16030

Blood, Plasma, and "Drawn Blood" Volumes in the Rat.

RICHARD W. LIPPMAN.* (Introduced by T. Addis.)

From the Department of Medicine, Stanford University School of Medicine, San Francisco, Calif.

The following simple method for determination of circulating blood and plasma volumes has been used in the normal albino rat. It can be applied usefully to control and experimental rats just before they are killed.

The rat was anesthetized with ether in a Mason jar. As soon as the animal ceased to move, the inferior vena cava was exposed rapidly by cutting the abdominal cavity widely open with scissors. Light anesthesia was continued by applying a small beaker containing ether-soaked cotton over the nose. A hemoglobin solution of known volume and concentration was injected slowly into the vena cava from a tuberculin syringe.† The volume was varied from 0.20 to 0.50 cc, depending upon the size of the rat, and the concentration varied from 7% to 8%. The needle was kept in place to prevent leakage for two minutes. It was then withdrawn and the animal was exsanguinated by severing the abdominal aorta. The first ml of blood was collected in a tube containing 2 mg of potassium oxalate, for a hematocrit determination by the Wintrobe method. The remaining blood was collected carefully, until all bleeding stopped, in an oiled centrifuge tube and allowed to clot. Under these conditions we had previously found that hemolysis did not occur.

The total "drawn blood' was recorded. The clotted blood was centrifuged and the serum separated for measurement of its oxyhemoglobin concentration by the method of Evelyn and Malloy.[1] From these data plasma and blood volumes were calculated and corrected by subtracting the volume of hemoglobin solution injected.

The procedure outlined was selected, in spite of known objections, because it happened to fit our experimental needs, and required simple analytical techniques with which we were fully familiar. Essentially, it is a dye method in which blood is drawn for measurements of concentration and hematocrit after a suitable mixing time. Preliminary experiments showed that a 2-minute mixing time gave the same results as longer mixing times. Since the interval was short and the dye was one that is essentially nondiffusible and relatively slowly metabolized, use of a mixing curve, such as that of Noble and Gregerson[2] seemed unnecessary and technically would be almost impossible to obtain.

It was also found that skill of the operator was of some importance in obtaining reproducible results. At first determinations will tend to be low, presumably due to shock resulting from slow and inexpert handling of the animals. However, with a reasonable amount of practice, relatively constant and repeatable results are obtained.

Results. Blood volumes (BV) and plasma volumes (PV) were determined in 125 rats (47 female, 78 male) ranging from 48 to 308 g. In 111 the "drawn blood' volume was recorded. As there did not appear to be any significant difference between the sexes, the results were pooled. Plots of PV and BV against body weight in grams (BW) and body surface in square centimeters (BS) do not produce a straight line, but when log BV and log PV are plotted against log BW (Fig. 1) the points fit a straight line reasonably well. From the data obtained, regression lines were

* The author wishes to make grateful acknowledgment of the assistance of Helen J. Ureen. This work was aided by a grant from the American Medical Association.

† Hemoglobin solution provided through the courtesy of Sharpe & Dohme, Inc., Philadelphia, Pa.

[1] Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.*, 1938, **126**, 655.

[2] Noble, R. P., and Gregerson, M. I., *J. Clin. Invest.*, 1946, **25**, 158.

