Cell Volume, Plasma Volume and Cell Percentage in Splanchnic Circulation of Splenectomized Dogs

By Shu Chien, B.M., Ph.D.

With the technical assistance of Samuel D. Huang

With the development of techniques for measuring cell volume and plasma volume separately, it has been shown that the percentage of blood cells in the overall circulation, including small as well as large vessels, is lower than that in the blood of only the large vessels, i.e., the arteries or veins. The \( F_{\text{cell}} \) value (overall cell percentage/large vessel cell percentage) averages 0.91 in man and 0.88 in splenectomized dogs, where the overall cell percentage is the cell volume as a percentage of the sum of cell volume and plasma volume, and the large vessel cell percentage is the arterial or venous hematocrit corrected for plasma trapping. Apparently, the blood in the minute vessels has a lower cell percentage than that in the arteries or veins. Such unequal distribution of cells between large and minute vessels has been demonstrated mainly for the entire circulation. In individual vascular beds, the determination of cell percentage under physiological conditions is rather difficult. Since the introduction of venous catheterization, a method has been devised to measure the blood volume in the splanchnic region. In this regional dilution technique, the use of different test substances for red cells and for plasma permits measurement of the splanchnic cell volume and splanchnic plasma volume simultaneously in an intact organism. From these measurements, the splanchnic cell percentage can be calculated. Preliminary reports on such studies in man have shown that the average ratio of splanchnic cell percentage to large vessel cell percentage varies from 0.775 to 0.91. In the dog, the spleen holds a variable amount of blood which is rich in red cells. The extent to which the blood volume in the spleen can be measured by the regional dilution technique is questionable. Therefore, the splanchnic cell percentage in the dog is more meaningful after the removal of the spleen. In the present experiments, the splanchnic cell and plasma volumes were measured simultaneously in splenectomized dogs under pentobarbital anesthesia. The cell percentage in the splanchnic circulation is calculated and compared with those in the overall circulation and in the large vessels.

Methods

Twenty-four healthy mongrel dogs were used. All of them had been splenectomized at least one month before the experiments were carried out. Each animal was fasted without restriction of water intake for 15 hours before the experiment. After the intravenous injection of sodium pentobarbital (33 mg/kg) the trachea was intubated. A catheter was inserted by way of an external jugular vein into the left hepatic vein under fluoroscopic control. The catheter, which was filled with a mixture of saline and heparin and connected to a pressure transducer, was left in place throughout the experiment. For the estimation of hepatic blood flow, sulfobromophthalein sodium (BSP) was infused into a femoral vein at a constant rate, after a priming injection of 3 mg/kg. A catheter identical with the one placed in the hepatic vein was introduced into the abdominal aorta via a
fenoral artery for the recording of arterial pressure and for sampling. Forty minutes after beginning the BSP infusion, simultaneous samples of arterial and hepatic venous blood were obtained. At least three pairs of such samples were drawn at about 10-minute intervals. One ml of each plasma sample was diluted with 2 ml of 0.9% saline and alkalinized with three drops of 40% KOH. The optical density, which was read in a Beckman spectrophotometer at a wave length of 580 mμ, was corrected for any minor degree of hemolysis according to Shoemaker's method. The estimated hepatic (or splanchnic) plasma flow (EHFP or ESPF) was computed from the readings of at least three pairs of samples. In cases where the arterial BSP concentration did not remain constant, corrections were made in the calculation according to Bradley et al. The estimated splanchnic cell flow (ESCF) and the blood flow (ESBF) were computed from ESPF and the arterial cell percentage. The arterial cell percentage was determined by centrifuging Wintrobe hematocrit tubes at 1500 x g for 30 minutes and using the plasma trapping factor of 0.96. After the first pair of blood samples for BSP determination had been taken, known amounts of I-albumin and Cr-labeled red blood cells were injected simultaneously into an external jugular vein for the determination of the splanchnic plasma and cell volumes by the regional dilution technique. Beginning with the injection of the radioisotopes, samples of blood were withdrawn simultaneously from the catheters placed in the aorta and the hepatic vein, at a constant rate of 3 ml/min. Three successive samples were taken from each catheter. A first pair of syringes was used to sample the blood during the first two minutes, and two more pairs of syringes were used to withdraw during the two succeeding one-minute periods. In order to calculate the total plasma volume and total cell volume, three more arterial samples were taken at ten-minute intervals after the injection of the radioisotopes. For the assay of the I131 activity in plasma and the Cr51 activity in cells, a well-type scintillation counter, which was connected to a pulse height analyzer and a scaler, was used. The plasma was counted directly. The cells were washed three times with 0.9% saline before counting. From the regional dilution of I131-albumin and Cr51-RBC respectively, the circulating splanchnic plasma volume (CSPV) and circulating splanchnic cell volume (CSCV) were calculated. Thus, CSPV = ESPF × \( \frac{(A_p - V_p) t_1 + (A_p - V_p) t_2}{\frac{1}{2} (A_p + V_p) t} \)

