Effects of Hepatic Venous Pressure on Transsinusoidal Fluid Transfer in the Liver of the Anesthetized Cat

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ABSTRACT

Arterial pressure, portal pressure, hepatic venous pressure, and hepatic volume were measured simultaneously in anesthetized cats. When hepatic venous pressure was raised, hepatic volume increased at first rapidly then slowly. After 20 minutes, the volume increased at a steady rate which continued for at least 4 hours. During this period, the hepatic blood volume (51Cr-tagged red blood cells) was constant, and fluid with a high protein content accumulated in the plethysmograph. When the hepatic lymphatics were tied, the rate of filtration (0.060 ± 0.003 ml min⁻¹ mm Hg⁻¹ 100 g⁻¹) was directly proportional to the hepatic venous pressure. When hepatic venous pressure was restored to zero, the filtered fluid was not reabsorbed by the liver. The data are discussed in relation to Starling’s hypothesis and the formation of ascites. It is concluded that transsinusoidal fluid filtration in the liver is dependent on the sinusoidal hydrostatic pressure and that no protective mechanisms are available to prevent filtration when hepatic venous pressure is raised for long periods.

ADDITIONAL KEY WORDS

plethysmograph    ascites
hepatic blood volume    Starling hypothesis    portal pressure

In cirrhosis of the liver, the primary site of vascular obstruction is postsinusoidal, and a major part of the ascitic fluid originates from the liver. Attempts have been made to correlate the formation of ascites with portal pressure, hepatic wedged venous pressure, and serum albumin levels. These data have been reviewed by Sedgwick and Poulantzas (1) and by Fomon and Warren (2). However in patients with hepatic cirrhosis, it is difficult to control the hepatic pressures and flows and to make accurate measurements of fluid formation. Studies in animals have also proved difficult. Brauer et al. (3) studied the effects of increased hepatic venous pressure in the isolated perfused liver of the rat. Hepatic volume increased and transudate with a protein concentration similar to that of plasma appeared on the surface of the liver. The red cell volume, Na space, and albumin space were increased. In dogs, partial occlusion of the thoracic inferior vena cava resulted in ascites (4, 5). There have been no quantitative studies on the relation between the rate of formation of ascites and hepatic venous pressure.

In a recent paper (6), we described a preparation to study the hepatic vascular bed in the anesthetized cat without interference with the hepatic artery or portal vein. We have now used this preparation to study the responses to increased hepatic venous pressure.

Methods

Cats were anesthetized with sodium pentobarbital (Nembutal), 30 mg/kg ip. When reflex limb and ear movements returned, additional doses of pentobarbital, 3 mg/kg, were given through a cannula in a forelimb vein. The cats were then prepared as previously described (6). Briefly, the liver was inserted into a plethysmograph and the
aperture was sealed with a plasticized hydrocarbon gel (Plastibase, Squibb). The portal vein and hepatic artery remained intact. Hepatic blood flow was drained through a cannula in the thoracic inferior vena cava and the abdominal inferior vena cava was occluded below the entrance of the hepatic veins. The blood entering the inferior vena cava below the occlusion, drained through cannulas in the femoral veins. The blood passed to a reservoir and was returned to the animal through cannulas in the jugular veins. These procedures allowed measurement of total hepatic flow and control of hepatic venous pressure. By raising or lowering the outlet of the hepatic venous cannula, the hepatic venous pressure was adjusted to any desired level, maintained there for any period, and then restored. In all experiments, the zero for the arterial, portal, and hepatic venous pressures was taken as the level at which the inferior vena cava emerged from the liver. During the periods of raised venous pressure, the volume of blood in the extracorporeal reservoir tended to decrease because of pooling of blood in the splanchnic bed and filtration of fluid into the plethysmograph. The reservoir volume was maintained by the addition of donor blood or 5% dextran (Rheomacrodex) in Ringer-Locke solution (see Discussion).

To separate filtration of fluid across the sinusoidal walls from changes in hepatic blood content, an isotope technique was used in five cats. Red blood cells in 10 ml blood were tagged with 0.2 me NaCr\(^{18}\)O\(_4\) in isotonic solution, according to the method of Veall and Vetter (7). The radioactivity from the liver was measured by placing a 5 × 5 cm NaI crystal with a collimator (5.4 cm wide and 11.5 cm long) directly over the top of the plethysmograph, and recorded with a scaler-timer (Baird Atomic Type 135) and a rate meter (t\(_w\) = 20 sec). Pulses of gamma energies above 100 kev were counted. The labeled red cells were infused until approximately 10,000 cpm were recorded over the liver and noradrenaline (1 to 2 \(\mu\)g/min) was infused to ensure mixing of the tagged cells with red cells in the spleen. It was not possible to place lead shielding between the plethysmograph and the animal; shielding over the abdomen and chest to the sides of the plethysmograph caused no significant change in the recorded radioactivity. The criticisms and difficulties of the technique are discussed later. Arterial pressure, portal pressure, total hepatic flow, hepatic volume, and radioactivity were recorded simultaneously on a Beckman Biomedical Recorder.

