J. Physiol. (1956) 132, 469–475

THE BLOOD VOLUME OF THE NORMAL GUINEA-PIG

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(*Received* 17 *June* 1955)

During the course of a departmental programme of quantitative studies on haemopoiesis it became necessary to obtain values for the blood volume of normal male guinea-pigs of the Mill Hill strain at varying stages of maturity. Although the blood volume of other small laboratory animals has been extensively studied, few data are available for the guinea-pig.

The earliest studies appear to have been those of Gscheidlen (1869), Ranke (1871) and Steinberg (1873), who obtained approximate values ranging from $\frac{1}{12}$ to $\frac{1}{22}$ of the body weight. Dreyer & Ray (1910), using a modification of Welcker's method, obtained a mean value of 4·3% of body weight, and Erlanger (1921) gave a range of 3·5–5·5%. These results are low when compared with those obtained by modern methods for other small laboratory mammals. Berlin, Huff, Van Dyke & Hennessy (1949), using the combined dye-marked cell method, obtained 7·60–7·98 ml./100 g for different breeds of rabbits. Comparable data for the guinea-pig have been obtained by modern techniques. Went & Drinker (1929) determined the blood volume of eighteen guinea-pigs by a micro-method using Vital Red and obtained a mean value of $6{\cdot}40 \pm 0{\cdot}98$ ml./100 g. Flexner, Gellhorn & Merrell (1942), using T1824, obtained a mean of 8·3 ml./100 g on four guinea-pigs, and Masouredis & Melcher (1951) obtained a mean of $7{\cdot}53 \pm 0{\cdot}71$ ml./100 g on a series of thirteen animals using the isotopic iodinated-globulin method. Rogers (Yoffey, 1954) obtained a value of 27 ml. for a 400 g guinea-pig (mean for six animals), i.e. 6·75 ml./100 g, using the T1824 method. In view of the small number of animals so far studied it was thought worth while to report the present series, since it has been selected to demonstrate the dependence of blood volume on body weight. Further, the method used includes certain modifications of the customary technique, which will be discussed below.

METHODS

Animals. Eighteen healthy male guinea-pigs of the Mill Hill strain were used, ranging in weight from 250 to 750 g.

Reagents. (i) T1824 dye. According to Gregersen (1951) some samples of this dye may contain up to 2·5% of a red or purple contaminant. The dye used in this investigation was B.D.H.

supravital grade. It was purified, first by salting out four times from aqueous solution with sodium acetate (A.R.), then by reprecipitation with 95 % ethanol. After filtering on a Büchner funnel it was then dried in a vacuum desiccator. Paper chromatography of the reprecipitated compound showed that it contained no component other than T 1824.

(ii) Injection solution, 1·00 g T 1824/100 ml. A solution of purified T 1824 was prepared containing 1·00 g in 100 ml. 0·9 % (w/v) sodium chloride (A.R.) in distilled water. This was sterilized by autoclaving in steam at 15 Lb./sq.in. for 20 min, and dispensed in rubber-capped ampoules.

(iii) Standard solution, 250 mg T 1824/l. A solution of 250 mg purified T 1824 was made up in 1 l. 0·9 % (w/v) sodium chloride (A.R.).

Calibration. In this method the T 1824 is determined spectrophotometrically in whole haemolysed blood. In the appropriate range of concentrations, the plot of mg T 1824/l. against optical density is linear and passes through the origin at zero concentration. In order to determine the calibration curve, solutions of T 1824 of concentration range from 2·5 to 25·0 μg/ml. in diluted haemolysed whole blood were prepared by placing 1·0 ml. heparinized guinea-pig blood in each

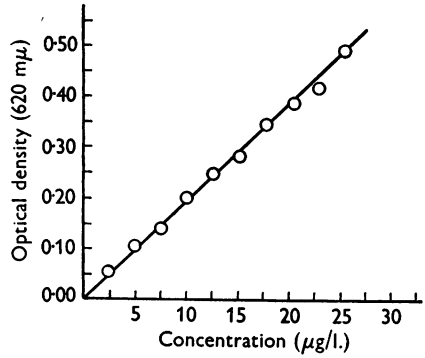

Fig. 1. Calibration curve of the optical density of T 1824 plotted against its concentration.

of ten graduated centrifuge tubes, adding 5 ml. distilled water, then quantities of standard T 1824 solution ranging from 0·1 to 1·0 ml. by means of a micrometer pipette, making up to the 10 ml. mark with distilled water and mixing. After completion of haemolysis the solutions were fully oxygenated with a stream of O_2 gas from a cylinder. The optical density of each was then determined at 620 mμ in a Hilger 'Uvispek' spectrophotometer against a blank of haemolysed blood prepared in the same way. The resulting calibration curve, the mean of ten determinations on blood from different animals, is shown in Fig. 1.

