times seem to warrant the conclusion that citrated plasma yields optimal values as compared with oxalated plasma. This, in conjunction with the aforementioned factors would make citrated plasma more suitable for the photoelectric determination of prothrombin time than oxalated plasma.

Summary. (1) In a series of 600 photoelectric determinations of the prothrombin time of 100 normal individuals, the mean value for citrated plasma was 12.6 seconds as compared to 13.8 seconds for oxalated plasma. (2) The standard deviation was 0.15 second for citrated as compared to 0.19 second for oxalated plasma. (3) The onset of clotting is more easily determined photoelectrically with citrated than with oxalated plasma. This is evidenced by a more distinct swing of the galvanometer lightbeam because no precipitate but a soluble complex of calcium citrate is formed upon addition of calcium chloride.

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Blood, Plasma and "Globulin" Space of Guinea Pigs Determined with I^{131} Rabbit Globulin. (19040)

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Iodine^{131} trace-iodinated-proteins have been used to determine vascular volumes in the dog(1), mouse(2) and man(3). In the course of studies of I^{131} trace-iodinated-rabbit antibody(4) it was necessary to determine the volume of guinea pig plasma in which this protein was diluted after intravenous administration. Since blood and plasma volumes have not been published for the guinea pig in the recent literature(5-7) except for a report of the plasma volume of 4 guinea pigs using T-1824(8), it was felt desirable to present the data.

TABLE I. Data on I^{131} Rabbit Globulin.

Amt protein nitrogen injected (mg)	12.63
" I^{131} injected (μc)	3.94
" protein bound iodine injected (μg)	200
No. iodine atoms per protein molecule	3.2
% activity not precipitable with trichloracetic acid	.51
Volume globulin injected (cc)	2.5

Methods. The globulin studied was obtained from immune rabbit serum and iodinated by a method described previously(9). Table I presents the composition and pertinent data of the preparation used in this study. The guinea pigs received the I^{131} rabbit globulin intravenously, and blood was obtained from the ear with a 20 mm^3 Sahli pipette at the times indicated in Table II. The blood was immediately delivered into an ointment tin sample dish lined with lens

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1. Krieger, H., Storaasli, J. P., Friedell, H. L., and Holden, W. D., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 511.

2. Wish, Leon, Furth, J. and Storey, R. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 644.

3. Crispell, K. R., Porter, B. and Nieset, R. T., *J. Clin. Invest.*, 1950, v29, 513.

4. Melcher, L. R., and Masouredis, S. P., *J. Immunol.*, In press.

5. Gardner, M. V., *J. Franklin Inst.*, 1947, v243, 498.

6. W. Junk. Ed., *Tabulae Biologicae, Band I Reine und physiologische Physik. Physikalische Chemie und biologische Anwendungen* p124, W. Junk, Berlin, 1925.

7. Went, S., and Drinker, C. K., *Am. J. Physiol.*, 1929, v88, 468.

8. Flexner, L. B., Gellhorn, A., and Merrell, M., *J. Biol. Chem.*, 1942, v144, 35.

9. Masouredis, S. P., Melcher, L. R., and Koblick, D. C., *J. Immunol.*, 1951, v66, 297.

TABLE II. 8-15 Min Dilution Vol of I^{131} Rabbit Globulin in the Guinea Pig, All Males.

Guinea pig No.	Wt, g	Time after inj for sample, min	Blood vol, cc/100 g body wt	Plasma vol, cc/100 g body wt
15	464	12	7.32	3.83
16	525	8	7.70	4.03
17	476*	9	7.28	3.81
18	479*	13	8.52	4.46
19	493*	11	7.32	3.83
20	490*	13	7.72	4.05
21	414	14	7.14	3.74
22	461	9	7.90	4.14
23	441	10	7.44	3.90
24	483*	13	6.72	3.52
25	505	12	6.70	3.51
26	543	15	9.24	4.84
27	529	8	6.92	3.62
Mean			7.53 ± .71†	3.94 ± .37†

* Only these animals were used for determination of the "globulin space."
† Stand. error of the mean.

paper, and the pipette washed with 1:10,000 heparin 2 to 3 times until free of blood, all washings being delivered into the sample dish. The samples were dried with an infra-red lamp and the beta activity determined with an end window Geiger-Mueller tube (about 2.0 mg/cm^2 mica window). No mass absorption correction was needed since the mass of the samples was less than 3×10^{-3} mg/cm^2. An aliquot of the total amount of radioactivity administered was counted simultaneously with the blood sample. The 8 to 15-minute dilution volume of the labelled foreign protein in whole blood was then determined. The plasma volume was calculated in terms of an average hematocrit of 47.6, which was obtained on 10 male guinea pigs weighing from 445 to 841 g. The hematocrits were determined on heparinized blood in a Wintrobe hematocrit tube which was centrifuged in an International clinical model centrifuge for 30 minutes at 3,800 rpm ($G_{max} = 1200$). No correction was employed for entrapped plasma.

Results. Table II presents the blood and plasma volumes per 100 g body weight in 13 guinea pigs. The blood volume was determined from the relationship,

$V = a/A - v$ where,

a = activity injected,

A = activity per unit vol. blood at time sample was obtained, and

v = vol. inj.

