) and static blood pressure (Starr & Rawson, 1940; Starr, 1940). Likewise, all these studies were directed to the circulatory disturbances caused by various heart diseases.

1.2.2 The mid-stage of MCFP measurement

Beginning in the 1950s, Guyton and his colleagues put MCFP research in the setting of integrated cardiac output regulation and introduced the current MCFP concept. Much of their efforts went into understanding the multilateral relationships among right atrial pressure, venous return, MCFP and left ventricular output. One of the principal outcomes of their intense investigations is what we now call the Guytonian relationship; that is, cardiac output and venous return equilibrate at a specific right atrial pressure and MCFP is a major determinant of cardiac output. (Guyton, 1955, 1987; Rothe, 1983a, 1983b). With respect to measuring MCFP, they established a set of well-organized laboratory procedures applicable to determine MCFP in the dog. By using this approach, they confirmed some of Starling's observations and advanced further theories. For instance, they concluded

that total body blood volume and total body vasomotor tone are two primary factors that can affect MCFP values (Guyton et al, 1973). Other extravascular determinants that they found to influence MCFP measurement are abdominal compression (Guyton et al, 1952), positive pressure ventilation (Guyton et al, 1952), muscular contraction (Guyton et al, 1962) and interstitial fluid shift (Guyton et al, 1952).

1.2.3 Recent developments in MCFP applications

Since the 1970s, much of the research involving MCFP measurement has utilized the concept of MCFP and its measurement as a tool to investigate other problems which may be outside cardiovascular physiology. This utilization was pragmatic and mainly based on the theory that MCFP is directly determined by total body blood volume and the total body vascular tone. MCFP value was used, for instance, as an index to estimate the effects of pharmacological agents on total body venous tone (Pang & Tabrizchi, 1986), the effects of a totally artificial heart on the resistance to venous return (Honda et al, 1976) and to assess total blood volume states (Rocha E Silva et al, 1987). Another development of MCFP measurement in recent years was the use of MCFP determination in investigating total body vascular compliance and this is to be discussed in this thesis elsewhere (Section 1.4.3 Applications of MCFP Measurement; Section 4.5 Possible Influence of the Pressure Disequilibrium to the

Application of MCFP Measurement).

1.3 Evolution of MCFP measurement techniques

1.3.1 Primitive techniques

According to the available evidence, Starling was the first person to determine MCFP experimentally. The basic techniques he applied were electrical stimulation of the vagus nerve or cardiac tamponade. By stimulating the peripheral end of the vagus or injecting oil into the pericardium to bring the heart to a standstill, he observed that there was significant rise in the inferior vena caval pressure and an acute drop in the femoral artery pressure following this circulatory cessation. Failing to identify an evident pressure change in the portal vein during the cardiac arrest, he then declared that the portal vein was the neutral point of the circulatory system (Bayliss & Starling, 1894; Starling, 1896, 1897). Starling was not the only investigator to determine MCFP by tamponade of the pericardium. A similar approach was used by Bolton (1903), but this time the heart was stopped by clamping the pericardium with forceps instead of oil injection.

In addition, Starr (1940) measured MCFP in the dog and in dead humans. He used the term "static blood pressure" to describe the pressure that "presents throughout the circulation when the heart had stopped". By puncturing a large vein or the right ventricle within 30 minutes following

the clinical death of the patient, or within 5 minutes after dogs were killed, he directly measured pressure and observed significantly higher static blood pressure in both patients who died from congestive cardiac failure and dogs that had received intravenous infusion of 6% acacia and 0.9% NaCl mixture solution. He concluded that this high static blood pressure was caused by plethora or hypervolaemia.

1.3.2 Guyton's technique

Systematic innovation in methodology applicable in determining MCFP occurred in the 1950s. Guyton et al (1952, 1962) developed a method which can be used repeatedly in anaesthetized dogs. Typically, the ventricles are brought to fibrillation electrically, to stop their output; blood is rapidly translocated from the femoral artery to the inferior vena cava to bring the pressures on the arterial and venous sides into equilibrium and the equilibrated inferior vena caval pressure is measured within 7 seconds of the cardiac arrest. The heart is defibrillated electrically immediately after the measurement (Guyton et al, 1973). As this experimental design satisfied some important principles of the MCFP definition, such as stopping circulation suddenly and equilibrating pressure apparently between the arterial and venous beds, it has therefore been accepted as a standard procedure for determining MCFP in the laboratory (Rothe, 1983a, 1983b).

1.3.3 Newly-developed techniques

During recent years, a number of novel, more flexible techniques have been developed. The modification was focused to either lessen the inconveniences associated with electrical ventricular fibrillation, or eliminate the species confines of Guyton's technique. Accordingly, these new techniques fall into the following three categories:

I. A non-electrical heart arrest method

Acetylcholine, when injected as a bolus into a large vein like the superior vena cava or the right atrium, can induce a recoverable cardiac arrest. Results using this method are reproducible in a variety of species including the dog, the calf (Gay et al, 1987, Goldman et al, 1984) and the guinea pig (Davis et al, 1989). Surgical procedures are simplified using this method and the undesired effects of deep anaesthesia are obviated.

Since acetylcholine itself is pharmacologically active, certain studies are required to demonstrate whether MCFP values determined by this method are comparable with those determined by electrical fibrillation. Lee et al (1988) did such a comparison in lightly tranquilized, ganglion-blocked dogs and found that there was no significant difference between MCFP measurements obtained separately from the two methods. A similar conclusion was reported in another study conducted by Gaddis et al (1986). Davis and associates

compared the MCFP determined by the method of acetylcholine injection with that determined by cardiac electrical pacing method in the guinea pig and obtained the correlation coefficient of 0.96 (Davis et al, 1989). Now acetylcholine injection has been accepted as an alternative to electrical fibrillation in determining MCFP and is widely used in research (Algeo et al, 1985; Appleton et al, 1985; Davis et al, 1989; Gay et al, 1987; Goldman et al, 1984; Pan & Young, 1982). However, results must be interpreted with caution in studies where this method is applied for protocols involving other pharmaceutical agents. As acetylcholine can effectively activate cholinergic receptors existing throughout the body, it has the potential to affect circulatory status profoundly, especially in the compliant splanchnic vasculature (Supple & Powell, 1981). There is no information in the literature on interactions between acetylcholine and other pharmacological agents when they are administered concurrently in animals whose MCFP is to be determined. In addition, it has been reported that repeated administration of acetylcholine to arrest the heart could exert a detrimental influence on pulmonary function, causing severe pulmonary congestion and atelectasis. This has been demonstrated in the reflex intact dogs anaesthetized by chloralose-pentobarbital or morphine-pentobarbital (Gaddis et al, 1986), although this effect was not reproducible in ganglion-blocked, pentobarbital-anaesthetized dogs (Lee et al, 1988). It is not clear why

this pulmonary function disturbance occurs only in the reflex intact dog. Further studies focusing on differentiating the combined effects of acetylcholine and anaesthetics from the combined effects of acetylcholine and ganglionic reflexes could help resolve this question as the main differences between these two studies are the uses of different anaesthetics and different animal preparations.

II. Determining MCFP through circulatory arrest

Techniques in this category are characterized by the determination of MCFP under conditions where the systemic circulation is stopped; whereas in the heart, a small portion of blood is not at rest at the time of MCFP determination. Measuring MCFP in this way is easily accomplished with cardiac bypass preparations by clamping the perfusion pump or occluding the pulmonary arteries in large animals (Green, 1975; Rocha E Silva et al, 1987). Samar and Coleman (1978) designed a hydraulic pulmonary artery occluder applicable in the rat and measured MCFP in anaesthetized and conscious rats. Yamamoto and associates (1980) developed an approach to determine MCFP in rats in which momentary circulatory arrest was induced by manual inflation of a balloon previously inserted in the right atrium through cannulation of the jugular vein. This method greatly simplifies the surgical procedures and can be used in either anaesthetized or conscious rats, thus it has become the most common

technique to determine MCFP in the rat (Edmunds et al, 1989; Gay et al, 1986; Pang & Tabrizchi, 1986; Trippodo, 1981).

The disadvantages of using mechanical interventions to stop the circulation, rather than arrest the heart itself, include: possible incompleteness of the circulatory cessation and abnormal blood redistribution in the pulmonary vasculature and the heart while MCFP is being measured. When the circulation is stopped by clamping the blood flowing from the right heart to the left heart, the heart continues to beat. This could result in ischemia in the vessels and cardiac chambers downstream and a volume accumulation in the vessels or cardiac chambers upstream. However, the impact on MCFP measurement of these blood redistributions has not been adequately evaluated. Although Samar and Coleman (1978) claimed that MCFP values measured after pulmonary arterial occlusion did not significantly differ from those measured following simultaneous occlusion of the aorta and the vena cava, it is not clear if this finding can be applied to other experimental models or other species due to its limited sample size (3 observations) and the relatively rarely-used animal model (open-chest rat). Additionally, the volume receptors are located in the right and left atria, so either inflating a right atrial balloon or blood accumulation in the atria can trigger a series of reflexes, as well as additionally releasing atrial natriuretic peptide. Although the majority of atrial reflexes are believed to be directed

towards chronic regulation of blood volume, one cannot rule out the possibility that such atrial disturbances and blood shifts will not influence MCFP values, especially when the MCFP manoeuvre is repeatedly applied over a relatively long period of time. Further comprehensive investigations in this area are required.

