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STUDIES OF PLASMA VOLUME, RED CELL VOLUME AND TOTAL BLOOD VOLUME IN YOUNG GROWING RATS

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Previous determinations of blood volume in the rat, with the exception of a few early studies in which attempts were made to estimate red cell volume by complete exsanguination, have been based on dilution analysis following the injection of substances labelling either the plasma or the red cells. However, the estimation of total blood volume from measurements either of plasma volume or red cell volume together with haematocrit determinations on samples of peripheral blood takes no account of inequalities in the distribution of cells and plasma in different parts of the circulation, and may be grossly misleading. The discrepancies in early determinations may be judged from Table 1 which surveys values reported up to the present time. Total blood volume can be accurately found only by the summation of cell volume and plasma volume individually determined (Reeve, 1948).

Simultaneous measurements of red cell volume and plasma volume in large rats have recently been reported by Huang & Bondurant (1956). However, this investigation, like most studies of blood volume in the rat, has been aimed at the establishment of a value for blood volume per unit body weight without regard to the dependence of this value on the weight or age of the animal. Since the relationship between blood volume and body weight may not be linear, it is inadmissible to use a value for blood volume per unit body weight obtained experimentally in animals of one weight range in order to calculate blood volumes of animals in a different weight range. A number of investigators (Chisholm, 1911; Metcoff & Favour, 1944; Lippman, 1947; Wang & Hegsted, 1949*a*) have studied the relationship between blood volume and body weight in the rat, but their studies are based either on cell volume measurements alone or plasma volume measurements alone; their conclusions must therefore be accepted with reserve. The possibility of variations due to strain, sex and diet must also be admitted.

The study here described has been carried out in order to obtain reliable data on the dependence of plasma volume and red cell volume on body weight

TABLE 1. Values reported for blood volumes in the rat

Investigators	Method	Weight range (g)	Haematocrit	Red cell volume (ml./100 g)	Plasma volume (ml./100 g)	Total blood volume (ml./100 g)
Jolly & Stini (1905)	Exsanguination	172–307	—	—	—	4–5
Chisholm (1911)	Exsanguination	53–148	—	—	—	7·84 – 4·73 6·28 (mean) $BV = 0{\cdot}099\ W^{0{\cdot}9}$
Berlin *et al.* (1949)	^{59}Fe *or* ^{32}P cells	150–320	45·8	2·16 ± 0·20	—	4·59 ± 0·57
Berlin *et al.* (1950)	^{32}P cells	61–73 180–186	45·0 45·8	2·87 (mean) 2·19 (mean)	— —	6·3 (mean) 4·81 (mean)
Sharpe *et al.* (1950)	^{55}Fe *or* ^{59}Fe cells	260–360	48·4	2·32 ± 0·16	—	4·95 ± 0·27
Montgomery (1951)	^{32}P cells	118–406	—	—	—	6·3 ± 0·7
Fryers (1952)	^{59}Fe cells	204–253	42·7	2·15 (mean)	—	4·97 (mean)
Contopoulos *et al.* (1954)	^{59}Fe cells	199–211	45·5	2·34 ± 0·05	—	—
Scott & Barcroft (1924)	Gasometric dilution, CO	95–154	—	—	—	5·4–7·9 6·13 (mean)
Cartland & Koch (1928)	Dye dilution, vital red	101–262	—	—	—	6·0–7·9 6·8 (mean)
Went & Drinker (1929)	Dye dilution, vital red	189–321	—	—	—	6·9–7·9 7·4 (mean)
Orten *et al.* (1933)	Dye dilution, vital red	184–310	—	—	3·09–4·05 3·43 (mean)	5·78–6·92 6·38 (mean)
Griffith & Campbell (1937)	Dye dilution, vital red	Adult	—	—	—	4·1–5·3 4·3 (mean)
Beckwith & Chanutin (1941)	Dye dilution, Evans blue	3–4 months	—	—	4·08 ± 0·49	7·98 ± 0·85
Metcoff & Favour (1944)	Dye dilution, Evans blue	41–68 73–89 137–336	37·1 35·8 43·9	— — —	5·58 (mean) 6·49 (mean) 4·03 (mean)	8·90 (mean) 10·7 (mean) 7·18 (mean)
Lippman (1947)	Dye dilution, haemoglobin	48–308	—	—	$PV = 0{\cdot}122W^{0{\cdot}778}$	$BV = 0{\cdot}195W^{0{\cdot}794}$
Wang & Hegsted (1949*a*, *b*)	Dye dilution, Evans blue	63–394	—	—	$PV = 0{\cdot}182W^{0{\cdot}723}$	$BV = 0{\cdot}191W^{0{\cdot}821}$
Huang & Bondurant (1956)	Dye dilution, Evans blue *and* ^{32}P cells	230–550	46·1	2·63 (mean)	3·13 (mean)	5·75 (mean)

PV = plasma volume (ml./100 g); BV = total blood volume (ml./100 g); W = body weight (g).

in a pure-line strain of rat—the August hooded strain—originally obtained from Dr Anna Goldfeder of New York City Cancer Research Laboratory, and now used in many investigations at the Institute of Cancer Research. Plasma volume has been measured by dilution studies with Evans blue dye and also with ^{131}I-labelled human serum albumin, red cell volume by dilution studies with ^{59}Fe-labelled red cells obtained from donor animals.