where \( A_p \) and \( V_p \) represent the plasma I131 activities (counts per minute) of the arterial and hepatic venous samples respectively, and \( t \) is the duration (in minutes) during which each sample pair was drawn. The subscripts 1, 2 and 3 represent the sequence of samples. CSPV is in ml and ESPF is in ml/min.

CSCV = ESCF × \( \frac{(A_c - V_c) t_1 + (A_c - V_c) t_2}{\frac{1}{2} (A_c + V_c) t} \)

where \( A_c \) and \( V_c \) are the Cr51 activities (counts per minute) per ml of blood cells in the arterial and hepatic venous samples respectively. The sum of CSCV and CSPV is the circulating splanchnic blood volume (CSBV). From the above measurements, the following can be calculated:

- Splanchnic cell % = \( \frac{CSCV}{CSBV} \times 100\% \)
- Splanchnic Fc100 values = \( \frac{\text{splanchnic cell %}}{\text{arterial cell %}} \)
- Overall cell % = \( \frac{\text{total cell volume}}{\text{total blood volume}} \times 100\% \)
- Overall Fc100 value = \( \frac{\text{overall cell %}}{\text{arterial cell %}} \)

The splanchnic mean circulation times (SMCT, in seconds) for the plasma and for cells are given as:

SMCT for plasma = \( \frac{60 \times \text{CSPV}}{\text{ESPF}} \)

SMCT for cells = \( \frac{60 \times \text{CSCV}}{\text{ESCF}} \)

Results

The results are summarized in table 1. The total plasma volume and the total cell volume have not been corrected for the amount of samples taken for the measurement of the circulating splanchnic blood volume. The overall Fc100 factor determined in these anesthetized, splenectomized dogs by using Cr51 and I131-albumin averaged 0.875 which agrees very well with the values obtained previously in this laboratory, when T-1824 was used to measure the plasma volume in unanesthetized, splenectomized dogs.

The circulating splanchnic cell volume averaged 19.6% (sE m = 1.6%) of the total.
### Table 1: Splanchnic Circulation of Splenectomized Dogs