III. Measuring MCFP without arterial to venous pumping

Guyton's technique utilizes a pump to facilitate blood translocation from the arterial compartment to the venous compartment; MCFP is then determined at the point of the recording chart where the declining systemic arterial pressure has met the rising vena caval or right atrial pressure. Some investigators consider that the use of a pump to translocate blood is a necessary measure to ensure experimental accuracy (Rothe, 1983a, 1983b). However, Young et al (1980) did not use a pump to translocate blood from the arterial to the venous side in dog experiments. Previous research had demonstrated that in dogs venous compliance was 30 times larger than arterial compliance ($\text{vascular compliance} = \text{changes in vascular blood volume} / \text{changes in transmural pressure}$). Therefore, the blood maintaining 1 mmHg transmural pressure on the arterial side, when completely translocated, could produce 1/30 mmHg transmural pressure on the venous side (Shoukas & Sagawa, 1973). Accordingly, these investigators calculated, instead of measured, the MCFP

valva. They first measured the inferior vena caval plateau pressure and the arterial residual pressure established during circulatory arrest. They then added 1/30 of the difference between the arterial residual pressure and the vena caval plateau pressure to the vena caval pressure plateau, making up the deficiency of not using arterial to venous pumping. Their calculation can be expressed as:

$$\text{MCFP} = \text{VPP} + 1/30 \times (\text{APP} - \text{VPP}) \quad (1)$$

where VPP is the venous plateau pressure; APP is the arterial plateau pressure after circulatory arrest and 1/30 is the arterial : venous compliance ratio.

Experimental results showed that the equilibrium pressure obtained by this method was almost identical to the equilibrium pressure determined by arterial to venous pumping in the anaesthetized dogs (Green, 1975). Similar assumptions and calculation procedures were applied to obtain MCFP values without pumping from calves (Gay et al, 1987; Goldman et al, 1984), rats (Samar & Coleman, 1978) and guinea pigs (Davis et al, 1989). In a comparative study conducted in the rat, a very high correlation coefficient ($r = 0.98$, $p < 0.01$) was found between the MCFP values determined by the traditional method and the MCFP values determined without using arterial to venous pumping (Yamamoto et al, 1980).

Initially, the procedure to obtain an arterial / venous

compliance ratio was composed of measuring corresponding changes in the blood volume contained in the relevant vasculatures after transmural pressure was arbitrarily altered, thus yielding respective compliances and the compliance ratio. The arterial : venous compliance ratio determined by this method was found to be approximately 1/30 in the dog (Shoukas & Sagawa, 1973). Later, an alternative procedure was developed to yield this ratio and this procedure is based on correlative analysis. Firstly, reference MCFP values are measured by using a pump; secondly, the vena cava plateau pressure and arterial plateau pressure are measured at a given time, usually less than 7 seconds after circulatory arrest, without the use of a pump; then this ratio is obtainable, because

$$\text{MCFP}_{(\text{reference})} = \text{VPP} + K \times (\text{APP} - \text{VPP}) \quad (2)$$

$$K = (\text{MCFP}_{(\text{reference})} - \text{VPP}) / (\text{APP} - \text{VPP}) \quad (3)$$

where $\text{MCFP}_{(\text{reference})}$ is the MCFP value determined under the condition of using a pump; VPP is the vena cava plateau pressure and APP is the arterial pressure plateau determined under the condition without pumping. K is the estimated arterial : venous compliance ratio.

Using Equation (3), Yamamoto and co-workers (1980) found that the arterial : venous compliance ratio was 1/60 in the rat. This compliance ratio is most frequently used in rat

experiments (D'Oyley et al, 1989; Edmunds et al, 1989; Waite et al, 1988; Tabrizchi & Pang, 1987; Trippodo, 1981)

1.4 Physiological significance, normal values and applications of MCFP

1.4.1 Physiological significance of MCFP

The cardiovascular system is a closed circuit filled with blood. If the quantity of blood in this circuit is too small to properly fill it, blood will not properly flow to the heart from peripheral vessels and the circulation will be abnormal due to the decrease in venous return. MCFP is a hydrostatic pressure produced by the blood in the vascular lumen pressing against the vascular walls when the hydrokinetic energy provided by the heart's beating is no longer present. Therefore, it can serve as a meaningful index of the total blood volume in the whole cardiovascular system (Guyton et al, 1973; Guyton, 1987).

Additionally, how much distending pressure a certain volume of blood in a vessel can create will depend upon the calibre of the vascular lumen. For a given blood volume, the larger the calibre, the smaller the distending pressure will be. The vascular calibre varies depending on vasomotor activity. Therefore, changes in vasomotor activity in terms of total body vascular tone can be reflected by fluctuations in MCFP measurement. In other words, MCFP is an indicator of total body vascular tone (Guyton et al, 1973; Rothe, 1983a,

1983b).

It has been demonstrated experimentally that as MCFP increases, the venous return increases (Guyton, 1955). As MCFP can be regarded as the pressure at sites somewhere on the venous side where the pressure is independent of cardiac function and as the right atrial pressure is a barrier to venous return, it is obvious that even without the heart pumping, the blood in the venous vessels will keep flowing to the right atrium until the pressure gradient between MCFP and the right atrial pressure disappears. The pressure difference between MCFP and right atrial pressure, or " the pressure gradient for venous return " as it is often called, is the driving force for venous return. Therefore, physiologically, MCFP is " one of the major factors that determines the rate at which blood flows from the peripheral vascular tree into the right atrium, which in turn determines the cardiac output " (Guyton, 1987).

1.4.2 Reported values for MCFP

MCFP has been reported as 6 - 10 mmHg in most laboratory animal species, including the calf, the dog, the guinea pig and the rat. Because of the technical difficulties, there is no direct information about the MCFP value in living humans at the present time. However man's MCFP value is believed to be in the same range as most laboratory animals' (Rothe, 1983a). Table 1 summarises MCFP normal values of common

laboratory animals.

It is noticeable that even though MCFP values fall only within a limited range in most species under normal conditions, its value can be greatly altered under some abnormal conditions. Ganglionic blockade can decrease MCFP by 25 - 35%, while a reflex with sympathetic origin, like the Cushing reflex, can elicit an average MCFP value as high as 17 mmHg (Guyton et al, 1952; Richardson & Feroso, 1964). In conscious calves with a totally artificial heart MCFP was reported as high as 20 - 30 mmHg (Honda et al, 1976).

Table 1. Normal values of MCFP for common laboratory animals

Species	Number	MCFP Values (mmHg)	Sources
Dog	9	8.2 \pm 0.5	Tipayamontri et al, 1987
	9	10.5 \pm 2.6	Drees & Rothe, 1974
	8	7.1 \pm 0.3	Lee & Goldman, 1989
Calf	3	8 \pm 2	Goldman et al, 1984
	12	8 \pm 1	Gay et al, 1987
Rat	18	7.5 \pm 0.5	Samar & Coleman, 1978
	6	7.7 \pm 0.2	Gay et al, 1986
	46	5.7 - 6.2	Pang & Tabrizchi, 1986
Guinea pig	9	5.8 \pm 0.5	Davis et al, 1989

Values are all means \pm standard errors except those reported by Pang & Tabrizchi which is the range of means.

1.4.3 Applications of MCFP measurement

MCFP is directly related to total body blood volume and total body vasomotor tone, two primary determinants of whole body capacitance. This makes MCFP measurement a uniquely convenient probe to investigate alterations in whole body vascular capacitance. The experimental method of measuring MCFP at various known blood volumes, then analyzing the total vascular MCFP-blood volume relationship is termed "effective vascular compliance" (Drees & Rothe, 1974; Trippodo, 1981). It is usually conducted by injecting or withdrawing known volumes of blood into or from the animal; then drawing a related MCFP-blood volume curve. Effective vascular compliance analysis is one of the most common methods to study whole body capacitance function. If the total body blood volume-MCFP curve is extrapolated to zero on the MCFP axis of a coordinate graph, one can obtain a corresponding point on the volume axis from the same coordinate. This point represents the unstressed volume, the volume of blood that still remains in the vascular lumen when the transmural pressure is zero (Greenway & Lutt, 1989; Hainsworth & Linden, 1979; Rothe, 1983b). Subsequently, the stressed volume, the portion of blood that can be mobilized in various situations, can be obtained by subtracting the unstressed volume from the total blood volume measured by other techniques. In recent years this has been a most common research area related to MCFP measurement (Davis et al, 1989;

Edmunds et al, 1989; Gay et al, 1986; Lee et al, 1987; Trippodo, 1981).

Other investigations applied the basic principle that MCFP values are directly dependent on the total blood volume and the total vascular tone; therefore changes in MCFP values represent changes in total blood volume or total vascular tone. Measuring MCFP alone has been used as an index to assess the pharmacological effects of vascular pressor (Pang & Tabrizchi, 1986; Tabrizchi & Pang, 1987) and vasodilator agents (D'Oyley et al, 1989), or to evaluate the circulatory status after severe haemorrhage (Rocha E Silva et al, 1987).

1.5 Concerns with MCFP measurement

Despite appearing to be a useful tool in cardiovascular research, the measurement of MCFP does possess some inherent problems. When utilized to evaluate the effects of various vasoactive agents on the circulation, MCFP measurement is even more problematic. The effects of gravity and body posture on MCFP measurement have never been examined, though it is obvious that these two factors can play a crucial role in determining the venous return under the condition of cardiac arrest. Without careful examination of such effects, the estimation of the blood redistribution direction and the amount of such a redistribution during circulatory cessation cannot be accurate, particularly if vasomotor activity has simultaneously been abolished. Therefore, it would not be

surprising if the results of MCFP measurements obtained from anaesthetized animals lying in a supine posture differ significantly from those obtained from conscious animals that are standing, even if other experimental conditions are the same.

For MCFP measurement, however, the overwhelming question is whether the expected pressure equilibrium required in its definition can develop throughout the whole body. Also it is unclear how long after circulatory arrest the pressure equilibrium can occur. Presently, 7 seconds following circulatory arrest is considered as the time for MCFP determination because autonomic reflexes may be evoked by circulatory arrest after this time. As the time available for development of pressure equilibrium is so limited, it is reasonable to question if equal pressure can really develop throughout the body in time. Obviously, these questions become particularly pertinent if MCFP is determined under conditions where rapid blood translocation by means of pumping is not available. However, even when such a pump is used and a pressure equilibrium is brought about between the central arterial and venous compartments by this time, it does not mean that pressure equilibrium has also occurred in the tributary vessels subordinated to the central compartments by this time. Rothe (1976) has shown that during the MCFP manoeuvre, pressures in the vessel segments of the jugular vein, carotid artery or femoral artery distal to the

cannulation catheters were unable to equilibrate with MCFP. This finding was explained as a result of reflex constriction in small arterioles of some vascular beds in response to circulatory cessation or vascular occlusion by the cannulation catheters. A pressure disequilibrium between MCFP and the vessels of a limb or the head may only be of theoretical importance because they are less compliant and thus can trap only small volumes of blood in these vascular beds. However, a pressure disequilibrium between MCFP and a highly compliant vascular bed, such as the splanchnic vasculature, may indeed have the ability to alter MCFP values significantly. Nevertheless, this potential problem has not been adequately addressed.