METHODS

Normal male brown-hooded rats of the August strain were used throughout. They were maintained on Medical Research Council diet No. 41 and water *ad libitum*.

Preparation of donor animals for red cell volume measurements. Rats of weight approximately 150 g were injected intravenously with 10 μc ^{59}Fe as ferric chloride in 1% sodium citrate solution. After an interval of 14 days to allow maximum uptake of radioactive iron by the circulating red cells, by which time plasma ^{59}Fe content was negligible, blood was withdrawn by cardiac puncture into a heparinized syringe and injected without delay into recipient animals.

Preliminary studies of mixing conditions. Rats of different weights were anaesthetized with 0·12 ml./100 g of a 60 mg/ml. solution of sodium pentobarbitone (Nembutal, Abbott Laboratories Ltd.) injected intraperitoneally. The right carotid artery was cannulated with a fine polyethylene cannula attached to a 1 ml. syringe by a No. 20 needle. A small quantity of heparin solution was injected intravenously into a lateral tail vein and the needle used for this injection was left in position in the vein. Through this needle was then injected either:

(*a*) 0·2 ml. of a solution of 0·5% Evans blue (Imperial Chemical (Pharmaceuticals) Ltd.) in isotonic saline, or

(*b*) 0·2 ml. of a solution of ^{131}I-labelled human serum albumin (Abbott Laboratories Ltd.) in isotonic saline containing about 1 μc/ml. ^{131}I, or

(*c*) 0·2 ml. heparinized blood from a donor animal containing about 1 μc/ml. ^{59}Fe as labelled red cells.

At 1, 2, 5, 10, 15, 20, 30, 45, 60 min after injection blood samples of volume approximately 0·25 ml. were withdrawn via the carotid cannula and transferred to weighed sample tubes $\frac{3}{8}$ in. in diameter. The tubes were reweighed to give the weight of each sample by difference. The radioactive samples were assayed directly for ^{131}I or ^{59}Fe in a well-type scintillation counter (Anger, 1951) after which the blood was transferred to Wintrobe tubes for haematocrit determination. The Evans blue samples were transferred directly to Wintrobe tubes for haematocrit determination; after reading the haematocrits the supernatant plasma was pipetted off and dye concentration estimated by diluting a portion of plasma to 3 ml. and measuring optical density at 605 mμ against a diluted plasma blank in a Unicam SP 600 spectrophotometer. Haematocrit determinations were performed by centrifugation at 3000 rev/min for 30 min at a radius of 15 cm.

Measurement of plasma volume. Rats were lightly anaesthetized with ether. Either 0·2 ml. of a solution of 0·5% Evans blue in isotonic saline or 0·2 ml. of a solution of ^{131}I-labelled human serum albumin, in isotonic saline containing 1 μc/ml. ^{131}I, was injected intravenously into each animal by the lateral tail vein. Using the same syringe, further 0·2 ml. portions of the injected solution were measured into 10 ml. volumetric flasks and diluted to 10 ml. for use as standards. Exactly 5 min after injection, a single 0·5 ml. sample of blood was withdrawn from each animal by cardiac puncture with a syringe moistened with heparin solution and transferred to a sample tube $\frac{3}{8}$ in. in diameter. 0·5 ml. portions of the standard dilution were similarly measured into other sample tubes. Samples and standards were assayed as already described.

Measurement of red cell volume. Rats were lightly anaesthetized with ether. 0·2 ml. of heparinized blood containing 3 μc/ml. ^{59}Fe as labelled red cells was injected intravenously into each animal by the lateral tail vein. Using the same syringe, further 0·2 ml. portions of the injected blood were measured into 10 ml. volumetric flasks and diluted to 10 ml. with water for use as

standards. Exactly 10 min after injection a single 0·5 ml. sample of blood was withdrawn from each animal by cardiac puncture with a syringe moistened with heparin solution and transferred to a sample tube $\frac{3}{8}$ in. in diameter. 0·5 ml. portions of the standard dilution were similarly measured into other sample tubes. Samples and standards were then assayed as already described.

