# SPLANCHNIC BLOOD FLOW IN MAN BY THE BROMSULFALEIN METHOD: THE RELATION OF PERIPHERAL PLASMA BROMSULFALEIN LEVEL TO THE CALCULATED FLOW

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In 1944 Warren and Brannon[1] described a safe method for catheterizing the hepatic vein in man. The following year, Bradley and co-workers[2] adopted this technique for the estimation of splanchnic blood flow by measuring hepatic extraction of bromsulfalein (BSP). Other workers used this method, but the estimated splanchnic blood flow (E.S.B.F.) has been followed for only relatively short periods.[3-6] The present report describes splanchnic blood flow over two and one-half to three hours in normal subjects. In particular, the relation of the BSP concentration in the peripheral blood to the calculated splanchnic blood flow is discussed.

## METHODS AND MATERIAL

The forty-nine normal subjects had no known hepatic dysfunction.

*Experimental Procedure.*—Observations were made after the subjects had been at rest in bed and had fasted for twelve hours. Sodium Amytal, 0.2 Gm. by mouth, was given thirty minutes before the observations were begun.

The technique for estimating splanchnic blood flow was essentially that of Bradley and associates.[2] A branch of the right hepatic vein was catheterized under fluoroscopic control. The catheter was kept in the same position throughout and this was fluoroscopically checked at the conclusion of the observation.

A priming dose of 150 mg. BSP was given and then BSP was infused in 0.5, 0.6, or 0.7 Gm. per cent solution. The infusion was maintained at a rate of about 1 ml. per minute, using a motor-driven 50 ml. syringe, modified from the model used by Allen and co-workers.[7] The mean dose of BSP was 0.090 (0.043 to 0.156) mg. per kilogram per minute.

Blood samples were taken simultaneously from the hepatic vein catheter and through an indwelling needle in an antecubital vein. The latter was used instead of an indwelling arterial needle because of the greater ease of repeated sampling over long periods. It was found that peripheral venous and arterial blood samples, taken simultaneously, showed identical BSP concentrations.

Blood samples were taken at intervals of five to twenty minutes. After the priming dose of BSP, at least twenty minutes elapsed before the first sample was taken. If the BSP concentration of the infusion had to be altered, then another twenty-minute stabilization period was allowed before further samples were withdrawn. The observations were continued for two and one-half to three hours.

*Analytical Methods:* Blood samples were taken into heparinized tubes and analyzed on the same day. After an initial centrifugation, the supernatant plasma was recentrifuged. Turbid and hemolyzed samples were discarded. Two milliliters of plasma were added to 4 ml. 0.9 Gm. per cent sodium chloride. Readings were made with an Evans photoelectric

