INTRAHEPATIC DISTRIBUTION OF BLOOD FLOW AND BLOOD VOLUME STUDIES IN THE LIVER

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PAGE

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Hepatic arterial blood flow was investigated in cats and dogs using the electromagnetic flowmeter technique. Stimulation of the hepatic nerves caused an immediate decrease in arterial flow in both species, however in the cat flow returned toward pre-stimulation levels even though nerve stimulation was maintained. This phenomenon is termed autoregulatory escape and it was not encountered in the canine liver where the decrease in flow was well maintained throughout the period of nerve stimulation.

In order to study the vascular events occurring during autoregulatory escape, the microsphere technique was chosen. Experiments were done to validate the technique as a measure of distribution of arterial and portal flows in the liver. One of the explanations put forward to explain autoregulatory escape in the intestine was a redistribution of flow away from the mucosa toward the submucosa. Although this explanation was not subsequently confirmed for the intestine, it still seemed possible that such a mechanism could be operative in the liver of the cat. To see if there was an intrahepatic redistribution of arterial and portal flows during autoregulatory escape, microspheres were injected into the hepatic artery and portal vein. There was no gross redistribution of intrahepatic arterial or portal flows during nerve stimulation in dogs and the results did not reveal any redistribution of intrahepatic flows during autoregulatory escape in cats. This phenomenon can best be explained as a relaxation of those vessels which were initially constricted by

nerve stimulation. Other theories on the mechanism of escape are discussed.

To study canine hepatic volume responses to nerve stimulation, the plethysmographic technique used in cats was modified for use in dogs. Maximal nerve stimulation was capable of expelling 36% of the blood volume of the liver, thus the liver appears to be an important reservoir of blood. The ability of the liver to act as a blood reservoir is compared with other organs of the body in cats and dogs.

The plethysmographic technique was also used to quantitate the increase in canine liver volume during outflow block caused by intra-arterial or intraportal infusions of histamine. Severe outflow block can result in an increase of liver volume by up to 50% and this is accompanied by a marked hypotension. To see what role hepatic pooling of blood played in the hypotension produced by intravenous histamine infusions, the increase in hepatic volume was prevented by nerve stimulation or phenoxybenzamine. The hypotension due to histamine infusion was not significantly different whether hepatic volume increased or not. Therefore it was concluded that hepatic pooling was not the cause of hypotension produced by intravenous infusions of histamine.

To disprove the theory that contraction of a sphincter at the junction of the hepatic vein and the inferior vena cava was responsible for outflow block, hepatic venous pressure measurements were made at different distances into an hepatic vein. When the tip

of the catheter for measuring pressure was just beyond this junction there was no significant increase in hepatic venous pressure during outflow block. These data with that in the literature suggest that outflow block is caused by contraction of sublobular veins and not a sphincter at the junction of the hepatic vein and inferior vena cava.

Further experiments showed that histamine caused a small redistribution of intrahepatic portal flow from the periphery toward the hilum. This was consistent with changes in hepatic venous and portal venous pressures measured during intra-arterial or intraportal infusions of histamine. Hepatic arterial flow distribution was not altered by histamine.

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DEDICATED TO HELEN AND TAMIS

HISTORICAL SURVEY

"For the king of Babylon stood at the parting of the way,
at the head of the two ways, to use divination:
he made his arrows bright, he consulted with images,
he looked into the liver".

Ezekiel 21:21

Of all the organs in the body, the liver contains the most blood and since blood was synonymous with life, the liver it was felt was the seat of the soul. Thus in early biblical times the livers of sacrificial animals were scrutinized carefully for signs from the gods. For some of us, this fascination with the ways of the liver has not subsided even after centuries of exhaustive study, and at times it may still seem that we are looking for signs from the gods.

The liver is a unique organ in terms of its vascularity. It is the only organ in higher vertebrates which has a high pressure arterial input and a low pressure venous input. Its position in the circulatory system, interposed between the gastrointestinal tract and spleen, and the heart has given it a major role in many body functions. Of these, two in particular are of importance to every other organ in the body. They are: 1) the regulation of energy metabolism; and 2) the regulation of plasma constituents (Shoemaker & Elwyn, 1969). More than anything else, it was the metabolism of the liver which first interested physiologists in this organ.

The liver is not the easiest of body organs to work with in situ, and consequently in 1855 Claude Bernard proposed that the study of isolated perfused livers might be a good means of studying liver function.

a) ISOLATED PERFUSED LIVERS

Researchers have always been at the mercy of the techniques available to them at the time of their studies and so it was not until 1868 that a technique for perfusing a mammalian muscle was developed by Ludwig and Schmidt. Luchsinger (1875) followed this seven years later with the first successful perfusion of the isolated liver. He used this technique to study glycogen formation as Bernard had suggested.

It seems odd that cardiovascular physiologists did not take up this technique immediately for hemodynamic studies of the liver. At first glance this technique offers several advantages over liver studies in the whole animal. In the isolated perfused liver, greater control can be exerted over inflow pressures. Actual measurements of flows are easily made. A greater range of concentrations of perfusate constituents is possible. Finally there are no extrahepatic influences such as blood flow from other organs to contend with (Shoemaker & Elwyn, 1969).

Although most of the early metabolic studies were done on isolated perfused cat livers, the early vascular studies were done with canine livers and human cadaver livers. Herrick (1907), perfused human livers by the hepatic artery or the portal vein and found that perfusion via the hepatic artery resulted in outflow from both the portal vein and the hepatic vein. When perfusion was by the portal vein, outflow was from the hepatic veins only. He concluded that there was much freer communication between the hepatic artery and portal vein

than between hepatic artery and hepatic vein.

In 1915, Mautner and Pick perfused the isolated dog liver via the portal vein. They immediately encountered a circulatory problem to which they gave the name "lebersperre". A loose translation of this term is liver barrier. It seems that perfusion of the dog liver was followed by marked congestion of the liver and serious diminution of outflow from the hepatic veins. Today this phenomenon is commonly referred to as outflow block of the liver.

The outflow block that Mautner and Pick encountered probably discouraged many investigators from using the isolated dog liver for hemodynamic studies. Thus it was not until 1932 that Bauer, Dale, Poulsson and Richards set out to specifically study this problem. Their technique was much improved over that used by Mautner and Pick, but usually within the first 30 minutes the liver became congested and the outflow of blood was greatly diminished. Further studies led them to conclude that the hepatic veins contained sphincters at their junction with the inferior vena cava and their powerful constriction was sufficient to block the outflow of blood from the canine liver.

In addition to perfusing the livers of dogs, they also used the cat and the goat. They did not encounter outflow block with these animals, but they observed that under the conditions of their experiments with the artificially adjusted perfusion pressures, they could not deduce the relative contributions of arterial and portal inflows to the liver under natural conditions. They did however observe that if the portal flow was reduced the arterial flow increased.

Even with improved perfusion techniques, the isolated liver still posed considerable problems, and it was not uncommon for the liver cells to lose their viability after 1 to 3 hours of perfusion. Brauer, Pessotti and Pizzolato (1951) rectified this matter and used a perfusion technique which allowed them to maintain an isolated rat liver for many hours. In 1956, Brauer, Leong, McElroy and Holloway used this technique to study the hemodynamics of the isolated perfused rat liver. Flow-pressure diagrams were constructed from their data, and it was found that at low perfusion pressures the portal perfusion rate increased from zero at a rate increasing with pressure so that the flow-pressure diagram was convex to the pressure axis. This they interpreted as autoregulation of portal flow. More recently however, Hanson and Johnson (1966) have produced convincing evidence that the portal flow in dogs does not demonstrate autoregulation.

Successful perfusions have now been developed for isolated canine livers and the problem of outflow block studied (Andrews, 1953; Kestens, Farrelly and McDermott, 1961; see also section V of this thesis).

b) WHOLE ANIMAL STUDIES

Whereas cardiovascular studies of the isolated perfused liver were for many years not very productive in establishing normal hemodynamic patterns, studies in the whole animal were much more productive. No doubt disease states produced stimulation for study of the liver. Cirrhotic livers have probably been in evidence since the advent of alcoholic spirits.

In 1863, Betz tried to discern if the amount of blood entering the two channels to the liver depended on their relative pressures. He injected a "hardening fluid" into dogs' livers and then measured the relative sizes of the arteries and portal veins. By measuring corresponding arteries and veins he found their relative sizes to be about 1:5. He calculated that the relative volumes of flow between artery and portal vein was 2:4 when the two vessels were flowing at different times and 1:50 when they were flowing at the same time. This work has been criticized (Herrick, 1907) because the arterial pressure was extremely low, but it is interesting to note that today, it is generally accepted that the portal vein contributes about twice the flow of the hepatic artery (Greenway & Stark, 1971).

In a dissertation presented in Berlin in 1873, Gad concluded that the hepatic arterial circulation performed two functions. It brought oxygen to the liver and mechanically controlled the portal flow. Herrick (1907), quotes earlier hemodynamic studies in which von Basch estimated portal pressure to be between 7 and 16 mm Hg. in a dog with the splanchnic nerves cut. Heidenhein found the portal pressure to be 5.2 to 7.2 mm Hg. with the splanchnic nerves intact. Cybulski estimated the portal vein flow in a small dog at 2.4 to 2.7 cc. per second. No details were given as to how these studies were carried out but the values quoted are within the range of experimental values accepted today.

In 1910, Butron-Opitz began publication of the most comprehensive studies of liver blood flow done to that time. All of

the experiments done by Burton-Opitz were on dogs anesthetised with a chloroform-ether mixture. Blood flows were measured with the aid of a stromuhr which he developed in 1908. With this technique, Burton-Opitz found resting hepatic arterial blood flow to be 26 cc/min/100 g of liver at a systemic arterial pressure of 99 mm Hg. He also gave the average weight of the dogs used and the average weight of the livers. A simple calculation reveals that dogs have about 29 grams of liver per kilogram of body weight. He went further and showed that stimulation of the hepatic plexus of nerves caused a marked reduction in blood flow through the hepatic artery and an increased systemic blood pressure. These results he felt, clearly showed that the liver was innervated by vasomotor nerve fibers. Upon testing the effect of raised bile duct pressure on hepatic arterial flow Burton-Opitz discovered that even pressures of 150 mm Hg. in the bile duct had no effect on the arterial blood flow.

Since he had a technique for measuring blood flow changes, Burton-Opitz (1911a) decided to test the theory of reciprocity of flow between arterial and portal channels. He expected that on occlusion of the portal vein the hepatic arterial flow would increase. However, on testing this he found that the systemic blood pressure decreased dramatically on portal occlusion and the arterial flow decreased accordingly. He realized that engorgement of the vascular beds normally drained by the portal vein was responsible for the decrease in blood pressure and thereby the decrease in hepatic arterial flow. Fortunately, portal congestion was a much thought about problem,

especially in association with cirrhotic livers and in 1877, N.V. Eck had proposed a method of diverting the portal flow directly into the inferior vena cava. Burton-Opitz devised a technique for diverting portal flow from its normal route to the inferior vena cava and back again. Without the ensuing portal congestion, he found that diversion of the portal blood to the inferior vena cava did indeed result in an increase in arterial flow.

Having established the volume of hepatic arterial flow in the dog, Burton-Opitz (1911c) turned his attention to the volume of portal inflow to the liver. This time the stromuhr was connected to the portal vein as near to the liver as possible. The resting portal flow he found to be 59 cc/min/100 g of liver. On combining this value with his former arterial flow studies, he gave a value of 84 cc/min/100 g total flow to the liver of the dog.

Whereas hepatic nerve stimulation caused a considerable decrease in arterial flow, nerve stimulation was without effect on the volume of portal flow. This led him to surmise correctly that flow of portal blood was more properly controlled by the organs giving rise to this blood than by the liver.

All the work previous to this time utilized the splanchnic nerves for stimulation when studying liver hemodynamics. When Burton-Opitz (1912a) tested the effects of splanchnic nerve stimulation on arterial and portal flows, he found that arterial flow increased and portal flow, after a transient increase, decreased. In light of today's knowledge, the transient increase in portal flow to splanchnic nerve

stimulation can be explained on the basis that constriction of the portal capacitance bed and spleen expelled blood into the portal vein. The following decrease in flow would result from the increased arterial resistance to flow offered by the portal organs. The increase in arterial flow was unexpected and harder to explain; however he noticed that the increase in flow always accompanied the increase in systemic pressure due to splanchnic nerve stimulation and he felt that rather than ascribing the increase in flow to vasodilatation of the hepatic arterioles, it was the increase in systemic pressure which caused the increased arterial flow. From more recent work, (Green, Hall, Sexton and Deal, 1959) we know that splanchnic nerve stimulation does in fact reduce arterial flow.

In his comprehensive study of liver circulation, Burton-Opitz (1911b; 1912c; 1912d; 1912e; 1913) included several other studies. He found that stimulation of a single nerve bundle from the hepatic plexus was capable of reducing arterial blood flow and speculated that the reduction in flow would be very specific to small areas of the liver. Afferent impulses from the hepatic plexus he noticed, resulted in an increased arterial flow which he thought was secondary to the increased systemic pressure caused by afferent stimulation. Adrenaline injections into the hepatic artery greatly reduced arterial flow, while on the other hand, adrenaline had little effect on portal blood flow when injected into the portal vein but did increase portal pressure markedly. Although portal flow was inconsistently affected by adrenaline, he felt that the increased portal pressure resulted from constriction of the

portal radicles and this he concluded was evidence for vasomotor innervation of these radicles.

In summary, Burton-Opitz produced values for the resting hepatic arterial and portal flows in the dog. The hepatic artery contributed about 25 to 30% of the total hepatic blood flow. Stimulation of the hepatic nerve plexus was capable of reducing arterial flow but not portal flow. Decrease in portal flow resulted in increased arterial flow. Stimulation of the splanchnic nerves decreased portal flow and increased arterial flow and he felt that the increased arterial flow was secondary to the rise in systemic pressure. Finally he observed that adrenaline was capable of reducing arterial flow to the liver but not portal flow. It is difficult to escape the conclusion that these studies were a massive contribution to the knowledge of the circulation of the liver.

TOTAL LIVER BLOOD FLOW MEASUREMENTS USING "FICK'S PRINCIPLE"

The surgical trauma associated with laparotomy and handling of the internal organs made techniques like those of Burton-Opitz unsuitable for measurement of hepatic blood flow in man. Even in animals no one was sure that the techniques used did not in some way alter the blood flow to the liver. Yet, changes in hepatic function due to hemodynamic changes cannot be differentiated from changes in cellular activity unless the state of perfusion is known. With this in mind, Bradley and his colleagues (1945) devised a method for measuring hepatic blood flow based on the "Fick Principle". This principle may be applied to any organ if three facts are known:

1) the concentration of a substance X in the blood entering the organ, 2) the concentration of X in the mixed venous blood leaving the organ, and 3) the total amount of X removed from or added to the blood each minute by the organ. Factors 1) and 2) constitute the arterial to venous gradient more commonly known as the A-V difference. The total blood flow to the organ can then be calculated by dividing the total removal rate of X by the amount of X removed from each ml of blood as it traverses the organ.

$$F = \frac{X}{(A-V)}$$

where X is the removal rate and (A-V) is the AV difference

The substance used for determination of this flow was Sulfobromophthalein, commonly known as BSP, whose removal from the blood depends to a great degree on the liver. Since the advent of the BSP method many studies have been completed on total liver blood flows (Greenway & Stark, 1971) and the mean value ranged from 100 to 130 ml/min./100 g of liver.

At first glance the BSP method of measuring total blood flow seems to be ideal, but it does have several disadvantages. The technique requires catheterization of one of the hepatic veins, a peripheral vein and an artery. The sampling procedures have to be simultaneous, rapid and accurate. As Grayson and Mendel (1965) have pointed out, catheterization of the hepatic vein requires considerable skill and the sampling, perfusion, and dye detection techniques require expensive equipment and the cooperation of a highly trained team of workers. Further studies brought to light other disadvantages

of the BSP clearance method. Brauer and Prescot (1950), found that there were extrahepatic mechanisms for removal of BSP from blood and that these mechanisms could account for 11 to 23% of the total dye extraction. Selkurt (1956) estimated extrahepatic uptake to be about 19% and felt that blood flow estimated with BSP would have an error of comparable magnitude. On the other hand, Casselman and Rappaport (1954) felt that extrahepatic uptake of BSP was negligible. Using the arterial concentration of the dye as 100%, they found hepatic venous concentration to be 61%, portal venous 98%, femoral venous 98%, renal venous 97% and jugular venous concentration 100%. Although these figures seem to bear them out, it must be remembered that they are purely percentages and tell very little about the extraction rates. Finally, BSP clearance can only be employed under steady state conditions and gives total liver blood flow which indicates nothing of the contribution of the arterial and portal channels. Nevertheless, keeping these disadvantages in mind, the BSP clearance method has been valuable in clinically assessing the hemodynamic state of the liver in man.

ELECTROMAGNETIC FLOWMETER TECHNIQUES

In 1936 Kolin introduced the electromagnetic flowmeter which is based on the principle that a voltage can be induced in a conductor moving at right angles to the lines of force of a magnetic field. The magnitude of the voltage induced is directly and linearly related to the velocity of the conductor. This induced voltage can be detected, amplified and recorded with the use of a galvanometer. One

great advantage of this technique is that the blood vessel walls have a sufficient electrical conductivity to permit the electrodes of the flow probe to be placed on the outer surface of the vessel to measure flow. With the introduction and subsequent refinement of this instrument another method became available for measuring blood flow to the liver.

The flowmeter probes are of two types; the cannulating and the non-cannulating type. The cannulating probe requires surgical intervention of the flow for a small period of time to allow placement in the vessel path. This technique can be employed in one of several ways. In the arterial long circuit for example, the femoral artery is long-circuited to the hepatic artery with the flow probe in between. Arterial long circuits are usually unsatisfactory because myogenic tone and neurogenic responses are reduced or abolished due to the appearance of vasoactive substances in the blood (Folkow, 1953; Dresel & Wallentin, 1966; Greenway, Lawson & Mellander, 1967). A more satisfactory technique places the long circuit on the venous side and the blood passes through the lungs before it reaches other peripheral vascular beds. The vasoactive substances are usually removed during passage of blood through the lungs. The non-cannulating flow probe, like Kolin's original model is placed around the blood vessel with a minimum of trauma to the vessel. In some cases the flow probes can readily be calibrated and this lends to their facility of use.

Green et al (1959) were among the first to use the electromagnetic flowmeter for measurement of hepatic blood flow. They studied

the effects of several autonomic drugs on the arterial and portal flows of the dog. The portal flow was measured with a non-cannulating flow probe while the arterial flow was long-circuited from the femoral artery through a cannulation-type flow probe and then to the hepatic artery. In their experiments, they found that portal flow contributed about 80% of the total hepatic flow and arterial peripheral resistance to flow was approximately 40 times the portal resistance.

Injections of adrenaline and noradrenaline into the hepatic artery they observed, always decreased flow and increased resistance in the arterial bed while portal flow sometimes increased and portal pressure always increased. Injections of the above drugs into the portal vein again resulted in reduced flow and increased portal resistance while the arterial bed was affected in the same manner as before but not to the same degree. Adrenergic blocking drugs abolished the constrictor actions of adrenaline and noradrenaline but did not unmask any dilator responses to splanchnic nerve stimulation. These results were not confirmed subsequently.

Since this work, many studies have been carried out on dogs using the electromagnetic flowmeter to discover the effects of a variety of vasoactive substances and various other stimuli, such as hemorrhage on liver blood flow (Greenway & Stark, 1971; Shoemaker & McElwyn, 1969).

In 1967 Greenway, Lawson and Mellander, carried out a comparable study in the cat. In contrast to Green et al, they used a non-cannulation type flow probe for the hepatic artery and long-

circuited the portal flow through a cannulating flow probe. This avoided the problems due to arterial long circuits and due to the lack of rigidity of the venous wall.

These workers reported a wide range of both arterial and portal control flows. The mean flow for the hepatic artery was 71 ml/min./100 g and 80 ml/min./100 g for the portal flow. The arterial flow is greater than that normally reported, but the spleen had been removed and this procedure could account for the abnormally high arterial flow.

