BLOOD VOLUME AND FLUID EXCHANGE RESPONSES IN THE LIVER OF THE ANESTHETIZED CAT

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Personal financial support during the course of this study was provided by a Medical Research Council of Canada Studentship and by my wife, Melanie. In the latter case I hope I may now, finally return the favor. Funds in support of this work were provided by the Medical Research Council of Canada.

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LIST OF FIGURES

For ease in location, all charts, illustrations, traces and tables are referred to as 'Figures'.

PUBLICATIONS

Portions of the work in this thesis have been published in the following journals.

1. Greenway, C.V., Lautt, W.W., and R.D. Stark. (1969) Capacitance responses and fluid exchange in the cat liver. J. Physiol. 205, 33-34P.

2. Greenway, C.V., Stark, R.D., and W.W. Lautt. (1969) Capacitance responses and fluid exchange in the cat liver during stimulation of the hepatic nerves. Circ. Res. 25, 277-284.

3. Greenway, C.V., and W.W. Lautt. (1970) Effects of hepatic venous pressure on transsinusoidal fluid transfer in the liver of the anesthetized cat. Circ. Res. 26, 697-703.

4. Greenway, C.V., and W.W. Lautt (1972) The effects of infusions of catecholamines, angiotensin, vasopressin and histamine on hepatic blood volume in the anaesthetized cat. British J. Pharmacol. (in press).

5. Greenway, C.V., and W.W. Lautt (1972) Effects of adrenaline, isoprenaline and histamine on transsinusoidal fluid filtration in the cat liver. British J. Pharmacol. (in press).

6. Lautt, W.W., and C.V. Greenway (1972) Hepatic capacitance vessel responses to bilateral carotid occlusion in anesthetized cats. Can. J. Physiol. Pharmacol. (in press).

ABSTRACT

Techniques are described to study the hepatic vascular bed in the anesthetized cat without interference with the hepatic artery or portal vein. Liver volume was recorded with a plethysmograph while simultaneous recordings were made of arterial and portal pressure and total hepatic blood flow. Red blood cells were tagged with ^{51}Cr and the blood content of the liver was continuously monitored. Any difference between total volume responses and changes in blood content were attributed to net trans-sinusoidal fluid shifts. Hepatic venous pressure was controlled and capillary filtration coefficients were determined by the use of a hepatic venous long-circuit.

About 14% of the blood volume of the cat was in the hepatic vascular bed. Stimulation of the hepatic nerves caused a well-maintained decrease in hepatic volume which was maximal at stimulation frequencies of 6-8 Hz and represented expulsion of up to 50% of the hepatic blood volume. The liver of the cat is thus an important blood reservoir. Bilateral occlusion of the carotid arteries did not mobilize blood from this large reservoir. The nervous supply to the capacitance vessels was not activated by carotid occlusion whereas reflex activation of the sympathetic nerves to the hepatic arterial resistance vessels did occur. This showed considerable selectivity in reflex activation of sympathetic nerves.

Dose-response curves for hepatic volume were obtained with intravenous infusions of adrenaline, noradrenaline, angiotensin, vasopressin and histamine. Adrenaline and noradrenaline decreased hepatic blood volume and did not differ significantly in potency. Up to 40%

of the hepatic blood volume was expelled by doses of adrenaline within the range secreted by the adrenal medullae. Isoproterenol, infused into the hepatic artery, had no significant effect on hepatic blood volume in doses which cause maximal vasodilation of the hepatic arterial bed; relaxation of hepatic capacitance vessels mediated by beta-adrenergic receptors did not occur. Angiotensin infusions in doses previously shown to cause intestinal and splenic vasoconstriction, decreased hepatic blood volume and on a molar or μg basis, angiotensin was the most potent of the agents tested. Doses within the probable physiological range of endogenous release of angiotensin decreased hepatic blood volume by up to 15%. The responses were not significantly different when the hepatic nerves were intact or sectioned. Vasopressin infusions produced only small decreases in hepatic blood volume; it is unlikely that endogenously released vasopressin causes significant capacitance responses. Histamine produced no change in hepatic blood volume in doses which readily produce outflow block in dogs; either the specific hepatic venous smooth muscle involved in outflow block is absent in the cat or it has no histamine receptors.

Elevation of hepatic venous pressure increased hepatic blood content and this became stable in 5-20 minutes. There was also a filtration of fluid which continued at a uniform rate for the duration of the pressure elevation. The rate of trans-sinusoidal fluid filtration was directly proportional to sinusoidal hydrostatic pressure (0.060 ± 0.003 ml.min^{-1}. mm Hg^{-1}.100 g liver^{-1}). No protective mechanisms existed within the liver to prevent filtration when hepatic venous pressure was elevated and the major factor controlling trans-

sinusoidal filtration was sinusoidal hydrostatic pressure.

The effects of intravenous infusion of adrenaline, intra-arterial infusions of adrenaline, isoproterenol and histamine, stimulation of the hepatic nerves and hepatic arterial occlusion on hepatic fluid exchange were evaluated at zero venous pressure and at elevated venous pressure. These stimuli did not induce filtration at zero venous pressure nor did they alter the rate of filtration induced by the elevated venous pressure. It is concluded that these procedures, which alter fluid exchange in other vascular beds, did not significantly alter sinusoidal hydrostatic pressure, surface area or permeability in the cat liver. The filtrate which formed as a result of elevated venous pressure had a protein content nearly identical to that of plasma and it is suggested that the colloid osmotic pressure gradient across the sinusoid is very small.

Thus, of the variables that might influence hepatic fluid exchange on the basis of the Starling hypothesis, (sinusoidal surface area and permeability and the hydrostatic and colloid osmotic pressures of the sinusoidal and interstitial fluids) only sinusoidal hydrostatic pressure appears to be an important control of fluid exchange in the liver.

INTRODUCTION

SERIES-COUPLED SECTIONS OF PERIPHERAL VASCULAR BEDS

By the mere possession of one small key, the door to a vast treasury that has been sealed since the beginning of time may be swung aside to reveal things that were hitherto unknown. A new approach to the study of peripheral vascular physiology began with the discovery of one such key. Mellander (1960) reported a technique of analysis of the vascular physiology of the cat hindlimb that revolutionized peripheral vascular physiology. The technique involved simultaneous recording of vascular resistance to blood flow, alterations in net fluid exchange, and changes in regional blood volume. The organ was sealed within a plethysmograph while the nerves and the arterial vessels remained intact. Any volume changes in the organ are due to either changes in the organ blood volume or to a filtration or reabsorption of fluids across the vascular walls. A refinement of the original technique, involving ^{51}Cr-tagged red blood cells, allows separation of these factors and will be described later. A schematic representation of the combined techniques is shown in Figure 1.

The techniques originally designed for use in vascular studies of the cat hindquarters (Mellander, 1960) were modified for use with skeletal muscle (without skin) (Kjellmer, 1965) and the intestine (Johnson, 1965; Johnson & Hanson, 1966; Wallentin, 1966). The data obtained in these experiments were interpreted by considering the vascular bed to be a series-coupled system where each segment is defined in functional terms rather than strictly anatomical terms. The functional classification allows simple physiological comparisons of various vascular beds without the necessity of detailed anatomical information.

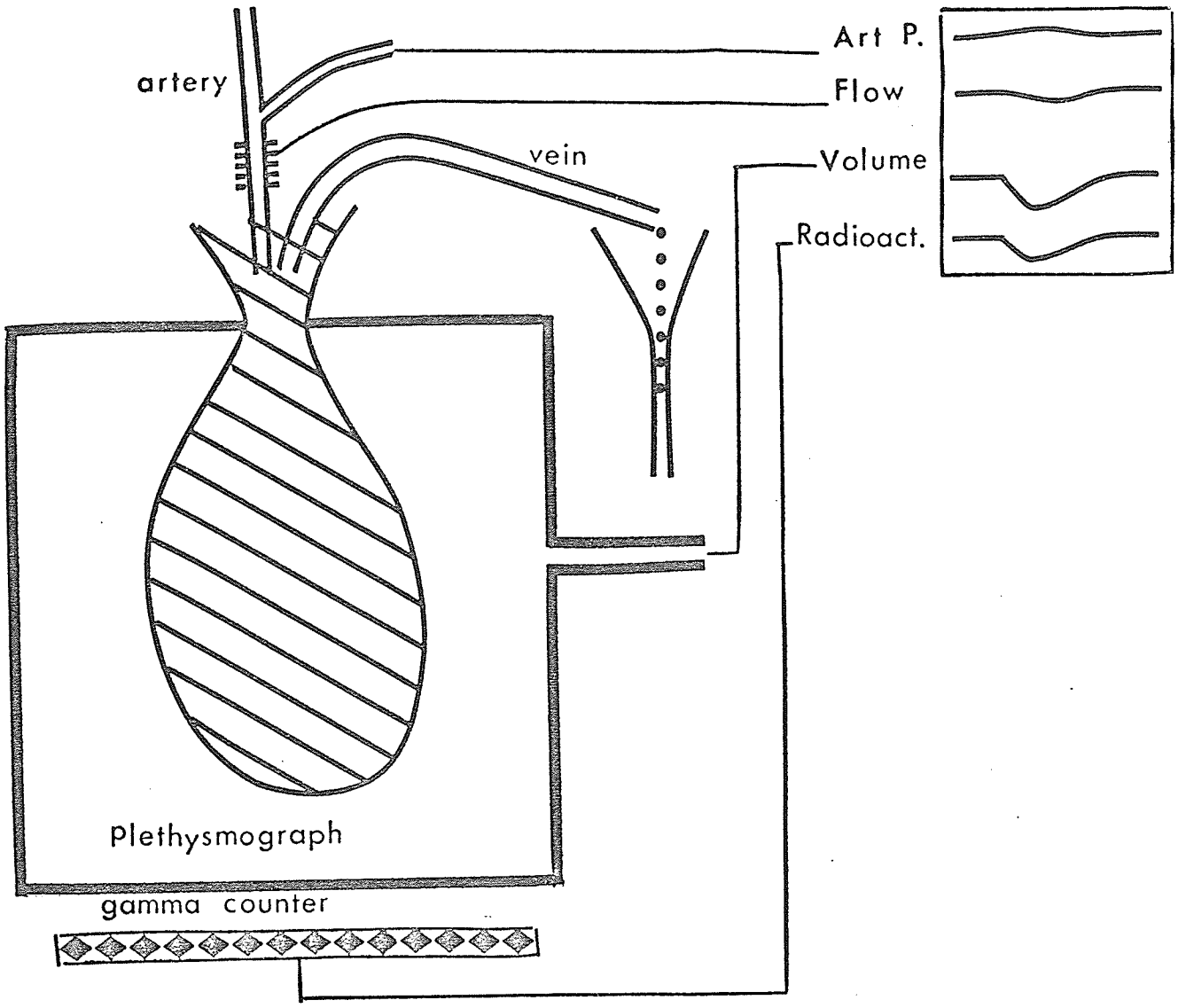


Figure 1. Schematic representation of plethysmograph techniques. Parameters recorded are arterial blood pressure, arterial blood flow, organ volume changes and level of radioactivity. Venous pressure is determined by the outflow level of the venous cannula. The venous blood is pumped back to the animal.

The functional regions of the series-coupled segments of the vascular bed have been classified as follows (Mellander, 1960): Windkessel vessels, precapillary resistance vessels, precapillary sphincters, exchange vessels, postcapillary resistance vessels and capacitance vessels. In skeletal muscle and the intestine, these functional divisions have well defined anatomical correlates. Windkessel vessels include the aorta and other large elastic arteries which tend to convert a pulsatile to a non-pulsatile flow. Small arteries and arterioles comprise the precapillary resistance vessels. Precapillary resistance vessel tone controls the total blood flow supplying an organ. Precapillary sphincters serve a precapillary resistance function but in particular they determine the number of capillaries that are available for exchange; dilation of precapillary sphincters allows blood to flow through the capillary and thus the surface area for diffusion and fluid exchange in that vascular bed is increased. The capillaries are the site of exchange.

Three types of capillaries are described on the basis of electron microscope studies (Folkow & Neil, 1971). Continuous or non-fenestrated capillaries are found in muscle, lungs, central nervous system, fat and connective tissue. They are a single layer of continuous endothelial cells which have intercellular pores in the range of 40 $\mathring{A}$ in width. The exception to this is in the central nervous system where no pores have been demonstrated. A basement membrane 200 - 600 $\mathring{A}$ in thickness lies on the outer surface of the endothelial cell. It consists of a fibrillar network embedded in a mucopolysaccharide matrix and it apparently causes little hindrance to transport. Fenestrated capillaries occur in the renal glomeruli, glands, the ciliary body,

choroid plexus and the intestinal mucosa. The countercurrent capillary systems of the kidney and intestinal villi are fenestrated. This system of capillaries has intercellular pores which can be varied in width. These capillaries permit large rapid fluxes of fluids and solutes. Discontinuous capillaries exist in bone marrow, liver and spleen and show large intercellular and intracellular gaps. Basement membranes are sparse or absent in discontinuous capillaries.

The postcapillary resistance vessels contribute little to total vascular resistance but the ratio between the pre- and postcapillary resistance determines the hydrostatic pressure of the capillaries. If the pre/postcapillary resistance is elevated, that is if a relative increase in precapillary resistance occurs, then the pressure drop proximal to the capillary is increased and the capillary pressure is reduced. Constriction of the small veins and venules adds little to the total peripheral resistance but it notably affects regional blood volume. Venous return and thus cardiac output are dependent on the venous capacity which effectively serves as an adjustable reservoir of blood for the heart.

A change in total volume recorded with the plethysmograph can be due to fluid shifts between tissues and blood or it can be an indication of a change in regional blood volume. In order to determine the role of each component, simultaneous recordings of ^{51}Cr radioactivity and plethysmograph volume are made. A record of these parameters is shown in Figure 2 (Wallentin, 1966) where the effect of raised venous pressure on intestinal volume was evaluated. By comparing the level of radioactivity in the intestinal segment with the change in total volume, it is seen that over the first minute the rapid volume change was due

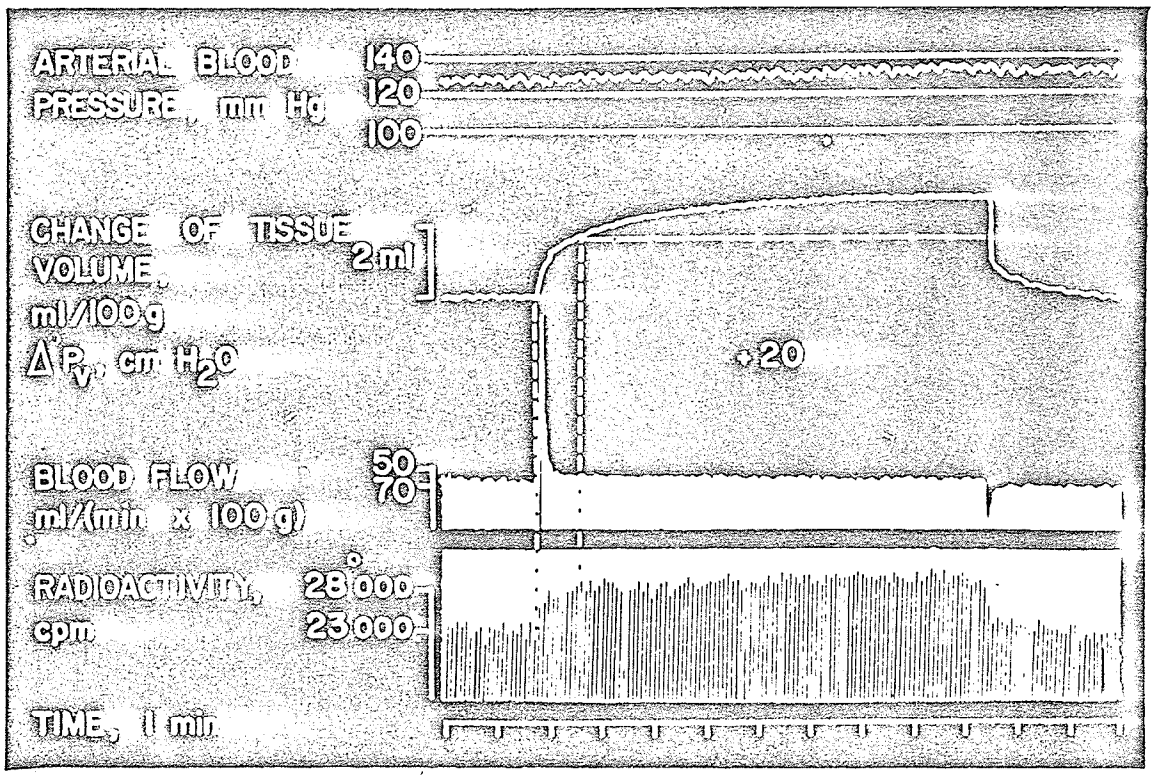


Figure 2. Effects of a prolonged period of raised venous pressure on regional blood volume (radioactivity). Note that the volume increase representing filtration of fluid decreases gradually, reaching a new isovolumetric state after about 8 minutes (Wallentin, 1966).

mainly to an increase in blood content, while after this time the blood volume remained stable. Further increases in total volume were due to fluid filtered across the vessel walls. In this case the fluid was filtered as a result of an elevated capillary pressure secondary to the elevated venous pressure. With these techniques, blood volume changes are reasonably straightforward whereas considerable complexity is encountered in interpretation of fluid exchange data. To understand the importance of being able to separate fluid filtration from altered blood volume we must first examine the factors that can affect fluid filtration across a vascular wall.

Fluid exchange across the capillary depends on a number of variables and their relationships were first described by Starling (1896) in what has become known as the 'Starling hypothesis'. The hydrostatic pressure across the capillary wall is the most obvious variable. However the colloid osmotic pressures are also important even though, under normal physiological conditions, they do not appear to play an important regulatory role. Two other variables which cannot be directly measured also play a major role; they are capillary permeability and the surface area of the exchange vessels across which fluid movements occur. Opening or closing precapillary sphincters effectively increases or decreases the total surface area since a capillary in which flow stops will rapidly equilibrate with the interstitial fluids. These variables are combined in the equation for fluid exchange.

$$F = CFC\ (Pc - Pt - coc + cot)$$

F = net fluid movement (positive value represents filtration)

CFC = a calculated value dependent on the surface area and the permeability of the exchange vessels

Pc = capillary hydrostatic pressure

Pt = interstitial hydrostatic pressure
coc = capillary colloid osmotic pressure
cot = interstitial colloid osmotic pressure

The roles of these variables are most easily described by examples of the results obtained in peripheral vascular studies.

Capillary surface area and hydrostatic pressure are influenced by a number of factors. Stimulation of the sympathetic nerves to skeletal muscle (Figure 3 from Mellander, 1960) caused a reduction in local blood volume (the rapid phase of volume change) and a slower continuous decline in volume which represents reabsorption from the interstitial fluids. Though arterial and venous pressures remained constant during this procedure, precapillary resistance increased relative to postcapillary resistance. This caused a greater pressure drop across the precapillary side of the vascular bed and reduced capillary hydrostatic pressure. Fluid was reabsorbed into the vascular bed.

The surface area of the exchange vessels cannot be evaluated under conditions of a steady state where rate of filtration equals the rate of reabsorption. Alterations in the surface area can be detected only when a net fluid movement is induced. This is illustrated in the following example. Evaluation of sympathetic nervous control on the vascular bed of the intestine is shown in Figure 4 (Dresel et al., 1966). After about 3 minutes from the onset of nerve stimulation, the volume was steady. This indicated that sympathetic nerve activity in the gut did not alter the pre/postcapillary resistance and thus no net fluid exchange occurred in either direction. This was in striking contrast to the situation in skeletal muscle (Figure 3) where sympathetic nervous

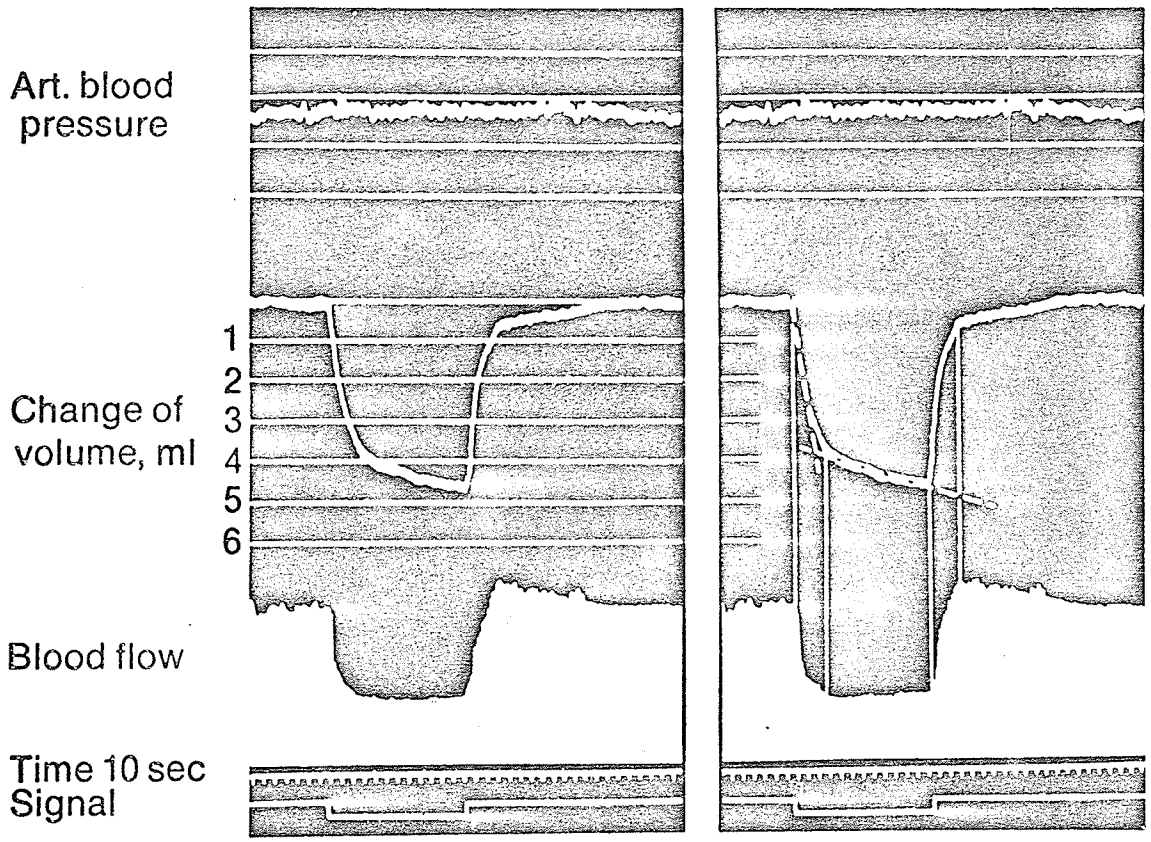


Figure 3. Blood flow and volume response in skeletal muscle on stimulation of sympathetic nerves. Slow component on volume trace represents reabsorption of tissue fluids (Mellander, 1960).

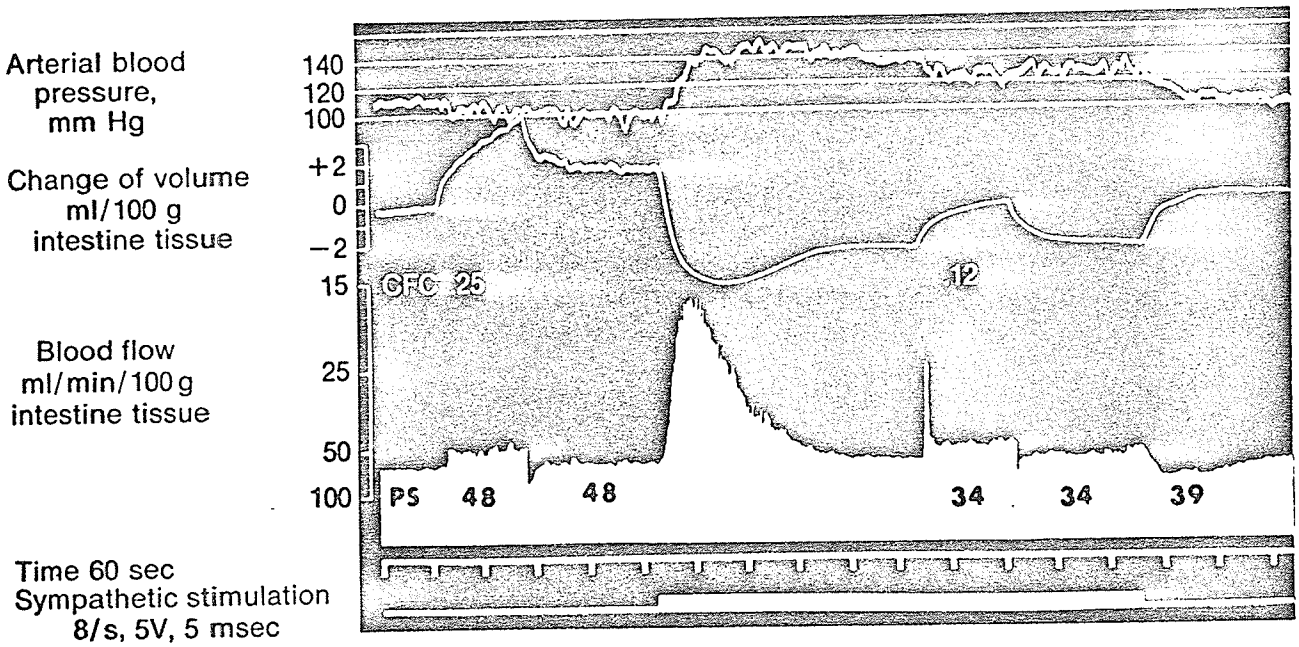


Figure 4. Effect of stimulation of sympathetic nerves on intestinal blood flow, blood volume, CFC and PS values in the cat. Regional blood volume, CFC and PS remain low throughout the period of stimulation whereas resistance displays 'autoregulatory escape' (Dresel et al., 1966). See text for definition of 'CFC' and 'PS'.

activation reduced capillary pressure and mobilized fluid from the tissues. The effect on blood flow was also markedly different in these two organs. Skeletal muscle flow remained reduced for the duration of nerve stimulation while intestinal blood flow showed a partial recovery toward control levels. This phenomenon is referred to as 'auto-regulatory escape' (Folkow et al., 1964), the mechanism of which is unknown. The effect of sympathetic nerves on capillary surface area is evaluated by comparing the rate of filtration induced by an elevation of venous pressure in the presence and absence of sympathetic nervous activity. The capillary filtration coefficient (CFC) is calculated from the slope on the trace seen immediately after the rapid change in blood volume has ceased. This was demonstrated earlier in Figure 2. The CFC represents the amount of fluid filtered across the vascular bed as a result of an increase in capillary hydrostatic pressure. The unit of the CFC is $ml.min^{-1}.mm\ Hg^{-1}.100\ g\ tissue^{-1}$. In Figure 4 it can be seen that the CFC was reduced when the sympathetic nerves were stimulated. This implies that the precapillary sphincters were activated by the nerves and effectively reduced the number of capillaries across which filtration occurred. These conclusions were confirmed by the use of another measurement referred to as the 'PS product' (Renkin, 1959). Briefly, this technique involves measuring the uptake of ^{86}Rb from arterial blood. ^{86}Rb diffuses only through the pores in the capillary wall and is handled by the tissues the same as is potassium. As ^{86}Rb is pore-restricted, its transcapillary diffusion depends upon the total pore area exposed to flow and this pore area depends on both capillary permeability factor 'P' and on the perfused capillary surface area 'S'. 'P'

and 'S' cannot be separated but together constitute the 'PS product'. At constant flow and arterial concentration of the tracer, any change in the venous concentration of the tracer reflects a change in PS. PS has the dimensions of ml. min^{-1}.100 g $tissue^{-1}$. In Figure 4 both CFC and PS were reduced, thus indicating that sympathetic nerves cause reduction of capillary surface area in the intestine. An alteration in permeability can be excluded as a variable in this instance, since, if permeability changes occurred during nerve stimulation, there should have been an increased filtration at zero venous pressure. This did not occur.

The use of Mellander's plethysmograph technique has demonstrated that stimulation of sympathetic nerves in skeletal muscle increases the pre/postcapillary resistance thus causing a reduced capillary pressure and reabsorption of fluid. Vascular resistance to flow is increased and precapillary sphincter constriction causes an initial reduction in CFC which is overcome by local accumulation of metabolites (Cobbold et al., 1963). In the intestine, stimulation of the sympathetic nerves causes a maintained reduction in volume; pre/postcapillary resistance is not altered and thus fluid exchange remains unaltered. Partial autoregulatory escape of flow occurs in the gut while CFC remains reduced throughout nerve stimulation.

The effects on fluid exchange of interactions between capillary hydrostatic pressure and CFC can further be illustrated in more complex situations. Vasodilation due to exercise in skeletal muscle of the cat resulted in a great decrease in pre/postcapillary resistance and the resultant increase in capillary pressure caused filtration of

fluid (Figure 5, Kjellmer, 1965). In this experiment the ^{51}Cr technique was employed to demonstrate that after the initial rapid increase in blood content the remaining increase in volume was due to filtration of fluid. Others (Cobbold et al., 1963) showed that CFC in the exercising muscle was increased depending on the extent of vasodilation. The CFC in both skeletal muscle and intestinal vascular beds increased as the degree of isoproterenol-induced vasodilation increased (Figure 6, Folkow et al., 1963). In these cases the increased filtration was due to both an increase in hydrostatic pressure and to an increase in surface area. Vasodilation can occur, however, with no increase in CFC. Stimulation of cholinergic vasodilator nerves to skeletal muscle caused a reduced vascular resistance while CFC was unaffected or decreased (Renkin & Rosell, 1962; Djojosugito et al., 1968). Thus the precapillary resistance function can be separated from the precapillary sphincter function.

