or the use of less isobutanol or replacement of phosphate by bicarbonate resulted in low heme protein content of the extract. When the extraction was carried out at 30-37°C for 1 hour no heme protein could be detected spectrophotometrically. This yellow extract had diaphorase activity. The fractionation of the extract on a larger scale and characterization of the components is now being attempted.

Summary. Washed rat liver particles were subjected to treatment with isobutanol in the presence of dilute solution of carboxymethylcellulose sodium salt and phosphate buffer at ice bath temperature. The resulting extract, after being freed from isobutanol, contained a water soluble catalytic system capable of transferring electrons from DPN • H_2 or TPN • H_2 to cytochrome C. The possible identity of a heme protein-like component, with cytochrome B and other enzyme properties of the extract (peroxidase, DPN-ase) are discussed.

I am deeply indebted to Dr. Brigit Vennesland for her continuous interest and criticism in this work. The generous gift of samples of DPN, TPN, alcoholdehydrogenase, Zwischenferment and cytochrome C from Dr. Eric Conn is greatly appreciated. My thanks are due to Dr. H. G. Williams-Ashman, who determined the enzymatic breakdown at DPN.

Received June 14, 1951. P.S.E.B.M., 1951, v77.

A Method for Determining Blood Volume of the Rat Using Radioactive Phosphorus.* (18808)

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With the advent of radioactive phosphorus, methods for the determination of the blood volume using the P^{32} labeled red cells have been worked out for a variety of mammals including man. The purpose of this paper is to present a method for measuring the blood volume of the rat using P^{32} labeled red cells, and to report the results obtained.

Method. The method used is a modification of the method employed by Reid and Orr(1) for human blood volume determinations using radioactive phosphorus labeled red cells. A normal adult ether-anesthetized rat is bled to death by either aortic or cardiac puncture and the blood heparinized and centrifuged. The serum and buffy coat are pipetted off, an equal volume of physiologic saline solution containing 12 μc of P^{32}† (2) is added and gently mixed. (Either carrier or carrier-free radioactive phosphorus may be used. If carrier P^{32} is used, care must be taken to prevent the K salt concentration from becoming too high, as hemolysis will result.) The cells are incubated for 45 minutes at 37°C; then are centrifuged and washed 3 times with normal saline. The final standard suspension of cells should be approximately equal to the original volume of blood—ordinarily about 8 ml. When the determination is to be made, the rat is weighed to the nearest half-gram and lightly anesthetized with ether. The jugular bulb is exposed and a half ml of the standard labeled cell suspension injected intravenously. (For this purpose we use 27 gauge needles and half ml tuberculin syringes.) Ten minutes is allowed to elapse and one-half ml of blood is withdrawn by cardiac puncture. This unknown rat sample is laked in 2 ml of tap water in a counting

* This work was done under U. S. Atomic Energy Commission Contract AT (30-1)-901 with the New England Deaconess Hospital.

1. Reid, A., and Orr, M. K., *J. Clin. Invest.*, 1950, v29, 313.

†The P^{32} used was supplied by the Oak Ridge National Laboratories, Oak Ridge, Tenn.

2. Cartland, G. F., and Kock, F. C., *Am. J. Physiol.*, 1928, v85, 540.

chamber cup.‡ One-half ml of the standard labeled cell suspension is diluted to 10 ml in physiologic saline. One-half ml of this diluted standard cell suspension is laked in 2 ml of tap water as the diluted standard sample. Both samples are evaporated to dryness at 95°C. The activity counts are then measured by a Geiger-Muller counter. The blood volume is calculated as follows: Example rat No. 7—wt 230.2 g. Background = 0.5 count per sec. Diluted standard sample = 8.7 counts per sec. (uncorrected). Corrected diluted standard sample = 8.2 counts per sec. per ½ ml. 20 x 8.2 = 164 counts per sec. per ½ ml = corrected standard sample. Corrected rat unknown sample = 5.7 counts per ½ ml per sec. $\frac{164}{5.7}$ = 28.8 ½ ml in blood volume. $\frac{28.8}{2}$ = 14.4 ml blood volume.

For the purpose of this experiment, the specific gravity of the blood is assumed to be 1—thus the percentage body weight = $\frac{14.4}{230.2}$ = 6% body weight.

This procedure may be repeated, as one does not need to sacrifice the animal. If a second determination is performed, a half ml sample of blood is obtained by cardiac puncture prior to giving the labeled red cells intravenously for the second time, and its count value subtracted from the sample obtained after the injection of the labeled cells—the calculation of the blood volume is otherwise the same.

Results. This study represents the blood volume determinations upon 34 normal adult white rats, seven of which were repeated 2 days after the first determination. The average blood volume for the whole series is 6.3% of body weight. The maximum variation in the repeated volumes is ±1.0% body weight, while the maximum variation from the average blood volume for the whole series is 1.7% body weight. The standard deviation is 0.7. 98% of the values fall within twice the standard deviation. The figures are given in Tables I and II.

Discussion. The results presented here agree with those previously presented in the literature by other workers using different methods. Their results are summarized in Table III. The radioactive red cell technic

‡ For this purpose we use screw cap bottle tops 1.7 cm diameter and 0.3 cm depth.