In some experiments, the lymphatic vessels from the liver and the hepatic nerves were tied by ligation of the connective tissue, nerves, and lymph gland round the hepatic artery. In other experiments, the plethysmograph was filled with petrolatum (USP) instead of Ringer-Locke solution. A small polyethylene tube was inserted into the lower part of the plethysmograph to allow collection of samples of the accumulating filtrate. The specific gravities of these samples and of plasma samples taken simultaneously were measured with copper sulphate solutions of various strengths (8, 9).

**Results**

**RESPONSE TO INCREASED HEPATIC VENOUS PRESSURE**

In 19 cats (2.0 to 2.8 kg body weight, mean 2.4 kg), after the preparation was completed, hepatic venous pressure was set to zero (the level of the emergence of the inferior vena cava from the liver). Mean arterial pressure was 112 ± 4.1 Hg (mean ± se), portal pressure was 8.8 ± 0.5 mm Hg, and total hepatic flow was 108 ± 6.5 ml/min/100 g liver.

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**FIGURE 1**

Response in one cat when hepatic venous pressure was increased to 4.68 mm Hg for 30 minutes.
Hepatic venous pressure was raised on 157 occasions to levels between 2.3 and 11.7 mm Hg. These pressures were maintained for periods between 20 minutes and 5 hours. The general picture is illustrated in Figure 1 and the quantitative data are discussed in the subsequent sections. Arterial pressure showed little consistent change while portal pressure increased rapidly and then remained at the new level throughout the period of raised venous pressure. Hepatic volume increased rapidly at first, then slowly. After a period of 5 to 20 minutes, the rate of increase in volume became steady and this rate was maintained, with minor brief fluctuations, for the duration of the period of raised venous pressure. On restoration of the venous pressure to zero, hepatic volume decreased. The decrease was larger than the rapid increase when venous pressure was raised but was much smaller than the total volume increase during the period of raised hepatic venous pressure. Thereafter the volume remained constant. In two cats, hepatic venous pressure was raised to 9.3 mm Hg for 4 to 5 hours. The steady increases in volume after 20 minutes were maintained for 4 to 5 hours.

In three experiments, the plethysmograph had been filled with petrolatum. Shortly after hepatic venous pressure was raised, a layer of clear fluid could be seen on the surface of the liver. This fluid gradually accumulated in the bottom of the plethysmograph and samples were taken at intervals. Its specific gravity was 85 to 94% (mean 90) of that of plasma samples taken simultaneously. The volume of this fluid which accumulated within the plethysmograph was similar to the residual increase in total volume after venous pressure was restored to zero.

PORTAL PRESSURE RESPONSE

The increment in portal pressure which occurred when hepatic venous pressure was raised to various levels on 157 occasions in 19 cats is shown in Figure 2. When hepatic venous pressure was raised by less than 5 mm Hg, 50% of the increase was reflected in portal pressure, and when hepatic venous pressure was raised to 12 to 14 mm Hg, 75% of the increase was reflected in portal pressure. On restoration of hepatic venous pressure, portal pressure returned to the control level. The increments were reproducible to within 1 mm Hg on repetition.

HEPATIC BLOOD VOLUME

In five cats, Cr\(^{31}\) - tagged red blood cells were injected as described in the Methods. On 14 occasions, the change in radioactivity of the liver was measured when hepatic venous pressure was increased by 4 to 10 mm Hg for periods of 60 minutes. On raising the venous pressure, radioactivity increased at first rapidly then slowly for 5 to 20 minutes. Thereafter little change or only small increases in radioactivity occurred. After 2 minutes, the mean increase in radioactivity was 51 ± 6% (mean ± se), and after 20 minutes, 90 ± 5% of the increase in the 60-minute period. The small increases in radioactivity between 20 and 60 minutes were sometimes accompanied by irregularities in the otherwise steady change in liver volume, due to movements of the animal or administration of supplementary doses of pentobarbital. Thus the progressive increase in total volume during the later part of the period of higher hepatic venous pressure was not due to a change in hepatic blood volume.

