Distribution of 'Extra Plasma' in the Blood of Some Tissues in the Dog as Measured With P^{32} and T-1824[1]

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In the splenectomized dog simultaneous measurements of cell and plasma volume (1, 2) show that if all the cells were distributed with plasma in the same proportions as in the venous or other large vessel blood only 87–90% of the measured blood volume would be accounted for and the remaining 10–13% would consist of plasma. An example will make this clear. Suppose that a splenectomized dog had a measured cell volume of 400 ml and a measured plasma volume of 600 ml and hence a measured blood volume of 1000 ml. Our observations (2) show that the venous cell percentage would then be 40 ÷ 0.87 or 46%. If all the cells were distributed with plasma in the proportions of 46:54, then the 400 ml cells would be distributed with 470 ml plasma leaving 130 ml of plasma or 13% of the total blood volume free from cells. This plasma may be termed 'extra' plasma, and provided our measurements are correct its presence indicates that the cells and plasma cannot be evenly distributed through the circulating blood of the splenectomized dog.

Microscopical observations suggest that blood in the minute vessels, where these can be seen, has a lower cell content than the blood in larger vessels, and Ebert and Stead (3) have provided supporting evidence. Thus, the extra plasma might be chiefly distributed in the small vessels feeding the tissues. However, there is little direct evidence on the relative amount of extra plasma in any living tissue. Further, it is uncertain if significant quantities of the extra plasma are found in all tissues or are restricted to one or a few (4).

In this paper we report observations made to clarify these problems. Tissues or organs were isolated from the circulation so that they contained all the blood flowing through them immediately before isolation. They were then analyzed for their cell and plasma contents and the proportions of cells and plasma in them were compared with those in venous blood. In this way the volume of extra plasma was ascertained. Thus, suppose that by analysis a given tissue contained 2 ml of cells and 8 ml of plasma and the venous cell percentage was 50. Then if the 2 ml of cells were distributed with plasma in the same proportions as in the venous blood, they would be distributed with 2 ml of plasma, and the remaining 6 ml of plasma would be the extra plasma in the tissue.

Attempts were made to measure the cell and plasma contents of a number of tissues, but these succeeded only with the liver, kidney and spleen. We find that about 30% of the liver blood and about 50% of the kidney blood is extra plasma, and this makes up about 60% of a dog's total extra plasma. Thus, the remaining 40% must be distributed in other tissues.

PROCEDURE

The experimental procedure consisted essentially in anesthetizing the dog, exposing the tissues to be removed, injecting the labeled cells and the T-1824 and finally the removing of blood and tissue samples.

Preparation of the Dog and Injection of Labeled Cells and T-1824. A small dog which had not previously received T-1824 or P^{32} was anesthetized with Nembutal. A long midline incision was made with cold cautery, the viscera were gently pushed aside and the pedicles of the kidneys were exposed by careful dissection. Blood loss was kept minimal. The spleen when present (see legend, fig. 2) was handled at intervals

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until its removal, to elicit contractions and promote red cell exchange.

After 15–30 minutes a blank sample was taken from an external jugular vein, and then injections of T-1824 and P^{32}-tagged cells were made into this vein. Every 8–10 minutes thereafter samples were withdrawn from the opposite jugular vein. The blood was immediately mixed with heparin, and portions were removed for measurement of hematocrit, hemoglobin and P^{32} (1). The plasma was analyzed for T-1824 by extraction (5) and for protein by refractometry.

Isolation of Tissues From Their Blood Supply. Our object was to apply hemostatic clamps with a minimum of disturbance so that the blood flow would be instantly halted and the tissues would contain their normal complement of blood. This was not difficult with the spleen and kidneys since the vessels reach them in a pedicle. One or two clamps were first placed across the pedicle. After a second set of clamps had been fixed in place to prevent blood loss from the animal the pedicle was severed between the clamps, and the organ was removed, rinsed with saline and then transferred and weighed in a beaker containing 20 ml of 1% bovine albumin in phosphate-citrate mixture (1).

TABLE 1. RECOVERY OF T-1824 AND P^{32} FROM TISSUES

Tissue	T-1824			P^{32}		
	N	Recovery	S.D.	N	Recovery	S.D.
		%			%	
Spleen	13	61.5	14.0	2	99.0	0.28
Kidney	8	71.4	2.85	4	103.2	4.89
Liver	11	79.5	5.76	5	101.2	1.90

The liver has no easily accessible pedicle. It was found, however, that edges of the lobes could be isolated with flat intestinal clamps, and if a second similar clamp was applied on the liver side, the isolated segment could be removed from the animal with negligible blood loss.

