Dye Dilution in Canine Heart-Lung Preparations

By C. W. SHEPPARD, PH.D., BILLY L. COUCH, M.D., AND BENNETT L. CROWDER, II

THE indicator dilution method continues to be useful in circulatory physiology and studies of the basic principles of the technic have been made by numerous workers at all levels, ranging from artificial physical models, such as tubes containing glass beads, to intact man. One of the most troublesome questions has been the validity of the estimation of "central volume" by analysis of the mean circulation time of arterial dye curves following bolus injection into the right heart. In glass bead columns it is possible, of course, to compare volumes obtained from the first moment of the distribution of dye outflow and from direct measurement in the physical system. At the other extreme, one cannot readily suggest an adequate method at present to determine the central volume in man. We have proceeded, therefore, to study it at the intermediate level of the isolated canine heart-lung preparation, since here it is possible to obtain volume measurements, both by curve analysis, and by equilibration of label in a recirculating system.

Methods

Approximately 1 L. of canine blood was collected in a large siliconized Erlenmeyer flask and kept in a water bath at 38 C. until needed, about 1½ to 2 hours after the death of the donor animal. Mepesulfate (approximately 30 mg./100 ml. blood) was used as an anticoagulant. A small portion of blood was centrifuged and the plasma added to 10 ml. of T 1824 dye (Matheson, Coleman and Bell) in a 10 ml. volumetric flask. The crystalline dye was partially dissolved in a few drops of saline before adding the plasma.

The heart-lung preparations were made on medium to large dogs (see table 1 for body weights). The hearts were opened and examined at the end of each experiment, and other precautions were taken to avoid the possible complication of intra-vascular filariform parasites in the animals. Other points noted at autopsy were completeness of occlusion of ligated vessels, particularly the aorta which usually required clamping in addition to ligation, general gross condition of the heart, coronary vessels, and lungs.

Figure 1 shows the experimental arrangement. Clean polyethylene or Tygon tubing was used and all glassware was siliconized. The arterial cannula was made of Teflon. Positive pressure respiration through a tracheal cannula was provided by a Stanton respirator connected to a cylinder of 5 per cent carbon dioxide in oxygen. Arterial blood pressure was recorded by a Sanborn strain-gage manometer connected into the stump of the left subclavian artery. A similar connection was made to the siliconized glass cannula inserted into the superior vena cava through which blood entered the system from the venous reservoir.

Pressure recording was primarily for the purpose of assessing the stability of the preparation through the constancy of mean arterial and venous pressures. The arterial pressure was recorded adjacent to the point in the system where blood flowed out through the arterial cannula, thus variations in cardiac output could be detected through variations in arterial pressure at this point. A uniform level of the outflow into the reservoir was maintained. A uniform level was also maintained for the resistance of the arterial outflow tube (90 mm. of 2.5 mm. I.D. polyethylene), but in some cases a clamp was applied to provide increased resistance.

After practice, the preparation could be completed in about 30 to 40 minutes after incising the thorax (fig. 1). The internal mammary arteries were bilaterally ligated and cut. The azygos vein was ligated with careful temporary retraction of the right lung, using saline-wet gauze. The phrenic nerves and vagi were bilaterally sectioned. The arterial pressure recorder was tied into the left subclavian artery. The brachiocephalic artery and its tributaries were carefully dissected free up to the point where the vessel bifurcates to form the right common carotid and subclavian. The left common carotid was ligated and the cannula tied into...
Experimental Results for Groups 2 and 3

<table>
<thead>
<tr>
<th>Animal weight Kg.</th>
<th>Hema-</th>
<th>Group</th>
<th>Tail correction factors</th>
<th>Cardiac output in whole blood ml/min.</th>
<th>Volume of preparation in ml. of whole blood</th>
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<tr>
<td></td>
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<td>no.</td>
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<td>For volume</td>
<td>From measured flow</td>
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<td>3</td>
<td>1.01</td>
<td>1.07</td>
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*Animals badly parasitized.
†Because of difficulties with dye standards in these 4 experiments, mean values of Δ
‡Flow very unsteady in this preparation.
DYE DILUTION IN HEART-LUNG

surface level was adjusted by raising the reservoir until it again approximated the pointer level. A 2 ml. sample of dyed plasma was introduced into the reservoir and allowed to equilibrate. The reservoir was gently shaken and blood returning through the arterial tubing was allowed to run down the wall sufficiently to permit thorough mixing of dye in the external volume. After 4 minutes, a small sample of blood was taken from the system, and this sampling was repeated at 5, 6 and 7 minutes.