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TABLE I. ESTIMATED SPLANCHNIC BLOOD FLOW IN 49 NORMAL SUBJECTS

| Subject | Sex and age | Surface area (sq. m.) | Weight (kg.) | BSP: Infusion rate (mg./kg./min.) | BSP: Peripheral level (mg./100 ml.) | E.S.B.F. (ml./min.) | E.S.B.F. (ml./sq.m./min.) | BSP (per cent hepatic extraction) |
|---|---|---|---|---|---|---|---|---|
| **A. Peripheral BSP level less than 1 mg./100 ml.** | | | | | | | | |
| C. M. | M, 42 | 1.93 | 76 | 0.043 | 0.48 | 5,260 | 2,720 | 25 |
| C. H. | M, 49 | 1.72 | 62 | 0.075 | 0.50 | 3,330 | 1,940 | 47 |
| T. R. | M, 29 | 1.60 | 50 | 0.099 | 0.50 | 2,410 | 1,500 | 70 |
| E. T. | M, 37 | 1.79 | 63 | 0.079 | 0.54 | 2,280 | 1,270 | 77 |
| J. J. | M, 40 | 1.49 | 50 | 0.091 | 0.62 | 2,570 | 1,720 | 51 |
| M. McG. | F, 34 | 1.54 | 53 | 0.094 | 0.65 | 2,040 | 1,321 | 49 |
| F. S. | M, 37 | 1.92 | 74 | 0.074 | 0.68 | 2,540 | 1,320 | 56 |
| G. F. | F, 45 | 1.66 | 60 | 0.097 | 0.70 | 1,960 | 1,182 | 69 |
| J. P. | M, 56 | 1.79 | 67 | 0.108 | 0.71 | 1,940 | 1,080 | 79 |
| J. H. | F, 34 | 1.44 | 48 | 0.104 | 0.72 | 1,635 | 1,135 | 67 |
| M. C. | F, 52 | 1.25 | 32 | 0.156 | 0.75 | 2,260 | 1,810 | 36 |
| M. F. | F, 42 | 1.36 | 40 | 0.093 | 0.76 | 2,760 | 2,030 | 27 |
| P. T. | M, 24 | 1.60 | 49 | 0.096 | 0.79 | 1,800 | 1,120 | 61 |
| J. H. | M, 26 | 1.59 | 51 | 0.098 | 0.81 | 1,640 | 1,020 | 69 |
| E. C. | M, 29 | 1.94 | 73 | 0.063 | 0.85 | 2,490 | 1,280 | 34 |
| W. W. | M, 38 | 1.72 | 63 | 0.082 | 0.85 | 1,962 | 1,140 | 58 |
| J. T. | M, 58 | 1.99 | 84 | 0.057 | 0.88 | 1,930 | 970 | 46 |
| Mean | | | | 0.089 | 0.68 | 2,411 | 1,530 | 54.1 |
| Standard error | | | | 0.0060 | 0.04 | 194 | 126 | 4.0 |
| **B. Peripheral BSP level 1 mg./100 ml. or more** | | | | | | | | |
| J. S. | M, 56 | 1.68 | 61 | 0.095 | 1.00 | 1,350 | 804 | 78 |
| J. K. | M, 40 | 1.87 | 67 | 0.075 | 1.00 | 1,550 | 830 | 52 |
| T. B. | M, 22 | 1.91 | 70 | 0.070 | 1.01 | 2,260 | 1,180 | 42 |
| C. S. | M, 47 | 1.75 | 60 | 0.088 | 1.02 | 1,561 | 895 | 58 |
| J. W. | M, 36 | 1.72 | 63 | 0.078 | 1.02 | 1,613 | 940 | 54 |
| G. D. | M, 68 | 1.53 | 46 | 0.094 | 1.05 | 1,120 | 723 | 63 |
| E. F. | F, 45 | 1.65 | 60 | 0.109 | 1.09 | 1,480 | 890 | 70 |
| P. H. | M, 34 | 1.93 | 72 | 0.094 | 1.14 | 2,500 | 1,290 | 43 |
| H. S. | M, 62 | 1.53 | 55 | 0.105 | 1.17 | 1,360 | 890 | 69 |
| T. D. | M, 67 | 1.56 | 48 | 0.106 | 1.18 | 1,240 | 795 | 43 |
| C. R. | M, 51 | 1.71 | 58 | 0.069 | 1.19 | 1,296 | 760 | 41 |
| J. B. | M, 46 | 1.78 | 65 | 0.090 | 1.19 | 1,940 | 1,090 | 48 |
| J. F. | F, 21 | 1.50 | 50 | 0.112 | 1.22 | 1,290 | 860 | 55 |
| J. M. | M, 57 | 1.57 | 60 | 0.086 | 1.22 | 1,120 | 715 | 70 |
| I. S. | M, 40 | 1.77 | 65 | 0.062 | 1.25 | 1,262 | 715 | 76 |
| F. B. | M, 64 | 1.74 | 64 | 0.072 | 1.28 | 1,600 | 920 | 41 |
| B. W. | M, 45 | 1.72 | 63 | 0.079 | 1.32 | 1,050 | 610 | 68 |
| T. O'N. | M, 20 | 1.66 | 57 | 0.100 | 1.40 | 1,760 | 1,060 | 41 |
| P. H. | M, 34 | 1.44 | 48 | 0.107 | 1.41 | 1,380 | 960 | 48 |
| J. M. | M, 57 | 1.55 | 54 | 0.065 | 1.42 | 912 | 588 | 50 |
| J. N. | M, 65 | 1.53 | 48 | 0.096 | 1.48 | 1,564 | 1,020 | 40 |
| D. M. | M, 50 | 1.56 | 49 | 0.114 | 1.52 | 1,226 | 788 | 50 |
| J. P. | M, 56 | 1.79 | 67 | 0.108 | 1.55 | 1,515 | 845 | 54 |
| C. M. | M, 33 | 1.79 | 69 | 0.083 | 1.57 | 1,500 | 850 | 50 |
| T. E. | M, 36 | 1.50 | 44 | 0.108 | 1.60 | 1,495 | 999 | 33 |
| A. A. | M, 75 | 1.57 | 50 | 0.100 | 1.62 | 908 | 578 | 57 |
| E. M. | M, 34 | 1.93 | 76 | 0.082 | 1.90 | 1,475 | 769 | 43 |
| J. T. | M, 56 | 1.46 | 47 | 0.131 | 1.91 | 1,038 | 710 | 38 |
| J. M. | M, 60 | 1.70 | 60 | 0.097 | 1.99 | 1,300 | 765 | 40 |
| J. F. | F, 45 | 1.64 | 60 | 0.091 | 2.39 | 1,270 | 775 | 31 |
| R. R. | M, 63 | 1.69 | 57 | 0.102 | 2.82 | 845 | 500 | 38 |
| M. H. | M, 60 | 1.56 | 49 | 0.112 | 3.25 | 1,030 | 660 | 28 |
| Mean | | | | 0.090 | 1.45 | 1,400 | 836 | 50.7 |
| Standard error | | | | 0.00028 | 0.10 | 63 | 31 | 2.18 |

