Direct measurement of blood pressures in minute vessels of the liver

K. NAKATA,[1] G. F. LEONG AND R. W. BRAUER
U.S. Naval Radiological Defense Laboratory, San Francisco, California

NAKATA, K., G. F. LEONG AND R. W. BRAUER. *Direct measurement of blood pressures in minute vessels of the liver.* Am. J. Physiol. 199(6): 1181–1188. 1960.—A method has been described which allows the insertion of microneedles into minute blood vessels of the transilluminated rat liver. Using dye injections under controlled pressures and direct observation, blood pressures in the terminal portal venules as well as in the central veins of this preparation have been determined and have been compared with simultaneous pressure measurements in the mesenteric veins and in the vena cava at the level of entry of the hepatic veins. The results indicate an unexpectedly large pressure drop in the portal venous tree (from 13 cm H_2O in the portal vein to approximately 6 cm in the portal venules), a significant pressure drop between portal venules and central veins (from 6 to 2.5 cm H_2O), and a paradoxical mean pressure relation between central vein and vena cava. Discussion of these results has been presented in terms of methodological problems, the implication of the results for the control of blood flow distribution in the liver, and the intermittency of hepatic vein flow in the rat.

EVIDENCE HAS BEEN accumulating to indicate that not only the over-all rate of hepatic blood flow, but also its macroscopic and microscopic distribution within the parenchyma of the liver can be subject to wide and diverse modification (1). The discussion concerning the nature of the mechanisms which bring about the several changes in hepatic hemodynamics, and their possible functional significance, has been hampered by lack of some of the basic data required, including in particular information concerning the pressure gradients in the various parts of the hepatic vascular tree. Even in the major vessels supplying the liver, existing methods of pressure measurement have not cleared up all uncertainty. While portal vein pressure measurements (whenever referred to a proper base line) have, on the whole, been fairly consistent, both as between different observers and as between different species (2), the results of hepatic vein pressure determinations have been far less uniform and their interpretation far less certain (3). In particular, however, extrapolation from such large vessel data as are available to a description of blood pressure gradients in the minute vessels of the liver has been abandoned by most experimenters for the lack of an adequate theoretical basis applicable to the special anatomical relations encountered in the liver (3).

The present report will describe the results of direct measurements of mean pressures in the minute vessels of a particular portion of the hepatic parenchyma. The technique developed for this purpose involves the insertion of sensing elements into the vessels to be studied under direct microscopic observation. To achieve this, it became necessary to develop improved methods of transillumination of liver parenchyma, avoiding possible difficulties inherent in uneven pressure distributions resulting from previously used transillumination methods, and to amplify these optical methods by suitable surgical procedures which should allow the introduction of sensing elements into the key portions of the hepatic parenchyma.

METHODS

The original description of the basic transillumination technique employed (previously presented by one of us (4)) is not likely to be readily accessible to English language readers. Hence, a brief résumé of the essential features of the original method will be presented below (see fig. 1).

Mice, used in (4), or, in the present investigation, Sprague-Dawley rats, weighing no more than 200 gm (and preferably between 70 and 100 gm), are deeply anesthetized, using 60 mg/kg of sodium pentobarbital intraperitoneally. (See below for methods used to assure adequate oxygenation.) A mid-line incision, beginning at the costal angle and extending caudally for 3 cm, is made, and the xyphoid process resected. The liver is partially freed from its attachment to the diaphragm by severing the falciform ligament. The clipped ventral skin of the subject, adjacent to the incision, is next coated with a cellulose acetate cement (Duco cement proved satisfactory for this purpose), and the prepared animal placed into the special holder shown in figure 1. The cement serves to make a water-tight seal between the stainless steel plate (no. *8* in fig. 1) and the shaved skin surrounding the laparotomy wound. The stainless steel plate forms one side of a chamber (no. *2*, fig. 1) through which warmed physiological saline is circulated

Received for publication May 31, 1960.

[1] Present address: Dept. of Pathology, Osaka Medical College, Takatsuki, Osaka, Japan.

(via the connectors *3* and *4* of fig. 1) in such a fashion as to maintain a temperature of 37.0° ± 0.2°C at the site which will be occupied by the liver lobe under observation. An elliptical opening in steel plate *8* is placed over the laparotomy wound, establishing connection between the chamber and the peritoneal cavity, and located so as to permit exteriorizing the left lateral lobe of the liver with a minimum of torsion or tension. The portion of the liver lobe within the transparent chamber is supported by means of a stretched strip of polyethylene film (Saran wrap), and gently fixed in position by means of a similar piece of plastic film in a 'U' shaped frame (*6* in fig. 1) placed on the upper surface of the liver tissue. This upper film layer has a small perforation (*5* in fig. 1) to permit subsequent introduction of the microneedle. The optical conditions in the transparent chamber now are as follows: at the bottom of the system is a plate of clear Lucite (1/16 in. thick), followed by a layer of saline 5–6 mm deep; next is the plastic film (0.07 mm thick) upon which rests the liver lobe which, in the region under observation, must not be more than 1.5 mm thick (i.e. observations are limited to a zone extending no more than 3 mm inward from the margin of a normal young rat's liver). This is covered by another layer of plastic film, and is overlaid in turn by about 10 mm of warm saline. This assembly, then, is placed on the stage of a microscope equipped with long focus (2–3 cm) condenser, and water immersion objectives (10–50 ×). In the work to be described, 10 × oculars were used throughout. The light source used for visual observation was a conventional 100-watt microscope illuminator (AO model 735 B). Under these conditions, thermocouple measurements have shown that temperatures in the liver lobe under observation are maintained between 37.5° and 37.7°C. The rectal temperature of the rats used in the present series fell slightly during the anesthesia and preparation of the animals, and reached a plateau between 36° and 37°C, maintained during the entire course of the experiments.