All blood samples were centrifuged; total and packed cell volumes were read, plasma samples were removed and diluted with saline until they could be read on the midscale of a Beckman Model B spectrophotometer at 620 mp, using appropriate prepared blanks. Dilutions of the initially injected dyed plasma were also prepared and read, recalling the necessity for adding small amounts of stabilizing "carrier" plasma.2

Computations and Data Processing

The principal aim of the investigation was to compare the volume of the system as determined from curve analysis, with the volume determined by the equilibrium method. In this comparison (fig. 2), the raw data were:

\[ D_i = \text{corrected optical density of the } i^{th} \text{ plasma sample, obtained as the product of observed optical density and dilution factor.} \]

\[ D_0 = \text{corrected optical density of dyed plasma initially injected, obtained as the mean product of density of 3 diluted samples and dilution factors.} \]

\[ D_{eq} = \text{mean density of plasma samples taken after equilibration in the second part of the experiment.} \]

\[ \Delta v_i = \text{volume of blood in the } i^{th} \text{ sample.} \]

\[ v_{eq} = \text{volume of dyed plasma introduced for equilibration.} \]

\[ v_{ext} = \text{external volume of equilibration system (reservoir and tubing).} \]

\[ H = \text{mean hematocrit of blood samples multiplied by 0.97 to correct for trapped plasma.} \]

Processing the data included the derivation of:

\[ v_1 = \text{cumulative volume of blood measured midway during collection of the } i^{th} \text{ sample (fig. 2).} \]

\[ v_{av} = \frac{\sum (D_1 v_1 \Delta v_i)}{\sum (D_1 \Delta v_i)}, \text{i.e., centroidal blood volume or system blood volume obtained from curve analysis.} \]

\[ v_{av} = \frac{v_0 - D_0 / (D_0 (1-H))}{D_{eq}}, \text{system blood volume determined by equilibration method.} \]

\[ \Delta_e = v_{av} - v_{ext}, \text{difference between result of 2 methods.} \]

The raw sample data were punched on Hollerith punched cards and the summation calculations were performed with standard IBM equipment including the Model 602A calculating punch (with the assistance of the Service Bureau Corporation). In some experiments, correction was made for prematurely truncated tails (fig. 2 and appendix). Correction factors were obtained by plotting the last few points on semilog paper and extrapolating.

An important test of the validity of the results was a comparison of the total volume of plasma actually collected with a similar value estimated from the dye curve. Data derived for this purpose were:

\[ v_p = \text{volume of dyed plasma injected in single pass experiment (usually 1 mg.)} \]

\[ v_{tot} = \text{sum of } \Delta v_i, \text{i.e., total volume of blood collected.} \]

\[ D = \frac{\sum (v_1 D_1 \Delta v_i)}{v_{tot}}, \text{i.e., mean plasma optical density of samples.} \]

\[ v_{pl} = D_0 \frac{v_0}{D}, \text{i.e., total volume of plasma collected as estimated from curve analysis.} \]

\[ \Delta_p = v_{pl} - v_{tot} (1-H), \text{difference between results of 2 methods.} \]

All the successful experiments were subjected to statistical analysis. Tests were applied to the means of \( \Delta_e \) and \( \Delta_p \) for significance of deviation from zero. In determining the variance of the data, experiments were subdivided into separate groups. Group 1 included the first 11 experiments, conducted during the earlier portion of the research at a time when techniques were still being perfected. Group 2 included 7 later experiments, in which dyed plasma was injected into the connecting tubing at the junction with the venous cannula. Group 3 included all later experiments, in which dyed plasma was injected through a long needle beyond the cannula tip.
Preliminary statistical analysis indicated that group 1 data were not in conflict with the other 2 groups, but because of the superiority of the techniques in the later experiments these data were removed from further consideration. In further analysis, 2 other factors besides group were considered to be of possible importance in interpretation of the results. One factor was steadiness or unsteadiness of flow, and the other largeness or smallness of the tail correction. Analysis of variance indicated that the \( \Delta c \) values contained a component of variance significantly greater between groups than among individuals. This was shown to lie primarily in the group 2 to 3 comparisons. The appreciable degree of interaction and serious disproportionation of the data required that this 2 x 2 x 2 system be analyzed by the method of fitting constants.3 The effect was found to lie predominantly in the group comparisons yielding an F ratio which was significant at the 5 per cent level. Group means and variance data were computed and tested by standard methods.

