This value is constant in the same animal in measurements made 15 to 30 days apart and with different periods of equilibration. These figures are substantially less than the volume of distribution of thiocyanate, bromide and radioactive sodium (Table II).

The volume of distribution of inulin in man (Table III) ranges from 15 to 16% of body weight and is not increased if the equilibration time is prolonged from 6 to 20 hours. This equilibration time was confirmed in a completely anuric patient whose inulin space after a single injection became constant after 6 hours. As in the dog, the simultaneous thiocyanate space was substantially larger than the inulin space.

Summary. The volume of distribution of inulin has been determined by a priming dose and a constant intravenous infusion to insure uniform distribution throughout the extracellular space, followed by collection of the total inulin excreted in the urine after the infusion is discontinued. The inulin space in the dogs ranges from 20.9 to 23.2% of the body weight (average 21.6) and in man from 15 to 16%, as compared with larger spaces obtained with thiocyanate, bromide and sodium.

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A Comparative Study of Blood Volume in Dogs.

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Although Evans Blue Dye (T-1824) is the most commonly used method for the determination of blood volume, there has been much controversy concerning the accuracy of the values obtained. While Gregersen[1] and others who have used the method for a long time uphold its validity, Hempden *et al.*[2] recently have offered experimental evidence to indicate inherent sources of error. The following experiments were carried out as part of a survey for a more accurate and rapid method of obtaining plasma and blood volumes.

In order to utilize constituents of the circulating blood which are known to remain in the vascular bed for relatively long periods, and to obtain a high degree of sensitivity, it was decided to use red cells and plasma protein tagged with radioactive elements. The red cells were labelled with P^{32} and the protein with I^{131}. Fe^{59} or Fe^{55} would undoubtedly be superior to P^{32}, but phosphorus has the advantage of permitting *in vitro* labelling without the necessity of metabolic incorporation into the cell. The methods for these studies were those of Hevesy,[3] who first described the use of red cells tagged with P^{32} to study blood volume, and those of Fine and Seligman,[4] who first reported blood volume studies employing plasma iodinated with I^{131}. Minor modifications were introduced by us.

The radioactive isotopes were obtained from the Isotope Division of the Atomic Energy Commission at Oak Ridge. Radioactive phosphorus has a half life of 14.3 days and emits a beta particle with a peak energy of 1.69 M.E.V. Radioactive iodine has a half life of 8.0 days. It emits a beta particle of .60 M.E.V. and two gamma rays of .367 and .08 M.E.V.

Healthy, full grown dogs weighing 9-22 kg were used. The tests were carried out in the

1 Gregersen, M. I., *J. Lab. and Clin. Med.*, 1944, **29**, 12.

2 Hempden, L., *et al.*, *Am. J. Physiol.*, 1947, **151**, 282.

3 Hevesy, G., and Zerahn, K., *Act. Physiol. Scand.*, 1942, **4**, 376.

4 Fine, J., and Seligman, A. M., *J. Clin. Invest.*, 1943, **22**, 285.

morning after an overnight fast of approximately 12 hours. Each animal was anesthetized with intravenous sodium pentobarbital, .44 cc/kg. No other medication was administered. The external jugular veins were used for injections of all test material and withdrawal of all blood samples.

Plasma volumes determined after the injection of T-1824 and iodinated plasma were done on the same day. Two or 3 days later the volumes were determined using red cells tagged with P^{32}.

Methods. T-1824—A control 5 cc blood sample was withdrawn into a Wintrobe hematocrit tube containing powdered heparin. The needle was left *in situ* and 5 cc of T-1824 injected. Blood was aspirated and reinjected 3 times in order to wash out any dye which adhered to the wall of the syringe. Exactly 10 minutes after injection of the dye, a second blood sample was withdrawn from the jugular vein on the opposite side. The plasma volume was determined using the Evelyn colorimeter. The specific gravities of the whole blood and plasma were determined by the copper sulfate method. The hematocrits and plasma protein values were obtained by reference to the charts provided by Phillips and co-workers.[5]

Iodinated plasma. The protein fraction of human plasma was activated with I^{131} according to the method described by Fine and Seligman.[4]* Exactly 20 cc of iodinated plasma were injected. In the calculations no correction was made for the injected plasma. Eight cc of blood were collected 5, 10, 15, and 20 minutes after the injection. Each sample was then centrifuged for 15 minutes at 3500 rpm. The activity of 1 cc of the plasma was determined with a Geiger-Müller counter. When the measurements on plasma were made without drying or ashing, immediate determination of the plasma volume was possible. (The standard and the samples were also counted after drying with no significant difference in the results.) The activity of 1 cc of the original iodinated plasma, diluted 1:100 was used as a standard. All samples were assayed using a bell shaped Geiger-Müller tube with a 3.0 mg per sq cm mica window. A conventional scale of 64 circuit was used. The 1 cc samples were placed in metal dishes of such dimensions that the surface area of the liquid counted was approximately 3.5 sq cm. The samples were counted at a 5 cm distance from the mica window. Each sample was assayed for that length of time necessary to reduce the counting error to less than 2%. The plasma volume was calculated as follows:

$$\frac{\text{Counts in Standard/cc} \times \text{Volume Injected} \times \text{Dilution of Standard}}{\text{Counts in Sample /cc}} = \text{Plasma Volume}$$

