The Dye Dilution Method for Describing the Central Circulation

An Analysis of Factors Shaping the Time-Concentration Curves

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In addition to providing accurate measurement of circulation times and cardiac output, the dye dilution curves may reveal the size and location of the volumes of blood with which the dye is mixed in the central circulatory system. A trivalent approach to the analysis of these curves is presented by a combination of theoretic analysis, mechanical system experimentation and clinical observation.

The method for measuring cardiac output based on the "dye dilution" principle of Stewart and Hamilton\(^1,2\) involves the injection of a known amount of colored substance into a vein and collection of serial samples of blood from an artery for determination of the concentration of dye. Other substances have been used for injection, such as salt solution and radioactive cells.\(^3,4\)

The validity of this method of measurement of flow depends on the assumption that the dye is distributed throughout a "central" pool of blood as it passes from the vein into the right heart chambers, the lungs, the left heart and out into the arterial system of vessels. The validity and accuracy of the method for determining rates of flow in mechanical systems and the cardiac output in animals and human subjects have been determined by other workers.\(^5,6\)

Our interest in the dye dilution curves has been in the theoretic and possible practical information to be derived from the shape of the curves. It was pointed out by Hamilton and others that the shape of these curves is governed not only by the flow, but also by the volume of blood in the central pool in which the dye is distributed. Previous analyses of these curves have tended to neglect the possibility that the anatomic characteristics of the pool might affect the shape of the curve, although it is obvious that, in the living organism, the central pool in which the dye is distributed is not a single volume, but a series of volumes made up of the veins through which the dye reaches the heart, the right heart chambers, the lung vessels, and the left heart chambers.\(^4,6,7\)

The purposes of our studies are (1) to derive a theory which will describe the dye concentration change in the outflow of systems made up of series of volumes or chambers, (2) to test the theory by comparison with dilution curves obtained from mechanical models in which the flow and volumes are known and (3) to apply the theory to the dilution curves obtained on human subjects.

In setting up theoretic and mechanical systems we have started with the simplest case and proceeded to more complex models as the differences between human curves and theoretic curves suggested lines on which the assump-
tions should be modified in order to approach more nearly the anatomic and physiologic conditions in vivo.

* Case 1. The simplest system assumed was that involving a single "central pool." An equation was derived relating the concentration of dye in time in terms of the volume of the chamber and the flow through the chamber, under the following assumptions:

1. the injection is instantaneous into the chamber, or any mixing of the dye with fluid before entering the chamber is negligible; 2. mixing of the dye is instantaneous and complete in the chamber volume; 3. the flow and volume of fluid are constant; 4. there is no loss of dye from the system; 5. after injection only dye-free fluid flows into the system.

The formula which relates the concentration to time after injection in this single chamber system is:

$$P = \frac{M}{V} e^{-(P/\dot{V})(t-t_0)}$$  \hspace{1cm} (1)

(The derivation of this and subsequent equations will be found in Appendix 2).

Figure 1 shows the results of an experiment with a model of such a "single volume" system. The points represented by dotted circles are the experimentally determined concentrations. The solid line represents the theoretic result expected from equation (1), knowing the flow and the volume of the bottle. (For details of the experimental procedure and apparatus see Appendix 1, Methods.) The agreement of the theoretic with the observed curve is evidence that our mechanical model fulfills the assumptions upon which the theory was based except for the slight duration of injection time. Since the resultant curve is a straight line on semi-logarithmic paper, and the slope, $S$, of the line in figure 1 is

$$S = \frac{F}{V}$$  \hspace{1cm} (1a)

it is apparent that it is possible to calculate the volume of the bottle in this system if one knows the flow and obtains two points on the concentration curve.

* Case 2. The second step in our analysis was to derive and test the expression for the concentration of dye in the fluid flowing out of the second of two bottles in series where the first volume is smaller than the second, under the same assumptions of complete and instantaneous mixing for this two chamber system as for a single chamber. It is assumed further that no mixing takes place in the connecting tubes.