Results

The cardiac outputs obtained from direct timing of blood flow are shown in column 6 of table 1. The rates computed from curve analysis are given in column 7. Values were obtained by reducing \( v_i \) to whole blood volumes and dividing by collection times. The preparation volumes (heart and lungs) determined by the 2 methods are also shown in the table. Column 8 shows the volumes computed from equilibrium dilution data and column 9 the "central" volumes computed from the curve centroidal values. Column 4 shows the estimated values of the correction factors applied to compensate for incomplete tail areas in some experiments. Column 5 shows the corresponding tail corrections for the centroid.

Further consideration of the differences between the elements of columns 6 and 7 provide an estimate of the reliability of the dye techniques. The mean of the differences (column 7 minus column 6) is 3.05, with a standard error of 3.0, indicating a nonsignificant difference between the 2 methods of flow determination.

Discussion

Principle of Flow Measurement by Label Techniques

The indicator dilution method for measuring fluid flow has not been completely understood by some workers who, on occasion, have even expressed skepticism as to its basic validity. In order to obtain a clear picture of the essential postulates, it will be convenient to consider the principle from the point of view of volume measurement rather than measurement of flow rate. The manner of injection of the label is immaterial as long as it all enters the circulation. Expressed in this manner, the principle is nothing more than an expression of the fact that all label injected must ultimately appear at the outflow of a system, provided that none is lost by an alternative route or by permanent attachment within the system. The theoretical analysis must, of course, be applied only to a single pass curve, or to data in which the single pass curve can be adequately constructed in the presence of multiple passes. Ignoring the difficulties which might occur in practice, let us imagine for the moment that such a single pass curve has been described from data (fig. 2). We will assume that the tail correction is negligible, or that if the data are actually as indicated in the figure, appropriate correction can be made. The optical density and concentration are proportional to one another and the constant of proportionality cancels out in computations of cardiac output and central.
volume. Thus, we may use concentrations in our further discussion. Let the ordinate values be label concentrations $C_i$ in units such as mg./ml. Let the abscissal values be volume collected and suppose that this represents volumes of total blood obtained in serial sampling containers such as the graduated tubes used in our present experiments. The volume in a given sample $i$ will be $\Delta v_i$. The amount of label in each tube is the product $C_i \Delta V_i$ obtained by multiplying the mg./ml. of label by the volume in ml. The total label recovered in all samples through sample $i$ is the sum of such values starting from the initial one, i.e., the area under the curve up to the end of the $i^{th}$ sample. If all injected dye is recovered, this total amount will be the area under the curve extending to infinite values of volume or time. Depending upon how slowly the dye appears, the total label injected may be recovered to a sufficient order of precision at relatively small abscissal values or much larger ones, but ultimately all label should be recovered, whether loss into extravascular spaces occurs or not (excluding binding or excretory loss). Furthermore, nothing is postulated with regard to such factors as the shape of the curve, or rate of flow. Actually, the shape of the curve can be changed into a pure rectangle, provided that the area is unaltered. If the horizontal dimensions of the rectangle are great enough to extend to $\nu_{tot}$ and embrace all abscissal values during which significant amounts of label are still being recovered, then the height is the mean value of the concentration $C$ taken over that interval. Whatever the means by which $C$ may be determined, if it is known and if the amount $I$ of injected label is known, then the width $\nu_{tot}$ of the rectangle may be computed without direct measurement from $\nu_{tot} = I/C$.

