VASCULAR CAPACITANCE AND THE CONTROL OF VENOUS RETURN: EFFECT OF HEAT STRESS, BARORECEPTOR STIMULATION, AND NEUROPEPTIDE Y

by

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ABSTRACT

The role of capacitance vessels and blood flow distribution (BFD) in the control of venous return and cardiac output were examined. First, saline infusion during exercise in humans maintained plasma volume, reduced heart rate and core temperature, but did not change endurance time. Second, the vascular mechanics of the skin were studied in dogs. The skin has a large venous compliance (C_v) and a long time constant of venous drainage (τ_v), and may act as a blood reservoir during heat stress. A rise in core or skin temperature increases C_v and decreases venous resistance (R_v), but does not change τ_v. The last three studies were performed in dogs on circulatory bypass. The third study examined the mechanisms of increase in venous return during heat stress. Splanchnic (SPL) unstressed volume (V_u) decreases with no change in R_v, C_v, τ_v or BFD during heat stress. This decrease in V_u is abolished by ganglionic blockade but not by α or ß-receptor blockade. The fourth study looked at the effects of the baroreflex on capacitance vessels and BFD. A decreases in carotid sinus pressure from 200 to 50 mm Hg increases SPL blood flow and C_v, and decreases SPL R_v, τ_v and V_u. The decrease in V_u is abolished by ganglionic blockade, but is only partially reversed by α-receptor blockade. The last study examined the effects of neuropeptide Y (NPY) on capacitance vessels and BFD. NPY decreases SPL V_u with no change in R_v, C_v, τ_v or BFD. Thus, NPY may play a role in the control of venous return.

RESUME

Nous avons examiné le rôle de la distribution du débit sanguin et de la circulation veineuse sur le control du retour veineux au coeur et du débit cardiaque. Premièrement, l'infusion de solution saline physiologique chez les humains faisant de l'exercise aide à maintenir un niveau de plasma constant, réduit les pulsations cardiaque et la température du corps, mais n'a pas d'effet sur la durée de l'exercise. Deuxièmement, les qualités mécaniques des vaisseaux cutanés ont été examinées avec une préparation canine. La compliance veineuse (C_v) de la peau est grande et sa constante temporelle de drainage veineux (τ_v) est longue, ce qui pourrait en faire un réservoir sanguin lors d'élévation du débit sanguin à la peau. Une élévation de la température du corps ou de la surface de la peau, augmente C_v et diminue la resistance veineuse (R_v), mais ne change pas τ_v. Les trois dernières études ont été réalisées avec une préparation canine de circulation extracorporelle à débit cardiaque constant. La troisième étude a examiné le mécanisme d'augmentation du retour veineux à température du corps élevé. Le volume passif (V_u) de la region viscérale est réduit, mais R_v, C_v et τ_v, ainsi que la distribution du débit sanguin ne change pas à haute température. Cette réduction du V_u peut être bloqué par un antagoniste ganglionaire, mais pas par des antagonistes de récepteurs adrénergiques de type α ou ß. La quatrième étude a examinée le contrôle des vaisseaux à capacité sanguine

élevée et de la distribution du débit sanguin par le réflexe des barorecepteurs. Une diminution de la pression dans les sinus carotidiens de 200 à 50 mm Hg augmente le débit sanguin viscéral et C_v, et réduit R_v, τ_v et V_u. Cette réduction du V_u peut être bloqué par un antagoniste ganglionaire, mais n'est que partiellement affectée par un bloquer de récepteurs de type α. La dernière étude a examinée l'effet du neuropeptide Y (NPY) sur la circulation veineuse et sur la distribution du débit sanguin. NPY réduit V_u de la région viscérale, mais n'a pas d'effet sur R_v, C_v ou τ_v, ainsi que sur la distribution du débit sanguin. Par conséquent, NPY pourrait jouer un rôle dans le contrôle du retour veineux au coeur.

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PREFACE

This thesis has been divided into eight chapters. The first chapter provides a background of information and a review of the literature pertinent to the thesis. In chapters 2, 3, 4, 5, 6 and 7, I have taken the advantage of the option provided by section 7 of the Guideline Concerning Thesis Preparation which states that "The candidate has the option, subject to the approval of the Department, of including as part of the thesis the text, or duplicated published text, of an original paper, or papers". The experiment in chapter 2 has already been published in the Journal of Applied Physiology, 66(6): 2799-2804, 1989. The experiment in chapter 3 is in press in the American Journal of Physiology, 259 (Heart Circ. Physiol. 28), 1990. The experiment in chapter 4 is under review by the American Journal of Physiology. The experiment in chapter 5 is under review by the Journal of Applied Physiology. The experiments in chapters 6 and 7 are under review by Circulation Research. Chapter 8 contains the conclusions and claim to originality.

In performing the experiments in chapter 2, I have received the assistance of Dr. Levy, Dr. Cosio and Dr. Marliss from the Royal Victoria Hospital, and their names appear as co-authors on this manuscript. I also received the assistance of Dr. A. Fournier of the INRS santé for the experiments in chapter 7 and he is co-author on this

manuscript. Dr. Magder, my thesis supervisor, is the senior author on all the manuscripts in this thesis.

The format of chapters 1, 2, 3, 4, 5, 6 and 7 as well as the units of measurements are those used by the American Physiological Society. The corresponding S.I. units (International System of Units) are referenced in Appendix I. Appendix II contains a list of the abbreviations used in the thesis.

CHAPTER 1

INTRODUCTION

A. DETERMINANTS OF VENOUS RETURN AND CARDIAC OUTPUT

The cardiovascular system is primarily a transport and exchange system. It supplies oxygen and nutrients to organs and tissues, and removes metabolic byproducts which can be recycled or excreted. Other functions of the cardiovascular system include the transport of hormones and peptides to target cells and thermoregulation.

The systemic circulation is composed of a series of vessels with differing functions and morphologies. The arteries, with thick muscular walls, divide into smaller arterioles which provide the resistance to blood flow to various organs and tissues. The arterioles further divide into tiny capillaries where materials are exchanged across thin walls. After the capillaries, the blood drains into venules which, under autonomic innervation, control the quantity of blood contained in the venous compartment. The venules progressively join together to form the large veins which return blood to the right atrium.

The veins and venules are approximately 18 to 30 times more compliant than the arteries (43) and therefore can hold large volumes at comparatively low pressures. For this reason, the veins and venules contain approximately 75% of total blood volume.

Traditionally the heart has been viewed as the energy generator for moving blood around the circulation. In a normal resting man of 70 kg, total blood volume is roughly 5

litres and cardiac output is approximately 5 $l \cdot min^{-1}$. The heart consequently pumps the entire blood volume once every minute. At a resting heart rate of 70 $beats \cdot min^{-1}$, nearly 72 ml of blood are ejected during every stroke and the energy generated by the ejection increases aortic pressure to 120 mm Hg. At maximal capacity, the heart of a young athlete can pump as much as 40 litres of blood per minute with a heart rate over 200 $beats \cdot min^{-1}$ and a stroke volume of nearly 200 ml (26). Clearly, the capability of the heart to pump blood is tremendous.

Nevertheless, the extent to which increases in heart rate and cardiac contractility can increase cardiac output is limited to the heart's ability to decrease right atrial pressure (P_{ra}). Decreases in P_{ra} increase venous return and cardiac output because P_{ra} is the effective back pressure of the entire systemic circulation. However, decreases in P_{ra} are limited by the collapse of the great veins. Once the great veins collapse, a vascular waterfall prevents any further increase in flow. This principle was clearly demonstrated in 1965 by Ross et al. (93) who varied the heart rate of human subjects by artificially pacing their right atrium. On average, increasing heart rate from 80 to 121 $beats \cdot min^{-1}$ increased cardiac output by only 0.05 $l \cdot min^{-1}$. A further increase to 148 $beats \cdot min^{-1}$ actually decreased cardiac output by 0.38 $l \cdot min^{-1}$. This is because as soon as heart rate increases, the time for diastole becomes

shorter. With falling diastolic time, end diastolic volume is reduced because the rate of venous return remains relatively constant. Thus, stroke volume also decreases. The end result of an increase in heart rate is a decrease in stroke volume in such proportion that cardiac output remains approximately constant. Any further increase in cardiac output with a rise in heart rate must be the result of an increase in venous return. In the same manner, an increase in cardiac contractility can increase stroke volume and cardiac output only by decreasing P_{ra}. Any further increase in cardiac output with an increase in contractility must be the result of an increase in venous return.

According to this analysis, changes in heart rate and cardiac contractility have little direct effect on cardiac output and on the rate of venous return. Consequently, the level of cardiac output and venous return must be determined by the characteristics of the circuit itself. There has to be a region in the systemic circulation, therefore, which serves as a reservoir from which blood continuously drains back to the heart. The pressure in this reservoir provides the energy for venous return, and the difference between this pressure and P_{ra} is the pressure gradient for venous return. The primary role of the heart in this system is to maintain the reservoir volume constant.

The region which serves as a reservoir can be assumed to be the veins and venules because they contain 75% of total

blood volume and have a compliance 18 to 30 times greater than that of the arteries. In order to understand the control of venous return and cardiac output therefore, one must study the factors which affect the pressure gradient for venous return. These are discussed in the next sections.

The Concept of Mean Systemic Pressure

In 1850, E. Weber was first to point out the importance of the circuit in the control of cardiac output (125). He constructed a model of the circulation in which the heart was replaced by two one-way valves in series, the arteries and veins were replaced by a loop of small intestine, and the microcirculation was represented by a sponge in the middle of the loop. The loop was filled with water, a portion of which was necessary to fill the tubing. This portion will be called "unstressed volume" because it does not stretch the elastic walls of the vessel to create pressure. The other portion of water will be called "stressed volume" because it creates pressure against the walls. After the loop was filled, the pressure was the same throughout and Weber called it the "the mean systemic pressure". By compressing a portion of intestine upstream from the valves, unidirectional flow occurred. The pressure on the side before the sponge increased (arterial) while the pressure on the side past the sponge decreased (venous).

Weber then measured the pressure at different segment lengths along the loop during flow. By adding the pressures together and dividing by the number of segments, he found that the resulting pressure was equal to the mean systemic pressure. Weber concluded that the heart cannot increase the pressure in the circulatory system, it can only redistribute the mean systemic pressure between the arteries and the veins. In order to increase the mean systemic pressure, volume must be added to the loop.

It took more than 40 years before the clinical significance of the mean systemic pressure was recognized. In 1897 Starling (118) recognized that after circulatory arrest, when the heart starts pumping to increase arterial pressure and decrease venous pressure, there must be a point in the circuit where the pressure does not change. As systemic flow decreases during heart failure, the pressure on the arterial side of this point will decrease while the pressure on the venous side will increase. If this point is located upstream from the capillaries, as the heart fails capillary pressure will rise and edema will ensue. If this point is located downstream from the capillaries, heart failure should result in a decrease in capillary pressure and the edema must be explained otherwise.

Using areflexic dogs, Starling found that portal pressure varied very little with circulatory arrest. Therefore, the pressure on the arterial side of the portal

vein will decrease with circulatory arrest whereas the pressure on the venous side will increase. Because the capillaries are located on the arterial side of the portal vein, Starling concluded that capillary pressure would decrease with heart failure and that the failure, by itself, could not be responsible for the increase in capillary filtration and edema.

When Starling performed the same experiment in dogs with intact reflexes, circulatory arrest resulted in a large increase in portal pressure and in mean systemic pressure due to active vasoconstriction. Starling concluded that vasoconstriction will inevitably accompany heart failure and will result in an increase in mean systemic pressure. This increase in mean systemic pressure will, in turn, increase capillary pressure and give rise to edema.

Forty years later, in 1940, Starr (119) used the concept developed by Starling and compared the mean systemic pressure of patients dying from prolonged congestive heart failure with that of patients dying without heart disease. In the former, mean systemic pressure was 20.3 cm of H_2O, and in the latter, it was 7.6 cm H_2O. This difference in mean systemic pressure could account for almost all of the difference in central venous pressure between these patients during life. Starr concluded that venous congestion with heart failure is not due to the mechanical weakness of the heart, but to the increase in mean systemic pressure through

vasoconstriction, increased blood volume or compression of the vessels from within.

Starr, with the help of Rawson (120), developed a model of the circulation which permitted the study of the effects of heart failure or changes in mean systemic pressure or both on cardiac output. The results of these studies expressed the first hint of the influence of mean systemic pressure on venous return and cardiac output. They found that an increase in what they called the "static blood pressure" was a method of partial compensation for the failing heart. For the same degree of heart failure, the circulating flow was much higher when mean systemic pressure was high than when it was low. It took another decade and the work of Arthur Guyton, however, before the relationship between mean systemic pressure and flow would be fully developed.

Cardiac Response and Venous Return Curves

Guyton emphasised the importance of the peripheral bed in the control of cardiac output by combining the cardiac response curve, i.e. the relationship between P_{ra} and cardiac output, and the venous return curve, i.e. the relationship between P_{ra} and venous return. Guyton reasoned that there can be one and only one point where the cardiac response curve and the venous return curve intersect, and this point gives the actual cardiac output for a given P_{ra}.

He constructed cardiac response curves by giving dogs large blood transfusions and studying right atrial pressure and cardiac output (45). The main factors affecting cardiac response curves have been well studied and are heart rate, contractility, preload and afterload. These factors can be influenced on a beat to beat level, or on a steady state basis, by the phase of respiration, oxygenation, sympathetic activity, myocardial damage and cardiac fatigue. Besides the normal cardiac response curve, Guyton studied the effect of sympathetic stimulation, which shifts the cardiac response curve up, and myocardial damage, which shifts the cardiac response curve down (Fig. 1.1).

Guyton's major contribution was to define and express mathematically the factors affecting the venous return curve. He constructed the curves by replacing the heart with a pump and measuring P_{ra} as the pump rate was varied (42). As venous return gradually decreases by slowing down the pump, P_{ra} increases until it is equal to the mean systemic pressure [or mean circulatory filling pressure (MCFP) in Guyton's terms]. At that point, venous return stops. Thus, MCFP is the driving pressure for the return of blood to the heart and the gradient for venous return is the difference between MCFP and P_{ra}. As P_{ra} decreases, venous return increases until P_{ra} is equal to approximately 0 mm Hg. At right atrial pressures below 0 mm Hg, venous return remains constant because the large veins entering the thorax

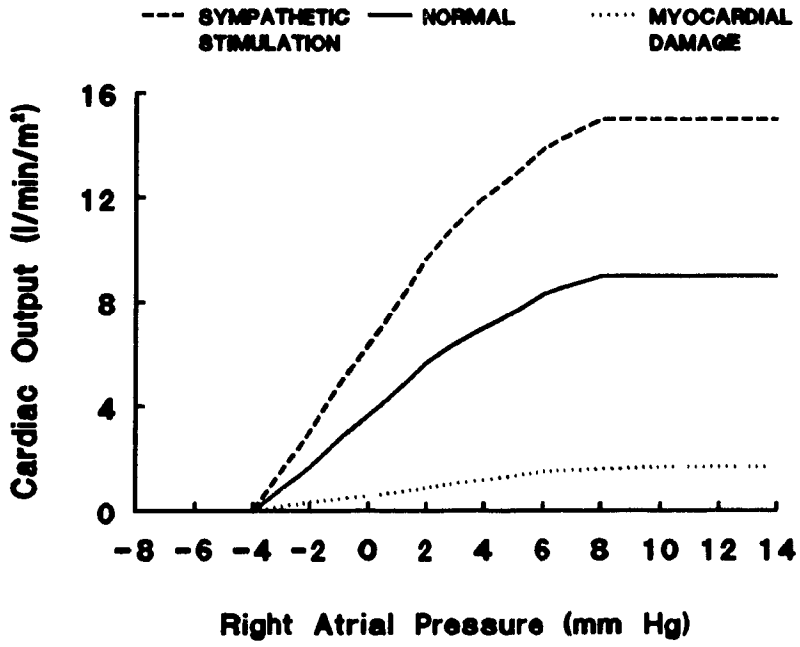

FIGURE 1.1: Relationship between right atrial pressure and cardiac output. The curves represent the cardiac response under normal conditions, under myocardial damage and under sympathetic stimulation.

collapse. When the pressure surrounding the veins exceeds the pressure inside the vessels, the walls collapse and a vascular waterfall prevents any further increase in flow. The maximum gradient for venous return (at constant MCFP) and the maximum venous return thus appears at a P_{ra} of 0 mm Hg or at the pressure of collapse (Fig. 1.2).

Mean systemic filling pressure can be increased by increasing stressed volume, by vasoconstricting the venules or by increasing the pressure surrounding the vessels. At constant P_{ra}, an increase in MCFP raises the pressure gradient and venous return rises (Fig. 1.2).

Another determinant of venous return is the resistance to venous return. Guyton demonstrated that increasing arterial resistance, which is located between the left ventricle and the venous blood reservoirs, greatly affects left ventricular pressure but has little effect on venous return. On the other hand increasing venous resistance, which is located between the blood reservoirs and the right atrium, greatly reduces cardiac output by decreasing the slope of the venous return curve (Fig. 1.2). Consequently, the level of venous resistance is an important determinant of venous return.

Mathematically, Guyton's analysis of the determinants of venous return can be expressed by the equation: $VR = (MCFP - P_{ra})/RVR$, where RVR is the resistance to venous return (43). Guyton and associates pointed out that the

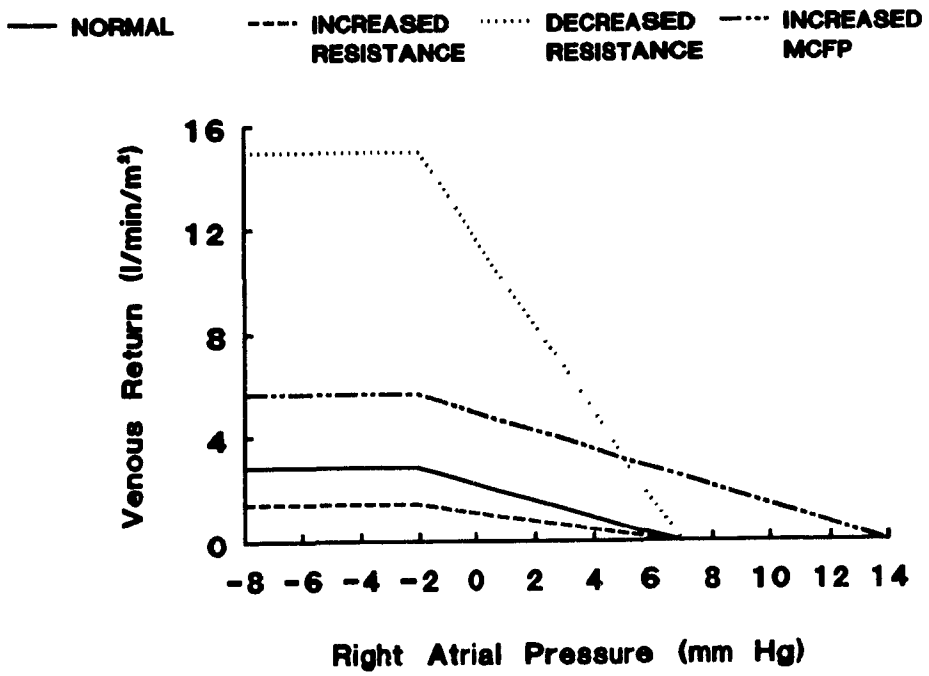

FIGURE 1.2: Relationship between right atrial pressure and venous return. The curves represent venous return under normal conditions, with an increase in mean circulatory filling pressure (MCFP), with an increase in venous resistance and with a decrease in venous resistance.

term RVR includes the venous resistance of each segment of the circulatory system as well as the weighted "capacitance" (compliance) of each portion of the circulatory system. They also pointed out that the size of the compliances are one of the most important factors which determine venous return and cardiac output. Examples of the combined effect of changes in cardiac response curves and venous return curves are shown in Figure 1.3.

Using this model of the circulation, Guyton and coworkers (44) asked whether the increase in cardiac output during epinephrine (EPI) infusion was due to an improved cardiac performance or to an increase in the return of blood from the periphery. Thus they constructed venous return curves in areflexic dogs using different concentrations of EPI. They found that, for a given P_{ra}, increases in cardiac output during EPI infusion are primarily due to an increase in MCFP. The direct effect of EPI on the heart, if MCFP remains constant, is only a marginal increase in cardiac output.

Two Compartment Model of the Circulation

In their analysis of the determinants of venous return, Guyton and colleagues (43) acknowledged the importance of differences in regional venous compliance. This concept, however, was developed as early as 1912 by August Krogh (68). He constructed a model of the circulation based on

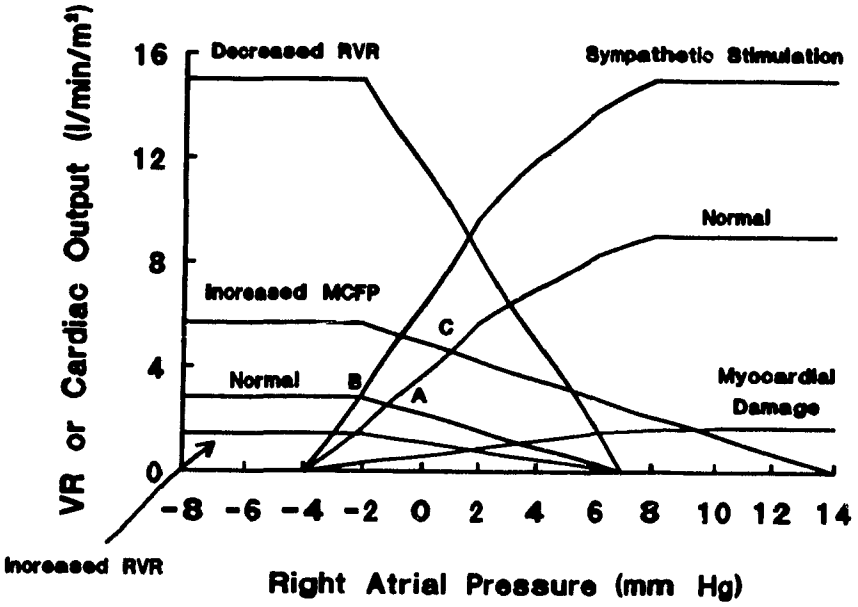

FIGURE 1.3: Relationship between right atrial pressure and venous return or cardiac output. The curves represent the venous return and cardiac responses as depicted in Figures 1.1 and 1.2. Point A represents normal cardiac output and right atrial pressure. Point B represents the effect of an increase in cardiac function only on cardiac output and venous return. Point C represents the effect of an increase in MCFP only on cardiac output and venous return. RVR, resistance to venous return.

the observation that dilatation of the arterioles to any organ must always result in a decrease in blood pressure unless compensated for by other mechanisms. Because blood pressure is tightly regulated, possible mechanisms which could restore blood pressure with regional vasodilatation include increases in cardiac performance and vasoconstriction in other regions. However, increasing the work of the heart is not enough to compensate for dilatation because, in Krogh's words, the heart "cannot do more than send out what it gets and what it gets by a (regional) vasodilatation is not sufficient to restore pressure". Also, contraction of arterioles in other organs would restore blood pressure only as long as total resistance remains constant. Krogh pointed out that during exercise there must be a decrease in total resistance since cardiac output increases while blood pressure remains relatively constant. He hypothesized, therefore, that there had to be a source of blood, independent of the dilatation in the working muscles, which will increase venous return to the level necessary during exercise. This source, he suggested, was the "portal system" (splanchnic bed).

Krogh described the portal system as a reservoir capable of holding varying amounts of blood, trapped between two resistances, a primary (arterial) and a secondary (venous) resistance. A decrease in the primary resistance increases the inflow to the portal system. The outflow from the

portal system, however, does not rise to the level of the inflow until volume accumulation in the compliant venules increases the pressure gradient for flow. This accumulation of volume in the portal system decreases the amount of blood available for the general circulation and results in a fall in venous return. On the other hand, an increase in the primary resistance decreases inflow to the portal system and results in a fall in volume and pressure. As volume leaves the portal region the amount of blood available for the general circulation and venous return increases.

To test his hypothesis, Krogh constructed a mechanical model of the circulation which permitted to control flow through two compliant beds in parallel. One bed had a large capacitance and represented the portal system, the other bed had a smaller capacitance and represented the "normal" veins. The arterial resistances of both regions could be controlled independently and there was a secondary resistance at the end of the bed representing the portal system. As Krogh predicted, increasing arterial resistance to the bed with a large capacitance, while maintaining total inflow constant, resulted in an increase in venous return. Decreasing arterial resistance to the same bed decreased venous return. Also, decreasing arterial resistance to the bed with a smaller capacitance increased venous return, and vice versa. With these experiments, Krogh demonstrated that changes in fractional flow to regions with differing venous

compliance can affect venous return without any change in MCFP.

In vivo evidence in favor of Krogh's model was obtained in 1935 by Barcroft and Samaan (6) who tried to explain the paradoxical increase in cardiac output with occlusion of the descending thoracic aorta. The increase in systemic flow could not be explained by reflex mechanisms because the central nervous system was destroyed. These authors attributed the increase to the mechanical effects of blood flow redistribution, i.e. from the portal bed to the less compliant bed. Aortic occlusions were also performed by others (3,28,59,60,115,121,122) with results similar to that of Barcroft and Samaan.

It was not until the middle of the 1970's before a mathematical model was developed by Caldini et al. (14) to describe Krogh's model and Barcroft and Samaan's results. In an attempt to evaluate the peripheral effects of epinephrine on cardiac output, Caldini and associates used a preparation in which the right ventricle was bypassed by a mechanical pump in order to control right atrial pressure and flow. Also, an external reservoir was interposed between the right atrium and the pump so that, at constant P_{ra} and flow, any active change in the periphery would be reflected by reciprocal changes in the reservoir blood level. With this preparation, it was possible to measure peripheral vascular compliance and resistance to venous

return. By analyzing the time course of the change in reservoir volume for a change in P_{ra} at constant flow, and plotting the results on semilogarithmic paper, these investigators found that the curves did not follow a single exponential decay. Better fits of the curves could be achieved with an analysis using two exponentials. These authors concluded that the systemic circulation behaved as if composed of two parallel compartments with differing time constants of venous drainage (defined as the product of venous resistance and venous compliance, τ_v), one fast and one slow. Further analysis showed that EPI resulted in an increase in fractional flow to the fast time constant bed with little effect on the venous parameters. They concluded that the steady state increase in venous return with EPI infusion is due to a redistribution of blood flow from the slow to the fast τ_v channel rather than a direct effect of EPI on the veins.

From these results, Caldini et al. developed a model in which the circulation is divided in two parallel compartments with differing time constants of venous drainage. In this model, Guyton's equation for venous return was applied to each of the two beds and the overall equation was $VR = (V - P_{ra}C_t)/(F_f\tau_f + F_s\tau_s)$, where, V is the stressed volume or total blood volume minus unstressed volume, C_t is total compliance, $(_f)$ is fast, $(_s)$ is slow and F is the fractional flow (for full derivation of the

equation see reference 14). An increase in fractional flow to the slow time constant bed decreases the slope of the venous return curve in the same way as an increase in resistance to venous return would in Guyton's model (Fig. 1.2). A decrease in fractional flow to the slow time constant bed increases the slope and venous return for a given P_{ra}. Changes in MCFP and resistance to venous return would affect the venous return curve in the same way as before.

Caldini and colleagues agreed with Krogh and considered the bed with a long time constant to be the splanchnic compartment. Since then, many investigators have divided the systemic vasculature in two compartments and all studies (36-38,75,78) but one (13) have found a longer τ_v for the splanchnic bed.

The Determinants of Venous Return

So far, the analysis of the determinants for venous return can be summarized as follows. The pressure gradient for venous return is determined by the difference between MCFP and P_{ra}. MCFP is a function of stressed volume and total vascular compliance, and P_{ra} is determined by the intersection of the cardiac response curve and the venous return curve. Resistance to venous return is a function of the regional values for τ_v and of the fraction of cardiac output going to these regions. The value of a regional τ_v

is determined by the product of its venous compliance and venous resistance. Finally, the entire circulation can be separated in two beds with differing τ_v, one faster, the extrasplanchnic bed, and one slower, the splanchnic bed.

Venous return can therefore be altered by changing one or more of the following determinants: stressed volume, right atrial pressure, venous resistance, venous compliance and the fractional flow to vascular beds with differing time constants.

B. EXPERIMENTAL MODELS FOR THE STUDY OF VENOUS RETURN

The factors affecting venous return have been investigated in a variety of animal models ranging from isolated organs to the intact conscious animal. These factors will be discussed in details shortly, but the general strategies used to study their effects can be divided in two groups. One approach has been to study the effect of interventions such as hypoxia, hypercapnia, hypothermia, thyrotoxicity or hypertension on the determinants of venous return. The contribution of endogenous responses such as autoregulation, baroreceptor reflex or lung inflation have been assessed also. Studies such as these attempt to define general responses to conditions which can be encountered clinically, and to identify the contribution of reflexes and local mechanisms to these responses.

Another approach has been to stimulate pharmacologically mixed or specific adrenergic receptors and cholinergic receptors to examine their effects on the determinants of venous return. In these studies, a variety of adrenergic, ganglionic and muscarinic blockers have been used in conjunction with the agonists to further define the specificity of the effects observed. Also, the effect of endogenous substances such as epinephrine, norepinephrine (NE), angiotensin II, vasopressin, histamine or atrial natriuretic peptide (ANP), and of other substances such as nitroglycerin, nifedipine and captopril have also been studied. These studies are performed in an attempt to manipulate the determinants of venous return, and have direct pharmacological and clinical applications.

A wide variety of techniques have been used to study the determinants of venous return. These techniques invariably measure one or more of the following: the movement of blood to and from vascular beds, MCFP, stressed or unstressed volume, blood flow distribution, venous compliance, venous resistance, time constant of venous drainage and pressure volume curves. The results from the various studies on venous return will therefore be summarized accordingly.

Changes in Volume

Because blood volume is an important determinant of venous return, many investigators have measured volume

changes in the total systemic circulation, in isolated vascular compartments, or in isolated organs under different conditions. This is usually done by using a bypass circuit in which venous outflow is drained into a reservoir and pumped back into the pulmonary artery or right atrium. Changes in reservoir blood volume at constant cardiac output thus mirror changes in systemic blood volume. Also, changes in venous return can be obtained by changing the pump rate while keeping reservoir volume constant. Variations of this technique can be used to measure changes in regional blood volume and regional blood flow.

Changes in the blood volume of specific organs have also been estimated by measuring changes in weight, in dimensions, in pressure, or recently, in radionuclide counts (7,29,80,91). An increase in volume of the organ usually means volume accumulation and is often interpreted as a decrease in venous return. This interpretation, however, could be inaccurate if the change in volume is due to a change in flow through the organ. An increase in flow to any organ almost always results in a passive accumulation of volume. Nonetheless, as seen in Krogh's model, an increase in fractional flow to organs with a fast time constant bed results in an overall increase in venous return.

In some preparations, such as isolated organs or in the intact conscious animal, total blood volume is measured and pressure-volume (P-V) curves are constructed. Extrapolation

of P-V curves to zero pressure gives a value for the unstressed vascular volume. As with any extrapolation, there is a possibility that the P-V curve becomes more curvilinear at low pressures and volumes, and that the unstressed volume does not represent the true intercept of the relationship. However, if the physiologically functional part of the relationship is linear, the calculated unstressed volume describes the position of the P-V curve for any given condition. Changes in the position of the P-V curve are then represented by changes in the calculated unstressed volume. Thus, the calculated unstressed volume is a measure of the capacitance of a given vascular bed. Decreases in unstressed volume are usually interpreted as a reciprocal increase in stressed volume, and an increase in venous return, and vice versa.

Volume shifts between an external reservoir and the systemic circulation are a fast and simple way to describe the effect of any given intervention on venous return. For this reason, the technique has been used more often than any other to study venous return, and the results from such studies will be described first. Nevertheless, these results give no information on whether the shifts in volume were due to a change in unstressed volume, in venous resistance, in venous compliance, or to a redistribution of blood flow. Thus, in order to understand the mechanisms of a change in venous return, a description of the studies

which measured the determinants of venous return will follow.

Variations in carotid sinus pressure and activation of the baroreceptor reflex result in shifts of volume between the total systemic vascular bed and an external reservoir in dogs (12,13,16,46,57,81,92,111-113). In such preparations, the pressure in the carotid sinuses is controlled independently of arterial pressure. An increase in carotid sinus pressure results in a decrease in arterial pressure, an accumulation of volume in the systemic bed and a decrease in reservoir volume. A decrease in carotid sinus pressure results in the reverse scenario. Thus, the baroreceptor reflex counteracts perceived hypertension by pooling blood and decreasing venous return, whereas hypotension is opposed by volume recruitment and increases in venous return. For this reason, the direct effect of any drug or endogenous substance on blood volume shifts should be separated from their effect on blood pressure and activation of the baroreceptor reflex (see chapter 6).

The volume shifts between the systemic bed and an external reservoir in response to a change in carotid sinus pressure range between 1.25 (81) and 12.9 $ml \cdot kg^{-1}$ (92) in dogs. One important factor responsible for this wide variation is the magnitude of the change in carotid sinus pressure studied. If arterial pressure is kept artificially constant during a change in carotid sinus pressure, the

magnitude of the shift in volume is much greater (113). On the other hand, if the spleen is removed, the magnitude of the change in volume decreases by up to 32% (112). In isolated preparations, the volume shift produced by a change in intrasinus pressure is similar in the splanchnic (12,13) and in the intestinal (46) circulation in dogs. This volume shift ranges between 5 and 7 $ml \cdot kg^{-1}$.

Pharmacological agents also shift volume between the systemic circulation and an external reservoir. In dogs, both epinephrine and norepinephrine infusion result in a dose dependant increase in external reservoir volume ranging from 0.4 (61) to 19 $ml \cdot kg^{-1}$ (27,92). Thus, EPI and NE infusions increase venous return. EPI and NE also increase arterial pressure. Therefore, baroreflex activation will resist the decrease in systemic volume produced by EPI and NE. Consequently, with combined baroreceptor denervation and EPI or NE infusion, one would expect a larger decrease in volume from the systemic bed. This is precisely what Müller-Ruchholtz et al. found during EPI infusion (83) but not during NE infusion (82). The results with NE are surprising and suggest that NE plays a role in the increase in systemic volume at high carotid sinus pressures (probably through an increase in venous resistance).

Epinephrine and NE are mixed α and ß-adrenergic receptor agonists; EPI is mostly ß and NE is mostly α. The respective contribution of these two receptor types to the

changes in volume produced by EPI and NE has been investigated by giving adrenergic blockers. Blockade of ß-receptors reduced the shift in volume from the systemic bed during EPI infusion (8,61,83), but not during NE infusion (82). Combining ß-receptor blockade with baro-denervation decreased the shift in volume with EPI (83) but not with NE (82) infusion. Combining α-receptor blockade with baro-denervation reduced the shift in volume from the systemic bed during EPI infusion (83), and abolished it during NE infusion (82). These results demonstrate that EPI has more of a mixed α and ß activation on the venous system whereas NE activates mostly α receptors.

In isolated preparations, norepinephrine infusion resulted in a decrease in volume of 170 ml per kg of liver in cats (41), of 25 $ml \cdot kg^{-1}$ in the intestine of dogs (96) and of 18.7 $ml \cdot kg^{-1}$ in the splanchnic bed of dogs (102). In the intestine of dogs, maintaining perfusion pressure constant by decreasing flow during NE infusion further decreased vascular volume (96). Removal of the splanchnic region before NE infusion completely abolished the shift in volume from the systemic bed to an external reservoir in dogs (102). This further supports the primary role of the splanchnic region as the body's vascular reservoir.

Alpha receptor stimulation with phenylephrine (8) or xylometazolin (82) in dogs results in a shift of volume out of the systemic bed into an external reservoir. Splanchnic

and splenic vascular volumes also decrease with phenylephrine (7). Stimulation with methoxamine, however, resulted in a shift of volume from an external reservoir to the systemic circulation (61). Activation of α-receptors results in an increase in arterial pressure but the decrease in volume with xylometazolin was not affected by baro-denervation (82). This effect is similar to what was seen with NE and baro-denervation (82) and suggests that the increase in systemic volume at high carotid sinus pressure is α-receptor mediated. Alpha-receptor blockade alone results in the pooling of 2.7 $ml \cdot kg^{-1}$ of blood in the systemic circulation in baro-denervated dogs (82).

Stimulation of ß-adrenergic receptors with isoproterenol results in a dose dependant recruitment of volume from the systemic bed in dogs ranging from 0.7 (61) to 12 $ml \cdot kg^{-1}$ (8). This shift in volume is abolished by ß-receptors blockade (8,83,102) or removal of the splanchnic bed (102). Arterial blood pressure decreases with isoproterenol infusion (8,36,61,83). One would expect activation of the baroreflex to result in the recruitment of volume from the systemic circulation and add to the effect of isoproterenol. Consequently, as Müller-Ruchholtz et al. (83) observed, baro-denervation reduced the change in volume mobilisation with isoproterenol. However, Imai et al. (61) saw no difference in the systemic volume change with isoproterenol and baro-denervation. This may indicate that the volume

mobilisation by the baroreflex during hypotension is achieved through ß-receptor activation. Thus, if isoproterenol results in the activation of most of the ß-adrenergic receptors, no decrease in volume change would be observed with baro-denervation. However, if isoproterenol results in partial activation of the ß-receptors, baro-denervation would result in a decrease in volume change. Müller-Ruchholtz et al. (83) also found that the decrease in systemic volume with isoproterenol is abolished with combined baro-denervation and α-receptor blockade. However, because arterial pressure was already below 50 mm Hg before isoproterenol infusion in these experiments, interpretation of the results becomes hazardous.

In isolated preparations, isoproterenol infusion in dogs resulted in a 14 $ml \cdot kg^{-1}$ decrease in splanchnic volume (102), no change in liver thickness (95) and an increase in intestinal volume (96). Finally, blockade of ß-receptors with prindolol alone decreases external reservoir volumes by 3.5 $ml \cdot kg^{-1}$ and, although arterial pressure was decreased, baro-denervation did not affect this shift in volume (83). These results add further evidence that at least part of the decrease in systemic volume at low carotid sinus pressure is ß-receptor mediated.

Ganglionic blockade alone results in a large (17.3 $ml \cdot kg^{-1}$) decrease in external reservoir volume in dogs on circulatory bypass (92).

Acetylcholine infusion results in a recruitment of volume from the systemic circulation (92), the splanchnic bed and the splenic bed (80) in dogs. The decrease in volume of the splanchnic and splenic beds was during blockade of nicotinic ganglionic receptors. There was no change in intestinal volume with acetylcholine infusion (96) but hepatic and extrasplanchnic volumes increased (80). Splenectomy diminished the acetylcholine induced change in splanchnic volume and abolished the changes in hepatic and extrasplanchnic volume. These results indicate that a large part of the change in splanchnic volume with acetylcholine occurs in the spleen. The change in splanchnic volume during acetylcholine infusion is abolished by muscarinic blockade. However, evidence that acetylcholine does not act directly on the splanchnic capacitance vessels but works through decreases in splanchnic flow was provided by Rutlen et al. (103). In a baro-denervated preparation, they showed that the changes in splenic and hepatic segment length (which represents volume changes) obtained by aortic constriction were equal to those obtained with acetylcholine infusion in the systemic circulation. Furthermore, acetylcholine infused directly into the portal vein had no effect on hepatic segment length.

The effets of angiotensin II and vasopressin on systemic volume changes have also been examined. In one study there was no change in reservoir volume with angiotensin II

infusion (27), whereas in another study external reservoir volume decreased by 4.5 $ml \cdot kg^{-1}$ (76). Vasopressin resulted in a 8 $ml \cdot kg^{-1}$ decrease external reservoir volume in cats (76) and in a 21 $ml \cdot kg^{-1}$ decrease in dogs (27).

Nitroglycerin decreases external reservoir volumes even in the presence of an α-adrenergic agonist (86). Nitroglycerin also increases splanchnic blood volume in man and in dogs (91). Thus, nitroglycerin results in a fall in venous return and of the work of the heart.

Morphine was found to decrease external reservoir volume by as much as 21 $ml \cdot kg^{-1}$ (38) and decrease venous return.

Hypoxia results in an increase in external reservoir volume (56). Splenectomy, baro-denervation and chemo-denervation as well as ganglionic blockade greatly reduce the decrease in systemic volume during hypoxia (56). These results suggest that most of the effects of hypoxia are mediated through reflexes.

Lung inflation to tracheal pressures of 20 mm Hg decreases external reservoir volume (17). These changes in volume are reduced by increases in carotid sinus pressure and vagotomy, and are abolished by combined vagotomy and ganglionic blockade. These results suggest that changes in volume with lung inflation are reflex mediated. Left ventricular distention also increases systemic volumes (57).

Using a bypass preparation Green (37) found that hypothermia decreases external reservoir volume by 31 $ml \cdot$

kg^{-1}. Thus venous return decreases significantly with hypothermia.

In summary, the factors which will result in a decrease in external reservoir volume include increases in carotid sinus pressure, lung inflation, left ventricular distention, hypothermia, ganglionic blockade, and infusion of angiotensin II, vasopressin, nitroglycerin and morphine. These factors decrease venous return in anesthetized animals. Factors which will recruit blood from the systemic venous bed include decreases in carotid sinus pressure, hypertension, hypoxia, and the infusion of epinephrine, norepinephrine, phenylephrine, isoproterenol and acetylcholine. These factors increase venous return in anesthetized animals.

The effect of a number of interventions on unstressed volume or total blood volume or both has also been investigated. Ganglionic blockade alone results in a 7.5 $ml \cdot kg^{-1}$ increase in unstressed volume (87) in intact anesthetized dogs without a spleen. This increase probably explains the decrease in reservoir volume with ganglionic blockade. In conscious rats, total blood volume was not affected by ganglionic blockade (127). Using the data from Green (36), it can be calculated that stressed volume increases with isoproterenol in dogs. Hypoxia increases in stressed volume in anesthetized dogs (75).

Infusion of atrial natriuretic peptide results in a

decrease in total blood volume and unstressed volume (123) in anesthetized and areflexic rats, and in anesthetized and areflexic dogs with splenectomy (69). These decreases in volume are maintained even if arterial pressure is maintained constant with EPI infusion, but are greatly diminished in anephric rats (123). These results suggest that, even though ANP results in a decrease in arterial pressure and venous return, it also decreases unstressed volume through venoconstriction.

In areflexic anesthetized dogs with splenectomy, Lee et al. (70) found no changes in total blood volume and unstressed volume with the infusion of angiotensin II or vasopressin. In conscious rats, angiotensin II did not affect total blood volume (127). Increases in reservoir volume with angiotensin II and vasopressin, therefore, do not appear to be due to a changes in unstressed volume.

Nifedipine, a dihydropyridine calcium antagonist, decreases unstressed volume without affecting total blood volume in areflexic anesthetized dogs, whereas captopril, an angiotensin converting enzyme inhibitor, does not affect volume (87). The decrease in unstressed volume during nifedipine infusion should increase venous return and, thereby, increase the work of the heart. On the other hand, captopril should not affect venous return.

Renal or spontaneous hypertension do not increase total blood volume but decrease unstressed volume in conscious

rats (23,106). This decrease in unstressed volume during hypertension should increase preload. With hypertension therefore, the heart must perform more work because of an increase in both preload and afterload.

Hypothermia increases unstressed volume by 7.7 $ml \cdot kg^{-1}$ in anesthetized dogs (37). The only data available on hyperthermia is on changes in total blood volume. Increases in total blood volume may be considered as an increased reserve for recruitment of volume in order to maintain high levels of venous return. Decreases in total blood volume may be considered the opposite. Changes in total blood volume during hyperthermia in intact animals are species dependant. Total blood volume increases in acclimatized and non-acclimatized rats during heat stress (58) whereas there is no change in the blood volume of dogs with splenectomy (77).