The equation relating the concentration of fluid leaving the second chamber to time is:

$$P_2 = \frac{M}{V_1 - V_2} e^{-(P/\dot{V}_1)(t-t_0)}$$

$$- \frac{M}{V_1 - V_2} e^{-(P/\dot{V}_2)(t-t_0)}$$

where $V_1$ and $V_2$ are the respective volumes of the two chambers and $P_2$ is the concentration in the second chamber.

In the special case where the two volumes are equal ($V_1 = V_2$), the equation reduces to:

$$P_2 = \frac{FM}{V_1^2} (t-t_0) e^{-(P/\dot{V}_1)(t-t_0)}$$  \hspace{1cm} (2a)

An illustration of the application of equation 2 to an experimental model is shown in figure 2. Again the theoretic curve (solid line) and the observed data (circles) agree well. It is of interest to note that when $V_1$ is small in proportion to $V_2$, the down stroke of the curve is governed only by two factors, the flow and $V_2$. Since in this case the first exponential term in equation 2 approaches zero rapidly, it is possible to estimate the size of $V_2$ from the slope of the downstroke by using equation (1a).
Case 3. The third step in building a simplified model was to add a third volume in series under the same assumptions previously stated.

![Diagram](image)

The equation expressing the concentration in the fluid flowing from the third bottle with time is:

\[ P_3 = \frac{MV_1}{(V_1 - V_2)(V_1 - V_3)} e^{-(P/V_1)(t - t_a)} - \frac{MV_2}{(V_1 - V_2)(V_2 - V_3)} e^{-(P/V_2)(t - t_a)} + \frac{MV_3}{(V_1 - V_2)(V_2 - V_3)} e^{-(P/V_3)(t - t_a)} \]

where \( P_3 \) and \( V_3 \) equal respectively the concentration and the volume of the third chamber.

This is the general equation where \( V_1, V_2, \) and \( V_3 \) are unequal. If the system is considered analogous to that of the flow from the right side of the heart through the lungs to the left side of the heart, the special case where \( V_1 \) and \( V_2 \)

![Graph](image)

Fig. 2. Two bottle experiment. The observed dye concentration (dots) is recorded with time after a single injection of 3.93 mg. of dye into the first of two bottles in series with volumes of 0.117 and 0.570 liter respectively with a flow through the system of 0.04033 liter per second. The solid line is determined entirely from theory (equation 2) except for the value of \( t_a \) = 5.07 seconds. This was estimated by fitting the observations on the downstroke of the curve with a straight line on log paper, the slope of the line being known theoretically. The values from \( t = 18 \) through \( t = 34.2 \) were used and \( t_a \) was determined by the method of least squares. Besides the nearly exact fit of the observed data to the theory, it is noted that the effect of the small volume in series with the larger is to delay the peak concentration. The latter part of the curve, however, is entirely the same as for the single larger volume. This is explained by the fact that the dye is rapidly washed out of the small bottle so that the latter part of the curve is governed only by the size of the larger bottle (see text). Furthermore the shape of the curve is identical, whether the small bottle is before or after the larger bottle.

The slight deviation of the observed points from the theoretic curve at the peak concentration is probably due to a small amount of streaming or incomplete mixing in the smaller bottle. We have found it difficult to obtain perfect mixing in bottles under 200 cc. volume when the flow is as high as 2.42 liters per minute.

The equation of the line is

\[ P_2 = \frac{3.93}{.453} e^{-0.00381/1.17}(t - 5.07) + \frac{.93}{.453} e^{-0.00381/1.70}(t - 5.07) \]

or

\[ P_2 = -49.86 e^{-3.46t} + 12.42 e^{-0.17t} \]
are equal volumes would be desired. This is given by:

\[ P_a = \frac{FM}{V_1(V_1 - V_3)} (t - t_a) e^{-(P/V_1)(t - t_a)} \]

\[ - \frac{MV_1}{(V_1 - V_3)^3} (e^{-(P/V_1)(t - t_a)} - e^{-(P/V_1)(t - t_a)}) \]  

(3a)

**Fig. 3.** Three bottle experiment. The observed dye concentration (dots) is recorded with time after a single injection of 4.80 mg. of dye into the first of three bottles in series with volumes of 0.124, 0.592, and 0.119 liters respectively with a flow through the system of 0.04833 liter per second. The solid line is obtained entirely from theory (equation 3) except for the value of \( t_a = 4.38 \) seconds for the observations on the straight line downstroke of the curve. This was estimated by fitting a straight line on log paper (the slope known theoretically) from 21.1 second through 46.1 second. The addition of another small bottle affects the shape of the upstroke and crown of the curve, but the slope of the downstroke line is again governed only by the volume of the largest bottle. Thus, by determining the slope of the downstroke and knowing the flow, the volume of the largest bottle can be calculated in such a system.

