Determination of Circulating Pulmonary Blood Volume in Dogs by an Arteriovenous Dye Equilibration Method

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The "equilibration" method introduced by Bradley to measure circulating "splanchnic blood volume" has been applied to the estimation of "pulmonary blood volume." The theoretic and practical aspects of the method are analyzed. Values for circulating "pulmonary blood volume" determined in anesthetized dogs were not significantly different from values obtained by the "mean circulation time output" method and were significantly greater than those determined by the "slope output" method. When experimental conditions were varied, relatively parallel changes were recorded by the three methods. It is believed that the "equilibration" method is a valid one for estimating circulating "pulmonary blood volume."

Determinations of the pulmonary blood volume in the intact subject have been based upon analysis of the arterial dilution curve of an intravascularly retained substance, usually dye, following its injection proximal to the pulmonary circuit. According to the method of Stewart 1 modified by Hamilton and colleagues, 2, 3 the volume of blood from the site of injection to the site of sampling has been considered to be the product of the pulmonary blood flow, or cardiac output, and the mean circulation time of the dye. The cardiac output and the mean circulation time may both be determined from the arterial dye concentration curve. This method will be referred to as the "mean circulation time output" method.

Recently Newman 4 has analyzed the theoretic and practical considerations involved in the dye dilution technic and has concluded that the magnitude of the volume of blood in the pulmonary vascular system can be calculated from the ratio of the cardiac output to the slope of the exponential part of the downstroke of the same dye dilution curve. Diluting volumes, such as the chambers of the heart and a portion of the arterial system, which are small in comparison with the pulmonary blood volume, are presumably not measured by this method, which will be referred to as the "slope output" method.

The mean circulation time output measurement includes only that volume of blood with which the dye mixed in its first passage through the lungs. In severe congestive heart failure or other conditions in which the mean circulation time may be greatly increased, an invalid dye-dilution curve may be obtained, because recirculating dye may obscure the final portion of the primary curve. 5 The slope output method relies upon the assumptions that simultaneous and homogeneous mixing of the injected substance with the pulmonary vascular volume takes place and that loss of dye from the pulmonary bed into the arterial system is an ex-
ponential function of the volume of the largest
diluting chamber. The validity of these
assumptions, particularly under abnormal cir-
stances, has not as yet been firmly

The approach introduced by Bradley to the
measurement of "circulating splanchnic blood
volume" has been applied to the pulmonary
circulation. This method allows sufficient time
for equilibration of dye to take place within the
pulmonary circulation and does not depend
upon assumptions of immediate and complete
mixing during one dye circulation. This method,
therefore, may be helpful in assessing the va-

PRINCIPLE OF METHOD

The amount of a substance remaining within a
segment of the vascular system following its
introduction into the general circulation may be
estimated as the difference between the total
amount entering and leaving that segment.
This can be calculated for any area in which the
blood flow can be measured and from which
continuous blood samples representative of
inflow and outflow can be obtained. The
amount of substance entering any vascular
compartment is equal to the blood flow multi-
plied by the mean concentration in the blood
entering that area; whereas the amount of
substance leaving is equal to the blood flow
multiplied by the mean concentration in the
emerging blood. The difference between these
values represents the amount retained. When
equilibration of the substance within the seg-
ment has been completed, the concentration
entering the segment will be the same as the
concentration leaving. At this time, the con-
centration within the entire segment is pre-
sumably essentially the same as that entering
and leaving. Dividing the amount of substance
retained within the segment by its concentra-
tion at equilibration yields the volume of dis-
tribution of the substance within that area.

This principle, established by Bradley, has
been applied to the measurement of "pul-
monary blood volume" in the following
manner:

When Evans blue dye is injected into the
systemic venous system, the amount entering
the pulmonary vascular tree during the period
of equilibration is equal to the cardiac output,
or pulmonary blood flow, multiplied by the
mean pulmonary arterial concentration of dye
during that period. The amount of dye leaving
the pulmonary vascular system during this
period equals the pulmonary blood flow multi-
plied by the mean pulmonary venous dye con-
centration. The difference between these values
is the amount of dye remaining within the pul-
monary vascular system at equilibration.

