Blood Volume in the Rat

H. B. Lee and M. D. Blaufox

Department of Nuclear Medicine, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, New York

The organ distribution of radiopharmaceuticals in the rat is usually estimated using 7% of body weight (BW) for blood volume (BV). In spite of its important impact on the evaluation of new agents, this value has not been validated adequately. We therefore studied blood volume in 70 awake Wistar rats (100 to 400 g BW) in which red blood cell volume (RBCV) and plasma volume (PV) were measured simultaneously. Red blood cell volume was measured by in vitro RBC-tagging with Tc-99m in Sn-pyrophosphate, 0.05 μg per ml of blood; plasma volume was measured with I-125 human serum albumin (HSA). Ten minutes after injection of the dose, 0.5 ml of blood was withdrawn from the carotid or femoral artery and duplicate samples of 0.025 ml of blood were counted after separating RBCs from plasma. Total blood volume was calculated by adding RBC volume and plasma volume. The relationship for the entire group was: BV (ml) = 0.06 × BW + 0.77 ($r = 0.99$, $n = 70$, $p < 0.001$). The difference between male and female rats was not statistically significant. The use of an arbitrary value of 7% for estimation of blood volume can lead to significant errors in calculating radiopharmaceutical distribution. The use of the general formula for the blood-volume calculation described here should improve the accuracy and reliability of estimates of radiopharmaceutical distribution.

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The estimation of blood volume is important in studies of radiopharmaceutical distribution. In spite of this, values used by various investigators differ enough to have a significant impact on reported results. The measurement of plasma volume (*1–4*) and of RBC volume (*5–8*) have been studied, but there are few reports on simultaneous measurements of plasma volume and RBC volume (*9–12*) for blood-volume estimation.

Estimates of blood volume in the rat derived from the plasma dilution method using T-1824 and I-131 human serum albumin vary from ~4.3 to 8.0 ml per 100 g BW, with a mean of ~7.0 ml. Blood volumes calculated on the basis of the dilution of labeled RBCs range from 4.5 to 6.3 ml per 100 g BW, with a mean of 5.7 ml. The errors encountered in making blood-volume determinations by dilution of either plasma or red cells alone have been pointed out by many investigators (*12–15*).

Although there are a few reports of the simultaneous measurement of plasma and RBC volumes for determination of total blood volume, the change in the relationship between blood volume and body weight as body weight increases has not been considered previously. Younger rats have a larger blood volume relative to their body weight than older rats (*4,6*). Many studies are carried out during periods of rapidly changing body weight, which emphasizes the importance of these relationships.

The present study was undertaken to determine the relationship between blood volume and body weight, using RBCs labeled with Tc-99m for RBC volume, and human serum albumin labeled with I-125 for plasma volume. These results yield the total exchangeable blood volume (TBV).

METHODS

Twenty-nine female and 41 male Wistar rats, (99.5–414 g), fed Purina Laboratory food and water ad libitum, were studied. Experiments were standardized with respect to time of day to avoid diurnal changes in blood volume (*12*).

The femoral (or carotid) artery and femoral vein were cannulated under light ether anesthesia using silastic tubing (medical grade, 0.012 i.d., 0.025 o.d., 10 in. long). The cannulae were filled with 20 units of heparinized saline and the free end brought through the tail. The rats were allowed to recover for an hour before study.

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For reprints contact: M. Donald Blaufox, MD, PhD, Dept. of Nuclear Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461.

PLASMA VOLUME DETERMINATION

Fifty or 100 μl of I-125 human serum albumin (0.5–0.1 μCi) was injected through the femoral-vein catheter. The actual injected dose was calculated from the change in weight of the dose syringe. A standard was prepared by diluting 0.025 ml of I-125 HSA with 10 ml of distilled water. The 0.025 ml of diluted standard was used for the dose calculation. Blood samples of 0.05 ml were drawn from the femoral or carotid artery 5 min and 10 min later, placed in two heparinized capillary tubes, 0.025 ml each, and analyzed to determine th hematocrit (Hct). Femoral arterial samples were drawn from larger rats and carotid samples from smaller rats.

