Changes in liver function and structure due to experimental passive congestion under controlled hepatic vein pressures[1]

R. W. BRAUER, R. J. HOLLOWAY AND G. F. LEONG
U.S. Naval Radiological Defense Laboratory, San Francisco, California

BRAUER, R. W., R. J. HOLLOWAY AND G. F. LEONG. *Changes in liver function and structure due to experimental passive congestion under controlled hepatic vein pressures.* Am. J. Physiol. 197(3): 681–692. 1959.—The effect of various hepatic vein pressures on hepatic hemodynamics, on fluid shifts within the liver, and on certain indices of liver function were studied in the isolated rat liver preparation. In general, venous pressures are scarcely perceived by the hepatic parenchyma unless they exceed the hydrostatic pressures acting at the liver surface, more particularly at the hilum of the liver. Venous pressures exceeding this value appear to distend the liver vasculature so that it acts like a system of rigid conduits; there is great expansion of the sodium space and of the colloid distribution spaces, and some evidence of partial sequestration of blood in the congested liver. All these changes impair oxygen supply to the tissue. To a lesser extent they interfere with the transfer of other substances from blood to parenchyma, and place considerable stress upon the mechanical framework of the liver. The resulting strain in turn tends to limit the extent of intrahepatic changes in passive congestion, and possibly the rate of fluid leakage from blood via parenchyma to transudate at the liver surface.

PASSIVE CONGESTION OF THE LIVER has been studied repeatedly in the past. Gross changes include increased liver volume and 'sweating' of the liver surface as well as greatly increased liver lymph flow (1–3). Liver histology, on the whole is changed to a surprisingly slight extent (1), and such changes as are observed appear to be readily reversible. The hepatic vein pressures needed to elicit these changes, however, the effects on splanchnic hemodynamics, and the alterations in liver function attendant on various degrees of passive congestion of the liver, have not been studied in any detail in the past.

The isolated perfused rat liver (4) is well suited to just such analysis since it allows independent modification of all circulatory parameters, and since methods for fluid compartment analysis and for the evaluation of a number of liver functions have been developed for this preparation. Studies of the effect of various venous pressures on liver hemodynamics, on liver function, and on liver structure have been conducted with this system. Venous pressures which are less than the hydrostatic pressure at the liver surface (especially at the hilum) scarcely affect the organ in any way. Higher venous pressures distend the vascular tree so that it rapidly assumes the properties of a rigid pipe system, and also result in a great expansion of the extravascular extracellular space. Liver function is affected partly by the barrier to oxygen transfer imposed by the edema fluid, and partly by the mechanical stress imposed on the structural framework of the liver. The experiments upon which these conclusions are based, and the bearing of these observations on the part played by the hepatic circulation in the regulation of liver function, will be discussed in the remainder of the present communication.

METHODS

Perfusion Techniques

Livers from male Sprague-Dawley rats, NRDL strain, weighing 350–400 gm, were perfused with rat blood through the portal vein, using essentially the same apparatus and procedure as previously described (4, 5). To control hepatic vein pressures, the surgical procedure was amended to include ligation of the caudal stump of the vena cava remaining attached to the liver; the assembly shown in figure 1 was substituted for the simple organ support funnel employed in previous studies.

Hepatic vein pressures could be raised by progressively closing the jaws of screw clamp *S*; venous pressures attained are indicated by the height of the fluid column in the side tube *M*. A cathetometer was employed to measure both portal and hepatic venous pressures, readings being referred to the level of the hilum of the liver as a common zero. Under basic conditions, then, the venous pressure is negative, usually -3 to -4 cm H_2O, while portal pressures are $+12.5$ to 13.5 cm H_2O unless otherwise specified.

In practice it was found convenient to defer connecting the T-tube assembly T-E-M to the vena cava can-

Received for publication March 20, 1959.
[1] This work was supported in part by a grant from the San Mateo County Heart Association.

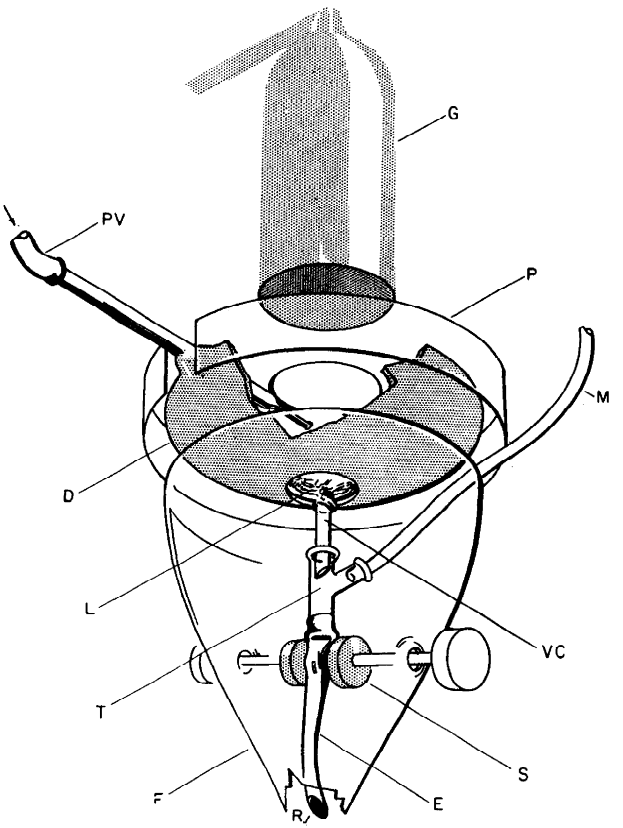

FIG. 1. A modified liver support arrangement for passive congestion experiments. PV—portal vein cannula and blood supply; L—liver; VC—vena cava cannula; T—T-tube; M—manometer tube; E—rubber tube extension of vena cava outflow; S—screw clamp for regulation of venous pressure; R—outflow toward blood reservoir; F—support funnel; D—organ support dish; P—Petri dish cover with central plastic covered opening for use with G—Geiger Müller tube for continuous radioactivity recording.

nula VC for about 30 minutes after beginning the perfusion, and to allow another 60 minutes for equilibration and removal of vasoconstrictor substances from the perfusate. Only then were experimental procedures begun. These were conducted at 38°C except in a few instances to be described under results. The standard protocol included a base line observation period under the standard pressure and temperature conditions listed above, a series of observations at the desired experimental pressures in portal and hepatic veins, and a final observation period after restoration of basal perfusion conditions.

Blood flow through the liver was measured by clocking the accumulation of a predetermined volume of blood in the collecting funnel after temporarily clamping the hose draining the funnel into the blood reservoir. At the end of each experiment the weight of the exsanguinated liver was determined and tissue specimens were placed in formalin for histological examination; hematoxylin and eosin staining was employed throughout.

Hepatic extraction efficiencies were determined for $CrP^{32}O_4$ colloid (6) and for $BS^{35}P$ (sodium phenoltetrabromphthalein di-S^{35}-sulfonate (7)). The test substance was injected into the reservoir, mixed thoroughly with the perfusate, and portal vein and hepatic vein samples were collected at 2, 4 and 8 minutes after injection, allowing 10 seconds for transit time across the liver. Blood concentrations of both isotopes were measured by count rate determinations on dried planchet samples, using an end window GM counter. Details of sample preparation, counting procedures, and calculations have been previously described, for BSP (8), for $CrPO_4$ (9). Calculated extraction efficiencies have a probable error of $\pm 3\%$.

Bile flow was determined by collecting bile in Wintrobe hematocrit tubes and recording the rate of accumulation. Bile secretion pressure (more correctly limiting bile pressure) was determined by observing the volume of bile rising in a vertical collecting catheter, and noting the level approached asymptotically (10).

Fluid Compartment Studies

To remove tissue samples without fluid loss from the specimen, a portion of the liver was separated from the rest by clamping it off with a narrow hemostat and severing it from the remaining liver by a scalpel incision along the side of the clamp away from the specimen. Clamp and sample were then blotted lightly and rapidly with tissue paper, and removed to a weighed beaker where the tissue was separated from the clamp by a second scalpel cut following closely along the surface of the clamp. The weighed tissue sample (0.5–1.5 gm) was either digested in fuming nitric acid, or transferred intact to a container fitting a well-type scintillation counter with appropriate geometry. Digestion was employed as preliminary to scintillation counting in the case of Na^{22} and Fe^{59} analyses, but was omitted for I^{131} analyses.

