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THE PLASMA, CELL AND BLOOD VOLUMES OF ALBINO RABBITS AS ESTIMATED BY THE DYE (T 1824) AND ^{32}P MARKED CELL METHODS

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At present the most satisfactory estimates of blood volume are obtained from summing simultaneous measurements of plasma volume, made by a dye method, and cell volume made by a marked cell method (Reeve, 1948). Such simultaneous determinations are time consuming. For work on the effects of haemorrhage and fat embolism in rabbits (Armin & Grant, 1951) measurements of blood volume were required, but a simpler technique was desired. Fairly satisfactory blood volume estimates can be derived from measurements of either plasma volume and haematocrit or cell volume and haematocrit, provided correction factors are used. We have obtained data for determining the correction factors from simultaneous estimates of plasma volume, made with T 1824, and cell volume, made with a modification of the ^{32}P method (Reeve & Veall, 1949) on a series of 29 rabbits. These estimates and the derivation of the correction factors are first reported. With the appropriate correction factor and measurements of cell volume by the ^{32}P method the blood volumes of a second series of 80 rabbits have been determined. The ^{32}P method has the advantages for the rabbit of requiring only small blood samples and of technical simplicity.

METHODS

General

The best method of injecting exact quantities of solutions and withdrawing blood samples was found to be through a fine polythene catheter passed via an ear vein into the great veins near the heart. The technique of catheterization, which has many applications, is described in an appendix. The catheter was maintained patent by a very slow infusion of heparinized saline. Injected fluids were washed in with a little saline; the first portion of a blood sample containing the saline from the catheter was discarded.

All animals were starved for 12–15 hr. before an experiment. A preliminary (blank) blood sample of 4 ml. was withdrawn. The suspension of marked cells and the T 1824 solution were then injected and washed in. About 8 min. later a 4 ml. blood sample was withdrawn, and after this at about

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8 min. intervals three 3·5 ml. blood samples were taken. All blood samples were heparinized. The plasma was separated from the blank blood sample and used to make the T 1824 standard. The ^{32}P content, the haemoglobin concentration and the haematocrit of each of the other whole blood samples were estimated, the plasma was then separated from these samples and its T 1824 content and total protein content were determined. Haemoglobin was estimated in duplicate by Method 1 (Reeve, 1944). The haematocrit was estimated by spinning 100 mm. columns of blood in 2 mm. bore haematocrit tubes at 3000 r.p.m. for 30 min. in a centrifuge head of 15 cm. radius. All packed cell estimates were multiplied by 0·95, since when the haematocrit method is used as stated 5% of the packed cell column consists of plasma (Leeson & Reeve, 1951). The results so corrected are reported here as ml. cells in 100 ml. venous blood and are termed '% cells in venous blood'. The total protein content was determined by the copper sulphate method (Van Slyke, Hiller, Phillips Hamilton, Dole, Archibald & Eder, 1950).

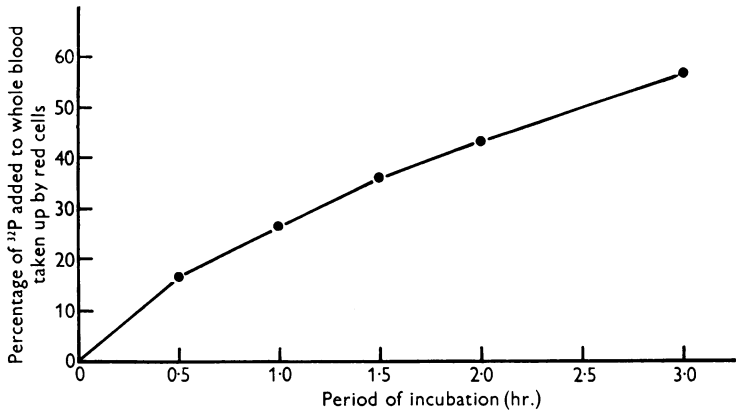

Fig. 1. The average rate of passage of ^{32}P into the red cells when rabbit's blood is incubated at 37° C. with 10 μc. of ^{32}P.

Blood volume technique

Estimation of cell volume with ^{32}P

Preparation of stock ^{32}P solutions. To get reliable estimates stock solutions must be carefully prepared and stored, for the presence of appreciable quantities of ^{32}P in a form that would be spun down during the washing of the red cells (for instance as an insoluble precipitate or concentrated in bacteria), might cause considerable errors in the estimation of cell volume. The ^{32}P was obtained from Harwell in sterile solution either as H_3PO_4 or as Na_2HPO_4, the latter solution containing added carrier phosphate in the proportion of 1 mg. P (4·6 mg. Na_2HPO_4) per millicurie ^{32}P. The H_3PO_4 solution was neutralized with a few drops of a weak NaOH (analytical grade) solution. Stock solutions were prepared by diluting either of the Harwell solutions with water till 1 ml. contained 100 μc., and were stored in small hard glass test tubes. The solutions were kept sterile by boiling the storage tubes after each exposure to the air and were stored in the refrigerator. They were discarded if they showed the least trace of cloudiness or precipitate.

Preparation of marked red cells. Before the experiment, 0·05 ml. of heparin solution (5000 i.u./ml.) and 0·1–0·2 ml. of stock ^{32}P solution containing about 10 μc. of ^{32}P were autoclaved at 15 lb./sq. in. for 30 min. in the stoppered centrifuge tubes described by Reeve & Veall (1949). 5 ml. of fresh blood were gently transferred into the centrifuge tube, which was then rotated for 1 hr. in an incubator at 37° C. During this period 2–3 μc. pass into the red cells (see Fig. 1). The centrifuge tube was cooled in iced water and the ^{32}P in the plasma was removed by three washings, each of about 25 ml. of ice-cold 0·9% sodium chloride solution. After each addition of saline the red cells were gently mixed and then spun down by 5 min. centrifugation at 2000 r.p.m. in a centrifuge head of

14 cm. radius. The saline was then sucked off and discarded. After removal of the third saline wash, 10 ml. of saline were added and the centrifuge tube was warmed to 25° C. The cells were then suspended by thorough gentle inversion and about 5 ml. of the cell suspension were injected into the rabbit via the catheter. The injection was usually made within 30 min. of completing the washing.