TABLE I.

Rat No.	Body wt, g	Blood vol, ml	% body wt
58	175	10	5.7
99	118.2	7.1	6
2	182.8	10.6	5.8
8	137.2	8	5.85
3	232	16.3	7
1	262.2	16	6.1
2	267.1	12.5	4.6
4	271.1	13.45	4.95
5	207.8	14.05	6.75
6	210.9	12.5	5.7
54	328.3	19.7	6
35	405.6	26.2	6.5
59	330.7	22.4	6.8
55	287.7	15.8	5.45
54	236.4	16.1	6.85
66	262.9	19.3	7.1
44	258	19.3	7.4
52	279.2	18.65	6.7
57	271.2	18.85	6.95
58	295.3	21.3	7.1
7	230.2	14.4	6
8	212.2	14.9	7
9	291.2	14.4	4.95
10	245.9	15.45	6.25
11	293.2	18.7	6
12	275.8	20.5	7.4
13	210.1	14.4	6.85
49	247.4	11.4	4.6
50	185.5	13.25	7.15
54	245	16.05	6.55
56	215.4	14.3	6.65
57	150	9.75	6.5
60	147.5	10.45	7.1
63	168	12.5	7.4
		Avg	6.3
		Max	7.4
		Min	4.6
		Std dev	.7

TABLE II.

Rat No.	Body wt, g	Initial blood vol, ml	Repeat blood vol, ml	Initial % body wt	Repeat % body wt
7	230.2	14.4	13.1	6.25	5.7
8	212.2	14.9	14	7.03	6.6
9	291.1	14.4	15.7	4.95	5.4
10	245.9	15.4	14.2	6.25	5.8
11	293.2	18.7	17.5	6	5.95
12	275.8	20.5	14	7.4	6.8
13	210.1	14.4	12.3	6.85	5.8

TABLE III.

Name	Method	Result
Cartland and Kock(2)	Dye	6 to 7.9% B.W.
Jolly and Stini(3)	Welcker	4 to 5 % B.W.
Chisolm(4)	Welcker	5.3 to 7.3% B.W.
Scott and Barcroft(5)	CO	5.4 to 7.9% B.W.
Griffith and Campbell(6)	Dye	4.1 to 5.3% B.W.
Cutting and Cutter(7)	"	4 to 5 % B.W.
Went and Drinker(8)	"	6.9 to 7.9% B.W.

for the measurement of the blood volume of the normal rat may thus be considered to be a reliable one.

Conclusions. 1. A method for the determination of blood volume of the rat by radio-isotope dilution is described. 2. In 34 normal adult white rats, average blood volume was 6.3% of body weight, with a stand. dev. of 0.7. 3. In 7 rats, the determination was repeated with a maximum error of ±1% body weight. 4. These results are in agreement with those reported in the literature using different methods.

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Received June 15, 1951. P.S.E.B.M., 1951, v77.

Human Ovarian Grafts onto the Chorio-Allantoic Membrane of Developing Chick Embryos.* (18809)

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Murphy(1) was the first to demonstrate that mammalian tissue (Jensen rat sarcoma) could live and grow actively on the chick embryo. Minoura(3) grafted ovary and testis from chick embryo and adult chickens onto the chorio-allantois of chick embryos. His conclusions indicated that these ovarian and testicular grafts remained functional and caused, in general, modification of the chick reproductive system in the direction of the type of gonadal graft. Oakley(4), studied grafts of liver from chicks, hens, and fetal rats on the chorio-allantois of chick embryos.

* The authors wish to express their appreciation to Dr. Marion Corrigan of the Dept. of Pathology, Michael Reese Hospital for her painstaking study of the microscopic sections and to Dr. Albert Milzer of the Dept. of Bacteriology and Virology, Michael Reese Hospital for his advice and generous use of his laboratory facilities. Mrs. Phyllis Adelman provided invaluable technical assistance.

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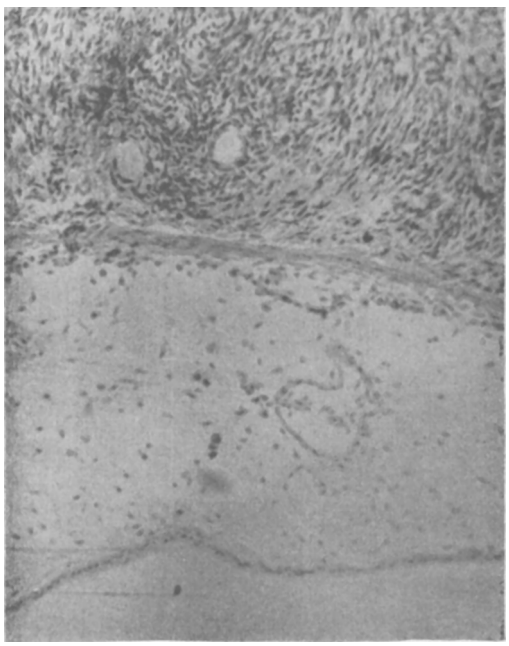

FIG. 1. Ovarian Graft, 4 Days, ×130. Follicular structures and ovarian stroma are identifiable. Contact is intimate. Vascularization absent.