For the purpose of the studies here envisioned, this basic observation technique required three amendments: *a*) stroboscope photography to record the anatomical relations with a satisfactory degree of resolution; *b*) elimination of respiratory movements during periods of close observation; and *c*) methods for insertion and guidance of microneedles.

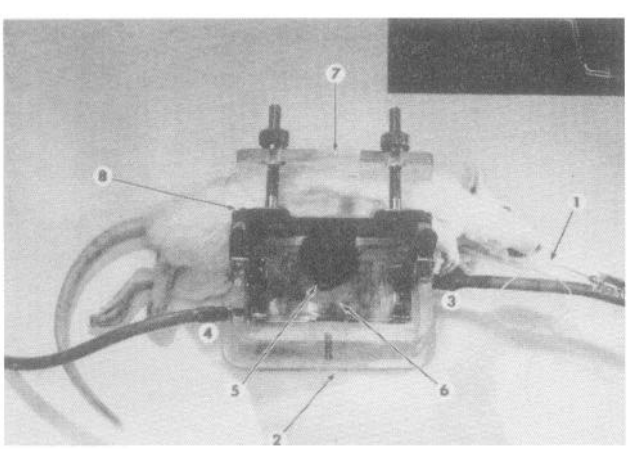


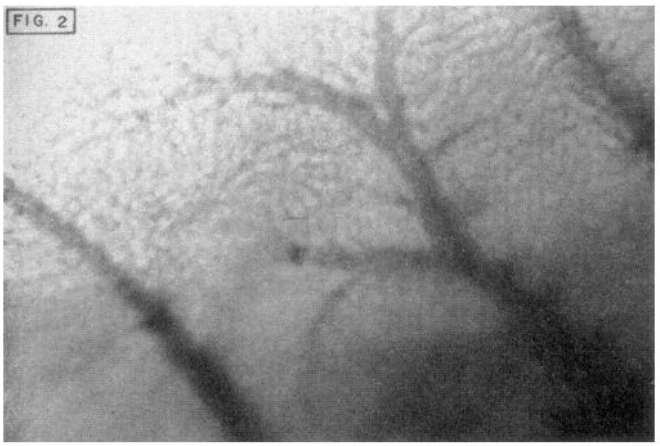
FIG. 1. Animal holder and transillumination chamber (see METHODS for significance of *numbers*). Insert shows microneedle employed for pressure measurements.

a) The photographic records were obtained by use of a stroboscopic light source (50 watt seconds) which allowed reducing the exposure time for each picture to 1/1100 second. For this purpose, a commercial Strobe flash unit (Graflex Strobomite flash unit) was modified by masking the reflector face down to a 2-inch diameter circular aperture, which in turn was covered by a Wratten gelatin gray filter.

b) To minimize respiratory excursions which interfere with the observation of specific segments of liver parenchyma, and to eliminate these completely for relatively brief periods of time during photography or special manipulations, the basic procedure was modified (cf. 5) by the introduction of a tracheal catheter for continuous oxygen insufflation. To secure complete suppression of respiratory excursions for periods of 10–15 minutes, 1 mg/kg of succinylcholine was administered by way of the femoral vein. This dose was found to suppress all respiratory excursions of the diaphragm for periods ranging from 5 to 10 minutes; by the end of 15 minutes, respiratory movements returned to presuccinylcholine levels. To avoid excessive carbon dioxide accumulation, these doses were never repeated at intervals of less than 30 minutes. Using these procedures, animals could be maintained in good condition for at least 6 hours; in some experiments the animals were carried for as long as 10 hours with no evident deterioration of the hepatic circulation or of the respiratory condition of the animal.

c) Micromanipulator-guided hollow glass microneedles to be inserted into the smallest branches of the portal or hepatic veins were prepared in the shape illustrated by the insert in figure 1 from soft glass tubing. For pressure measurements, tips of 10–15 μ diameter were found most suitable. Such tips are small enough not to produce marked local tissue disturbances, and at the same time large enough not to be excessively liable to occlusion by minute fibrin clots and leukocytes. Heparin (1.0 mg/kg) was given via femoral vein prior to micropuncture.

Blood pressures in the minute vessels were determined by an adaptation of the technique used by Landis and co-workers (6). The distal end of the microneedle was connected by a polyethylene catheter to a T tube to which, in turn, a capillary manometer and microburette were attached. The whole apparatus was filled with 0.05–0.10% T-1824 in physiological saline containing 0.2 mg/100 cc of heparin sodium. After placing the needle orifice in the desired location, and under continuous microscopic observation, the pressure in the needle was gradually increased by means of the microburette until dye solution could be seen to be extruded into the blood stream; the pressure at which this flow