The washing removes almost all the ^{32}P from the saline. Analysis showed that only 1–2% of the total activity was in the saline, and that in the few experiments in which intervals longer than 30 min. elapsed between completion of washing and injection, the ^{32}P content of the saline did not exceed 2·5% of the total.

Other technique. Standards were prepared in triplicate by adding 1 ml. of a 1 in 50 dilution of the remainder of the suspension of radioactive cells to 12 ml. of a phosphate citrate mixture (1 part of a 2·5% tri-sodium citrate solution and 9 parts of Sorensen M/15 phosphate buffer solution of pH 7·3). Samples of rabbit's blood were prepared by adding 0·5 ml. of whole blood to 12 ml. of the phosphate-citrate mixture. Before making the radioactive counts the red cells were haemolysed with saponin. The cell volume, C, was determined from

$$C = \frac{v \times C_{st.} \times d \times h}{C_{sa.} \times D},$$

where v = volume of radioactive red cell suspension injected,

$C_{st.}$ = counts/min. of dilute standard,

d = dilution of the dilute standard,

h = haematocrit packed cell volume in unit volume of blood $\times 0{\cdot}95$,

$C_{sa.}$ = counts/min. of the samples, corrected for the loss rate of ^{32}P from the blood and for spontaneous dilution of the blood,

D = dilution of the samples.

No correction was made for the small proportion of ^{32}P in the saline of the injected red cells. At most this caused an overestimate by 2% and ordinarily probably by 1% of cell volume. The packed cell columns were read to the top of the white cell layer. Thus the cell volume estimates are 1–2% greater than the red cell volumes. Though the distribution of white cells may not be constant through all the circulating blood, errors caused by uneven distribution are probably negligible. Blood was withdrawn from a donor rabbit to provide the activated red cells for the majority of the estimations. In 13 animals marked *S* in Tables 1 and 2 their own red cells were activated and injected. If, for instance, as a result of a blood group difference, injected activated red cells were rapidly removed from the circulating blood this would result in an overestimate of cell volume. All blood volume estimations here reported were made on rabbits not previously transfused. Examination of the loss rates of ^{32}P from the blood after the injection provided no evidence of abnormally rapid removal of the injected donor cells from the circulation. There was no significant difference in the estimates of cell and blood volume obtained when the rabbits' own activated cells were injected and when activated cells from a donor rabbit were injected. We therefore think it unlikely that much error has resulted from using activated donor rabbit cells.

Plasma volume estimation

1 ml., measured by weight, of a 200 mg./100 ml. T 1824 solution was injected through the catheter, either just before or just after the suspension of marked red cells. With the undyed plasma a standard was prepared of nearly the same dye concentration as the dyed samples, and the dye contents of standard and samples were estimated with a photoelectric photometer using the red filters described by Gibson & Evelyn (1938). The observed optical densities were transformed into dye concentrations from calibration curves.

Plasma volume, V, was obtained from $V = \frac{C_{st.} \times d \times v}{C_{sa.}}$,

where $C_{st.}$ = dye concentration of standard,

d = dilution of standard,

v = volume of dye injected,

$C_{sa.}$ = dye concentration of samples corrected for dye loss and for spontaneous dilution.

Correction for spontaneous dilution of the blood

When successive samples of blood are drawn from the rabbit over a short period they often show some fall in haemoglobin and plasma protein concentration. The concentrations of dye in plasma and of marked red cells in blood will be altered if fluid is removed from or added to the blood. Assuming that the proportion of T 1824 labelled protein to total protein, and the proportion of labelled red cells to total red cells remains constant during the course of an experiment, corrections for fluid shifts can be made by a formula of the type suggested by Noble & Gregersen (1946). The formula is $C = C_n \times \frac{A_1}{A_n}$,

where C = corrected concentration of the nth sample of dye in plasma or marked red cells in blood,

C_n = the observed concentrations of dye or marked red cells in the nth sample,

A_1 = the total protein concentration of the first dyed plasma sample, or the haemoglobin concentration of the first radioactive whole blood sample,

A_n = the total protein concentration of the nth dyed plasma sample, or the haemoglobin concentration of the nth radioactive whole blood sample.

With this formula the plasma and cell volumes are estimated in terms of the fluid content of the blood at the time of withdrawal of the first dyed and radioactive samples.

RESULTS

Loss of ^{32}P from the blood after the injection of radioactive red cells, and of T 1824 from the plasma after the injection of the dye

Rate of ^{32}P loss. When for each rabbit the ^{32}P concentrations in the blood are plotted against the time after injection of the radioactive red cells, the changes in concentration over the first 35 min. after the injection are within the error of measurement. However, when the changes in concentration over time in all 29 rabbits of the first group of experiments are summed, a mean loss rate from the blood of about 5 % of the ^{32}P/hr. is found. This is similar to the loss rate of about 6 %/hr. from man's circulating blood reported by Reeve & Veall (1949). Analysis shows that a mean concentration of labelled red cells in the venous blood of the rabbit is reached by 8 min. after their injection.