Changes in Venous Compliance

The pressure in the systemic venous compartment for a given blood volume (MCFP) is determined by its venous compliance (C_v). Therefore, increases in C_v, for a given blood volume and right atrial pressure, will decrease MCFP and venous return, whereas decreases in C_v will increase MCFP and venous return. Changes in C_v have been measured in a variety of ways. In bypass preparations, this can be done by altering the pressure of the venous outflow draining into

the reservoir and measuring the resulting change in vascular volume. In intact animals, MCFP can be obtained before and after injection of a known amount of volume into the circulation. In dogs and rats, the two species most studied, values for total systemic C_v range between 1 and 3.5 $ml \cdot mm\ Hg^{-1} \cdot kg^{-1}$. In isolated organs, values for the dog intestine fall within the above range (96) whereas Greenway et al. (41) found a value between 25 and 30 $ml \cdot mm\ Hg^{-1}$ per kg of liver in cats. Splanchnic C_v is approximately twice as large as that of the extrasplanchnic bed (13,75,78).

Studies on the effect of changes in carotid sinus pressure on C_v have produced conflicting results. In one study, Shoukas et al. (113) found that changes in carotid sinus pressure between 75 and 200 mm Hg had no effect on total C_v. The same group, however, later found an increase in total C_v with increases in intrasinus pressure (13,111, 112). The reason for this discrepancy is not known. The increase in C_v was not affected by splenectomy (112), but was abolished by epinephrine infusion (111). On the other hand, Ross et al. (92) found a decrease in total C_v with increases in carotid sinus pressure. Alexander (2) obtained the same results using isolated intestinal loops. Hoka et al. (57) and Brunner et al. (13) found no change in splanchnic and extrasplanchnic C_v with changes in intrasinus pressure. It is difficult, therefore, to draw any definitive conclusions about the effect of the baroreceptor

reflex on venous compliance. More studies are needed to clarify this subject.

Most studies have found a decrease in total C_v with EPI or NE infusion. These studies were performed in anesthetized dogs (78,111) and cats (76) as well as conscious dogs (8) and rats (127). C_v also decreased when NE was combined to ganglionic blockade in anesthetized rats (123), and with NE infusion in the liver of cats (41). One study found no change in C_v with NE infusion (22) whereas two studies found an increase in C_v with EPI or NE infusion in anesthetized dogs (92,102). An increase in C_v with EPI was also found in isolated intestinal loop in dogs (2). The reason for this discrepancy is not known. So far therefore, the evidence indicates that C_v decreases with EPI and NE infusion. This may explain the increase in reservoir volume with NE and EPI infusions and would tend to increase MCFP and venous return in the intact conscious animal.

Ganglionic blockade by itself decreases total C_v in anesthetized dogs (22,92) but there was no change in C_v when hexamethonium was combined with atropine infusion (87). Ganglionic blockade did not change C_v in conscious rats (127). Thus, the effect of ganglionic blockade on C_v remains to be clarified.

The effect of α-adrenergic agonists on C_v has not been investigated. However, ß-adrenergic stimulation with isoproterenol does not alter total C_v in anesthetized dogs

(36). Acetylcholine alone was found to increase total C_v (92) in anesthetized dogs but there was no change in compliance with combined acetylcholine and baro-denervation in anesthetized pigs (103). The effect of acetylcholine on C_v , therefore, appears to be reflex mediated. Angiotensin II infusion decreases C_v in anesthetized cats and dogs whereas vasopressin, captopril and nifedipine infusion have no effect on C_v (70,76,87). ANP in anesthetized rats (123) and dogs (69), as well as morphine (38) and left ventricular distension (57) in dogs have no effect on C_v. Nitroglycerin increases the compliance of the slow time constant bed in dogs and decreases that of the fast time constant bed (86). These effects were abolished by infusion of an α-adrenergic agonist. There was no difference in the total C_v of conscious hypertensive rats compared to the normotensive animal (23,106).

Hypothermia decreases the compliance of the splanchnic and extrasplanchnic beds (37) whereas hypoxic hypoxia increases total and splanchnic C_v in dogs (75). In one study, the effect of heat stress on C_v was measured in conscious rats (58). Total vascular compliance was measured from the relation between changes in blood volume and central venous pressure during an infusion of 1.6% of body weight of saline over 10 min. Neither cardiac output nor heart rate was controlled during this measurement. There was no change in compliance during heat stress in non-

acclimatized rats but there was a decrease in compliance during heat stress in acclimatized rats.

In summary, the factors which decrease C_v and which would tend to increase MCFP and venous return include, EPI, NE, angiotensin II and hypothermia. The factors which increase C_v and would tend to decrease MCFP and venous return include acetylcholine, nitroglycerin and hypoxic hypoxia. The factors which have no effect on C_v include isoproterenol, nifedipine, captopril, vasopressin, ANP, morphine and left ventricular distension. The factors which still need to be investigated are the baroreceptor reflex, α-adrenergic receptor agonists and ganglionic blockade. Conclusions about the effects of a change in C_v, or lack of, on MCFP and venous return should not be made hastily, however, for a given intervention may affect more than one of the determinants of venous return.

Changes in Venous Resistance

Venous resistance (R_v) is an important determinant of venous return because it directly affects the amount of blood contained in a vascular region for a given flow. For example, an increase in R_v at constant inflow will result in a decrease in outflow and a passive accumulation of blood in the vascular region until the pressure in the venous region is raised enough to restore the equilibrium between inflow and outflow. Decreases in R_v will result in the opposite,

an increase in outflow and loss of regional volume until the pressure in the venous region is low enough to restore the equality between inflow and outflow. These shifts of volume in or out of vascular regions are translated in decreases and increases in steady state venous return.

Measurements of R_v for the whole animal are usually made by subtracting right atrial pressure from the measurement of MCFP and dividing the difference by cardiac output. In isolated regions, R_v can be obtained by dividing the difference between venous pressure and the pressure in the region when flow is stopped, by the initial flow through the region.

There are no assessments of the influence of the baroreceptors on R_v and only two studies examined the influence of EPI or NE on venous resistance in anesthetized dogs. Mitzner and Goldberg (78) found an increase in total R_v with EPI infusion. Rutlen et al. (102) found that NE infusion decreases transhepatic R_v calculated by dividing the difference between splenic and hepatic venous pressures by hepatic venous outflow. This decrease may be due to ß-receptor activation by NE.

Appleton et al. (4) found a dose dependant increase in R_v with infusion of phenylephrine in anesthetized dogs. This increase was present even after ganglionic blockade and, therefore, is not dependant on reflexes. Activation of ß-receptors with isoproterenol decreases R_v of the

splanchnic bed (36,102) in dogs. Ganglionic blockade by itself decreases R_v in anesthetized dogs (4), but does not change R_v when combined with an infusion of atropine in splenectomized dogs (87) or in conscious rats (18).

Angiotensin II increases R_v in conscious rats. Both angiotensin II and vasopressin infusions result in dose dependant increases in R_v in anesthetized, splenectomized and ganglionic blocked dogs (70). Angiotensin II and vasopressin, therefore, appear to have a direct effect on R_v, independent of reflexes.

Treatment with ANP results in an increase in R_v in anesthetized rats (18). ANP increases R_v in anesthetized dogs even if combined with hexamethonium and splenectomy (69) which suggests a direct effect of ANP on R_v.

Neither nifedipine nor captopril has any effect on R_v in splenectomized dogs treated with hexamethonium and atropine (87). In conscious calves, thyrotoxicity decreased R_v (35). In anesthetized dogs, morphine (38) increases splanchnic and extrasplanchnic R_v, and hypothermia (37) increases only splanchnic R_v. Finally, hypoxic hypoxia decreases total as well as renal and splanchnic R_v (75). There are no assessments of R_v during hyperthermia.

In summary, the factors which increase R_v and will tend to decrease venous return include epinephrine, phenylephrine, angiotensin II, vasopressin, ANP, morphine and hypothermia. The factors which decrease R_v and will

tend to increase venous return include isoproterenol, ganglionic blockade, thyrotoxicity and hypoxic hypoxia. The factors which do not affect R_v are nifedipine and captopril. The influence of the baroreceptor reflex and hyperthermia on R_v remain to be investigated. Again, the factors just mentioned may affect more than one of the determinants of venous return and their overall effect on venous return may be different than what can be predicted from their effect on R_v.

Changes in Time Constant of Venous Drainage

Changes in the time constant of venous drainage require measurements of both R_v and C_v. Because changes in R_v and C_v can have opposite effects on venous return, τ_v gives a better reflection of the overall effect of an intervention on venous return. With an increase in τ_v at constant flow, blood volume will accumulate and venous return will tend to decrease. With a decrease in τ_v at constant flow the opposite will occur, a decrease in volume and an increase in venous return.

All measurements of τ_v have been made in isolated regions of the systemic circulation in dogs. However, since τ_v is the product of R_v and C_v, the time constant of the total systemic bed can be calculated from studies where measurements of R_v and C_v were made. In all studies (36-38,75,78) but one (13), the τ_v of the splanchnic bed was

orders of magnitude greater than that of the extrasplanchnic region.

Decreases in splanchnic τ_v were found with Isoproterenol (36) and hypothermia (37) and these factors would tend to increase venous return. Splanchnic τ_v increases with morphine (38) and total systemic τ_v increases with ANP (69) and vasopressin (70). These factors would tend to decrease venous return. There was no change in splanchnic, extrasplanchnic and renal τ_v with hypoxic hypoxia (75). Also, total systemic τ_v did not change with nifedipine (87), captopril (87) and angiotensin II (70). Although a change in total systemic τ_v will directly affect venous return, the end result of a change in regional τ_v on venous return depends on the fraction of cardiac output going to regions with fast or slow τ_v.

Changes in Fractional Flow to Fast and Slow Time Constant Beds

As discussed in the previous section, the splanchnic bed has a greater τ_v than the extrasplanchnic bed. According to Krogh's analysis an increase in fractional flow to the splanchnic bed will result in a decrease in venous return whereas an increase in fractional flow to the extrasplanchnic bed will increase venous return. Most of the studies on blood flow distribution to fast and slow τ_v beds have been done in anesthetized dogs.

So far, studies on the influence of the baroreceptors on blood flow distribution have not been conclusive. In one study, decreases in carotid sinus pressure resulted in increases in splanchnic fractional flow (13) whereas in another study splanchnic fractional flow decreased (57). In both of these studies, however, the total change in fractional flow was less than 5% of total flow.

Caldini et al. (14) found a redistribution of blood flow to the fast time constant bed with epinephrine infusion. Mitzner and Goldberg (78), however, showed that if arterial pressure is maintained constant by decreasing cardiac output, EPI results in an increase in fractional flow to the slow τ_v bed. Further support for the influence of the baroreflex in the distribution of cardiac output during adrenergic stimulation comes from Rutlen et al. (102) who found an increase in fractional flow to the splanchnic bed during combined NE infusion and ganglionic blockade. This issue will be further discussed in chapter 6.

Severe hypoxia increases the fractional flow to the extrasplanchnic bed (1,56) whereas moderate hypoxia does not change the distribution of flow (75). The redistribution of flow during severe hypoxia was maintained in the face of baro- or chemo-denervation and ganglionic blockade. Thus reflexes do not play a role in this response. Morphine resulted in an increase in splanchnic fractional flow (38), but isoproterenol (36), ANP (18,69), nitroglycerin (86),

hypothermia (37) and left ventricular distention (57) had no effect on blood flow distribution.

In most species studied, exposure to heat results in an increase in fractional flow to heat dissipating organs. Compensatory decreases in blood flow to other organs, however, differs between species. In dogs, blood flow decreases in the thyroid glands, brain and spinal cord and, although there is no change in total splanchnic blood flow, the fraction of flow to the splanchnic bed decreases (48). In baboons (49), sheep (47) and man (99), there is a decrease in total as well as fractional flow to the splanchnic bed during heat stress. The impacts of these changes in fractional flow on venous return remain to be assessed.

In summary, only NE (in ganglionic blocked dogs) and morphine resulted in an increase the fraction of flow to the splanchnic bed. Severe hypoxia and EPI (in intact animals) increase extrasplanchnic fractional flow.

Changes in MCFP and Venous Return

In a number of studies, the effects of pharmacological agents, physiological stimuli, reflexes, and endogenous substances on venous return were evaluated by repeated measurements of MCFP or venous return or both and cardiac output. For example, MCFP is increased by epinephrine in anesthetized dogs (22,44,95) and conscious rats (127),

phenylephrine (4), hypoxia (75,94), hypercapnia (94), hypoxic hypercapnia (94) and nifedipine (87) in anesthetized dogs, angiotensin II in conscious (128) and anesthetized dogs (70), thyrotoxicity in conscious calves (35) and hypertension (23,106) in conscious rats. These factors, therefore, should tend to increase venous return. The increases in MCFP with phenylephrine, thyrotoxicity, hypoxic hypercapnia, hypoxia and hypercapnia are reflex mediated because they are either reduced or abolished by ganglionic blockade.

Decreases in MCFP were observed with ganglionic blockade alone in anesthetized (4,22,87,95) and conscious dogs (128), ß-adrenergic receptor stimulation (95) in anesthetized dogs, ANP in anesthetized dogs (69) and rats (123), spinal cord transection (123) in anesthetized rats, and pentobarbital anesthesia (105) in rats. However, no change in MCFP were found by Chien et al. with ANP or trimetaphan in anesthetized rats (18). No change in MCFP were observed either in anesthetized dogs with histamine (95), vasopressin (70) and captopril (87), and in anesthetized rats with furosemide (18).

Usually, the factors which increase MCFP also increase venous return. Thus, there is a rise in both MCFP and venous return with hypoxia (1,75,94), hypercapnia (94) and hypoxic hypercapnia (94) in anesthetized dogs, and with thyrotoxicity in conscious calves (35). The effect of

hypoxia, hypercapnia and hypoxic hypercapnia (94) on venous return is reflex mediated and is abolished by ganglionic blockade. Although there is a rise in MCFP with phenylephrine infusion, venous return increased in anesthetized dogs (7) but not in conscious calves (35). There was even a decreased in venous return with phenylephrine infusion in thyrotoxic calves (35). In a third study on anesthetized dogs, venous return increased with phenylephrine but only after ganglionic blockade (4). These results may be explained by the fact that phenylephrine increases venous resistance directly and reflexly, through increases in arterial pressure. Depending on the balance between increases in MCFP and R_v, venous return may increase, decrease or stay constant. Angiotensin II infusions increases MCFP but decreases venous return in anesthetized (70) and conscious dogs (128). This decrease is probably due to the increase in R_v with angiotensin II.

Isoproterenol decreases MCFP but increases venous return in anesthetized dogs (36). The large decrease in R_v with ß-receptor activation is probably the cause. ANP, which generally decreases MCFP, lowers venous return in anesthetized dogs (69) and rats (18), probably through its increase in R_v. Vasopressin has no effect on MCFP but decreases venous return in anesthetized dogs (70). This is probably due to the) -ease in total systemic r_v with vasopressin. Acetyl ine increases venous return in dogs

whose ganglionic nicotinic receptors have been blocked (80). This increase persists even after splenectomy and evisceration. More work on the effect of acetylcholine on the determinants of venous return needs to be done to explain these results.

There are species differences in the cardiac output and venous return responses to heat stress. In sheep (47) and baboons (49), cardiac output does not increase with heat stress whereas in rats (77), dogs (48) and humans (100), there are substantial increases in cardiac output.

C. HUMAN STUDIES

There has been no study in humans on the factors affecting venous return since Starr measured the MCFP of dying patients in 1940. Although radionuclide techniques have been used to measure changes in regional blood volumes in humans (29,91), an understanding of the mechanisms responsible for these changes is still lacking.

Because firm data on venous return is not available, information about the control of venous return and cardiac output must be deduced from the study of factors which affect the common cardiovascular response to physiological or pathological stimuli. For example, the typical cardiovascular response to exercise or heat stress can be obtained by measuring cardiac output, regional blood flows, blood volume, regional blood volume changes, heart rate,

stroke volume, core temperature, skin temperature, and arterial pressure in healthy individuals of different sex and age groups. The same measurements can be repeated with subjects under hypovolemic or hypervolemic conditions, subjects affected with disease such as myocardial damage, hypertension, emphysema, fever, hyperthyroidism or others. By comparing the normal cardiovascular response to that of the altered condition, some information can be obtained on how venous return and cardiac output are affected and regulated in these states.

One of the favorite strategies used by many investigators is to measure the cardiovascular response of normovolemic subjects during conditions of multiple demands for the cardiovascular system, such as exercise or heat stress or both. This response is then compared to the cardiovascular response when the subjects are hypovolemic or hypervolemic. Since blood volume is one of the most important determinants of venous return, changes in cardiovascular response between normovolemia, hypovolemia and hypervolemia can give a fair indication of alterations in venous return or regional blood flows or both. The data obtained can also indicate which vascular beds are protected in situations of limited resources and which organs are most likely to be affected first.

Cardiovascular Responses to Heat Stress at Rest

Increases in core temperature are centrally perceived in the hypothalamus, adjacent preoptic area (34,50,53,55) and in the spinal cord (34,63). Other sites of thermosensitivity include the splanchnic organs (90), skeletal muscles (62,65) and skin (9,55,109). The efferent response to centrally perceived hyperthermia is the onset of sweating and cutaneous vasodilatation. The increased skin blood flow transfers heat from the core to the surface and evaporation of the sweat on the skin maintains the temperature gradient between the core and the surface.

Cardiac output increases linearly with core temperature (T_c) until it reaches a plateau of 12 to 13 $l \cdot min^{-1}$ at core temperatures above 39°C (67,97,100). Mean arterial pressure decreases by 10 to 15 mm Hg at the onset of heating but gradually returns to normal as heating proceeds. Right atrial mean pressure also decreases to values close to 0 mm Hg and remains low until heating is stopped (97,100).

Blood flow to the splanchnic (99,101) and renal (99) beds decreases during heat stress. Forearm muscle blood flow was found to decrease by Detry et al. (20) in subjects heated to core temperatures of 38.1°C. Henriksen et al. (54) found increases in forearm muscle blood flow at body temperatures above 39°C.

Skin vasodilatation during heat stress depends on the withdrawal of sympathetic vasoconstrictor tone as well as

active sympathetic vasodilation in non-acral regions (5,39,110). The presence of sweat glands is required for the active vasodilatation (11), and the mediator is thought to be vasoactive intestinal peptide (VIP) released from the cholinergic nerve terminals (66,71,124). Total cutaneous blood flow measurements are difficult to obtain for the skin is a large diffuse organ. However, measurements of regional skin blood flows can be obtained. The two methods of choice are venous occlusion plethysmography and laser Doppler flowmetry. These two techniques provide estimates of regional volume changes in the skin as well. Whereas venous occlusion plethysmography is limited to the arms and legs and automatically includes underlying muscle blood flow, the Doppler flow meter can be applied anywhere but measures changes in skin or subcutaneous blood flow only. Laser doppler flowmetry has the added advantage of measuring red blood cell velocity which can be used to study the opening of arterio-venous anastomosis (116). Forearm skin blood flow increases from around 3 or 4 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ to over 25 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ during heat stress (84).

Estimates of total skin blood flow have been made by Detry et al. (20) by subtracting the changes in splanchnic, renal and muscle blood flow from the change in cardiac output during heat stress. They obtained values in the range of 7 to 8 $l \cdot min^{-1}$ for maximal skin blood flow during heat stress. This estimate becomes somewhat exaggerated if,

as Henriksen et al. (54) observed, muscle blood flow increases at core temperatures above 39°C. Nevertheless, it would be correct to assume that most, if not all, of the increase in cardiac output during heat stress goes to the skin and, because the oxygen extraction of the skin is very low, all of this increase in flow is for heat dissipation. Values for maximal skin blood flow during heat stress can therefore be estimated to be anywhere between 4 and 7 $l \cdot min^{-1}$.

Sweat rates during heat exposure can vary from 1.5 $l \cdot hr^{-1}$ to 2-3 $l \cdot hr^{-1}$ depending on skin temperature, core temperature, relative humidity, convective heat loss, level of acclimatization and other factors (88). Maximal daily sweat production has been estimated at 10-15 litres (88). The immediate vascular response to heat stress is hemodilution, which is thought to be due to skin vasodilatation and decreases in capillary pressure (51). As heat stress progresses sweat production results in a loss of plasma volume proportional to the degree and the duration of the heat exposure (51). This is true until plasma volume is decreased by 16 to 20%, at which point it stops. Thus, this level appears to represent the lower limit for plasma volume loss (21,51,52).

The combined increase in skin blood flow and loss of plasma volume during heat stress have important consequences on the determinants of venous return. The skin has been

described as a compliant vascular region (14,97) and increases in flow to the skin should result in the pooling of the blood in the periphery and a decrease in venous return. The concomitant decrease in blood flow to the splanchnic region will help counteract this situation but because total cardiac output increases during heat stress, the decrease in splanchnic flow is not sufficient to compensate for the increase in skin flow.

Decreases in intravascular volume resulting from sweat losses will have a direct effect on venous return because stressed volume must be maintained by recruitment of unstressed volume. Thus if the volume losses exceed the limits of unstressed volume recruitment, venous return and cardiac output will fall.

The mechanisms responsible for the increase in venous return during heat stress at rest have not been examined yet. However, the effects of blood volume changes on the cardiovascular response to heat stress have been studied. Mild dehydration (2% of body weight) has no effect on the rate of plasma volume loss, on forearm blood flow or on heart rate (64). Nevertheless, core temperature rises faster and reaches higher values in hypohydrated individuals exposed to heat. Hyperhydration per se has not been studied during heat stress at rest. Acclimatization to a hot environment results in an increase in plasma volume and sweating, as well as a decrease in heart rate, core

temperature and skin temperature (88). The extent to which these cardiovascular changes are due to expansion of plasma volume is speculative.

Cardiovascular Responses to Exercise

Exercise places tremendous demands on the cardiovascular system. Cardiac output and venous return can increase up to eight times resting values, heart rate can quadruple and stroke volume can double at maximal exercise in endurance athletes (26). Because the efficiency of the exercising muscles is anywhere between 0 and 25%, at least three quarters of the energy produced is converted into heat. Consequently, exercising at 50% of maximal capacity for a period longer than 10 minutes results in a thermal stress (89). For this reason, physiological adjustments to exercise and heat stress are intrinsically linked.

The elevated demand for muscle blood flow at the onset of exercise is partially accommodated by a decrease in blood flow to the splanchnic (98), renal (126), skin (10,11) and inactive muscle beds (10). Vasoconstriction to these beds is sympathetically mediated and is directly proportional to the intensity of exercise and to environmental temperatures (97). The onset of exercise is also associated with a loss of plasma water which can reach up to 15% of plasma volume (400 ml) and is also proportional to exercise intensity (19). This loss of plasma water is due to increased

filtration of fluid from the vascular compartment into active muscle as a result of vasodilatation (114) and to elevated tissue osmolality (74). As exercise continues and core temperature increases, the skin vasodilates and more of the plasma water is lost from evaporation of sweat.

Maintenance of exercise over prolonged periods of time places three major difficulties on the cardiovascular system. One, there is competition for blood flow between the active muscles and the skin requiring higher total flows as core temperature increases. Two, there is an initial loss of plasma volume due to exercise itself and then a continuous loss of plasma volume through sweat evaporation. The loss of plasma volume reduces the amount of unstressed volume that can be recruited to maintain elevated venous return. Finally, if the skin is a slow time constant bed, increases in cutaneous flow will decrease the slope of the venous return curve and maximal venous return.

The determinants of venous return, as reviewed above, have not been studied during exercise in man. However, the performance of the cardiovascular system in states of hypovolemia and hypervolemia can give a good indication of the influence of blood volume on venous return during exercise. Dehydration can be achieved by limited drinking and repeated exposures to heat or exercise or both (15,40,104,108), or by ingestion of diuretics (32,85). Hypovolemia during exercise results in a larger decrease in

plasma volume (15,32,40,85), in higher core temperatures (15,30,32,40,85,104,108) and heart rates (15,32,40,85,104, 108), and in a decrease in cardiac output (32,85,104), sweat rates (40,108) and forearm blood flow (85). Skin temperature is not affected by dehydration (15,40). Saltin (104) found a decrease in performance time during maximal exercise in dehydrated subjects. The severity of the changes in cardiovascular response appears to be proportional to the level of dehydration (108).

Hypervolemia is difficult to achieve during exercise because equilibration of fluid between the intravascular and extravascular space is very rapid. A number of studies succeeded in changing plasma volume before exercise but failed to maintain plasma volume constant during exercise (40,85,107). These studies did not find differences in cardiac output, heart rate, core temperature and endurance time with hyperhydration. In studies where plasma volume was successfully maintained or increased during exercise, cardiac output was higher (30,32) and core temperature and heart rate were lower (15,30-32) at the end of exercise. Fortney et al. (31) also found a tendency for higher forearm blood flow during hypervolemia. Others did not measure plasma volume but gave fluids before (79) and during (79, 117) exercise and found decreases in heart rate and core temperature. Staff and Nilsson found increases in endurance time with fluid ingestion during exercise (117).

D. PURPOSE OF THESIS EXPERIMENTS

The experiments presented in this thesis were designed to answer specific questions concerning the control of venous return by the sympathetic nervous system and the influence of blood volume and blood flow distribution on temperature regulation and endurance time. The rationale behind these questions is described in the next sections.

Temperature Regulation and Endurance Time

Blood volume has a direct influence on cardiovascular performance and on the defence against hyperthermia during exercise. This influence appears to be significant enough to affect fatigue and exhaustion time in humans. Studies dealing with exhaustion and endurance time, however, should be conducted in a double blind fashion to reduce the risk of contamination of the results by motivational factors. Of the two studies which looked at the effect of hydration levels on endurance time in humans (40,117), neither used a double blind protocol.

In chapter 2 of the thesis, I tested the hypothesis that maintenance of plasma volume during exercise would reduce core temperature, improve cardiovascular performance and increase endurance time using a double blind protocol.

Control of Venous Return During Heat Stress

Very little is known about the mechanics of the skin vascular bed. Yet, knowledge of the mechanical parameters of the skin vasculature is essential to evaluate the impact of increases in cutaneous blood flow on overall venous return.

In chapter 3 of the thesis, I tested the hypothesis that the skin is a compliant vascular bed with a long time constant of venous drainage and potential blood reservoir during heat stress.

Central to the study of the determinants of venous return lies the problem of measuring absolute regional blood volumes as well as changes in regional volumes. For this purpose I developed a technique which permits repeated measurements of splanchnic and extrasplanchnic blood volumes in dogs under circulatory bypass. This method is described in chapter 4 and will be used for the studies in chapters 5 to 7.

Heat stress was used in this thesis because it results in an endogenous stimulation of the sympathetic nervous system and an increase in venous return. Therefore, the mechanisms of increase in venous return during heat stress could be studied for the first time and the sympathetic pathway responsible for this increase could be examined with ganglionic blockade and specific adrenergic antagonists.

In chapter 5 of the thesis, I tested the hypothesis that

blood flow redistribution and decreases in splanchnic capacitance are responsible for the increase in venous return during heat stress. Also, I used ganglionic, α and β-receptor blockade to identify the neuro-humoral mediators responsible for changes in venous return during heat stress. In this chapter, I provided the first analysis of the venous system in which changes in all four determinants of venous return (i.e. R_v, C_v, unstressed volume and fractional flows) are assessed simultaneously.

Control of Venous Return by the Baroreceptor Reflex

As mentioned previously, the results concerning the effect baroreceptor stimulation on regional blood flow distribution and on total or regional venous compliance are conflicting. Furthermore, there are no assessments of venous resistance at different carotid sinus pressure levels even though venous resistance is an important determinant of venous return. Consequently, in chapter 6 of the thesis I tested the hypothesis that changes in carotid sinus pressure affect regional blood flow distribution and pressure-volume relationships, as well as splanchnic venous resistance. Also, ganglionic and α-receptor blockade were used to identify the neuro-humoral factors responsible for the changes in the determinants of venous return with variations in carotid sinus pressure.

The Influence of Neuropeptide Y on Venous Return

As will become evident from chapter 5, whereas neither α nor ß-adrenergic receptor blockade could abolish the change in splanchnic unstressed volume during heat stress, ganglionic blockade did. Consequently, we hypothesized that a vasoconstricting substance, released from the adrenergic nerve terminal, was responsible for the change in the splanchnic unstressed volume during heat stress. Further evidence in favor of this hypothesis was obtained in chapter 6 where α-receptor blockade could block only half of the change in unstressed volume produced by a decrease in carotid sinus pressure.

Neuropeptide Y (NPY) has attracted our attention as a good candidate for this vasoconstriction for the following reasons. NPY is a 36 amino acids peptide present in sympathetic nerve terminals and is co-released with norepinephrine upon nerve stimulation (33,72). Unlike norepinephrine, NPY produces a slowly developing, long-lasting vasoconstriction which is resistant to alpha adrenergic blockade (24,73). NPY-like immunoreactivity has been established on both arteries and veins (25) and the plasma level of NPY increases during stress (non-thermal) in rats (129) and after exercise in humans (72). Very little is known about the in vivo systemic effect of NPY, especially on the capacitance vessels. Unfortunately, there are no specific blockers for NPY. The best option was

therefore to infuse NPY and to measure its effects on venous return.

In chapter 7 or the thesis, I tested the hypothesis that NPY infusion can produce a change in capacitance similar to that observed during heat stress and thus could have an important function in the control of venous return.

E. REFERENCES

1. ADACHI, H., W. STRAUSS, H. OCHI, AND J.N.,JR. WAGNER. The effect of hypoxia on the regional distribution of cardiac output in the dog. *Circ. Res.* 39(3): 314-318, 1976.

2. ALEXANDER, R.S. The participation of the venomotor system in pressor reflexes. *Circ. Res.* 2: 405-409, 1954.

3. ALLBAUGH, E., AND S.M. HORVATH. Effect of total occlusion of thoracic aorta on blood pressure, splanchnic blood pressure, splanchnic blood flow, and metabolic state in dogs. *Am. J. Physiol.* 180: 451-455, 1955.

4. APPLETON, C., M. OLAJOS, E. MORKIN, AND S. GOLDMAN. Alpha-1 adrenergic control of the venous circulation in intact dogs. *J. Pharmacol. Exp. Ther.* 233(3): 729-734, 1985.

5. BARCROFT, H. Sympathetic control of vessels in the hand and forearm skin. *Physiol. Rev.* 40 (Suppl. 4): 81-91, 1960.

6. BARCROFT, H., AND A. SAMAAN. The explanation of the increase in systemic flow caused by occluding the descending thoracic aorta. *J. Physiol. Lond.* 85: 47-61, 1935.

7. BELL, L., J. HENNECKEN, B.L. ZARET, AND D.L. RUTLEN. Alpha-adrenergic regulation of splanchnic volume and cardiac output in the dog. *Acta Physiol. Scand.* 138: 321-329, 1990.

8. BENNETT, T.D., C.R. WYSS, AND A. SCHER. Changes in vascular capacity in awake dogs in response to carotid sinus occlusion and administration of catecholamine. *Circ. Res.* 55: 440-453, 1984.

9. BENZINGER, T.H. Heat regulation and homeostasis of central temperature in man. *Physiol. Rev.* 49: 671-759, 1969.

10. BEVEGÅRD, S., AND J.T. SHEPHERD. Studies on the regulation to the circulation in man. *Physiol. Rev.* 47: 178-213, 1967.

11. BRENGELMANN, G.L., J.M. JOHNSON, L. HERMANSEN, AND L.B. ROWELL. Altered control of skin blood flow during exercise at high internal temperatures. *J. Appl. Physiol.* 43: 790-794, 1977.

12. BRUNNER, M.J., A.S. GREENE, A.E. FRANKLE, AND A.A. SHOUKAS. Carotid sinus baroreceptor control of splanchnic resistance and capacity. *Am. J. Physiol.* 255(24): H1305-H1310, 1988.

13. BRUNNER, M.J., A.A. SHOUKAS, AND C.L. MACANESPIE. The effect of the carotid sinus baroreceptor reflex on blood flow and volume redistribution in the total systemic vascular bed of the dog. *Circ. Res.* 48: 274-285, 1981.

14. CALDINI, P., S. PERMUTT, J.A. WADDELL, AND R.L. RILEY. Effect of epinephrine on pressure, flow, and volume relationships in the systemic circulation of dogs. *Circ. Res.* 34: 606-623, 1974.

15. CANDAS, V., J.P. LIBERT, G. BRANDENBERGER, J.C. SAGOT, C. AMOROS, AND J.M. KAHN. Hydration during exercise. Effects on thermal and cardiovascular adjustments. *Eur. J. Appl. Physiol.* 55: 113-122, 1986.

16. CARNEIRO, J., AND D. DONALD. Blood reservoir function of dog spleen, liver, and intestine. *Am. J. Physiol.* 232(1): H67-H72, 1977.

17. CHENG, E.Y., J. KAY, S. HOKA, Z.J. BOSNJAK, R.L. COON, J.L. SEAGARD, AND J.P. KAMPINE. Influence of lung inflation reflex on vascular capacitance in the systemic circulation. *Am. J. Physiol.* 257: R1004-R1011, 1989.

18. CHIEN, Y.W., E.D. FROHLICH, AND N.C. TRIPPODO. Atrial natriuretic peptide increases resistance to venous return in rats. *Am. J. Physiol.* 252(21): H894-H899, 1987.

19. CONVERTINO, V.A., L.C. KEIL, E.M. BERNAUER, AND J.E. GREENLEAF. Plasma volume, osmolality, vasopressin and renin activity during graded exercise. *J. Appl. Physiol.* 50: 123-128, 1981.

20. DETRY, J-M.R., G.L. BRENGELMANN, L.B. ROWELL, AND C. WYSS. Skin and muscle components of forearm blood flow in directly heated man. *J. Appl. Physiol.* 32(4): 506-511, 1972.

21. DIAZ, F.J., D.R. BRANDSFORD, D. KOBAYASHI, S.M. HORVATH, AND R.G. MCMURRAY. Plasma volume changes during rest and exercise in different postures in a hot humid environment. *J. Appl. Physiol.* 47: 796-803, 1979.

22. DREES, J.A., AND C.F. ROTHE. Reflex venoconstriction and capacity vessel pressure-volume relationships in dogs. *Circ. Res.* 34: 360-373, 1974.

23. EDMUNDS, M.E., G.I. RUSSELL, AND J.D. SWALES. Vascular capacitance and reversal of 2-kidney,1-clip hypertension in rats. *Am. J. Physiol.* 256(25): H502-H507, 1989.

24. EDVINSSON, L., P. EMSON, J. MCCULLOCH, K. TATEMOTO, AND R. UDDMAN. Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat. *Neurosci. Lett.* 43: 79-84, 1983.

25. EDVINSSON, L., P. EMSON, J. MCCULOCH, K. TATEMOTO, AND R. UDDMAN. Neuropeptide Y: Immunocytochemical localization to and effect upon feline pial arteries and veins in vitro and in situ. *Acta Physiol. Scand.* 122: 155-163, 1984.

26. EKBLOM, B. Effect of physical training on oxygen transport system in man. *Acta Physiol. Scand.* Suppl. 328: 1969.

27. EMERSON, T.E., JR. Effects of angiotensin, epinephrine, norepinephrine, and vasopressin on venous return. *Am. J. Physiol.* 1015: 933-942, 1966.

28. FERRAND, E.A., AND S.M. HORVATH. Cardiovascular and hemodynamic functions in dogs subject to prolonged aortic occlusion. *Arch. Surg.* 76: 951-955, 1958.

29. FLAMM, S.D., J. TAKI, R. MOORE, S.F. LEWIS, F. KEECH, F. MALTAIS, M. AHMAD, R. CALLAHAN, S. DRAGOTAKES, N. ALPERT, AND H.W. STRAUSS. Redistribution of regional and organ blood volume and effect on cardiac function in relation to upright exercise intensity in healthy human subjects. *Circulation* 81: 1550-1559, 1990.

30. FORTNEY, S.M., E.R. NADEL, C.B. WENGER, AND J.R. BOVE. Effect of acute alteration of blood volume on circulatory performance in humans. *J. Appl. Physiol.* 50(2): 292-298, 1981.

31. FORTNEY, S.M., N.B. VROMAN, W.S. BECKETT, S. PERMUTT, AND N.D. LAFRANCE. Effect of exercise hemoconcentration and hyperosmolality on exercise responses. *J. Appl. Physiol.* 65(2): 519-524, 1988.

32. FORTNEY, S.M., C.B. WENGER, J.R. BOVE, AND E.R. NADEL. Effects of blood volume on forearm venous and cardiac stroke volume during exercise. *J. Appl. Physiol.* 55(3): 884-890, 1983.

33. FRIED, G., L. TERENIUS, T. HOKFELT, AND M. GOLDSTEIN. Evidence for differential localization of noradrenaline and neuropeptide Y in neuronal storage vesicles isolated from rat vas deferens. *J. Neurosci.* 5: 450-458, 1985.

34. GERBER, R L., F.W. KLUSSMANN, ANDH.C. HELLER. *Preoptic/anterior hypothalamic neural responses to thermal stimulation of the hypothalamus and spinal cord in unanesthetized unrestrained rabbits*. In: *Thermal Physiology*. N.Y.: edited by HALES, J.R.S. Raven Press, 1984, p. 83-86.

35. GOLDMAN, S., M. OLAJOS, AND E. MORKIN. Control of cardiac output in thyrotoxic calves. *J. Clin. Invest*. 73: 358-365, 1984.

36. GREEN, J.F. Mechanism of action of isoproterenol on venous return. *Am. J. Physiol*. 232(2): H152-H1566, 1977.

37. GREEN, J.F., AND A.P. JACKMAN. Mechanism of the increased vascular capacity produced by mild hypothermia in the dog. *Circ. Res*. 44: 411-419, 1979.

38. GREEN, J.F., A.P. JACKMAN, AND G. PARSONS. The effects of morphine on the mechanical properties of the systemic circulation in the dog. *Circ. Res*. 42(4): 474-478, 1978.

39. GREENFIELD, A.D.M. *The circulation through the skin*. In: *The Handbook of Physiology*. Washington,DC.: edited by HAMILTON, W.F., AND P. DOW. American Physiological Society, 1963, p. 1325-1351.

40. GREENLEAF, J.E., AND B.L. CASTLE. Exercise temperature in man during hypohydration and hyperhydration. *J. Appl. Physiol*. 30: 847-853, 1971.

41. GREENWAY, C.V., K.L. SEAMAN, AND I.R. INNES. Norepinephrine on venous compliance and unstressed volume in cat liver. *Am. J. Physiol*. 248(17): H468-H476, 1985.

42. GUYTON, A.C. Determination of cardiac output by equating venous return curves with cardiac response curves. *Am. J. Physiol*. 35: 123-129, 1955.

43. GUYTON, A.C., G.G. ARMSTRONG, AND P.L. CHIPLEY. Pressure volume curves of the arterial and venous systems in live dogs. *Am. J. Physiol*. 184: 253-258, 1956.

44. GUYTON, A.C., A.W. LINDSEY, B. ABERNATHY, AND J.B. LANGSTON. Mechanism of the increased venous return and cardiac output caused by epinephrine. *Am. J. Physiol*. 192(1): 126-130, 1958.

45. GUYTON, A.C., A.W. LINDSEY, AND B.N. KAUFMANN. Effect of mean circulatory filling pressure and other peripheral circulatory factors on cardiac output. *Am. J. Physiol*. 180: 463-468, 1955.

46. HAINSWORTH, R., AND F. KARIM. Responses of abdominal vascular capacitance in the anaesthetized dog to changes in the carotid sinus pressure. *J. Physiol. Lond.* 262: 659-677, 1976.

47. HALES, J.R.S. Effect of exposure to hot environments on the regional distribution of blood flow and cardiorespiratory function in sheep. *Pflügers Arch.* 344: 133-148, 1973.

48. HALES, J.R.S., AND R.A.L. DAMPNEY. The redistribution of cardiac output in the dog during heat stress. *J. Therm. Biol.* 1: 29-34, 1975.

49. HALES, J.R.S., L.B. ROWELL, AND R.B. KING. Regional redistribution of blood flow in awake heat stressed baboons. *Am. J. Physiol.* 237(6): H705-H712, 1979.

50. HAMMEL, H.T. Regulation of internal body temperature. *Ann. Rev. Physiol.* 30: 641-710, 1968.

51. HARRISON, M.H. Effects of thermal stress and exercise on blood volume in humans. *Physiol. Rev.* 65(1): 149-209, 1985.

52. HARRISON, M.H., R.J. EDWARDS, L.A. COCHRANE, AND M.J. GRAVENEY. Blood volume and protein responses to skin heating and cooling in resting subjects. *J. Appl. Physiol.* 54: 515-523, 1983.

53. HELLON, R. *Thermoreceptors.* In: *Handbook of Physiology. The cardiovascular System. Peripheral Circulation and Organ Blood Flow.* Bethesda, MD: edited by SHEPHERD, J.T., AND F.M. ABBOUD. American Physiological Society, 1983, p. 659-673.

54. HENRIKSEN, O., J. BüLOW, J.K. KRISTENSEN, AND N.A. LASSEN. *Local tissue temperature: An important factor for regulation of blood flow in peripheral tissues during indirectly induced hyperthermia.* In: *Thermal Physiology.* N.Y.: edited by HALES, J.R.S. Raven Press, 1984, p. 255-258.

55. HENSEL, H. Neural processes in thermoregulation. *Physiol. Rev.* 53: 948-1017, 1973.

56. HOKA, S., Z.J. BOSNJAK, H. ARIMURA, AND J.P. KAMPINE. Regional venous outflow, blood volume, and sympathetic nerve activity during severe hypoxia. *Am. J. Physiol.* 256(25): H162-H170, 1989.

57. HOKA, S., Z.J. BOSNJAK, D. SIKER, R. LUO, AND J.P. KAMPINE. Dynamic changes in venous outflow by baroreflex and left ventricular distension. *Am. J. Physiol.* 254(23): R212-R22 1988.

58. HOROWITZ, M., E. SUGIMOTO, T. OKUNO, AND T. MORIMOTO. Changes in blood volume and vascular compliance during body heating in rats. *Pflügers Arch.* 412: 354-358, 1988.

59. HORVATH, S.M., AND E.A. FERRAND. Aortic obstruction and cardiac output. *Am. Heart. J.* 55: 631-636, 1958.

60. ILLEBEKK, A., J. THORVALDSON, AND F. KIIL. Left ventricular function during acute elevation of aortic blood pressure in dogs. *Am. J. Physiol.* 231: 1476-1484, 1976.

61. IMAI, Y., K. SOTOH, AND N. TAIRA. Role of the peripheral vasculature in changes in venous return caused by isoproterenol, norepinephrine, and methoxamine in anaesthetized dogs. *Circ. Res.* 43: 553-561, 1978.

62. JESSEN, C., G. FEISTKORN, AND A. NAGEL. Temperature sensitivity of skeletal muscle in the conscious goat. *J. Appl. Physiol.* 54: 880-886, 1983.

63. JESSEN, C., AND E. SIMON. Spinal cord and hypothalamus as core sensors of temperature in the conscious dog. III. Identity of functions. *Pflügers Arch.* 324: 217-226, 1971.

64. KENNEY, W.L., C.G. TANKERSLEY, D.L. NEWSWANGER, D.E. HYDE, S.M. PUHL, AND N.L. TURNER. Age and hypohydration independently influence the peripheral vascular response to heat stress. *J. Appl. Physiol.* 68: 1902-1908, 1990.

65. KNIFFKI, D.D., S. MENSE, AND R.F. SCHMIDT. Muscle receptors with fine afferent fibres which may evoque circulatory reflexes. *Circ. Res.* 48 (Suppl. I): I-25-I-31, 1981.

66. KOLKA, M.A., AND L.A. STEPHENSON. Cutaneous blood flow and local sweating after systemic atropine administration. *Pflügers Arch.* 410: 524-529, 1987.

67. KOROXENIDIS, G.T., J.T. SHEPHERD, AND R.J. MARSHALL. Cardiovascular response to acute heat stress. *J. Appl. Physiol.* 16: 869-872, 1961.

68. KROGH, A. The regulation of the supply of blood to the right heart. *Skand. Arch. Physiol.* 27: 227-248, 1912.

69. LEE, R.W., AND S. GOLDMAN. Mechanism for decrease in cardiac output with atrial natriuretic peptide. *Am. J. Physiol.* 256(25): H760-H765, 1989.

