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BLOOD VOLUME DETERMINATION IN THE MOUSE

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SUMMARY

1. The blood volume of the mouse has been measured using ^{59}Fe-labelled red cells to determine the red cell volume and ^{131}I-labelled human serum albumin to determine the plasma volume.

2. Values for the blood volume of $95{\cdot}0 \pm 1{\cdot}5$, $96{\cdot}3 \pm 2{\cdot}7$ and $84{\cdot}7 \pm 1{\cdot}2$ ml./kg body wt. were found for CSl female, CBA female and CBA male mice respectively.

3. A marked discrepancy was observed between the venous (cardiac) haematocrit and the whole body haematocrit.

4. The blood volume of the mouse must be determined from the red cell volume *and* the plasma volume, measured using appropriate labels, and not from the red cell volume *or* the plasma volume using the venous haematocrit.

INTRODUCTION

The mouse is used more extensively than any other laboratory animal in experimental biology and detailed information about mice is consequently required during the course of a variety of investigations. In many experiments it is necessary to know the volume of the blood or the volume of the plasma in the animals which are being studied so it is desirable to provide reliable values for the blood volume and the plasma volume of the mouse.

Values measured by exsanguination (Dreyer & Ray, 1910; Furth & Sobel, 1946; Grüneberg, 1941; Oakley & Warrack, 1940; Taylor, 1945) can be disregarded, as this method can provide no more than a very rough estimate, and the use of dye dilution techniques to measure the plasma volume (Bond & Leonard, 1959; Furth & Sobel, 1946; Wish, Furth & Storey, 1950) has been superseded by far more satisfactory methods which use radioactive isotopes. Although several investigators have taken advantage of these last methods to estimate the blood volume of the mouse

(Friedman, 1955; Kaliss & Pressman, 1950; Wish *et al.* 1950) very small groups of animals have been studied in most instances.

An extensive survey has been reported by Paxson & Smith (1968), who have estimated the blood volume in several strains of mice. They have assumed that the discrepancy between the venous haematocrit and the whole body haematocrit, which has been observed in other species (Chaplin, Mollison & Vetter, 1953; Everett, Simmons & Lasher, 1956), can be disregarded. The validity of this assumption is questionable, however, as the values for the blood volume which have been calculated by investigators who have used labelled serum albumin (Friedman, 1955; Kaliss & Pressman, 1950; Wish *et al.* 1950) are appreciably higher than the values calculated by investigators who have used labelled erythrocytes (Greenfield, Godfrey & Price, 1958; Keighley, Russell & Lowy, 1962; Paxson & Smith, 1968). This would be expected to be the case if the venous haematocrit was appreciably higher than the whole body haematocrit.

In the present investigation the plasma volume and the red cell volume have been measured and used to estimate the blood volume as well as to determine the relationship between the venous (cardiac) haematocrit and the whole body haematocrit in young adult mice.

METHODS

Measurements using iodinated human serum albumin (^{131}IHSA)

The plasma volumes of specific pathogen free, female, albino mice (CSI/ASH), weighing between 20 and 28 g, and of CBA mice of both sexes, weighing between 15 and 21 g, were measured using an isotope dilution technique. ^{131}IHSA (1·4 mCi/ml., protein concentration 20 mg/ml., 20–80 μCi/mg, protein free iodine probably less than 2%) was diluted in sterile saline to 100 μCi/ml. and 0·1 ml. was injected I.V. using a microlitre syringe. A small volume of Evans Blue dye was added to ensure the immediate recognition of any leakage which might occur at the injection site, and if any leakage was observed the animal was excluded from the series. Animals were anaesthetized with ethyl ether vapour 10 min after the administration of such injections. Samples of blood collected from the right atrium before death were weighed and counted on a Panax Gamma, One-Sixty Scintillation Spectrometer.

The plasma volumes of CSI female mice were also measured 10 and 40 min after an injection of ^{131}IHSA to assess the effects of removal of the protein from the plasma.

Measurements using ^{59}Fe-labelled red cells

CSI females, CBA males and CBA females each received 2 μCi of ^{59}Fe by I.V. injection (ferric citrate, specific activity 3–30 mCi/mg iron). After 5–6 days the mice were anaesthetized with ethyl ether vapour and samples of blood, collected from the right atrium using heparinized syringes, were pooled for each group. The blood was centrifuged at 3500 rev/min for 10 min on a bench centrifuge and the plasma was removed. The cells were washed three times in sterile isotonic saline and resuspended to the original volume. Blood samples were continuously agitated on a Matburn Wheel. Labelled cells from appropriate donor groups were injected I.V. into CSI

females, CBA males and CBA females. Each animal received 0·1 ml. of the labelled red cell suspension. After 10 min the animals were anaesthetized with ethyl ether vapour and samples of blood were collected before death from the right atrium using a heparinized syringe, weighed and counted on a Panax Gamma, One-Sixty Scintillation Spectrometer.

Blood density determinations

The density of blood collected from the right atrium was determined by weighing known volumes of blood in 200 cu. mm pipettes. Blood was drawn quickly into a pipette up to the graduation mark, and the pipette was then wiped and weighed. The temperature was recorded.