RED-BLOOD-CELL VOLUME DETERMINATION

Half a ml of blood was withdrawn from the femoral artery and placed in a heparinized test tube. The blood was incubated for 10 min at room temperature with 0.01 ml of normal saline, containing 0.05 μg of stannous pyrophosphate (Sn-PPi), per ml of blood. After incubation, 10 μCi $^{99m}TcO_4$ in 0.01 ml of normal saline was added and incubated for 10 min. Following incubation period, 25 μl of the blood labeled with Tc-99m RBCs was pipetted into two heparinized capillary tubes (1.2 mm i.d., 1.4 mm o.d., 75 mm long) for the dose calculations. The remaining Tc-99m-RBC blood was weighed before injection. The RBC tagging method is a modification of the technique described by Korubin (*16*). Ninety-five percent of the Tc-99m is on the RBCs at time zero and the counts/ml RBC do not change significantly up to ~30 min, when elution of the label becomes detectable. The blood radioactivity is 96.5% on the RBC at 5 min and 97.2% at 10 min. A known volume of the Tc-99m-RBC blood was injected through the femoral-vein catheter immediately after I-125 HSA injection, using a tuberculin syringe and a 27-gauge needle. The catheter was flushed with 0.02 ml of normal saline. The femoral catheter was removed at the end of the study and counted. Any tracer left in the catheter was subtracted from the injected dose counts.

Five and ten minutes after I-125 HSA and Tc-99m RBC blood injections, ~0.06 ml of arterial blood was obtained. Using a micropipet, 0.025 ml of blood was transferred into another capillary tube. Its tip was sealed and the tube centrifuged for 4 min.

The hematocrit was calculated and the packed RBC in the capillary tube were carefully separated from plasma and placed in a 5-ml counting tube. Samples were counted in a well scintillation counter with a 20% window peaked at 140 keV. After 3 days (for Tc-99m decay), samples were counted again for 2 min for I-125 (20% window centered on 35 keV).

CALCULATIONS

RBC volume was obtained by dividing the cpm of the total injected Tc-99m RBC dose by the cpm of RBC tracer. The mean of the 5- and 10-min samples was used.

$$\text{RBCV} = \frac{\text{cpm of Tc-99m RBC injected dose}}{\text{cpm/ml of Tc-99m RBC}},$$

$$\text{Estimated BV by RBCV} = \frac{\text{RBCV} \times 100}{\text{Hct} \times \text{CF}},$$

where CF = hematocrit correction factor (0.96);

$$\text{and PV} = \frac{\text{cpm of I-125 HSA injected dose}}{\text{cpm/ml of plasma}}$$

$$\text{Estimated BV by PV} = \frac{\text{PV} \times 100}{(100 - \text{Hct})}$$

The estimated blood volume was calculated from plasma volume without considering the plasma trapped in the packed red cells after centrifugation. The true blood volume was calculated by adding RBC volume and plasma volume. Statistical analysis was performed by group t-test and one-way analysis of variance.

TABLE 1
Body Weight and Blood Volumes in Wistar Rats

		BW* g	HCT† (%)	PV‡ (ml/100 g)	RBCV§ (ml/100 g)	BV¶ (ml/100 g)	n
1	BW < 120 (g)	114.22 ± 8.74**	40.00 ± 2.46	4.68 ± 0.57	2.12 ± 0.17	6.80 ± 0.50	22
2	BW > 120 (g)	270.19 ± 80.40	43.94 ± 2.77	3.92 ± 0.32	2.27 ± 0.17	6.19 ± 0.40	48
3	Female	175.4 ± 46.86	43.28 ± 2.98	4.06 ± 0.41	2.26 ± 0.2	6.33 ± 0.39	29
4	Male	253.5 ± 112.6	42.30 ± 3.39	4.22 ± 0.64	2.22 ± 0.19	6.44 ± 0.60	41
5	Total	221.18 ± 98.7	42.70 ± 3.24	4.16 ± 0.54	2.24 ± 0.19	6.40 ± 0.52	70

* BW = body weight.
† HCT = hematocrit.
‡ PV = plasma volume.
§ RBCV = red-blood-cell volume.
¶ BV = PV + RBCV.
** ± = s.d.