70. LEE, R.W., S. STANDAERT, L.D. LANCASTER, D. BUCKLEY, AND S. GOLDMAN. Cardiac and peripheral circulatory responses to angiotensin and vasopressin in dogs. *J. Clin. Invest.* 82: 413-419, 1988.

71. LUNDBERG, J.M., A. ANGGARD, J. FAHRENKRUG, T. HOKFELT, AND V. MUTT. Vasoactive intestinal polypeptide in cholinergic neurons of exocrine glands: functional significance of coexisting transmitters for vasodilation and secretion. *Proc. Natl. Acad. Sci. USA* 77: 1651-1655, 1980.

72. LUNDBERG, J.M., A. MARTINSSON, A. HEMSEN, E. THEODORSSON-NORHEIM, J. SVEDENHAG, B. EKBLOM, AND P. HJEMDAHL. Co-release of neuropeptide Y and catecholamines during physical exercise in man. *Biochem. and Biophys. Res. Comm.* 133: 30-36, 1985.

73. LUNDBERG, J.M., AND K. TATEMOTO. Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. *Acta Physiol. Scand.* 116: 393-402, 1982.

74. LUNDVALL, J. Tissue hyperosmolality as a mediator of vasodilation and transcapillary fluid flux in exercising skeletal muscle. *Acta Physiol. Scand.* 379(suppl.): 1-142, 1972.

75. MALO, J., H. GOLDBERG, R. GRAHAM, H. UNRUH, AND C. SKOOG. Effect of hypoxic hypoxia on systemic vasculature. *J. Appl. Physiol.* 56(5): 1403-1410, 1984.

76. MARTIN, D.S., AND J.R. MCNEILL. Arginine vasopressin increases apparent whole body capacity in anesthetized cats. *Am. J. Physiol. Heart Circ. Physiol.* 258(27): H722-H728, 1990.

77. MIKI, K., T. MORIMOTO, H. NOSE, T. ITOH, AND S. YAMADA. Canine blood volume and cardiovascular function during hyperthermia. *J. Appl. Physiol.* 55(2): 300-306, 1983.

78. MITZNER, W., AND H. GOLDBERG. Effects of epinephrine on resistive and compliant properties of the canine vasculature. *J. Appl. Physiol.* 39(2): 272-280, 1975.

79. MOROFF, S.V., AND D.E. BASS. Effects of overhydration on man's physiological responses to work in the heat. *J. Appl. Physiol.* 20(2): 267-270, 1965.

80. MORSE, M.A., L. BELL, AND D.L. RUTLEN. Influence of splanchnic intravascular volume changes on cardiac output during muscarinic receptor stimulation in the anaesthetized dog. *Acta Physiol. Scand.* 138: 331-336, 1990.

81. MüLLER-RUCHHOLTZ, E.R., E. GRUND, F. HOUER, AND E.R. LAPP. Effect of carotid pressorreceptor stimulation on integrated systemic venous bed. *Basic Res. Cardiol.* 74: 467-476, 1979.

82. MüLLER-RUCHHOLTZ, E.R., H.M. LÖSCH, E. GRUND, AND W. LOCHNER. Effect of alpha adrenergic receptor stimulation on integrated systemic venous bed. *Pflügers Arch.* 370: 241-246, 1977.

83. MüLLER-RUCHHOLTZ, E.R., H.M. LÖSCH, E. GRUND, AND W. LOCHNER. Effect of beta adrenergic receptor stimulation on integrated systemic venous bed. *Pflügers Arch.* 370: 247-251, 1977.

84. NADEL, E.R., E. CAFARELLI, M.F. ROBERTS, AND C.B. WENGER. Circulatory regulation during exercise in different ambient temperatures. *J. Appl. Physiol.* 46(3): 430-437, 1979.

85. NADEL, E.R., S.M. FORTNEY, AND C.B. WENGER. Effect of hydration state on circulatory and thermal regulations. *J. Appl. Physiol.* 49(4): 715-721, 1980.

86. OGILVIE, R.I. Effect of nitroglycerin on peripheral blood flow distribution and venous return. *J. Pharmacol. Exp. Ther.* 207: 372-380, 1978.

87. OGILVIE, R.I., AND D. ZBOROWSKA-SLUIS. Effects of nifedipine and captopril on vascular capacitance of ganglion-blocked anesthetized dogs. *Can. J. Physiol. Pharmacol.* 68: 431-438, 1990.

88. PANDOLF, K.B., M.N. SAWKA, AND R.R. GONZALEZ. *Human performance physiology and environmental medicine at terrestrial extremes.* Indianapolis: Benchmark Press, Inc., 1988,

89. RAVEN, P.B., AND G.H.J. STEVENS. *Cardiovascular function and prolonged exercise.* In: *Perspectives in exercise science and sports medicine Volume I: Prolonged exercise.* Indianapolis, Indiana: edited by LAMB, D.R., AND R. MURRAY. Benchmark Press, Inc., 1988, p. 43-74.

90. RAWSON, R.O., AND K.P. QUICK. Evidence of deep-body thermoreceptor response to intra-abdomen heating in ewe. *J. Appl. Physiol.* 28: 813-820, 1970.

91. ROBINSON, V.J.B., O.A. SMISETH, N.W. SCOTT-DOUGLAS, E.R. SMITH, J.V. TYBERG, AND D.E. MANYARI. Assessment of the splanchnic vascular capacity and capacitance using quantitative equilibrium blood-pool scintigraphy. *J. Nucl. Med.* 31: 154-159, 1990.

92. ROSS, JR., J., C.J. FRAHM, AND E. BRAUNWALD. Influence of carotid baroreceptors and vasoactive drugs on systemic vascular volume and venous distensibility. *Circ. Res.* 9: 75-82, 1961.

93. ROSS, JR., J., J.W. LINHART, AND E. BRAUNWALD. Effects of changing heart rate by electrical stimulation of the right atrium in man: studies at rest, during muscular exercise and with isoproterenol. *Circulation* 32: 549-558, 1965.

94. ROTHE, C.F., A.D. FLANAGAN, AND R. MAASS-MORENO. Reflex control of vascular capacitance during hypoxia, hypercapnia, or hypoxic hypercapnia. *Can. J. Physiol. Pharmacol.* 68: 384-391, 1990.

95. ROTHE, C.F., A.D. FLANAGAN, AND R. MAASS-MORENO. Role of ß-adrenergic agonists in the control of vascular capacitance. *Can. J. Physiol. Pharmacol.* 68: 575-585, 1990.

96. ROTHE, C.F., B.L. JOHNS, AND T.D. BENNETT. Vascular capacitance of dog intestine using mean transit time of indicator. *Am. J. Physiol.* 234 (1): H7-H13, 1978.

97. ROWELL, L.B. Human cardiovascular adjustments to exercise and thermal stress. *Physiol. Rev.* 54(1): 75-159, 1974.

98. ROWELL, L.B., J.R. BLACKMON, AND R.A. BRUCE. Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. *J. Clin. Invest.* 43(8): 1677-1690, 1964.

99. ROWELL, L.B., G.L. BRENGELMANN, J.R. BLOCKMON, AND J.A. MURRAY. Redistribution of blood flow during sustained high skin temperature in resting man. *J. Appl. Physiol.* 28(4): 415-420, 1970.

100. ROWELL, L.B., G.L. BRENGELMANN, AND J.A. MURRAY. Cardiovascular responses to sustained high skin temperature in resting man. *J. Appl. Physiol.* 27(5): 673-680, 1969.

101. ROWELL, L.B., J-M.R. DETRY, G.R. PROFANT, AND C. WYSS. Splanchnic vasoconstriction in hyperthermic man - role of falling blood pressure. *J. Appl. Physiol.* 31(6): 864-869, 1971.

102. RUTLEN, D.L., E.N. SUPLE, AND JR.,W.J. POWELL. ß-Adrenergic regulation of total systemic intravascular volume in the dog. *Circ. Res.* 48: 112-120, 1981.

103. RUTLEN, D.L., O.A. VENGEN, AND A. ILEBEKK. Regulation of splanchnic organ size during muscarinic receptor stimulation in the anaesthetized pig. *Acta Physiol. Scand.* 138: 337-344, 1990.

104. SALTIN, B. Circulatory response to submaximal and maximal exercise after thermal dehydration. *J. Appl. Physiol.* 19(6): 1125-1132, 1964.

105. SAMAR, R.E., AND T.G. COLEMAN. Measurement of mean circulatory filling pressure and vascular capacitance in the rat. *Am. J. Physiol.* 234(1): H94-H100, 1978.

106. SAMAR, R.E., AND T.G. COLEMAN. Mean circulatory filling pressure and vascular compliance in the spontaneously hypertensive rat. *Am. J. Physiol.* 237(5): H584-H589, 1979.

107. SAWKA, M.N., R.W. HUBBARD, R.P. FRANCESCONI, AND D.H. HORSTMAN. Effect of acute plasma volume expansion on altering exercise-heat performance. *Eur. J. Appl. Physiol.* 51: 29301-312, 1983.

108. SAWKA, M.N., A.J. YOUNG, R.P. FRANCESCONI, S.R. MUZA, AND K.B. PANDOLF. Thermoregulatory and blood responses during exercise at graded hypohydration levels. *J. Appl. Physiol.* 59(5): 1394-1401, 1985.

109. SCHäFER, K., H.A. BRAUN, ANDH. HENSEL. *Temperature transduction in the skin.* In: *Thermal Physiology.* N.Y.: edited by HALES, J.R.S. Raven Press, 1984, p. 1-11.

110. SHEPHERD, J.T. *Physiology of the circulation in human limbs in health and disease.* Philadelphia: W. B. Saunders Co. , 1963,

111. SHOUKAS, A.A., AND M.J. BRUNNER. Epinephrine and the carotid sinus baroreceptor reflex. *Circ. Res.* 47: 249-257, 1980.

112. SHOUKAS, A.A., C.L. MACANESPIE, M.J. BRUNNER, AND L. WATERMEIER. The importance of the spleen in blood-volume shifts on the systemic vascular bed caused by the carotid sinus baroreceptor reflex in the dog. *Circ. Res.* 49: 759-766, 1981.

113. SHOUKAS, A.A., AND K. SAGAWA. Control of total systemic vascular capacity by the carotid sinus baroreceptor reflex. *Circ. Res.* 33: 22-33, 1973.

114. SJOGAARD, G., AND B. SALTIN. Extra-and intra-cellular water spaces in muscles of man at rest and with dynamic exercise. *Am. J. Physiol.* 243: R271-R280, 1982.

115. SMITH, R.F. Effect of acute partial and complete obstruction of the thoracic aorta in the dog. *Am. Surg.* 19: 1096-1103, 1953.

116. SONG, C.W., L.M. CHELSTROM, AND D.J. HAUMSCHILD. Changes in human skin blood flow by hyperthermia. *Int. J. Radiat. Oncol. Biol. Phys.* 18: 903-907, 1990.

117. STAFF, P.H., AND S. NILSSON. Fluid and glucose intake during prolonged severe physical activity. *T. norske Laegeforen.* 91: 1235-1239, 1971.

118. STARLING, E.H. Some points in the pathology of heart disease. *The Lancet* 1: 652-655, 1897.

119. STARR, I. Role of the "static blood pressure" in abnormal increments of venous pressure, especially in heart failure. II. Clinical and experimental studies. *Am. J. Med. Sci.* 199: 40-55, 1940.

120. STARR, I., AND A. RAWSON. Role of the "static blood pressure" in abnormal increments of venous pressure, especially in heart failure. I. Theoretical studies on an improved circulation schema whose pumps obey Starling's law of the heart. *Am. J. Med. Sci.* 199: 27-39, 1940.

121. STENE, J.K., B. BURNS, S. PERMUTT, P. CALDINI, AND M. SHANOFF. Increased cardiac output following occlusion of the descending thoracic aorta in dogs. *Am. J. Physiol.* 243: R152-R158, 1982.

122. STOKLAND, O., M.,M. MILLER, A. ILLEBEKK, AND F. KIIL. Mechanism of hemodynamic responses to occlusion of the descending thoracic aorta. *Am. J. Physiol.* 238(H7): H423-H429, 1980.

123. TRIPPODO, N.C., F.E. COLE, E.D. FROHLICH, AND A.A. MACPHEE. Atrial natriuretic peptide decreases circulatory capacitance in areflexic rats. *Circ. Res.* 59. 291-296, 1986.

124. VAALASTI, A., H. TAINIO, AND L. RECHARDT. Vasoactive intestinal peptide (VIP)-like immunoreactivity in the nerves of human axillary sweat glands. *J. Invest. Derm.* 85: 246-248, 1985.

125. WEBER, E. *Ueber die Anwending der Wellenlehre auf die Lehre vom Kreislaufe des Blutes*. In: *Berichte ueber die Verhandlungender Konigl. Sachsiseher Gesellschaften der Wissen-Schaften zu Leipzig. Weidmanische Buchandlung*, 1850.

126. WHITE, H.L., AND D. ROLF. Effects of exercise and of some other influences on the renal circulation in man. *Am. J. Physiol*. 152: 505-516, 1948.

127. YAMAMOTO, J., N.C. TRIPPODO, S. ISHISE, AND E.D. FROHLICH. Total vascular pressure-volume relationship in the conscious rat. *Am. J. Physiol*. 238(7): H823-H828, 1980.

128. YOUNG, D.B., R.H. MURRAY, R.G. BENGIS, AND A.K. MARKOV. Experimental angiotensin II hypertension. *Am. J. Physiol*. 239(8): H391-H398, 1980.

129. ZUKOWSKA-GROJEC, Z., M. KONARSKA, AND R. MCCARTHY. Differential plasma catecholamine and neuropeptide Y responses in acute stress in rats. *Life Sci*. 42: 1615-1624, 1988.

CHAPTER 2

EFFECT OF SALINE INFUSION ON BODY TEMPERATURE AND ENDURANCE TIME DURING HEAVY EXERCISE

A. ABSTRACT

We tested the hypothesis that volume infusion during strenuous exercise, by expanding blood volume, would allow better skin blood flow and better temperature homeostasis, and thereby improve endurance time. Nine males exercised to exhaustion at 84.0 ± 3.14% (SE) of maximum O_2 consumption on a cycle ergometer in a double-blind randomized protocol with either no infusion (control) or an infusion of 0.9% NaCl (mean volume 1,280.3 ± 107.3 ml). Blood samples and expired gases (breath-by-breath), as well as core and skin temperatures, were analyzed. Plasma volume decreased less during exercise with the infusion at 15 min (-13.7 ± 1.4% control vs. -5.3 ± 1.7% infusion, $P < 0.05$) and at exhaustion (-13.6 ± 1.2% vs. -1.3 ± 2.2%, $P < 0.01$). The improved fluid homeostasis was associated with a lower core temperature during exercise (39.0 ± 0.2°C for control and 38.5 ± 0.2°C for infusion at exhaustion, $P < 0.01$) and lower heart rate (194.1 ± 3.9 beats·min^{-1} for control and 186.0 ± 5.1 beats·min^{-1} for infusion at exhaustion, $P < 0.05$). However, endurance time did not differ between control and infusion (21.96 ± 3.56 min and 20.82 ± 2.63 min, respectively), and neither did [H^+], peak O_2 uptake and CO_2 production, end-tidal partial pressure of CO_2, blood lactate or blood pressure. In conclusion, saline infusion increases heat dissipation and lowers core temperature during strenuous exercise but does not influence endurance time.

B. INTRODUCTION

During physical activity at levels sufficient to raise core temperature, competition could develop between the skin and muscles for blood flow (4,27) through a number of mechanisms. First, increased skin blood flow reduces the flow available for the muscles, and second, it could reduce the cardiac output. This latter effect would occur because the skin has a compliant vasculature (28) and increased skin blood flow would increase this region's vascular volume, thereby decreasing both central blood volume and the maximum cardiac output (29). The greater the increase in core temperature, the greater is the vasodilatory drive to skin vessels (4,14 17,24), and the greater the importance of these factors. Meanwhile, sweat production and loss of fluid to the interstitial space also contribute to the depletion of vascular volume and further limit peak cardiac output (7,8,11, 21,26).

We therefore hypothesized that preventing blood volume losses through fluid intake may reduce the competition for blood flow between skin and exercising muscles. Better skin perfusion should result in better thermoregulation, and better muscle perfusion should result in better maintenance of adequate energy supplies for the muscle. Summation of the two should improve endurance performance. To test this we compared the endurance time, skin and core temperature, gas exchange and blood volumes in subjects exercising at near

maximal load, with either no fluid given, or with an intravenous infusion of isotonic saline. The protocol was double-blind and randomized.

C. METHODS

Subjects. Nine healthy males gave informed consent to participate after being instructed as to the purpose of the experiment and its possible risks. Their age ranged from 17 to 29 years old (mean 21.7). Their mean height, weight and peak O_2 uptake ($\dot{V}O_2$) were 177.6 ± 6.6 cm (SD), 73.5 ± 12.3 Kg and 53.7 ± 10.5 $ml \cdot min^{-1} \cdot Kg^{-1}$, respectively. We purposely chose subjects with differing activity levels to represent a wide range of peaks $\dot{V}O_2$.

Equipment. Subjects exercised on an electrically braked cycle ergometer (MIJNI, Hardt, Holland) while breathing through a low resistance mouthpiece for breath-by-breath analysis of expired gases (Medical Graphics Corp., St. Paul, MI). Ventilation ($\dot{V}E$, $l \cdot min^{-1}$ BTPS), $\dot{V}O_2$ ($ml \cdot min^{-1}$ STP) and CO_2 output ($\dot{V}CO_2$, $ml \cdot min^{-1}$ STP) were measured by a computer from the areas under the curves of the flow and gas signals. Heart rates (HR) were recorded continuously from a three-lead electrocardiogram and were averaged over six beats.

Mean skin temperature (T_s) was continuously computed from a weighted mean of eight local skin measurements (37). The thermocouples (series 400, Yellow Spring Instruments, Yellow Springs, OH) were partially covered for proper support.

Esophageal temperature (T_{es}) was measured every minute with a thermocouple (Mon-a-Therm, St. Louis, MO) at the level of the left atrium. The probe was placed in the esophagus at a distance of 0.25 times the height of the subject from the tip of the nose. The probe was positioned at the level of the left atrium by retracting and advancing it until the highest stable temperature was obtained (37).

Experimental preparation. The mean room temperature, percent humidity and barometric pressure for the control and saline infusion studies are summarized in Table 2.1. There were no differ nces in these parameters between the days these trials were performed.

Exercise protocol. Subjects dressed in light trunks and running shoes performed three exercise tests at least one week apart and between 0700 and 0900 hours. An 8 hour fast preceded each test. They were instructed not to do any heavy exercise the day before the experiment and to eat and drink as they would normally. The subject's hydration state were similar in the two exercise tests as shown by the small differences in Hematocrit [Hct, 0.44 ± 0.65% (SD)] and hemoglobin (Hb, 0.09 ± 0.31 g) between tests. An 18 gauge cannula was inserted in a right forearm vein to obtain blood samples and a second cannula was inserted in the left forearm for the infusion of saline.

In order to assess the subjects's aerobic capacity, they exercised with a work load which increased at 200 $kpm \cdot min^{-1}$

TABLE 2.1 Infusate volume, environmental conditions, changes in weight and sweat production.

	CONTROL	INFUSION
VOLUME OF SALINE INFUSED, ml	0.0	1280.3 ± 107.3
ROOM TEMPERATURE, °C	24.1 ± 0.39	23.9 ± 0.42
% HUMIDITY	27.1 ± 1.91	25.1 ± 1.73
BAROMETRIC PRESSURE, Torr	758.8 ± 2.68	758.7 ± 1.91
CHANGE IN WEIGHT, kg	-0.54 ± 0.10	0.63 ± 0.09
AMOUNT OF SWEAT PRODUCED, ml	542.2 ± 103.6	645.9 ± 142.3
VOLUME OF BLOOD WITHDRAWN, ml	164.0 ± 10.8	171.7 ± 10.8
$\dot{V}O_2$ AT EXHAUSTION, $ml \cdot min^{-1}$	3376.5 ± 174.7	3481.8 ± 194.3
$\dot{V}CO_2$ AT EXHAUSTION, $ml \cdot min^{-1}$	3638.4 ± 161.4	3875.6 ± 240.4

Values are mean ± SE.

until exhaustion. The other two studies were randomized double-blind protocol. For each test, subjects cycled until exhaustion at 73.2 ± 1.6% (SE) of the maximum work load that they achieved during the incremental test. A Borg scale (3) of perceived exertion, scored from 6 to 20, was used to estimate the degree of discomfort. Every two minutes subjects were asked to score their sensation until they reached 19, and then the scale was removed. Verbal encouragement was always provided by the same investigator and was directly proportional to the level reached on the scale, with the most forceful encouragement given after the scale was removed. At the end of the constant load test, subjects reached an average of 84.0 ± 3.14% of their peak $\dot{V}O_2$. During one trial, 0.9% sodium chloride was infused (Table 2.1) as rapidly as possible (SAL). The rate of infusion was 69.0 ± 9.0 $ml \cdot min^{-1}$. In the other trial a negligible amount of saline was infused (CON). The temperature of the infusate was 24°C (room temperature). It was calculated that the infusate temperature would decrease core temperature by only 0.011°C. In both trials, a screen covered the intravenous bags and foil paper covered the intravenous tubing to ensure that neither subject nor investigator knew whether saline was being infused. In addition, a cold wet towel was placed around the subject's forearm to eliminate any sensation induced by rapid fluid infusion. All clocks and stop watches were removed from the subject's view.

The value of $\dot{V}O_2$ at the same work load during CON and SAL were different by > 500 $ml \cdot min^{-1}$ in two subjects, suggesting technical errors in these tests. These data were therefore rejected and the subjects were asked to repeat these two experiments. The values of $\dot{V}O_2$ in the repeated protocols were similar, and the results were used in the analysis.

Sample analysis. Blood samples were drawn with free-flowing technique at regular intervals during exercise, at -10, 0, 15 minutes, and exhaustion, and then at 5 (R5) and 20 (R20) minutes of recovery. The right arm was kept in a fixed position for at least 25 minutes before the blood samples at time 0 were taken. The blood samples were put in ice and single analyses were done within one hour after the end of the experiment. pH was analyzed on a Corning 175 automatic pH/blood gas system (Corning Inc., Medfield, MA). The pH was converted for statistical purposes to hydrogen ion concentration ($nmol \cdot l^{-1}$). Lactate concentration was assayed by an automated enzymic microfluorimetric method on supernatants of blood immediately deproteinized in cold 10% ($wt \cdot vol^{-1}$) perchloric acid (22). Hct and Hb concentration were obtained with a Technicon H6000. Plasma, total protein and albumin concentrations were obtained with a Technicon SMAC II.

Because subjects did not void, weight losses were assumed to be due to sweat production and respiratory evaporation, so that changes in weight could be used to calculate the total amount of sweat produced. The weight in Kilograms of the

dried naked subject after exercise was subtracted from the weight before exercise. The difference was then multiplied by 1,000 assuming that one Kg of weight loss equals 1,000 ml of sweat. The weight of saline infused was accounted for by adding it to the weight of the subject before exercise. The accuracy of the scale was ± 0.05 Kg.

Percent changes in plasma volume (%PV) at selected intervals were calculated by applying the formula (13) $\%PV = \{[([Hb]_{before} \times (1-Hct_{after}))/([Hb]_{after} \times (1-Hct_{before}))]-1\} \times 100$, where before represents before and after represents after exercise was started.

Blood samples in one subject were hemolyzed at 15 min and at exhaustion and his data were excluded for those times. Two subjects did not complete 15 min during the constant work load studies and the data at this time includes only 6 subjects.

Statistics. Data are presented as mean ± SE unless otherwise stated. A repeat measurement analysis of variance was used, followed by a Duncan's new multiple-range test for the analysis of the temperature measurements, heart rates and expired gases (5). Paired *t* tests with Bonferroni corrections were used for the analysis of the changes in plasma volume, plasma total protein albumin concentrations, $[H^+]$ and lactate because of incomplete data sets.

D. RESULTS

After 15 minutes of exercise, the mean percent change in plasma volume during SAL was -5.28 ± 1.68% as compared to -13.67 ± 1.35% ($P < 0.05$) in CON (Fig. 2.1). The corresponding values at exhaustion were -1.27 ± 2.23% in SAL and -13.61 ± 1.19% in CON ($P < 0.01$). The plasma volume throughout exercise during SAL was not significantly different from the baseline level. After 20 minutes of recovery, plasma volume returned to the baseline level for CON (-0.59 ± 1.34%) and did not change significantly in SAL (+6.05 ± 1.45%). The pattern of change in plasma total protein and albumin concentration in whole blood also supported a significant alteration in circulating volume during CON (Fig. 2.2). The total protein concentration at exhaustion was 7.52 ± 0.14 $g \cdot dl^{-1}$ for CON and 6.48 ± 0.14 $g \cdot dl^{-1}$ ($P < 0.005$) for SAL, whereas for albumin at exhaustion it was 5.21 ± 0.07 $g \cdot dl^{-1}$ and 4.50 ± 0.08 $g \cdot dl^{-1}$ ($P < 0.005$), respectively.

Mean T_{es} at rest for CON and SAL were similar, 36.74 ± 0.08°C and 36.57 ± 0.03°C respectively (NS, Fig. 2.3). With saline infusion, T_{es} was lower [$P < 0.05$, analysis of variance (ANOVA)] at 30% of maximum endurance time ($P < 0.05$) and until exhaustion. T_{es} at exhaustion was 39.00 ± 0.21°C and 38.46 ± 0.15°C for CON and SAL, respectively ($P < 0.01$).

For clarity, differences in T_s were normalized by assigning the value of zero to T_s at rest (Fig. 2.4). After a small initial drop, T_s rose progressively until 70% of maximum

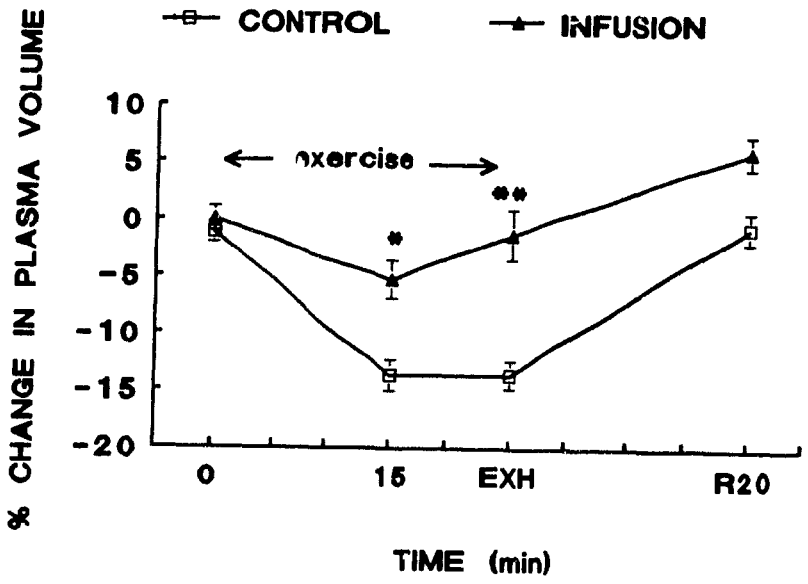

FIGURE 2.1 Percent change in plasma volume as a function of time during constant load exercise. EXH, exhaustion; R20, 20 min of recovery. * $P < 0.05$; ** $P < 0.01$ for control vs. saline. Data are mean ± SE. R 20 is plotted from EXH time, which varied between subjects. $n = 9$, except for 15 min where $n = 6$ and EXH where $n = 8$.

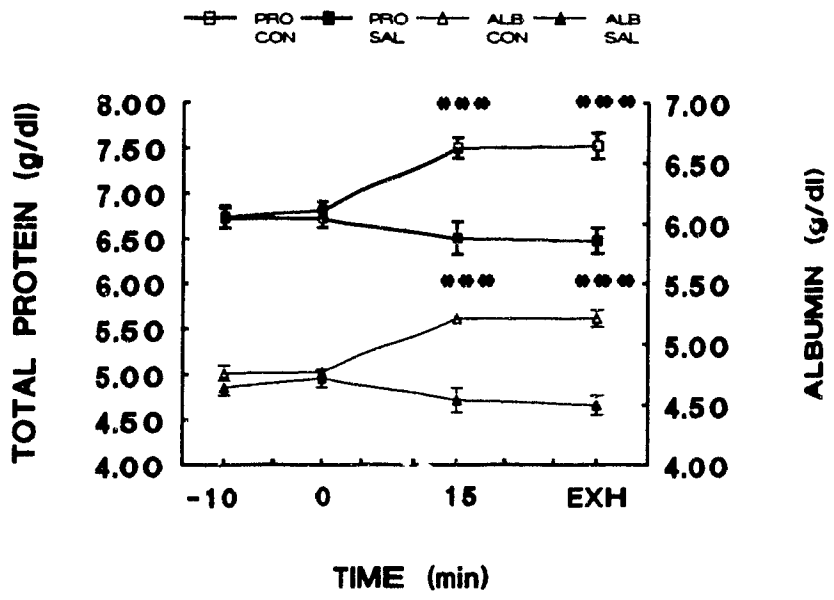

FIGURE 2.2 Plasma total protein (PRO) and albumin (ALB) concentrations as a function of time during constant load exercise. CON, control; SAL, saline infusion. Data are mean ± SD, *** $P < 0.005$ for control vs saline. $n = 9$, except for 15 min where $n = 6$ and EXH where $n = 8$.

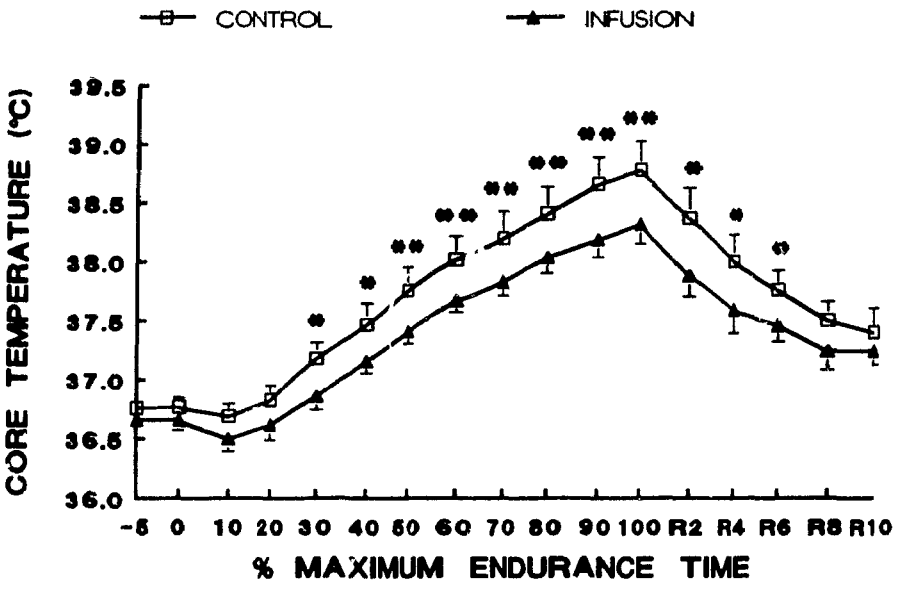

FIGURE 2.3 Esophageal (core) temperature as a function of percent maximum endurance time during constant load exercise. Percent maximum endurance time was used to normalize time to exhaustion. Symbols as in Fig. 2.1. Data are mean ± SE. R2, R4, R6, R8, R10 indicate recovery times at 2, 4, 6, 8 and 10 minutes respectively. n = 9.

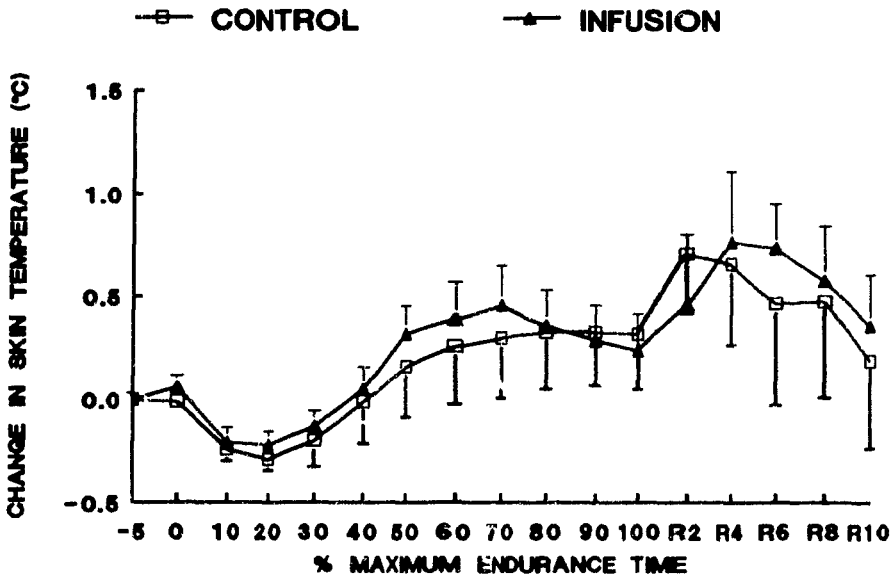

FIGURE 2.4 Changes in skin temperature as a function of percent endurance time during constant load exercise. Skin temperature at rest was used as reference, with change from reference temperature presented. Data are mean ± SE. There was no difference between control and infusion. See legend of Fig. 2.3 for definition and abbreviations.

endurance time and then reached a plateau. There was no significant difference in the change or in absolute values of mean T_s between CON and SAL. In the first 4 min of recovery, T_s increased at a time when core temperature was decreasing.

Heart rate was lower during exercise with saline infusion as compared to control ($P < 0.025$, ANOVA, Fig. 2.5). Mean heart rate at exhaustion was 194.1 ± 3.9 beats·min^{-1} for CON and 186.0 ± 5.1 beats·min^{-1} ($P < 0.05$) for SAL. One subject was excluded for technical reasons.

The calculated amount of sweat produced by each subject was not significantly different between CON and SAL (Fig. 2.6, Table 2.1).

Blood [H^+] decreased with exercise in both conditions ($P < 0.02$). [H^+] at exhaustion was 63.0 ± 13.0 nmol·l^{-1} in both protocols. Lactate levels increased by similar magnitudes in both CON and SAL. The mean lactate concentration at exhaustion was 10.96 ± 0.35 mmol·l^{-1} for CON and 10.19 ± 0.38 mmol·l^{-1} for SAL (NS). The values for $\dot{V}CO_2$ and $\dot{V}O_2$ at exhaustion were not significantly different between CON and SAL (Table 2.1). $\dot{V}E$ was not significantly different between CON and SAL; the values at exhaustion were 138.8 ± 7.0 and 141.5 ± 8.5 l·min^{-1}, respectively.

The endurance times did not differ between SAL and CON and averaged 20.82 ± 2.63 and 21.96 ± 3.56 min respectively (Fig. 2.7).

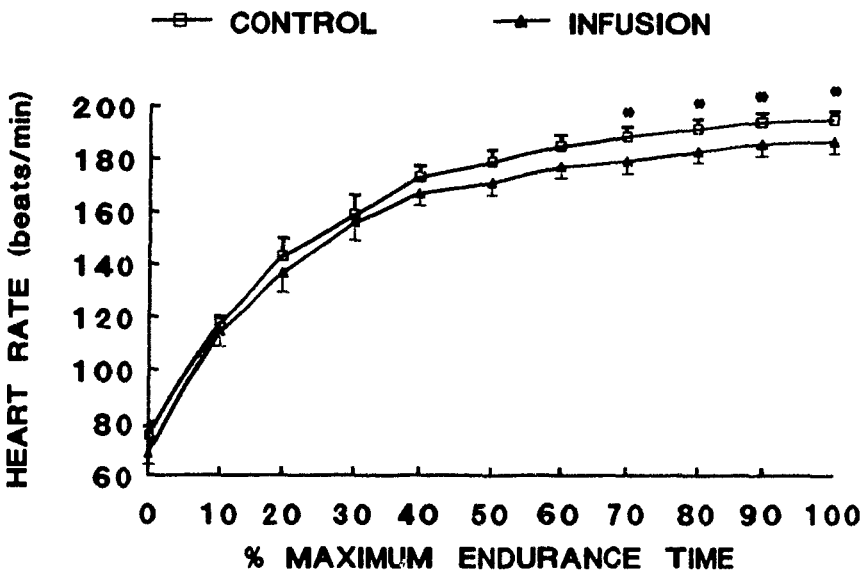

FIGURE 2.5 Heart rate as a function of percent maximum endurance time during constant load exercise. Symbols as in Fig. 2.1. n = 8. Heart rate was significantly lower during the infusion after 60% of maximum endurance time.

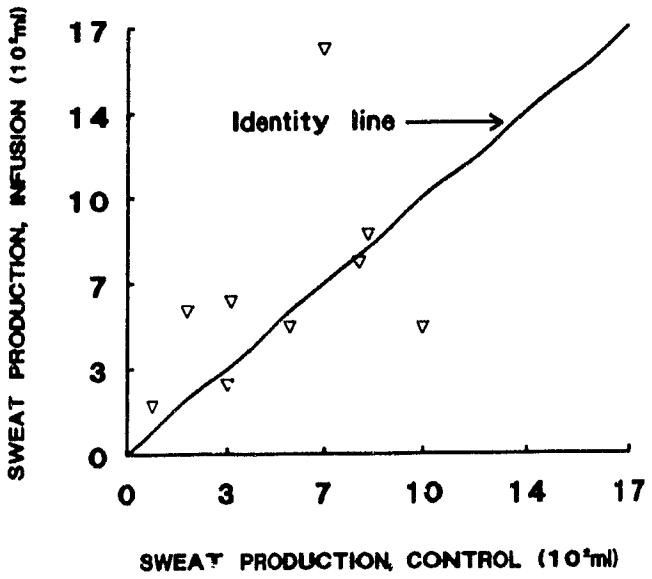

FIGURE 2.6 Identity plot of calculated sweat losses during CON and SAL. There was no significant difference between tests.

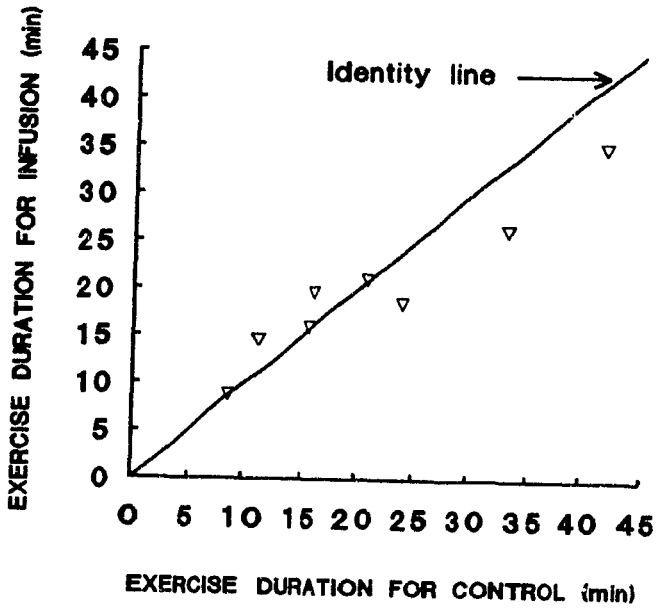

FIGURE 2.7 Identity plot of time to exhaustion during CON and SAL. There was no significant difference between tests.

E. DISCUSSION

The hypothesis tested in this study was that maintenance of blood volume during exercise would improve temperature regulation, which in turn would improve exercise endurance. We successfully maintained plasma volume levels throughout exercise with the infusion of saline (Figs. 2.1 and 2.2), and this was associated with improved thermoregulation and lower increments in heart rates (Figs. 2.3 and 2.4). Endurance time, however, was not affected by saline infusion (Fig. 2.7).

Before discussing the results, certain aspects of the methodology warrant discussion. Motivation can be highly variable. We tried to control this by using a Borg scale of perceived exertion to estimate how early during the test subjects began to fatigue, and to what extent verbal encouragements were needed. There was no difference in the rate of rise of perceived exertion between CON and SAL (slope = 0.21 ± 0.03 and 0.21 ± 0.02 point/percent endurance time, respectively). The scale was taken away on average at 68.9 ± 7.6% of maximum endurance time in CON and at 66.2 ± 6.5% in SAL. These results indicate that the lower core temperatures with saline infusion did not alter the perception of work performed by the subjects in the first two-thirds of the exercise period.

Termination of exercise was allowed only after significant signs of exhaustion such as grunting through the mouthpiece

and irregular pedalling rate. The high lactate concentrations at end exercise were very similar during CON and SAL (10.96 and 10.12 $mmol \cdot l^{-1}$, respectively), indicating that subjects performed equally hard in both studies. We therefore believe that motivational factors were minimized as determinants of the end point of exercise.

When asked after the completion of the studies whether or not they could differentiate between CON and SAL, only 1 of 9 subjects answered affirmatively and, although he was correct, his endurance time between CON and SAL was the same. All subjects stated that they had no tangible notion of time during the experiments, we consequently believe that subjects were adequately blinded. The investigator directly involved was only apprised of the protocol after the completion of each subject's tests.

We chose high exercise loads because we wanted a steady increase in core temperature throughout the experiment, but enough time to permit multiple measurements. This allowed us to test whether or not increased core temperature and decreased blood volume are potentially limiting factors in endurance performance.

A potential error in the calculation of sweat production is the time taken after exercise to make weight measurements of "dried off" subjects (usually 20-25 min), for some subjects could continue to sweat during recovery while others might stop sweating. This factor could not be controlled.

Hydration level and thermoregulation. Strategies used by other investigators to compare temperature regulation at different blood volumes during exercise have varied. Blood volumes have been manipulated by hypohydrating (6,34) and hyperhydrating (12,23,24) subjects before exercise or by giving fluids (sometimes of differing osmolality) during exercise (34,36). In all but two studies (12,36), a warm environment was superimposed upon exercise. In all of these studies a reduction in blood volume or restriction of fluid intake resulted in a higher core temperature during exercise as well as lower skin blood flow (24) and a higher heart rate (6,23, 34,36). Recently, Fortney et al. (9), using techniques similar to ours, maintained plasma volume during exercise in the heat and showed improved thermoregulation, lower heart rate and a tendency towards increased forearm blood flow. Endurance was not examined. Our results are consistent with these studies but differ from Francesconi and Hubbard's (10).

Francesconi and Hubbard examined the effects of saline and bicarbonate infusions on thermoregulation in exercising rats. Although the ambient temperature was high (35 ^{o}C), they found no difference in core temperature between control and infused rats. They did not calculate changes in plasma volume, but a comparison of the hematocrit and plasma protein concentrations before and after exercise suggests similar decreases in volumes for the control and infusion experiments. Thus, the amount infused may not have been large enough to compensate

for the exercise induced volume loss. In our study, we successfully limited the decrease in plasma volume with an infusion of at least 1 l of saline, which is one fifth of the total blood volume of a 70 kg man. Everything being equal, if we assume that Francesconi and Hubbard's rats had a total blood volume of 24 ml (mean weight of rats = 310 g), an infusion of at least 4.8 ml would have been necessary to be the equivalent of the amount infused in the present study, but they only gave 2 ml. It is therefore not unexpected that thermoregulation was not altered.

Because the environmental temperature, relative humidity, work load, $\dot{V}O_2$, $\dot{V}E$ and sweat production were the same in both conditions, the only mechanism of heat dissipation left to explain the difference in core temperature and heart rate between CON and SAL is increased skin blood flow. Although we did not measure skin blood flow, evidence from Fortney et al. (9) indicate blood flow to the skin is augmented with maintenance of plasma volume during exercise. In their study, plasma volume was allowed to decrease in the first 10 min of exercise but thereafter infusion restored plasma volume to control. Even with this initial decrease in plasma volume, forearm blood flow had a tendency to be larger with infusion. Because we started the infusion immediately at the onset of exercise, this effect, if anything, was greater in our study.

Endurance performance. The most interesting result of the present study is that even though saline infusion resulted in

a lower core temperature and lower heart rate, endurance time was not improved. This issue has been previously addressed in both animal (10,20) and human studies (32,35,36).