Because of practical difficulties in obtaining
mixed pulmonary venous blood, samples are
taken from a peripheral artery. Equilibration
of dye within this system is considered to be
complete when pulmonary arterial and periph-
eral arterial dye concentrations become
identical. At this time the concentration of dye
within the pulmonary vascular system is as-
sumed to be identical with the concentration
in the blood entering and leaving the system.
The pulmonary blood volume is therefore
determined by dividing the calculated amount
of dye remaining in the system at equilibration
by the concentration of dye at equilibration.

Stated mathematically,

\[ PBV = \frac{CO \times (PA_{mt} - FA_{mt})}{C_{eq}} \]

where \( PBV \) = circulating blood volume within
the pulmonary vascular system, left atrium,
left ventricle, and large arteries, in cubic
centimeters; \( CO \) = cardiac output, in cubic
centimeters per second; \( t \) = time, in seconds,
until equilibration; \( PA_{mt} \) = mean pulmonary
arterial dye concentration during time \( t \), in
milligrams per cubic centimeter; \( FA_{mt} \) = mean
femoral arterial dye concentration during time
\( t \), in milligrams per cubic centimeter; \( C_{eq} \) =
dye concentration at equilibration, in milli-
grams per cubic centimeter.

PROCEDURE

The circulating pulmonary blood volume was
measured by the "equilibration" method 23 times in
17 dogs anesthetized with Nembutal and compared
with volumes obtained by the mean circulation
time output and slope output methods. A no. 7
cardiac catheter was inserted into the pulmonary
artery, a short-length no. 8 catheter into the right
atrium, and an indwelling Cournand needle into each femoral artery. A no. 7 catheter was connected to one femoral arterial needle in order to minimize dead space differences between femoral and pulmonary arterial collections. One minute following the collection of arterial and mixed venous bloods and of a five-minute expired air sample for a direct Fick cardiac output determination, 3 to 5 mg. of Evans blue dye were injected directly into the jugular vein or, by catheter, into the right atrium. Dye injection took approximately 0.5 seconds. The Gibson dye-injection syringes were calibrated by weight to deliver standard volumes through a catheter or needle. In the first four determinations blood samples were collected manually; thereafter specimens were collected continuously by means of a constant suction pump* from pulmonary and femoral arteries at 25-second intervals for 200 to 300 seconds. Collection tubes were changed by hand after each period.

The average variation in the volume of the samples collected with the pump was 1.7 per cent. The mean maximal variation in any determination was 4.1 per cent. The variation was largely due to error in determining the precise time of collection. This variation in sampling is correctable and introduces no appreciable error in the pulmonary blood volume determinations, whereas errors would be introduced if flow were inconstant. Flows and tubing dead space were adjusted so that the calculated delay times from pulmonary and femoral arteries to the sampling sites were similar.

Samples at two-second intervals were collected at the same time in a multiple fraction collector† from the opposite femoral artery for dye curve analyses. Flows approximately 0.7 to 0.8 cc. per second were obtained through a polyethylene segment 1.0 cc. in volume. Ten- and 20-minute blood samples for total plasma volume determinations were also analyzed. All samples were collected in tubes calibrated to 0.1 cc. containing purified dry heparin‡ which added no color to the specimens. Hematocrits were spun in Wintrobe tubes for 30 minutes at 3,000 r.p.m.

Dye concentrations were determined in undiluted plasma with a Beckman DU spectrophotometer at 620 Å. Microuettes§ which permitted analysis of samples with volumes as small as 0.1 cc. were used for the small two-second specimens, whereas the larger samples collected for the equilibration method study were read in standard Beckman Corex cells.

Density values were essentially identical using either type of cuvette; however, it was believed that greater precision was possible with the Beckman macrocuvette cells. Standard dye curves were constructed using dilutions of dye in undiluted dog plasma. Oxygen analyses were performed by the method of Van Slyke and Neill.