The same sampling and digestion procedures were followed for tissue P^{32} analyses, but here the digest was boiled down, made up to volume, and aliquots were pipetted onto filter paper covered planchets. These were dried and counted under a 1.4 mg/cm² end window counter.

The forms in which the isotopes were administered, the standardization procedures employed, and the manner of expressing the data for each isotope were as follows:

Na^{22} was administered as NaCl in 0.9% saline and standardized for each experiment by comparison with a plasma sample collected just prior to obtaining the tissue specimen. From the results, the plasma equivalent Na^{22} space was calculated as the volume which would contain the same amount of Na^{22} as 100 gm of tissue, if the Na^{22} concentration in the fluid equaled that in the corresponding plasma sample. The Na^{22} does not enter rat erythrocytes to a significant degree during 60 minutes in the perfusion system; thus the actual, corrected hematocrit (Wintrobe) was not significantly different from the calculated hematocrit based on whole blood and blood plasma Na^{22} determinations and the assumption of an exclusively extracellular Na^{22} distribution.

Radioiodinated human serum albumin (IHSA) free of

dialyzable radioactivity was standardized for each experiment by reference to the corresponding plasma samples. Plasma equivalent albumin space was determined in the same way as the Na^{22} space, substituting IHSA for Na^{22}. Time of contact, more critical in the case of albumin than in that of Na^{22}, is taken as 30 minutes after the last previous change in circulatory parameters, and 30–60 minutes after IHSA administration. (See Scanning results, below.)

Fe^{59}-labeled rat erythrocytes were obtained from donor rats injected with 100 μc of $Fe^{59}Cl_3$, 7 days previously. Data are standardized against whole blood collected just prior to removing the tissue specimen. Results are converted to 'erythrocyte mass' by multiplication with the corrected hematocrit of the standard blood sample and are expressed per 100 gm tissue.

$CrP^{32}O_4$ colloid was used to introduce a firmly fixed marker into the tissue. If sufficient time was allowed for substantially complete removal of injected $CrPO_4$ from the circulation (about 30 min. result in 98–99 % uptake), the total quantity of P^{32} in the liver becomes a constant, and changes in tissue volume can be calculated from tissue P^{32} concentration, by applying the relation

$$\frac{V_1}{V_2} = \frac{(CrPO_4)_2}{(CrPO_4)_1} \qquad (1)$$

where V_1 and V_2 are the liver volumes at *times 1* and *2*, and $(CrPO_4)_1$ and $(CrPO_4)_2$ are the corresponding concentrations of $CrPO_4$ (measured as P^{32}) in representative aliquots of liver. Two conditions which must be fulfilled to warrant such calculations are: *a*) even, or at least sufficiently even, distribution of the colloid throughout the liver and *b*) the absence of superimposed alterations in tissue volume due to the sampling procedure. The first of these conditions is satisfied in the isolated rat liver preparation provided the tissue samples weigh at least 2.0 gm; in control experiments it could be shown that, if samples of this size were taken, variations in $CrPO_4$ concentration between samples were less than ± 2 %. The second condition was tested by collecting two successive tissue samples, 40 minutes apart, from each of a series of preparations perfused under standard conditions, leaving perfusion pressures unchanged between samples. To permit hemostasis a whole lobe (usually the left lateral) was removed as the first tissue aliquot, the pedicle being ligated at once after resection. In this series it was found that $CrPO_4$ concentrations in the second aliquot were 2–3 % (average 2.4 %) below those in the first tissue sample. These results were sufficiently regular to warrant making the appropriate correction in the experimental samples. The standard procedure adopted was to collect the first tissue sample when venous pressures were elevated, then restore normal perfusion conditions, and, after a period of 30 minutes or more, to collect the second tissue aliquot. The reverse order was followed in three experiments, but the results obtained were not distinguishable from those derived by the standard sequence of pressures.

Time course of changes in concentration of γ emitting tracers in the tissue following venous pressure changes was observed by placing a shielded 1-inch diameter end-window GM tube (400 mg/cm^2) 1 inch from the surface of the perfused liver, and recording the count rate in the tissue by means of a count rate meter-recording ammeter system. The base line count rate not due to the liver was obtained at the termination of the experiment, after removal of the liver from its position in the apparatus, all other elements of the perfusion circuit being left in place. Using successive doses of isotope it could be shown that the relation between count rate recorded in the tissue and tissue total isotope content was linear within the limits of reliability of these measurements (± 10 %) for Na^{22}, Fe^{59} and I^{131}.

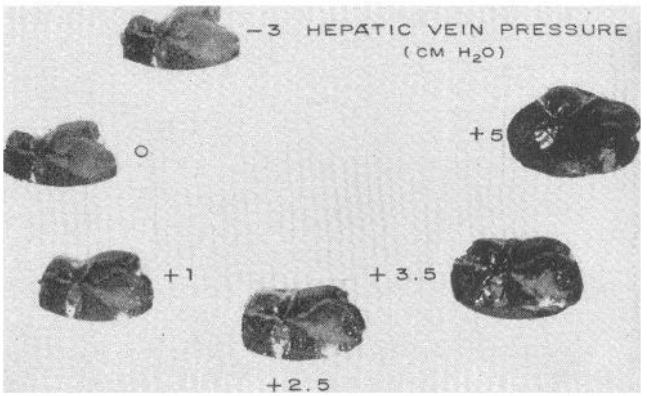

FIG. 2. Appearance of 1 liver (*58-89*) at successive venous pressures (the experiment progressed clockwise). Note beads of transudate if venous pressure > +1 cm.

Liver blood volume was estimated not only by tissue analysis, which yields maximum values for the distribution spaces of erythrocytes and plasma proteins, but also by transient analysis, to yield values referred to below as single passage distribution spaces. For this purpose small volumes (0.3 ml or less) of blood containing either I^{131}-labeled serum albumin or Fe^{59}-labeled erythrocytes were injected rapidly into the portal vein cannula close to the liver; the blood from the vena cava cannula was temporarily diverted onto a rotating sample collector which divided the effluent stream into 40 equal samples during the 90-second period following the injection pulse. The isotope content of the separate samples was determined as described previously, and plotted on semilogarithmic coordinates as a function of time. The single passage dilution space was then calculated from the slope of this curve and the known perfusion rate, using the relation

$$D_x = -\frac{100 b_t}{\left(\frac{d \log x}{d_t}\right)} \qquad (2)$$

where D_x is the single passage dilution space for x in milliliters, b_t is the erythrocyte or the plasma flow rate in milliliters per second, and $(-d \log x)/d_t$ is the negative slope of the descending portion of the dilution curve, the natural logarithm of the blood concentration of x being plotted as a function of time in seconds. The limitations of this method, and the probable interpretation of the compartments derived will be discussed elsewhere (11).

TABLE 1. *Total Protein and Albumin Distribution Between Transudate and Blood Plasma*

	Plasma	Transudate	Transudate / Plasma
Total protein content, %	4.48 (3.8–3.8)	4.52 (3.7–5.1)	1.02 (0.98–1.07)
IHSA content (relative)	1.00	1.02 (0.96–1.07)	1.02 (0.96–1.07)
$\frac{\text{IHSA}}{\text{Total protein}}$ (rel.)	1.00	1.00 (0.98–1.02)	1.00 (0.98–1.02)

The probable error of the single passage distribution spaces here reported is 8%.

For intercomparison of fluid compartment data all values are expressed as percentage of tissue weight under basal perfusion conditions. In the case of single passage dilution spaces, changes are measured directly, and the base line value can be computed from the absolute volumes measured, together with the tissue weight at sacrifice, or it can be derived from mean values obtained in previous studies under basal perfusion conditions which were not altered between the compartment determination and final weighing of the liver (to be published by R. J. Holloway, G. F. Leong and R. W. Brauer). Actually both procedures yield identical results due to the reversiblity of tissue volume changes in passive congestion. Tissue analyses, on the other hand, yield results in terms of plasma equivalent space per 100 gm tissue weight at the time of sampling. To convert these results to absolute volumes which can then be expressed as percent of original tissue weight requires three steps: *a*) multiplication by the final tissue weight at sacrifice under basal perfusion conditions, followed by: *b*) multiplication by a factor expressing the volume change between basal conditions and perfusion conditions at the time of sampling. This factor is the factor V_1/V_2 of *equation 1*, provided *1* and *2* refer to sampling and sacrifice times, respectively. This factor was never larger than 1.3 in the present experiments, and could be derived either directly from the $CrPO_4$ concentration data or from the combined Na^{22} and erythrocyte space data (see DISCUSSION, fig. 5); *c*) division of the absolute volume calculated in steps *a* and *b* by the tissue weight under basal conditions, a step which obviously reverses step *a*. Thus, in actual practice plasma equivalent spaces derived from tissue analysis were multiplied only by the volume correction factor to obtain directly results expressed as percent of tissue weight under basal conditions.

