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Sluggish Clearance of Red Blood Cells From Microcirculation in Spleen, Cardiac and Skeletal Muscles

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ABSTRACT

In three isolated organs, spleen, cardiac and skeletal muscles, kinetic studies of red cell washout were carried out by using perfusion of the cell-free, oxygenated Ringer's solution. It is found that in each organ there are slow components for red cells to be emptied out from the vascular lumens ranging 30 to 50 minutes as the desaturation half-time. The slowest decay constants (K) are -1.48×10^{-3} for spleen, -2.33×10^{-3} for gastrocnemius muscle, and -4.0×10^{-3} for cardiac muscle.

INTRODUCTION

It was 1628 when William Harvey published a text in which he clearly demonstrated that "Blood flows through the circulatory system in a living body." Now we are aware that the main functions of the cardiovascular system are to exchange O_2 with CO_2 in tissues and cells, and to supply nutrients to organs. An impressive feature of this circulatory system is the rate at which blood moves through a vascular bed. In a man who has five liters of total blood volume and at least five liters per minute of cardiac output at rest, the total circulation time will be an order of one minute. In other words, any tracer injected at any particular point of the vascular system would appear at the same place within a minute, or any red cell marked will circulate through whole vascular beds and reach the same location within a minute.

The vascular beds, im many different organs, are not so simply or homogeneously built that all the red cells which traverse through different parts of the organ circulate and emerge from there at the same time. In addition, the architecture of capillaries even in one organ is so complicated that red cells which enter the capillaries at one time may flow through different lengths of passages and reach the vein at different times. For instance, the spleen which contains abundant red cells was shown to have two different mixing kinetics of ^{51}Cr-labelled red cells in man (Harris et al, 1958, Motulsky et al, 1958) In an isolated spleen perfused with Ringer's solution, red cells which traverse in different vascular routes in the spleen can be detected in the effluent. The red cells which traverse the shortest passage appear first, and those which take the longest route would reach the

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vein last. With this approach we have determined the kinetics of red cell washout from the spleen and have found that in the spleen there are three compartments for red cells stored or flowing through the red pulp. (Song and Groom. 1971)[3].

In this series of studies, we have also demonstrated that the morphological counterparts which are responsible for the fast (Ⅲ) and intermediate (Ⅱ) compartments, are indeed those free red cells in the vascular lumens and in the red pulp respectively (Song and Groom, 1971)[4]. Only the slow compartment which had a desaturation half-time of over 50 minutes could not be anatomically demonstrated. Rather, the long time to clear the red cells from the red pulp was due to the stickiness of red cells or adherence of red cells to reticulum fibers or macrophages in the red pulp (Song and Groom, 1971)[4] In fact, red cells of the slow (Ⅰ) compartment are found to be immature or abnormal red cells and microscopic observations with supravital stains on the slides of sample smears confirmed that the majority of those cells are reticulocytes (Song and Groom, 1972)[5].

This finding is surprising, particularly in two aspects; one is that reticulocytes have such a long half-time of washout and another is that a high proportion of red cells (up to ten percent of total red cells in the spleen) are reticulocytes. Therefore, it seemed to be desirable to investigate the red cell washout from other organs to show if red cells may behave similarly to those in the spleen. We have chosen the heart because it is generally accepted that the coronary vessels have the shortest circulation time, and the skeletal muscle in which inhomogeneity of blood perfusion is well known. Interestingly, in all cases we have also found that the washout curves consist of more than three compartments including the slow (Ⅰ) compartment, which has the desaturation half-time longer than 30 minutes in each case. In the present study, comparisons are made for the slow compartments of the three different organs, spleen, heart and gastrocnemius muscle.

METHODS

Healthy, adult cats were used in these experiments. All animals were anesthetized with sodium pentobarbital (30 to 40mg/Kg intraperitoneally) and were heparinized before removing the organs.

From 25 cats (10 with norepinephrine contraction of the spleen, 15 for control) the spleen was removed after vessels surrounding the organ were tied off except for the main splenic artery and vein. Cannulae were inserted into both splenic vessels for the perfusion and collection of samples.

For the perfusion of coronary vessels, the chest was opened by left thoracotomy and the heart was exposed. A cannula was inserted into the right auricle via the superior vena cava for the collection of venous outflow and another into the aorta for the infusion after tying off the pulmonary vessels and arterial branches arising from the aortic arch. Before beginning the perfusion, the residual blood from both right and left ventricular lumens was sucked out. Ten isolated hearts were used.

