An Improved Method for the Calibration of Continuously Recorded Dye Dilution Curves


A simplified method is described for the calibration of continuously recorded dye dilution curves. The accuracy of this method is demonstrated in vitro and in vivo.

One of the main problems in the use of continuously recorded dye dilution curves has been the calibration of the obtained curves. Methods involving measurements from the "tail" of the dilution curve are often inaccurate because in this portion of the curve the concentration of dye is low. Other methods of calibration involve determinations from a sample of blood withdrawn before or after the dilution curve has been recorded.

A method of calibration has been developed which avoids many of these difficulties. It involves the use of a sample of the same blood which is being withdrawn through the cuvette for the inscription of the dye curve and is therefore of a much higher concentration. The calibration is carried out by determining the mean concentration of dye in the sample and determining the mean deflection over the period of the collection.

Method

To obtain a constant rate of withdrawal and for the collection of an integral sample, a syringe pump was designed (fig. 1). The pump is electrically driven and a four speed transmission drives a horizontal shaft (ram) either forward or backward. The end of the shaft is attached to the syringe plunger, so that the pump may be used for either injection or withdrawal of fluid. Interchangeable syringe holders for 20, 50, and 100 ml. syringes allow a selection of 12 rates of flow which vary from .00018 to 2.1 ml./sec. The pump is designed so that two syringes mounted in parallel can be attached to the horizontal shaft and these in turn are connected through a special crossover stopcock to either polyethylene tubing carrying the cuvette and intra-arterial needle or to a mercury containing reservoir (fig. 1). The stopcock is designed so that the switch over from one syringe to the other can be achieved in less than 0.2 sec. The mercury reservoir is necessary because air in the syringe which is not initially collecting blood acts as a cushion that, at the changeover, alters the rate of withdrawal. The efficiency of the apparatus and the accuracy of this method of calibration was tested by in vitro and in vivo experiments.

In Vitro Tests. Experiments were carried out with a system of 3 bottles similar to those used by Newman et al.1 The changing optical density was measured by a densitometer2 and recorded on a twin-channel Poly Viso (Sunborn). This densitometer gives a deflection that is directly proportional to the dye concentration over the entire range needed for dye dilution curves. The flow through this bottle system was kept constant by a centrifugal pump. Twenty consecutive observations measuring the collected flow varied between 4.97 and 5.04 L./min. with an average of 5.01 L./min. (2 S.D. ± .036 L./min.). Known quantities of dye (indigo carmine)3 were injected using a syringe that was calibrated to deliver a fixed amount. This amount was checked spectrophotometrically (Coleman Junior) at the end of each group of experiments. The dye was injected into the stream proximal to the first bottle and the dilution curve was obtained by withdrawing fluid from the system distal to the third bottle. A constant rate of withdrawal was assured by the syringe pump described. The stopcock at first was turned into position A (fig. 2), so that the initial portion of the dilution curve is collected in syringe 1 while syringe 2 is drawing from the mercury reservoir. Watching the inscription of the curve the operator selects a point near its peak, turns the stopcock to position B, so that the sample for calibration is now drawn into syringe 2, syringe 1 being connected to the mercury reservoir. After a few seconds the stopcock is again turned to position A so that the remainder of the blood, not needed for calibration, is collected in syringe 1. The sample from syringe 2 is used for calibrating the curve. On turning the stop-

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* Designed and built by Mr. Bailey F. Moore in the Apparatus Shop of Vanderbilt University, School of Medicine. Further details can be obtained from Dr. Elliot V. Newman, Department of Medicine, School of Medicine, Nashville, Tenn.
CALIBRATION OF CONTINUOUSLY RECORDED DYE DILUTION CURVES

Fig. 1. Syringe pump, showing two 50 cc. syringes in place which are attached through the crossover stopcock to the densitometer photohead and mercury reservoir. On the top of housing on right containing the transmission and motor is mounted a 4-speed selector. Parallel to this housing is the bearing block for the ram. The knob operates a sliding rack pinion which may be used to disengage the power head and position the ram. Below ram on front and rear surfaces of the bearing block are safety microswitches preventing excessive withdrawal or injection of fluid and protecting syringes from the force of the pump after the pistons have reached the end of their traverse. Rear one operated by the adjustable stop at the end of the ram; front one, by fixed stop on the ram just behind the syringe yoke.

Fig. 2. Diagram of crossover stopcock.

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Cuvette Reservoir Cuvette Reservoir

cock from either position a microswitch marks the Poly-Viso tracing. In addition, a small spike of about 0.1 to 0.2 sec. duration appears on the curve itself and is due to the brief cessation of flow at the time the stopcock is turned. The over-all shape of the curve however is not distorted (fig. 3).

