PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 92-97 (1976)

Plasma Volumes and Entrapment of Plasma in Tissues of Normal and Insulin-Immunized Guinea Pigs[1] (39487)

PETER H. WRIGHT[2] AND VIVIAN WILLIAMS

Department of Pharmacology, Indiana University School of Medicine, 1100 West Michigan Street, Indianapolis, Indiana 46202

After intravascular injection, a substance will disappear from the blood to accumulate or to be metabolized within one or more tissues or to be excreted from the body. If found within a given tissue after death, that substance could be situated within the cells or in the extracellular spaces of that tissue. When assessing the fate of insulin injected into guinea pigs it therefore became important to know the plasma volumes of the injected animals and the amounts of plasma trapped within the livers, kidneys, and spleens in which the hormone was found after death. The following is an account of a study in which 115 guinea pigs were injected with mixtures of radio-iodinated albumin and insulin. The fate of the albumin was followed for periods of up to 2 hr. The results show that previous estimates of plasma volume in the normal guinea pig were probably low and that significant amounts of plasma do remain trapped in the livers, kidneys, and spleen after death.

Materials and methods. Guinea pigs. Male albino guinea pigs (493–1445 g) were fed a standard diet *ad libitum* and had free access to water at all times. They included 67 animals which had received no previous treatment (normal) and 48 (immune) which had been immunized by a standard method (1) with bovine or porcine insulin.

Inocula. Each animal received an inoculum which contained a commercial preparation of ^{131}I-labeled human serum albumin (Albutope, E. R. Squibb and Sons, Inc., N.J.) with a specific activity of 20 to 40 μCi/mg of protein. This was added in a final concentration of about 10 μCi/ml to a buffered solution (3% NGPS; see below) which also contained commercially labeled ^{125}I-labeled porcine insulin (approx 10 μCi/ml) and normal or anti-insulin guinea pig serum (0.2–0.7 ml of serum/ml of inoculum).

Experimental procedure. Each animal was anesthetized with pentobarbital (25–50 mg/kg) and given a single subcutaneous injection of antihistamine (chlorphenyramine maleate injection, N.F.; 10 mg). Catheters (PE 90, id, 0.034 in.; Clay Adams, N.J.) were inserted in the external jugular vein and carotid artery, both being connected by way of three-way taps to a pump used to infuse heparinized saline (10 U/ml, sodium heparin injection, U.S.P.) at a steady rate (0.1 ml/min). After about 15 min, the inoculum (1.0 ml) was injected through the venous catheter, the volume being carefully measured and residual inoculum in the catheter being flushed into the animal with saline immediately after injection. From the arterial catheter a total of three to 11 measured samples of blood (0.8 or 1.0 ml) was drawn at timed intervals from 1 min to 2 hr after each injection. Care was taken to ensure that blood samples were not contaminated with saline from the catheters or taps and that plasma was rapidly separated by centrifugation. At the end of each experiment, blood was drawn from the arterial catheter (more than 25 ml) until all respirations ceased; a lethal dose of barbiturate was given if signs of life persisted. The abdomen was then opened and the liver, kidneys, and spleen were removed and weighed.

Assay of radioactive contents of inocula and tissues. Weighed portions of liver, kidney, and spleen (ca. 1.0 g) were placed in ice-cold distilled water (10 ml) and homogenized (Polytron, Kinematica GMBH, Lucerne, Switzerland). Aliquots of inocula and plasma were diluted in a neutral phosphate buffer (0.05 *M*, Na_2HPO_4/KH_2PO_4; pH 7.0) containing sodium chloride (0.4%, w/

[1] This study was supported by U.S. Public Health Service Grant AM-16534.