Precapillary sphincters show myogenic responses, that is the sphincter tone varies directly with the transmural pressure. Arterial occlusion caused a marked dilation of the sphincters in skeletal muscle which was reflected in the rise in CFC (Cobbold, et al., 1963). This effect is important in man where postural changes may cause large fluctuations in transmural pressure. The effect on CFC of shifting from supine to erect posture in eight human subjects is shown in Figure 7 (Mellander et al., 1964). The increased transmural pressure caused the precapillary sphincters to constrict and thus reduced the number of capillaries open for circulation. This is one factor which slows the massive shifts of fluid from the vessels into the tissues which tend to occur due to the

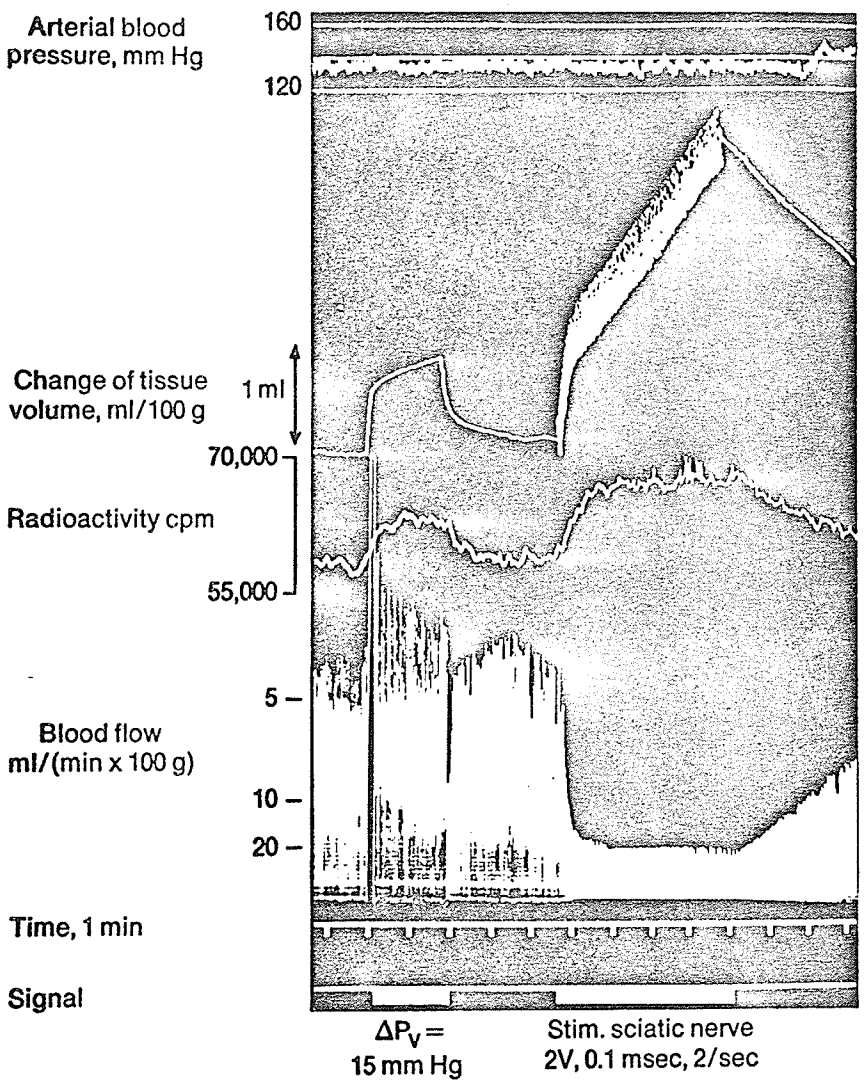


Figure 5. Effect of exercise on cat calf muscle (Kjellmer, 1965).

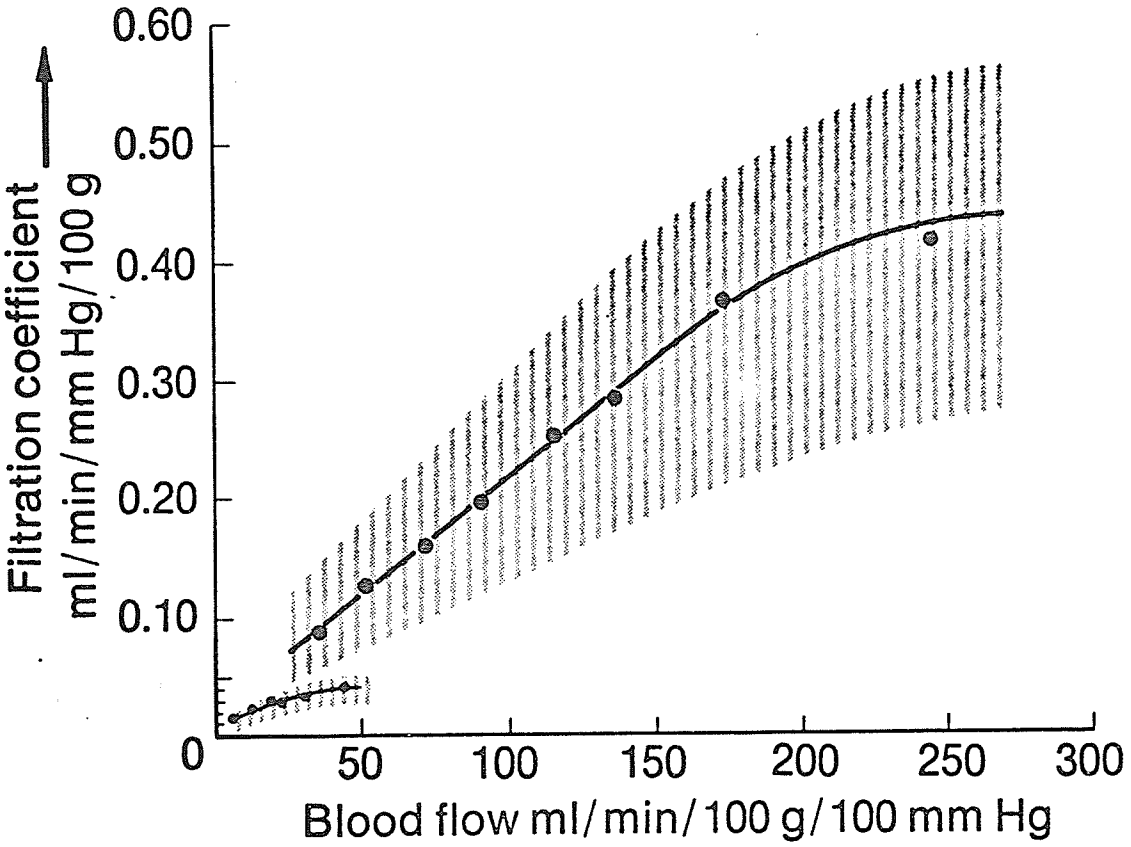


Figure 6. Correlation between blood flow and CFC in the intestine of the cat. The lower left curve shows the same correlation for skeletal muscle. Vasodilation was achieved by infusions of isoproterenol (Folkow et al., 1963).

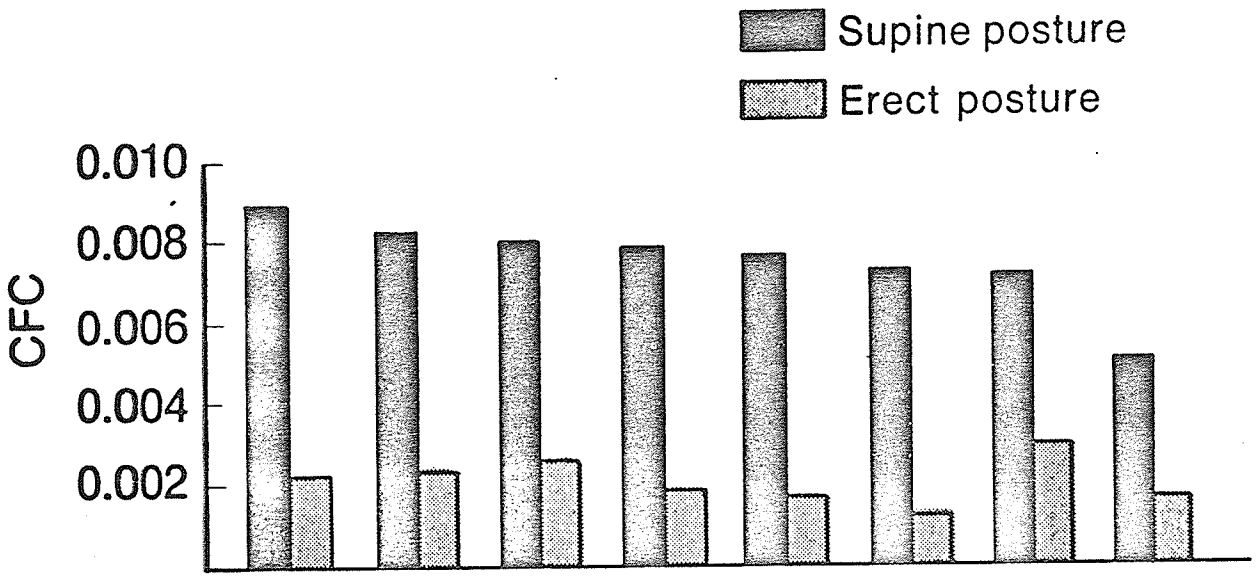


Figure 7. Average change in CFC in the feet of eight human subjects on shifting from supine to erect posture (Mellander et al., 1964).

increased capillary hydrostatic pressure.

Another method of altering net fluid exchange is achieved by altering the capillary permeability to large molecular weight solutes. This affects the gradient of colloid osmotic pressure across the endothelium and can have marked effects on fluid movements. Altered permeability will affect fluid exchange at normal venous pressure as well as at elevated venous pressure. Confirmation of permeability changes can be obtained by measuring diffusion-uptake of solutes of various molecular weights (e.g. dextrans). Histamine reduces the arterial resistance in skeletal muscle and intestinal vascular beds (Kjellmer & Odelram, 1965; Dietzel et al., 1969; Shehadeh, 1969) but has in addition been shown to increase capillary permeability in skeletal muscle (Kjellmer & Odelram, 1965; Appelgren et al., 1966; Dietzel et al., 1969).

Summary

The approach by the Swedish workers allows us to consider the functions of the peripheral vascular bed in terms of series-coupled sections. The precapillary resistance primarily determines total flow of blood to the organ. Precapillary sphincters determine the distribution of this flow within the organ and the capillary surface area available for exchange. This exchange is also affected by the pre/postcapillary resistance ratio which determines hydrostatic pressure, and the structure of the capillary which determines their permeability. The capacitance vessels determine the organ blood content. Examples have been given of the measurement and interaction of these variables in skeletal muscle and intestine.

THE HEPATIC VASCULAR BED

Hepatic vascular studies have been most numerous in the areas of hepatic anatomy and the physiology of the resistance vessels. The knowledge of the hepatic capacitance and fluid exchange functions in the intact liver is extremely limited. Fortunately for those of us working on hepatic vascular physiology, there are two recent and extremely thorough reviews of the anatomy (Elias & Sherrick, 1969) and of the physiology (Greenway & Stark, 1971) of the hepatic vascular bed. The work in this thesis was included in the second of these reviews (except for sections II and IV which were not then completed).

Anatomy of the Liver

Because of the nature of the work described here, a firm understanding of the basic anatomy of the hepatic vascular bed is useful. Elias & Sherrick (1969) have presented a brilliant outline of the structure of the liver with an historical summary and numerous stereograms. An example of their stereograms (three-dimensional representations based on serial sections) is shown in Figure 8. The anatomical information presented here is taken directly from their text.

The Sinusoid

The sinusoid is a capillary lined by littoral cells which are intermediate between endothelial cells and phagocytes. The littoral cells of the liver are also called Kupffer cells and can phagocytise particulate matter. The littoral cells do not adhere to one another but overlap loosely like shingles, leaving many open gaps. The littoral cell cytoplasm forms thin perforated sheets (Figure 9) allowing free

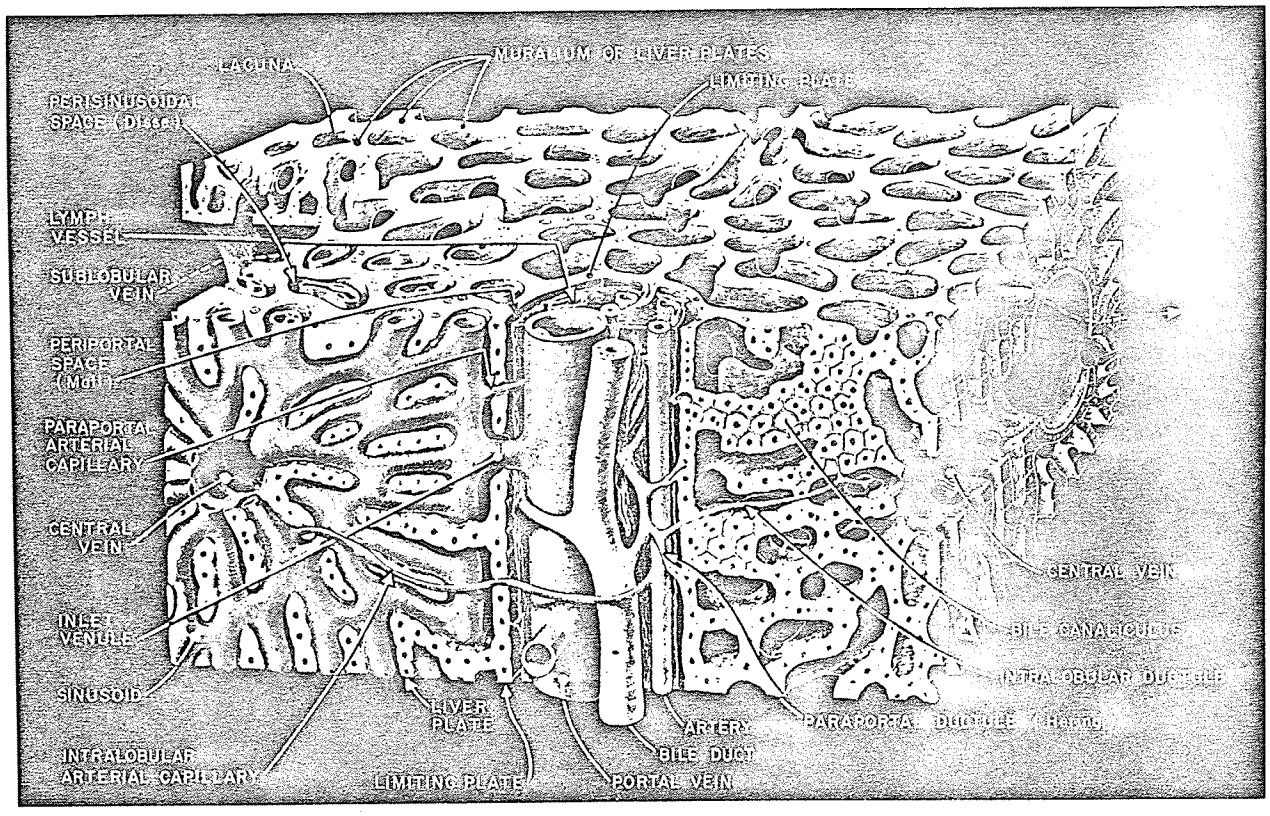


Figure 8. Summary of liver structure (From Elias & Sherrick, 1969).

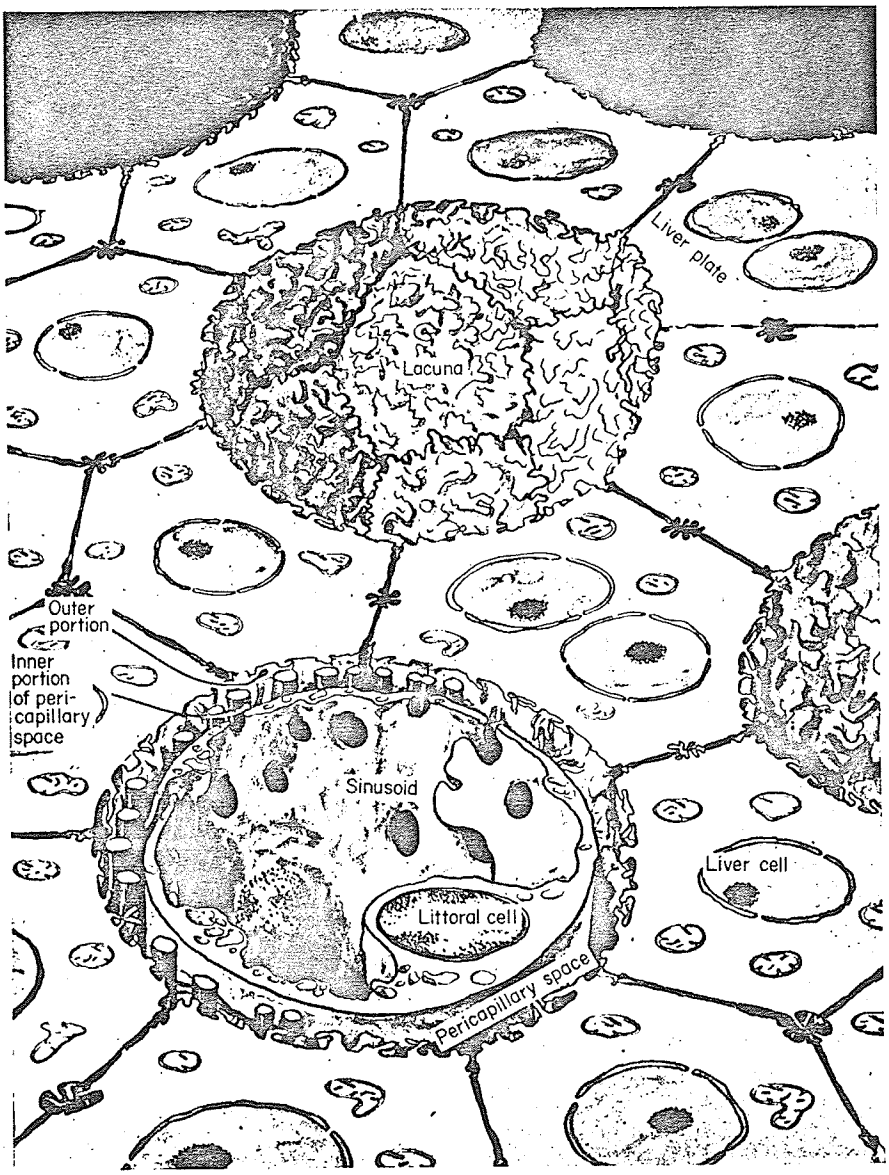


Figure 9. Basic concepts of the liver plate, the lacuna, the sinusoid and the pericapillary space with its inner and outer portion. (From Elias & Sherrick, 1969).

communication between the sinusoidal space and pericapillary space. This type of capillary was previously defined as a discontinuous capillary (sinusoid).

The Lacunae

The lacunae (Figure 9) consist of the sinusoidal space and the pericapillary space (space of Disse) which are separated by the single layer of littoral cells. The interconnecting community of lacunae are referred to as the hepatic labyrinth and are surrounded by single layers of hepatic cells which are collectively referred to as the hepatic muralium. The pericapillary space contains a reinforcing network of reticular fibers which supports the sinusoid. The bile canaliculi surround each liver cell like a wire in a mesh system and this adds considerable structural strength to the muralium.

The Vasculature

The hepatic sinusoids receive blood from the portal vein and from the hepatic artery. Inlet venules arise from small sub-branches of the portal vein. The inlet venules are perpendicular to the portal venule and pass between the cells of the limiting plate (a single layer of hepatic cells surrounding the bundle of small branches of portal vein, hepatic artery, bile duct and lymphatics) (Figure 8). The inlet venules terminate in the sinusoids.

The major arterial supply to the liver is the hepatic artery, a branch of the coeliac artery. Some arterioles enter the sinusoids at the periphery while others enter much closer to the central vein (Figure 8), thus conveying oxygenated blood to the cells in the immediate proximity of the central veins. Portal inlet venules, on the other hand,

enter the sinusoid only at the periphery. The proposed existence of sphincters at the junction of the sinusoid with both the inlet and outlet vessels lacks supporting evidence. This point will be discussed later. The sinusoids drain into the central venules which enter sublobular veins, finally draining into the hepatic veins.

Vascular Physiology of the Liver

I have made no attempt to review the entire literature in this field. Greenway and Stark (1971) compiled a comprehensive review of hepatic vascular physiology and the following synopsis relies heavily on their labors. Only those data relevant to the work to be described in the thesis have been presented.

Blood Pressures and Flows

The hepatic sinusoids receive blood from the portal vein and the hepatic artery. The total hepatic blood flow ranges from 100 to 130 ml/min per 100 g liver (about 25% of the cardiac output) and is relatively constant between species. Roughly 25-30% of this flow is supplied by the hepatic artery and 70-75% is supplied by the portal vein which drains blood from the intestine, stomach, spleen, pancreas and omentum.

The estimated sinusoidal pressure is 2 to 5 mm Hg higher than that of the central venous pressure in the rat (Nakata et al., 1960). Equivalent data are not available for the cat, dog or man. Mean arterial pressures for cats and dogs range from 100 to 130 mm Hg, portal venous pressure from 7 to 10 mm Hg and hepatic venous pressure from 1 to 2 mm Hg. From these pressures the calculated hepatic arterial resistance is in the order of 40 times that of the portal venous resistance. The

majority of sinusoids are perfused by a mixture of arterial and portal venous blood while some small portions of the liver may receive blood from only one source (Greenway & Stark, 1971).

The hepatic arterial smooth muscle is affected by changes in transmural pressure, adrenergic innervation and blood-borne vasoactive substances in the hepatic artery or portal vein. Smooth muscle in the portal vein controls portal vascular resistance. Since portal flow is determined by the sum of the outflow from other organs, changes in portal resistance affect portal pressure much more than portal flow. The portal resistance does not show autoregulation in response to changes in transmural pressure but it is affected by adrenergic nerves and humoral agents.

Autoregulation

The hepatic arterial bed shows myogenic autoregulation (Greenway et al., 1967b). Increases in the hepatic arterial pressure, or portal pressure and flow, or hepatic venous pressure, result in increased resistance in the hepatic arterial system. No such autoregulation is observed in the portal system. The effect of myogenic stimuli on pre-capillary sphincters in the liver is unknown.

Hepatic Nerve Stimulation

In response to adrenergic nerve stimulation the hepatic arterial flow shows a maximal increase in resistance at a stimulation frequency of around 8 Hz (Figure 10). The flow reaches a minimum after about 30 seconds and then recovers toward control levels over 2-5 minutes. The mechanism causing this autoregulatory escape is unknown. The neurally mediated constriction occurs by activation of alpha-adrenergic

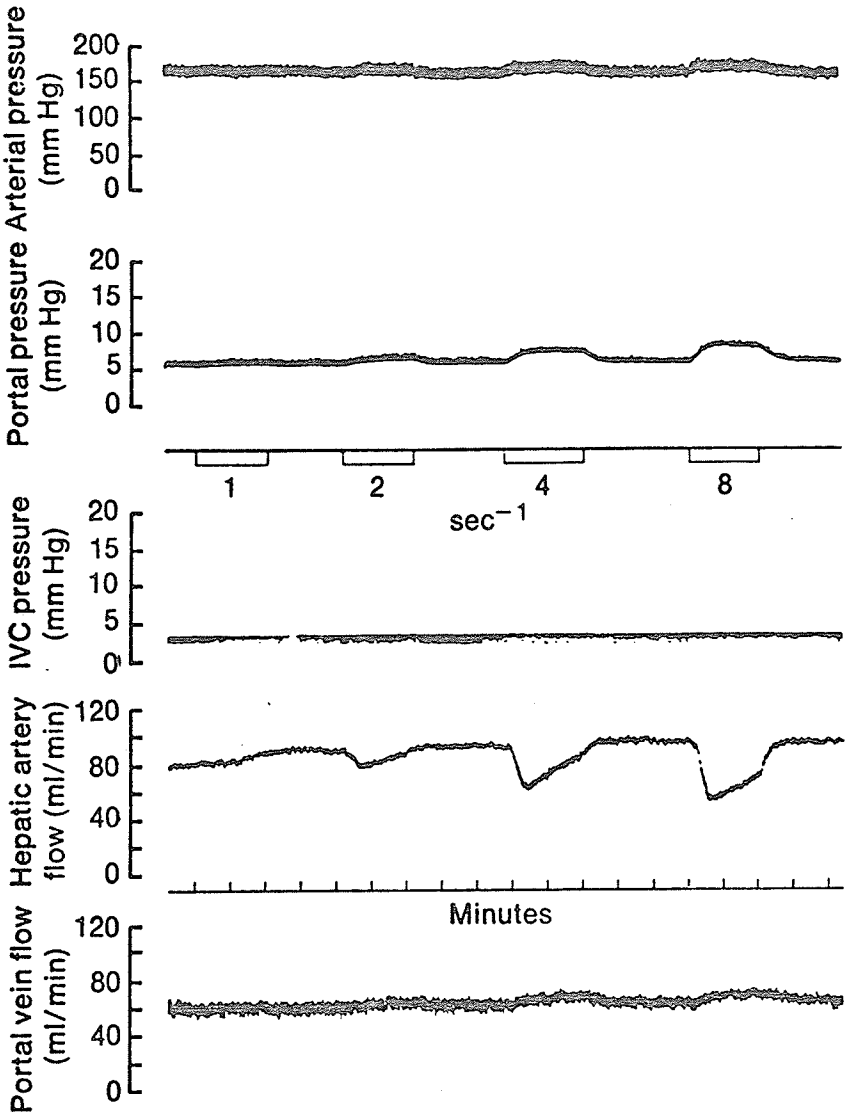


Figure 10. Response of hepatic artery and portal vein to various frequencies of sympathetic nerve stimulation. Note the portal venous response is maintained throughout the period of stimulation while the hepatic artery displays 'autoregulatory escape' (Greenway et al., 1967a).

receptors; however, by blocking these receptors with phenoxybenzamine, a beta-adrenergic vasodilator response can be elicited (Greenway et al., 1967a).

Since the portal flow is determined by the sum of the flows of the organs draining into this vessel, stimulation of the hepatic nerves causes an increase in portal resistance which is seen as an increase in portal pressure with little change in blood flow (Greenway et al., 1967a). Neither the portal vein nor the hepatic artery appears to be innervated by parasympathetic cholinergic fibers.

The capacitance and fluid exchange responses to sympathetic nerve stimulation in the liver are unknown. Griffith & Emery (1930) stated that the liver volume is reduced during hemorrhage but the quantitative involvement and the mechanism of the response are not known.

The Carotid Baroreceptor Reflex

The baroreceptor reflexes are best studied by perfusion of the carotid sinuses but combining this technique with plethysmography was not feasible and therefore the effects of bilateral carotid occlusion were examined. This is a complex stimulus involving baroreceptor and chemoreceptor responses and cerebral ischemia (Brown et al., 1963) but it has been used frequently as a simple means of producing inhibition of the carotid sinus baroreceptors.

Reflex activation of the hepatic adrenergic nerves, accomplished by bilateral occlusion of the carotid arteries, caused a transient elevation of hepatic arterial resistance (Greenway et al., 1967a). The mesenteric artery supplies those organs which drain into the portal vein, and since baroreceptor activity has little effect on mesenteric arterial

blood flow, carotid occlusion causes only small decreases in portal venous flow (Löfving, 1961; Bond & Green, 1969).

Carotid occlusion was thought, for many years, to cause a significant constriction of the capacitance vessels throughout the body (Heymans & Neil, 1958). However in recent years serious doubt has been raised as to whether the venous compartment is affected by carotid occlusion (see Discussion, Section II). Heymans et al., (1931) suggested that the liver volume decreased during the baroreceptor reflex but the extent of this involvement was not made clear.

Pharmacological Agents

The effect of humoral agents on hepatic vascular resistance has been examined both in isolated and intact liver preparations. Serious objections have been raised as to the physiological state of the vascular beds of isolated organs or organs perfused by arterial long circuits (Folkow, 1953; Johnson, 1960; Dresel & Wallentin, 1966; Greenway et al., 1967a). Vascular resistance and reactivity in general are reduced in these preparations. Thus only the results from preparations with intact arterial systems will be considered here.

Infusion of noradrenaline directly into the hepatic artery or portal vein in cats caused increased resistance in these vessels and the hepatic artery showed autoregulatory escape as it did to nerve stimulation (Greenway et al., 1967a; Ross & Kurrasch, 1969). Intravenous infusion of noradrenaline also increased vascular resistance but if arterial pressure was uncontrolled the flow remained constant. Adrenaline in small doses caused inconsistent effects on hepatic arterial resistance and this appeared to result from the combined response of

both alpha and beta-adrenergic receptors. The portal resistance was elevated and intravenous infusions of adrenaline increased total hepatic blood flow while the hepatic arterial flow showed only a slight change. The increased hepatic flow is due to intestinal and splenic vasodilation and can be blocked by propranolol (Greenway & Lawson, 1966b; Ross, 1967a, 1967b; Greenway & Lawson, 1968; Greenway & Stark, 1970). Isoproterenol caused vasodilation of the hepatic artery and a marked increase in portal vein flow due to intestinal and splenic dilation (Greenway & Stark, 1971). Catecholamines are known to cause constriction of capacitance vessels in a number of vascular beds (Mellander & Johansson, 1968) though data for the liver do not exist.

Histamine infused into the hepatic artery or the portal vein caused a marked vasodilation of the hepatic arterial bed in cats and dogs. Portal pressure in the dog was elevated (Chien & Krakoff, 1963) and hepatic venous outflow block occurred in response to small doses of histamine (Oshiro & Greenway, 1971). Smooth muscle in the portal vein of the cat is relatively insensitive to histamine (Hughes & Vane, 1967) and the occurrence of outflow block has not been reported.

Intravenous angiotensin caused a decrease in total hepatic blood flow and usually a slight elevation of portal pressure (Chiandussi et al., 1963; Bashour et al., 1963). Hepatic arterial flow was reduced and portal flow declined due to intestinal and splenic vasoconstriction (Cohen et al., 1970).

The splenic capsule contracted in response to angiotensin (Greenway & Stark, 1970) and, although the potency of angiotensin in this response was high, it was estimated that the quantities of

angiotensin endogenously released were not sufficient to cause significant splenic constriction. The effect of angiotensin on capacitance vessels and fluid exchange throughout the body has not been thoroughly evaluated though it is generally considered that angiotensin is not a venoconstrictor nor does it alter fluid exchange significantly (Mellander & Johansson, 1968).

Vasopressin reduced total hepatic blood flow as well as portal venous flow and pressure (Cohen et al., 1970). Hepatic arterial resistance was slightly elevated by vasopressin on direct arterial infusion but after i.v. infusion, hepatic arterial resistance did not change or even decreased (Cohen et al., 1970). This may have been due to a myogenic response to the decrease in portal flow (Hanson & Johnson, 1966).

Vasopressin demonstrates considerable regional differences in vascular responses as does angiotensin. Vasopressin is not considered to elicit significant capacitance or fluid exchange responses in gut or skeletal muscle (Mellander & Johansson, 1968). The response of the splenic capsule was weak and it was suggested that endogenous release of vasopressin is not of sufficient quantity to cause expulsion of blood from the spleen (Greenway & Stark, 1970).

The response of the hepatic vascular resistance to these various stimuli has received considerable attention; however, it is only total vascular resistance that has been evaluated. The effect of these stimuli on the pre- to postcapillary resistance ratio and on presinusoidal sphincter resistance has remained unexplored as have the capacitance responses. From the data discussed earlier, obtained from skeletal muscle and intestinal vascular beds, it might be expected that

many of the stimuli cited above would have effects on sinusoidal pressure and surface area in the liver as well as on hepatic blood volume.

Hepatic Blood Content and Fluid Exchange

The most poorly understood and least studied areas of hepatic vascular physiology are those of capacitance and fluid exchange. The earliest attempts to measure hepatic volume involved crude air filled plethysmographs (Francois-Franck & Hallion, 1896, 1897; Thompson, 1899). Later plethysmograph studies (Heymans et al., 1931; Griffith & Emery, 1930) on the effects of carotid occlusion and hemorrhage on liver volume included no quantitative data. The plethysmographs used in these early studies had unstable base-lines and apparently leaks were frequent. The state of the animal with the plethysmograph in place was not evaluated in detail but it must be assumed that many problems beset the experimentors since in vivo recording of the hepatic volume utilizing the plethysmograph was discontinued.