Kozlowski et al. (20) tested whole body cooling in dogs by placing ice bags around their chests and found that endurance time was increased when they were cooled. Francesconi and Hubbard (10) studied volume infusion and found no difference in endurance time when bicarbonate or saline were infused in rats compared to control runs. However, as noted above, the volume infusion may not have been sufficient to improve thermoregulation.

In humans, Sawka et al. (35) found no difference in endurance time during exercise in the heat when plasma volume was expanded before exercise but core temperature and heart rate were not affected. Saltin (32) found that endurance time decreased in dehydrated subjects exercising at a maximum level for an average of 6 minutes. Staff and Nilsson (36) examined endurance performance during running at 70% $\dot{V}O_2max$ on a treadmill until exhaustion with or without fluid intake in a thermoneutral environment and they found a 21% improvement in endurance time with hydration. One important factor in these studies differs from ours. We used a double-blind randomized protocol, but Staff and Nilsson could not blind their trials since subjects had to drink at fixed intervals during the experiment. Similarly, Saltin's dehydrated subjects were not blinded. Therefore it is quite possible that the increase in

endurance in these studies was due to motivational factors.

An estimate of cutaneous blood flow during moderate exercise in a thermoneutral environment is in the order of 3 $l \cdot min^{-1}$ (19). Redistribution of flow from visceral organs (27) and inactive muscle (19) cannot account for this increase in skin blood flow. Hence it is assumed that at constant cardiac output, active muscle blood flow would have to be compromised (27,28) or cardiac output would have to increase. We hypothesized that at a critical core temperature the need for temperature homeostasis would take precedence over muscle blood flow. As skin flow increased and blood pooled in the compliant skin vasculature the peak cardiac output would decrease and thereby decrease muscle blood flow. Exercise would cease as muscle blood flow becomes inadequate for its energy demands. Evidence in favour of this hypothesis is found in Bell et al.'s study (2) who found a reduction in active muscle blood flow in sheep walking on a treadmill in the heat (40 °C), whereas the flow to the heat dissipating areas markedly increased. Rowell (30) also found a lower cardiac output in human subjects exercising in a hot environment when compared to exercise in a thermoneutral environment. Although these studies were performed in hot environments and ours in a thermoneutral environment, we tried to match the thermoregulatory drive of these studies by using an exercise level high enough to raise core temperature markedly. We further predicted that fluid infusion would

prevent the normal decrease in blood volume, allow a higher cardiac output, better skin and muscle blood flow and therefore a greater endurance time.

The rejection of our hypothesis is most likely because the demand for skin blood flow was not high enough to exhaust the reserves in cardiac output. This reserve can be estimated using the formula cardiac output = (0.06 weight) + 5.5 $\dot{V}O_2$ ($l \cdot min^{-1}$) (18). The average predicted maximum cardiac output in our study is then 26.0 $l \cdot min^{-1}$. At plateau, the average $\dot{V}O_2$ was 3.3 $l \cdot min^{-1}$ and the estimated cardiac output was 22.6 $l \cdot min^{-1}$. Subjects should therefore still have had 3.4 $l \cdot min^{-1}$ reserve in cardiac output. If 85% of cardiac output can go to the muscle, muscle blood flow at plateau can be estimated at 18.6 $l \cdot min^{-1}$. As noted above, skin blood flow during this level of exercise has been estimated at $\approx$3 $l \cdot min^{-1}$. If 2.4 $l \cdot min^{-1}$ is left for the perfusion of all other regions (1), the cardiac output needed to maintain exercise at the end of our study would be 24 $l \cdot min^{-1}$, a value approaching but still below the maximum of 26 $l \cdot min^{-1}$ estimated for our population. It can therefore be argued that the stress on the vascular system was not high enough for the competition for blood flow between the skin and active muscles to be a problem.

The question therefore arises as to why the subjects stopped exercising. The most likely explanation is that the accumulation of muscle metabolites such as lactate limited endurance performance. This stress is the same with or

without saline infusion if muscle blood flow is not altered. This should be the case in our study, since energy demands were the same in both trials. It is quite possible, however, that the effects of saline infusion on exercise endurance would be more important at high ambient temperatures, because under these conditions high sweat production and therefore large reductions in blood volume could decrease cardiac reserves.

In conclusion, saline infusion during exercise can maintain blood volume at base-line levels. As has been previously shown (9), this maintenance in blood volume is reflected in a lower HR, lower core temperature, and, most likely, increased skin blood flow. Surprisingly, and contrary to what was predicted this increase in heat dissipation capacity did not influence performance time in this protocol.

F. REFERENCES

1. Astrand, P., and K. Rodahl. *Textbook of Work Physiology*. New York N. Y.: McGraw-Hill Book Company, 2nd edition, 1977.

2. Bell, A. W., J. R. S. Hales, R. B. King, and A. A. Fawcett. Influence of stress on exercise-induced changes in regional blood flow in sheep. *J. Appl. Physiol.* 55(6): 1916-1923, 1983.

3. Borg, G. A. V. Psychological bases of perceived exertion. *Med. Sci. Sports Exercise*. 14: 377-381, 1982.

4. Brengelmann, G. L., J. M. Johnson, L. Hermansen, and L. B. Rowell. Altered control of skin blood flow during exercise at high internal temperatures. *J. App. Physiol.* 43: 790-794, 1977.

5. Bruning, J. L., and B. L. Kintz. *Computational handbook of statistics*. Glenview, IL and London USA: Scott, Foresman & Co., 2nd Edition, 1977.

6. Candas, V., J. P. Libert, G. Brandenberger, J. C. Sagot, C. Amoros, and J. M. Kahn. Hydration during exercise. Effects on thermal and cardiovascular adjustments. Eur. *J. App. Physiol.* 55: 113-122, 1986.

7. Costill, D. L. Sweating: its composition and effects on body fluids. *Ann. N. Y. Acad. Sci.* 301: 160-174, 1977.

8. Fortney, S. M., E. R. Nadel, C. B. Wenger, and J. R. Bove. Effects of blood volumes on sweating rate and body fluids in exercising humans. *J. App. Physiol.* 51: 1594-1600, 1981.

9. Fortney, S. M., N. B. Vroman, W. S. Beckett, S. Permutt, and N.D. LaFrance. Effect of exercise hemoconcentration and hyperosmolality on exercise responses. *J. Appl. Physiol.* 65(2): 519-524, 1988.

10. Francesconi, R., and R. Hubbard. Exercise in the heat: Effects of saline or bicarbonate infusion. *J. App. Physiol.* 57: 733-738, 1984.

11. Grande, F., H. L. Taylor, J. T. Anderson, E. Buskirk, and A. Keys. Water exchange in men on a restricted water intake and low calorie carbohydrate diet accompanied by physical work. *J. App. Physiol.* 12: 202-320, 1958.

12. Greenleaf, J. E., and B. L. Castle. Exercise temperature in man during hypohydration and hyperhydration. *J. App. Physiol.* 30: 847-853, 1971.

13. Harrison, M. H. Effects of thermal stress and exercise on blood volume in humans. *Physiol. Rev.* 65(1): 149-209, 1985.

14. Hammel, H. T. Regulation of internal body temperature. *Ann. Rev. Physiol.* 30: 641-710, 1968.

15. Hellon, R. Thermoreceptors. In: *Handbook of physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow*, J. T. Shepherd and F. M. Abboud, eds. Bethesda, MD: American Physiological Society, 1983, Sect. 2, Vol. III, Part II, Chapter 18, pp. 659-673.

16. Hensel, H. Neural processes in thermoregulation. *Physiol. Rev.* 53: 948-1017, 1973.

17. Jessen, C., and E. Simon. Spinal cord and hypothalamus as core sensors of temperature in the conscious dog. III. Identity of functions. *Pflügers Arch.* 324: 217-226, 1971.

18. Jones, N. L., and E. J. Campbell. *Clinical exercise testing*. Toronto, Ont.: W. B. Saunders Co., 2nd Edition, 1982.

19. Johnson, J. M., and L. B. Rowell. Forearm skin and muscle vascular response to prolonged leg exercise in man. *J. App. Physiol.* 39: 920-924, 1975.

20. Kozlowski, S., Z. Brzezinska, B. Kruk, H. Kaciuba-Uscilko, J. E. Greenleft, and K. Nazar. Exercise hyperthermia as a factor limiting performance: Temperature effect on muscle metabolism. *J. Appl. Physiol.* 59(3): 766-773, 1985.

21. Ladell, W. S. S. The effects of water and salt intake upon the performance of men working in hot and humid environments. *J. Physiol (Lond.)* 127: 11-46, 1955.

22. Lloyd, B., J. Burrin, P. Smythe, and K. G. M. M. Alberti. Enzymatic fluorometric continuous flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. *Clin. Chem.* 24: 1724-1729, 1978.

23. Moroff, S. V., and D. E. Bass. Effects of overhydration on man's physiological responses to work in the heat. *J. App. Physiol.* 20: 267-270, 1965.

24. Nadel, E. R., S. M. Fortney, and C. B. Wenger. Effect of hydration state on circulatory and thermal regulations. *J. App. Physiol.* 49: 715-721, 1980.

25. Nadel, E. R., E. Cafa Relli, M. F. Roberts, and C. B. Wenger. Circulatory regulation during exercise in different ambient temperatures. *J. Appl. Physiol.* 46: 430-437, 1979.

26. Pearcy M., S. Robinson, D. I. Miller, J. T. Thomas Jr., and J. DeBrota. Effects of dehydration, salt depletion and pitressin on sweat rate and urine flow. *J. App. Physiol.* 8: 621-626, 1956.

27. Rowell, L. B. Cardiovascular adjustments to thermal stress. In: *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow.* Bethesda, MD: American Physiological Society, 1984, Sect. 2, Vol. III, Part 2, Chapter 27, p. 967-1023.

28. Rowell, L. B. Human cardiovascular adjustments to exercise and thermal stress. *Physiol. Rev.* 54(1): 75-159, 1974.

29. Rowell, L. B. *Human circulation. Regulation during physical stress.* New York, N. Y.: Oxford University Press, 1986.

30. Rowell, L. B., J. A. Murray, G. L. Brengelmann, and K. K. Kraning. Human cardiovascular adjustments to rapid changes in skin temperature during exercise. *Circ. Res.* 24: 711-724, 1969.

31. Rowell, L. B., H. J. Marx, R. A. Bruce, R. D. Conn and F. Kusumi. Reduction in cardiac output, central blood volume and stroke volume with thermal stress in normal men during exercise. *J. Clin. Invest.* 45: 1801-1816, 1966.

32. Saltin B. Circulatory response to submaximal and maximal exercise after thermal dehydration. *J. App. Physiol.* 19: 1125-1132, 1964.

33. Savard, G. K., B. Nielsen, J. Laszczynska, B. E. Larsen, and B. Saltin. Muscle blood flow is not reduced in humans during moderate exercise and heat stress. *J. Appl. Physiol.* 64(2): 649-657, 1988.

34. Sawka, M. N., A. J. Young, R. P. Francesconi, S. R. Muza, and K. B. Pandolf. Thermoregulatory and blood responses during exercise at graded hypohydration levels. *J. Appl. Physiol.* 59(5): 1394-1401, 1985.

35. Sawka, M. N., R. W. Hubbard, R. P. Franscesconi, and D. H. Horstman. Effect of acute plasma volume expansion on altering exercise-heat performance. *Eur. J. Appl. Physiol.* 51: 303-312, 1983.

36. Staff, P. H., and S. Nilsson. *Fluid and glucose intake during prolonged severe physical activity*. Oslo, Norway: Arbeidsfysiol. Inst., 1971, p. 1235-39.

37. Wenger, C. B., M. F. Roberts, J. A. J. Stolwijk, and E. R. Nadel. Forearm blood flow during body temperature transients produced by leg exercise. *J. App. Physiol*. 38: 58-63, 1975.

CHAPTER 3

THE SKIN VASCULAR BED IS A POTENTIAL BLOOD RESERVOIR DURING HEAT STRESS

A. ABSTRACT

To determine the potential role of the skin vasculature as a blood reservoir we measured venous compliance (C_v), resistance (R_v) and their product, the time constant of venous drainage ($\tau_{sk} = R_vC_v$), in skin flaps from the hindlimbs of 15 dogs anesthetized with pentobarbital sodium at different core temperatures (T_c 37-42°C), skin temperatures (T_s, 25.3-50.0°C) and during an infusion of papaverine (5%). The vasculature of the flaps was isolated and a double occlusion technique was used to measure the static pressure in the venous compartment. The blood volume of the flap was altered by changing either flow or outflow pressure (P_v). The change in volume was estimated from the change in weight of the flap with a force transducer. At T_c = 37°C, R_v was 2.27 ± 0.81 mm Hg·min·ml^{-1}·100g^{-1} (mean ± SD), C_v was 0.17 ± 0.06 ml·mm Hg^{-1}·100g^{-1} and τ_{sk} was 28.0 ± 8.8 sec. R_v decreased with elevated T_c, T_s and with papaverine. C_v increased with a rise in T_c and T_s. Increasing T_c and T_s did not change τ_{sk} but the papaverine infusion shortened it. The lowest skin time constant (20 sec) occurred during maximal vasodilatation. This long τ_{sk} indicates that the skin could serve as a blood reservoir during heat stress.

B. INTRODUCTION

If a vascular bed is assumed to consist of a resistive element (arterial) in series with a compliant element (venules and veins, C_v) which empties through another smaller resistive element (veins, R_v) (14,25), then the product of C_v and R_v (i.e. the time constant of venous drainage, τ_{sk}), determines the accumulation of blood in this vascular region for a given increase in flow or venous pressure (P_v). τ_{sk} represents the time taken for 63% of the total change in volume to occur. The greater the venous time constant, the greater the accumulation of blood. It has been postulated that the time constant of the skin (τ_{sk}) is long (2) for this would be favorable for heat dissipation. If so, a substantial volume of blood will accumulate in the skin as cutaneous blood flow increases with heat stress. This in turn will reduce the slope of the venous return curve, and therefore, maximal venous return (2). As a result, maximal cardiac output will be lower and perfusion of other organs could be compromised (20). Heretofore there has been no measurement of τ_{sk}. To do so, we used the double occlusion technique of Townsley et al. (25) in vascularly isolated skin flaps from dog hindlimbs to measure the pressure in the compliant region. Flow or venous outflow pressure were changed to produce changes in vascular volume. This allowed the calculation of C_v, R_v and τ_{sk}. The effect on C_v and R_v of central versus local heating was also studied to determine if heat stress alters τ_{sk}. Finally, we

injected papaverine to determine the mechanical characteristics of skin vessels without tone.

C. METHODS

Fifteen mongrel dogs of either sex (28 ± 12.5 Kg, SD) were anesthetized with 25 $mg \cdot Kg^{-1}$ of pentobarbital sodium and supplementary doses were given when necessary. They were intubated and ventilated with a Harvard respiratory pump. If they shivered or panted, a muscle relaxant (pavulon 1 - 2 mg) was given.

Figure 3.1 is a representation of the experimental preparation. A flap of skin and subcutaneous tissue was isolated from the inner right thigh of the hindlimb and extended from the top of the inner thigh to the ankle. The average weight of the flaps was 121.8 ± 32.5 g (SD). The saphenous vein and artery as well as any other blood vessels crossing the boundaries of the flap were ligated. The blood supply to the flap was isolated by following the femoral artery and vein and tying off all but one branch supplying and draining the flap. The nerve which ran along the artery supplying the flap was left intact.

Polyvinyl catheters were inserted in the right and left femoral vein and artery. Blood was drawn from the left femoral artery to supply the flap through the distal end of the right femoral artery. The right carotid artery was cannulated with a polyvinyl catheter in line with a heat

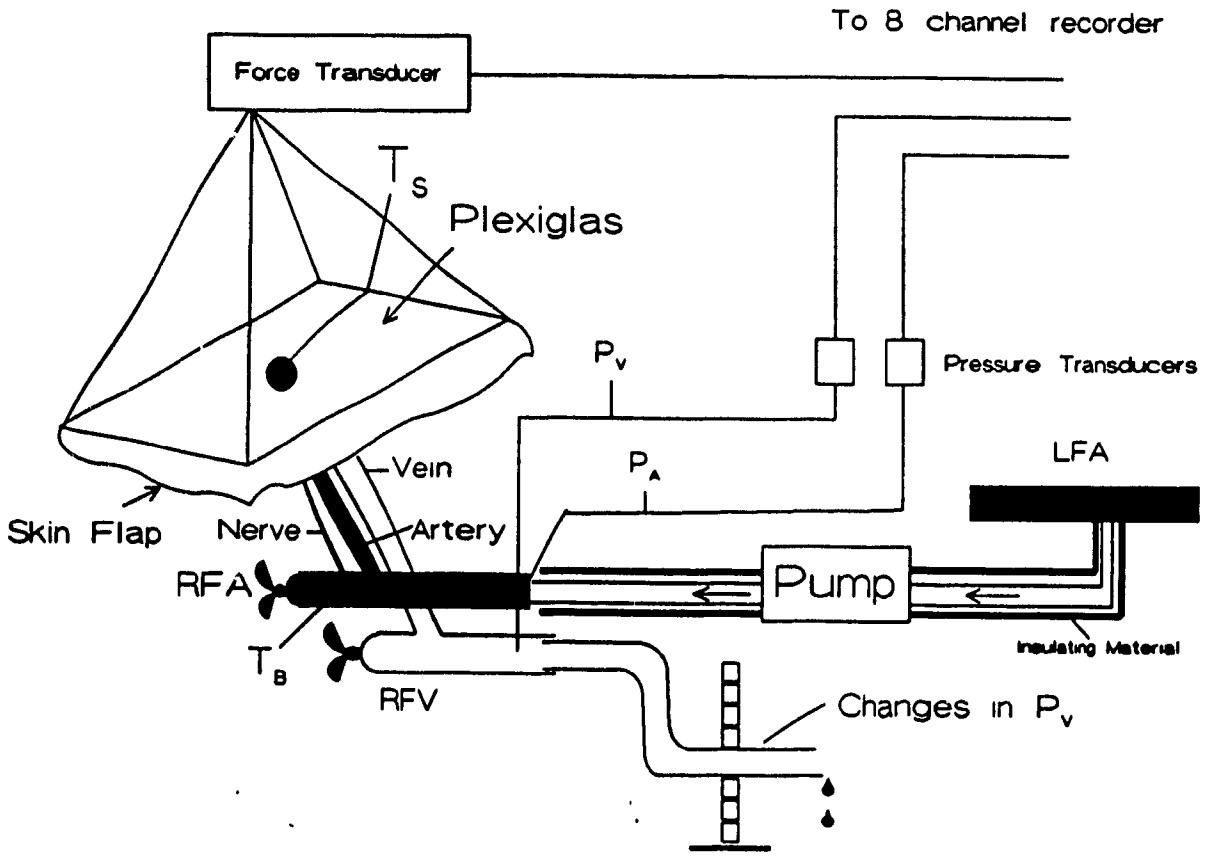

FIGURE 3.1 Experimental preparation. Skin flaps were attached to a Plexiglas rectangle and hooked to a force transducer. Blood from the left femoral artery (LFA) was pumped into right femoral artery (RFA) to supply the flaps and was collected from the right femoral vein (RFV) to measure flow. Height of venous tubing could be changed to control venous pressure (P_v). Arterial pressure (P_a) and P_v were measured at flap level. Skin (T_s) and blood (T_B) temperatures were also measured.

exchanger to control core temperature and the blood was returned via the left femoral vein. Every hour 1,000 units of heparin were added for anticoagulation.

INSTRUMENTATION

Trantec pressure transducers (California, USA) connected to a Gould Universal preamplifier with the output to an eight channel recorder were used for all pressure measurements. The pressure of the right femoral vein and artery (P_a) were referenced to the level of the flap. Systemic arterial pressure was measured from a catheter in the right carotid artery and referenced to the mid thorax. In order to avoid changes in compliance due to distortion of skin vessels, the flap was glued to a plexiglass rectangle which was suspended from a force transducer (Grass Instrument, Mass., USA). Care was taken that the flap hung loosely from the force transducer. The transducer was connected to a Gould carrier with output to an eight-channel recorder and an analog to digital converter. The signal was stored on a personal computer for later analysis. Blood flow through the flap was controlled by a Masterflex pump (Cole-Palmer instruments Co., Illinois, USA). The circuit was covered with insulating material and foil paper from the pump to the entry of the flap to reduce heat loss. Flows were measured by timed collections of venous blood. The tubing from the venous exit was open to atmosphere and the venous outflow pressure was controlled by the height of the tubing. The compliance of the venous tubing

was 0.006 ± 0.001 ml·mm Hg^{-1} (SD).

The arterial blood temperature was monitored with a Mon-a-Therm thermocouple (St. Louis, USA); core temperature (T_c) was measured per rectum. In 4 dogs, skin temperature (T_s) was recorded with a thermocouple on the surface of the flap between the skin and plexiglass.

PROTOCOL

A period of 15 min was allowed after the completion of the surgical preparation before blood flow was set with the pump at a level which produced a P_a between 100 and 130 mm Hg (range 1.5-3.5 ml·min^{-1}) and P_v was set at ≈ 3 mm Hg. To obtain the pressure in the compliant region, flow was stopped by simultaneously turning off the pump and clamping the venous exit (i.e. double occlusion procedure) (25). P_v reached a plateau within 4 to 10 seconds. This plateau pressure is the mean static elastic recoil pressure of the flap vasculature (P_{el}). The first few occlusions were maintained for 30 to 40 sec in order to establish the stability of the plateau, the absence of leaks and the presence of vascular reflexes (Fig. 3.2).

The volume in the vascular region was changed by altering P_v or flow and was estimated by measuring the digitized change in weight of the flap (1g = 1ml of blood). Double occlusions were performed before and after every change in volume. Two to three step increases in P_v or flow were performed followed by step decreases to baseline. Since the values obtained from

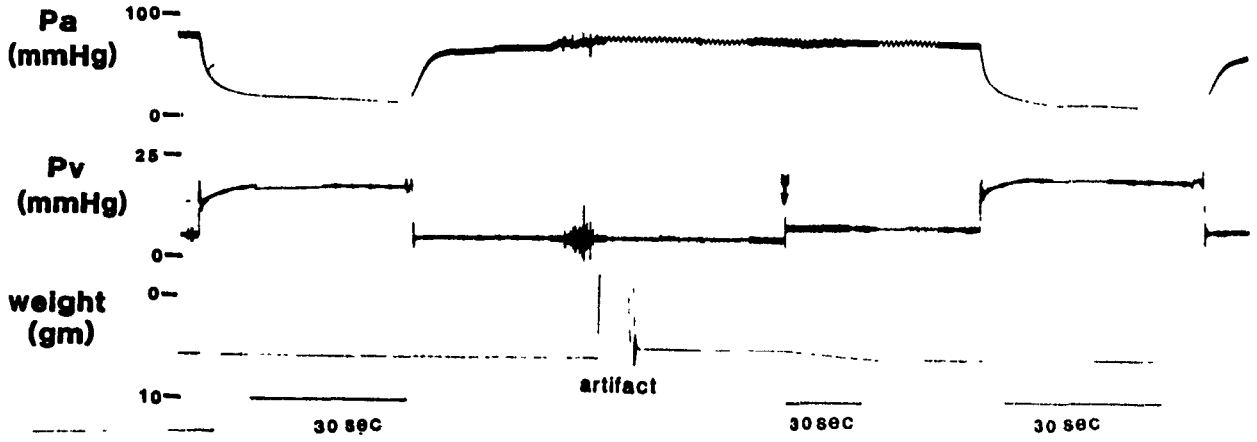

FIGURE 3.2 Sample tracing of double-occlusion technique and of a step increase in venous pressure (P_v). With occlusion of the inflow and outflow, arterial pressure (P_a) decreases and P_v increases rapidly to plateau while the weight of flap remains constant. With a step increase in P_v, weight of flap increases (scale of weight signal is inverted) and P_a remains constant. Second double occlusion shows an increase in venous plateau pressure. Weight signal is a mean signal on zero suppressor. Paper speed was faster during double occlusion, thus two time scales are indicated.

step decreases in P_v or flow did not differ from increases and since there was a time dependant deterioration of the preparation, only a few step decreases were performed. The double occlusion and step change procedures were repeated at core temperatures of 37°C and 42°C (n = 9), at skin temperatures of 25.3 ± 3.0°C and 50.0 ± 1.7°C (n = 4), and with infusion of 5% papaverine directly into the flap vasculature (n = 4).

Blood gases (Corning Medical, Mass., USA) and hematocrit (Readacrit centrifuge, Clay Adams, USA) assessments were made at the beginning, middle and end of every experiment.

Occasionally, following step changes in P_v or flow, there was an increase or decrease in P_a to a new stable value within 5 to 10 seconds. Since this reflex adjustment did not affect P_v or flows, we included these trials in the analysis. For trials with step changes in flow, the venous collection for the flow measurement was taken after the transient change in P_a. We rejected any recordings in which P_{el} fell after occlusion and, in the few cases in which P_{el} rose after the plateau was reached, we used the values within 10 sec of occlusion for analysis.

MEASUREMENT OF FLAP WEIGHT AND CALCULATIONS

The weight of the plexiglass sheet was recorded first with the force transducer. The skin flap was then glued onto the plexiglass and both were suspended on the force transducer. The flow and venous pressure were adjusted to initial levels.

The combined weight of the plexiglass and flap was recorded and the weight of the plexiglass sheet was subtracted from the total weight to obtain the flap weight. At the end of the experiment, the flow and venous pressure were adjusted to initial levels and the weight of the flap was obtained.

Venous compliance was calculated from the change in volume (ΔV) divided by the difference between P_{el} before (P_{elb}) and after (P_{ela}) a step change in P_v or flow ($C_v = \Delta V/(P_{ela} - P_{elb})$). Venous resistance was calculated, before and after a step change, from the difference between P_{el} and P_v divided by flow ($R_v = (P_{el} - P_v)/flow$). The venous time constant was calculated from the product of the average R_v (before and after a step change) and C_v. τ_{sk} was also calculated from $\Delta V/\Delta flow$. Arterial resistance (R_a) was calculated from the difference between arterial pressure and P_{el} divided by flow ($R_a = (P_a - P_{el})/flow$). The influence of a critical closing pressure on R_a was ignored (15).

The loss of plasma fluid to the interstitium during step changes in flow or P_v was calculated for each measurement by multiplying the duration of the change in weight (sec) by the rate of plasma fluid loss to the interstitium (gm/sec) over the duration of the experiment. This rate was obtained by dividing the total weight gain of the skin flaps by the duration of the experiments. On average, the loss of plasma fluid volume to the interstitium was 0.03 ± 0.02 gm (SD). This weight gain represented 3.6 ± 3.1% of the total weight

gain for a given change in flow or P_v and was considered to be small.

STATISTICAL ANALYSIS

Data are presented as mean ± SD, unless otherwise stated. A linear regression of least square method was used to assess the relationship between step changes in P_v and changes in P_{el} and between step changes in flow and changes in P_{el}. The regression analysis was also used to determine if R_a, R_v, C_v and τ_{sk} were affected by P_{el}. The R values of these regressions analyses were 0.04, 0.09, 0.29 and 0.18 respectively and were not significant (all data were included in the regressions). There was also no statistical difference in the mean P_{el} in the different conditions at which these measurements were made. A *t*-test for independent means could therefore be used to compare the values of R_a, R_v, C_v and τ_{sk} between the two different T_c and T_s and between control and papaverine infusion. Significance was taken at $P < 0.05$. A Bonferonni correction was made for repeated tests on the same variable.

D. RESULTS

Mean arterial PO_2, PCO_2, pH, bicarbonate concentration and hematocrit values before, during and at the end of the experiment are shown in Table 3.1. By the end of the experiment there was a mild mixed metabolic acidosis and respiratory alkalosis; oxygenation was adequate.

There was a linear relationship between step changes in P_v

Table 3.1 Blood gases, bicarbonate concentration, pH, and hematocrit before, during and at the end of the experiments.

	PO_2	PCO_2	HCO_3^-	pH	Hct
BEFORE	90.1	27.6	19.2	7.4	43.3
	(12.5)	(5.8)	(1.95)		(6.9)
DURING	89.8	22.4	18.6	7.4	42.3
	(3.1)	(7.4)	(6.9)		(11.1)
END	76.0	26.2	14.2	7.3	32.1
	(15.1)	(7.1)	(2.2)		(5.0)

Values are means ± SD (numbers in parentheses are SD); n = 15 experiments. Hct, hematocrit.

and changes in P_{el} (ΔP_{el} = 1.04 ΔP_v + 0.07, R = 0.96, Fig. 3.3, upper). The relationship was also linear between changes in flow and changes in P_{el} (ΔP_{el} = 1.25 Δflow - 0.19, R = 0.89, Fig. 3.3 lower).

At a T_c of 37°C, R_a was 34.2 ± 23.0 mm Hg·min·ml^{-1}·100g^{-1}, R_v was 2.27 ± 0.81 mm Hg·min·ml^{-1}·100g^{-1}, C_v was 0.17 ± 0.06 ml·mm Hg^{-1}·100g^{-1} and τ_{sk} was 28.0 ± 8.8 sec.

INCREASE IN CORE TEMPERATURE

When the core temperature was raised to 42°C, R_a decreased by 43% to 19.5 ± 8.5 mm Hg·min·ml^{-1}·100g^{-1} (Fig. 3.4, $P < 0.05$). R_v decreased by 32% to 1.52 ± 0.62 mm Hg·min·ml^{-1}·100g^{-1} ($P < 0.05$) and C_v increased by 65% to 0.29 ± 0.13 ml·mm Hg^{-1}·100g^{-1} $P < 0.02$) so that τ_{sk} did not change significantly.

HEATING OF THE SKIN

When the flap was directly heated from 25.3 ± 3.0°C (T_c = 37°C) to 50.0 ± 1.7°C (T_c = 37°C), R_a decreased by 72% from 35.8 ± 26.8 to 10.2 ± 3.2 mm Hg·min·ml^{-1}·100g^{-1} ($P < 0.05$, Fig. 3.5), R_v decreased by 42% from 2.33 ± 0.80 to 1.35 ± 0.36 mm Hg·min·ml^{-1}·100g^{-1} ($P < 0.025$) and C_v increased by 47% from 0.19 ± 0.02 to 0.28 ± 0.03 ml·mm Hg^{-1}·100g^{-1} ($P < 0.025$). There was no change in τ_{sk}.

PAPAVERINE

With papaverine infusion R_a decreased by 66% from 19.2 ± 10.7 to 6.6 ± 3.2 mm Hg·min·ml^{-1}·100g^{-1} ($P < 0.05$, Fig. 3.6). Maximal vasodilatation was verified by the stability of P_a after giving additional doses of papaverine and by the absence

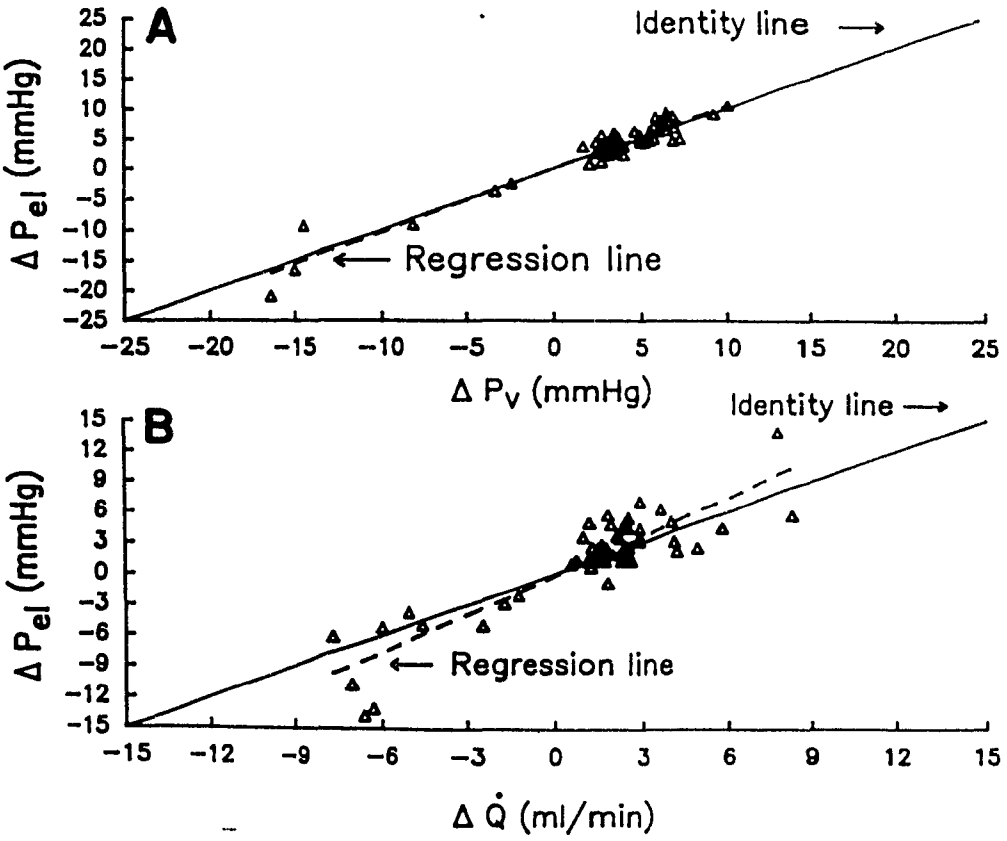

FIGURE 3.3 A: changes in elastic recoil pressure of flap vasculature (ΔP_{el}) as a function of step changes in outflow pressure (ΔP_v). Values > 0 on abscissa represent step increases in P_v whereas values < 0 represent step decreases in P_v. B: ΔP_{el} as a function of step changes in blood flow ($\dot{Q}$). Linearity of these two relationships demonstrates that, for a given change in P_v or flow vascular reflex mechanisms were minimal because P_{el} varied at a constant rate.

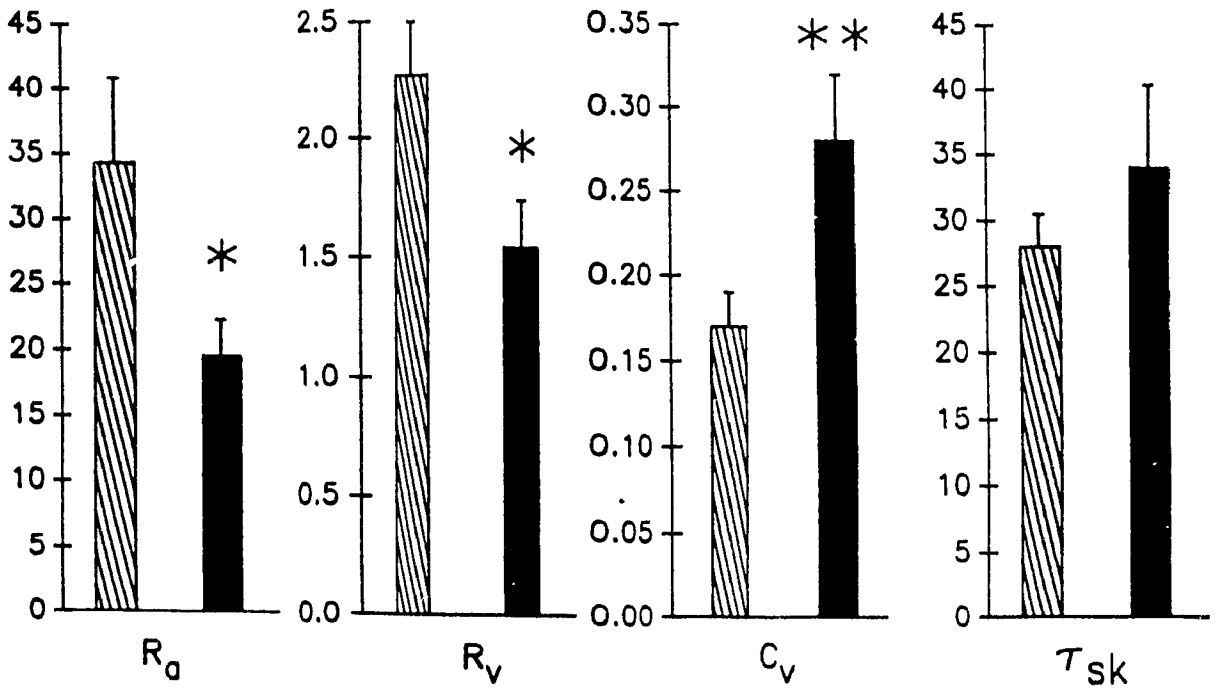

FIGURE 3.4 Bar graph of arterial resistance (R_a; mm Hg·min·ml^{-1}·100g^{-1}), venous resistance (R_v; mm Hg·min·ml^{-1}·100g^{-1}), venous compliance (C_v; ml·mm Hg^{-1}·100g^{-1}) and time constant (τ_{sk}; in sec) at core temperatures (T_c) of 37 (stippled bars) and 42°C (solid bars). * $P < 0.05$; ** $P < 0.025$. Values are means ± SE.

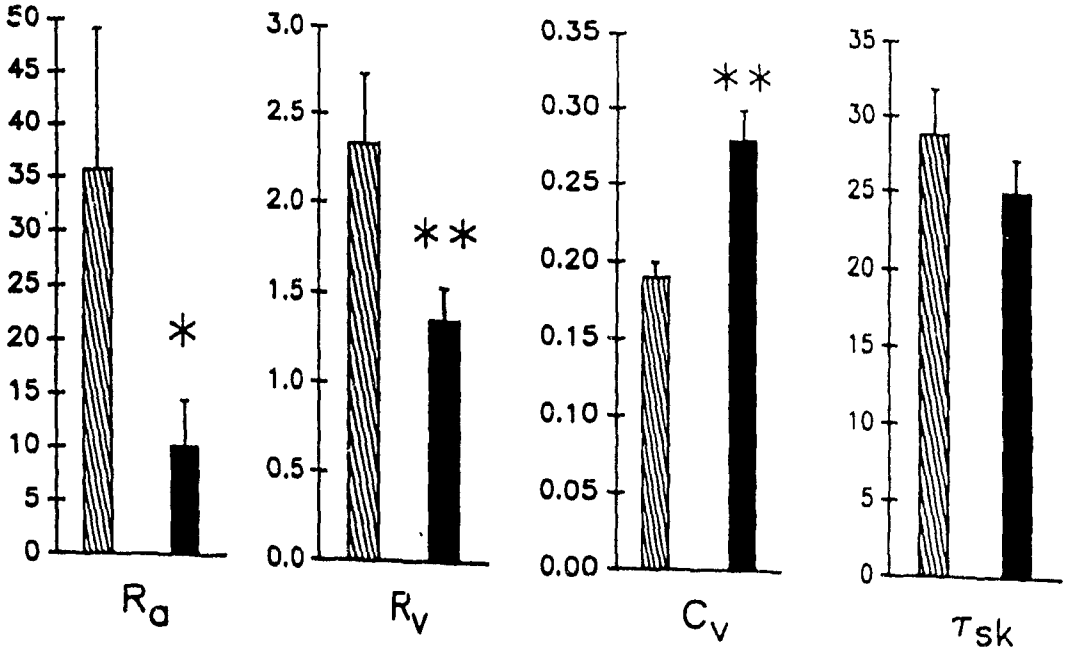

FIGURE 3.5 Bar graph of arterial resistance (R_a), venous resistance (R_v), venous compliance (C_v) and time constant (τ_{sk}) at control skin temperature (T_s) and at T_s = 51.0 ± 1.7°C. Units as in Fig. 3.4. Stippled bars, T_s = 25.5 ± 0.7°C; solid bars T_s = 50.0 ± 1.7°C. * $P < 0.05$; ** $P < 0.025$. Values are mean ± SE.

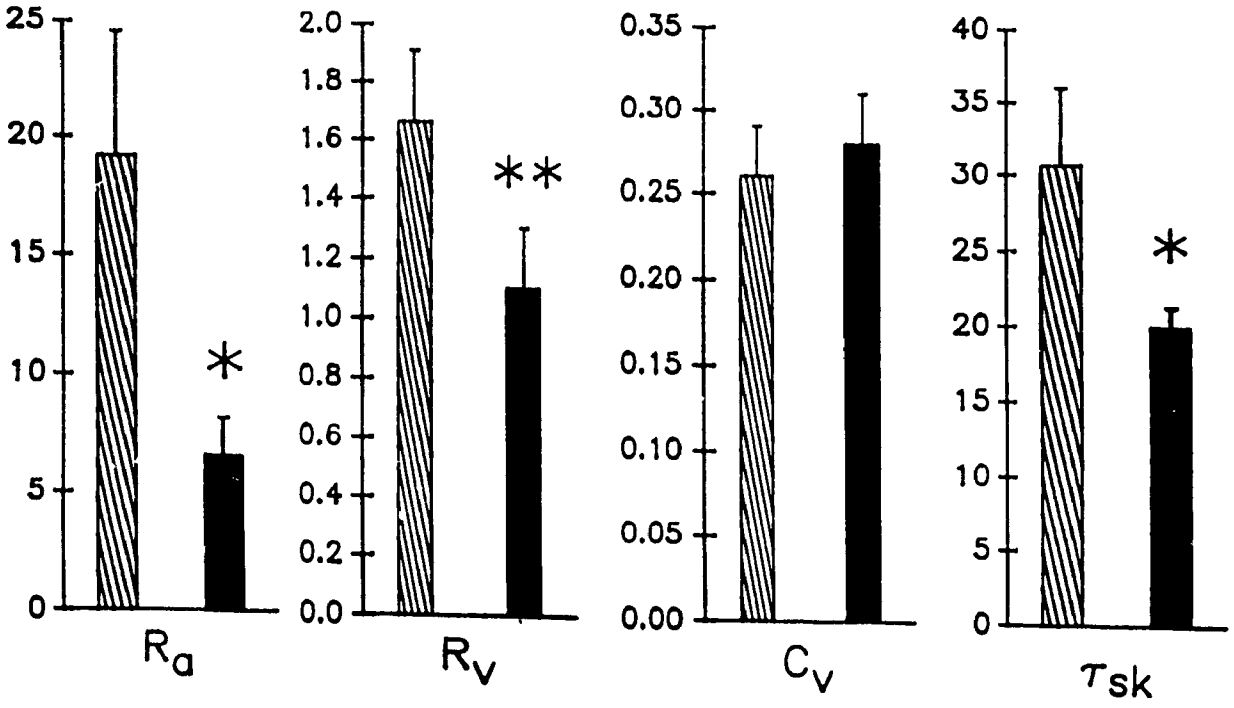

FIGURE 3.6 Bar graph arterial resistance (R_a), venous resistance (R_v), venous compliance (C_v) and time constant (τ_{sk}) during control (stippled bars) and with papaverine (solid bars) infusion. Units as in Fig. 3.4. * $P < 0.05$; ** $P < 0.025$. Values are mean ± SE.

of reactive hyperemia after occlusion of the arterial supply. R_v decreased by 34% from 1.66 ± 0.51 to 1.10 ± 0.44 mm Hg·min·ml^{-1}·100g^{-1} ($P < 0.025$) whereas C_v did not increase significantly. τ_{sk} decreased from 30.7 ± 10.6 to 20.0 ± 2.5 sec ($P < 0.05$).

CHANGES IN FLOW VERSUS CHANGES IN P_v

The time constant of the skin calculated by R_vC_v and by $\Delta V/\Delta flow$ were compared within every condition studied (Table 3.2). The values for $\Delta V/\Delta flow$ were significantly smaller than the values for R_vC_v at core temperatures of 37 and 42°C. There was no significant difference in any of the other conditions.

Venous compliance obtained by step changes in P_v and by step changes in flow were also compared for the three conditions (Table 3.3). C_v was significantly higher with step changes in flow at control and high skin temperatures. There was no significant difference in any of the other conditions.

E. DISCUSSION

This study represents the first measurements of the compliance, resistance and time constant of venous drainage of the skin vasculature. As Caldini et al. (2) suggested, the time constant of venous drainage of the skin is long (28 sec) compared to the time constant of the peripheral and splanchnic vasculature (all under 25 sec; 2,5,6,16,17). The skin of the dog has therefore the longest time constant measured to date.

