A Method for the Continuous Measurement of Plasma Volume in the Dog *

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THE CONTINUOUS measurement of plasma volume is desirable in a variety of experimental situations. Both "direct" and "indirect" applications of the dilution principle have been employed for this purpose. In the direct method repeated single determinations of plasma volume are obtained using multiple injections of a tracer substance (dye or isotope). In contrast, the indirect method is based on the plasma disappearance curve of a tracer material given in a single injection. The latter procedure was introduced by Gibson and Evans [4] in 1937, using the azo dye T-1824. The rate of dye disappearance from plasma was established during a control period. In an ensuing experimental situation (e.g., fluid infusion), deviations of dye concentration from a prolongation of the control disappearance slope were considered to reflect changes in plasma volume. It is important to note that this slope was projected linearly on a rectangular coordinate plot. Thus an increase or decrease in plasma concentration of dye above or below that of a synchronous point on the projected control disappearance curve indicated a fall or a rise in plasma volume.

This indirect procedure is based on the assumption that a constant amount of T-1824 is removed per unit time and that experimentally-induced deviations from a stable control disappearance curve result primarily from a shift of fluid into or out of the vascular compartment rather than from alterations in the rate at which the tracer substance leaves the plasma (diffusion into lymph, excretion, phagocytosis). There are two flaws in this technic. It is now well established that a rectangular coordinate plot of the concentration of tag in the plasma against time is not linear. A semilogarithmic plot is not linear either over a period of several hours. It must be stated, however, that the deviations from linearity in either case are small and that short term projections do not introduce a great error. Secondly, it is clear that deviations from the disappearance slope must be minimal during control periods so that an accurate projection of the slope can be established. This is not always possible, e.g., previous studies of the indirect method in this laboratory,[14] using radioactive iodinated human serum albumin (RIHSA) as the tracer substance in anesthetized, splenectomized dogs, demonstrated that in some animals control disappearance slopes over periods of 120 to 240 minutes after injection of tag were not even approximately linear despite efforts to maintain stable experimental conditions. Parallel fluctuations in hematocrit, hemoglobin concentration and plasma specific gravity supported the view that deviations from the expected disappearance curve were due to fluctuations in

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plasma volume. Under such circumstances the accurate projection of a control disappearance slope was hardly feasible. However, in the great majority of animals which were followed up to six hours, it was possible to divide the disappearance curve into two slopes which were approximately linear on a semilog plot. It must be admitted that the division of a general disappearance curve into two separate exponential components is both artificial and arbitrary. This suggested that the empirical establishment of the means of these two slopes might be an expedient approach to the problem. A reliable mean control slope would then permit accurate continuous calculations of changes in volume by the indirect method in any given animal, without the prior delineation of a stable control disappearance curve. A decrease or an increase in plasma volume would be determined from the rise or fall in the measured plasma concentration of the tracer substance above or below that of a synchronous point on the mean disappearance slope of the tracer. It is clear that this method can be used only when whole blood or plasma being lost from the circulation can be accurately measured by volumetric means.

This paper describes the mean plasma disappearance slope of RIHSA measured in a group of anesthetized, splenectomized dogs. The validity of the use of this slope has been tested by comparing plasma volumes estimated from this mean slope with volumes obtained by simultaneous direct measurement in a variety of situations.

Materials and Methods

The Experimental Preparation. Mongrel dogs were used. All animals were splenectomized at least one month prior to these studies and maintained in a healthy condition with a good diet. Anesthesia was induced with intravenous pentobarbital (30 mg. per kg.) and carefully maintained thereafter according to respiratory rate and reflex responses by supplemental intravenous doses (12 mg. or less per dose). No premedication was given. Anesthesia was maintained in a stable state for at least 45 minutes before blood volume determinations were begun. Following induction, one polyethylene catheter was inserted into the abdominal aorta via the femoral artery, through which all analytical blood samples were taken and through which measured experimental hemorrhage was performed. A cannula was also placed in the opposite femoral artery for injection of tracer substances. The method of slow continuous bleeding used has been described elsewhere.[7]

Plasma Volume Determinations (Direct). Plasma volumes were determined by various dilution technics using RIHSA or T-1824. RIHSA methods of plasma volume determination used in this laboratory previously have been described.[14] T-1824 was extracted by the method of Allen [1] and read in a Coleman spectrophotometer. *In vitro* recovery of known amounts of the dye was carried out on 38 blood samples; the mean recovery was 96 ± 2.8 per cent.