Stimulation of the hepatic nerves at frequencies ranging from 1 to 15 Hz. always caused an abrupt reduction in flow which reached a maximum in 30 seconds. With continued stimulation however, the flow began to recover toward the control flow level. This was similar to the response to constrictor fiber stimulation of the small intestine (Folkow, Lewis, Lundgren, Mellander and Wallentin 1964 a, b) which had been termed autoregulatory escape. Portal flow on the other hand, was little affected by hepatic nerve stimulation but portal pressure was elevated during the period of stimulation.

For the first time a frequency-resistance pattern was established for the hepatic artery and portal vein. Greenway et al found that during the first 30 seconds of stimulation the resistance increased with increasing frequency of stimulation and was maximum at 6 to 10 Hz. for both arterial and portal vascular beds. Control resistance in the hepatic arterial bed was found to be about 30 times that in the portal bed.

In experiments where arterial flow was kept constant during nerve stimulation, the resistance still reached a maximum in 30 seconds and then began to decrease. When pressure in the hepatic artery was kept constant during stimulation the resistance was maximum at 30 seconds and again decreased with this. This showed that the arterial vessels escaped from constrictor fiber stimulation during either constant flow or constant pressure perfusion.

On construction of pressure-flow curves for the arterial bed, they found, as had Torrance (1961) that autoregulation occurred. Autoregulation of portal flow was not tested.

Like Green et al (1959), Greenway Lawson and Mellander found that noradrenaline given into the hepatic artery reduced arterial flow but had little effect on portal flow. However, in contrast to the studies of Green and his colleagues, they found that the arterioles escaped from the constrictor effects of small doses of noradrenaline given intra-arterially.

Finally, it was confirmed that the liver of the cat could participate in baroreceptor reflexes, for when the carotid arteries were occluded the resistance to arterial flow increased and this increase could be eliminated by section of the hepatic nerves.

In summary, the liver has an arterial input and a venous input. The resting portal pressure ranges between 7 and 12 mm Hg. while the hepatic arterial pressure ranges between 100 and 130 mm Hg. The total hepatic blood flow is in the neighborhood of 100 to 130 ml/min./100 g of liver and of this total the hepatic artery contributes

between 25 and 30% of the flow. The arterial resistance is some 30 to 40 times the portal resistance. The body contains approximately 30 g of liver per kilogram of body weight. These were similar for cats, dogs and man.

The arterial vessels can participate in baroreceptor reflexes and the occurrence of autoregulation of arterial flow was established. Autoregulation of portal flow probably does not occur. Finally, autoregulatory escape to neurogenic vasoconstriction has been demonstrated in the cat but has not been studied in any other species.

INTRAHEPATIC DISTRIBUTION OF FLOW

The studies quoted previously have been concerned mainly with blood flow by either the portal or arterial channel or total blood flow to the liver. Many workers however, have been concerned with the distribution of blood once it enters the liver.

Hyrtl (1864) studied the intrahepatic distribution of flow in the arterial and portal circuits using "injection preparations" of livers of amphibians and reptiles. He concluded that the terminal branches of the artery did not form a capillary network of their own but emptied directly into the large capillary network of the portal veins. From this he suggested that the hepatic cells were bathed in a mixture of portal and arterial blood. With the use of a similar technique in frogs, Leonard (1887) disagreed with Hyrtl. She concluded that the hepatic artery supplied blood only to the supporting connective tissues of the liver and to the bile duct walls. Leonard's

studies however, were incompatible with the fact that if the portal vein gradually became occluded, the artery was capable of assuming the functions of the portal vein (Cohnheim & Litten, 1876). Cohnheim and Litten along with Tischner (1904) felt that part of the hepatic arterial blood in the liver was used to provide nourishment to the walls of the portal blood vessels and disturbances of the arterial blood supply led to thrombosis of the portal branches.

Intrahepatic distribution of portal blood flow was also studied from another angle. Interest evolved early as to whether the blood flowing in the portal vein was streamlined in nature. Sérégé (1901) found that when he injected india ink into the mesenteric veins, the particles inevitably ended up in the right lobes of the liver, whereas injections into the splenic vein always appeared in the left lobes. These results seemed to indicate that streamlining did occur in the portal vein.

Mall (1906) found that upon injecting defibrinated blood into the hepatic artery of an isolated dog liver, 75% emerged from the portal vein and only 25% from the hepatic veins. This led him to conclude that the hepatic artery communicated much more freely with the portal vein than with the hepatic vein. In this regard the work of Herrick has already been mentioned (page 2).

In the early 1920's workers began to visualize the blood flow in liver edges with microscopes and transillumination techniques. Wertheimer (1922) was the first to use this technique with frog livers but he was unable to maintain the circulation of the intact liver.

McQueen (1927) was the first to note that blood flowing in the sinusoids of the intact toad liver had a definite pulse and he attributed this to auricular systole. He thought that each auricular systole would momentarily reduce hepatic venous outflow and thus produce the observed pulse pattern.

Copher and Dick (1928) using transillumination techniques confirmed the observations of Sénégé (1901) that streamlining occurred in the portal vein. They detected three main streams in the portal vein and noted that individual lobes of the liver received blood from specific areas of abdominal viscera.

Loeffler (1936) felt that hepatic artery capillaries fed the portal vein radicles and bile ducts but never entered sinusoids directly. He concluded that the liver could survive on portal blood alone; the reason for liver necrosis upon hepatic artery ligation was thrombosis of the portal and hepatic veins due to lack of arterial blood to their walls.

As transillumination techniques became more refined, the reports of liver structure and blood flow became more detailed. Knisely (1939), reported the presence of sphincters at the exit of each sinusoid in the frog liver and these sphincters determined the amount of blood in each sinusoid. Using the technique of Knisely, Wakim and Mann (1942), noted that blood flow in the liver sinusoids was not constant but intermittent and the degree of activity was not the same in all sinusoids. They observed that in some sinusoids the flow volume was large with blood cells packed tightly together, while in other

sinusoids the flow could be quite rapid but the volume was small due to the narrow lumen as indicated by single file passage of blood cells. In other sinusoids still flow was very sluggish.

Their final conclusion was that the liver could function as a storage reservoir for blood because they noted that inactive sinusoids went through two phases. In the storage phase they were packed with motionless blood cells and in the non-storage phase the sinusoids contained few if any blood cells in their potential lumens.

The preparation of corrosion casts of the liver and the transillumination studies are more concerned with anatomical than with hemodynamic aspects of the liver circulation. It is therefore appropriate to refer to the work of Elias at this point for it is on the basis of these histological studies that the more dynamic studies of blood flow are now explained. Previous to the work of Elias (1949 a, b), the liver was thought to be composed of cords of hepatic cells. This structure was first proposed by Gerlach (1849) and subsequently modified by others (Braus, 1924; McIndoe, 1928; Stohr & Shultze, 1918). Elias demonstrated as had Hering (1866) almost 80 years previously that the liver was a continuous mass of cells tunnelled by a three-dimensional network of sinusoids and these sinusoids are separated from each other by walls of hepatic cells one cell thick. The structures giving rise to the sinusoids and the manner of branching of the intrahepatic vessels is conveniently summarized in Fig. 1 which is taken from Elias and Sherrick (1969).

Daniel and Pritchard (1951 a, b) used cine-radiography to

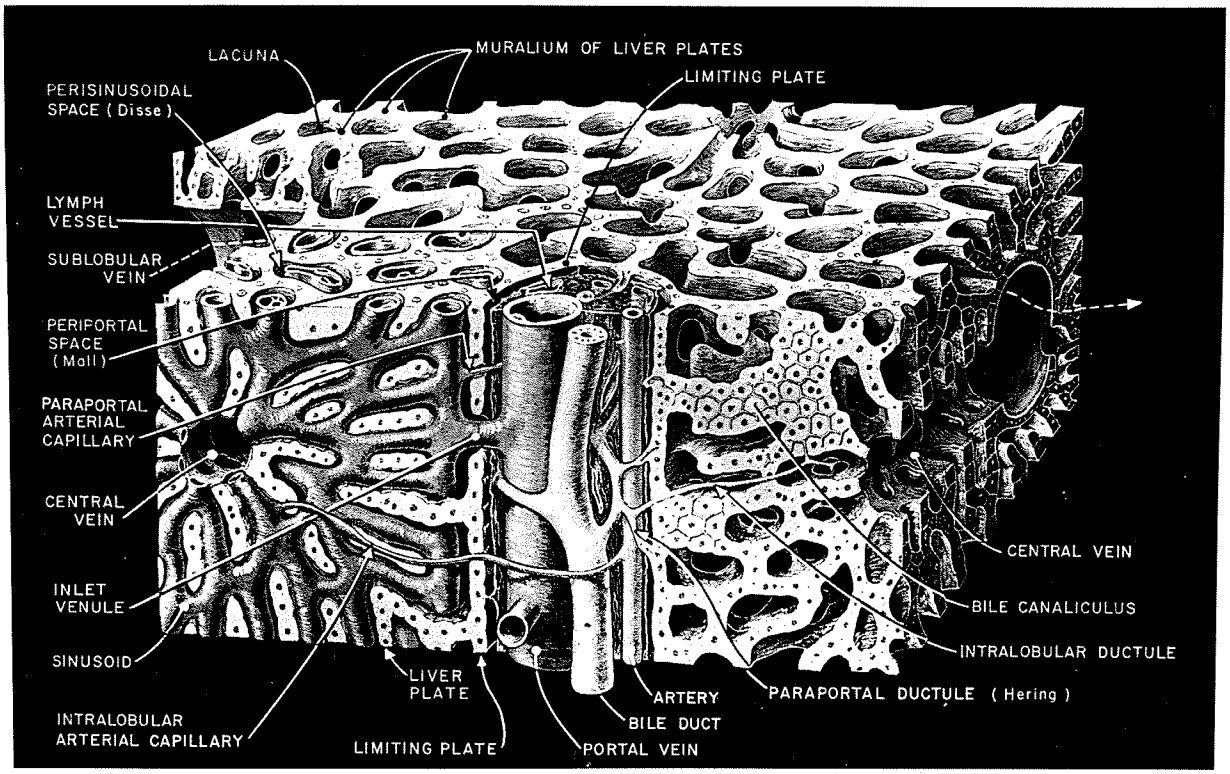

Figure 1

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

follow the flow of blood through the portal beds of several animals. They cannulated a small tributary of the portal vein and then following the injection of small volume of thorotrast, they took photographs every 0.5 seconds to follow the passage of this contrast medium through the portal bed of the liver. This technique also allowed an estimation of the transit time of thorotrast from the portal venous bed to the hepatic venous bed.

In rats and young cats in which nothing other than the operative procedure for cannulation and injection was carried out, they observed two types of portal perfusion. In one group, which they referred to as normal, the contrast medium seemed to perfuse the whole liver with the right lobes appearing to get a greater amount of the contrast medium then the left lobes. The second group they termed as one with a "restricted intrahepatic circulation". In this group the more peripheral segments of the portal venous branches did not seem to fill with thorotrast. The sinusoidal shadow was restricted to the area immediately around the shortened portal branches and the whole liver was never outlined as it was in the first group. In animals with this restricted intrahepatic circulation the transit time of thorotrast was significantly decreased.

In their second study (Daniel & Pritchard, 1951 b), they found that stimulation of the hepatic plexus reduced the caliber of the large and small intrahepatic portal vessels and sometimes caused a restricted intrahepatic circulation. Injections of adrenaline into the portal vein had much the same effect as nerve stimulation except

that the smaller intrahepatic portal vessels seemed to be affected more than the large ones.

On the basis of their studies, they concluded that the intrahepatic portal blood could be redistributed from a circuitous peripheral route to a more direct centrilobar path. However, they could not ascribe a mechanism for this redistribution.

Intrahepatic distribution of blood flow has also been measured with diffusible indicators. This technique assumes that the distribution of an indicator and its washout from the tissue is to a large degree flow limited. Hollenberg and Dougherty (1966) theorized that if portal and arterial channels meet in the sinusoids, then there should be no difference in the clearance rates of ^{85}Kr when given by either route. However, the portal route always cleared ^{85}Kr faster than the arterial route thus suggesting functionally separate pathways. To account for their observations they suggest that some sinusoids are perfused solely by one or the other channel but the great majority of sinusoids are perfused with a mixture of portal and arterial blood.

While my own work was in progress, Griffen, Levitt, Ellis and Lifson (1970) and Lifson, Levitt, Griffen and Ellis (1970) perfused dog livers with blood containing D_2O and/or T_2O as the diffusible indicators. After given amounts of perfusion time, they measured the isotope concentrations in different areas of the liver. These two papers are very difficult to read and understand, consequently the interpretation of their work is not clear; however, they seem to indicate that no area of the liver is consistently overperfused or underperfused.

This probably means that from animal to animal the same area of the liver did not receive the same amount of portal or arterial perfusion. Due to their methods of calculation of perfusion, if one area were underperfused it would mean that another area of the liver would have to be overperfused and thus they concluded that the liver was non-uniformly perfused.

Although there have been many studies on the intrahepatic distribution of blood flow, few of these studies have brought forth quantitative results. Preparation of corrosion casts no doubt gives some idea of the distribution of portal and arterial vessels but no one knows exactly how these "hardening fluids", affect the vessels and the conditions under which they were injected was far from physiological.

Transillumination studies of liver edges give quantitative results only on vessel size and the extreme edge of the liver may not necessarily behave in the same manner as the deeper parts of the liver.

Streamlining in the portal vein has been criticized by Groszmann, Kotelanski and Cohn (1971), because all demonstrations of streamlining have been done with the use of dyes which have a higher specific gravity then blood. Groszman et al injected radio-iodinated serum albumin into the splenic or superior mesenteric arteries of human volunteers. Hepatic venous blood samples from both the right and left lobes gave no indication of preferential streaming in the portal vein.

Even the work of Griffen et al (1970) and Lifson et al (1970),

which gave more quantitative data then any previous study used a very artificial perfusion system which gave total liver blood flows barely half that which is considered normal. The results they obtained in single input studies were consistent with a flow-limited model but the double input studies were grossly inconsistent with this type of model.

The anatomical studies by Elias and the studies on the intrahepatic distribution of blood flow have established that the sinusoids to a great degree are perfused by a mixture of portal and arterial blood. Streamlining of blood flow may be a feature of the portal vein at least in anesthetized animals. The sinusoids show intermittency of flow and a blood storage function is attributed to them by some. The portal blood may be redistributed from the periphery toward the hilum of the liver. Finally, intrahepatic distribution of arterial and portal blood appears to be non-uniform.

HEPATIC VOLUME STUDIES

In order to gain a more complete functional picture of a vascular bed, one must know something of its capacitance function along with its anatomy, blood flow and resistance to blood flow. The capacitance function can be studied by plethysmography or by observance of weight changes in the intact or isolated organ. The liver, unlike some skeletal muscles does not lend itself easily to measurement of volume changes but this has not prevented attempts to measure these changes.

Francois-Franck and Hallion (1896) were the first to attempt measurement of liver volume changes and from their work came the fact

that stimulation of the sympathetic nerves to the liver caused a decrease in volume. They were followed by Thompson (1899), who discovered that intravenous injections of peptones and proteoses caused marked engorgement of the canine liver. This was the initial observation of what is now termed outflow block.

Neubauer (1914) found that intravenous injections of adrenaline in rabbits caused decreases in liver volume as did stimulation of the central end of the vagus and the splanchnic nerves. Edmunds (1914) recorded an initial decrease and then an increase in the volume of the liver in anaphylaxis in dogs. Mautner and Pick (1915) showed that the volume of the canine liver increased in shock from peptone or histamine and the outflow from the hepatic veins decreased as the liver volume increased. The injection of glucose, levulose and maltose was followed by increases in the volume of the liver (Mautner, 1927).

In 1929, Mattson was able to record liver volume changes in the intact conscious dog and confirmed that intravenous adrenaline decreased liver volume while glucose and levulose increased it. Small doses of peptone he found decreased liver volume while large doses caused shock and increased liver volume.

In an extensive study of liver volume changes in the cat Griffith and Emery (1930) found that direct stimulation of the hepatic nerve plexus resulted in a profound decrease in liver volume. Stimulation of the splanchnic nerves also caused decreases in liver volume but stimulation of the peripheral end of the vagus was without effect

on liver volume. Upon examining the effects of hemorrhage they found it always to decrease liver volume.

Heymans, Bouckaert and Dautrebande (1931) and Gollwitzer-Meier and Schulte (1931) showed that the volume of the liver can be modified by reflexes originating in the carotid sinus. A rise in sinus perfusion pressure caused a decrease in liver volume and conversely a decrease in pressure was followed by a decrease in volume. They concluded that the liver was an important blood depot which could make blood available to the general circulation upon reflex activation. This work however, was not subsequently confirmed (Lautt & Greenway (1972).

Brauer, Holloway and Leong (1959) increased hepatic venous pressure in isolated rat livers and observed increased liver volumes with increased filtration of fluid from the surface of the liver.

In most of the studies mentioned above the authors did not produce any calibrations for the volume changes so the results are qualitative only. The use of isolated liver preparations has already been criticized (see page 12). In 1960, Mellander developed a method to study blood flow, blood pressures, capacitance changes and filtration simultaneously in cat skeletal muscle. This technique was modified by Greenway, Stark and Lautt (1969) for use with the cat liver. They examined the effects of hepatic nerve stimulation on liver volume in cats and found that maximal nerve stimulation could expel up to 50% of the blood volume of the liver. These nerves, however could not be reflexly activated by occlusion of the carotid arteries (Lautt &

Greenway, 1972).

In two further studies (Greenway & Lautt 1970) they confirmed that increased hepatic venous pressure increased hepatic volume and fluid filtration. They also demonstrated that noradrenaline and adrenaline were capable of decreasing hepatic blood volume by up to 40%. On the other hand, isoprenaline and histamine in doses which caused maximal vasodilatation produced no significant changes in hepatic volume in the cat. Angiotensin caused hepatic volume to decrease and on a molar basis was the most potent of these agents. Finally, they showed that vasopressin in doses which might be released from the posterior pituitary caused only slight decreases in hepatic blood volume.

In summary, sympathetic nerve stimulation, noradrenaline, adrenaline and angiotensin caused a decrease in liver volume. Histamine increased volume in the dog liver as did peptones, proteoses and anaphylaxis. Some sugars also increased liver volume but the mechanism of this is unknown. Maximal nerve stimulation could expel up to 50% of the hepatic blood volume in the cat.

STATEMENT OF THE PROBLEM

The results of the following thesis work are presented in five sections for ease and clarity of reading. Each section contains an introduction, methods, results and discussion of the results.

In section I an electromagnetic flowmeter technique was used to measure hepatic arterial flow in both the cat and dog to record the responses to hepatic nerve stimulation. This was done to confirm the

results of earlier workers in the cat and to investigate whether autoregulatory escape also occurred in the hepatic arterial bed of another species.

In order to study the vascular events occurring during autoregulatory escape in the liver, the microsphere technique as applied by Rudolf and Heymann (1967) was chosen. Before the microsphere technique could be justifiably used, its validity for measurement of intrahepatic distribution of arterial and portal flow had to be established (Section II). The technique was then used to study the effects of hepatic nerve stimulation on the intrahepatic distribution of flow (Section III).

To compare the volume responses of the canine liver to hepatic nerve stimulation with those already established for the cat, the plethysmographic technique was modified for use in the dog. The role of the liver as a blood reservoir was studied and evaluated (Section IV).

This technique was also used to determine changes in canine liver volume during outflow block produced by histamine and an attempt was made to settle the controversies over the cause of outflow and its role in the hypotension produced by i.v. histamine infusion (Section V).

GENERAL METHODS

GENERAL METHODS

All animals were without food but had water ad libitum 18-24 hours prior to the beginning of each experiment. Dogs and cats of either sex were anesthetized with sodium pentobarbital (Nembutal, Abbott, 30 mg/kg iv in dogs and ip in cats). A forelimb cutaneous vein was cannulated to give supplementary injections of the anesthetic agent (2 mg/kg) each time reflex corneal, limb and ear movements returned. In dogs the trachea was intubated with a cuffed endotracheal tube and in cats the trachea was cannulated to allow a clear airway. A femoral artery was cannulated to allow measurement of systemic arterial pressure. Pressure was recorded with a Statham Pressure Transducer (Model P23AC) connected to a Grass Polygraph (Model 5D).