Hemodynamic studies, including those on blood content and fluid exchange have been attempted in isolated livers where blood or a substitute was pumped through the hepatic artery or portal vein or sometimes both vessels. Brauer et al., (1959) examined the effects of elevated venous pressure on the isolated perfused liver of the rat. Hepatic volume increased and transudate with a protein content similar to that of plasma protein appeared on the liver surface. Serious objection to the use of isolated preparations for hemodynamic studies has already been mentioned (p. 26). Greenway et al., (1967a) showed that insertion

of an arterial long circuit into an otherwise intact hepatic preparation, markedly reduced the vascular reactivity of the hepatic bed. It is therefore imperative that any examination of fluid exchange be done in a vascular bed with normally functioning resistance segments.

THE PROBLEM

In the present work I decided to examine the effects of a variety of stimuli on blood content and fluid exchange in the intact hepatic vascular bed. The techniques developed by Mellander (1960) and reviewed earlier in this thesis were used with appropriate adaptations. The criteria to be fulfilled for proper use of the Swedish techniques were straightforward.

1. The organ had to be enclosed in a plethysmograph and sealed completely without interference with the nervous supply or the blood supply from the hepatic artery and portal vein, and without causing occlusion of the hepatic venous outflow. It was also essential that changes in plethysmograph volume not cause changes in the pressure within the plethysmograph.

2. The ^{51}Cr-tagged red blood cell techniques had to be adapted for use in the liver in such a way that the radioactivity of the liver could be monitored.

3. Establishment of a hepatic venous long-circuit was required in order to control the venous pressure for CFC measurements. This was complicated by the inaccessibility of the hepatic veins, thus a long circuit preparation had to be devised which involved cannulation of the inferior vena cava (Greenway & Lawson, 1966a), a modification of techniques reported by Grundy & Howarth (1957).

For ease of reading, the thesis has been divided into four sections. The results in each section are discussed before the next set of data are presented. In Section I the effects of stimulation of the hepatic nerves and bilateral carotid occlusion on hepatic blood volume and fluid exchange are examined. Section II examines the hepatic blood volume responses to infusions of adrenaline, noradrenaline, angiotensin, vasopressin and histamine. Section III demonstrates the effects of hepatic venous pressure on trans-sinusoidal fluid exchange while the effects of infusions of isoproterenol, histamine and adrenaline and hepatic arterial occlusion on this fluid exchange are reported in Section IV. The methodology involved in obtaining these results is described as one unit since many of the techniques were used in all four sections.

METHODS

SURGICAL METHODOLOGY

Anesthesia and Minor Surgical Preparation

Cats of either sex were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg Nembutal, Abbott). Additional doses (3 mg/kg) were administered intravenously when reflex limb, ear or eye movements returned. The trachea was cannulated and mean femoral arterial pressure was recorded from a cannula in the right femoral artery.

Insertion of the Liver into the Plethysmograph

An abdominal incision was made along the midline (6-8 cm long) and along the right subcostal margin (4-5 cm). The cutaneous and muscle layers along the incision were tied separately. Great care taken at this stage avoided blood loss throughout the experiment. In cats weighing less than 2.3 kg or in cats with a narrow chest, a 2 cm incision was also made along the left subcostal margin. The anterior ligaments connecting the left medial and quadrate lobes to the diaphragm and the dorsal ligaments connecting the left lateral lobe of the liver to the diaphragm were ligated and cut.

In experiments where the hepatic nerves were stimulated, the procedure described under the appropriate heading below was followed at this time. Unless otherwise indicated, the nerves and lymphatics were dissected free from the hepatic artery, ligated and cut. A branch of the hepatic artery, the gastroduodenal artery, was cannulated in some experiments to allow close intra-arterial infusions into the hepatic artery without obstruction of the flow. Portal pressure was

recorded from a cannula inserted (to within 1 cm of the hilum of the liver) through a small vein from the appendix. During this procedure the liver and intestines were kept moist with Ringer-Locke solution. In experiments in which the venous long-circuit was used, a loose ligature was placed around the inferior vena cava above the adrenal veins and below the hepatic vein.

A carrier, lubricated with paraffin oil (Figure 11), was then slid under the entire liver except for the right lateral and caudate lobes, and, when the liver was securely in place on the carrier, it was lifted and the lower plate of the plethysmograph (Figure 12) was slid under the carrier which was then removed. In this way the liver was not exposed to hard or sharp edges or to undue manipulation.

A plasticized hydrocarbon gel (Plastibase, Squibb) was squirted by syringe around the aperture and all around the perimeter of the lower plate where the second section (the sides) of the plethysmograph would rest. The second section of the plethysmograph was then lowered over the bottom plate and held in place by pins cemented onto the bottom plate and inserted through the side plate (Figure 12). Again Plastibase was injected around the aperture of the plethysmograph as well as on the upper surface of the second section. The lid (Figure 13) was then passed over the securing pins and held in place by wing nuts which were connected to these pins.

By this method the liver was sealed within the plethysmograph and the hepatic artery, portal vein and hepatic veins passed, intact, through a 2 cm aperture. The plethysmograph was filled with warm

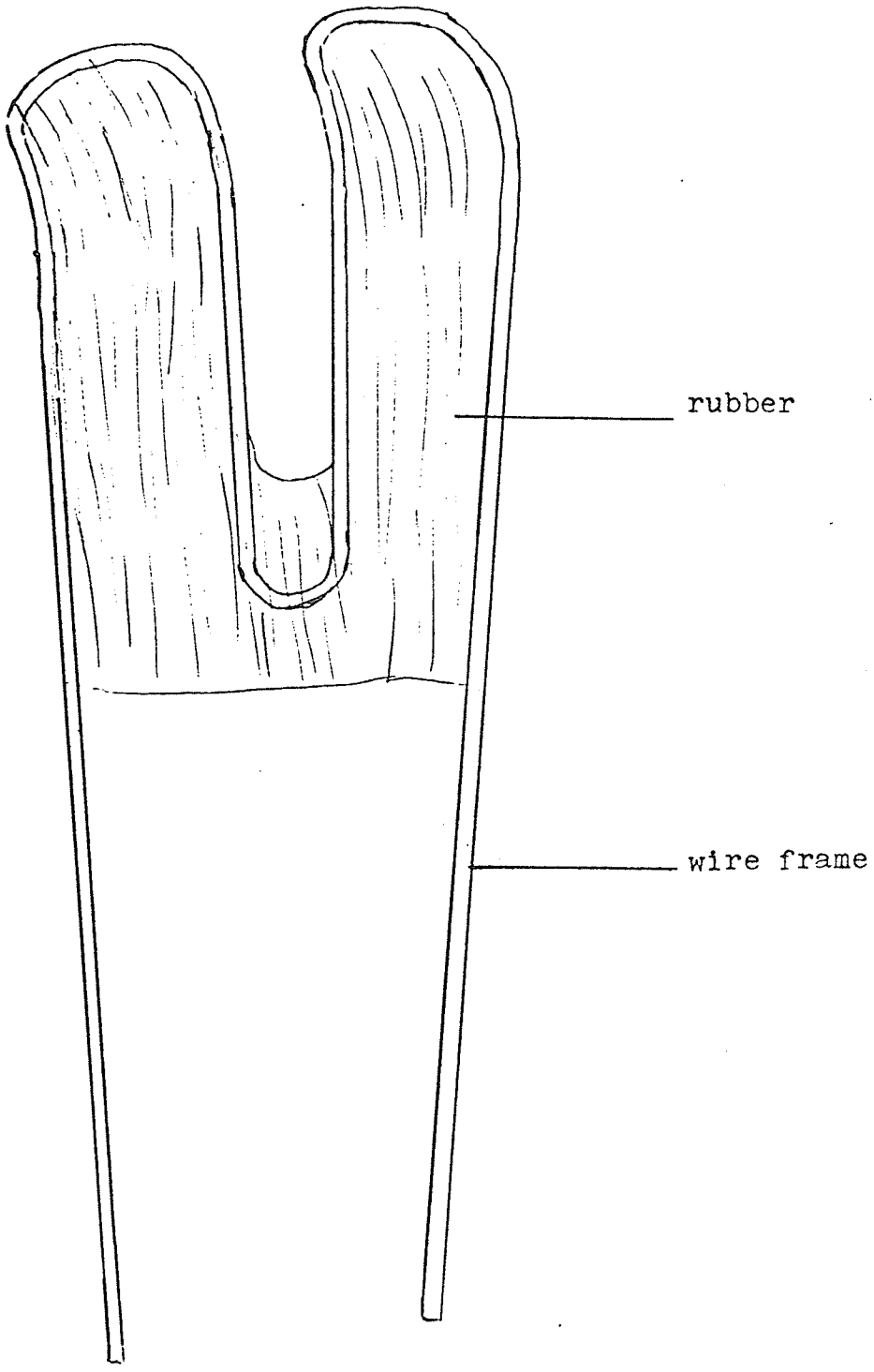
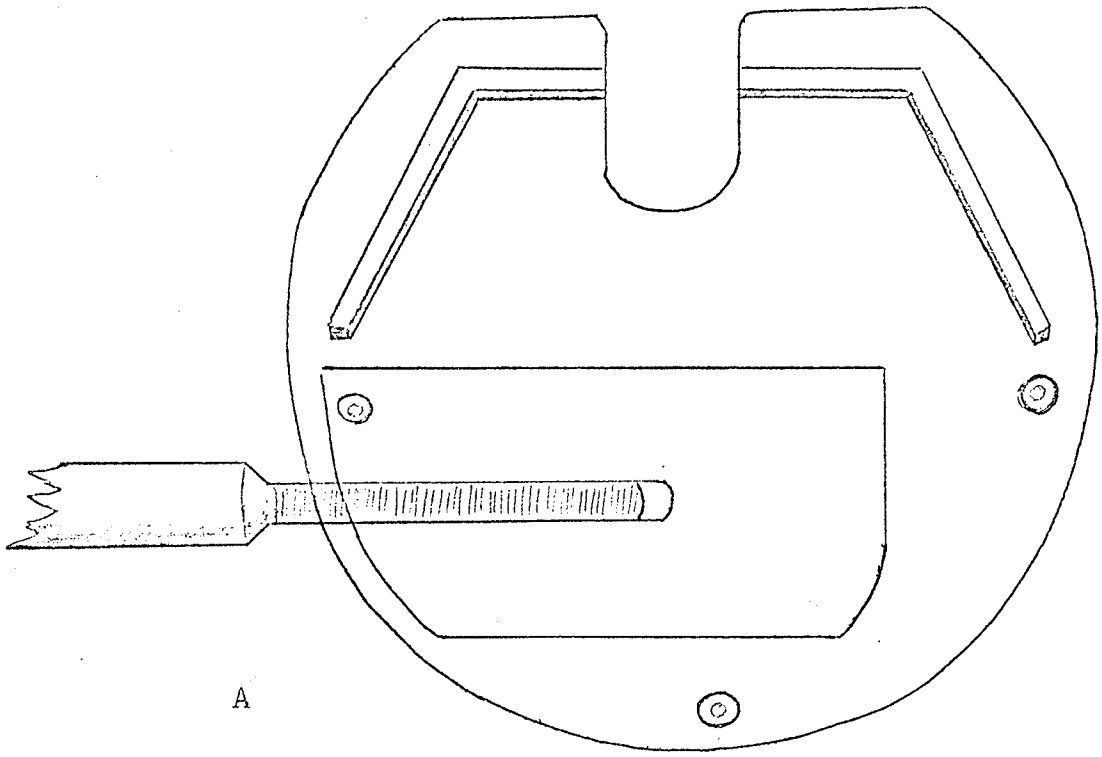
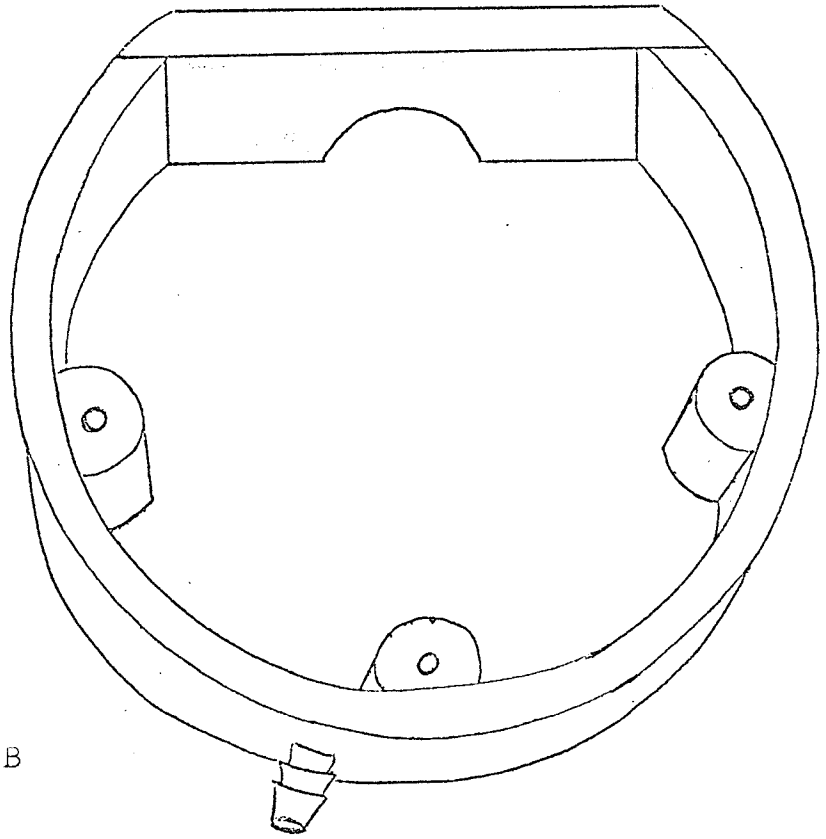


Figure 11. Lubricated carrier - used to elevate liver to allow insertion into the plethysmograph.



A



B

Figure 12. A. Bottom plate
B. Side segment of plexiglass plethysmogrph

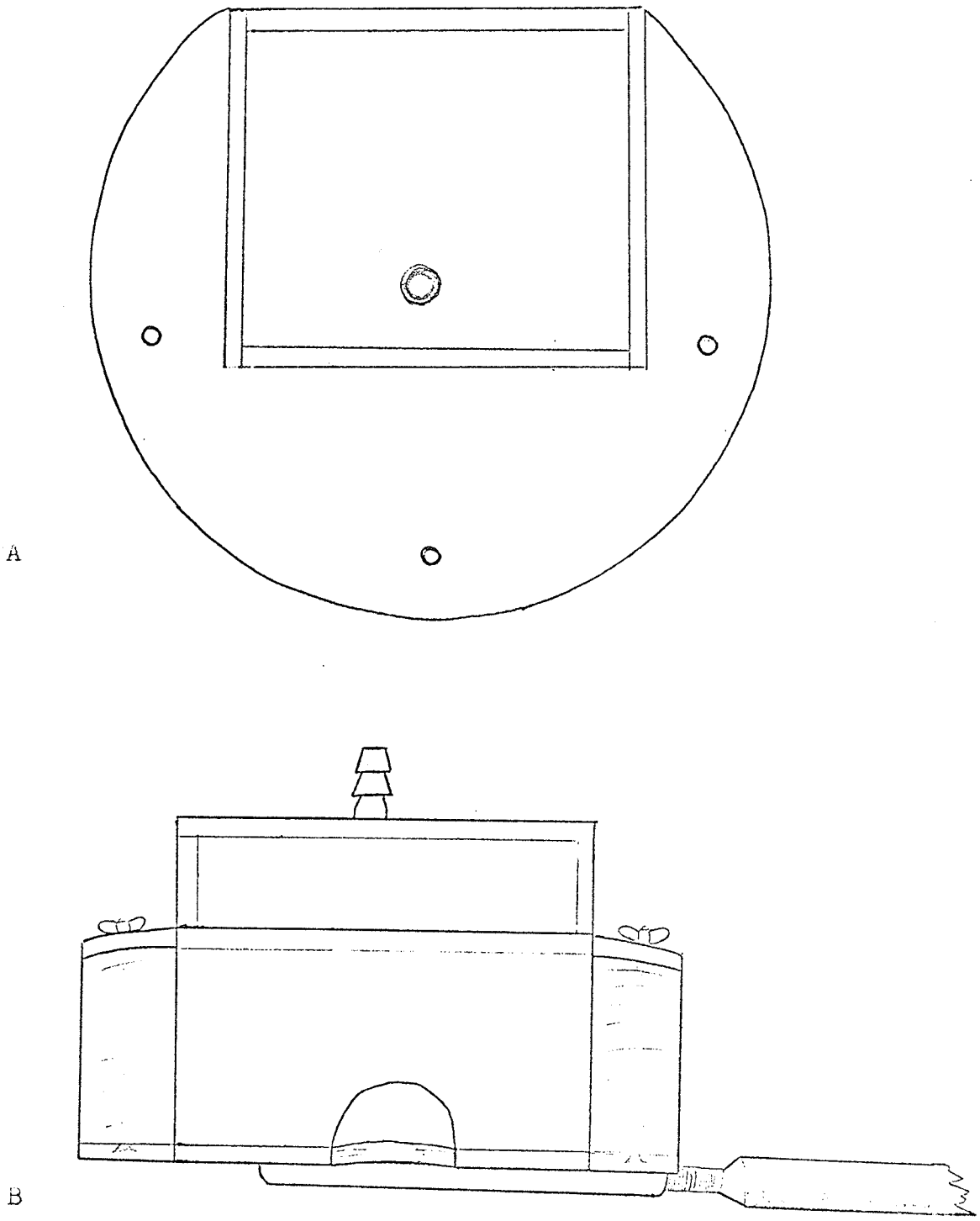


Figure 13. A. Lid of plethysmograph
B. Assembled plethysmograph

(37°C) Ringer-Locke solution and connected to a float recorder which was attached to an isotonic transducer (Harvard Apparatus Co. Model 356) (Figure 14). The pressure in the plethysmograph was set at zero relative to the atria of the heart.

Hepatic Nerve Stimulation

The hepatic nerve plexus was carefully dissected free of the hepatic artery immediately proximal to the branch of the gastroduodenal artery. The longest section of nerve which could be freed by this method was 3-4 mm. The nerves were tied and cut and the distal ends were inserted through a 2 mm diameter ring electrode. The electrode was made of a ring of plexiglass with two parallel circles of platinum wire mounted on the inner surface to serve as bipolar stimulating electrodes. The electrodes were connected to a Grass stimulator (Model SD5) through an isolation unit. Maximal stimulus parameters of 1 msec duration and 15 volt square wave pulses were used (Greenway et al., 1967a). Only the frequency of stimulation was varied.

The Venous Long-Circuit

In experiments where the long-circuit was to be used a ligature had been placed around the inferior vena cava between the renal and hepatic veins before insertion of the liver into the plethysmograph. The cat was given positive pressure ventilation and an incision was made into the 6th or 7th intercostal space. A retractor was used to widen the 2 cm long incision so as to allow access to the inferior vena cava. The inferior vena cava was cleared of the phrenic nerve and connective tissue. After this last major

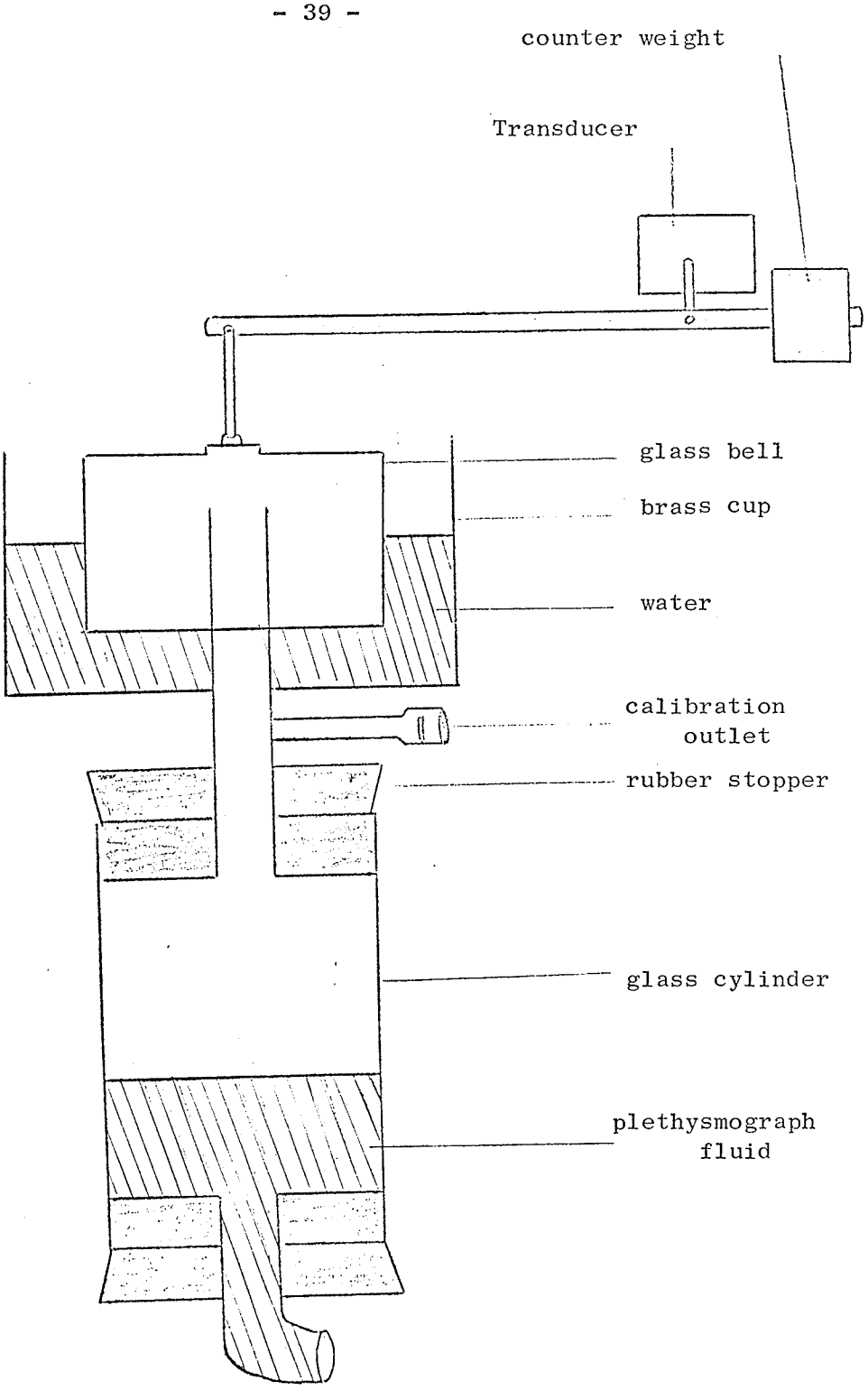


Figure 14. Volume recording apparatus - attached to plethysmograph - transducer output goes to pen recorder.

incision had been made, 15-20 minutes was allowed for hemostasis then heparin (10 mg) was administered. The femoral and jugular veins were cannulated with teflon cannulae. Teflon tubes have thinner walls than conventional tubes and were therefore used to allow the largest possible internal diameter in the venous cannulae.

[A large donor cat was anesthetized with ether and the femoral vein was cannulated. Blood was withdrawn using 20 ml syringes and the collected blood was placed into a glass container with 10 mg of heparin. Part of this blood was used to fill the reservoir and the perfusion lines (72 ml); the rest was used during the experiment to replace blood and fluid loss.]

The clamps were removed from the femoral venous cannulae and as blood filled the drain tubes and flowed into the reservoir, the exact volume of blood drained from the femoral veins was pumped back to the animal via the jugular veins. The thoracic inferior vena cava was then tied and all the blood that had previously reached the heart via the inferior vena cava was now drained from the femoral veins and pumped back to the heart via the jugular veins.

The inferior vena cava was cannulated through the thoracic incision and the glass cannula (Figure15) was sutured into place along with another tube used to produce a small negative intrathoracic pressure. The skin edges were sealed around the cannula and vacuum tube and a negative intrathoracic pressure of 5 cm of water was applied. Artificial respiration was discontinued and the cat breathed spontaneously.

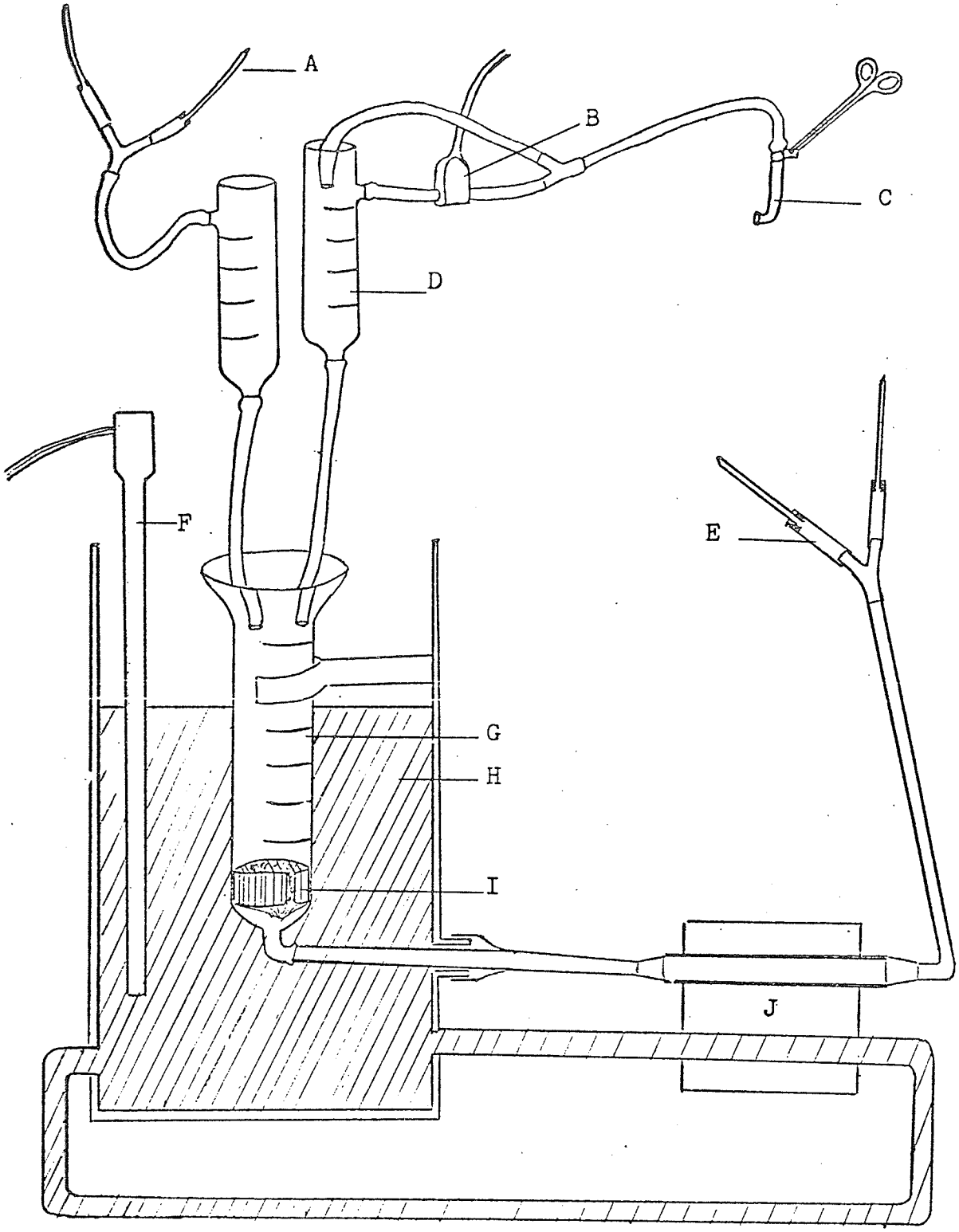


Figure 15. Long-circuit assembly. A. femoral vein cannulae. B. electromagnetic flow probe. C. inferior vena cava cannula. D. auxiliary reservoir. E. jugular cannulae. F. heater and thermostat. G. reservoir. H. warming bath. I. filter. J. peristaltic pump.

The loose ligature which had previously been placed around the vena cava between the renal and hepatic veins was tightened by suspending a weight from it. Thus all the flow below the occlusion of the vena cava was drained via the femoral veins. This included all the blood normally passing via the inferior vena cava to the heart except for the hepatic outflow which was drained by the cannula in the thoracic vena cava (Figure 16). An electromagnetic flow probe was used to obtain a continuous record of total hepatic blood flow.

The hepatic venous pressure was controlled by raising or lowering the outlet level of the hepatic venous cannula. Unless specifically stated otherwise the hepatic venous pressure was set at zero relative to the hilum of the liver. Femoral arterial pressure, portal venous pressure, hepatic venous blood flow and hepatic volume were recorded on a Beckman polygraph recorder.

The blood reservoir in the long-circuit was supplied with a filter made of fiberglass and surgical gauze in order that any clots formed in the long-circuit would be trapped and excluded from the circulation. The glass reservoirs were siliconized prior to each experiment and the cat was heparinized to further reduce clotting. The circuit was assembled (Figure 15) and washed through with a 10% formaldehyde solution followed by a weak ammonia solution to precipitate any remaining formaldehyde. Finally the circuit was washed four times with distilled water and then sealed until it was used the next morning. Such rigid sterilization procedures are essential to prevent bacterial contamination of the preparation (Greenway and Howarth, 1963).