Rate of dye loss. Fig. 2 shows a number of graphs of the plasma T 1824 concentration plotted against time after dye injection. There is a fairly rapid fall in dye concentration. The mean dye loss rate in the group was about 25 %/hr. which is 3–4 times that from human blood. In a few instances amongst the earliest estimates when faults in the catheterization technique may have caused some disturbances to the rabbits' circulations, the graphs of dye disappearance were L shaped. An example is shown in Fig. 2.

Determination of $C_{sa.}$. $C_{sa.}$, the concentration of marked red cells or dye that would be found if the marked red cells were distributed without loss through the circulating red cells, or the T 1824 through circulating plasma, is required for the equation used in determining cell and plasma volume. For estimation of both cell and plasma volume, $C_{sa.}$ was usually determined by extrapolation back to zero time on graphs of the type shown in Fig. 2. When the scatter of the

points made extrapolation uncertain (as with the L-shaped dye disappearance graphs), the mean concentration of the four samples was corrected for the mean loss rate of the group of rabbits.

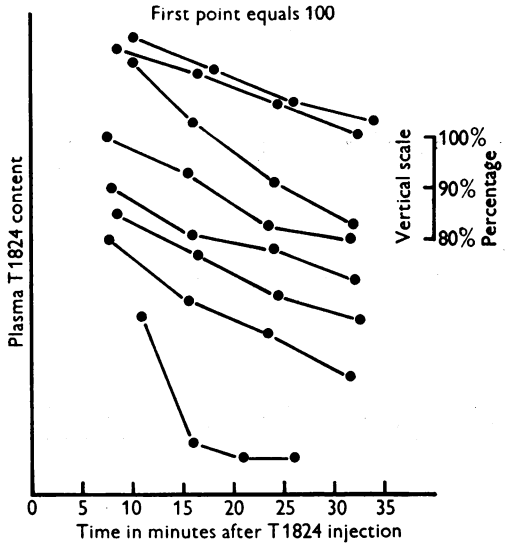

Fig. 2. Representative rates of loss of T 1824 from rabbits' circulating plasma. The T 1824 concentrations were corrected for spontaneous dilution as described in the text. For ease of comparison the dye concentration of the first sample drawn after the injection in each case is called 100, and the dye contents of the subsequent samples are expressed as percentages of this value. The vertical scale, to which each loss is plotted, is shown on the right.

Simultaneous estimates of plasma and cell volume

Two series of such estimates were made, one at the beginning and one at the end of the main series of ^{32}P estimates of blood volume reported later. The results of both series are shown in Table 1. The only differences in technique between the two series were (i) that in the first series catheterization technique was less skilful than in the second and therefore there was a little blood loss before the start of the blood volume estimation, and sometimes more disturbance of the rabbit's circulation, and (ii) that in the first series a sample of T 1824 supplied by Imperial Chemical (Pharmaceuticals) Ltd., and in the second a sample supplied by the Warner Institute was used. The means of the two series show that the animal weights, haematocrit values, plasma volumes, cell volumes and blood volumes were comparable. Further there was no significant difference between two ratios (discussed shortly) shown in columns 8 and 10 of the table for the two series. For analysis the two series can therefore be combined.

It was earlier noted that the best estimate of blood volume is obtained from the sum of simultaneous estimates of cell and plasma volume. Column 6 of

TABLE 1. Simultaneous estimates of plasma and cell volume in albino rabbits

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Rabbit (sex)	Weight (kg.)	% cells in venous blood*	T 1824 plasma volume (ml.)	^{32}P cell volume (ml.)	Blood volume =(4)+(5)	% cells in total blood $=\frac{(5)\times 100}{(6)}$	Ratio (7)/(3)	T 1824 haematocrit cell volume $=(4)\times\frac{(3)}{100-(3)}$	Ratio (5)/(9)	Calculated blood volume† (ml.)
Series 1										
30 –	3·15	30·8	157·2	54·0 *S*	211·2	25·6	0·83	70	0·77	206
25 –	2·95	36·2	93·0	40·4 *S*	133·4	30·3	0·84	52·7	0·77	131
35 –	2·35	37·6	88·5	42·1	130·6	32·3	0·86	53·3	0·79	131
36 –	2·85	35·2	103·5	44·3	147·8	30·0	0·85	56·2	0·79	148
23 –	3·25	34·0	104·8	48·5	153·3	31·6	0·93	54·0	0·90	167
41 F.	2·65	37·2	104·8	50·2	155·0	32·4	0·87	62·0	0·81	158
37 F.	2·85	42·6	79·0	46·0 *S*	125·0	36·8	0·86	58·6	0·78	126
39 F.	3·1	35·8	106·8	43·8	150·6	29·1	0·81	59·6	0·74	144
43 M.	3·3	34·2	116·1	47·3	163·4	29·0	0·85	60·3	0·78	162
47 F.	3·05	29·2	157·0	56·7	213·7	26·6	0·91	64·6	0·88	228
48 F.	2·3	39·8	84·7	40·1	124·8	32·1	0·81	56·0	0·72	118
54 M.	2·6	33·8	101·3	41·1	142·4	28·9	0·85	51·8	0·79	143
59 F.	2·7	34·2	113·8	48·0	161·8	29·7	0·87	59·0	0·81	164
64 F.	2·6	37·2	99·4	46·0	145·4	31·6	0·85	58·8	0·78	145
69 M.	2·2	31·9	106·0	41·8	147·8	28·3	0·89	49·6	0·84	154
Means	2·8	35·3	107·7	46·0	153·7	30·3	0·86 ±0·0329	57·8	0·80 ±0·0473	
Series 2										
203 M.	2·4	34·0	89·5	34·2	123·7	27·7	0·81	46·1	0·74	118
202 M.	2·8	32·3	111·0	39·6	150·6	26·3	0·81	52·9	0·75	144
201 M.	2·6	33·2	98·7	38·2	136·9	27·9	0·84	49·0	0·78	135
198 M.	2·7	37·2	97·2	44·2	141·4	31·2	0·84	57·6	0·77	139
205 F.	3·0	38·2	118·7	51·8	170·5	30·4	0·80	73·4	0·71	159
206 F.	2·9	34·8	113·8	48·8	162·6	30·1	0·86	60·6	0·80	164
207 F.	2·4	34·6	91·3	41·0	132·3	31·0	0·90	52·9	0·85	139
208 F.	2·8	33·4	119·8	44·2	164·0	27·0	0·81	60·0	0·74	156
209 F.	3·4	36·1	134·3	55·8	190·1	29·3	0·81	76·0	0·74	181
210 F.	2·95	39·0	104·8	52·4	157·2	33·3	0·85	66·9	0·78	157
211 F.	3·2	38·8	96·0	49·8	145·8	34·2	0·88	60·8	0·82	151
212 M.	2·4	42·0	98·0	61·2	159·2	38·4	0·91	71·0	0·86	171
213 F.	2·3	34·4	102·0	40·8	142·8	28·6	0·83	53·5	0·76	139
215 F.	2·4	32·3	106·2	40·8	147·0	27·8	0·86	50·7	0·80	149
Means	2·73	35·7	105·8	45·9	151·7	30·2	0·84 ±0·0352	59·39	0·78 ±0·0436	
Means of both series	2·76	35·52	106·8	45·97	152·8	30·26	0·85 ±0·034	58·55	0·79 ±0·046	