[2] Author to whom reprint requests should be addressed.

v) and normal guinea pig serum (3%, v/v); this solution is here termed 3% NGPS. Plasma (0.3 ml) was diluted in 3% NGPS (2.5 ml). Using a micropipet, (Eppendorf, Bio-Rad Laboratories, N.Y.) a small aliquot of inoculum (10 μl) was diluted in 3% NGPS (5.0 ml). Aliquots (0.5 ml) of these diluted samples of plasma and inoculum and of the well-mixed homogenates were placed in small culture tubes (10 × 75 mm) and most (3/4 or 6/8, 75%) of the replicate samples were treated with an equal volume of trichloracetic acid (0.5 ml; 20 TCA, w/v). The resultant precipitates, after centrifugation and aspiration of supernatent fluid, were washed once with more dilute acid (1.0 ml; 10% TCA, w/v). The radioactive contents of these washed precipitates (TCA-precipitable radioactivity) and of the untreated diluted samples of inocula, plasma, and homogenates (total radioactivity) were measured in an automatic gamma counter (Series 1195, Searle Analytic Inc., Des-Plaines, Ill.). All values for radioactivity (counts) quoted below refer to ^{131}I in TCA precipitates from inocula, plasma, and homogenates and accounted, in each case, for more than 95 to 97% of the total radioactive contents of these tissues. ^{125}I in the labeled insulin did not interfere with these estimates.

Calculations. Plasma volumes (PV) were calculated from the amount of injected TCA-precipitable radioactivity (C_i) and the estimated concentration of radioactivity in the plasma at zero time (C_p^0/v):

$$PV = C_i \times v/C_p^0, \quad (1)$$

where v is the volume of plasma (0.0536 ml) assayed for radioactive content at each time interval. The value of C_p^0 was determined by extrapolation to zero time of the line relating the radioactive contents of plasma samples (C_p^t) to the times (t, min) at which they were collected:

$$\log_{10} C_p^t = \log_{10} C_p^0 - bt, \quad (2)$$

where b is a regression coefficient.

The volume of distribution of injected albumin (AV) was calculated on the assumption that the only losses of albumin from the body were those due to successive bleedings. The amount lost at each such bleeding (C_{bl}) is given by the expression:

$$C_{bl} = (100 - \text{Hct})C_p^t \times V_{bl}/100v, \quad (3)$$

where V_{bl} is the volume of blood drawn (0.8 or 1.0 ml) and Hct is the hematocrit. At any time t after injection of the inoculum, the volume of distribution of the residual labeled albumin (AV^t), the albumin space, is

$$AV^t = (C_i - \sum C_{bl}) \times v/C_p^t \quad (4)$$

The hematocrit (Hct) was not measured at any time but was assumed to be 40%, a value somewhat less than the range usually quoted (41 to 48%) for normal guinea pigs (2–4) but reasonable for animals that were bled repeatedly over periods of up to 2 hr. For comparative purposes, the albumin spaces of individual animals are expressed as percentages of their plasma volumes (100 × AV^t/PV).

The plasma contents of individual tissues examined after death (PV_T) are given by the expression:

$$PV_T = \text{Total radioactive content of tissue} - \text{radioactive content of plasma}$$
$$= [C_H \times W(10 + w)/0.5 \times w]/[C_p^d/v], \quad (5)$$

where w is the weight of the portion of tissue homogenized in water (10 ml), W is the weight of the whole tissue, and C_H and C_p^d are the radioactive contents (respectively) of the aliquot (0.5 ml) of tissue homogenate and of the sample of plasma (volume, v) taken at the time of death. It was assumed, therefore, that all radioactive albumin found in a tissue is confined to the plasma.

Correlations and comparisons based on such data were carried out by methods described by Snedecor and Cochran (5).

Results. Plasma volumes. Of 112 determinations, 15 have been arbitrarily excluded from present consideration because in these instances the standard errors of estimates of $\log_{10} C_p^0$ [see Eq. (2)] exceeded 1.5% of the estimates themselves. Of the remainder, 60 were for normal and 37 for insulin-immunized guinea pigs.

The plasma volumes of normal and insulin-immunized guinea pigs increased with

total body weight but when related to unit body weight (ml/100 g) they decreased (Table I). Linear relationships were established in both groups of animals between plasma volume and body weight (Table II, lines 1 and 2) but in neither case did the line pass through the origin; this accounts for the decrease in the ratio of plasma volume to body weight as body weight increases (Table I). The slopes (b) of these two regression lines do not differ significantly ($P > 0.05$) but, as illustrated in Fig. 1, the elevation of the line for immune animals is significantly higher ($P < 0.01$) than that for normal guinea pigs.

