A Rapid Micro Blood Volume Method in Laboratory Animals.* (28781)

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In connection with other investigations in our laboratory, a rapid method for determining blood volumes and serial hematocrits was required. A micro-adaptation of the standard method(1) for determination of blood volume in humans, using I^{131}-labeled human serum albumin, was developed. The procedure requires only a single 25 μl sample of blood for obtaining the blood volume, plasma volume and hematocrit. Normal values by the micro-method for blood volume, plasma volume and hematocrit of the mouse, hamster, rat, guinea pig and rabbit are presented. Comparative normal values by a macro-method, performed simultaneously in the same animal with the micro-method, for these parameters in the rat, guinea pig and rabbit are also given.

Materials and methods. The mice used in these studies were adult male mice of the Swiss Webster strain. Adult male Sprague-Dawley rats were employed as were adult golden, albino or banded male and female hamsters. These animals were fed Purina Laboratory Chow Checkers and water *ad libitum*. The guinea pigs were adults of a mixed breed. Adult male New Zealand rabbits were used. The guinea pigs and rabbits were fed Purina Rabbit Chow Checkers and water *ad libitum*. The diet of the guinea pigs was supplemented daily with fresh lettuce.

With the exception of the rabbits, the animals were anesthetized with ether. The mice and rats were injected with I^{131}-human serum albumin† by way of the lateral tail vein. The exposed femoral vein was used for the injection in the hamsters and guinea pigs, and the marginal ear vein was used in the rabbit. The dose of I^{131}-human serum albumin was approximately 2.5 μc/100 g body weight for each species.

The elapsed time between injection of the I^{131}-human serum albumin and withdrawal of the blood sample was 2 minutes for the mouse, hamster and rat and 5 minutes for the guinea pig and rabbit(2,3). Blood samples were withdrawn from the exposed inferior vena cava into heparinized syringes or from a peripheral vein for the micro-method. Blood was obtained from the rabbit by cardiac puncture or from the marginal vein of the opposite ear.

Micro-method. A 25 μl pipette‡ was used to transfer the blood sample to a heparinized capillary tube§ having dimensions of 1.2 to 1.4 mm O.D. and 75 mm long. The flat tip of the pipette was placed to the red tip of the capillary tube. The blood was transferred to the capillary tube by applying gentle, positive pressure to a piece of rubber tubing equipped with a mouthpiece, the open end of the tubing being attached to the upper end of the micropipette. Care was taken that no air space existed in the tip of the capillary tube that was to be sealed.‖ The length occupied in the capillary tube by the 25 μl of blood was 31.3 $\pm$ 0.35 mm in 42 observations.

The sealed capillary tube containing the blood sample was positioned in a 5 ml counting vial. The counting vial was then placed in a well-type scintillation counter having a 2-inch sodium iodide-thallium activated crystal. A dose of I^{131}-human serum albumin equivalent to that given to the animal was dispensed into a 10 ml volumetric flask. The standard was protected from adsorption to

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† I^{131}-human serum albumin (catalog no. 6703) was obtained from Abbott Laboratories, Oak Ridge, Tenn.

‡ Volumetric transfer Lambda-pettes (TC), Research Specialties Co., Richmond, Calif.

§ Heparinized red tip capillary tube (A-2930), Clay-Adams Inc., New York.

‖ A polyethylene closure (Critocap), Biological Research Inc., St. Louis, Mo., was used to seal the capillary tube.

TABLE I. Comparison of Micro Blood Volume, Plasma Volume and Hematocrit with Corresponding Values Obtained by the Macro-Method.

Species	Body wt, g	Blood vol, ml/100 g		Plasma vol, ml/100 g		Hematocrit	
		Micro	Macro	Micro†	Macro‡	Micro	Macro
Mouse (12)*	32 ± .74	13.3 ±.63	—	8.8 ±.45	—	34.6 ± 1.21	—
Hamster (8)	102 ± 1.19	7.4 ±.31	—	3.9 ±.36	—	48.9 ± 2.69	—
Rat (12)	189 ± 1.32	8.3 ±.09	8.1 ±.08	5.0 ±.03	4.7 ±.02	40.2 ± .47	41.6 ± .34
Guinea pig (8)	598 ± 33.0	7.3 ±.22	7.4 ±.18	4.0 ±.10	4.1 ±.20	45.2 ± .88	46.2 ± .64
Rabbit (4)	2200 ±300	7.0 ±.63	6.7 ±.51	4.5 ±.62	4.3 ±.43	37.2 ± 3.0	36.5 ±2.2

* No. of animals in each group. † Indirect method. ‡ Direct method. ± standard error of mean.

glass by addition of carrier human serum albumin(4). The 25 μl of the standard was pipetted into a capillary tube as above, capped and counted. Free I^{131} in the I^{131}-human serum albumin was less than 5% as determined by trichloroacetic acid precipitation.