* 0·95 × haematocrit packed cell volume in 100 ml. venous blood (see text).

† Blood volume calculated from the formula $\frac{C\times 100}{0{\cdot}858\,H-0{\cdot}2}$ (see text).

S indicates animals injected with their own ^{32}P labelled cells. The other animals received labelled cells from donor rabbits.

Table 1 shows such estimates. Examination of the data of Table 1 shows that the blood volumes of column 6 cannot be obtained either from the formula

$$\frac{\text{Cell volume} \times 100}{\text{\% cells in venous blood}'}$$

or from

$$\frac{\text{Plasma volume} \times 100}{100 - \text{\% cells in venous blood}}$$

where '% cells in venous blood' is ml. cells/100 ml. venous blood, without the use of correction factors. Thus, in column 7 are shown a series of percentages obtained from the formula

$$\frac{\text{Cell volume } (^{32}\text{P}) \times 100}{\text{Cell volume } (^{32}\text{P}) + \text{plasma volume (dye)}}$$

which expresses the cell volume as a percentage of the total blood volume. This percentage has also been termed 'body haematocrit' and is hereafter abbreviated to '% cells in total blood'. With the percentage of column 7 and the cell volume of column 5 the blood volume of column 6 is correctly determined from the formula

$$\frac{\text{Cell volume} \times 100}{\text{\% cells in total blood}}.$$

The error in the estimate of blood volume from the conventional formula

$$\frac{\text{Cell volume} \times 100}{\text{\% cells in venous blood}}$$

is then given by

$$\frac{\text{\% cells in total blood}}{\text{\% cells in venous blood}}.$$

The values for this last ratio are shown in column 8; the mean value is 0·85, and therefore on the average the conventional estimate is only 0·85 of the combined estimate. The mean error in the estimate given by the formula

$$\frac{\text{Plasm volume} \times 100}{100 - \text{\% cells in venous blood}}$$

can be determined similarly from the mean of the values of the ratio

$$\frac{100 - \text{\% cells in total blood}}{100 - \text{\% cells in venous blood}'}$$

(not shown in the table), which is 1·08. Therefore on the average the blood volume obtained from the conventional formula

$$\frac{\text{Plasma volume}}{100 - \text{\% cells in venous blood}}$$

is 1·08 of the combined estimate. For comparison with other published data we show in column 9 of the table the estimates of cell volume obtained from the formula

$$\frac{\text{Plasma volume} \times \%\text{ cells in venous blood}}{100 - \%\text{ cells in venous blood}},$$

and in column 10 the value for the ratio

$$\frac{\text{Cell volume } (^{32}\text{P}) \text{ (column 5)}}{\text{Cell volume (dye haematocrit) (column 9)}}.$$

On the average the ^{32}P cell volume is only 0·79 of the dye haematocrit estimate of cell volume.

Correction factors

Let V = blood volume, C = cell volume, P = plasma volume, H = cell volume in 100 ml. venous blood, and $S = 100 - H$. Then V may be determined from C and H with the correction factor f_1 thus, $V = C \times \frac{100}{f_1 H}$ or V may be determined from P and S with the correction factor f_2, thus $V = P \times \frac{100}{f_2 S}$. In this paper we require to determine V from C and H, and therefore f_1 is the appropriate factor. This factor is the correction factor required to change H, the % cells in venous blood, into the % cells in total blood. Its value is best determined from the data of Table 1 with the regression equation relating H, the % cells in venous blood, to the % cells in total blood, which is % cells in total blood = $0{\cdot}858\,H - 0{\cdot}2$. The standard error of the regression coefficient, 0·858, is $\pm 0{\cdot}075$. In the blood volume results reported for the second series of rabbits, blood volume, V, has been calculated from the formula $V = C \times \frac{100}{0{\cdot}858\,H - 0{\cdot}2}$.