Linear relationships were established for 18 normal and 18 insulin-immunized guinea pigs between albumin space and time for all samples of blood drawn more than 10 min after inoculum injection (Table II, lines 3 and 4); they do not differ significantly in any respect ($P > 0.05$). When the results for the two groups of animals are combined (Fig. 2), the albumin space is seen to increase

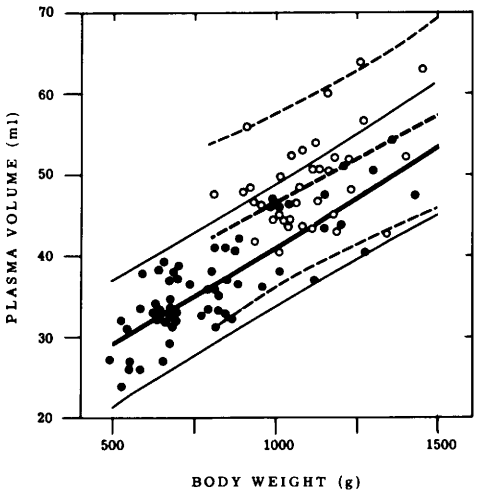

FIG. 1. Plasma volumes (ml) and body weights (g) of normal (closed circles) and insulin-immunized (open circles) guinea pigs. Also shown are the corresponding regression lines (heavy print) and limits of confidence (95%) for individual observations (light print) for normal (continuous lines) and immune (interrupted lines) animals.

TABLE I. PLASMA VOLUMES AND BODY WEIGHTS OF NORMAL AND INSULIN-IMMUNIZED GUINEA PIGS

GP[a] group	Normal[b]				Immune[b]			
			Plasma volume				Plasma volume	
	n	Body wt (g)	ml	ml/100 g	n	Body wt (g)	ml	ml/100 g
A	28	627 ± 12	32.5 ± 0.8	5.18 ± 0.10				
B	17	814 ± 10	36.1 ± 0.8	4.43 ± 0.09	2	854 ± 46	42.7 ± 5.2	4.97 ± 0.33
C	6	997 ± 11	43.3 ± 2.0	4.33 ± 0.18	18	1006 ± 13	46.6 ± 1.0	4.65 ± 0.12
D	7	1200 ± 25	44.7 ± 2.0	3.73 ± 0.15	14	1174 ± 14	51.2 ± 1.6	4.36 ± 0.12
E	2	1395 ± 35	50.8 ± 3.4	3.65 ± 0.33	3	1395 ± 32	52.6 ± 5.8	3.76 ± 0.33

[a] Animals are grouped (n) according to ranges of body weight in grams (A, 500-700; B, 701-900; C, 901-1100; D, 1101-1300; and E, over 1300).
[b] All quoted values refer to means (± SE).

TABLE II. LINEAR INTERRELATIONSHIPS FOR GUINEA PIGS INJECTED WITH ^{131}I-LABELED HUMAN SERUM ALBUMIN[a]

Line	Animal group	Ordinate (y)	Abscissa (x)	Observations (n)	Intercept (c)	Regression coefficient ($b \pm S_b$)	Correlation coefficient (r)
1	Normal	Plasma volume	Body weight	60	17.11	24.0984 ± 2.1468	0.8275
2	Immune	(ml)	(kg)	37	24.88	21.7165 ± 6.1127	0.5148
3	Normal	Albumin space	Time (min)	149	1.9951	0.00114 ± 0.00009	0.7201
4	Immune	(Log_{10}% of PV)		162	1.9955	0.00104 ± 0.00008	0.7358
5	All*	Liver weight	Body weight	87	6.96	22.3768 ± 1.3078	0.8803
6		Kidney weight	(kg)	87	0.80	4.4297 ± 0.3628	0.7980
7		Spleen weight (g)		82	0.50	0.6598 ± 0.1470	0.4485
8	All*	Liver plasma	Tissue weight	87	0.28	0.0972 ± 0.0057	0.8804
9		Kidney plasma	(g)	87	−0.25	0.2026 ± 0.0098	0.9135
10		Spleen plasma (ml)		80	0.00	0.0951 ± 0.0084	0.7897

