Washout kinetics of red cells and plasma from the spleen

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Levesque, M. J., and A. C. Groom. Washout kinetics of red cells and plasma from the spleen. Am. J. Physiol. 231(6): 1665-1671, 1976—Radioiodinated (125I) serum albumin was injected intravenously in cats and allowed to equilibrate in the circulation. Red cell and plasma washout from the isolated spleens were then compared during perfusion with oxygenated Ringer solution, the respective concentrations in the outflow being measured by celsoscope and scintillation counters. Washout kinetics yielded three exponential components for cells (perfusate volumes for 50% washout (V1,2) being 0.067, 4.70, and 97 ml/g spleen) but only two for plasma (V1,2: 0.14 and 2.40 ml/g). There is no plasma counterpart to the slowly released cells, i.e., they do not represent a separate vascular space. This is in accord with a previous view that these are immature cells, delayed through adherence to fine structures of the red pulp. Compartment analysis indicates that the plasma and two remaining cell components represent washout from two separate vascular spaces, containing 0.09 ml/g blood at arterial hematocrit 37% and 0.42 ml/g blood at hematocrit 75%, perfused by 0.9 and 0.1 of the arterial inflow respectively. Evidence suggests these spaces are i) blood vessels, and ii) red pulp.

splenic circulation; splenic blood stores; intrasplenic hematocrit; splenic washout kinetics

Compartmental analysis has become widely used as a means of interpreting the kinetics of distribution of tracer substances within the body and within even a single organ or tissue (13). The outcome of this approach is always a simple equivalent model, sufficient to represent the kinetics of distribution but not necessarily in the sense of being the only valid model. For the investigator there always remains the obligation to determine what, if any, the morphological counterparts to the various "compartments" of the model might be.

Previous studies from this laboratory have shown that the washout of red cells from the cat spleen, perfused with cell-free Ringer solution, is equivalent to that from a system of three compartments (16). These compartments were referred to as fast, intermediate and slow, respectively, because of their widely different half times of washout (30 sec, 8 min, and 54 min). Evidence suggesting that there are indeed morphological counterparts to these three compartments has come from three separate lines of investigation. First, studies of red cell washout from contracted spleens showed that 82% of the cells mobilized on contraction had come from the intermediate compartment (9). This suggested that the intermediate compartment constituted the splenic reservoir. Second, histological studies of the distribution of red cells within the spleen, made after different periods of washout, indicated that the fast compartment consisted of red cells in the splenic vessels, the intermediate compartment of free cells in the red pulp, and the slow compartment of cells adhering to the fine structures of the red pulp (17). By means of both optical and scanning electron microscopy the cells of the slow compartment were identified as immature and abnormal cells (18, 20). Third, by collecting the venous outflow at three particular stages of the washout fairly pure samples (>85%) of the cells from each compartment were obtained. Examination of cell volume and specific gravity showed that cells of the fast and intermediate compartments were not significantly different from those of arterial blood, whereas the cells of the slow compartment were larger in volume and lighter in specific gravity, i.e., they were predominantly younger cells (10). By use of supravital stains these latter cells were found to consist largely of reticulocytes (19).

From these results the following hypothesis was formulated. There are only two vascular compartments within the spleen, i) the vessels constituting the rapid pathway, and ii) the red pulp; the distinction between the intermediate and slow compartments is purely the result of a cellular factor.

One way to test this hypothesis would be to compare the washout kinetics of radioactively labeled plasma with those of red cells, the two being studied in the same preparation simultaneously. If the hypothesis were true, then one might expect to find in the plasma washout only two compartments, the two corresponding to the fast and intermediate components of cellular washout. Any result other than this would indicate the need for a different interpretation of the previous experimental findings. If the hypothesis were to be confirmed, however, then there would be the additional bonus that the red cell and plasma volumes of both vascular compartments could be computed from the data. Hence, the hematocrit of the blood contained in each compartment could be derived. The present paper reports the results of such an investigation.

