17123. The Blood Volume of the Adult Rat, as Determined by Fe^{59} and P^{32} Labelled Red Cells.*

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The measurement of the blood volume of the rat by the use of plasma diluents has been reported by several investigators,[1-6] but there have been no reports on the use of labelled red blood cells for this purpose.

Fe^{59} given enterally or parenterally has been shown by Hahn and co-workers[7] to be incorporated into the red blood cell as an integral part of the hemoglobin molecule. The plasma iron does not exchange with the iron incorporated into the hemoglobin.[8] Hevesy and Zerahn[9] have modified the Hahn and Hevesy[10] method for determining the total red cell volume by using red blood cells labelled by the incorporation *in vitro* of P^{32}. Red blood cells may be labelled by both of these methods for use in the determination of the circulating blood volume.

Method. The first of the 2 groups of animals used consisted of 12 adult Slonaker rats, of which 3 were lactating, the second group of 30 adult rats (the first generation of a cross-breeding of highly inbred Slonaker and Curtis Dunning rats). In each group the blood type of all animals was the same. The individuals of Group I were given doubly labelled red blood cells, those of Group II, cells labelled only with P^{32}.

A single animal was injected intraperitoneally with a solution of Fe^{59}, as ferric chloride, buffered with sodium citrate and adjusted to pH 6. After sufficient time had elapsed for the Fe^{59} level in the blood to reach a constant value, approximately 5 cc was withdrawn into a heparinized syringe by cardiac puncture, placed in a paraffined 25 cc Erlenmeyer flask and incubated with P^{32} as ($Na_2HP^{32}O_4$) at 37°C for 2 hours with constant rotation. The cells were washed 3 times by adding isotonic saline solution, centrifuging and removing the supernatant fluid. Then plasma containing no P^{32} was added to the washed cells to reconstitute whole blood. Ether anesthetized animals in Group I and nembutal anesthetized animals in Group II were injected into the tail vein with 0.2 cc of this reconstituted blood. After 3 minutes approximately 1.0 cc of blood was withdrawn by cardiac puncture, 0.1 cc being used for the determination of P^{32} content, 0.5 cc for the determination of the Fe^{59} and 0.25 cc for the measurement of the hematocrit in Smith hematocrit tubes. Standards were prepared by diluting 0.2 cc of the reconstituted blood in 25 cc of distilled water.

The P^{32} was assayed by drying 0.1 cc of blood on a piece of lens paper on a thin aluminum foil, wrapping the lens paper and aluminum foil in cellophane and counting on an Eck-Krebs counter-tube. In a similar manner, 0.1 cc of the diluted standard was mounted and counted. The probable error of

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TABLE I.
The Blood Volume of the Adult Rat as Determined with Red Blood Cells Doubly-labelled with Fe^{59} and P^{32}.

Rat No.*	Body wt in g	Hematocrit	Blood vol. (Fe^{59}) cc/100 g body wt	Blood vol. (P^{32}) cc/100 g body wt	Total red cell vol. (P^{32}) cc/100 g body wt
1	205	45.	5.06	5.26	2.36
2	213	45.5	4.69	5.58	2.54
3	185	44	4.97	5.13	2.26
4	198	43.5	5.81	5.95	2.58
5	190	49	3.63	3.94	1.93
6	180	53	4.28	4.10	2.17
7	185	48	4.11	4.32	2.07
8	174	50	4.36	4.31	2.16
9	170	50	4.41	4.12	2.06
10	233	46	4.98	5.36	2.46
11	202	50	4.95	5.10	2.55
12	218	48	5.74	5.27	2.53
Avg	196.3	47.7	4.75	4.87	2.31

* Rats 1-9 were males; 10-12 lactating females.

counting was 1%. The Fe^{59} was assayed by a modification of Peacock's method.[11] The blood samples and the diluted standard were placed in 25 cc Erlenmeyer flasks; 10 mg of carrier iron, as ferric chloride, were added to the sample which was then dried at 70°C. After drying, 3-5 cc of concentrated nitric acid were added and evaporated to dryness. The ashing was completed with concentrated sulfuric acid and 30% hydrogen peroxide. The sulfuric acid solution of the ash was transferred to a centrifuge cone and the iron precipitated with concentrated sodium hydroxide. After centrifuging, the supernatant was discarded, the precipitate was redissolved in 0.25 cc of concentrated hydrochloric acid, the precipitation and centrifugation repeated, and the supernatant again discarded. The final precipitate was dissolved in 0.25 cc concentrated hydrochloric acid. The solution was transferred to an electrodeposition cell containing 35 cc of plating solution (one part saturated oxalic acid and five parts saturated ammonium oxalate). The iron was electro-deposited at 0.8 amperes and 12 volts in an apparatus designed by Dunn.[12] The samples and the standard were counted on a thin end window Geiger-Mueller counter. The probable error of counting was 1%.