Extraction of T-1824 From Tissues. The tissues were snipped with scissors, comminuted for 3–5 minutes in a Waring blender with 150–200 ml of 1% albumin in phosphate-citrate and then poured into graduated cylinders. More fluid was added until for each gram of tissue there were 10–15 ml of the albumin-buffer. The total volume was noted after the foam had been broken with a few drops of capryl alcohol and the tissue mixture was stored in stoppered bottles at 4°C. Next morning this was shaken up and *ca.* 50 ml were removed and centrifuged for 10 minutes at 2000 rpm. To a measured volume of the supernatant was added a one-fifth volume of 15% sodium dodecyl sulfate, and the mixture was poured into the flared tubes used for extraction of T-1824 (5), where it slowly filtered through a 0.5-cm layer of ground glass on top of 3 cm of paper pulp. The paper column with the sorbed T-1824 was washed with saline, then with methanol-saline, and the dye was eluted with acetone-water.

T-1824 was measured with a Beckman spectrophotometer, Model DU, by reading the optical density at 620 mμ. The purity of the eluted dye was checked by noting also the optical density at 450 mμ and at 700 mμ.

Plasma Volume in Isolated Tissues. The total quantity of T-1824 was computed from $(D \times 10 \times V) \div (0.103 \times r \times v)$, the product of the optical density, the volume of the dye-tinged eluate and the total volume of comminuted tissue divided by the product of the specific absorption coefficient, the fraction recovered from the given tissue (table 1) and the volume from which the dye was extracted. Hence, the total T-1824 in a tissue divided by the T-1824 concentration of the venous plasma is the apparent plasma volume of the isolated tissue.

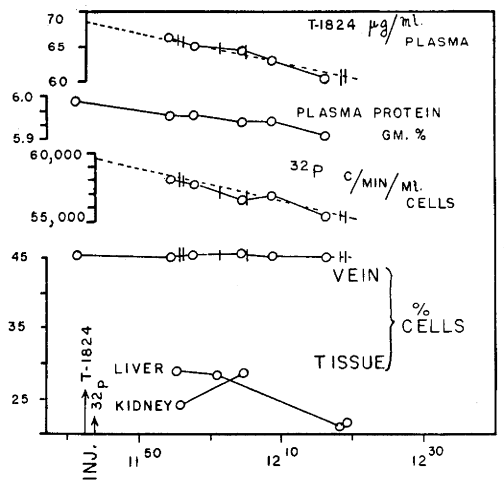

FIG. 1. Time-concentration data for venous blood of splenectomized *dog 6* which, with the quantities of labeled materials in isolated tissues (table 2), are needed to compute the percentage cells in tissue blood.

Volume of Blood Cells in Tissues. After 4–5 days the bottles were removed from the refrigerator, vigorously shaken up and then allowed to sediment briefly before decanting some of the supernatant into Veall's liquid counting chamber (6). The radioactivity in three or more samples from each tissue was counted and at least 3000 and usually 10,000 counts were recorded. To express the total tissue radioactivity in terms of the volume of contained red cells, the total counts/min were corrected for radioactive decay and divided by the number of counts/min in 1 ml of cells in the venous blood.

RESULTS

Test of Recovery of P^{32} and T-1824 Added to Tissue Samples. To state the quantity of P^{32} and T-1824 in tissue blood of animals injected with these substances, it is necessary to determine the extent of recovery of these

substances when added to freshly removed samples of tissue which are treated exactly as those from the experimental animal. Table 1 shows that P^{32} from added tagged cells is recovered quantitatively but some of the T-1824 is lost. Though the dye was added with excess albumin and plasma, attempts to increase T-1824 recovery were unsuccessful, and it became necessary to establish the extent and consistency of recovery by running a number of tests. These are summarized in table 1 which shows the mean dye recovery and its standard deviation for each tissue.

