Comparison of the Bromsulphalein Method with Simultaneous Direct Hepatic Blood Flow

By EWALD E. SELKURT, Ph.D.

A method has been devised for directly measuring hepatic outflow in dogs so as to permit simultaneous comparison with the indirect method, employing the hepatic removal of Bromsulphalein (BSP). The results show that the indirect method averages 7.3 per cent higher than the direct. This difference is attributed to a small degree of extrahepatic removal of the dye.

The Bromsulphalein extraction method developed by Bradley, Ingelfinger, Bradley, and Curry is being applied with increasing frequency to problems of hepatic hemodynamics. However, estimates of the validity of the method have been controversial.

A method for measuring hepatic venous outflow directly has been developed and has been employed in the present investigation to evaluate critically the Bromsulphalein (BSP) extraction method for measuring effective hepatic blood flow (EHBF).

METHOD

Dogs were anesthetized with pentobarbital sodium (30 mg. per kilogram, intravenously) to permit the necessary surgical procedures. Approach to the vena cava inferior to the hepatic veins was made through a right flank incision, beginning at the rib margin. Loose ligatures were placed around the vena cava between the right adrenal vein and the right renal vein. All cannulas, manometers, and parts of the external circuit were filled with saline before insertion and connection. The animals were heparinized (4 mg. per kilogram) before any vessels were cannulated. Supplementary heparin was administered in a dose of 10 mg. every half hour. In three experiments in which the rotameter was used, an additional dose of 250 mg. of the heparinoid manuronate* was given, plus 50 mg. total every half hour.

Prior to insertion of the hepatic vein cannula into the inferior vena cava, a shunt circuit originating in

* Supplied through the courtesy of Dr. Joseph Seifter, Wyeth Institute of Applied Biochemistry, Philadelphia, Pa.
the femoral veins was opened permitting blood from the hind portions of the animal to return by an external jugular vein (fig. 1). Insertion of the jugular cannula forward into the chest took advantage of the negative intrathoracic pressure which aided in overcoming resistance in the external circuit. The inferior vena cava was then ligated and incised, and the hepatic cannula introduced and tied into place. As illustrated in the diagram, this cannula was provided with a small balloon tied to polyethylene tubing, the latter passing snugly through a narrow metal sleeve at the rear of the cannula.

Donor blood in sufficient amount to fill the pump circuit was now introduced by way of the reservoir. A quantity of blood was kept in reserve to replace that removed for dye analysis. The collapsed balloon was pushed up the inferior vena cava by advancing the polyethylene tubing to which it was attached to an orifice, so that venous pressure would remain unaltered during the flow recording. Small aliquots of the hepatic venous blood were retained for chemical analysis simultaneously, the key opened the reservoir permitting an equal volume of the animal’s blood to enter the circumflex chambers D₁ and D₂ minimized pressure variations created by the pump action.

Bromsulphalein was infused by motor driven syringe at rates based on previous experience, supplemented by an initial priming dose of 50 mg. The rates of infusion are given in table 1. An average interval of 52 minutes elapsed between the beginning of the infusion and the start of flow determinations. The intervals, except for one instance of 21 minutes, were at least of 30 minutes duration.

Direct flow measurements in 11 experiments were made with the volume recorder illustrated in figure 1. Depression of the shunt key diverted about 40 ml of blood to a graduated cylinder through an outflow orifice adjusted by a clamp so that venous pressure remained unaltered during the flow recording. Simultaneously, the key opened the reservoir permitting an equal volume of the animal’s blood to enter the circulation, thus minimizing arterial blood pressure fluctuations. The blood entering the cylinders of the volume recorder caused a deflection of the optical manometer C in a manner linearly related to the accumulated volume. The rate of outflow was subsequently measured by calibration of this manometer against known volumes and relating the degrees of deflection on the record to the time signal of the photokymograph. Small aliquots of the hepatic venous blood were retained for chemical analysis and the remainder returned to the reservoir. Simultaneous arterial blood samples were taken, so that Bromsulphalein A-V differences could subsequently be measured. Accompanying the records of flow, the venous pressure was recorded, and also the arterial pressure with manometer A. All manometers were calibrated against static pressures at the close of the experiment. In three experiments an optically recording rotameter of the type described by Shipley

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<th>No. Obs.</th>
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<th>V.P. cm. H0</th>
<th>Dye inf. mg/min</th>
<th>Art. conc. mg.%</th>
<th>A-V mg.%</th>
<th>EHBF ml/min</th>
<th>Direct ml/min</th>
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and Wilson was used for continuous measurement of flow, replacing the shunt key and volume recorder. Calibration curves were made at the conclusion of the experiment with the animal's blood.

Flow measurements were made approximately every three minutes in groups of four with an average of eight minutes between groups, to permit calibration of records and to make any necessary adjustments. This was continued as long as the condition of the animal remained satisfactory. The time during which the observations were made averaged 88 minutes, and varied from 27 to 144 minutes.