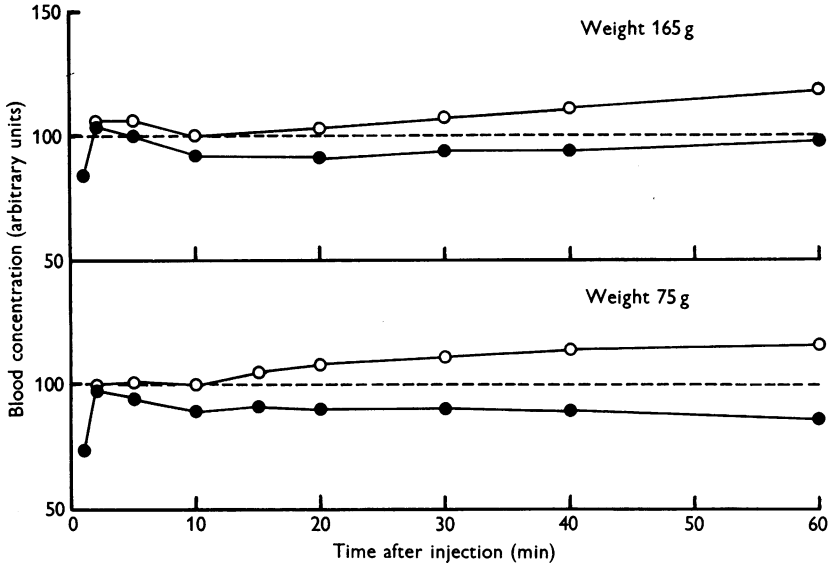

Fig. 1. Mixing curves for ^{59}Fe-labelled red cells injected intravenously into August rats. ●, Observed ^{59}Fe concentration in circulating blood; ○, ^{59}Fe concentration corrected for activity removed in previous samples.

RESULTS

Preliminary studies of mixing conditions

In order to study the mixing of the injected materials in the circulating blood and to establish a valid sampling technique for routine blood volume determinations, preliminary studies were made using animals with one carotid artery cannulated for serial blood sampling. Injection was performed by tail vein and 0·2 ml. blood samples withdrawn for analysis at intervals during the first hour after injection. Fig. 1 shows typical results for two animals of different weights injected with ^{59}Fe-labelled red cells. Figs. 2 and 3 show similar results for animals injected with Evans blue and ^{131}I-labelled human serum albumin respectively. The experimental values for concentration of the injected material in the circulating blood are shown both uncorrected and corrected for material removed from the circulation in previous samples. This correction has been performed using the relationship

$$\text{Corrected concn.} = \text{Observed concn.} \times \frac{T_I}{T_I - T_R},$$

where T_I = total amount injected, T_R = total amount removed in previous samples.

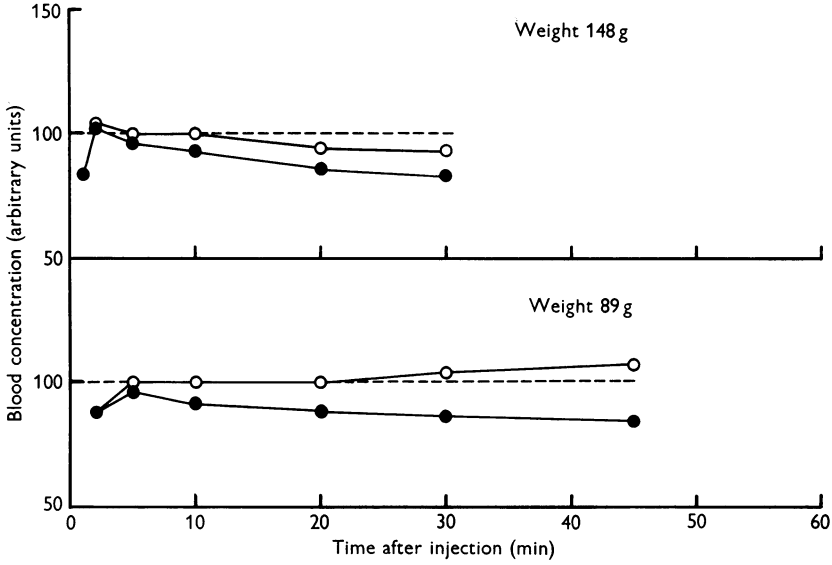

Fig. 2. Mixing curves for Evans blue injected intravenously into August rats. ●, Observed concentration of dye in plasma; ○, dye concentration corrected for amount removed in previous samples.