colorimeter and an Ilford green (625) filter. After the initial "blank" reading, a drop of 40 per cent sodium hydroxide was added and a further reading taken. The concentration of BSP in the blood was then calculated. The concentration of BSP in the solution used for infusion was determined in every experiment. The hematocrit value was determined on one of the first three hepatic venous samples, using the Wintrobe technique. The oxygen unsaturation of the hepatic vein blood was estimated on 5 ml. samples using a Haldane blood gas apparatus.[8]

*Calculation.*—If the infusion rate of BSP (Q) is adjusted to maintain a constant blood level (P), then Q is also the rate of removal of BSP by the liver and the Fick principle is applicable. E. S. B. F. may then be calculated by the method of Bradley and co-workers[2] according to the following formulas.

Estimated splanchnic plasma flow $= \dfrac{100\ Q}{P\text{-}H}$ ml./min. and Estimated splanchnic blood flow $= \dfrac{100\ Q}{P\text{-}H} \times \dfrac{1}{100\text{-Ht.}}$ ml./min. where Q = infusion rate of BSP ml./min.

P = mg. BSP/100 ml. plasma (peripheral vein).
H = mg. BSP/100 ml. plasma (hepatic vein).
Ht. = Hematocrit value.

(Allowance was made for changing BSP values in the peripheral blood provided the rate of change was not greater than 0.0005 mg. per milliliter per minute.)

*The percentage extraction of bromsulfalein* was calculated.

$$\text{Per cent extraction} = \frac{P\text{-}H}{P} \times 100.$$

## RESULTS

The subjects were divided into two groups depending on whether the peripheral BSP concentration was greater or less than 1 mg. per 100 milliliters. The mean infusion rate of BSP in both groups was similar.

In thirty-two subjects the peripheral BSP concentration was greater than 1 mg. per 100 ml. and the mean E.S.B.F. was found to be 836 ± 31 ml. per minute per square meter (Table I). This corresponds with values previously reported in the literature (Table II). There was no correlation between the peripheral venous BSP level and the E.S.B.F. (Fig.1).

TABLE II. COMPARISON OF PRESENT RESULTS WITH THOSE PREVIOUSLY REPORTED

| AUTHOR | DATE | NUMBER OF SUBJECTS | PERIPHERAL PLASMA BSP CONCENTRATION (MG. 100 ML.) | ESTIMATED SPLANCHNIC BLOOD FLOW (ML./SQ.M.) |
|---|---|---|---|---|
| Bradley and co-workers | 1945 | 23 | 0.94 ± 0.04* | 865 ± 24 (640-1070) |
| Myers | 1947 | 12 | 1.61 ± 0.22 | 850 ± 71 (600-1160) |
| Bondy and co-workers | 1949 | 7 | — | 851 ± 71 (641-1210) |
| Sherlock and co-workers | 1950 | 32 | 1.45 ± 0.10 | 836 ± 31 (500-1290) |
| | | 17 | 0.68 ± 0.04 | 1530 ± 126 (970-2720) |

*Throughout this paper in the expression of ± values the standard error is used.