METHODS

Fifteen cats weighing 1.5-4.5 kg were used in these experiments. All were healthy animals and were anesthetized with sodium pentobarbital (40 mg/kg ip).

Splenic washout procedure. In 10 animals weighing
1.5-3.5 kg, the spleen was isolated surgically except for the main splenic vessels and, then, before cannulation, 20 μCi of radioiodinated (125I) human serum albumin (Frosst Pharmaceuticals) was injected into the general circulation via a femoral vein. A period of 90 min was allowed for intravascular equilibration of the labeled material, and the animals were then heparinized (1,000 USP U/kg iv). The splenic artery and vein were cannulated, and the spleen, placed in a Lucite chamber containing saline at 37°C, was perfused in the manner we described previously (16), except that we used a phosphate-buffered Ringer solution of pH 7.4, equilibrated at 37°C with 5% CO₂ in O₂ (Fig. 1). The composition of this solution was as follows, in grams per liter: NaCl, 8.0; KCl, 0.2; Na₂HPO₄ (anhydrous), 1.15; KH₂PO₄, 0.2; CaCl₂, 0.1; MgCl₂·6H₂O, 0.1; glucose, 1.0. This solution was prepared free of particulate matter by filtration through Millipore filters (GSPW 04700, 0.22 μm) 3 times before use.

In all experiments the perfusion was carried out at a constant-flow rate of 6 ml/min by means of a Cole-Parmer pump. The perfusion pressure at the splenic artery was monitored continuously by a pressure transducer (Statham P23DC) and the splenic weight was measured throughout with a force transducer (Grass Instrument Co. F703c). Both sets of data were recorded by a Beckman type R Dynograph from the start of the perfusion until the end of the experiment. The distal end of the venous cannula was open to the atmosphere and was maintained at a constant level, lower than that of the splenic vein, so that with a constant-flow rate the splenic venous pressure was held constant throughout the experiment. The outflow from the splenic venous cannula was collected continuously in a measuring cylinder except that, at a series of prearranged time intervals, samples were collected in small glass test tubes for assay of red cell and radioactive albumin concentrations. The cellular concentration of each sample was measured by a Celloscope counter (Particle Data, Inc., series 112) and the 125I-labeled content was measured with a well-type scintillation counter (Nuclear-Chicago Corp.). For the radioactive assay the whole sample was counted, and then 20-100 μl were added to a known volume of a commercially available, particle-free, isotonic solution (Diluton, BDH Chemicals) to give a final cell concentration of 12,000-60,000 cells per milliliter for Celloscope counting. For the terminal portion of the albumin washout, concentrations were measured by precipitation of the protein from 100-ml samples with trichloracetic acid and subsequent dissolution of the precipitate in 5 ml of 2 N NaOH. This permitted more accurate measurement of low-radioactive concentrations than could be obtained by counting 5-ml samples of the outflow.

**Splenic drainage procedure.** In five animals, weighing 2.5-4.5 kg, a different approach was used as an independent check on values of the intrasplenic hematocrit determined by the washout procedure. After isolation of the spleen the animals were heparinized (1,000 USP U/1/kg) and the splenic artery and vein were cannulated. The arterial cannula was clamped and the spleen was permitted to empty its contents passively, via the splenic venous cannula, into a series of graduated test tubes. When the outflow ceased a small quantity of norepinephrine (0.5 μg in 0.1 ml saline) was injected via a polyethylene tube (PE-50) inserted through the arterial cannula to the level of the arterial bifurcation. This was sufficient to induce active contraction of the spleen. Further blood samples were then collected until the outflow finally ceased. During this whole procedure the splenic weight was recorded continuously, as described earlier. The hematocrit of each blood sample was measured in duplicate with a microhematocrit centrifuge.