The equation of the line is

\[ P = -254.4 e^{-3.96(t - 4.38)} + 12.84 e^{-0.827(t - 4.38)} + 241.5 e^{-0.469(t - 4.38)} \]

\[ = -1403 e^{-3.914} + 18.36 e^{-0.827} + 1430 e^{-0.469} \]

Figure 3 shows the observed results of a model experiment for the system to which equation (3) applies, and the theoretic equation (solid line).

Again it can be seen that, if the volumes of \( V_1 \) and \( V_2 \) are small in proportion to \( V_3 \), the down stroke of the curve is governed only by the flow and volume of the largest chamber. It should be noted that these equations are valid for any sequence of the volumes. In this illustration the volumes were placed in order of small \( \rightarrow \) large \( \rightarrow \) small, because we were consciously seeking to imitate the normal right heart \( \rightarrow \) lungs \( \rightarrow \) left heart system of volumes.

To illustrate how closely the equation for three chambers in series may imitate the curves obtained on the human subject, we have illustrated in figure 4 a mechanical model and a human curve. The model was constructed with \( V_1 \) and \( V_3 \) containing 120 cc. each to represent roughly the total right and left heart volumes, and with \( V_2 \) of 500 cc. to represent roughly the volume of blood in the lungs with which dye may be mixed. In order to imitate the recirculation which takes place in the human a pump was inserted after \( V_3 \) in the mechanical model, which returned a fraction of the flow back into \( V_1 \). The similarity of the two curves is obvious. The downstroke shows a sharp deviation at the point where a portion of the dye is recirculated, but it is still possible to calculate the volume.

**Fig. 4.** Comparison of a human dye dilution curve (x) with a "model" curve (dots) shows close similarity. The flow or the cardiac output was calculated by Hamilton's method from the human curve. Using the straight line part of the downstroke of the human curve the largest volume was calculated using equation (1a) where the flow \((F)\) is obtained from Hamilton's formula for cardiac output.

The model was then constructed with three bottles, the largest being the same volume as calculated from the human curve (550 cc.) and with the same flow. The two small bottles were arbitrarily chosen at 120 cc. in an attempt to imitate roughly the effect of the right and left heart volumes. The deviation from the straight lines at about 19 to 20 seconds is due to recirculation. In the mechanical model recirculation was accomplished by a pump which returned a fraction of the outflow from the third to the first bottle slowly enough to provide a 16 second recirculation time.
of \( V_2 \) in this case from the slope of the downstroke before recirculation.

In figures 5 and 6 are illustrations of the application of the theory to a patient who recovered from cardiac failure, in whom it is reasonable to suppose that marked changes in the volume of blood in the lungs occurred. Similar calculations of the volume of blood in the pulmonary vessels in normal subjects yield a value which is in the range of magnitude suggested by

![Image](Fig. 5A)

**Fig. 5A**

The patient was a 58 year old white man. At the time of the first dye curve and chest x-ray (4/4/49) the patient was dyspneic and orthopneic with slight cyanosis. Examination of the chest showed dullness over the lower third of both lung bases and extensive loud inspiratory and expiratory rales over both lung fields particularly at the bases. He expectorated white frothy sputum. The neck veins were obviously abnormally full and the liver was enlarged and tender. There was moderate peripheral dependent edema. Pulse was 140, temperature 101 F., and respiration 48. Weight was 137 pounds. The patient was treated with bed rest, low salt diet and digitalis administration.