Details of the chemical methods and the method of calculation of effective hepatic blood flow have been described elsewhere. A modification of previously described methods was to prepare standard curves with aliquots of blank plasma in the amount used for unknown. Such a procedure permitted analysis of slightly hemolyzed samples against standards mixed with blank plasma samples of a comparable degree of hemolysis. Blood levels of Bromsulphalein were steady enough (changes of less than 0.00009 mg. per minute per milliliter) so that no correction for changing levels appeared warranted except for one experiment (no. 11) in which an increase of 0.000136 mg. per minute per milliliter occurred.

RESULTS

The results of the investigation are summarized in Table 1, in which data have been arbitrarily arranged in relation to mean arterial blood pressure. The level of the arterial pressure, in the ranges observed, apparently had no influence on the ratio: $EHBF/direct$ blood flow. The combined average of the ratio was 1.073 with range of 0.93 to 1.36.

The question of the validity of the method during experimental variation of hepatic flow can be answered by detailed presentation of two representative experiments.

In figure 2 is shown an experiment in which blood flow was kept reasonably steady during 35 minutes by replacing blood drawn for chemical analysis with donor blood. No flow measurements were made during the next 20 minutes. During this interval vascular resistance increased and hepatic flow diminished from 500 ml. per minute to 330 ml. per minute, and blood flow as measured by the Bromsulphalein method followed the direct measurements closely. As anticipated, during the period of slower blood flow the Bromsulphalein A-V difference increased from 0.35 to 0.475 mg. per cent at arterial dye concentrations of 1.20 and 1.275 mg. per cent respectively.

Another experiment is presented in figure 3. Flow and arterial pressure were kept reasonably constant for an hour by replacement of blood removed for dye analysis with donor blood. Then 200 ml. of blood to which Bromsulphalein was added in an amount of dye estimated to produce a concentration equal to that in the animal's blood were rapidly infused. This transfusion increased arterial blood pressure from 130 to 170 mm. Hg. Direct hepatic blood flow rose from 500 to 700 ml. per minute. Concomitantly, effective hepatic blood flow increased from 542 to 612 ml. per minute, but was not as well maintained as the simultaneous direct blood flow. As anticipated for a more rapid blood flow, Bromsulphalein A-V differences decreased during this interval from 0.18.
MEASUREMENTS OF HEPATIC BLOOD FLOW

Fig. 4. The relationship of A-V Bromsulphalein concentration in the present series to the simultaneous direct hepatic blood flow. Each point is the average of all observations made in each animal.

to 0.16 mg. per cent at arterial dye concentrations of 0.81 and 0.90 mg. per cent, respectively.

In fulfillment of the fundamental premise that the rate of dye removal should be inversely related to the rate of blood flow, the data relating average differences to rate of direct blood flow in all experiments are presented in figure 4. It can be seen that the expected relationship prevails.

DISCUSSION

The average value of 1.073 for EHBF/direct flow is in approximate conformity with the previous finding of 1.1, arrived at by computations involving a consideration of extrahepatic A-V differences for Bromsulphalein, and of rate of extrahepatic dye removal during hepatic ischemia. It is somewhat higher than Bradley’s estimate of less than 5 per cent, based on comparison of dye removal before and after hepatectomy and evisceration.

The average error of 7.3 per cent is undoubtedly attributable to extrahepatic dye removal. The fact that the ratios of individual experiments ranged from 0.93 to 1.36, and that individual simultaneous comparisons at times varied more widely, indicates that minor errors creep in, for example, in (1) analysis for Bromsulphalein; (2) direct measurement of flow; (3) small changes in plasma dye levels; (4) irregular uptake by the liver, prehepatic removal, and enterohepatic recirculation of the dye. Although individually insignificant, as determined previously, these negligible sources of error may compound to account for the observed range of variation.

It is believed that the Bromsulphalein method for measuring hepatic blood flow will prove to be as adequate as other indirect methods used for measuring blood flow provided that proper attention is given to obvious necessary details such as infusion of the correct amount of dye to maintain constant blood levels, the correct placement of the hepatic vein catheter, and care in withdrawal of the hepatic vein sample so as not to contaminate it with inferior vena cava blood. The method should be particularly reliable if based on the average of a series of consecutive measurements.

SUMMARY AND CONCLUSIONS

A method has been devised for direct measurement of total hepatic venous outflow in anesthetized dogs. Comparison of measurements made with this direct method with those made by the indirect Bromsulphalein method have served as a basis for the evaluation of the validity of the latter method.

A total of 274 comparisons in 14 experiments yielded an average ratio of the effective hepatic blood flow (EHBF) to simultaneous direct flow of 1.073. In individual experiments the average ratio varied from 0.93 to 1.36. Individual flow comparisons showed greater variability. The difference between effective hepatic blood flow and direct flow is attributed to extrahepatic dye removal.

It is concluded that the Bromsulphalein method when correctly performed reliably measures hepatic blood flow, based on a well-maintained correspondence to direct flow under varying physiologic conditions.

ACKNOWLEDGMENT

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REFERENCES

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