Cardiac output was calculated by the dye dilution method according to Hamilton's modification of the principles introduced by Stewart.

\[
CO = \frac{60I}{C_i} \times \frac{1}{1 - hct}
\]

where \(CO\) = cardiac output, in cubic centimeters per minute; \(I\) = amount of dye injected, in milligrams; \(C_i\) = mean plasma concentration of dye during its first complete circulation, in milligrams per cubic centimeter; \(t\) = seconds for first circulation of dye; \(hct\) = hematocrit.

The pulmonary blood volume was calculated by the mean circulation time output method from the formula

\[
PBV = \frac{CO \times MCT}{60}
\]

where \(PBV\) = volume of blood from point of injection to point of sampling, in cubic centimeters; \(CO\) = cardiac output, in cubic centimeters per minute; \(MCT\) = mean circulation time of dye in seconds.

"Central blood volume" was also calculated by the slope output method analyzed extensively by Newman and colleagues, by the formula

\[
CBV = \frac{CO}{m}
\]

where \(CBV\) = pulmonary blood volume, in cubic centimeters; \(CO\) = cardiac output, in cubic centimeters per minute; \(m\) = slope of the exponential downstroke of the arterial dye dilution curve in milligrams per cubic centimeter per minute.

"Circulating pulmonary blood volume" was calculated by the equilibration method according to the formula as previously stated.

**RESULTS**

The cardiac output, as determined by the dye dilution technic, agreed well with that determined with the direct Fick method (fig. 1). The average ratio of the cardiac output obtained in individual animals by the dye dilution

\[
\frac{\ln C_2 - \ln C_1}{t_2 - t_1} \times 60
\]

where \(C_1\) and \(C_2\) are dye concentration values on the exponential downstroke at times \(t_1\) and \(t_2\).
procedure to the output obtained with the Fick method was 1.02 ($\sigma = 0.18$). Evidence for a systematic error could not be discerned. In all but four of the 23 measurements, the cardiac output measured with the dye dilution technic fell within $\pm 25$ per cent of the result obtained with the direct Fick method. The four instances in which the difference was more than 25 per cent were eliminated from the analyses of pulmonary blood volume because of the possibilities of lack of steady state or of faulty technic.

In the remaining 19 measurements in 15 dogs, determinations of pulmonary blood volume with the equilibration method yielded results which were not significantly different from those measured with the mean circulation time output method (fig. 2) but were probably significantly higher than those determined with the slope output method (fig. 3).

The average value for pulmonary blood volume by the equilibration method was 535 cc., or 227 cc. per 10 Kg. body weight ($\sigma = 92.5$ cc. per 10 Kg.) (table 1). This represented 27.2 per cent of the total blood volume. The rather large standard deviation was caused in part by the varied levels of anesthesia and by the changes in total blood volume produced by a prior determination, as well as by the rather large variability inherent in these determinations. Several determinations gave results which were disproportionately small and were probably in error, although the source of such error could not be detected.

The average value for pulmonary blood volume obtained with the mean circulation time output method was 603 cc. or 256 cc. per 10 Kg. ($\sigma = 59.7$ cc. per 10 Kg.) representing 30.7 per cent of the total blood volume (table 1). The values obtained by the mean circulation time output method are compared
Table 1.

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Mean, cc. ................................................. 398 ± 693 535 1965
Mean, cc./10 Kg. ....................................... 160 ± 256 227 833
Standard Deviation, cc./10 Kg. ...................... 51.2 ± 59.7 92.5
% Total Blood Vol. ..................................... 20.3 ± 30.7 27.2

Experiments Eliminated from Statistical Analysis Because of Poor Check Between Cardiac Outputs Determined by the Dye Dilution and Fick Methods

3B  23.2  3.43  2.62  4.41  18.0  0.2628  0.2349  0.204  116.4  778  1029  910  2888
13A 28.7  2.65  4.09  5.04  16.0  0.5353  0.4606  0.357  109.6  538  746  1013  2732
16  26.8  3.53  2.90  11.10  9.42  0.4857  0.4611  0.411  88.5  318  554  313  1170
17  26.8  3.53  2.82  10.00  10.2  0.5482  0.4832  0.403  105.5  388  651  1086  1865

PA = Pulmonary arterial. FA = Femoral arterial.
* 9 per cent body weight.

with those obtained with the equilibration method in figure 2. A statistically significant difference could not be demonstrated between these methods ($P = 0.1$). The positive coefficient of correlation ($r = +0.60$) was statistically significant.