At the time of the second dye dilution curve and chest x-ray (4/19/49) the patient had no dyspnea or cyanosis and the neck veins emptied. The chest revealed absence of dullness at the bases and only a few scattered rales on deep inspiration just above the diaphragm. The liver was less enlarged and the weight had dropped to 109 pounds because of loss of 28 pounds of edema fluid. Temperature was 98.8 F., pulse 96 and respiration 24. Blood pressure remained between 120 to 90 systolic and 80 to 60 diastolic. The clinical diagnosis was arteriosclerotic heart disease with cardiac failure and chronic bronchitis.

The central volume \((C.V.)\) was calculated from formula (1a) which relates the slope of the straight line downstroke of the curves to the flow \(F\) (or the cardiac output, \(C.O.\)) and to the largest volume in the chest. In this case the slope increased greatly as the patient recovered from cardiac failure and the signs of pulmonary congestion even though the cardiac output dropped. This is interpreted as being due to a marked diminution in the volume of blood in the lungs.

\[ Ht = \text{hematocrit}, \quad B.V. = \text{blood volume}, \quad C.O. = \text{cardiac output}, \quad C.V. = \text{central volume from formula (1a)}, \quad M.C.T. = \text{mean circulation time measured from time of injection of dye}, \quad \text{"N. to N.\"} = \text{the volume of blood calculated by multiplying the cardiac output times the mean circulation time} \]

The ordinate is the concentration of dye \((T-1824)\) in mg. per liter of arterial serum on a logarithmic scale. The abscissa is seconds after the appearance of the dye. In the top curve the dye first appeared 23 seconds after injection into an arm vein, and in the bottom curve, 17.5 seconds after injection.

![Image](Fig. 5B)
estimates made by anatomic measurements and by estimates made from the circulation time and flow through the lungs. These results will be reported later.8

Case 4. One obvious discrepancy between both the mechanical and theoretic models previously considered and the human subject lies in the assumption of constant volumes within the chambers. In the contracting heart there is a changing volume with each beat. The theory next considered was for the dilution curve from a single chamber which intermittently contracts. It is assumed for this derivation that the chamber has two constant volumes, the ejection volume \( V_e \), and the residual volume after ejection \( V_R \).

Also for purposes of this analysis we assume that \( V_R \) and \( V_e \) are the same for each cycle and that there is complete mixing of the fluids in \( V_R \) and \( V_e \) before each ejection. Thus \( V_R \) plus \( V_e \) is the maximum diastolic volume of the chamber, and \( V_R \) is the volume at the end of systole.

![Diagram](https://via.placeholder.com/150)

Fig. 6. Simultaneous determination of dye concentration from arterial plasma samples and from a continuous recording of the density change in the patient’s ear after a single injection of T-1824 (Evans blue). This demonstrates agreement in the concentration changes from two sites in the circulation and shows no significant deviation of the serial sample method from that of continuous photoelectric recording. The circulation in patient’s ear was “arterialized” by vasodilation with heat.

The equation representing the concentration of dye in the outflow fluid at time \( t \) is

\[
P = P_0 \left( \frac{V_R}{V_R + V_e} \right)^{k t}
\]  

or

\[
P = P_0 \left( \frac{V_R}{V_R + V_e} \right)^R
\]  

where \( V_R \) is residual volume in liters; \( V_e \) is ejection volume in liters; \( F \) is total flow through the chamber or the sum of the ejection volumes in a time unit, expressed in liters per minute; \( R \) is number of ejections per minute; \( t \) is time in minutes following first ejection; \( P \) is concentration in fluid ejected from chamber in units per liter; \( P_0 \) is concentration at \( t = 0 \).

The slope, \( S \), of the line obtained by plotting the logarithm of \( P \) against time is

\[
S = \frac{\log P - \log P_0}{t} = R \log \left( \frac{V_R}{V_R + V_e} \right)
\]

The slope, \( S \), of the concentration curve can be obtained experimentally, and \( V_e \) can be determined from an independent method of measurement of \( F \) and \( R \) (since \( V_e = \frac{F}{R} \)).

