The Influence of Injection Site upon the Form of Dye-Dilution Curves

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The validity of the serial compartment analysis of dye dilution curves was tested by injecting dye into the right auricle, pulmonary artery, a pulmonary vein and the aorta consecutively while sampling blood from the carotid artery. It was found that the serial compartment analogy is valid and that the lungs are the largest volume in the series. In curves obtained by injecting into the pulmonary artery and sampling from the left auricle it was shown that under the conditions of the experiments, the lungs act like a single volume from which dye is washed out in exponential fashion.

In previous analyses of the form of the dye dilution curve it has been assumed that the dye enters a single central pool of blood. We have presented theoretic and model experiments that mixing actually occurs as a serial dilution in the right heart, lungs and left heart. The present experiments were designed to test this hypothesis on living animals and to demonstrate the contribution of each segment of the central circulatory system to the final arterial curve. To accomplish this, dye was injected into various segments of the central circulation such as the right auricle, pulmonary artery, pulmonary vein and aorta. If the lungs contain the largest central volume (which we predict from the relative sizes of the volumes in the heart and lungs and from the inspection of normal human curves) the downslope of curves resulting from dye injections into the pulmonary artery should be the same as the downslope in curves from injections into the right auricle as long as cardiac output is the same. To determine the form of the curve through the lungs alone, dye was injected into the pulmonary artery and sampled from the left auricle.

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Methods

An adaptation of the Morgan-Sturm photomultiplier densitometer permits the rapid performance of repeated dye curves on the same dog. The response of this instrument is directly proportional to optical density. As a cuvette for the densitometer, one of the dog's carotid arteries was divided and bridged with a piece of polyethylene tubing. This in turn was inserted through a black plastic block through which a window was cut. The internal diameter of this tubing was 2.7 mm. and the length 50 cm. The maximal distance from the proximal end of the carotid artery to the cuvette was 15 cm. The black plastic block was clamped between the light source and the photomultiplier tube of the densitometer. The carotid artery was chosen because it was found that when an artery such as the femoral was employed, peripheral vasoconstriction slowed flow and clotting eventually resulted. Arterial concentration curves with peak deflections from 20 to 40 mm. were obtained with single instantaneous injections of 0.3 to 1.5 mg. of T-1524; the sensitivity of the instrument to dye density was kept constant throughout each experiment. Calibrations of densitometer deflections in terms of dye concentration were performed on each dog. The response of the densitometer was calibrated by injecting 2.5 to 5 mg. of dye, measuring the plateau deflection in the baseline after equilibrium was reached and determining the ratio of this value to the concurrent change in blood concentration. Arterial blood samples were obtained before and after the calibrating injection and serum T-1524 concentrations were determined at 620 mju and then corrected for plasma hematocrit. In separate experiments, increments of dye were injected and serum concentrations of T-1524 were plotted against the cumulative deflections of the densitometer. These in vivo calibrations showed the response to be linear to a serum level of 80 mg. per liter, a level which was not exceeded in these experiments.

In many published experiments employing the dye-dilution technic it is apparent that the dilution...
curves are distorted by slow flow through the side-arm or sampling tube. Experiments were performed on a model to determine the effect of the sampling tubing used in our experiments on the form of the dye dilution curves. The model consisted of a blood filled reservoir, a rotary pump and a length of polyethylene tubing with the same internal diameter used in the dog experiments. Dye was injected into the inflow of the pump and the optical density of blood and dye was recorded as a function of time by two densitometer phototubes, one next to the pump outflow and the other 68 cm. distal to it. The rate of flow was 2.96 ml. per second and the volume in the tubing between the two points was 3.65 ml. It can be seen (fig. 1) that with this ratio of flow to volume the upstroke and crown of the curve are distorted whereas the downstroke is the same. The curve itself represents the "washout" of the rotary pump. The experiment was repeated injecting the dye directly into the tubing proximal to the first of the two densitometers. The curve recorded in the proximal recorder was a steep hyperbola-like curve, not unlike curves obtained by injecting at the root of the aorta and sampling at the carotid cuvette.

Dye-filled cardiac catheters were inserted under fluoroscopic control. The catheters were kept filled with dye so there would be no problem of calibration for the dead space of the catheters. One catheter was inserted high in the right auricle and another into a pulmonary artery branch both via the external

![Densitometer records recorded from the carotid cuvette showing examples of curves from various sites of injection.](image)

**Fig. 2.** Densitometer records recorded from the carotid cuvette showing examples of curves from various sites of injection.
jugular vein. A third catheter was passed retrograde from a carotid artery into a pulmonary vein. The time of injection of dye was signaled automatically by an electrical impulse discharged by metal contacts between the barrel and plunger of the syringe when it was emptied. The duration of injection was 0.1 to 0.2 second. Representative curves are shown in figure 2.