In all animals the initial plasma volume was measured directly with RIHSA using a single 10-minute sample. This method was chosen deliberately after the following study of methods had been made. Two variations of the direct technic have been widely used. In one, serial determinations of the plasma concentration of radioactivity are obtained over a period of 30 to 60 minutes and plotted on a semilogarithmic scale against time; backward extrapolation provides the concentration of the tracer at zero time for purposes of calculating plasma volume. In the other procedure, plasma volume is derived from the concentration of radioactivity measured in a single sample withdrawn 10 minutes after injection of RIHSA. A comparison of these two technics was performed in 46 dogs. Table 1 shows the means of the estimated volumes are very close—946 and 953 ml, respectively.

TABLE 1. *Plasma Volumes (RIHSA) Determined by Extrapolation and by 10-Minute Sample (46 Dogs)*

Mean volume—extrapolation	946.0 ml.
Mean volume—10-minute sample	953.0 ml.
Mean of Differences	+6.5 ml. ± 26.8 (+0.6 ± 2.5%)

TABLE 2. *Plasma Volumes Measured Simultaneously with T-1824 and RIHSA (45 Dogs)*

Mean T-1824 volume	668.4 ml.
Mean RIHSA volume	688.6 ml.
Mean of Differences	+22.1 ± 69.8 ml. (+1.8 ± 10.2%)

Thus statistics show, in a crude manner, that the two estimate methods yield essentially identical volumes. The next statistic more precisely validates the same conclusion. The mean of the differences between estimates of volumes on the same animal was only 6.5 ml. The standard deviation of the mean of the differences was ± 26.8. The statistic in parentheses is included for two reasons: it gives the reader an impression of the order of magnitude of the differences regardless of animal size; and it is a commonly-employed statistic in comparisons of this type. It should be pointed out that the use of the standard deviations of percentages usually leads to speciously-narrow degrees of dispersion not because the data is better, but because percentage differences are not normally distributed. The above methods of presenting the tabular data are used throughout this paper.

Since the single 10-minute sample procedure is simpler, requires fewer blood samples and is uniquely suitable for the practical application of the mean disappearance slope, it was used routinely.

Following the initial direct measurement of plasma volume with RIHSA, it was necessary in most experiments to obtain a second direct determination of plasma volume. For the latter T-1824 was used. If a third measurement was later needed, RIHSA was again used. Thus it was essential to demonstrate that the two methods provide equivalent measures of the same plasma volume. In 45 anesthetized, splenectomized dogs, duplicate measurements of plasma volume were obtained using RIHSA and T-1824 injected simultaneously. The results are summarized in Table 2. The mean plasma volumes were 668.4 ml. and 688.6 ml. as determined with T-1824 and RIHSA, respectively. The mean of the differences was 22.1 ± 69.8 ml. (1.8 ± 10.2 per cent). Therefore, since the results were in essential agreement, it seemed reasonable to use either or both agents to serve as an index of the accuracy of continuous indirect measurements of plasma volume.

Calculations and Definitions. The real plasma volume is obviously that volume of plasma present in the animal at any given time during the experiment. This has been estimated by both direct and indirect methods. The following definitions are offered for the sake of clarity.

The Plasma Volume (Direct). This is the plasma volume determined directly by the dilution technic with RIHSA or T-1824, using either the sample withdrawn 10 minutes after injection of the tracer, or backward extrapolation of additional measured concentrations over a period of time.