It is then possible to calculate the residual volume of the chamber \( (V_R) \) which is the only remaining unknown in the equation. A dye injection method for the determination of residual volume in a heart chamber is suggested by this analysis.9

**Discussion of Assumptions Used**

1. The Assumption of Constant Volume

A consideration of case 4 in which the contractile nature of the heart chambers is approached, makes it seem desirable to extend the three chamber theory to include this as-
assumption. It seems doubtful that the volume diluting the lungs varies greatly with each pulse and therefore equation (3) might be modified to allow for the contractile nature of \( V_1 \) and \( V_3 \). It should be noted, however, that since the downstroke of the concentration curve is determined by the largest volume in the series, and if, as seems likely in the normal, this represents the lung volume, the modification of the theory to account for the contractile nature of the heart may not affect the downstroke appreciably.

2. The Assumption of Complete Mixing

The theory developed made use of the assumption that the dye is completely mixed with all the fluid in each chamber. The effect upon the curve of incomplete mixing can be easily demonstrated in our mechanical model (see Appendix 1, Methods) by omitting the precautions taken to insure complete and uniform mixing. In which case the calculation of the volume of a single chamber from the formula \( S = \frac{F}{V} \) gives erroneously low results. The calculation in this case represents the apparent volume with which the dye was mixed. The formation of streams of flow within the heart chambers and lung vessels might prevent complete and uniform mixing. How much error in the calculation of volumes is introduced by incomplete mixing can be determined only by experimental observations on the intact circulation. It seems likely however, that good mixing within the heart chambers does occur, since the Fick principle of flow measurement gives reproducible results from samples taken to insure complete mixing, in which the right ventricle. In mechanical models using bottles of from 100 to 1000 cc. volume the turbulence created only by the inflow is adequate to mix the dye with 85 to 95 per cent of the volume in the bottles.

It may be questioned whether the blood in the lungs can be treated theoretically as a single mixing chamber. It is obvious that the blood in the lungs is contained in a multitude of parallel channels. So long as the flow is proportional to the volume in each separate channel, the theory for a single volume will apply to the net effect of a multitude of parallel channels. How much the circulatory system of the lungs deviates from the theory in this respect remains a problem for experimental determination. An answer to this problem could be obtained by determining simultaneous dilution curves from various segments of the lung.

3. The Assumption of Instantaneous Injection

Experimental conditions may deviate from the theory by failure to fulfill the assumption that the injection of the dye is instantaneous. In mechanical experiments, virtually instantaneous injection can be achieved as shown by the agreement between theory and observations in the models (figs. 1, 2, 3).

However, in clinical practice the dye is usually injected into a peripheral vein and some delay in entering the right heart may occur due to mixing of the dye with blood in the veins. Precautions can be taken to minimize this delay, and the delay can be eliminated if the dye is injected into the heart through an indwelling catheter. This error would in most cases affect only the first part of the dye curves but would not alter the final downstroke of the curve after all the dye had entered the lungs or the largest volume.

4. The Assumption of No Recirculation

The assumption that only dye-free fluid enters the system after injection of the dye is valid only until recirculation of dye occurs in the intact closed circuit. However, in most cases it may be possible to determine the constants which describe the curve before significant recirculation occurs. Recently Nylin has shown that recirculation into the right heart of man is earlier than had been previously supposed.

5. The Assumption of Constant Flow

A variation in flow during the observation period would change the shape of the curve and impair the usefulness of the curves for the calculation of the volumes in the system.

6. The Assumption of Representative Sampling

Under ideal conditions the theory requires continuous recording of the concentration of
dye in the outflow fluid. While our mechanical collection system gives only successive samples, the interval between samples is so short that the error is trivial. Furthermore, comparison of dye curves obtained from human subjects by collecting consecutive two-second samples of arterial blood with curves obtained from a continuous photoelectric recording device on the ear has shown substantial agreement (fig. 7).8

It might be questioned whether the same curve would be obtained from all points in the peripheral arterial circulation. The identity of the simultaneous curves from two different sites supports the assumption that the concentration curve is not modified in the arterial pathways after leaving the heart.