Table 3.2 Time constant of venous drainage calculated as R_vC_v or as $\Delta V/\Delta flow$ at T_c of 37 and 42°C, at control and high T_s, and during control (for papaverine) and with papaverine infusion.

Condition	R_vC_v (sec)	$\Delta V/\Delta flow$ (sec)	P value
$T_c = 37^oC$	30.6 ± 8.0	17.7 ± 6.6	<0.01
$T_c = 42^oC$	36.7 ± 8.9	20.2 ± 9.3	<0.01
Control T_s	41.2 ± 16.0	16.9 ± 6.1	NS
High T_s	36.1 ± 5.3	30.1 ± 7.3	NS
Control	37.7 ± 24.3	27.8 ± 22.2	NS
Papaverine	23.5 ± 4.7	20.3 ± 7.3	NS

Values are means ± SD. Values in table do not compare with values in text, because values in table represent only half of the measurements, R_v, venous resistance; C_v, venous compliance; ΔV, change in volume; $\Delta flow$, change in flow; T_c, core temperature; T_s, skin temperature.

Table 3.3 C_v obtained by ΔP_v or Δflow at T_c of 37 and 42°C, at control and high T_s, and during control (for papaverine) and with papaverine infusion.

Condition	C_v with ΔP_v	C_v with Δflow	P value
$T_c = 37^oC$	0.16 ± 0.08	0.20 ± 0.07	NS
$T_c = 42^oC$	0.18 ± 0.11	0.27 ± 0.09	NS
Control T_s	0.13 ± 0.03	0.29 ± 0.05	<0.02
High T_s	0.15 ± 0.05	0.44 ± 0.05	<0.01
Control	0.21 ± 0.06	0.35 ± 0.14	NS
Papaverine	0.19 ± 0.10	0.36 ± 0.12	NS

Values are means ± SD. C_v, venous compliance; ml.mm Hg^{-1}. $100g^{-1}$; ΔP_v, step changes in venous pressure; Δflow, step changes in flow; T_c, core temperature; T_s, skin temperature.

METHODOLOGICAL CONSIDERATIONS

Before discussing the results, a few points concerning the methodology deserve consideration. Estimating variations in blood volume from changes in weight has a potential for error because there is a steady weight gain from the accumulation of interstitial fluid. The loss of fluid to the interstitial space for any given change in flow or P_v however was small (see method section).

An error in the estimation of the mean elastic recoil pressure of the flap could occur because the model places compliances and resistances into discrete regions whereas the compliances and resistances are actually distributed continuously. When flow stops, some arterial blood redistributes to the venous region and the plateau pressure may overestimate the pressure in the venules. At the same time, some of the blood in venules will redistribute to the larger veins and may lead to an underestimation of the plateau pressure in the compliant region. All in all, the changes in elastic recoil pressure caused by these shifts in volume should be small and should balance one another.

MEASUREMENT OF VENOUS TIME CONSTANT

Although the difference between τ_{sk} calculated by $\Delta V/\Delta flow$ and by R_vC_v was significant only at core temperatures of 37 and 42°C, the value of τ_{sk} from $\Delta V/\Delta flow$ was lower than that with R_vC_v whenever they could be compared (Table 3.2). To explain this, one must first realize that $\Delta V/\Delta flow$ is a dynamic

measurement of the time constant whereas R_vC_v is a static measurement. Hence, for similar changes in volume, a loss of volume to the interstitial space after an increase in flow would not affect $\Delta V/\Delta flow$ but would affect the measurement of P_{el} and consequently the τ_v calculated from the product R_vC_v. As explained previously, this effect should be small. Second, step increases in flow resulted in a decrease in R_v. From the equation for venous resistance, this means that after an increase in flow, the difference between P_{el} and P_v is proportionally smaller than the difference in flow. Since P_v does not change with increases in flow, it is P_{el} which does not increase proportionally with flow. When this non-proportional increase in P_{el} is applied to the equation for venous compliance, $\Delta V/\Delta P_{el}$, it results in a larger C_v for a given change in flow. This, added to the fact that mean R_v was used in the product R_vC_v, results in a value of τ_{sk} from $\Delta V/\Delta flow$ which is smaller than the value from R_vC_v. The effect of the decrease in R_v on the venous compliance is the dominant factor in the equation. The value of τ_{sk} determined by R_vC_v therefore reflects more precisely the mechanical changes in the vascular bed produced by a change in flow because it takes into account small variations in venous resistance and volume transfers to the interstitium.

Another interesting and closely related observation was that C_v measured from step changes in P_v were usually smaller than those obtained from step changes in flow. This

difference was significant only at control and high skin temperatures but a trend could be seen in all conditions (Table 3.3). As mentioned previously, step increases in flow generally resulted in a decrease in R_v. With step changes in P_v, however, the direction of changes in R_v varied greatly [R_v increased or was maintained constant in 60% of the cases with increases in P_v (n = 55)]. Accordingly, changes in plateau pressure either exactly matched, were greater, or were lower than changes in P_v. Since changes in P_{el} were the same or greater than changes in P_v in 60% of the cases, and since the reverse was true for step changes in flow, C_v for a given change in volume tended to be smaller for step changes in P_v than for changes in flow. The variation in the direction of change in R_v with changes in venous pressure explains why this difference is not statistically different in all cases (Table 3.3). A possible explanation for the decrease in R_v with increases in flow is that recruitment of capillary vessels occurs with step increases in flow because of the increase in vascular pressures.

C_v OF OTHER REGIONS

Since the C_v of the skin has not been measured before, we can only compare this value to the C_v of other regions and of the whole systemic circulation. Of note, the values for total systemic, splanchnic and peripheral compliances are usually normalized per total weight of animal rather than per weight of tissue. The venous compliance of the skin obtained from

the average of all conditions studied (2.4 ml.mm Hg^{-1}·kg of $skin^{-1}$) is larger than the compliance of the total systemic vasculature (<1.35 ml.mm $Hg^{-1}.kg^{-1}$) (1,16), of the splanchnic vasculature (<1.26 ml.mm $Hg^{-1}.kg^{-1}$) (1,5,6,16,17), and of the peripheral vasculature (<1.21 ml.mm $Hg^{-1}.kg^{-1}$) (1,5,6,17) in dogs. It is comparable to the systemic compliance in dogs in one study (2.0 ml.mm $Hg^{-1}.kg^{-1}$) (24) and to the compliance of the dog intestine (2.67 ml.mm Hg^{-1}·kg of $intestine^{-1}$) (18). C_v of the skin was smaller than the total venous compliance of conscious rats (3.13 ml.mm $Hg^{-1}.kg^{-1}$) (22) and of the cat liver (26 ml.mm Hg^{-1}·kg of $liver^{-1}$) (7).

Changes in vascular compliance and resistance with temperature variations may not be limited to the skin. Green and Jackman (6), found a decrease in venous splanchnic and peripheral compliances with hypothermia in dogs. In association with the decrease in C_v there was an increase in R_v in the splanchnic bed but not in the periphery. These variations in C_v and R_v of the splanchnic region with hypothermia mirror very well the events occurring in the skin.

EFFECTS OF CENTRAL versus LOCAL HEATING

Heat is sensed both centrally by spinal or hypothalamic receptors (4) and peripherally through skin sensors (23). We stimulated central receptors by heating the blood perfusing the entire animal at constant T_s and the peripheral receptors were stimulated by heating the skin flap at constant T_c. A possibly confounding factor could have occurred if the heated

blood, which perfused the entire animal at high T_c, had increased the temperature of the blood in the skin flap to a greater extent than with local heating of the flap. However, the temperature of the blood perfusing the skin at high T_c was the same as with skin surface heating (i.e. $\approx$ 36°C). The difference between surface and central heating could therefore be compared.

Although the decrease in R_a and R_v and increase in C_v appeared to be greater with skin heating than with heating of the core, these differences were not statistically significant. Central and peripheral heating were therefore considered to have similar effects on arterial resistance, C_v and R_v. Evidence from Webb-Peploe et. al (26) suggests that changes in venous tone caused by central or peripheral temperature sensors may be additive.

MAXIMAL SKIN BLOOD FLOW

The total resistance (R_t) during maximal vasodilatation with papaverine was used to estimate the maximal blood flow achievable at an arterial pressure of 90 mm Hg. With R_t of 7 mm Hg$\cdot$min$\cdot$ml$^{-1}\cdot$100g^{-1} blood flow was 13 ml$\cdot$min$^{-1}\cdot$100g^{-1} which agrees well with the value obtained by Hales and Dampney (11) in the skin of the legs of heat stressed Greyhound dogs.

BLOOD VOLUME ACCUMULATION WITH CHANGES IN TEMPERATURE

The volume of blood that accumulates in a vascular region with increases in flow depends on the venous time constant (i.e. $\Delta V = \Delta flow \cdot \tau_v$) and on the size of the vascular bed.

Hales and Dampney (11) found that the total skin blood flow in conscious Greyhound dogs under heat stress was approximately 144 $ml \cdot min^{-1}$ or 1.8% of peak cardiac output. Using their value for skin blood flow at rest (62 $ml \cdot min^{-1}$) and the τ_{sk} obtained in the present study at high T_s (assuming a τ_{sk} of 24.9 sec throughout the skin), the total amount of blood that would accumulate in the skin for a change in blood flow of 82 $ml \cdot min^{-1}$ is 33.6 ml, a quantity of blood too small to challenge normal circulatory homeostasis. If the same analysis is used to estimate the accumulation of blood in the human skin during heat stress however, the results are staggering.

Rowell et al. (20) estimated the maximum skin blood flow in heated resting man to be as much as 7-8 $l \cdot min^{-1}$. If we assume that the total mass of skin is 2 kg (19), the blood flow would be 350 to 400 $ml \cdot min^{-1}$ per 100g of skin. In a thermoneutral environment total skin blood flow is 350 $ml \cdot min^{-1}$ (19). Using a τ_{sk} of 24.9 sec and an increase in skin blood flow to 8 $l \cdot min^{-1}$, 3.2 l of blood would accumulate in the skin of maximally vasodilated men, a value too large to allow the cardiac output of 13 $l \cdot min^{-1}$ observed by Rowell and coworkers in heat stressed subjects.

Possible explanations are that the τ_{sk} of human skin is much lower than the dog's under normal conditions, or that τ_{sk} of humans decreases markedly during heat stress. Since we cannot measure the time constant of the skin in humans, some of the possible mechanism by which τ_{sk} can decrease during heat

stress will be explored.

In order to maintain an increase in skin blood flow of 7.6 $l \cdot min^{-1}$ and an accumulation of blood in the skin of 1.5 l at a cardiac output of 13 $l \cdot min^{-1}$, human τ_{sk} would have to decrease to less than 12 sec. This decrease in τ_{sk} could hypothetically be obtained by diverting flow through arteriovenous anastomoses (AVA) which are thought to be important in heat transfer (21). The diversion of blood flow to AVA's during heat stress differs between species. Hales and colleagues estimated AVA flow to be 6% in Greyhounds (11), 8.6% in baboons (12) and 30% in sheep (9). Even if we apply the larger flow through the AVA's of the sheep to man, there would still be an accumulation of 2.3 l of blood in the skin during heat stress. Furthermore, skin AVA's in humans are thought to be prominent only in acral regions (19). It therefore seems unlikely that diversion of blood through AVA's could decrease human skin τ_{sk} sufficiently to allow an increase in blood flow of 7.6 $l \cdot min^{-1}$.

Another possible explanation is that skin blood flow is overestimated in humans during heat stress. Detry et al. (3) measured a decrease in forearm muscle blood flow in men subjected to heat stress. They applied this decrease to all muscles and added it with the decrease in blood flow to visceral organs and with the increase in cardiac output during body heating to calculate a skin blood flow of 7-10 $l \cdot min^{-1}$. However, a 1.4 to 2.4 fold increase in blood flow to non-

respiratory muscles has been shown by Hales (10) in sheep under severe heat stress. Furthermore, Henriksen et al. (13) measured forearm muscle blood flow in humans using Xenon-133 clearance and found increases in flow during heat stress. Therefore some of the large increases in cardiac output calculated by Rowell (20) may have gone to muscle and not just to the skin.

Henriksen et al. (13) estimated the maximal skin and subcutaneous tissue blood flow to be close to 80 $ml \cdot min^{-1}$ per 100 g which would result in an accumulation of around 550 ml of blood.

In summary, the vascular bed of the skin of dogs is characterized by a venous compliance as large as that of the intestine and a time constant of venous drainage which is the longest of any organ measured to date. If human skin has similar characteristics, the skin could be a major blood reservoir during heat stress and large increases in skin blood flow could compromise maximum venous return and therefore maximum cardiac output.

F. REFERENCES

1. Brunner, J. M., A. A. Shoukas, and C. L. MacAnespie. The effect of the carotid sinus baroreceptor reflex on blood flow and volume redistribution in the total systemic vascular bed of the dog. *Circ Res*. 48:274-285, 1981.

2. Caldini, P., S. Permutt, J. A. Waddell, and R. L. Riley. Effect of epinephrine on pressure, flow, and volume relationships in the systemic circulation of dogs. *Circ Res*. 34:606-623, 1974.

3. Detry, J-M. R., G. L. Brengelmann, L. B. Rowell, and C. Wyss. Skin and muscle components of forearm blood flow in directly heated resting man. *J. Appl. Physiol*. 32(4):506-511, 1972.

4. Gerber, R. L., F. W. Klussmann, and H. C. Heller. Preoptic/anterior Hypothalamic neural responses to thermal stimulation of the hypothalamus and spinal cord in unanesthetized unrestrained rabbits. In: *Thermal Physiology*. Edited by J.R.S. Hales, N.Y.: Raven Press, 1984, p. 83.

5. Green, J. F. Mechanism of action of isoproterenol on venous return. *Am J Physiol*. 232(2):H152-H156, 1977.

6. Green, J. F. and A. P. Jackman. Mechanism of the increased vascular capacity produced by mild perfusion hypothermia in the dog. *Circ Res*. 44:411-419, 1979.

7. Greenway, C. V., K. L. Seaman, and I. R. Innes. Norepinephrine on venous compliance and unstressed volume in cat liver. Am J Physiol. 248(17):H468-H476, 1985.

8. Guyton, A. C., G. G. Armstrong, and P. L. Chipley. Pressure volume curves of the arterial and venous systems in live dogs. *Am J Physiol*. 184:253-258, 1956.

9. Hales, J. R. S. Effect of exposure to hot environments on the regional distribution of blood flow and on cardiorespiratory function in sheep. *Pflügers Arch*. 344:133-148, 1973.

10. Hales, J. R. S., L. B. Rowell, and R. B. King. Regional redistribution of blood flow in awake heat stressed baboons. *Am J Physiol*. 237(6):H705-H712, 1979.

11. Hales, J. R. S. and R. A. L. Dampney. The redistribution of cardiac output in the dog during heat stress. *J Therm Biol*. 1:29-34, 1975.

12. Hales, J. R. S. Effects of heat stress on blood flow in respiratory and non-respiratory muscles in the sheep. *Pflügers Arch.* 345:123-130, 1973.

13. Henriksen, O., J. Bülow, J. K. Kristensen, and N. A. Lassen. Local tissue temperature: an important factor for the regulation of blood flow in peripheral tissues during indirectly induced hyperthermia. In: *Thermal Physiology*, editor J.R.S. Hales, N.Y.: Raven Press, 1984, pp. 255-258.

14. Magder, S. The vascular mechanics of venous drainage in the dog hindlimb. *Am J Physiol.* In press.

15. Magder, S. Starling resistor versus compliance: which explains the zero-flow pressure of a dynamic arterial pressure flow relation. *Circ. Res.* 67:209-220, 1990.

16. Malo, J., H. Goldberg, R. Graham, H. Unruh, and C. Skoog. Effect of hypoxic hypoxia on systemic vasculature. J Appl Physiol. 56(5):1403-1410, 1984.

17. Mitzner, W. and H. Goldberg. Effect of epinephrine of resistive and compliant properties of the canine vasculature. *J Appl Physiol.* 39(2):272-280, 1975.

18. Rothe, C. F., B. L. Johns, and T. B. Bennett. Vascular capacitance of dog intestine using mean transit time of indicator. *Am J Physiol.* 234(1):H7-H13, 1978.

19. Rowell, L. B. *Human circulation. Regulation during physical stress.* New York, N.Y.: Oxford University Press, 1986.

20. Rowell, L. B., G. L. Brengelmann, and J. A. Murray. Cardiovascular response to sustained high skin temperature in resting man. *J Appl Physiol.* 27(5):673-680, 1969.

21. Rübsamen, K. and J. R. S. Hales. Role of arteriovenous anastomoses in determining heat transfer across the hindleg skin of sheep. In: *Thermal Physiology*. Edited by J.R.S. Hales, N.Y.: Raven Press, 1984, p. 259.

22. Samar, R. E., and T. G. Coleman. Mean circulatory pressure and vascular compliances in the spontaneously hypertensive rat. *Am J Physiol.* 237(5):H584-H589, 1979.

23. Schäfer, K., H. A. Braun, and H. Hensel. Temperature transduction in the skin. In: *Thermal Physiology*. Edited by J.R.S. Hales, N.Y.: Raven Press,1984, p. 1.

24. Shoukas, A. A., and K. Sagawa. Control of total systemic vascular capacity by the carotid sinus baroreceptor reflex. *Circ Res*. 33:22-33, 1973.

25. Townsley, M. I., R. J. Korthuis, B. Rippe, J. C. Parker, and A. E. Taylor. Validation of double vascular occlusion method for Pc,i in lung and skeletal muscle. *J Appl Physiol*. 61(1):127-132, 1986.

26. Webb-Peploe, M. M., and J.T. Shepherd. Response of dogs' cutaneous veins to local and central temperature changes. *Circ. Res*. 23:693-699, 1968.

CHAPTER 4

MEASUREMENT OF PERIPHERAL AND SPLANCHNIC BLOOD VOLUME IN INTACT ANESTHETIZED DOGS

A. ABSTRACT

We developed an experimental method which enables repeated measurements of blood volume in the splanchnic and extrasplanchnic beds in intact anesthetized dogs on circulatory bypass. Regional volumes are calculated from the product of flow and mean transit time obtained with first pass dilution curves of indocyanine green. Independent flows from electromagnetic flow meters are used to validate the flows from the dilution curves and to calculate the uptake of indicator by tissues. The uptake was less than 5%. Applications of our method to the study of the determinants of venous return are also discussed.

B. INTRODUCTION

The importance of the splanchnic vascular bed as a blood reservoir which can be used to assist cardiovascular homeostasis is well recognized (8,16). Although assessments of splanchnic volume under a variety of physiological conditions could greatly help our understanding of the role of capacitance vessels in the maintenance of cardiac output, such measurements have proved to be difficult to obtain. Some splanchnic blood volume measurements have been made in men and dogs (5,13), but it has not been feasible to obtain repeated measurements of total splanchnic and extrasplanchnic blood volumes in a preparation in which physiological conditions can be altered at will. We present a method which allows repetitive measurements of total splanchnic and extrasplanchnic volumes in intact anesthetized dogs.

C. METHODS

Surgical Procedures

Nineteen mongrel dogs of either sex with a mean weight of 29.6 ± 3.5 kg (SD) were anesthetized with either 25 $mg \cdot kg^{-1}$ of pentobarbital sodium (n = 9) or 100 $mg \cdot kg^{-1}$ of α-chloralose (n = 10); additional doses were administered as necessary. An endotracheal intubation was performed and the dogs were ventilated at an appropriate tidal volume and frequency for their size. After performing a ventral laparotomy, an external circulatory circuit was achieved as follows (Fig. 4.1). The superior vena cava (SVC), the inferior vena cava,

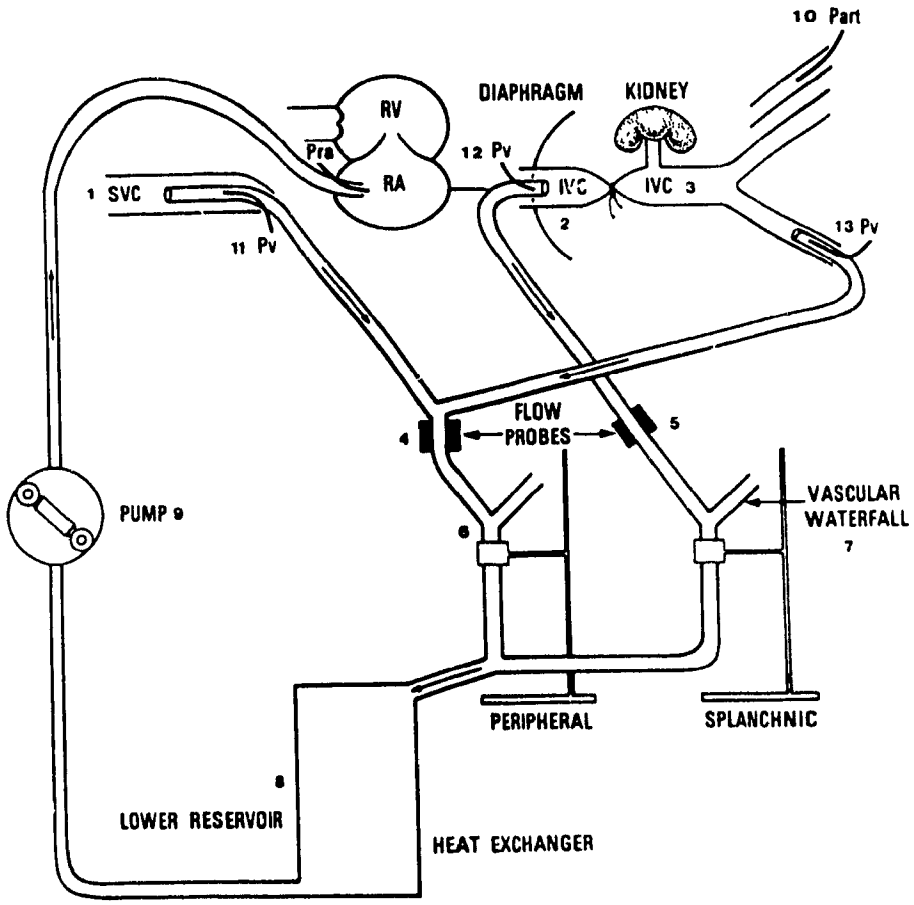

FIGURE 4.1 Experimental preparation. 1, SVC, superior vena cava. 2, IVC, inferior vena cava (above kidneys). 3, IVC (below kidneys). 6 and 7, venous outflows. 10, Part, arterial pressure. 11, 12 and 13, Pv, venous pressure. See text for details.

above the diaphragm (splanchnic bed, SPL), and the inferior vena cava above the bifurcation into the iliac veins (IVC) were cannulated with 3/8" polyvinyl catheters. The SPL and IVC regions were separated by tying an umbilical tape around the inferior vena cava just above the renal veins. The venous outflows from the SVC and IVC were combined and called the peripheral (PER) outflow. The azygous vein was ligated. PER and SPL venous outflows drained by gravity through separate polyvinyl catheters and electromagnetic flowmeters (Carolina Medical). The blood then passed through separate Y connectors and collected in a common reservoir. The Y connectors had one arm open to atmosphere to create a waterfall, and their height determined venous outflow pressures. A roller pump directed the blood from the reservoir through a heat exchanger and a filter into the right atrium. This circuit was primed with 1.5 to 2.5 litres of blood from a donor dog. The pump was adjusted to maintain constant flow. The left femoral and carotid arteries were cannulated and arterial pressure could be obtained from either site. Twenty to 25 minutes was allowed to assure that the reservoir volume stayed relatively constant and that the preparation was stable.

Regional Blood Volume Measurement

Mean transit times (17) obtained from indicator dilution curves, were used to determine regional blood volumes. The inflows to the SVC, IVC and SPL regions were sequentially and temporarily isolated to permit the measurement of regional blood volume. When a region was isolated, the speed of the

pump was reduced to maintain arterial pressure and flow at the same level as before the occlusion. Direct inspection of the tracings was used to achieve this.

Blood flow to the SVC region was isolated by occluding the descending aorta below the left subclavian artery and the venous outflow of the two other regions. Enough time was allowed for the pressure and flow to equilibrate. The indicator (Cardiogreen, Beauty Creations, Missassagua, CAN) was injected in the ascending aorta, through a catheter inserted in the left carotid artery. A blood sample was then collected from the venous outflow at a rate of 20 $ml.min^{-1}$ and passed through a densitometer cuvette (DC-410, Waters Instruments, Minnesota, USA). The densitometer signals were passed through an analog to digital converter and the data was stored on a personal computer for later analysis.

Blood flow to the SPL region was isolated by occluding the right carotid artery and the venous outflow of the two other beds. The venous pressures in SVC and IVC increased to the level of the arterial pressure. The indicator was injected in the ascending aorta and a blood sample was collected as previously described. Efforts to further isolate the SPL region by occluding the abdominal aorta (just above the kidneys) as well as injections of the indicator in the thoracic aorta did not significantly alter the SPL volume measurement and were abandoned.

Blood flow to the IVC region was isolated by occluding the right carotid artery and the venous outflow of the other

regions. The indicator was injected in the abdominal aorta, just above the kidneys and a blood sample was collected as previously described.

Between blood volume measurements, all occluders were released and the pump speed was returned to the initial level. Steady state values of flows and pressures were established before the next measurement was made.

In two additional dogs, SPL blood flow was isolated and the indicator was injected as previously described. However, instead of permitting recirculation, all blood leaving the splanchnic bed was collected. The volume of the collected blood was measured and, after mixing, a sample of the blood was passed through the densitometer to measure the indicator concentration. The total amount of indicator recovered could then be calculated from the product of the concentration and volume collected. In one of these animals, the first blood collection totally emptied the reservoir and only one measurement could be made. In the other animal, three measurements were obtained before the reservoir emptied.

The product of the mean transit time (MTT) and blood flow from the indicator dilution curve (Q_D) was used to calculated regional blood volumes (Fig. 4.2) (17). Blood flow was obtained with the equation $Q_D = (amt \cdot 60)/(K \cdot \int cdt)$, where amt is the amount of indicator injected, K is the calibration constant of indicator concentration and $\int cdt$ is the area under the indicator dilution curve (corrected for recirculation, see below). MTT was obtained with the equation, $MTT = \int tcdt/\int cdt$,

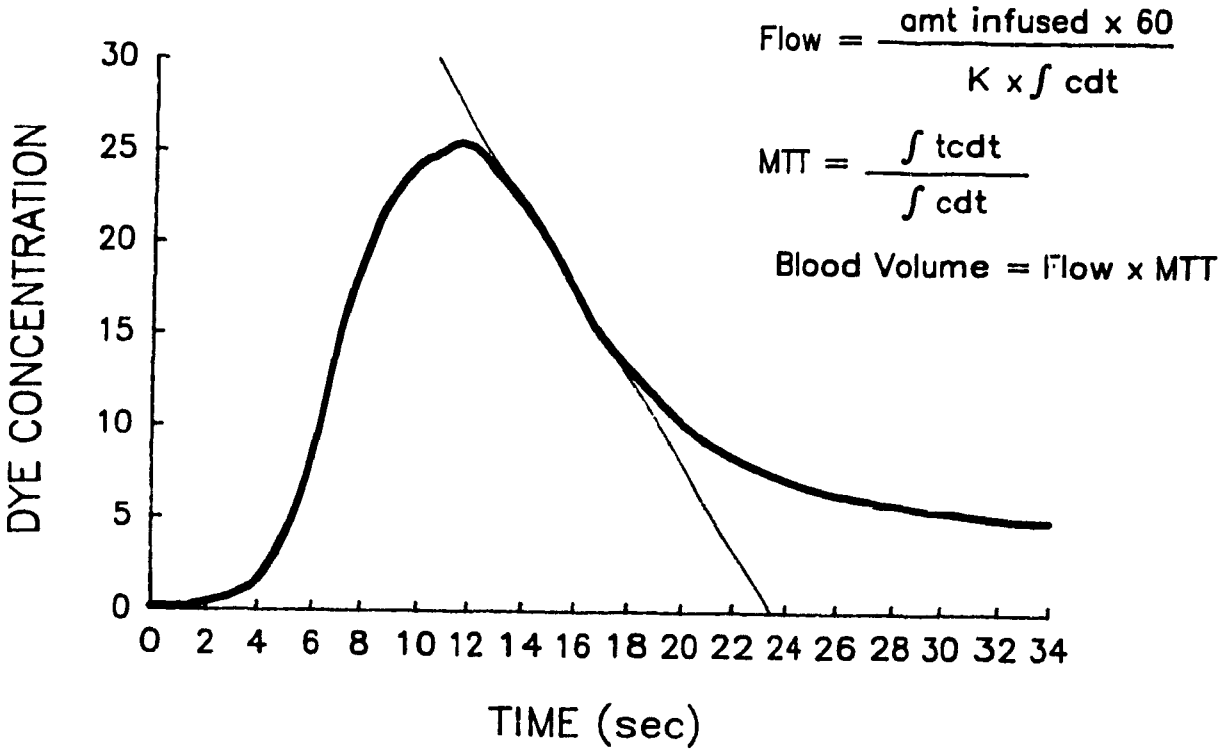

FIGURE 4.2 Indicator dilution curve. Example of the time course of indicator concentration used for the measurement of regional blood volumes. The straight thin line represents an extrapolation to zero of the linear portion of the descending curve. Also shown are the equations for flow (Q_D), mean transit time (MTT) and blood volume. Abbreviations: amt is the amount of indicator injected; c is the concentration or indicator; K is the constant of indicator concentration; t is time.

where $\int tcdt$ is the area under the curve of time multiplied by the indicator concentration over time. Recirculation of the indicator was corrected by extrapolating to zero the linear portion of the logarithmic down slope of the indicator concentration versus time (17).

Analysis

If the equation for flow is rearranged to amt = $Q_D \cdot K \cdot \int cdt/60$ and if the independent signal from the flow meters (Q_p) is substituted for Q_D, the amount of indicator recovered for any given indicator dilution curve can be calculated. The difference between the amount recovered and the amount injected then becomes an estimate of the loss of indicator to the tissues and to hemorrhage. As mentioned previously, in two additional dogs the total amount of indicator recovered during blood collections from the splanchnic bed were compared to the amount injected. Finally, a linear regression of least square method was used to assess the identity plot between Q_D and Q_p. Data are presented as means ± standard deviations unless otherwise specified.

D. RESULTS

A total of 58 regional blood volume measurements were made, 25 in SVC, 9 in IVC and 24 in SPL. The amount of indicator injected was either 1, 1.5, 2, 3 or 3.5 mg.

In the SVC region, the average blood volume was 13.6 ± 4.5 $ml \cdot kg^{-1}$. One injection of 2.5 mg of indicator was given and the calculated amount recovered was 2.44 mg or 97.6%. Twenty

four injections of 1 mg of indicator were given and the calculated amount recovered was 0.96 ± 0.1 mg. The slope for the regression analysis of the identity plot for Q_D against Q_P was 1.01 and the r value was 0.98 (Fig. 4.3).

In the IVC region, the blood volume was 18.6 ± 8.7 $ml \cdot kg^{-1}$. One injection of 2.5 mg of indicator was given and the calculated amount recovered was 2.41 mg or 96.4%. Two injections of 1.5 mg were given and the mean estimated recovery was 1.5 ± 0.03 mg. Six injections of 1 mg were given and the calculated amount recovered was 0.96 ± 0.17 mg. The slope of the regression analysis between Q_D and Q_P was 0.93 and the r value was 0.97 (Fig. 4.3).

In the SPL region, the average blood volume was 27.0 ± 9.9 $ml \cdot kg^{-1}$. One injection each of 3.5 and 2 mg of indicator were given and the calculated amounts recovered were 3.2 and 1.9 mg respectively. Eighteen injections of 1.5 mg were used and the calculated recovery was 1.44 ± 0.15 mg. Four injections of 1 mg of indicator resulted in a calculated recovery of 0.95 ± 0.09 mg. The regression analysis of Q_D over Q_P gave a slope of 1.04 and a r of 0.99 (Fig. 4.3).

Reproducibility of the volume measurements in the same animal for the SVC and SPL region was assessed in four dogs and the values are shown in Table 4.1. The measurements of SVC and SPL blood volumes were generally reproducible.

When all of the blood draining the SPL bed was collected and the concentration of indicator was measured, the amounts of indicator recovered from injections of 1.5, 1.5, 2, and 3

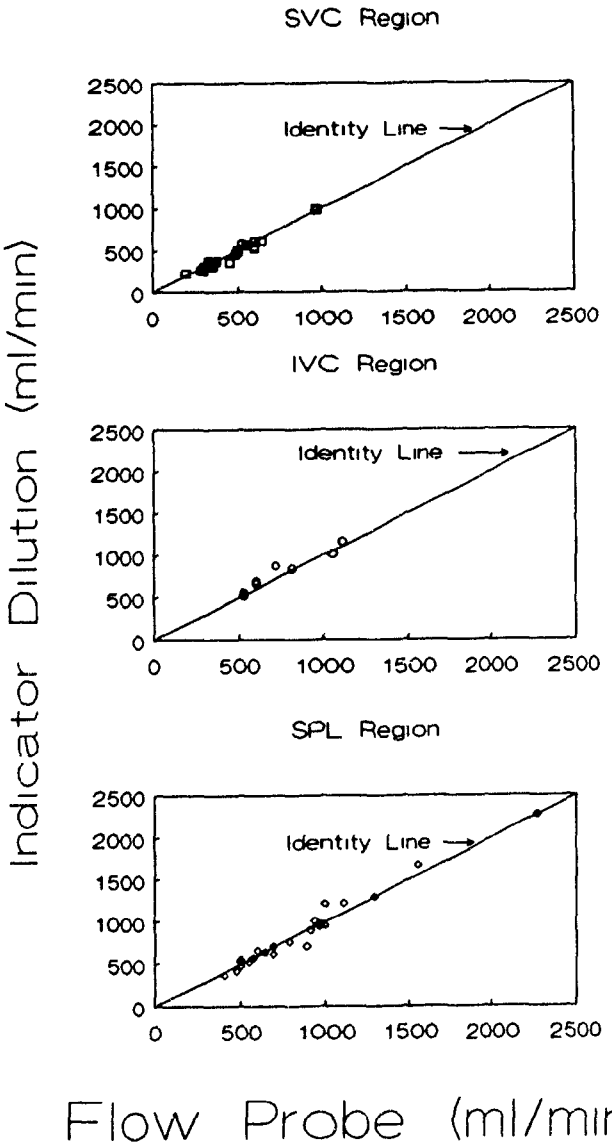

FIGURE 4.3 Comparison between flows from indicator dilution and from flowmeters. The three graphs represent the identity plots of the flows obtained with electromagnetic flowmeters (abscissa) and the flows calculated from the indicator dilution curves (ordinate) for the blood volume measurements in the SVC region (top), IVC region (middle), and SPL region (bottom).

Table 4.1 Repeated measurements of splanchnic and upper peripheral volumes.

DOG	SPL Volumes (ml)	SVC Volumes (ml)
1	611.8	323.2
	502.6	360.8
2	1007.0	-----
	1076.8	-----
3	670.9	279.4
	690.8	342.1
	668.2	396.3
	579.4	355.2
	604.9	-----
4	-----	322.9
	-----	323.4

SPL, splanchnic bed; SVC, superior vena caval bed.

mg were 1.5, 1.43, 1.85 and 2.98 mg respectively.

In the animals in which the blood volumes in all three regions were measured (n = 9), the sum of these volumes was 62.1 ± 15.5 $ml \cdot kg^{-1}$.

E. DISCUSSION

Blood volumes in the central compartment of the circulation (7,11) and in isolated organs (1-3,14) have been measured previously from the product of mean transit time and blood flow. However, this technique has not been used to measure major peripheral regions with an intact arterial system. The purpose of the present study was to develop a reliable technique for repeated measurements of splanchnic and peripheral blood volumes in intact anesthetized dogs. Using a bolus injection of indocyanine green (ICG), first pass indicator dilution curves were obtained from which blood flows, mean transit times and blood volumes could be calculated. As a validating measure, the calculated blood flows were compared to measured blood flows from electromagnetic flow probes. A number of theoretical considerations must be met to keep the variability and error of the volume measurements within an acceptable range.

1) ***There must be little or no loss of indicator.*** If ICG is taken up by the tissues, or, if some of the indicator is lost through hemorrhage, the area under the dilution curve will be reduced because not all of the indicator will leave the region. From the equation for Q_D, a decrease in the

denominator for a given amount of indicator injected, results in an overestimation of the flow. But, the flows calculated from the dye curves were compared to the independent flow measurements and were found to be similar (Fig. 4.3). Moreover, Q_p was used to quantify the total amount of indicator lost during each volume measurement. By substituting Q_p for Q_D in the equation, and solving for the amount of indicator injected, more than 95% of the indicator was calculated to be recovered. These values were confirmed by collecting blood from the splanchnic region after injections of indicator.

Uptake of ICG by the liver has been extensively used to estimate hepatic blood flow and hepatic function (4,6,10,12). Consequently, the use of indicator dilution curves to estimate splanchnic flows and volumes may, at first, seem unsuitable. However, the relatively small uptake of ICG in our first pass technique may be explained by the fact that the doses of indicator used are 20 to 100 times smaller than the doses used by the traditional methods. Consequently, most of the ICG in plasma will be bound to protein and little will be available for uptake by the liver. Nevertheless, the loss of indicator is probably the major source of variability in the volume measurements, but it was found to be less than 5%.

2) ***The region must have a single inflow or single outflow.*** In the present preparation, a single outflow site for all three regions was guaranteed by occluding the venous outflows of the two other beds.

3) ***There must be adequate mixing.*** Rapid injections (< 1 sec) of the indicator were made in the proximal aorta and in the abdominal aorta where flows and turbulence are high.

4) ***Flow through the region must be constant.*** Flow was constantly monitored and the volume measurement was made only when flow was steady. To insure that inflow and outflow through the region was constant, a steady reservoir volume was also required before the measurements were made.

Another concern in this study is that increases and decreases in regional volumes through physiological stimuli could change the rate of uptake of indocyanine green and invalidate comparison between volume measurements. Greenway et al. (9) studied indocyanine green uptake with changes in hepatic blood volume in the cat. These authors concluded that the effects of large changes in hepatic blood volume on indocyanine green uptake were small and that uptake kinetics are independent of hepatic blood volume.

A comparison of the sum of the volumes with other published data of total blood volume also supports the validity of the measurements. Since we did not measure the volume in the central compartment, we assumed that the heart and lungs contain 20% of total blood volume. When this volume is added to the sum of the blood volumes in SVC, IVC and SPL, total blood volume equals 77.6 ± 19.2 $ml \cdot kg^{-1}$. This value correlates well with the value for total blood volume of 77 $ml \cdot kg^{-1}$ obtained by Rothe et al. using ^{51}Cr-labelled erythrocytes and ^{125}I-labelled albumin (15).

Only 9 measurements of blood volume were made in the IVC region because these measurements resulted in a rapid deterioration of the preparation and a congestion of the splanchnic region. The reason for these difficulties is that it is impossible to isolate the inflow to the IVC region from the inflow to the SPL region. As described in METHODS, in order to isolate the inflow to the IVC, the outflows of both the SVC and the SPL regions are occluded. To reduce the inflow and the pressure in the SVC region, the right carotid artery is also occluded. The inflow to the SPL region however cannot be reduced and, in order to maintain constant inflow and blood pressure in the IVC, the splanchnic bed must fill with blood until the pressures equilibrate. This greatly reduces reservoir volume and produces congestion in the SPL region. After the volume measurement and the release of the SPL outflow, a long time is needed for the reservoir volume to return to initial volume and for a steady state to be reached. Such difficulties do not occur for the measurement of the volume in the SPL region because the IVC is a non-compliant region and the pressure equilibrates readily when the IVC outflow is occluded.

The main advantage of our method is that it allows repeated measurements of regional blood volumes under varying physiological conditions. With animals under circulatory bypass, regional pressure volume curves can be constructed which permit assessments of regional unstressed volume. In a closed system, a change in unstressed volume results in a

reciprocal change in stressed volume. Since stressed volume is a major determinant of venous return, the technique can be used to quantify changes in venous return under different physiological conditions.

In conclusion, we have developed a reliable technique which permits repeated measurements of blood volume in the splanchnic and extrasplanchnic beds and which can be used to assess the response of regional capacitance vessels to alterations in cardiovascular homeostasis.

F. REFERENCES

1. ANDRES, R., K.L. ZIERLER, H.M. ANDERSON, W.N. STAINSBY, G. CADER, A.S. GHRAYYIB, AND J.L.,JR. LILIENTHAL. Measurement of blood flow and volume in the forearm of man; with notes on the theory of indicator-dilution and on production of turbulence, hemolysis, and vasodilatation by intra-vascular injection. *J. Clin. Invest.* 33: 482, 1954.

2. BAKER, C.H. Vascular volume changes resulting from vasodilatation of the dog forelimb. *Am. J. Physiol.* 209(1): 60-64, 1965.

3. BAKER, C.H. Blood flow and volume distribution with elevated venous pressure. *Am. J. Physiol.* 218(3): 674-680, 1970.

4. BANASZAK, E.F., W.J. WILLIAM, J. STEKEIL, R.A. GRACE, AND J.J. SMITH. Estimation of hepatic blood flow using a single injection of dye clearance. *Am. J. Physiol.* 138(4): 877-880, 1960.

5. BRADLEY, S.F., P.A. MARKS, P.C. REYNELL, AND J. MELTZER. The circulating splanchnic blood volume in dog and man. *Trans. Assoc. Am. Physicians* 66: 294-302, 1953.

6. BURCZYNSKI, F.J., K.L. PUSHKA, D.S. SITAR, AND C.V. GREENWAY. Hepatic plasma flow: accuracy of estimation from bolus injections of indocyanine green. *Am. J. Physiol.* 252: H953-H962, 1987.

7. DOW, P. Estimations of cardiac output and central blood volume by dye dilution. *Physiol. Rev.* 36: 1956, 1956.

8. GREENWAY, C.V. Role of splanchnic venous system in overall cardiovascular homeostasis. *Federation Proc.* 42: 1678-1684, 1983.

9. GREENWAY, C.V., I.R. INNES, K.L. PUSHKA, G.D. SCOTT, AND D.S. SITAR. Changes in hepatic blood volume on galactose an indocyanine green uptake by cat liver. *Am. J. Physiol.* 256: G524-G531, 1989.

10. HUNTON, D.B., J.L. BOLLMAN, AND H.N. HOFFMAN,II. Studies of hepatic function with indocyanine green. *Gastroenterology* 39: 713-724, 1960.

11. LEWIS, A.E. Estimation of plasma volume of the heart. *Am. J. Physiol.* 172: 203, 1953.

12. REEMTSMA, K., G.C. HOTTINGER, A.C.,JR. DEGRAFF, AND O.,JR. CREECH. The estimation of hepatic blood flow using indocyanine green. *Sur. Gyn. Obst.* 3: 353-356, 1960.

13. ROBINSON, V.J.B., O.A. SMISETH, N.W. SCOTT-DOUGLAS, E.R. SMITH, J.V. TYBERG, AND D.E. MANYARI. Assessment of the splanchnic vascular capacity and capacitance using quantitative equilibrium blood-pool scintigraphy. *J. Nucl. Med.* 31: 154-159, 1990.

14. ROTHE, C.F., B.L. JOHNS, AND T.D. BENNETT. Vascular capacitance of dog intestine using mean transit time of indicator. *Am. J. Physiol.* 234 (1): H7-H13, 1978.

15. ROTHE, C.F., R.H. MURRAY, AND T.D. BENNETT. Actively circulating blood volume in endotoxin shock measured by indicator dilution. *Am. J. Physiol.* 236(2): H291-H300, 1979.

16. ROWELL, L.B. Importance of scintigraphic measurements of human splanchnic blood volume. *J. Nucl. Med.* 31: 160-162, 1990.

17. ZIERLER, K.L. Theoretical basis of indicator-dilution methods for measuring flow and volume. *Circ. Res.* 10: 393-407, 1962.