Radioactive red cells. Approximately 50 cc of heparinized dog blood, containing 5 microcuries of P^{32} were placed in a paraffin lined flask, and incubated for 2 hours at 37°C. The mixture was gently agitated every 5-15 minutes. Hevesy[6] stated that these are the optimum conditions for maximum penetration of P^{32} into the red cell. At the end of the incubation period the blood was centrifuged at 2500 rpm for 15 minutes. The supernatant plasma was then decanted off. The packed red cells were washed once in normal saline and resuspended in sufficient normal plasma to restore the original volume. (In these experiments, a control blood sample was withdrawn from the animal for determination of residual activity from the iodinated plsama.) Then exactly 20 cc of the activated red cell

* Preparation of I^{131} plasma:

1. A solution containing 5 mg of KI plus the desired amount of carrier free I^{131} and 30% HNO_3 were rapidly distilled into 25 cc of cold redistilled CCl_4.

2. The CCl_4 solution of iodine was washed once with distilled H_2O and then added to 50 cc of chilled plasma which had previously been extracted with CCl_4. 10 cc of 25% Na_2CO_3 were added and the mixture shaken until the color disappeared.

3. The plasma was then separated by centrifugation and dialyzed for 48 hours with cold running water.

4. The precipitated globulin was centrifuged down and the supernatant fluid was ready for use.

5 Phillips, R. A., *et al.*, *Bull. U. S. Army Med. Dept.*, 1943, **71**, 66.

6 Hevesy, G., *et al.*, *Act. Med. Scand.*, 1943-44, **116**, 561.

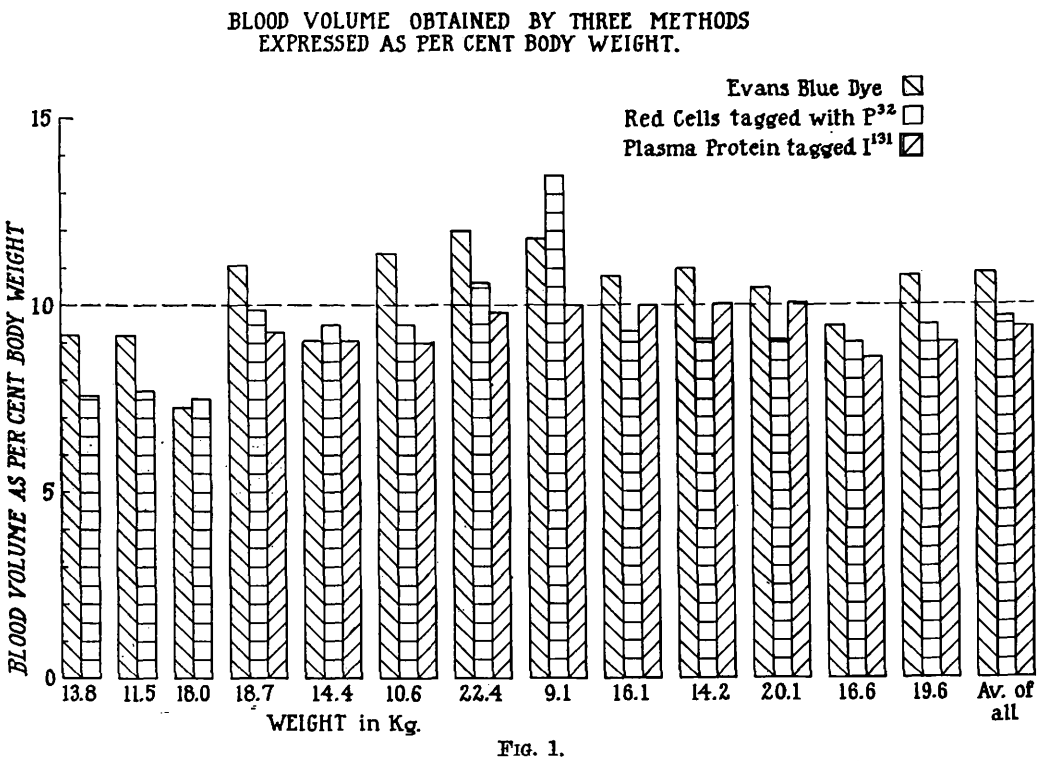

FIG. 1.
Blood volume obtained by 3 methods expressed as per cent body weight.