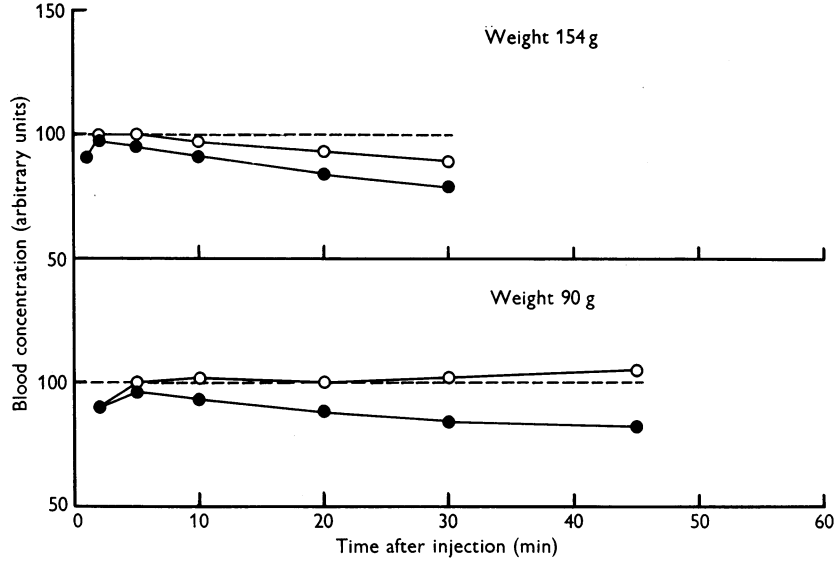

Fig. 3. Mixing curves for ^{131}I-labelled human serum albumin injected intravenously into August rats. ●, Observed ^{131}I concentration in circulating blood; ○, ^{131}I concentration corrected for activity removed in previous samples.

The uncorrected mixing curves following the injection of labelled red cells and labelled plasma are similar in form. The low concentration of injected material in the first sample withdrawn in each experiment is due to dead space blood in the cannula. After the second sample, the blood concentration falls. This fall is at first mainly attributable to the mixing of the injected material in the circulation, but may later be due in part to the effects of repeated sampling and in part to the loss of injected material to extravascular compartments.

The results for ^{59}Fe-labelled cells are of especial interest since no significant loss of labelled cells from the circulation is to be expected in the duration of the experiment. Inspection of Fig. 1 shows that the initial mixing phase is complete after 10 min. Thereafter the uncorrected curves of blood ^{59}Fe concentration fall only slightly despite repeated sampling, showing that the homoeostatic mechanisms maintaining the constancy of blood volume are not able to compensate for the loss of successive samples. The corrected curves therefore show an apparent rise in concentration, reflecting a reduced blood volume. These effects are particularly clear in the smaller animal studied, in which the blood volume has fallen significantly after 15 min. In the larger animal blood volume is approximately constant up to 20 min after injection.

Consideration of Fig. 1 suggests that a sampling technique in which a single sample is taken at the end of the initial mixing period 10 min after injection is satisfactory for the estimation of blood volume with ^{59}Fe-labelled red cells in the rat.

The results for Evans blue and for ^{131}I-labelled albumin are similar save that the uncorrected mixing curves show a steady fall after the initial mixing phase due to the removal of injected material from the circulation. If the blood volume is assumed constant in the larger animals up to 20 min after injection, the rate of loss of Evans blue and ^{131}I-labelled albumin may be estimated from Figs. 2 and 3 to be 22 %/hr and 25 %/hr respectively. To correct for loss during mixing, the mixing curve should properly be extrapolated back to zero time. From Figs. 2 and 3 it is however considered that a single sample withdrawn 5 min after injection gives a sufficiently accurate estimate of the extrapolated blood concentration of Evans blue or ^{131}I-labelled albumin at zero time in the rat, loss of injected material during the first 5 min being approximately compensated by the slightly incomplete mixing after this time.

Measurements of plasma volume and red cell volume

Following these initial studies, plasma volume and red cell volume measurements were performed on a large number of rats. Animals were injected with Evans blue, ^{131}I-labelled human serum albumin or ^{59}Fe-labelled red cells by tail vein. Single blood samples were taken by cardiac puncture 5 min after

injection with Evans blue and ^{131}I-labelled albumin or 10 min after injection with ^{59}Fe-labelled red cells, and analysed as described.