The average value obtained by the slope output method was 398 cc., or 169 cc. per 10 Kg. ($r = 51.2$ cc. per 10 Kg.), representing 20.3 per cent of the total blood volume. Volumes measured by the slope output method were probably statistically significantly lower than those measured by the equilibration method ($0.02 > P > 0.04$) and definitely significantly lower than volumes measured by the mean circulation time output method ($P < 0.001$). This is in accord with the theoretic consideration that the slope output method probably measures a smaller volume than that measured by the other two methods. A comparison of the values determined by the slope output and equilibration methods appears in figure 3. Except for the four anomalously low values obtained by the equilibration method, the smaller magnitude of the slope output measurement is evident. Statistically significant
positive correlations were obtained between these two methods \( r = +0.66 \) and between the slope output and mean circulation time output methods \( r = +0.79 \).

Determinations of cardiac output and pulmonary blood volume were performed twice in succession in the same animal on six occasions. The values for cardiac output and pulmonary blood volume obtained during the repeated determination differed from the initial results because of alterations in the depth of anesthesia and because of the removal of 150 to 200 cc. of blood during the previous determination. These changes in cardiac output and pulmonary blood volume as recorded by the three methods are illustrated in figure 4. In five of the six sets of observations, the pulmonary blood volume as measured by these methods varied together and changed in the same direction as the changes in cardiac output. In the sixth animal, the pulmonary volume obtained by the slope output method decreased paradoxically, whereas the values by the other two methods and for the cardiac output remained nearly constant.

**DISCUSSION**

The basic assumptions upon which the equilibration method rests are as follows:

1. It is assumed that at equilibration the dye is evenly distributed throughout the total volume of blood to be measured. The criterion for equilibration is the identity of dye concentration in the pulmonary and peripheral arteries. Only if there is even distribution of dye at this time will pulmonary and peripheral arterial dye concentrations be representative of the dye concentration within the volume between these points. In a system which normally virtually replaces its volume five times a minute, mixing of dye within the actively circulating blood would seem adequate to validate this assumption. However, if there exist blood depots within the lungs which are not in direct communication with the circulating blood volume and with which exchange of dye cannot occur, the volume of these depots will not be included in the measurement. Despite this limitation, the method allows a longer time interval for mixing of dye to occur within the accessible pulmonary blood volume than meth-

![Fig. 4. Initial values for cardiac output (liters per minute per 10 kilograms body weight) measured by the dye dilution technic and for pulmonary blood volume (cubic centimeters per 10 kilograms) as determined by the equilibration, mean circulation time output, and slope output methods, and the changes in these values following the removal of 150 to 200 cc. of blood and the alteration of the depth of anesthesia. Asterisk denotes poor checks obtained between the cardiac output measured by dye and Fick methods in the initial determination in dog 3 and in the final determination in dog 13.](http://circres.ahajournals.org/)
ods currently in use, which rely upon dye curve analyses involving a single circulation of dye through the lungs. The time required for equilibration by this method is sufficiently long to encompass several recirculations and may be extended for as long a period as is necessary.

2. It is assumed that significant loss of dye from the pulmonary capillaries or phagocytosis by the pulmonary reticuloendothelial system does not occur during the period of equilibration. In the normal subject, this is probably a valid assumption.10-13 When pulmonary capillary pressures are elevated to pulmonary edema levels, however, the possibility of significant transcapillary dye loss must be considered. Although this objection also applies to other dye methods, the presumed advantage of more adequate time for mixing afforded by this method also allows longer time for extravascular loss. If red cell instead of plasma tags were used under these conditions, more accurate approximation of pulmonary blood volume might be obtained.

3. It is assumed that the hematocrit of the pulmonary vascular bed is essentially the same as that in the pulmonary and femoral arteries. This assumption is also implicit in the other dye methods. However, it has been demonstrated that the hematocrit within the small vessels of the lung is considerably lower than that in the large peripheral vessels.14 The mean circulation time for radioactive red cells also has been shown to be shorter than that for Evans blue.12-13 The error introduced by the assumption of equal hematocrit can be minimized by simultaneously determining both pulmonary red cell and plasma volumes using tagged cells and dye.

