Effect of the Sampling System on the Shape of Indicator Dilution Curves


This paper describes the distorting effects produced by various sizes of polyethylene tubing and various flow rates on a square wave input of dye concentration. The importance of an accurate sampling system when recording dilution curves for analysis of their shape is emphasized and the distortion caused by inadequate sampling systems is demonstrated in vivo.

INDICATOR dilution curves for quantitative estimations of volumes and valve incompetence have come into increasing use in recent years. Since any system of analysis depending on the shape of the curve (i.e., slope of the ascending or descending limb) requires accurate reproduction of the time-concentration curve actually occurring within the vessel, it is important to assess errors in the curves caused by the sampling system. Distortion of the curve by the sampling system departs from the concept of "representative sampling" and might account for many of the variations in some of the reported "central volumes," as measured from slope of the descending limb.

In order to assess the effect of variations in diameter and length of tubing and the rate of flow in a sampling system, experiments were carried out in vitro and in vivo.

Methods

In Vitro

The apparatus which was used to test the distortion, caused by the sampling tubing, of a dye concentration square wave input consisted of a two-chambered lucite well in which the chambers were connected through a two-way stopcock to a common outlet. Polyethylene tubing led from the stopcock through a phototube cuvette to a mechanical pump capable of withdrawing fluid at various constant rates. The optical density of fluid flowing in the system was measured by a photoelectric densitometer, and recording circuit was 95 per cent response (3 time constants) in 0.15 sec.

The two well chambers were filled with blood and continuous mixing was assured by mechanical stirrers. Dye (indigo carmine) was added to one chamber. The undyed blood was withdrawn from the chamber past the phototube at a constant rate, and baseline density established. A square wave input of dye was obtained by turning the stopcock rapidly to connect the withdrawal system to the chamber containing dyed blood. The time-density curves were recorded and, for comparison, replotted as per cent of maximum density change against time (time zero being time of initial deflection from baseline).

Observations were made on polyethylene tubing with internal diameters of 1.19 and 1.77 mm., and at distances of 6, 12, and 18 cm. from outflow to center of the cuvette, and at flow rates of 0.282, 0.445, 0.715, 1.39, and 2.1 ml/sec. Observations on blood having different hematocrits were also obtained.

In Vivo

Part I. Mongrel dogs were anesthetized with Nembutal and a tracheal cannula inserted for the administration of 100 per cent oxygen, in order to eliminate changes in density due to changes in arterial oxygen saturation. A no. 8 or no. 10 cardiac catheter was introduced under fluoroscopic control into the right heart. The left femoral artery was exposed and a polyethylene cannula (internal diameter 2.69 mm.) was inserted so that the tip lay at or near the aortic bifurcation. Polyethylene tubing carried a cuvette across which the densitometer phototube was attached. A polyethylene catheter (internal diameter 1.19 mm.) was introduced percutaneously into the opposite femoral artery. This cannula was of sufficient length so that the tip lay at the aortic bifurcation. To this cannula was attached additional polyethylene of the same size, carrying a cuvette and phototube and was connected to a syringe and mechanical withdrawal pump so that constant withdrawal rates could be obtained.

Dye was injected into the right heart and simultaneous time-density curves were recorded from the two systems. Free flow was allowed from the large cannula in the left femoral artery and was measured.
by timed collection of blood, while the flow from the right femoral artery was controlled by the constant rate withdrawal pump.

The distance from the center of the cuvette to tip of polyethylene cannula in the controlled flow system was 14.5 cm. and internal diameter was 1.19 mm. On the left the large free flow cannula measured 10 cm. from the center of cuvette to tip of the cannula with an internal diameter of 2.69 mm.

Part II. A polyethylene cannula about 30 cm. long was inserted into the left femoral artery of a dog. Two cuvettes with phototubes were placed on the same tube, one 20 cm. from the tip of cannula and the second 20 cm. from the first. Simultaneous recordings of the time-density curves were made from the two positions after injection of dye into the right heart. Four pairs of simultaneous curves were obtained at various flows through the tube. The curves were replotted after correction to make the areas under the two curves equal.