Determination of blood volume. After induction of light ether anaesthesia, 0·50 ml. blood was withdrawn by cardiac puncture into a heparinized 10 ml. graduated centrifuge tube and diluted to the 5 ml. mark with distilled water. 0·50 ml. of 1·00 g/100 ml. T 1824 solution was injected into the heart, using a micrometer syringe. Exactly 5 min later, using a different needle, 0·50 ml. blood was withdrawn by the same route and placed in a graduated heparinized tube. Distilled water was added to the 5 ml. mark, and after mixing and oxygenating as before the optical density was determined in the 'Uvispek' spectrophotometer at 620 mμ, using the previously withdrawn blood as a blank. The blood volume was calculated after converting optical density to concentration by means of the calibration chart.

RESULTS

The results of the determination of blood volume on eighteen normal male guinea-pigs are shown in Fig. 2. For comparison the results obtained by Went & Drinker (1929) and Masouredis & Melcher (1951), after conversion to

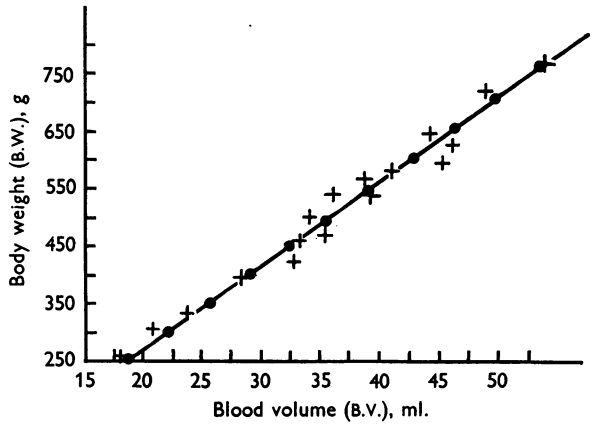

Fig. 2. Blood volume of normal male guinea-pig (Mill Hill strain: T 1824 whole blood method) +. Regression line B.V. = 1·300 + 0·0692 B.W. Mean B.V. (ml./100 g) = 7·20; σ = 0·31; N = 18. ●, regression line.

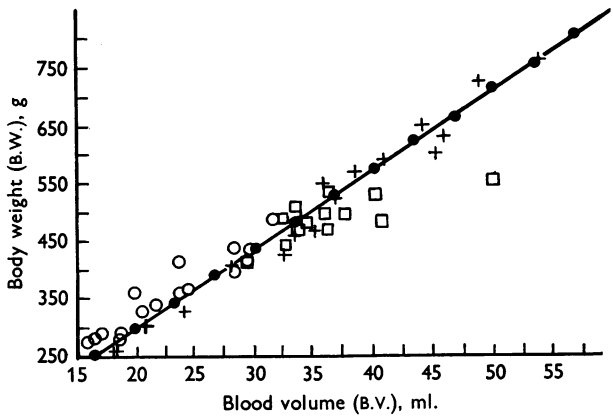

Fig. 3. Blood volume of normal male guinea-pig (Mill Hill strain) combined results. Regression line B.V. = 0·07642 B.W. − 2·57. Mean B.V. (ml./100 g) = 7·06; σ = 0·50; N = 49. +, this paper; □, Masouredis & Melcher, 1951; ○, Went & Drinker, 1929; ●, Regression line.

TABLE 1. Mean blood volume for guinea-pigs

Authors	Blood volume (ml./100 g)	σ	ϵ_m	No. of animals
(*a*) Ancill (this paper)	7·20	0·31	0·07	18
(*b*) Masouredis & Melcher (1951)	7·53	0·71	0·20	13
(*c*) Went & Drinker (1929)	6·40	0·49	0·12	18
Overall mean	7·06	0·50	0·07	Total 49

absolute figures, have also been plotted, and the combined results are shown in Fig. 3. Regression lines have been fitted in each case. The mean blood volume per 100 g body weight, with standard deviation (σ) and standard error of the mean (ϵ_m), has been determined in each case, and with the grand mean is shown in Table 1.

DISCUSSION

The values in Table 1 have been obtained by three different methods, i.e. (*a*) T1824 dye method (haemolysed whole blood); (*b*) ^{131}I labelled globulin method; (*c*) Vital Red dye method. It is of interest to compare the significance of the differences which exist between the mean values. Table 2 shows the results of such a comparison, using 'Student's "*t*" test'.

TABLE 2. Analysis of results of Table 1

Comparison	n*	Δ_m	ϵ_Δ	t	P
a–*b*	29	−0·33	0·21	1·56	<0·50, >0·10
a–*c*	34	+0·80	0·14	5·76	<0·01
b–*c*	29	+1·13	0·23	4·85	<0·01

* In this table n*, t, P have their customary meaning: Δ_m = difference between means; ϵ_Δ = standard error of difference between means.

