A Rapid, Simplified Method for Serial Blood Volume Determinations in the Rat.* (20878)

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The extensive use of the rat in experimentation has emphasized the value of having a rapid, simple method of blood volume determination. The desirability of such a procedure was recognized as early as 1905 by Jolly and Stini(1) who employed the Welker Method. Scott and Barcroft(2) in 1924 developed a technic using carbon monoxide. The application of these methods was limited, however, because they required the sacrificing of the animal. Cartland and Koch(3) in 1928 modified the Keith-Rowntree plasma dye method(4) for use in the rat and made possible, for the first time, repeated observations in the same animal. In the light of subsequent advancements, however, their procedure has now become unnecessarily tedious and involved. Further modifications have therefore been undertaken and a much simplified version using Evans blue dye and the spectrophotometer is presented here.

Method. After withholding food, but not water, for the preceding 12 hours, the rat is lightly anesthetized, weighed and fastened to a ratboard. Using a 22-gauge needle and a calibrated syringe, 0.9 cc of blood for the standard sample is withdrawn by means of cardiac puncture. The needle is left in place and exactly 0.1 cc of 0.5% Evans blue dye is injected directly into the ventricle. The syringe is rinsed until all of the dye is removed, and the gentle flushing is continued over a 2-minute period to prevent clotting. At the end of that time, a second 0.9 cc sample of blood containing the dye is withdrawn. Each specimen is placed in an appropriately numbered Wintrobe hematocrit tube and centrifuged at 3000 RPM for 30 minutes. Dry heparin is used in both syringes and tubes to prevent clotting. Both hematocrits are recorded, and exactly 0.3 cc of plasma is withdrawn from each hematocrit tube. All of the syringes used for injecting dye and withdrawal of plasma and blood are previously calibrated with mercury. The dye free sample to be used for the standard is diluted to exactly 3.0 cc with a 1:1000 solution of the 0.5% Evans blue dye in normal saline. The unknown sample containing the injected dye is also diluted to exactly 3.0 cc with normal saline giving it a dilution factor of 10. With the wave length setting at 620 mμ, the respective densities are then determined using the Beckman spectrophotometer. The total blood volume and blood volume per 100 g of body weight are determined using the following formulae:

$$PV = \frac{D_1}{D_2} \times 10$$

PV = plasma vol; D_1 = density reading of standard; D_2 = density reading of plasma containing unknown amount of dye.

$$TBV = \frac{PV}{1 - (.96 \times Hct.)} + .8$$

TBV = total blood vol; PV = plasma vol; Hct = hematocrit.

$$BV/100\ g = \frac{TBV}{\frac{Wt\ of\ rat\ in\ g}{100}}$$

BV = blood vol; TBV = total blood vol.

Results. In the first 100 determinations, the blood volume ranged between 4.00 and 6.43 cc/100 g body weight. An example of the findings is given in Table I. The average value of 5.04 cc was significantly lower than the findings of Cartland and Koch(3) (6.9 cc/100 g), Orten *et al.*(5) (6.38 cc/100 g), and Went and Drinker(6) (7.4 cc/100 g) who used vital red dye, and Metcalf and Favour(7) (7.6 cc/100 g), and Beckwith and Chanutin(8) (7.98 cc/100 g) who used Evans

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TABLE I. Example of Results Obtained in Blood Volume Determinations on 10 Different Animals.

Wt, g	Hematocrit	D_1	D_2	Plasma vol, cc	TBV, cc	BV/100 g, cc
400	.460	.348	.252	12.45	23.10	5.77
405	.476	.363	.314	10.04	19.24	5.04
390	.480	.365	.294	11.20	21.40	5.49
366	.490	.312	.288	9.75	18.50	5.29
421	.500	.333	.263	11.40	21.90	5.39
416	.505	.360	.310	10.48	22.03	5.30
403	.554	.370	.336	9.94	22.10	5.55
326	.440	.497	.470	9.53	17.30	5.31
432	.515	.355	.282	11.35	23.23	5.36
468	.500	.344	.254	13.58	27.18	5.81

blue dye. It does, however, compare favorably with those found by Griffith and Campbell(9) (4.3 cc/100 g) who used vital red dye, and by Berlin and his associates(10) who used P^{32} (3.94-5.95; avg 4.59 $\pm$ 0.57 cc/100 g) and Fe^{59} (3.63-5.81 cc/100 g). Although those methods employing radioactive tracers are at present too intricate for mass application, they are probably the most accurate that are currently available.

The use of intracardiac puncture was not particularly hazardous. The mortality rate for the first 148 determinations was 4.05%. All of the animals were eventually sacrificed and at autopsy only a focal organized pericarditis was present over the injection site.

Discussion. Depending upon the individual needs, several satisfactory methods for determining the blood volume of the rat have been devised. When volumetric studies are required on large numbers of animals, however, they have proven to be too elaborate in their procedure and not readily applicable to mass production. With reasonable attention to detail, the method described above has been found to be relatively simple and accurate, and with planning as many as 15 animals can be processed during one determination period. It is also useful when serial determinations are required. An example of these findings is given in Table II.

TABLE II. Example of Comparative Results of Serial Determinations Obtained at Time Intervals Varying from 1 Week to 3 Months on 10 Different Animals.

Determinations		
1st	2nd	3rd
4.34	4.74	4.35
5.75	5.32	5.78
5.14	5.15	5.16
4.47	4.74	5.05
4.15	4.68	4.67
4.13	4.73	4.14
5.15	5.45	5.18
4.65	4.39	4.65
4.87	5.76	5.28
4.34	4.39	4.42

The usual sources of error native to this type of procedure were controlled in the following manner: 1) the 2-minute time interval between injection of the dye and withdrawal of the final sample was sufficient to permit complete mixing of the material without permitting significant extravascular loss; 2) as the absorption of hemoglobin is negligible in the maximum spectral absorption range of Evans blue dye (620 mμ), slight hemolysis was of no consequence and 3) the problem of lipemia was eliminated by diet.

Summary. A relatively rapid, simplified method for the determination of the blood volume in the rat using Evans blue dye and the spectrophotometer is described. It has been found to be satisfactory for both mass production and serial determinations. A comparison of the results with those obtained by other methods is given.

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