In seventeen subjects the peripheral venous BSP concentration was less than 1 mg. per 100 ml. and the mean E.S.B.F. was 1530 ± 126 ml. per minute per square meter (Tables I and II). There was a positive inverse correlation between peripheral venous BSP concentration and E.S.B.F. (Fig. 1).

A statistical difference (p = 0.02) was established between the mean E.S.B.F.'s corresponding to peripheral BSP values greater or less than 1 mg. per 100 milliliters.

*Effect of Changing the Peripheral Bromsulfalein Level on Estimated Splanchnic Blood Flow.*—In twenty-two subjects the peripheral BSP value showed a slow consistent rise during the two- to three-hour period of the experiment, despite the BSP infusion rate's remaining constant. The calculated splanchnic blood flow at the beginning of the observation period was compared

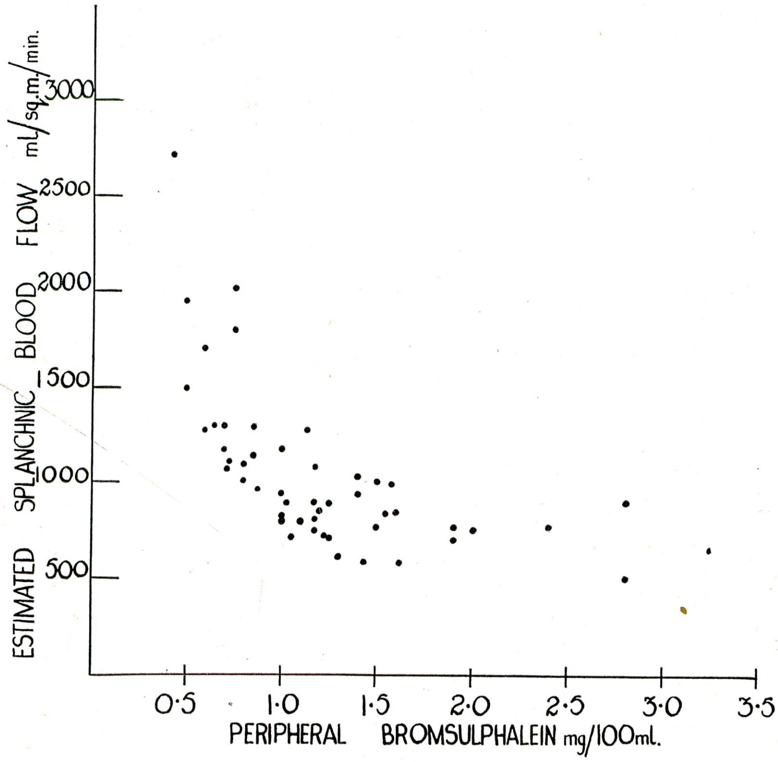


Fig. 1.—The relation of peripheral venous bromsulfalein values to estimate splanchnic blood flow.

with that at the end (Fig. 2). When the peripheral BSP value was below 1 mg. per 100 ml., an increase in peripheral BSP resulted in an apparent fall in E.S.B.F. If the initial levels were greater than 1 mg. per 100 ml., then further increase in the peripheral BSP level did not lead to any significant change in the calculated splanchnic blood flow.

In two subjects a more detailed study of the effect of changing peripheral venous BSP values was made. The results in one of these subjects are illustrated (Fig. 3). Reduction of the peripheral BSP level by altering the amount of BSP infused resulted in an apparent increase in E.S.B.F. This rise in E.S.B.F. persisted while the peripheral BSP level remained low, even when the original rate of infusion was resumed. Finally, a further "priming" dose of BSP was given and the infusion rate of BSP was again increased. With the resultant high peripheral BSP value, E.S.B.F. returned to a value comparable with those initially recorded. During the course of these observations, the subject's general condition, pulse, and blood pressure did not change. Moreover, there was no significant change in the percentage saturation of the hepatic venous blood with oxygen. These data suggest that, despite the calculated results, splanchnic blood flow did not in fact alter significantly during the period of observation.