Results. The blood volume as determined by both Fe^{59} and P^{32}, together with the total red cell volume, the hematocrit and the body weight of the Group I animals are presented in Table I. The same determinations for the Group II animals, except the Fe^{59} volumes, are recorded in Table II. The coefficient of correlation between the blood volume and hematocrit was 0.64. The standard deviation for all of the animals was not significantly different from the standard deviation for groups of litter mates. The average blood volume as determined by P^{32} for both groups, excluding the lactating females, was 4.59 ± 0.57 cc‖/100 g body weight; the total red cell mass 2.16 ± 0.20 cc‖/100 g body weight and the hematocrit 45.8 ± 2.9.

Discussion. The values reported using vital red are 4.3 cc/100 g body weight (1937)[1]; 6.38 cc/100 g body weight (1933)[2]; 6.9 cc/100 g body weight (1928)[3]; and 7.4 cc/100 g body weight (1929)[4]; with the use of Evans blue (T-1824)-7.6 cc/100 g body weight (1944)[5] and 7.98/100 g body weight (1941).[6] The total red cell volume was reported as 2.95 cc/100 g body weight;[2] 3.45 cc/100 g body weight;[5] and 3.90 cc/100 g body weight.[6]

The good agreement between the values of

[11] Peacock, W. C., Evans, R. W., Irvine, J. W., Jr., Good, W. M., Kip, A. F., Weiss, S., and Gibson, J., II, *J. Clin. Invest.*, 1946, **25**, 605.

[12] Dunn, R., Donner Laboratory, Univ. of Calif.

‖ Standard deviation.

TABLE II.
Blood Volume of the Adult Rat as Determined by P^{32} Labelled Red Blood Cells.

Rat No.*	Body wt in g	Hematocrit	Blood vol. (P^{32}) cc/100 g body wt	Total red cell vol. (P^{32}) cc/100 g body wt
13	260	49	4.27	2.08
14	240	45	4.92	2.20
16	180	46	4.77	2.22
17	165	43	5.02	2.18
18	150	45	4.17	1.88
19	230	46	4.87	2.26
20	240	47	4.34	2.04
21	230	54	3.96	2.13
22	260	47	3.92	1.85
23	230	48	4.13	1.98
24	185	50	3.46	1.78
25	225	45.5	5.50	2.31
26	232	47	5.39	2.54
27	237	47	4.33	2.10
28	190	45	4.84	2.30
29	257	44	4.82	2.17
30	225	47	5.11	2.08
31	210	44	4.24	2.04
32	205	50	4.14	2.12
33	215	50	4.41	2.51
34	235	50	4.94	2.26
35	230	44	4.52	1.99
36	295	43	4.00	1.72
37	315	46	4.23	1.95
38	320	48	4.66	2.24
39	240	46	4.50	2.07
40	200	44	4.35	1.91
41	235	43	4.81	2.07
42	245	48	4.65	2.23
43	260	45	4.99	2.25
44	240		3.79	
Avg	231.7	45.0	4.52	2.12

* Rats 13-14, 16-17, 19-23, 25-26, 27-30, 31-32, 33-34, 35-37, 39-40, 40-42, 24 and 44 were litter mates; 16, 17, 18, 24 and 44 were females.

the blood volume as obtained by Fe^{59} and by P^{32} labelled red blood cells permits the use of the P^{32} labelled red blood cells for the determination of the blood volume of the rat. The use of P^{32} thus eliminates the comparatively lengthy and tedious processes required for the accurate assay of Fe^{59}. In addition it eliminates the need for building up in a donor animal red blood cells with a concentration of Fe^{59} sufficient so that a large dilution of a small amount of the donor cells may be accurately determined by measurement of the amount of Fe^{59} present in the diluted sample.

Conclusions. 1. The blood volume of normal rats determined by the use of Fe^{59} labelled red blood cells is essentially the same as with P^{32} labelled cells.

2. The use of P^{32} labelled red blood cells is hence satisfactory for the determination of blood volume.

3. Determinations with P^{32} indicate that the blood volume of the normal rat is 4.59 $\pm$ 0.57/cc 100 g body weight; the total red cell volume 2.16 $\pm$ 0.20 cc/100 g body weight; and the hematocrit 45.8 $\pm$ 2.6.

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