Let it be assumed that values of cumulated volume specifically enter into the determination of $C$, we may recall that concentration may be expressed as a function of any abscissal variable and its mean value will be determined on the basis of equal area between the equivalent rectangle and the actual curve irrespective of the horizontal scale units. When, as is usually the case, the abscissa is in units of time measured, perhaps from the timing signals on a recorder chart, this provides the necessary scale factor to convert volume into flow rate or cardiac output. In the present case, this was the time $\Delta t$ required to collect all of the blood. When divided into the volume values obtained by the 2 methods, this yielded the data in columns 5 and 6 of the table. The fact that these 2 values do not differ significantly is merely an expression of adequate technique and the validity of the postulate that all of the label went into the system and was recovered.

This approach yields 1 or 2 obvious general consequences in the extension of the technique to situations where the basic postulates have not been adequately met. One is encountered where the complete recovery of label at the outflow has not been achieved. Here, if the percentage of unrecovered label is known, it will be sufficient to increase the measured value of $C$ by this percentage, thus increasing the area of the equivalent rectangle to its estimated proper value. This is the basis for applying the factors in column 4 of table 1. In the experiment in line 4, columns 6 and 7 would have been highly discrepant without the use of the factor in column 4. Other instances, though less successful, frequently yielded a significant improvement in agreement.

Another consequence of the theoretical analysis permits an estimate to be made of the effect of irregular flow on the result of indicator dilution measurements of cardiac output. In the experiment in line 5 of the table, flow was irregular but good agreement was still obtained between columns 6 and 7. This was because the present determinations were based on measured and computed volumes rather than on rates of flow. In practical dye studies, this will not be the case. Since the principle is based on the assumption of uniform flow rate, the extension to variable flow rates would be of interest. Again, from the over-all relation that dye injected equals dye...
recovered, as computed for the area under the dye curve,

\[ I = \int_0^\infty C(t) Q(t) \, dt. \]

Since \( Q(t) \) is no longer constant, it cannot be factorized out in front of the integral sign except by defining the quantity \( \bar{Q} \) as a weighted arithmetic mean value of the variable \( Q \)'s, the weighting factor being \( C(t) \).

The generalized expression equivalent to the conventional one now is:

\[ \bar{Q} = \frac{1}{\infty} \int_0^\infty C(t) \, dt, \]

and since the value of \( \bar{Q} \) is weighted in favor of the peak concentrations, if flow happened to be high during the peak period, the result would be erroneously high.

**Central Volume Measurements**

The measurement of the volume of an isolated heart-lung preparation differs from the measurement of "central volume" in an intact organism, but the present results are nevertheless instructive in several respects. First of all, it is clear that the differences between the 2 methods are subject to more fluctuation than the cardiac output differences. On the average, column 8 values in group 3 exceed column 9 by only 3.9 ml., but the least significant difference at the 5 per cent level is 13.3 ml. The origin of a significant difference in group 2 is not clear. The mean of the \( \Delta e \)'s is −37 with a standard error of 10.9 ml. The group included several of the largest animals studied. We may also speculate that perhaps simple injection of dyed plasma into the tube leading to the venous cannula is a less satisfactory procedure, and that the results in group 3 favor the use of a long injection needle. It is also true that group 3 included a larger number of experiments in which flow was more satisfactorily controlled and a more complete curve was obtained.

Particular attention was paid to the possible effects of variable flow in the volume determinations. Studies of the competence of the preparations repeatedly showed that cardiac output readily responded to changes in venous pressure. Thus, the best criterion of steadiness of this pressure was uniform cardiac output and with it uniformity in the pressure drop across the arterial catheter, as shown by uniformity of pressure in the aortic strain gage record. When variation occurred in this pressure, particularly between the single pass and equilibration stages in the experiment, the possibility existed that variable distention might occur on the venous side and with it differences in the volumes determined by the 2 methods. Statistical comparison between experiments where flow was deemed unsteady with those showing steady flow records failed to indicate any significant effect related to this cause.

No significant difference occurred between experiments in which the tail correction factor was large and those where a smaller correction was required. If, by premature truncation, only 5 per cent of the total area of the curve is lost, even an inaccurate correction procedure for \( C \) may suffice to reduce the error in the result. However, a 5 per cent loss in area may represent a larger loss in the moment of this area taken about the origin of the curve.