From 15 cats, the gastrocnemius muscle was surgically isolated and the Achilles' tendon was tied and cut. Then the vessels running to and from the muscle were ligated, except the popliteal artery and vein. The muscle was

separated from the animal, carefully having cut both tendon attachments to the femur.

All the organs isolated in this way were placed in a lucite chamber containing mineral oil at 37°C. For each organ the infusion flow was maintained constant throughout the experiment and varied between 2 and 12 ml/min, depending on the weights of the animals and the type of organs. The perfusion pressure in the inflow cannula was measured continuously, using a Statham pressure transducer (P23DC) and a Beckman type R Dynograph.

The perfusate which contained 100 mg% of glucose in the modified Ringer solution was prepared to have normal pH (7.4) when equilibrated with 5% CO_2 in O_2. Therefore, we could perfuse the isolated organ with normal physiological conditions; isotonic (300 mos/L), isothermal (37°C), normal pH, and a sufficient amout of O_2 supply. The solution was also prepared free of particulate matters by filtration through Millipore filters (GSWP04700, 0.22μ) three times before use.

The cellular concentrations in 0.5ml diluted samples of the venous outflow were determined by means of an electric sensing-zone cell counter (Celloscope: Particle Data Inc., series 112). For the confirmation of red cells, one drop of "redout" (Becton, Dickinson) was used and also smears of samples obtained from venous outflow on the microscopic slides were observed under a microscope. Reticulocyte counts were done by staining these slides with supravital stains (new Methylene Blue or Brilliant Cresyl Blue) and the counterstain with wright stain.

RESULTS

During the perfusion of the isolated organ with cell-free Ringer solution, the infusion pressures recorded from three different organs were within a normal range, from 20cm H_2O to 200cm H_2O approximately. The individual pressure tracing was always steady during the whole experimental period after a short period of an initial drop in a few cases of skeletal muscle.

Red cell concentrations in the samples of venous blood were around 6.0 and 9.0×10^9 cells per ml at the beginning of perfusion, and dropped to values of 10^6 or 10^5 cells per ml after a considerable amount of perfusion, usually over one or two hours perfusion, in all organs studied. As the orders of cellular concentrations were so large, the concentrations were plotted on a semilogarithmic scale against volumes of Ringer solution perfused. Interestingly, the curve of mean cell concentrations vs volume perfused showed a smooth curved relationship rather than a single straight line on a semilogarithmic scale. However, the last part of the washout curve could be treated as a straight line with a very slow decay constant. In all three different organs, the regression equations were computed and the slope constant as well as the intercept values on the Y axis were estimated. The correlation coefficients were highly significant with values of over 0.7 in all cases.

The last part of the straight line in the washout curve is very important since any attempts to establish "compartmentalization" in biological systems must be based on a linear function of the washout curve. Therefore, applying the classical curve-peeling method, a model consisting of these compartments for both spleen[3] and cardiac muscle[6] was proposed, and a model of four compar-

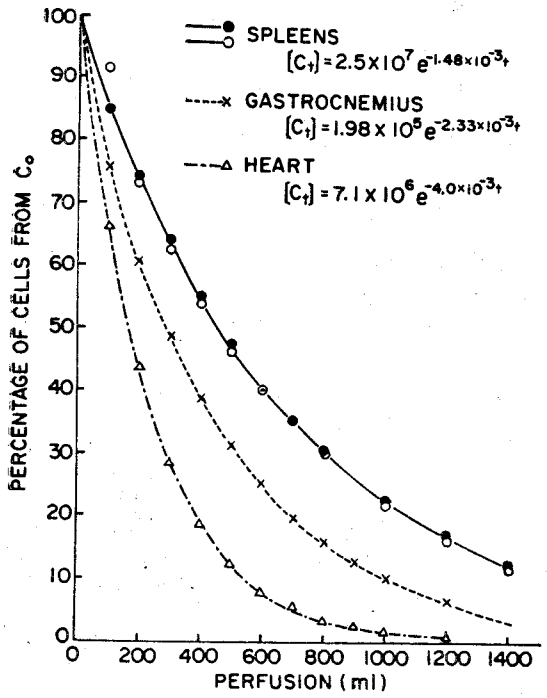

Fig. 1. The percentages of red cells in the slow compartments during washout from various organs
(• control spleen, ○ contracted spleen, × gastrocnemius muscle, △ cardiac muscle) are plotted on a logarithmic scale against the cumulative volumes of fluid perfused.