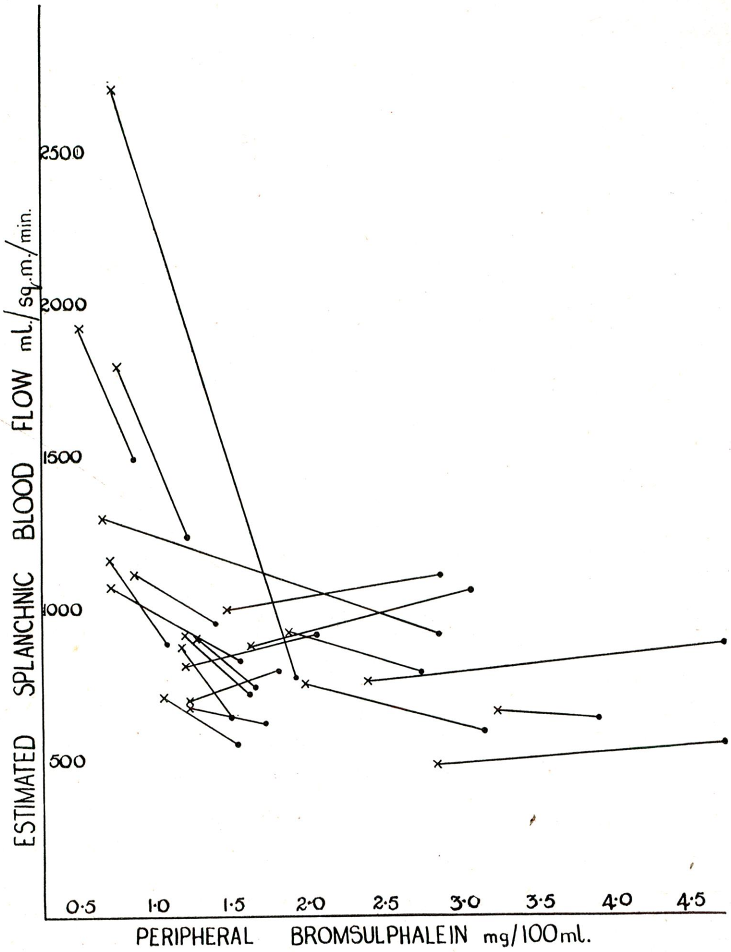


Fig. 2.—Changes in calculated splanchnic blood flow related to alteration in peripheral venous bromsulfalein. × represents initial reading and • the value obtained 2 to 3 hours later.

*Hepatic Bromsulfalein Extraction.*—The percentage extraction of BSP was greater at the lower peripheral BSP levels. The peripheral level was plotted on semilog paper against percentage extraction (Fig. 4). The values obtained are comparable with those given by Myers[9] for his control series and provide evidence that hepatocellular function with respect to BSP was normal in the present group.

*Constancy of the Estimated Splanchnic Blood Flow Over Long Periods.*—In fifteen subjects the initial peripheral BSP value was greater than 1 mg. per 100 ml. and the calculated splanchnic blood flow was compared with that obtained two hours later (Fig. 2). E.S.B.F. showed little change.