CHAPTER 5

REGIONAL PRESSURE-VOLUME RELATIONSHIP DURING HEAT STRESS WITH OR WITHOUT NEURAL BLOCKADE

A. ABSTRACT

To determine the mechanisms of increase in venous return during heat stress, we measured blood volumes (V_b), unstressed volumes (V_u), blood flow distribution, venous compliance (C_v), venous resistance (R_v) and the time constant of venous drainage (τ_v) of the splanchnic (SPL) and extrasplanchnic (PER) vascular beds in 20 dogs anesthetized with chloralose. PER was divided into superior vena caval (SVC) and inferior vena caval (IVC) regions. A circulatory bypass was used with constant cardiac output. V_b was measured in the SVC and SPL region with indicator dilution curves and mean transit times. Changes in venous pressures and stop flow techniques were used to construct the pressure volume (P-V) curves and to obtain C_v, R_v and τ_v. V_u was extrapolated from the P-V curves. When core temperature was increased from 38°C to 42°C, there was a 23% decrease in SPL V_b and a 38.5% decrease in SPL V_u. When ganglionic blockade was applied during heat stress, SPL V_b increased by 24.7% and SPL V_u by 106%. Surprisingly, however, neither ß nor α-blockade reversed the effect of heat stress on splanchnic V_b and V_u. In conclusion, increases in venous return during heat stress are due to a decrease in SPL V_u and, although mediated through the sympathetic ganglions, this decrease cannot be abolished by α or ß receptor blockade.

B. INTRODUCTION

In heat stressed baboons (14) and sheep (15), the increase in blood flow to heat dissipating organs is completely compensated for by decreases in blood flow to other regions. Consequently, cardiac output does not rise. Men (26) and dogs (13) subjected to heat stress, however, redistribute regional blood flows less efficiently and cardiac output rises as total flow demands increase. Since under steady state conditions venous return equals cardiac output, the vascular adjustments to heat stress must include mechanisms by which the return of blood to the heart also increases (8,18). Assuming a two parallel compartment model of the circulation (4), venous return can increase in four ways: (1) by decreasing unstressed volume (ie. position of the pressure-volume (P-V) curve), (2) by decreasing venous compliance (ie. slope of the P-V curve), (3) by decreasing venous resistance, or (4) by redistributing blood flow to vascular beds with a fast time constant of venous drainage. The present study was designed to determine which of these mechanisms is responsible for the increase in venous return during heat stress in dogs and to identify the efferent pathways.

C. METHODS

Surgical Procedures

Twenty mongrel dogs of either sex with a mean weight of 30.2 ± 3.4 Kg (SD) were anesthetized with 10 $mg \cdot kg^{-1}$ of thiopental, followed by 100 $mg \cdot Kg^{-1}$ of α-chloralose; additional

doses of chloralose were administered as necessary. An endotracheal intubation was performed and the dogs were ventilated at an appropriate tidal volume and frequency for their size. A midline thoracotomy and a ventral laparotomy were performed after which an external circulatory circuit was established as explained in details in chapter 4.

During the normothermic period, body temperature was maintained at 37.8 ± 0.2°C (control). To produce hyperthermia within twenty to thirty minutes, warm water was circulated through the heat exchanger until rectal temperature reached 41.9 ± 0.1°C (heat stress). A thermocouple (Mon-a-Therm) was placed in the right atrium, to ensure that the blood temperature did not exceed 43°C. If panting occurred, the phrenic nerves were cut.

Instrumentation

Arterial pressure (Trantec transducers) was measured in the carotid artery. SVC, IVC and SPL venous pressures were measured with catheters placed at the junction of the cannula and vein. The transducers were referenced to the right atrium by direct inspection. Pressure, flow and densitometer signals were recorded on a Gould eight channel recorder. Flow and densitometer signals were further processed through an analog to digital converter (Data Translation DT2801) and sampled at 26 Hz/channel to the hard disk of a personal computer for later analysis.

Blood gases and hematocrit were taken before every series of measurements. Ventilatory rate was adjusted and

bicarbonate sodium was given or both to keep PCO_2 between 35 and 40 and pH between 7.3 and 7.4. The average hematocrit was 39.7 ± 0.77%.

Protocol

Venous pressures were set between 0 and 3 mm Hg by adjusting the height of the waterfalls and regional blood volumes were measured (see below). Next, regional mechanical parameters were measured (see below) and the venous pressures were adjusted to the initial level. The regional blood volume measurements were then repeated. For each region, the average of the blood volumes before and after the mechanical measurements was used for analysis. The variation between these volumes was 1.6 ± 1.2 $ml \cdot kg^{-1}$ (SD).

In the first series of experiments (n = 8), control and heat stress conditions were studies and the starting condition was randomized.

In the second series of experiments, an attempt was made to identify the efferent pathway responsible for the increase in venous return during heat stress. Accordingly, heat stress was maintained throughout the experiment and the measurements were made with heat stress only, and then with ganglionic blockade (n = 4), ß-receptor blockade (n = 4) or α-receptor blockade (n = 4). Ganglionic blockade was achieved using hexamethonium chloride (5 $mg \cdot kg^{-1}$ first and 2$mg \cdot kg^{-1}$ every ½ hr, n = 3) or trimetaphan (5 $mg \cdot min^{-1}$, n = 1). Ganglionic blockade was considered effective when additional doses did not affect blood pressure and when reactive hyperhemia was

abolished. Propranolol and phentolamine, 0.2 $mg \cdot kg^{-1}$, were used for ß-receptor and α-receptor blockade respectively. ß-blockade was considered to be effective when heart rate decreased by at least 20 $beats \cdot min^{-1}$. In one dog it was necessary to give 5 times the dose of propranolol to get this decrease in heart rate. Blockade of α-adrenergic receptors was considered effective when additional doses of phentolamine had no effect on arterial pressure and when reactive hyperemia was abolished.

Regional Blood Volume Measurement

Regional blood volumes were measured in the SPL and in the IVC regions as described in details in chapter 4. The amounts of indicator injected for the volume measurements were 1 mg for the SVC region and 1.5 mg for the SPL region. After the completion of every indicator dilution curve a stop flow was performed (see below) to measure the elastic recoil pressure at which the volume measurement was made. Blood volumes in the IVC region were not measured.

Measurement of Regional Mechanical Parameters

After the blood volume measurements were made and steady state values of flows and pressures returned, the technique of Mitzner and Goldberg (21) and Malo et al. (20) were used to measure venous compliance and resistance. Venous pressures were set at 0 to 3 mm Hg by adjusting the height of the waterfalls. Systemic flow was stopped by occluding all venous outflows and the ascending aorta, and by turning off the pump simultaneously. The occlusion was held until the venous

outflow pressure started to plateau (5-13 sec). The venous plateau pressures (P_{vpl}) are considered to be the regional static elastic recoil pressures which determine the pressure gradient for venous return since the outflow pressures (waterfalls) are fixed. Flow was then reestablished and all pressures returned to initial levels. Outflow pressures were increased 3 mm Hg by simultaneously and abruptly lifting the waterfalls. A second stop-flow procedure was performed to obtain new P_{vpl}. The increase in outflow pressure resulted in a transient decrease in outflow. If inflow to each region is constant during this period, the integral of the flow transient is equal to the change in volume in the region. Constant regional inflows were assumed if the outflows returned to initial levels. Another increase in P_v was performed followed by two step decreases, alternating with stop flows. Assessments of vascular mechanics were made for the combined SVC and IVC regions (PER) and for the SPL region.

Analysis

Changes in regional volumes (ΔV) with step changes in P_v were obtained by integrating the area under the transient change in flow for a given change in venous pressure (Fig. 5.1). Venous compliance (C_v) was calculated from the equation, $C_v = \Delta V/\Delta P_{vpl}$. Where ΔP_{vpl} is the difference in P_{vpl} (during occlusion) before and after a change in P_v. Venous resistance (R_v) was calculated from the equation, $R_v = (P_{vpl} - P_v)/flow$. The time constant of venous drainage (τ_v) was calculated with the equation, $\tau_v = R_vC_v$.

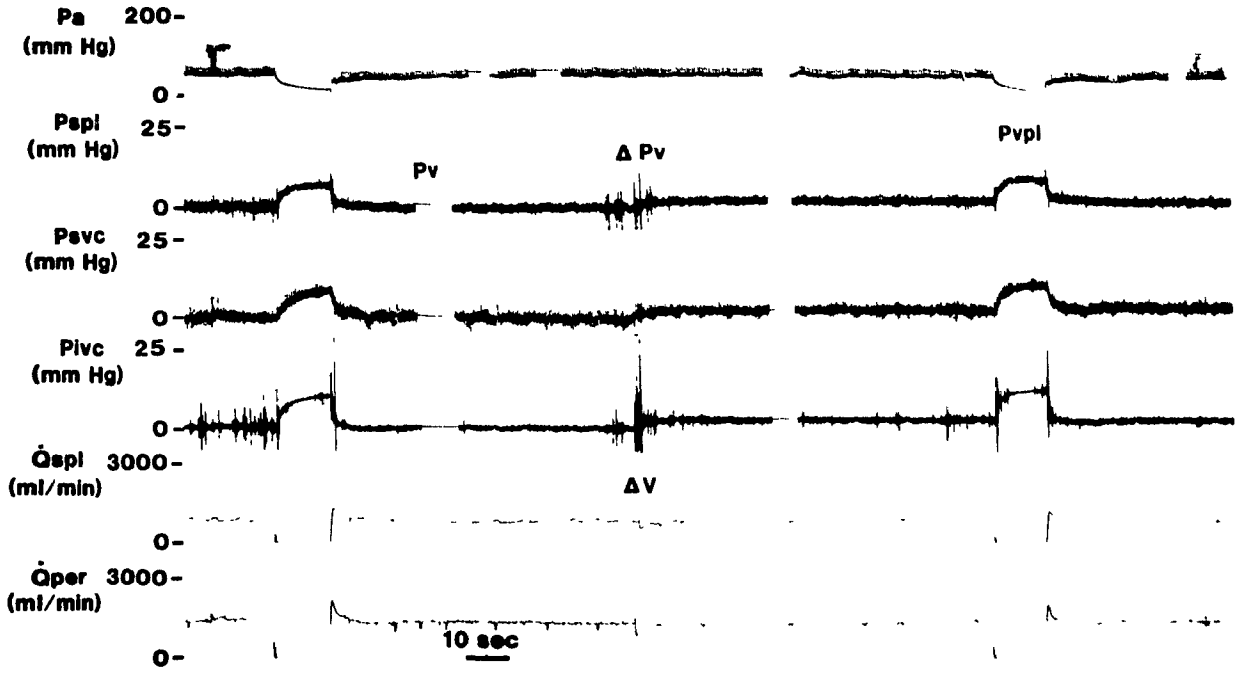

FIGURE 5.1 Sample tracing showing arterial pressure (Pa), splanchnic venous pressure (Pspl), superior vena caval pressure (Psvc), inferior vena caval pressure (Pivc), splanchnic flow ($\dot{Q}$spl) and peripheral flow ($\dot{Q}$per) during a step increase in venous pressure (ΔPv) and during stop flow measurements. Changes in volume (ΔV) in the splanchnic and peripheral regions were obtained by integrating the area under the flow curves as flow changes transiently with a change in Pv. Regional venous compliance was calculated by dividing the change in volume by the difference between venous plateau pressures (Pvpl) before and after a change in flow. Regional venous resistances were obtained before and after a changes in Pv by dividing the difference between Pvpl and Pv by flow.

For the blood volume measurements, regional flows (Q_D) were calculated with the equation, $Q_D = (inf \cdot 60)/(\int cdt \cdot K)$. Where inf is the amount of indicator injected, $\int cdt$ is the area under the indicator dilution curve and K is the constant of calibration of the indicator. Mean transit time (MTT) was calculated from the equation, $MTT = \int tcdt/\int cdt$. Where $\int tcdt$ is the area under the curve of time multiplied by the indicator concentration against time. Regional blood volumes (V_b) were obtained by multiplying MTT by Q_D.

An independent flow signal (flow probe, Q_p) was used to calculate the amount of indicator recovered for any given dilution curve. By substituting Q_p in the equation for Q_D, we obtain the calculated amount of indicator recovered for each indicator dilution curve. The difference between the amount recovered and the amount injected estimates the uptake of the indicator by the tissues. In the SVC region, the calculated amount of indicator recovered was 101.0 ± 1.2%, while in the SPL region it was 95.4 ± 0.8%. A linear regression of least square method was used to assess the identity plot between Q_D and Q_p (Fig. 5.2). When all data was pooled together, the R value was 0.99.

The relationship between plateau pressure and blood volume (P-V curves) was assessed by a linear regression of least square method. The P-V curves were constructed for the SVC and SPL regions for every conditions. Extrapolations of the individual curves to zero pressure were used to calculate unstressed volumes (V_u). The P-V curves were linear for both

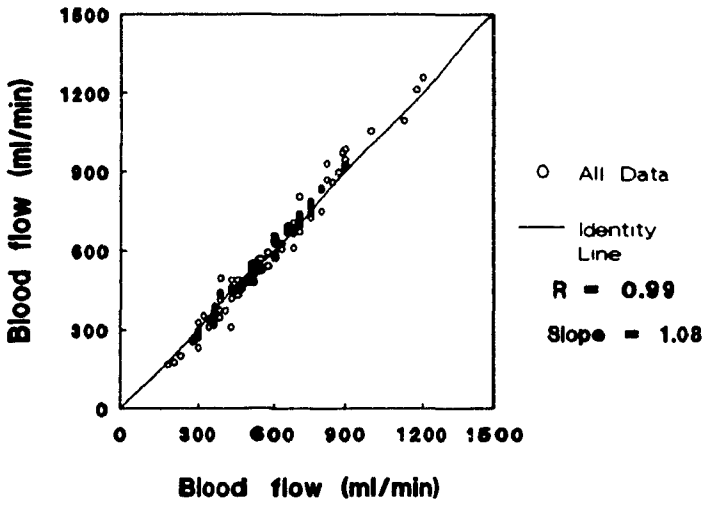

FIGURE 5.2 Identity plot of the flows during blood volume measurements obtained with electromagnetic flowmeters (abscissa) and using indicator dilution curves (ordinate). All data from all conditions were pooled together.

regions over the range studied. The mean R values were 0.93 ± 0.03 for SPL and 0.94 ± 0.02 for PER. Attempts to fit the P-V curves with second and third order polynomials did not improve the R value. As with any extrapolation, there is a possibility that the P-V curve becomes more curvilinear at low pressures and volumes and that V_u does not represent the true intercept of the relationship. However, the physiologically functional part of the relationship was linear and hence V_u describes the true position of the P-V curve under a variety of physiological conditions.

Statistical Analysis

Data are presented as mean ± SE, unless otherwise stated. The differences in R_v, C_v, τ_v, V_b and V_u between control and heat stress, heat and ganglionic blockade, heat and α-blockade and heat and ß-blockade were assessed with a paired *t* test for repeated measures (Bonferonni correction). The differences were considered to be significant at $P < 0.05$.

D. RESULTS

EFFECT OF HEAT STRESS

Splanchnic blood volume decrease by 4.6 ± 1.4 $ml \cdot kg^{-1}$ during heat stress. Table 5.1 shows that the change in volume was region specific because blood volume did not change in the SVC region. There was a parallel shift to the left of the splanchnic P-V curve with heat stress (Fig. 5.3). This shift resulted in a decrease in SPL unstressed volume (Table 5.1).

Cardiac output was 1.87 ± 0.12 $l.min^{-1}$ for control and 1.71

TABLE 5.1 Effect of heat stress on regional blood volume, unstressed volume and vascular mechanical parameters.

Parameter	Region	Condition	
		Control	Heat Stress
Blood Volume	SPL	22.7 ± 2.3	17.5 ± 1.6[a]
	SVC	11.8 ± 0.8	11.5 ± 1.0
Unstressed Volume	SPL	14.8 ± 2.3	9.1 ± 1.9[a]
	SVC	7.6 ± 1.1	7.7 ± 1.0
Arterial Pressure		71.7 ± 3.0	75.0 ± 4.0
Arterial Resistance	SPL	0.18 ± 0.03	0.20 ± 0.02
	PER	0.13 ± 0.01	0.18 ± 0.03
Venous compliance	SPL	0.91 ± 0.15	1.03 ± 0.11
	PER	0.36 ± 0.06	0.38 ± 0.02
Venous Resistance	SPL	0.02 ± 0.003	0.02 ± 0.002
	PER	0.02 ± 0.003	0.02 ± 0.002
Time Constant	SPL	16.3 ± 2.5	14.7 ± 1.8
	PER	5.9 ± 0.7	6.9 ± 0.4

Values are means ± SE. Control, core temperature of 37.8 ± 0.2°C. Heat stress, core temperature of 41.9 ± 0.1°C. SPL, splanchnic region. SVC, superior vena caval region. PER, extrasplanchnic region. The volumes are in $ml \cdot kg^{-1}$, arterial pressure is in mm Hg, the resistances are in mm $Hg \cdot sec \cdot ml^{-1} \cdot kg^{-1}$, the compliances are in $ml \cdot mm\ Hg \cdot kg^{-1}$ and the time constants of venous drainage are in seconds. [a] $P < 0.01$ vs. control, $n = 8$.

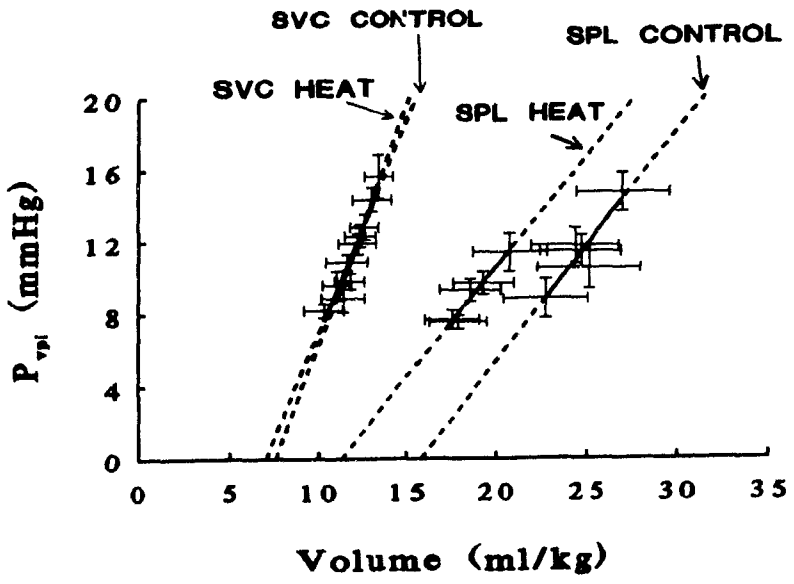

FIGURE 5.3 Pressure-Volume (P-V) curves of the splanchnic (SPL) and superior vena caval (SVC) regions during control (38°C) and during heat stress (41.9°C). The solid lines are linear regressions and the dashed lines are extrapolated to zero venous plateau pressure (P_{vpl}).

± 0.26 $l.min^{-1}$ for heat stress. The distribution of blood flow between SPL and PER was not affected by heat stress. During control, 43 ± 4% of flow went to SPL and 57 ± 4% to PER. With heat stress, 47 ± 3% of flow went to SPL and 53 ± 3% to PER. None of the other parameters were affected by heat stress (Table 5.1).

EFFECT OF GANGLIONIC BLOCKADE DURING HEAT STRESS

Arterial pressure and resistance decreased markedly with ganglionic blockade (Table 5.2). Splanchnic blood volumes increased by 5.2 ± 1.6 $ml \cdot kg^{-1}$. This increase is very similar to the 4.6 $ml \cdot kg^{-1}$ decrease in volume observed with an increase in core temperature (see previous section), and indicates that ganglionic blockade completely reversed the effect of heat stress on SPL V_b. Again, the change in volume was region specific (Table 5.2). There was a parallel shift to the right of the SPL P-V curve with ganglionic blockade (Fig. 5.4). This shift is exactly the reverse of the change in P-V curve observed with increases in core temperature. None of the other parameters were affected by ganglionic blockade (Table 5.2).

EFFECT OF ß-BLOCKADE DURING HEAT STRESS

Heart rate decreased from 170 ± 13.2 to 145 ± 12.6 $beats.min^{-1}$ ($P < 0.005$) with propranolol. Unlike ganglionic blockade, ß-receptor blockade had no effect on SPL blood volume (Table 5.3) and on the SPL P-V curve (Fig. 5.5), suggesting that the decrease in SPL V_b during heat stress is not mediated though ß-receptors. None of the other parameters were affected by propranolol.

TABLE 5.2 Effect of ganglionic blockade on regional blood volume, unstressed volume and vascular mechanical parameters during heat stress.

Parameter	Region	Condition	
		Heat stress	G-Blockade
Blood Volume	SPL	22.6 ± 3.0	29.4 ± 1.5[c]
	SVC	12.2 ± 0.8	13.0 ± 0.7
Unstressed Volume	SPL	8.9 ± 4.5	18.3 ± 2.0[c]
	SVC	9.7 ± 1.1	10.3 ± 0.5
Arterial Pressure		96.5 ± 7.6	71.4 ± 4.7[b]
Arterial Resistance	SPL	0.33 ± 0.06	0.25 ± 0.04[c]
	PER	0.20 ± 0.02	0.14 ± 0.01[a]
Venous compliance	SPL	1.69 ± 0.58	1.68 ± 0.26
	PER	0.39 ± 0.08	0.42 ± 0.09
Venous Resistance	SPL	0.01 ± 0.002	0.01 ± 0.002
	PER	0.02 ± 0.003	0.02 ± 0.003
Time Constant	SPL	15.4 ± 4.6	15.3 ± 1.8
	PER	6.0 ± 0.9	5.8 ± 1.3

Values are means ± SE. Heat stress, core temperature of 41.9 ± 0.1°C. G-Blockade, ganglionic blockade (hexamethonium chloride, 5 $mg \cdot kg^{-1}$, $n = 3$, and trimetaphan, 5$mg \cdot min^{-1}$, $n = 1$). The units are the same as in TABLE 5.1. [a] $P < 0.01$, [b] $P < 0.005$ and [c] $P < 0.05$ vs. heat stress.

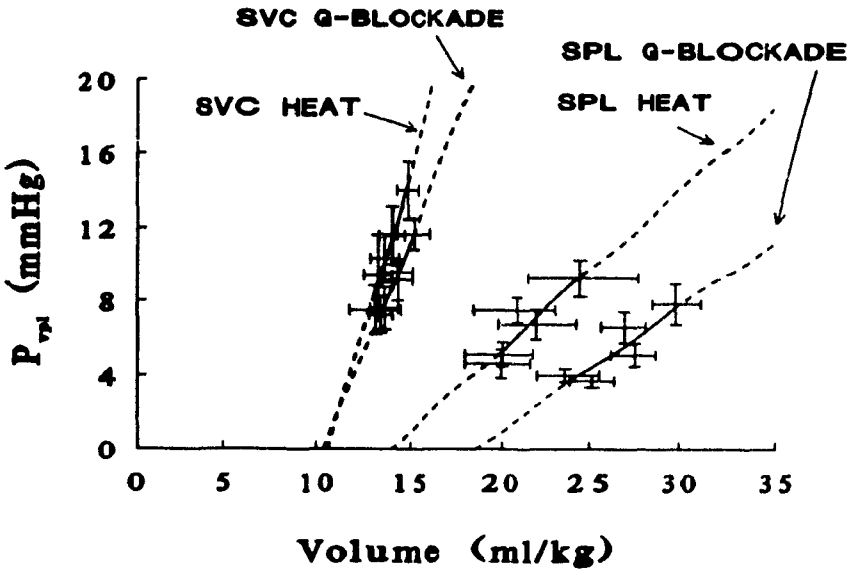

FIGURE 5.4 P-V curves of the splanchnic (SPL) and superior vena caval (SVC) regions during heat (41.9°C) and with ganglionic (G) blockade with either trimetaphan (n = 1) or hexamethonium chloride (n = 3). The solid lines and dashed lines are the same as in Fig. 5.3. P_{vpl}, venous plateau pressure.

TABLE 5.3 Effect of ß-receptor blockade on regional blood volume, unstressed volume and vascular mechanical parameters during heat stress.

Parameter	Region	Condition	
		Heat stress	ß-Blockade
Blood Volume	SPL	21.8 ± 0.2	22.6 ± 1.2
	SVC	12.4 ± 1.5	9.2 ± 1.6
Unstressed Volume	SPL	11.0 ± 1.2	12.8 ± 0.9
	SVC	9.2 ± 1.6	11.4 ± 1.6
Arterial Pressure		102.4 ± 12.7	91.9 ± 15.6
Arterial Resistance	SPL	0.31 ± 0.13	0.25 ± 0.04
	PER	0.21 ± 0.06	0.17 ± 0.03
Venous compliance	SPL	1.96 ± 0.67	1.44 ± 0.18
	PER	0.46 ± 0.08	0.31 ± 0.05
Venous Resistance	SPL	0.02 ± 0.001	0.02 ± 0.005
	PER	0.02 ± 0.002	0.02 ± 0.005
Time Constant	SPL	17.6 ± 0.7	16.9 ± 3.0
	PER	6.0 ± 0.6	4.5 ± 1.4

Values are means ± SE. Heat stress, core temperature of 41.9 ± 0.1°C. ß-Blockade, ß-receptor blockade (0.2 $mg \cdot kg^{-1}$). The units are the same as in TABLE 5.1, $n = 4$.

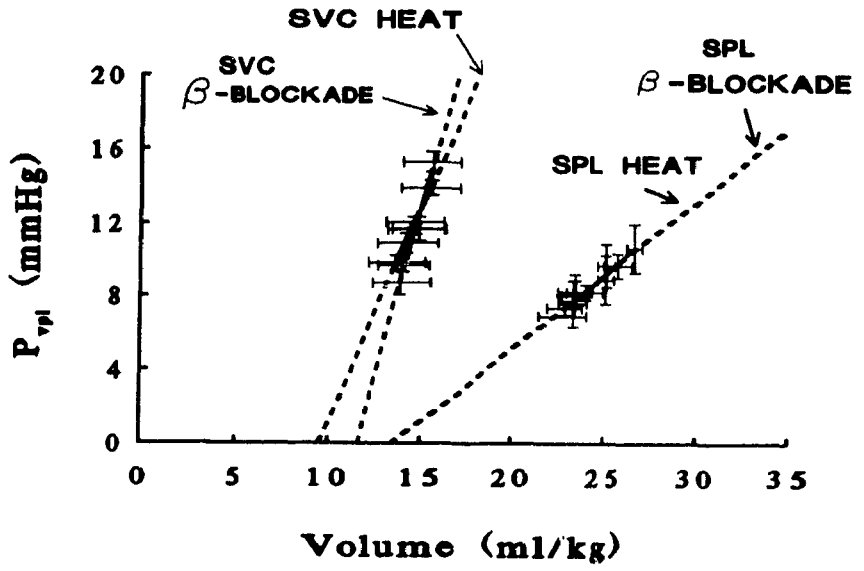

FIGURE 5.5 P-V curves of the splanchnic (SPL) and superior vena caval (SVC) regions during heat (41.9°C) and with ß-receptor blockade with propranolol (n = 4). The solid lines and dashed lines are the same as in Fig. 5.3. P_{vpl}, venous plateau pressure.

EFFECT OF α-BLOCKADE DURING HEAT STRESS

Arterial pressure and resistance decreased markedly with phentolamine (Table 5.4). Similar to propranolol, α-receptor blockade could not reverse the effect of heat stress on SPL blood volume and P-V curve (Table 5.4 and Fig. 5.6). These results suggest that the decrease in splanchnic V_b and V_u during heat stress are not mediated through α-receptors. None of the other parameters were affected by phentolamine.

E. DISCUSSION

The preparation used in this study deviates greatly from the intact conscious animal. The anesthesia, surgical procedure, homologous donor blood, pump and tubing inevitably alter the physiological response to external stimuli. Despite these disadvantages, the preparation allowed measurements of venous pressures, flows, absolute blood volumes, venous compliance, resistance, time constant of venous drainage and P-V relationship of the splanchnic and extrasplanchnic beds. Although bleeding is inevitable during a bypass preparation, replacement with dextran did not significantly alter the hematocrit value. Complete recovery from heat stress with return to control P-V relationship was also possible (Fig. 5.7).

Efforts were made to estimate the error in the measurements of regional blood volume due to the loss of cardiogreen to the liver, to other tissues, or to hemorrhage. The calculated amounts of indicator recovered showed that all

TABLE 5.4 Effect of α-receptor blockade on regional blood volume, unstressed volume and vascular mechanical parameters during heat stress.

Parameter	Region	Condition	
		Heat stress	α-Blockade
Blood Volume	SPL	19.5 ± 1.5	21.0 ± 2.4
	SVC	9.8 ± 1.3	10.0 ± 1.9
Unstressed Volume	SPL	11.2 ± 0.7	10.4 ± 1.9
	SVC	7.7 ± 1.1	7.2 ± 1.4
Arterial Pressure		128.0 ± 3.6	71.3 ± 2.4[a]
Arterial Resistance	SPL	0.34 ± 0.09	0.19 ± 0.04[c]
	PER	0.16 ± 0.03	0.11 ± 0.02[c]
Venous compliance	SPL	1.15 ± 0.15	1.67 ± 0.21
	PER	0.30 ± 0.08	0.41 ± 0.05
Venous Resistance	SPL	0.02 ± 0.004	0.01 ± 0.002
	PER	0.02 ± 0.006	0.02 ± 0.006
Time Constant	SPL	17.2 ± 1.1	14.9 ± 0.6
	PER	4.3 ± 0.8	5.1 ± 0.8

Values are means ± SE. Heat stress, core temperature of 41.9 ± 0.1°C. α-Blockade, α-receptor blockade, phentolamine (0.2 mg/kg). The units are the same as in TABLE 5.1. [a] $P < 0.01$ and [c] $P < 0.05$ vs. heat stress.

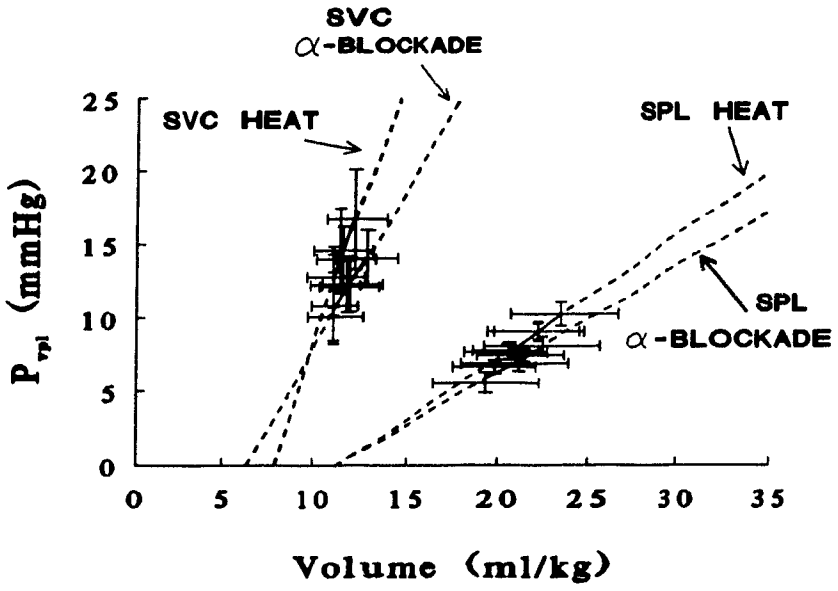

FIGURE 5.6 P-V curves of the splanchnic (SPL) and superior vena caval (SVC) regions during heat (41.9°C) and with α-receptor blockade with phentolamine (n = 4). The solid lines and dashed lines are the same as in Fig. 5.3. P_{vpl}, venous plateau pressure.

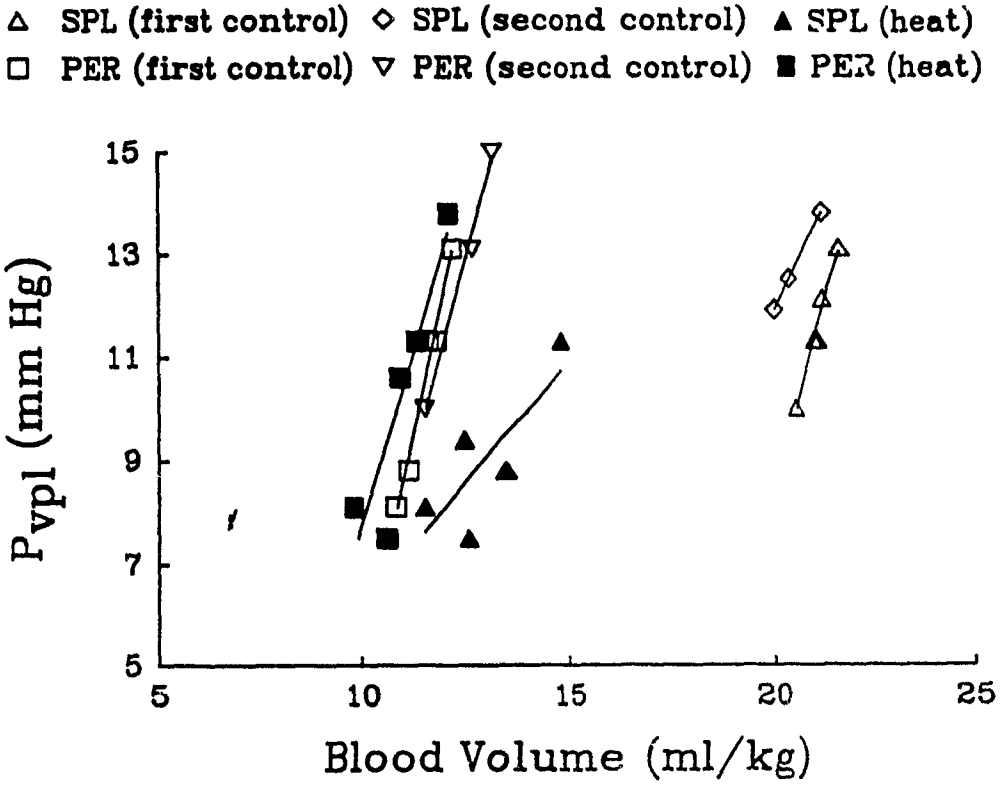

FIGURE 5.7 P-V curves of the splanchnic (SPL) and peripheral (PER, superior vena caval only) regions of one animal during control (37°C), heat exposure (41.9°C) and then back to control conditions. The solid lines are linear regressions. P_{vpl}, venous plateau pressure.

of the cardiogreen was recovered from the SVC region and over 95% was recovered from the SPL region.

Concerns may be raised as to whether increases in temperature alter the uptake of indocyanine green by the liver. We found no difference, however, in the calculated amount of indicator recovered during control and heat stress, 95.4 ± 0.4% and 95.1 ± 1.3% respectively. Another concern is that changes in liver volume could affect hepatic uptake of indicator. Greenway et al. (12) studied indocyanine green uptake with change in hepatic blood volume in cats. They concluded that the effects of large changes in hepatic blood volume on indocyanine green uptake were small. Although uptake of indicator by the liver is probably the greatest source of error in the volume measurements, the above discussion demonstrates that this error is small and should remain constant for consecutive measurements of volumes under the conditions described in this study.

Two important findings are presented here. First, heat stress resulted in a decrease in splanchnic unstressed volume and did not affect any of the three other mechanisms of increase in venous return. Second, this decrease in SPL volume can be reversed by ganglionic blockade but not by α or ß-receptor blockade. A comparison of our findings with the work of others, the implications of our findings and whether or not they can be applied to the intact animal is discussed next.

Venous Parameters

The C_v, and τ_v calculated for the normothermic condition can be compared with the values obtained by other investigators. The splanchnic C_v of 0.91 ml.mm $Hg^{-1}.kg^{-1}$ agrees well with the results of other authors (3,9,10,20,21). Peripheral C_v (0.36 ml.mm $Hg^{-1}.kg^{-1}$) is also comparable to the results from other studies (3,20,21), but is one half to one third that of Green and Jackman (10) and Green (9) (1.05 and 1.2 ml.mm $Hg^{-1}.kg^{-1}$ respectively). The reason for this discrepancy is unknown.

Peripheral τ_v (5.9 sec) was similar to that observed by most authors (9,10,20,21). Values in the literature for splanchnic τ_v, however, vary greatly ranging from ≈ 8 and 9.4 sec (10,21) to 24 and 24.5 sec (4,9). Our value of 16.3 sec agrees best with the value of 18 sec obtained by Malo et al. (20) and is concomitant with the belief that the vasculature can be divided into two parallel circuits with very different time constants of venous drainage (4).

Arterial Pressure and Blood Flow Distribution

We found no decrease in P_a with heat stress. Although P_a usually decreases slightly in humans during heat stress (17,25), it has been shown to increase (13,19), decrease (6,29) or remain constant (6,19) in anesthetized and conscious dogs depending on the degree of heating. On the opposite end of the scale, Green and Jackman (7) also found no change in arterial pressure and resistance in hypothermic dogs with a preparation similar to ours.

We found no redistribution of blood flow from the splanchnic to the peripheral region with heat stress. In humans, splanchnic blood flow decreases as peripheral flow increases at high core temperatures (25). In intact dogs, heat stress increases blood flow in the skin of the lower legs and ears, in the tongue, in the maxillo turbinals, in the nasal mucosa, in the respiratory muscles and in the spleen (13). Blood flow to the respiratory muscles most likely did not increase in the present study since the animals were not allowed to pant. This may explain the lack of change in peripheral blood flow.

Once again, the results from the study by Green and Jackman (10) on hypothermia can be used to draw general conclusions on the changes in blood flow distribution at varying core temperature in anesthetized dogs. The lack of flow redistribution in the latter study and ours suggests that anesthesia may hinder blood flow redistribution with changing body temperature.

Changes in P-V Curve

To the best of our knowledge, decreases in splanchnic unstressed volume during heat stress have not been demonstrated previously. Furthermore, these changes in capacitance did not result from variations in venous compliance or resistance but from a parallel shift to the left of the P-V curve.

Green and Jackman (10) observed an increase in total unstressed volume during mild hypothermia in anesthetized

dogs. This change was mainly due to a decrease in venous compliance of the splanchnic and peripheral regions and therefore implies a change in the slope of the P-V curves of the entire vasculature. The discrepancy between their study and ours may be due to differences in the methods used for the measurement of venous compliance and the fact that P-V curves were not constructed by Green and Jackman.

A change in capacitance without a change in the slope of the P-V curve has been observed in the liver of anesthetized cats after an infusion of norepinephrine (11), in the entire vasculature of conscious hypertensive rats (7,28) and in conscious rats after the infusion of hexamethonium (32). Drees and Rothe (5) also describe a reflex compensation for changes in blood volume which alters the unstressed volume more than the slope of the P-V curve. Shoukas and Sagawa (30) found that total vascular capacity changed without a change in compliance when carotid sinus pressure was varied. Thus changes in the position of the P-V curve appears to be more important for increasing venous return than changes in the slope of the P-V curve. Greenway et al. suggested that since the amount of blood that can be mobilized as blood pressure falls is smaller with a change in the slope of the P-V curve than with a change in the position of the P-V curve, the latter mechanism gives the animal the best chances of survival in situations where mobilization of blood is most vital (11).

Mechanisms of Volume Mobilisation

Traditionally, recruitment of unstressed volume has been thought to be mediated through α-adrenergic stimulation. However, a growing number of investigators have argued that ß-activation is the main effector for volume recruitment (2,9, 16,22-24,27). Bennett et al. (2) found that both α and ß receptors seem to be activated for volume recruitment. Nevertheless, these authors concluded that ß stimulation produces the largest changes in capacitance.

We consequently tested the hypothesis that the decrease in capacitance during heat stress is mediated through the sympathetic nervous system by infusing ganglionic blockers. Since hexamethonium and trimetaphan readily abolished this decrease (Fig. 5.4), sympathetic outflow may be regarded as the main effector of the recruitment in unstressed volume during heat exposure. We next examined whether this effect was mediated through ß or α stimulation. We began with ß-blockade based on the evidence presented above, but propranolol had no effect on capacitance during heat stress. Even more surprising, α-blockade was also ineffective. Since heart rate decreased by 15% with propranolol and arterial pressure by 45.5% with phentolamine, we consider the doses of α and ß blockers used in this study to be adequate. Therefore, neither α nor ß-adrenergic stimulation appear to be responsible for volume recruitment during heat stress. Furthermore, none of the other determinants of venous return (C_v, R_v and fractional distribution of blood flow) were

affected by heat stress. Consequently, we are left in search of a sympathetically released constricting agent that mediates changes in unstressed volume without affecting venous resistance, compliance or distribution of blood flow.

Increase in Venous Return

Caldini et al.'s developed an equation for venous return, which involves stressed volume, regional time constants and fractional flows to the fast and slow time constant beds (4). Since only unstressed volume changed during heat stress in our study, this equation can be used to estimate the increase in venous return if all of the decrease in V_u (6 $ml.kg^{-1}$) is transferred to stressed volume in the intact animal. This analysis showed that venous return, and thus cardiac output, would increase by 54% during heat stress. Since Hales et al. measured a 70% increase in cardiac output during heat stress in conscious dogs (13), the results presented here can explain 77% of the increase in cardiac output in their animals. Therefore, decreases in SPL unstressed volumes can be considered to be the major mechanism of increase in venous return during heat stress.

In summary, heat stress reduces splanchnic capacitance without changing the capacitance of the peripheral region and has little influence on splanchnic and peripheral venous compliances, resistances and time constants of venous drainage. This decrease in unstressed volume can be abolished by ganglionic blockade but not by α or ß-receptor blockade and is the major mechanism of increase in venous return during

heat stress.

F. REFERENCES

1. Banaszak, E. F., W. J. Stekiel, R. A. Grace, and J. J. Smith. Estimation of hepatic blood flow using a single injection dye clearance method. *Am. J. Physiol.* 198: 877-880, 1960.

2. Bennett, T. D., C. R. Wyss, and A. Scher. Changes in vascular capacity in awake dogs in response to carotid sinus occlusion and administration of catecholamine. *Circ. Res.* 55: 440-453, 1984.

3. Brunner, J.M., Shoukas, A.A., and MacAnespie, C.L. The effect of the carotid sinus baroreceptor reflex on blood flow and volume redistribution in the total systemic vascular bed of the dog. *Circ Res.* 48:274-285, 1981.

4. Caldini, P., Permutt, S., Waddell, J.A., and Riley, R.L. Effect of epinephrine on pressure, flow, and volume relationships in the systemic circulation of dogs. *Circ. Res.* 34:606-623, 1974.

5. Drees J. A. and C. F. Rothe. Reflex venoconstriction and capacity vessel pressure-volume relationships in dogs. *Circ. Res.* 34: 360-373, 1974.

6. Ederstrom H.E. Blood flow changes on the dog during hyperthermia. *Am. J. Physiol.* 176: 347-351, 1954.

7. Edmunds, M.E., G.I. Russedd, and J.D. Swales. Vascular capacitance and reversal of 2-kidney, 1-clip hypertension in rats. *Am. J. Physiol.* 256: H502-H507, 1989.

8. Guyton, A.C., Determination of cardiac output by equating venous return curves with cardiac response curves. *Physiol. Rev.* 35:123-129, 1955.

9. Green, J.F. Mechanism of action of isoproterenol on venous return. *Am. J. Physiol.* 232(2):H152-H156, 1977.

10. Green, J.F. and A.P. Jackman. Mechanism of the increased vascular capacity produced by mild perfusion hypothermia in the dog. *Circ. Res.* 44: 411-419, 1979.

11. Greenway, C.V., Seaman, K.L., and Innes, I.R. Norepinephrine on venous compliance and unstressed volume in cat liver. *Am. J. Physiol.* 248(17):H468-H476, 1985.

12. Greenway, C.V., I.P. Innes, K.L. Pushka, G.D. Scott, and D.S. Sitar. Changes in hepatic blood volume on galactose an indocyanine green uptake by cat liver. *Am. J. Physiol.* 256: G524-G531, 1989.

13. Hales, J.R.S. and Dampney, R.A.L. The redistribution of cardiac output in the dog during heat stress. *J. Therm Biol*. 1:29-34, 1975.

14. Hales, J.R.S., Rowell, L.B., and King, R.B. Regional redistribution of blood flow in awake heat stressed baboons. *Am. J. Physiol*. 237(6):H705-H712, 1979.