4. It is assumed that cardiac output and pulmonary blood volume remain constant during the determination. The measurements, as performed in this study, required the sampling of 30 to 45 cc. of blood a minute during the two- to three-minute period required for equilibration. This may constitute a not insignificant proportion of the total blood volume of a small animal. Alterations of cardiac output and pulmonary blood volume probably occur during the procedure. However, these changes are of significance only during the first 30 to 60 seconds, at which time the difference between pulmonary arterial and femoral arterial dye concentrations is large. The cardiac output measured by the dye dilution technic simultaneously performed with the pulmonary blood volume determinations represents the flow during the first 20 to 30 seconds of that determination. This has checked well with flows obtained by direct Fick oxygen analysis immediately preceding the dye injection. The cardiac output, therefore, remained stable at least during the first half minute. After this period, changes of flow and pulmonary volume may occur without greatly influencing the determination of the volume, since the arteriovenous dye concentration difference has become small.

5. Another assumption is that there exist no abnormal or anastomotic vascular pathways by which blood may bypass the catheter sampling from the pulmonary artery. The presence of significant right-to-left intracardiac shunts through an atrial or ventricular septal defect would invalidate the method because dye, undetected in pulmonary arterial samples, might greatly influence the femoral arterial concentration. Only a minimal error, however, is introduced by the presence of anastomotic flow between the bronchial and pulmonary vascular system, a flow which may assume significant proportions in pathologic situations.15 The amount of dye deposited or removed by the bronchial circulation would depend on both the magnitude of the anastomotic flow and the difference between the mean dye concentration entering the pulmonary volume by way of the bronchial arteries and the mean dye concentration leaving the pulmonary volume. It is improbable that mean bronchial arterial concentrations, that is, the concentration of dye entering the pulmonary volume, differs significantly from mean femoral arterial dye concentrations, that is, the concentration of dye leaving. Therefore, it can be concluded that the errors introduced by this type of anastomotic flow upon the calculation of pulmonary blood volume can be safely neglected.

The applicability of this method also depends
upon the ability to measure accurately the factors entering the formula:

\[ PBV = \frac{CO \times (PA_{mt} - FA_{mt})}{C_{RQ}} \]

The cardiac output can be determined with reasonable precision with either the Fick or dye methods.14-20

Reliable results for mean pulmonary and femoral arterial dye concentrations can be obtained only if each sample represents an accurate time integration for the period of collection. A constant rate of collection is essential if the samples are to be truly integrated with respect to time. Varying rates of flow during the early seconds of collection when dye levels are rapidly changing may produce great errors. With the constant suction pump, satisfactorily constant flows can be obtained.

Pearce and collaborators21 have emphasized that a sampling system with a long time constant, like that used in obtaining the mean pulmonary and femoral arterial dye concentrations in these experiments, produces distortion of the dye dilution curve. Little or no error is introduced in the determination of mean dye concentration, since sampling is continued for a considerable period after the slope of the falling dye concentration curve has become quite gradual. The true configuration of the arterial dye dilution curve was simultaneously obtained from the opposite femoral artery using a sampling system with a time constant of less than one second.

The most serious practical difficulty in the actual determination is measuring the arteriovenous dye differences with sufficient accuracy. These arteriovenous differences may represent only 10 per cent of the dye concentration levels analyzed. An error of one per cent may therefore be magnified tenfold. It is for this reason that all dye analyses are performed on undiluted plasma using standard Beckman cuvettes. In this way analytic errors can be reduced to 1 per cent or less.

We have arbitrarily selected equilibration as that period during which the pulmonary and femoral arterial concentrations are identical or no further than 1 per cent apart, and following which they do not significantly diverge. It is apparent, however, that after pulmonary and femoral arterial concentrations have approximated each other, the calculation of circulating pulmonary blood volume will vary but little, no matter what time of equilibration is selected, since the opposite effects of added time upon the total blood flow and mean arteriovenous difference cancel each other.