Plasma and red cell volumes were calculated using the following relationships:

$$PV = \frac{10 \times EB_S \times 100}{EB_P \times W},$$

where PV = plasma vol. (ml./100 g),
EB_S = Evans blue concn. in standard,
EB_P = Evans blue concn. in plasma,
W = body wt. (g);

or

$$PV = \frac{10 \times I_S \times Pt \times 100}{I_B \times W},$$

where I_S = ^{131}I counting rate from 0·5 ml. standard,
I_B = ^{131}I counting rate from 0·5 ml. blood sample,
Pt = corrected % plasma in blood sample;

$$RCV = \frac{\left[\frac{10 \times Fe_S \times Ht}{Fe_B} - RC_I\right] \times 100}{W},$$

where RCV = red cell vol. (ml./100 g),
Fe_S = ^{59}Fe counting rate from 0·5 ml. standard,
Fe_B = ^{59}Fe counting rate from 0·5 ml. blood sample,
Ht = corrected % red cells in blood sample,
RC_I = volume of red cells injected (ml.).

Haematocrit readings were corrected for trapped plasma assuming a value of 4% of plasma in the total packed cell column (Huang & Bondurant, 1956). Red cell volumes were corrected for the total volume of labelled red cells injected; this was usually 0·1 ml. No such correction was thought necessary in plasma volume measurements where homoeostasis may be expected to maintain constancy of blood volume after injection. Results are shown in Fig. 4.

Measurements of plasma volume with ^{131}I-labelled human serum albumin usually showed good agreement with those made by the Evans blue method. However, two batches of ^{131}I-labelled albumin gave anomalous results; simultaneous determinations of plasma volume by the ^{131}I-labelled albumin and dye methods in which samples from these batches were injected mixed with Evans blue showed over-estimation of plasma volume by the ^{131}I-labelled albumin, whilst studies of the mixing curves showed a rapid initial loss of ^{131}I from the circulation amounting to some 20% of the injected material. For this reason

less confidence is placed in the results obtained with ^{131}I-labelled human serum albumin than those with Evans blue, and the former have not been included in the detailed analysis of data.

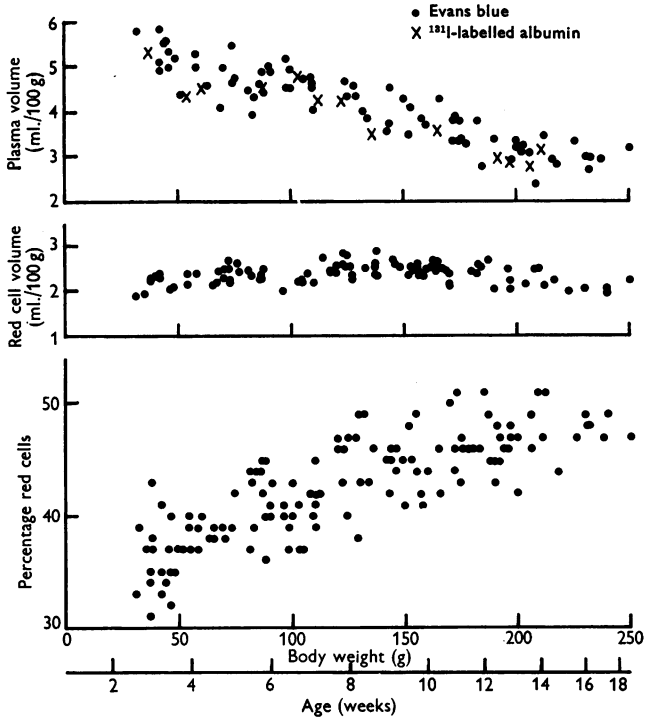

Fig. 4. Plasma volume, red cell volume and haematocrit values in August rats.

Table 2 and Fig. 5 show the results grouped according to body weight. Total blood volume was calculated as the sum of plasma volume and red cell volume, the small contribution (1–2%) of the white cells being neglected. The final column of Table 2 gives the ratio of mean body haematocrit, calculated from the experimentally derived values for red cell volume and total blood volume to the haematocrit measured on a sample of cardiac blood; both haematocrits are expressed as the percentage of red cells in total blood excluding white cells.

DISCUSSION

A fundamental difficulty in the measurement of blood volume in small animals by dilution methods arises from the time required for uniform mixing of the injected material and the loss of injected material to extra-vascular compartments during the mixing phase. In large species this difficulty may be overcome by serial blood sampling and extrapolation of the blood concentration curve back to the time of injection. This method has been applied to the

determination of plasma volume and red cell volume in large rats by Huang & Bondurant (1956) using a double sampling technique. It is questionable, however, whether such a procedure is valid in small animals where the volume of each sample withdrawn is a significant fraction of the total blood volume, since the concentration of injected material in later samples may be decreased by virtue of the amount removed in earlier samples. If it be assumed that the total blood volume and haematocrit remain constant, then it is possible to

TABLE 2. Plasma volumes, red cell volumes and total blood volumes in male August rats