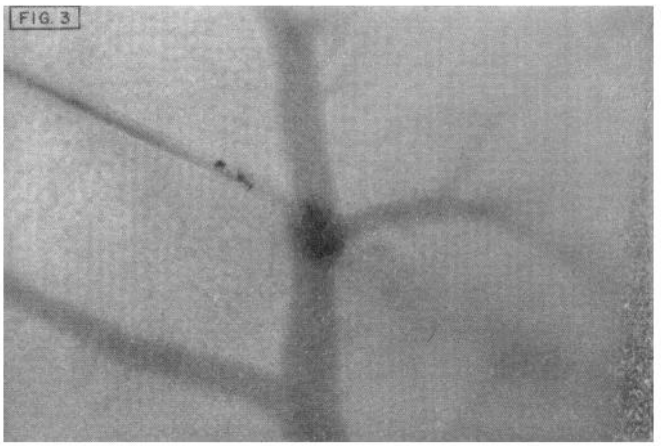


FIG. 2. Vascular pattern seen at margin of rat liver. Portal venules and hepatic venules at this level have diameters of 30–40 μ and 40–60 μ, respectively. These vessels occur in a ratio close to 1.0 and, more often than not, follow a parallel course. Terminal twigs of portal venules in general are located at a small distance from liver margin, while recognizable hepatic vein branches are often seen parallel to and almost at extreme edge of this margin. Sinusoids have diameters between 6 and 13 μ. In the particular illustration one can recognize a series of 'main' sinusoids through which blood is coursing rapidly, uniformly and in a nearly direct line from portal to hepatic venule; in addition, one can recognize the network of 'ladder' capillaries not in direct path of blood stream and displaylng intermittency of flow (see text). Hepatic arterioles do not penetrate this zone.

FIG. 3. Central venule puncture, showing position of microneedle, relative size of vessel and needle and minimal disturbance of blood flow. Note stream of dye extruded into vessel and marking direction and streamlined character of blood flow.

first started was recorded, and the pressure raised 5–10 mm higher. The pressure was next reduced gradually until the dye ceased to flow, and the blood could be seen to begin to ascend in the needle lumen. This point was also recorded. An experienced observer can reproduce each end point within less than ± 1 mm of saline. The difference between the ascending and descending readings was found to average 1.0 mm. The mean of two such determinations was taken as one pressure reading; results reported below constitute the mean value of at least three successive pressure readings, and can be considered as having a standard error of ± 0.5 mm for each situation. The apparatus was calibrated by inserting the needle into a plasma-filled chamber under the microscope and determining the pressure reading by the usual procedure. The difference between the level of the manometer and the surface of the solution in the chamber, determined by cathetometer, was 1.4 ± 0.1 cm of saline. This value has to be deducted from all measurements to be reported below to convert them to absolute pressures.

The relation of orifice of the microneedle tip to the blood vessel lumen will be discussed in greater detail below; the desired position in most cases represented perforation of the vessel at right angles to the vessel axis, the needle being inserted in such a fashion as to minimize disturbance of the blood stream as shown by direct observation. Failure to establish a seal between the puncturing glass capillary and the punctured vessel results in extravasation and gross local circulatory disturbances. Since dye injections were used in connection with the manometry, it was a simple matter to recognize any such situations and to eliminate them from the series here reported. A troublesome source of interference with pressure measurements by the method described is the occasional formation of fibrin clots around the tip of the catheter, providing, in effect, a valve that obstructs the flow of fluid back into the glass capillary. This situation, too, can be diagnosed readily, since the dye T-1824 will promptly stain such fibrin clots. All cases in which fibrin clots did form around the tip of the vessel, and could not be moved by manipulation of pressures in the microneedle, were likewise eliminated from the present series.

In addition to measuring minute vessel pressures by micropuncture under microscopic guidance, venous pressures were determined in the mesenteric veins in a number of transillumination experiments, and at several positions in the vena cava in a series of separate studies. A branch of the vena mesenterica inferior was catheterized as far distally as possible, the catheter advanced to the nearest bifurcation (giving rise to the next lower order vessel), and mean blood pressures at this point were recorded by means of a saline manometer. Segments of the intestine, the venous return of which had become impaired as a result of the catheterization, were resected. Catheterization of the vena cava was performed either by way of one femoral vein, or by way of a jugular vein past the right auricle. The position of the catheter tip in the vena cava proved to be critical (see RESULTS) and was checked both under x-ray (using Urocon to fill and visualize the catheter, and an image amplifier-cinefluorographic camera to record the results), and at autopsy.

All pressures are recorded as cm physiological saline referred to the hilum of the liver as a common zero level. Correction for capillary action was found to be the same for the large vessel as for the micropuncture measurements, i.e. 1.4 ± 0.1 cm.

To estimate path-lengths traversed by blood from the

TABLE 1. *Mesenteric Vein Pressures Before and After Preparation of Rats for Transillumination*

Time of Observation, Min.			
3*	30	35†	70
12.7±0.42	11.6±0.31	13.3±0.45	13.0±0.34
ΔP (70 - 30 min.)			1.3±0.11 cm
Δh (saline level in chamber, relative to hilum of liver)			1.4±0.01 cm

Values given are mean mesenteric vein pressures (cm saline, rel. to hilum, 7 rats) ± S.D. * Rat in dorsal position, laparotomy wound clipped closed. † Rat inserted in transillumination position in animal holder, left lateral lobe inserted in chamber, and chamber as well as abdominal cavity filled with saline immediately prior to this measurement.

junction of the mesenteric veins to the several points where pressure measurements were made, the following procedures were used: mesenteric vein to hilum of the liver, by direct measurement in a series of animals; hilum of the liver to terminal distributing portal venules, by direct measurements of several possible vascular paths from corrosion casts prepared on livers of the same size animals as the ones here employed[3]; sinusoidal path-lengths, measured on photomicrographs obtained in the present series by tracing several possible vascular paths with a cartographer's wheel; central vein to hepatic vein (this figure, too, represents an estimate based on anatomical data derived from corrosion casts of the vascular tree of the livers of rats weighing 150–200 gm). In each case, the mean of three determinations was employed and values are to be construed as having a ±10% uncertainty at each level.