After counting, the blood sample was centrifuged in an International Micro-capillary centrifuge, model MB, at 11,000 rpm for 5 minutes. The total column of blood and the length of the packed red blood cells and plasma were measured on a millimeter scale. The hematocrit was calculated, and the packed red blood cells in the capillary tube were carefully separated from the plasma by use of an ampule file. The red blood cells were recounted, and the per cent of plasma trapped in the cells was calculated. In a series of 12 blood samples, only 2.30 ± 0.12% of the plasma remained in the packed red cells. No correction for the trapped plasma was made in calculating plasma volume. Direct plasma volume, which could be obtained by counting the plasma, was not determined. Rather, plasma volume was determined from the blood volume and hematocrit by the indirect method.

Blood volume, plasma volume and hematocrit were determined on samples of blood from 2 different sites in the rat. The inferior vena cava in the anesthetized rat was exposed as before. Approximately 2 minutes after giving the dose of I^{131}-human serum albumin, the tip of the tail was cut. The first drop of blood was discarded, then 25 μl of blood from the tail was transferred to the capillary tube, capped and counted as above. Simultaneously, a sample of blood was withdrawn from the exposed vena cava, and 25 μl of the heparinized blood was pipetted into the capillary tube, capped and counted.

Macro-method. One ml of heparinized blood was diluted with 0.9% NaCl, and an aliquot was counted in a well-type scintillation counter. An aliquot of the diluted standard was also counted.

Hematocrits were obtained using a 1 ml Wintrobe hematocrit tube. The tubes were centrifuged at 2,000 rpm for 30 minutes, and the hematocrit was read directly from the tube.

The remaining blood sample was centrifuged at 2,000 rpm for 15 minutes. One ml of the plasma was diluted with 0.9% NaCl, and an aliquot was removed for counting.

All of the values for each micro determination were obtained from a single 25 μl sample of blood from each animal. On the other hand, 3 separate aliquots of blood totaling approximately 5 ml from each animal were used to obtain the complete data reported for the macro-method.

The formula used for calculating blood or plasma volume was:

$$\text{Total volume} = \frac{\text{cpm/ml of diluted Std.} \times \text{dilution of Std.}}{\text{cpm/ml of blood or plasma}}.$$

The blood volume was calculated without considering the unequal distribution of cells throughout the circulation(5).

Results. Table I presents the normal values for total blood volume, plasma volume and hematocrit in the mouse, hamster, rat, guinea pig and rabbit. Normal values were obtained by both the macro- and micro-methods in the rat, guinea pig and rabbit. Good agree-

ment exists between the micro values and the corresponding macro values in the same group of animals.

When blood samples were taken from a central site (the inferior vena cava) or from a peripheral site (the tip of the tail), no difference in the values for the foregoing parameters was noted in the rat. In 9 rats having an average body weight of 179 ± 3.08 g, a blood volume of 7.4 ± 0.03 ml/100 g of body weight was obtained when the blood was sampled from the vena cava. When the blood was taken from the tip of the tail in these animals, the blood volume obtained was 7.1 ± 0.07 ml/100 g of body weight. Plasma volume was 4.5 ± 0.12 ml/100 g of body weight for the central site and 4.3 ± 0.05 ml/100 g of body weight for peripheral blood. Hematocrit values were 38.4 ± 0.56% from the central site and 39.5 ± 0.55% from the peripheral site.

Discussion. A number of procedures using radioisotopes for determination of blood and plasma volume in laboratory animals have been reported(6-9). However, these methods have either been tedious to perform(6,8) or have required moderately large samples of blood. Some(7,9) required determining hematocrits in a different series of animals. In the present method, only a single 25 μl sample of blood is required for determination of blood volume, plasma volume and hematocrit. This is of considerable advantage, particularly if one does not wish to sacrifice the animal. These determinations require less than 15 minutes total to perform. Repeated samplings of blood from a peripheral site, such as the ear or tail of an animal, can be performed without anesthesia. Removal of this small volume of blood does not alter the blood volume of the animal nor does this small amount of blood loss affect body fluid shifts.