[a] The symbols refer to those for a linear regression ($y = C + bx$), the number of paired observations used for calculation (n), the standard errors of the regression coefficients (S_b), and the correlation coefficients (r). Where Regression lines for normal and immune animals were not significantly different ($P > 0.05$), they have been combined (*). Other statistical comparisons are considered in the text.

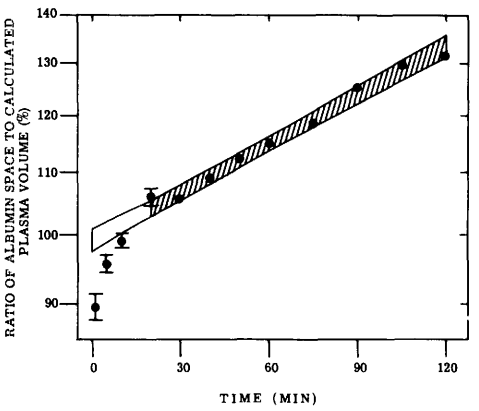

FIG. 2. Ratios of albumin space to plasma volume (100 × AV/PV) at timed intervals after injection of radio-iodinated albumin. Each point represents the mean of 25 to 36 observations for 18 normal and 18 insulin-immunized guinea pigs. Also shown are the standard errors for early mean values (1 to 20 min) and a shaded area covering the limits of confidence (95%) for the combined observations after 10 min for normal and immune animals (see Table II, lines 3 and 4).

after 10 min at a rate of about 17%/hr. Mean values (± SEM) for albumin space (relative to a plasma space of 100) at 1 (90.3 ± 1.3), 5 (95.8 ± 1.0), and 20 (106.1 ± 1.3) or more minutes after injection are all significantly different from the calculated plasma volume ($P < 0.001$). Only at 10 min is the albumin space (99.0 ± 1.0) equal to the plasma volume ($P > 0.05$).

Plasma entrapment by tissues. Linear relationships were established for 87 guinea pigs between the weights of the livers, kidneys, and spleens and the body weights of the animals from which they were obtained, and between the plasma contents and weights of the organs themselves. Since no distinction could be made between the various regression lines for normal and insulin-immunized animals, the results have been combined (Table II, lines 5 to 10). One or other of the organs from the remaining 25 guinea pigs, 12 of which were normal and 13 of which were immunized, were arbitrarily considered to have abnormally high plasma contents; contents (milliliters of plasma per gram of tissue) exceeded the mean values for animals of comparable weight by more than two standard deviations. As illustrated in Fig. 3, the livers, kidneys, and spleens of these 25 guinea pigs, with minor exceptions,