Correspondence between calculated and measured blood volumes

The data of Table 1 may be used to determine how closely a blood volume calculated from the cell volume, the % cells in venous blood and the above formula will be expected to lie to a blood volume obtained from the combined ^{32}P and dye measurements. Column 11 of Table 1 shows such calculated values. The standard deviation of the differences between these calculated values and the measured values of column 6 is 6·5 ml.; on the basis of these data in 95% of cases the calculated value would be expected to be within ± 13 ml. of the measured value. The blood volumes of Table 1 range from 124 to 214 ml. and the differences between the values of columns 11 and 6 tend to increase with the size of the blood volume. If these differences are expressed as percentages of the values of the blood volumes of column 6, then the standard deviation of the differences expressed as percentages is 4·15. It is probable therefore that in 95% of cases the calculated blood volume will lie within $\pm 8{\cdot}3$ ml./100 ml. of the combined estimate.

Estimates of cell and blood volume in albino rabbits with the ^{32}P *method*

For the estimates given below the venous catheterization technique was the same as in the two series just reported, but no T1824 estimates of plasma volume were made, and only two 3 ml. samples were withdrawn at intervals of 10 and 15 min. after the injection of the radioactive red cells. To determine $C_{sa.}$ the mean ^{32}P content of the two samples was multiplied by 1·01 to allow for ^{32}P loss. Table 2*a* and *b* summarizes the results of these observations.

TABLE 2. Estimates of cell and blood volume in albino rabbits with the ^{32}P method

(*a*) In animals with more than 34% cells in venous blood

Rabbit (sex)	Weight (kg.)	Haemoglobin (g./100 ml.)	% cells in venous blood	Cell volume (ml.)	Cell volume (ml./kg. body wt.)	Blood volume (ml.)	Blood volume (ml./kg. body wt.)
46 F.	2·6	12·85	34·7	38·9	15·0	132	50·8
44 M.	2·3	14·35	38·4	37·8	16·4	116	50·4
51 F.	2·5	12·5	35·9	39·8	15·9	130	52·0
52 M.	2·5	13·0	36·3	38·7	15·5	125	50·0
57 M.	2·6	13·8	39·8	47·1	18·1	139	53·5
63 M.	2·9	13·3	37·9	52·3	18·1	162	55·9
71 M.	2·25	12·8	36·2	40·0	17·8	130	57·8
73 M.	1·9	13·3	37·8	36·0	19·0	112	59·0
80 F.	2·05	13·3	38·6	41·2	20·1	125	61·0
81 M.	2·25	12·8	36·9	43·8	19·5	140	62·2
82 F.	2·25	12·9	36·5	37·9	16·9	122	54·2
88 F.	2·65	12·4	35·2	44·4	16·8	148	55·8
93 F.	2·8	13·1	34·0	47·3	16·9	163	58·2
94 M.	2·9	14·4	39·2	53·6	18·5	161	55·5
95 F.	2·6	13·5	37·2	48·2	18·6	152	58·5
97 F.	2·7	12·5	35·9	43·4	16·1	142	52·5
98 M.	3·45	13·5	37·0	55·0	16·0	175	50·7
101 M.	2·9	12·9	35·0	49·3	17·0	166	57·2
102 M.	3·5	14·0	37·6	63·1	18·1	198	56·5
105 F.	2·5	15·3	41·0	57·0	22·8	163	65·1
108 M.	2·35	13·0	34·4	43·4	18·5	149	63·5
110 M.	2·5	13·3	39·4	48·7	19·5	145	58·0
127 M.	2·2	13·8	39·1	35·6	16·2	107	48·6
129 F.	2·0	13·3	36·7	34·3	17·2	110	55·0
135 F.	2·6	12·9	36·4	49·9	19·2	161	62·0
137 F.	2·7	13·2	36·6	49·3	18·3	158	58·5
138 F.	2·95	12·8	35·0	44·3	14·9	149	50·0
139 F.	2·8	13·9	35·9	50·4	18·0	165	59·0
124 M.	2·2	13·8	38·6	40·0	18·2	122	55·5
117 F.	2·7	12·4	36·5	45·6	16·9	147	54·5
149 F.	2·5	12·8	35·6	37·7	15·1	124	49·6
128 M.	2·75	13·4	37·6	45·7	16·7	143	52·0
154 M.	2·3	12·9	35·5	38·5	16·8	128	55·6
110 M.	2·7	12·8	35·3	48·6	18·0	162	60·0
164 F.	2·7	12·6	36·3	52·3	19·4	169	62·6
165 F.	2·45	13·2	35·9	51·5	21·0	169	69·0
166 M.	3·3	12·5	34·2	54·6	16·6	187	56·6
167 M.	2·7	14·9	40·4	53·6	19·9	156	57·7
170 F.	2·55	11·7	34·6	48·0	18·8	163	64·0
174 F.	2·65	13·9	37·6	47·0	17·8	146	55·0
176 F.	2·7	12·9	37·6	45·0	16·7	144	53·4
179 M.	3·0	13·4	37·6	55·8	18·6	174	59·0 *S*
183 F.	2·8	13·4	38·1	50·0	17·9	154	55·0 *S*
186 M.	2·75	12·5	35·8	42·3	15·4	139	50·5 *S*
195 M.	2·4	13·2	37·1	46·8	19·5	148	61·7 *S*
Mean	2·61	13·2	36·86	46·1	17·74±1·64	147	56·51±4·62

TABLE 2 (*cont.*)