The abdomen was opened by a midline incision and the skin edges stitched back over the exposed muscle edges. Portal pressure was measured from a small cannula inserted into the portal vein by way of an accessory splenic vein or the cecal vein. Pressure was recorded with a Statham Pressure Transducer (Model P23BC). All other cannulae used to measure pressure or to calibrate flow were as large as the vessels would conveniently allow.

MEASUREMENT OF HEPATIC ARTERIAL FLOW

In most cats the hepatic artery was too small to fit into the flow probe and consequently the coeliac artery was used to measure arterial flow to the liver. To accomplish this, all branches of the coeliac other than the hepatic branch were ligated and the spleen removed to make room for the flow probe. This same technique was used in dogs to keep the methods uniform between the two species.

The fascia and connective tissue overlying the coeliac artery were gently separated away from the vessel until a 1-1.5 cm length was cleared. A 2 or 3 mm non-cannulating flow probe (Nycotron) was then placed around the coeliac artery along with an arterial micrometer clamp. This clamp placed downstream from the flow probe served to hold the probe in place and allowed frequent checks of zero flow without disturbing the probe (see Fig. 2). The splenic artery was cannulated to allow measurement of pressure beyond the flow probe. Pressure measurements beyond the probe would indicate if the flow probe or clamp were in any way reducing the flow through the artery. The gastroduodenal artery was tied off so that the coeliac artery supplied blood only to the liver. A loose ligature was placed around the hepatic artery and threaded through a short piece of polyethylene tubing. This tubing was slid down the ligature to occlude flow in the artery so that a true hepatic artery zero flow could be obtained if any small coeliac branches were missed. The Nycotron flowmeter was connected to the Grass Polygraph to record flow changes. At the end of each experiment the hepatic artery was occluded and timed volumes of blood were collected from the splenic artery cannula. These volumes and times were correlated with the amount of pen deflection on the Grass and in this manner the flow probe was calibrated.

PREPARATION OF HEPATIC NERVES FOR STIMULATION

The hepatic nerve plexus originates from the coeliac ganglion and the nerves travel in the sheath surrounding the hepatic artery to the liver. Access to the nerves was gained by separating the mesentery over the lesser curvature of the stomach, transecting the hepatoduodenal

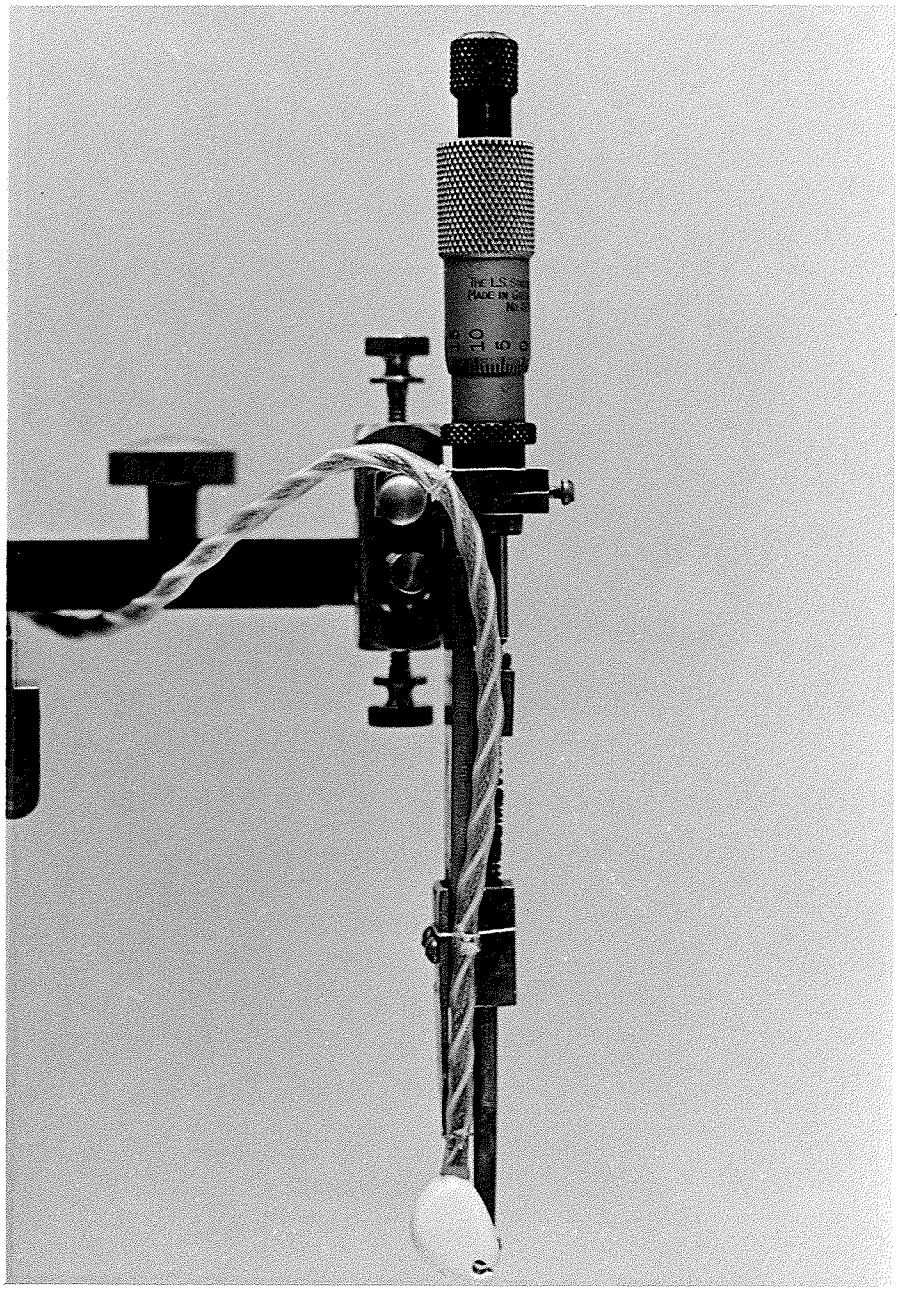

Figure 2

Flow probe and micrometer clamp.

ligament and gently pulling the stomach caudad. If this is done properly the artery will be seen to run parallel to the edge of the pancreas as it travels toward the liver. The nerves are teased away from the artery, transected and placed into a ring electrode for stimulation (Greenway et al., 1967).

PREPARATION AND INJECTION OF MICROSPHERES

Two sets of carbonized microspheres (15 ± 5 u diameter) were obtained (3M Company). One set was labelled with radioactive ^{141}Ce and the other with radioactive ^{51}Cr. These labels are incorporated directly into the microspheres and it has been shown that there is an insignificant amount of leaching of the label over a prolonged period of storage in saline or blood (Rudolf & Heymann, 1967; Ohlsson et al., 1969). Each mg of the 15 ± 5 u size microspheres contains approximately 440,000 spheres. The specific activity for both ^{141}Ce and ^{51}Cr was 10 mC/g. The microspheres were purchased suspended in 10% dextran.

Prior to the injection of microspheres, they were shaken vigorously on a vortex mixer for 5 minutes. 0.2 cc of ^{141}Ce or 0.4 cc of ^{51}Cr was drawn into a disposable plastic syringe and injected via the portal or arterial cannula. This was immediately followed by injection of 2 cc of saline to flush the microspheres through the cannula. The elapsed time between withdrawal from the bottle and injection never exceeded 15 seconds so sedementation did not occur.

When microspheres were to be injected into the portal vein, a small bore polyethylene cannula (PE 90) was used. This cannula had a sealed tip with small backward pointing holes for 3 mm from this tip.

This type of cannula prevented any streamlining of the microspheres in the portal vein. Microspheres were injected into the coeliac artery and thus into the hepatic arterial flow by the cannula in the splenic artery, used to measure pressure beyond the flow probe. In the majority of experiments ^{141}Ce microspheres were given during the control period and ^{51}Cr microspheres during the test period.

CALCULATION OF INTRAHEPATIC DISTRIBUTION OF FLOW

At the end of each experiment the arterial and portal vessels were tied off to prevent back flow of blood and the animal was killed. The liver was removed and weighed and the lungs were also removed and weighed. The gallbladder was separated from the liver, emptied of its contents and placed in a small plastic tube. The liver was next divided into its seven lobes which are designated; left lateral, left medial quadrate, right medial, right lateral, caudate and papillary process of the caudate lobe (Miller, Christensen and Evans, 1964). The last mentioned lobe is termed the papillary process throughout this thesis. Each lobe starting with the left lateral was placed on a paper towel soaked in saline and transverse slices 2 mm thick (1-4 g) were cut, beginning at the end furthest from the hilum. The tubes were weighed and counted in a 2 channel auto-gamma spectrometer (Packard Inst. Co.). The settings of the counter to cover the two peaks of radioactivity were determined from measurements of the energy spectra of the microspheres and in each experiment pure samples of each type of spheres were counted to determine the overlap of radioactivity between the two channels. The overlap of ^{141}Ce into the ^{51}Cr channel was $0.29 \pm 0.12\%$ (mean $\pm$ S.E.) and no correction was made for this. The overlap of

^{51}Cr into the ^{141}Ce channel was 23 $\pm$ 1.1% and this overlap was subtracted to obtain the true ^{141}Ce count. The total radioactivity due to each type of microsphere was obtained by summing the radioactivity in all the samples. Samples of lung tissue were also counted to estimate the extent to which microspheres passed through the liver and were trapped in the lungs.

The cpm/g for each type of microsphere for each tissue sample, for each lobe and for the liver as a whole were calculated and the cpm/g for each sample and lobe were expressed as a ratio of the cpm/g for the whole liver. The following is a sample calculation for Cr microsphere in one slice, one lobe and the whole liver:

One slice - cpm 4,800 weight 2.00 g	2400 cpm/g
One lobe - cpm 52,000 weight 20.0 g	2700 cpm/g
Whole liver - cpm 500,000 weight 200 gm	2500 cpm/g
Relative flow to slice 2400/2500 = 0.96	
Relative flow to lobe 2700/2500 = 1.08	

These values are designated as relative flows throughout the thesis. Thus a value of 0.5 or 2.0 designates an area of the liver which received half or double respectively, the mean flow through the liver. Using the relative flow data and the known position of each tissue slice in the liver, maps of the gross intrahepatic distribution of flow were constructed.

AUTORADIOGRAPHIC STUDIES

Since ^{141}Ce emits a beta particle upon radioactive disintegration and ^{51}Cr does not, only ^{141}Ce-labelled microspheres were used

for the autoradiographic studies. After injection of the microspheres into either the hepatic artery or portal vein, the inflow and outflow vessels of the liver were simultaneously tied and the animal was killed. The liver was removed from the animal and representative slices were cut from different areas of the liver. Each slice was laid on a glass microscope slide and wrapped in a thin sheet of plastic. The liver slice was abutted to the emulsion surface of a Type NTB 3 Nuclear Plate (Kodak Ltd.). To minimize shrinkage of the tissue the preparation was kept at -20^{o}C. After a 24 hour exposure period, the plates were developed for 10 minutes in Kodak D-19 developer fixed for 1 hour and washed for 3 hours in running water. The plates were examined after they had dried.

The methods are elaborated further in each section of the thesis.

SECTION I

EFFECTS OF HEPATIC NERVE STIMULATION ON HEPATIC ARTERIAL FLOW

INTRODUCTION: In a recent review by Greenway and Stark (1971), considerable emphasis was placed on the fact that autoregulatory escape from nerve stimulation was a mechanism for protecting the liver from hypoxia due to sustained vasoconstriction. This emphasis was based on data from the cat and it seemed important to investigate this problem in another species. For this the dog was chosen.

METHODS: The hepatic arterial flow, portal pressure and femoral arterial pressure were recorded in 10 cats and 10 dogs while the hepatic nerves were stimulated at frequencies in the range of 0.5-12 Hz.

RESULTS: Since some of the animals used were common to Sections I, II and III the control values for all of the animals used in the first three Sections are presented here. A total of 23 cats was used and they had a mean body weight of 2.6 $\pm$ 0.1 kg (mean $\pm$ S.E.) with 70.1 $\pm$ 2.9 g of liver or an average of 27 g of liver per kg of body weight. After completion of surgery and 20-30 minutes of equilibration time, the femoral arterial pressure was 122 $\pm$ 3.3 mm Hg while the portal pressure was 7.5 $\pm$ 0.3 mm Hg. In those experiments in which hepatic arterial flow was measured, it was found to be 63.0 $\pm$ 9.0 ml/min per 100 g of liver.

For 28 dogs the mean body weight was 7.2 $\pm$ 0.3 kg (mean $\pm$ S.E.) with a mean of 234 $\pm$ 11.7 g of liver. These data give an average of 33 g of liver per kg of body weight. After surgery and equilibration time the mean femoral arterial pressure was 118 $\pm$ 2.8 mm Hg, while the portal pressure was 8.6 $\pm$ 0.4 mm Hg. The liver received 57.0 $\pm$ 6.0 ml/min per 100 g of arterial blood.

The responses of the pressures and flows to a range of frequencies of nerve stimulation in one experiment in a cat and one in a dog are shown in Fig. 3. In both the dog and cat the responses of femoral arterial and portal pressure were very similar. Both are elevated and maintained throughout the duration of stimulation. On the other hand, the flow responses of the two species are very different. The dog showed a well maintained decrease in arterial flow at all frequencies tested while in the cat, the initial decrease in flow to nerve stimulation was followed by a recovery of flow toward the control level. This also occurred at every frequency tested and this recovery of flow has been termed autoregulatory escape (Folkow et al., 1964; Greenway et al., 1967). Fig. 4 shows the effect on hepatic arterial flow of a more prolonged period of stimulation at 4 Hz in the dog. Note that flow does not show any escape even after 10 minutes of stimulation.

The pressure and flow data from these experiments were used to calculate hepatic arterial conductance (flow divided by arterial minus hepatic venous pressure) and Fig. 5 is a plot of this conductance, expressed as a percentage of control against frequency of nerve stimulation. The 10 dogs all showed a decrease in conductance with nerve stimulation. This decrease in conductance was not significantly different after 3 minutes from that at 30 seconds after the onset of stimulation (paired t-test $P > 0.1$).

In the 10 cats, arterial conductance also decreased and at 30 seconds the decrease was not significantly different from that seen in dogs (unpaired t-test $P > 0.1$). However, at all frequencies tested, the arterial conductance significantly recovered during 3 minutes of

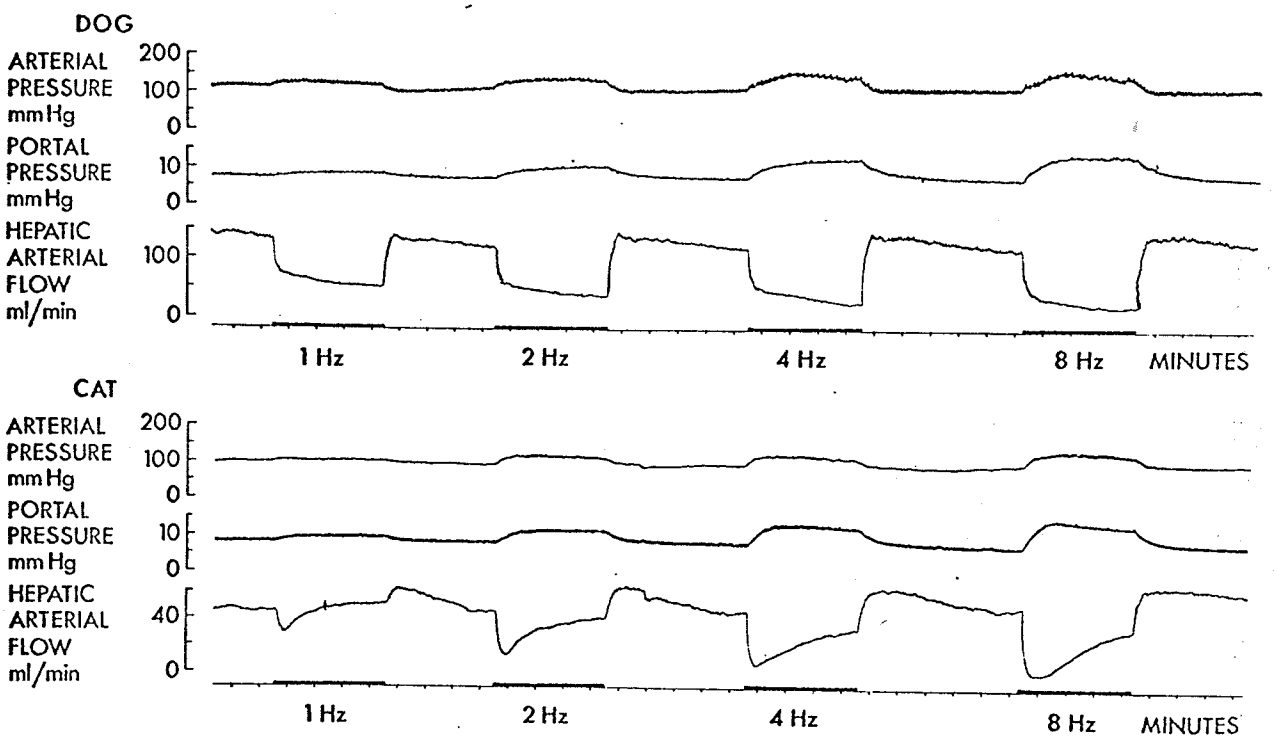

Figure 3

The arterial pressure, portal pressure and hepatic arterial flow responses to stimulation of the hepatic nerves in a cat and a dog. Note the occurrence of autoregulatory escape of the flow responses in the cat but not the dog.

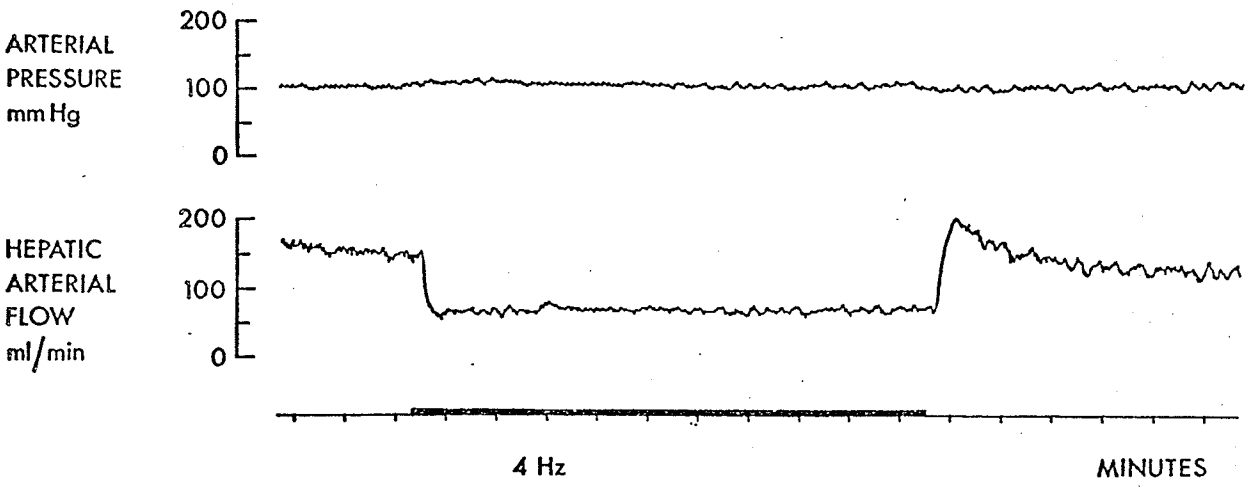

Figure 4

The response to a 10 min period of hepatic nerve stimulation at 4 Hz. in a dog. The decrease in hepatic arterial flow was well maintained for the period of stimulation.