The centroidal volume may be taken as the horizontal abscissal distance from the origin to the centroid of the curve (fig. 2). The summing of the products of the areas of the elementary strips by their distances from the origin is analogous to computing the turning moment of these strips about the origin, if the curve were cut in a vertical plane from some uniform material of constant thickness. In this case the centroid would be the point where all of the mass of the curve could be concentrated to produce the same turning moment, or alternatively the point at which the curve would just balance. Clearly the moments exerted by the elements in the tail depend not only on their areas but also on their relatively large horizontal distances from the origin. In this respect, then, the tail error may be considered as more important in the case of determining "central volume." It is surprising that errors in estimating the correction factors did not usually produce sig-
significant differences, even when the corrections were rather large. Only the experiment in line 4 of table 1 seemed to be inadequately corrected. One may regard the difficulty in estimating the tail effect as a serious one in the intact animal. Not only is the tail important, but its effect is difficult to assess in the presence of recirculation. It is also particularly subject to distortion by recording devices.

By dividing the values in columns 8 or 9 in table 1 by the animal weights in column 1, the volumes may be expressed as ml./Kg. The mean value of 12.3 compares very favorably with 11.6 obtained by Schlant et al. Their values and ours apply to a highly artificial system. We must reserve judgment concerning volumes in the intact animal which, as observed by some methods, seem to be considerably larger.

Verification of the Central Volume Principle

Since the results in table 1 can be accepted as indicating, in this instance at least, that the centrolid volume of a single pass indicator curve obtained from a bolus injection can be used as a measure of the volume of a circulatory labyrinth, it is pertinent to consider what factors might have produced disagreement. Excluding methodology which seems adequate, discordant results can arise through the violation of one or more basic postulates of the method. We review these as follows: 1. All dye which enters the labyrinth must be recovered. The amount need not be known. 2. The dye curve must provide a record equivalent to or proportional to the concentration of uniformly mixed samples at the outflow. This is equivalent to a record of mean label flux. 3. Label and substance being traced (in this case blood) must behave identically. 4. The horizontal scale of the dye curve must be in units of volume throughput or else adequate conversion to these units must be possible. 5. The concentration of label must be uniform over the cross section of fluid entering the system. If this is true, the amount of label entering the system will be distributed among the flow laminae in proportion to their individual velocities.

Summary

In a large series of canine heart-lung preparations, system volumes were determined by the T 1824 dye bolus technic of Hamilton. Results were compared with volumes independently determined by equilibration of label in a recirculating system. The mean differences of values in the largest group of experiments was 3.9 ml., and was not significantly different from zero. A small but significant mean difference in a second smaller group is discussed relative to the basic postulates of the method.

Appendix

Estimation of Tail Correction Factors

Suppose that the experimental data terminally follow the relation:

\[ R = R_o e^{-0.693 v/v_{tot}} \]

A semilog plot will then be linear and \( R_o \) can be obtained from its intersection with the axis \( v = 0 \). At the point where \( R = 0.5 R_o \), the abscissa will be \( v_{1/2} \), thus determining this parameter. The missing tail area is obtained by integration from the truncation point \( v_{tot} \) to infinity, thus

\[ A_t = R_o \int_{v_{tot}}^{\infty} e^{-0.693 v/v_{1/2}} \, dv \]
The missing component of moment is similarly
\[ M_1 = R_0 \int_{v_{1/2}}^{\infty} v e^{-(0.693 v^2/v_{1/2})} \, dv, \]

If \( A \) and \( M \) are the truncated area and moment, respectively, of the curve, the factors to be applied are \((A + A_1)/A\) and \((M + M_1)/M\), respectively.

In determining the centroid, the factor is \((1 + M_1/M)/(1 + A_1/A)\).

Acknowledgment

The writers are indebted to Dr. David L. Yudilevich for a stimulating discussion of the results of this work.

Summario in Interlingua

In un grande serie de preparatos cardio-pulmonar canin, le volumenes del systema esseva determinate per le teehnica de Hamilton con le colorante T 1824. Le resultatos esseva comparate con le volumenes de-terminate independentemente per le equilibration del marca in un systema recirculante. Le differentia medie del valores in le plus grande gruppo de experi-mentes esseva 3,9 ml, i.e., non significativemente differente de zero. Un miere sed significative differentia medie obtenite in un altere serie que esseva minus extense es discutite in relation al postulates fundamental del metodo.

References

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