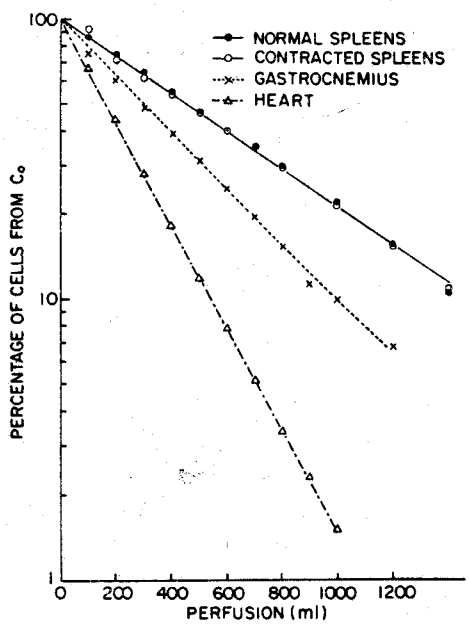

Fig. 2. The red cell washout curves are plotted on a linear scale as a function of volumes perfused. C_t represents red cell concentrations at a given time, t of the washout. C_o indicates the initial red cell concentrations of each slow compartment in different ograns.

tments was suggested for skeletal muscle[7].

The initial concentrations of red cells which are represented as intercepts of each strarght line on a semilogarithmic scale are 2.5×10^7 cells per ml for control spleen, 1.20×10^7 cells per ml for contracted spleen, 7.1×10^6 for cardiac muscle and 1.98×10^5 for skeletal muscle. Since the values of initial concentrations (Co) differ from one another between different organs, it is necessary to have a normalization in order to compare the kinectics of red cell washout. The Co values are considered as 100% and the consecutive cell concentrations thereafter are computed as a fraction of Co in each organ. In Figure 1 the percentages of cells obtained during the perfusion are plotted on a semilogarithmic scale showing straight lines.

After 200 ml of perfusion the samples of venous outflow from both control and contracted spleens showed over 70% of Co while only 44% of Co was found in the sample from heart. The washout of slow compartmental cells from skeletal muscle lay between spleen and cardiac muscle. The cell concentration in the washout from the heart was below 10% after 500 ml perfusion, whereas over 1000 ml was required for skeletal muscle and over

1400 ml perfusion from the spleen to reach this concentration. With the semilogarithmic scale the ends of each line seem to separate further for the different organs. In order to avoid this faulty impression the data are replotted on a linear scale in Figure 2.

The percentages of slow compartmental cells will approach zero after a long perfusion in all organs. However, the rate of decrease in cell concentration is quite distinct for different organs as indicated by the "K" constants of the exponents. Both spleens have the lowest value(-1.48×10^{-3}), while cardiac muscle has the highest value (-4.0×10^{-3}), and skeletal muscle has a value between the two.

DISCUSSION

All of the kinetic studies of clearance or washout using various substances as markers have some advantages and some disadvantages. Radioisotopes or dye indicators were commonly used whether they were diffusible or not. To eliminate the problem of diffusion, ^{51}Cr-labelled red cells are excellent as long as the red cells used can maintain physiological conditions while going through all the labelling procedure. However, the technique used to detect the radioactivity is again limited. In our experiments, the washout curve was accurate over a range of 100,000-fold. This accuracy cannot be accomplished by any other means at present.

Our results of red cell washout studies from the spleen(Sung and Groon, 1971, 1972)[3,4], skeletal muscle[7] and cardiac muscle[6] revealed a slow compartment which had not been shown by any of the methods or techniques used previously. With isolated organs, all the neural or humoral feedback mechanisms can be avoided to give a reasonably good steady state condition. In addition, using autologous red cells in situ as a marker, wehave not introduced any possible artefacts due to react ions with foreign materials.

This is the first suggestion, to our knowledge, that there may be a three-compartment model for red cells passing through various organs. The washout curves suggest a *biophysical* model which predicts how red cells may circulate in these organs. The *anatomical* correlates can be demonstrated by studying the morphology at different stages of the washout (i.e. near the end of the washout of each compartment).