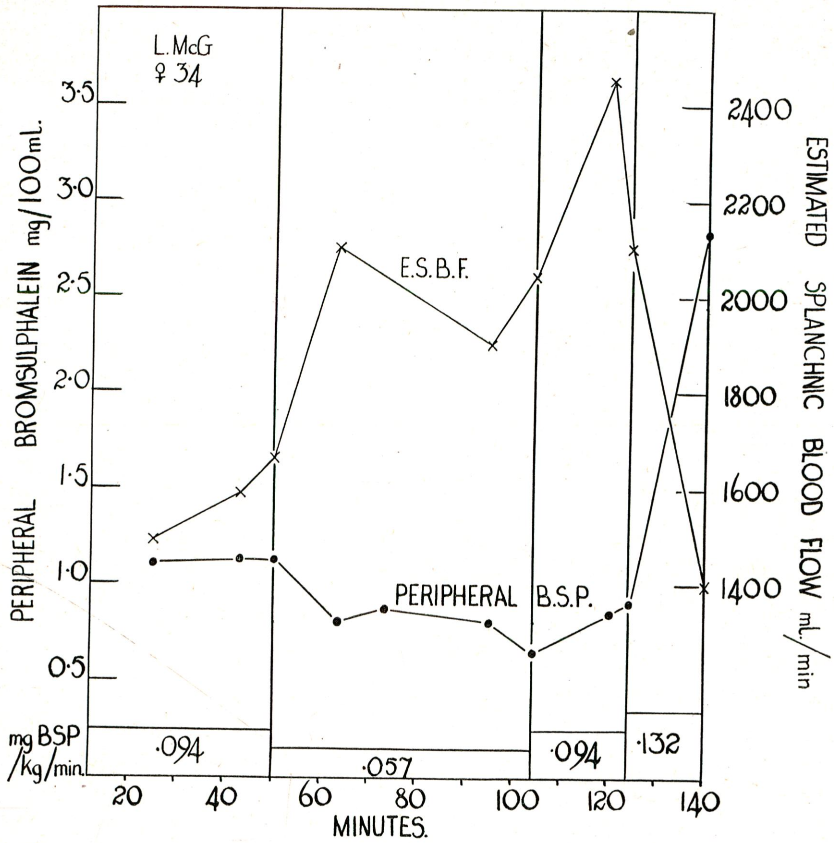


Fig. 3.—Effect of changing dose of bromsulfalein on peripheral bromsulfalein values and calculated blood flow.

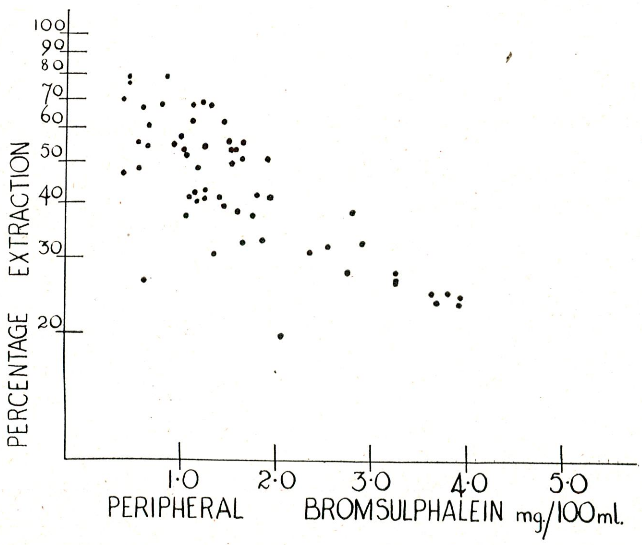


Fig. 4.—The relation of peripheral bromsulfalein values to the percentage extraction by the liver.

TABLE III. REPEATED ESTIMATIONS OF SPLANCHNIC BLOOD FLOW IN TWO NORMAL SUBJECTS

| SUBJECT | AGE | SEX | WT. (KG.) | S.A. (SQ.M.) | BSP: INITIAL PERIPHERAL VENOUS VALUE (MG./100 ML.) | BSP: DOSE (MG./KG./MIN.) | E.S.B.F. (ML./MIN.): TIME IN MINUTES FROM START OF BSP INFUSION: 35 | 45 | 60 | 75 | 90 | 110 | 130 | 150 | 170 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M. H. | 60 | M | 49 | 1.56 | 2.07 | 0.112 | 1250 | 1050 | 1030 | — | 1200 | 1070 | 1000 | 990 | — |
| A. B. | 40 | M | 64 | 1.74 | 1.15 | 0.072 | — | 1600 | — | 1510 | 1530 | 1380 | 1350 | 1650 | 1330 |

