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Blood Volume in Relation to Body Weight of the Male Rat Using Radio-Iodinated Serum Albumin.* (29915)

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An accurate means of estimating total blood volume in the rat is useful in laboratory investigation. This report is the result of blood volume determinations in 122 normal male albino rats of varying body size. The method described is simple and the results provide a constant means of estimating total blood volume in relation to body weight.

Method. One hundred twenty-two white male rats ranging in weight from 140 to 294 g were used. The animals were anesthetized with 2% sodium pentobarbital given intraperitoneally in dosage of 0.2 cc per 100 g body weight and the femoral artery was then cannulated with a no. 50 polyethylene catheter. Radio-iodinated serum albumin (RISA) was used to determine blood volume. Approximately 1.0 microcurie of RISA in a volume of 0.25 to 0.5 cc was injected into the femoral artery, then the cannula was flushed with 0.4 to 0.5 cc of heparinized saline to insure complete injection of RISA. Initially a few animals received the RISA intravenously, but it was soon found that intraarterial injection gave equal values and satisfactory curves. Arterial injections eliminated the additional step of cannulating a vein. Samples of blood of 0.2 cc each were obtained from the femoral artery at 5, 10 and 15 minute intervals following injection. Before each sample was collected the arterial cannula was drained of its heparinized saline and after each collection the tube was flushed with 0.2 cc of saline. The blood samples were pipetted into test tubes containing 1 cc of saline and counted in a well-type scintillation counter. Standards for each determination were prepared by injecting from the same syringe used in the rat, an equal amount of RISA in a dilution of 100 ml into a volumetric flask. After thorough mixing of this standard, 2 cc was pipetted into a test tube and this was counted in the well counter. The counts per minute of the 3 blood samples were plotted on linear graph paper. The curve was extrapolated to zero time and this zero

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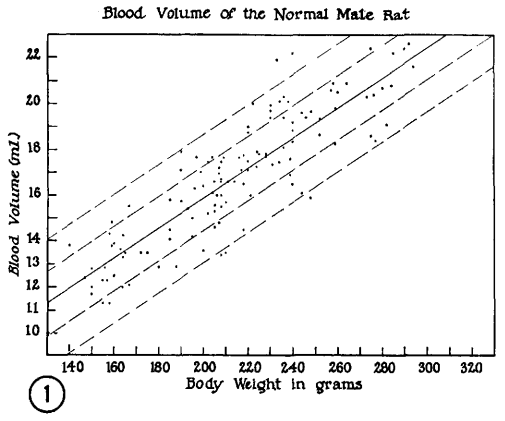

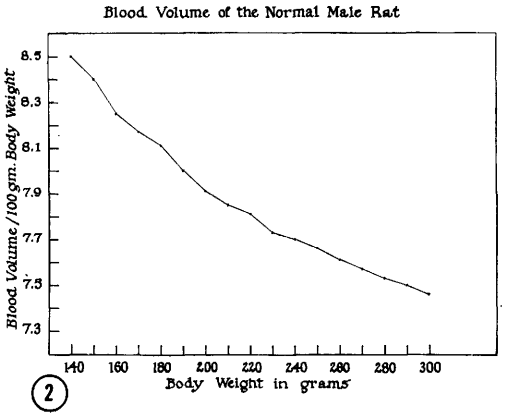

FIG. 1 and 2

value was used in the calculation. Blood volume was calculated from the following formula:

Total Blood Volume in cc = (C/M Standard × 10)/(C/M Blood Sample).

Data of blood volume and body weight with the volume as ordinate and the weight as abscissa are shown in Fig. 1. A standard regression line was derived from the data. Of 122 determinations all but 7 fell within 2 standard deviations from the mean regression. Fig. 2 shows the relationship of blood volume/100 g body weight as a function of body weight. This figure demonstrates that as weight increases the blood volume/100 g body weight slowly decreases.