In Vivo Experiments. Mongrel dogs were anesthetized with Nembutal and a tracheal cannula inserted for the administration of 100 per cent oxygen. A no. 8 cardiac catheter was introduced under fluoroscopic control into the right heart. Through this known quantities of dye were injected as above. Both femoral arteries were exposed and cannulated with polyethylene tubing (internal diameter 0.177 cm.). The cannula from the left leg was arranged so that multiple arterial samples could be collected in tubes attached to a rotating lucite disc.1 The flow from this side was approximately 2 to 3 ml./sec. The cannula in the right femoral artery was attached to additional polyethylene tubing which carried the cuvette and photohead. This in turn was connected through the crossover stopcock to the syringes mounted on the pump. The withdrawal speed of 1.39 cc./sec. was used throughout these experiments. No attempt was made to minimize the dead space in the two withdrawal systems as the intention was to compare the cardiac output obtained by calibration from the integral sample with those calculated from multiple arterial samples. These observations would be uninfluenced by any distortion imposed on the curve by excessive or unequal volumes of the withdrawal systems.

RESULTS

In Vitro. Using the in vitro system with a constant flow, 70 consecutive observations were made. The flows calculated from the dilution curves obtained were compared with the measured flow through the system. The
FIG. 3. Dye dilution curve obtained from the femoral artery of a dog. Paper markings, 1 cm. and 0.2 cm. squares. Time marker, seconds; continuous thick line, automatic signal denoting the period over which integral sample was collected. Spikes on curve, points where the stopcock was turned. In this instance, 80 nig. indigo carmine injected; mean concentration of integral sample, 34.3 mg./L. and mean deflection, 13.1 mm. Total area of original dilution curve, 122.3 mm. sec. Therefore cardiac output = $80 \times 60 = 1.50$ L. plasma/min. (table 1, experiment no. 18).

Table 1.—Comparison of Cardiac Output by Continuous Recording and Multiple Sampling Techniques

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>C.O. from multiple arterial samples (L. plasma/min.)</th>
<th>C.O. from integrated sampling (L. plasma/min.)</th>
<th>Percentage error</th>
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<tr>
<td>1</td>
<td>3.49</td>
<td>3.50</td>
<td>+2.9</td>
</tr>
<tr>
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<td>2.68</td>
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<tr>
<td>20</td>
<td>1.04</td>
<td>0.94</td>
<td>−0.6</td>
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</table>

Mean error, −0.385 S.D., ±3.6 per cent.

percentage error ranged from +6 to −6 per cent. The mean error was −0.045 and S.D. was ±1.10 per cent.

In Vivo. Twenty consecutive pairs of simultaneously recorded dilution curves were obtained from the dog preparations already described. The percentage error ranged from +4.6 to −9.6 per cent. The mean error was −0.387 and the S.D. ±3.6 per cent (table 1). In two observations the percentage error was −9.5 and −9.6 per cent. In these instances the curves obtained from the collection of multiple arterial samples were not entirely satisfactory and the error was thought to be in the multiple sample collection rather than the integral sampling. Excluding these two pairs of curves the percentage error of the remaining 18 observations range from +4.6 to −3.4 per cent with an S.D. of ±2.16 per cent.

Discussion

When collecting the integral sample for calibration, we have found that collecting from the peak of the curve to a point towards the end of the descending limb gives the optimum results. On the whole we have avoided starting sample collection on the rapidly changing ascending limb of the curve, although in cases where this has been done the results have been satisfactory. The sample may be collected during inscription of the whole curve or any part of it. McNeely et al.7 started their sample collection before the appearance of dye was recorded, similarly collection may be extended into the portion of the curve that is inscribed during recirculation. As long as the mean plasma concentration of dye in the sample collected is known and the mean deflection caused during the time of sampling can be calculated, the dilution curve can be calibrated.

One of the main advantages of integral sampling is that the plasma used for calibration contains a high concentration of dye. In addition, the sample for calibration is actually collected while the dilution curve is being inscribed, thus eliminating any inaccuracies caused by changes of oxygen saturation, instrument drift, etc., that may develop in the interval between the inscription of the dilution curve and collection of the sample for calibration. It is for this reason that we limit our sample collection to the curve itself, and for the reasons already given favor collecting the sample during inscription of the descending limb of the curve.
Clinical experience with this method of calibration has been entirely satisfactory.

**Summary**

A method for the calibration of continuously recorded dye dilution curves, which involves the collection of an integral sample has been described. Experimental work shows that this method gives a high degree of accuracy, when compared with the measured flow through a hydraulic system and with the cardiac output in dogs measured synchronously by the collection of multiple arterial samples. A syringe pump designed to withdraw or inject at various constant rates is described.

**Acknowledgment**

We wish to acknowledge the help of Dr. John H. Brewer, Director of Biological Research, Hynson, Westcott, and Dunning, Baltimore, Md., for further supplies of indigo carmine.

**Summario in Interlingua**

Es describite un methodo pro le calibration de curvas de dilution de colorante a registra- tion continue. Le metodo require le collection de un specimen integral. Labores experimental ha monstrate que le metodo resulta in alte grados de exactitude in comparation con le mesuration del fluxo in un sistema hydraulic e con le rendimento cardiac in canes mesurate synchronemente per le collection de multiple specimens arterial. Un pumpa syringa es describite que es construite pro le retraction o injection a varie rapiditates constante.

**REFERENCES**

An Improved Method for the Calibration of Continuously Recorded Dye Dilution Curves
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