The splanchnic vascular bed has distinctive features that may be able to prevent the establishment of pressure equilibrium, yet this has not been assessed by those using MCFP measurement. For instance, Samar and Coleman (1978) speculated that the portal pressure was able to equilibrate with MCFP because it had a higher pressure than the vessels downstream. This speculation may not be true. Gaddis et al (1986) reported that the portal venous pressure was higher than MCFP at the seventh second following heart arrest in anaesthetized dogs. This suggests that a pressure disequilibrium may exist in an important vascular bed. It is important then to consider the splanchnic circulation, more particularly the hepatic circulation, when investigating

MCFP.

1.6 Profile of the hepatic circulation

1.6.1 An overview of the hepatic circulation

The hepatic circulation is comprised of the portal vein, hepatic artery, hepatic vein and hepatic sinusoids. The sinusoids connect terminal portal venules and terminal hepatic arterioles at one end and terminal hepatic venules at the other end and are functionally specialized capillaries. With a normal range of pressure between 7 - 10 mmHg in most laboratory animals, the portal vein is a low-pressure, low-resistance system, providing two-thirds of the liver's blood supply. In contrast, the hepatic artery is a high-pressure, high-resistance system. It normally supplies about a third of the blood that the liver needs, but this quota increases as the portal flow decreases, so the total hepatic flow is held steady (Lautt, 1985; Greenway & Lautt, 1989). The hepatic vein originates from the sinusoids and empties directly into the inferior vena cava. This vein may not function only as a canal of venous return, but also be involved in control of the hepatic circulation (Greenway & Stark, 1971; Greenway & Lautt, 1989). It is estimated that the hepatic vasculature receives about 25% of the total cardiac output although the liver normally accounts for only 2.5% of the total body weight (Greenway & Lautt, 1989).

1.6.2 Hepatic outflow resistance

Any obstruction or narrowing in the circuit from which blood leaves the liver can constitute resistance to hepatic outflow. But, in many situations the major site of resistance is not clear and current reviews about outflow resistance sites are not conclusive. Early work showed that injection of bacterial toxins or live Gram-negative bacteria into the dog can induce hepatic outflow block, causing blood pooling in the liver and an elevation in portal pressure (Atik et al, 1968; McLean et al, 1956; McLean & Meil, 1956; Meil & Spink, 1957). These signs can also be found in anaphylactic shock (Meagraith et al, 1949; Meil & Spink, 1957). As these hepatic responses were similar to that induced by hepatic vein obstruction (Hindshaw et al, 1966), it was then considered as a consequence of obstruction in the intrahepatic venous system and led to speculation that there were outflow sphincters at the caval ostia of the hepatic vein that controlled the hepatic volume (Meagraith et al, 1949; Greenway & Stark, 1971). Greenway and Lutt (Lutt & Greenway, 1987; Greenway & Lutt, 1989) further postulated that, in relation to portal flow, the transhepatic resistance was fundamentally post-sinusoidal and there were hepatic venous outflow sphincters localized in third-order branches of the hepatic veins over a length of < 0.5 cm in the cat (Lutt et al, 1986; Lutt et al, 1987; Lutt & Greenway, 1987; Greenway & Lutt, 1989) and within 2 cm from the outlet

of the hepatic vein into the inferior vena cava in the dog (Legare & Lutt, 1987; Lutt & Legare, 1987; Greenway & Lutt, 1989; Lutt & Greenway, 1987). They additionally suggested that hepatic outflow resistance governed by these sphincters was not a classic waterfall resistance, but a pressure-distensible resistance (Lutt et al, 1987).

The principal experimental evidence supporting this hypothesis is that a sudden rise in hepatic venous pressure can be observed if a recording catheter is advanced to pass through a certain zone of the lobar veins, bringing the pressure close to that of the portal pressure upstream. This sudden rise in hepatic venous pressure is not thought to result from wedging the catheter tip in the lumen of the vessel (Lutt et al, 1986; Legare & Lutt, 1987; Lutt et al, 1987; Lutt & Legare, 1987). Furthermore, Mitzner (1974) observed that portal pressure was only about 1 mmHg higher than the estimated sinusoidal pressure but about 5 mmHg higher than hepatic venous pressure, implying that the resistance is mainly post-sinusoidal. Additionally, infusion of histamine into the hepatic artery or the portal vein can selectively increase hepatic volume and portal pressure (Lutt & Legare, 1987; Greenway & Oshiro, 1973); whereas infusion of norepinephrine or angiotensin, and electrical stimulation of the hepatic nerves induces a generalized active vasoconstriction, presenting a reduction in hepatic volume and a increase in portal pressure in a dose-related or

frequency-related manner (Lautt et al, 1987; Lautt & Legare, 1987). The appearance of generalized, passive hepatic congestion strongly suggests that histamine caused a confined active vasoconstriction in the intrahepatic venous system. Vasoconstriction at the outlets of the lobar hepatic veins or the hepatic veins likely would be one of mechanisms for the effects of histamine.

However, this hypothesis is not universally accepted. Although it is known that there are some sphincter-like smooth muscle bands in the hepatic vein (Thomas & Essex, 1949), anatomically-well-defined sphincters have never been demonstrated. The lack of solid anatomical proof for the existence of these sphincters makes this hypothesis somewhat vulnerable. In addition, the effects of histamine on hepatic circulation show strict species-selectivity. Although histamine-induced hepatic outflow block is easily initiated in the dog, it has never been demonstrated in the cat (Greenway & Lautt, 1972) or any other species. As hepatic outflow block has been observed in species other than the dog (Greenway & Lautt, 1989), the rigid species-selectivity of histamine is difficult to interpret. Furthermore, their hypothesis also conflicts with results obtained by direct measurement of intrahepatic pressures that demonstrated that the transhepatic resistance to be basically pre-sinusoidal in the rat (Nakata et al, 1960). Yet it is unknown if this is due to species differences as, quite coincidentally, " in the

livers of mouse and rat there are few nerves that extend deep into the liver lobule. (Forssmann & Ito, 1977)."

Besides, it is noticeable that early studies also suggested the existence of outlet and inlet sphincters in the junctions of the sinusoid and terminal hepatic venule, or the sinusoid and terminal portal venule (Knisely et al, 1957; Bloch, 1955). With a transillumination technique, using monochromatic light and a binocular microscope, McCuskey (1966) was even able to show that these sinusoid sphincters consisted of the endothelial cells in the liver of living frogs. Contrary to Lautt and Greenway's views, but somewhat consistent with direct measurement results in the rat, his direct measurement results also showed that, even when the inside diameters of the examined vessels were comparable, terminal portal venule pressure was about 3.8 mmHg higher than the terminal hepatic venule pressure, indicating there was a significant pressure drop across the sinusoid.

1.7 Objectives of the present study

As MCFP measurement is widely used to investigate changes in vascular capacitance, and as pressure equilibrium regarding MCFP measurement has not been properly investigated, the present study was designed to assess the hypothesis that the tributary venous vasculatures can equilibrate pressure with the central venous compartment; and that a universal pressure equilibrium therefore develops

throughout the venous system in the anaesthetized rat when MCFP is being measured by the technique that is in current use.

Specific hypotheses under investigation in this study were as follows:

1. that during circulatory arrest, the hepatic portal venous pressure (HPVP) and the renal vein pressure (RVP) can equilibrate with the inferior vena caval pressure (IVCP), which represents the central venous pressure and from which MCFP is to be estimated by a current technique;

2. that pressure equilibration develops by the 8th second following circulatory arrest which is close to the time of MCFP determination used by most investigators;

3. that in cases where these pressures fail to equilibrate during MCFP manoeuvres, the pressure disequilibrium may be attributed to the incomplete circulatory arrest in the vasculatures investigated; therefore, bringing the circulation of these beds to a zero-flow condition will eliminate the pressure disequilibrium;

4. that in cases where these pressures fail to equilibrate during MCFP manoeuvres, the pressure disequilibrium may be attributed to the effects of intraperitoneal route of anaesthesia on the splanchnic vasculature. Therefore, alteration in the route of anaesthesia induction from intraperitoneal administration to intravenous administration will eliminate the pressure

disequilibrium;

5. that in cases where these pressures fail to equilibrate during MCFP manoeuvres, the pressure disequilibrium may be attributed to reflex vasoconstriction in the regions concerned, initiated by certain experimental procedures; therefore, abolishing vasoconstriction reflexes by ganglionic blockade will eliminate the pressure disequilibrium.

Chapter 2

MATERIALS AND METHODS

2.1 The experimental animals and grouping

All experiments of this study were carried out on male Sprague-Dawley rats weighing 300-450 g (Charles River, Quebec). These animals were allocated to the following groups to meet the objectives of this study:

Group I: 7 rats subjected to sham surgery for the determination of the stability of haemodynamic variables during the entire period of the experimental protocol;

Group II: 16 reflex intact rats anaesthetized with urethane intraperitoneally for the determination of the pressure equilibrium during the MCFP manoeuvre;

Group III: 8 ganglion-blocked rats anaesthetized with urethane intraperitoneally for the determination of the effects of reflex vasoconstriction on the establishment of the pressure equilibrium during the MCFP manoeuvre;

Group IV: 6 reflex intact rats anaesthetized with urethane intravenously for the determination of the effects of intraperitoneal injection of urethane on the establishment of the pressure disequilibrium during the MCFP manoeuvre;

Group V: 5 reflex intact rats with both a right atrial balloon and an abdominal aortic snare in position, used for the determination of the influence of the residual arterial

pressure on the portal venous pressure during the MCFP manoeuvre.