The functional significance of different red cell compartments can be determined by modifying these in various ways. For example, we[8] found that the intermediate compartment of the spleen was the reservoir. The spleen contracted with norepinephrine, and expelled large numbers of red cells into the circulation, This was associated with a dramatic decrease in volume of the intermediate compartment as assessed with the washout curves.

Red cells are generally assumed to move through vascular beds with the rate measured by the circulation time, i.e. a transit time of less than a minute. If this does not occur, sludging may develop, and local circulation in the area may stop. We believe this assumption is valid for the fast compartment (i.e. the large channels) in all organs studied, since some cells traversed these vascular beds within one minute. However, there are distinct slow compartments in all three organs. Our evidence suggests the reticulocytes, which must be stickier than mature cells, are primarily responsible for these slow compartments (Song and Groom 1972, 1973)[5,6]. At least in

the spleen there is good evidence that reticulocytes stick to reticulum fibers or macrophages. Young red cells also adhere to structures in the red pulp of the spleen (Groom et al 1973)[4].

The similarity of the washout curves for control and contracted spleens is very interesting. with contraction, half of the red cells in the slow compartment can be released and the initial concentration, *Co*, dropped to half the value of the control spleen(Song and Groom 1971)[8]. Nevertheless, the rate constants are the same, which might suggest that the rate constant is not dependent upon the content of reticulocytes, but on the reaction of the reticulocytes with the surface area available in the red pulp.

It is assumed the capillary endothelium of both skeletal muscle and cardiac muscle is the same, but the geometry (length and diameter) of the capillaries may cause the difference in clearance of red blood cells from these organs.

The skeletal and cardiac muscle experiments were not comparable since the skeletal muscle was in a resting condition (Groom et al, 1973)[7] while the heart was beating continuously(Song and Groom 1972)[6]. We plan to repeat the study with exercising skeletal muscle to see if the washout curve approaches that of cardiac muscle. This may reduce the sluggish clearance of reticulocytes from the vascular beds and eventually result in a more efficient circulation during exercise. This could be another possible beneficial factor in physical exercise.

In healthy conditions, the slow compartment may not cause significant troubles for perfusion of blood, because the peripheral blood contains less than one percent of reticulocytes. However, in a case such as the spleen where over 10% of reticulocytes are found, the slow clearance of reticulocytes may cause a disturbance in a normal pattern of blood flow, with sluggish movement of red cells through vascular beds. Reticulocytosis may occur due to compensatory mechanisms or pathological conditions where the reticulocyte count exceeds 10%. If the sluggishness caused by high reticulocyte counts is severe enough to reduce the shear rate, or to decrease the axial streaming of red cells, the red cell membranes may interact with each other to cause aggregation. This could cause sludging or even form a plug to prevent blood flow. As yet we do not have any evidence to prove if this situation occurs.

Red cell flows through vascular beds may be important in the formation of thrombus (and obstructive disease), especially in cases of reticulocytoses. More work should be done on this.

REFERENCES

Groom, A. C., Song, S. H. and Campling, B.: *Clearance of red blood cells from the vascular bed of skeletal muscle with particular reference to reticulocytes. Microvascular Res.* **6**:*51, 1973.*

Groom, A. C. and Song, S. H.: *Effect of norepinephine of the washout of red cells from the cat spleen. Am. J. Physiol.* **221**:*255, 1971.*

Harris, I. M., McAlister, J. M., and Prankerd, T. A. J.: *Splenomegaly and the circulating red cell. Brit. J. Haematol.* **4**:*97, 1958.*

Motulsky, A. G., Casserd, F., Giblett, E. R., Brown, G. Ø., Jr. and Finch, C. A.: *Anemia and the spleeen. New Engl. J. Med.* **259**:*1164, 1215, 1958.*

Song, S. H. and Groom, A. C.: *Storage of blood cells in spleen of the cat. Am. J. Physiol.* **220**:*779, 1971.*

Song, S. H. and Groom, A. C. : *The distribution of red cells in the spleen. Can, J. Physiol. Pharmacol.* **49**:*734, 1971.*

Song, S. H. and Groom, A. C. : *Sequestration and possible maturation of reticulocytes in the normal spleen. Can. J. Physiol. Pharmacol.* **50**:*400, 1972.*

Song, S. H. : *Red cell washout from the coronary vessels of isolated feline hearts. Presented at Internat. Symp. on O_2 Transport to Tissues. April. 1973.*