Discussion. Many different methods have been used to estimate total blood volume of the rat. Jolly and Stini(1) tried complete exsanguination as did Chisholm(2). Later attempts centered on using some substance such as vital red or Evans blue and performing a dilution analysis. These earlier works present a wide range of values for the blood volume. Reeve(3) stated that total blood volume can be accurately found only by summation of the cell volume and the plasma volume individually determined.

Recent work has been more precise, but still the majority of investigations are aimed at finding a mean blood volume per unit body weight and applying this value to all weights. Huang and Bondurant(4), using Evans blue for plasma volume and P^{32} for RBC volume, found an average blood volume of 5.75 ml/100 g body weight in 35 rats with an average weight of 395 g. They obtained good correlation for plasma volume using Evans blue and RISA. Jorgensen, Voigt, and Jensen (5) found an average blood volume of 5.92 ml/100 g body weight in 31 rats weighing 177 to 318 g. Their range was 3.68 ml to 8.24 ml/100 g. Wang(6), using Evans blue and P^{32}, found a mean total blood volume in 11 rats of 16.26 cc. Anthony and Kreider (7) using T 1824 found a mean total blood volume of 63 ml/kg body weight. Hsu and Kawin(8) using RISA found a mean total blood volume of 6.82 ± .72 ml/100 g body weight. The above studies were not correlated directly with body weight and their data were expressed as ratios/100 g.

Other studies utilizing a variety of methods have been undertaken to determine the ratio of blood volume to body weight. Belcher and Harriss(9) measured plasma volume with Evans blue and red cell volume with Fe^{59} and found that with increasing weight the plasma volume decreases per 100 g body weight while the RBC volume remains stable. Garcia(10), using Fe^{59}, showed that the volume per unit body weight showed a continuous fall with increasing body weight from 7.2 ml/100 g body weight in the newborn to a plateau of 4.4 ml/100 g body weight after 200 days of age. With a modified CO-method, Tribukait(11) showed that blood volume decreased from 8.5 to 6.7 ml/100 g body weight with increasing age and weight. The present study is more in agreement with the latter studies, although our range of values is much narrower than others reported. We found that although the blood volume per unit falls with

increasing weight this fall is not as great as other studies have shown.

Because of some difference in whole body and large vessel hematocrit the most accurate method of determining total blood volume is separately measuring plasma and red cell volumes, but this requires two different labeling substances to be diluted. The methods using hematocrit and dilution analysis of either plasma or cell volume are subject to the criticism of inaccurate hematocrit and trapped plasma values. Our method circumvents this latter objection with sacrifice of only a minor degree of accuracy. Funkhouser *et al*(12) measured blood volumes in humans both by plasma and red cell labels and their data show that blood volume determined by addition of the plasma volume and red cell volume gave a value which was regularly only 5% lower than values obtained using only RISA and counting whole blood. Thus with only minimal loss of accuracy great simplicity of determinations is achieved.

From a comparison of Fig. 1 and 2 it is apparent that as body weight increases in the rat, as in man, blood volume expands more slowly. Thus in experiments wherein corrections for body weight are important, ratios may give false interpretations of data since blood volume/body weight ratio is not a proportional function. Greater accuracy will be obtained by plotting blood volume *vs* body weight which allows a direct reading of the expected blood volume at any given body weight.

Summary. Total blood volume was determined in 122 male rats using RISA. The values were related to body weight and a least squares regression line was plotted. This regression line enables one to predict total blood volume for any given body weight. The method utilizing RISA and whole blood counting has the advantage of simplicity with minimal loss of accuracy.

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Microcirculatory Effects of Cortisol. Protective Action Against Na_4EDTA Damage.* (29916)

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For the most part, the biological effectiveness of the adrenal corticosteroids has been ascribed either to changes in carbohydrate and protein metabolism, electrolytes, or to some direct influence on cell membranes. These effects become manifest after some hours or even days following administration of the steroid. In contrast, the anti-inflammatory action of corticoids develops quickly.

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