In order to determine the form of the dye-dilution curve through the lungs, excluding the right and left heart, a separate group of experiments was performed on six dogs. These animals were prepared as were those of the group described above except that only one dye-filled catheter was passed. A second catheter was passed into the left auricle via the carotid artery. Just before dye injection and during the inscription of the curve, blood was continuously withdrawn by means of constant negative pressure produced by a mercury filled gas sampling tube and leveling bulb. There was a layer of heparinized saline on top of the mercury in the sampling tube so that the dog’s blood could be

Table 1

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Wt. Kg.</th>
<th>Cardiac output liters/min.</th>
<th>Central volume (ml.) calculated from $V = F/S$</th>
<th>Site of Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pulmonary Vein</td>
</tr>
<tr>
<td>24</td>
<td>13.2</td>
<td>1.64 2.05 1.60 1.94 1.71 1.77</td>
<td>27.4 47.5</td>
<td>Pulmonary Artery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right Auricle</td>
</tr>
<tr>
<td>25</td>
<td>14.0</td>
<td>3.78 4.29 4.31 4.28 4.22 4.11</td>
<td>54.7 82.5</td>
<td>86.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>73.5</td>
</tr>
<tr>
<td>27</td>
<td>16.3</td>
<td>2.08 2.49 2.18 2.36 2.30 2.36</td>
<td>36.2 85.0</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76.2</td>
</tr>
<tr>
<td>28</td>
<td>11.8</td>
<td>1.53 1.66 1.53 1.96 1.84 1.70</td>
<td>24.4 36.6</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43.6</td>
</tr>
<tr>
<td>32</td>
<td>18.2</td>
<td>1.63 1.96 1.54 1.88 1.61 1.72</td>
<td>28.9 42.0</td>
<td>46.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>46.3</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>14.7 2.33 35.2 61.5 62.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean of Cardiac output.
± One standard deviation. Mean coefficient of variation 6.6%.
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The method by which we calculated central blood volumes was described previously.1

Calculations of volume from the product of mean circulation time and flow are not included because we are uncertain about the physiologic significance of this "needle to needle" volume.

RESULTS

The cardiac outputs and calculated volumes of five dogs are recorded in table 1. The starred numbers are the mean cardiac output values plus or minus one standard deviation. The central volumes calculated from curves produced by pulmonary vein injections are considerably smaller than the ones from pulmonary artery and right auricle injections. On the other hand, there is no significant difference between the central volumes calculated from right auricle and pulmonary artery injection curves.

A semilogarithmic replot of the data of figure 2 is shown in figure 4. This shows the similarity of the slopes from right auricle and pulmonary artery injections as compared with the steeper slope resulting from a pulmonary vein injection. The right auricle and pulmonary artery injection curves have a slow build-up time while the curves from pulmonary vein and aorta injections have a fast one. Although the downslopes of the curves from right auricle, pulmonary artery and pulmonary vein injections follow a straight line when the logarithm of concentration is plotted against a linear time scale, curves resulting from injections at the root of the aorta have the appearance of hyperbolas, suggesting that they are the result of laminar flow. However, these aortic root to carotid cuvette curves have a fast rise and fall and thus contribute only slightly to the upstroke and crown of the slower curves from the large preceding volumes (see tubing experiment, fig. 1).

In the second group of five dogs, curves, obtained by injecting into the pulmonary artery and sampling from the left auricle, gave straight lines on the downslope when the logarithm of concentration was plotted against time (fig. 3).

DISCUSSION

The forms of the curves and the volumes calculated from their slopes support the serial compartment analogy. They show that the blood in the lungs, or some part of the lungs, is the largest volume in the series, that is, the volume which controls the downslope of curves resulting from injections into the blood stream at points before the blood reaches the lungs.

The short build-up times of pulmonary vein to carotid cuvette and pulmonary artery to left auricle, shown in the curves, indicate that individual segments of the central circulation do behave like single mixing volumes.

Several possible explanations can be suggested for the small values obtained for central volume. Under the conditions of the experiment mixing of dye with the blood in the lungs may not have been complete. Some parts of the lung blood volume may have received proportionately more dye than others and the downslope accordingly may have been steeper. Such factors as laminar flow and variable proportions of flow to vessel volumes in the lungs would
introduce errors in the calculation of central volume from the slope of the time-concentration curves.

Another possible explanation is that the central volume may represent only a portion of the blood between the pulmonary artery and veins, for example, the blood in the alveolar capillaries and smaller vessels. A line of evidence supporting this argument is that some patients with mitral stenosis and pulmonary small vessel disease have had normal central volume values associated with low cardiac outputs.4

A third explanation is that the serial compartment analogy does not apply and that the apparent "washout" curves in actuality represent some other mechanism. It has been suggested5 that a more valid interpretation is in terms of "random walk." In this interpretation a series of probability distributions is assigned to the population of dye molecules from instant to instant after injection. The parameters of the probability distribution represent F/V (the fraction of bed-volume displaced per unit time) and the "randomizing power" of the vascular pattern through which flow occurs. The time-dilution curve thus represents the build up and decay at one point along the "walk." However, the fact that dilution curves from pulmonary artery to left auricle, as well as right auricle to carotid artery, are exponential shows the serial compartment analogy is at least approximately valid.

SUMMARY

1. The purpose of the experiments reported here was to test the validity of the analysis of dye-dilution curves in which an analogy was made between the central circulation and serial compartments from which dye was consecutively washed out in exponential fashion.

2. The method was to obtain repeated dye curves from the same animal when injecting dye consecutively into the right auricle, pulmonary artery, a pulmonary vein and the aorta, and when sampling at the carotid artery, or from the left auricle.

3. To make possible the performance of repeated determinations, changes in dye concentration were measured with a photomultiplier densitometer which was attached to a piece of polyethylene tubing bridging a carotid artery. The distortion due to sampling technic was demonstrated.

4. It was found that (a) the central circulation does behave as do serial volumes, (b) the lungs are the largest volume in the series, and (c) the lungs act like a single volume from which dye is washed out in an exponential fashion.

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