RESULTS

In Vitro

Examination of the time-response curves following a "square wave" input reveals the amount of distortion produced by changes in

![Figure 1](image1)

**Fig. 1.** Top. Time-density curves showing the effect of rate of withdrawal (ml./sec.) on a "square wave" input. Internal diameter of polyethylene, 1.77 mm.; distance from outflow to center of cuvette, 18 cm. Bottom. Time-density curves showing the effect of changing the length of polyethylene (outflow to center of cuvette) from 6 cm. to 18 cm. at four rates of withdrawal. Internal diameter of polyethylene tube, 1.19 mm.

![Figure 2](image2)

**Fig. 2.** Top. Time-density curves showing the effect of changing diameter of tubing with length constant at three rates of withdrawal. Bottom. Time-density curves showing the effect of packed cell volume at four rates of withdrawal. Tubing internal diameter, 1.19 mm.; length, 18 cm.

![Figure 3](image3)

**Fig. 3.** Comparison of the slopes of the straight line portion of the downstroke of simultaneous pairs of curves, plotted as ratio: sampling system slope to free flow slopes. Slopes are calculated as: $S = \frac{\ln C_1 - \ln C_2}{\text{seconds}}$. 

**Results**

In Vitro

Examination of the time-response curves following a "square wave" input reveals the amount of distortion produced by changes in
the variables of the sampling system. Figure 1 Top shows the effect of changing the rate of withdrawal, length and diameter remaining constant. Figure 1 Bottom shows the effect of the length of the tubing at various rates of withdrawal and reveals an increasing distortion by the longer tubing at slower rates, this difference being eliminated by fast rates. In figure 2 Top, the effect of changing the diameter with length constant is again observed to produce greater distortion at the slower flow rates although the change from 1.19 to 1.77 mm. internal diameter still causes some distortion even at the highest rates of flow. Figure 2 Bottom shows the increasing distortion with increase in hematocrit.

In Vivo

Part I. Fifty pairs of simultaneously recorded curves were obtained from dog preparations as described. The degree of distortion of the curves drawn through the sampling system were quantitated by comparison of the downslope of the curve obtained through this system to the downslope of the curves from the free-flowing cannula. The ratio of the sampling system slopes to the free-flowing cannula slopes are given in figure 3. It can be seen that similar slopes (i.e., no distortion) are obtained when the sampling system draws at rates of 0.71 ml./sec. and above, whereas the slopes of the sampling system curves show progressively larger errors at lower rates of withdrawal when compared with the slopes obtained from the free-flowing system. This effect can be seen in figure 4 where examples of typical simultaneous curves are reproduced.

The distortion of time-concentration curves is such that rapidly changing curves will be affected to a greater degree than more slowly changing curves. This can be demonstrated by using a bottle system and comparing the curves from a cuvette placed directly on the outflow tube to the curves obtained from a constant sampling system as described (diameter 1.77 mm. and length 18 cm. at a sampling rate of 0.7 ml./sec.) and the downslope of the free-flowing curves.

\[
\text{Slope} = \frac{\ln C_1 - \ln C_2}{\text{seconds}}
\]

Fig. 4. Distortion of dye dilution curves by sampling system. o curves from free-flowing cannula, x curves from sampling system. The rate of sampling was progressively decreased from A through D:

<table>
<thead>
<tr>
<th>Flow (ml/sec.)</th>
<th>Free</th>
<th>Sampling system</th>
<th>Free</th>
<th>Sampling system</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.2</td>
<td>1.39</td>
<td>.523</td>
<td>.523</td>
</tr>
<tr>
<td>B</td>
<td>6.3</td>
<td>0.71</td>
<td>.375</td>
<td>.355</td>
</tr>
<tr>
<td>C</td>
<td>5.9</td>
<td>0.44</td>
<td>.850</td>
<td>.635</td>
</tr>
<tr>
<td>D</td>
<td>5.5</td>
<td>0.28</td>
<td>.779</td>
<td>.443</td>
</tr>
</tbody>
</table>

* Calculated as slope = \( \frac{\ln C_1 - \ln C_2}{\text{seconds}} \).
Par II. The increasing distortion caused by 20 cm. of tubing can be readily seen in figure 5. At very rapid flows little, if any, distortion can be seen, whereas slowing of flow in the tubing brings out progressively greater distortion due to the effect of the 20 cm. of tubing between the cuvettes.