The Plasma Volume (Indirect). This is the plasma volume determined by an indirect method. As conceived by Gibson and Evans,[4] it was calculated from the difference between the actually measured plasma concentration of tracer on the projected control disappearance slope of T-1824 in the individual subject. This required a control period to obtain the measurements which allowed projection. In the studies reported here, this volume is derived from the difference between the measured plasma concentration of tracer (RIHSA) at any given time and the plasma concentration of tracer *predicted* by the mean disappearance slope of RIHSA—a curve, previously obtained in a group of control subjects. (The derivation and proof of this curve is the essence of this paper.) Its derivation requires the following definitions:

Predicted Disappearance Curve. Analysis of the disappearance of RIHSA from the plasma of several animals led us to suspect that a mean disappearance curve could be established for all dogs in a similar experimental situation. This mean disappearance curve could then be applied to any given animal and would be a measure of the expected rate of loss of RIHSA in that animal. Such a curve was constructed from actual measured RIHSA concentrations in nine dogs. When used thereafter as a measure of the expected rate of loss in similar experimental preparations, it was termed the *Predicted Disappearance Curve.*

The Initial Plasma Volume. This is the plasma volume present at the beginning of the experiment. It may be measured by various applications of the dilution technic; in fact, it was determined here in three different ways, all of which provided equivalent values.

The Virtual Plasma Volume. This is the volume of plasma which would be present under various experimental conditions if no intravascular fluid shifts occurred. For example, in these experiments it is the volume of plasma which would remain in the vascular compartment following an experimental hemorrhage if compensatory hemodilution did not occur:

Virtual Plasma Volume = Initial Plasma − Volume-plasma removed.*

The *concentration* of tracer present in this *Virtual Plasma Volume* at any given time can be obtained from the *Predicted Disappearance Curve.*

Since, in fact, compensatory alterations in plasma volume ordinarily do occur following hemorrhage, it is clear that the undiluted residual volume is virtual rather than real. On the other hand, under circumstances in which no compensatory fluid shift occurs, the virtual and real plasma volumes are equal.

Thus, using these concepts, the *Plasma Volume (Indirect)* can be derived on the assumption that a deviation in plasma concentration of the tracer material from the *Predicted Disappearance Curve* is due to the movement of fluid into or out of the vascular compartment. First, this fluid shift at a given time is calculated *and expressed as a fraction* of the Virtual Plasma Volume at that time:

$$\text{Fluid Shift} = \frac{CP_{m_p} - CP_{m_m}}{CP_{m_p}}$$

when CP_{m_p} equal counts per minute obtained from the *Predicted Disappearance Curve* of RIHSA, and CP_{m_m} equals counts per minute *measured* in the plasma at that time,* then plasma volume is calculated:

Plasma Volume (Indirect) = *Virtual Plasma Volume* ± (Fluid Shift × *Virtual Plasma Volume*)

Results

Estimation of the Predicted Disappearance Curve. Figure 1 is a semilog plot of RIHSA activity against time. The ordinate is in percentage of the activity at 10 minutes, with the activity at 10 minutes being assigned the value of 100 per cent. This adjustment of data units allows a common basis for the comparison of the nine animals studied. Note also that the ordinate has a log scale. The abscissa is time in minutes from the injection of RIHSA.

Although there is considerable variation among the plotted points of Figure 1, inspection shows that the trends of the individual lines have remarkably similar slopes; that is to say, the changes in rates of disappearance in all animals essentially are the same. The lines have different positions on the graph because their intercepts with the ordinate vary, probably because of

* The plasma removed is calculated in the usual way from the volume of blood withdrawn; the hematocrit was determined with RIHSA according to the method of Hlad and Holmes.[5]

* Of course, the fluid shift can be converted to an actual volume in ml. instead of a fraction, if one desires.