Transudate appearing at the liver surface was collected for chemical analysis by aspiration into small capillary pipettes. Total protein content (by the biuret method (11)), and I^{131} content (in preparations to which radioiodinated human serum albumin had been added previously) were determined in such transudate as well as in simultaneously collected blood plasma samples. Electrophoretic patterns for semiquantitative comparison were prepared by filter paper electrophoresis (R. M. Garver, unpublished method). Approximate evaluation of transudation rates was achieved by mopping well defined portions of the liver surface with small weighed tissue paper sponges for a specified period of time and reweighing the sponges.

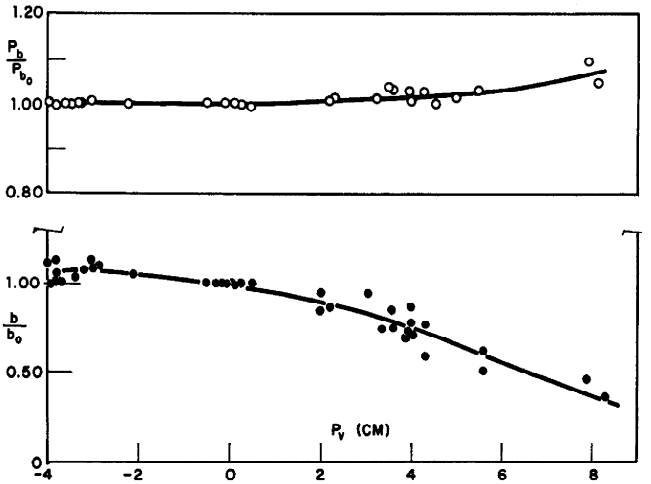

FIG. 3. Effect of venous pressure changes on perfusion rate and portal pressure in a series of isolated liver preparations. b, b_0—perfusion rates at the experimental venous pressure and at 0 cm H_2O venous pressure, respectively; P and P_0 are the corresponding portal pressures, where P_0 ranged from +12.4 to 13.2 cm H_2O.

RESULTS

Appearance of Liver Surface

If hepatic vein pressures are increased by gradual steps from the basal level (−3 cm H_2O relative to the hilum of the liver) to +7 or +8 cm H_2O, portal pressure being held nearly constant at +12.5 to +13.0 cm H_2O, the appearance of the liver undergoes the changes typified by figure 2. There is little visible effect until venous pressures exceed the zero level. As little as +1 cm H_2O, however, produces visible congestion of the liver surface; transudate collects in droplets until the entire liver surface appears uniformly wet; the sharp liver margins become rounded as the organ seems to swell. When venous pressures exceed +5 cm, the lobular architecture is no longer recognizable, and the degree of congestion is such that one might suspect extensive hemorrhages. Histopathological examination however, shows that extravasation has not occurred and within a few minutes after restoring negative venous pressures, the abnormal appearance of the liver surface has either disappeared entirely or shows only a few minute red areas where congestion persists for some time. Only if venous pressures exceed +10 cm H_2O is there positive evidence of disruption of the structural framework of the liver.

Transudate Formation

There is a rapid increase in transudate formation as venous pressures are raised from 0 to +4 or +5 cm, and an apparent leveling out with further pressure increase: at +7 cm transudate formation is only 10–15% greater than at 5 cm H_2O. Erythrocytes do not appear in the transudate until venous pressures exceed +4 cm; at higher venous pressures the transudate may appear definitely 'hemorrhagic' with hematocrits which may run, in an exceptional case, as high as 10% (at +8 cm H_2O).

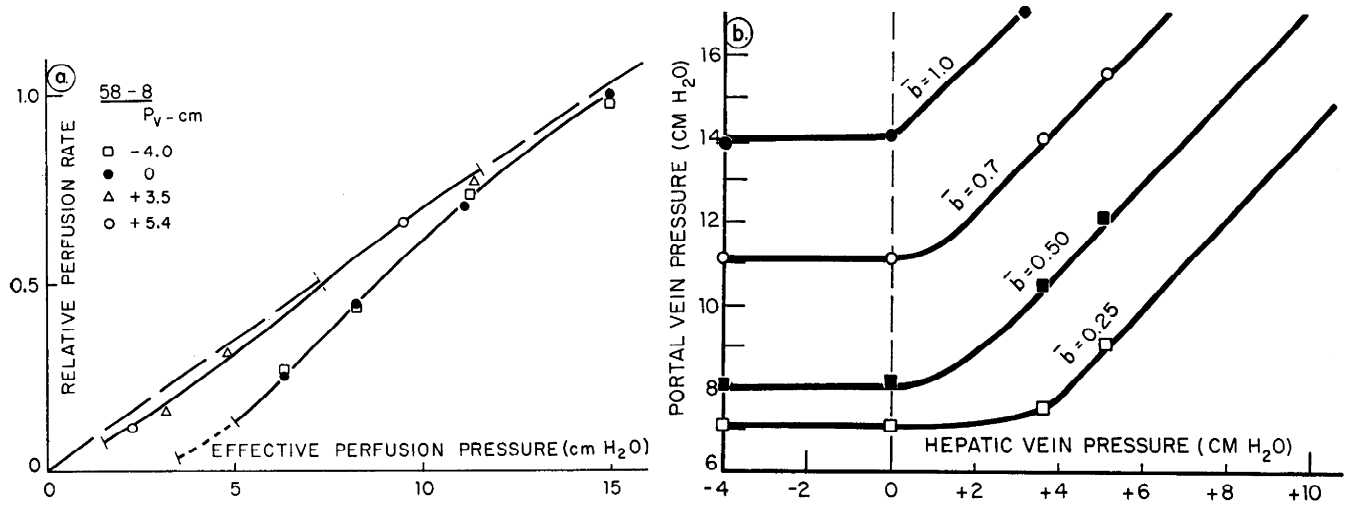

FIG. 4. Flow pressure relations for the isolated rat liver preparation at different venous pressures. *a*, effective perfusion pressure vs. perfusion rate (relative to perfusion rate at a portal pressure of 14.0 cm H_2O, venous pressure = −4.0 cm) at venous pressures indicated under P_v (see text for convention applied to determine effective perfusion pressure). *b*, portal pressure, P_b, required to maintain the perfusion rate b indicated as hepatic vein pressure, P_v, is varied. Same data as figure 4*a*.

Filter paper electrophoresis failed to indicate marked differences between the proteins contained in the transudate and those of the blood plasma. Quantitative analysis confirmed this impression: not only total protein content, but also iodinated albumin concentration was substantially the same in perfusate plasma as in transudates collected at venous pressure of +4 cm H_2O (table 1).

Histological Observations

Liver specimens collected during exposure to venous pressures up to +8 cm, when compared to livers perfused under basal perfusion conditions showed evidence of congestion, including distended central veins and enlarged sinusoids, edema of the connective tissue of the portal areas and dilatation of the lymphatic channels. Frequently centrilobular areas showed less than ordinary staining of the cytoplasm of the hepatic cells, and evidence of some increase of cytoplasmic volume. In such areas one frequently encountered numerous cells containing vacuoles not due to lipid droplets. In about 20% of the preparations there was evidence of separation of the sinusoidal endothelia from the underlying parenchymal cells, giving the appearance of a serous perisinusoidal space. Frank hemorrhages into the tissue were not seen except for a few isolated subcapsular areas which may have been due to surgical trauma. The overall impression gained is that the observed changes are of a low order of intensity, and of such evanescent character that chemically fixed specimens may well give an incomplete picture of the appearance of the tissue while exposed to positive venous pressure.