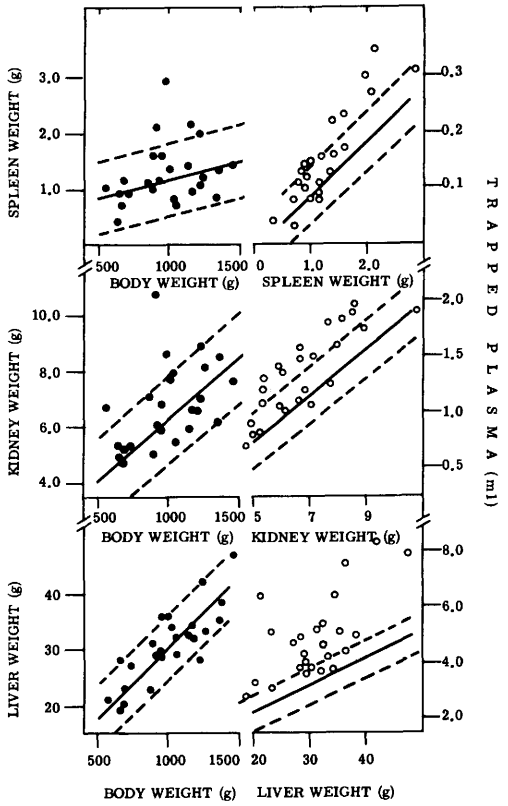

FIG. 3. The organ weights (g, closed circles) and the volumes of trapped plasma (ml, open circles) for the livers, kidneys, and spleens of 25 guinea pigs considered to have high plasma tissue contents. The regression lines (continuous) and limits of confidence (95%) for individual observations (dotted lines) refer to values obtained from 87 other animals. In each case, tissue weight or trapped plasma have been related to body weight (g) or tissue weight (g). The values shown here are derived from information shown in Table II.

were not excessively heavy relative to body weight. However, the vast majority had tissues whose plasma contents exceeded the mean values for livers, kidneys, and spleens from the other 87 guinea pigs. Contents were abnormally high in 17 livers, 11 pairs of kidneys, and 8 spleens. In eight animals the liver was the only affected organ, but in nine other animals the kidneys ($n = 3$), spleen ($n = 5$), or both of these organs ($n = 1$) were also affected. In five animals the kidneys only were affected, but in two others the kidneys and the spleen were involved. The spleen was never the only organ affected. Of the 17 animals whose livers were affected, eight had died (usually from

respiratory complications) before they could be exsanguinated; no note of such complications was made in the cases of the other nine animals with affected livers.

Discussion. Investigation of the vascular spaces in guinea pigs began in 1929 when Went and Drinker (6) first used Vital Red to estimate blood volume. Since then, others (2, 3, 7, 8) have used dyes, radioiodinated proteins, and radiolabeled red cells to estimate blood volume but have reported only indirect estimates of plasma volume. Constable (9) bled guinea pigs 4 min after injection of Evans Blue (T1824) and reported that plasma volume falls from a value of 5.73 ml/100 g body wt at birth to 3.0 ml/100 g body wt in guinea pigs weighing 800 to 900 g. Edmondson and Wyburn (10) bled their guinea pigs 5 min after injection of radioiodinated albumin. For animals weighing about a kilogram, they found that mean plasma volumes (± SEM) for males (3.31 ± 0.07 ml/100 g body wt) and females (3.59 ± 0.07 ml/100 g body wt) were about the same. Using the same technique, Wiseman and Irving (4) got a slightly larger value (4.1 ± 0.2 ml/100 g body wt) for animals weighing 600 g. Rieke, in an unpublished observation reported by Osmond and Everett (3), without experimental details, obtained a similar plasma volume (4.1 ml/100 g body wt) for even smaller animals weighing about 300 g. By comparison, as shown in Table I, the plasma volumes of the present animals were greater and decreased from about 5.2 ml/100 g body wt for those weighing about 600 g to a significantly lower value of 3.7 ml/100 g of body wt for guinea pigs weighing about 1200 g. The discrepancy between these and previously published findings is probably due, at least in part, to differences in technique. Thus, the present results are based on multiple observations made between at least 10 and as much as 120 min after injection of labeled albumin, a method which is "to be preferred and recommended" (11) for the estimation of plasma volume in man. Plasma volumes determined by this method, as illustrated in Fig. 2, are likely to exceed any which depend upon single observations on blood samples drawn less than 10 min after injection, the time of sampling used by all the investigators quoted above. It should also be noted that the present relatively larger volumes are of the same order as those (4 to 6 ml/100 g body wt) reported for other small mammals (12).

No explanation can yet be given for the slightly but significantly larger plasma volumes of insulin-immunized guinea pigs. The difference does not appear to be due to increased permeability of capillary membranes induced by insulin, which was present in all inocula. Had this been so, the labeled albumin should have escaped from the plasma more rapidly and so increased the albumin space more rapidly in immune than in normal guinea pigs. This did not occur, as illustrated in Table II (lines 3 and 4), and was probably prevented by the administration of antihistamine. It was also considered possible that the immune animals may have been more lean (less obese) than normal animals of comparable total body weight and that this could have led to a relative increase in their plasma volumes. This, however, also seems unlikely because the livers, kidneys, and spleens of the immune animals were no larger than those of the normal guinea pigs of comparable weight (Table II, lines 5 to 7).