Discussion

Our analysis of the factors shaping the dye dilution curves is meant to be used as a rational guide to interpretation and to further investigation. The validity of the theories set forth depends entirely upon the fulfillment of the assumptions by the flow system being analyzed. In the model systems the theory can be substantiated. In the human flow system proof of the validity of these theories requires further investigation, and the analysis seems valuable because it suggests experiments which will either substantiate the theory or will allow modification of the theory to fit the system. For example, it may be possible to obtain further evidence as to whether the lung volume governs the slope of the downstroke of the dilution curves. If dye is injected into a peripheral vein, the right auricle, the right ventricle and the pulmonary artery in the same individual under constant conditions of flow, the slopes of the downstrokes should be identical, if the present analysis is correct.

Besides the analysis of normal curves, there may be important and useful information to be obtained by a consideration of the effects of shunts upon the curves. We have begun preliminary observations on model systems and patients on theoretic analysis of the shape of curves obtained when shunts of different sizes, directions and locations are inserted into the system. It seems certain that accurate information concerning the size, direction, and location of intracardiac shunts may be obtained from arterial dye dilution curves.
The theory of the dilution curve obtained from contractile chambers may be useful for determining the size of the residual volume in individual chambers of the heart if one can obtain the dilution curve emerging from the chamber. Preliminary observations make it reasonable to expect that dilution curves may be obtained from isolated chambers of the heart by determining the dilution of injected radiopaque materials with a photofluorographic technic. Our experience with contractile model systems indicates that it is necessary to determine accurately the dilution of injected material which occurs with each heartbeat in order to calculate the residual volume of contractile chambers.

SUMMARY AND CONCLUSIONS

1. A theoretic analysis has been made of some factors governing the time concentration curve in flow systems made up of a series of volumes into which dye is injected, under certain fixed assumptions as to the characteristics of the flow system for one, two, and three chamber systems.

2. A technic and an apparatus are described for a rapid serial sampling from flow systems for determination of rapid concentration changes. This apparatus has been used in mechanical model experiments set up under the conditions of the flow system postulated for the theory. Agreement between the experimental and theoretic results is shown.

3. There is general similarity obtained from a three-chambered model flow system imitating roughly the right heart, lung, and left heart chambers, and curves obtained from the heart-lung flow system of the normal human subject. The extent to which the basic assumptions are fulfilled in the human subject is discussed.

4. Agreement between curves from serial arterial blood samples and from continuous photoelectric recording of dye density in the arterialized human ear is demonstrated.

5. Our analysis suggests that the time concentration curves after a single injection of dye may be used to obtain information concerning the relative size of the volumes of blood in the heart chambers and lungs and the residual volume of the heart.

APPENDIX 1: METHODS

Mechanical Models. The models consisted of bottles connected in series to a water supply by flexible tubing. The connections between bottles were as short as possible. The inflow tube was led to the bottom of the bottle and the outflow was near the opening. In order to insure complete mixing, about 20 cc. of mercury were placed in the bottle and the system was mounted on a shaking machine. The mercury split into fine droplets which moved vigorously. That complete mixing was obtained by this method is demonstrated by the agreement between the theoretic curves and the observed curves. The outflow was led to a large calibrated container so that an accurate measure of the flow was obtained during the experiment. The dye was injected by syringe through a needle inserted into the flexible inflow tubing near the first bottle. The serial samples for determination of dye concentration were obtained from a side arm on the outflow tubing. (fig. 7). The serial samples were collected continuously by means of the illustrated rotating lucite disk into which there are drilled a continuous series of funnels of constant diameter. The disk rotated at constant speed, making possible the collection of accurately timed serial samples with no spillage of fluid, which is essential for accurate results. The concentration of dye in each tube was determined by photoelectric colorimetry. The amount of dye injected was determined by weighing the syringe before and after injection. A check on the amount of dye actually injected was obtained by collecting all the outflow fluid in a large container and determining the amount in the total flow through the system after injection.

At first we attempted to use the dye T-1824 (Evans blue) for the model experiments, but found this unsatisfactory because tap water caused an instantaneous change in the color. Satisfactory colorimeter results were obtained with phenolsulfonphthalein (PSP) as the dye. The samples were alkalized by a small drop of concentrated potassium hydroxide solution before reading.