Weight range (g)	Plasma volume (ml./100 g)	Red cell volume (ml./100 g)	Total blood volume (ml./100 g)	Cardiac haematocrit (% red cells) A	Mean body haematocrit (% red cells) B	$\frac{B}{A}$
26–50	5·38±0·26	2·21±0·14	7·59±0·27	36·5	29·1	0·80
51–75	4·76±0·31	2·34±0·10	7·10±0·30	39·1	33·0	0·84
76–100	4·66±0·22	2·38±0·13	7·04±0·24	41·8	33·8	0·81
101–125	4·55±0·25	2·47±0·14	7·02±0·25	42·6	35·2	0·83
126–150	4·13±0·30	2·54±0·10	6·67±0·28	45·5	38·1	0·85
151–175	3·86±0·20	2·45±0·07	6·31±0·19	45·8	38·8	0·84
176–200	3·29±0·19	2·39±0·18	5·68±0·24	46·7	42·7	0·90
201–225	3·02±0·31	2·25±0·22	5·27±0·34	48·5	42·7	0·88
226–250	3·03±0·24	2·07±0·22	5·10±0·26	48·5	40·7	0·84

The errors quoted are fiducial limits for $P = 0{\cdot}05$.

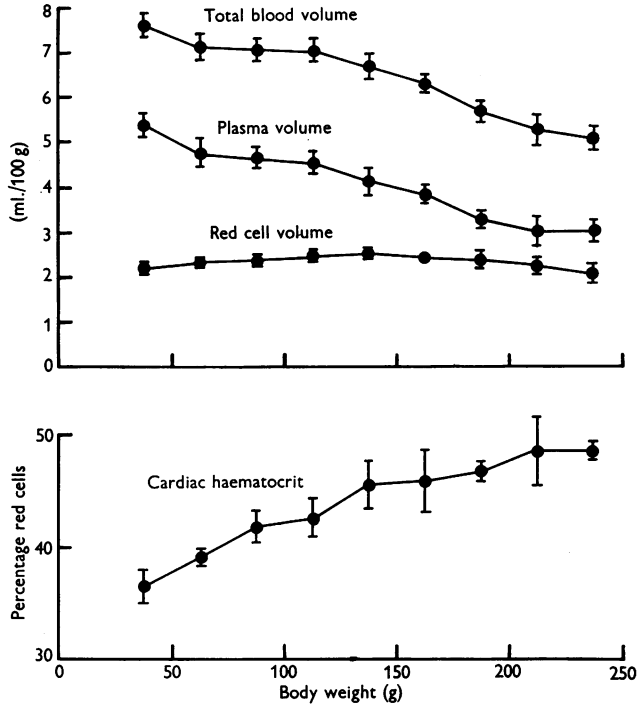

Fig. 5. Mean values of total blood volume, plasma volume, red cell volume and haematocrit in August rats. The errors shown are fiducial limits for $P = 0{\cdot}05$.

correct the measured concentration of later samples for the amount of injected material previously removed. If, on the other hand, the blood volume is not maintained but decreases in proportion to the amount of blood removed, then no correction is necessary. In practice, as is shown by Figs. 1–3, homoeostatic mechanisms operate, but the blood volume may still fall during repeated sampling, particularly in small animals. As a result, a systematic underestimation of the blood volume may result if the uncorrected data are extrapolated, while correction of the data based on the assumption that the blood volume remains constant may lead to an overestimation. It is therefore considered that a single sampling technique is to be preferred; the errors that result from such a technique are in any case small compared with the variation arising from biological and other causes. The interval between injection and sampling must depend on the nature of the injected material; intervals have been quoted ranging from 2 min for injections of haemoglobin solution (Lippman, 1947) to 2 hr for ^{59}Fe-labelled red cells. The studies reported here indicate that a mixing time of 5 min is satisfactory for Evans blue or ^{131}I-labelled albumin, but that 10 min should be allowed for ^{59}Fe-labelled red cells.

The measurements of plasma volume with ^{131}I-labelled albumin reported here are not sufficient in number for a detailed comparison of this with the Evans blue technique to be made, though no serious discrepancy between the two sets of results can be observed. Huang & Bondurant (1956) found good agreement between simultaneous measurements of plasma volume with Evans blue and ^{131}I-labelled albumin mixed before injection. By contrast, Wish, Furth & Storey (1950), in simultaneous measurements in mice, found that the plasma volume estimated with Evans blue was consistently higher by a factor 1·2 than that estimated with ^{131}I-labelled protein; they attributed the discrepancy to a rapid early loss of dye from the circulation, and corrected their Evans blue data by this factor. A similar rapid initial loss of Evans blue was reported by Cruickshank & Whitfield (1945) in the cat. No such fall in the Evans blue concentration of the plasma can however be observed in the mixing curves of Fig. 3, though it may be mentioned that the concentrations used were considerably higher than those used by the latter workers.