<table>
<thead>
<tr>
<th>Dog no. &amp;</th>
<th>Body wt</th>
<th>Art. cell %</th>
<th>Overall circulation</th>
<th>Circulating splanchnic volume</th>
<th>Est. splanchn. flow</th>
<th>Splanchn. mean circ. time sec</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FV ml</td>
<td>CV ml</td>
<td>CSPV ml</td>
<td>CSCV ml</td>
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<tr>
<td>1</td>
<td>12.0</td>
<td>42.5</td>
<td>591</td>
<td>357</td>
<td>0.857</td>
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<tr>
<td>2</td>
<td>10.0</td>
<td>40.0</td>
<td>508</td>
<td>276</td>
<td>0.880</td>
<td>189</td>
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<tr>
<td>3</td>
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<td>45.2</td>
<td>427</td>
<td>267</td>
<td>0.853</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>10.6</td>
<td>43.3</td>
<td>479</td>
<td>307</td>
<td>0.899</td>
<td>119</td>
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<td>10.3</td>
<td>46.3</td>
<td>420</td>
<td>281</td>
<td>0.865</td>
<td>76</td>
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<tr>
<td>6</td>
<td>11.1</td>
<td>44.0</td>
<td>572</td>
<td>331</td>
<td>0.840</td>
<td>110</td>
</tr>
<tr>
<td>7</td>
<td>22.1</td>
<td>43.6</td>
<td>1121</td>
<td>696</td>
<td>0.878</td>
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<td>45.8</td>
<td>468</td>
<td>281</td>
<td>0.824</td>
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<td>42.2</td>
<td>334</td>
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<td>41.1</td>
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<td>280</td>
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<td>45.7</td>
<td>557</td>
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<td>296</td>
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<tr>
<td>15</td>
<td>8.9</td>
<td>48.3</td>
<td>338</td>
<td>281</td>
<td>*</td>
<td>100</td>
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<td>14.7</td>
<td>40.6</td>
<td>649</td>
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<td>567</td>
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<td>40.7</td>
<td>563</td>
<td>308</td>
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<td>89</td>
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<tr>
<td>20</td>
<td>12.4</td>
<td>42.3</td>
<td>615</td>
<td>362</td>
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<tr>
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<td>695</td>
<td>307</td>
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<tr>
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<td>656</td>
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<td>83</td>
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<tr>
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<td>10.8</td>
<td>40.0</td>
<td>500</td>
<td>294</td>
<td>0.925</td>
<td>87</td>
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<tr>
<td>24</td>
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<td>41.2</td>
<td>648</td>
<td>394</td>
<td>0.917</td>
<td>95</td>
</tr>
<tr>
<td>Mean</td>
<td>11.5</td>
<td>42.4</td>
<td>485</td>
<td>285</td>
<td>0.875</td>
<td>98</td>
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<tr>
<td>SD</td>
<td>2.82</td>
<td>3.25</td>
<td>50.9</td>
<td>35.5</td>
<td>0.855</td>
<td>35.2</td>
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<tr>
<td>SFem</td>
<td>0.58</td>
<td>0.74</td>
<td>10.4</td>
<td>7.2</td>
<td>0.007</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*Not determined.
1 CSBV calculated from CSPV and splanchnic cell %, which is assumed to be 0.86 × arterial cell %.
2 CV calculated from PV and overall cell %, which is assumed to be 0.85 × arterial cell %.
3 CSPV calculated from CSPV and splanchnic cell %, which is assumed to be 0.86 × arterial cell %.
4 Mean values, standard deviations and standard errors of the mean expressed in terms of ml/10 kg or ml/min/10 kg.
cell volume, and the circulating splanchnic-plasma volume averaged 20.5% (SE = 1.5%) of the total plasma volume. The circulating splanchnic blood volume as a percentage of total blood volume had a mean value of 20.1% (SE = 1.4%). In 20 dogs, the splanchnic cell percentage and the splanchnic $F_{cell}$ value were calculated. The splanchnic cell percentage was lower than the large vessel cell percentage in 17 out of these 20 dogs, resulting in splanchnic $F_{cell}$ values of smaller than unity. A comparison of the large vessel cell percentage with the splanchnic cell percentage in individual dogs showed that the difference is significant at the 99% level (Student $T$ test, $P < 0.01$). The average splanchnic $F_{cell}$ factor of 0.862 was very close to the average $F_{cell}$ value of the overall circulation, and no statistically significant difference could be demonstrated between these two values.

