Blood Volume of Obese (*ob/ob*) and Diabetic (*db/db*) Mice (34462)

TERENCE T. T. YEN, JEAN STIENMETZ, AND PATRICK J. SIMPSON
(Introduced by I. S. Johnson)
The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206

The *in vivo* turnover of various substances in the obese mouse has been studied at great length. Since most investigators dosed their animals on a weight basis, each obese mouse generally received twice as much chemical as its normal control. In the course of our respirometric study of the utilization of various metabolites by the obese and diabetic mouse, we found that a dose based on weight gave results which did not agree with our *in vitro* observations (unpublished data). Realizing that it is not requisite for *in vivo* and *in vitro* experiments to be compatible, we nonetheless felt that the disparity of these results might be attributed to the administration of an inappropriate dose to the obese animals.

The excess weight of the obese mouse has been reported to be due entirely to increased fat content (1, 2). The active body mass of these mice in terms of body nitrogen is actually less than that of normals. Since adipose tissue is not highly vascular, it is clearly possible that the blood of the obese mouse may constitute an abnormal percentage of its total body weight. We, therefore, undertook a comparative study of the blood volume of the fat and normal mouse.

Materials and Methods. Male mice affected by mutations at either the obese (*ob*) locus (3) or the diabetes (*db*) locus (4) were supplied by the Jackson Laboratory. Four strain-genotype combinations were used in this study: C57BL/6J-obese (*ob/ob*) and normal (ob^+/ob^+) mice, and C57BL/KsJ-diabetic (*db/db*) and normal (db^+/db^+) mice. All mice were 3½ months of age and were given food and water *ad libitum*.

Chromate-^{51}Cr ($Na_2{}^{51}CrO_4$, New England Nuclear, act. 165 mCi/mg Cr)-labeled mouse blood was used in measuring the blood volume. The method was essentially that of Gordee and Simpson (5). The mouse blood was prepared as follows: 10 ml of heparinized blood from ob^+/ob^+ mice was centrifuged at 2000 rpm for 10 min. The cells were incubated for 1 hr at 37° in 4 ml of Strumia's solution containing 200 μCi of $Na_2{}^{51}CrO_4$, washed in 3 × 4 ml Strumia's solution, and then reconstituted with the refrigerated original plasma. Of the 22% of the added radioactivity which did not bind to the red blood cells, only 1.3% was present in the third wash.

Each mouse was weighed and injected intravenously with 0.2 ml of ^{51}Cr-labeled blood containing about 425,000 dpm. After a 5-min period for thorough mixing of blood, each mouse was anesthetized lightly with ether and bled by severing the axillary brachial vessels. Blood was collected from a pool in the cavity formed by the reflected thoracic skin and the chest wall. With a Nuclear Chicago Model 4216 automatic Gamma-Spectrometer at 2.5% efficiency, radioactivity was determined on 0.2 ml of blood. Total blood volume was calculated from the isotope dilution. Three microhematocrit determinations were performed on each mouse.

Results and Discussion. The volume of the whole blood is presented in Table I. Body surface area as well as body weight of these mice is included in the table, since the former represents a third parameter on which dose is sometimes based (6). The normal mice of the two substrains of C57BL showed no significant differences in body weight, surface area, or blood volume. Our value of about 8 ml blood per 100 g body weight for a normal mouse was in the upper range of the blood volume values reported in the literature (7), agreeing quite well with the data reported by Gordee and Simpson (5), Friedman (8),

TABLE I. Blood Volume of Obese and Diabetic Mice.