Typical Estimation of Cell and Plasma Volume in Tissue Blood. The time concentration data (fig. 1) of an experiment with a splenectomized 6.6-kg female under light Nembutal anesthesia indicate that intravascular mixing of labeled materials was completed by 10 minutes. Extrapolation through these data to the time of injection (fig. 1, *dotted lines*) gives a total cell volume of 175 ml and a total plasma volume of 298 ml. Note that the venous cell percentage remains constant, the plasma protein level almost constant, and that the isolation of the tissues scarcely alters the progressive decline in levels of P^{32} and T-1824. The short vertical strokes intersect the venous blood levels of T-1824 and P^{32} at the time of the isolation of the tissues (fig. 1). These levels, together with the total T-1824 and P^{32} in each tissue, are listed in Table 2. The volumes of apparent plasma and cells in the tissues are derived (columns 3 and 6) on the likely assumption that the jugular blood levels are equivalent to those of the blood flowing through the tissues. The cell percentages are plotted in figure 1, from which it can be seen that the tissue blood is richer in plasma than the venous blood.

Liver. In figure 2 the data consist of 17 points connected by lines numbered according to each of the six dogs from which liver samples were removed. The dotted line which intersects the ordinate at 0.69 was fitted to the data by the method of least squares. If a reasonable explanation can be offered for the significantly negative slope of this line, 0.69 is the ratio of the percentage cells in liver blood to that in venous blood. The slope indicates an apparent decrease in the cell percentage in liver blood, for the venous cell percentages remained nearly constant. This might be caused by either a progressive diminution of total liver P^{32} compared with liver T-1824 or a progressive increase in total liver T-1824 compared with liver P^{32}. The only likely explanation of the former hypothesis is that plasma flows in while red cells are actively lost from the liver blood, but this is improbable because during the course of the experiments the liver red cell content did not fall significantly. The postulated explanation is that the liver *in situ* accumulates T-1824 at a regular rate. The evidence favoring this view is now examined.

TABLE 2. CELL AND APPARENT PLASMA VOLUME IN TISSUE BLOOD (*Dog 6*, see fig. 1)

Tissue	Tissue T-1824, μg	Plasma T-1824, μg/ml	Tissue Plasma, *ml*	Tissue P^{32} CPM·10^{-3}	Cell P^{32} CPM·10^{-3}/ml	Tissue Cells, ml	Cells, %
Liver 1	125	66.0	1.89	47.9	57.9	0.77	29.0
Liver 2	172	64.6	2.66	59.6	57.0	1.05	28.3
Liver 3	155	60.4	2.56	37.4	54.8	0.68	21.1
Liver 4	231	60.4	3.82	58.5	54.8	1.07	21.9
Kidney, lft.	378	65.7	5.75	106.0	57.9	1.83	24.2
Kidney, rt.	416	64.4	6.47	148.0	56.6	2.62	28.8

Evidence of Accumulation of T-1824 in the Liver. Two lines of evidence other than the data of figure 2 show that the liver accumulates T-1824. Table 3 summarizes measurements made on the viscera of a 10-kg dog which in other experiments (7) over a period of 12 weeks had received 228 mg of T-1824. Three months after the last injection part of the liver and spleen and one entire kidney were analyzed by the methods here described. The liver contained about 10% of the total T-1824 injected. The kidneys and spleen in contrast retained only negligible quantities. Thus, the liver can accumulate T-1824 and retain it over a long period of time. Elsewhere (8) it has been shown that surviving liver slices incubated at 38°C with T-1824 albumin accumulate signifi-

cant quantities in the course of 1 hour. Thus it is probable that the liver *in situ* begins to take up T-1824 soon after an intravenous injection of the dye.

Computation of the T-1824 Uptake by the Liver From the Data of Figure 2. *Equation 1* relates the F_{cells} factor at 1 hour to that at the time of injection, where F_{cells} is defined as the liver blood cell percentage divided by

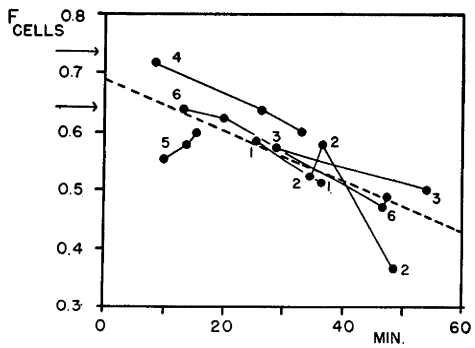

FIG. 2. Effect of T-1824 accumulation on estimates of apparent plasma in liver and hence on the illustrated relation between cell percentages in hepatic and venous blood. When corrected for dye uptake by the liver, F_{cells} = cell percentage hepatic ÷ cell percentage venous = 0.69 as indicated by the intercept of the *dotted line*. This intercept has a S.D. = 0.047 as indicated by two *arrows*. Data are numbered according to experiments; *dogs 1, 2, 3* were normal, *dogs 4, 5, 6* splenectomized at least 1 month previously.