RESULTS

General observations. Figure 2 illustrates a typical microscopic field in the rat liver margin as observed by the methods described above, showing the relations between afferent and efferent vessels, as well as typical vessel dimensions. The limits of variability between animals with respect to these several dimensions, as observed in a series of 25 rats, are indicated in the legend to figure 2.

Attention may be called here to several important features which characterize this vascular bed.

a) Blood flow in the absence of demonstrable external stimuli appears to be steady in all channels down to the larger sinusoids (>10 μ). However, in the smaller sinusoids which, like the rungs of a ladder, appear to form an anastomosing network between the larger sinusoids, blood flow is constant neither in velocity nor in direction. Intermittency of flow in larger sinusoids, or in portal or hepatic vein twigs is interpreted by the present observers as certain evidence of injury to the animal.

b) Figure 2 does not include any reference to the hepatic artery. This is typical of the actual vascular relations for the liver margin in the rat, as well as in the mouse. In these species, the hepatic arterial blood joins the portal blood stream at the level of the portal vein branches having diameters greater than 100 μ. Since vessels of this size do not penetrate to within 3 or 4 mm of the liver margin (K. Nakata and R. Kinosita, to be published), and therefore are not seen in the fields studied here. Blood flow in the liver margin of rat and mouse is fully described in terms of portal vein inflow and hepatic vein outflow.

Under no circumstances have the present observations revealed special constrictions of the sinusoids (either at their proximal or their distal ends) which could be interpreted as corresponding to inlet or outlet sphincters.

PRESSURE GRADIENT MEASUREMENTS

Mesenteric vein pressures. Mesenteric vein pressures for the anesthetized rat, lying on its back, are shown in table 1. The results, in accord with previous reports (2) indicate 'portal vein pressure' of about 12 cm saline in the anesthetized rat. There is often an initial decline in portal pressure during the first 10 minutes, but thereafter pressure fluctuations are small with even levels of anesthesia. Placing these same animals on their sides and inserting one liver lobe into the chamber as for transillumination results in slightly increased mesenteric vein pressures, as shown in table 1. The mean difference in venous pressure levels before and after this procedure is substantially equal to the depth of saline above the liver hilum in the observation chamber (table 1, bottom lines), which under conditions of these experiments determines not only the pressure exerted upon the exteriorized lobe of the liver, but also the intra-abdominal pressure.

Intrahepatic portal vein pressures. Table 2 shows the results of a series of measurements of intrahepatic portal venule and central vein pressures, together with the corresponding mesenteric vein pressure measurements. The microneedles were inserted into the highest order of conducting branch, just proximal to its breaking up into the terminal distributing branches of the portal venule; the diameters at the point of insertion ranged from 30 to 40 μ. The direction of insertion in eight experiments was nearly at right angles to the vessel axis (fig. 3). In five experiments, the microneedle was introduced into the main portal branch by way of one of the distributing branches, and was oriented at an obtuse angle to the main stream of blood flow. Table 2 shows that, as might be expected, a pressure differential of 0.9 cm saline exists between lateral and countercurrent orientation of the measuring orifice.

In each experiment, a large pressure differential was recorded between mesenteric venous pressure and the portal venule pressure. The values presented in table 2 indicate that about half of the portal venous pressure is dissipated in passage from the major extrahepatic branches of the portal vein to the terminal distributing radicals of this vessel near the liver margin.

Central vein pressures. In this case, microneedles were inserted into the first accessible collecting branch of a hepatic vein at a level at which it had a diameter ranging from 40 to 60 μ. Because of the slightly larger diameter of these vessels, it was invariably possible to place the needles very nearly at right angles to the axis

[3] The authors express their thanks to Dr. L. M. Julian, School of Veterinary Science, University of California, Davis, for preparing the specimens used in our experiments.

TABLE 2. *Blood Pressure Along Vascular Path Through Liver*

Location	Mean Pressure,† cm Saline Rel. to Hilum of Liver	ΔP (Mean), cm Saline
Mesenteric vein*	12.7±0.11	6.5±0.24
Extralobular portal venule (needle at right angles to blood stream)	6.3±0.20	
(needle pointing into blood stream)	7.2±0.30	5.4±0.39
Hepatic venules (smallest collecting branches)	2.3±0.15	3.7±0.16

* Rat in transillumination position. † To correct for over-all capillary effect of measuring system subtract 1.4 cm from all values; each value represents 7 animals.

of the blood stream. The results of these measurements, shown in table 2, line 4, indicate a mean pressure drop between portal venules and central vein of 3.7 cm saline.