Genotype	N	Body weight (g)	Surface area (cm^2)[a]	Blood vol (ml)[b]	Hematocrit (%)
ob⁺/*ob*⁺	5	25.7 ± 0.9[c]	78.4 ± 1.8	2.04 ± 0.08	46.2 ± 0.66
ob/*ob*	6	45.9 ± 1.8	114.6 ± 3.8	2.46 ± 0.12	50.0 ± 0.90
% of *ob*⁺/*ob*⁺		179	148	121	
db⁺/*db*⁺	5	25.4 ± 0.4	77.8 ± 0.9	2.10 ± 0.07	47.4 ± 0.70
db/*db*	5	49.3 ± 2.9	121.0 ± 4.9	2.66 ± 0.18	49.2 ± 0.40
% of *db*⁺/*db*⁺		194	155	127	

[a] Surface area calculated according to Benedict's formula (6).

[b] The blood volume calculations included no correction for differences in the hematocrit value of venous and average body blood, since the 0.2-ml sample was taken from a well-mixed pool of at least 1 ml blood obtained by severing both the branchial vein and artery.

[c] Mean ± standard error.

Storey *et al.* (9), and Wish *et al.* (10).

The obese and diabetic mice were quite similar to each other with respect to body weight, surface area, and blood volume. Their blood volume to weight and blood volume to surface area ratios were quite unlike those of normal mice. Although the mutants were nearly twice as heavy and had 50% greater surface area than their controls, their blood volume was only about 25% greater. Conceivably, either type of fat mouse dosed according to weight would receive enough compound to make its initial blood level 150% that of a similarly dosed normal control. Likewise, dosing on a surface area basis would result in a mutant with an initial blood level 125% as high as its normal control. Our findings differ from those of Mayer and Hagman (11), who found the blood volume to be constant over a weight range of 20–80 g.

The best way to determine dosage levels in metabolic studies is to measure the pool size of the substance of interest and dose accordingly. Since this is often not practical because of the technical difficulties involved in labeling pools of different sizes equally, we would suggest the possibility of dosing the animal on a blood volume basis in hopes of creating a specific blood level of the compound being studied. Such an approach is especially helpful in comparing animals which differ greatly in size. It is apparent that the blood levels would not necessarily be equal if the lean and fat animals were dosed on a body-weight basis. The results of this study indicate that *in vivo* doses for obese or diabetic mice might be adjusted more effectively according to blood volume rather than body weight or surface area.

Summary. Mouse blood labeled with ^{51}Cr was used to determine the blood volume of the obese and the diabetic mouse. While mutant mice were twice as heavy as normal controls, their blood volume was only 25% greater than normal. This finding suggests the possibility that blood volume rather than body weight should be used as the basis for *in vivo* dosage calculations.

The authors thank Dr. Robert S. Gordee for his valuable consultation and Mrs. Jean Pieper and her associates for radioactivity determinations.

1. Bates, M. W., Nauss, S. F., Hagman, N. C., and Mayer, J., Am. J. Physiol. **180**, 301 (1955).

2. Wrenshall, G. A., Andrus, S. B., and Mayer, J., Endocrinology **56**, 335 (1955).

3. Ingalls, A. M., Dickie, M. M., and Snell, G. D., J. Heredity **41**, 317 (1950).

4. Hummel, K. P., Dickie, M. M., and Coleman, D. L., Science **153**, 1127 (1966).

5. Gordee, R. S. and Simpson, P. J., J. Bacteriol. **94**, 6 (1967).

6. Benedict, F. G., Yale J. Biol. Med. **4**, 385 (1932).

7. Paxon, C. L. and Smith, L. H., Exptl. Hematol. **11**, 42 (1968).

8. Friedman, J. J., Am. J. Physiol. **196**, 420 (1959).

9. Storey, R. H., Wish, L., and Furth, J., Proc. Soc. Exptl. Biol. Med. **74**, 242 (1950).

10. Wish, L., Furth, J., and Storey, R. H., Proc. Soc. Exptl. Biol. Med. **74**, 644 (1950).

11. Mayer, J., and Hagman, N. C., Proc. Soc. Exptl. Biol. Med. **82**, 647 (1953).

Received July 9, 1969. P.S.E.B.M., 1970, Vol. 133.