(*b*) In animals with less than 34% cells in venous blood

Rabbit (sex)	Weight (kg.)	Haemoglobin (g./100 ml.)	% cells in venous blood	Cell volume (ml.)	Cell volume (ml./kg. body wt.)	Blood volume (ml.)	Blood volume (ml./kg. body wt.)
49 M.	2·6	9·8	28·6	37·4	14·4	154	59·2
55 F.	2·2	11·0	31·4	37·1	16·9	139	63·7
61 F.	2·4	12·3	32·6	40·0	16·7	144	60·0
72 M.	2·0	11·5	32·0	32·0	16·5	118	59·0
74 M.	2·4	11·3	31·8	45·2	18·8	167	69·5
75 F.	2·3	11·7	32·9	38·9	16·9	139	60·5
76 M.	2·45	12·2	33·4	41·3	16·9	146	59·6
77 F.	2·05	11·7	31·8	34·0	16·6	125	61·0
114 ?	1·6	11·1	30·8	26·7	16·7	102	63·9
115 F.	2·8	12·0	33·4	47·4	17·0	167	59·6
116 ?	4·1	11·7	32·8	66·4	16·2	237	57·8
130 M.	2·4	11·5	32·7	36·4	15·2	131	54·5
133 F.	3·2	11·5	31·6	50·6	15·7	189	59·0
151 M.	3·2	11·7	32·8	47·7	14·9	171	53·4
153 F.	2·5	11·9	31·7	34·6	13·9	128	51·2
155 F.	2·3	11·5	31·5	36·1	15·7	135	58·6
162 F.	2·8	11·7	33·9	50·3	18·0	174	62·1
168 M.	3·3	12·3	33·6	53·3	16·2	187	56·7
171 F.	2·9	11·6	33·3	54·0	18·6	190	65·5
178 M.	2·6	11·5	32·5	40·8	15·7	147	56·5 *S*
191 F.	2·7	11·2	33·9	48·6	18·0	168	62·2 *S*
193 F.	3·2	11·9	32·3	49·8	15·0	181	56·5 *S*
194 F.	2·35	10·9	32·0	38·6	16·4	142	60·4 *S*
196 F.	2·95	12·1	33·2	40·0	13·5	141	47·8 *S*
197 F.	2·55	10·9	30·8	40·5	15·9	155	60·7 *S*
125 F.	2·50	11·1	32·6	33·2	13·4	119	48·0
Mean	2·63	11·5	32·3	42·3	16·14 ± 1·42	154	58·73 ± 4·93
Mean of all values	2·62	12·6	35·22	44·7	17·15 ± 1·74	149·5	57·32 ± 4·82

S indicates animals injected with their own P^{32} labelled cells. The other animals received labelled cells from donor rabbits.

Wintrobe (1946) gives 39·8 (S.D. ± 4·3) as the normal percentage haematocrit value for male rabbits. The mean % cells in venous blood for our 71 albino rabbits was 35·22, or adding 5% trapped plasma to make these results comparable with those of Wintrobe, 37·0. It is thus possible that some of our animals were anaemic. To test if there was any significant difference in blood volume between those with lower and those with higher haematocrit levels we have arbitrarily divided the rabbits into two groups, those with more than 34·0% cells in venous blood (Table 2, section (*a*)) and those with less than 34·0% cells in venous blood (Table 2, section (*b*)).

The first group contains 45 rabbits. The mean body weight is 2·61 kg., the mean % cells in venous blood 36·9 (range 34·0–41·0), the mean cell volume 17·74 ± 1·64/kg. and the mean blood volume 56·51 ± 4·62/kg. The second group of 26 rabbits contains animals with percentages of cells in venous blood ranging between 28·6 and 33·9, mean 32·3, with a mean body weight of 2·63 kg. The mean cell volume is 16·14 ± 1·42 ml./kg. and the mean blood volume 58·73 ± 4·93 ml./kg. Hence though the mean blood volume of the second group is slightly higher,

there is no significant difference between the blood volumes of the two groups. The cell volume of the second group is lower as expected from the lower haematocrit levels. If both groups of rabbits are combined the mean cell

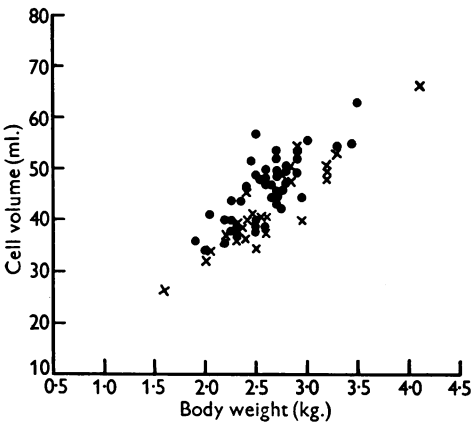

Fig. 3.

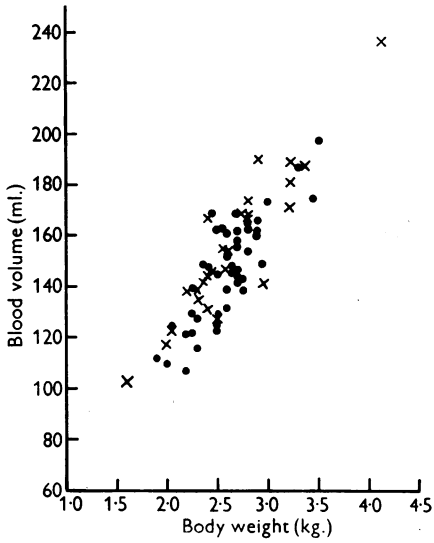

Fig. 4.

Fig. 3. The cell volumes of albino rabbits plotted against body weights. The data are from Table 2. Data from Table 2*a* (more than 34·0% cells in venous blood) are indicated by ●, data from Table 2*b* (less than 34·0% cells in venous blood) by ×.

Fig. 4. The blood volumes of albino rabbits plotted against body weights. The data are from Table 2. Data from Table 2*a* (more than 34·0% cells in venous blood) are indicated by ●, data from Table 2*b* (less than 34·0% cells in venous blood) by ×.