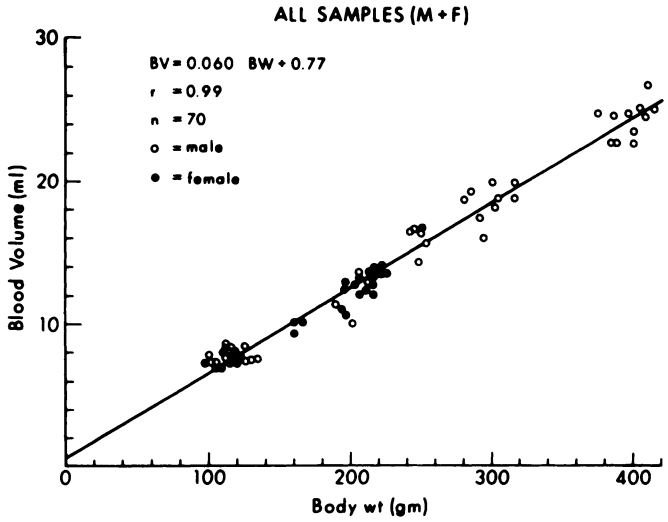

FIGURE 1
Relationship between total blood volume and body weight of entire study group of 70 animals is shown above. Linear correlation among all animals is highly significant. There is no significant difference between male (O) and female (●) animals. Regression equation given in text can be used if animals used are >120 g

RESULTS

The blood volume determined by both RBC volume and plasma volume simultaneously, and the hematocrit and body weight are presented in Table 1. The average total blood volume, RBC volume, plasma volume (all per 100 g BW), and the Hct (%) are 6.40 ± 0.52 (s.d.), 2.23 ± 0.19, 4.16 ± 0.54 ml and 42.70% ± 3.24, respectively (n = 70, male + female). The figures for male and female total blood volume were 6.44 + 0.6 and 6.33 + 0.39, ml per 100 g BW (NS). The total blood volume in the entire group of 70 animals had the following relationship to body weight (BW): TBV (ml) = 0.06 × BW + 0.77 (r = 0.99, Fig. 1). The blood volumes calculated from RBC volume or plasma volume using the hematocrit are 5.24 ± 0.41 (s.d.) and 7.38 ± 0.88 ml per 100 g BW, respectively; these are significantly different ($p < 0.0005$, n = 70).

Rats weighing less than 120 g have a greater relative total blood volume than those that are heavier ($p < 0.0005$, Fig. 2). The relationship between TBV and body weight in the 48 animals weighing 120 g or more was: TBV = 0.062 × BW + 0.0012. The RBC volume per 100 g body weight in the animals >120 g is not significantly different from that in the smaller animals, but plasma volume is significantly different ($p < 0.0025$).

DISCUSSION

Gregersen and Leeson (*15,17*) reported that the hematocrit value is not identical with the volume percentage of cells in blood samples. In order to obtain the true volume percentage of cells, a correction must be made for the plasma trapped in the packed cell column. The correction factor for this packed cell volume has been reported as 0.96 or 0.95 (*15,17,18*).

It also has been pointed out by many investigators that blood volume estimated from plasma volume is generally greater than that calculated from RBC volume. The simultaneous study of RBC volume and plasma volume reported here confirms that the blood volumes estimated from RBC volume and plasma volume are significantly different ($p < 0.0005$). The differences between mean blood volumes per 100 g body weight reported in the past