suspension were injected. Five cc of blood were collected in a heparinized syringe 5, 10, 15, and 20 minutes after the injection. One cc samples were then counted for activity without drying. An aliquot of the original suspension was diluted 1:100 with 5% hydrochloric acid, and 1 cc of this was used as the standard. The blood volume was calculated as follows:

$$\frac{\text{Counts in Standard/cc} \times \text{Volume Injected} \times \text{Dilution of Standard}}{\text{Counts in Sample/cc}} = \text{Blood Volume}$$

Discussion. Blood volume studies by all the 3 methods described above were done on each of 10 dogs. P^{32} and T-1824 volume determinations were completed on 4 additional dogs. Using the plasma volume determined by iodinated protein and T-1824, and the jugular hematocrit, the total blood volume was calculated for each method. With the red cells tagged with P^{32}, the dilution figures alone provided the total blood volume. In Fig. 1 the total blood volumes are shown in terms of per cent of body weight. From this chart it is seen that in most of the animals, the blood volume with T-1824 was larger than that obtained with the other 2 methods. The average blood volume (expressed as per cent of body weight) for the 10 animals using T-1824 was 10.5%; with P^{32}, 9.7%; and I^{131}, 9.4%.

The values obtained were all calculated using data from the 10 minute sample. After 10 minutes, T-1824 determinations err on the high side because of "dye leakage" from the vascular system. With the labelled red cell method, a sample taken earlier than 5 minutes does not allow adequate time for mixing, while after 10 minutes there is a "leakage" of P^{32} into the plasma and tissue fluids (Hevesy[3]). When iodinated protein is used, the blood samples can be drawn from 10 to 25 minutes following the injection with no evidence of significant decrease of iodinated protein in the plasma and thus no apparent change in the blood volume.

In Fig. 2 are examples of the variations in

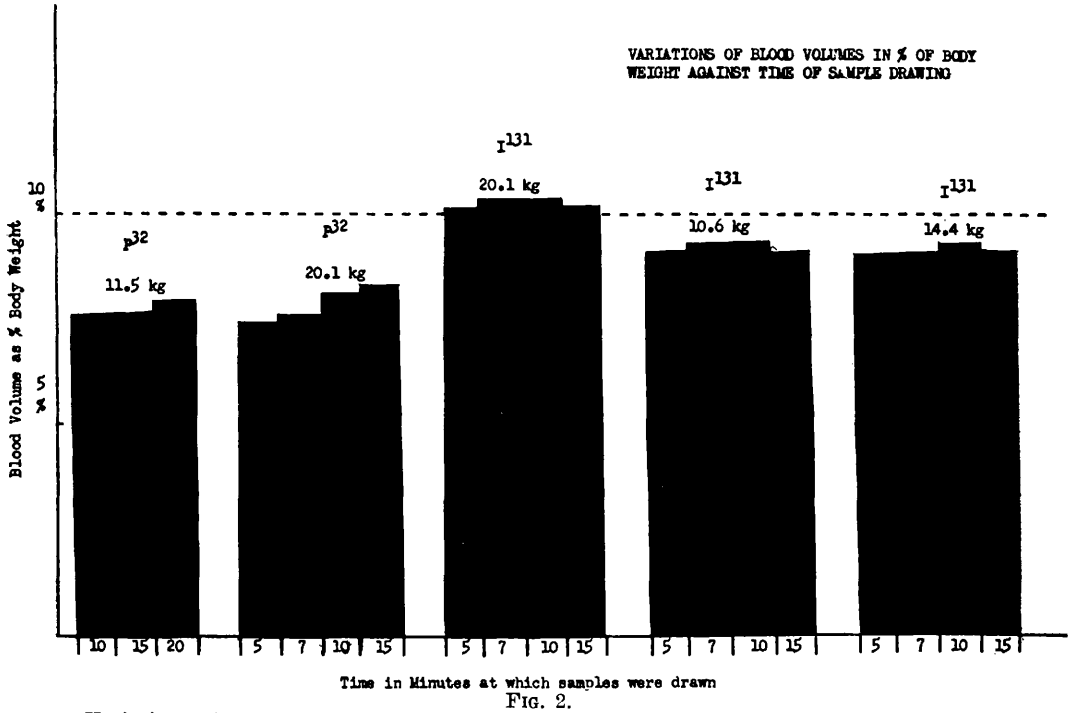

FIG. 2.
Variations of blood volumes in per cent of body weight against time of sample drawing.

blood volume (expressed as per cent body weight) with samples drawn at different time intervals. With the P^{32} method, an increase in volume is noted after 10 minutes, while with iodinated protein the blood volume remains more or less constant. All the animals were tested *in vivo* with a gamma ray counter for radioactivity over the thyroid gland without evidence of selective uptake of iodine.