2.2 Anaesthesia

Two separate methods as described below were used to introduce anaesthesia to the animals.

In all groups but Group IV, the rats were anaesthetized with 40% urethane (weight / volume; Sigma Chemical Company, St. Louis, USA) intraperitoneally. The initial dose of urethane was 1.0 g / Kg body weight and a maintenance dose of 0.1 g / Kg body weight was given intraperitoneally as required.

In order to examine if intraperitoneal administration of urethane could influence MCFP measurement significantly, in the rats of Group IV, 20% urethane (weight / volume) at a dose of 1.0 g / Kg body weight was administered intravenously following induction of anaesthesia by Fluothane inhalation (Ayerst Laboratories, Montreal, Canada). A maintenance dose of 20% urethane (0.1 g / Kg body weight) was given intravenously as required.

2.3 Surgical procedures

Anaesthetized rats were positioned supinely on a table constantly warmed to 36.0° - 37.0° C. A length of polyethylene PE 50 tubing (outside diameter 0.965 mm, inside diameter 0.58 mm; Becton Dickinson & Co.) was introduced into the left

femoral artery and advanced to the iliac artery, allowing measurement of systemic arterial pressure and rapid alteration of blood volume as required. Three 20 cm lengths of polyethylene PE 10 tubing (outside diameter 0.61 mm, inside diameter 0.28 mm) were introduced to measure inferior vena cava, hepatic portal vein and renal vein pressures as follows: the inferior vena cava was cannulated via the left femoral vein and the tip of this catheter was positioned near the right renal vein opening. After cannulation of the inferior vena cava, the abdominal cavity was opened through a 3 cm long mid-line incision to enable cannulation of the hepatic portal vein via a fine vein located in the mesoileum; the catheter tip was positioned in the hilus of the liver. A length of PE 10 tubing was introduced into the right external jugular vein, then manipulated to pass the heart and the liver along the vena cava and into the left renal vein. The tip of this catheter was carefully positioned close to the hilus of the left kidney. The renal vein was not cannulated in the rats of Group I, III, IV and V.

Circulation was arrested by the method of Yamamoto and co-workers (Yamamoto et al, 1980). A saline-filled, balloon-tipped catheter was inserted into the right atrium via the right external jugular vein. The catheter position was checked by inflating the balloon to reduce the arterial pressure to below 25 mmHg and form an inferior vena caval pressure plateau rapidly. This right atrial balloon was not

inflated during the observation period in those rats that received sham surgery (Group I).

In the 5 rats of Group V, a length of PE 50 tubing was also placed around the abdominal aorta at the site between the diaphragm and the coeliac artery to form a snare. Both ends of the tubing were brought out of the back of the animal and a piece of fine surgical silk tied to the middle of the PE 50 tubing brought out of the anterior wall of the abdomen.

When the two ends of the PE 50 tubing were pulled together, the abdominal aorta could be snared against the posterior wall of the abdomen; when the silk was pulled, the snare could be released. The simultaneous use of snaring the abdominal aorta and inflating the right atrial balloon can effectively decrease the arterial pressure to less than 10 mmHg.

After placement of the abdominal catheters and the snare, the abdominal incision was then closed with sutures. Measurements were started after a 30 minute equilibration period.

2.4 Calibration and recording

All vascular catheters were connected to physiological pressure transducers (Gould; Model No. P 23XL), which were previously calibrated using a mercury manometer and zeroed at the level of the inferior vena cava. The pressure changes during the circulatory arrest of 20 seconds were recorded on

a Gould TA 2000 recording system and the heart rate was automatically calculated from the femoral arterial pulsation by this system. Figure 1 is a sample of such recording.

2.5 Experimental protocols

Except the rats of Group I which were designated for sham surgery, the remaining four groups of animals were all subjected to blood volume alteration prior to the MCFP manoeuvre. Based upon the observations of other workers (Trippodo, 1981; Yamamoto et al, 1980), the total blood volume of the rat can be assumed to approximate 60 ml / Kg body weight and 10% of estimated blood volume was depleted and expanded for these experiments. Volume expansion or depletion was randomly assigned and alteration of blood volume was carried out by rapidly injecting or withdrawing blood through the left femoral arterial catheter. Volume expansion was carried out by the means of rapidly injecting the blood from donor rats. Almost immediately after the accomplishment of 10% blood volume change (less than 15 seconds), the circulation was arrested by injecting approximately 0.3 ml saline into the balloon inserted in the right atrium and the inflation of the balloon was maintained for 20 - 30 seconds during which vascular pressures were recorded; the animal's blood volume was restored to normal shortly after recording was finished. In rats where the abdominal snare was used (Group IV), the abdominal aorta was

occluded immediately following inflation of the right atrial balloon; the aortic snare was released after deflating this balloon.

Except for the sham surgery rats and the rats of Group V, each animal of the remaining three groups was subjected to three MCFP manoeuvres under the conditions of normal blood volume, estimated 10% blood volume depletion and estimated 10% blood volume expansion, respectively; the interval between two MCFP manoeuvres was 15 minutes. In Group V rats, right atrial balloon inflation alone or right atrial balloon inflation plus abdominal snare was used alternately and the interval was 10 minutes. Each of the two circulatory arrest manoeuvres comprises a paired measurement. Circulatory arrest manoeuvres in units of pairs were conducted randomly-assigned as normal blood volume, 10% blood volume depletion and 10% blood volume expansion. Each rat in this group received five pairs of MCFP manoeuvres.

For ganglionic blockade of Group III, a bolus injection of 2% hexamethonium (weight / volume; 20 mg / Kg body weight; Sigma Chemical Company, St. Louis, USA) and 0.15% atropine (weight / volume; 0.5 mg / Kg; Sigma Chemical Company, St. Louis, USA) was given intravenously 30 minutes prior to the start of the protocol. Ganglionic blockade was confirmed by absence of the rebound rise in arterial pressure plateau of the MCFP manoeuvre (Yamamoto et al, 1980). Figure 2 shows a comparison of the arterial pressure plateaus recorded without

ganglionic blockade (panel A) and during ganglionic blockade (panel B).

2.6 Data analyses

Heart rate, systemic arterial pressure, hepatic portal venous pressure and renal vein pressure were obtained from the recording chart at 4-second intervals. MCFP was calculated according to the following equation (Yamamoto et al, 1980):

$$\text{MCFP} = \text{IVCP} + 1/60 \times (\text{APP} - \text{IVCP}) \quad (4)$$

where IVCP was the inferior vena caval pressure measured at the 8th second following circulatory arrest; APP was the residual systemic arterial pressure measured from the iliac artery catheter at the 8th second following circulatory arrest; 1/60 was the arterial : venous compliance ratio. The MCFP determination time was set at the 8th second of circulatory arrest because it was close to the MCFP determination time (7 seconds following circulatory arrest) that was frequently used in previous investigations (Guyton et al, 1973).

For statistical analysis, unpaired Student-t tests were used to compare each tributary venous pressure with the IVCP at five sampling times during the three blood volume states. This procedure was applied only to the pooled data of Group

II due to the inequality of sample sizes in this group. Other statistical comparisons were conducted using paired Student-t tests unless otherwise indicated. The time courses of changes in haemodynamic variables over the circulatory arrest period in Group II and III rats, or over the period of 3 hours post-surgery observation in Group I rats were initially analyzed using one-way analyses of variance (ANOVA). Post hoc Dunnett's t tests were used to identify differences from the control values (Dunnett, 1955; Steel & Torrie, 1980; Wallenstein et al, 1980). For the sham surgery rats (Group I), the controls were the measurements made at the 30th minute after the completion of sham surgery because the animal was assumed to have recovered after 30 minutes' equilibration. The controls for changes in venous pressures over the 20 seconds' circulatory arrest were the first measurement during this period (i.e., the 4th second measurements), since one of the interests of this study was to observe the timing for the establishment of pressure equilibrium within the venous system. The significance level for rejection of the hypotheses of this study was set at 0.05.

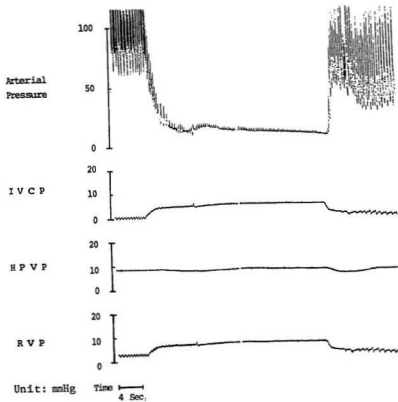


Figure 1. Recordings of iliac arterial pressure (AP), inferior vena caval pressure (IVCP), hepatic portal venous pressure (HPVP) and renal vein pressure (RVP) during circulatory arrest by the inflation of a right atrial balloon in a reflex intact rat in 10% blood volume expansion state.

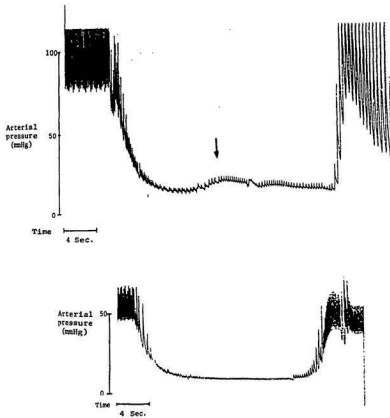


Figure 2. Comparison of the arterial pressure plateaus following circulatory arrest obtained from a reflex intact rat in Group II (panel A) and a ganglion-blocked rat in Group III (panel B) under the condition of normal blood volume. Note the pressure rebound (arrow) in panel A and the elimination of the pressure rebound in panel B.