On a 10-kg weight basis, the average value for ESPF was 148 (SE = 7.7) ml/min and that for ESCF was 108 (SE = 5.3) ml/min. The ESBF reported here is comparable with the values found by Bradley on dogs under sodium pentobarbital, but is significantly lower than the results obtained on unanesthetized dogs. The splanchnic mean circulation time averaged 42 seconds for the plasma and 33 seconds for the cells. The application of Student $T$ test to the difference between these two circulation times in individual dogs showed a $P$ value of smaller than 0.01.

**Discussion**

As early as 1921, the work of Smith et al. suggested that there is an unequal distribution of cells in the overall circulation. With the advances in the techniques for measuring cell volume and plasma volume, it has become established that there is "extra plasma" present in the minute vessels, making the overall cell percentage lower than the large vessel cell percentage. Since the splanchnic and the overall $F_{cell}$ values both average between 0.86 and 0.88, the "extra plasma" seems to be present in the splanchnic bed with the same proportion as in the rest of the circulation. In man, the overall $F_{cell}$ value of 0.91 is also the same as the ratio: splanchnic-cell percentage/venous cell percentage, found by Lathem and Gordon. Using anatomical data in the literature, Bradley has calculated a theoretical splanchnic $F_{cell}$ value of 0.82 for the dog. In a preliminary report by Cominsky et al., the splanchnic cell percentage of eight splenectomized dogs under pentobarbital anesthesia was found to average 0.718 (SD 0.18) of the large vessel cell percentage. Although their results also indicate the presence of extra plasma in the splanchnic circulation, the average value of 0.718 is significantly lower than our average splanchnic $F_{cell}$ value of 0.862. There is no apparent explanation for this discrepancy. Since there are many potential sources of error (see below) in the determination of splanchnic cell percentage by the regional dilution technique, any minor difference in the techniques used in two series of experiments may cause considerable deviation in results. The lack of constancy in the speed of sampling in the early phase after the injection of isotopes may especially contribute significant errors to the measurements. It is noteworthy that in our experiments (dogs 9 through 24) where the sampling rate was controlled by constant withdrawal pumps, the standard deviation of the splanchnic $F_{cell}$ value was reduced to 0.08 as compared to 0.11 for the entire series and 0.18 for the results of Cominsky et al. The finding that evisceration did not change the overall $F_{cell}$ value in the splenectomized dog is in agreement with our result that the splanchnic $F_{cell}$ value is close to the overall $F_{cell}$ value of 0.88. The results in the literature on the direct determination of cell percentage in organs removed from the splanchnic area are not entirely in accord with one another. In acutely sacrificed dogs, Gibson et al. used radio-iron labeled red blood cells and iodine-albumin to measure the blood volume in the "minute vessels" of individual organs after letting the blood drain out of the "large vessels." The minute vessel cell percentage thus determined in the liver was almost the same as the large vessel cell percentage, and the minute vessel cell percentage of the in-
testine was less than one-half of the large vessel cell percentage. Using P³⁸-labeled red blood cells and T-1824, Allen and Reeve determined the cell percentage of the blood in the liver from serial tissue samples obtained by biopsy. Their value for the cell percentage of the blood in the liver of such open-abdomen preparations was about 0.7 x large vessel cell percentage.

As a corollary to our findings on the splanchnic cell percentage and F cell value, the splanchnic mean circulation time for the plasma is longer than that for the cells.