**RESULTS**

This investigation involved perfusion of isolated, decervated cat spleens with Ringer solution for a period of several hours. It was important to maintain the spleen in a steady state throughout and, therefore, as many parameters as possible were kept within the physiological range. The mean perfusion pressure was 83 ± 12 (SD) cmH₂O and the splenic venous pressure was approximately 4 cmH₂O. The mean value of portal venous pressure in cats has been reported (6) as 6 mmHg (range, 3.5-11 mmHg). In our experiments both inflow and outflow pressures were, therefore, on the low side of normal. We used a phosphate-buffered Ringer solution rather than the bicarbonate-buffered solution used previously (16), since changes in pH of the perfusate with time have recently been found with the latter. In the present study measurements of perfusate pH, at both inflow and outflow, were made at regular intervals throughout each experiment. The pH at the outflow was always less than at the inflow, the mean values being 7.38 ± 0.03 SD (inflow) and 7.20 ± 0.03 SD (outflow). In all experiments the perfusate was equilibrated at 37°C with 5% CO₂ in O₂. Splenic weight remained constant throughout all perfusions, the mean value being 11.6 ± 1.3 (SE) g/kg body wt. This is similar to the value 11 g/kg (range, 5.8-19) reported by Greenway et al. (6).

**Splenic washout experiments.** The mean values for concentration of red cells and 125I-count rates per milliliter in samples of the splenic outflow, taken at different stages of the washout, are shown plotted on a semilogarithmic scale versus the volume of fluid per gram splenic weight that had perfused the spleen (Figs. 2 and 3). In both cases a rapid decrease by an order of magni-
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FIG. 2. Mean cell washout curve from 9 spleens during perfusion at constant flow (6 ml/min) with cell-free, phosphate-buffered Ringer solution. Cell concentration (± SE) in outflow is plotted against cumulative volume of fluid perfused per gram of splenic weight. Washout curve may be expressed as sum of 3 exponential components (1, 2, 3; see text).

This analysis showed that the washout curves of 125I-labeled albumin and red cells could be resolved into the sum of two and three exponential components, respectively. These are shown as solid lines in Figs. 2 and 3. Each component may be characterized by the volume of perfusate per gram splenic weight needed in order to reduce the concentration of albumin or cells by one-half ($V_{1/2}$). For the plasma components the values of $V_{1/2}$ were 0.14 and 2.4 ml/g, and for the red cells the values were 0.067, 4.7, and 97 ml/g, respectively. It is clear that there is a close correspondence between the $V_{1/2}$ values of the plasma components and those of the fast and intermediate components of red cells.

From the foregoing results it follows that the washout of plasma from the spleen corresponds to that from a system of two separate compartments, whereas the washout of red cells is equivalent to that from a combination of three. By use of these models we may extract additional information from the data, as shown in Table 1. Thus, the initial rates of washout from the compartments are proportional to their perfusion rates. On this basis the fraction of the total initial concentration, in the venous outflow, of cells or albumin deriving from each compartment is equal to the fraction of the total flow of cells or albumin perfusing that compartment in the blood-perfused steady state immediately prior to washout. For the fast compartment of plasma and red cells these are equivalent to 90.1 and 90.3%, respectively; for the intermediate compartment the corresponding values are 9.9 and 9.6%, respectively, and for the slow compartment of red cells the value is 0.15%. It is evident, therefore, that with regard to the fractional perfusion of the compartments there is again a close correspondence between the two plasma compartments and the fast and intermediate red cell compartments.

The total radioactivity (counts per minute per gram) or total number of red cells (cells/gram) within a compartment is given by the quotient of the initial concentration and the rate constant, i.e., by $C_0/k$, alternatively...
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VOLUME PERFUSED/ SPLENIC WEIGHT

(ml/g)

FIG. 3 Mean 125I-labeled albumin washout curve from 8 spleens during perfusion at constant flow (6 ml/min) with cell-free, phosphate-buffered Ringer solution. Radioactive concentration (± SE) in outflow (counts/ml per min) is plotted against cumulative volume of fluid perfused per gram of splenic weight. Washout curve may be expressed as sum of 2 exponential components only (1,2; see text).

expressed as $C_0 \cdot V_{1/2}/\log_2$. From measurements of the plasma radioactivity, red cell count, and hematocrit of femoral venous blood, these stores may be expressed in terms of volume (ml/g) of plasma or cells, respectively (Table 1). The mean total blood store of the spleen was 0.51 ml/g, of which 0.35 ml/g was red cells. This indicates that the mean splenic hematocrit was 70%.