TABLE 3. Estimates of cell and blood volume in brown rabbits with the ^{32}P method

Rabbit (sex)	Weight (kg.)	Haemoglobin (g./100 ml.)	% cells in venous blood	Cell volume (ml.)	Cell volume (ml./kg. body wt.)	Blood volume (ml.)	Blood volume (ml./kg. body wt.)
147 F.	3·2	12·7	34·4	50·6	15·8	173	54·0
148 M.	2·8	14·8	38·9	51·9	18·5	156	55·7
157 F.	2·65	13·3	35·8	57·5	21·7	188	71·0
173 F.	2·8	13·6	37·9	54·1	19·4	168	60·0
84 F.	2·65	11·5	33·1	38·2	14·4	135	51·0
156 F.	2·6	11·4	32·2	49·4	19·0	180	69·2
158 F.	2·65	11·7	32·2	48·0	18·2	175	66·0
159 F.	2·8	9·7	28·8	60·2	21·5	246	87·9
160 F.	2·75	9·7	28·8	45·8	16·7	187	68·0
Means	2·77	12·0	33·6	50·6	18·35 ±2·44	179	64·75 ±11·27

volume is 17·15 ± 1·74 ml./kg., and the mean blood volume 57·32 ± 4·82 ml./kg. Graphs of the data from Table 2 are shown in Figs. 3 and 4. We have not included the data from Table 1 in the figures since a number of the rabbits on whom the combined estimates were made had lost amounts of blood ranging

from 5 to 15 ml. before the withdrawal of the first dyed and radioactive blood samples, and therefore the average cell and blood volumes are a little low.

A few observations, shown in Table 3 were made on brown rabbits. The mean cell volume, 18·35 ml./kg., and blood volume 64·75 ml./kg., were higher than in albino rabbits and the blood volumes showed a greater range of values (S.D. ± 11·27). It therefore seems unsafe to apply the results on albino to brown rabbits.

DISCUSSION

There is now much evidence that in man (Hevesy, Koster, Sorensen, Warburg & Zerahn, 1944; Gibson, Peacock, Seligman & Sack, 1946; Meneely, Wells & Hahn, 1947; Barnes, Loutit & Reeve, 1948; Reeve & Veall, 1949; Nachman, James, Moore & Evans, 1950; Mollison, Veall & Cutbush, 1950), and dog (Hahn, Ross, Bale, Balfour & Whipple, 1942; Gibson *et al.* 1946; Nickerson, Sharpe, Root, Fleming & Gregersen, 1950), when estimates of cell volume are made with cells labelled with radioactive iron, radioactive phosphorus or by a blood group difference, and estimates of plasma volume are made by T 1824 or Brilliant Vital Red, the % cells in total blood averages nine-tenths or less of the % cells in venous blood. The results presented in this paper show that the same is also true for the rabbit. The evidence indicates that the radioactive iron, radioactive phosphorus and group difference methods give similar estimates of cell volume (Barnes *et al.* 1948; Reeve & Veall, 1949; Berlin, Huff, Van Dyke & Hennessy, 1949). Some confusion has arisen because the estimates of cell volume given by improved carbon monoxide methods agree closely with those given by the T 1824 haematocrit method (Root, Roughton & Gregersen, 1946; Courtice & Gunton, 1949*a*, *b*). But it seems almost certain that the carbon monoxide method gives an overestimate of cell volume (Barnes *et al.* 1948; Reeve & Veall, 1949; Nickerson *et al.* 1950).

The rate of loss of T 1824 from the rabbit's circulating plasma, estimated to average 25 % during the first hour, is about 4 times that from man's and 3 times that from the dog's circulating plasma (for review of loss rates, see Reeve, 1948). Similar loss rates from the rabbit's circulating plasma may be calculated from some of Courtice's (1943) observations. In estimating $C_{sa.}$, the denominator of the equation used for calculating plasma volume, allowance must be made for the loss of dye between the injection of dye and the withdrawal of samples. In general, the faster the rate of loss the less certain is the correction for loss. Thus T 1824 is less satisfactory for estimating the plasma volume of the rabbit than for estimating that of man or dog. We therefore do not claim high precision for the T 1824 estimates of plasma volume given here, but have no reason to believe them much in error.

Courtice (1943, and personal communication) found in 60 healthy, muscular, brown rabbits an average of 70 ml. blood/kg. body weight. For blood volume estimations he used the T 1824 and haematocrit method, but his haematocrit

values were not corrected for trapped plasma and he used no other correction factors. His results can be made comparable with ours by correcting his haematocrit values for 5% trapped plasma and using a value for the correction factor f_2 derived from the data of our Table 1. The regression equation relating 100 − % cells in venous blood (S), to 100 − % cells in total blood, derived from the data of Table 1, is 100 − % cells in total blood = 0·858 S + 14·4. This provides the best value for f_2. V, the blood volume is therefore best determined from the equation $V = \frac{P \times 100}{0{\cdot}858\,S + 14{\cdot}4}$, where P = plasma volume. Courtice's Table 8 shows an average plasma volume of 50 ml./kg. and an average haematocrit of 28·5%. From this we recalculate that the average blood volume of his animals was 65 ml./kg. body weight. This compares well with our average of 64·75 ml. on brown rabbits, but is considerably higher than our average of 57·32 ml./kg. body weight on albino rabbits. Courtice & Gunton (1949*b*) report further observations on the blood volumes of a group of rabbits since ascertained to have been of mixed breed. We calculate from the T 1824 plasma volume and haematocrit estimates given in their Table 6 and applying our correction factor that their rabbits averaged 54 ml./kg. body weight. This is close to our mean value for albino rabbits. Courtice & Gunton point out that the rabbit's blood volume is much affected by the type of diet, and dietary differences may explain some of the difference between the earlier observations of Courtice and the later ones of Courtice & Gunton. However they cannot explain the significant mean difference of 7·43 ml./kg. between our estimates on brown and on albino rabbits, since both groups of animals were fed on a grass concentrate and water diet. Thus it is probable that the rabbit's blood volume varies with the breed of the rabbit as well as with the type of diet.