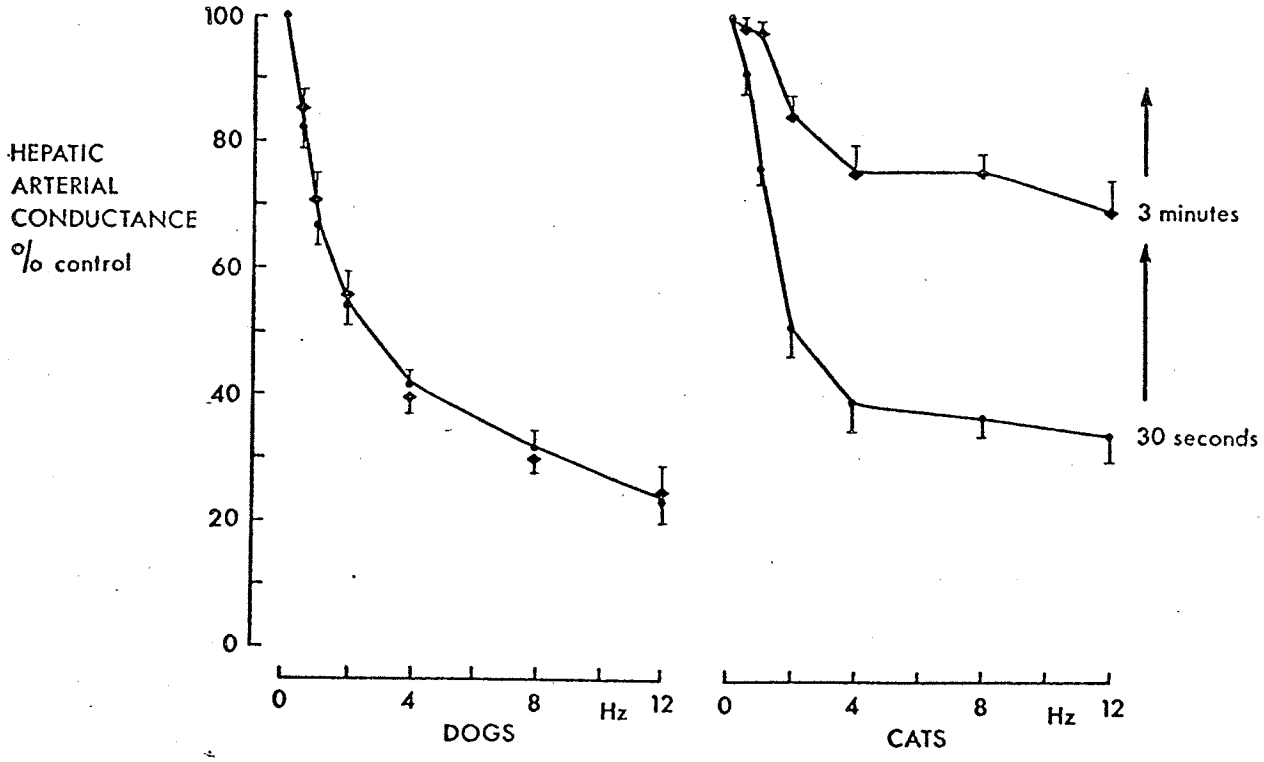

Figure 5

The mean (± S.E.) changes in the hepatic arterial conductance during hepatic nerve stimulation in 10 dogs and 10 cats. The circles show the response 30 seconds, and the diamonds the response 3 minutes after beginning stimulation. Note the occurrence of autoregulatory escape in the cats but not the dogs.

continued stimulation (paired t-test $P < 0.001$). If the period of stimulation was extended past 3 minutes the flow or conductance often tended to recover further. In all experiments, whether in cats or dogs, a small post-stimulatory hyperemia followed cessation of stimulation (Fig. 3 & 4).

The effects of hepatic nerve stimulation on portal pressure are plotted in Fig. 6. Changes in portal pressure are expressed as a percentage of control portal pressures. Analysis of the data by the unpaired t-test showed no significant differences to hepatic nerve stimulation between the cat and dog even though autoregulatory escape occurred in the cat but not in the dog. The portal pressure changes were maximum between 6 and 8 Hz.

DISCUSSION: In this comparison between the dog and cat with regard to nerve stimulation, the techniques were as similar as possible and the same equipment was used for both studies. In this manner variations due to major technical discrepancies were eliminated and yet autoregulatory escape occurred in cats but not in dogs. The escape in cats can in no way be accounted for by failure of the nerve after 3 minutes of stimulation because the portal pressure rise was as well maintained in cats as it was in dogs.

These results confirm the earlier observations in cats by Greenway et al (1967), but they do not support the conclusion that autoregulatory escape is a mechanism for protecting all livers from hypoxia. Protection from hypoxia is a result of escape in the cat but in the dog hypoxic areas appear on the surface of the liver on gross examination during hepatic nerve stimulation. This species difference

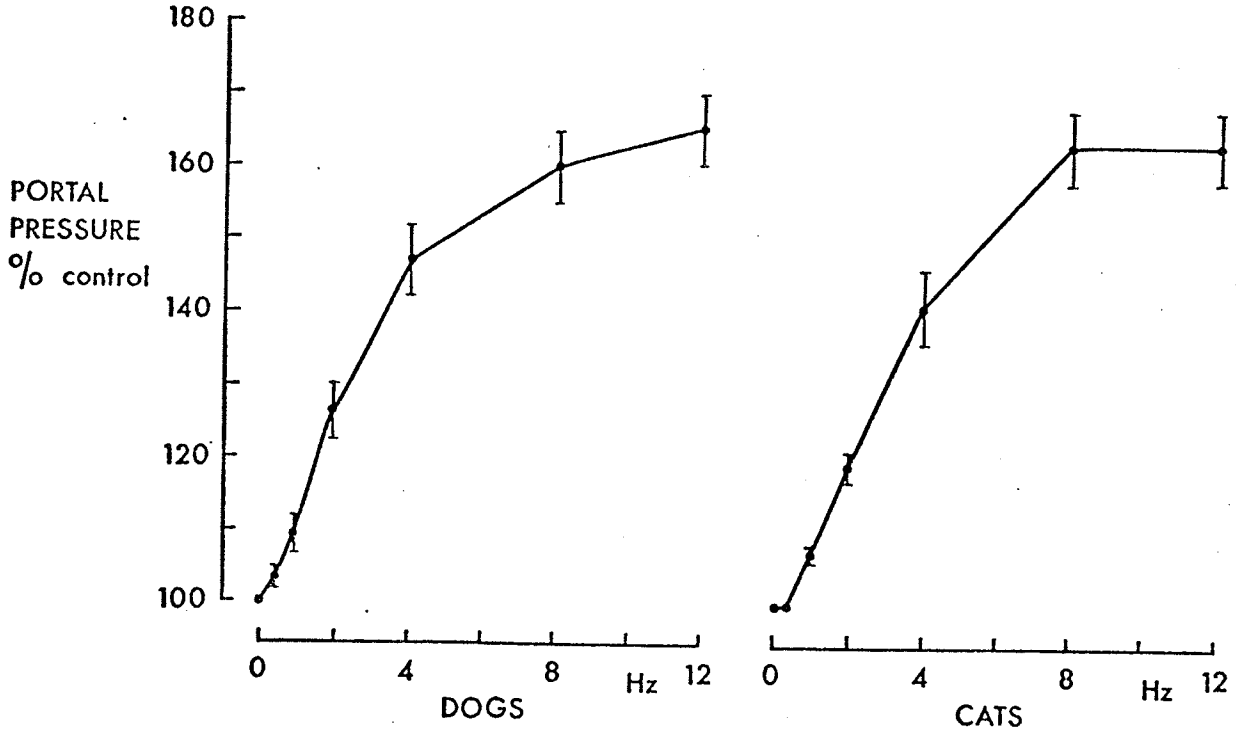

Figure 6

The mean (± S.E.) changes in portal pressure during hepatic nerve stimulation in 10 dogs and 10 cats.

in the occurrence of escape might explain the conflicting data between cats and dogs on the role of the sympathetic nervous system in the response to hemorrhage and endotoxin (Chien, 1967; Jacobson & Swan, 1966; Lillihei, Longerbeam, Block & Mannax, 1964; McNeill, Stark & Greenway, 1970; Nickerson, 1970b; Stark, McNeill & Greenway, 1971).

If autoregulatory escape occurred in one vascular bed or solely in one species, its importance in general circulatory physiology might be questionable. But in cats, escape during sympathetic nerve stimulation is marked in the intestine (Folkow et al., 1964; Dresel & Wallentin, 1966), in intestinal lymph glands (Lundgren & Wallentin, 1964), in the kidney (Johansson, Sparks & Biber, 1970) and in the liver. It is less marked in the spleen (Greenway, Lawson & Stark, 1968) and absent in skeletal muscle (Mellander, 1960) and in adipose tissue (Oberg & Rosell, 1967). In dogs, escape is absent in the liver during nerve stimulation and absent in the kidneys at low frequencies of stimulation (Disalvo & Fell, 1971), but it does occur in the intestinal bed (Oshiro & Greenway, unpublished observations). Others (Richardson & Johnson, 1969; Ross, 1971a, b) have shown that escape occurs in the canine intestinal bed during noradrenaline infusion. Since escape is not confined to one vascular bed or to one species, I decided to study the vascular changes which result in autoregulatory escape during nerve stimulation.

Folkow and co-workers (1964a, b) on the basis of india ink injections, proposed that blood flow was redistributed away from the mucosa toward the submucosa in the intestine during nerve stimulation. Although this theory was not subsequently confirmed (Ross, 1971a; Greenway & Murthy, 1972), it still seemed possible that such a mechanism

could be operative in the liver of the cat. If a redistribution of blood flow in the cat liver was responsible for autoregulatory escape, it could be studied with the aid of radioactive microspheres.

SUMMARY: The electromagnetic flowmeter technique was used to study the effects of hepatic nerve stimulation on hepatic arterial flow in cats and dogs. Both species demonstrated a frequency-dependent decrease in arterial flow during nerve stimulation. However, the hepatic arterial flow in the cat escaped from the effects of nerve stimulation while the decreased flow in dogs was well maintained. This escape of arterial flow in cats was observed at every frequency tested and it could not be explained by failure of the nerves since portal pressure increased and remained increased throughout the period of stimulation.

SECTION II

VALIDATION OF THE MICROSPHERE TECHNIQUE

INTRODUCTION As Wagner, Rhodes, Sasaki and Ryan (1969) have pointed out, there are at least three basic criteria that the microsphere method must satisfy in order for their use to be valid in the study of blood flow distribution. They are: 1) the microspheres must be uniformly mixed with blood and have essentially the same rheology as red blood cells; 2) the microspheres themselves do not alter blood flow; and 3) the microspheres become impacted in the microcirculation and in this manner are prevented from recirculating.

Several studies have been done to satisfy the first criteria. In 1967, Phibbs, Wyler and Neutze checked the mixing of microspheres with blood by rapid freezing of the artery into which the spheres had been injected. Upon mapping the location of spheres they found fewer spheres close to the artery wall, but they concluded that the flow of microspheres from a medium sized artery into a smaller artery was similar to the flow of blood. In a further study by Phibbs and Dong (1970), they showed that the smaller the spheres the more closely they approximate the distribution of erythrocytes.

Rudolf and Heymann (1967), found that 50 μ microspheres, when injected into fluid flowing in a physical model of branching tubes were distributed in direct proportion to the blood flow in each tube. When Ohlsson (1971), injected microspheres into the left atrium, the two kidneys received equal amounts of the spheres. Finally, Tow, Wagner, Lopez-Majano, Smith and Migita (1966), demonstrated good correlation between simultaneously injected labelled red cells and microspheres in

their distribution to the tissues. The above studies tend to confirm that the microspheres behave rheologically similar to red blood cells in the circulation.

Kaihara, Van Heerden, Migita and Wagner (1968), observed that the distribution of two sets of microspheres was similar when one set was given shortly after the first set. Greenway and Murthy (1972), gave one set of microspheres ten minutes before and also two hours before a second set of microspheres and found no significant differences in the distribution between the two sets of spheres in the splanchnic organs. Thus the first set of microspheres does not seem to alter the circulation in any discernable way.

If the microspheres are shunted from the inflow circulation to the outflow circulation, their use is no longer valid for a study of the inflow distribution. Kaihara et al (1968), showed that the lungs virtually trap all 15 $\pm$ 5 μ spheres so in the present study the existence of shunt vessels in the liver was studied by simply checking the amount of radioactivity in the lungs at the end of each experiment.

The study of Greenway and Murthy (1972) also points out the importance of microsphere size and the structure of the vascular bed in determining the validity of the use of microspheres. If the vascular sections are coupled in parallel, the distribution of flow can be studied with the use of microspheres. However, if the vascular sections are coupled in series, the distribution of microspheres to a great degree is determined by their size and is not a valid measure of flow. For example, portal flow cannot be measured by the intra-aortic administration of

microspheres. Under the present circumstances this problem did not arise since the microspheres were given into the inflowing arterial or portal flows.

The major problems encountered in this study were the expression of distribution of microspheres in an organ as large as the liver and proof of the complete mixing of the spheres with the inflowing blood. It had to be shown that counting the radioactivity in transverse slices of liver was a valid method of determining the actual distribution of microspheres. If the microspheres were found to concentrate in different areas of each slice then counting the radioactivity in transverse slices might be questionable. In order to satisfy this problem, autoradiography was performed on a number of liver slices. The adequacy of mixing was studied by simultaneous injections of two differently labelled sets of microspheres into the hepatic artery or the portal vein. The variation in relative flows to each area of the liver would then yield information about these mixing errors.

It is now well established that a decrease in total portal flow will result in an increase in total arterial flow to the liver. The manner in which the microspheres were used in this study could not detect an increase in total flow to the liver, however if the portal flow to a given area could be reduced then the change in arterial flow to that area could be measured. If the method could detect an altered arterial flow in this way it would add weight to the validity of the use of microspheres for this study.

Once it was established that the criteria for the use of microspheres in the present study had been satisfied, the control distribution of the hepatic artery and the portal vein were studied.

METHODS The methods for autoradiography have been discussed previously (page 34). To check the adequacy of mixing of the microspheres in the inflowing blood, simultaneous injections of the two types of microspheres were made into the portal vein (4 experiments) and into the hepatic artery (2 experiments). The simultaneous injections were accomplished by two separate cannulae placed into the portal vein or the coeliac artery. To study the control distribution of flows microspheres were injected into the portal vein and the hepatic artery at the same time.

RESULTS Autoradiography was carried out on 86 liver slices in 11 experiments, which included control, nerve stimulation, and histamine treatments. Although the autoradiography showed that some liver slices contained more microspheres than other slices, the microspheres in each case were uniformly distributed throughout the slice. There were no areas of gross concentration of microspheres in any slice. Thus the counting of radioactivity in transverse slices is justified.

The results of two simultaneous portal injections of microspheres in the cat is shown in Fig. 7. The relative flow values for alternate slices have been omitted for clarity. The variation between the relative flows determined from the two isotopes in each slice of liver is a measure of the error due to incomplete mixing of the spheres

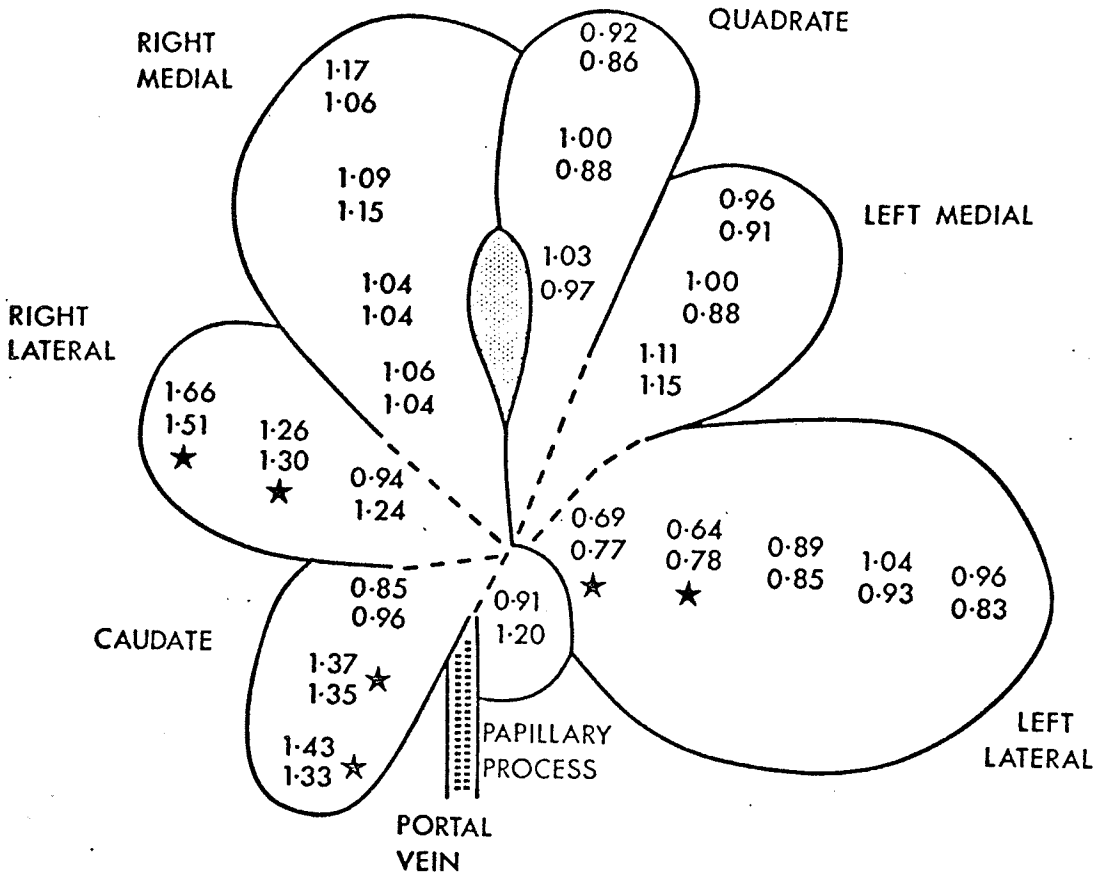

Figure 7

Map of gross intrahepatic distribution of portal flow during the control period in one cat. Each pair represent the relative flows in a tissue slice determined from the two types of microspheres given simultaneously through different cannulas in the portal vein. The values for alternate slices have been omitted for clarity. The relative flows in the areas marked with an asterisk are significantly different ($P < 0.01$) from the mean flow for the whole liver (1.0).

in the inflowing blood. The coefficient of variation (Steel & Torrie, 1960) as determined from the data in these six experiments was 15%. The 99% confidence interval was 0.82 to 1.18 and the areas marked with an asterisk received a significantly higher or lower flow/g than the liver as a whole.

The data from these experiments was also analysed by Tukey's procedure (Steel & Torrie, 1960) to determine the honestly significant difference. The areas with relative flows below or above the 99% confidence limits were also significant by this procedure ($P < 0.01$). In any one area from a cat or a dog, some areas received significantly low or high relative flows. The highest relative flow in this set of experiments was 3.80 and the lowest flow was 0.40, however large differences between adjacent slices were never seen.

In every experiment, two samples of lung tissue were counted to determine the amount of radioactivity which passed through the liver and became trapped in the lungs. The radioactivity in the lungs never exceeded 0.3% of the total injected and the mean value was $0.14 \pm 0.03\%$ (mean $\pm$ S.E.). The proportion of microspheres which passed through the liver to the lungs was negligible in every case.

In order to see if any microspheres passed through all capillary beds and continued to circulate, blood samples were taken from the inferior vena cava in 4 experiments 10 minutes after the injection of microspheres. No radioactivity was detected in these blood samples.

In 5 experiments in the dogs, the major branch of the portal

vein to the left lateral lobe was ligated. Then ^{141}Ce microspheres were given into the portal vein while ^{51}Cr spheres were given into the hepatic artery at the same time. Fig. 8 shows the mean results for 4 of the 5 experiments. Since the results show a marked reduction in relative portal flow at the free end of the lobe, it is clear that the ligated portal branch fed mainly the free end of the lobe. The flow to the hilar end was not significantly affected. In those areas of the lobe where the portal flow was much reduced, the arterial flow was significantly increased (unpaired t-test, $P < 0.01$). The relative portal and arterial flows in the other lobes was not significantly different from control animals. In the fifth dog the arterial flow did not increase in the free end of the lobe even though the portal flow was reduced.