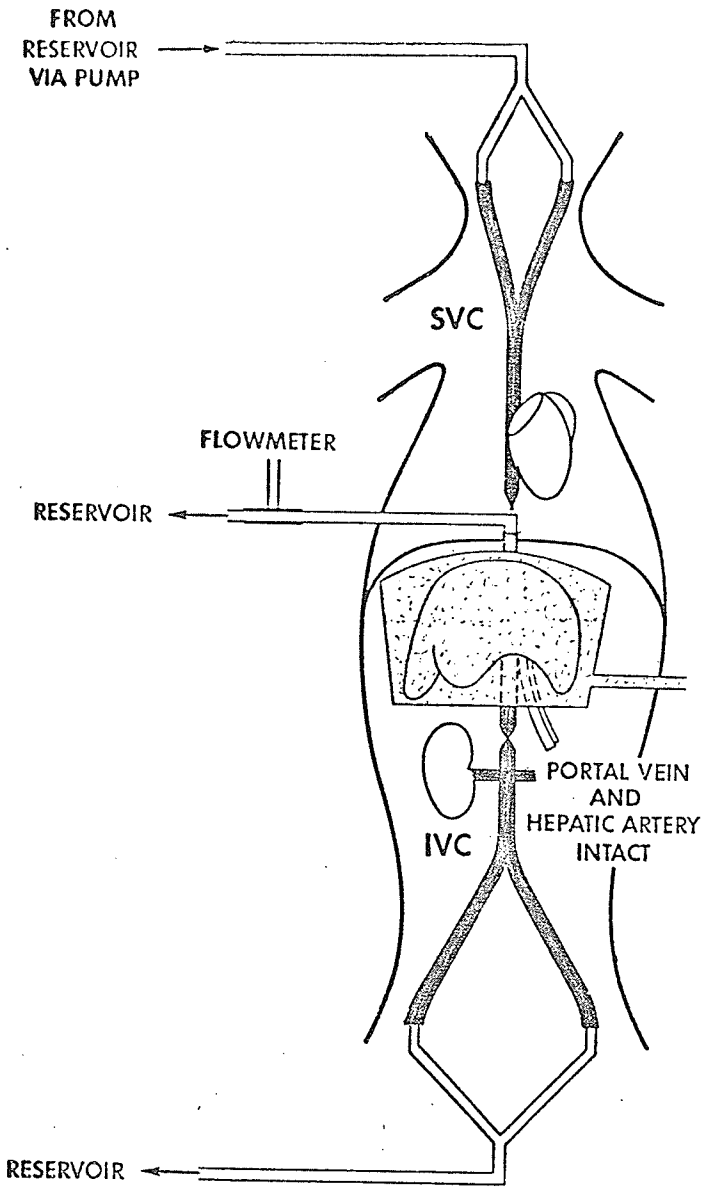


Figure 16. The hepatic venous long-circuit with the liver in a plethysmograph. The IVC is occluded above the renal veins such that all blood entering the IVC below the occlusion drains backward via the femoral cannulae while the blood entering above the occlusion (only hepatic blood) is drained via the thoracic cannula. Hepatic venous pressure is regulated by the position of the outlet of the thoracic cannula. Total hepatic blood flow is measured from this outlet. The blood from the outlet cannulae is drained to a reservoir, warmed and pumped back to the cat via the jugular vein cannulae.

NON-SURGICAL METHODOLOGY

Infusions

Close intra-arterial infusions into the hepatic bed were accomplished by the use of a cannula in the gastroduodenal artery. The common trunk of the hepatic artery divides to form the hepatic artery and the gastroduodenal artery, thus infusions into this side branch of the hepatic artery will be carried directly to the liver. Intravenous infusions were made directly into the blood reservoir in experiments in which the long circuit was established. In other experiments drugs were infused into a femoral vein.

Infusions were made with an infusion pump (Harvard apparatus, Model 940) and the volume of infusion was kept to a minimum by infusing at rates of 0.136 to 0.0136 ml/min. Only when dose-response curves were being obtained did the rate of infusion vary from this range. The extreme range of infusion rates was 0.68 to 0.0034 ml/min.

Stock solutions of all the drugs used except angiotensin and vasopressin were dissolved (1 mg/ml) in distilled water and then frozen. The stock solutions were made up in 50 ml quantities which were re-frozen after use. Isoproterenol-HCl (British Drug Houses), (-)-Adrenaline-HCl (Sigma Chemical) and (-)-noradrenaline tartrate (Wintrop Laboratories) stock solutions were prepared for infusion by dilution of 1 ml stock to 68 ml in saline and ascorbic acid (20 mg/100 ml). Angiotensin amide (Hypertensin^{R}, Ciba), vasopressin (Pitressin^{R}, Parke, Davis Co.) and histamine acid phosphate (British Drug Houses) were made up in 0.9% NaCl solution. All doses of catecholamines and histamine are expressed as free base.

Calculation of Hepatic Blood Volume

At the end of an experiment a sample of hepatic venous blood was taken and a cord was tied around the outlet from the plethysmograph thereby simultaneously clamping all vessels. The vessels were cut below the cord and the weight of the portion of the liver in the plethysmograph was determined as was the total weight of the liver. A cannula was inserted into the hepatic veins and the liver was flushed with 0.9% saline. The fluid which was drained from the portal vein was collected and its volume recorded.

An ammonia solution was prepared by diluting 1.4 ml of stock solution (28% ammonia) to 100 ml with deionized water. Forty ml of this solution was placed in each of four plastic containers and 1 ml of washout sample was added to one, duplicate samples of 0.1 ml blood were added to two other containers and the fourth served as a blank. Light absorbance was determined with a spectrophotometer at wave length 540 λ. Hepatic blood volume was calculated by the equation:

Blood Volume = (Washout reading/Mixed venous reading) X

(volume of washout/10.)

Radioactive tagging of Red Blood Cells

A 5% Rheomacrodex solution was given as replacement for a 10 ml blood sample which was withdrawn into a syringe that had previously been filled with 3 ml ACD solution (2g disodium citrate, 3g dextrose, water to 120 ml). The blood sample was centrifuged at slow speed for about 15 min and 3 ml of the plasma supernatant was added to 100 ml sterile isotonic saline to be used later as a wash solution. A sterile

isotonic solution of $Na_2Cr^{51}O_4$ (0.2 mc) was added to the red cells, shaken and left for 30 minutes at 37°C. The red cells were washed twice and recentrifuged at < 1000 rpm and the final packed cells were re-suspended in 10 ml of the plasma-saline solution.

The radioactivity from the liver was measured by placing a 5 x 5 cm NaI crystal with collimator (5.4 cm wide and 11.5 cm long) directly over the top of the plethysmograph, (10 cm) and recorded with a scaler-timer (Baird Atomic Type 135) and a rate meter (t 1/2 = 20 sec). Pulses of gamma energies above 100 kev were counted. The labeled red cells were infused until approximately 10,000 cpm were recorded over the liver and noradrenaline (1-2 μg/min) was infused to ensure mixing of the tagged cells with red cells in the spleen.

Protein Content of Filtrate

In three experiments the plethysmograph was filled with petrolatum (USP) instead of Ringer-Locke solution. A small polyethylene pipe was inserted into the lowermost part of the plethysmograph and samples of filtrate were collected through this tube. If the slightest trace of red blood cells was present the data were excluded. A 1 ml sample of filtrate and mixed venous blood were taken simultaneously. The blood sample was centrifuged and the plasma was treated in an identical manner to the filtrate.

The technique of protein estimation involved measurement of the specific gravity of the sample with copper sulfate solutions of various strengths. A single drop of sample was added to the copper sulfate solution and the specific gravity of the sample was calculated

from the concentration of the solution that it remained suspended in, neither rising to the surface nor sinking. For full description and evaluation of this technique see Phillips et al. (1950), and Van Slyke et al. (1950). The protein content of the filtrate was expressed as a percentage of the serum protein.

Calibrations

The femoral arterial and portal venous pressure transducers were calibrated with a mercury and water manometer respectively once every month. The transducers and recorder were found to be extremely stable. The hepatic venous flowmeter was easily calibrated since the flow could be manually checked by a direct measurement of the time taken to fill the auxiliary reservoir (see Figure 15) to a pre-measured level. Zero flow was also checked by clamping the thoracic cannula in front of the flow probe and allowing the blood to pass through a side branch and thus to be shunted around the flow probe with free access to the reservoir (Figure 15). The hepatic volume recording was checked for linearity and calibrated before every experiment. The full range of operation was checked by stepwise injections of 2 ml to a total of 10 ml into the plethysmograph.

EVALUATION OF METHODS

Anesthetics and General State of Animals

The cats were anesthetized with pentobarbital since barbiturate anesthesia has been shown to cause no significant alteration in hepatic vascular parameters (Fisher et al., 1956; Gilmore, 1958; Evringham, 1959; Galindo, 1965; Katz, 1969). Greenway et al. (1967a)

reported that hemodynamic effects of stimulation of the hepatic nerves were the same in cats under pentobarbital anesthesia as in those under chloralose and urethane.

The general state of the animals was good. Corneal, ear flick and swallowing reflexes returned repeatedly as the effects of each supplementary dose of pentobarbital wore off. The arterial pressure was well maintained and liver volume remained stable. Dose-response curves obtained 3 hours apart were not significantly different and consistent responses to nerve stimulation were obtained for at least 4 hours after completion of the preparation. The cats breathed spontaneously. It was hoped that we could avoid the hypocapnia frequently arising from excessive artificial ventilation. Blood gas tensions were not measured.

Laparotomy produces little change in total hepatic blood flow (Restrepo, 1960); however, Greenway et al. (1967b) showed that with even mild hemorrhage the hepatic artery dilated and portal venous flow was reduced. Though blood loss during surgery was always very small the cumulative effects of minor blood loss and variable trauma could possibly have altered the ratio of arterial and portal flows while not altering the total hepatic blood flow.

The Plethysmograph

The edges of the plethysmograph were smoothed and rounded to avoid tissue damage. When the wall and lid of the plethysmograph were slid into place over the metal bolts, care had to be taken to avoid catching an edge of the liver between segments of the plethysmograph.

The right lateral and caudate lobes of the liver are separate in the cat and could be excluded from the plethysmograph without causing tissue damage. In a few cats isolation of these lobes was difficult and resulted in hepatic congestion seen as dark blotchy areas on the liver surface. If, for any reason, the liver took on this appearance the experiment was terminated unless the condition could be corrected and the liver congestion relieved. Bleeding of the liver surface as a result of clumsy handling was rare but when it occurred it was obvious since the blood pooled in the transparent plethysmograph. These experiments were excluded.

Plastibase proved to be an excellent sealant without obstructing the intact hepatic vessels and the liver did not herniate through the aperture. The effect of the plethysmograph on portal venous pressure was an indicator of the extent of obstruction to the portal or hepatic veins. Minimal obstruction occurred. Control pressures and flow were within normal range (see general observations during control periods). The volume base-line was stable for extended periods of time suggesting that the plethysmograph did not leak. On the two occassions that Evans blue dye was introduced into the plethysmograph, no color appeared in the peritoneal space. The volume of the liver returned to control levels on cessation of nerve stimulation or drug infusion, indicating that the plethysmograph had not leaked during the maneuver.

It was not possible to determine the site of volume changes within the liver. Large vessels of the portal vein and hepatic venous

channels within the liver may contain 40% of the blood volume of the liver with 56% of the blood volume being in the small vessels (Greenway and Stark, 1971) but the relative contribution of each segment toward the total capacitance response is unknown.

The gall bladder was not a factor in the volume response measured. The gall bladder was visible through the transparent wall of the plethysmograph. Its volume was usually 2 to 3 ml and no obvious changes in this volume occurred during the experiment. The lymphatics were routinely tied in all experiments except those in which the effects of i.v. angiotensin, elevated hepatic venous pressure or carotid arterial occlusion were compared in the presence and absence of the hepatic nerves. Routine occlusion of the lymphatics did not result in an increasing base-line and was unlikely to affect the results. The lymphatics have been ligated in similar experiments on the intestine and skeletal muscle (Mellander, 1960; Folkow et al., 1963).

Hepatic Blood Volume Determinations

In the calculation of hepatic blood volume the assumption has been made that the intrahepatic hematocrit is the same as that of the arterial blood. The organ hematocrit may in fact be less than that of venous blood (Lewis et al., 1952; Allen and Reeve, 1953). The blood volume estimated in this way could represent an underestimate of from 10% to 30% based on the lower organ hematocrit determined from the rat and dog respectively. However, the error is probably less than this since the present determination includes all the intrahepatic vessels and not just the sinusoids and small vessels.

GENERAL OBSERVATIONS DURING CONTROL PERIODS

The control values presented here (Figure 17) are means ± standard errors of the data from every cat which was used. All of the experiments involved insertion of the liver into a plethysmograph. Some animals also had the venous long-circuit established. Animals in which the venous long-circuit was established underwent grossly different surgical preparation and the control results for each group are presented separately. Group 1 only had the liver inserted into the plethysmograph while group 2 also had the venous long circuit. Control values for each series of experiments are presented in the appropriate 'Results' sections.

All control pressures and flows were measured after the surgery was completed, immediately before the first experimental maneuver. Hepatic blood volume was determined at the conclusion of the experiments. The blood volume in group 2 was measured after venous pressure had been elevated at least once in all but two cats. In these two cats the long-circuit was established but venous pressure remained at zero for the duration of the experiments (the hepatic blood volume for these animals was 23 and 29 ml/100 g). Insertion of the liver into the plethysmograph resulted in an elevated portal venous pressure. The elevation was significantly less in the presence of the hepatic venous long-circuit. The hepatic blood volume was greater in those animals in which the long-circuit was established and this may be related to the fact that during cannulation of the inferior vena cava, when all of the caval blood was drained via the femoral venous cannulae, there was a large temporary elevation in hepatic venous pressure.

	Group 1 (n=23)	Group 2 (n=32)
Cat body weight (Kg)	2.5 ± 0.03	2.5 ± 0.4
Liver weight (g)	89.4 ± 2.4	91.3 ± 3.7
Liver weight in pleth. (g)	71.9 ± 2.5	76.0 ± 2.8
Arterial pressure (mmHg)	135.6 ± 4.3	122.4 ± 5.3
Portal venous pressure (mmHg)		
Before insertion in pleth.	6.4 ± 0.4	5.7 ± 0.3
After insertion in pleth.	9.7 ± 0.4	7.7 ± 0.4*
Mean change on insertion	+3.2 ± 0.3	+1.8 ± 0.4*
Hepatic blood flow (ml/min/100g)	-	101.1 ± 4.7
Hepatic blood volume (ml/100g)	19.7 ± 3.0	28.3 ± 3.3*

Figure 17: General observations during control periods. Group 1 had the liver in the plethysmograph while Group 2 had the venous long-circuit in addition. Hepatic blood flow was not measured in Group 1.

* Statistically significant difference between Group 1 and 2 ($p < 0.01$).

SECTION I

BLOOD VOLUME RESPONSES AND FLUID EXCHANGE IN THE CAT LIVER DURING STIMULATION OF THE HEPATIC NERVES AND BILATERAL OCCLUSION OF THE CAROTID ARTERIES

RESULTS (SECTION I)

Venous Long-Circuit Control Results

The mean control results from all experiments have been tabulated and presented earlier; the following control data are means and standard errors of the values recorded in 15 cats of this series (exclusive of the carotid occlusion series in which the long-circuit was not used). The mean body weight was 2.6 kg and the liver weight was 72 ± 3.8 g. The portion of the liver within the plethysmograph was 85% (61 ± 3.1 g) of the total. Mean portal venous pressure was 8.0 ± 0.4 mm Hg; arterial pressure was 112 ± 2.4 mm Hg and hepatic blood flow was 128 ± 12 ml/min per 100 g liver. Hepatic blood volume determined at the end of the experiments was 27 ± 3.3 ml/100 g liver.

Capacitance Response to Hepatic Nerve Stimulation

Square wave pulses (15 v, 1 msec duration) were delivered to the hepatic nerves at frequencies of 1-10 per second for periods of 3-20 minutes on 107 occasions. Figure 18 shows the responses of one cat to stimulation of the hepatic nerves at frequencies of 2 and 4 per second. Arterial pressure remained virtually unchanged while portal venous pressure showed a maintained increase as described by Greenway *et al.* (1967b). The liver volume decreased rapidly at first and then reached a plateau by about 4 minutes. The decrease was greater and more rapid and the plateau was reached earlier as the frequency of stimulation increased. Maximal responses occurred at frequencies of 6 to 8 per second.

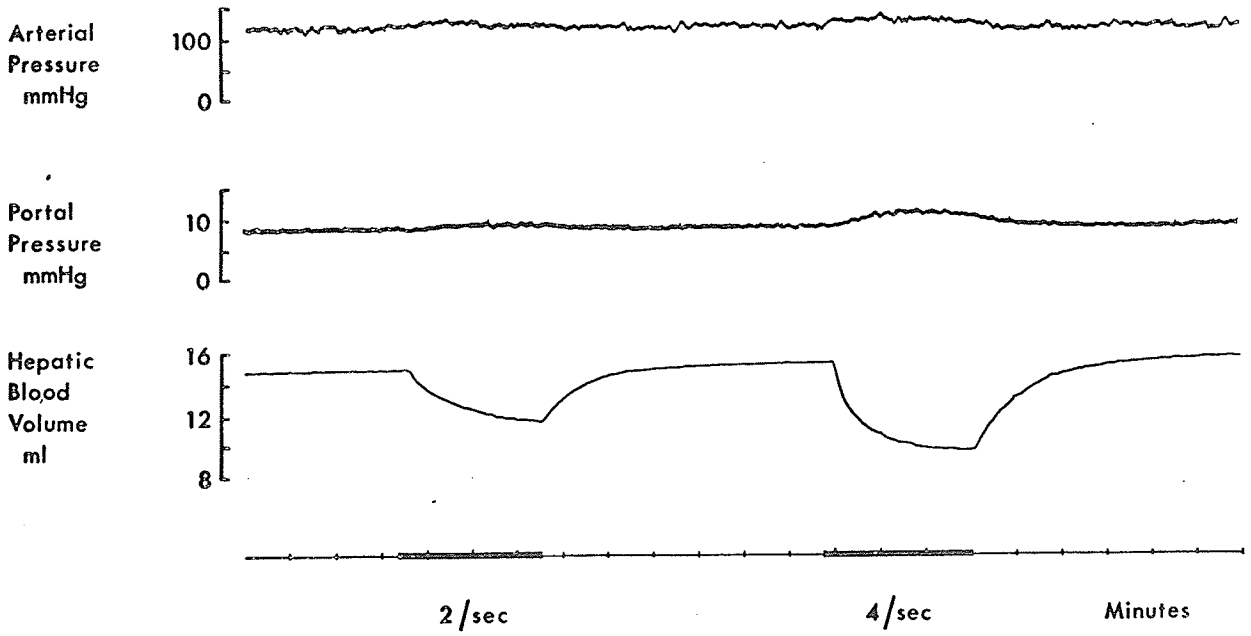


Figure 18. Responses in one cat to stimulation of the hepatic nerves (15v, 1 msec) at frequencies of 2 and 4/sec. Hepatic blood volume was determined immediately after these responses to allow calibration of the capacitance responses in terms of hepatic blood volume.

Complete frequency-response curves were obtained in 12 cats. The mean and standard error of these results are shown in Figure 19. The frequency-response curve previously obtained by Greenway et al. (1967b) for the hepatic arterial resistance is plotted on the same graph for comparison. The portal venous resistance graph was similar to that for the hepatic artery.

The maximal decrease in volume in response to nerve stimulation was expressed as a percent of the measured blood volume determined at the end of each experiment. Maximal stimulation of the sympathetic nerves expelled 49 $\pm$ 4% of the blood content of the liver. This response was well maintained for up to 20 minutes (the longest period tested) and on cessation of stimulation the volume returned to the control level.

Capillary Filtration Coefficient (CFC)

Hepatic venous pressure was determined by the position of the hepatic outflow into the reservoir. (The pressure could thus be varied by adjusting the level of the cannula outlet). In these experiments the hepatic venous pressure was set below the level of the right atrium and after the preparation was set up the pressure was increased in steps of 1 cm blood until a continuous rise in hepatic volume occurred. To obtain an isovolumetric state the pressure was then reduced 1 cm and kept at that level (usually 0 to 2 cm above the right atrium) except when varied for a specific purpose. To determine the capillary filtration coefficient the hepatic venous pressure was increased by 4.7 mm Hg for 1 1/2 minutes (Mellander, 1960; Folkow et al., 1963). The CFC was determined on 6 to 22 occasions in

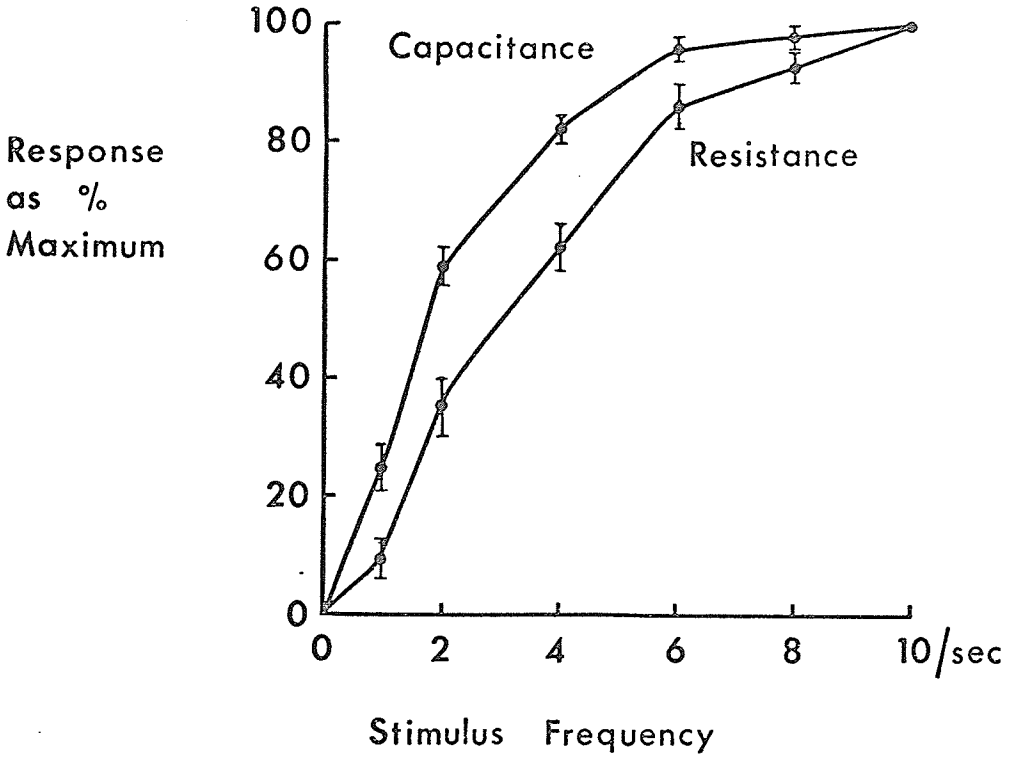


Figure 19. Frequency-response graph (mean ± S.E.) for the capacitance responses to stimulation of the hepatic nerves (15v, 1 msec). The graph for the hepatic arterial resistance responses, taken from Greenway et al. (1967) is also shown.

each of 7 cats. The determination in one cat is shown in Figure 20. Raising the hepatic venous pressure by 4.7 mm Hg caused an increase of 3.0 $\pm$ 0.4 mm Hg in the portal pressure. The CFC was calculated from the slow component of the volume record as shown by the broken line in Figure 20. In these calculations it was assumed that 100% of the rise in hepatic venous pressure was transmitted to the sinusoids (see Discussion). The mean CFC for each cat was 0.20 - 0.51 ($ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 100\ g\ liver^{-1}$) and the standard error in each animal did not exceed $\pm$ 0.01. The mean value for the 7 cats was 0.30 $\pm$ 0.03 $ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 100\ g\ liver^{-1}$. On 9 occasions the CFC was determined with an increase in venous pressure of 2.3 and 7.0 mm Hg and these values were not statistically different from those determined with increases of 4.7 mm Hg.

Capillary Filtration Coefficient (CFC) During Hepatic Nerve Stimulation

Stimulation of the hepatic nerves resulted in a rapid decrease in liver volume that reached a plateau after a few minutes. CFC could not be measured during the rapid change, thus all determinations were made once the plateau had been reached. On 28 occasions in 4 cats the coefficients were determined 2 to 5 minutes after the onset of nerve stimulation at frequencies of 1 to 10 per second. One such determination is shown in Figure 20. The coefficient thus determined did not differ from that obtained during the control period (paired t-test $p > 0.8$). On 14 occasions the coefficients were determined 5 to 10 minutes after the onset of nerve stimulation and these values did not differ from the control values or those determined 2 to 5 minutes after onset of stimulation ($P > 0.8$).

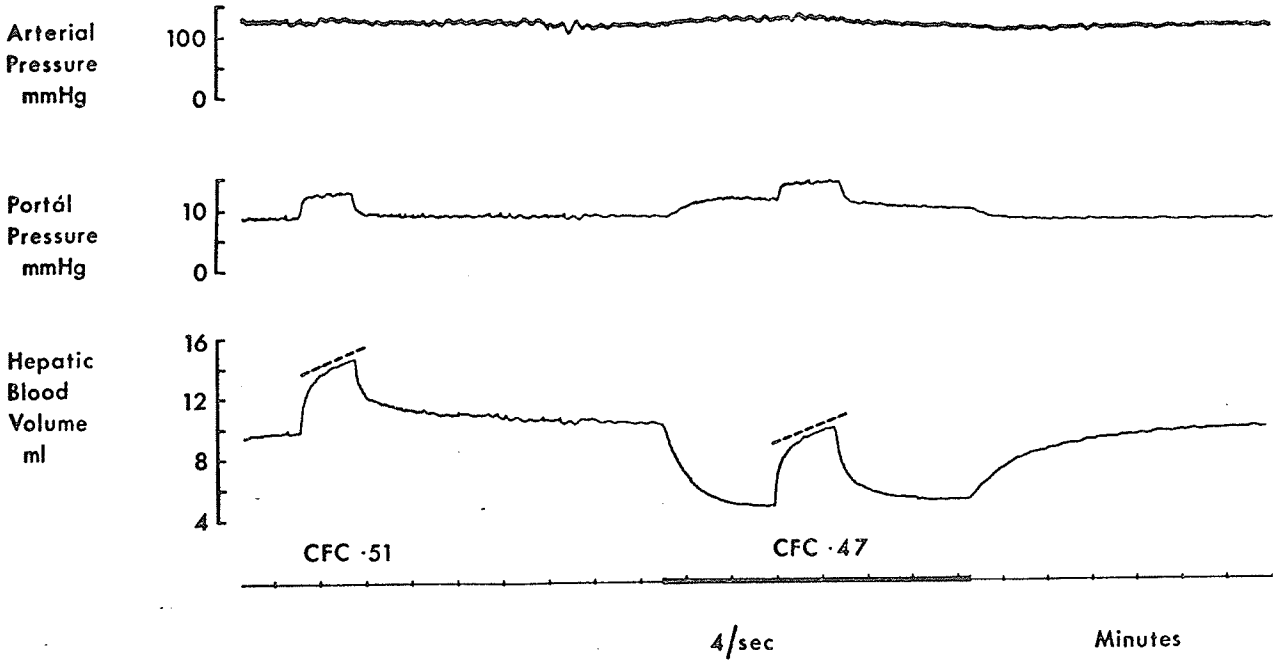


Figure 20. Determination of the capillary filtration coefficient (CFC) by raising hepatic venous pressure 4.7 mm Hg, before and during stimulation of the hepatic nerves at a frequency of 4/sec. The broken lines represent the slopes taken for the calculation. The hepatic blood volume was determined shortly after these responses to allow calibration of the capacitance response to nerve stimulation in terms of hepatic blood volume.

On cessation of nerve stimulation the volume recovered slowly (Figure 20) and the coefficient could not be determined until 2 to 5 minutes after cessation. These values did not differ from those during the control period or during nerve stimulation ($p > 0.8$). Thus CFC did not change either during stimulation of the hepatic nerves for up to 10 minutes or after cessation of stimulation.

Carotid Occlusion Series - Control Results

In 8 cats (weight 2.5 $\pm$ 0.04 kg) the liver weight was 87 $\pm$ 2.5 g with 71.4 $\pm$ 3.3 g in the plethysmograph. The arterial and portal venous pressures were 126 $\pm$ 2.3 and 10.7 $\pm$ 0.1 mm Hg respectively. Hepatic blood volume determined at the conclusion of the experiments was 21.2 $\pm$ 2.2 ml/100 g liver. The hepatic venous long-circuit was not established in these experiments.

Reflex Capacitance Effects of Bilateral Carotid Arterial Occlusion

The carotid arteries were occluded on 13 occasions in 8 cats with intact hepatic nerves and on 8 occasions in 5 cats with the nerves interrupted. The data from each cat were averaged and treated as a single value (Figure 21). Carotid occlusion resulted in increases in arterial pressure of 20-60 mm Hg and small increases in portal venous pressure of 0.7 $\pm$ 0.1 and 0.8 $\pm$ 0.5 mm Hg in the intact and denervated preparations respectively.

A typical response of the innervated liver to bilateral carotid arterial occlusion is shown in Figure 22. Arterial pressure was elevated for the duration of the occlusion while portal venous pressure and hepatic volume showed initial small increases which

Cat number	Increase in arterial pressure (mm Hg)	Change in liver volume (ml/100 g)	Change in hepatic blood volume (%)
LIVER INNERVATED			
1	45	-1.7	-7.0
2	35	0	0
3	50	-1.7	-7.4
4	40	-1.2	-7.9
5	25	-0.7	-3.0
6	22	0	0
7	32	+0.8	+3.3
8	28	0	0
Mean	35 ± 3.5	-0.56 ± 0.32	-2.75 ± 1.50
LIVER DENERVATED			
1	25	-0.8	-3.3
2	50	+0.2	+0.9
9	56	-0.8	-3.9
10	45	-0.1	-0.2
11	54	-0.5	-1.9
Mean	45 ± 5.6	-0.40 ± 0.20	-1.68 ± 0.91

Figure 21. The effects of carotid occlusion in each cat.

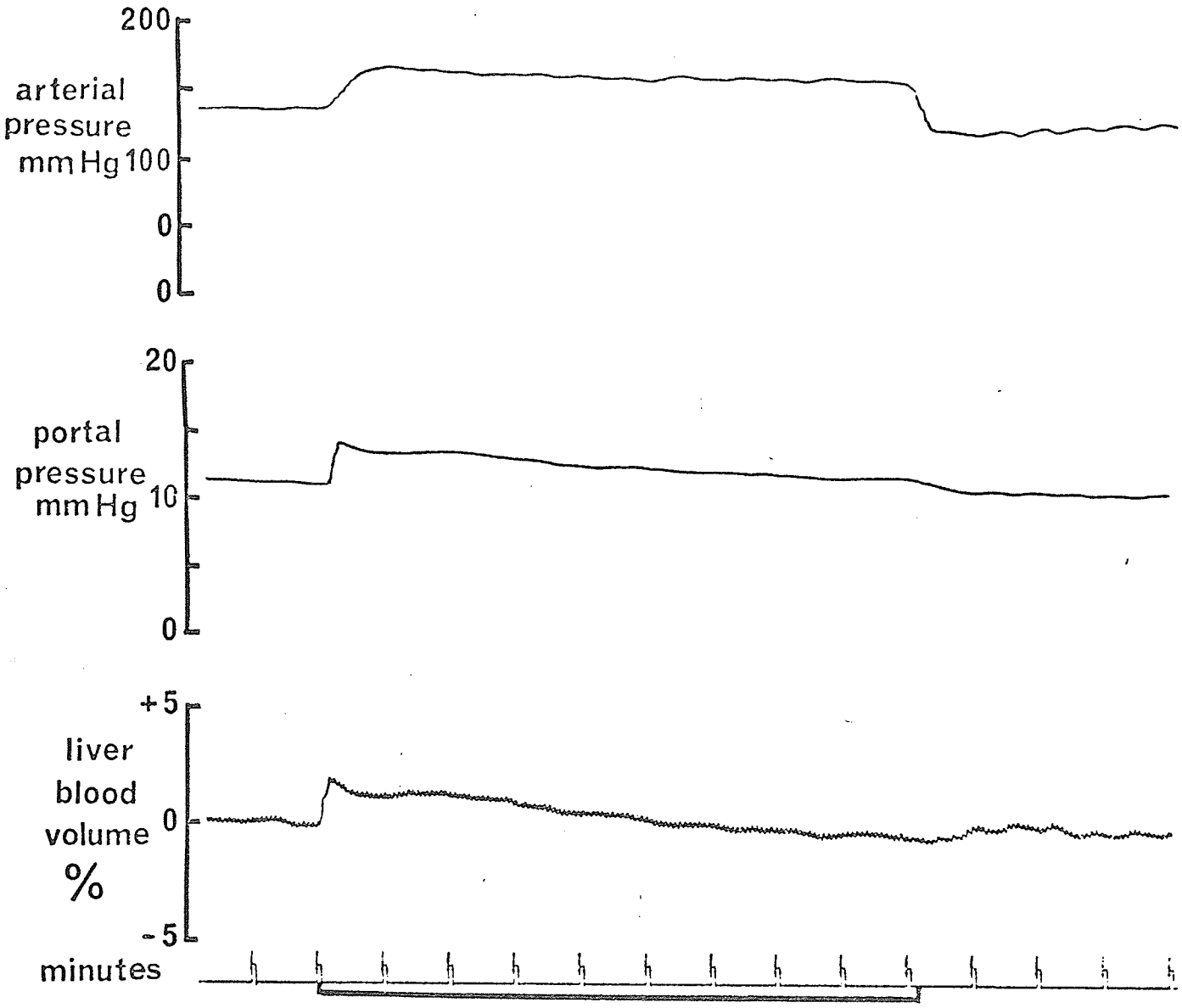


Figure 22. Response in one cat to bilateral carotid arterial occlusion. Hepatic blood volume in this cat was 25 ml/100 g.

returned toward (or slightly below) control levels, eventually becoming stable. Occlusion was maintained until the hepatic volume stabilized and all variables were measured at this time. The capacitance response ranged from -8 to +4% of the hepatic blood volume. The mean response for the innervated liver was $-2.7 \pm 1.5\%$ (Figure 21). There was no significant difference ($p > 0.6$) in the capacitance response between denervated and innervated livers. The response of all preparations to i.v. angiotensin or noradrenaline or direct nerve stimulation was within the normal range (Section II).