For reasons discussed later, it proved...
extremely difficult to calibrate the changes in radioactivity in terms of changes in hepatic blood volume (in milliliters). An approximate calibration was made on five occasions in four cats when hepatic venous pressure was raised to 9.3 mm Hg for 1 hour. It was assumed that the change in total volume during the first 2 minutes was due only to a change in hepatic blood content, that is, the increase in radioactivity and the change in total volume at 2 minutes were equated. This may involve an overestimation of the blood volume changes (see Discussion). The results for these experiments are shown in Figure 3. After 20 minutes, the total volume increased at a steady rate while the blood content was almost constant. Subtraction of these two curves gives a measure of the amount of fluid filtered across the sinusoidal walls. The rate of filtration remained constant from 20 to 60 minutes after raising hepatic venous pressure. Calculation of the rate of filtration during the first 20 minutes was not justified due to the difficulties in accurate calibration of the blood volume and the small differences between blood volume and total volume. On restoration of hepatic venous pressure to zero, blood content returned almost to the control level while total volume remained increased by an amount approximately equal to the calculated volume of filtrate. The large standard errors in this figure reflect the variation in the slopes in different animals and not marked irregularities in the individual recordings (see Fig. 1).

Thus it is clear that when hepatic venous pressure is raised, the increased total volume is due to an increase in hepatic blood volume which becomes almost constant in 5 to 20 minutes and a filtration of fluid which continues at a steady rate for the period of raised venous pressure. An accurate measurement of the steady-state filtration rate is obtained after 20 minutes.

RELATION BETWEEN FILTRATION RATE AND HEPATIC VENOUS PRESSURE

In four cats, the hepatic nerves and lymphatics were tied. Hepatic venous pressure was increased in steps of 2.34 mm Hg up to 11.7 mm Hg and then decreased in the same steps. Each venous pressure was maintained for 30 minutes and the steady-state filtration rate was determined from the slope of the volume recording during the last 5 minutes of each period. Seven complete curves were obtained and the relation between filtration rate and hepatic venous pressure is shown in Figure 4A. The steady-state filtration rate is a linear function of the hepatic venous pressure with a slope of $0.060 \pm 0.003 \text{ ml} \cdot \text{min}^{-1} \cdot \text{mm}^{-1} \text{Hg} \cdot 100 \text{ g}^{-1} \text{ liver (mean} \pm \text{ SE).}$ The values during the stepwise lowering of the venous pressure were very similar to the values during the stepwise raising of the pressure. The relatively large standard errors again reflect
the fact that although the relationship was linear in each cat, the slope varied quite markedly in different animals.

In six cats, the lymphatic vessels and hepatic nerves were not tied. The results are shown in Figure 4B. The values in these preparations were not significantly different statistically from those in animals whose lymphatics were tied due to the large variation between animals. However, the mean values suggest that with lower hepatic venous pressures, the measured filtration rates were somewhat smaller when the lymphatics were open. Unfortunately it was not possible to study both situations in the same cat since the lymphatics could not be tied after the liver was placed in the plethysmograph.

Discussion

The general state of the animals and the stability of the preparations were discussed previously (6). The major difficulty experienced in the experiments reported here was continuous measurement of the hepatic blood volume. The radioactivity technique has been used previously for studies on the vascular beds of skeletal muscle (10) and intestine (11), but application of the technique to the liver was relatively unsatisfactory. It was not possible to place lead shielding underneath the plethysmograph and radioactivity from the tissues below the liver was recorded. However it seems unlikely that this radioactivity changed markedly when hepatic venous pressure was increased. Changes in radioactivity from the intestine and spleen were not recorded since these organs lay outside the collimated area. The changes in radioactivity, therefore, reflected changes in hepatic blood volume but the absolute level of radioactivity could not be correlated with hepatic blood volume. Calibration was difficult and to obtain the data shown in Figure 3, the total volume change in the first 2 minutes after venous pressure was increased. Such a procedure overestimates the change in blood volume since some fluid is filtered during this period. The change in blood volume is large, however, and the error is unlikely to exceed 25% (if the initial filtration rate were four times the steady-state filtration rate). Another difficulty was also experienced. Throughout, and especially towards the end of each experiment, the radioactivity over the liver increased slowly even when hepatic venous pressure was zero. At the end of each experiment, some radioactivity remained even when most of the blood was removed by perfusion of the liver with saline. It seems likely that some tagged red cells were destroyed and phagocytosed by the reticuloendothelial system of the liver. The slow increase in radioactivity during prolonged increases in hepatic venous pressure may have been due to this factor and the delayed compliance in the hepatic vascular bed may have been overestimated. However, the data on radioactivity in this paper were obtained within 2 hours after administration of the labeled red cells, and at this time the error in the hepatic blood volume measurements due to slow accumulation was less than 5%. In the intestine the increase in blood volume was almost complete after 2 minutes (11), but in the liver it took 5 to 20 minutes before the blood volume became almost steady. Since after 2 minutes the blood volume had increased to only 50% of its final level, determination of the slope of the total volume increase at this time was not an accurate measure of the initial filtration rate, and determinations of capillary filtration coefficient (6) overestimate the true value. However, the error may not be as serious as it at first appears, since after repeated brief increases in hepatic venous pressure the delayed compliance was much less marked than in the experiments reported here.