Vena cava pressures. Vena cava pressures at levels corresponding to the drainage of the several hepatic veins, were obtained in a series of 18 rats. Catheterization from the distal end of the vena cava (i.e. through the femoral vein) gave the results shown in table 3, *column 2. Column 3* in the same table shows the corresponding set of results obtained when approaching the same points from the cardiac side (i.e. through the jugular vein). Both sets of results clearly show a marked pressure gradient along the short segment of the vena cava within the liver. The signs of the small differences between the readings obtained with the catheter pointing either cephalad or caudad are shown in *column 4* of table 3. The result is that expected from the Pitot effect when values in the region between heart and diaphragm are examined. On passing through the diaphragm toward the abdominal vena cava, however, this difference in recorded pressure disappears at first, and then reappears with a reversed sign. This suggests that the presence of the catheter in the intrahepatic flexure of the vena cava affects the observed pressure gradients in this part of the vena cava in a manner suggesting a decrease in the effective resistance to blood flow through this vessel segment.

Based on these several observations, one can conclude that the pressure against which the hepatic vein from the left lateral lobe must discharge is not less than 2.8 cm H_2O, and not more than 3.4 cm H_2O, probably closer to the former. Thus, the mean hepatic venule pressures recorded are slightly below the mean vena cava pressures against which this vessel is supposedly draining, though probably above the mean lateral pressure in the vena cava immediately above the diaphragm.

DISCUSSION

Before considering the implications of the present observations and their bearing on problems of liver function, it is pertinent to inquire to what extent the procedures employed for observation affect the physiological state of the tissue under study. It was suggested above that the technique employed is as gentle as, or even more gentle than, any method of direct observation of the liver described to date. Gross evidence supporting this contention is the absence of demonstrable pathological effects in experiments in which the liver lobe after several hours of observation was returned to its normal position, the abdomen closed, and the animal allowed to recover. Histological as well as transillumination studies performed at successive 24-hour intervals in a series of such preparations failed to reveal any evidence of tissue injury (K. Nakata, unpublished observations).

The manipulations required to exteriorize a liver lobe for observation do not appear to interfere seriously with venous drainage of the tissue. Transillumination observations confirm other experiments indicating extraordinary sensitivity of the hepatic parenchyma to elevations of venous pressure (cf. 7); in the present series local obstructions of a hepatic venule (as by a fibrin clot) resulted in readily observable congestive changes, even when venule pressures were increased by no more than 10 mm of saline. The venule pressures actually recorded in satisfactory experiments are below rather than above those recorded for the pertinent segment of the vena cava, a seeming anomaly that will require further discussion at a later point in this communication. Thus, venous outflow in these preparations can be considered either as wholly undisturbed, or as impaired to such a slight degree as not to produce visible congestion—i.e. venous pressure changes considerably smaller than 10 mm saline.

Could vasomotor reflexes or mechanical obstruction of the afferent blood supply to the lobe under study have resulted from the operative manipulations? Recent observers who have used the quartz rod transillumination technique in some form—thus avoiding the necessity of seriously altering the position of any parts of the liver—concur on the conclusion that whenever the great majority of the sinusoidal channels in the observed tissue are flowing freely, one is looking at undisturbed, or at least uninjured liver (8); controversy, if it still exists, centers on the question of whether intermittency of flow in any part of the major channels is ever observed in the healthy, undisturbed liver. Since, as mentioned in the presentation of the results, the present technique results in a tissue where all major sinusoidal channels are flowing freely, while only the ladder sinusoids, though active for the most part, show some evidence of intermittency of flow, it seems safe to conclude that these preparations correspond closely with those accepted by others as intact and undisturbed. Vasomotor mechanisms in this tissue were tested (K. Nakata, G. F. Leong and R. W. Brauer, to be published) and were found active and responsive. Finally, studies to be reported elsewhere (J. S. Krebs, G. F. Leong and R. W. Brauer, to be published) have shown that the early intrahepatic distribution of such substances as S^{35} BSP between liver lobes, or between portions of a liver lobe, is not modified perceptibly by the manipulations required to prepare an animal for transillumination of

TABLE 3. *Mean Pressures Recorded From Catheters Opening at Several Levels of Inferior Vena Cava in the Rat*

Position	Pressure in cm Saline (Rel. to Hilum of Liver) When Catheter Inserted		
	Cephalad from rt. femoral vein	Caudad from left jugular vein	ΔP
Rt. renal vein	5.36±0.08	5.06±0.15	+0.30†
Hepatic vein from caudate lobe	4.37±0.09	4.41±0.07	−0.04
Hepatic vein from medial lobe*	3.46±0.07	3.49±0.14	−0.03
Diaphragm*	2.70±0.04	3.06±0.12	−0.36‡
2–3 mm Cephalad of diaphragm	2.25±0.05	2.63±0.15	−0.38‡

Position indicates location of catheter orifice in vena cava at level of various points listed. Values given for cephalad from rt. femoral are means of 10 rats; for caudad from left jugular, 8 rats. * Left lateral lobe draining into vena cava between these two points. † $P < 0.02$ that $\Delta P > 0$. ‡ $P < 0.02$ that $\Delta P < 0$.

the left lateral liver lobe by the procedures employed in the present study. Under the conditions employed for those experiments, the distribution of test substance is a measure of relative tissue perfusion, and the results allow the deduction that this has not been altered in the exteriorized liver lobe. Thus, it seems proper to conclude that the blood supply to the tissue segment under observation is essentially intact and is not significantly disturbed by the manipulative procedures.

The fact that mesenteric vein pressures corrected for intra-abdominal pressure are substantially the same in the transilluminated preparation as in trained unanesthetized rats may be taken as further evidence of the satisfactory physiological status preserved under the conditions of the present experiments.