The results obtained in dogs indicate that the equilibration method may be applied to the approximation of pulmonary blood volume. That approximately 27 per cent of the total blood volume is located in the heart, lungs, and large arteries is consistent with most experimental data.1-3, 22, 23 The fairly close agreement obtained by this method with the mean circulation time output method would tend to substantiate both approaches, at least under the conditions of these experiments. Both methods rely upon basic assumptions which are not completely validated; these assumptions are different, however, for each method. Although it is possible that systematic errors arising from different incorrect assumptions might coincidentally lead to good agreement, it is more probable that similar volumes are being measured. Until an absolute method is devised for measurement of pulmonary blood volume, the validity of either method cannot be unequivocally decided.

Theoretically, the mean circulation time output method should result in a somewhat higher figure than the equilibration method, since the volume of the right atrium and right ventricle is measured by the first method, as performed in these experiments, but not by the second method. The slightly lower mean value observed with the equilibration method, however, was not significantly different statistically from the mean value obtained with the mean circulation time output method.

The smaller volumes measured with the slope output method are to be expected on theoretic grounds. On the basis of mathematical and mechanical considerations, it is postulated that only the largest diluting volume, that is, the volume of the pulmonary vascular system or a portion of it, is measured by this method.4, 5, 21 The comparison data in these experiments are consistent with this view.
The pulmonary blood volume determinations before and after the production of a small hemorrhage or following changes in depth of anesthesia indicate that changes in pulmonary blood volume are well reflected by the equilibration method. In all instances the values obtained by the equilibration technic varied in the same direction as the cardiac output and almost always paralleled changes in volume recorded by the other two techniques.

From the analysis of the assumptions upon which the equilibration method depends and from the fairly good correlation with the mean circulation time output method, it would appear that the equilibration method is a valid approach for the approximation of pulmonary blood volume in particular and of regional blood volume in general. These results would therefore also tend to confirm the validity of Bradley’s measurements of circulating splanchnic blood volume. No standard exists with which results obtained by the equilibration method for circulating splanchnic blood volume can be compared. Although the standards available for comparison of pulmonary volume determinations have admittedly not been completely substantiated, the correlations obtained in this study provide inferential evidence that the equilibration approach is valid.

**SUMMARY**

A method of estimating circulating “pulmonary blood volume,” involving continuous sampling of blood from the pulmonary and femoral arteries following proximal dye injection, has been described.

Circulating pulmonary blood volume was measured by the “equilibration” method in anesthetized dogs. The values determined were similar to those obtained with the “mean circulation time output” method and were statistically significantly higher than those obtained with the “slope output” method.

When experimental conditions were varied, relatively parallel changes in pulmonary blood volume were recorded by the three methods.

It is concluded that the equilibration method is a valid one for estimating circulating pulmonary blood volume.

**APPENDIX**

**Calculations**

Circulating pulmonary blood volume was determined by the use of the formula

\[ PBV = CO \times \frac{t(PA_{mt} - FA_{mt})}{C_F} \]

A sample calculation, using hypothetical but characteristic figures, is presented in table 2. The mean pulmonary arterial concentration from the time of injection to the time of equilibration is first calculated.

In the first column appears the dye concentration of each pulmonary arterial sample, and in the second the concentration of simultaneously drawn femoral arterial samples. Equilibration was considered complete at the end of that 25-second interval at which the pulmonary and femoral arterial concentrations were identical or within 1 per cent of each other and after which they did not significantly diverge. In the third column is recorded the volume of each pulmonary arterial sample, as measured in the calibrated collection tubes. The time of collection of each sample is listed in the fourth column. This time, however, must be corrected for the fact that capacity of the catheter and of the tubing of the pump introduces a “delay time error,” that is, blood from the pulmonary artery does not actually appear in the collecting tube until some time after the beginning of collection. The magnitude of the delay time may be calculated as follows:

\[ \text{Delay time} = \frac{\text{Interval of collection (sec.)} \times \text{Vol. of dead space}}{\text{Av. vol. of samples}} \]

**Example:**

\[ \text{Delay time} = \frac{25 \times 3.5}{7.0} = 12.5 \text{ seconds} \]

Each sample usually represented a 25-second collection interval (Column V). If the volumes of the samples were not constant, each specimen would truly represent an interval somewhat greater or less than 25 seconds. Variations in volume of samples may be caused either by variation in the rate of sampling or by errors in the measurement of the time of collection. The method to be used to correct the collection interval depends upon which of these factors is responsible.