Chapter 3

RESULTS

3.1 Baseline measurements and sham surgery results

The baseline measurements of pooled data from reflex intact rats (Group II) are: heart rate, 419.4 ± 8.1 beats / minute (mean \pm 1 standard error); mean systemic arterial pressure, 82.8 ± 2.7 mmHg; inferior vena caval pressure, 2.0 ± 0.3 mmHg; renal vein pressure, 3.8 ± 0.3 mmHg and hepatic portal venous pressure, 7.2 ± 0.3 mmHg. The effects of blood volume changes on the haemodynamic variables of reflex intact rats are shown in table 2. Time course of changes in heart rate, mean systemic arterial pressure, inferior vena caval pressure and hepatic portal venous pressure of Group I rats over three hours' post-sham surgery observation is shown in Figure 3. During the first two hours after surgery, these basic cardiovascular variables, especially IVCP and HPVP which are key pressures of this study, are stable in the sham surgery rats.

3.2 Time course of venous pressures during a 20-second circulatory arrest in reflex intact rats

Figure 4 shows the time-course of IVCP, RVP and HPVP measurements over a 20-second circulatory arrest by right atrial balloon inflation in reflex intact, urethane-

intraperitoneally-anaesthetized rats (Group II). HPVP is significantly higher than IVCP during the entire period of 20 seconds under the condition of 10% blood volume depletion ($p < 0.01$; Fig. 4A). HPVP is also significantly higher than IVCP at the 4th, 8th, 12th and 16th second measurement during 10% blood volume expansion ($p < 0.01$ for the former two comparisons and $p < 0.05$ for the latter two comparisons; Fig. 4C). Under normal blood volume conditions, however, HPVP is significantly higher than IVCP only at the 4th second recording time ($p < 0.01$; Fig. 4B). Results of ANOVA show that only IVCP changes significantly during the 20-second recording period in the three blood volume states. Dunnett's t testing of the time-course of changes in IVCP indicates that IVCP measured at the 16th and 20th second of the circulatory arrest is significantly higher than that measured at the 4th second in the normal blood volume and 10% volume expansion states ($p < 0.05$ for each of these comparisons); and highly significantly higher at the 12th, 16th and 20th second of the arrest during the 10% blood volume depletion state ($p < 0.01$ for each of these comparisons).

3.3 Comparison of venous pressures at the 8th second of circulatory arrest in reflex intact rats

Table 3 gives the values of HPVP, IVCP and RVP at the 8th second of circulatory arrest by right atrial balloon inflation in Group II rats. At the 8th second following

circulatory arrest, HPVP is significantly higher than IVCP under the conditions of 10% blood volume depletion and 10% blood volume expansion ($p < 0.01$ for the two comparisons); while RVP is not significantly different from IVCP in the three blood volume states.

Figure 3. Time course of changes in vascular variables following sham surgery

The time-course of changes in heart rate, the systemic arterial pressure, the inferior vena caval pressure and the hepatic portal venous pressure after surgery and vascular cannulation in 7 sham surgery rats (Group I). Each point represents the mean and the vertical bar represents ± 1 S.E.M. Dunnett's t test is used to test the differences between the means measured at the 30th minute sampling time (control) and the means measured subsequently for each variable. Only the means of heart rate of the 150th and the 180th minute are found significantly different from the control. Statistical significance of these differences is expressed as: *, $p < 0.05$; **, $p < 0.01$.

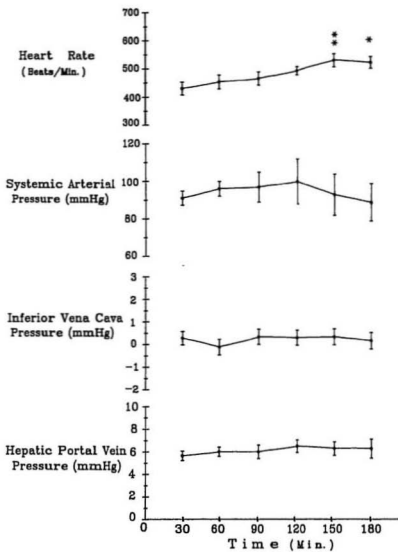


Table 2. Effects of blood volume changes on cardiovascular variables

Values are means \pm 1 S.E.M. of pooled data from Group II rats. Variables are denoted as follows: HR, heart rate; AP, mean systemic arterial pressure; IVCP, inferior vena caval pressure; RVP, renal vein pressure and HPVP, hepatic portal venous pressure. Other abbreviations and symbols are denoted as follows: BV, blood volume; n, the number of samples pooled; *, differences between before and after volume alterations in paired Student-t test, $p < 0.05$; **, $p < 0.01$.

Table 2. Effects of blood volume changes on cardiovascular variables in Group II rats

Variable	Pre-withdraw 10% BV	Post-withdraw 10% BV	Pre-inject 10% BV	Post-inject 10% BV
HR (b./min) (n = 16)	425.1±14.1	427.3±20.1	394.5±25.6	387.3±19.9
AP (mmHg) (n = 16)	86.9±4.1	46.4±3.1 **	79.2±3.2	92.3±3.6 **
IVCP (mmHg) (n = 16)	1.6±0.2	1.2±0.2 *	1.2±0.2	1.9±0.2 *
RVP (mmHg) (n = 11)	3.2±0.2	2.4±0.2 **	3.1±0.2	3.7±0.3 *
HPVP (mmHg) (n = 12)	6.8±0.3	5.1±0.3 **	7.0±0.5	8.3±0.5 *

Figure 4. Time-course of changes in venous pressures following circulatory arrest during three blood volume states in reflex intact rats

The time-course of changes in hepatic portal venous pressure (HPVP), inferior vena caval pressure (IVCP) and renal vein pressure (RVP) following a 20-second circulatory arrest under the conditions of 10% blood volume depletion (panel A), normal blood volume (panel B) and 10% blood volume expansion (panel C) in reflex intact rats. Each point represents the mean and vertical bars represent ± 1 S.E.M. of the pooled data from Group II rats. Statistically significant differences between HPVP or RVP and IVCP for each timed measurement in unpaired Student-t test are represented as follows: *, differences between IVCP and HPVP, $p < 0.05$; **, differences between IVCP and HPVP, $p < 0.01$; +, differences between IVCP and RVP, $p < 0.05$.

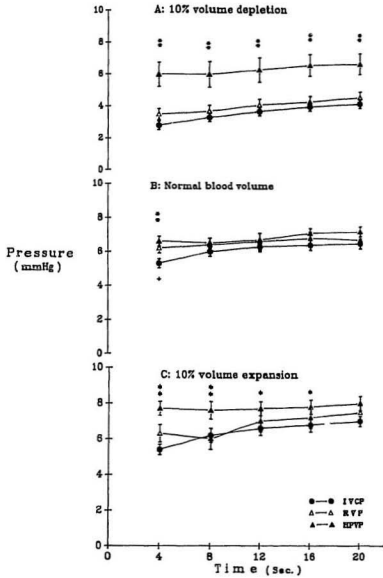


Table 3. Measurements and comparisons of vascular pressures at the 8th second of circulatory arrest during three blood volume states in reflex intact rats (Group II)

Values are means \pm 1 standard error. Statistical comparisons of IVCP with HPVP, or RVP, are made by unpaired Student-t test and denoted by **, $p < 0.01$. Variables are denoted as follows: AP, mean arterial pressure; IVCP, inferior vena caval pressure; HPVP, hepatic portal vein pressure; MCFP, mean circulatory filling pressure estimated from IVCP; RVP, renal vein pressure. B.V. is the abbreviation for blood volume.

Table 3. Measurements and comparisons of vascular pressures at the 8th second of circulatory arrest during three blood volume states in reflex-intact rats (Group II)

Pressures (mmHg)	10% B. V. Withdrawal	Normal Blood Volume	10% B. V. Expansion
AP (n=16)	12.8±0.7	17.8±1.0	16.3±1.0
MCFP (n=16)	3.55±0.23	6.20±0.30	6.57±0.43
IVCP (n=16)	3.4±0.2	6.0±0.3	6.2±0.4
RVP (n=11)	3.7±0.4	6.5±0.4	6.8±0.6
HPVP (n=12)	6.2±0.8 **	6.5±0.3	7.7±0.5 **

3.4 Comparison of circulatory arrest by balloon inflation alone and balloon inflation plus abdominal aortic snare

Venous pressures and MCFP measured at the 8th second of circulatory arrest by the balloon inflation method or by balloon inflation plus abdominal aortic snare are shown in Table 4. This data was collected from 22 pairs of MCFP manoeuvres, each consisting of balloon inflation alone and balloon inflation plus abdominal aortic snare, in which circulatory arrest was carried out during randomly-assigned normal blood volume, 10% blood volume depletion or expansion. Paired-t tests show that the only significant difference between the two methods inducing circulatory arrest is that balloon inflation plus abdominal aortic snare results in lower residual arterial pressure during circulatory arrest than balloon inflation alone. (9.8 ± 0.7 vs 17.9 ± 1.1 mmHg, $p < 0.01$) Furthermore, pressure disequilibrium between HPVP and IVCP can also be seen with circulatory arrest by balloon inflation alone or by balloon inflation plus abdominal aorta snare such that IVCP is significantly lower than HPVP under both conditions ($p < 0.01$, Table 4). Calculated MCFP value is not affected by addition of an abdominal aortic snare.

3.5 Effects of ganglionic blockade on measurements of venous pressures during the MCFP manoeuvre

In the 8 rats of Group III after induction of ganglionic blockade, the resting mean arterial pressure was 59.5 ± 2.7 mmHg and heart rate was 428.5 ± 16.8 beats / min. In these ganglion-blocked rats, HPVP was still significantly higher than IVCP upto 12 seconds following circulatory arrest during both 10% blood volume depletion and 10% blood volume expansion ($p < 0.01$ at the 4th second recording and $p < 0.05$ at the 8th and 12th second recordings in comparisons between HPVP and IVCP; Fig. 5A and 5C). During the normal blood volume state, HPVP is also significantly higher than IVCP ($P < 0.01$; Fig. 5B). Results of ANOVA show that there is no statistically significant change in either of the pressures over the 20-second circulatory arrest in any of the three blood volume states. At the 8th second measurement during 10% blood volume depletion, HPVP was significantly higher than IVCP (3.6 ± 0.3 vs 2.6 ± 0.4 , $p < 0.05$; Table 5). At the same scheduled measurement during 10% blood volume expansion, HPVP was also significantly higher than IVCP (6.5 ± 0.3 vs 5.3 ± 0.4 , $p < 0.05$; Table 5).