The curves on human subjects were obtained from serial samples of arterial blood from the brachial or femoral artery collected with the same apparatus. The dye (T-1824, 10 to 20 mg. in 2 to 4 cc.) was injected as rapidly as possible into a large antecubital vein. A needle (size 16 to 19) was placed in the brachial or femoral artery and the blood flowed freely through a short (10 to 15 cm. in length) piece of flexible plastic tubing (internal diameter 0.4 cm.) into the funnels on the rotating disk (fig. 7). The end of the tubing was held in a fixed position over the center of the line of rotating funnels.

The blood samples in the test tubes were then covered and allowed to clot and the tubes centrifuged. The serum was then placed in a microcell
(0.1 to 0.3 cc. of serum) and the density of the dye read in a Beckman spectrophotometer at a wave length of 620Å. A standard curve was made for each patient with known dilutions of T-1824 dye in serum obtained from the subject before the injection of dye. In this way, any interference from blank substances and possible alteration of T-1824 by the patient's blood was eliminated. Hemolysis was eliminated by coating the inside of the test tubes with silicone oil.

The detailed description of the apparatus and method of continuous photoelectric recording of the density of T-1824 from the human ear (fig. 6) will be described elsewhere. The curve in figure 6 was replotted from a curve written by a direct-writing electrocardiograph machine (Sanborn Visocardiette Model 51) coupled by suitable amplifiers to a phototube multiplier earpiece. The amplifiers are arranged so that the deflection of the writing pen is directly proportional to the concentration of T-1824. This apparatus is similar to that divided by Morgan and Sturm.

**APPENDIX 2: DERIVATION OF EQUATIONS**

1. *Equation (1) of Text.* If $M$ units of dye are injected into a chamber of volume $V_1$, the rate of change in the number of units of dye, $M_1$, remaining in the chamber at any time, $t$, is dependent upon the concentration, $P_1$, at that time and the flow, $F$, under the assumptions stated in the text. Since no dye is contained in the fluid flowing in and each of the $F$ units flowing out per unit of time contains

$$P_1 = \frac{M_1}{V_1} \text{ units of dye,}$$

the rate of change in amount of dye is given by

$$\frac{dM_1}{dt} = - \frac{FM_1}{V_1} = - FP_1 \quad (1)$$

Dividing both sides of equation (1) by $V_1$ gives the rate of change in concentration of dye as

$$\frac{dP_1}{dt} = - \frac{FP_1}{V_1} \quad (2)$$

which on integration gives

$$P_1 = a_1 e^{-\left(\frac{F}{V_1}\right)t} \quad (3)$$

where $a_1$ is the constant of integration.

To evaluate $a_1$ we have the condition that when $t = t_a$, $P_1 = \frac{M}{V_1}$, which gives

$$P_1 = \frac{M}{V_1} e^{-\left(\frac{F}{V_1}\right)(t-t_a)} \quad (4)$$

2. *Equations (2) and (2a) of Text.* If $M_1$ units of dye remain in chamber 1 at any time $t$, and $M_2$ units are in chamber 2 at this time, the rate of change in $M_2$ will depend on the concentration in the inflow from the first chamber, $P_1$, and in the outflow from the second, $P_2$. Since each unit of volume flowing in contains $P_1 = \frac{M_1}{V_1} \text{ units of dye and each unit flowing out contains } P_2 = \frac{M_2}{V_2} \text{ units, the net change in the amount of dye in the second chamber per time unit, which involves } F \text{ volume units of flow is given by:}$$

$$\frac{dM_2}{dt} = \frac{FM_1}{V_1} - \frac{FM_2}{V_2} \quad (5)$$

Dividing equation (5) by $V_2$ to get the change in concentration per unit of time, we have:

$$\frac{dP_2}{dt} = \frac{F}{V_2} P_1 - \frac{F}{V_2} P_2 \quad (6)$$

Since $P_1$ is not influenced by $P_2$ because of the direction of flow, $P_1$ as given by equation (4) may be substituted in equation (6), giving

$$\frac{dP_2}{dt} + \frac{F}{V_2} P_2 = - \frac{FM}{V_1 V_2} e^{-\left(\frac{F}{V_1}\right)(t-t_a)} \quad (7)$$