Hemodynamics

As hepatic vein pressures are gradually raised, initial portal pressures being between 12.5 and 13.0 cm H_2O, the perfusion rate is not measurably affected until venous pressures rise above the hilar level (fig. 3). Further increases in venous pressure decrease the perfusion rate, gradually at first, and at a progressively faster rate as venous pressures exceed +3 cm H_2O. Portal pressure changes, also shown in figure 3, are small in this particular setting; they merely reflect the inability of the pressure regulating system of the perfusion apparatus to completely buffer out such portal pressure fluctuations.

If portal pressures, as well as hepatic vein pressures are each set at several levels, a series of flow pressure diagrams can be constructed (fig. 4). Such curves must be compared on the basis of the effective perfusion pressures driving blood through the hepatic vasculature. If venous pressures are zero or above, effective perfusion pressure is equal to the pressure difference between portal and hepatic veins. No a priori assumption can be made, however, regarding the degree to which 'negative' venous pressures are transmitted to the hepatic vasculature. The shape of the blood flow—venous pressure curve of figure 3, and the appearance of the liver shown in figure 2 suggest as a reasonable hypothesis that negative venous pressures are not transmitted to the parenchyma at all; in this region effective perfusion pressure may be assumed, therefore, to equal portal pressure alone. Figure 4*a* shows a typical experiment plotted using this dual definition of effective perfusion pressure. In this as in five other experiments, the flow pressure diagram for zero venous pressure was not distinguishable from that for venous pressures of −3 and −4 cm plotted using portal pressure as the sole measure of effective perfusion pressure, vindicating the above hypothesis.

At hepatic vein pressures above the hilar zero level, the sigmoid flow pressure diagram typical of the negative venous pressure zone is straightened out to approximate a straight line such as would represent the constant resistance to nonturbulent flow of a rigid conduit system. The slope of the particular line approached by these points, furthermore, seems to be the same as that of the line defining the constant limiting resistance of the

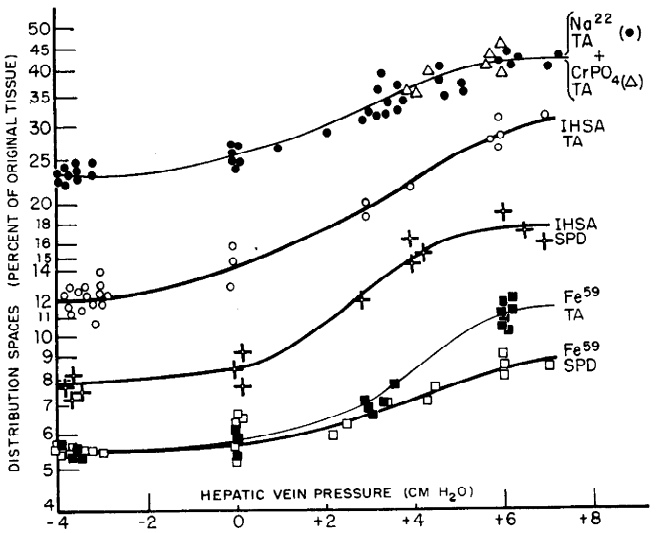

FIG. 5. Fluid compartment analysis of a series of isolated liver preparations perfused at portal pressures of 12.5–13.2 cm H_2O, and variable hepatic vein pressures. Test substances used indicated at the right. TA refers to tissue analysis data, SPD refers to single passage distribution analyses. In the top curve, the *triangles* represent the calculated sodium space based on $CrP^{32}O_4$.

hepatic vascular tree at high portal pressures in the same preparations (5). The points obtained for several positive venous pressures in figure 4*a* fall on a common flow pressure curve, confirming the impression that this is the flow pressure diagram for the fully distended, and therefore rigid, hepatic vascular tree. Similar results were obtained in the remaining experiments of this series, although the degree of 'straightening out' of the flow pressure curve is somewhat variable from one liver to the next.

Figure 4*b* shows another way of looking at the same data. The question posed here is: how much must the portal pressure be changed to maintain perfusion rates constant at one of several levels when hepatic vein pressures are increased progressively. (This, after all, is close to being the actual situation expected *in vivo*.) The points plotted are derived from figure 4*a* by drawing horizontals at constant relative perfusion rates of 1.0, 0.75, 0.50 and 0.25, determining the intercepts with the flow pressure diagrams, and subtracting the proper venous pressures from the effective perfusion pressures to obtain the corresponding portal pressures. As before, negative venous pressures are without measurable effect. To maintain relative perfusion rates of 1.0 or 0.75, portal pressures must be increased at about the same rate as venous pressures virtually as soon as the latter exceed zero. At lower perfusion rate levels, portal pressures can lag a good deal behind hepatic vein pressures; thus, the centimeter-for-a-centimeter relation of portal to hepatic vein pressure increments is not established at a relative perfusion rate of 0.25 until hepatic vein pressure has reached +3 to +4 cm H_2O.

Fluid Compartments

Hepatic vein pressure changes result in fluid compartment changes which in turn underlie the altered appearance of the congested livers shown in figure 2. The time course of these changes can be conveniently studied by administering suitable isotopic tracers (Na^{22}, Fe^{59} erythrocytes or IHSA), allowing time for attainment of equilibrium distributions under basal perfusion conditions, and then continuously recording radioisotope content changes in the tissue as venous pressures are increased. Thus, following an increase in venous pressure from 0 cm H_2O to +4.0 cm H_2O, the tissue Fe^{59} increases promptly, reaching a new steady level within 3–5 minutes. The iodinated albumin concentration rises equally rapidly at first, but continues to increase for at least 15 minutes; thereafter, the increase becomes very slow, so that between 30 and 60 minutes after the venous pressure has increased, the tissue albumin content increases by no more than one-twentieth of its value. Sodium-22 accumulation follows a time course rather similar to that for Fe^{59} erythrocytes; it tends to be rapid immediately after venous pressure has been increased, and to reach near equilibrium within less than 5 minutes. Occasionally this initial change is followed by a more gradual increase of small magnitude which may last for another ten minutes, and add another twentieth to the tissue sodium content. All told, then, 30 minutes after the venous pressure change, tissue concentrations of Fe^{59}-labeled erythrocytes, and of Na^{22} ion represent substantially true equilibrium values, while the albumin distribution at this time is in a quasi equilibrium, probably largely representative of its maximum extracellular distribution.

Return to negative venous pressures is reflected by prompt return of radioisotope contents to or to near the original values. In particular, Na^{22} levels have returned to within a small percentage of their precongestive levels within 3 minutes after negative venous pressures have been restored; Fe^{59} erythrocyte levels likewise retain no perceptible imprint of passive congestion episodes unless venous pressures have been raised to destructive levels of +10 cm or more.

The results of more precise analytical determinations by tissue analysis or by dilution curve analysis, are compiled in figure 5. The logarithmic scale of the ordinate allows visual intercomparison of relative degrees of change. Inspection of the figure as a whole reveals two general trends: the first, already familiar from the surface appearance and the hemodynamic data, is that venous pressure changes in the range below hilar pressure cause far smaller changes—or none at all—in the parenchyma than corresponding venous pressure changes between 0 and +6 or +7 cm. A second point, not noted previously except possibly in connection with transudation rates, is that liver volume changes appear to have approached their limit when venous pressures have risen to about +6 cm H_2O. Na^{22} space, for instance, increases only a small percentage between +5 and +8 cm, and the same is suggested by the single passage dilution space data.

More detailed examination of figure 5 may well start with the question whether the total observed liver volume increase can be accounted for in terms of the fluid com-

TABLE 2

	% Increase Between $P_V = -4$ and $P_V = +6$ cm H_2O	
	Dynamic	Equilibrium
IHSA	110 (100–150)	160 (150–170)
Fe^{59} erythrocytes	55 (42–65)	100 (90–120)

partments recorded in this figure. For this purpose the total volume change as measured by the dilution of $CrP^{32}O_4$ previously fixed in the tissue, reduced by the amount of erythrocytes accumulated in the congested liver, is to be compared with the tissue volume change represented by the observed expansion of the sodium space. The $CrPO_4$ results are plotted accordingly in figure 5 in terms of a 'hypothetical sodium space' based on a mean sodium space of 23.6% for the rat liver under basal perfusion conditions; the values for positive venous pressures are then calculated from the $CrPO_4$ concentration data by subtracting the erythrocyte mass increment, and ascribing the entire remaining volume change to an expansion of the 'hypothetical sodium space'.[2] As shown by the relative positions of the points representing the observed Na^{22} space, and the Na^{22} space calculated from the $CrPO_4$ dilution data, the results obtained indicate that the liver volume increase in passive congestion can actually be accounted for by the combined increase of tissue erythrocyte mass and tissue Na^{22} space.