Spleenic drainage experiments. Mean hematocrit values of successive blood samples, collected from the splenic vein when predetermined volumes of blood had drained from the organ, were plotted versus the volume drained (Fig. 4). The initial hematocrit (33.5%) was not significantly different from that of femoral venous blood (35.8 ± 2.5%). However, after the first few milliliters the hematocrit rose steadily to a value of 78.5%, at which stage no further blood left the spleen. In these five animals the mean splenic weight was 40.3 ± 2.1 g (SE) and the mean volume of blood drained was, therefore, 18.1 ml. The mean hematocrit of the blood drained may be calculated from Fig. 4 and was found to be 56%.

DISCUSSION

There can be no doubt from Figs. 2 and 3 that the washout curves of plasma and red cells from the spleen are very different. After perfusion by 15 ml/g, the cellular washout showed evidence of an extremely slow component that was completely absent in the case of albumin. This became apparent when the concentration of cells in the outflow had fallen by only slightly more than 2 orders of magnitude. Thereafter the concentration fell by only 1 more order of magnitude, in spite of perfusion by a further 90 ml/g of Ringer solution. Concentrations of 125I-labeled albumin were followed through a total range of 4 orders of magnitude, until sample counts could no longer be detected above background.

The compartmental models for red cells and for plasma, derived from these data, are shown in Fig. 5. The red cell flows to the fast and intermediate cell compartments, are, in fact, 90.25 and 9.6%, respectively, of the total (Table 1). If these compartments do indeed represent red cells in distinctive vascular spaces (see above) then the plasma flow should also be distributed to these same spaces in the same proportion. This is evidently so, because the plasma flow is distributed to two compartments in amounts of 90.1 and 9.9%, respectively, of the total flow (Table 1). The values of the washout half volumes of cells and plasma for either space differ only by a factor of approximately 2.0, whereas the ratio of the sum of these half volumes ($V_{1/2}$ cells + $V_{1/2}$ plasma) for the two spaces is 34:1, leaving no possibility whatever that these kinetics could be reproduced by blood flowing through a single vascular compartment with a Gaussian distribution of transit times (21). Therefore, we conclude that the fast compartments of both cell and plasma models represent washout of blood from the same vascular space. Similarly, the slower plasma compartment and the intermediate cell compartment arise from the washout of a separate vascular space in which blood is moving more slowly.

No plasma counterpart to the very slow red cell compartment was found. This compartment, representing in these experiments 22% of the red cells in the spleen, cannot, therefore, be due to perfusion of a third vascular space but must indicate some delay process peculiar to these red cells, i.e., it must be the result of a purely cellular factor. This confirms the results of our earlier histological studies (17) that the cells comprising the slow compartment are those that adhere to the fine structures of the red pulp ("bound" cells) whereas those comprising the intermediate compartment are "free" cells within the red pulp. These bound red cells, which have been shown to be predominantly immature and abnormal (18–20) will, when released from the bound state, join the free cells of the intermediate compartment in the red pulp. Moreover, evidence has been provided (19) that the immature red cells within the spleen could not have originated there but must have been accumulated from the blood flowing through the red pulp. For these reasons the slow cell compartment in Fig. 5 has been shown as exchanging with the intermediate compartment.