A. Correction of Interval for Collection Errors (Rate of Flow Constant). When the constant suction pump was used, as was the case in the illustrative table, the flow was presumed to remain constant and the small variations in the volume of samples to be
Table 2.—Calculation of Pulmonary Blood Volume by the Equilibration Method. (Calculation of Mean Pulmonary Arterial Concentration)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (c) of PA Sample mg. %</th>
<th>Concentration of FA Sample mg. %</th>
<th>Vol. of PA Sample cc.</th>
<th>Time of Collection of PA Sample sec.</th>
<th>Time Interval (Uncorrected) sec.</th>
<th>Time Interval (Corrected for Sampling Variation sec.)</th>
<th>Time of Collection Corrected for Delay &amp; Sampling Variation sec.</th>
<th>Concentration X Corrected Time Interval (c/t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.800 (1.578)*</td>
<td>0.350</td>
<td>7.1</td>
<td>0-25</td>
<td>25</td>
<td>12.9</td>
<td>0-25</td>
<td>20.356</td>
</tr>
<tr>
<td>2</td>
<td>0.400</td>
<td>0.625</td>
<td>7.0</td>
<td>26-50</td>
<td>25</td>
<td>25.0</td>
<td>13.0-37.0</td>
<td>10.000</td>
</tr>
<tr>
<td>3</td>
<td>0.350</td>
<td>0.370</td>
<td>6.9</td>
<td>51-75</td>
<td>25</td>
<td>24.6</td>
<td>38.0-62.5</td>
<td>8.610</td>
</tr>
<tr>
<td>4</td>
<td>0.340</td>
<td>0.350</td>
<td>6.8</td>
<td>76-100</td>
<td>25</td>
<td>24.3</td>
<td>62.6-86.8</td>
<td>8.262</td>
</tr>
<tr>
<td>5</td>
<td>0.325</td>
<td>0.325</td>
<td>7.0</td>
<td>101-125</td>
<td>25</td>
<td>25.0</td>
<td>86.9-111.8</td>
<td>8.125</td>
</tr>
<tr>
<td>6</td>
<td>0.320</td>
<td>0.318</td>
<td>7.1</td>
<td>126-150</td>
<td>25</td>
<td>25.4</td>
<td>111.9-137.1</td>
<td>ct = 55.353</td>
</tr>
<tr>
<td>7</td>
<td>0.315</td>
<td>0.316</td>
<td>7.1</td>
<td>161-175</td>
<td>25</td>
<td>25.7</td>
<td>137.2-162.8</td>
<td>t = 111.8 sec.</td>
</tr>
<tr>
<td>8</td>
<td>0.314</td>
<td>0.311</td>
<td>7.0</td>
<td>176-200</td>
<td>25</td>
<td>24.6</td>
<td>102.9-157.4</td>
<td></td>
</tr>
</tbody>
</table>

Mean PA concentration = \( \frac{55.353}{111.8} = 0.4956 \)  
Mean FA concentration = \( \frac{50.500}{111.8} = 0.4517 \)  
Cardiac output = 8.10 L/min.  
PBV = Cardiac output \times \frac{mean PA concentration}{mean FA concentration} = 780 cc.

Dead space, PA catheter and pump tubing = 3.5 cc.  
Delay time = 12.5 sec.

PA = Pulmonary arterial. FA = Femoral arterial.  
* Corrected for dilution in dead space.

Caused by errors in collection. These collection errors are correctable in the following manner.

Corrected interval = Uncorrected time interval (sec.) \times \frac{Vol. of sample}{av. vol. of samples}

Example (table 2, sample 1):

Corrected interval = 25 \times \frac{7.1}{7.0} = 25.4 seconds

The true time of collection (column VII) of each sample can now be calculated. For the first period, the delay time is subtracted from the corrected time interval (25.4 - 12.5 = 12.9 seconds). For the subsequent periods, the corrected time interval is added to the time of completion of the previous period.