Twenty minutes after the hepatic venous pressure was increased, the hepatic blood volume had reached a steady level. However the total volume continued to increase and this was associated with accumulation of fluid in the plethysmograph. In these experiments, extra fluid was added to the vascular system to compensate for the fluid filtered into the plethysmograph. If this were not done, the rate of filtration would undoubtedly become progressively slower.
It was shown that 50 to 75% of the increment in hepatic venous pressure was transmitted to the portal vein. Since the greatest pressure drop between the portal and hepatic vein appears to occur in the portal radicals within the liver (12), it is probable that sinusoidal pressure is almost equal to hepatic venous pressure and that changes in hepatic venous pressure cause quantitatively similar changes in sinusoidal pressure. Our data (Fig. 4A), therefore, suggest that the steady-state filtration is directly proportional to sinusoidal hydrostatic pressure. These results are in marked contrast to the results observed in the intestinal vascular bed where the filtration ceased after 5 to 8 minutes. It was suggested that the cessation of filtration was principally due to an increase in tissue hydrostatic pressure and possibly also to a decrease in the interstitial colloid osmotic pressure (11, 13, 14). The hepatic sinusoidal wall appears to be permeable to substances of high molecular weight (15) and this is confirmed by the high specific gravity and hence protein content (9) of the filtered fluid in these experiments. It would, therefore, appear that the colloid osmotic pressure across the sinusoidal wall is near zero and plays no significant role in hepatic transsinusoidal fluid exchange. Further work to test this suggestion is in progress. Since the filtration at any given venous pressure continues for at least 4 to 5 hours, increases in tissue hydrostatic pressure in the liver are not sufficient to abolish the transsinusoidal hydrostatic pressure gradient. Because we could not accurately measure the initial filtration rate, it is not possible to decide whether an initial high filtration rate is reduced to a lower steady rate by some rise in tissue pressure.

When lymphatic drainage was prevented, the filtration rate was directly proportional to the hepatic venous pressure in all the cats although the absolute rate of filtration varied in different animals. When the lymphatic drainage was intact, the accumulation of fluid in the plethysmograph during small venous pressure increments may have been reduced. This would be reasonable since distention of the lymphatics and increased hepatic lymph flow during periods of raised hepatic venous pressure have been reported (3, 16). The role of lymphatic drainage may be underestimated in these experiments since insertion of the liver into the plethysmograph may have resulted in some obstruction to the hepatic lymph vessels. This aspect requires further study.

On restoration of hepatic venous pressure to zero, the hepatic blood volume returned towards the control level and 80% of the increase during the period of raised venous pressure drained from the liver. The filtered fluid remained in the plethysmograph and no evidence of reabsorption of this fluid was obtained. Reduction of hepatic venous pressure below zero had no effect on the hepatic volume or portal pressure and this confirms previous reports (3).

The data suggest a reasonable mechanism for the formation of ascites in hepatic cirrhosis and congestive heart failure. Raised hepatic venous pressure causes continuous transsinusoidal filtration of a fluid of high protein content. Some of this fluid is drained by the hepatic lymphatics while the remainder accumulates in the peritoneal cavity. Some of the fluid may also originate from other sources (5). It is difficult to understand why the formation of ascites from the liver should be related to a reduction in plasma colloid osmotic pressure (17) and it seems more likely that this factor plays a role in formation from other sources or in reabsorption of the ascitic fluid.

In conclusion the data suggest that transsinusoidal filtration of fluid in the liver is dependent on the sinusoidal hydrostatic pressure and that no protective mechanisms are available to prevent this filtration when hepatic venous pressure is raised for prolonged periods.

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HEPATIC VENOUS PRESSURE AND FLUID TRANSFER


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