In the 4 experiments which showed an increase in arterial flow, the relative arterial flows in all areas of the left lateral lobes were plotted against the relative portal flows in the same areas. A regression line was calculated (Steel & Torrie, 1960) and the regression intercept was 1.93. The slope was 1.05 and the correlation coefficient was 0.71 ($P < 0.001$). Thus at zero portal flow, the mean relative arterial flow was 1.93 in the left lateral lobe and this increase was highly significant.

In 8 experiments (4 in cats and 4 in dogs) ^{141}Ce microspheres were administered into the portal vein at the same time that ^{51}Cr microspheres were given into the hepatic artery. In these experiments there were 81 slices of liver where the relative portal flow (0.42 ± 0.08

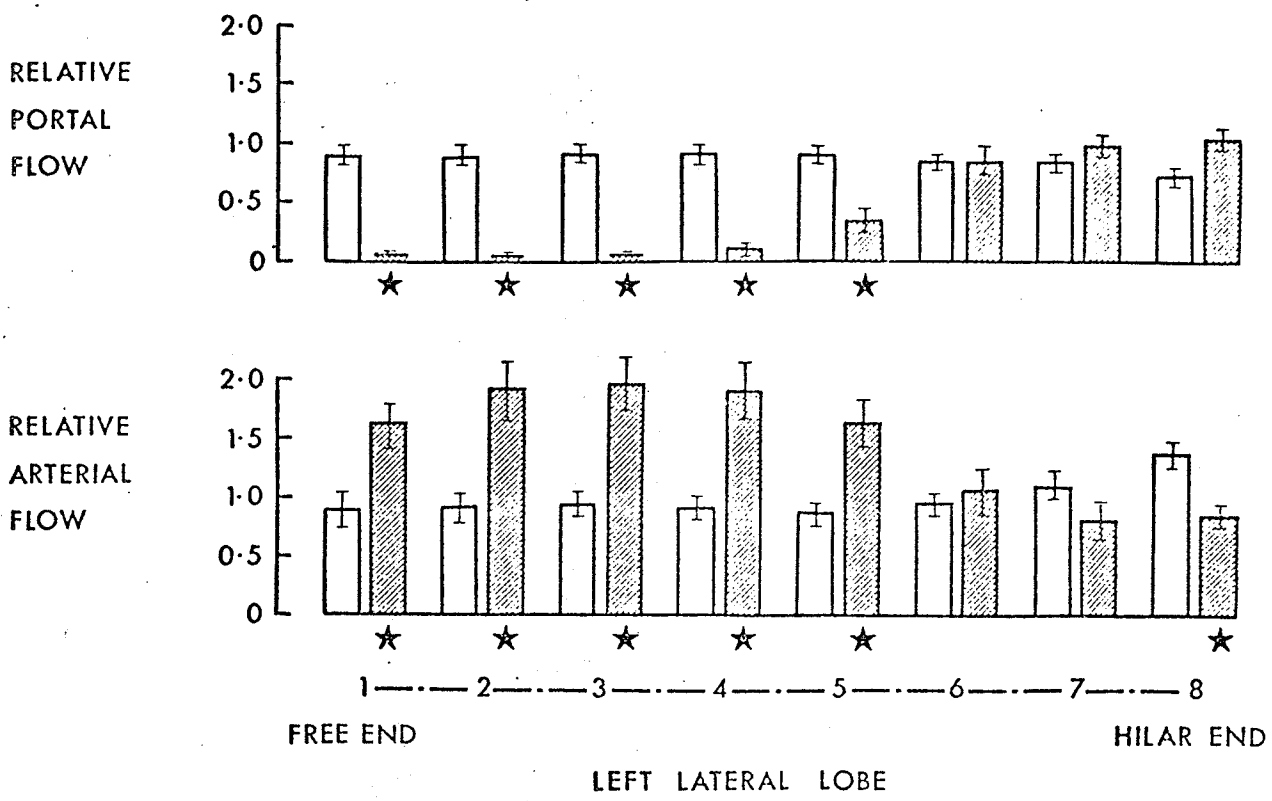

Figure 8

The means (± S.E.) of relative portal and arterial flows in 8 transverse slices of the left lateral lobe determined by simultaneous injection of microspheres into the portal vein (141_{Ce}-spheres) and hepatic artery (51_{Cr}-spheres) in 8 control dogs (open bars) and in 4 dogs after occlusion of a branch of the portal vein (shaded bars). The asterisks indicate $P < 0.01$, t-test for unpaired data.

mean ± S.E.), was significantly less than the mean portal flow through the whole liver ($P > 0.01$). The mean relative arterial flow in these areas was 1.07 ± 0.07 and this value is not significantly greater than the mean arterial flow through the whole liver. Thus the areas which received a spontaneously low portal flow did not receive a significantly high arterial flow.

The mean values of the relative flows for each area and each lobe were calculated for the control injections in all the experiments and in cats (arterial flow n = 11; portal flow n = 12) and dogs (arterial flow n = 15; portal flow n = 15). In cats, these mean values were not significantly different from the mean flow through the whole liver (1.0) in any area or lobe (unpaired t-test $P > 0.05$). In dogs, the portal flow to the caudate lobe and the arterial flow to the papillary process were significantly higher than the mean flow to the whole liver. The mean values for the lobes are shown in Table 1. The gall bladder received a much lower portal flow than the hepatic parenchyma in both cats and dogs but its relative arterial flow was variable in different animals and ranged from 0.5 to 6.0 in both cats and dogs. The causes of this variability were not clear and it was still present after acute hepatic denervation in 6 cats.

DISCUSSION The most accurate method of locating the microspheres in the tissue is no doubt by autoradiography. However, it is difficult to precisely quantitate the distribution of spheres in this manner and comparison between two slices is even more difficult. Since the distribution of spheres within each slice was always uniform, it was

Table 1

The means (± S.E.) of 22 cats and 23 dogs for body weight, liver weight, weights of individual liver lobes (as % total liver weight) and relative portal and hepatic arterial flows in each lobe.

	CAT			DOG		
Body Weight	2.3 ± 0.09 kg			7.1 ± 0.3 kg		
Liver Weight	62 ± 2.6 g			200 ± 11 g		
	Lobe weight as % total liver weight	Relative flows		Lobe weight as % total liver weight	Relative flows	
		Portal	Arterial		Portal	Arterial
Left lateral	30 ± 0.7	0.86 ± 0.08	0.90 ± 0.05	29 ± 0.7	0.91 ± 0.04	1.01 ± 0.05
Left medial	12 ± 0.7	1.14 ± 0.07	0.98 ± 0.06	14 ± 0.4	0.94 ± 0.04	0.90 ± 0.05
Quadrate	12 ± 0.5	0.97 ± 0.09	1.07 ± 0.08	7 ± 0.4	0.87 ± 0.06	1.21 ± 0.09
Right medial	21 ± 0.9	0.96 ± 0.13	0.98 ± 0.04	17 ± 0.5	0.91 ± 0.06	0.92 ± 0.06
Right lateral	11 ± 0.6	1.20 ± 0.16	1.12 ± 0.12	16 ± 0.6	1.24 ± 0.09	0.89 ± 0.09
Caudate	9 ± 0.4	1.38 ± 0.24	1.23 ± 0.13	10 ± 0.5	1.34 ± 0.07*	0.97 ± 0.08
Papillary process	3 ± 0.1	1.20 ± 0.14	1.27 ± 0.17	6 ± 0.1	0.94 ± 0.09	1.68 ± 0.10*
Gall bladder (empty)	2 ± 0.1	0.04 ± 0.01*	3.14 ± 0.70	1 ± 0.1	0.06 ± 0.01*	1.87 ± 0.58

* indicates significantly different from 1.0 (t-test, $P < 0.01$).

felt that counting the radioactivity in transverse slices was a valid method of determining the distribution of microspheres to each slice and this method was chosen to study the distribution of flow to an organ as large as the liver.

Buckberg, Luck, Payne, Hoffman, Archie and Fixler (1971), have shown that the accuracy with which the distribution of flow to an organ or segment of that organ can be determined is related to the number of microspheres which reach that organ or segment. They calculated that in order for the flow to be expressed within 10% of the mean, each sample should contain at least 384 microspheres. In these experiments a minimum of 10^5 microspheres of each type was always given and this amount was adequate for maximum precision.

Most of the studies involving the use of microspheres have been done on the arterial distribution of blood. With plastic microspheres of the 15 ± 5 μ size evenness of mixing with arterial blood has not presented a great problem. It is imperative however that mixing errors of this type be checked so that one realizes the limitations within which he is working. In these experiments the coefficient of variation was 15% and the 99% confidence interval represented flows of 80 to 120% of the mean flow to the liver. Thus the method appeared sufficiently precise to show redistributions which were likely to be of physiological significance.

The radioactivity in the lungs was virtually negligible in all experiments. Thus there were no arterio-venous or porto-venous anastomoses large enough to allow 15 ± 5 μ diameter microspheres to

pass through them. In a separate study on these microspheres (Greenway & Murthy, 1972) it was shown that the ^{141}Ce spheres were 12 $\pm$ 0.15 μ and the ^{51}Cr spheres 17 $\pm$ 0.16 (mean $\pm$ S.E.). Thus the size range was very small. This confirms other evidence that there are no shunt vessels larger than 12μ in the normal liver (Gordon, Flasher & Drury, 1953; Ohlsson, Rutherford, Boitnott, Haalebos & Zuidema, 1970). Since there was also no radioactivity detected in the inferior vena cava blood 10 minutes after the injection of microspheres, the problem or recirculation of microspheres resulting in erroneous data did not present itself.

A decrease in portal flow causes hepatic arteriolar vasodilatation, probably by a myogenic response of the arteriolar smooth muscle due to a reduction in sinusoidal pressure (Hanson & Johnson, 1966). The experiments on portal branch occlusion demonstrate that arteriolar vasodilatation can occur in relatively small areas of the liver when portal flow to that area is selectively reduced. The relative arterial flow could increase up to 3.00 but the average increase was 1.93. This agrees with previous data (Greenway & Lawson, 1969) that maximal arteriolar vasodilatation in the splenectimized cat increased arterial flow up to 300% (mean 200%) of control flow. In the one animal which did not show the increase in arterial flow, it seems probable that the hepatic arterial bed had already been maximally dilated due to the reduction in portal flow after splenectomy (Greenway & Lawson, 1968).

The detection of the increased arterial flow to a localized

area coupled with the previous mentioned facts shows that the microsphere technique as used in these and subsequent studies was valid and sensitive enough to detect relative flow changes of physiological significance.

Areas of hepatic parenchyma can receive spontaneously high or low flows and the mechanism for this remains to be elucidated. But the areas which received low portal flows did not necessarily receive high arterial flows so there was no reciprocity of flow involved in spontaneous reductions of portal flow.

SUMMARY Autoradiography was used to show that counting the radioactivity in transverse slices of liver was a valid method of determining the distribution of microspheres in the tissue. Simultaneous injections of microspheres into the hepatic artery or portal vein of one liver revealed that some areas of the liver received a higher or lower flow relative to the flow through the liver as a whole. Examination of the lungs in each experiment demonstrated that there were few if any anastomotic vessels greater than 12 μ in diameter in the liver. It was established that the microsphere technique was sensitive enough to reveal an increase in arterial flow to a given area when portal flow to that area was decreased. Flow changes likely to be of physiological significance could be detected by the microsphere method. Areas of spontaneous high or low flows can occur in the liver.

SECTION III

INTRAHEPATIC DISTRIBUTION OF BLOOD FLOWS DURING HEPATIC NERVE STIMULATION

INTRODUCTION The autoregulatory escape in the liver was very similar to that seen in the intestinal vascular bed (Folkow et al. 1964 a, b; Ross, 1971 a, b). In both organs, escape occurs during constant flow perfusion and the initiating factor cannot be the decrease in total arterial flow (Dresel & Wallentin, 1966; Greenway et al. 1967). However, as Dresel and Wallentin pointed out, these experiments did not exclude the possibility that a local accumulation of metabolites or the opening of shunt vessels results in a redistribution of the flow through certain areas of the organs. Evidence for a redistribution was presented for the intestine (Folkow et al. 1964 b) but since that time other workers have suggested alternative explanations. Richardson and Johnson (1969) felt that the most tenable hypothesis based on their experiments was a secondary relaxation of those vascular elements which were initially constricted. Ross (1971a) could not demonstrate any significant change in blood flow distribution during autoregulatory escape and concluded that escape could not be explained by the method of redistribution proposed by Folkow et al.

In the liver, a redistribution of portal flow during nerve stimulation was postulated by Daniel and Prichard (1951 a, b), but their interpretation has been criticized since the radiographic method is more likely to show changes in blood volume than flow distribution (Folkow & Neil, 1971; Greenway & Stark, 1971). Since the microsphere technique was shown to be satisfactory for determining intrahepatic distribution

of blood flow, I decided to study the vascular changes involved in autoregulatory escape during sympathetic nerve stimulation in the cat liver and compare it to the vascular changes seen in the dog liver which does not show any escape.

METHODS ^{141}Ce microsphere were injected before and ^{51}Cr microspheres 4 minutes after the onset of nerve stimulation at 6 Hz in 10 dogs. In 5 of these experiments the spheres were given into the coeliac artery and in the other 5 they were given into the portal vein. To analyze the data from these experiments, the slices of liver from each lobe were pooled into 3 groups. Each lobe consisted of the outer third or free end, the middle third or middle, and the inner third or hilar end. Since the papillary process was quite small and lay close to the hilum it was considered as a whole. All data were analyzed by the paired t-test (Steel & Torrie, 1960).

In a similar series of experiments in cats, microspheres were injected before and after nerve stimulation began. However, since arterial flow escaped from the effects of nerve stimulation, the relative arterial flow was measured at 30 seconds in one series of 5 cats, and in a second series of 6 cats at 4 minutes after the onset of stimulation. The data from these 11 experiments was treated in the same way as that for the dogs.

RESULTS The intrahepatic distributions of both arterial and portal flows before and after sympathetic nerve stimulation in the dogs are given in Table 2. Although the flows for the middle of each lobe were

Table 2

The relative arterial and portal flows in areas of canine liver before (C) and during (N) stimulation of the hepatic nerves at 6 Hz. Each set of data (± paired S.E.) are the means for 5 dogs.

	RELATIVE ARTERIAL FLOWS			RELATIVE PORTAL FLOWS		
	C	N	S.E.	C	N	S.E.
Left Lat. - whole	1.15	1.14	(0.06)	0.92	0.58*	(0.06)
free end	1.04	0.95	(0.08)	1.11	0.59*	(0.10)
hilar end	1.33	1.57	(0.15)	0.57	0.42	(0.10)
Left medial - whole	0.91	0.84	(0.06)	0.98	0.89	(0.06)
free end	0.89	0.94	(0.14)	1.11	0.96	(0.11)
hilar end	0.97	0.80	(0.05)	0.79	0.77	(0.04)
Quad. - whole	1.15	1.19	(0.08)	0.75	0.57	(0.06)
free end	1.02	1.17	(0.11)	0.81	0.58	(0.10)
hilar end	1.27	1.21	(0.12)	0.72	0.58	(0.10)
Right medial - whole	0.79	0.80	(0.06)	0.83	0.94	(0.02)
free end	1.06	1.05	(0.17)	0.66	0.67	(0.05)
hilar end	0.73	0.82	(1.07)	0.95	1.14	(0.05)
Right Lat. - whole	0.84	0.93	(0.07)	1.45	1.73	(0.07)
free end	0.90	1.11	(0.13)	1.38	1.29	(0.10)
hilar end	0.80	0.85	(0.09)	1.58	2.02	(0.12)
Caud. - whole	0.91	0.77	(0.05)	1.19	1.47	(0.11)
free end	0.82	0.94	(0.10)	1.24	1.37	(0.24)
hilar end	1.05	0.81	(0.06)	1.28	1.33	(0.15)
Pap. Proc.	1.60	1.60	(0.15)	0.69	0.85	(0.07)
Gall Bladder	2.79	3.66	(0.38)	0.06	0.07	(0.02)

* $P < .01 > .001$ paired t-test.

calculated, they do not add any additional experimental information and consequently have been omitted from these tables. Only the values for the free and hilar ends and for the whole lobe are given. With the exception of a reduction of portal flow to the free end of the left lateral lobe and to the left lateral lobe as a whole, there were no significant changes in either portal or arterial distribution of flow in the canine liver during nerve stimulation.

The arterial flow to the gall-bladder varied widely from animal to animal but was not changed in any significant way by nerve stimulation. The portal flow to the gall-bladder was consistently low and is probably negligible.

Table 3 gives relative arterial and portal flows in the cats. As before the relative flows for the middle of each lobe have been omitted. There were no significant alterations of flow distribution in any area of the liver after nerve stimulation. As in the dog, the arterial flow to the gall-bladder varied widely while the portal flow was extremely low.

In both cats and dogs, the lungs were checked for radioactivity to see if nerve stimulation resulted in the opening of shunt vessels. In no case did the radioactivity exceed 0.3% of the total injected radioactivity for each isotope.

DISCUSSION These experiments do not demonstrate any major redistribution of flow during the maximum decrease in flow in cats or dogs, nor is there any redistribution of flow in the cat during autoregulatory escape. Since there was no redistribution of arterial flow and no

Table 3

The relative arterial and portal flows in areas of feline liver before (C) and during (N) stimulation of the hepatic nerves at 6 Hz. The relative arterial flows were determined 30 sec and 4 min after the onset of nerve stimulation. Each set of data (± paired S.E.) are the means for 5 or 6 cats.

	RELATIVE ARTERIAL FLOW						RELATIVE PORTAL FLOW		
	at 30 seconds			at 4 minutes					
	C	N	S.E.	C	N	S.E.	C	N	S.E.
Left Lat. - whole	1.01	1.14	(0.05)	1.04	0.95	(0.04)	0.96	0.88	(0.07)
free end	1.02	1.13	(0.07)	0.94	0.96	(0.06)	1.06	0.85	(0.10)
hilar end	1.08	1.30	(0.11)	1.09	0.98	(0.07)	0.89	0.96	(0.08)
Left. Med. - whole	1.10	1.20	(0.05)	0.93	0.93	(0.06)	1.43	1.34	(0.36)
free end	1.09	1.30	(0.09)	0.93	1.03	(0.16)	1.48	1.06	(0.24)
hilar end	1.10	1.13	(0.09)	0.96	0.94	(0.05)	1.50	1.48	(0.24)
Quad. - whole	1.07	0.90	(0.06)	1.11	1.22	(0.06)	1.12	0.95	(0.08)
free end	1.05	0.85	(0.06)	1.33	1.37	(0.08)	1.06	0.80	(0.13)
hilar end	1.10	1.00	(0.11)	1.01	1.12	(0.11)	1.14	0.93	(0.11)
Right Med. - whole	0.89	0.78	(0.04)	0.78	0.89	(0.04)	0.91	1.18	(0.19)
free end	0.93	0.77	(0.07)	0.86	0.97	(0.10)	1.05	1.32	(0.16)
hilar end	0.85	0.81	(0.06)	0.75	0.92	(0.04)	0.95	1.08	(0.09)
Right Lat. - whole	1.12	1.08	(0.08)	1.11	1.20	(0.06)	0.84	0.71	(0.19)
free end	1.22	1.27	(0.11)	1.02	1.32	(0.11)	0.87	0.80	(0.14)
hilar end	1.06	1.07	(0.14)	1.16	1.08	(0.06)	0.91	0.72	(0.15)
Cand. - whole	1.18	1.23	(0.10)	1.15	1.05	(0.05)	1.05	0.94	(0.14)
free end	1.12	1.29	(0.16)	1.08	1.07	(0.06)	1.44	1.30	(0.13)
hilar end	1.20	1.09	(0.17)	1.19	1.00	(0.08)	0.91	1.06	(0.08)
Pap. Proc.	1.37	1.27	(0.10)	1.38	1.22	(0.14)	1.02	0.89	(0.09)
Gall Bladder	1.16	2.18	(0.61)	4.63	3.68	(1.10)	0.09	0.10	(0.01)

The distribution during nerve stimulation was not significantly different from the control in any case ($P > 0.05$ paired t-test).

microspheres traversed the liver to reach the lungs, the opening of shunt vessels during escape is excluded.