3.6 Experimental results of the rats anaesthetized with urethane intravenously

An average of 1.5 ml of 20% urethane solution was injected intravenously during the experimental protocol in

the 6 rats of Group IV. The mean systemic arterial pressure of these rats was significantly higher than the reflex intact rats (Group II) anaesthetized with urethane intraperitoneally (105.0 ± 3.8 vs. 82.8 ± 2.7 mmHg, $p < 0.01$ in unpaired t test). The heart rate of these rats did not differ significantly from the rate observed in the rats of Group II (428.7 ± 5.5 vs 419.4 ± 8.1 beats / minute). The abdominal vessels of the rats in Group IV appeared healthier than those receiving the intraperitoneal injection of urethane. Under direct observation with no magnification, no congestion was observed in the mesenteric vessels and there was little exudation seen in the abdominal cavity in the rats of Group IV. In contrast, these two signs were fairly common in the rats that received intraperitoneal injection of urethane. However, as shown in Figure 6, HPVP measured at the 8th second of circulatory arrest was still significantly higher than IVCP ($p < 0.01$) determined simultaneously in 10% volume depletion and 10% volume expansion.

Table 4. Comparison of haemodynamic responses to circulatory arrest by balloon inflation alone and balloon inflation plus abdominal aortic snare in Group V rats

Values are means \pm 1 S.E.M. calculated from 22 pairs of measurements in Group V rats. AP, IVCP and HPVP are arterial plateau pressure, inferior vena caval pressure and hepatic portal venous pressure, respectively, measured at the 8th second following circulatory arrest by balloon inflation alone (B), or by balloon inflation plus abdominal aortic snare (B&S) under randomly-assigned normal blood volume, 10% blood volume depletion or expansion conditions. MCFP represents the mean circulatory filling pressure calculated from the equation:

$$\text{MCFP} = \text{IVCP} + 1/60 \times (\text{AP} - \text{IVCP}).$$

p represents the results of statistical comparisons between B and B&S in paired Student-t test. NS indicates no significant difference. Statistical comparisons of IVCP with HPVP under the conditions of balloon inflation alone or balloon inflation plus abdominal aortic snare are separately made by paired Student-t test and comparison results are expressed as: **, p < 0.01.

Table 4. Comparison of haemodynamic responses to circulatory arrest by balloon inflation alone and balloon inflation plus abdominal aortic snare in Group V rats

Pressures (mmHg)	Balloon Alone(B)	Balloon Plus Snare (B&S)	B - B&S	p
AP	17.8±1.1	9.8± 0.7	8.0± 1.3	p < 0.01
MCFP	4.70±0.28	4.57±0.29	0.13±0.18	NS
IVCP	4.5±0.3	4.5±0.3	-0.004±0.2	NS
HPVP	6.0±0.3 **	5.9±0.3 **	0.2±0.12	NS

Figure 5. Time-course of changes in venous pressures following circulatory arrest during three blood volume states in ganglion-blocked rats

Shown is the time-course of changes in hepatic portal venous pressure (HPVP) and inferior vena caval pressure (IVCP) during a 20 - second circulatory arrest under the conditions of 10% volume depletion (Panel A), normal blood volume (Panel B) and 10% volume expansion in ganglion-blocked rats (Group III). Each point represents the mean and bar represents ± 1 standard error for 8 rats. Statistically significant differences between IVCP and HPVP from each timed measurement in paired Student-t test are represented as follows: * - $p < 0.05$; ** - $p < 0.01$.

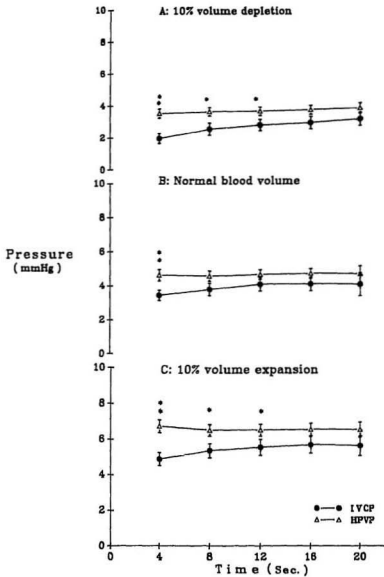


Table 5. Measurements and comparisons of vascular pressures at the 8th second of circulatory arrest during three blood volume states in ganglion-blocked rats

Values are means \pm 1 S.E.M. from 8 rats. Statistical comparisons of IVCP with HPVP are made by paired Student-t test and denoted by *, $p < 0.05$. Variables are denoted as follows: AP, mean arterial pressure; IVCP, inferior vena caval pressure; HPVP, hepatic portal venous pressure; MCFP, calculated mean circulatory filling pressure. BV is the abbreviation for blood volume.

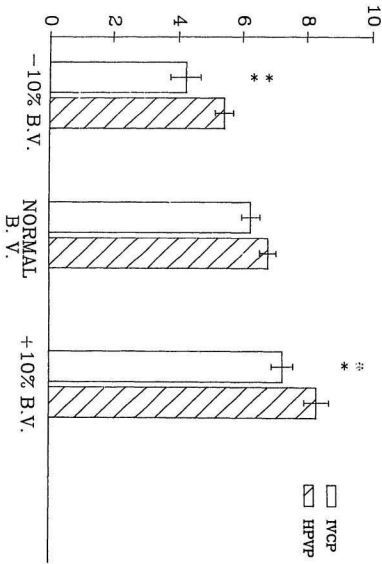
Table 5. Measurements and comparisons of vascular pressures at the 8th second of circulatory arrest during three blood volume states in ganglion-blocked rats

Pressures (mmHg)	10% B. V. Withdrawal	Normal Blood Volume	10% B. V. Expansion
AP	9.5±0.8	10.3±1.0	13.6±0.9
MCFP	2.66±0.38	3.87±0.37	5.74±0.38
IVCP	2.6±0.4	3.8±0.4	5.3±0.4
HFVP	3.6±0.3 *	4.6±0.3	6.5±0.3 *

Figure 6. Comparison of HPVP with IVCP measured at the 8th second of circulatory arrest during three blood volumes in rats anaesthetized with urethane intravenously

Hepatic portal venous pressure (HPVP; hatched columns) and inferior vena caval pressure (IVCP; open columns) measured at the 8th second following circulatory arrest under the conditions of 10% blood volume depletion (-10% BV), normal blood volume (Normal BV) and 10% blood volume expansion (+10% BV) in the rats anaesthetized with urethane intravenously (Group IV). Each column bar represents the mean and each bar represents ± 1 S.E.M. of this mean of 6 rats. Statistical significance between IVCP and HPVP in paired Student-t test is expressed as: **, $p < 0.01$.

Venous Pressure (mmHg)



Chapter 4

DISCUSSION

4. . Pressure disequilibrium during the MCFP manoeuvre

Experimental determination of MCFP is based on an assumption that every vascular bed subordinate to the central vena cava has the same opportunity to equilibrate pressure, so the pressure equilibrium can develop throughout the entire cardiovascular system during MCFP manoeuvres. But, as revealed by this series of experiments, this assumption is only hypothetical. In fact, even within the venous system the ability to reach this pressure equilibrium varies between different vascular beds. For individual vascular beds it also varies with experimental protocols. In this series of experiments, renal vein pressure was found not significantly different from inferior vena cava pressure. This could be explained that this vessel opens directly into the inferior vena cava; thus pressure changes in the vena cava are easily transmitted upstream towards it. However, the hepatic portal vein did not equilibrate pressure with the inferior vena cava when blood volume had been rapidly changed, though it did equal IVCP under normal blood volume conditions. This relatively high hepatic portal venous pressure appeared not to result from vasoconstriction following this circulatory disturbance as it remained after blocking ganglionic

reflexes. Neither did it appear to result from the relatively high residual arterial pressure after circulatory arrest caused by balloon inflation, as it was not abolished when the aortic inflow to the splanchnic bed is already stopped by a snare. Furthermore, both HPVP and IVCP were virtually unchanged when the arterial residual pressure was significantly decreased by addition of the abdominal aortic snare, indicating that changes in arterial pressure have little effects on venous pressures when arterial pressure is lower than 20 mmHg. If changes in arterial pressure were transmittable to the venous side and if this higher portal venous pressure during MCFP manoeuvres was caused by a relatively high arterial residual pressure, reduction in the residual arterial pressure by half should have decreased pressure disequilibrium by a reasonable proportion.

It might be argued that this higher portal venous pressure was created by a reverse flow from the abdominal aorta distal to the snare to the coeliac artery, as this residual aortic pressure recorded in the iliac artery was still about 10 mmHg following aortic snaring, which is significantly higher than portal venous pressure. However, because the pressure gradient between the abdominal aorta and the portal vein is only about 4 mmHg after snaring, it may not be big enough to allow the portal vein to hold about 1.5 mmHg pressure surplus over the inferior vena cava (Table 4; page 54). Although the possibility of reverse flow could not

be ruled out directly with this protocol, studies in dogs where arterial to venous pumping was used showed that higher portal pressure existed during MCFP manoeuvres (Gaddis et al, 1986; Rothe et al, 1990). Nevertheless, as there was a pressure difference between the portal vein and the inferior vena cava during the MCFP manoeuvre, it at least indicates that this technique, when used in the anaesthetized rat, does not satisfy the definition of MCFP, which states that all pressures should be equal at the time of MCFP measurement.