the venous cell percentage, H, and is the mean value derived from the dotted line of figure 2.

$$F_{1hr} = \frac{C \text{ liver}}{\frac{C \text{ liver}}{F_{0hr} \times H} + \frac{\text{Gain}}{\text{p.l.}}} \times \frac{1}{H}. \quad (1)$$

C liver is the total volume of red cells in the liver. $F_{0\,hr}$ is 0.69, and $F_{1\,hr}$ is 0.43 as derived in figure 2 by the method of least squares. Gain is defined as the quantity of T-1824 in milligrams accumulated by liver substance, excluding liver blood, in 1 hour; p.l. is the plasma level in milligrams/milliliter of T-1824 in venous blood at the end of the hour. Thus, gain/p.l. has the dimensions of a volume of plasma, and the sum of this plasma volume equivalent and the liver blood volume can be used to compute the apparent cell percentage in liver blood. The above equation is readily tested by letting gain = 0, whereupon $F_{1\,hr} = F_{0\,hr}$. In other words, if there were no accumulation of dye, the estimated percentage cells in hepatic blood divided by that in venous blood would remain constant, and the dotted line in figure 2 would be parallel to the abscissa.

The equation may be rewritten thus,

$$\text{Gain} = \frac{(F_{0hr} - F_{1hr})(C \text{ liver} \times \text{p.l.})}{F_{0hr} \times F_{1hr} \times H} \quad (2)$$

Provided that the total blood volume of the intact liver is known, the average quantity of dye accumulated in the liver can be computed by substituting the mean values of the six experiments. These were $F_{0\,hr} = 0.69$; $F_{1\,hr} = 0.43$; $H = 0.43$; p.l. = 61.5 μg T-1824/ml. The red cell content averaged 6.6 ml cells/100 gm liver (range 5.7–7.5 ml/100 gm); Cizek (7) finds in 5 small dogs given an overdose of Nembutal that the liver averages 5.8% of the body weight (range 4.5–6.6%), and from this the average value of C liver becomes 23.2 ml red cells. Solving for the gain with these values gives 2.91 mg T-1824/hr as the average gain of T-1824 by liver substance in the six experiments.

TABLE 3. RETENTION OF T-1824 IN SOME VISCERAL ORGANS

Tissue	T-1824 in Sample, μg	Sample, Wt., gm	Estimated Organ Wt., gm	Total injected T-1824, %
Liver	1370	19	300	10
Kidney	370	32	60	0.3
Spleen	58	9	20	0.06

Plasma level is 0.2 μg T-1824/ml.

Proportion of T-1824 Leaving the Plasma That is Accumulated by the Liver. The loss of T-1824 from the plasma in the first hour after injection can be calculated from the quantity of T-1824 injected and from the rate of dye loss as determined by linear extrapolation. The average loss in the hour after injection for the six experiments was 3.80 mg. Hence the 2.91 mg, which we estimate as the average accumulation of T-1824 in the liver during this period, was 76% of the total loss. This is a high proportion of the total loss, but the variance of the slope of the dotted line in figure 2 indicates that if our premises are valid the loss to the liver must lie between 55–95% of the total leaving the plasma. Thus the liver would

appear to be the tissue chiefly responsible for the removal of T-1824 from the plasma.

The Value of F_{cells} in Liver Blood. In the three previous subsections evidence that T-1824 is accumulated by the liver has been given, and making the assumption that T-1824 accumulation by the liver is solely responsible for the negative slope of the dotted line in figure 2 the average quantity of T-1824 accumulated by the liver in the six experiments has been calculated. Making allowance for this loss of dye to the liver the value of F_{cells} in liver blood is computed as 0.69 as shown in figure 2.

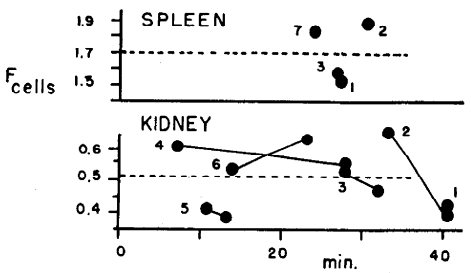

FIG. 3. Ratio of cell percentage in splenic and renal blood to that in venous blood. In *dog 7* the spleen alone was isolated and measured.