Turning to the estimation of liver blood volumes, it should be observed that for albumin as well as for erythrocytes, the single passage dilution spaces (or pools) increase considerably less rapidly with venous pressure than the distribution measured by tissue analysis after 30 minutes of equilibration (table 2). Albumin space, furthermore, increases far more rapidly than erythrocyte mass, regardless of which method of measurement is adopted. In analyzing these results, it seems relevant to note two additional facts: *1*) at subhilar venous pressures, single passage dilution analysis and tissue analysis yield the same result for erythrocyte mass. This equality breaks down at positive hepatic vein pressures, the single passage space increasing more slowly than the equilibrium erythrocyte mass. *2*) At subhilar venous pressures the hematocrit of the perfusate (av. 43.5%) equals the hematocrit of the rapidly moving blood in the liver

[2] The calculations involved in this procedure are the following: calculated expansion of the total sodium space is given by the relation

$$Na_2 = \frac{\overline{(C_1)}}{\overline{Na_1}\,\overline{(C_2)}} + I\left(\frac{(C_1)}{(C_2)} - 1\right) + (R_1 - R_2)\cdot\frac{C_1}{C_2}$$

where Na_2 and $\overline{Na_1}$ are the calculated sodium space at venous pressure 2, and the actual sodium space (23.5 ± 2.2% of the original tissue volume) at basal venous pressure. I is the intracellular (or better the nonsodium) space, assumed independent of venous pressure; R_1 and R_2 are the erythrocyte concentrations at the two venous pressures; and (C_1) and (C_2) are the chromic phosphate concentrations in the tissue at the 2 venous pressures.

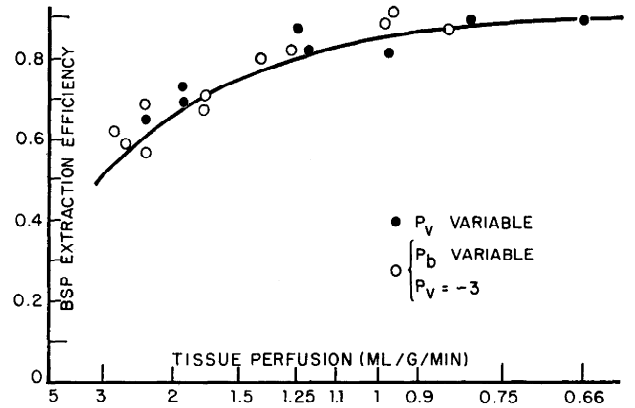

FIG. 6. BSP extraction efficiency as a function of perfusion rate, when this is varied by portal pressure changes at constant venous pressure ($P_V = -3$ cm H_2O), or by venous pressure (P_V variable) while portal pressure is held at 12.5–13.3 cm H_2O.

(42.5% av.), while at positive venous pressures a far lower hematocrit would be calculated from the transient analysis data (about 35% at +6 cm H_2O).

Removal of Test Substances From the Perfusate

Sodium phenoltetrabromphthalein di-S^{35}-sulfonate (BS^{35}P). The BSP extraction efficiency of the isolated rat liver is shown in figure 6 as a function of perfusion rate. The results fall on the same curve whether perfusion rate is modified by increasing venous pressure while maintaining substantially constant portal pressures between 12.5 and 13.0 gm, or whether venous pressure is held at −3 or −4 cm while the perfusion rate is controlled through the portal pressure.

Chromic phosphate colloid. Figure 7 shows the relation between perfusion rate and colloid extraction efficiency for livers perfused while maintaining venous pressures at levels 2–3 cm below the hilum (solid points), and for preparations in which portal pressures were held constant while hepatic vein pressures were raised to levels of +3.0 cm (open points with center dot) and +6 cm H_2O (open points). In addition each of these points carries a marker indicating the direction toward that point for the same preparation which represents the same portal pressure, but a venous pressure of −3 to −4 cm H_2O. In general the points obtained under positive hepatic vein pressures fall within the range of values derived from livers perfused under standard conditions. The rate of change of $CrPO_4$ extraction efficiency due only to changing perfusion rate is represented for the experiments with constant negative venous pressures by the slope of the least square line drawn through the solid points at perfusion rates greater than 1.2 ml/gm/min; the corresponding values for the positive venous pressure experiments are represented by the slopes indicated for each of the open points, and gathered together in the insert. The slopes which result on raising venous pressure from negative values to +6 cm are but slightly smaller than those due to modification of the perfusion rate by varying portal pressure, hepatic venous pressures being negative. On the other hand, the transition from −3 to +3.0 cm H_2O venous pressure is not, in general,

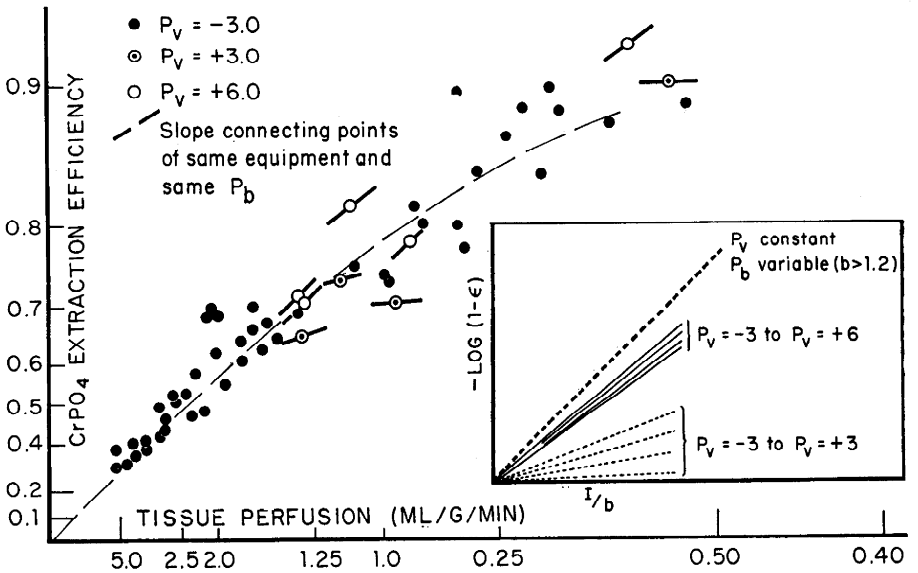

FIG. 7. $CrPO_4$ colloid extraction efficiency, $\varepsilon CrPO_4$, as a function of perfusion rate (b). The actual functions plotted are: log $(1 - \varepsilon)$ vs. 1/b (see (9) for justification of this choice of coordinates). *Solid points:* venous pressure constant at −3 to −4 cm H_2O, portal pressure variable; open points: venous pressure +6 cm H_2O; *open points with center dot:* venous pressure +3.0 cm H_2O. The lines drawn through each positive venous pressure point represent the direction in which (toward the lower left) the corresponding point for negative venous pressure (same preparation, same portal pressure) is located. Slopes of these lines are gathered together in the insert for comparison with the common slope of the extraction efficiency vs. perfusion rate curve for negative venous pressure experiments when the hepatic vasculature is fully perfused.

accompanied by as marked an increase in extraction efficiency as a portal pressure change producing an equivalent change in perfusion rate.

Bile Secretion

In the isolated rat liver perfused with portal pressures of 12.5–13.5 cm H_2O, venous pressures below the hilar level have but little effect on bile flow, as indicated by the open square on the pressure axis: between −4 cm and 0 cm H_2O, mean bile flow in twenty experiments decreased by only 7%. Positive venous pressures lead to increasingly pronounced reduction in bile flow rate (open circles of fig. 8). Effects tend to be fully reversible up to venous pressures of about +4 cm; after higher venous pressures, bile flow rates often are not restored to basal levels by a return to subhilar venous pressures; the proportion of organs permanently impaired increases rapidly with the maximum venous pressure.