4.2 Portal venous pressure and MCFP

The results of this study conflict with the initial assumption about the relationship between portal pressure and MCFP. Starling (Bayliss & Starling, 1894; Starling, 1897) observed that portal venous pressure was virtually unchanged and there was pressure equilibrium between the portal vein and the inferior vena cava after the heart was stopped. He then concluded that the portal vein was the neutral point of the whole circulatory system and its pressure was equal to the mean systemic pressure, or MCFP as it is now termed. Although Starling's conclusion was not further tested, the view that the portal vein can equilibrate pressure with the central vena cava during the MCFP manoeuvre has since been taken for granted (Samar & Coleman, 1978). However, recent studies have shown that the portal vein may not always be able to equilibrate pressure with the inferior vena cava

under various conditions. Gaddis et al (1986) found that portal pressure was significantly higher than inferior vena caval pressure at the 7th second following heart-arrest in dogs whose blood volume was reduced by 10 - 20%. This is basically consistent with the results of this study except that this study was carried out in rats during atrial balloon inflation-induced circulatory arrest; also, the pressure disequilibrium was observed following volume expansion. In addition, Rothe et al (1990) reported a significantly higher portal pressure during the MCFP manoeuvre in the presence of histamine. As shown in this and other studies, the portal vein does not always equilibrate pressure with the inferior vena cava following sudden circulatory arrest. Therefore, Starling's conclusion that portal venous pressure could serve as an equivalent to mean systemic pressure does not appear to be valid in all species or under all conditions, particularly under conditions of volume alteration or histamine intervention.

In this study, portal pressure did not change over 20 seconds of circulatory arrest. During this period, portal pressure appeared to fall only slightly at the 8th second after inflation of the balloon, but this pressure decrease was not statistically significant by ANOVA. This can be considered as a common point between this study and that conducted by Starling. A major difference between the results of this study and those reported by Starling is that

he constantly observed pressure equilibrium between the portal vein and the inferior vena cava after circulatory arrest, while this study did not. Although the precise factors causing this discrepancy are unclear, differences in experimental procedures are certainly relevant. In Starling's experiments, the heart was arrested by injecting oil into the pericardium in a stepwise manner and the pressure equilibrium was observed after the heart had been arrested for more than three minutes; whereas in this study the heart or the circulation was arrested suddenly and the whole process of measuring MCFP lasted 20 - 30 seconds. In addition, other experimental steps of surgery, anaesthesia and volume alteration, were all possible sources for this apparent contradiction.

4.3 Vasoconstriction and ganglionic blockade

The use of hexamethonium and atropine in this study was designed to block ganglionic reflexes in order to ascertain whether the pressure disequilibrium observed was produced by reflex vasoconstriction. For the protocols of this study, there were at least two sources that could induce nervous vessel constriction: the acute circulatory arrest and the volume disturbances. Either sudden collapse in total circulation with circulatory arrest or acute alterations in total blood volume will stimulate the baroreceptors located in the carotid arteries and the aortic arch, and initiate

noticeable vasoconstriction via baroreflexes. Although volume alterations in this study were only 10% of estimated total blood volume, they did influence circulatory status as can be observed in Table 2 of page 45. This "excessive" vascular response to relatively small volume alterations may result from the rapid rate of blood withdrawing / injecting and the early measurement of circulatory variables when the actual recording may have preceded the establishment of full cardiovascular compensation. Nervous vasoconstriction, originating either from stimulation of the central nervous system or from activation of peripheral sensors is conducted by the autonomic nervous system, mainly the sympathetic nerves. Therefore, such neurally mediated vasoconstriction is sensitive to ganglionic blockade.

It is known that the splanchnic vascular bed can rapidly respond to nervous stimulation resulting in increased vascular resistance and reduced vascular capacitance (Lautt & Legare, 1987; Lautt et al, 1987; Rothe, 1983a, 1983b). In some species, such as dogs and cats, it is proposed that there are hepatic outflow sphincters situated in the hepatic lobar veins. These sphincters respond to nervous stimulation and certain pharmacological agents and serve as a gate to control hepatic outflow and consequently the portal pressure (Greenway & Lautt, 1989). If there was vasoconstriction induced by circulatory arrest or acute volume alterations, particularly constriction of these proposed hepatic venous

sphincters, it would likely result in high portal pressure.

Hexamethonium is commonly used to block autonomic ganglia. In most reported cases a bolus injection of 10 mg / Kg body weight can produce satisfactory ganglionic blockade (Harris et al, 1989; Rochford & Henry, 1990). However, the rat preparation used in this study seemed resistant to this dose of hexamethonium. In preliminary experiments, 10 - 20 mg / Kg body weight hexamethonium failed to eliminate the baroreceptor reflexes induced by bilateral carotid artery occlusion. As the existence of muscarinic cholinergic neurons in the autonomic ganglia is well documented (Hammer & Giachetti, 1982; Wess et al, 1987), atropine was chosen to cooperatively block the ganglionic reflexes. Concurrent use of atropine (0.5 mg / Kg) and hexamethonium (20 mg / Kg) did lead to satisfactory ganglionic blockade. Under this combined ganglionic blockade, reflex vasoconstriction in the splanchnic vascular bed may reasonably be assumed to have been eliminated. Supportive evidence for successful ganglionic blockade was derived from the observation that the secondary pressure rise in the arterial plateau, which is believed to be a sign of activation of sympathetic reflexes following circulatory arrest (Yamamoto et al, 1980), and the acceleration in heart rate during MCFP manoeuvring, were eliminated (Fig. 2; page 38). However, even with such a ganglionic blockade, the pressure differences between HPVP and IVCP still existed, although the degree and duration of

these pressure differences were reduced (Figures 4 and 5; pages 47 and 56). This indicates that the pressure disequilibrium, at least in part, came from a resistance independent of autonomic reflexes. However, as the apparent pressure disequilibrium did decrease after the use of ganglionic blockade, there remains another possibility, that reflex vasoconstriction in the splanchnic bed might have been partially involved in the constitution of the pressure disequilibrium. It is hard to clarify this situation as ganglionic blockade itself can reduce the baseline of haemodynamic variables; therefore, it may reduce the pressure disequilibrium proportionally. If this study had additionally monitored the total circulating blood volume and the splanchnic blood volume during the MCFP manoeuvre before and after ganglionic blockade, then the vascular compliance of the total body and the splanchnic vasculature at zero flow could have been obtained under reflex intact and ganglionic blockade conditions. Carefully comparing the altered degree of splanchnic compliance after ganglionic blockade with the altered degree of total body compliance to see if they were changed proportionally during ganglionic blockade would provide important information to clarify this situation.

4.4 Hepatic outflow resistance

It is convenient to simply divide the hepatic outflow resistance into two mechanisms according to their responses

to ganglionic blockade. The first mechanism can be termed functional resistance as it will be adjusted mainly by nervous activities and this has been discussed in the previous section. However, it should be emphasized that these sphincters suggested by Lauth and Greenway have never been identified in the rat (Lauth & Greenway, 1987). The second mechanism might be called physical resistance as it still remains, even after ganglionic blockade has been initiated; this may be an alternative mechanism responsible for the pressure disequilibrium.

Mitzner (1974) reported that there was a vascular waterfall resistance in the hepatic vascular bed. His experiments were conducted in dogs where a shunt was established between the portal vein and abdominal inferior vena cava and hepatic arterial flow was controlled by a perfusion pump. This allowed portal flow to be completely diverted to the inferior vena cava and arterial flow to be varied over a wide range. He found that the hepatic sinusoid pressure was 1.6 mmHg higher than hepatic venous pressure when the portal flow was diverted to the inferior vena cava and the hepatic arterial perfusing rate was set at zero. He concluded that there was a "closing pressure" in the sinusoid at zero flow that helped maintain a higher portal pressure; this is similar to a waterfall or Starling resistor. If hepatic outflow resistance is mainly a pre-sinusoidal resistance in the rat (Nakata et al, 1960), and if there are

only few nerve fibres travelling deeply into the lobule of the rat's liver (Forssman & Ito, 1977), the vessels inside the liver may not respond to ganglionic blockade. Closing pressure at zero inflow around the sinusoid site causing the pressure disequilibrium during the MCFP manoeuvre may be a more likely explanation than that of activation of hepatic venous sphincters, which should respond well to ganglionic blockade and present as a post-sinusoidal resistance.

The portal pressure higher than inferior vena caval pressure at zero flow was also seen in the report by Price et al.: Dynamics of blood through the normal canine liver. In one of their experimental animals, the portal venous pressure and central venous pressure were about 5 and 1 mmHg, respectively, when hepatic arterial pressure was 20 mmHg and portal flow was zero (Price et al, 1964). This may also be caused by closing pressure. From the data of this study it is difficult to interpret what induced the pressure disequilibrium and why it was associated with volume alterations. McCuskey (1966) showed that the sinusoidal Kupffer cells were capable of bulging into the sinusoidal lumen, causing sinusoid closure. If rapid volume alterations could provide direct physical stimulation to these endothelial cells and could thus cause them to bulge toward the sinusoidal lumen through their intracellular activities, this may be a mechanism underlying this pressure disequilibrium.

4.5 Possible influences of the pressure disequilibrium to the applications of MCFP measurement

The presence of pressure disequilibrium raises some questions for current uses of MCFP measurement, especially in situations where the applications are directed to evaluate the effects of a given pharmacological agent; problems could arise because both the distribution of total body blood volume and the activity of the hepatic outflow resistance could be altered under such conditions. MCFP measurement has been used alone to assess changes in total body venous tone or total body blood volume (Pang & Tabrizchi, 1986; Tabrizchi & Pang, 1988; Rocha E Silva et al, 1977), but more frequently it has been measured in combination with volume alteration to assess changes in total body vascular compliance (Trippodo, 1981; Gay et al, 1986; Gay et al, 1988). These applications have all been established on the basic assumption of equal pressures throughout the entire cardiovascular system during MCFP manoeuvres, as well as the assumed parallel relationship between the MCFP value and the total blood volume. The former has been proved untenable by studies in this thesis and other studies (Gaddis et al, 1986; Rothe et al, 1990). The latter is also in doubt because, if the hepatic outflow resistance is changeable and variable volumes of blood can be trapped in the splanchnic bed, the measured MCFP values may not correctly reflect the actual whole blood volume. As the value of MCFP relies on the total volume of blood that is

actually circulating around the whole body, and as this relatively high portal pressure during MCFP manoeuvring may result from a volume retention in the splanchnic vasculature after circulatory arrest, the influences of this pressure disequilibrium on the applications of MCFP measurement appear to depend on how much blood is trapped in the splanchnic vasculature at the time of MCFP determination.