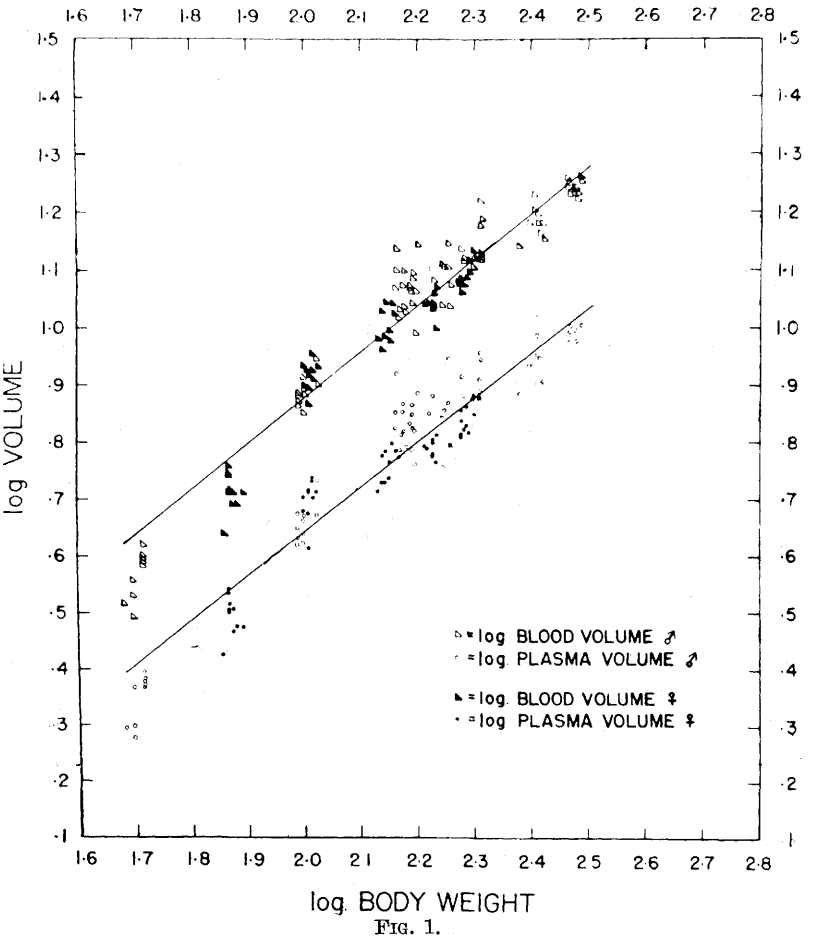

FIG. 1.

calculated, and the following formulae were established as applicable to the observed weight range:

$BV = 0.195\ BW^{0.794}$

$PV = 0.122\ BW^{0.778}$

Nearly all of the observations fell within a reasonably small zone on either side of the line, the values falling below the line at weights below 100 g. Although the relation of blood and plasma volume to a power of body weight was not perfect, it was a better fit than any simple alternative, and is presented for purposes of comparison with other investigators' results.

The general relationship of organ size to a power of body size has been established by Huxley,[3] who used body size from which the size of the organ has been subtracted. The use of total body weight is of considerably greater convenience and in the present instance, where the weight of the blood is a relatively small proportion of the total body weight, the difference introduced by such use was found to be insignificant. Failure of the regression line to fit more perfectly may be due to increased deposition of fat, a relatively avascular tissue, as the rats approach maturity and senescence.

Comparison of our results with those published previously is complicated by the various methods of presenting data. Table I summarizes in chronological order the results of several different observers and, for purposes of comparison, gives the blood and plasma volumes calculated by us for a rat of 150 g. In the references cited, body surface

[3] Huxley, J. S., *Problems of Relative Growth*, New York, The Dial Press, 1932.

TABLE I.

Author	Formulæ BV	Formulæ PV	150 g rat BV cc	150 g rat PV cc
Chisolm[5]	.099 $BW^{0.9}$		9.0	
Went and Drinker[6]	.074 BW		11.1	
Cutting and Cutter[7]		.014 BS		4.5
Griffith and Campbell[8]	.043 BW		6.5	
Beckwith and Chanutin[9]	.054 BS	.027 BS	17.2	8.7
Metcoff, Favour and Stare[10]	.047 BS	.028 BS	15.2	8.7
Lippman	.195 $BW^{0.794}$	.122 $BW^{0.778}$	10.4	6.0

BV is blood volume in cc, PV is plasma volume in cc, BW is body weight in grams, BS is body surface in sq.cm.

was assumed to have been calculated by the formula of Carman and Mitchell[4] where not otherwise stated.

The "drawn blood" volumes obtained by measurement have been plotted in Fig. 2 against the blood volumes determined by the hemoglobin method described. The curve obtained indicates that the "drawn blood" volume is related to actual blood volume, being somewhat below 50% of the actual blood