Three explanations have been proposed for autoregulatory escape in the intestine (Richardson & Johnson, 1969). The first is the parallel circuit concept (Folkow et al. 1964 b) which suggests that low resistance vessels dilate in one circuit when the blood flow to a parallel circuit is reduced. This concept was put forward to explain why nerve stimulation failed to reduce total flow to the gut and why reactive hyperemia followed cessation of nerve stimulation. Since no redistribution of flow occurred in the cat liver this hypothesis is untenable in the present situation.

The second explanation is the series concept which postulates that some elements undergo vasodilatation in series with vasoconstrictor elements. This concept could also explain the type of adjustments responsible for autoregulation in a vascular bed and it would suggest that autoregulation and autoregulatory escape are interrelated phenomena. Contrary to what Dresel and Wallentin (1966) observed, Richardson and Johnson (1969) proved that the two phenomena were not interrelated and could work independently of each other. This argues against the second concept as being the explanation for autoregulatory escape.

The third concept and the one most likely to explain these results is that of relaxation of those same vessels which initially underwent constriction. Recent work (Ross, 1971 a; Greenway & Murthy, 1972) suggests this is the mechanism in the intestine and this hypothesis would also account for the fact that there is no significant

redistribution of blood flow during escape in the cat's liver.

This relaxation is unlikely to be myogenic for 3 reasons: a) the escape and post-stimulatory hyperemia are relatively slow, b) hepatic myogenic responses appear to be controlled by sinusoidal pressure (Hanson & Johnson, 1966; Greenway & Stark, 1971) and this does not change during hepatic nerve stimulation (Greenway, Stark & Lautt, 1969) and c) failure of the arteriolar smooth muscle to maintain its contraction due to some unknown myogenic mechanism, such as failure of cell to cell conduction does not readily explain the occurrence of post-stimulatory hyperemia.

Escape cannot be due to accumulation of vasodilatory metabolites secondary to reduction in total flow since it was present during constant flow perfusion (Greenway, Lawson & Mellander, 1967). Nor is escape due to this mechanicm consistent with the fact that there is no escape from angiotensin II infusion (Greenway & Lautt, 1972). It cannot be due to accumulation of metabolites in certain underperfused areas of the liver because there was no redistribution of flow within the liver.

A third theory has been put forward by Shanbour and Jacobson (1971), and it suggests that the receptors become refractory or desensitized. This theory is supported by the work of Shehadeh, Price and Jacobson (1969), which demonstrated that even dilating agents such as bradykinin could evoke autoregulatory escape. It is difficult to imagine how this could occur other than by refractory receptors. There are however, at least three factors which are not consistent with this

theory. Escape occurs at all frequencies tested (0.5 - 12 Hz). If escape were due to desensitization, it might be expected to occur only at the higher frequencies of stimulation. Secondly, if it were simply a case of receptors being desensitized, there is no good reason why post-stimulatory hyperemia should occur. Finally Shehadeh et al. even while infusing noradrenaline continuously in successively higher doses, found that each increase in dose caused a greater decrease in flow than the previous dose before escape set in. This would suggest that the receptors are losing their refractoriness while being continuously in the presence of noradrenaline. This is unlikely.

There is one more theory to consider which might explain the mechanism of autoregulatory escape. Hepatic nerve stimulation, besides constricting arterioles could increase the production of vasodilatory metabolites. This would explain why escape occurs even during constant flow perfusion. It would also explain the occurrence of post-stimulatory hyperemia. However, metabolic effects of nerve stimulation are quite similar in cats and dogs (Edwards, 1972) but the dog liver does not escape from hepatic nerve stimulation.

If the above theory were modified slightly to state that a specific vasodilator rather than just a secondary metabolite were produced during nerve stimulation, this would readily explain auto-regulatory escape and the ensuing hyperemia. Davies, Horton and Witherington (1967) have shown that stimulation of the sympathetic nerves to the spleen increased the splenic venous concentration of prostaglandin E_2. Hedqvist and Brundin (1969) produced evidence that

prostaglandins of the E series inhibit the vasoconstrictor action of noradrenaline. More recently, McGiff, Crowshaw, Terragno, Malik and Lonigro (1972) showed that recovery from the initial constrictor effects of a continuous noradrenaline infusion in the dog kidney was accompanied by an increase in the release of a prostaglandin E-like substance. They also found that angiotensin II infusions in the kidney produced the same phenomenon (McGiff, Crowshaw, Terragno & Lonigro, 1970). Of interest is the fact that they found no increase in the release of prostaglandins during renal nerve stimulation, however flow tended to recover very little during nerve stimulation in dogs. The release of prostaglandin E as the mechanism of autoregulatory escape from nerve stimulation or noradrenaline infusion in the cat liver and intestine is an attractive but still unproven hypothesis. Moreover, experiments being conducted at present in this laboratory suggest that aspirin and indomethacin which are purported to block the release of prostaglandin, do not prevent autoregulatory escape in the cat liver or intestine (Lister & Greenway, 1972, unpublished observations).

SUMMARY The microsphere technique was used to study the intrahepatic distribution of arterial and portal flows during hepatic nerve stimulation in cats and dogs. This technique revealed that there was no redistribution of flows in cats and dogs during the maximum decreases in flow to nerve stimulation nor during autoregulatory escape in cats. It was concluded that autoregulatory escape could best be explained as a secondary relaxation of those vessels which were initially constricted by nerve stimulation due to the increased production of a vasodilator factor. Other theories on the mechanism of escape were also discussed.

SECTION IV

EFFECTS OF HEPATIC NERVE STIMULATION ON HEPATIC VOLUME

INTRODUCTION As mentioned in the historical survey, changes in hepatic blood volume in cats during sympathetic nerve stimulation have recently been described (Greenway et al. 1969) and these results suggest that the liver can function as an important blood reservoir. On the other hand, the work of Guntheroth and Mullins (1963) suggests that only the dog spleen and not the liver functions as a blood reservoir. In the dog, the adrenergic innervation is unusual in that a dense plexus of nerves penetrates the media of the hepatic veins (Ungvary & Donath, 1969). Since Mellander (1960) proved that alterations in venous caliber were mainly responsible for capacitance changes it was of interest to study the volume changes in the dog liver and to compare them with those changes already established for the cat (Greenway, Stark & Lautt, 1969).

METHODS The general methods for measurement of pressures and preparation of the hepatic nerves for stimulation have already been described (see pages 29, 30).

In order to gain space for the large plethysmograph required for the dog, positive pressure ventilation was instituted (16/sec. and volume regulated to almost suppress spontaneous diaphragm movements) and the lower end of the sternum was split for about 10 cm. The ligaments connecting the liver to the diaphragm and stomach were ligated and transected and the base plate of the plethysmograph was placed under the liver with the exception of the right lateral and caudate lobes. The middle portion of the plethysmograph was then placed around the liver and a plasticized hydrocarbon gel, Plastibase ^{R} (Squibb) was

layered around all joints and the orifice through which the intact vessels passed. The edges of the top of the plethysmograph were also layered with Plastibase and the top set in place. Wing nuts on pins molded into the base of the plethysmograph held the entire assembly together (Fig. 9).

The plethysmograph was filled with Ringer-Locke solution at 37°C and the outlet from the plethysmograph was connected to a float recorder which in turn operated an isotonic transducer (Harvard Apparatus Co., Model 356). The pressure in the plethysmograph was adjusted to zero relative to the hilum of the liver.

Inserted in the line between the plethysmograph and the float recorder was a T piece which allowed calibration of the plethysmograph (Fig. 10). Volume changes were calibrated in 5 ml increments over a range of 40 ml. All recordings were made on a Grass Polygraph Model 5D.

In 7 separate experiments the blood content of the liver was determined by the hemoglobin washout technique (Greenway et al. 1969). After the dog was anesthetized and placed on artificial ventilation the abdomen and chest were opened along a midline incision. The animal was heparinized (4 mg/kg) and a mixed venous blood sample taken from the right atrium. All inflow and outflow vessels of the liver were ligated and the liver was removed from the animal and weighed. The portion of the inferior vena cava still attached to the liver was cannulated and saline flushed through the liver until the solution emerging from the portal vein and the hepatic artery contained no more

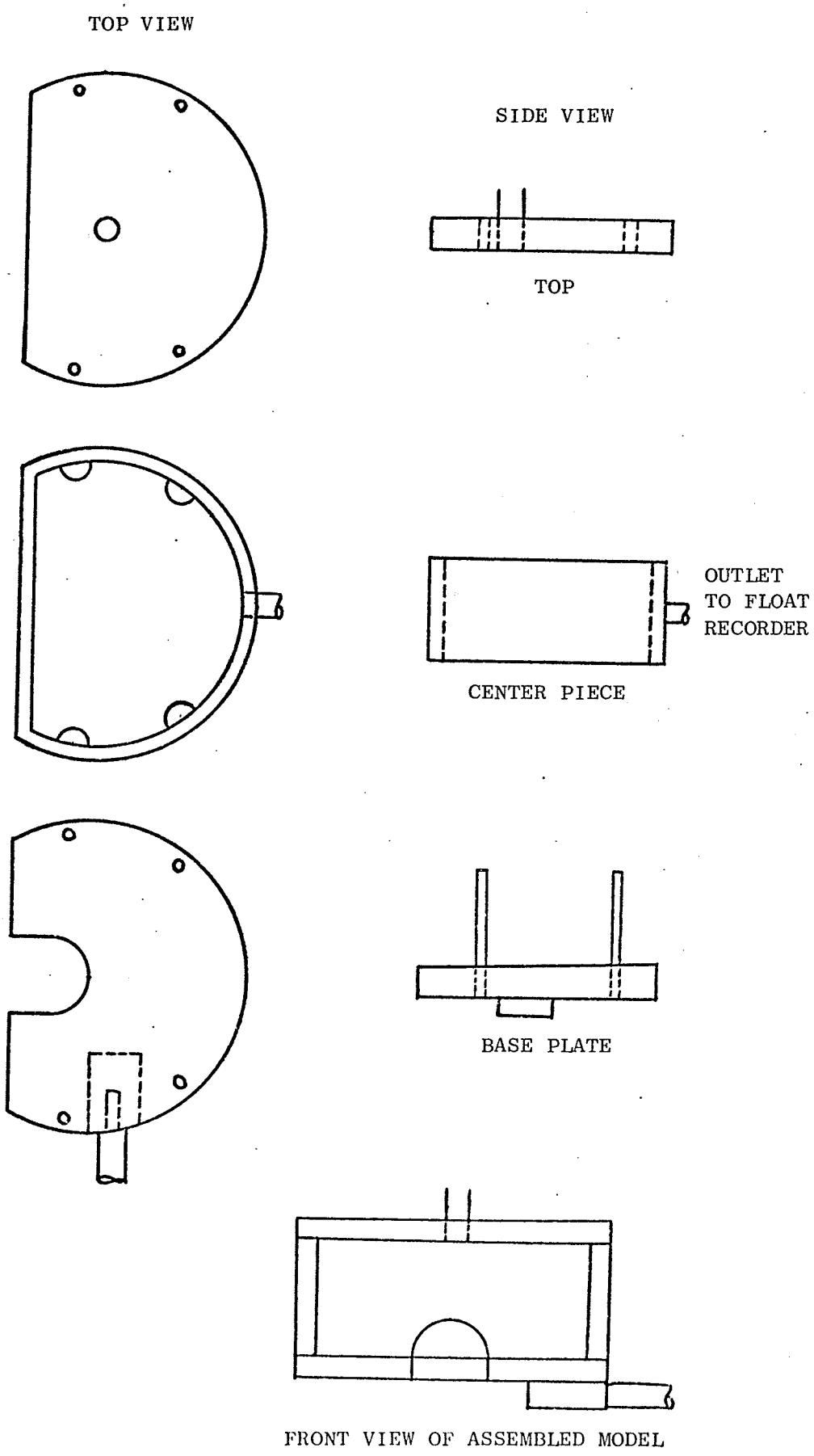

Figure 9

Diagram of sections of plethysmograph

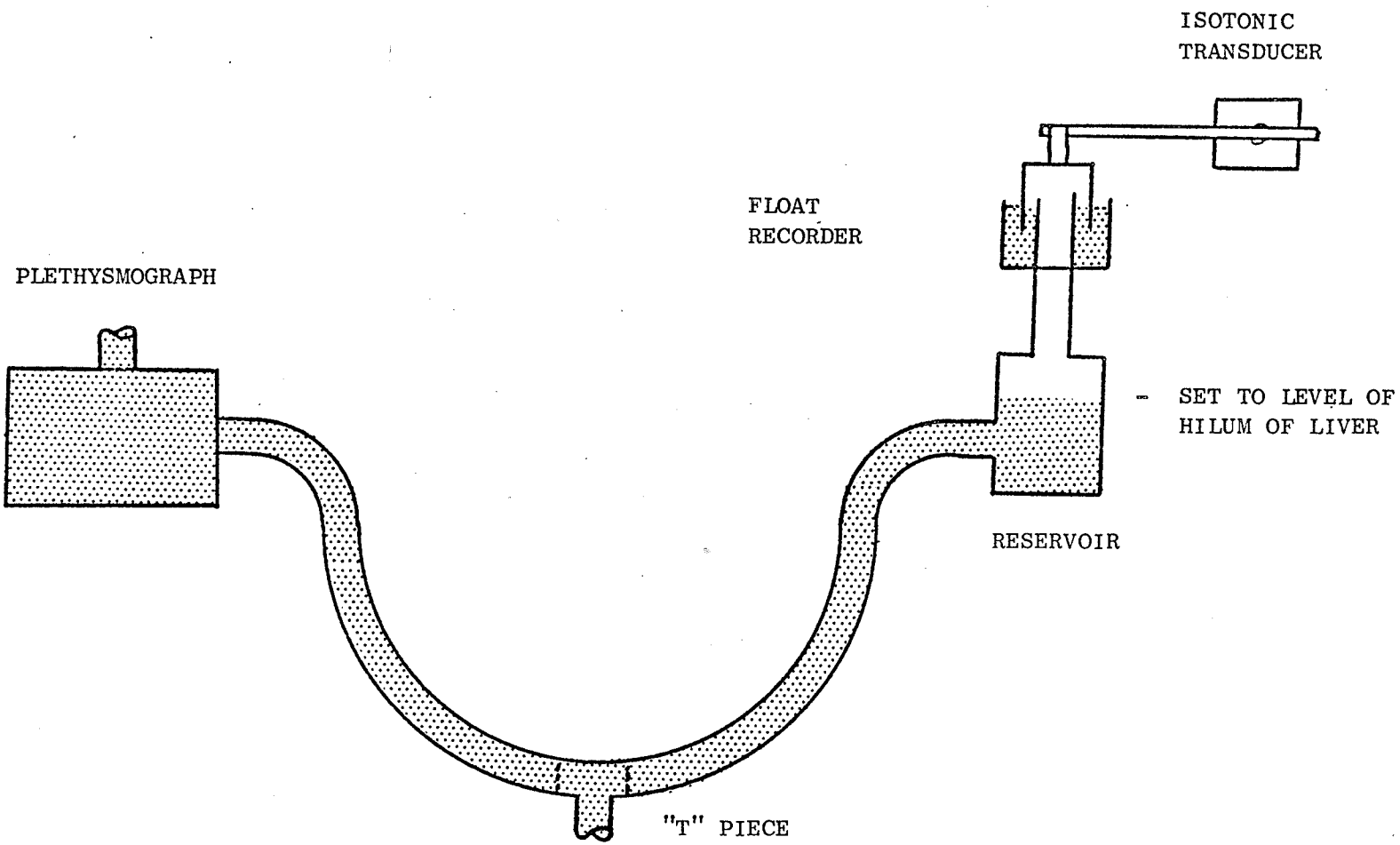

Figure 10

Diagram of plethysmograph connected to float recorder.

blood. The portions of diaphragm remaining attached to the liver were cut away and weighed and this weight was subtracted from the original liver weight. The washout volume was recorded.

Beforehand, an ammonia solution was prepared (1.4 ml of ammonia diluted in 1000 ml of distilled H_2O). 40 ml of this solution was placed in each of two flasks then 39 and 38 ml of solution were placed in each of two other flasks respectively. 0.1 ml of mixed venous blood was placed in one 40 ml flask and 0.2 ml of mixed venous blood in the other 40 ml flask. 1.0 ml of the washout sample was put into the 39 ml flask and 2.0 ml was placed into the 38 ml flask. The absorbance of these four solutions was read on a spectrophotometer using a wavelength of 540 A.

The formula used to determine the blood volume of the liver is:

$$\frac{\text{Abs. Read. Washout Samp.}}{\text{Abs. Read. Mix. Ven. Samp.}} \times \frac{\text{Vol. Mix. Ven. Samp.}}{\text{Vol. Washout Samp.}} \times \frac{\text{Washout Vol.}}{\text{Liver wt.}} \times 100$$

This expression gives the volume in ml/100 g of tissue.

RESULTS Control values for dogs were as follows: body weight 7.4 $\pm$ 0.5 kg. (mean $\pm$ S.E.), femoral arterial blood pressure 123 $\pm$ 6.0 mm Hg., portal pressure 7.5 $\pm$ 0.7 mm Hg., liver weight 252 $\pm$ 22 g, and the percentage of the liver in the plethysmograph was 70 $\pm$ 2.0%. In dogs the hepatic blood volume was 31 $\pm$ 2.4 ml/100 g of liver. This is the total hepatic blood volume at physiological pressures. Blood volumes determined when the animal is dead and the liver drained are much smaller than this.

The data for hepatic volume changes in the cat were taken from a previous paper (Greenway et al. 1969) and in those experiments, liver blood volume was 27 ± 3 ml/100 g of liver.

Hepatic volume responses to stimulation of the hepatic nerves at frequencies of 1 to 12 Hz. were studied in 13 dogs. Nerve stimulation produced a frequency-dependent decrease in hepatic volume. This decrease was slower than the decrease in arterial flow and was well maintained for the duration of stimulation. A record of one experiment is shown in Fig. 11 . The mean responses in all the dogs is shown in Fig. 12 and the data for cats taken from a previous paper (Greenway et al. 1969) are shown for comparison. When the changes were expressed per 100 g of liver, there were no significant differences between the responses in dogs and cats at any frequency (unpaired t-test, $P > 0.1$). The maximum response occurred at 4 to 8 Hz. and represented expulsion of 36% (dogs) and 47% (cats) of the total hepatic blood volume.

DISCUSSION The hepatic blood volumes determined for cats and dogs - about 30 ml/100 g of liver, are very large. Earlier studies were reviewed by Greenway and Stark (1971) and in most cases the hepatic blood volumes were not determined at normal physiological pressures. Since a high proportion of this content can be expelled by sympathetic nerve stimulation in cats and dogs, it is clear that the liver is an important potential blood reservoir, if we define this as an area from which a significant volume of blood can be rapidly redistributed in a precise and controlled way.

Comparable data for other organs are fragmentary and attempts

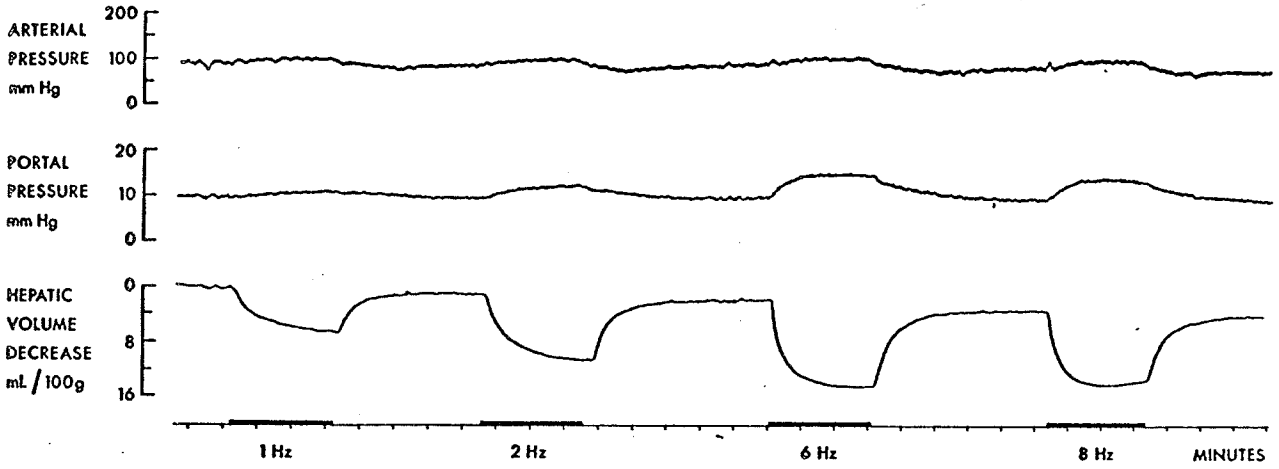

Figure 11

The effects of hepatic nerve stimulation on hepatic volume in one dog.