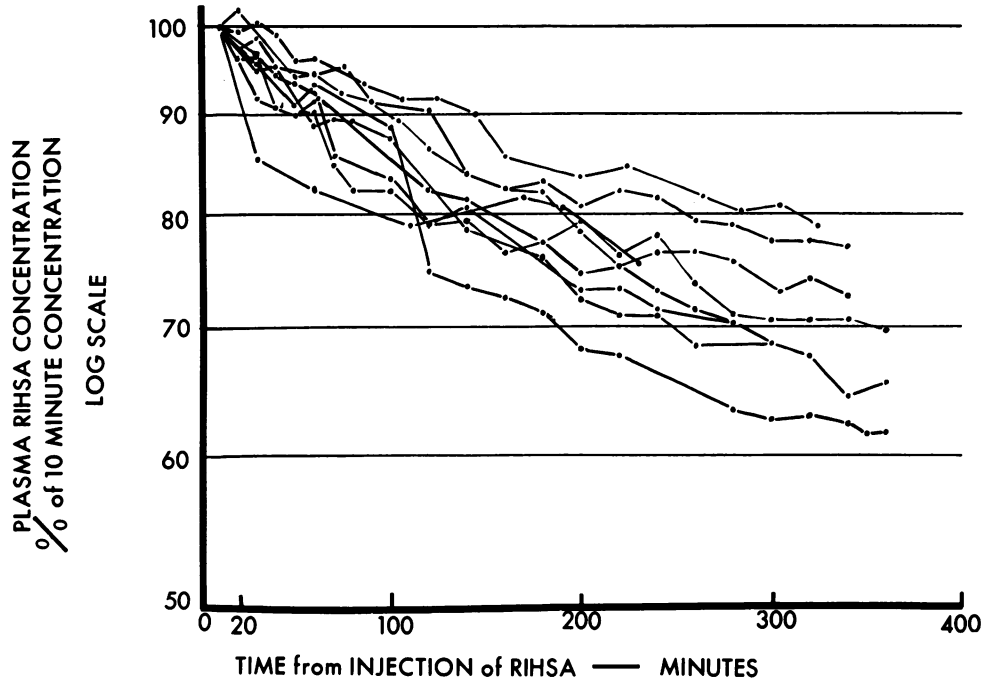

FIG. 1. Disappearance of RIHSA in nine dogs.

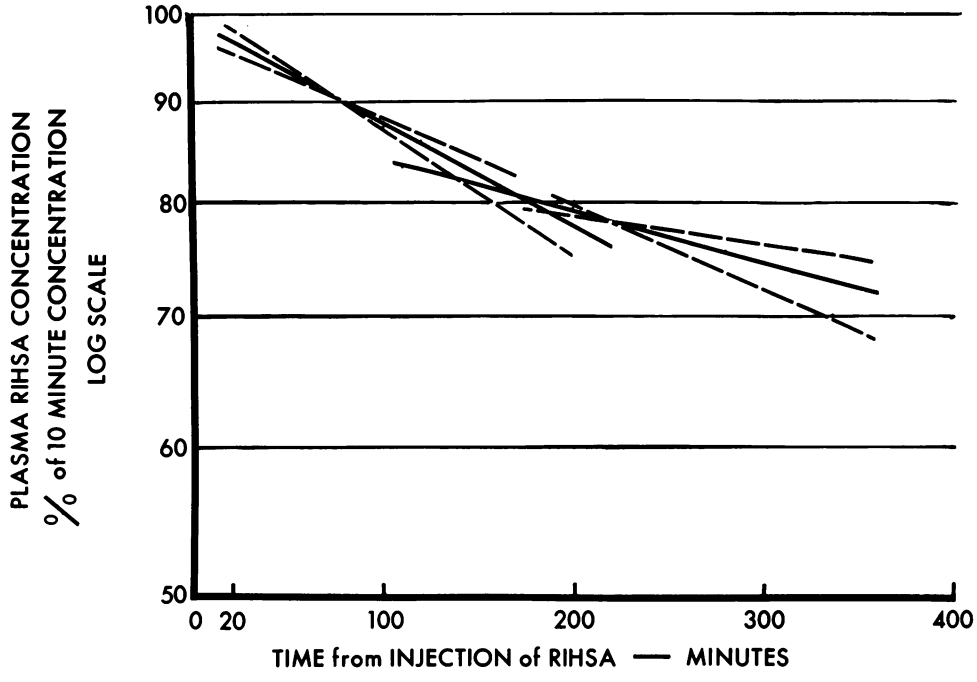

FIG. 2. Disappearance curves of RIHSA in nine dogs.

variations in the mixing times of different animals. Since the curves are so similar, they were divided in two parts.