Freinkel, Schreiner, Athens, Hiatt & Breese (1954) found that the success of simultaneous determinations in man depended on the ratio of the amounts of Evans blue and labelled albumin injected; they postulated a reaction between the two substances leading to a rapid clearance of the latter under certain conditions. Similar effects have not been observed in the present studies. The anomalous results obtained with certain batches of labelled albumin in these studies may have been due to incomplete protein binding of the isotopic label, to partial denaturation of the protein during the labelling process or to rapid clearance of foreign protein from the circulation. No attempt was made to identify the nature of the observed effects. It is, however, recommended in

view of the results reported that ^{131}I-labelled albumin and Evans blue should only be used for blood volume studies in rats if detailed mixing curves have been followed and show no rapid early loss of the labelling substance from the circulation.

The results of the investigations show that it is incorrect to apply a single value for blood volume per unit weight to rats of different weights. The total blood volume falls steadily with increase in weight over the weight range studied, but this fall is not paralleled by a similar fall in red cell volume; on the contrary, the red cell volume is seen to be approximately constant over the weight range 40–250 g falling only slightly at the upper and lower ends of this range. The anaemia of the young growing rat in this weight range is thus seen to be mainly artifactual and to arise from the increased plasma volume per unit body weight in young animals. The slight fall in red cell volume observed in animals of body weight less than 100 g may reflect some degree of neo-natal anaemia. The fall observed at weights greater than 200 g may be related to the increased proportion of adipose tissue in adult rats; similar findings in man have been reported (Huff & Feller, 1956). The red cell volume data reported here are in reasonable agreement with those of Berlin, Huff, Van Dyke & Hennessy (1949), Sharpe, Culbreth & Klein (1950), Fryers (1952) and Contopoulos, Ellis, Simpson, Lawrence & Evans (1954) but show a somewhat lower value in small animals than that reported by Berlin, Van Dyke, Siri & Williams (1950). The value of 2·87 ml./100 g in rats of weight 61–73 g quoted by the latter authors is however based only on six observations.

A recent study of red cell volume and plasma volume as a function of age in the rat has been made by Garcia (1956) on the basis of red cell volume measurements with ^{59}Fe-labelled cells and haematocrit determinations. Since the latter were made not on cardiac or venous blood, but on blood obtained by exsanguinating the animals as completely as possible, the plasma volumes reported are probably not greatly in error despite the fact that they are based on measurements with labelled red cells. Both the red cell and plasma volumes reported are in reasonable agreement with those of Table 2, save for a marked fall in red cell volume in rats of weight less than 100 g, which was not observed in the present investigation. This may represent differences due to strain or diet; such differences are also suggested by the published growth data.

Metcoff & Favour (1944), Lippman (1947) and Wang & Hegsted (1949*a*) have made measurements of plasma volume by dye dilution methods in animals of different weights. These workers attempted to demonstrate a linear regression between the logarithms of body weight and plasma volume or total blood volume, representing a power law relationship between blood volume and body weight. Thus, Lippman (1947) found his data to fit the relationship:

$$\text{Plasma volume} = 0{\cdot}122\ (\text{body weight})^{0{\cdot}778}.$$

On the other hand, both Metcoff & Favour (1944) and Wang & Hegsted (1949*a*) found that the relation between blood volume and body weight changed at puberty and that their data could not adequately be represented by a single regression line. The plasma volume data reported here are in reasonable agreement with the values quoted by Lippman (1947) and Wang & Hegsted (1949*a*). Since, however, there seems no adequate physiological basis for a simple power relationship between either plasma volume or total blood volume and body weight or body surface area, attempts to express experimental data in terms of such a relationship appear unprofitable, and indeed misleading. Moreover, because the workers cited have studied whole blood volume in terms of plasma volume measurements alone their results fail to demonstrate the relative constancy of red cell volume per unit body weight during growth.

It seems probable that the increased plasma volume of young rats is related to differences in distribution of cells and plasma in vessels of different size, and in particular to the haemodynamic requirements for a marginal layer of plasma in small vessels (Krogh, 1929; Gibson, Seligman, Peacock, Aub, Fine & Evans, 1946). If this explanation is correct, then it is to be expected that the pattern observed in these studies will be found to be a general one, and that the total blood volume per unit body weight will fall and mean body haematocrit will rise with increase in body weight in any chosen species, particularly in small animals. Confirmation of this hypothesis must await detailed investigations of plasma and red cell volume in small species, which are at present lacking. Ancill (1956) reports a constant value of 7·20 ml./100 g body weight for total blood volume in guinea-pigs in the weight range 250–750 g on the basis of plasma volume studies with Evans blue, but gives no data relating to smaller animals. Wish *et al.* (1950) describe simultaneous measurements of red cell volume with ^{32}P-labelled red cells and plasma volume with Evans blue and ^{131}I-labelled albumin in mice, quoting a mean red cell volume of 2·99 ml./100 g and a plasma volume of 5·06 ml./100 g in normal animals, but give no information regarding the weights of their animals.