In addition to the manipulations required to establish the transilluminated preparations, consideration must be given to the possible effects of the micropuncture upon the local circulatory status of the liver segment under observation. Since all punctures were performed under continuous visual observation, the sequence of local changes elicited is known and can be described as follows: insertion of the needles causes a slight congestive response confined to the immediate vicinity of the needle body. This effect does not usually extend to the needle tip, once this has come to rest; blood flow in the vessel segments punctured at right angles is not visibly disturbed unless the needle occludes a significant fraction of the vessel lumen, a situation avoided in all experiments labeled 'lateral puncture' in the present series. In experiments in which the needle was pointing into the blood stream, the perforated branch was totally occluded so that the needle tip was, in effect, extended upstream by the vessel segment between puncture site and the nearest branching point. Visual observation failed to indicate perceptible congestion in the non-obstructed vessel under those conditions. For this reason, the slight pressure differential seen between the two series of portal venule pressures in table 2 was ascribed to different orifice orientation, rather than to direct or reflex vascular obstruction.

A final point to be mentioned in connection with discussion of the methodology here employed is the possible effect of succinylcholine upon the hepatic circulation. In the majority of experiments, this factor was eliminated by restricting the use of this drug to the brief period immediately preceding the insertion of the microneedles into the vessels. At least 15 minutes were allowed to elapse between insertion of the microneedles and the start of the measurements, for the double purpose of allowing dissipation of vasoactive products possibly resulting from the tissue injury, and dissipation of the effect of the small dose of succinylcholine. Thus, all measurements reported were obtained at a time when succinylcholine effects were negligible. In a few experiments, a deliberate attempt was made to make pressure measurements during a period of succinylcholine action. If any differences in hepatic vein or portal vein pressures resulted from the action of this drug, they were so small as to not be apparent from the data obtained (but cf. 9).

Anatomically, the liver region here examined is somewhat specialized; the liver margin differs from a large portion of the remaining substance of the rat liver, and presumably from the bulk of the liver of species with more rounded liver margins, in the fact that the afferent vessels—portal veins exclusively—are all located in a single plane, while efferent vessels follow the upper and lower liver surfaces. One result of this arrangement is that in this situation the so-called typical liver lobule—an intrinsically three-dimensional structure—cannot be recognized, and that those blood flow patterns specifically dependent upon extensive three-dimensional collateral flow cannot be expected to materialize in the present situation. However, the tissue sampled here is sufficiently deep to allow observation of the majority of vascular reactions typical of the hepatic parenchyma; one may conceive of the parenchyma of the deep liver substance as composed of several superposed layers, each having the same type of basic vascular structure as the tissue segments which are the subject of the present communication. The blood flow per unit weight of liver lobe margin, as estimated by the indirect method alluded to above (J. S. Krebs, G. F. Leong and R. W. Brauer, to be published) was found to be only 5–10% below that of the deep (core) portion of the same lobes in well oxygenated pentobarbital anesthetized rats.

With these considerations in mind then, one may turn to a discussion of the substantive results of the present series of experiments represented in table 2. In order to appreciate the significance of observed venous pressures along the path of the blood from mesenteric vein to vena cava, these are represented as a function of the length of path traversed (fig. 4). A number of interesting features emerge from such correlation: A negligible pressure gradient in the region between the roots of the

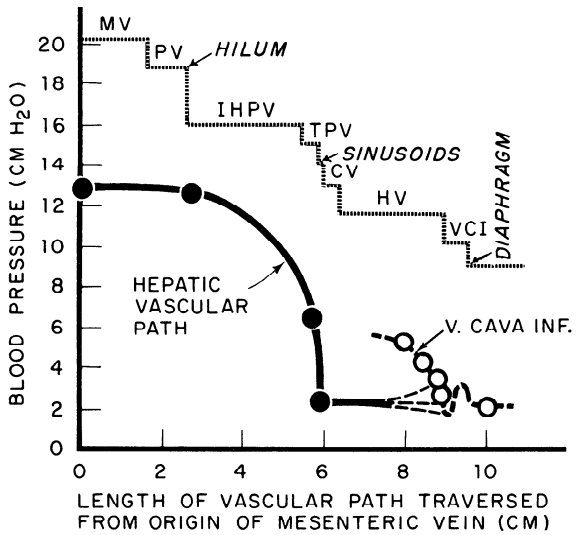


FIG. 4. Changes in pressure along path of blood through vascular tree of rat liver from mesenteric vein to hepatic vein to vena cava inferior.

mesenteric veins and the hilum of the liver; a marked pressure gradient between mesenteric vein and the terminal branches of the portal vein; a very steep loss of pressure from this point, across the sinusoidal bed, and to the smallest central veins; and finally, a pressure gradient along the hepatic vein which, to say the least, is shallow and precarious.

The rather unexpectedly great pressure drop in the intrahepatic portions of the portal vein lends substance to the views of those who would attribute a significant regulatory role to vasomotor tone in this part of the vascular tree (10). One may well wonder whether this gradient is not in fact less uniform for different parts of the liver than has been assumed in the past. Thus, could not the transition from diffuse to restricted flow patterns as described years ago on the basis of angiographic studies (11) find its explanation in an increase of the pressure differential between central and marginal distributing branches of the portal vein? Epinephrine (which also induces a restricted circulation in the rat liver (12)) was found, in fact, to increase the resistance of this part of the portal tree, enhancing the pressure gradient between mesenteric veins and liver margin (K. Nakata, G. F. Leong and R. W. Brauer, to be published). Present methods, unfortunately, do not permit resolution of this question by direct measurements of portal venule pressures in liver regions fairly close to the hilum of any liver lobe and away from the margin.