Figure 23 shows the response to carotid occlusion before and after denervation of the liver in the same cat. The hepatic volume decreased by 1.7 ml in this cat and the total hepatic blood volume was 24 ml, thus the decrease in volume during carotid arterial occlusion represents 7% of the hepatic blood volume. After the liver was denervated, carotid occlusion decreased hepatic blood volume by 0.8 ml (3.3% of the hepatic blood volume). The hepatic nerves were then stimulated at a frequency of 1 Hz (15V, 1 msec duration); hepatic volume decreased 5.8 ml (24% of the hepatic blood volume) and the response was not yet complete by the end of the 4 minute stimulation period. It is clear that the responses to carotid arterial occlusion were very similar before and after denervation of the liver and the responses were much smaller than the response to hepatic nerve stimulation at a frequency of 1 Hz. Thus the data obtained in the same cat from innervated and denervated hepatic beds confirm data obtained in separate cats (Figure 21) in which no statistical difference in the response of hepatic blood

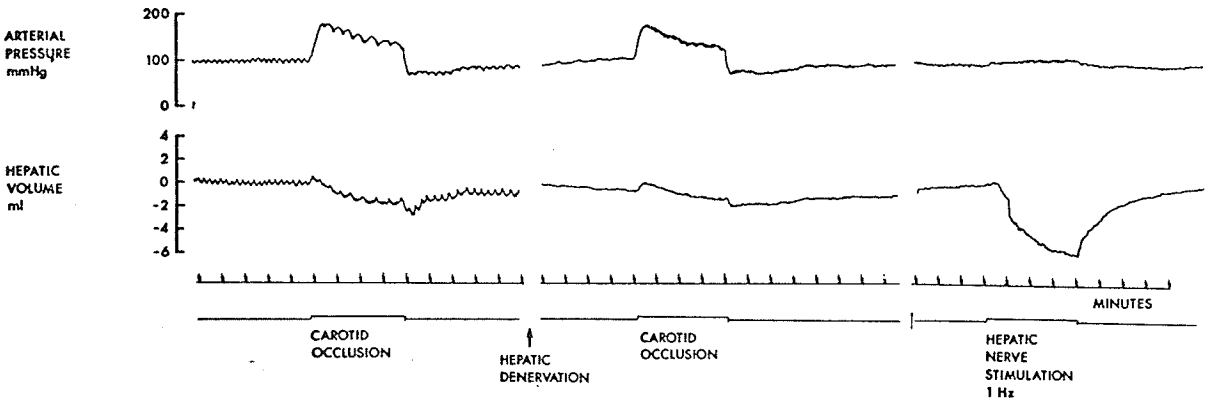


Figure 23. Cat 3.0 kg, liver weight 101 g. The hepatic volume response to occlusion of the carotid arteries before and after section of the hepatic nerves and to stimulation of the hepatic nerves at a frequency of 1 Hz. The hepatic blood volume in this cat was 24 ml.

volume to bilateral carotid arterial occlusion could be demonstrated in the presence or absence of the hepatic innervation.

DISCUSSION (SECTION I)

Capacitance Response to Hepatic Nerve Stimulation

The hepatic blood volume for this series of animals was 27 ml/100 g liver or 7.5 ml/kg body weight. Since the blood volume of the cat is 53 ± 7 ml/kg body weight (Groom et al., 1965) the hepatic blood volume represents 14% of the total blood volume. Maximal stimulation of the hepatic nerves expelled half of the blood volume of the liver (13 ml/100 g liver). This represents 7% of the blood volume of the cat. The fact that hepatic blood flow was transiently reduced in response to nerve stimulation (Greenway et al., 1967a) while the capacitance response was well maintained indicates that the expulsion of blood could not have been a passive consequence of flow reduction.

Maximal nerve stimulation in the cat expels 25-30% of the 3 ml blood/100 g in skeletal muscle and 35% of the 8 ml/100 g in the intestine (Mellander & Johansson, 1968). Figure 24 shows a comparison of the response in the liver with that of other vascular beds. Though skeletal muscle and skin contain the largest volume of blood they do not constitute the largest blood reservoir. To be considered a reservoir, some physiological stimulus must be able to result in redistribution of the blood from the organ into the general circulation. Skeletal muscle blood volume can be reduced 28% by nerve stimulation resulting in a redistribution of about 5% of the blood volume of the cat. This calculation assumes a maximal capacitance response simultaneously in all skeletal muscle vascular beds, a situation which seems unlikely to occur. The liver and spleen are capable of mobilizing 7 and 9% of the

	blood content as % of total blood volume	% of contents expelled by sympathetic nerves	% total blood volume expelled
Muscle & Skin	17	28	4.7
Intestine	6	35	2.1
Liver	14	50	7
Spleen	12	75	9

Figure 24: The effect of local nerve stimulation on regional blood volumes and the percent of the total blood volume of the cat that can be expelled from each organ. (Mellander & Johansson, 1968; Greenway et al., 1968 ; Present data).

total blood volume respectively. The spleen has long been recognized as an important blood reservoir in the cat but it is much less so in man. The liver in cats and man contains a large blood volume and if the hepatic capacitance response in man is similar to that in cats, the liver may represent the largest single blood reservoir in the human body.

Capacitance Response to Bilateral Occlusion of the Carotid Arteries

Though the liver volume decreased in response to direct nerve stimulation, bilateral carotid arterial occlusion did not result in a significant capacitance response ($-2.7 \pm 1.5\%$ of the hepatic blood volume). It remained possible however that the large increases in arterial pressure caused a passive engorgement of the liver which exactly compensated for a neurally mediated constriction of the capacitance vessels. This possibility was negated by cutting the sympathetic nerves to the liver and repeating the experiments. The response was not significantly different ($p > 0.6$) in absence of the hepatic nerves. The ability of the capacitance vessels to respond to appropriate stimuli was confirmed by infusions of angiotensin or noradrenaline or direct stimulation of the nerves to the liver. The capacitance responses to the drugs occurred in the presence of large elevations in arterial pressure (Section II).

Direct stimulation of the hepatic nerves caused a marked but transient increase in hepatic arterial resistance. A similar response occurred to bilateral occlusion of the carotid arteries. The mean hepatic arterial resistance response that occurred during carotid arterial occlusion was similar to that seen with a stimulation frequency

of 10 pulses per second (Greenway et al., 1967a). In contrast to the responses of the resistance vessels, the capacitance vessels showed a large, well-maintained contraction to direct stimulation but no response to carotid arterial occlusion. This implies great selectivity in reflex activation of the sympathetic nervous system.

It had previously been reported that the carotid sinus reflex involved a hepatic capacitance response (Heymans et al.,1931) and, though no quantitative data were reported, the qualitative observation is nevertheless opposed to those reported here. The reason for the discrepancy is not clear but the single trace of hepatic volume change in the original publication shows an extremely small response which may not constitute a significant biological response.

The role of the venous system in the carotid sinus reflex has been long debated. Carotid occlusion elicited a decrease in splenic blood volume which was mediated by sympathetic nerves though again no quantitative measures of expelled blood volume were reported (Driver & Vogt, 1950). Hadjiminas & Oberg (1968) analyzed the response of the intestine and skeletal muscle vascular beds to carotid occlusion by comparing the relative degrees of resistance and capacitance response with those recorded during direct nerve stimulation. The reflexly mediated capacitance response was significantly less than the resistance response in skeletal muscle while the degree of reflex stimulation of capacitance and resistance vessels in the intestine was similar.

The response of the hepatic vascular bed to reflex stimulation appears to be one extreme of a continuum,the intestinal response being the other

extreme. The relative capacitance and resistance responses of the intestine are equally activated by carotid occlusion and direct nerve stimulation while the capacitance vessels of skeletal muscle are activated to a lesser extent and the hepatic capacitance vessels are not activated at all by reflex stimulation through the carotid sinus baroreceptors. The position of the spleen on this continuum appears to be towards the intestine, that is, the splenic volume does show some reflex reduction (Hadjiminas & Oberg, 1968; Driver & Vogt, 1950).

Though some vascular beds may reflexly expel some portion of their blood volume, the net increase in venous return as a result of venous constriction in response to the carotid baroreflex is negligible (Polosa & Rossi, 1961; Groom et al., 1962; Corcondilas et al., 1964; Browse et al., 1966). The lack of any significant hepatic capacitance component in this reflex tends to support the contention that net venous involvement in the reflex is insignificant. The afferent pathways which are involved in reflexes causing contraction of the hepatic capacitance vessels, as for example after hemorrhage (Griffith and Emery, 1930), remain to be elucidated.

Since the hepatic capacitance vessels respond to small infusions of noradrenaline and adrenaline (0.1-0.2 $\mu g \cdot min^{-1} \cdot kg^{-1}$ see Section II), the amounts of these catecholamines released from the adrenal medullae during bilateral carotid occlusion must be less than these infusion rates. This confirms the conclusions of Hodge et al. (1969) from studies in dogs and man. Release of catecholamines plays only a small part in arterial baroreceptor reflex responses.

Fluid Exchange Responses to Stimulation of the Hepatic Nerves

In response to direct stimulation of the hepatic nerves, hepatic volume decreased rapidly and reached a plateau after about 4 minutes. The absence of any changes in hepatic volume after this time indicated that net fluid movements had not occurred. Thus, stimulation of the hepatic nerves does not alter the pre- to postsinusoidal resistance ratio and sinusoidal hydrostatic pressure is unchanged. In this respect the liver resembles the intestine (Folkow *et al.*, 1964) and spleen (Greenway *et al.*, 1968) rather than skeletal muscle (Mellander 1960) which reabsorbs fluids into the vascular compartment on nerve stimulation.

Altered precapillary (presinusoidal) sphincter tone can affect either the sinusoidal hydrostatic pressure or sinusoidal surface area. A change in sphincter tone which might alter only the surface area would not cause a change in net fluid movements at zero venous pressure. Though the effect of nerve stimulation on sinusoidal pressure can be evaluated at normal venous pressure, the effect of nerve stimulation on sinusoidal surface area must be examined at an elevated venous pressure. The capillary filtration coefficient, defined earlier, is a measure of sinusoidal surface area and sinusoidal permeability.

Calculation of the CFC requires knowledge of the proportion of the rise in venous pressure which is transmitted back to the sinusoids. This value is not known for any vascular bed; however, in skeletal muscle it has been assumed that 80% of the increment in venous pressure is transmitted to the capillary (Cobbold *et al.*, 1963). A rise in hepatic

venous pressure of 4.7 mm Hg caused an increase of 3.0 mm Hg in portal venous pressure; thus, since approximately 64% of the rise in hepatic venous pressure is transmitted back to the portal vein, and since the greatest drop in pressure between the portal and hepatic veins occurs in the portal venous radicals (Nakata et al., 1960), the proportion transmitted to the sinusoids is probably very high. One hundred per cent transmission has been assumed for the present calculations. (Further discussion of this assumption is given in Section III).

The CFC was determined by a method similar to that used in skeletal muscle (Mellander, 1960) and intestine (Folkow et al., 1963). In these organs the early, rapid increase in volume following a rise in venous pressure is due to passive engorgement of the vessels with blood. In the liver, elevations in hepatic venous pressure cause a similar increase in blood volume but this rise continues for 5 to 20 minutes (See Results, Section III). Since the present CFC values were calculated based on the volume slopes measured after only 1 1/2 minutes of elevation in venous pressure, the absolute value of the CFC is not accurate. The same problem occurred in determinations of CFC in the intestine (Wallentin, 1966).

The CFC determined here is 0.3 $ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 100\ g\ liver^{-1}$ which is large compared to that of the intestine ($0.1\ ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 100^{-1}\ g$) and skeletal muscle ($0.01\ ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 100\ g$) (Mellander and Johansson, 1968). This suggests that the vascular bed of the liver has a higher permeability to small molecules or a larger surface area than the intestine or skeletal muscle. Though these data can only be

regarded as suggestive due to the previous objection, data to be presented later support this conclusion. In spite of the unreliability of the absolute value of the CFC, comparison of the CFC obtained before and during nerve stimulation does provide useful data.

The CFC obtained before, 2 to 5 minutes and 5 to 10 minutes after onset of nerve stimulation, were not significantly different ($p > 0.8$). Values obtained 2 to 5 minutes after cessation of nerve stimulation were also not different ($p > 0.8$). This suggests that a maintained contraction of the presinusoidal sphincters, as occurs in the intestinal vascular bed (Folkow et al., 1964) is not produced in the hepatic vascular bed by sympathetic nerve stimulation. However, it does not exclude the possibility that short-lasting changes occur at the onset of stimulation, as in skeletal muscle (Cobbold et al., 1963).

The data presented here are consistent with the following conclusions. Stimulation of the hepatic nerves causes a transient increase in hepatic arterial resistance and a reduction in blood flow. Portal resistance increases but flow does not change unless there is a concomitant change in intestinal or splenic blood flow (Greenway et al., 1967b). The autoregulatory escape of the hepatic arterial flow does not affect the capacitance response of the liver which is well maintained for the duration of the stimulation. Sinusoidal pressure and surface area remain unchanged during nerve stimulation. Bilateral occlusion of the carotid arteries causes a neurally mediated, transient elevation of the hepatic vascular resistance to blood flow while having no capacitance effect on the hepatic vessels.

SUMMARY (SECTION I)

1. Stimulation of the hepatic nerves causes a well maintained decrease in hepatic blood volume of up to 50%. Maximal effects occur at a frequency of 8 Hz resulting in a rapid expulsion of about 7% of the total blood volume of the cat from the liver.

2. The capacitance response seen with nerve stimulation is not affected by the autoregulatory escape of the blood flow in the hepatic artery.

3. Stimulation of the hepatic nerves produces a capacitance response which becomes steady with no evidence of fluid accumulation or reabsorption. Since net fluid exchange is unaffected we conclude that sympathetic nerve stimulation does not alter the pre- to postcapillary resistance ratio and sinusoidal hydrostatic pressure remains unchanged.

4. CFC is not affected by sympathetic nerve activation; therefore, sinusoidal surface area is not under sympathetic neural control.

5. Bilateral occlusion of the carotid arteries does not mobilize blood from the large hepatic blood reservoir. Since reflex activation of the hepatic nerves by carotid occlusion has been shown to result in a hepatic arterial resistance response equivalent to direct stimulation of the sympathetic nerves at a frequency of 10 Hz., we conclude that reflex sympathetic discharge is capable of great selectivity.

SECTION II

EFFECTS OF INFUSIONS OF ADRENALINE, NORADRENALINE ANGIOTENSIN, VASOPRESSIN AND HISTAMINE ON HEPATIC BLOOD VOLUME IN THE ANESTHETIZED CAT

RESULTS (SECTION II)

Control Values and Calculation of Results

Twenty-two cats were used. The mean liver weight was 91 ± 2.3 g (mean ± S.E.) and the hepatic blood volume was 20 ± 1.1 ml/100 g liver. Before infusion of the drugs mean arterial pressure was 132 ± 4.7 mm Hg and mean portal pressure was 9.5 ± 0.5 mm Hg. In all the experiments the hepatic volume became steady a few minutes after onset of drug infusion and no significant changes occurred during the remainder of the infusion. On cessation of the infusion the hepatic volume returned to pre-infusion levels with no consistent under- or overshoot. The results therefore represent reversible changes and are interpreted as changes in blood volume. (This point is more fully discussed in Section III). For each infusion the volume change was calculated as a percentage of the hepatic blood volume determined at the end of the experiment. For all experiments, except those in which angiotensin was infused, hepatic nerves and lymphatics were ligated and cut. All drugs except histamine and isoproterenol were infused intravenously.

Effect of Catecholamines on Hepatic Capacitance

A representative record showing the effects of two infusions of adrenaline is reproduced in Figure 25. Arterial pressure rose rapidly then decreased slightly within 5-8 minutes and remained steady. Portal pressure was elevated and hepatic blood volume declined. The infusions were continued until the hepatic volume became stable. All parameters quickly returned to control levels on cessation of the infusion. Liver volume recovered to control levels within 15 minutes. The changes in arterial pressure, portal pressure and hepatic volume in response to i.v. infusions of a range of doses of adrenaline and noradrenaline (0.1 - 2.0 μg/kg/min) were examined in 4

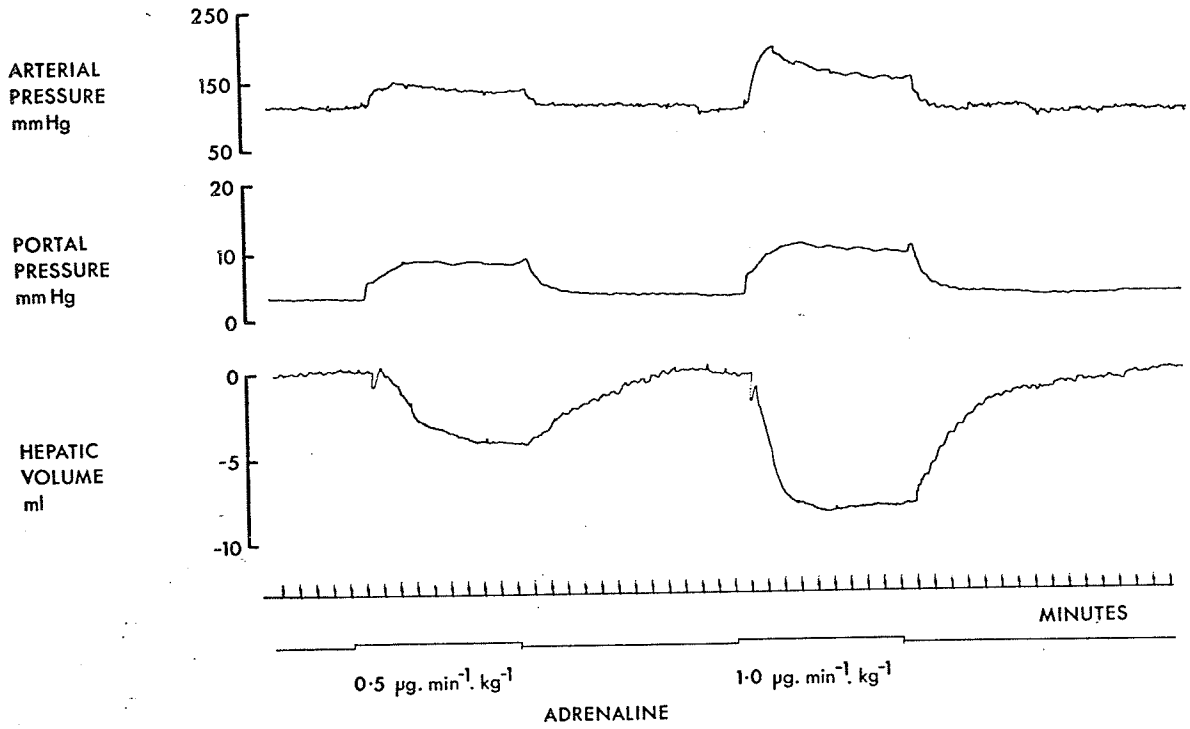


Figure 25. A representative record of the responses to infusions of adrenaline in one cat. At the end of the experiment, the hepatic blood volume was 29.3 ml and the liver weight 112 g. Thus the change in liver volume during adrenaline infusion represents a decrease in blood volume of 15% and 27% for the first and second infusions respectively.

cats. The mean and standard error of these changes from control values were calculated for the 4 experiments, and the results, expressed as dose-response curves are shown in Figure 26. The responses to the two agents were not significantly different (paired t-test, $p > 0.5$) at any dose. The catecholamines expelled about 40% of the blood volume of the liver at the highest dose (2 μg/kg/min) and this dose raised arterial pressure by 30-35 mm Hg and portal venous pressure by 4-5 mm Hg. In one cat low doses (0.1 and 0.2 μg/kg/min) caused small elevations of liver volume; however, on infusion of adrenaline into the portal vein all effective doses caused a decrease in liver volume.

To further examine the possibility that an increase in hepatic blood volume might result from stimulation of beta adrenergic receptors in the capacitance vessels, isoproterenol was infused into the hepatic artery on 7 occasions in 3 cats in doses of 0.1 or 0.2 μg/kg/min. These doses have been shown to cause maximal vasodilation of the hepatic arterial bed without a significant reduction in arterial pressure (< 5 mm Hg) (Greenway & Lawson, 1969). There was no significant change in portal pressure (+ 0.1 ± 0.1 mm Hg) or hepatic volume (- 1.3 ± 3.7%) and arterial pressure decreased by only 2.6 ± 1.0 mm Hg.

Effect of Angiotensin on Hepatic Capacitance

The dose-response curves of 4 cats with innervated livers were compared with those of 4 cats with the nerves cut. The response of all measured parameters to the various doses (.005 -0.5 μg/kg/min) were not significantly different (unpaired t-test, $p > 0.5$) in the presence or absence of nerves. The 8 curves from separate cats were thus pooled and are shown in Figure 27. In response to 0.5 μg/kg/min angiotensin

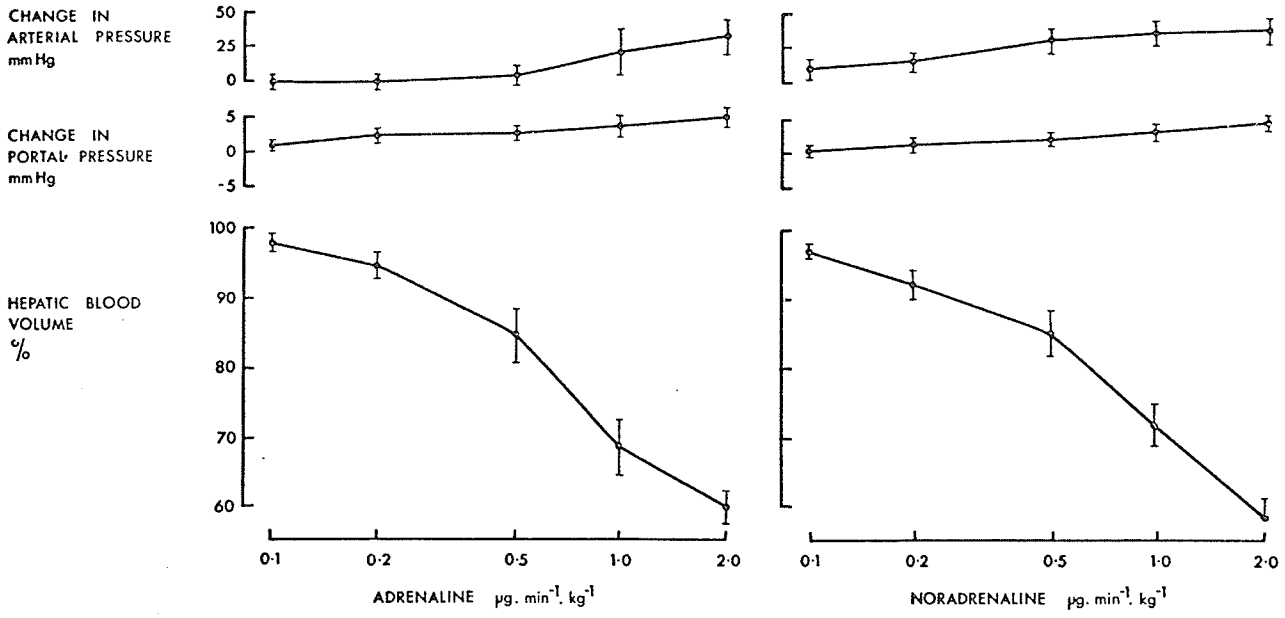


Figure 26. Dose-response curves for adrenaline and noradrenaline (i.v.). Each point represents the mean (± S.E.) for 4 cats.

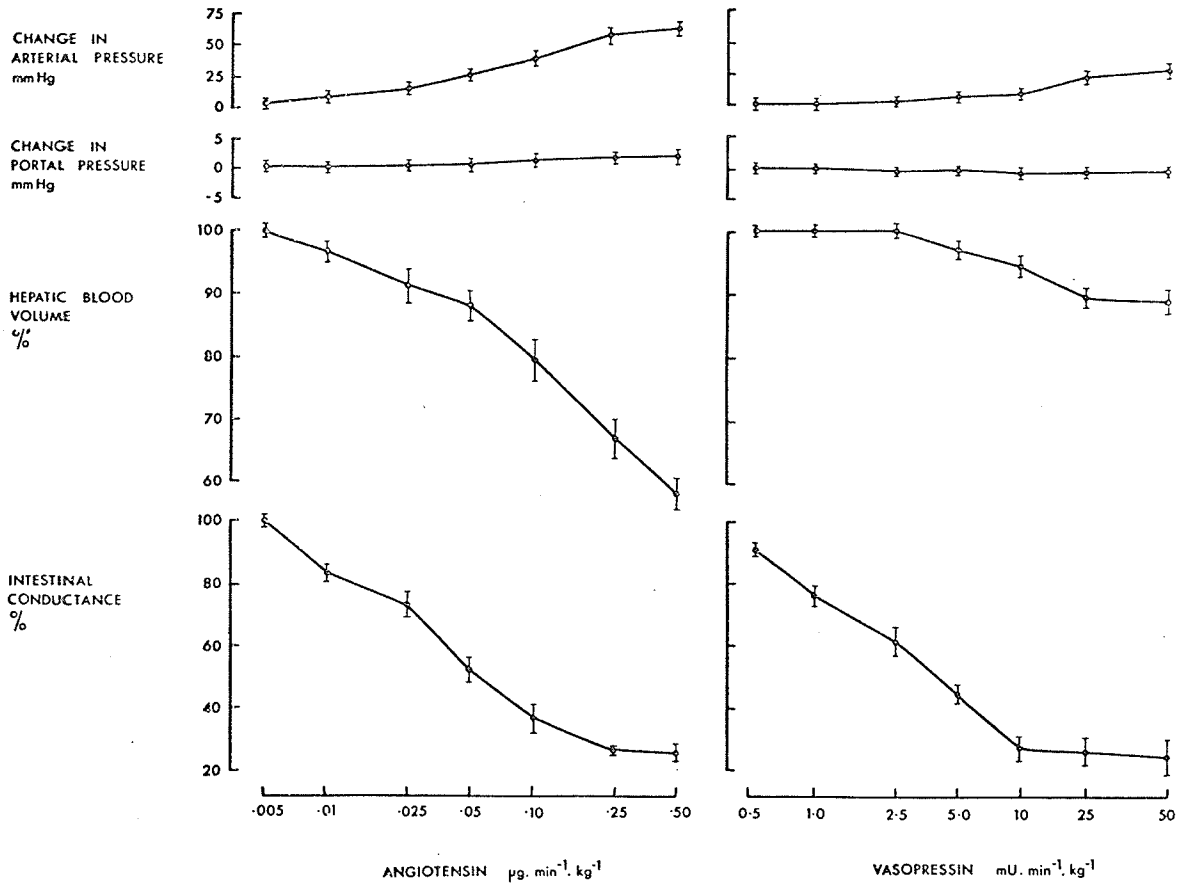


Figure 27. Dose-response curves for angiotensin and vasopressin (i.v.). The angiotensin curves represent the means (± S.E.) for 8 cats and the vasopressin curves for 4 cats. The intestinal conductance responses are reproduced from a publication of Cohen et al., (1970).

i.v. the hepatic blood volume decreased by 42.8 ± 2.9%, arterial pressure increased by 61 ± 2.0 mm Hg and portal venous pressure increased by 1.6 ± 0.3 mm Hg. For the purposes of comparison and discussion, the dose-response curves for angiotensin and vasopressin on the resistance vessels of the intestine (Cohen et al., 1970) are included in Figure 27.

Effect of Vasopressin on Hepatic Capacitance

The mean of 4 dose-response curves obtained in 4 cats is shown in Figure 27. The maximum dose used (150 mU/kg/min) caused a reduction in hepatic blood volume of 18 ± 1.3%, an elevation of 34 ± 6.3 mm Hg in arterial pressure and a reduction in portal venous pressure of 0.95 ± 0.23 mm Hg. Vasopressin did not cause a change in liver volume at a dose of 2.5 mU/kg/min or lower, although at this dose the intestinal blood flow was reduced to 60% of control (Cohen et al., 1970).

Effect of Histamine on Hepatic Capacitance

Histamine (0.4 - 10 μg/kg/min) was infused on 30 occasions in 3 cats. The infusions were made directly into the hepatic artery to allow these large doses to be administered to the hepatic vascular bed in the absence of gross systemic effects. The hepatic blood volume was not significantly affected by doses up to 10 μg/kg/min, which caused arterial pressure to decrease by 22 mm Hg. In no instance did histamine cause an increase in hepatic blood volume even when 40 μg (3 cats) and 100 μg/kg/min (1 cat) were infused.

Occlusion of the Hepatic Artery

In 6 cats the capacitance effects of hepatic arterial occlusion were examined. Occlusion resulted in an elevation of arterial pressure from 124 to 137 mm Hg ($\pm$ 2.8) and a reduction of portal pressure from 9.0 to 8.7 mm Hg ($\pm$ 0.25). (Paired-t-analysis indicated $p < 0.025$ and $p > 0.3$ respectively). Blood volume of the liver decreased by 0.97 $\pm$ 0.50 ml/100 g tissue which represented 4.9 $\pm$ 2.5% of the hepatic blood volume.