Kidney. Figure 3 shows that the relationship between the cell content of renal and venous blood remains fairly constant. Though the ratio of the percentage cells in kidney blood to that in venous blood shows appreciable differences between the two kidneys of the same animal, the results as a whole show no significant decrease with time. The mean ratio is 0.51 with S.D. 0.096. Thus we have no evidence that the kidney accumulates T-1824 within the hour after injection, and the experiment of table 3 bears this out. Hence, the kidney blood with 49% less cells than the venous blood is distinctly rich in plasma, and this probably occurs because of the large proportion of small vessels. The blood which is isolated in both kidneys by clamping the renal pedicle is a remarkably uniform fraction of the whole, being 3.2–3.6% of the measured blood volume.

Spleen. Figure 3 shows that the cell content of splenic blood was 1.7 times greater than that of venous blood. The blood content was 60–68 ml/100 gm of spleen, but depending on the apparent dilation the spleens contained 4–10% of the total blood in the body. The rather low proportions of total measured blood in the spleens in these animals were due to lightness of anesthesia and the contractions of the spleen caused by handling.

DISCUSSION

These observations show that the splenic blood has from 50–90% more cells and the hepatic and renal blooc has 31 and 49% less cells than the venous blood. Thus at least two tissues, liver and kidney, contain 'extra plasma.' Our previous findings (1, 2) indicated that 13% of the total blood in the splenectomized dog consisted of extra plasma. In the six experiments reported here on the average the liver contained 78.5 ml of blood or 18.9% of the blood volume. This blood consisted of 23.3 ml of cells and 55.2 ml of plasma. Since the average venous cell percentage was 43, about 24.3 ml of the liver plasma or 31% of the blood in the liver was extra plasma. The two kidneys contained an average of 14.1 ml blood (3.4% of the blood volume) of which 3.1 ml was cells and 6.9 ml was extra plasma. This extra plasma made up 49% of the kidney blood. Thus on the average 31.2 ml of extra plasma was located in the liver and kidneys. The average blood volume of the six dogs was 415 ml, and the total extra plasma would be 13% of this or 54 ml. Thus the liver and kidneys contained approximately 60% of the total extra plasma and 24% of the total blood. Hence, if our conclusions are approximately correct, about 40% of the total extra plasma is distributed elsewhere than in the liver and kidneys, and it is therefore probable that the extra plasma is distributed through a number of tissues.

We do not wish to claim high accuracy for the numerical results reported here, though we think they are approximately correct, for though the experiments were made with much care the technical difficulties to be surmounted are considerable. Essentially the problem is to measure exactly the quantity of the cells and plasma in the blood vessels of a living tissue. This requires the careful isolation of the tissue with its contained blood, and the exact measurement of the agents used for cell and plasma labeling. Before tissue isolation these agents

must be evenly distributed through the circulating blood, and ideally these should not be lost from the blood, because if they move into the tissue fluids or cells they will still be measured as if they were in the blood. Removal of the tissues requires anesthesia and some manipulation which may lead to alterations in the distribution of cells and plasma. An indication of such a possibility is a slight decline in liver blood content on the average from 21.7 to 18.0 ml/100 gm during the period of tissue sampling. Unless organs have a pedicle containing their vascular supply it is technically difficult to isolate them without losing blood. The technique used for removing liver samples proved satisfactory except in *dog 5*, and here a 20-ml loss from a cut liver lobe did not change the proportions of cells in other tissues.

The two chief difficulties met with in our experiments were the incompleteness of T-1824 extraction from the tissues and the accumulation of T-1824 by the liver substance. We have sought to surmount these difficulties by establishing the level of recovery of T-1824 from the various tissues (table 1) and by correcting for the T-1824 loss to the liver substance by extrapolation as shown in figure 2.

The only previous work on dogs available for reference (9) in which the cells and plasma of tissue blood were measured with separate methods, was performed after death and cessation of the circulation and then only on the blood that would not trickle out of a tissue sample. The results of this earlier work are therefore not comparable with those reported here. Lewis, Goodman and Schuck currently report measurements similar to ours of tissue blood in organs removed from anesthetized rats (10).

SUMMARY

Tissues were isolated so as to contain their normal complement of circulating blood. The volumes of cells and plasma in them were determined from the contained quantities of P^{32} in labeled cells and T-1824 in plasma. Hepatic and renal blood had 31 and 49% less and splenic 70% more cells than venous blood. The former tissues with 22.3% of the measured blood volume contained 60% of the 'extra plasma.' The remaining 40%, therefore, was distributed in other tissues.

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