Since plasma volumes determined with iodinated protein remain constant for a comparatively long period, this may be a method for studying rapid changes in effective circulating blood volume which occur under various circumstances such as vasodilatation and vasoconstriction after administration of drugs or during the course of surgical procedures.

The blood volume with red cells tagged with P^{32} is actually a measure of the dilution of red cells in the vascular system. If all the red cells under normal conditions are assumed to be in active circulation, the circulating total red cell volume can be calculated as the product of the (P^{32}) volume and the jugular hematocrit. In Fig. 3 the total cell volumes have been calculated. The total blood volume may be calculated as the sum of total cell volume and the plasma volume. Table I shows a comparison of blood volumes when the calculated cell volume is added to the plasma volume determined by T-1824, and to plasma volume determined by iodinated protein. In Fig. 3, the blood volumes (expressed as per cent body weight) are compared when (a) cell volume determined by P^{32} is added to plasma volume estimated by I^{131}, (b) plasma volume obtained with I^{131} and jugular hematocrit, (c) plasma volume obtained with T-1824 and jugular hematocrit. The volumes by the first two methods are of similar magnitudes.

Conclusions. 1. A comparison of 3 methods for the determination of blood volume was made on 10 dogs. 2. The average blood volumes expressed as per cent of body weight for the 3 methods were: T-1824, 10.5%; for red cells tagged with P^{32}, 9.7%; and iodinated protein, 9.4%. 3. The iodinated protein method permitted accurate determinations of plasma volumes for a much longer period after injection than did the other two methods. This method was simple and direct. It per-

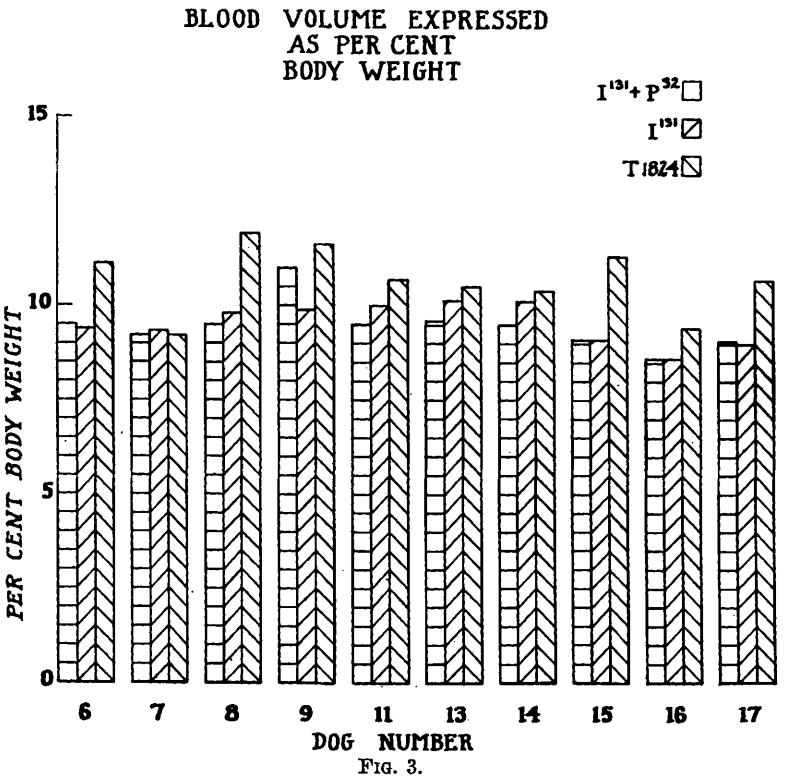

FIG. 3.
Blood volume expressed as per cent body weight.

TABLE I.
Comparison of Blood Volume Using Plasma Volume Obtained with T-1824 and I^{131}.

Dog	Wt, kg	Cell vol. P^{32} cc	Plasma vol. T-1824 cc	Blood vol. cc	Plasma vol. I^{131} cc	Blood vol. cc
6	18.7	801	1070	1871	986	1787
7	14.4	515	805	1320	811	1326
8	22.4	1015	1163	2178	1114	2129
9	9.1	590	534	1124	450	1040
11	16.1	668	928	1596	869	1537
13	14.2	550	911	1461	806	1356
14	20.1	908	1100	2008	1000	1908
15	10.6	415	648	1163	550	965
16	16.6	638	877	1515	795	1433
17	19.6	720	1296	2016	1056	1776

mitted calculation of the volume within a few minutes after the samples were obtained. It appeared to have a decided advantage over blood volume estimation with P^{32} impregnated red blood cells and the T-1824 dye method.

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