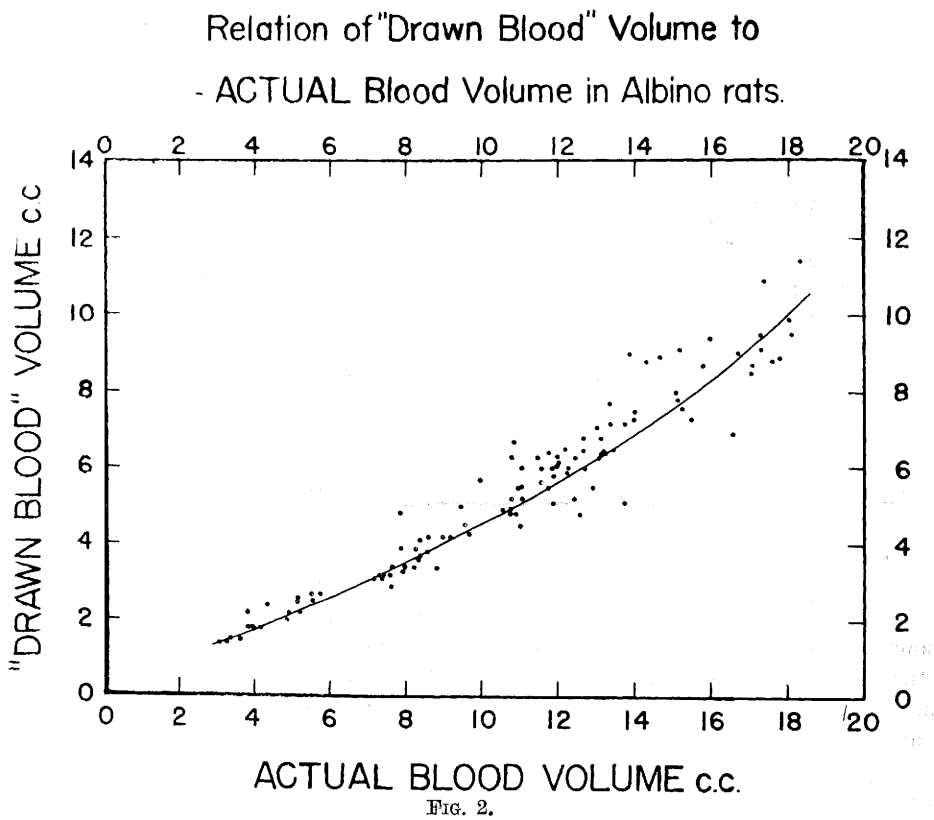

FIG. 2.

[4] Carman, G. G., and Mitchell, H. H., *Am. J. Physiol.*, 1926, **76**, 380.

[5] Chisolm, R. A., *Quart. J. Exp. Physiol.*, 1911, **4**, 207.

[6] Went, S., and Drinker, C. K., *Am. J. Physiol.*, 1929, **88**, 468.

[7] Cutting, W. C., and Cutter, R. D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1053.

[8] Griffith, J. P., Jr., and Campbell, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 38.

[9] Beckwith, J. R., and Chanutin, A., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 66.

[10] Metcoff, J., Favour, C. B., and Stare, F. J., *J. Clin. Invest.*, 1945, **24**, 82.

volume at weights below 100 g and somewhat above 50% at high weights. This confirms the impression acquired incidentally in this laboratory, in the process of accumulating data in numerous other rat experiments, that an indication of the direction of change in actual blood volume can be obtained by measuring the "drawn blood" volume.

Summary. 1. Blood and plasma volumes have been determined in the normal albino rat, and have been found to be approximately related to a power of body weight, the deviation being ascribed to deposition of avascular fat as the rat matures. There appeared to be no significant difference in this regard between the sexes.

2. An estimation of blood volume changes may be made from the "drawn blood" volumes.

16031

Effect of Chloroform on the Antitryptic Activity of Blood Plasma.

H. CROXATTO AND R. CROXATTO.

From the Laboratorio de Fisiología, Universidad Católica, Santiago, Chile.

It was shown in a previous paper[1] that treatment of plasma with 10% chloroform, decreased its hypertensinase activity instead of increasing it. This was a remarkable finding because: (a) hypertensin is a very sensitive reagent to test for proteolytic activity, and (b) the characteristic clotting and fibrinolytic properties of chloroform treated plasma have been attributed to an increased tryptic activity.

Previous experiments have also shown that hypertensin is an excellent substrate for the detection of antiproteolytic substances. Using hypertensin as a reagent, we have been able to show that plasma protects hypertensin against the destructive action of trypsin and to a lesser degree from that of chymotrypsin. On the contrary, the hypertensinase activity of crystalline carboxypeptidase and pure aminopeptidase (from yeast) remained unaffected (Croxatto and Croxatto[2]).

The degree of protection afforded by plasma, against the destruction of hypertensin by an appropriate dose of trypsin, may be used as a measure of its antitryptic activity. By using a technique based on this assumption, we have studied the effect of chloroform treatment on the antitryptic activity of plasma.

Experimental. Dog, ox, horse and human plasmas were treated with 10% chloroform, with the technique already described. Chloroform was separated by centrifuging and vacuum distillation, usually after 24 hours contact with the plasma. Aliquots of treated and untreated plasma were added to hypertensin (3 units) plus 0.1 ml of a solution of crystalline trypsin. A freshly prepared solution of one mg of trypsin* per milliliter of 0.9% sodium chloride was used. The amount of this solution used (0.1 ml) was enough to produce more than 80% destruction of hypertensin in 2 hours, with no plasma present.

The action of plasma on hypertensin, in absence of trypsin, was also tested. All experiments were carried out at pH 7.4, obtained by adding 0.2 ml of a solution of 0.02N sodium phosphate buffer, pH 7.4. The incubation period was of 2 hours at a temperature of 37°C.

The hypertensin activity present after incubation was determined by injecting the solutions into the femoral vein of a cat, under Dial anesthesia and recording with the

[1] Sainz, N., and Croxatto, H., *Bol. Soc. Biol.*, Santiago, Chile, **1944, 2, 261.**

[2] Croxatto, H., and Croxatto, R., *Bol. Soc. Biol.*, Santiago, Chile, in press.

* Plant's crystalline trypsin containing 85% of magnesium sulfate.