DISCUSSION

These data demonstrate the effect of variations in dimensions of a sampling system and rate of withdrawal on a time-concentration curve. The distortion of a square wave input as it traverses the tubing is probably related to the character of the flow (laminar or turbulent). Each of the variables causes changes in relation to the other variables, and critical values for each cannot be established independently. We have been unable to establish any precise relation of improved response time to any one of the following: ratio of volume to flow, internal surface area to flow, or linear velocity.

The possibility that the distorted response at slower rates of withdrawal may be due to slower passage of the wave front through the length of the cuvette was considered. However, the longest transit time through the cuvettes of 2.4 cm. length was 0.16 sec. and the shortest 0.019 sec. Any distortion caused by this would be only a very small fraction of the total observed.

The distorting effect of a sampling system is shown by the in vivo curves obtained in dogs. These curves varied in duration and slope of the downstroke but showed that a sampling system of the dimensions described produces a significant change if the sampling rate is slower than 0.71 ml./sec. From the in vitro analysis it is apparent that sampling systems with different dimensions would have their own requirements as to the flow necessary to prevent distortion. Throughout this work we have found some form of withdrawal pump necessary to assure an adequate and constant sampling rate.

We have demonstrated distortion in continuously recorded dye dilution curves and from previous reports it is apparent that this also applies to multiple sampling technics and is probably true for any indicator used.

In the analysis of dye dilution curves the accurate reproduction of the curve is essential. Distortion of either the ascending or descending limb can profoundly affect calculations based on the shape of the curve. This is particularly important in the calculation of “central volume” from the ratio of flow to slope. The variation in rates of sampling and dimensions of sampling systems reported in the literature necessitate the exercising of great caution when comparing slopes and volumes obtained by different investigators. This is particularly important in animal work and in curves obtained from left heart injections since a sampling system which produces only slight
distortion in slow curves may greatly distort more rapidly changing curves.\textsuperscript{9-13} The distortion changes only the shape and not the total area under the curve, therefore, calculations of outputs will be unaffected irrespective of the sampling system.

**Summary**

Distortion of a "square wave" input of indicator concentration (indigo carmine) by passage through polyethylene tubing of various dimensions has been demonstrated. Such distortion can be prevented by increasing flow, decreasing length, or decreasing diameter. Since no critical values of any 1 variable could be established every system must be evaluated as an entity.

The distortion caused by the sampling system of the time-concentration curve occurring in the arterial system of the dog, following injection of indicator dye into the right heart is demonstrated. The rate of withdrawal needed for accurate reproduction of the curve is established for one system.

Comparisons of values for appearance to peak times, "central volume," valvular insufficiency, etc., cannot be made unless it is certain that the systems used do not distort the curves obtained.

**Summario in Interlingua**

Esseva demonstrate le distortion del entra de "unda rectangular" de un date concentration del indicator (carmino indigo) in consequentia de su passage per tubos de polyethylene de varie dimensiones. Iste distortion pote esser prevenite per accelerar le fluxo del indicator o per reducir le longor o le diametro del tubos. Proque nullo valor critic del un o del altre de iste tres variabiles poteva esser establete, omne systema debe esser evaluatate come entitate.

Esseva demonstrate le distortion del curva de tempore e concentration in le systema arterial del can post injection del indicator in le corde dextere. Le rapiditate del retraction require pro le exacte reproduction del curva es establete un inter le multe possibile systemas.

Comparationes inter le valores pro temores culminal, "volumine central," insufficientia valvular, etc. non es possibile si le systemas usate resulta in un distortion del curvas.

**REFERENCES**

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