DISCUSSION (SECTION II)

The hepatic volume before infusion of drugs was always constant. A stable volume trace indicated that there was neither a fluctuating blood volume nor a net trans-sinusoidal movement of fluids. During drug infusion the volume attained a new stable level and again no evidence of fluid shifts was seen. Cessation of the infusions resulted in a recovery of the volume to pre-infusion levels. These data suggest that none of the doses of the drugs used resulted in altered sinusoidal hydrostatic pressure or permeability. This is discussed more fully in Section IV. The dose range of drugs varied from the smallest effective dose to the largest dose that did not cause irreversible changes in control parameters or produce signs of vascular shock.

Effect of Catecholamines on Hepatic Blood Volume

The amount of catecholamines reflexly released from the adrenal medullae has been estimated to seldom exceed 2-3 μg/kg/min (Celander, 1954). Infusion of 2 μg/kg/min of noradrenaline or adrenaline in the present experiments caused a decrease of hepatic blood volume of about 40%. This is similar to the maximum expulsion of 50% of the blood volume in response to electrical stimulation of the hepatic nerves (Section I). The effects of noradrenaline and adrenaline were not significantly different (paired-t analysis $p > 0.05$) at any dose tested; however, this does not necessarily imply equal potency on the smooth muscles of the capacitance vessels. Noradrenaline causes little change in hepatic blood flow whereas adrenaline causes a marked elevation due to intestinal vasodilation (Greenway & Lawson, 1968). This increase in flow might cause a passive increase in volume which could oppose an

active constriction, though maximal vasodilation of the hepatic arterial bed, resulting from i.a. infusion of isoproterenol, did not produce a passive increase in liver blood volume. This also indicates that isoproterenol did not have a direct effect on the capacitance vessels of the liver. In vivo studies demonstrate that isoproterenol causes a small elevation in vascular hindleg volume in the dog (Abboud et al., 1965; Johnsson & Oberg, 1968) while the splenic volume is unaltered by beta-adrenergic agonists (Davies et al., 1969; Greenway & Stark, 1970). Relaxation of hepatic capacitance vessels mediated by beta-adrenergic receptors did not occur.

Effect of Angiotensin and Vasopressin on Hepatic Blood Volume

The effect of angiotensin and vasopressin on the intestine and spleen during hemorrhage has recently been evaluated. Both agents cause marked intestinal and splenic vasoconstriction (Cohen et al., 1970) and it has been suggested that they play a major role in the intestinal and splenic vasoconstriction following hemorrhage (McNeil et al., 1970; Stark et al., 1971). At a dose of angiotensin 0.1 μg/kg/min the intestinal blood flow was reduced to 40% of control while the same dose caused expulsion of only 20% of hepatic blood volume. The splenic blood volume was affected to approximately the same extent as hepatic volume (Greenway & Stark , 1970) at equal doses and it has been shown that the quantities of angiotensin produced during hemorrhage in the cat are insufficient to decrease splenic volume (Greenway et al., 1968; Stark et al., 1971). Angiotensin is formed during hemorrhage in the dog at rates of about 0.25 - 1.5 μg/min (Regoli & Vane, 1966; Hodge et al., 1966). Assuming similar release per unit body weight, the cat may produce

0.05 - 0.15 μg/min. Infusions over this dose range (0.02 - 0.06 μg/kg/min) caused hepatic blood volume to decrease by up to 15%. It therefore appears that, even though angiotensin can result in considerable hepatic capacitance responses, the quantity of angiotensin released by physiological stimuli is unlikely to alter hepatic blood volume significantly. The possibility of indirect capacitance effects of infused angiotensin, mediated by sympathetic nerve activity, has been excluded by the observation that responses of the denervated liver were not significantly different from those of the liver with nerves intact. It remains possible that catecholamines released from the adrenal glands or from the sympathetic terminals within the liver could play a role although removal of the adrenal glands did not alter the response to i.v. angiotensin in the spleen (Greenway & Stark, 1970).

Vasopressin produced relatively minor capacitance responses in the liver even in very large doses. It had previously been concluded from data obtained using an isolated, perfused liver that there were no specific vasopressin receptors in the rat liver (Noguchi & Plaa, 1970). This apparently is not the case with the liver of the cat. It is unlikely that the decreased hepatic volume was a passive consequence of portal flow reduction, since the volume was unaltered by a dose of vasopressin (2.5 mU/kg/min) that reduced intestinal flow by 60%.
Altered arterial pressure also appears to have minor effects on the volume of the liver since even large elevation of arterial pressure as seen with carotid arterial occlusion (Section I) caused no passive increase in the volume of the denervated liver and hepatic arterial occlusion resulted in a reduction in hepatic volume of only 5%. The

autoregulatory escape of blood flow seen during hepatic nerve stimulation and carotid arterial occlusion (Greenway et al., 1967a) also caused no significant change in liver blood volume (Section I). Thus it appears that passive capacitance changes in the liver as a result of hemodynamic alterations of the hepatic artery or portal vein are minimal, and the weak capacitance response to vasopressin is the result of an active vascular constriction. Though the present data suggest that the cat liver has vasopressin-sensitive receptors, the maximum response is small when compared with the hepatic capacitance effects of the catecholamines and direct nerve stimulation. McNeill et al. (1970) showed that hemorrhage, in the cat in which the adrenals, kidneys and intestinal nerves were removed, resulted in a reduction of intestinal blood flow to 40% of control flows. When the pituitary was also removed, hemorrhage caused no intestinal vasoconstriction, suggesting that the release of vasopressin during hemorrhage was of sufficient quantity to cause reduction of intestinal blood flow to 40% of control flow. From Figure 27 it is seen that an infusion of vasopressin of 5-10 mU/kg/min causes the same degree of constriction in the intestine. The errors in such an estimation are large but it suggests that the maximum range of endogenous secretion of vasopressin lies somewhere in the middle of our range of doses. Doses over this range produce hepatic blood volume changes of less than 5%. Thus it is unlikely that endogenously secreted vasopressin causes significant reduction in hepatic blood volume.

Twenty percent of the hepatic blood volume was expelled by 0.7 μg/kg/min adrenaline and noradrenaline, 0.1 μg/kg/min angiotensin and approximately 0.7 μg/kg/min vasopressin (170 mU/kg/min ; 250 U/mg

(Sawyer, 1961)). Thus angiotensin is the most potent agent on the basis of molar or μg quantity infused intravenously.

Effect of Histamine on Hepatic Blood Volume

The total absence of hepatic capacitance effects of histamine in the cat is in marked contrast to the effect seen in the dog. In the dog, histamine caused hepatic outflow block and the hepatic blood volume increased markedly (Oshiro & Greenway, 1972), while the cat showed no increase in hepatic volume at doses from 0.4 to 100 μg/kg/min (i.a.). The lack of histamine-sensitive smooth muscle in the liver of the cat represents an important species variation. It is this insensitivity of the cat liver to histamine that is partially responsible for the markedly different hemodynamic consequence of anaphylactic reactions and the acute reaction to endotoxin in the cat and dog. In the dog, histamine released within the liver (Oshiro & Greenway, 1972) results in severe outflow block with hepatic and intestinal pooling of blood and the resultant decrease in venous return causes severe systemic hypotension (Hinshaw et al., 1962). In the cat, splanchnic congestion does not occur in anaphylactic reactions in spite of an elevated plasma histamine level and the initial hypotension seen in that species is attributed to intense constriction of the pulmonary vascular bed (Kuida et al., 1961).

SUMMARY (SECTION II)

1. Dose-response curves were obtained for intravenous infusions of adrenaline, noradrenaline, angiotensin, vasopressin and histamine. The recorded parameters were arterial and portal venous pressure and hepatic blood volume.

2. Adrenaline and noradrenaline decreased hepatic blood volume and did not differ significantly in potency. Up to 40% of the hepatic blood volume was expelled by doses within the range secreted by the adrenal medullae.

3. Isoproterenol, infused into the hepatic artery, had no significant effect on hepatic blood volume in doses which caused maximal vasodilation of the hepatic arterial bed. Relaxation of hepatic capacitance vessels mediated by beta-adrenergic receptors did not occur.

4. Angiotensin infusions in doses previously shown to cause intestinal and splenic vasoconstriction, decreased hepatic blood volume and on a molar or μg basis, angiotensin was the most potent of the agents tested. Doses within the probable physiological range of endogenous production decreased hepatic blood volume up to 15%. The responses were not significantly different when the hepatic nerves were intact or sectioned.

5. Vasopressin infusions produced only small decreases in hepatic blood volume. It is unlikely that endogenously released vasopressin is of sufficient quantity to cause significant hepatic capacitance responses.

6. Histamine produced no change in hepatic blood volume in doses which readily produce outflow block in dogs. Either the specific hepatic venous smooth muscle involved in outflow block is absent in the cat or it has no histamine receptors.

7. After the rapid change in hepatic blood volume at the onset of the infusion, hepatic volume remained steady for the duration of each infusion. There was no evidence that these agents caused net transsinusoidal fluid movements.

SECTION III

EFFECTS OF HEPATIC VENOUS PRESSURE ON TRANS-SINUSOIDAL FLUID EXCHANGE IN THE LIVER OF THE CAT

RESULTS (SECTION III)

Responses to Increased Venous Pressure

The effects of elevated hepatic venous pressure were evaluated in 19 cats. Mean arterial pressure was 112 ± 4.1 mm Hg (mean ± S.E.), portal pressure was 8.8 ± 0.5 mm Hg and total hepatic blood flow was 108 ± 6.5 ml . min^{-1} .100 g $liver^{-1}$. Control values were determined immediately before the first experimental maneuver.

Hepatic venous pressure was raised on 157 occasions to levels between 2.3 and 11.7 mm Hg. These pressures were maintained for between 20 minutes and 5 hours. Figure 28 shows the general effects of elevating hepatic venous pressure by 4.68 mm Hg for 30 minutes. Arterial pressure showed little change while portal pressure rapidly increased to a new level which was maintained for the duration of the raised venous pressure. In the series of experiments in which the effect of drug infusions on the rate of filtration was examined, the venous pressure was elevated by 7 mm Hg (Section IV). This series consisted of 10 cats and was the largest series in which the venous pressure was elevated to the same extent. The mean effect of elevated hepatic venous pressure on a number of parameters was evaluated. Mean arterial pressure before venous pressure elevation was 134 ± 12 mm Hg (mean ± S.E.) and 130 ± 11 mm Hg after; portal pressure was 7.3 ± 0.7 mm Hg before and 10.8 ± 0.6 mm Hg after; total hepatic blood flow was 98 ± 5.4 ml . min^{-1} .100 g^{-1} before and 91 ± 4.6 ml min^{-1} .100 g^{-1} after.

Hepatic volume increased rapidly at first and by 5-20 minutes the increase in volume continued at a constant but lower rate. This steady-state increase was maintained with minor fluctuations for the

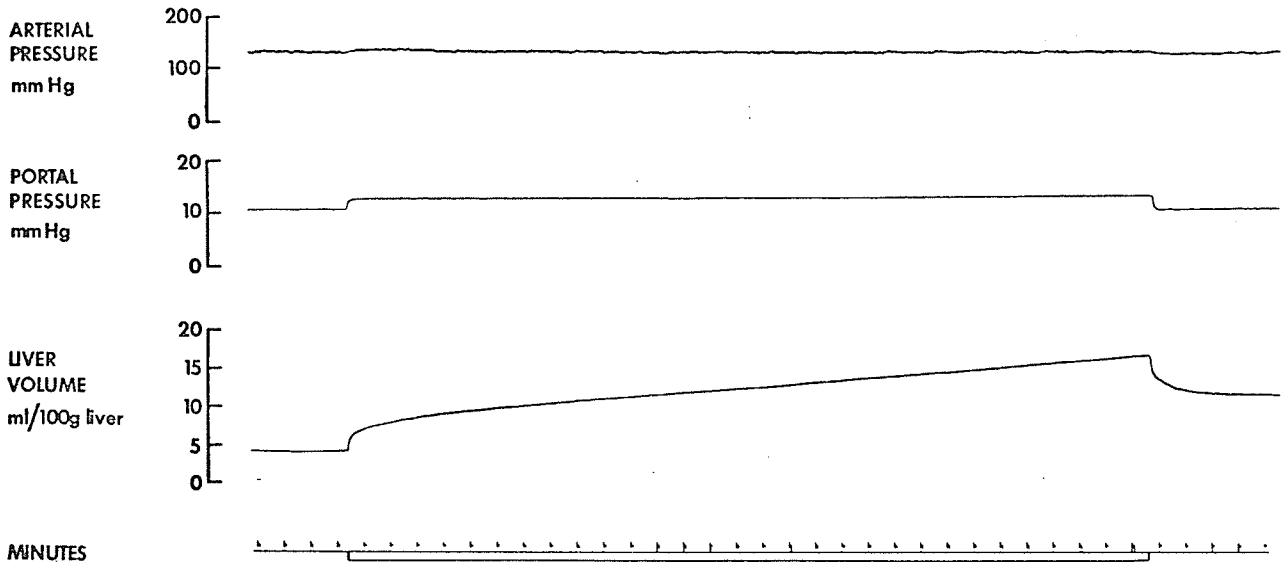


Figure 28. Response in one cat when hepatic venous pressure was increased to 4.68 mm Hg for 30 minutes.

duration of the elevation in hepatic venous pressure. Returning the hepatic venous pressure to zero resulted in a rapid reduction in hepatic volume which leveled off and became steady after a few minutes. The resultant reduction in hepatic volume was larger than the rapid increase seen on elevation of the venous pressure but it was much smaller than the total volume change during the period of raised venous pressure. In 2 cats the venous pressure was raised to 9.3 mm Hg for 4-5 hours. The steady increase in volume after 20 minutes was maintained for the entire duration of elevated pressure. The change in total volume over this period (over 150 ml) was greater than the original volume of the liver and since no such increase in volume was observed, it was possible that the slow steady rate of volume increase represented filtration of fluid into the plethysmograph.

The technique of separation of total volume changes into the two components, blood volume and net trans-sinusoidal fluid shifts, is adapted from the procedures used in the gut and skeletal muscle (Figure 2). ^{51}Cr-tagged red blood cells were injected in 5 cats and radioactivity from the liver was monitored. The effects of elevations of hepatic venous pressure of 4-10 mm Hg for 60 minutes, on the levels of radioactivity and total liver volume, were examined on 14 occasions. Increasing the venous pressure resulted in a rapid increase in radioactivity which continued for 5-20 minutes after which time any changes were small. Two minutes after the increase in pressure, the radioactivity was 51 $\pm$ 6% (mean $\pm$ S.E.) and 20 minutes after, 90 $\pm$ 5% of the total increase over 60 minutes. The small increase in radioactivity that occurred between 20-60 minutes was usually accompanied by small

irregular changes in liver volume during movements of the animal or administration of supplementary doses of pentobarbital.

For reasons outlined in the discussion, accurate calibration of changes in radioactivity in terms of the change in blood volume was difficult. It was assumed that the change in total volume during the first 2 minutes was due only to a change in hepatic blood content (the possible error in this estimate is discussed). Figure 29 represents the mean data from 5 cats in which hepatic venous pressure was elevated to 9.3 mm Hg for 1 hour. The blood content of the liver remained nearly constant after about 20 minutes while total volume continued to increase at a steady rate. The difference between the two curves gives a measurement of the amount of fluid filtered across the sinusoidal walls. The rate of filtration calculated by this means remained constant for the duration of the 60 minute period of elevated venous pressure. Though the difference between the two slopes could not be calculated with any degree of precision at times before 20 minutes, extrapolation of the filtration component in Figure 29 back to zero time shows a net filtration of zero. This strongly suggests that the filtration rate over the first 20 minute period was not significantly different from that measured after 20 minutes.

Extrapolation of the total volume trace back to zero time (dotted line, Figure 29) allows an accurate estimate of the change in blood volume that occurred in response to the elevated venous pressure (see Discussion). The effect of venous pressure on liver blood volume was measured on 33 occasions in 26 cats at 4 venous pressure levels. Figure 30 shows the mean and standard errors of these data.

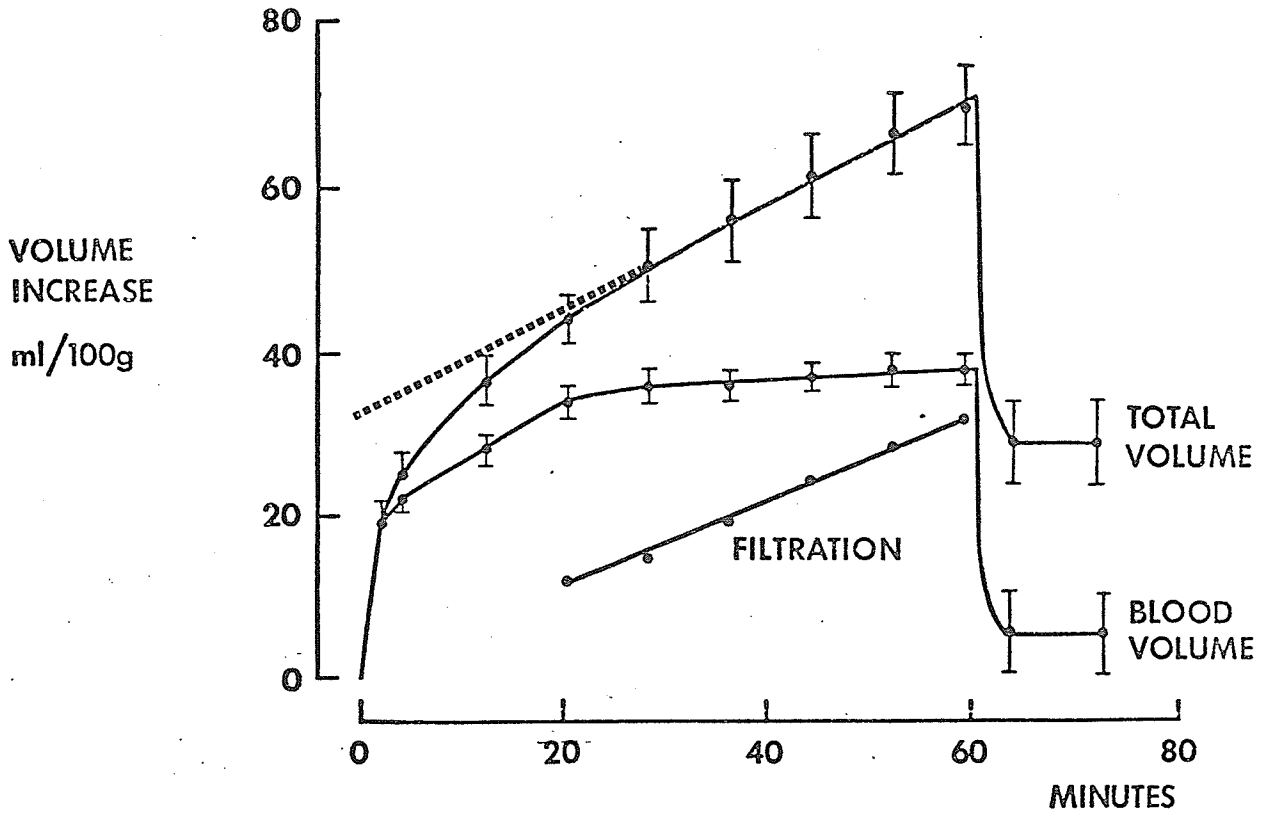


Figure 29. Changes in total hepatic volume and blood volume (mean ± S.E.) in five cats when hepatic venous pressure was increased to 9.35 mm Hg for 60 minutes. Trans-sinusoidal fluid filtration was calculated by subtracting the mean values of these two curves. Extrapolation of the total volume trace back to zero time (dotted line) gives an estimate of blood volume changes.

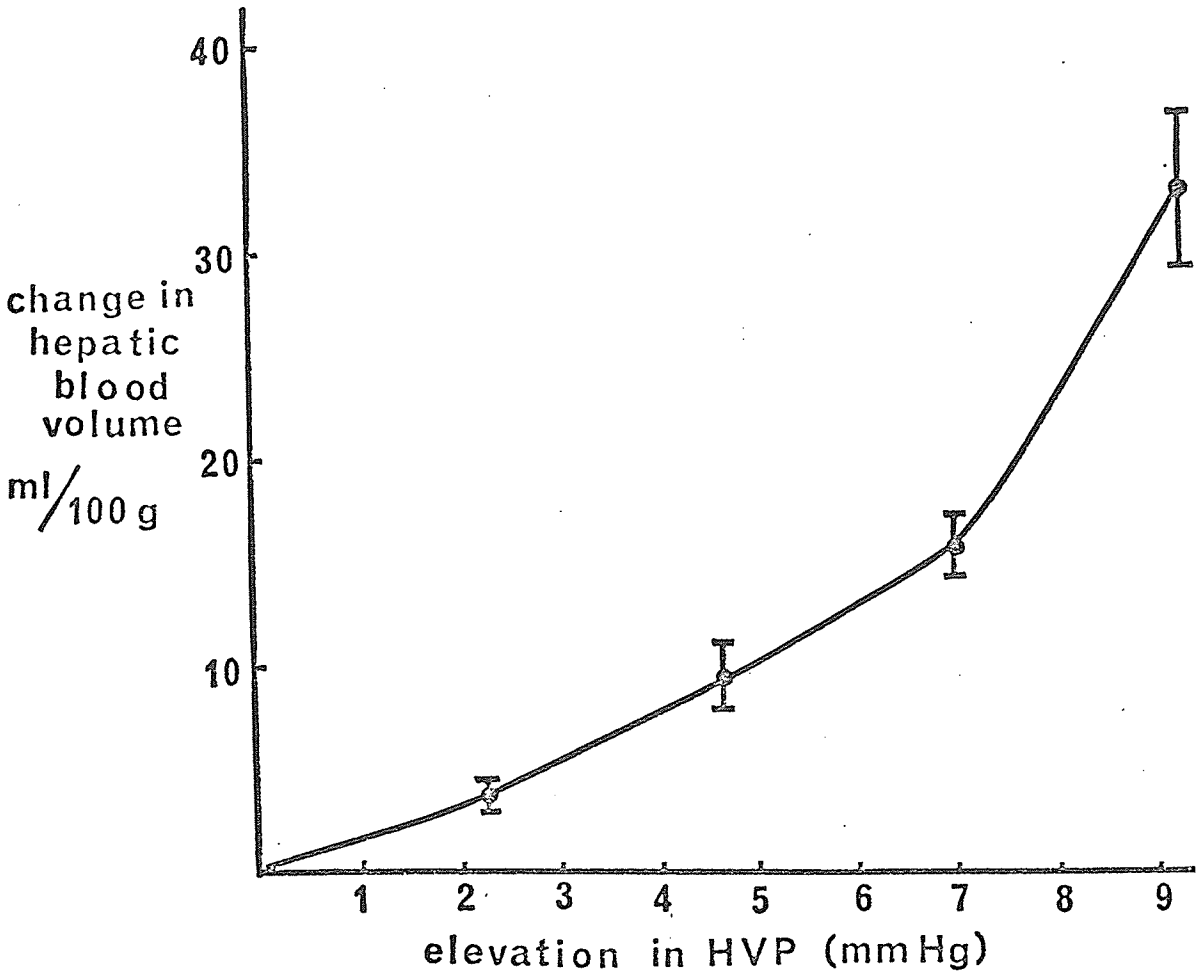


Figure 30. Effect of elevation of hepatic venous pressure (HVP) on hepatic blood volume (mean ± S.E.). HVP was raised on 33 occasions in 26 cats. (The mean liver weight in cats with the venous long circuit established is 91.3 ± 3.7 g in a 2.5 kg cat).

Restoration of the pressure to zero resulted in a return of hepatic blood content to very near the control level (Figure 29). The small residual increase in radioactivity seen after the pressure was returned to zero was roughly the same as that which accumulated over the period of from 20 to 60 minutes during raised venous pressure. This point will be discussed later. The large standard errors shown in Figure 29 represent variations in slopes between different animals and not irregularities within individual experiments.

Thus, when hepatic venous pressure is elevated, the increase in total volume is due to an increase in hepatic blood content, which becomes stable after 5-20 minutes, and a filtration of fluid which continues at a steady rate for the duration of pressure elevation. The steady-state filtration can be accurately measured from the slope of the total volume trace after 20 minutes and extrapolation of the steady slope back to zero time provides an estimate of the change in hepatic blood volume.

Relation between Filtration Rate and Hepatic Venous Pressure

Hepatic venous pressure was raised in steps of 2.34 mm Hg up to 11.7 mm Hg and decreased in the same manner as represented by Figure 31. Each venous pressure was maintained for 30 minutes and the steady-state filtration was determined from the last 5 minutes of each period as shown by the dotted lines in the figure. In 4 cats the hepatic nerves and lymphatics were tied and 7 complete curves were obtained (Figure 32A). The steady-state filtration rate is a linear function of the hepatic venous pressure with a slope of 0.060 ± 0.003 ml.min^{-1} mm Hg^{-1} .100 g liver^{-1} (mean $\pm$ S.E.). The values obtained during

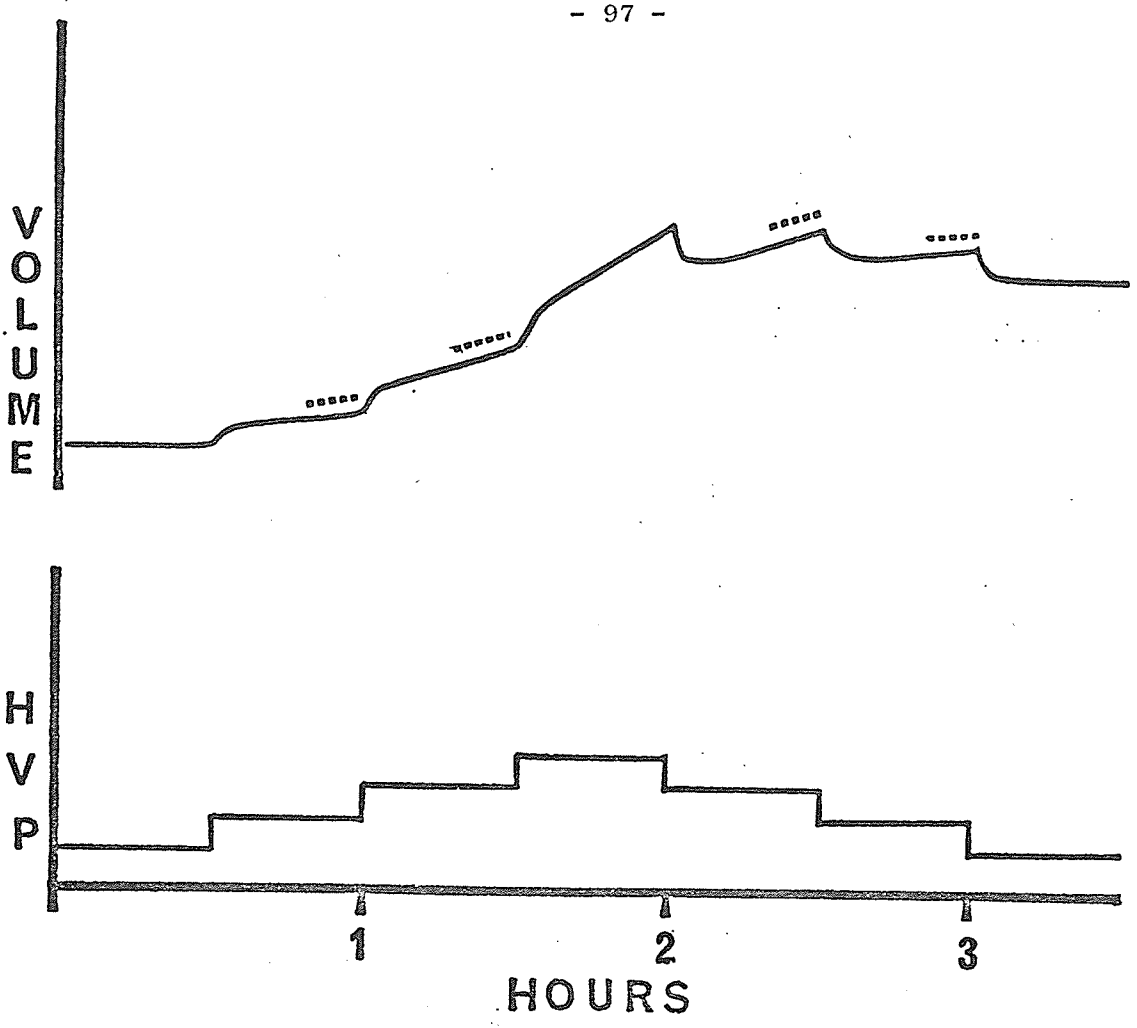


Figure 31. Effects of stepwise elevations of hepatic venous pressure (HVP) on hepatic volume. The traces are hand drawn and not taken from any specific experiment and are meant to be purely instructive.

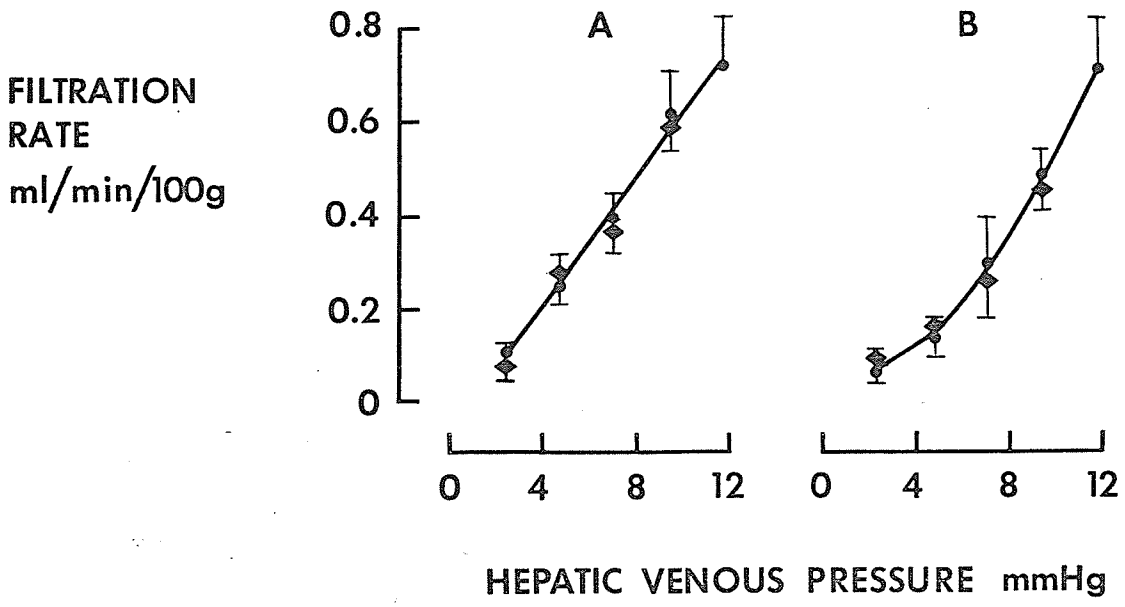


Figure 32. Relation between steady state filtration rates (mean ± S.E.) and hepatic venous pressures in cats in which the hepatic lymphatic drainage was occluded (A) or intact (B). ● = values during stepwise increases in venous pressure; ◆ = values during stepwise decreases.

stepwise lowering of the venous pressure were similar to those obtained during stepwise elevation of the pressure. Once again the large standard errors reflect variations between animals rather than within each experiment.