B. Correction of Interval for Variations in Flow.

When the flow is not constant throughout the determination, a different type of calculation is necessary to correct the interval and to determine the true time of collection. It should be emphasized that this calculation is valid only if each sample is drawn at a constant rate, although that rate differs from the constant rate at which other samples are collected. When specimens are drawn manually, the constant rate of withdrawal of each sample in-variably varies from sample to sample. This situation may also be duplicated, using the constant suction pump, if the rate of collection is changed during the determination at the junction of one collection with another. However, if a progressive change in the rate of pump flow ensues, as might occur if a partial obstruction develops in the tubing, the determination is considered invalid. Under these circumstances each sample would not represent a true integrated mean concentration for the period of collection.

The corrected interval of collection for varying flows is calculated as follows: The delay time for each different flow rate must be calculated independently by the formula.

Delay time = Collection interval (sec.) \times \frac{Vol. of tubing}{Vol. of individual sample}

After calculation of each delay time, the true time of collection may be determined.

When the volume of sample for any period decreases, the delay time, that is, the time it takes for blood to pass from the artery to the test tube, is increased. That sample, therefore, must represent an interval of time which is smaller than the actual collection interval. The decrement in the true in-
terval which the sample represents is equal to the magnitude of the increase in delay time. In a similar manner, if the rate of collection is increased, the delay time is decreased and the sample represents an interval that is greater than the actual collection time by the decreased delay time.

Example (Catheter dead space = 1.0 cc):

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Vol. of Sample</th>
<th>Delay Time</th>
<th>Interval</th>
<th>Corrected Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.0</td>
<td>2.8</td>
<td>25</td>
<td>25 - 2.8 = 23.2</td>
</tr>
<tr>
<td>2</td>
<td>7.5</td>
<td>3.3</td>
<td>25</td>
<td>25 - (3.3 - 2.8) = 24.5</td>
</tr>
<tr>
<td>3</td>
<td>8.5</td>
<td>2.0</td>
<td>25</td>
<td>25 - (2.0 - 3.3) = 25.4</td>
</tr>
</tbody>
</table>

After the corrected interval is calculated, the actual time of collection is determined as before by adding the corrected interval to the time of termination of the previous period. It should be noted that this correction can be made only if the volume of each sample exceeds the dead space of the system. In addition, corrections for both rate of flow variations and collection errors cannot be made simultaneously. It must be assumed that collection errors are negligible when changes in rate of flow are considered to be responsible for large variations in the volume of the samples.

After the corrected time interval is determined by one of the two methods described above, the mean concentration of dye during equilibration is calculated as follows:

\[
\text{Corrected concentration} = \frac{\text{Observed concentration}}{\text{Vol. of sample} - \text{Vol. of dead space}}
\]

Example (table 2, sample 1):

\[
\text{Corrected concentration} = 0.800 \times \frac{7.1}{7.1 - 3.5} = 1.578
\]

The mean concentration of dye is then determined from the formula:

\[
\text{Mean concentration} = \frac{c_1 \times t_1 + c_2 \times t_2 + \ldots + c_{\text{eq}} \times t_{\text{eq}}}{t_1 + t_2 + \ldots + t_{\text{eq}}}
\]

where \(c_1, c_2, \ldots, c_{\text{eq}}\) are the dye concentrations of each sample representing corrected time intervals \(t_1, t_2, \ldots, t_{\text{eq}}\). \(c_{\text{eq}}\) is the concentration at equilibration.

Thus, in 111.7 seconds to equilibrium, \((c_{\text{eq}} + c_{\text{eq}})\) equals 55.353. Mean concentration equals 55.353/111.8 = 0.4956 mg./liter.

A similar calculation is then performed to obtain mean femoral arterial concentration.

Pulmonary blood volume is then calculated by substituting into the formula:

\[
PBV = \frac{CO \times (FA_{\text{ml}} - FA_{\text{eq}})}{c_{\text{eq}}}
\]

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