Turning next to the pressure drop between portal venule and central vein, almost 40 % of the total pressure gradient across the liver was observed in this very short segment of vascular path. Considering the high branching ratio, and the relatively slight decrease in vessel diameter associated with the progression from one order of portal vein to the next, and from the terminal distributing portal venules to the sinusoids, one would predict a decrease rather than increase of resistance per unit path-length (i.e. a flattening rather than a steepening of the curve in figure 3) as one progressed downstream in the liver (13). The fact that such a prediction proves wholly false, at least for the division into segments of the vascular tree which is feasible here, implies either gross deviations from the assumptions underlying the Poiseuille relation, or the existence of short vessel segments of very high resistance somewhere between portal venule and central vein. Such sphincter activity has indeed been postulated by some (cf. 14). In the rat, the most careful scrutiny by photographic methods employing very short exposure times to obtain sharp pictures has failed in our hands as in those of other groups (cf. 8) to reveal any indication of such constrictions at either the proximal or the distal end of the sinusoids. On the other hand, deviation from the Poiseuille assumptions in the sinusoidal bed of the liver is clearly indicated; collisions between erythrocytes and vessel wall are by no means uncommon and represent the most direct kind of evidence of that "interaction of the blood stream with the vessel wall" which is incompatible with the sort of streamline fluid flow to which the Poiseuille relation is applicable. The present authors feel, therefore, that the sinusoidal bed may well represent by itself the vascular element responsible for the steep pressure gradient shown in figure 3.

Intermittency of flow in the 'ladder-rung' sinusoids also is readily compatible with such a view. Since these sinusoids often link corresponding segments of several main sinusoids, that is, segments which occupy nearly equal positions on the pressure path diagram, minor flow irregularities would suffice to shift the pressures at either end of such cross-connecting channels and turn the flow now in one direction and now in the reverse. It may well be that these ladder-rung sinusoids are also the ones responsible for the changes in sinusoid surface, and in vascular bed cross-section deduced previously from physiological measurements (15). At the present stage, it is clear, however, that definite resolution of these several questions is not feasible, but must await the outcome of more refined micropuncture studies, which are to provide values of blood pressures in the actual sinusoids.

The final vessel segment to be considered is that between central vein and the point of effusion of hepatic blood into the vena cava. The pressure in this segment (see table 2) is low, even at the point furthest upstream. The actual value here obtained is well in accord with results obtained in dogs using transhepatic catheters, the tips of which were left in small hepatic veins, the orifice facing downstream (16). Data obtained by the more conventional venous catheterization methods have been somewhat variable, and have tended to be higher (3); to what extent this reflects an orifice orientation effect, and to what extent it reflects partial obstruction of the catheterized vessels by the catheter itself (and consequently conversion of the portion of the vascular bed in the line of obstructed flow into a mere inert conduit leading to the portal vein) cannot be assessed. The present data, however, lend further weight to the

views of those who are reluctant to overlook the effects of intravascular catheters upon hemodynamics in a low pressure vascular bed like that of the liver.

The observations regarding pressures at the distal end of the hepatic vein focus attention upon the existence of a considerable pressure drop across a relatively short segment of a large blood vessel, the vena cava. In the absence of any sphincter-like obstruction, this gradient must be the direct result of distortion of the vessel in its course through the liver; the existence of such a diaphragmatic flexure of the vena cava, and of jet-like flow of blood through it, has been described long ago for several mammalian species (17), and has recently been confirmed for the rat (18). The importance of a steep pressure gradient in the vena cava may be twofold: in the first place, it causes the different lobes of the rat liver to drain against different pressures; in view of the extreme susceptibility of the fluid compartment framework of the rat liver to even small venous pressure changes (7), the different sites of hepatic vein orifices for the several liver lobes in the rat might well result in important structural or compositional differences between the several parts of the organ. In the second place, the pressure gradient in this segment of the vena cava should vary with the respiratory movements of the diaphragm and contribute to a flow in the hepatic veins which, on the basis of the mean pressures shown in figure 3, must be discontinuous to escape a paradoxical 'flowing uphill' of hepatic venous blood. Intermittency of hepatic vein flow linked to respiratory excursions was surmised long ago by Franklin and Janker on the basis of observations of 'notching' of the stream of contrast medium in the hepatic segment of the vena cava (17). More recently, by injecting via the transhepatic route directly into a hepatic vein, intermittency of flow in this vessel could be conclusively demonstrated in the rat (18), and was found correlated with the respiratory cycle; inspiration corresponded with 'no flow', expiration, especially near the peak of the cephalad excursion of the diaphragm, with maximal hepatic vein flow. Retrograde flow in the hepatic veins of the median and left lateral lobes of the rat liver was observed during the inspiratory phase of the respiratory cycle. The pressure relations here presented thus are in accord with the unstable pressure gradients indicated by the angiographic studies.