The lymphatic and hepatic nerves were left intact in 6 cats and the stepwise pressure changes were made as above. Figure 32B shows the mean and standard error of these data. The values were not significantly different from those in animals with the lymphatics tied. It appears that the fluids filtered across the sinusoid are not drained by the lymphatics, except possibly at lower filtration rates.

Collection of Filtrate

In 3 experiments the plethysmograph was filled with petrolatum. On raising hepatic venous pressure, clear droplets of fluid could be seen forming on the surface of the liver. These droplets accumulated at the bottom of the plethysmograph and could be removed for analysis. The specific gravity was 85-94% (mean 90%) of that of plasma samples taken simultaneously. The volume of the accumulated fluid could not be measured with great precision; however, it was always similar to the residual increase in total volume seen after venous pressure was returned to zero.

Portal Pressure Response to Elevated Hepatic Venous Pressure

On 157 occasions in 19 cats the effect of various elevations of hepatic venous pressure on portal pressure was examined. Figure 33 shows the mean and standard error of these data. When hepatic venous

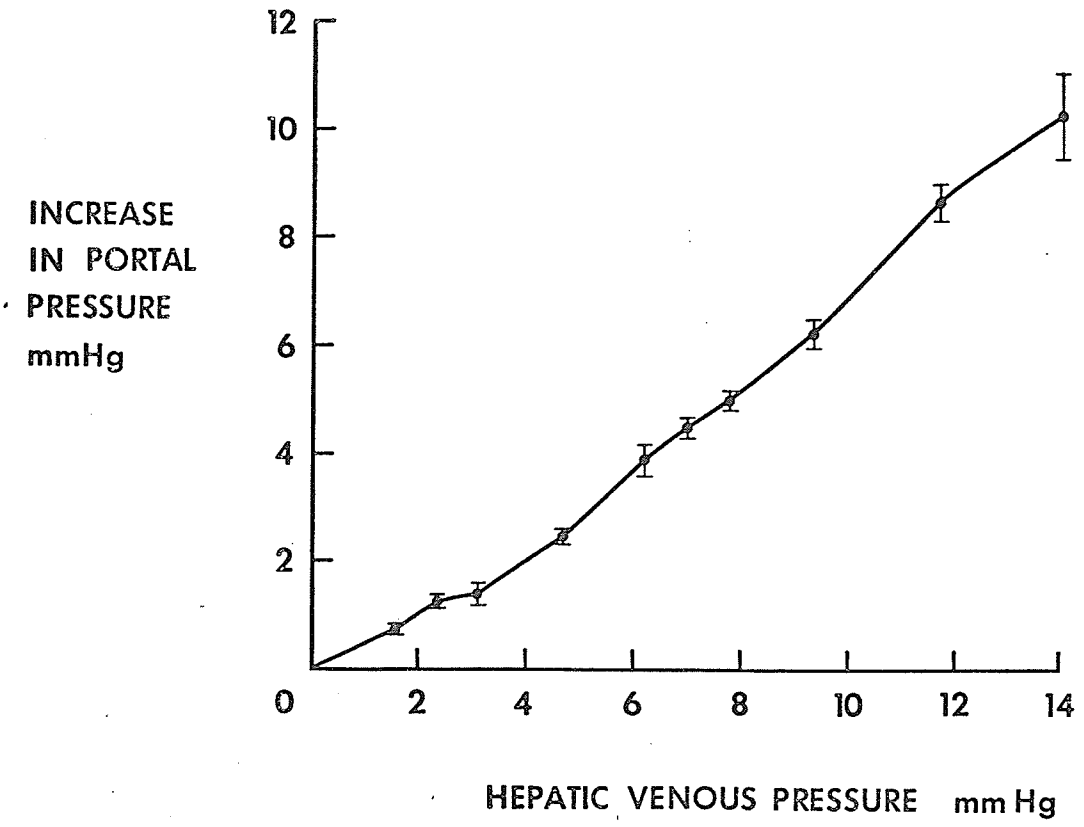


Figure 33. Increases (mean ± S.E.) in portal pressure (from the control value of 8.8 ± 0.5 mm Hg) which occurred when hepatic venous pressure was increased.

pressure was elevated by small amounts, (< 5 mm Hg) 50% of the increment was reflected in portal pressure while elevations in the range of 10-14 mm Hg were 75% transmitted to the portal vein. Restoration of hepatic venous pressure resulted in a rapid reduction in portal pressure (Figure 28). The increments caused by altered hepatic venous pressures were reproducible to within 1 mm Hg on repetition in each cat.

DISCUSSION (SECTION III)

Measurement of Blood Volume Changes

Elevation of hepatic venous pressure initially resulted in a rapid increase in liver volume; after 5-20 minutes the volume increase continued at a lower but steady rate for up to 5 hours. It was obvious that not all of the volume response could be attributed to changes in blood volume. From data obtained in the gut and skeletal muscle, we suspected that at some time after the pressure increase vascular engorgement became stable and any further volume change could be attributed to fluid filtration. However we could not assume that the slow, steady volume increments seen after 20 minutes were due to trans-sinusoidal filtration. It was necessary to separate the two components of the volume response by use of ^{51}Cr-tagged red blood cells, as outlined earlier. The radioactivity technique, which has been used previously for studies on the vascular beds of skeletal muscle (Ablad & Mellander, 1963) and intestine (Wallentin, 1966), were not as readily applicable to the hepatic vascular bed.

Because of the inability to place lead shielding around the plethysmograph and thus isolate the liver, radioactivity from tissues other than the liver was recorded. It seems unlikely that the tissue not beneath the collimator contributed significantly to the total counts of radioactivity from the liver. Changes in radioactivity from the intestine and spleen were not recorded since these organs were well outside the collimated area.

Calibration of the counts per minute in terms of blood content was difficult since the exact blood volume of the area counted could

not be determined. To obtain the data shown in Figure 29 it was assumed that the total volume change in the first 2 minutes after raised venous pressure was due to changes in blood volume. That is, the increase in radioactivity over that time was equated with the total volume change within the plethysmograph. This assumption results in a slight over-estimation in blood volume since fluid was also being filtered over this period. If the same rate of filtration as occurred over the measurable range (> 20 min) occurred during the first 2 minutes, then blood volume changes were over-estimated by 1 ml (2 min X 0.5 ml/min/100 g) per 100 g of liver in this series of experiments. However this represents an error in blood volume estimation of less than 5%.

Another difficulty occurred with this preparation. Throughout the experiments radioactivity over the liver increased slowly even when hepatic venous pressure was zero. On restoration of venous pressure to zero after elevation for a period of time, there remained a residual radioactivity which was slightly above that recorded during the control period. The residual radioactivity was very similar to the small increases that occurred in radioactivity between 20 and 60 minutes after venous pressure was raised. At the end of the experiments some radioactivity remained even when as much blood as possible was removed by perfusion of the liver with saline. It seems likely that some tagged red blood cells were destroyed and the membranes were removed by phagocytosis by the reticuloendothelial system of the liver. The slow increase in radioactivity during the experiments may have been due to this factor; however, the total error seen at the end of a one hour period of elevated venous pressure due to the slow accumulation of

radioactivity was less than 5% (Figure 29).

In the intestine the increase in blood volume was almost complete after 2 minutes (Wallentin, 1966) (Figure 2) and CFC values measured at this time were not distorted to any great extent by delayed venous compliance. In the liver, blood volume became steady only after 5-20 minutes and thus measurement of CFC at 2 minutes included a large component of delayed compliance. After 2 minutes the hepatic blood volume has risen to only 50% of its final level, thus determination of the slope of the total volume increase at this time is not an accurate measure of the initial filtration rate and a determination of the CFC involves considerable over-estimation. Though the absolute value cannot be regarded as accurate, maneuvers such as stimulation of the hepatic nerves (Section 1) would still be expected to show a demonstrable effect on this value if, in fact, initial filtration was affected by stimulation of the nerves.

Extrapolation of the total volume slope along the steady-state filtration portion of the curve (from 20 minutes on) back to the time at which venous pressure was elevated (zero time) allows an estimate of hepatic blood volume changes that incorporates an error of less than 2% when compared with the blood volume measured in Figure 29. Thus the technique of measuring the effect of hepatic venous pressure on hepatic blood volume as described in Figure 30 is justified. The present discussion thus justifies equating the rate of filtration with the steady rate of total volume change seen after 20 minutes of elevated venous pressure, and extrapolation of this slope to zero time provides an accurate estimate of blood volume changes.

Relation of Hepatic Venous Pressure to Net Filtration

In order to relate pressure changes to fluid exchange, the proportion of the change in venous pressure that is transmitted to the sinusoids must be known. It was shown (Figure 28) that 50-75% of the increment in hepatic venous pressure was transmitted to the portal vein. Since the greatest drop between the portal and hepatic veins appears to occur in the portal radicals within the liver (Nakata et al., 1960), it is probable that sinusoidal pressure is almost equal to hepatic venous pressure and that changes in hepatic venous pressure cause quantitatively similar changes in sinusoidal pressure. Our data show that the steady state filtration is directly proportional to sinusoidal hydrostatic pressure, which is in accordance with the Starling equation. The steady-state filtration rate is a linear function of hepatic venous pressure (and thus sinusoidal pressure) with a slope of 0.060 ± 0.003 ml . min^{-1} . mm Hg^{-1} .100 g $liver^{-1}$ and this rate of filtration is maintained for however long the venous pressure is elevated. The prolonged filtration is in contrast to the observation in the intestine that filtration ceases after 5-8 minutes (Figure 2). It has been suggested that the filtration in the intestine stopped primarily as a result of an increase in tissue hydrostatic pressure and possibly also due to a decrease in the interstitial colloid osmotic pressure (Johnson, 1965; Wallentin, 1966; Johnson & Hanson, 1966).

The Role of Tissue Hydrostatic Pressure

The role of tissue hydrostatic pressure changes in the liver cannot be completely evaluated. However since the filtration that ensues with any given venous pressure continues undiminished for at least 5 hours,

it may be stated that increases in tissue hydrostatic pressure are not sufficient to alter the trans-sinusoidal pressure gradient after the initial 20 minute period of delayed compliance of the capacitance vessels has elapsed. Because of the delayed compliance, the initial rate of filtration cannot be accurately assessed and it remains possible that an initial high rate of filtration is reduced to a lower steady state by some rise in tissue pressure which then remains constant. This appears unlikely since, if the linear filtration trace (Figure 29) is extrapolated back to time zero, this filtration rate is seen to account for all the volume changes (i.e. intercepts the abscissa at zero volume). If the initial filtration rate were higher than the steady-state rate, extrapolation to zero time would indicate a small volume of filtered fluid in excess of that expected if the rate for the first 20 minutes were the same as that measured after that time. It is thus probable that tissue hydrostatic pressure changes in the liver do not play a significant role in the balance of fluid exchange across the sinusoids.

The Role of the Lymphatics

The role of the lymphatics was evaluated by repeating the experiments relating hepatic venous pressure to net fluid filtration without ligation of the lymphatics and hepatic nerves. Though there was no statistical difference between the two groups, a trend appeared that might have been confirmed statistically if the experiment could be repeated in the same preparation (thus allowing paired-t analysis). When the lymphatics were intact it appeared that accumulation of fluid in the plethysmograph may have been reduced slightly. Inclusion of the liver in the plethysmograph was accompanied by an elevation of portal

pressure from 5.7 to 7.7 mm Hg and lymph drainage may have been less than would have occurred in the absence of such an obstruction. This would be reasonable since distension of the lymphatics and increased lymph flow during periods of raised hepatic venous pressure have been reported (Nix et al., 1951; Hyatt et al., 1955; Brauer et al., 1959).

Trans-sinusoidal Reabsorption

Restoration of the hepatic venous pressure to zero after a period of elevation resulted in a return of blood volume to very near the control level while there was no evidence of reabsorption of any of the filtered fluids. Reduction of venous pressure below zero had previously been reported to have no effect on hepatic volume or portal pressure (Brauer et al., 1959). This was confirmed by our data. The fact that portal pressure was unaffected by negative venous pressure suggests that the vessels collapse at these pressures and this could account for the lack of reabsorption seen. In the intact animal, abdominal pressures may be slightly negative and it may be possible under these conditions to impose a small negative pressure on the hepatic veins which should be transmitted to the sinusoids and result in reabsorption of fluids into the vascular compartment. This possibility could not be evaluated in the present preparation. Creation of a negative pressure within the plethysmograph by lowering the glass cylinder shown in Figure 14 caused frequent leaks in the system. Even with the plethysmograph pressure set below zero, the hepatic vein was still exposed to positive pressure and so did not represent the conditions of negative pressure within a sealed abdomen.

Ascites

The present data suggest a mechanism for the formation of ascites in hepatic cirrhosis and congestive heart failure. In congestive heart failure the central venous pressure increases, resulting in an equivalent increase in hepatic sinusoidal pressure. The hepatic circulation, in cirrhosis of the liver, is distorted by fibrosis and nodules of regenerating cells. The primary vascular lesion is post-sinusoidal, a condition that results in elevated sinusoidal pressure (Fomon & Warren, 1969). Filtration rates in the cat liver are directly related to sinusoidal pressure (Figure 32). Myogenic responses, changes in interstitial pressure and changes in colloid osmotic pressure do not serve as protective mechanisms against prolonged filtration. Thus, in these clinical situations, large volumes of fluids of high protein content filter across the liver and may result in ascitic volumes of up to 15 liters with intra-abdominal pressures as high as 40 mm Hg (Hyatt & Smith, 1954). The increase in intra-abdominal pressure may slow filtration but for this to occur, large volumes of ascites must already exist.

For illustrative purposes, if we assume that the data obtained here apply quantitatively to the human liver, elevation of hepatic venous pressure by only 4 mm Hg for 1 hour would result in formation of over 150 ml of ascitic fluid (From Figure 32, 0.2 ml/min/100 g x 1500 g liver x 60 min = 180 ml). Some of this fluid is drained by hepatic lymphatics while the remainder accumulates in the peritoneal cavity. Fluid reabsorption occurs from the peritoneal surfaces and eventually filtration and reabsorption become balanced and the volume of ascitic fluid remains constant though turning over rapidly (Prentice et al., 1952).

The elevation in hepatic venous pressure is transmitted to the portal vein (Figure 33) and thus to the spleen and intestine which also contribute to ascites formation (Hyatt & Smith, 1954). The role of these vascular beds in ascites formation is beyond the scope of the present work.

SUMMARY (SECTION III)

1. Elevation of hepatic venous pressure results in an increase in hepatic blood content, which becomes constant in 5-20 minutes, and filtration of fluid which continues at a uniform rate for the duration of the pressure elevation.

2. The rate of trans-sinusoidal fluid filtration (0.06 ± 0.003 ml . min^{-1} .mm Hg^{-1}.100 g $liver^{-1}$) is directly proportional to sinusoidal hydrostatic pressure.

3. No protective mechanisms exist to prevent filtration when hepatic venous pressure is elevated. Filtered fluids pass freely across the surface of the liver and continue to pool in the peritoneal cavity (plethysmograph) as long as the venous pressure is elevated.

4. Tissue hydrostatic pressure does not appear to play a role in hepatic fluid exchange. The filtration rate remains uniform after the blood volume changes cease and there is suggestive evidence that the rate of filtration over the first 20 minutes is the same as that which occurs after the blood volume is stable. This suggests that fluid filtration did not alter tissue hydrostatic pressure significantly.

SECTION IV

EFFECTS OF INFUSIONS OF ISOPROTERENOL, HISTAMINE AND ADRENALINE AND OF HEPATIC ARTERIAL OCCLUSION ON TRANS-SINUSOIDAL FLUID EXCHANGE

RESULTS (SECTION IV)

Control Results

In 12 cats, after surgery was complete, hepatic venous pressure was set at zero relative to the level of the vena cava as it emerged from the liver. Mean arterial pressure after the preparation was set up was 143 ± 12 mm Hg (mean ± S.E.), portal pressure was 6.9 ± 0.5 mm Hg, total hepatic blood flow was 102 ± 5 ml/min/100 g liver and there was a net filtration of 0.04 ± 0.03 ml/min/100 g liver. This small filtration indicates some mild degree of elevation of sinusoidal pressure caused by the plethysmograph.

In 10 cats the effects of drug infusions on a steady-state filtration caused by elevation of hepatic venous pressure by 7 mm Hg was examined. After the venous pressure was raised, mean arterial pressure was 130 ± 11 mm Hg, portal pressure was 10.8 ± 0.6 mm Hg and hepatic blood flow was 91 ± 5 ml/min/100 g liver. The mean steady-state filtration during the raised venous pressure was 0.33 ± 0.06 ml/min/100 g liver.

The Effect of Adrenaline on Trans-sinusoidal Fluid Exchange

Initially the effect of infusion of adrenaline on filtration at zero venous pressure was evaluated. Figure 34 represents the mean and standard error of 5 intra-arterial dose-response curves obtained in 4 cats (the effect of adrenaline on arterial and portal pressure and hepatic capacitance has been described in the previous section). The direct effects of adrenaline, independent of altered hepatic blood flow are seen at doses below 2.0 μg/kg/min. Higher doses result in re-circulation of adrenaline and increased hepatic blood flow due to intestinal vaso-

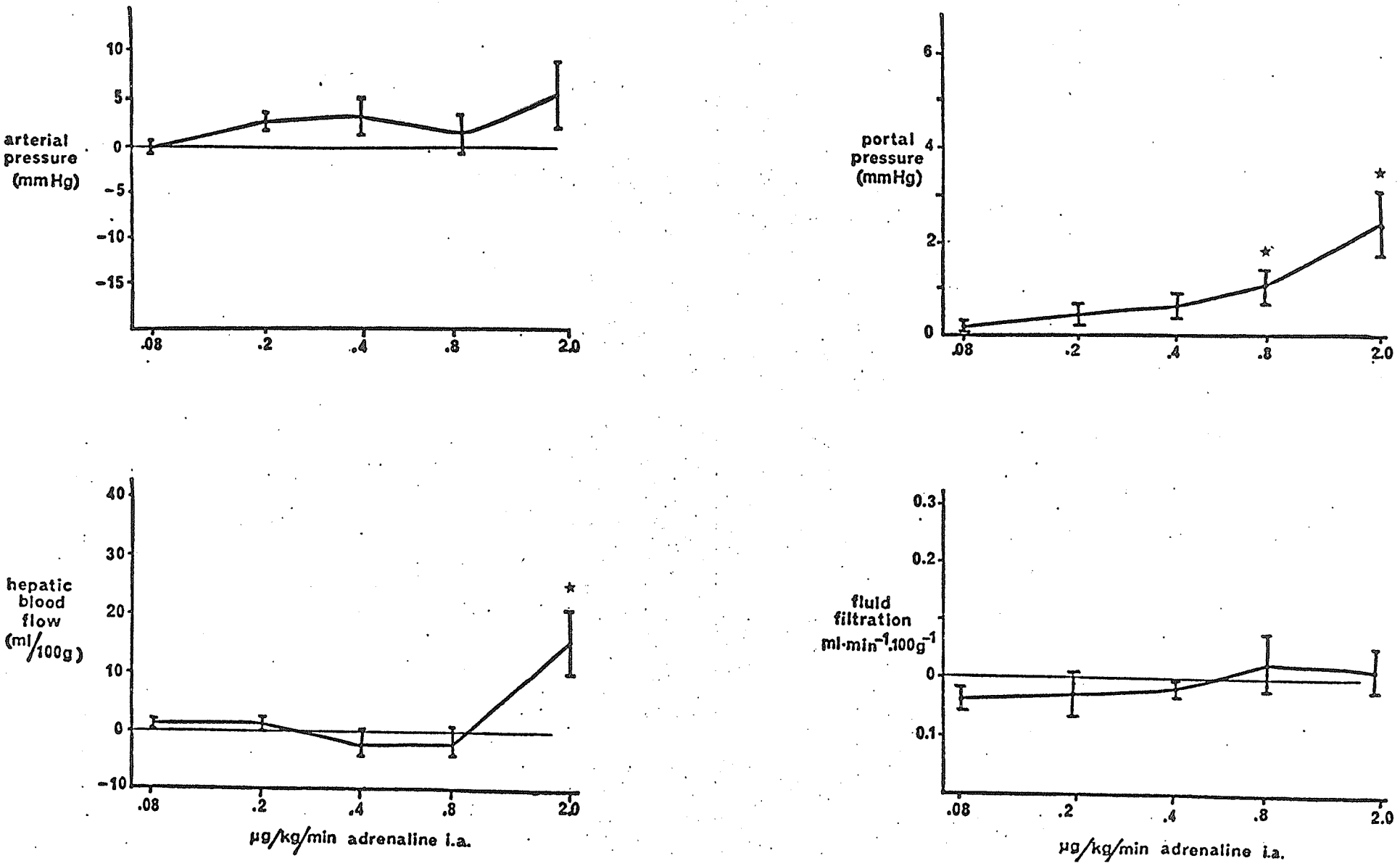


Figure 34. Dose-response curves for adrenaline infused into the hepatic artery. Effects are shown as the mean change from the pre-infusion control value.
*= statistical difference ($p < .05$ paired-t test).

dilation. At all doses tested, adrenaline did not result in significant alterations in net fluid exchange.

The mean of 3 dose-response curves for intravenously infused adrenaline (0.08 - 2 μg/kg/min) is shown in Figure 35. The infusions were made directly into the blood reservoir and the drug reached the liver via both hepatic artery and portal vein as would occur during discharge by the adrenal medullae. These doses did not alter arterial pressure but portal pressure and hepatic venous flow were elevated at all but the lowest dose. The small reductions in filtration rate over the middle dose range were less than that caused by a reduction in venous pressure of 1 mm Hg (Section III).

The effect of stimuli on sinusoidal pressure and permeability can be evaluated at normal venous pressure while alterations in sinusoidal surface area can only be demonstrated in the presence of a net 'base-line' flux of fluids. Trans-sinusoidal fluid filtration was established by elevation of the hepatic venous pressure. This constant rate of filtration then served as a base-line which could be altered by a change in sinusoidal surface area.

Clarification of Experimental Procedure

To clarify the experimental procedure the results obtained in one cat in which adrenaline was infused are shown in Figure 36. The data are plotted from the graph which could not be reproduced directly due to the length of the record. Control venous pressure was set at the level of the hilum of the liver. At time zero, hepatic venous pressure was increased by 7 mm Hg and the initial rapid increase in hepatic volume previously described is seen. After 15-20 minutes the volume increased

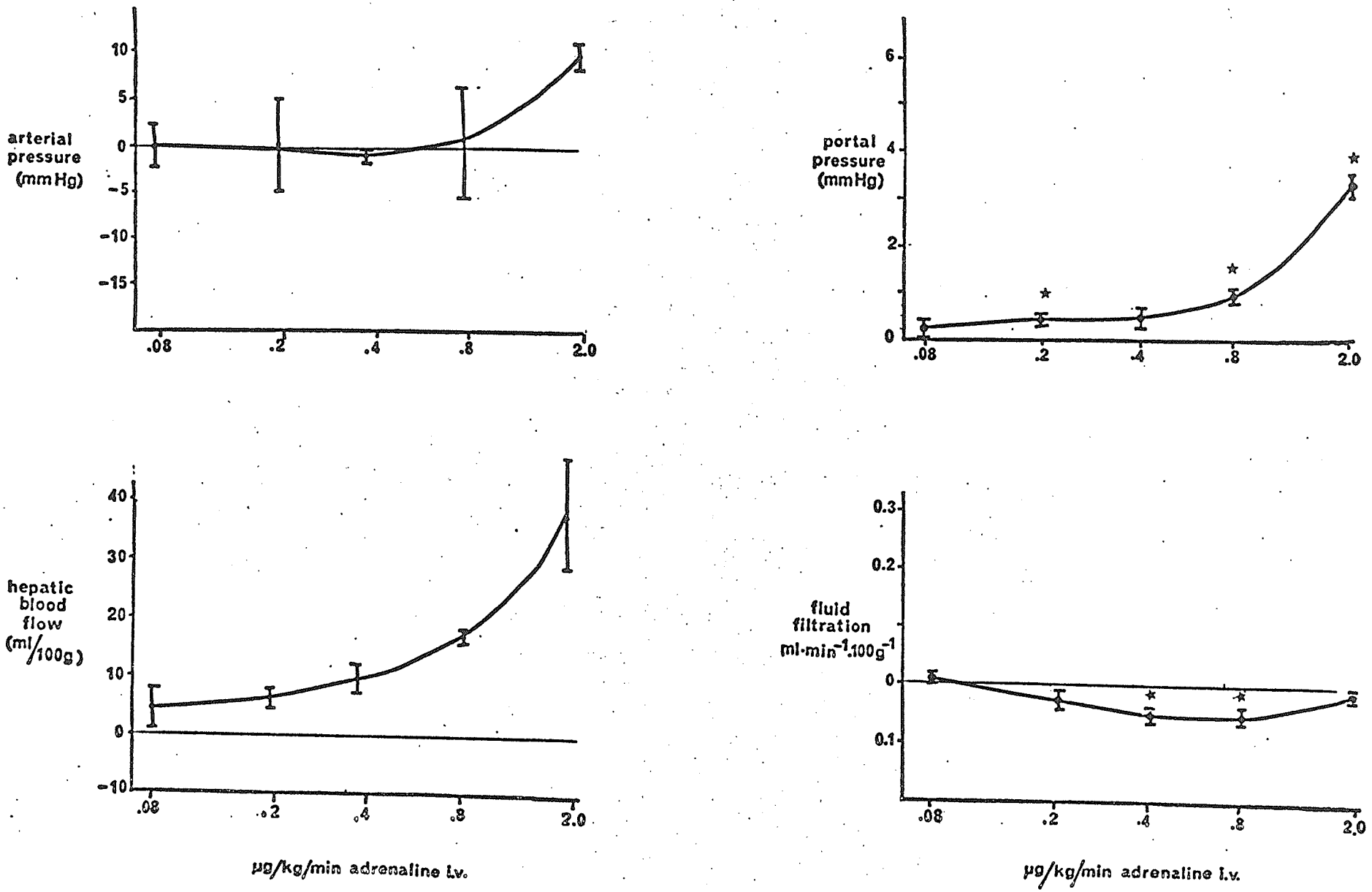


Figure 35. Dose-response curves for adrenaline infused intravenously. Effects are shown as the mean change from the pre-infusion control value.
*= statistical difference ($p < .05$ paired-t test).

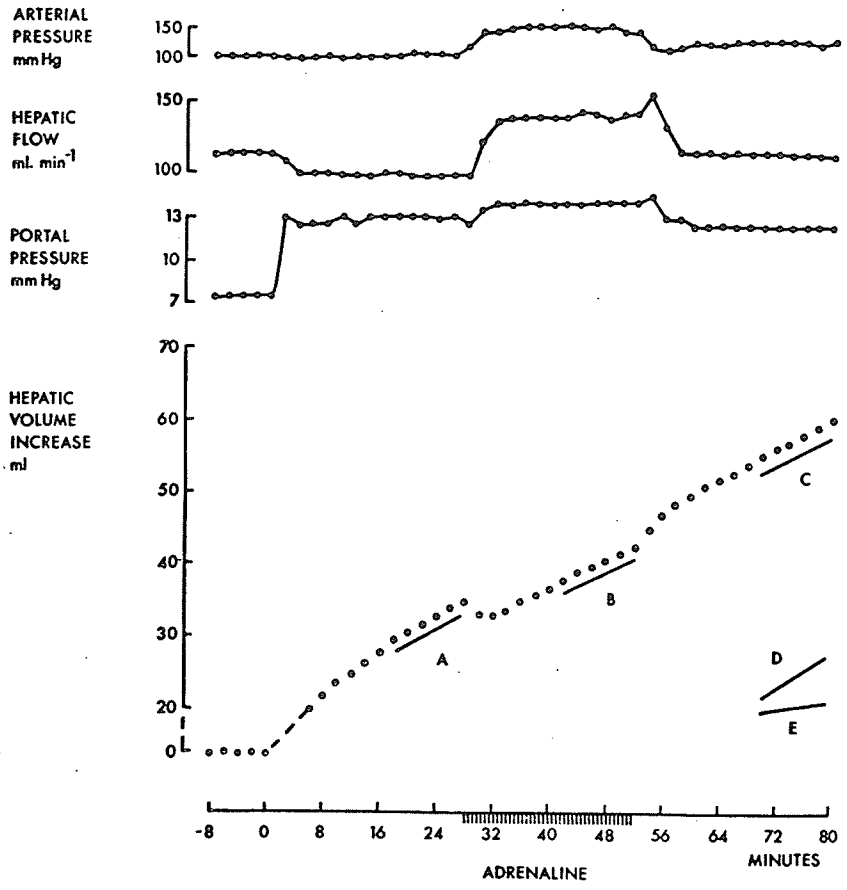


Figure 36. The data for an experiment in one cat (2.2 kg body weight, 83 g liver) replotted on scales suitable for publication. At zero time hepatic venous pressure was increased to 7 mm Hg. The steady-state volume increase (slope A) was 0.52 ml/min. Adrenaline (2 μg/min/kg) was infused intravenously and the steady-state volume increase (slope B) was 0.48 ml/min. After cessation of the adrenaline the steady-state volume increase (slope C) was 0.48 ml/min. When hepatic venous pressure was subsequently increased to 8.5 mm Hg and reduced to 5.5 mm Hg the steady-state volume increases were 0.60 (slope D) and 0.15 (slope E) ml/min respectively.

at a steady rate (slope A). At 28 minutes, adrenaline was infused intravenously for 24 minutes. Arterial and portal pressure increased and hepatic blood volume increased due to intestinal vasodilation (Greenway & Lawson, 1966b; 1968). The steady increase in volume was interrupted at the onset of adrenaline infusion due to the effects of adrenaline on the hepatic blood volume (Section II) but these were complete in less than 15 minutes after which time a steady-state increase in volume resumed (slope B). (Blood volume changes did not occur with isoproterenol or histamine infusions (Section II)). When the adrenaline infusion was discontinued, an initial increase in hepatic blood content occurred which was then followed by a steady volume increase (slope C). The filtrations before and after the drug (slopes A & C) were not significantly different and the mean of these values was compared with the slope obtained during drug infusion by the student t-test for paired data (Steel & Torrie, 1960).

The sensitivity of this preparation was demonstrated by elevation of the venous pressure by 1.5 mm Hg to 8.5 mm Hg and by reduction of the same degree to 5.5 mm Hg. The steady-state slopes obtained during these maneuvers are shown in Figure 36. Analysis of the paired data from several experiments shows that the slopes at these various pressures are significantly different ($p < 0.001$).