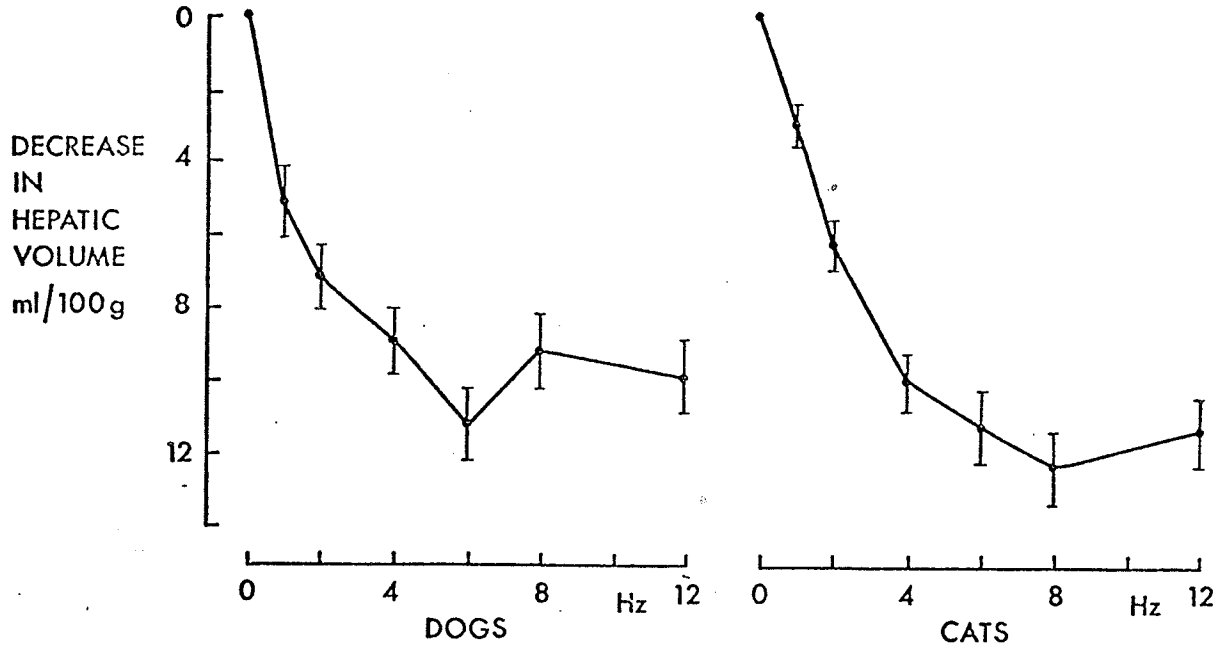

Figure 12

The mean (± S.E.) changes in hepatic volume during hepatic nerve stimulation in 13 dogs and 15 cats. Mean hepatic blood volume was 31 ml/100 g. liver in dogs and 27 ml/100 g. in cats.

to find in the literature a table of regional blood volumes and the proportions which could be expelled by sympathetic nerve stimulation were unsuccessful. Since no table could be found, one was prepared from the data available in the literature. The figures in table 4 were calculated mainly from the following references but many other papers were also studied (Brooksby & Donald, 1971; Documenta Geigy, 1962; Farnswork, Paulino-Gonzalez & Gregersen, 1960; Folkow & Neil, 1971; Greenway, Lawson & Stark, 1968; Greenway & Stark, 1971; Groom, Rowlands & Thomas, 1965; Jodal & Lundgren, 1970; Kerr & Kirklin, 1970; Mellander, 1960; Mellander & Johansson, 1968; Oberg & Rosell, 1967). This table is not presented as a definitive conclusion but for the sake of comparisons in the present study. The table suggests that the largest proportions of the blood volume are in skeletal muscle, liver, spleen (in cats and dogs but not in man - Ayers, Davies & Withrington, 1972), adipose tissue and lungs. Up to 26% of the blood volume can be mobilized from these reservoirs by maximal nerve stimulation. This represents the "blood volume reserve", of Groom et al (1965) and is the theoretical amount of blood which can be removed without causing marked hypotension. Since the sympathetic response to hemorrhage is modified in anesthetized animals subjected to surgical trauma (Chien, 1967; Groom et al. 1965), the volume of blood which can in fact be removed before hypotension occurs will usually be smaller than this. More than half of this blood volume reserve is in the splanchnic vascular bed and Brooksby and Donald (1971) have shown that 54% of the volume of a small hemorrhage (7.2 ml/kg) was mobilized from the

Table 4

Regional distribution of blood volume - a tentative tabulation for the cat and dog.

ORGAN	% BODY WT	% TOTAL BLOOD VOLUME	% TOTAL BLOOD VOLUME MOBILISED BY SYMPATHETIC NERVES
Splanchnic organs			
Liver	3	14	6
Spleen	1	12	9
Intestine	3	5	2
Muscle	45	14	4
Adipose tissue	14	11	4
Lungs	1.5	10	?
Skin	3	2	1
Heart - chambers		5	-
- muscle	0.5	1	-
Kidneys	0.5	1	-
Remainder	28.5	25	?
	100%	100%	26%

splanchnic circulation in dogs. These data give support to the generally accepted but not well documented view of the importance of the liver and splanchnic bed as a blood reservoir.

SUMMARY The plethysmographic technique was used to measure the decreases in canine liver volume produced by hepatic nerve stimulation. The volume decrease was found to be frequency-dependent and maximal at 6-8 Hz when 35% of the blood volume of the liver was expelled. It was concluded that the liver is an important blood reservoir and its ability to act in this fashion was compared with other body organs in cats and dogs.

SECTION V

EFFECTS OF HISTAMINE ON HEPATIC VOLUME, BLOOD FLOW AND BLOOD FLOW DISTRIBUTION

INTRODUCTION: In dogs, histamine and agents which release endogenous histamine cause the liver to engorge with blood. Ever since Mautner and Pick (1915) described this phenomenon, it has been the subject of two controversies which have been summarized in a review by Rocha e Silva (1966). The first controversy concerns the relative roles of hepatic pooling of blood and peripheral vasodilatation in the hypotension produced by intravenous infusion of histamine. Although general opinion now favours peripheral vasodilatation as the major cause of hypotension, hepatic pooling has never been studied quantitatively and therefore its contribution remains unanswered.

The second controversy concerns the mechanism of hepatic pooling due to histamine. Earlier workers could not agree on whether the increase in liver volume was due to generalized hepatic venous constriction or to contraction of sphincters residing at the junction between the hepatic veins and the inferior vena cava (Arey, 1941; Bauer, Dale, Poulsson & Richards, 1932; Thomas & Essex, 1949; Walker, MacDonald & Pickard, 1960). The histological studies of Elias (1953), suggested that histamine contracted the sublobular veins. Since the decrease in liver volume due to sympathetic nerve stimulation was attributed to widespread contraction of intrahepatic vessels (Greenway et al., 1969), the interesting situation arises where venous constriction caused by nerve stimulation results in a decrease in volume while that induced by histamine caused an increase in liver volume. Clearly the sites of action of noradrenaline and histamine cannot be identical throughout the liver.

This final section of the thesis attempts to clarify and quantitate the response to histamine in the canine liver, to resolve these two controversies and to study the intrahepatic distribution of blood flow during histamine infusion.

METHODS: The methods for recording canine liver volume and for stimulating the hepatic nerves have previously been described (see pages 67, 68). In addition the gastroduodenal branch of the hepatic artery or an accessory splenic vein was cannulated to allow infusions of histamine.

In some experiments, hepatic venous pressure was recorded by a catheter introduced through the right jugular vein and passed down until its tip could be felt within the vena cava at the level of the diaphragm. The tip was then guided into an hepatic vein for a variable distance and the mean pressure recorded. At the end of each experiment, the exact location of the catheter tip was determined relative to the junction of the hepatic vein and the inferior vena cava. The diameter of the hepatic vein was always much larger than that of the catheter (external diam. of catheter, 1.2 mm) and the pressures recorded were not wedged pressures. In other experiments, arterial flow was recorded by the methods already described (see page 29), during histamine infusion.

In experiments on isolated strips of portal and hepatic veins the dogs were anesthetized with sodium pentobarbitone and the veins removed into cold Krebs-Henseleit ($4^{o}C$). Uniform circular strips (10 mm x 4 mm) were cut and suspended in 10 ml glass organ baths filled with Krebs-Henseleit at $37^{o}C$ and bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs-Henseleit solution was, NaCl 118 mM/L, KCl 4.7 mM/L, $CaCl_2$ 2.5 mM/L, $MgSO_4$ 1.2 mM/L, KH_2PO_4 1.1 mM/L, $NaHCO_3$ 25 mM/L and glucose 11 mM/L. Isotonic recordings under 1 g tension

were made on a Kymograph by a lever with a magnification of 10. Results are expressed as actual shortening of the strips.

Histamine acid phosphate (British Drug Houses) and (-)-noradrenaline tartrate (Winthrop Laboratories) were dissolved in 0.9% w/v NaCl immediately before use. All doses of the above drugs were expressed as the free base. Phenoxybenzamine (Dibenzyline, Smith, Kline & French) was dissolved in propylene glycol (250 mg in 25 ml) acidified with 4 drops of 1N HCl. Phenotolamine HCl (Aldrich Chemical Co.) was dissolved in 0.1N HCl (5 mg/ml). Before injection, the required doses of phenoxybenzamine and phentolamine were diluted with 5 volumes of 0.9% NaCl solution. One hour was allowed to elapse after phenoxybenzamine administration before responses were tested.

To determine the intrahepatic distribution of blood flow during histamine infusion, ^{141}Ce microspheres were injected into the hepatic artery or portal vein before and ^{51}Cr microspheres 5 minutes after the onset of a continued intraportal histamine infusion (4 ug/kg/min). The data were calculated in the same manner as the data for intrahepatic distribution of flow during nerve stimulation (see page 59).

RESULTS: Control values. The dogs had a body weight of 7.8 $\pm$ 0.4 kg. (mean $\pm$ S.E.). The femoral arterial pressure was 114 $\pm$ 5.0 mm Hg. while the portal pressure was 7.7 $\pm$ 0.4 mm Hg. The mean liver weight was 263 $\pm$ 13 g and the percentage of the liver included in the plethymograph was 67.5 $\pm$ 1.5%.

The hepatic volume responses to intra-arterial or intraportal infusions of histamine were studied in 9 dogs. The responses to each dose by either route of administration did not differ significantly and the data were pooled. Dose-response curves were obtained over the

range of 1 to 20 ug histamine/kg/min. At each dose level, the infusion was continued for 5 minutes and 20 minutes elapsed between each infusion. The responses to infusion of histamine and to stimulation of the hepatic nerves in one animal are shown in Fig. 13. Histamine infusion caused an increase in hepatic volume while nerve stimulation caused a decrease. The mean dose-response curve for histamine in all the animals is shown in Fig 14 . With small doses of histamine, the response was reproducible and the volume recovered on cessation of the infusion. With large doses, the recovery was prolonged (Fig. 13) and repeated infusions of the same dose gave progressively smaller responses. Also, the response to larger doses were accompanied by decreases in arterial pressure.

In 4 dogs, control responses to intra-arterial infusions of histamine 4 μg/min/kg and stimulation of the hepatic nerves (6 Hz), were first obtained and then nerve stimulation and histamine infusion were carried out simultaneously. The results in one experiment is shown in Fig. 13 . Hepatic volume always decreased during the simultaneous procedure but the decrease was smaller than when the nerves were stimulated alone. In all the experiments, the decrease in hepatic volume during simultaneous stimulation and infusion was 58 $\pm$ 4% (mean $\pm$ S.E.) of the decrease during nerve stimulation alone. Arterial pressure did not decrease during simultaneous infusion and stimulation.

In 6 dogs, the hepatic volume responses to nerve stimulation and to histamine infusions were studied before and after administration of phenoxybenzamine or phentolamine. After small doses (2 mg/kg) of either of these agents, the response to nerve stimulation was reduced to 54 $\pm$ 10% of the control while the histamine response was reduced to

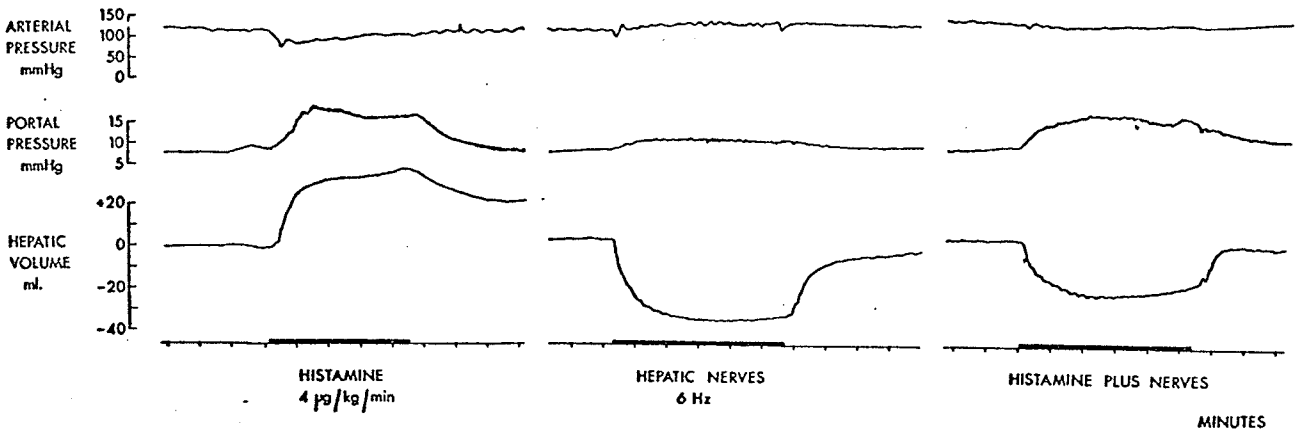

Figure 13

The responses of arterial and portal pressures and hepatic volume in one dog to infusion of histamine into the hepatic artery, stimulation of the hepatic nerves and simultaneous infusion and nerve stimulation.

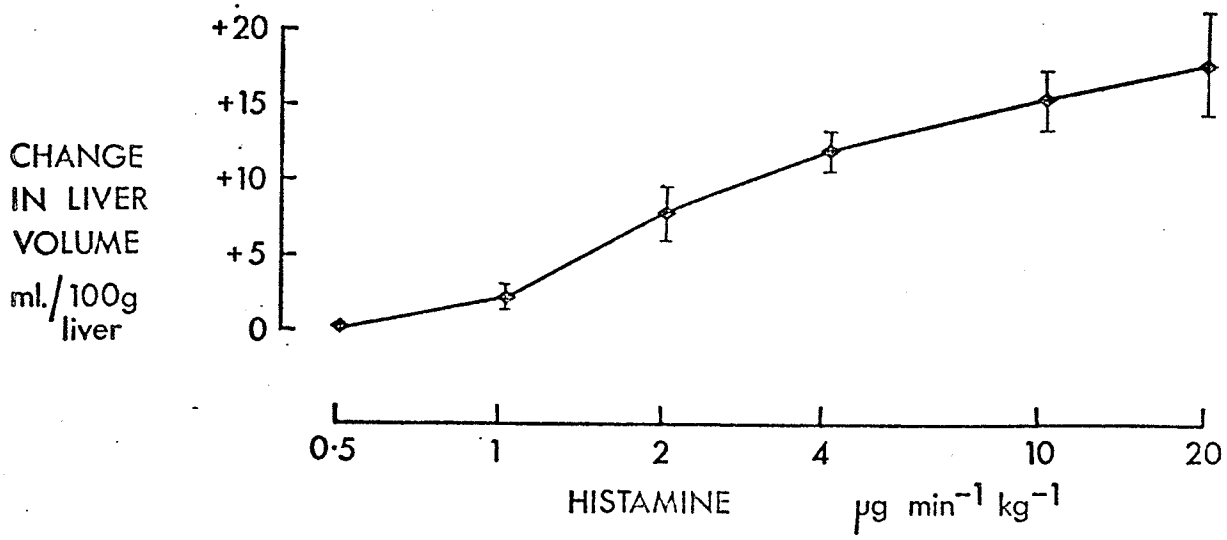

Figure 14

Dose-response curve for intra-arterial infusions of histamine on hepatic volume. Each point represents the mean ± S.E. of results in 9 dogs.

22 ± 13% of control. When the dose was increased (5-10 mg/kg), the nerve response was reduced to 31 ± 6% of control while the histamine response was reduced to 19 ± 5% of control. Thus the responses to histamine were blocked at least as effectively as the responses to nerve stimulation.

Nine experiments were carried out to exclude the possibility that the increase in hepatic volume produced by histamine was due to contraction of a sphincter in the large hepatic veins. Hepatic venous pressure was recorded at various distances from the junction of the hepatic vein and inferior vena cava. The pressure recorded during the control period was greater when the catheter tip lay further within the liver. During intra-arterial infusions of histamine ($2\mu g/min/kg$) and during hepatic nerve stimulation (6 Hz), hepatic venous pressure did not increase significantly (paired t-test $P > 0.1$) when the catheter tip lay 0-4 cm from the junction of the hepatic vein and inferior vena cava but when the catheter tip was 4-8 cm within the hepatic vein, the increases were significant (paired t-test $P < 0.01$). The results are shown in Fig. 15 . The increases in hepatic venous pressure were very similar for both histamine and hepatic nerve stimulation.

To obtain further data on whether the responses of hepatic venous smooth muscle to histamine and noradrenaline were different, isolated strips of canine hepatic veins were studied in vitro. Four strips from each of 6 dogs were prepared. For 2 strips, cumulative dose-response curves were obtained for histamine and 2 hours later for noradrenaline. For the other 2 strips the order of the drugs was reversed. The results are shown in Fig. 16 . The dose-response curves to histamine and noradrenaline were not significantly different.

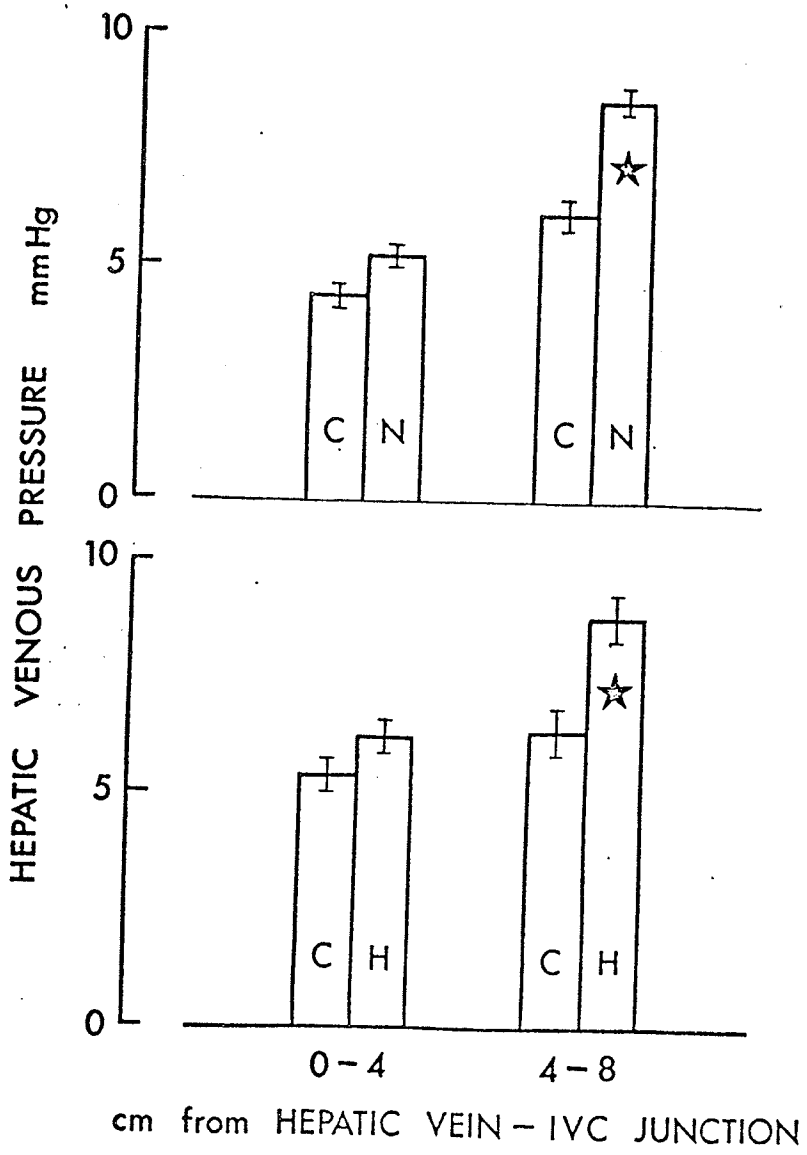

Figure 15

The hepatic venous pressures recorded from catheters 0-4 or 4-8 cm from the junction of the hepatic vein and inferior vena cava before (C) and during hepatic nerve stimulation (N) or histamine infusion (H). Each value represents the mean $\pm$ S.E. of 9 experiments and the asterisks denote $P < 0.01$, paired t-test.