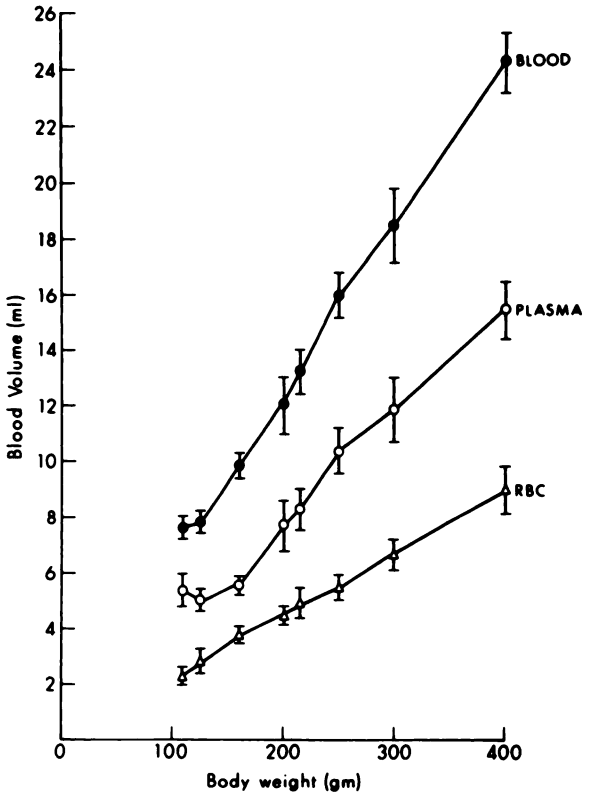

FIGURE 2
Relationship for RBCV, PV and total BV (RBCV plus PV) for animals studied is plotted against their body weight. Volumes are expressed in ml and weight in g. Volumes increase with body weight but smaller animals have relatively greater volume than very large ones

TABLE 2
Previous Studies of Blood Volumes

n	BW (g)	Hct	Method used for RBCV	RBCV (ml) per 100 g BW	Method used for PV	PV (ml) per 100 g BW	BV (ml) per 100 g BW	Ref.
	BV from PV (ml)							
12	189.0*	40.2			I-131 HSA	5.0	8.3	1
12	53.4*	37.1			CO	5.6	8.9	2
9	81.2*	35.8			CO	5.9	10.6	2
13	197.9*	43.9			CO	4.0	7.2	2
50	305.0*	47.8			T-1824	3.75	6.0	10
43	241.0*	46.1			I-125 HSA	3.96	7.47	3
	BV from RBCV (ml)							
12	196.3*	47.7	P-32	2.31			4.87	5
31	231.7*	45.0	P-32	2.21			4.52	5
—	<100.0	—	Fe-59	—			7.2	6
—	>100.0	—	Fe-59	—			4.4	6
34	239.0*	—	P-32	—			6.3	7
16	326.0*	48.4	Fe-55	2.3			4.98	8
	BV from RBCV + PV (ml)							
11	254.0*	50.3	P-32	2.36	T-1824	4.0	6.38	10
35	393.0*	46.1	P-32	2.63	T-1824	3.16	5.77	11
10M	180–250	36.0	Fe-59	1.91	I-125 HSA	3.4	5.3	12
18F	180–250	36.7	Fe-59	2.04	I-131 HSA	3.5	5.56	12
– M	101–125	42.6	Fe-59	2.47	T-1824	4.6	7.02	19
– M	226–250	48.6	Fe-59	2.07	T-1824	3.03	5.10	19

* = mean.

may be explained in part by the weight of animals used and differences in methods. Several studies of blood volume in rats by other investigators are listed in Table 2. In general, the mean blood volume per 100 g body weight is lower than the value reported here. Garcia (*6*), Lippman (*4*), and Belcher (*19*) reported that animals weighing <100 g have relatively larger blood volume. The present study confirms that the group of rats with mean BW 114 ± 8.7 have higher blood volume than the group with mean BW 270 ± 80 g ($p < 0.0005$). The female rat weight reported here did not exceed 250 g because virgin rats rarely exceed this weight.

The accurate estimation of blood volume is critical to the performance of radiopharmaceutical distribution studies. These data demonstrate that the generally accepted figure of 7% overestimates the blood volume in most animals. Such an overestimate would lead to errors in determination of organ distribution and blood background estimates. The significant differences in relative blood volume in animals of different size are important in reconciling results of different investigators.

The linear regression described here appears to be reliable for estimating rat blood volume, and is recommended for general use in radiopharmaceutical distribution studies in the rat.

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