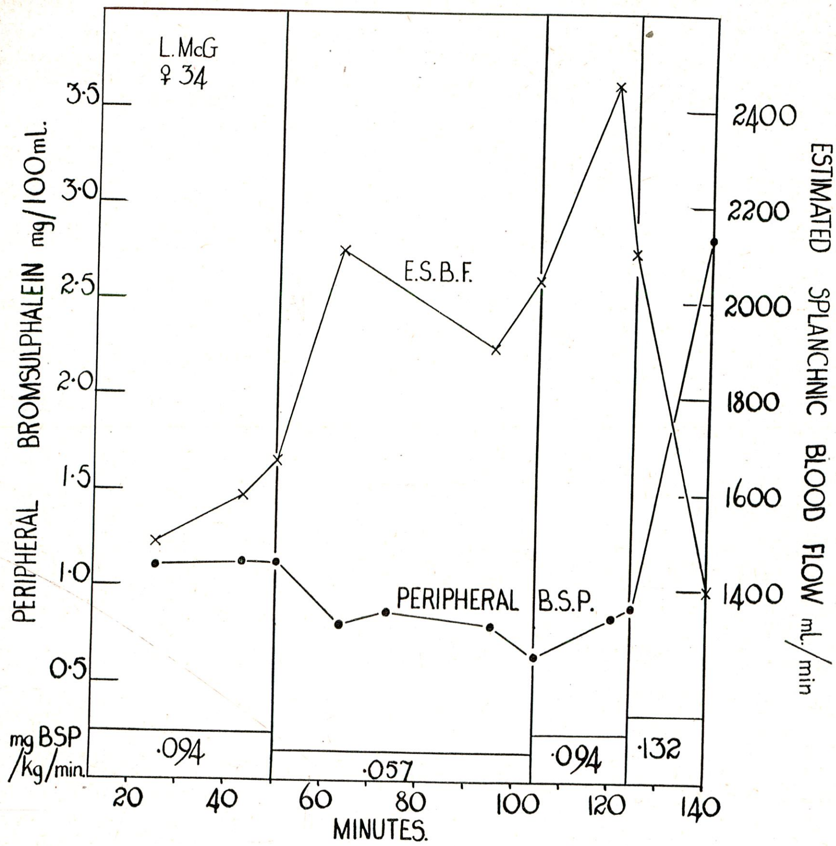


Fig. 3.—Effect of changing dose of bromsulfalein on peripheral bromsulfalein values and calculated blood flow.

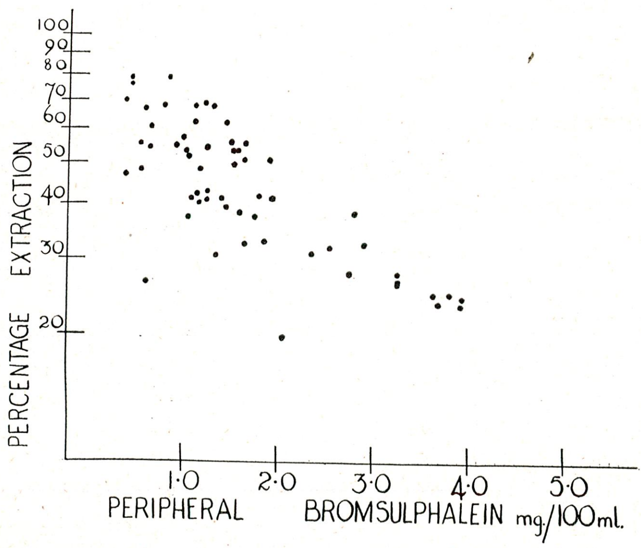


Fig. 4.—The relation of peripheral bromsulfalein values to the percentage extraction by the liver.

TABLE III. REPEATED ESTIMATIONS OF SPLANCHNIC BLOOD FLOW IN TWO NORMAL SUBJECTS

| | | | | | BSP | | E.S.B.F. (ML./MIN.) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | TIME IN MINUTES FROM START OF BSP INFUSION | | | | | | | | |
| SUBJECT | AGE | SEX | WT. (KG.) | S.A. (SQ.M.) | INITIAL PERIPHERAL VENOUS VALUE (MG./100 ML.) | DOSE (MG./KG./MIN.) | 35 | 45 | 60 | 75 | 90 | 110 | 130 | 150 | 170 |
| M. H. | 60 | M | 49 | 1.56 | 2.07 | 0.112 | 1250 | 1050 | 1030 | — | 1200 | 1070 | 1000 | 990 | — |
| A. B. | 40 | M | 64 | 1.74 | 1.15 | 0.072 | — | 1600 | — | 1510 | 1530 | 1380 | 1350 | 1650 | 1330 |

In two normal subjects more detailed studies were made. The results are tabulated in Table III. It is seen that, with the catheter remaining in the same branch of the hepatic vein, range of variability in E.S.B.F. is of the order of 300 ml. per minute.

## DISCUSSION

The very high calculated splanchnic blood flow, encountered when the peripheral BSP value was low, was an unexpected finding. Bradley and co-workers,[2] using a similar technique, did not describe this difficulty, even though in thirteen of their twenty-three observations the BSP level was less than 1 mg. per 100 milliliters. It must be emphasized that both the high and low peripheral BSP values reported in the present work resulted from BSP infusion, in amounts comparable to those used by Bradley and associates.