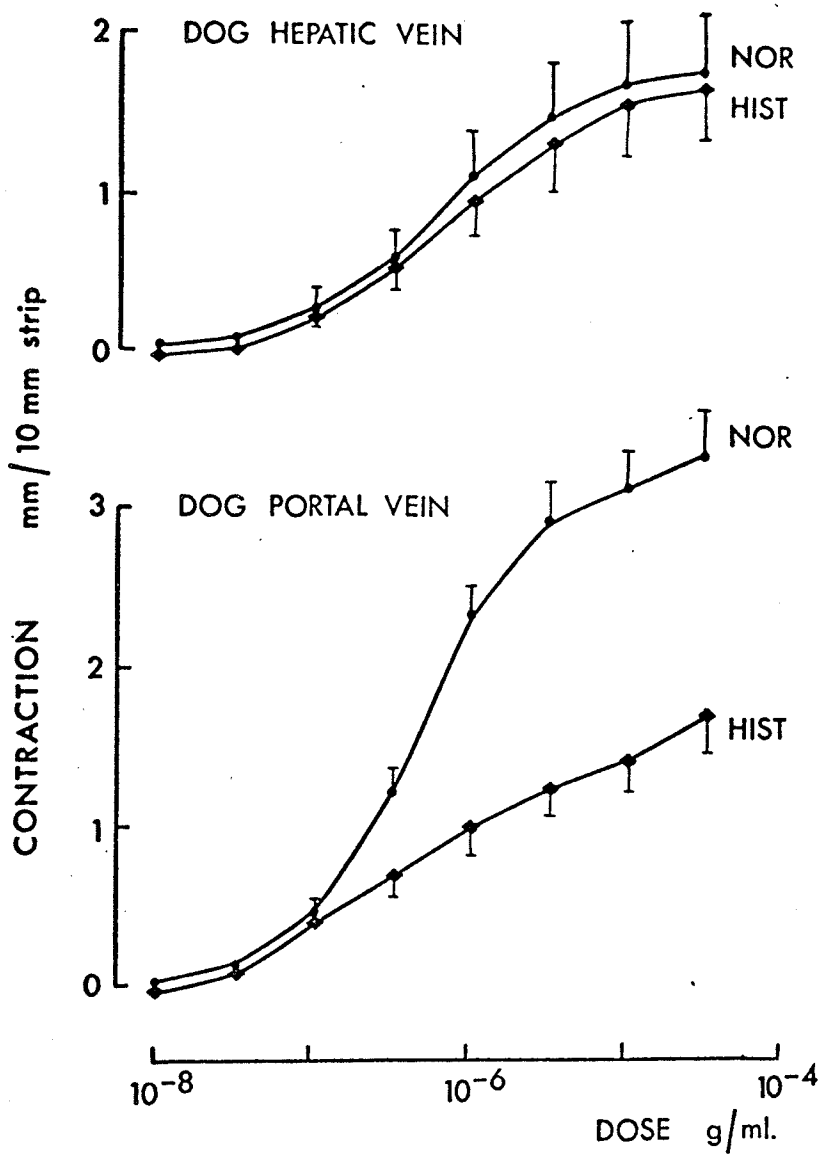

Figure 16

Dose-response curves to histamine (HIST) and noradrenaline (NOR) on hepatic venous and portal strips. The response is the actual contraction by the 10 mm strips and each point is the mean $\pm$ S.E. of 24 strips from 6 dogs.

Similar experiments were carried out with strips from the portal veins of 6 dogs and the results are shown in Fig.16 . The minimal doses of histamine and noradrenaline required to elicit a response were similar but the maximal response to histamine was smaller than that to noradrenaline.

Hepatic arterial flow responses to intra-arterial infusions of histamine were recorded in 7 dogs. Histamine caused hepatic arterial vasodilatation and maximal responses were obtained at 1 μg/min/kg when the flow increased to 150 ± 5% of control. With larger doses of histamine, (greater than 10 μg/min/kg) the increase in flow was smaller but these doses caused a fall in arterial pressure. The decrease in hepatic resistance (arterial-hepatic venous pressure divided by flow) was the same at all doses above 1 μg/min/kg.

To determine whether the increase in arterial flow was necessary for histamine to increase hepatic volume, the response to infusions of histamine into the portal vein were recorded before and during complete occlusion of the hepatic artery in 6 dogs. The increase in hepatic volume during occlusion was 54 ± 9% of the increase before occlusion.

Intravenous infusions of histamine (2-20 μg/min/kg.) were given in 6 dogs. In 3 dogs, all doses caused a decrease in hepatic volume, in 1 dog all doses caused an increase and in 2 dogs the hepatic volume decreased at low doses and increased at 20 μg/min/kg . In all 6 dogs, histamine caused a dose-dependent fall in arterial pressure. The mean results are shown in Table 5 . It is clear that the fall in arterial pressure was not caused by pooling of blood in the liver. On 8 occasions in 3 dogs where histamine increased hepatic volume at least in high doses, the histamine infusion was repeated while the hepatic

Table 5

Effects of intravenous infusions of histamine on arterial pressure and hepatic volume (means ± S.E.) in 6 dogs.

Dose (μg/min)/kg	Arterial pressure (mm Hg)			Change in hepatic volume (ml/100g)
	Before	During	(Paired S.E.)	
2	111	99 *	(2.3)	-0.2 ± 2.0
4	103	80 *	(4.0)	-0.8 ± 3.8
10	106	65 *	(4.9)	-2.6 ± 2.5
20	109	48 *	(5.3)	6.3 ± 5.9

* $P < .05$ paired t-test.

volume increase was prevented by simultaneous nerve stimulation. The fall in arterial pressure was not significantly altered by prevention of the increase in hepatic volume.

The hypotension produced by these intravenous infusions of histamine was not significantly different (paired t-test $P > 0.1$) after administration of phenoxybenzamine or phentolamine (5-20 mg/kg).

In 4 dogs, microspheres were injected into the portal vein before and 5 minutes after the infusion of histamine (4 μg/min/kg) was begun. The mean relative portal flow in each area and each lobe for the 4 experiments before and during histamine infusion were compared (paired t-test). Fig.17 shows a diagramatic map of the liver. In the striped areas, relative portal flow decreased during histamine infusions, in the stippled areas it increased while in the blank areas it showed no change. It can be seen that there was a redistribution of flow toward the hilum of the liver. The data from the slices of liver in each lobe were pooled into 3 groups - the outer third or free end, middle third and the inner third or hilar end. The mean data for the free and hilar ends are presented in Table 6 . Again the papillary process was considered as a whole since it is small and lies close to the hilum. The relative portal flow in the free ends showed a significant decrease except in the caudate lobe. The flow in the hilar ends and papillary process showed a relative increase and these increases were significant in 5 of 7 lobes. The centers of the lobes showed no significant changes and there were no changes in the distribution between lobes (except for the papillary process).

Although this redistribution towards the hilum was significant, it represents small changes in the relative flows. The largest changes

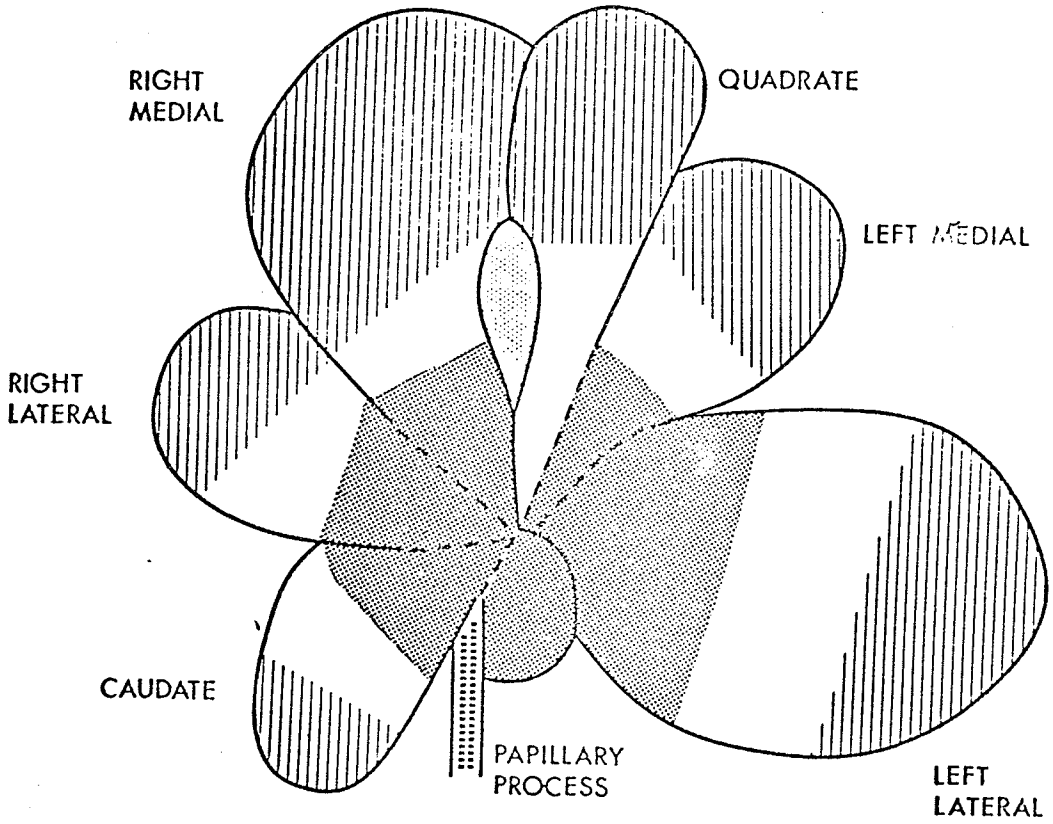

Figure 17

The changes in the intrahepatic distribution of portal flow in dogs during infusion of histamine (4 μg/min/kg) into the portal vein. The relative flow decreased in the areas shaded by the diagonal lines and increased in the stippled areas.

Table 6

The means (± paired S.E.) of the relative portal flows in the free and hilar ends of each lobe of the liver before and during infusion of histamine (4 μg/min/kg) in 4 dogs.

LOBE	RELATIVE PORTAL FLOW					
	Free end			Hilar end		
	Control	Histamine	(S.E.)	Control	Histamine	(S.E.)
Left lateral	0.93	0.55 *	(0.03)	0.79	1.28 *	(0.08)
Left medial	0.84	0.40 *	(0.02)	0.90	1.37 *	(0.19)
Quadrate	0.77	0.49 *	(0.04)	0.87	0.75	(0.12)
Right medial	0.89	0.71 *	(0.04)	0.95	1.49 *	(0.09)
Right lateral	1.43	0.97 *	(0.07)	1.18	1.47 *	(0.09)
Caudate	1.56	1.39	(0.11)	1.18	1.46	(0.18)
Papillary process				1.22	2.53 *	(0.16)

* indicates $P < 0.01$, t-test for paired data

were to half or double the mean flow through the whole liver and in many areas the redistribution was smaller than this.

In 4 dogs, the same procedures were carried out except that the microspheres were given into the hepatic artery. There were no significant changes in the distribution of hepatic arterial flow during histamine infusions.

DISCUSSION: Although the occurrence of outflow block in the dog liver is well known, the mechanism has been difficult to study due to the problem of recording hepatic volume quantitatively and continuously. In the dog, the plethysmographic technique has the disadvantage that the chest must be opened but the responses to hepatic nerve stimulation were very similar to those in cats breathing spontaneously (Greenway, Stark & Lautt, 1969; see page 73).

Histamine infusions into the hepatic artery or portal vein produced a dose-dependent increase in hepatic volume in dogs. This response was not seen in cats (Greenway & Lautt, 1972). The fall in arterial pressure which accompanied the response in the dogs appeared to be secondary to the pooling of blood in the liver and not due to re-circulation of the histamine, since it was absent when the pooling was prevented by simultaneous stimulation of the hepatic nerves. The intense congestion during histamine infusion cannot be explained on the basis of relaxation of the venous bed and the response must involve active constriction of some part of the vascular smooth muscle. Since the stimulation of sympathetic nerves decreases hepatic volume and histamine infusions increase it, the sites of action of the two cannot be the same. Several possible sites of action of histamine may be considered.

The possibility of a sphincter at the junction of the hepatic vein and inferior vena cava has never been conclusively disproved. The existence of sphincter-like smooth muscle bands in the dog but not the cat is well known (Arey, 1941; Thomas & Essex, 1949; Elias & Sherrick, 1969) but it has never been shown that this smooth muscle responds to histamine but not to hepatic nerve stimulation. Fluorescence studies have demonstrated that this smooth muscle has a rich adrenergic innervation which is unusual in that the nerve fibers penetrate the media of the vessels (Ungvary & Donath, 1969). In these experiments, the control hepatic venous pressure and the pressure during histamine infusions progressively increased as the catheter was introduced up the hepatic vein. It is not clear whether this was due to obstruction to flow by the catheter but it was clear that both histamine and hepatic-nerve stimulation produced qualitatively and quantitatively similar responses. Thus the different effects on hepatic volume cannot be accounted for on the basis of responses in the large hepatic veins. The similarity of the responses of isolated hepatic vein strips to histamine and noradrenaline supports this conclusion. The absence of any significant increases in hepatic venous pressure when the catheter tip was 0-4 cm within the hepatic veins specifically excluded the possibility of a sphincter at the junction of the hepatic vein and inferior vena cava.

It has been clearly shown that the site of action of histamine is post-sinusoidal. Mahfouz and Geumei (1967) perfused the canine liver through the hepatic artery and allowed the effluent to drain through both portal and hepatic veins. Histamine caused the proportion draining through the portal vein to increase markedly while noradrenaline did

not alter this proportion. This suggested that histamine caused a marked increase in post-sinusoidal resistance while noradrenaline had almost equal effects on pre- and post-sinusoidal venules. The response of the isolated portal strips confirm that portal smooth muscle is relatively insensitive to histamine. Since the large hepatic veins have been excluded as a specific site for histamine, it is reasonable to suggest that the constriction occurs in the smaller sublobular veins as shown in histological studies by Elias (1953). If histamine also constricted the small hepatic and portal venules of the liver, congestion would not occur. Thus this data with that in the literature, suggest that histamine causes a specific and intense constriction of the sublobular veins with resultant congestion of the vessels up-stream, while the sympathetic nerves cause a uniform constriction of both hepatic and portal venules with expulsion of blood from the liver. If histamine and nerve stimulation are applied simultaneously, the passive congestion is prevented except possibly in the sinusoids which are non-contractile and the hepatic volume response is similar but somewhat smaller than that during nerve stimulation alone.

The hepatic volume responses to intravenous infusions of histamine were small and variable even though arterial pressure decreased by up to 50 mm Hg. If any hepatic volume increase was prevented by simultaneous nerve stimulation, the hypotension was not significantly altered. The decreases in hepatic volume which were often seen may have been responses to release of adrenal medullary hormones during histamine infusion (Staszewska-Barczak & Vane, 1965). Therefore it seems clear that hepatic pooling plays no significant part in the hypotension due to intravenous infusions of histamine. Conversely,

it follows that circulating histamine is unlikely to be a cause of hepatic outflow block which occurs in dogs under a variety of conditions such as endotoxin administration, anaphylactic reactions and hypoxia (Greenway & Stark, 1971). Since histamine is the only vasoactive substance known to produce outflow block and since the liver of the dog contains large amounts of histamine (Ojers, Holmes & Dragstedt, 1941; Riley & West, 1953), the hypothesis that histamine release within the liver is the final common mechanism for outflow block seems likely but not proved.

In these experiments, hepatic volume increased by up to 17 ml/100 g of liver during histamine infusion. This represents a 50% increase over the normal blood content of 31 ml/100 g, and is about 7% of the total blood volume of the dog. Dale (1929) attempted to attribute some physiological significance to this phenomenon but in the intervening years no evidence has been brought forward to substantiate it.

The effects of phentolamine and phenoxybenzamine are of some interest although it is well known that phenoxybenzamine has antihistaminic actions (Nickerson, 1970a). Phenoxybenzamine and phentolamine blocked the effects of histamine and nerve stimulation in parallel and both responses were substantially reduced after doses of 2 mg/kg. However, even large doses (20 mg/kg) did not block the hypotension due to the vasodilator action of histamine. Thus the receptors mediating the excitatory vascular actions of histamine appear to differ from those mediating the inhibitory vascular actions. Outflow block can be blocked by mepyramine (Oshiro, unpublished observations) which suggests that H_1-receptors are involved while the vasodilator actions of histamine

are partly antagonized by mepyramine and appear to involve both H_1 and H_2-receptors (Black, Duncan, Durant, Ganellin, & Parsons, 1972). These problems are difficult to study since neither the arteriolar smooth muscle which controls the vasodilator actions nor the hepatic venular smooth muscle involved in outflow block can be set up in an organ bath. In the intact animal it is difficult to obtain reproducible dose-response curves before and in the presence of progressively increasing doses of blocking agents and controls after washout of the blocking agents cannot be done.

The results of the microsphere studies during infusion of histamine suggest that a redistribution of portal flow occurs from the free ends toward the hilar ends of the lobes. This redistribution is consistent with observations on hepatic venous pressures during histamine infusions. The increase in pressure during histamine infusion was greater near the free end of the lobe than at the hilar end. Therefore the pressure gradient for flow (portal minus hepatic venous pressure) was smaller at the free end of the lobe than at the hilar end during histamine infusion. Since arterial pressure is much greater than portal pressure, this increase in venous pressure is not sufficient to alter the distribution of arterial flow. It seems unnecessary to postulate any pre-sinusoidal actions of histamine to explain its effects on intrahepatic flow distribution.

SUMMARY: The plethysmograph was used to measure the increase in canine liver volume during the outflow block caused by intra-arterial or intraportal infusions of histamine. In severe outflow block the volume of the liver could increase by 50% and this was accompanied by marked hypotension. To discover what role hepatic pooling of blood played in

the hypotension caused by intravenous infusions of histamine hepatic volume was recorded during such infusion and any increases in liver volume were prevented by nerve stimulation, phenoxybenzamine or phentolamine. Since there was no significant difference in the hypotension produced by histamine before and after the prevention of the increase in liver volume it was concluded that pooling of blood in the liver was not responsible for the hypotension seen during intravenous histamine.

The mechanism responsible for outflow block was investigated. Hepatic venous pressure measurements just beyond the junction of the hepatic vein and inferior vena cava showed that pressure was not significantly increased during outflow block. Isolated hepatic vein experiments revealed that this smooth muscle was equally sensitive to noradrenaline and histamine. This data along with that in the literature suggested that contraction of sublobular veins was mainly responsible for outflow block and that there was no sphincter at the junction of the hepatic vein and inferior vena cava.

The microsphere technique was used to show that histamine caused a small redistribution of intrahepatic portal flow from the periphery toward the hilum. This finding was consistent with changes in hepatic venous and portal venous pressures during intraportal histamine infusions. Histamine did not alter hepatic arterial flow distribution.

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