SUMMARY

1. Simultaneous estimates of plasma volume with the dye T 1824 and cell volume with red cells labelled with ^{32}P in 29 rabbits are reported.

2. On the average, blood volume calculated from the formula

$$\frac{\text{Plasma volume} \times 100}{100 - \%\text{ cells in venous blood}}$$

is 1·08 times, and calculated from the formula

$$\frac{\text{Cell volume} \times 100}{\%\text{ cells in venous blood}}$$

0·85 times the value obtained from the combined dye and marked cell estimate.

3. Estimates of blood volume lying within ±8% of the combined estimate can be obtained in 95% of cases from measurements of cell volume by the ^{32}P method and of the % cells in venous blood, from the formula,

$$\frac{\text{Cell volume} \times 100}{0{\cdot}858 \times \%\text{ cells in venous blood} - 0{\cdot}2}.$$

Such estimates are reported for 71 albino and 9 brown rabbits.

4. The average cell volume/kg. body weight was 17·15 ml., S.D. ± 1·74, in the albino, and 18·35 ± 2·44 in the brown rabbits. The average blood volume was 57·32, S.D. ± 4·82 ml. in the albino and 64·75 ± 11·27 in the brown rabbits.

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APPENDIX

Technique of catheterization

The catheter is inserted through a needle into the central or marginal vein at the base of the ear, and is then passed along the venous tree for a distance of 15–20 cm. till its tip reaches the great veins. The catheter is long enough to allow freedom of head movement and is well tolerated by the conscious rabbit.

The needle for inserting the catheter has an internal diameter of about 1·27 mm. and a shaft about 4 cm. long. To reduce the external diameter, the wall of the distal centimetre is ground so thin that it will just support a cutting edge on its bevelled point.

The catheter is made from a 90 cm. length of polythene tubing of an external diameter that will just allow the catheter to pass easily through the needle. To allow rapid withdrawal of blood its internal diameter should not be less than 0·6 mm. The polythene tubing supplied by Allen and Hanbury (Sterivac cannulae No. 1) varies considerably in both external and internal diameters and suitable portions require to be selected.

A few millimetres of one end of the catheter is rendered solid. To do this, the tubing is passed into a metal tube of the same bore as the needle used for insertion, so that its tip just projects beyond the end of the tube; the other end is connected to a 20 ml. syringe. The tip of the metal tube is heated in a small flame till the polythene melts and then melted polythene is sucked a short way into the catheter. After the polythene has set the needle is gently reheated and the catheter withdrawn.

Two holes, one at each end of a diameter, are bored with a small hypodermic needle immediately above the solid portion, the tip of which is then bevelled with a razor blade (see Fig. 5). This form of catheter tip obviates the difficulty caused by the end of the catheter being sucked against the vein wall and thus obstructed when blood is withdrawn. On the other end of the catheter a thickened rim is formed by inserting a needle into it and heating sufficiently to melt the polythene. This reinforced end is fitted to a needle.

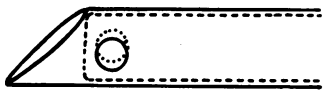

Fig. 5. The polythene catheter tip with solid end and two holes (diagrammatic). The internal bore of the catheter should not be less than 0·6 mm.

Perfusing the catheter. The needle of the catheter is connected to a motor-driven syringe containing heparinized saline (2 mg. heparin to 100 ml./isotonic sodium chloride) and delivering about 10 ml./hr. This slow perfusion suffices to keep the tip of the catheter free from clot. Before insertion the catheter is filled with the saline.

Inserting the catheter. The hair of the rabbit is removed from the basal portion of the outer surface of the ear. The animal is warmed on a heated table until the ear vessels remain dilated when the ear is handled. Procaine (0·25 ml. of 5% solution) is injected around the nerves accompanying the vessels at the base of the ear. A section of the straight part of the central vein is exposed through a 15 mm. skin incision and its upper surface freed from adventitia. The bevelled tip of the catheter is passed into the inserting needle so that its bevel is parallel to and a little within that of the needle. An assistant compresses the vein at the base of the ear and the operator inserts the tip of the needle into the congested vein, and pushes 5–10 cm. of the catheter into the vein. The motor syringe is started and the needle withdrawn from the vein along the catheter. The vein is tied distal to the point of insertion and proximally, and loosely, around the catheter. The catheter is then pushed for a distance of 15–20 cm. into the vein which usually brings the tip into the region of the heart. The ligature round the catheter is then tightened.

Sometimes the catheter does not at once pass beyond the point to which it is first inserted. After an interval of a few minutes' further manipulation, especially when the rabbit stretches its head forward, the catheter almost always slips in easily. The catheter is more liable to stick in the left than in the right veins probably because the right veins pursue a more direct course to the heart.

Injecting and withdrawing through the catheter. The catheter holds about 0·3 ml. of solution. After injection through the catheter 0·5 ml. saline is sufficient to wash in the injected fluid. To remove the saline from the catheter so that a true blood sample can be obtained, 0·8 ml. (saline followed by blood) is withdrawn and discarded and then the sample is withdrawn. After withdrawal of the sample the blood remaining in the catheter is washed in with saline.