These data are summarized in Figure 2 in which the individual points are the means of the several groups at each time interval in Figure 1. Since the line exhibits two slopes, two lines of regression were calculated with the separation point at 140 minutes. In calculating the linear regression, the 140-minute point was included in both series. These regression lines describe the mean disappearance curve of RIHSA in these nine anesthetized, splenectomized dogs.

The slope, *b*, from 20 to 140 minutes is − 0.109 per cent per minute.* The 99 per cent confidence lines for this interval are indicated by the dashed lines. The slopes of the confidence lines are − 0.084 and − 0.134. This indicates that the mean rate of decrease in plasma concentration of RIHSA was 0.109 per cent per minute, and that there is one chance in 100 that the true mean lies outside the limits of − 0.084 per cent per minute and − 0.134 per cent per minute. From 140 to 340 minutes the mean slope, b, is − 0.058 per cent per minute; the 99 per cent confidence lines indicate that the true mean lies between − 0.053 per cent per minute and − 0.063 per cent per minute. When this curve is utilized for the direct or indirect measurement of plasma volume in these experiments, it is called the *Predicted Disappearance Curve.*

Validity of the Predicted Disappearance Curve. Since the 99 per cent confidence intervals of the mean regression slope are relatively narrow, it would appear feasible to apply this line to other animals studied under comparable experimental conditions. It should be possible to use this curve to predict the disappearance slope of RIHSA in a single animal with reasonable accuracy. In the latter situation any deviation from the *Predicted Disappearance Curve* should reflect a shift of fluid into or out of the vascular compartment; the degree and direction of deviation should be a measure of the magnitude and direction of this fluid shift. To test this hypothesis, a variety of comparisons were made.

Direct Measurement of Initial Plasma Volume by Use of the 10-Minute Point and by Extrapolation from the Predicted Disappearance Curve. As described in "Methods," the *Initial Plasma Volume* was measured in 46 dogs simultaneously by two technics: measurement of the plasma concentration of RIHSA in a single 10-minute post-injection sample; and by extrapolation of the actual RIHSA disappearance slope of each animal. The mean difference between these two procedures was not significant. If the *Predicted Disappearance Curve* is valid, it should provide an equally accurate comparison with the 10-minute sample procedure. First, the mean points shown in Figure 2 were transposed to semilogarithmic paper and a transparent template was constructed, the left-hand margin lying along the ordinate and the superior margin following the plotted line. This, then, represents the *Predicted Disappearance Curve.*

Then known amounts of RIHSA were injected intravenously into 17 dogs, and blood samples were withdrawn 10 minutes and again 20 minutes, later, when mixing of the tracer was assumed to be complete. Plasma volumes were calculated from the 10-minute sample by the usual method. Then, instead of extrapolating for each animal from the tracer concentrations at 20, 30, 40, 50 and 60 minutes as was done before, the *Predicted Disappearance Curve* positioned on the 20-minute sample was used for extrapolation. The template was placed on the individual semilogarithmic graphs for each animal in such fashion that the upper border of the template coincided with the RIHSA concentration of the 20-minute sampel, and the ordinate margin of the tem-

* The formula used here for the straight line is $y = a - bx$, where x and y are the coordinates of the points on the line, a is the intercept of the line on the ordinate and b is the slope of the line.

TABLE 3. *Initial Plasma Volumes as Measured by RIHSA at 10 Minutes and by Extrapolation from the* Predicted Disappearance Curve

Dog No.	10-Min. Point	Extrapolation from Predicted Curve	Difference	% Difference
1	819	806	−13	−1.6
2	1,176	1,128	−48	−4.1
3	741	740	−1	−0.1
4	872	844	−28	−3.2
5	798	785	−13	−1.6
6	568	557	−11	−1.9
7	914	914	0	0
8	605	632	+27	+4.5
9	880	868	−12	−1.4
10	907	877	−30	3.3
11	688	693	5	0.7
12	777	782	5	0.6
13	1,266	1,255	−11	−0.9
14	970	968	−2	−0.2
15	651	660	9	1.4
16	862	847	−15	−1.8
17	1,112	1,097	−15	−1.4
Mean	859.2	838.2		