Sample volumes were subsequently calculated from sample weights using the blood density and the packed cell volume measured with a Hawksley microhaematocrit centrifuge.

RESULTS

The results are summarized in Table 1. The density of blood collected from the right atrium was found to be $1{\cdot}057 \pm 0{\cdot}004$ g/cm^3 (mean and S.E.) at 23° C.

The whole body haematocrit has been calculated from the red cell volume and the plasma volume using the relationship:

$$\text{whole body haematocrit} = \frac{\text{red cell volume}}{\text{red cell volume} + \text{plasma volume}}.$$

The values of the whole body haematocrit and the venous (cardiac) haematocrit are presented in Table 1, together with the blood volume of the mouse calculated by adding the red cell volume, measured using labelled red cells, to the plasma volume measured using labelled human serum albumin.

TABLE 1. The plasma volume, red cell volume, blood volume, whole body haematocrit and venous haematocrit of the mouse

Mouse strain and sex	Number per group	Plasma volume (ml./kg body wt.)	Red cell volume (ml./kg body wt.)	Blood volume (ml./kg body wt.)	Whole body haemato-crit	Venous haemato-crit
CS1 ♀	55	$59{\cdot}4 \pm 1{\cdot}2$	$35{\cdot}6 \pm 0{\cdot}9$	$95{\cdot}0 \pm 1{\cdot}5$	$37{\cdot}5 \pm 2{\cdot}9$	$50{\cdot}0 \pm 0{\cdot}6$
CBA ♀	17	$65{\cdot}6 \pm 2{\cdot}7$	$30{\cdot}7 \pm 0{\cdot}9$	$96{\cdot}3 \pm 2{\cdot}7$	$31{\cdot}9 \pm 3{\cdot}9$	$48{\cdot}1 \pm 0{\cdot}9$
CBA ♂	20	$56{\cdot}1 \pm 1{\cdot}1$	$28{\cdot}6 \pm 0{\cdot}4$	$84{\cdot}7 \pm 1{\cdot}2$	$33{\cdot}7 \pm 2{\cdot}1$	$47{\cdot}5 \pm 0{\cdot}5$

Values are means ± S.E.

There is no difference between the mean blood volume per unit body weight of CSI females and that of CBA females, but the mean value for CBA males is lower. There is however a considerable difference between the whole body and venous haematocrits.

Between 10 and 40 min after an injection of ^{131}IHSA no significant

increase was demonstrated in the proportion of activity localized in the spleen. A 6·5 % increase in the estimated plasma volume from 60·3 ± 0·5 ml./kg at 10 min to 63·9 ± 2·3 ml./kg at 40 min was observed.

DISCUSSION

Discrepancies between the values for the blood volume estimated using the plasma volume and values estimated using the red cell volume in man (Davies & Topley, 1959; Sjostrand, 1953) have been attributed to differences between the whole body haematocrit and the venous haematocrit (Chaplin *et al.* 1953). Differences between the whole body haematocrit and venous haematocrit are now reported in the mouse also. The blood volume can thus be calculated only by adding together the red cell volume and the plasma volume each measured separately using appropriate labels.

In males the blood volume per unit weight is less than it is in females reflecting the sex difference in body weight, which does not appear to be associated with a corresponding sex difference in blood volume.

The value for the blood density agrees well with that given by Schermer (1967).

As labelled murine serum proteins are not commercially available, labelled human serum albumin has been used to estimate the plasma volume. There does not appear to be any serious objection to doing so because it has been demonstrated that the value of the plasma volume of the rat estimated by the use of labelled homologous serum albumin is not appreciably different from that estimated by the use of labelled heterologous serum albumin (Everett *et al.* 1956) and in our studies no increase was demonstrated in the proportion of activity localized in the spleen between 10 and 40 min after an injection of ^{131}IHSA.

The technique which has been employed will over-estimate the plasma volume if labelled serum albumin is lost from the blood immediately after being injected. The possibility that such loss occurs even before the labelled protein is uniformly distributed throughout the blood cannot be excluded but is difficult to take into account. It is unlikely, however, that an appreciable discrepancy between the measured value and the true value for the plasma volume will result, as it has been demonstrated that, between 10 and 40 min after injection loss of labelled protein results in an increase of only 6·5% in the estimated value for the plasma volume. Kaliss & Pressman (1950) also found little difference in the estimated plasma volume at 15 and 60 min after injection.

The blood volumes calculated from the venous haematocrit and the red

cell volume measured using ^{59}Fe-labelled red cells (71·2, 63·8 and 60·2 ml./kg body wt.) agree well with the value of 53–63 ml./kg body wt. found by Paxson & Smith (1968) who did not use ether anaesthesia. The blood volumes calculated from the venous haematocrit and the plasma volume, measured using labelled human serum albumin (118·8, 126·4 and 106·9 ml./kg body wt.), correspond with the value of 121 ml./kg body wt. found by Kaliss & Pressman (1950) who used chloroform anaesthesia. Thus the differences in the values provided by the two methods reported by various investigators using different strains of mice have been shown to reflect pronounced discrepancies between the whole body haematocrit and the venous (cardiac) haematocrit. It is therefore totally unjustifiable to estimate the blood volume of the mouse either from the plasma volume or from the red cell volume using the venous haematocrit.

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