The bile flow decrease is not due primarily to the reduced perfusion rates which result from the elevated hepatic vein pressures. Thus, if the same perfusion rates were established by lowering portal pressures while holding venous pressure below zero, bile flow rate would only change to the extent indicated by the dashed line of figure 8. This line is derived from the data of reference (10), in combination with the flow pressure data of figure 4.

If the reduced bile flow in the livers under positive venous pressures were due to impaired oxygen transfer from erythrocytes to tissue, reduction of the Q_{O_2} by lowering the perfusion temperature should decrease this effect (12). The solid points of figure 8 represent results obtained at 30°C instead of at 38°C. Up to hepatic vein pressures of +5 cm H_2O the hypothermic livers appear wholly unaffected by venous pressure changes. At still higher venous pressures the protection is less complete, although the data suggest that even here bile flow in the hypothermic livers is less affected by the high venous pressures than in the normothermic ones.

Limiting bile secretion pressures were determined in four preparations at hepatic vein pressures of −3.5 to −4.5, 0, and +4 to +5 cm H_2O. Figure 9 shows the results of one typical experiment of this series. The time course of bile ascending in a vertical catheter was not altered by raising venous pressures from negative to zero levels. Positive venous pressures slow the rate of secretion somewhat, as expected on the basis of figure 8. The point at which net bile flow rate approached zero, the limiting bile pressure, was not affected by venous pressures below the hilar level, and was decreased slightly by the positive venous pressures. In no case was an increased limiting bile pressure observed, and the decreases seen did not exceed 1.0 cm H_2O. Bile pressure invariably exceeds portal pressures by at least 8 cm H_2O (10).

DISCUSSION

The hepatic circulation, with its low mean transmural pressures, and large vascular volume in an organ which normally has little structural rigidity, is subject to greater modification by external physical forces than almost any other regional circulation in the mammalian body. Before attempting to describe or to analyze active vasomotor control in the liver, the influence of these external physical factors upon hepatic blood flow and upon the efficiency of contact between blood and parenchyma must be well understood. The present communication contributes two pieces of information to such an analysis.

In the first place, the data presented indicate that in the rat liver hepatic vein pressures are hardly perceived by the parenchyma if they are less than the external pressure at the liver surface. This conclusion can be drawn from every figure presented above: invariably pressure changes in the hepatic vein elicit slight responses or none at all if they occur in the region below

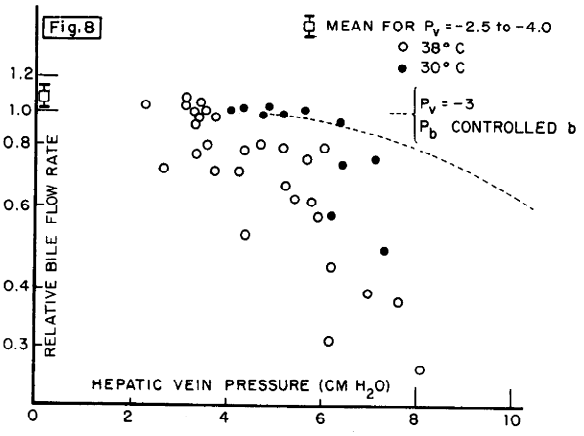

FIG. 8. Bile flow (relative to basal perfusion conditions) as a function of venous pressure at 38°C (*open circles*) and at 30°C (*solid points*). For comparison the dashed line indicates bile as a function of perfusion rate at negative venous pressure and variable portal pressure. (*Ordinate* in this case was chosen so that perfusion rates correspond with those at the venous pressures indicated as determined from Fig. 3. Bile flow rates from (10).)

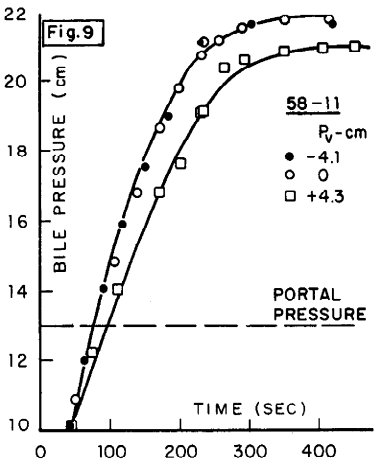

FIG. 9. Limiting bile pressure determination at several hepatic vein pressures (P_v, as indicated). *Abscissa* represents time required for bile to ascend to the level indicated (on the *ordinate*) in a vertical catheter.

0 cm H_2O relative to the hilum of the liver. One may conjecture that this isolation of the liver from 'negative' venous pressures would be complete if the hepatic vasculature were even slightly more collapsible than it actually appears to be, and that maintenance of this isolation may be the real physiological role of the various types of sphincter-like structures described in the venous portion of the hepatic vascular tree (13). The net effect of these isolation mechanisms is to establish the pressure differential between portal vein and peritoneal cavity at the hilum of the liver as the primary pressure gradient which in a normal liver regulates hepatic blood flow and hepatic resistance unless pressures in the thoracic vena cava are abnormally high.

A second consideration emerging on the basis of the present data, especially perhaps those of figure 4*b*, concerns the extent to which elevated hepatic vein pressures are transmitted to the portal vein, or conversely the extent to which portal vein pressures allow deductions regarding hepatic vein pressures. If the normal hepatic circulation were maximally perfused with blood, i.e. if all available channels were open and contained blood in active movement toward the venous side, any elevation of hepatic vein pressure above the hilar level would be reflected pari passu in portal pressure changes if hepatic blood flow remained constant. On the other hand, a lag in the portal pressure-venous pressure curve like that shown in figure 4*b* at low relative perfusion rates (0.5 or less) is evidence of an incompletely perfused vascular bed, one in parts of which transmural pressures do not exceed the critical opening pressures of the vascular channels. In view of the small effect of hepatic resistance on splanchnic blood flow in the normal subject, these observations suggest a new method for evaluating the circulatory status of the liver *in vivo*.

Turning to the consequences of the hemodynamic changes discussed so far, the effects of passive congestion on fluid balance between liver parenchyma and the blood stream claim first place. A very slight increase in venous pressure—as little as 1 cm of water above the hilar level—suffices to induce a ready flow of transudate across the vascular endothelia, and into the tissue spaces whence it reaches the surface of the liver. The pressures available to drive this fluid across the vessel walls can be clearly delimited in the isolated system and must be very far below the 30 cm H_2O which represent the total osmotic pressure of the plasma proteins. This is possible only if the effective colloid osmotic pressure due to the plasma proteins is but a fraction of the total, i.e. if the hepatic endothelia are highly permeable to these molecules. This is borne out by the high total protein content of the transudate. More important yet, even at the high transudation rates which result from venous pressures of +4 or +5 cm H_2O the proportion of small to large protein molecules in transudate (as indicated by IHSA to total protein ratio) is the same as in the blood plasma. The hepatic endothelia, therefore, are shown to be not only highly permeable to plasma proteins but to have a pore population which does not even exert a sieving effect on the plasma proteins at very rapid transmural fluid flow rates. The picture which emerges is of an organ in which a large blood-tissue interface is lined by a membrane which is readily and nearly equally permeable to all plasma proteins. This picture accords well with observations by other workers who, in livers of intact animals, have failed to find selective protein filtration in fluid passing at various rates from blood to lymph or to transudate in the liver (14, 15). It also accords with the extraordinarily high rate of lymph formation in the normal liver (16).

In the absence of protein restraining hepatic endothelia, the fluid balance within the tissue should be extraordinarily labile, and should be restrained only by such mechanical factors as tissue tension, which in turn should reflect largely the mechanical rigidity of connective tissue scaffolding within and of the capsule surrounding the hepatic parenchyma. Attention has already been called, in the presentation of the results, to the fact that just such a type of control of tissue expansion is suggested by the results of quantitative tissue analyses presented in figure 5. Apparently, resistance to further volume increase of the rat liver rises rapidly when the tissue has

expanded by about 15%, corresponding to a stretch of 5–7% for the linear elements within the liver substance, or of a roughly similar amount for the area of the (non-spherical) liver capsule. These values are comparable to values derivable from the stress strain diagrams for cylindrical liver specimens under compression (17), so that the interpretation here proposed would appear to fit the known mechanical properties of the tissue. The strain imposed upon tissue structure when these values are approached is manifest in a number of ways, including erythrocyte leakage into transudate, and partially irreversible liver function changes.