15. Hales, J.R.S. Effect of exposure to hot environments on the regional distribution of blood flow and on cardio-respiratory function in sheep. *Pflügers Arch*. 344:133-148, 1973.

16. Imai, Y., K. Sotoh, and N. Taira. Role of the peripheral vasculature in changes in venous return caused by isoproterenol, norepinephrine, and methoxamine in anaesthetized dogs. *Circ. Res*. 43: 553-561, 1978.

17. Koroxenidis G.T., J.T. Shepherd, and R.J. Marshall. Cardiovascular response to acute heat stress. *J. Appl. Physiol*. 16(5): 869-872, 1961.

18. Krogh A. The regulation of the supply of blood to the right heart. *Skand. Arch Physiol*. 27: 227-248, 1912.

19. Kullmann, R., W. Schönung, and E. Simon. Antagonistic changes in blood flow and sympathetic activity in different vascular beds following central thermal stimulation. *Pflügers Arch*. 319: 146-161, 1970.

20. Malo, J, H. Goldberg, R. Graham, H. Unruh and C. Skoog. Effect of hypoxic hypoxia on systemic vasculature. *J. Appl. Physiol*. 56(5):1403-1410, 1934.

21. Mitzner, W. and H. Goldberg. Effect of epinephrine on resistive and compliant properties of the canine vasculature. *J. Appl. Physiol*. 39(2):272-280, 1975.

22. Muller-Ruchholtz E. R., H-M. Losch, E. Grund, and W. Lochner. Effect of alpha adrenergic receptor stimulation on integrated systemic venous bed. *Pflügers Arch*. 370: 241-246, 1977.

23. Muller-Ruchholtz E. R., H-M. Losch, E. Grund, and W. Lochner. Effect of beta adrenergic receptor stimulation on integrated systemic venous bed. *Pflügers. Arch*. 370: 247-251, 1977.

24. Ross, J. Jr, C. J. Frahm, and E. Braunwald. Influence of carotid baroreceptors and vasoactive drugs on systemic vascular volume and venous distensibility. *Circ. Res*. 9: 75-82, 1961.

25. Rowell, L.B., G.L. Brengelmann, J.R. Blackmon, and J.A. Murray. Redistribution of blood flow during sustained high skin temperature in resting man. *J. Appl. Physiol.* 28(4): 415-420, 1970.

26. Rowell, L.B. Cardiovascular adjustments to thermal stress. In: *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow.* Bethesda, MD: American Physiological Society, 1984, Sect. 2, Vol. III, Part 2, Chapter 27, p. 967-1023.

27. Rutlen, [illegible]., E.N. Supple, and W.J. Jr Powell. Beta-adrenergic regulation of total systemic intravascular volume in the dog. *Circ. Res.* 48: 112-120, 1981.

28. Samar, R.E., and T.G. Coleman. Mean circulatory pressure and vascular compliancies in the spontaneously hypertensive rat. *Am. J. Physiol.* 237(5):H584-H589, 1979.

29. Schönung, W., H. Wagner, C. Jessen, and E. Simon. Differentiation of cutaneous and intestinal blood flow during hypothalamic heating and cooling in anaesthetized dogs. *Pflügers. Arch.* 328: 145-154, 1971.

30. Shoukas, A.A. and K. Sagawa. Control of total systemic vascular capacity by the carotid sinus baroreceptor reflex. *Circ. Res.* 33: 22-33, 1973.

31. Vatner, S.F., C.B. Higgins, R.W. Millard, and D. Franklin. Role of the spleen in the peripheral vascular response to severe exercise in untethered dogs. *Cardiovasc. Res.* 8: 276-282, 1974.

32. Yamamoto J., N.C. Trippodo, S. Ishise, and E.D. Frohlich. Total vascular pressure-volume relationship in the conscious rat. *Am. J. Physiol.* 238: H823-H828, 1980.

CHAPTER 6

BARORECEPTOR REFLEX CONTROL OF REGIONAL CAPACITANCE AND BLOOD FLOW DISTRIBUTION WITH OR WITHOUT ALPHA ADRENERGIC BLOCKADE

A. ABSTRACT

To determine the effects of changes in carotid sinus pressure (P_{cs}) on regional vascular capacitance, we measured regional blood volumes (V_b), unstressed volumes (V_u), blood flow distribution, venous compliances (C_v), resistances (R_v) and time constants of drainage (τ_v) in dogs anesthetized with chloralose and on circulatory bypass at constant cardiac output. The circulation was divided into splanchnic (SPL) and extrasplanchnic (PER) regions. PER was divided into superior vena caval (SVC) and inferior vena caval (IVC) regions. V_b was measured in each region from indicator dilution curves and mean transit times. Outflow pressures were changed to alter regional volumes and flow was stopped to measure regional elastic recoil pressures. V_u was extrapolated from the pressure-volume (P-V) curves. P_{cs} of 50 and 200 mm Hg were maintained in random order. With a decrease in P_{cs}, arterial pressure increased from 58.7 ± 4.1 to 104.6 ± 6.4 mm Hg ($P < 0.01$), PER fractional blood flow decreased from 69.8 ± 3.8 to 55.8 ± 3.9% ($P < 0.001$), SPL V_b decreased form 28.3 ± 1.9 to 19.3 ± 1.2 $ml \cdot kg^{-1}$ ($P < 0.01$) and SPL V_u decreased from 19.6 ± 1.4 to 6.3 ± 2.1 $ml \cdot kg^{-1}$. SPL R_v and τ_v also decreased whereas SPL C_v increased. Phentolamine (0.2 $ml \cdot kg^{-1}$) at low P_{cs} partially reversed the decrease in capacitance whereas hexamethonium completely reversed it. In conclusion, changes in regional volumes and blood flow distribution are important components of the carotid sinus reflex and can be only partially explained by endogenous α-receptor activation.

B. INTRODUCTION

Previous studies have examined the ability of the carotid sinus reflex to mobilize blood from the total systemic circulation (6,10,28,34) as well as from isolated vascular regions (5,13,14,16), and have demonstrated a fall in total systemic and splanchnic volumes with decreases in carotid sinus pressure. Consequently, changes in venous tone have been thought to play a role in the regulation of arterial blood pressure through mobilization or sequestration of regional blood reserves (1,13,20). It remains unclear, however, whether the ability of the carotid sinus reflex to alter regional blood volumes results from changes in venous compliance, venous resistance, redistribution of blood flow or by shifts of the entire pressure-volume (P-V) curve (5,6,16). Furthermore, no attempt has been made to identify the neuro-humoral pathways responsible for these adjustments. In the present study, we examined the effect of a 150 mm Hg change in intrasinus pressure on regional 1) blood volumes, 2) P-V relationships and unstressed volumes, 3) blood flow distribution, 4) venous compliance and 5) venous resistance. We also studied the effect of α-adrenergic receptor blockade at low intrasinus pressure in order to determine the role of endogenous α-receptor agonists in the carotid sinus reflex.

C. METHODS

Surgical Procedures

Thirteen mongrel dogs of either sex with a mean weight of 32.9 ± 5.8 Kg (SD) were anesthetized with 10 $mg \cdot kg^{-1}$ of thiopental, followed by 100 $mg \cdot Kg^{-1}$ of α-chloralose. Additional doses of chloralose were administered as necessary. An endotracheal tube was inserted and the dogs were ventilated with O_2 enriched room air at an appropriate tidal volume and frequency for their size.

The right and left carotid sinuses were isolated by ligating the internal carotid artery approximately 1 cm distal to the sinus and the external carotid artery just distal to the lingual artery. The proximal parts of the common carotid arteries were cannulated with a Y connector and the pressure within the carotid sinus region (P_{cs}) was controlled by a non pulsatile pump with inflow from the right common carotid. The cervical vagosympathetic trunks were exposed and cut to eliminate the buffering effect of the aortic arch baroreceptor reflex and the cardiopulmonary receptor reflexes.

After a midline sternotomy and a ventral laparotomy were performed, heparin sodium was administered. A circulatory bypass was then achieved as explained in details in chapter 4. Twenty to 25 min were allowed before any measurements were made.

Instrumentation

Arterial pressure was measured in the left carotid artery. SVC, SPL and IVC venous pressures (P_v) were measured with

catheters placed at the junction of the veins and cannulae. The zero pressure reference point of the transducers was the midpoint of the right atrium (direct inspection). The flow probes were calibrated with a stop watch and a graduated cylinder at the beginning of the experiments and mechanical zeros were verified during every stop flow procedure (see below). Pressure, flow and densitometer (Waters Instruments, Minnesota, USA) signals were recorded on a Gould eight channel recorder. Flow and densitometer signals were further processed through an analog to digital converter (Data Translation DT2801) and sampled at 26 Hz/channel to the hard disk of a personal computer for later analysis.

Regional Blood Volume Measurement

Regional blood volumes were measured in the SPL and in the IVC regions as described in details in chapter 4. The amounts of indicator injected for the volume measurements were 1 mg for the SVC region and 1.5 mg for the SPL region. After the completion of every indicator dilution curve a stop flow was performed to measure the elastic recoil pressure at which the volume measurement was made. Blood volumes in the IVC region were not measured.

Measurement of Regional Mechanical Parameters

Regional mechanical parameters were measured using the technique of Mitzner and Goldberg (27) and Malo et al. (25) as described in details in chapter 5.

Protocol

In the first series of experiments (n = 7), intrasinus pressure was randomly set at either 50 (low P_{cs}) or 200 (high P_{cs}) mm Hg. Measurements of regional blood volumes were made first, followed by the measurement of regional mechanical parameters and a second measurement of blood volumes. Then, intrasinus pressure was changed to either 50 or 200 mm Hg and the measurements were repeated.

In the second series of experiments (n = 6), intrasinus pressure was fixed at 50 mm Hg. Regional blood volumes were measured, followed by regional mechanical parameters and a second measurement of blood volumes. An α-adrenergic receptor blocker, Phentolamine (0.2 $mg \cdot kg^{-1}$), was then given and the measurements were repeated. In two animals of this series, a third set of measurements was performed after ganglionic blockade with hexamethonium chloride (5 $mg \cdot kg^{-1}$).

Arterial blood gases were obtained before every set of measurements. The O_2 mixture and ventilatory rate were adjusted and/or bicarbonate sodium was given to maintain arterial PO_2 > 100 Torr, PCO_2 between 35 and 40 Torr, and pH between 7.35 and 7.40. The hematocrit values were > 38%. Rectal temperature was maintained at 38°C with the heat exchanger and a heating pad.

Analysis

SPL and PER venous compliances (C_v), venous resistances (R_v), time constant of venous drainage (τ_v), arterial resistances (R_a) and regional blood volumes (V_b) were

calculated as described in details in chapter 5.

The average of two blood volume measurements, before and after mechanical parameters measurements, was used. The average variation between these two measurements for the SVC region was 14.3 ± 7.2% (SD) and for the SPL region it was 12.8 ± 6.3%. An independent flow signal from the flow probe (Q_p) was used to calculate the amount of indicator recovered for any given dilution curve. By substituting Q_p in the equation for Q_D, we obtained the calculated amount of indicator recovered from a given indicator dilution curve. The difference between the amount recovered and the amount injected estimates the uptake of the indicator by the tissues and liver. In the SVC region, the calculated amount of indicator recovered was 101.9 ± 4.2% (SD), while in the SPL region it was 98.9 ± 6.8%.

Statistical Analysis

Data are presented as mean ± SE, unless otherwise stated. A t-test for repeated measures was used to assess the differences between the arterial pressures, fractional flows, venous resistances, venous compliances, time constants, blood volumes and unstressed volumes at carotid sinus pressures of 50 and 200 mm Hg as well as at P_{cs} of 50 mm Hg and with α-receptor blockade. The differences were considered to be significant at $P < 0.05$.

D. RESULTS

A linear regression analysis was used to assess the relationship between the flows obtained from indicator dilution curves (Q_D) and the flows obtained with the flow probes (Q_P) during the volume measurements (Figure 6.1). In both the SVC and SPL regions, the R value of the regression was 0.99. The slopes of these curves were not different from identity.

The relationship between plateau pressure and blood volume (P-V curves) was assessed by a linear regression and unstressed volume (V_u) at zero pressure was extrapolated from this relationship. The relationship between P_{vpl} and blood volume was linear for both regions over the ranges and under the conditions studied (Table 6.1). Attempts to fit the P-V curves with second and third order polynomials did not improve the R values.

Changes in Carotid sinus Pressure

The pattern and magnitude of the responses to changes in P_{cs} were similar whether P_{cs} was increased from 50 to 200 mm Hg or decreased from 200 to 50 mm Hg. Hence, the results were pooled together for analysis and are expressed as a decrease in P_{cs}.

At an intrasinus pressure of 200 mm Hg, splanchnic blood volume was 28.3 ± 1.9 $ml \cdot kg^{-1}$ (Table 6.2). With a decrease in P_{cs}, SPL blood volume fell by 9.0 ± 3.9 $ml \cdot kg^{-1}$ (SD) and SVC blood volume did not change. The elastic recoil pressures (P_{vpl}) at which the blood volumes were measured were 15.3 ± 1.1

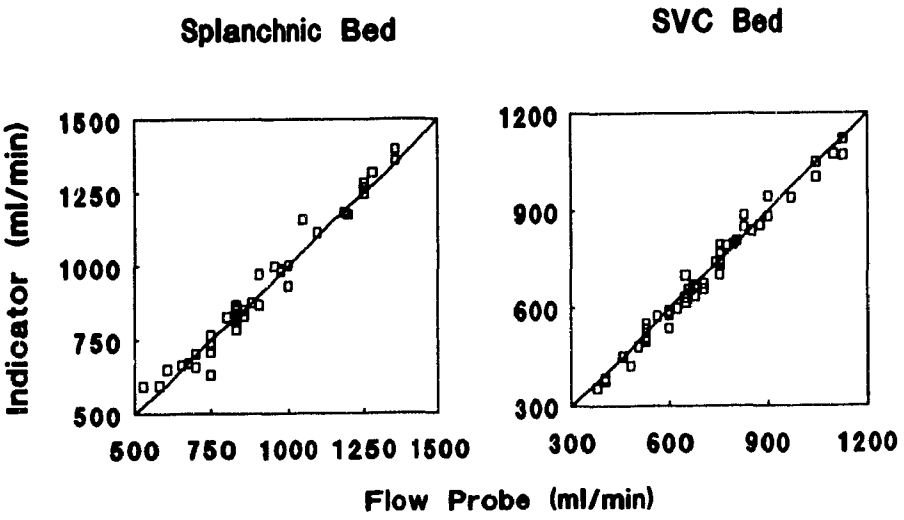

FIGURE 6.1 Relationship between blood flow measured with electromagnetic flow probes and by indicator dilution technique in the splanchnic and superior vena caval (SVC) regions. The solid lines represent identity. The slope of the regression analyses for these data were not significantly different from identity.

TABLE 6.1 Regression coefficients for the regression analyses of the pressure-volume curves of the splanchnic and superior vena caval regions.

Condition	SPL R Values	SVC R Values
P_{cs} = 50, mm Hg	0.91 ± 0.05	0.90 ± 0.02
P_{cs} = 200, mm Hg	0.92 ± 0.03	0.92 ± 0.02
P_{cs} = 50, mm Hg*	0.92 ± 0.03	0.94 ± 0.01
α-Receptor Blockade	0.96 ± 0.01	0.94 ± 0.03

Values are means ± SD. SVC, superior vena caval region; SPL, splanchnic region; P_{cs}, carotid sinus pressure. *, regression coefficients for the analyses of the two series of experiments are presented separately.

TABLE 6.2 Effect of changes in carotid sinus pressure on regional blood volume, unstressed volume and vascular mechanical parameters.

Parameter	Region	Condition	
		P_{cs} = 200	P_{cs} = 50
Blood Volume	SPL	28.3 ± 1.9	19.3 ± 1.2†
	SVC	12.5 ± 0.8	11.5 ± 0.9
Unstressed Volume	SPL	19.6 ± 1.4	6.3 ± 2.1‡
	SVC	9.1 ± 0.8	7.9 ± 0.8
Arterial Pressure		58.7 ± 4.1	104.6 ± 6.4†
Venous Compliance	SPL	0.54 ± 0.09	1.04 ± 0.18†
	PER	0.21 ± 0.05	0.27 ± 0.03
Venous Resistance	SPL	0.048 ± 0.009	0.021 ± 0.006†
	PER	0.017 ± 0.005	0.017 ± 0.003
Time Constant	SPL	23.9 ± 3.4	17.4 ± 0.5*
	PER	3.4 ± 0.6	4.7 ± 0.5
Arterial Resistance	SPL	0.150 ± 0.021	0.202 ± 0.025*
	PER	0.066 ± 0.011	0.156 ± 0.026†
Fractional Flows	SPL	30.2 ± 3.8	44.2 ± 3.9‡
	PER	69.8 ± 3.8	55.8 ± 3.9‡

Values are means ± SE. SVC, superior vena cava region; P_{cs}, carotid sinus pressure, mm Hg; volumes, $ml \cdot kg^{-1}$; pressures, mm Hg; compliances, $ml \cdot mm\ Hg^{-1} \cdot kg^{-1}$, resistances. $mm\ Hg \cdot ml^{-1} \cdot min^{-1} \cdot kg^{-1}$; time constant, sec; flows, $ml \cdot min^{-1} \cdot kg^{-1}$. *, †, ‡, significant level vs. P_{cs} = 200 mm Hg at $P < 0.05$, $P < 0.01$ and $P < 0.001$ level, respectively.

and 12.5 ± 1.3 mm Hg for SPL at high and low P_{cs} respectively. The corresponding values for the SVC were 14.4 ± 1.0 and 12.9 ± 1.3 mm Hg. These pressures were not significantly different. There was a shift to the left and a decrease in slope of the splanchnic P-V curve with a fall in P_{cs} (Figure 6.2). Little change occurred in the P-V curve of the peripheral region. At high P_{cs}, unstressed volume of the splanchnic region was 19.6 ± 1.4 $ml \cdot kg^{-1}$ and fell by 68% at an intrasinus pressure of 50 mm Hg (Table 6.2). SVC unstressed volume did not change.

Splanchnic venous compliance was more than double that of the peripheral region at a P_{cs} of 200 mm Hg (Table 6.2). Since the peripheral bed is actually two to three time larger then the splanchnic bed, SPL C_v is 4 to 6 times more compliant than PER C_v. The baroreflex efferent pathways appear to specifically regulate the splanchnic venous region since a decrease in P_{cs} increased SPL C_v by 93% and decreased SPL R_v and τ_v by 56% and 27% respectively, whereas the peripheral region was not affected (Table 6.2).

On average, a 150 mm Hg decrease in P_{cs} resulted in an increase in P_a of 43.4 ± 8.0 mm Hg (Table 6.2). The gain of the carotid sinus reflex control of total peripheral resistance was calculated by dividing the steady state change in P_a by the total change in P_{cs}. The average gain was 0.31 ± 0.06.

Splanchnic arterial resistance at high P_{cs} was twice as large as peripheral R_a (Table 6.2). Due to the difference in

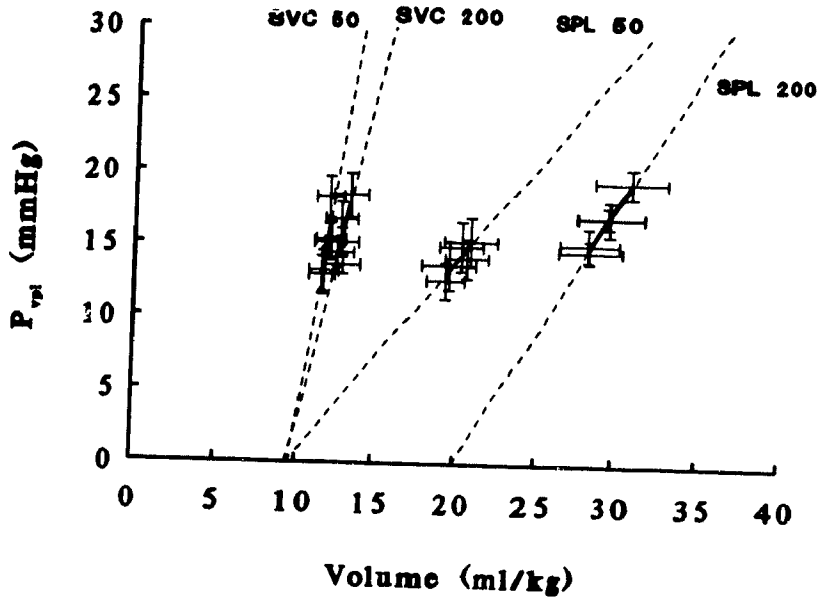

FIGURE 6.2 Relationship between venous plateau pressure (P_{vpl}) and blood volume for the splanchnic (SPL) and superior vena caval (SVC) regions at carotid sinus pressures of 50 and 200 mm Hg. The curves were determined by varying regional venous outflow pressure alternating with stop flow to obtain venous plateau pressures. The broken lines represent extrapolation to zero pressure of the curves to estimate unstressed volume. Values are means ± SEM; n = 7.

size of the two beds, SPL and PER arterial resistances were, in fact, similar. The baroreflex predominantly controlled arterial resistance of the peripheral bed since decreasing P_{cs} resulted in a 136% increase in PER R_a, whereas SPL R_a increased by only 35% (Table 6.2). In every single dog, when P_{cs} was decreased to 50 mm Hg, splanchnic blood flow increased and peripheral blood flow decreased reciprocally (Figure 6.3). The fractional flow to the splanchnic region was 30.2 ± 3.8% at high P_{cs} and increased to 44.2 ± 3.9% at low P_{cs} (Table 6.2).

Alpha Adrenergic Blockade

With intrasinus pressure held at 50 mm Hg, phentolamine resulted in a 5.1 ± 3.7 $ml \cdot kg^{-1}$ (SD) increase in SPL blood volume (elastic recoil pressure of 10 mm Hg, Table 6.3). SVC blood volumes did not change. There was a parallel shift to the right of the splanchnic P-V curve with α-receptor blockade (Figure 6.4). The values for SPL unstressed volume were 8.4 ± 3.2 $ml \cdot kg^{-1}$ at a P_{cs} of 50 mm Hg and 12.9 ± 3.2 $ml \cdot kg^{-1}$ with α-receptor blockade. These values were not significantly different. Phentolamine did not affect SVC unstressed volume and P-V curve.

Venous compliance did not change with α-receptor blockade but the venous resistances of both the splanchnic and peripheral regions decreased (Table 6.3). Only the time constant of venous drainage of the SPL region changed because of this decrease in resistance.

Arterial pressure decreased by 41% with phentolamine. This decrease was the result of comparable reductions in SPL

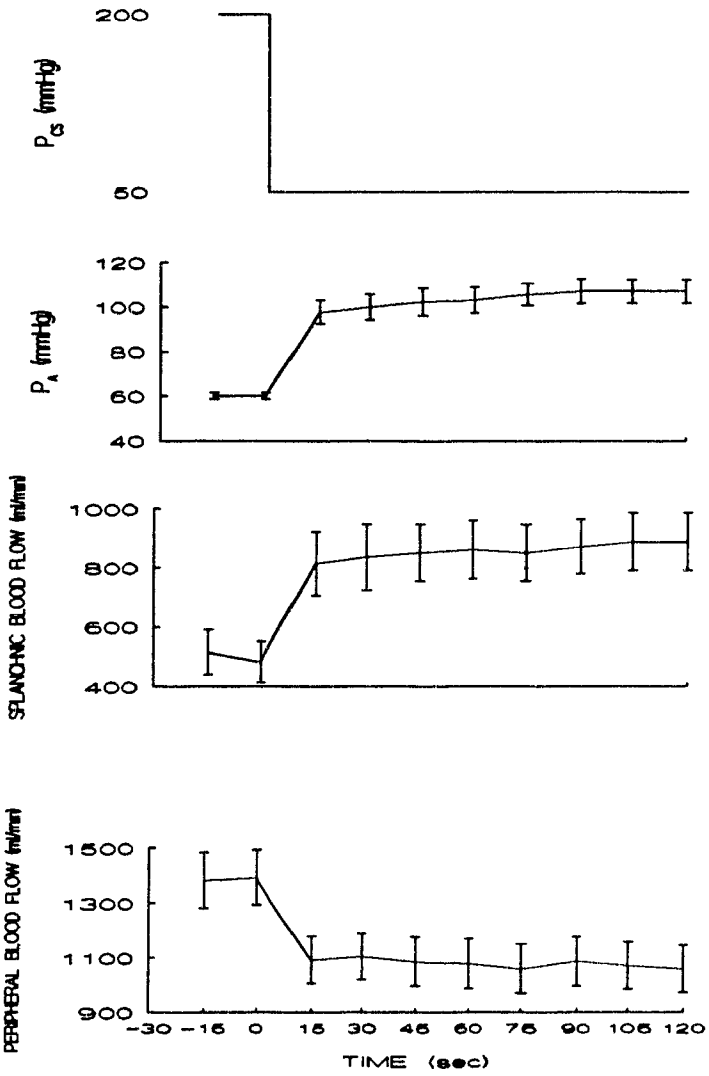

FIGURE 6.3 Time course of changes in mean arterial pressure (P_a) and venous return flows from splanchnic and peripheral vascular beds in response to decreasing carotid sinus pressure (P_{cs}). Decreasing P_{cs} caused an increase in P_a and splanchnic blood flow and a decrease in peripheral flow. Values are means ± SEM; n = 7.

TABLE 6.3 Effect of α-adrenergic receptor blockade at a carotid sinus pressure of 50 mm Hg on regional blood volume, unstressed volume and vascular mechanical parameters.

Parameter	Region	Condition	
		P_{cs} = 50	α-Receptor Blockade
Blood Volume	SPL	22.0 ± 2.7	27.6 ± 2.9*
	SVC	15.5 ± 0.8	16.2 ± 0.4
Unstressed Volume	SPL	8.4 ± 3.2	12.9 ± 3.2
	SVC	11.4 ± 1.2	11.9 ± 0.6
Arterial Pressure		156.8 ± 11.7	92.8 ± 5.2†
Venous Compliance	SPL	1.43 ± 0.18	1.58 ± 0.25
	PER	0.40 ± 0.09	0.40 ± 0.05
Venous Resistance	SPL	0.016 ± 0.003	0.009 ± 0.001*
	PER	0.017 ± 0.004	0.012 ± 0.003*
Time Constant	SPL	18.3 ± 1.8	12.6 ± 1.1*
	PER	5.0 ± 0.8	3.8 ± 0.5
Arterial Resistance	SPL	0.215 ± 0.036	0.124 ± 0.018*
	PER	0.192 ± 0.027	0.115 ± 0.019†
Fractional Flows	SPL	48.0 ± 4.7	48.1 ± 3.8
	PER	52.0 ± 4.7	51.9 ± 3.8

Values are means ± SE. SVC, superior vena cava region; P_{cs}, carotid sinus pressure, mm Hg; volumes, $ml \cdot kg^{-1}$; pressures, mm Hg; compliances, $ml \cdot mm\ Hg^{-1} \cdot kg^{-1}$, resistances, $mm\ Hg \cdot ml^{-1} \cdot min^{-1} \cdot kg^{-1}$; time constant, sec; flows, $ml \cdot min^{-1} \cdot kg^{-1}$. *, †, significant level vs. P_{cs} = 50 mm Hg at $P < 0.05$ and $P < 0.01$ level, respectively.

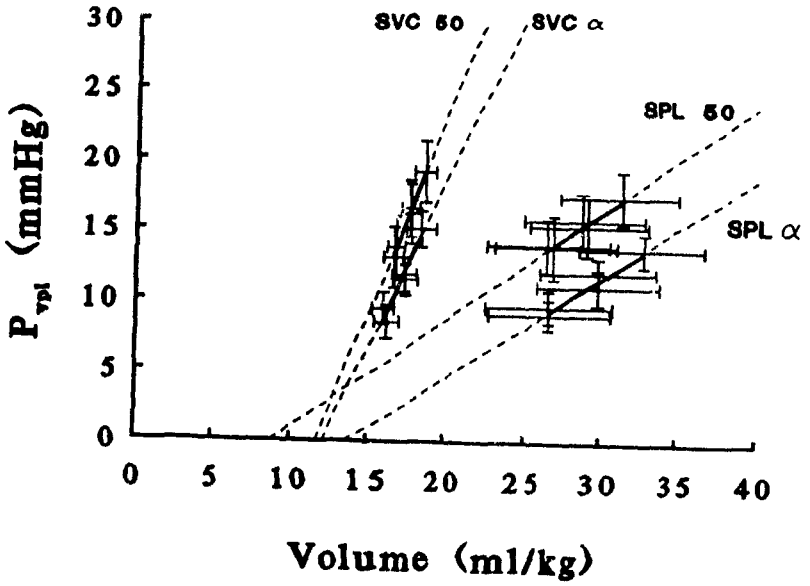

FIGURE 6.4 Relationship between venous plateau pressure (P_{vpl}) and blood volume for the splanchnic (SPL) and superior vena caval (SVC) regions at carotid sinus pressures (P_{cs}) of 50 mm Hg and with α-adrenergic receptor blockade. The curves were determined in the same way as in Figure 6.2. The broken lines represent extrapolation to zero pressure of the curves to estimate unstressed volume. Values are means ± SEM; n = 6.

and PER arterial resistances and, therefore, the fractional flow to these beds did not change (Table 6.3).

In two animals, hexamethonium was superimposed on α-receptor blockade. In each of these animals, hexamethonium injection resulted in a further shift to the left of the splanchnic P-V curve. SVC P-V curve did not change. The data from the two animals were grouped and are shown in Figure 6.5.

E. DISCUSSION

We examined the open-loop responses of the carotid sinus reflex to intrasinus variations of 150 mm Hg and the effect of α-receptor blockade at low intrasinus pressure. With decreases in intrasinus pressure (hypotension), there was a 75% increase in arterial resistance, but the rise was greater in the extrasplanchnic than in the splanchnic bed. As a result, the fraction of flow to the splanchnic bed increased. In the intact animal, this increase in flow would reduce venous return and cardiac output because the splanchnic bed is more compliant than the extrasplanchnic bed. The decrease in cardiac output would further decrease arterial pressure. Concurrently, however, splanchnic venous resistance decreased by 56% and the splanchnic time constant of venous drainage, as a result, was reduced. This reduction in venous resistance would increase venous return and cardiac output in the intact animal and would help maintain arterial pressure. Additionally, there was a shift to the left of the splanchnic P-V curve and a consequent decrease in unstressed volume.

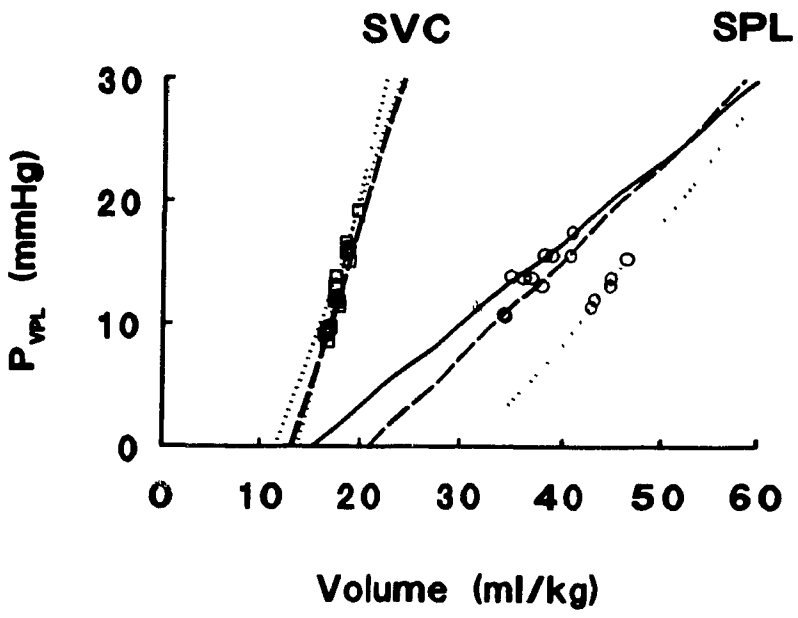

FIGURE 6.5 Relationship between venous plateau pressure (P_{vpl}) and blood volume for the splanchnic (SPL) and superior vena caval (SVC) regions at carotid sinus pressures of 50 mm Hg (solid), with α-adrenergic receptor blockade (long dash) and with ganglionic blockade (dots). The curves were determined in the same way as in Figure 6.2. Values are means; n = 2.

This decrease in V_u would increase stressed volume (total volume is constant) which would raise mean systemic pressure and venous return. The net effect of all these changes would have been a 110% increase in venous return. When this ability to increase venous return is added to the 78% increase in arterial pressure (through the direct effect on the arterial bed), the potential of the carotid sinus reflex to restore arterial pressure during hypotension becomes tremendous.

In the second series of experiments, the α-receptor antagonist phentolamine completely reversed the increase in arterial resistance but only partially reversed the change in splanchnic capacitance. On the other hand, ganglionic blockade further increased splanchnic capacitance. Thus, endogenous α-receptor agonists seem to be responsible for only part of the open-loop response. Before discussing these results, some methodological considerations will be addressed.

The present preparation deviates greatly from the intact conscious animal. The anesthesia, surgical procedure, homologous donor blood, pump and tubing inevitably alter the physiological response to external stimuli. Notwithstanding these limitations, we were able to repeatedly measure regional blood flows, blood volumes, unstressed volumes and vascular mechanical parameters at different carotid sinus pressures. Although bleeding is inevitable with a bypass preparation, volume replacement with dextran did not significantly alter the hematocrit value.

Efforts were made to estimate the error in the

measurements of regional blood volume due to the loss of indocyanine green in the liver, other tissues, or by hemorrhage. The calculated amounts of indicator recovered showed that all of the cardiogreen was recovered from the SVC region and over 98% was recovered from the SPL region.

Our preparation combined two open-loop systems. An isolated carotid sinus preparation was used to look at the control of the circulation by the baroreflex, independent of negative feedback on the carotid sinus receptors and without the influence of the cardiopulmonary receptors. Also, the bypass preparation eliminated the influence of changes in right atrial pressure, cardiac contractility and heart rate on the periphery. Although these changes are a functional part of the circulatory response of the baroreceptor reflex in the intact animal, the purpose of the study was to examine the effect of changes in carotid sinus pressure on venous return parameters. Allowing changes in right atrial pressure and cardiac output would affect regional flows and volumes and would contaminate the measurements. Thus, in order to study the direct effect of the baroreceptor reflex on venous return it is necessary to maintain right atrial pressure and cardiac output constant.

Blood Flow Distribution

The increase in arterial resistance of the extrasplanchnic region was greater than that of the splanchnic regions with decreases in intrasinus pressure. Others have also found nonuniformities in the distribution of sympathetic

outflow with changes in carotid sinus pressure (2,24,26,29, 35-37). Selective sympathetic outflow to the extrasplanchnic region has a potential adaptive value for the animal because the skeletal muscle bed is roughly 2 to 3 times larger than the splanchnic bed, has a high resting arterial resistance and is metabolically inactive at rest. Thus, changes in extrasplanchnic arterial resistance will have a large impact on arterial resistance and arterial pressure with less metabolic consequences than similar changes in splanchnic arterial resistance.

Because of the larger increase in extrasplanchnic arterial resistance, the fraction of blood flow to the splanchnic region will increase. Since the splanchnic region has a larger venous compliance (and time constant of venous drainage) than the extrasplanchnic region, an increase in the fractional flow to the splanchnic bed will produce a passive increase in volume of this region and venous return will decrease. However, the negative impact of this increase in splanchnic blood flow was more than compensated for by decreases in venous resistance and capacitance.

Others have not found a consistent redistribution of blood flow with changes in P_{cs} (6,16). Of importance is that, unlike in our study, these investigators included renal blood flow in the splanchnic compartment. This could obscure changes in blood flow redistribution because the renal bed has a fast time constant of venous drainage (25) and, when included in the splanchnic circulation, accounts for almost

half of total splanchnic flow. Furthermore, renal blood flow is autoregulated during baroreceptor stimulation in conscious (15,19,21) and anesthetized (4,21,22,26,29) animals, so that half the change in splanchnic blood flow could be counteracted by autoregulation in the kidneys.

Changes in Venous Resistance

Measurements of R_v at different P_{cs} have not been obtained before. Total resistance of the splanchnic region increases with decreases in P_{cs} (4,6,8,16,21,22,26,29). Although these measurements have included both R_a and R_v, they represent mostly changes in arterial resistance since R_a is up to 10 times larger than R_v.

We found a large decrease in venous resistance with decreases in P_{cs}. This change in R_v is functionally important because a decrease in R_v reduces the capacity of the splanchnic bed to retain volume and is mechanically similar to a decrease in downstream pressure. Thus, blood leaves the splanchnic bed without active contraction of the venous smooth muscles and there is a downward movement on the P-V curve (see Fig. 6.4). Furthermore, increases in the fractional flow to the splanchnic bed will result in less accumulation of volume than otherwise predicted.

Changes in venous resistance have been attributed to activation of ß-receptor agonists. Isoproterenol decreases vascular volume (12,17,18,31) through a decrease in splanchnic R_v (12,31). Our results demonstrate that reflexly induced decreases in R_v can reduce splanchnic blood volumes. Whether

or not the decrease in venous resistance is mediated through ß-activation or through other mechanisms remains to be verified.

Changes in Venous Compliance

Splanchnic C_v doubled when carotid sinus pressure was decreased. Changes in splanchnic C_v of this magnitude have not been observed before (6,16) and are in the opposite direction of what may have been predicted. One explanation may be that at high intrasinus pressures and high vascular volumes the splanchnic bed is on the linear part of the P-V curve whereas at low P_{cs} and decreases in volume, the splanchnic bed is on the curvilinear part of the P-V curve (23). At least in this study, therefore, decreases in splanchnic venous compliance are not part of the mechanism of volume mobilization by the carotid sinus reflex.

Changes in Capacitance

Decreases in vascular volumes with decreasing carotid sinus pressure have been described previously (3,5,6,8,14,16, 28,30,32-34). The shift in volume in these studies ranged from 1.25 (28) to 12.9 $ml \cdot kg^{-1}$ (30). Differences in the magnitude of the change in P_{cs} are mainly responsible for this wide range. In studies in which the splanchnic bed was separated from the rest of the circulation, decreases in P_{cs} over the full range of sensitivity reduced splanchnic volumes by 4 to 6.5 $ml \cdot kg^{-1}$ (5,6,8,14). We found a decrease in total capacitance of 9 $ml \cdot kg^{-1}$ with a decrease in P_{cs}, all of which was from the splanchnic bed. This decrease in capacitance was

due to active constriction of venous smooth muscles and can be visualized as a shift to the left of the P-V curve (Figure 6.2).

Changes in splanchnic and peripheral unstressed volumes at different intrasinus pressures have not been measured before. These measurements are crucial because in the intact animal changes in unstressed volume result in reciprocal changes in stressed volume, a major determinant of venous return. A fall of 150 mm Hg in intrasinus pressure reduced splanchnic unstressed volume by $\approx$ 13 ml$\cdot$kg^{-1} but did not affect extrasplanchnic V_u.

Effect of α-Adrenergic Receptor Blockade

Alpha receptor blockade was used to examine contribution of endogenous α-receptor agonists to the carotid sinus reflex. To quantify the effect of α-receptor blockade on the arterial and venous components of the baroreflex, the vasodilatory effects of phentolamine at low P_{cs} were compared to those of an increase in intrasinus pressure. On the arterial side, a rise in intrasinus pressure decreased arterial pressure by 44% whereas phentolamine decreased P_a by 41%. Therefore, the arterial component of the increase in sympathetic outflow was completely abolished by α-receptor blockade suggesting endogenous α-adrenergic receptor activation.

On the venous side of the circulation, splanchnic capacitance increased by 47% with a rise in intrasinus pressure. However, α-receptor blockade at low P_{cs} increased splanchnic capacitance by only 25%. Thus, it appears that α-

adrenergic receptor activation is responsible for only half of the baroreflex induced venoconstriction. The mediator for the other half of the venoconstriction is unknown. Nevertheless, this mediator appears to be released through sympathetic activation since ganglionic blockade shifted the splanchnic P-V curve further to the right (Figure 6.5). Possible candidates include norepinephrine, through ß-receptor vasoconstriction, or more likely, the release of angiotensin II or neuropeptide Y from the sympathetic nerve terminals.

The injection of phentolamine resulted in a further decrease in splanchnic venous resistance. As can be seen on the P-V curve (Figure 6.4), the fall in resistance resulted in a decrease in volume and pressure and, therefore, in a downward movement on the curve. This suggests that at least part of the changes in venous resistance are mediated through α-receptor activation. When intrasinus pressure was decreased from 200 to 50 mm Hg, there was a concomitant vasoconstriction of the capacitance vessels (half of which was mediated through α-receptors) and decrease in venous resistance (probably through vasodilatation of larger veins or of hepatic sphincters). One way to explain this dual constriction and dilatation at high sympathetic tone is through the presence of a greater density of ß-receptor in the larger resistive veins as compared to the small capacitance venules. Norepinephrine released from nerve terminals would then result in the vasoconstriction of the capacitance vessels (predominant α-effect) and the vasodilatation of the larger veins

(predominant ß-effect). The further decrease in resistance with α-blockade by phentolamine can be explained by unopposed ß-receptor activation in the large veins.

Baroreceptor Reflex Control of Arterial Pressure

In order to evaluate the overall baroreflex control of arterial pressure, it is necessary to examine the response of both, resistance and capacitance vessels. Since the direct effect of a decrease in intrasinus pressure on the arterial bed has already been discussed, we will focus here on the potential increase in venous return resulting from the changes in venous parameters. Assuming a two compartment model of the circulation and a right atrial pressure of 0 mm Hg, venous return is determined by the equation $VR = V/(F_f\tau_f+F_s\tau_s)$ where V is stressed volume (total blood volume minus unstressed volume), F is the fractional flows to the slow ($_s$) and fast ($_f$) time constant beds and τ is the product of venous resistance and compliance (7,9,11). The decrease in intrasinus pressure would have affected all of the determinants in this equation. By altering the value of these determinants one at a time and calculating the resulting change in venous return, their respective contribution to the control of arterial pressure can be evaluated.

Decreasing splanchnic τ_v from 23.9 to 17.4 sec would increase venous return by 26%, increasing the fractional flow to the splanchnic bed would decrease venous return by 23% and increasing stressed volume by 13 $ml \cdot kg^{-1}$ would increase venous return by 111%. If all of the changes are applied at the same

time venous return would increase by 110%. This analysis is enlightening because it demonstrates that the trade-off for increasing extrasplanchnic arterial resistance (increased splanchnic blood flow) is counterbalanced by a decrease in the splanchnic venous time constant.

Changes in Fractional Flows and Changes in Venous Return

The paper by Caldini, Permutt, Waddell and Riley (7) brought about some controversy because it inferred that the major mechanism of increase in cardiac output with epinephrine is the redistribution of blood flow to fast time constant beds and the increase in venous return is due to a passive shift of blood from the slow time constant bed. In their analysis, venous tone had little or no effect on venous return. In other studies, epinephrine was found to have a large effect on venous tone. We think the results obtained here may resolve some of this controversy. In the study by Caldini et al., arterial pressure increased by close to 50% of control values during the infusion of epinephrine. Therefore, according to our results, the increase in carotid sinus pressure should have produced a greater decrease in arterial resistance in the extrasplanchnic bed than in the splanchnic bed. With constant cardiac output, extrasplanchnic blood flow should have increased while splanchnic blood flow should have decreased. This is exactly what these authors observed. Therefore, we believe that the redistribution of blood flow was not due to the effect of epinephrine per se, but to the baroreceptor reflex. In further support for this statement,

they found a significant decrease in venous compliance, a 112% increase in venous resistance and a 52% increase in time constant of the slow time constant bed. These results are very similar to our observations in the splanchnic bed with an increase in carotid sinus pressure. In contrast, Mitzner and Goldberg (27) kept a constant arterial pressure and found an increase in splanchnic blood flow and a decrease in extrasplanchnic flow with epinephrine infusion.