In the experiments reported here, the variation in the splanchnic F cell value (SD = 0.111) is much greater than that of the overall F cell value (SD = 0.031). This is at least partially due to the larger error involved in the determination of the splanchnic cell percentage. In the measurement of the total plasma and cell volumes, the quantity of the test agent dispersed in the overall circulation can be determined accurately by using a calibrated syringe or by weighing. In the determination of the splanchnic plasma and cell volumes, however, the quantity of the test agent dispersed in the splanchnic bed is computed by integrating the instantaneous concentration differences between the arterial blood and the hepatic venous blood. With the techniques used in our experiments, any fluctuation in the rate of sampling, especially from the arterial catheter in the early phase after the injection of the radioisotopes, can introduce a significant error. Furthermore, in calculating the difference between two numbers (arterial and hepatic venous concentrations), the errors involved in the individual determinations are magnified many times. In using the regional dilution technique, it is assumed that the cumulative difference between the quantities of test agent entering and leaving the splanchnic bed is equal to the amount dispersed in this area. This requires that the test agent does not leave the splanchnic circulation and diffuse into the extravascular space. Such requirement is met when one uses the Cr³¹-labeled red cells, but probably not when I¹³¹-albumin is used, since the latter is known to disappear from the circulation at a rate of about 9% per hour. However, the amount of I¹³¹-albumin leaving the splanchnic circulation during the equilibration period is probably negligible. Although the indirect equilibration method employed here is subject to these potential sources of error, it offers the advantages over any direct method in that a more physiological condition is maintained and that repeated determination on the same subject is possible.

Besides the measurement errors mentioned above, it is also possible that the splanchnic F cell is truly more variable than the overall F cell value. If in the living dog, the minute vessel cell percentage in the liver is different from that in the intestine, as was found by Gibson et al. in freshly sacrificed dogs, it is conceivable that a variation of the distribution of blood between these two compartments can cause a change in the splanchnic cell percentage. During the determination of CSBV, the amount of blood in the large vessels that is included depends on the position of the sampling catheters. The CSBV measured is that from the arterial sampling site and all equitemporal points, to the hepatic venous sampling site and all equitemporal points. A variation in the inclusion of the large vessel blood in the determination of CSBV can also cause a corresponding fluctuation in the measured splanchnic cell percentage. Finally, it should be pointed out that, with the regional dilution technique, one measures the circulating cell or plasma volume in the splanchnic region. If there are portions of cells or plasma in which the test agents equilibrate using the figure of 9% per hour as the loss rate of I¹³¹-albumin from the circulation of the dog, assuming that the exponential loss rate is constant even during the early phase after injection, one can show that approximately 0.3% of the injected dose is lost from the total circulation during the first 2 postinjection minutes which constitute the "equilibration period" for the measurement of CSBV. Even if this transcapillary leakage were to occur wholly in the splanchnic circulation, it can contribute an error of only 4-15% in the measurement of splanchnic plasma volume, since the latter contains 20% of the injected dose of I¹³¹-albumin.

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very slowly, these portions may not be included in the measurement.

Despite these possible sources of error in the determination of splanchnic cell percentage and splanchnic $F_{\text{cells}}$ value, it is interesting that the splanchnic cell percentage found in the splenectomized dog is significantly ($P < 0.01$) lower than the larger vessel cell percentage, and is almost the same as the overall cell percentage. Therefore, the splanchnic circulation contains a fair share of "extra plasma" in proportion to the amount of blood present in this region.

**Summary**

The circulating splanchnic cell and plasma volumes were measured with the regional dilution technique in splenectomized dogs under sodium pentobarbital. For a 10-kg dog, the average circulating splanchnic cell volume is 56 (SE$_{\text{m}} = 5.0$) ml and the average circulating splanchnic plasma volume is 98 (SE$_{\text{m}} = 7.2$) ml. These values represent 20% of the total cell volume and 20% of the total plasma volume respectively. The splanchnic cell percentage, averaging 36.3% (SE$_{\text{m}} = 1.32$%), is significantly lower than the large vessel cell percentage (average 42.4%) and is almost equal to the overall cell percentage. The splanchnic $F_{\text{cells}}$ value averages 0.86 and is almost the same as the overall $F_{\text{cells}}$ value. Therefore, the splanchnic circulation contains a portion of the total "extra plasma" in proportion to the blood volume in this region. The splanchnic mean circulation time of the plasma (average 42 seconds) is significantly longer than that of the cells (average 33 seconds).

**Acknowledgment**

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SHU CHIEN

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