Mean of Differences −0.0 + 16.4 ml. (0.8 ± 2.0%)

plate coincided with the ordinate of the graph. The intersection of the template of the *Predicted Disappearance Curve* with the ordinate of the graph was noted, and this value (counts per minute per ml.) was used in the calculation of the *Initial Plasma Volume.*

Table 3 shows *Initial Plasma Volumes* as determined from the 10-minute sample and as calculated from the *Predicted Disappearance Curve.* The mean difference between the two procedures was 9.0 ± 16.5 ml. (0.8 ± 2.0%). This is directly comparable to the previously demonstrated mean difference of 6.5 ± 26.8 ml. (0.6 ± 2.5%) between plasma volumes measured with RIHSA by the 10-minute sample and the standard extrapolation procedure. In effect, then, the *Predicted Disappearance Curve* gave results as good as the actually measured disappearance when used for computing *Initial Plasma Volume.*

Plasma Volume (Indirect) Compared with Plasma Volume (Direct) in Nonbled Animals.

At 200 Minutes. As a further test of the validity of the *Predicted Disappearance Curve, Plasma Volumes (Direct)* at 200 minutes after the original injection of RIHSA were performed using T-1824 and compared to the simultaneous values obtained for *Plasma Volume (Indirect)* derived from the *Predicted Disappearance Curve.*

After stabilization had been achieved in 10 animals, known amounts of RIHSA were injected. Samples were withdrawn at 10 and 20 minutes and at 20-minute intervals thereafter. The amount of blood removed was carefully measured and recorded. At 200 minutes a *Plasma Volume (Direct)* was done using T-1824. A simultaneous sample of blood was also taken to determine the residual concentration of RIHSA. From the latter and the *Predicted Disappearance Curve* the *Plasma Volume (Indirect)* was calculated as described above. This value was then compared with that for the *Plasma Volume (Direct)* obtained from T-1824.

Results are shown in Table 4. The mean difference was 7.3 ± 8.1 ml. (1.2 ± 6.7%). The correspondence between the two methods is at least as good as that obtained when T-1824 and RIHSA are used for simultaneous *Plasma Volumes (Direct).*

TABLE 4. *Comparison of Plasma Volumes as Obtained Simultaneously from the* Predicted Disappearance Curve *and as Determined by Direct T-1824 Measurement at 100 Minutes*

Dog No.	P_0*	P_w**	P_0†	P_d‡	Difference	% Difference
1	806	11	760	770	−10	−1.3
2	1,128	13	1,113	1,127	−14	−1.4
3	740	17	669	609	60	9.9
4	844	14	833	860	−27	−3.3
5	785	13	763	754	9	1.2
6	557	35	499	514	−15	−2.9
7	914	16	876	866	10	1.2
8	632	13	605	583	22	3.8
9	868	13	891	862	29	3.2
10	877	23	809	800	9	1.1

Mean of Differences 7.3 + 8.1 ml. (1.2 ± 6.7%)

* P_0 = initial plasma volume (at time zero).
** P_w = plasma withdrawn from animal.
† P_1 = Plasma Volume (Indirect).
‡ P_d = Plasma Volume (Direct).

At 350 Minutes. In this group of animals, comprising seven of the 10 dogs from the above section, a *Plasma Volume (Direct)* was determined using a second injection of RIHSA 350 minutes after the initial injection of the isotope. The *Plasma Volume (Indirect)* was ascertained from the *Predicted Disappearance Curve* using the measured residual activity of the original RIHSA at this time. The two values were then compared. Results are given in Table 5. The mean difference was − 8.4 ± 106 ml. (0.1 ± 4.6%). This again demonstrates the close correspondence of the *Plasma Volume (Indirect)* with the *Plasma Volume (Direct)* and furnishes further evidence in favor of the validity of the *Predicted Disappearance Curve.*

Plasma Volume (Indirect) Compared with Plasma Volume (Direct) in Animals Undergoing Slow Removal of 35 Per Cent of their Blood Volume.