The 8 to 15-minute dilution volume in whole blood was found to be 7.53 ± 0.71 cc† per 100 g body weight, and the plasma volume was 3.94 ± 0.37 cc† per 100 g body weight. The direct experimentally determined value in these data is the blood volume, whereas the plasma volume is a derived value in terms of the hematocrit obtained in this experiment. Consequently the "true" plasma volume will differ from the value reported, depending on the deviation of the centrifuged hematocrit from the "true" hematocrit. Since there is a great deal of controversy concerning the correction of the centrifuged hematocrit(10,11) no attempt has been made to correct our centrifuged hematocrit.

Discussion. Fig. 1 is a plot of the per cent of the administered radioactivity remaining per ml serum as a function of time over a period of 5 days in 5 guinea pigs with an average weight of 480 g. Analysis of this curve reveals 2 principal components, an initial rapid decrease of activity followed by a slow single exponential rate of degradation of the rabbit globulin in the guinea pig (4). This interpretation is based on the fact that the radioactivity remaining in these animals, as obtained from excretion data, follows a single exponential function with about the same half life as the slower component in the serum activity-time curve. The initial rapid decrease of activity in the serum in the first 20 hours therefore represents either a dilution of the labelled foreign protein into a space larger than the 8 to 15-minute dilution volume or an initial pooling of the labelled protein in some tissue. Since the activity in the thyroids of animals sacrificed at 4 hours was less than 0.06% and the highest tissue concentration attained was 0.48% in the kidney(12), this initial rapid decrease in serum activity cannot be accounted for by I^{131} leaving the blood and

† Standard error of the mean.

10. McLain, P. L., and Rure, C. H. W., *Am. J. Physiol.*, 1949, v156, 12.

11. Chapin, M. A., and Ross, J. F., *Am. J. Physiol.*, 1942, v137, 447.

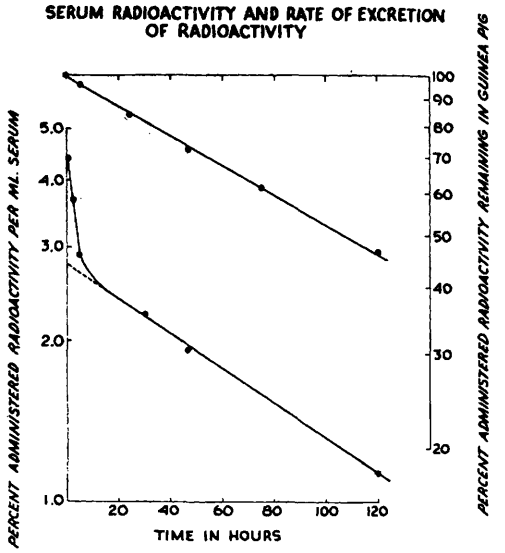

FIG. 1. Serum radioactivity and rate of excretion of radioactivity in 5 guinea pigs following intravenous administration of I^{131} trace iodinated rabbit globulin.

being pooled in the thyroid or in other tissues. Kreiger *et al.*(13) have shown that I^{131} labelled protein slowly enters the thoracic duct lymph and has correlated this initial rapid rate of disappearance of activity with the dilution of the labelled protein in the lymph space. In view of these findings extrapolation of the slower rate to zero time should yield the total volume into which the protein mixes. This calculation, based on a composite curve of 5 guinea pigs, yields a final protein dilution volume of 7.58 cc/100 g body weight, if the concentration of the foreign protein in this larger volume is the same as in the blood. The estimated value gives a minimum volume, since this volume is inversely proportional to the concentration of protein found in this fluid. The 8 to 15-minute dilution volume (peripheral plasma volume) therefore constitutes a maximum value of 59% of the total body fluid volume into which the protein is diluted. The "globulin space" is probably made up of an undetermined number of compartments, which include liver lymph, thoracic duct lymph and other extravascular globulin spaces, since the initial rapid decrease in the serum activity curve is not a single exponential rate.

Summary. (1) The blood and plasma volumes of guinea pigs were determined by the dilution of I^{131} trace iodinated rabbit globulin. (2) The average 8 to 15-minute dilution blood volume was found to be 7.53 $\pm$ 0.71 cc† per 100 g body weight, and the average plasma volume was 3.94 $\pm$ 0.37 cc† per 100 g body weight. (3) The labelled protein was found to mix slowly over a period of 20 hours into a larger body fluid volume which was found to have a minimum value of 7.58 cc/100 g body weight, which includes the peripheral plasma volume and a number of undetermined compartments.

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12. Mascuredis, S. P., *The Use of I^{131} Trace Iodination as a Technique for Labelling Proteins.* Thesis, University of California, 1951.

13. Kreiger, H., Holden, W. D., Hubay, C. A., Scott, M. W., Storaasli, J. P., and Friedell, H. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 124.

Shortening of Uterine Muscle at Different Temperatures. (19041)

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The experiments here reported show that the amount of shortening of uterine muscle when contracting *in vitro* is a function of temperature. There are no previous reports on this question as regards uterine muscle. On smooth muscle in general there is only one report(1), on the dog's retractor penis, and this deals with only a narrow temperature range (16°-35°C). The literature on the relation between temperature and shortening of

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1. Winton, F., *J. Physiol.*, 1927, v63, 28.