It is clear, therefore, that from the morphological
TABLE 1. Analysis of washout of red cells and albumin from spleen of cats during perfusion with Ringer solution

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Red Cells (n, 9)</th>
<th>Albumin (n, 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Component 1</td>
<td>Component 2</td>
</tr>
<tr>
<td>Intercept C₀, cells ml⁻¹ or counts min⁻¹ ml⁻¹</td>
<td>8.5 x 10⁸</td>
<td>9.0 x 10⁸</td>
</tr>
<tr>
<td>± SE</td>
<td>0.7 x 10⁹</td>
<td>0.4 x 10⁹</td>
</tr>
<tr>
<td>Desaturation V₁,₂, ml g⁻¹</td>
<td>0.067</td>
<td>4.70</td>
</tr>
<tr>
<td>± SE</td>
<td>0.007</td>
<td>0.33</td>
</tr>
<tr>
<td>Total flow, %</td>
<td>90.3</td>
<td>9.6</td>
</tr>
<tr>
<td>Store, cells g⁻¹ or counts min⁻¹ g⁻¹</td>
<td>8.15 x 10⁸</td>
<td>6.1 x 10⁸</td>
</tr>
<tr>
<td>± SE</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Capacity, cells or plasma ml g⁻¹</td>
<td>0.033</td>
<td>0.242</td>
</tr>
<tr>
<td>± SE</td>
<td>0.004</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Total splenic capacity (ml g⁻¹) ± SE, of red cells, 0.35 ± 0.04; plasma, 0.16 ± 0.05; whole blood, 0.51 ± 0.07. Mean splenic weight, 26.7 ± 2.3 g (SE).

standpoint there are only two distinct vascular compartments within the spleen. From previous histological studies at different stages of cell washout (18) we believe these to be i) blood vessels, and ii) the red pulp. On this basis we may combine the two separate models for cells and plasma into one model for the morphological distribution of blood within the spleen (Fig. 6). For this purpose the cells of the intermediate and slow compartments, which are both in the red pulp, are lumped together.

Since the appropriate volumes of red cells and plasma are known, we may calculate the hematocrit of the blood in each vascular compartment (Fig. 6). For the fast compartment this turns out to be 37%, almost identical to the mean value 35.8% ± 2.5 (SD) for femoral venous blood in this group of animals. The agreement between these two values supports the hypothesis that

![Fig. 4. Splenic drainage method (see text). Mean value (+ SE) of hematocrit (%) of successive venous samples is plotted against cumulative volume of blood drained per gram of splenic weight. Arrow denotes point at which 0.5 μg of norepinephrine was injected into splenic artery.](http://ajplegacy.physiology.org/)

![Fig. 5. A: 3-compartment model, derived from cell washout kinetics, for distribution of red cells in spleen. B: 2-compartment model, derived from kinetics of 125I-labeled albumin washout, for distribution of plasma in spleen. In both A and B, qi is flow to compartment (% of total inflow); CAP is capacity of compartment (milliliters cells or plasma per gram spleen weight); V₁,₂ is desaturation half volume of compartment (milliliters perfusate per gram splenic weight).](http://ajplegacy.physiology.org/)

![Fig. 6. Compartment model for distribution of whole blood in spleen (see text). Nine-tenths of splenic arterial blood passes through smaller compartment that contains blood of similar hematocrit (37%). One-tenth of total blood flow passes through red pulp (the major compartment) that contains blood of hematocrit 75%.](http://ajplegacy.physiology.org/)
the fast compartment represents blood contained in the splenic blood vessels. For the larger compartment the hematocrit is 75%, suggesting very strongly that this represents blood in the red pulp. It has been known for many years that in dogs the hemoglobin concentration of splenic blood is much higher than that of peripheral blood (2, 11). More recently, measurements on blood drained from excised dog spleens, contracting under electrical stimulation, have shown the hematocrit to be as high as 90% (14). To our knowledge there are no comparable data available for cat spleen. For this reason, therefore, we measured the hematocrit of blood in the splenic pulp of five cats by a drainage method.

Instead of collecting all the blood expelled from the spleen as one mixed sample, the outflow was fractionated and the hematocrit of each successive sample measured (Fig. 4). The initial hematocrit was comparable to that of femoral venous blood but, as drainage continued, the hematocrit rose gradually, reaching a final value of 78.5%. This value is very similar to the hematocrit of 75% determined from the washout experiments for the larger of the two vascular compartments, and the agreement lends support to the hypothesis that this larger compartment does indeed represent the blood contained in the splenic pulp.