Values for blood volume, plasma volume and hematocrit obtained by the micro-method in the rat, guinea pig and rabbit agreed with these parameters using the macro-method when conducted simultaneously in the respective animals. In the mouse and hamster, it was not possible to obtain comparable values by the macro-method due to the small amount of blood obtainable from the vena cava.

In general, values obtained in these studies agreed with those reported by others for the appropriate species of animals(7-9). However, Wish *et al*(10), using I^{131}-labeled plasma, reported an average plasma volume in the mouse of 5.1 ml/100 g of body weight, which is considerably lower than the average plasma volume of 8.8 ml/100 g of body weight found in the present studies. Kaliss and Pressman(7), using I^{131}-bovine serum albumin, obtained the following average values in the mouse: blood volume, 12.1 ml/100 g body weight; plasma volume, 6.7 ml/100 g body weight; and hematocrit, 44.6%. Although the value for the blood volume in the mouse is in close agreement with that found in the present studies, their value for the hematocrit is considerably higher, hence the plasma volume is lower than that found in the studies above.

Summary. A micro-method of blood volume determination using commercially available I^{131}-human serum albumin has been described. From a single sample of 25 μl of blood withdrawn from a peripheral site, it is possible to obtain total blood volume, plasma volume and hematocrit in not more than 15 minutes. Normal values are presented for these parameters in the mouse, hamster, rat, guinea pig and rabbit. The normal value for total blood volume by this method was 7.0 to 8.3 ml/100 g of body weight in the rat, hamster, guinea pig and rabbit. A value of 13.3 ml/100 g of body weight was found for the mouse. The plasma volume for all the animal species tested was 3.9 to 5.0 ml/100 g of body weight, with the exception of the mouse. The plasma volume found in the mouse was 8.8 ml/100 g of body weight. There was good agreement of these results when compared to the macro-method which requires a minimum of 2 ml of blood.

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Microsomal Lipids of Various Tissues in the Rat.* (28782)

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Considerable data indicates the functional importance of lipid components of protoplasm in normal, physiological activities of cells and tissues (Fleischer and Klounen(1); Gray and MacFarlane(2); Johnson and Albert(3)). Believing that the fundamental lesion involved in the malfunction of organs and tissues must lie within the functional mechanisms of constituent cells, it is pertinent that a base line of lipid constituents in normal cells must be accurately defined by quantitative analysis.

The lipid composition of rat liver cell sap has been described by Getz(4). MacFarlane *et al*(5) have described the fatty acids of phospholipids from mitochondria and microsomes of rat liver. A study similar to that herein described has recently been published by Getz *et al*(6).

Materials and methods. Sprague-Dawley rats 300-400 g fed standard purina laboratory chow were fasted for 24 hours and sacrificed by decapitation. Liver was perfused *via* the dorsal aorta with Locke's solution and homogenized in cold, 0.25 M sucrose(7).

Cell debris, mitochondria and nuclei were separated from soluble phase proteins and microsomes by low temperature centrifugation at 11,000 × *g* for 15 minutes. Fatty layer on the surface of supernatant was aspirated and treated separately. Microsomes were isolated by centrifugation of remaining supernatant in the Spinco "L" at 105,000 × *g* for 1 hour, washed 3 times with 0.25 M sucrose, lyophilized and subsequently extracted.

Microsomes were extracted overnight in chloroform-methanol (2:1 v/v) and washed according to Folch *et al*(8). Total lipid/extract was determined gravimetrically and a sample (300-400 mg) diluted in 10 ml of hexane, placed on a 16 gram silicic acid (Unisil 200-325 mesh, Clarkson Chem. Co., Williamsport, Pa.) column apparatus described by Hirsh and Ahrens(9). Lipid classes were eluted in accordance with a scheme devised by E. C. Horning, modified by D. A. Turner, Nat. Inst. Health, Sinai Hospital, Baltimore, Md., shown in Table I. Only data on lipid classes and fatty acid constituents of liver microsomes is here reported. All solvents analytical reagent grade. Hexane was redistilled.

Eluate was monitored by phosphomolybdic acid, spot test and fractions pooled accordingly. Purity of fractions collected by column chromatography was checked in the following way. Each pooled fraction was analyzed quantitatively for cholesterol, ester linkages, glycerol and phosphorus by methods of Hanel and Dam(10), Snyder and Stephens(11), Lambert and Neish(12), and Bartlett(13) respectively. Di-, mono- and triglycerides were determined by calculation of glycerol ester ratios. Following purity check of all fractions, total mass of each was determined gravimetrically. Aliquots of each

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