At 100 Minutes. The experiment in this group of animals was identical to that above except that the animals were slowly bled 35 per cent of their initial blood volume in such manner that the removal of blood was at as continuous a rate as possible over a 3½-hour period. Samples used for determining radioactivity are included in the bleeding volume. *Plasma Volume (Direct)* was determined by T-1824 at 100 minutes, and the *Plasma Volume (Indirect)* was computed as before. Results obtained in seven dogs are shown in Table 6. The mean difference was 39.8 ± 24.4 ml. (4.9 ± 4.5%). Again the correspondence between the two methods is striking when it is re-

TABLE 5. *Comparison of Plasma Volumes as Obtained Simultaneously from the* Predicted Disappearance Curve *and as Determined by Direct Measurement with RIHSA at 350 Minutes*

Dog No.	P_0	P_w	P_1	P_d	Difference	% Difference
1	806	25	725	776	−51	−6.6
2	1,128	28	946	1,034	−88	−7.7
3	790	32	642	654	−12	−1.8
5	785	27	662	700	−38	−5.4
8	632	29	559	554	5	0.7
9	868	28	978	679	281	32.1
10	877	42	763	855	−92	−10.8

Mean of Differences −8.4 ± 99 ml. (−0.1 ± 4.6%)

membered the complexities involved in the experiment.

At 200 Minutes. In this group of six dogs, which were also members of the preceding group, the *Plasma Volume (Direct)* was determined at 200 minutes by a second injection of RIHSA. The *Plasma Volume (Indirect)* was calculated in the usual fashion and the two values compared. The results may be seen in Table 7. The mean difference was -2.3 ± 34.7 ml. ($0.7 \pm 6.8\%$).

Discussion

The singular advantage of the method presented here for studying changes in intravascular fluid volume is that it permits accurate determinations of the magnitude and direction of such changes with a single injection of RIHSA. In this manner it differs from previously described technics [2, 3, 6, 10, 11, 12] which utilize repeated direct determinations of plasma volume to estimate the variations in intravascular fluid volumes. It thus saves time, serves to eliminate the errors inherent in successive determinations of plasma volume by the direct method due to accumulation of the tag,[9] and, most important, allows an almost limitless number of observations in a single animal.

In order to achieve optimum results with this method, it is essential that the experimental animal be a standard preparation. Our experience is similar to that of Reeve,[10] i.e., that best results are obtained in the splenectomized dog. This eliminates any possible auto-transfusion with its attendant errors in blood volume.[13] In addition, we recommend that the animal should have a recovery period of at least one month following splenectomy before any blood volume determinations are undertaken. This serves to eliminate unhealthy animals and permits the survivors to regulate their blood volume at a normal level prior to the experiment.

It also is essential for the animal to be in a stable state with regard to blood pressure, respiration and reflexes before determining the blood volume, inasmuch as variations in these functions may change its value. It is our conviction that these physiological variables should be stable for at least 45 minutes following induction of anesthesia and placement of cannulae. It has been shown by Price [8] that once stable levels are reached, they tend to remain so. Blood volume, however, is not stable, as shown by our results in animals in which variations in concentration of tag in the plasma were observed even though the blood pressure, respirations and reflexes remained steady throughout.

The template used in these experiments for the *Predicted Disappearance Curve* was constructed from data obtained in the time interval of six hours following injection of the tag, and is, therefore, usable only for that period. During this time the disappearance is proceeding in such a fashion that a plot of the actual data may be resolved into two components, both of which, in practice, approximate exponential decay rates. The rates of decay of the two portions, as defined by their slopes, diverge sufficiently to make it expedient to incorporate them separately into the *Predicted Disappearance Curve*, particularly if the curve is to be used for studying any events occurring in the first two hours. We have used this portion of the curve to compute *Initial Plasma Volume*. This is in contrast to most studies done on disappearance of plasma tags over similar intervals of time in which the rate of loss of the tag has been taken as occurring continuously at the same rate. Further refinements of the *Predicted Disappearance Curve* are, of course, possible, but in practice they add little to the accuracy of the predictability of the magnitude of intravascular fluid shifts during this six-hour period. We want to emphasize that the division of the disappearance curve into two exponential portions is purely arbitrary. At the sacrifice of some degree of precision, a single straight line could have been used.