Our anomalous results for E.S.B.F. can be related to the mechanisms for the removal of BSP from the blood. Although the liver may be the principal organ involved,[2, 10, 11, 12] there is other evidence that extrahepatic sites and, in particular, the reticuloendothelial system play an important part in its disposal.[13-17] If BSP is removed from the blood by sites other than the liver, the quantity infused would not correspond with that excreted by the liver. The numerator in the equation used to calculate E.S.B.F. would thus be too high and falsely high values for splanchnic blood flow would be obtained. This might explain the incredibly high values for E.S.B.F. found when the peripheral BSP level is low.

Wirts and Cantarow[15] have shown that 67 to 100 per cent of injected BSP is recoverable in the bile within two hours, and although the reticuloendothelial system may participate initially in the withdrawal of the substance from the blood stream, the liver is ultimately responsible for its removal from the body. When the peripheral BSP level is increased, then the extrahepatic sites of removal may become saturated with the dye. The rate of entry of BSP into these sites then equals the rate of removal and the liver receives and excretes the exact quantity that is being infused. The Fick principle becomes applicable and correct values for E.S.B.F. are obtained.

Provided the peripheral BSP level is greater than about 1 mg. per 100 ml., raising the level further will not produce a further depression of the calculated splanchnic blood flow. Saturation of any extrahepatic removal mechanisms appears to be complete. This is in contrast to the findings of Cohn and associates[18] in the eviscerated-hepatectomized-nephrectomized dog. With this preparation these authors could demonstrate removal of BSP from the blood by other tissues in increasing amounts, even with plasma concentrations as high as 100 mg. per 100 milliliters. It is impossible, however, to compare the results obtained from this preparation with those obtained from man where the liver must be playing the major role in BSP removal.

Mendeloff and co-workers[19] refer to saturation of the liver cells with BSP at high peripheral concentrations, particularly in patients with liver disease. They believe that it is under these circumstances that extrahepatic removal of the dye may occur. The present work shows that increasing the peripheral

BSP level from 1 to 4 mg. per 100 ml. does not lead to any change in E.S.B.F. (Fig. 2). Extrahepatic withdrawal of the dye is therefore unlikely to be a serious source of error at these levels.

Anomalously high calculated splanchnic blood flows are obtained whether the low peripheral BSP value occurs initially or follows a previous high value (Fig. 3). Saturation, therefore, is probably controlled by the peripheral BSP level.

Reliable estimates of E.S.B.F. by this technique will not be obtained unless the peripheral venous BSP is greater than 1 mg. per 100 milliliters. The first venous blood sample obtained should, therefore, be analyzed immediately; if the BSP concentration obtained is not adequate, then the concentration of the infusion fluid must be increased. Prediction of the strength of infusion required in any one subject cannot be assessed entirely on the age and size of the subject and the preliminary estimation is essential. The rate of increase should be small so that the necessary correction can be made. Under these conditions, the liver and extrahepatic sites of removal are working at maximal capacity in the disposal of BSP from the blood stream. The conditions are similar to those employed in measuring the maximal tubular capacity (Tm) of the kidney. All measurements of E.S.B.F. by this technique therefore necessarily measure maximal hepatic capacity for BSP (Lm).[20]

Provided these conditions are satisfied, the BSP extraction technique has proved a satisfactory method of measuring splanchnic blood flow for periods of as much as three hours.

## SUMMARY

The estimated splanchnic blood flow (E.S.B.F.) has been determined in forty-nine normal subjects by the bromsulfalein (BSP) extraction technique using hepatic vein catheterization.

In thirty-two subjects, the peripheral venous BSP level was greater than 1 mg per 100 ml. and the E.S.B.F. was 836 ± 31 ml. per minute per square meter. In twelve subjects the peripheral BSP value was less than 1 mg. per 100 ml. and the E.S.B.F. was 1530 ± 126 ml. per minute per square meter.

When the peripheral BSP level was less than 1 mg. per 100 ml., significant diminution in the calculated splanchnic blood flow could be induced by raising the peripheral concentration. Increases to values greater than 1 mg. per 100 ml. failed to produce any further significant change in E.S.B.F. The reverse finding, that reduction of peripheral BSP values from above 1 mg. per 100 ml. to below that value increased the apparent E.S.B.F., was also true.

These results suggest that, unless extrahepatic removal mechanisms for BSP are saturated, values for E.S.B.F. are falsely high. With adequate peripheral BSP levels, changes in E.S.B.F. have been followed for periods of three hours and have shown only small fluctuations.

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