The effects of single intravenous infusions of adrenaline at a dose equivalent to maximal physiological release (2 μg/kg/min) from the adrenal medullae (Celander, 1954) were examined at elevated venous pressure on 7 occasions in 4 cats (Figure 37). The effects of this dose at zero venous pressure are shown for comparison. At the raised venous

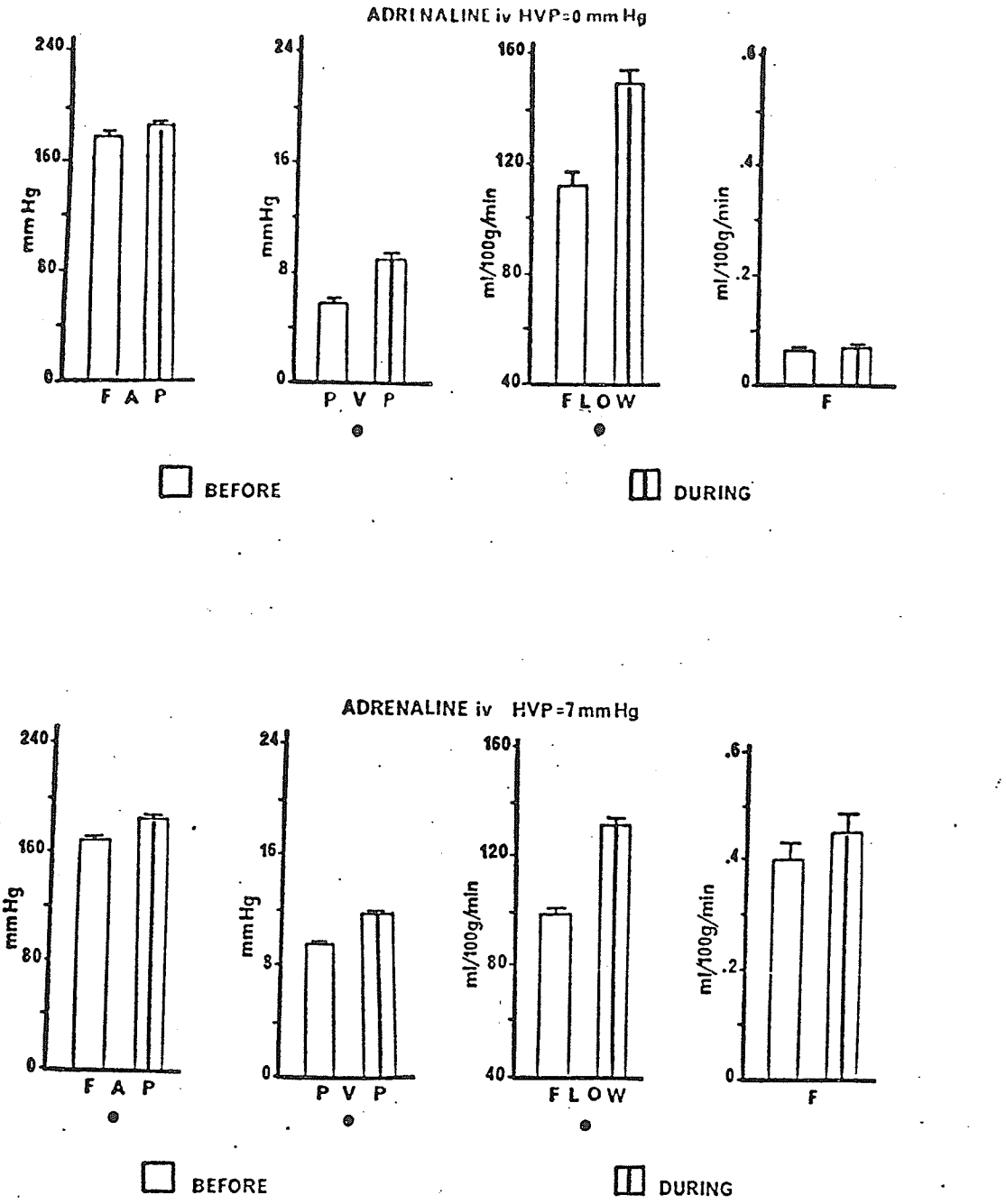


Figure 37. Effect of intravenous infusion of adrenaline (2 μg/kg/min) at zero and at elevated hepatic venous pressure. The mean values of femoral arterial pressure (FAP), portal venous pressure (PVP), total hepatic blood flow and rate of fluid filtration (F) are shown before and during infusion. • = statistical difference ($p < .05$, paired-t test).

pressure, infusion resulted in elevation of all parameters except for net fluid exchange which remained unaltered. It is concluded that adrenaline does not induce net filtration at zero venous pressure nor does it alter the rate of trans-sinusoidal fluid filtration induced by elevated venous pressure.

The Effect of Isoproterenol on Trans-sinusoidal Fluid Exchange

Isoproterenol (0.08 - 0.2 μg/kg/min) was infused into the hepatic artery at zero venous pressure in 3 cats on 5 occasions and at elevated venous pressure (7 mm Hg) in 5 cats on 9 occasions (Figure 38). The dose used was the highest dose that did not result in a reduction in arterial pressure of more than 5 mm Hg. This dose caused maximal vasodilation of the hepatic arterial bed (Greenway & Lawson, 1969). In response to infusions of the drug, total hepatic blood flow was increased at control and elevated venous pressures, and portal pressure was mildly increased at the higher venous pressure. Net fluid exchange was not altered by infusion of the drug. It is concluded that isoproterenol does not induce net filtration at zero venous pressure and does not alter the rate of trans-sinusoidal fluid filtration induced by elevated venous pressure.

The Effect of Histamine on Trans-sinusoidal Fluid Exchange

A mean of 6 dose-response curves obtained in 3 cats on infusion of histamine (0.4 - 10 μg/kg/min) into the hepatic artery is shown in Figure 39. For this set of experiments only, the hepatic long circuit was not established, thus hepatic blood flow was not recorded. Arterial pressure was significantly reduced at doses of $\geq$ 4 μg/kg/min.

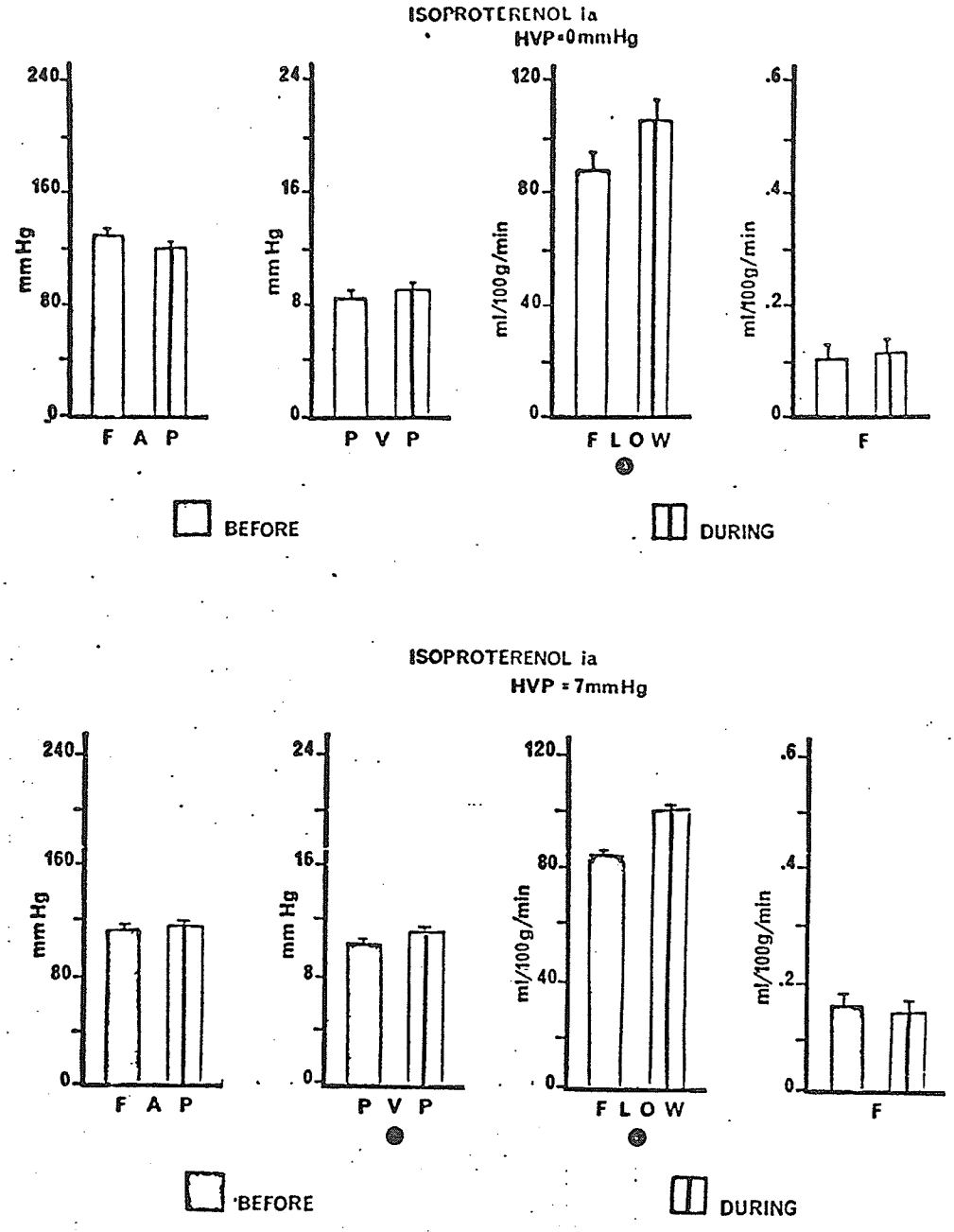
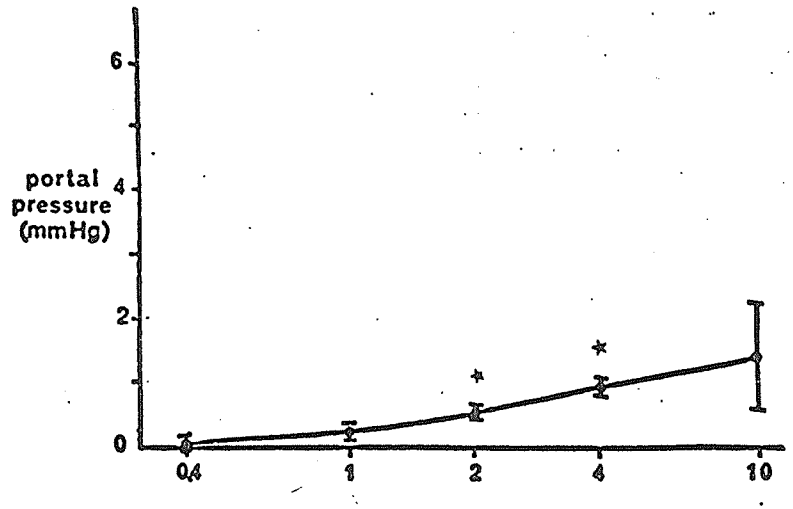
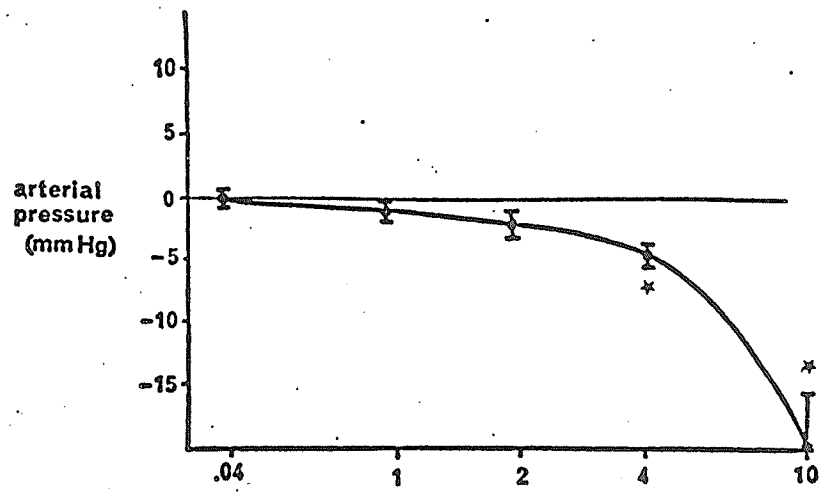


Figure 38. Effect of i.a. infusion of isoproterenol (0.08-0.2 μg/kg/min) at zero and at elevated hepatic venous pressure. The mean values of femoral arterial pressure (FAP), portal venous pressure (PVP), total hepatic blood flow and rate of fluid filtration (F) are shown before and during infusion. ● = statistical difference ($p < .05$ paired-t test).





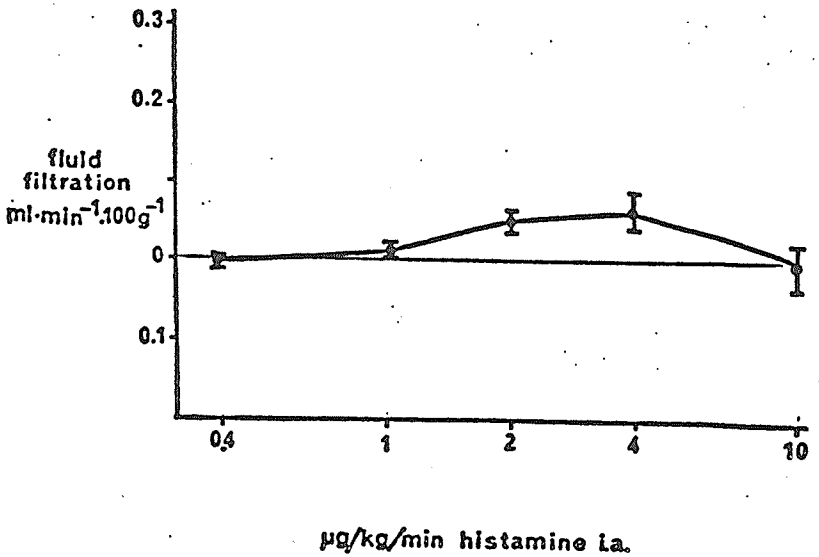


Figure 39. Dose-response curves for histamine infused into the hepatic artery. Effects are shown as the mean change from the pre-infusion control value. Blood flow was not measured. *= statistical difference ($p < .05$ paired-t test).

while portal pressure was elevated only slightly at 2 and 4 μg/kg/min. At 10 μg/kg/min, when arterial pressure had decreased markedly, portal pressure was variably affected but the effect was not statistically significant. The mild elevation in portal venous pressure at lower doses was consistent but biologically insignificant. At no dose tested was fluid exchange altered ($p > 0.05$). The highest dose of histamine (2 μg/kg/min) that did not reduce arterial pressure by more than 5 mm Hg was infused into the hepatic artery at zero venous pressure in 6 cats on 11 occasions and at elevated (7 mm Hg) venous pressure in 3 cats on 10 occasions. This dose produced an increase in CFC in skeletal muscle (Kjellmer & Odelram, 1965). The results are summarized in Figure 40. Total hepatic blood flow showed small but significant ($p < 0.01$) increases due to hepatic arterial vasodilation (Greenway et al., 1967b). No recorded parameter was notably affected by these infusions though paired-t analysis demonstrated some consistent minor changes. It is concluded that histamine does not induce net filtration at zero venous pressure and does not alter the rate of trans-sinusoidal fluid filtration induced by elevated venous pressure.

The Effect of Hepatic Arterial Occlusion on Trans-sinusoidal Fluid Exchange

Mechanical occlusion of the hepatic artery was accomplished by suspending a weight from a ligature which had previously been loosely looped around the artery. This maneuver was always done at the conclusion of a drug infusion experiment. Figure 41 shows the effect of occlusion of the hepatic artery at zero venous pressure on 2 occasions and at elevated venous pressure on 3 occasions. None of the recorded

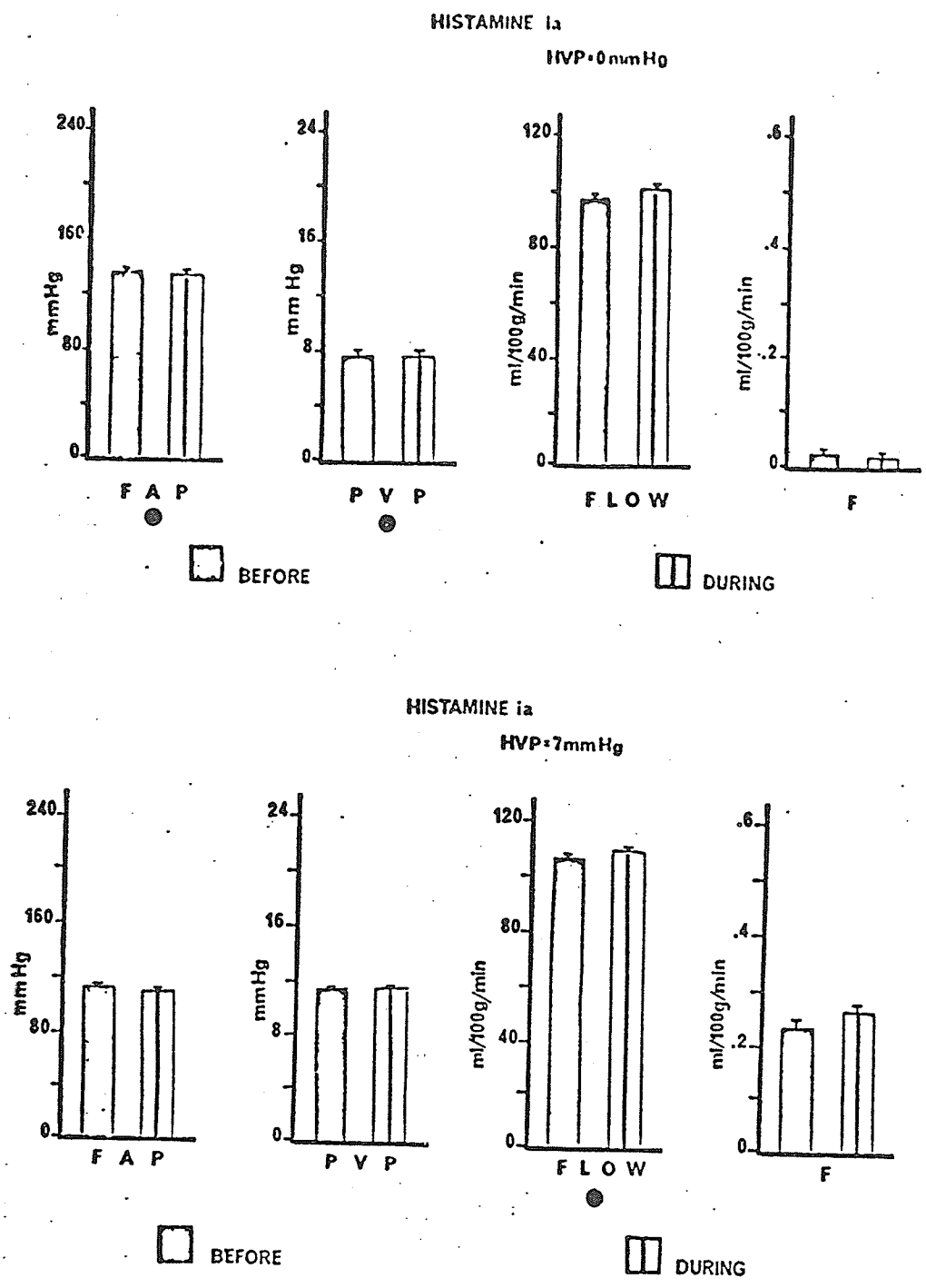


Figure 40. Effect of i.a. infusion of histamine (0.08-0.2 μg/kg/min) at zero and at elevated hepatic venous pressure. The mean values of femoral arterial pressure (FAP), portal venous pressure (PVP), total hepatic blood flow and rate of fluid filtration (F) are shown before and during infusion. ● = statistical difference ($p < .05$ paired-t test).

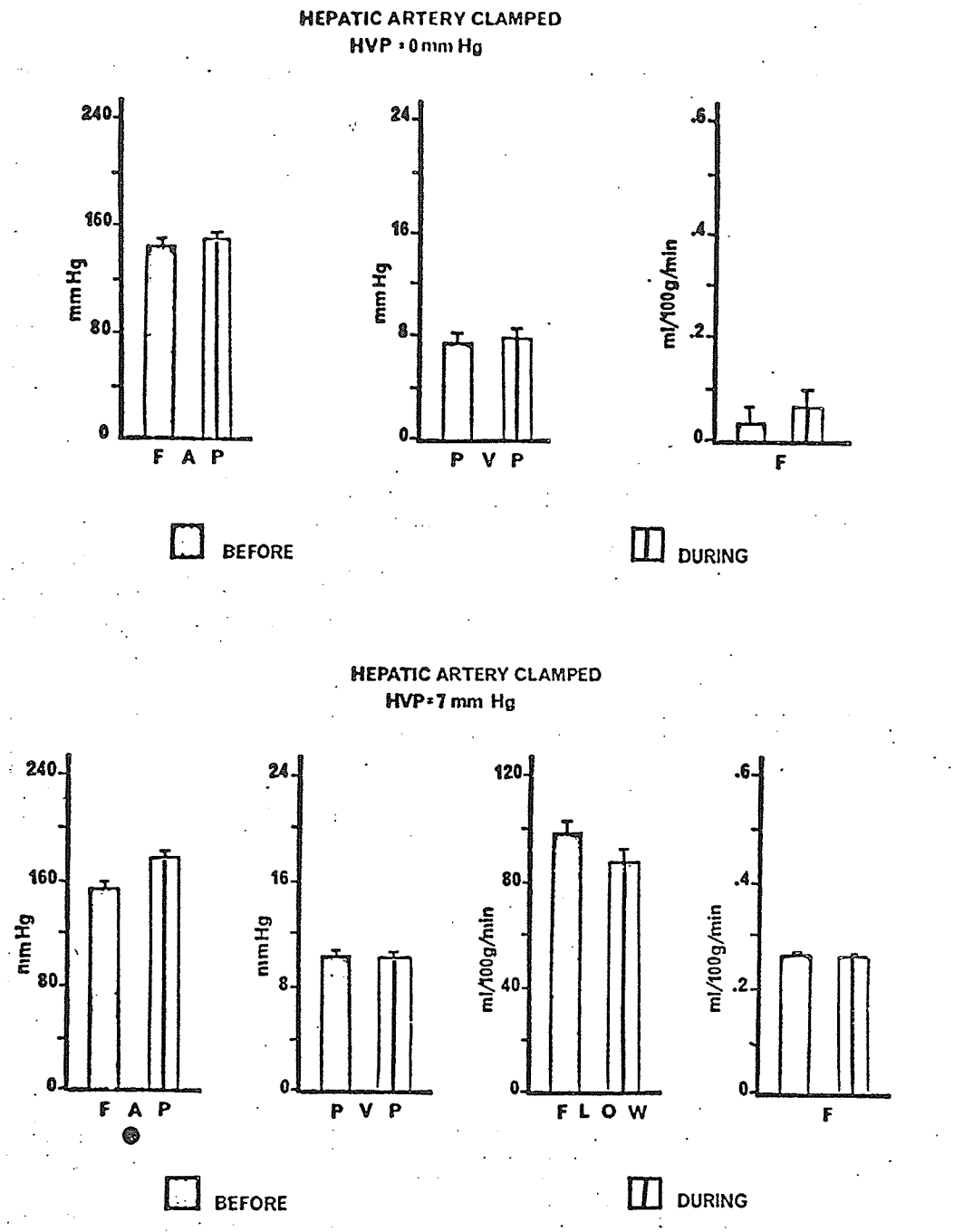


Figure 41. Effect of clamping the hepatic artery at zero and elevated hepatic venous pressure. The mean values (± S.E.) of femoral arterial pressure (FAP), portal venous pressure (PVP), total hepatic blood flow and rate of fluid filtration (F) are shown before and during infusion.
● = statistical difference ($p < .05$, paired-t test).

variables were altered by this procedure except arterial pressure which rose at the elevated venous pressure. (For the effects of hepatic arterial occlusion at zero venous pressure see Section II where n=6: only femoral arterial pressure was significantly altered). The experiments, in which the effect of arterial occlusion was evaluated at zero venous pressure, did not have the venous long-circuit established and therefore hepatic venous flow was not recorded. It is concluded that hepatic arterial occlusion does not induce net filtration at zero venous pressure and does not alter the rate of trans-sinusoidal fluid filtration induced by raised venous pressure.

DISCUSSION (SECTION IV)

Effect of Hemodynamic Alterations on Sinusoidal Pressure, Surface Area and Permeability

Sinusoidal pressure is determined by the pre- to postcapillary resistance ratio. If, for instance, the precapillary resistance decreases relative to postcapillary resistance then the pressure drop across the precapillary site will be reduced, resulting in an elevation in capillary pressure and filtration of fluid out of the vascular compartment. A number of stimuli that were reported to alter capillary pressure in the intestinal or skeletal muscle vascular beds (see Introduction) have been examined in the liver.

The dose of adrenaline and isoproterenol infused into the hepatic vascular bed in these fluid exchange studies was sufficient to cause marked elevations in total hepatic blood flow. Intravenous administration of adrenaline caused intestinal vasodilation and a large increase in portal venous flow while intra-arterial infusions of isoproterenol and histamine were made in doses that increase hepatic arterial flow (Greenway et al., 1967b; Greenway & Lawson, 1969). A final method of altering the pre- to postcapillary resistance ratio was attempted by occluding the hepatic artery. Reducing the arterial pressure had been demonstrated to cause precapillary dilation in skeletal muscle (Cobbold et al., 1963). The fact that all these maneuvers failed to produce any change in fluid exchange at zero venous pressure suggests that sinusoidal pressure had not been altered. The same procedures were repeated in a preparation in which the hepatic venous pressure had been raised to 7 mm Hg. In this preparation an alteration in the steady-state

filtration could occur as a result of an altered pre/postcapillary resistance, a change in the sinusoidal surface area or a change in sinusoidal permeability.

Changing the sinusoidal pressure from 7 to 8.5 mm Hg results in an increase in mean filtration rate from 0.33 to 0.40 ml/min/100 g. Such a change is readily detectable by the present techniques (Figure 36). If the surface area of the exchange vessels were effectively increased by 20% this would also result in a change in filtration rate from 0.33 to 0.40 ml/min/100 g. This indicates that the method would readily detect a change in sinusoidal pressure of 1.5 mm Hg or a change in sinusoidal surface area of 20%. Even smaller changes than this would be expected to show statistical differences. Thus the data suggest that isoproterenol, adrenaline, histamine and hepatic arterial occlusion do not alter sinusoidal pressure by more than 1.5 mm Hg, do not change sinusoidal surface area by more than 20% and do not alter sinusoidal permeability.

Physiological Implications of the Results

Postsinusoidal Resistance

The data cited here offer supportive evidence for the conclusions that postsinusoidal resistance in the hepatic vascular bed is very low. In a vascular bed such as that in skeletal muscle where the pre- to postcapillary resistance ratio is 4:1, a small vasodilation would reduce this ratio to 3:1. Assuming constant arterial and venous pressures of 100 and 0 mm Hg respectively, the first situation results in a capillary pressure of 20 mm Hg while the second situation results

in a capillary pressure of 25 mm Hg. Maximal vasodilation in this bed results in larger increments in capillary pressure and will result in considerable filtration of fluids (Figure 5, Kjellmer, et al., 1965). In the case of the hepatic vascular bed the pre- to postsinusoidal resistance ratio, as calculated from data obtained by Nakata et al., (1960) in an isolated rat liver, is estimated to be in the range of 49:1. Using the same pressures as in the example above, the sinusoidal pressure in this system is 2 mm Hg. The maximum vasodilation known to occur in the liver (Greenway & Lawson, 1969) would reduce the ratio to 30:1 at the most, assuming all of the reduced resistance occurred at the precapillary sites. The increase in sinusoidal pressure as a result of this altered ratio is less than 1.5 mm Hg. Thus the maximum possible effect on sinusoidal pressure as a result of vasodilation would be minimal. The existence of a very low postsinusoidal resistance is compatible with the present observations that an increase in portal flow (adrenaline i.v.) and maximal vasodilation of the hepatic arterial bed (isoproterenol) do not alter sinusoidal pressure. Contraction of the capacitance vessels also occurs with no significant increase in sinusoidal pressure. If postsinusoidal resistance is very low then one would also expect that a reduction in arterial pressure would not alter the steady state filtration rate, a hypothesis which was confirmed by occlusion of the hepatic artery.

Presinusoidal Sphincters

Occlusion of the hepatic artery at an elevated venous pressure might also have been expected to result in myogenic sphincter dilation and thus increase surface area as seen in skeletal muscle (Cobbold et al.,

1963). No such change occurred. Infusion of isoproterenol which increased capillary surface area in skeletal muscle and intestine (Figure 6, Folkow et al., 1963) had no effect on fluid exchange in the liver. In other vascular beds the precapillary sphincters serve to distribute the available blood flow. At a given time some capillaries are excluded from the circulation while others receive nutritive flood flow. In skeletal muscle, only about 1/3 of the capillaries have blood flowing through them at one time and this proportion increases during increased activity. The liver is never in a condition that may be regarded as a resting state and it is possible that the sinusoids are never completely excluded from the circulation. In fact there may be no presinusoidal sphincters in the liver. This conclusion is supported by histological data which demonstrate a lack of sphincter-like smooth muscle in the liver (Elias & Sherrick, 1969).

Sinusoidal Permeability

The hepatic sinusoidal wall is permeable to substances of high molecular weight (Mayerson et al., 1960). This is confirmed by the high specific gravity and hence protein content (Van Slyke et al., 1950) of the filtered fluid in these experiments (Section III). The sinusoids are lined by discontinuous endothelium (Elias & Sherrick, 1969) and it is not surprising that histamine does not increase permeability and fluid filtration as it does in skeletal muscle capillaries which are lined by continuous endothelium. It therefore appears that the colloid osmotic pressure across the sinusoidal wall is near zero and plays no significant role in hepatic trans-sinusoidal fluid exchange.

SUMMARY (SECTION IV)

1. Sinusoidal hydrostatic pressure is unaltered by intravenous infusions of adrenaline, intra-arterial infusions of adrenaline, isoproterenol and histamine or by hepatic arterial occlusion.

2. Post-sinusoidal vascular resistance is very low and sinusoidal pressure is therefore nearly the same as hepatic venous pressure. The only hemodynamic maneuver that appears to alter sinusoidal pressure significantly is elevation of hepatic venous pressure. Such venous pressure increments are quantitatively similar in the sinusoids.

3. Sinusoidal surface area was unaffected by intravenous infusions of adrenaline, intra-arterial infusions of adrenaline, isoproterenol and histamine or by hepatic arterial occlusion. It is suggested that the liver does not possess presinusoidal sphincters capable of excluding any region of the vascular bed from nutritive blood flow.

4. Colloid osmotic pressure is unlikely to play any role in fluid exchange regulation in the liver. Since the filtrate had 90% of the protein content of plasma, any osmotic pressure gradient across the sinusoidal wall would be very small.

5. The hepatic sinusoids appear maximally permeable to plasma proteins, and histamine causes no further increase in vascular permeability.

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