TABLE 6. *Comparison of Plasma Volumes as Obtained Simultaneously from the* Predicted Disappearance Curve *and by Direct T-1824 Measurement at 100 Minutes in Dogs Undergoing Slow Removal of 34 Per Cent of Their Blood Volume*

Dog No.	P_0	P_w	P_1	P_d	Difference	% Difference
1	693	80	614	598	16	2.7
2	786	105	689	670	19	2.8
3	1,255	55	1,186	1,148	38	3.3
4	968	129	867	853	14	1.7
5	600	92	603	527	76	14.4
6	847	119	749	728	21	2.9
7	1,097	152	967	907	60	6.6

Mean of Differences 34.8 ± 24.4 (4.9 ± 4.5%)

Conversely, it could have been divided into smaller segments. The latter procedure, however, does not give any greater precision and adds to the complexity. For the practical purpose of following changes in plasma volume, two separate slopes seem to be the best compromise.

This technic does not permit one to determine plasma or blood volume in situations in which unmeasured blood loss is occurring. It does, however, enable one to follow the intravascular fluid volume for a period of five or six hours, *provided the amount of blood removed is known.* For this reason it is essential to collect and measure accurately all blood withdrawn, including that used as samples.

If the foregoing protocol is followed, it is readily seen from the data presented that the accuracy of predictability of intravascular fluid volume by this method under varied experimental conditions is extremely good. Extension of this technic to the study of intravascular fluid volume changes under other experimental conditions is warranted. It would appear to offer promise of defining the specific role of intravascular fluid shift in the response of the organism to hemorrhage; such studies are in progress.

Summary

1. A uniformly stable animal preparation for use in studying changes in intravascular fluid volume has been developed which permits the acquisition of statistically significant data regarding this parameter under controlled experimental variations.

2. Data have been presented showing the essential equivalence of the plasma volumes obtained from the 10-minute count after the injection of RIHSA with those computed by the standard technic of extrapolation.

3. Simultaneous comparison of T-1824 and RIHSA as indicators for determining

TABLE 7. *Comparison of Plasma Volumes as Obtained Simultaneously from the* Predicted Disappearance Curve *and by Direct RIHSA Measurement at 200 Minutes in Dogs Undergoing Slow Removal of 35 Per Cent of Their Blood Volume*

Dog No.	P_0	P_w	P_1	P_d	Difference	% Difference
1	693	225	512	450	62	13.8
2	786	275	535	532	3	0.6
3	1,255	443	765	795	−30	−3.8
4	968	338	672	692	−20	−2.9
6	847	293	609	639	−30	−4.7
7	1,097	373	765	764	1	1.3

Mean of Differences −2.3 ± 34.7 ml. (0.7 ± 6.8%)

plasma volume demonstrate the equivalence of the two methods.

4. A *Predicted Disappearance Curve* of RIHSA was computed from data on nine standard animals under pentobarbital anesthesia. The accuracy of this curve in predicting intravascular fluid volume changes in similar standard preparations was then assessed.

a. A transparent template was constructed from this *Predicted Disappearance Curve*. Initial plasma volumes obtained by extrapolation with this template from a 20-minute sample show excellent correlation with the volumes obtained from a single 10-minute sample.

b. Variations in plasma volume occurring over a six-hour period in hemorrhaged and nonhemorrhaged dogs were assessed by means of this template; the accuracy of the method was checked by comparison with direct determinations of plasma volume at suitable intervals in the experiment. The correlation of the intravascular fluid volume obtained from the *Predicted Disappearance Curve* with that found by direct determination was surprising in its accuracy.

5. It is concluded that this method provides an effective means of following changes in intravascular fluid volume in the described standard preparation under controlled variations in experimental design.

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