The volume increase of the liver in passive congestion, as has been pointed out in presenting the results, can be accounted for numerically in terms of the increases in erythrocyte mass and in plasma equivalent Na^{22} space. The most direct manner of explaining such behavior is to conclude that there is actual accumulation in these preparations of fluid with the sodium content of extracellular fluid, and that ionic and fluid shifts altering the intracellular compartment do not contribute in an important way to the weight increase due to increased hepatic vein pressures. In the absence of direct observations bearing on the anatomical distribution of sodium ion in these tissues, or on the extent of the so-called intracellular compartment, the above view cannot be accepted as more than a plausible (because economical) hypothesis. It accords well not only with numerical results, but also with the rapid course of sodium accretion to or removal from these livers when venous pressures are changed up or downward.

This hypothesis receives further support from the albumin space measurements. The albumin content of the liver increases somewhat less rapidly in time than the Na^{22} content on increasing the venous pressure, but after 30 minutes an equilibrium appears to have been reached which is not greatly altered by further lapse of time. At this point, the added 'plasma equivalent albumin space' is just about equal to the added 'plasma equivalent sodium space.' Thus, raising the venous pressure from -3 to $+6$ cm H_2O results in the accumulation of 23 ml of extra volume per 100 gm liver, of which about 18 ml are not due to extra erythrocytes. At the same time the tissue accumulates extra Na^{22} equivalent to 18 ml of blood plasma, and IHSA equivalent to about 17 ml of blood plasma. Such remarkable agreement, surely is not readily explained unless one assumes that these results represent the accumulation in the liver of actual fluid substantially identical in composition with blood plasma, or with liver lymph, and hence (in the liver) with extracellular fluid.

The fact that the entire extra sodium space is accessible to plasma colloids differentiates this fluid phase from the Na^{22} space measured in the same livers when venous pressures are below the hilar level: only about 42% of this 'basal' sodium distribution space is accessible to IHSA (fig. 5 and to be published by Holloway, Leong and Brauer). It amounts to a mere restatement of these relations to point out that the fluid accumulated in the liver in passive congestion, as a whole, bears a closer relation to the vascular tree of the liver than the fluid representing the Na^{22} distribution volume in the liver under basal perfusion conditions.

The studies of erythrocyte distribution contribute two additional points to the dissection of the fluid compartments in the congested liver. In the first place, at positive venous pressures a discrepancy develops between single passage dilution space results (representing erythrocytes undergoing rapid turnover in the vascular tree) and tissue analyses 10 or 30 minutes after administration of labeled erythrocytes (representing the total erythrocyte mass in the tissue): the total erythrocyte mass increases faster than the dynamic erythrocyte mass, so that at $+6$ cm H_2O venous pressure the latter accounts for only three-fourths of the total red cells present. This discrepancy is less marked at lower venous pressures and disappears entirely when venous pressures fall below the hilar zero level. These observations suggest that one effect of passive congestion is a rearrangement of pressure gradients so that the movement of blood in some parts of the circulation becomes quite slow; whether movement actually ceases anywhere cannot be determined from the present data. At any rate this effect is not the result of extravasation, as proved by the prompt reversibility of the erythrocyte accumulation when the passive congestion is relieved.

A second result of the erythrocyte mass determinations is to establish an upper limit for the intravascular plasma volume. Under basal perfusion conditions, the hematocrit of the rapidly circulating blood, calculated from single passage dilution space analyses for albumin and red blood cells, is equal to that of the perfusate (10). Since tissue analyses and dynamic measurements yield the same erythrocyte content under these conditions, it seems plausible to deduce that the blood moving in the hepatic vasculature actually has the same composition as the perfusate, and that the intravascular volume is measured by the sum of dynamic plasma and erythrocyte spaces. Expansion of these under positive venous pressure entails widening of mean vessel lumen, and slowing of linear flow rate under the double influence of widened cross sections and diminished pressure gradients; as pointed out above, there is even some evidence of stagnation under these conditions. Every one of these factors would tend to increase rather than to decrease the mean hematocrit of a moving heterogeneous suspensoid like blood, if plasma margination and core flow had been important previously (as they do not appear to be in the normal liver). The effect of the circulatory changes in passive congestion should, therefore, be either nil as far as the mean liver hematocrit is concerned, or the cell concentration in the blood vessels might actually be increased by sieving and transudation. Thus, the 30 minute distribution of erythrocytes, as determined by tissue analyses, sets an absolute upper limit to the plasma volume within the vascular tree of the congested liver. This maximum volume, calculated from perfusate hematocrit and total liver erythrocyte mass, is found to be

substantially equal to the single passage dilution space for IHSA at all venous pressures studied. It is not possible at present to determine whether this implies that blood within the hepatic vascular tree has a constant hematocrit regardless of venous pressure, or whether there is merely a fortuitous balance of high cell concentrations in stagnant regions and perivascular circulation of some of the plasma proteins.

These considerations, then, prove conclusively that a considerable amount of fluid, equal at least to the difference between the sodium space increment and the single passage IHSA space increment, must accumulate in the extravascular spaces of the liver when venous pressures are raised.

Histological observation shows edema of the portal spaces, and distended lymphatics in the same region. At the same time serous spaces between sinusoidal endothelia and parenchymal cells were observed in one-fifth of these tissues. Considering the rapidity with which extra sodium leaves the tissue when venous pressures are dropped from +4 or +6 cm to −3 or −4 cm, it seems justifiable to question the reliability of negative findings relating solely to fluid accumulation in the tissue (18) and to give considerable weight to such positive observations as the dilatation of the Disse spaces just mentioned. The authors feel that fluid accumulation in these perisinusoidal spaces is real and represents an important part of the pathophysiology of passive congestion.

It is a striking fact that the rather extensive distortion of the fluid matrix surrounding the cells of the hepatic parenchyma results in slight, and, in general in only transitory changes in liver function. Thus, neither the uptake of $CrPO_4$ colloid by the Kupffer cells of the sinusoidal endothelia, nor the uptake of the albumin bound and water soluble dye BSP is greatly altered when venous pressures are raised to positive values. In the case of the colloid, the changes in $CrPO_4$ extraction efficiency appear wholly accounted for by perfusion rate changes if sufficiently high venous pressures are attained. A venous pressure of only +3 cm however, produces a smaller increase in extraction efficiency than would be predicted from the change in perfusion rate (insert, fig. 7). This effect may reflect of changes in the blood flow distribution through the various possible vascular channels, but this hypothesis cannot be tested in terms of morphological and pressure gradient data available at present.

The shape of the BSP extraction efficiency versus perfusion rate curve is altogether different from that for $CrPO_4$, and suggests an uptake mechanism which, except at excessive perfusion rates, is limited by transfer equilibria at the liver cell surface rather than by the rate of delivery of dye to the surface of the active cells (12). In line with this view, passive congestion was shown not to modify the BSP extraction efficiency of the isolated liver.

The bile secretion data have been discussed in part in the process of presenting the relevant results. The conclusion reached is that impaired oxygen transfer to the tissue is important in restricting bile flow at venous pressures of +4 or +5 cm. This is in line with observations indicating that oxygen supply to the secreting cells is the major factor in the interaction of hepatic circulation and bile secretion. The effect of passive congestion on bile flow can be explained with a minimum of hypothesis if one accepts the parenchymal cell as the site of bile secretion (19), and if one accepts the sinusoidal portion of the vascular bed as the main site of lymph and transudate formation in the liver. Excessive transudate formation, then, would lead to the opening up of the perisinusoidal spaces, and to the formation of a physical barrrier between oxygen carrying erythrocytes and oxygen consuming hepatic cells, which in turn would respond with decreased bile secretion. Hypothermia, by decreasing oxygen utilization to a greater extent than oxygen diffusion rates, should tend to minimize such an effect, and does so in actuality as shown in figure 8. At venous pressures above 5 cm an additional factor comes in to play, namely severe mechanical strain within the hepatic parenchyma. This is evidenced, for instance, by the appearance of erythrocytes in the previously clear transudate, and by the increasing incidence of irreversible changes in various parameters. In the case of bile secretion, then, this effect expresses itself in marked reduction of bile flow rate, a reduction which can no longer be fully counteracted by hypothermia.