Integrating gives

$$P_2 = a_2 e^{-\left(\frac{F}{V_2}\right)t} + \frac{M}{V_1 - V_2} e^{-\left(\frac{F}{V_1}\right)(t-t_a)} \quad (8)$$

where $a_2$ is the constant of integration. Evaluating $a_2$ from the condition that when $t = t_b$, $P_2 = 0$ gives

$$P_2 = - \frac{M}{V_1 - V_2} e^{-\left(\frac{F}{V_1}\right)(t-t_a)} + \frac{M}{V_1 - V_2} e^{-\left(\frac{F}{V_1}\right)(t-t_a)} \quad (9)$$

In the special case where $V_1 = V_2$, equation (7) becomes:

$$\frac{dP_2}{dt} + \frac{F}{V_1} P_2 = \frac{FM}{V_1^2} e^{-\left(\frac{F}{V_1}\right)(t-t_a)} \quad (10)$$

Integrating and evaluating the constant of integration from the initial condition gives:

$$P_2 = \frac{FM}{V_1^3} (t - t_a) e^{-\left(\frac{F}{V_1}\right)(t-t_a)} \quad (11)$$

3. *Equations (3) and (3a) of Text.* The change per unit of time in the amount of dye $M_3$ in the third of
three chambers is determined by the inflow from the second chamber and the outflow. This is given by:

$$\frac{dM_3}{dt} = F \frac{M_2}{V_2} - \frac{M_3}{V_3}$$  \hspace{1cm} (12)

Dividing equation (12) by $V_3$ to get change in concentration per unit of time gives:

$$\frac{dP_3}{dt} = \frac{F}{V_3} P_2 - \frac{P_3}{V_3} P_2$$  \hspace{1cm} (13)

Substituting for $P_2$ its value from equation (9), integrating and imposing the condition that $P_3 = 0$ when $t = t_a$ gives

$$P_3 = \frac{M V_1}{(V_1 - V_2)(V_1 - V_3)} e^{-\left(\frac{F}{V_1}\right)(t-t_a)}$$

$$- \frac{M V_2}{(V_1 - V_2)(V_2 - V_3)} e^{-\left(\frac{F}{V_2}\right)(t-t_a)}$$

$$+ \frac{M V_3}{(V_1 - V_3)(V_3 - V_2)} e^{-\left(\frac{F}{V_3}\right)(t-t_a)}$$  \hspace{1cm} (14)

For the special case of $V_1 = V_3$, substitution of $V_1$ for $V_3$ in equation (13), and integration, gives:

$$P_3 = \frac{M}{V_1 - V_2} \left[ \frac{F}{V_1} (t - t_a) - \frac{V_2}{V_1 - V_2} \right] e^{-\left(\frac{F}{V_1}\right)(t-t_a)}$$

$$+ \frac{M V_2}{(V_1 - V_2)^2} e^{-\left(\frac{F}{V_2}\right)(t-t_a)}$$  \hspace{1cm} (15)

4. Equations (4) and (4a) of Text. In a single contractile chamber with a fixed number of contractions, $R$, per unit of time and fixed residual and ejected volumes, $V_R$ and $V_E$, (see assumptions stated in text) if the concentration in the fluid ejected by the first contraction is $P_0$, (time $t = 0$), then the concentration in the chamber following expansion and in the fluid ejected by the second contraction is:

$$P_2 \left( \frac{V_R}{V_E + V_R} \right)$$

The concentration following the next expansion and therefore that of the fluid ejected by the third contraction is

$$P_3 \left( \frac{V_R}{V_E + V_R} \right)^2$$

In general the concentration following each successive contraction and expansion will be diluted by the factor $\frac{V_R}{V_E + V_R}$, and the concentration in the ejected fluid at the $x$th ejection will be

$$P = P_0 \left( \frac{V_R}{V_E + V_R} \right)^{x-1}$$  \hspace{1cm} (16)

Expressing this concentration as a function of time units from first ejection we have

$$P = P_0 \left( \frac{V_R}{V_E + V_R} \right)^{Rt}$$  \hspace{1cm} (17)

or

$$P = P_0 \left( \frac{V_R}{V_E + V_R} \right)^{IV_{KE}t}$$  \hspace{1cm} (18)

Where $F$ is the volume flowing through the system per time unit, that is the sum of the ejected volumes per time unit.

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