Unequal distribution of cells and plasma in vessels of different size is also indicated by the values quoted in Table 2 for the ratio of mean body haematocrit to cardiac haematocrit, which shows a mean value of $0{\cdot}84 \pm 0{\cdot}025$ over the entire weight range. This is in general agreement with measurements of the ratio in other species but not with the figure of 0·987, reduced to 0·942 after splenectomy, reported by Huang & Bondurant (1956) in rats of 230–550 g weight. It may be that the high ratios reported by these workers arise, as they suggest, from a rapid loss of unbound ^{32}P from the circulation after injection, leading to an over-estimation of red cell volume. Values for the haematocrit ratio in other species have been deduced from simultaneous estimation of plasma volume and red cell volume by a number of workers; values for man and dog are reviewed by Reeve (1948). Wish *et al.* (1950) found a mean value

of 0·88 in mice, whilst Armin, Grant, Pels & Reeve (1952) obtained a value of 0·85 in albino rabbits. In dogs, Gibson, Peacock, Seligman & Sack (1946) found a ratio of 0·91, but Reeve, Gregersen, Allen & Sear (1953) found 0·988, this being reduced to 0·899 after splenectomy. In man, the ratio has been quoted as 0·88 by Gregersen (1951) and as 0·91 by Chaplin, Mollison & Vetter (1953). Earlier reported values are reviewed by Reeve (1948).

The data of Table 2 suggest that the haematocrit ratio increases slightly with body weight over the range studied. Detailed analysis of the data shows

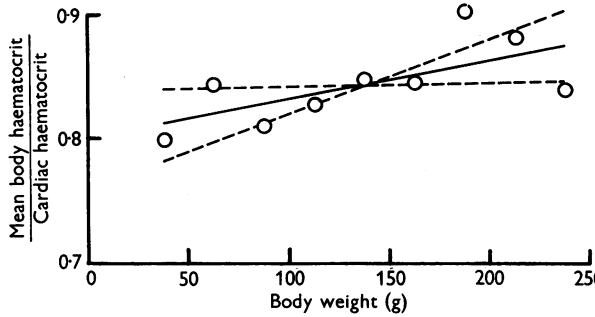

Fig. 6. Variation of haematocrit ratio with body weight in August rats. The regression line has the equation

$$\text{Haematocrit ratio} = 0{\cdot}80 + 0{\cdot}00031 \text{ body weight.}$$

The dotted lines show fiducial limits to the slope corresponding to $P = 0{\cdot}05$.

that the linear regression of the value of the ratio on body weight in fact has a slope which is just significantly greater than zero at the 5% level of probability (Fig. 6). The value of the ratio determined from the regression line ranges from 0·81 at 37·5 g body weight to 0·87 at 237·5 g body weight. This finding provides further evidence that the changes observed in blood volume per unit body weight are related to the haemodynamic requirements of small blood vessels. In small animals, the proportion of the total blood volume contained in smaller vessels is increased, and the haematocrit ratio is thereby reduced.

SUMMARY

1. Measurements were made of plasma volume, red cell volume and cardiac haematocrit in male rats of the 'August' strain of weight range 31–250 g. Plasma volume was measured by dilution analysis with Evans blue dye and also with ^{131}I-labelled human serum albumin. Red cell volume was measured by dilution analysis with ^{59}Fe-labelled red cells.

2. Preliminary studies of mixing conditions indicated that satisfactory estimation of both plasma and red cell volume is possible by single sampling techniques, a sample of cardiac blood being taken 5 min after injection for the plasma volume measurements and 10 min after injection for the red cell volume measurements.

3. Detailed values of plasma volume, red cell volume and haematocrit of animals in different weight ranges are presented. These data demonstrate that it is invalid to use a single value for blood volume per unit body weight to calculate blood volumes of animals of different weights. Red cell volume per unit body weight is approximately constant over the weight range studied but plasma volume per unit body weight falls continuously with increase in weight. It is suggested that the increased plasma volume observed in small animals is related to haemodynamic factors governing blood flow in small vessels.

4. The value of the ratio of calculated mean body haematocrit to the observed cardiac blood haematocrit increases slightly with increase in body weight, rising from 0·81 at body weight 37·5 g to 0·87 at body weight 237·5 g, with a mean value of $0{\cdot}84 \pm 0{\cdot}025$ over the entire weight range.

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