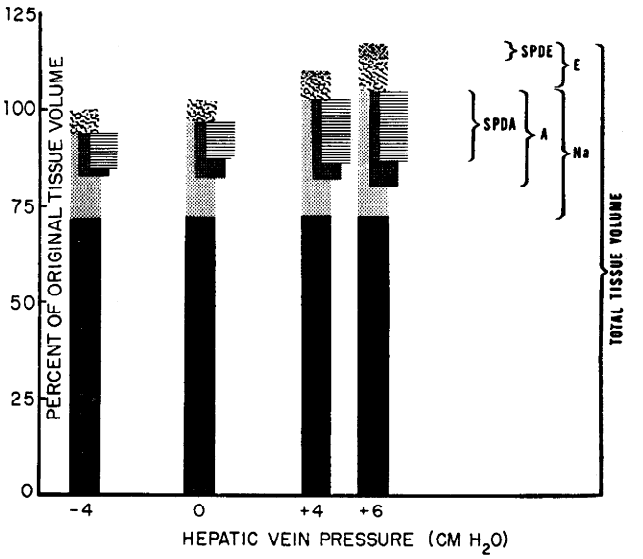

FIG. 10. Summary of fluid compartment changes due to venous pressure changes. The test substances are indicated at the right (E—Fe^{59} erythrocytes, A—I^{131}-labeled plasma albumin, Na^{22}—sodium ion, SPD refers to single passage distribution analyses; E + SPDA represents the maximum intravascular volume).

The bile secretion pressure data, finally, seem to answer a further question of physiological anatomy. The limiting bile pressures have been shown to represent the point of balance between bile pressure independent bile secretion into, and bile pressure dependent bile leakage out of the biliary tree (10). Since the pressure

controlling the loss of bile must of necessity be the transmural pressure acting at the bile duct wall rather than the bile pressure alone, interstitial fluid pressures in the critical leakage zone must affect the limiting bile pressure. The data of figure 10, therefore, imply that the main site of leakage should be far enough upstream so that venous pressure changes are not significantly transmitted to the tissue spaces into which leakage takes place. This pretty well localizes this site within the portal tracts, and coincides satisfactorily with anatomical evidence suggesting the small bile ducts or the ductular region as the most likely site for this process (20).

Reviewing the information gained from the experiments reported above, results will be found to bear on the following general areas:

a) Hepatic hemodynamics. A variety of data have been presented to indicate that venous pressures are not effectively transmitted to the hepatic parenchyma unless they exceed the hydrostatic pressure at the liver surface, or more specifically at the hilum of the liver. Venous pressures above this level rapidly distend and fill the hepatic vascular tree, so that it acts like a rigid system of conduits. The transition from the normal to the distended state is marked by a lag in the response of portal pressures to hepatic vein pressure increases when constant hepatic blood flow is maintained.

b) Fluid balance. The extreme permeability of the hepatic vascular tree is illustrated by the fact that hepatic vein pressures only 1 cm H_2O above the hilar level result in the appearance of transudate at the liver surface; the IHSA and total protein concentrations in this transudate are indistinguishable from the corresponding values in blood plasma. Liver volume increases in passive congestion, and there is a suggestion that the extent of such swelling is limited to about 25% by the mechanical properties of liver capsule and connective tissue elements. The liver volume increase due to elevated hepatic vein pressures can be wholly accounted for in terms of increased erythrocyte mass and increased sodium space. The sodium space expansion, furthermore, is equal to the expansion of the 30 minute IHSA space. At least half of this fluid volume increase has been shown to be extravascular. The erythrocyte mass also increases rapidly with hepatic vein pressure; in passive congestion a difference appears between single passage distribution space for erythrocytes, and tissue erythrocyte mass as found by tissue analysis. The discrepancy suggests a quasi sequestration of as much as 25% of the total erythrocyte mass at venous pressures of +6 cm H_2O (fig. 10).

c) Liver function. Transfer of test substances like BSP or $CrPO_4$ colloid from blood to hepatic parenchyma or to Kupffer cells is hardly affected by passive congestion once the effect of blood flow changes is eliminated. Only at low positive venous pressures is there any indication that $CrPO_4$ uptake may be slightly impaired. Bile flow, relative to that under basal perfusion conditions, is reduced by positive venous pressures to a far greater extent than can be explained by perfusion rate changes. Lowering the perfusion temperature to 30°C eliminates this effect entirely up to venous pressures of +5 cm H_2O, and decreases the relative bile flow reduction at higher venous pressures. Bile secretion pressure is not significantly effected by increasing hepatic vein pressures, or it is slightly reduced thereby.

These various changes are discussed in the light of the possible morphological changes in the hepatic parenchyma in passive congestion. It is suggested that the two most important morphologic effects of passive congestion, both due to fluid accumulation in the tissue, are distention of the tissue and consequent strain upon its structural elements, and the interposition of a fluid barrier between oxygen carrying erythrocytes and oxygen consuming cells in certain key regions. The reversibility of those changes not due to excessive mechanical strain was illustrated by numerous examples.

The authors are greatly indebted to Dr. E. L. Dobson, University of California, Berkeley, Calif., for making available the $CrP^{32}O_4$ colloid used in these experiments, and to Dr. H. Brown Dunning, Hynson, Westcott & Dunning, Inc., Baltimore, Md., for the phenoltetrabromphthalein used in the synthesis of $S^{35}BSP$. We express our thanks to our colleague, Lt. R. M. Garver, for performing the paper electrophoretic studies and to Dr. James Arnold, our colleague, for evaluating the histological material collected in the course of this study.

REFERENCES

1. Bolton, C. and W. G. G. Barnard. *J. Path. & Bact.* 34: 701, 1931.
2. Volwiler, W., J. H. Grindlay and J. L. Bollman. *Gastroenterology* 14: 40, 1950.
3. McKee, F. W., H. Schloerb, J. G. Schilling, G. H. Tishkoff and G. H. Whipple. *J. Exper. Med.* 87: 457, 1948.
4. Brauer, R. W., R. L. Pessotti and P. Pizzolato. *Proc. Soc. Exper. Biol. & Med.* 78: 174, 1951.
5. Brauer, R. W., G. F. Leong, R. F. McElroy, Jr. and R. J. Holloway. *Am J. Physiol.* 186: 537, 1956.
6. Dobson, E. L. and H. B. Jones. *Acta med. scandinav.* 144 Suppl.: 273, 1951.
7. Krebs, J. S. and R. W. Brauer. USNRDL TR-67, 1955.
8. Brauer, R. W., R. L. Pessotti and J. S. Krebs. *J. Clin. Invest.* 34: 35, 1955.
9. Brauer, R. W., G. F. Leong, R. F. McElroy, Jr. and R. J. Holloway. *Am. J. Physiol.* 184: 593, 1956.
10. Brauer, R. W., G. F. Leong and R. J. Holloway. *Am. J. Physiol.* 177: 103, 1954.
11. Levin, R. and R. W. Brauer. *J. Lab. & Clin. Med.* 38: 474, 1951.
12. Brauer, R. W., G. F. Leong, R. J. Holloway, J. S. Krebs and H. H. Carroll. In: *The Physiology of Induced Hypothermia*, edited by R. D. Dripps, Nat. Acad. Sci.—NRC publication No. 451, Washington, D. C., 1956, p. 235.
13. Knisely, M. H., F. Harding and H. Debacker. *Science* 125: 1023, 1957.
14. Bollman, J. L., J. C. Cain and J. H. Grindlay. *J. Lab. & Clin. Med.* 33: 1349, 1948.
15. Nix, J. F., F. C. Mann, J. L. Bollman, J. H. Grindlay and G. V. Flock. *Am. J. Physiol.* 164: 119, 1951.
16. Brauer, R. W. *Gastroenterology*. In press.
17. Harkness, R. D. In: *Liver Function*, edited by R. W. Brauer. Am. Inst. Biol. Sci. Monograph No. 4, 1958, p. 59.
18. Popper, H. *Arch. Path.* 46: 132, 1948.
19. Brauer, R. W. *J.A.M.A.* 169: 1462, 1959.
20. Popper, H. and F. Schaffner. *Liver: Structure and Function.* New York: Blakiston, 1957, p. 106.