From the washout experiments the total blood volume of the spleen was 0.51 ± .07 (SE) ml/g splenic wt and the mean hematocrit was 70.0%. The drainage experiments indicated that, after maximal contraction of the spleen by 0.5 µg norepinephrine, a volume of blood equivalent to 0.45 ± .03 ml/g (SD) and of mean hematocrit of 56% can be discharged from the spleen. This indicates that nine-tenths of the blood in the organ can be expelled on contraction. It has been reported, from measurements of changes in splenic weight, that during contraction up to 5 ml blood/kg wt can be discharged into the splenic vein of the cat (6). This value corresponds to 0.45 ml/g splenic wt, since the mean weight of the organ in those experiments was 11 g/kg body wt, and is in good agreement with the present results. Although the volume and hematocrit of the blood discharged has been estimated in various species, this, to our knowledge, the first time that a measurement of the total volume and hematocrit of the blood in the spleen has been reported.

It is of interest that the rate constant for clearance of red cells from the fast compartment was roughly twice that for plasma (V₁₁₂ = 0.067 and 0.14 ml/g, respectively; Table 1) whereas in the case of the intermediate compartment the opposite was true (V₁₂ = 4.7 and 2.4 ml/g, respectively; Table 1). Radioactive tracer studies have shown that red cells travel slightly faster than plasma in blood of normal hematocrit traversing the heart and lungs (8, 15), the kidney (3, 12), and the liver (5). Studies of this phenomenon made in an isolated, blood-perfused gastrocnemius muscle of cat, under conditions in which recirculation of labeled cells and plasma was avoided, showed that the magnitude of the effect was greatly dependent on the hematocrit of the blood (7).

Thus, the ratios of the mean velocities of cells to plasma at hematocrits of 20, 27, and 49% were 1.27, 1.12, and 0.98, respectively, while at a higher hematocrit (87%) a reversal occurred, cells traveling more slowly than plasma (velocity ratio 0.80). The present observations on spleen are thus qualitatively in agreement with these values.

From the classic work of Fahraeus (4) and the more recent work of Barbee and Cokelet (1), it is clear that when blood flows through tubes 100 µm or less in diameter the hematocrit of blood within the tube is less than that in the feed reservoir (due to a nonuniform distribution of red cells in the radial direction). In particular, when blood of hematocrit 10% is passed through vessels 29 µm in diameter the tube hematocrit is found to be approximately half of this (1). From the conservation of mass it follows that, under these conditions, the mean velocity of red cells passing through the tube will be twice that of plasma. Now perfusion of the fast compartment of the spleen by the first few milliliters of Ringer solution will be accompanied by a manyfold reduction in hematocrit of the blood contained therein. Therefore, if this compartment represents blood contained in the vessels, a ratio of 2.1 for the clearance-rate constants of cells and albumin becomes, under the conditions of these experiments, entirely reasonable. It must be emphasized, however, that in the blood-perfused spleen the hematocrit of the fast pathway would be constant at 35-40% throughout, and, therefore, a ratio of clearance-rate constants much nearer 1.0 would be expected. No such measurement has, to our knowledge, been reported.

The reversal of the cell:plasma velocity ratio found in gastrocnemius muscle (7) does not occur in glass tubes and no satisfactory explanation for it has yet been advanced. However, the larger vascular compartment of the spleen does not correspond to a series of cylindrical tubes but, rather, to a tortuous array of paths through the red pulp possessing, somehow, a remarkable capacity for plasma skimming and hemoconcentration (22). That the rate of clearance of plasma from the red pulp should exceed that of cells by a factor of 2 is, therefore, not entirely unexpected.

The V₁₁₂ for the slow red cell compartment is of particular interest to us because it provides a measure of the rate of release of immature and abnormal cells from adherence to the fine structures of the pulp. This process appears to be pH- and flow rate-dependent and is the subject of an investigation to be reported in a separate paper. As the pH of the perfusate is reduced below 7.4 the V₁₁₂ (slow) cells is reduced considerably and it is this that accounts for the much larger (5 times) value found in the present study than the value reported previously (16).

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