To summarize the several problems, hitherto in the realm of speculation, which are brought into focus by the micropuncture studies reported here: *a*) the large pressure drop recorded in the portal tree, between hilum of liver and terminal venule twigs, has given substance to speculations regarding the importance of this vessel segment in the control of liver blood flow, and in the observed transition in blood flow distribution in the liver; *b*) the pressure gradient across the sinusoidal bed has been defined clearly, although pinpointing of the site of resistance by actual experiment will require further refinement of method; the considerations associated with the analysis of this part of the vascular bed of the liver have provided a testable hypothesis regarding the problem of intermittency of flow in certain sinusoids; *c*) finally, analysis of the pressure gradients in the hepatic vein, revealing a paradoxical mean pressure gradient, have provided the beginnings of a physical basis for the observed relation of hepatic venous outflow to the respiratory cycle in the rat.

REFERENCES

1. BRAUER, R. W. (ed.). *Liver Function.* Washington, D. C.: American Inst. Biol. Sci., 1958, Chapt. 2, Monograph no. 4.
2. HOFFBAUER, F. W., J. L. BOLLMAN AND J. H. GRINDLAY. *Gastroenterology* 16: 194, 1950; VOLWILER, W., J. H. GRINDLAY AND J. L. BOLLMAN. *Gastroenterology* 14: 40, 1950; FRIEDMAN, E. W., S. MILROD, II. A. FRANCK AND J. FINE. *Proc. Soc. Exper. Biol. & Med.* 82: 636, 1953; JOHNSON, D. H. *J. Physiol.* 120: 1P, 57P, 1953; WELCH, G. E., R. EMMETT, C. C. CRAIGHEAD, G. HOEFFLER, D. C. BROWNE AND J. ROSEN. *Am. J. M. Sc.* 228: 643, 1954.
3. FRIEDMAN, E. W. AND R. S. WEINER. *Am. J. Physiol.* 165: 527, 1951; MYERS, J. D. AND H. J. TAYLOR. *J. Clin. Invest.* 30: 662, 1951; BROWNE, D. C. AND G. E. WELCH. *J.A.M.A.* 158: 106, 1955; WERNER, A. Y., D. M. MACCANNON AND S. M. HORVATH. *Am. J. Physiol.* 170: 624, 1921; WELCH, G. E., R. EMMETT, C. C. CRAIGHEAD, G. HOEFFLER AND D. C. BROWNE. *Am. J. M. Sc.* 228: 643, 1956; REYNOLDS, T. B., D. C. BALFOUR, E. C. LEVINSON, W. R. NICKELSON AND B. C. PATTERSON. *J. Clin. Invest.* 34: 213, 1955; REYNOLDS T. B., A. G. REDEKER AND H. M. GELLER. *Am. J. Med.* 22: 341, 1957; PATON, A., T. B. REYNOLDS AND S. SHERLOCK. *Lancet* 1: 918, 1953.
4. NAKATA, K., M. SUGIURA AND T. NAKATSUBO. *Acta Haematol. Jap.* 18: 92, 1955.
5. IRWIN, J. W. AND J. MACDONALD. *Anat. Rec.* 117: 1, 1953.
6. LANDIS, E. M. *Am. J. Physiol.* 75: 548, 1925.
7. BRAUER, R. W., R. J. HOLLOWAY AND G. F. LEONG. *Am. J. Physiol.* 197: 681, 1959.
8. KNISELEY, M. H., E. F. HARDING AND H. DEBACKER. *Science* 125: 1023, 1957; MAEGRAITH, B. G. In: *Liver Function*, edited by R. W. BRAUER. Washington, D. C.: American Inst. Biol. Sci., 1958, Monograph no. 4, p. 135; ANDREWS, W. H., R. HECKER AND B. G. MAEGRAITH. *J. Physiol.* 122: 53P, 1953; SENEVIRATNE, R. P. *Quart. J. Exper. Physiol.* 35: 77, 1949; CHENG, K. K. *J. Path. & Bact.* 67: 89, 1954 and 71: 265, 1956.
9. PETERS, R. *Acta Hepatologica* 4: 28, 1956.
10. ANDREWS, W. H. H. *Lancet* 166: 1955; ANDREWS, W. H. H. In: *Liver Function*, edited by R. W. BRAUER. Washington, D. C.: American Inst. Biol. Sci., 1958, Monograph no. 4, p. 175.
11. PRITCHARD, M. M. L. AND P. M. DANIEL. In: *Ciba Symposium on Visceral Circulation*, G. E. W. WOSTENHOLME, editor, 1952, p. 60.
12. DANIEL, P. M. AND M. M. L. PRITCHARD. *J. Physiol.* 114: 52, 538, 1951.
13. ZOPFF, G. *Arch. klin. Chir.* 197: 319, 1940.
14. KNISELEY, M. H. *Science* 125: 1023, 1957; MAEGRAITH, B. G., W. H. H. ANDREWS AND C. E. M. WENYON. *Ann. Trop. Med.* 43: 225, 1949.
15. BRAUER, R. W., G. F. LEONG, R. F. MCELROY, JR. AND R. J. HOLLOWAY. *Am. J. Physiol.* 186: 537, 1956.
16. BOLLMAN, J. L., M. KHATTAB, K. THORS AND J. H. GRINDLAY. *A. M. A. Arch. Surg.* 66: 562, 1953 (personal communication, 1959).
17. FRANKLIN, K. J. AND R. JANKER. *J. Physiol.* 86: 264, 1936 and 89: 160, 1937.
18. BRAUER, R. W., G. F. LEONG, R. L. PESSOTTI AND R. F. MCELROY, JR. *Fed. Proc.* 18: 15, 1959.