Among the 87 guinea pigs which had been adequately exsanguinated at the time of death in the present studies, significant volumes (mean ± SEM, milliliters per gram of tissue) of plasma remained in the livers (0.108 ± 0.001), kidneys (0.158 ± 0.002), and spleens (0.090 ± 0.002). A similar distribution was reported in dogs by Gibson *et al.* (13), who also found higher concentrations in the kidneys (0.17 ml/g) than in the livers (0.12 ml/g) or spleens (0.07 ml/g) under similar circumstances. These levels probably do not reflect the entrapment of plasma alone because it is known that albumin escapes from the plasma into the lymph, especially in and around the liver (14); such leakage also accounts for the increase in the albumin space seen over 2 hr in the present studies (Fig. 2). Significantly higher levels of entrapment were found in the livers (0.160 ± 0.008), kidneys (0.198 ± 0.006), and spleens (0.131 ± 0.018) of the other 25 guinea pigs used here. The liver was the organ most affected (17/25) and in

many such cases (8/17) it was noted that the animal had died before it could be exsanguinated. Such increased entrapment of plasma was not reflected in the weights of the affected organs and was only detected by direct measurement (Fig. 3). The present study therefore shows that significant amounts of blood can become trapped at the time of death in the livers, kidneys, and spleens of guinea pigs and that such entrapment can become excessive if the animal is not exsanguinated at the time of death. These facts should be taken into account when studying the fate of any substance, endogenous or exogenous, which is present in the blood in high concentrations and which appears to become concentrated in a tissue, such as the liver, where blood could accumulate after death.

Summary. Relative to body weight, the plasma volumes of 60 normal guinea pigs fell from 5.2 to 3.7 ml/100 g body wt as body weight increased from about 600 to 1200 g. Lower values reported by others are attributed to differences in methodology. Slightly higher plasma volumes were found in 37 insulin-immunized guinea pigs. After exsanguination at the time of death, trapped plasma accounted for 10 to 20% of the weights of the livers, kidneys, and spleens, more being trapped when death occurred before exsanguination could be effected.

1. Wright, P. H., Makulu, D. R., and Posey, I. J., Diabetes **17,** 513 (1968).
2. Masouredis, S. P., and Melcher, L. R., Proc. Soc. Exp. Biol. Med. **78,** 264 (1951).
3. Osmond, D. G., and Everett, N. B., Quart. J. Exp. Physiol. **50,** 1 (1965).
4. Wiseman, R., and Irving, C. C., Proc. Soc. Exp. Biol. Med. **114,** 728 (1963).
5. Snedecor, G. W., and Cochran, W. G., "Statistical Methods." Iowa State University Press, Iowa (1967).
6. Went, S., and Drinker, C. K., Amer. J. Physiol. **88,** 468 (1929).
7. Ancill, R. J., J. Physiol. (London) **132,** 469 (1956).
8. Flexner, L. B., Gellhorn, A., and Merrell, M., J. Biol. Chem. **144,** 35 (1942).
9. Constable, B. J., J. Physiol. (London) **167,** 229 (1963).
10. Edmondson, P. W., and Wyburn, J. R., Brit. J. Exp. Path. **44,** 72 (1963).
11. International Committee for Standardization in Haematology. Brit. J. Haematol. **25,** 801 (1973).
12. Altman, P. L., and Dittmer, D. S., eds., "Respiration and Circulation," pp. 381-383. F.A.S.E.B., Bethesda, Maryland (1971).
13. Gibson, J. G., Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J., and Evans, A. D., J. Clin. Invest. **25,** 848 (1946).
14. Mayerson, H. S., Wolfram, C. G., Shirley, H. H., and Wasserman, K., Amer. J. Physiol. **198,** 155 (1960).

Received March 15, 1976. P.S.E.B.M. 1976, Vol. 153.