Using MCFP value as an index to evaluate changes in whole body venous tone or total body blood volume after a given treatment may be less vulnerable to this pressure disequilibrium providing that such use of MCFP measurement is only qualitative. In such a case, as long as volume retention in the splanchnic vasculature is not large enough to upset the total circulating volume significantly, changes in MCFP value can still reflect changes in whole body venous tone or total body blood volume, although this measurement may not be precise because the portion of blood that is trapped in the splanchnic vasculature is not accounted for in the total circulating volume. Therefore, it appears that what the pressure disequilibrium affects is mainly the quantitative sensitivity of MCFP measurement in such use.

However, for the application of MCFP measurement as an index of total vascular capacitance, this pressure disequilibrium may create more serious problems. Two features of this application, measuring MCFP following acute blood volume alterations and relying on linear extrapolation along

an MCFP-volume curve to obtain the unstressed volume, make the suitability of such application particularly questionable as the pressure disequilibrium is triggered by volume alterations. Also, it seems more severe during volume depletion than during volume expansion, indicating that it may not demonstrate a linear relationship with volume alterations. Figure 7 suggests some potential problems related to the pressure disequilibrium and the use of measuring MCFP in this aspect. Theoretically, MCFP should be estimable from any vessel. However, when pressure disequilibrium exists and if the portal vein rather than the inferior vena cava happens to be chosen to estimate MCFP, the MCFP values so estimated will be higher than that estimated from the inferior vena cava and, consequently, will give an MCFP-blood volume relationship displaced to the right of the MCFP-blood volume relationship drawn according to the data from the inferior vena cava (e.g., line A and line B of Figure 7). The MCFP values used to draw line B are calculated by using Equation (4) of page 35 and the MCFP values used to draw line A are calculated by using the same equation, except that IVCP is replaced by HPVP. However, because of the pressure difference between the two vessels, two different sets of MCFP values as well as MCFP-volume relationships are derived. This indicates that the technique currently used to determine MCFP in the anaesthetized rat is not appropriate because different MCFP values are measured from different

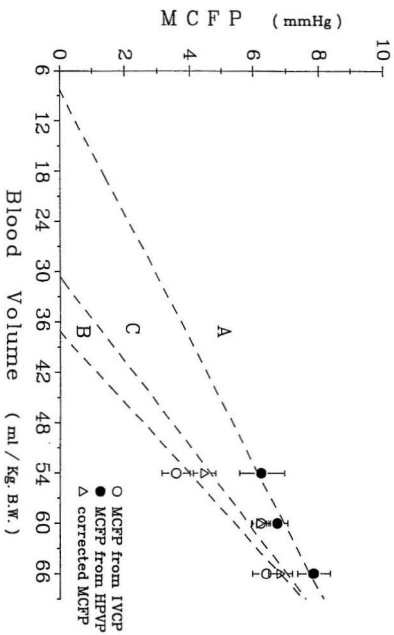
vessels. It also suggests that using MCFP measurement to assess total vascular capacitance is easily affected by the pressure disequilibrium because it can apparently shift the MCFP-volume relationship.

Although line B is drawn in a way that is widely used currently and although the extrapolated unstressed volume (37.1 ml / Kg body weight / mmHg) of this line is close to the values reported in the literature (Trippodo, 1981; Gay et al, 1986; Gay et al, 1988), it may not be truly representative of the MCFP-total blood volume relationship as it does not consider the blood trapped in the splanchnic bed. The line that represents the true MCFP-total blood volume relationship should lie between line A and line B. As the total blood volume and splanchnic volume had not been monitored during MCFP manoeuvre in this study, it is impossible to locate the exact position of this true MCFP-volume line. However, if the total effective vascular compliance is 3.30 ml / Kg body weight / mmHg in the rat (Trippodo, 1981) and if the total splanchnic vascular compliance is 1.1 ml / Kg body weight / mmHg (Brooksby & Donald, 1972), the location of such a curve can be estimated: As a 1 mmHg pressure reduction in the portal vein will transfer 1.1 ml / Kg body weight blood from the splanchnic bed to the inferior vena cava, thus MCFP value will be increased by 0.33 mmHg. Line C of Figure 7 is drawn after correcting the MCFP values of line B by balancing the net

difference between the MCFP value determined from IVCP and corresponding HPVP values with the factor of 0.33. Comparing line C with line B, one can clearly see that, due to the existence of pressure disequilibrium, the MCFP-volume curve determined by currently-used techniques (Line B; Fig. 7) is actually shifted to the right. This, in general, will overestimate the effective vascular compliance and the unstressed volume, and subsequently underestimate the stressed volume and predicted MCFP value. As the pressure disequilibrium manifests more significantly during volume depletion than during volume expansion, this also seriously affects the slope of the MCFP-blood volume relationship and the extrapolated unstressed volume. Therefore, when the difference between corrected MCFP and the MCFP estimated from IVCP is only about 0.8 mmHg at 10% blood volume depletion (4.37 vs 3.55 mmHg), the difference between the extrapolated unstressed volume of line C and that of line B is as high as 5.0 ml / Kg body weight (32.1 vs 37.1 ml / Kg body weight). Thus the extrapolated unstressed volume is highly vulnerable to the pressure disequilibrium.

Figure 7. Comparison of MCFP-blood volume curves derived from three different sources

The filled circle (\bullet) represents the MCFP calculated from the equation: $MCFP_{(b)} = HPVP + 1/60 \times (APP - HPVP)$; the open circle (\circ) represents the MCFP calculated from the equation: $MCFP_{(b)} = IVCP + 1/60 \times (APP - IVCP)$; the open triangle (Δ) represents the corrected MCFP, calculated from the equation: $MCFP_{(c)} = MCFP_{(b)} + 0.03 \times (HPVP - MCFP_{(b)})$. In these equations, MCFP denotes mean circulatory filling pressure; HPVP denotes hepatic portal venous pressure; IVCP denotes inferior vena caval pressure and APP denotes to systemic arterial plateau pressure. The correction factor is 0.03. All the pressures are measured at the 8th second of circulatory arrest. Because there is pressure disequilibrium between the portal vein and the inferior vena cava during MCFP manoeuvre, the true MCFP-volume curve is predicted to lie in line C's position, which generates an extrapolated unstressed volume of 32.1 ml / Kg body weight, 5.0 ml / Kg body weight less than the unstressed volume extrapolated from line B.



4.6 Anaesthesia and pressure disequilibrium

Urethane as a laboratory anaesthetic is commonly used in rat experiments. But it has also been reported to cause a number of unwanted effects. It can cause acute disturbances in energy metabolism (Sanchez-Pozo et al, 1988), increase plasma renin activity (Pettinger et al, 1975; Sonkodi & Nafradi, 1979) and alter the ability to regulate hydration (Severs et al, 1981). In this study, the route of urethane administration into these animals was particularly important because anaesthetics are usually given to rats intraperitoneally. This route can specifically irritate the splanchnic vascular bed where the special interest of the study lies. Intraperitoneal injection of urethane caused evident irritation of regional circulation in the abdomen, which may be a factor affecting the portal pressure value during the MCFP manoeuvres. However, as the pressure disequilibrium was still present after substituting intraperitoneal injection of urethane with intravenous injection, it is unlikely that local stimulation of urethane was a major factor responsible for the pressure disequilibrium.

It is possible that anaesthesia itself was a factor involved in the pressure disequilibrium. Bloch (1955) observed that the depth of surgical anaesthesia can disturb sinusoid sphincters. The deeper the anaesthesia, the more the sinusoidal channels were shut down. Thus, portal pressure

should be elevated. Obviously, changing the anaesthesia route is not much of help to eliminate involvement of anaesthesia in the pressure disequilibrium. To clarify this, a comparison with conscious animals would be necessary.

4.7 The value of maintaining normal systemic arterial pressure after ganglionic blockade

Because it abolishes total body vascular tone, ganglionic blockade can induce a severe fall in systemic arterial pressure which tends to reduce blood perfusion to the organs. Therefore, many investigators correct this arterial pressure drop to re-establish the level at the commencement of their experiments. While it might have been useful to maintain a normal systemic arterial pressure by infusing blood or norepinephrine after ganglionic blockade, this did not prove viable in this study. The main problem linked with volume expansion was that the infusion of a huge quantity of blood to elevate the arterial pressure to the pre-blockade value often led to death of the animals. Alternatively, it was found that the use of norepinephrine infusion produced a markedly higher portal pressure; that made the increase in portal pressure incompatible with the increase in other pressures. It was quite common in our pilot experiments with norepinephrine infusion that portal pressure exceeded 12 mmHg, approximately twice the control value, while the systemic arterial pressure was similar to pre-

blockade values. Subsequently, this resulted in more obvious pressure disequilibrium during MCFP manoeuvring. As the aim to block ganglionic reflexes was to observe if the pressure disequilibrium resulted from the reflex vasoconstriction, failure to restore the arterial pressure to normal should not detract from the main experimental objectives of this study.

4.8 Conclusions

According to the experimental results of this study, it can be concluded that in urethane-anaesthetized rats under the condition of blood volume alterations, hepatic portal venous pressure is significantly higher than the inferior vena caval pressure for at least 16 seconds during the MCFP manoeuvre of right atrial balloon inflation. This pressure disequilibrium between the portal vein and the inferior vena cava persisted after effective reduction in the residual arterial pressure by addition of an abdominal aortic snare, after ganglionic blockade or with intravenous administration of urethane and is likely the result of a physical hepatoportal resistance at zero inflow. Therefore, the basic assumption of equal pressures throughout the entire cardiovascular system during MCFP manoeuvring appears not to hold true when MCFP is determined by this technique. The presence of this pressure disequilibrium in the anaesthetized rat causes underestimation in the MCFP values based on the measurements of inferior vena caval pressure. Thus, it will

affect the application of MCFP-blood volume relationship to determine changes in whole body vascular capacitance by shifting the MCFP-blood volume curve to the right.

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