It will be seen that, at the conventional significance level ($P = 0{\cdot}05$), there is no significant difference between the results with the T1824 and those with the isotopic dilution method, though there is a highly significant difference between the results with these methods and those with the Vital Red dye method of Went & Drinker (1929). Since there is no difference between the distribution of T1824 and ^{131}I labelled serum albumin (Freinkel, Schreiner & Athens, 1953), any difference between these results, apart from experimental error, should be due to the fact that whilst T1824 is bound to plasma albumin (Rawson, 1943), ^{131}I is bound to plasma globulin. Since albumin is lost from the circulation into the lymph more rapidly than is globulin, as indicated by the high albumin/globulin ratio of lymph (Drinker & Yoffey, 1941), any difference due to this factor would be reflected in a relatively lower concentration of marker substance when albumin is marked. In fact, the reverse is the case, though the difference between the two methods is not significant. This is in agreement with the results of Lewis & Goodman (1950) who found that the effect due to any difference between the rate of loss of T1824 from the blood stream and the rate of gain from lymphatic return is negligible. Gregersen (1951) also concluded that within the limits of experimental error, the volume distribution of antigenic albumin and globulin is the same as that of T1824.

In several cases dye concentration was estimated at 10, 20, 30 min after injection and plotted semi-logarithmically as a time-concentration curve. On extrapolation to zero time the theoretical dye dilution at the moment of injection was determined. It was found that the error due to mixing time was negligible if the sample were taken at 5 min after injection. This finding

is in agreement with those of Metcoff & Favour (1944), Lawson, Overbey, Moore & Shadle (1947) and Lewis & Goodman (1950).

The figures of Masouredis & Melcher (1951) were not corrected for plasma entrained by the sedimented corpuscles. Since this factor does not enter into determinations based on haemolysed whole blood, it should be allowed for in comparing the present results with those of these authors. If the customary factor of 0·96 is assumed to apply to the values recorded by Gregersen, Gibson & Stead (1935), their mean result becomes 7·23 and is almost identical with the values reported in this paper. The discrepancy between the present results and those of Went & Drinker (1929) can probably be accounted for by errors in their estimations due to the use of direct visual comparisons in capillary tubes, and to the use of Vital Red instead of T1824. Vital Red is suitable only for spectrophotometric estimation (Gregersen, 1937; Gregersen & Gibson, 1937).

The usual procedure in T1824 methods involves removal of the corpuscles by centrifuging before colorimetric estimation of the dye in plasma. Gregersen *et al.* (1935) pointed out that haemolysis of moderate degree adds little to the optical density of the sample at 620–630 mμ, the range of $\lambda_{max.}$ for T1824 in plasma. In fact the molecular extinction coefficients ϵ at 620 mμ are respectively 54,000 approx. for T1824 (Brenner, 1952) and 300 for oxyhaemoglobin (Lemberg & Legge, 1949). Many workers quoted by Horecker (1943), and Falholt (1955), have shown that the Lambert-Beer Law is applicable to solutions of oxyhaemoglobin of high concentration, and that the spectral characteristics of oxygenated haemolysed whole blood are nearly the same as those of equimolar oxyhaemoglobin solutions. Nicholson (1952) and Nicholson, Nahas & Wood (1952) were the first to use T1824 in whole haemolysed blood for the determination of human blood volume, thus eliminating the variable error due to dye held in the plasma between the packed cells. It appears that this method may be successfully applied to small laboratory animals such as the guinea-pig.

Reeve (1948), in a comprehensive review on blood volume, states that the most satisfactory estimate is obtained by summing plasma volume determined by the dye method and corpuscular volume determined simultaneously by the isotopic marked cell method. But such methods are time-consuming and for many purposes unnecessary. It seems to be generally recognized (Gregersen, 1951; Robinson & McCance, 1952) that the T1824 method gives an accurate estimate of plasma volume, but that an error due to variations of the haematocrit in different parts of the body is introduced when these haematocrit values are used in estimating blood volume. Gregersen (1951) estimates this error to be of the order of 12%. In the haemolysed whole blood method this error is reduced, since the loss of dye by corpuscular entrainment is avoided.

In order to compare plasma volume estimates made by conventional methods

with those made on haemolysed samples, the haematocrit was determined on the blood samples before haemolysis in ten cases, and the plasma volume calculated by difference, thus avoiding the necessity for a correction factor. The mean value obtained was 3·86 ml./100 g, $\sigma = 0{\cdot}28$, which is very close to that of Masouredis & Melcher (1951) (3·94 ml./100 g, $\sigma = 0{\cdot}37$, N = 13.).

SUMMARY

1. The literature on the blood volume of the guinea-pig is reviewed.

2. A method for the determination of the blood volume of the guinea-pig, based on the estimation of T1824 in whole haemolysed blood, is presented and discussed.

3. The mean value obtained for blood volume is 7·20 ml./100 g body weight as compared with 7·53 obtained by Masouredis & Melcher (1951) by the ^{131}I dilution method. When this latter figure is corrected for error due to incomplete separation of plasma in the haematocrit it becomes identical with the former within the limits of experimental error. When the present results are combined with those of Masouredis & Melcher the figure obtained for mean blood volume is 7·14 ml./100 g ($\sigma = 0{\cdot}41$, N = 30) and for mean plasma volume 3·91 ml./100 g ($\sigma = 0{\cdot}33$, N = 23).

I wish to thank Prof. J. M. Yoffey for constant advice and encouragement during this investigation, which was undertaken during the tenure of a grant from the British Empire Cancer Campaign Research Council; Messrs Reckitt and Colman Ltd., Hull, for a grant for laboratory expenses and Miss Pat Hill for invaluable technical assistance.

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