In summary, the maintenance of constant arterial pressure by the carotid sinus baroreflex is achieved through the control of resistance as well as capacitance vessels. On the arterial side, preferential increases in extrasplanchnic arterial resistance provide a fast and effective defence against hypotension. This control of arterial resistance is achieved through α-adrenergic receptors. On the venous side, the story is more complex. The baroreflex appears to control only the splanchnic region. Carotid hypotension results in a shift to the left of the splanchnic P-V curve (venoconstriction) and a decrease in venous resistance (relaxation). Only half of this shift in the P-V curve can be explained through α-receptor activation.

F. REFERENCES

1. ALEXANDER, R.S. The participation of the venomotor system in pressor reflexes. *Circ. Res.* 2: 405-409, 1954.

2. BAGSHAW, R.J., AND R.H. COX. Baroreceptor control of regional haemodynamics during halothane anaesthesia in the dog. *Br. J. Anaesth.* 49: 535-543, 1977.

3. BENNETT, T.D., C.R. WYSS, AND A. SCHER. Changes in vascular capacity in awake dogs in response to carotid sinus occlusion and administration of catecholamine. *Circ. Res.* 55: 440-453, 1984.

4. BOND, R.F., AND H.D. GREEN. Cardiac output redistribution during bilateral common carotid occlusion. *Am. J. Physiol.* 216: 393-403, 1969.

5. BRUNNER, M.J., A.S. GREENE, A.E. FRANKLE, AND A.A. SHOUKAS. Carotid sinus baroreceptor control of splanchnic resistance and capacity. *Am. J. Physiol.* 255(24): H1305-H1310, 1988.

6. BRUNNER, M.J., A.A. SHOUKAS, AND C.L. MACANESPIE. The effect of the carotid sinus baroreceptor reflex on blood flow and volume redistribution in the total systemic vascular bed of the dog. *Circ. Res.* 48: 274-285, 1981.

7. CALDINI, P., S. PERMUTT, J.A. WADDELL, AND R.L. RILEY. Effect of epinephrine on pressure, flow, and volume relationships in the systemic circulation of dogs. *Circ. Res.* 34: 606-623, 1974.

8. CARNEIRO, J., AND D. DONALD. Blood reservoir function of dog spleen, liver, and intestine. *Am. J. Physiol.* 232(1): H67-H72, 1977.

9. COLEMAN, T.G., JR,R.A. MANNING, R.A. NORMAN, AND A.C. GUYTON. Control of cardiac output by regional blood flow distribution. *Ann. Biomed. Eng.* 2: 149-163, 1975.

10. DREES, J.A., AND C.F. ROTHE. Reflex venoconstriction and capacity vessel pressure-volume relationships in dogs. *Circ. Res.* 34: 360-373, 1974.

11. GREEN, J.F. Pressure-flow and volume-flow relationships of the systemic circulation of the dogs. *Am. J. Physiol.* 229: 761-769, 1975.

12. GREEN, J.F. Mechanism of action of isoproterenol on venous return. *Am. J. Physiol.* 232(2): H152-H1566, 1977.

13. HADJIMINAS, J., AND B. ÖBERG. Effects of carotid baroreceptor reflexes on venous tone in skeletal muscle and intestine of the cat. *Acta Physiol. Scand.* 72: 518-532, 1968.

14. HAINSWORTH, R., AND F. KARIM. Responses of abdominal vascular capacitance in the anaesthetized dog to changes in the carotid sinus pressure. *J. Physiol. Lond.* 262: 659-677, 1976.

15. HIGGINS, C.B., S.F. VATNER, D.L. EKBERG, AND E. BRAUNWALD. Alteration in the baroreceptor reflex in the conscious dog with heart failure. *J. Clin. Invest.* 51: 716-724, 1972.

16. HOKA, S., Z.J. BOSNJAK, D. SIKER, R. LUO, AND J.P. KAMPINE. Dynamic changes in venous outflow by baroreflex and left ventricular distension. *Am. J. Physiol.* 254(23): R212-R221, 1988.

17. IMAI, Y., K. SOTOH, AND N. TAIRA. Role of the peripheral vasculature in changes in venous return caused by isoproterenol, norepinephrine, and methoxamine in anaesthetized dogs. *Circ. Res.* 43: 553-561, 1978.

18. KAISER, G.A., JR.,J. ROSS, AND E. BRAUNWALD. Alpha and beta adrenergic receptors mechanisms in the systemic venous bed. *J. Pharmacol. Exp. Ther.* 144: 155-162, 1964.

19. KIRCHHEIM, H. Effect of common carotid occlusion on arterial blood pressure and on kidney blood flow in unanesthetized dogs. *Arch. Ges. Physiol.* 306: 119-134, 1969.

20. KIRCHHEIM, H. Systemic arterial baroreceptor reflexes. *Physiol. Rev.* 56: 100-176, 1976.

21. KIRCHHEIM, H., AND R. GROSS. Das verhalten der nierendurchblutung und des neirenumfangs bei blurdruckstegerungen durch doppelsetigen corotisverschlu beta oder schritmachertachykardiw. Untersuchungen zur autoregulation der neirenurchbluntung am wachen hund. *Arch. Ges. Physiol.* 315: 159-172, 1970.

22. KRAMER, K. Die stellung der niere im gesamtkreislauf. *Verhandl. Deut. Ges. Innere. Med.* 65: 225-241, 1959.

23. LEE, R.W., L.D. LANCASTER, R.G, GAY, M. PAQUIN, AND S. GOLDMAN. Use of acetylcholine to measure total vascular pressure-volume relationship in dogs. *Am. J. Physiol.* 254(23): H115-H119, 1988.

24. LÖFVING, B. Differentiated vascular adjustments reflexly induced by changes in the carotid baro- and chemoreceptor activity and by asphyxia. *Med. Exp.* 4: 307-312, 1961.

25. MALO, J., H. GOLDBERG, R. GRAHAM, H. UNRUH, AND C. SKOOG. Effect of hypoxic hypoxia on systemic vasculature. *J. Appl. Physiol.* 56(5): 1403-1410, 1984.

26. MCGIFF, J.G., AND D.M. AVIADO. Differential response of renal and femoral blood flow and vascular resistance. *Circ. Res.* 9: 1327-1335, 1961.

27. MITZNER, W., AND H. GOLDBERG. Effects of epinephrine on resistive and compliant properties of the canine vasculature. *J. Appl. Physiol.* 39(2): 272-280, 1975.

28. MÜLLER-RUCHHOLTZ, E.R., E. GRUND, F. HOUER, AND E.R. LAPP. Effect of carotid pressorreceptor stimulation on integrated systemic venous bed. *Basic Res. Cardiol.* 74: 467-476, 1979.

29. POLOSA, C., AND G. ROSSI. Cardiac output and peripheral blood flow during occlusion of the carotid arteries. *Am. J. Physiol.* 200: 1185-1190, 1961.

30. ROSS, JR., J., C.J. FRAHM, AND E. BRAUNWALD. Influence of carotid baroreceptors and vasoactive drugs on systemic vascular volume and venous distensibility. *Circ. Res.* 9: 75-82, 1961.

31. RUTLEN, D.L., E.N. SUPLE, AND JR.,W.J. POWELL. ß-Adrenergic regulation of total systemic intravascular volume in the dog. *Circ. Res.* 48: 112-120, 1981.

32. SHOUKAS, A.A., AND M.J. BRUNNER. Epinephrine and the carotid sinus baroreceptor reflex. *Circ. Res.* 47: 249-257, 1980.

33. SHOUKAS, A.A., C.L. MACANESPIE, M.J. BRUNNER, AND L. WATERMEIER. The importance of the spleen in blood-volume shifts on the systemic vascular bed caused by the carotid sinus baroreceptor reflex in the dog. *Circ. Res.* 49: 759-766, 1981.

34. SHOUKAS, A.A., AND K. SAGAWA. Control of total systemic vascular capacity by the carotid sinus baroreceptor reflex. *Circ. Res.* 33: 22-33, 1973.

35. VATNER, S.F., D. FRANKLIN, R.L. VAN CITTERS, AND E. BRAUNWALD. Effects of carotid sinus nerve stimulation on the coronary circulation of the conscious dog. *Circ. Res.* 28: 11-21, 1970.

36. WALLIN, G., W. DELIUS, AND K.E. HAGBARTH. Comparison of sympathetic nerve activity in normotensive and hypertension subjects. *Circ. Res.* 33: 9-21, 1973.

37. WENNERGREN, G. Aspects of central integrative and efferent mechanisms in cardiovascular reflex control. *Scand. Suppl.* 428: 1-53, 1975.

CHAPTER 7

THE INFLUENCE OF NEUROPEPTIDE Y ON REGIONAL CAPACITANCE IN DOGS

A. ABSTRACT

To determine the effect of neuropeptide Y on regional vascular capacitance, we measured regional blood volume (V_b), unstressed volume (V_u), blood flow distribution, venous compliance (C_v), venous resistance (R_v) and the time constant of venous drainage (τ_v) in 9 dogs anesthetized with chloralose and on circulatory bypass; cardiac output was kept constant. The systemic circulation was divided into a splanchnic (SPL) and an extrasplanchnic (PER) region. PER was further divided into a superior vena caval (SVC) and an inferior vena caval (IVC) region. V_b was measured in the SPL and SVC regions using indicator dilution curves and the mean transit times. Changes in venous outflow pressures were used to construct pressure volume (P-V) curves and to calculate C_v, R_v and τ_v. V_u was extrapolated from the P-V curves. Hexamethonium chloride was infused throughout the experiment to prevent changes in baroreceptor activity and the effects of endogenous release of epinephrine, norepinephrine and NPY. NPY (300 to 600 μg bolus and 15$\mu g \cdot min^{-1}$) increased arterial blood pressure from 98.4 $\pm$ 4.2 to 121.3 $\pm$ 6.9 mm Hg ($P < 0.001$), decreased SPL V_b from 31.6 $\pm$ 2.1 to 24.9 $\pm$ 1.8 $ml \cdot kg^{-1}$ ($P < 0.05$) and decreased V_u from 21.8 $\pm$ 2.6 $ml \cdot kg^{-1}$ to 12.1 $\pm$ 2.2 ($P < 0.001$). SVC volumes were not affected by NPY. Peripheral C_v increased with NPY but SPL C_v did not change. Thus, exogenous NPY infusion can actively decrease splanchnic capacitance which suggests a role for this neuropeptide in circulatory homeostasis.

B. INTRODUCTION

Neuropeptide Y (NPY) is a 36 amino acids peptide present in sympathetic nerve terminals and co-released with norepinephrine upon nerve stimulation (8,11). Unlike norepinephrine, NPY produces a slowly developing, long-lasting vasoconstriction which is resistant to alpha adrenergic blockade (5,12). NPY like immunoreactivity has been established on both arteries and veins (6) and the plasma level of NPY increases during stress in rats (18) and after exercise in humans (11). Although these findings suggest a role for NPY in the control of peripheral vascular tone, very little is known about its effect on total circulatory homeostasis and more particularly, on capacitance vessels.

We have recently found a decrease in the capacitance of the splanchnic bed during heat stress. This decrease could not be blocked by α or ß-adrenergic antagonists but was reversed by ganglionic blockade (4). Consequently, we hypothesized the release of a non-adrenergic substance from the peripheral nerve endings of venous smooth muscles and turned our attention to NPY as a possible constrictor of capacitance vessels. Therefore, the present experiments were undertaken to determine the effect of NPY on a) regional pressure-volume curves, b) unstressed volume, c) venous compliance and resistance and d) distribution of cardiac output.

C. METHODS

Animal Preparation

Nine mongrel dogs of either sex with a mean weight of 30.1 ± 5.4 kg (SD) were anaesthetized with 10 $mg \cdot kg^{-1}$ of thiopental and 100 $mg \cdot kg^{-1}$ α-chloralose; additional doses of chloralose were administered as necessary. Body temperature was maintained at 38°C with a heating pad and a heat exchanger. An endotracheal intubation was performed and the dogs were oxygenated with a mixture of 100% O_2 and room air at an appropriate tidal volume and frequency for their size. A median sternotomy and laparotomy were performed. The spleen was functionally removed by injecting 2 mg of epinephrine in the splenic artery and, after contraction, tying off all arteries and veins leading to and from this organ. Heparin sodium was administered before cannulation of the veins and right atrium. A circulatory bypass was then achieved as described in detail in chapter 4. Twenty to 25 min were allowed before any measurements were made.

Instrumentation

Arterial pressure (Trantec transducers) was measured in the left carotid artery. SVC, SPL and IVC venous pressures were measured with catheters placed at the junction of the veins and cannulae. The transducers were referenced to the right atrium by direct inspection. The flow probes were calibrated with a stop watch and a graduated cylinder at the beginning of the experiments and mechanical zeros were verified during every stop flow procedure. Pressure, flow and

densitometer (Waters Instruments, Minnesota, USA) signals were recorded on a Gould eight channel recorder. Flow and densitometer signals were further processed through an analog to digital converter (Data Translation DT2801) and sampled at 26 Hz/channel to the hard disk of a personal computer for later analysis.

Regional Blood Volume Measurement

Regional blood volumes were measured in the SPL and in the IVC regions as described in details in chapter 4. The amounts of indicator injected for the volume measurements were 1 mg for the SVC region and 1.5 mg for the SPL region. After the completion of every indicator dilution curve a stop flow was performed to measure the elastic recoil pressure at which the volume measurement was made. Blood volumes in the IVC region were not measured.

Measurement of Regional Mechanical Parameters

Regional mechanical parameters were measured using the technique of Mitzner and Goldberg (16) and Malo et al. (15) as described in details in chapter 5.

Protocol

Two conditions were studied. In the first, hexamethonium chloride (HEX, 5 $mg \cdot kg^{-1}$ bolus, and 2 $mg \cdot kg^{-1}$ every ½ hour) was given and vascular measurements were made, then NPY (300 to 600 μg followed by 15 $\mu g \cdot min^{-1}$, porcine NPY, INRS Santé, Montreal, CAN) was superimposed on HEX and the measurements were repeated. Under every condition, measurements of regional blood volumes were made first, followed by the

measurement of regional mechanical parameters and a second measurement of blood volumes.

Arterial blood gases were obtained before every set of measurements. The O_2 mixture and ventilatory rate were adjusted and/or bicarbonate sodium was given to maintain arterial PO_2 > 100 Torr, PCO_2 between 35 and 40 Torr, and pH between 7.35 and 7.40. The hematocrit values were > 38%.

Analysis

SPL and PER venous compliances (C_v), venous resistances (R_v), time constant of venous drainage (τ_v), arterial resistances (R_a) and regional blood volumes (V_b) were calculated as described in details in chapter 5.

The average of two blood volume measurements, before and after mechanical parameters measurements, was used. The average variation between these two measurements was 44.5 ± 30.1 ml (SD) which represents 6.4 ± 3.8% variation between measurements. An independent flow signal from the flow probe (Q_p) was used to calculate the amount of indicator recovered for any given dilution curve. By substituting Q_p in the equation for Q_D, we obtained the calculated amount of indicator recovered from a given indicator dilution curve. The difference between the amount recovered and the amount injected estimates the uptake of the indicator by the tissues and liver. In the SVC region, the calculated amount of indicator recovered was 104.4 ± 6.1% (SD), while in the SPL region it was 95.9 ± 5.7%.

Statistical Analysis

Data are presented as mean ± SE, unless otherwise stated. A t-test for repeated measures was used to assess the differences between the arterial pressures, venous resistances, venous compliances, time constants, blood volumes and unstressed volumes during hexamethonium and with NPY infusion. The differences were considered to be significant at $P < 0.05$.

D. RESULTS

A linear regression was used to assess the relationship between Q_D and Q_p (Figure 7.1). In the SVC region, the r value was 0.98, in the SPL region, the r value was 0.99. The slopes of these curves were not different from identity.

The relationship between plateau pressure and blood volume (P-V curves) was assessed by a linear regression and extrapolation to zero pressure of this relationship was used to calculate unstressed blood volume. The relationship between P_{vpl} and blood volume was linear for both regions over the range studied (Figure 7.2). The mean r values were 0.96 ± 0.04 (SD) for SPL and 0.95 ± 0.04 for SVC with hexamethonium and 0.94 ± 0.05 and 0.91 ± 0.08 with NPY, respectively. Attempts to fit the P-V curves with second and third order polynomials did not improve the r values.

Arterial blood pressure increased during the infusion of NPY from 98.4 ± 4.2 to 121.3 ± 6.9 mm Hg ($P < 0.001$). The mean cardiac output with hexamethonium was 98.7 ± 5.0 $ml \cdot min^{-1}$.

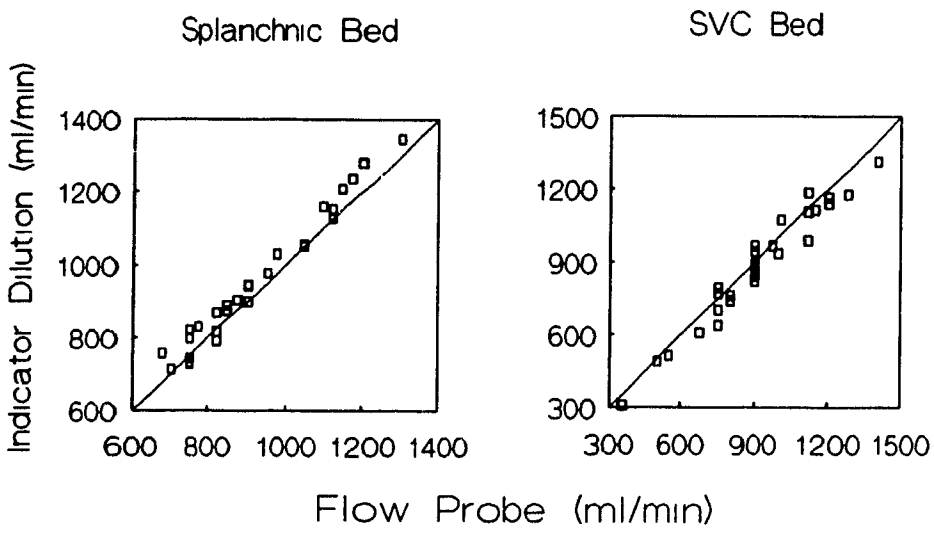

Figure 7.1 Relationship between blood flow measured with electromagnetic flow probes and by dye dilution technique in the splanchnic (SPL, left panel) and superior vena caval (SVC, right panel) regions. The solid lines represent identity.

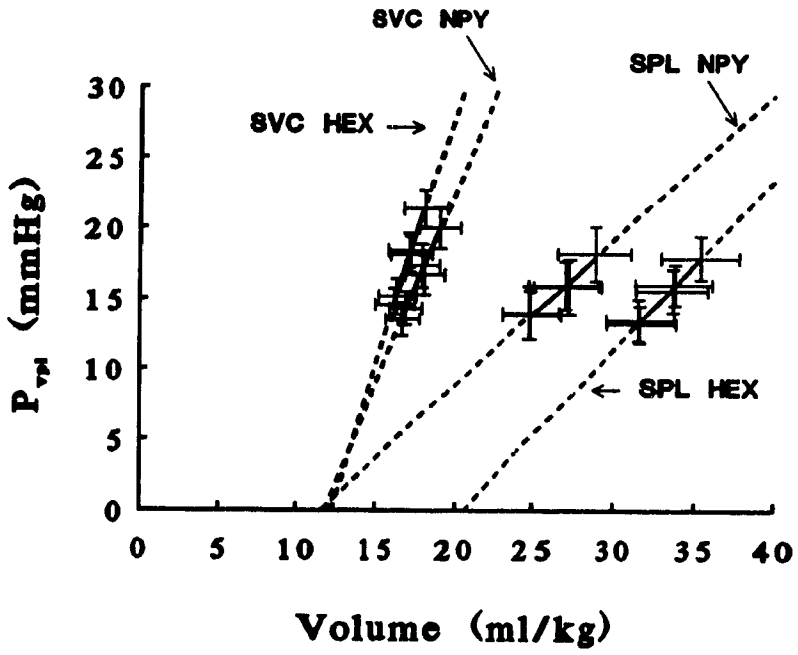

Figure 7.2 Relationship between venous plateau pressure (P_{vpl}) and blood volume for the splanchnic (SPL) and superior vena caval (SVC) regions during hexamethonium (HEX) infusion only, and during Neuropeptide Y (NPY) infusion superimposed on hexamethonium. The curves were determined by varying regional venous outflow pressure alternating with stop flow to obtain venous plateau pressures. The broken lines represent extrapolation to zero pressure of the curves to estimate unstressed volume. Values are means ± SEM; n = 9.

kg^{-1}; 30% of this flow was directed to the splanchnic region and 70% to PER (Table 7.1). Cardiac output was the same with NPY and the distribution of flow did not change.

Splanchnic blood volume was 31.6 ± 2.1 $ml \cdot kg^{-1}$ during hexamethonium infusion and decreased by 21.1% with NPY (Table 7.1). In contrast, SVC blood volume did not change. NPY infusion also resulted in a decrease in the intercept of the splanchnic P-V curve (Fig. 7.2). Splanchnic unstressed volume was 21.8 ± 2.6 $ml \cdot kg^{-1}$ with hexamethonium and decreased by 44.5% with NPY to 12.1 ± 2.2 (Table 7.1). The intercept of the P-V curve of the SVC region did change with NPY.

NPY infusion resulted in an increase in peripheral (SVC + IVC) venous compliance from 0.3 ± 0.03 to 0.43 ± $ml \cdot mm\ Hg^{-1} \cdot kg^{-1}$ (Table 7.1) which also increased PER time constant. None of the other mechanical parameters changed with NPY.

E. DISCUSSION

This study is the first evidence that NPY can selectively decrease vascular capacitance through a shift to the left of the P-V curve of the splanchnic area. This decrease in capacitance would result in an increase in venous return and thus cardiac output in the intact animal. MacLean and Hiley (14) found an increase in cardiac output and total peripheral resistance with infusion of NPY in pithed rats. The increase in cardiac output was mainly due to an increase in stroke volume since heart rate did not change. In the steady state condition, cardiac output equals venous return. Thus

Table 7.1 Regional Effects of Neuropeptide Y on Blood Volume, Unstressed Volume, Mechanical Parameters and Blood Flow Distribution.

Parameter	Region	Condition	
		HEX	NPY
Blood Volume	SPL	31.6 ± 2.1	24.2 ± 1.8*
	SVC	16.4 ± 1.2	17.0 ± 1.1
P_{vpl}	SPL	13.5 ± 1.5	13.8 ± 1.7
	SVC	15.2 ± 1.3	14.3 ± 1.2
Unstressed Volume	SPL	21.8 ± 2.6	12.1 ± 2.2†
	SVC	11.9 ± 1.5	11.1 ± 1.0
Venous Compliance	SPL	0.91 ± 0.13	1.07 ± 0.16
	PER	0.30 ± 0.03	0.43 ± 0.04*
Venous Resistance	SPL	0.029 ± 0.005	0.028 ± 0.005
	PER	0.014 ± 0.001	0.014 ± 0.001
Time Constant	SPL	19.0 ± 2.1	20.1 ± 2.2
	PER	3.4 ± 0.3	4.7 ± 0.4*
Fractional Flows	SPL	30.2 ± 1.2	31.9 ± 2.1
	PER	69.8 ± 1.2	68.1 ± 2.1

Values are means ± SEM. HEX, hexamethonium alone; NPY, neuropeptide Y superimposed on HEX; SPL, splanchnic bed; SVC, superior vena cava region; PER, peripheral region (SVC + IVC). Volumes, $ml \cdot kg^{-1}$; P_{vpl}, venous plateau pressures for blood volumes measurement, mm Hg; compliance, $ml \cdot mm\ Hg^{-1} \cdot kg^{-1}$; resistance, $mm\ Hg \cdot min \cdot ml^{-1} \cdot kg^{-1}$; time constant, sec; flows, $ml \cdot min^{-1} \cdot kg^{-1}$. * $P < 0.01$, † $P < 0.001$ vs. HEX.

peripheral adaptations must have occurred in the latter study to increase venous return. Our results provide a mechanism for this increase in venous return. Before discussing the results further, some methodological considerations must be considered.

The extent to which the anesthesia, surgical procedure, homologous donor blood and tubing affected the integrity of the vascular system is difficult to assess. Nevertheless, the preparation allowed measurements of venous pressures, flows, absolute blood volumes, venous compliances, resistances, time constants of venous drainage and P-V relationships of the splanchnic and extrasplanchnic beds. Although bleeding is inevitable during a bypass preparation, replacement with dextran did not significantly alter the hematocrit value.

Efforts were made to estimate the error in the measurements of regional blood volume due to the loss of indocyanine green in the liver, other tissues, or by hemorrhage. The calculated amounts of indicator recovered showed that all of the cardiogreen was recovered from the SVC region and over 95% was recovered from the SPL region. Concerns may be raised however as to whether changes in liver volume could affect hepatic uptake of indicator. Greenway et al. (9) studied indocyanine green uptake with change in hepatic blood volume in cats. They found that large decreases in hepatic volume resulted in small but significant decreases in indocyanine green uptake and concluded that the "uptake kinetics (of the cat liver) are independent of hepatic blood

volume". Accordingly, we did not find a difference in the calculated amount of dye recovered from the splanchnic area between the two conditions (96.0 ± 6.3% for HEX and 95.8 ± 6.4% for NPY). Although uptake of indicator by the liver is probably the greatest source of error in the volume measurements, the above discussion demonstrates that this error is small and remains constant for consecutive measurements of volumes under the conditions described in this study.

So far, the role of NPY in the cardiovascular system has remained elusive. NPY is released peripherally under a number of physiological conditions (10,11,13,18) and, within the brain, it is co-localised with epinephrine in a group of cells considered essential for the normal maintenance of blood pressure (3). Consequently, it has been suggested that NPY could play an important role in the modulation of circulatory homeostasis. The systemic effects of NPY have been difficult to study because of the lack of a specific antagonist. Furthermore, the release of NPY is potentiated by ß-adrenergic stimulation and inhibited by α-adrenergic stimulation of presynaptic receptors. Also, infusions on NPY inhibit the release of norepinephrine through a presynaptic mechanism and potentiates the vasoconstrictor response to norepinephrine through a posjunctional mechanism (2). In order to eliminate some of these interactions between NPY and the adrenergic transmitters and to minimize the baroreceptor activation of resistive and capacitance vessels, hexamethonium was given to

block the endogenous release of adrenergic agents and NPY and to be able to examine the effect of exogenous NPY on regional arterial and venous beds.

At low doses, NPY does not induce direct vasoconstriction but potentiates the vasoconstrictor responses to norepinephrine, α-adrenoreceptor agonists and sympathetic nerve stimulation (2,7). Higher doses of NPY produce direct vasoconstriction (2,14). We used doses of NPY which would result in a direct pressor response since we were interested in the direct effect of NPY on capacitance vessels.

Infusion of NPY during hexamethonium blockade resulted in a 23.3% increase in arterial blood pressure. This effect was due to the direct action of NPY on arterial smooth muscles rather than an ionotropic effect of NPY on the heart since cardiac output was fixed during the study. The arterial vasoconstriction produced by NPY was the same in the splanchnic and peripheral regions since the distribution of cardiac output did not change with NPY. NPY decreased unstressed volume of the splanchnic region by 44.5% (≈10 $ml \cdot kg^{-1}$). This large decrease in capacitance is comparable to the total capacitance changes obtained by altering carotid sinus pressure by 150 mm Hg (17). Decreases in capacitance can occur through decreases in compliance, shifts to the left of the P-V curve or by decreases in venous resistance. The only mechanism of decrease in capacitance seen in the present experiment was a shift to the left of the P-V curve which is most likely to be due to a constriction of venous smooth

muscles.

In order to compare the magnitude of the effect of NPY on the arteries and veins, one can calculate the effect of the decrease in unstressed volume on venous return and estimate the change in arterial pressure due to this increase in flow. Caldini et al. (1) developed the equation for venous return, $VR = (V - P_{ra}C_t)/(F_s\tau_s + F_f\tau_f)$, where V is the stressed volume, P_{ra}, is right atrial pressure, C_t is total vascular compliance, $\tau_{s,f}$ are the regional time constants to fast (f) and slow (s) time constant beds and $F_{s,f}$ are the fractional flows to the fast and slow time constant beds. This equation, can be applied to our analysis since most of the parameters of the equation were measured. The fast and slow time constant beds correspond to the SPL and PER vascular beds in our preparation (Table 7.1) and for simplification we assume P_{ra} to be equal to zero. Now, if all of the decrease in unstressed volume with NPY ($\approx$10 $ml.kg^{-1}$) is transferred to stressed volume in the intact animal, from the above equation we find that venous return, and thus cardiac output, would increase by approximately 55% for a 30 kg dog. During hexamethonium infusion, arterial blood pressure was 98 mm Hg for a flow of 2,793 ml/min. This gives us a total resistance of 0.0351 mm $Hg \cdot ml^{-1} \cdot min^{-1}$. Keeping this value for resistance constant, if we now increase flow by 55%, the arterial blood pressure would increase to $\approx$152 mm Hg which is a 55% increase. By comparing this 55% increase in pressure to the 23.3% increase seen from the direct effect of NPY on the arterial bed, we suggest that

NPY has a more potent influence on capacitance vessels than on the arterial bed.

In summary, NPY can selectively decrease the capacitance of the splanchnic vascular bed which suggests an important role for this peptide in circulatory homeostasis.

F. REFERENCES

1. CALDINI, P., S. PERMUTT, J.A. WADDELL, AND R.L. RILEY. Effect of epinephrine on pressure, flow, and volume relationships in the systemic circulation of dogs. *Circ. Res.* 34: 606-623, 1974.

2. DAHLÖF, P. Modulatory interactions of neuropeptide y (NPY) on sympathetic neurotransmission. *Acta Physiol. Scand.* suppl. 586: 1-85, 1989.

3. DAMPNEY, R.A.L., A.K. GOODCHILD, AND R.M. MCALLEN. Vasomotor control by subretrofacial neurons in the rostral ventrolateral medulla. *Can. J. Physiol. Pharmacol.* 65: 1572-1579, 1987.

4. DESCHAMPS, A., R. NAAMANI, I. SHRIER, AND S. MAGDER. Effect of beta adrenergic blockade on regional capacitance and unstressed volume in dogs during heat stress. *Am. Physiol. Soc.* 32(4): 152, 1989.(Abstract)

5. EDVINSSON, L., P. EMSON, J. MCCULLOCH, K. TATEMOTO, AND R. UDDMAN. Neuropeptide Y: cerebrovascular innervation and vasomotor effects in the cat. *Neurosci. Lett.* 43: 79-84, 1983.

6. EDVINSSON, L., P. EMSON, J. MCCULOCH, K. TATEMOTO, AND R. UDDMAN. Neuropeptide Y: Immunocytochemical localization to and effect upon feline pial arteries and veins in vitro and in situ. *Acta Physiol. Scand.* 122: 155-163, 1984.

7. EVEQUOZ, D., B. WAEBER, J-F. AUBERT, J-P. FLUCKIGRE, J. NUSSBERGER, AND H.R. BRUNNER. Neuropeptide Y prevents the blood pressure fall induced by endotoxin in conscious rats with adrenal medullectomy. *Circ. Res.* 62: 25-30, 1988.

8. FRIED, G., L. TERENIUS, T. HOKFELT, AND M. GOLDSTEIN. Evidence for differential localization of noradrenaline and neuropeptide Y in neuronal storage vesicles isolated from rat vas deferens. *J. Neurosci.* 5: 450-458, 1985.

9. GREENWAY, C.V., I.R. INNES, K.L. PUSHKA, G.D. SCOTT, AND D.S. SITAR. Changes in hepatic blood volume on galactose an indocyanine green uptake by cat liver. *Am. J. Physiol.* 256: G524-G531, 1989.

10. LUNDBERG, J.M., A. HEMSEN, G. FRIED, E. THEODORSSON-NORHEIM, AND H. LAGERCRANTZ. Co-release of neuropeptide Y (NPY)-lide immunoreactivity and catecholamines in newborn infants. *Acta Physiol. Scand.* 126: 471-473, 1986.

11. LUNDBERG, J.M., A. MARTINSSON, A. HEMSEN, E. THEODORSSON-NORHEIM, J. SVEDENHAG, B. EKBLOM, AND P. HJEMDAHL. Co-release of neuropeptide Y and catecholamines during physical exercise in man. *Biochem. and Biophys. Res. Comm.* 133: 30-36, 1985.

12. LUNDBERG, J.M., AND K. TATEMOTO. Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. *Acta Physiol. Scand.* 116: 393-402, 1982.

13. LUNDBERG, J.M., L. TORSELL, A. SOLLEV, J. PERNOW, E. THEODORSSON-NORHEIM, A. ANGGARD, AND B. HAMBERGER. Neuropeptide Y and sympathetic vascular control in man. *Regul. Pept.* 13: 41-42, 1985.

14. MACLEAN, M.R., AND C.R. HILEY. Effect of neuropeptide Y on cardiac output, its distribution, regional blood flow and organ vascular resistances in the pithed rat. *Br. J. Pharmacol.* 99: 340-342, 1990.

15. MALO, J., H. GOLDBERG, R. GRAHAM, H. UNRUH, AND C. SKOOG. Effect of hypoxic hypoxia on systemic vasculature. *J. Appl. Physiol.* 56(5): 1403-1410, 1984.

16. MITZNER, W., AND H. GOLDBERG. Effects of epinephrine on resistive and compliant properties of the canine vasculature. *J. Appl. Physiol.* 39(2): 272-280, 1975.

17. ROSS, JR., J., C.J. FRAHM, AND E. BRAUNWALD. Influence of carotid baroreceptors and vasoactive drugs on systemic vascular volume and venous distensibility. *Circ. Res.* 9: 75-82, 1961.

18. ZUKOWSKA-GROJEC, Z., M. KONARSKA, AND R. MCCARTHY. Differential plasma catecholamine and neuropeptide Y responses in acute stress in rats. *Life Sci.* 42: 1615-1624, 1988.

CHAPTER 8

GENERAL CONCLUSIONS

The primary objective of this thesis was to examine the endogenous control of the determinants of venous return. In the first study, exercise and saline infusion in humans was examined because the competition for blood flow between the muscles and the skin represents a unique condition where the limits of the cardiovascular system may be reached, and where blood volume manipulations can be used to alter these limits. Next, the vascular mechanics of the skin in dogs were investigated in order to understand the full impact of an increase in cutaneous blood flow on venous return. Heat stress was used in the third study as a means to increase venous return through sympathetic activation. In this way, not only could the effect of heat stress on the determinants of venous return be studied, but also, the sympathetic control of these determinants could be examined. Changes in carotid sinus pressure were used in the fourth study to determine the effect of the baroreflex on the determinants of venous return, and to compare the sympathetic control of venous return under these condition to the sympathetic control under heat stress. In the last study, the effects of NPY on the determinants of venous return were investigated because neither α nor ß-receptor blockade could reverse the effect of heat stress on splanchnic capacitance.

In the first project, I tested the hypothesis that maintenance of blood volume during exercise would maintain cardiac output and improve temperature regulation, as a result of which endurance time would increase. The use of a double

blind protocol was essential because personal motivation is an important factor determining endurance time. The negative result from this study is important because it suggests that, under the conditions studied, temperature regulation and heart rate are not as important as other factors, like muscle acidosis, in determining exercise endurance time.

In the second project, I tested the hypothesis that the skin is a compliant region with a long time constant of venous drainage. There are no previous reports of measurements of skin time constant, venous compliance or venous resistance. The long time constant of venous drainage of the skin in dogs indicates that increases in skin blood flow could substantially impair venous return. A comparison between the τ_{sk} of the dog and that of humans will be possible only when a reliable assessment of total human skin blood flow is available because the product of an increase in skin blood flow and τ_{sk} gives the amount of blood which accumulates in the skin. Thus by knowing the magnitude of increase in total skin blood flow in humans during heat stress, it will be possible to estimate a value for τ_{sk} which would result in a reasonable accumulation of blood in the skin to allow the high level of cardiac output observed during heat stress.

In the third project, the mechanisms of the increase in venous return during heat stress were investigated. For this, simultaneous measurements of splanchnic and extrasplanchnic capacitance were obtained in dogs for the first time. The shift to the left of the pressure-volume curve of the

splanchnic region with no change in slope demonstrates that constriction of the capacitance vessels to reduce unstressed volume does not necessarily affect the compliance of the vessels. Ganglionic blockade during heat stress was then used to differentiate between central and local mechanisms of control. Because ganglionic blockade abolished the response, adrenergic receptor blockers were given to further investigate the sympathetic control of venous return during heat stress. Failure of phentolamine and propranolol to reverse the effect of heat stress on splanchnic capacitance indicates that neither EPI nor NE is at the origin of this effect.

Although not mentioned in chapter 5, I also used an angiotensin II receptor inhibitor and α_2-receptor blocker during heat stress in dogs on bypass, but neither of these agents was able to block the decrease in unstressed volume (n = 1 for each). We therefore turned our attention to NPY as a possible candidate for the control of splanchnic venous tone during heat stress. Since there are no specific blockers for NPY receptors, the peptide was infused to observe its effects on the determinants of venous return. NPY infusion mimics the effect of heat stress on splanchnic unstressed volume. These results are of importance because they indicate a potential role for NPY in the control of venous capacitance. Also, the difference between the effect of NPY on splanchnic venous tone and that of EPI or NE is remarkable. In general, EPI or NE infusion affects both venous compliance and resistance (see introduction). NPY, however, has no effect on these

parameters and only affects capacitance.

The influence of changes in carotid sinus pressure on the determinants of venous return were also examined. This study was performed for a number of reasons. As mentioned previously, there are no measurement of venous resistance with changes in carotid sinus pressure and the data on redistribution of blood flow is conflicting. Also, the response of the capacitance vessels to carotid sinus stimulation is sympathetically mediated and thus could be compared to the response observed with heat stress.

The results from this study indicate that the response of capacitance vessels to increases in sympathetic outflow is not homogeneous. Increases in sympathetic outflow through a decrease in carotid sinus pressure significantly affect splanchnic venous resistance and compliance. These effects are not seen with sympathetic stimulation during heat stress. Also, the sympathetic control of the capacitance vessels appears to compensate for the changes in arterial tone and redistribution of blood flow which occur with a fall in carotid sinus pressure. The role of venous resistance in this reflex is crucial. Finally, only half of the change in splanchnic capacitance from decreases in carotid sinus pressure could be blocked by phentolamine. This further supports our hypothesis that a mediator other than EPI and NE controls splanchnic venous capacitance.

CLAIMS OF ORIGINALITY

The following observations are original contributions to the understanding of the factors which affect endurance time during exercise in humans, and of the sympathetic control of venous return.

1) Maintenance of plasma volume during heavy exercise in humans in a thermoneutral environment lowers core temperature and heart rate but does not improve endurance time.

2) The skin vascular bed in dogs has a large venous compliance and venous resistance, and thus a long time constant of venous drainage. Therefore, increases in blood flow to the skin will result in a pooling of blood in the periphery and a decrease in venous return unless compensatory mechanisms are activated.

3) Skin venous compliance increases and venous resistance decreases with core or skin surface heating in dogs. As a result, the time constant of venous drainage of the skin does not change during heat stress and the amount of peripheral blood accumulation for a given change in skin blood flow remains constant.

4) Repeated absolute measurements of splanchnic and extrasplanchnic blood volume can be obtained using indicator dilution techniques. These measurements can be combined with

stop flow techniques to construct regional pressure-volume curves and obtain regional unstressed volumes under different experimental conditions.

5) Splanchnic capacitance decreases during heat stress in dogs. The change in unstressed volume can explains 80% of the increase in cardiac output in the conscious heated dog. Heat stress does not affect splanchnic venous compliance and resistance as well as extrasplanchnic volumes, venous compliance and venous resistance.

6) Ganglionic blockade reverses the decrease in splanchnic capacitance during heat stress which implies that sympathetic activation is at the origin of the response.

7) Alpha-adrenergic receptor blockade with phentolamine, or ß-receptor blockade with propranolol does not reverse the decrease in splanchnic capacitance during heat stress. These results imply that a sympathetically released mediator other than EPI and NE is responsible for the response. This mediator remains to be identified.

8) With a fall in carotid sinus pressure from 200 mm Hg to 50 mm Hg, there is a 14% redistribution of blood flow from the extrasplanchnic bed to the splanchnic bed. This redistribution of flow increases the volume in the splanchnic bed which tends to decrease venous return.

9) Venous resistance of the splanchnic region decreases with a drop in carotid sinus pressure. This decrease in resistance is responsible for a passive decrease in splanchnic volume and tends to increase venous return.

10) Unstressed volume of the splanchnic region decreases with a fall in carotid sinus pressure. This fall in unstressed volume is accompanied by a decrease in the slope of the splanchnic pressure-volume curve and tends to increase venous return.

11) The time constant of venous drainage of the splanchnic region decreases with a fall in carotid sinus pressure. This change is mainly due to a large decrease in venous resistance.

12) With a fall in carotid sinus pressure, the increase in venous return due to a decrease in venous resistance nearly exactly matches the decrease in venous return due to a rise in splanchnic blood flow. These results suggest that the baroreceptor reflex compensates for the negative effects of changes in arterial tone by decreasing splanchnic venous resistance.

13) Ganglionic blockade with hexamethonium reverses the effect of decreases in carotid sinus pressure on splanchnic capacitance. These results indicates that the response is

sympathetically mediated.

14) Only half of the change in splanchnic unstressed volume caused by a fall in carotid sinus pressure can be blocked by phentolamine. Thus, the other half of the response is either due to ß-receptor vasoconstriction, which is unlikely, or to a mediator other than EPI and NE.

15) Infusion of neuropeptide Y decreases splanchnic capacitance in dogs. Splanchnic venous compliance and resistance are not affected by NPY. NPY does not affect extrasplanchnic volumes and venous resistance, but venous compliance increases slightly. These results represent the first evidence that NPY can actively decrease splanchnic capacitance and thus increase venous return and cardiac output.

APPENDIX I

S.I. UNIT EQUIVALENTS

1 Hertz (Hz) = 1 cycle/min

1 Pascal (Pa) = 0.075 mm Hg

= 0.01 cm H_2O

APPENDIX II

ABBREVIATIONS

Δ	delta
τ_{sk}	time constant of venous drainage of the skin
τ_v	time constant of venous drainage
ANOVA	analysis of variance
ANP	atrial natriuretic factor
BTPS	
C	celsius
CO_2	carbon dioxide production
CON	control
C_v	venous compliance
dl	decilitres
EPI	epinephrine
EXH	exhaustion
Fig.	figure
g	grams
Hb	hemoglobin
HCO_3^-	bicarbonate concentration
Hct	hematocrit
HR	heart rate
IVC	inferior vena caval region
kg	kilograms
kpm	kilograms per minutes
l	litres
LFA	left femoral artery

APPENDIX II (cont.)

ABBREVIATIONS

MCFP	mean circulatory filling pressure
mg	milligrams
min	minutes
ml	millilitres
n	number
NE	norepinephrine
NPY	neuropeptide Y
P-V	pressure volume
P_a	arterial pressure
PCO_2	partial pressure of carbon dioxide
P_{el}	elastic recoil pressure
PER	peripheral region
PO_2	partial pressure of oxygen
P_{ra}	right atrial pressure
PV	plasma volume
P_v	venous pressure
P_{vpl}	venous plateau pressure
Q_D	flow from indicator dilution technique
Q_P	flow from electromagnetic flow probe
R	recovery
R_a	arterial resistance
RFA	right femoral artery
RFV	right femoral vein
R_v	venous resistance
RVR	resistance to venous return

APPENDIX II (cont.)

ABBREVIATIONS

SAL	saline infusion
SD	standard deviation
SE	standard error of the mean
sec	seconds
SPL	splanchnic region
STP	standard temperature and pressure
SVC	superior vena caval region
T_b	blood temperature
T_c	core temperature
T_{es}	esophageal temperature
T_s	skin temperature
V	stressed volume
V_b	blood volume
$\dot{V}E$	ventilation
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_2max$	maximal oxygen uptake
$\dot{V}CO_2$	carbon dioxide production
V_u	unstressed volume
$[H^+]$	hydrogen ion concentration