EVALUATION OF A DYNAMIC METHOD FOR CALIBRATION OF DYE DILUTION CURVES

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Dilution techniques were first used for the measurement of the cardiac output in man by Hamilton *et al.* (1928). The original method involved multiple arterial samples and subsequent determination of the dye concentration in each sample.

The introduction of continuously recorded dilution curves (Friedrich, Heimbecker, and Bing, 1950; Nicholson and Wood, 1951; Milnor *et al.*, 1953) overcame the need for multiple samples but introduced the problem of calibrating the time concentration curve obtained. Milnor *et al.* (1953) drew attention to the inherent errors in the practice of calibrating from a single venous sample obtained some minutes after the dye curve had been obtained, and this led Emanuel, Lacy, and Newman (1957) to develop the technique of integral sampling. This overcame many of the previous problems, but the concentration of dye in the sample for calibration still had to be determined.

Sparling *et al.* (1960) devised a technique that eliminated the need for a calibration sample, thus avoiding any form of chemical or spectrophotometric analysis. They compared the area of the dilution curve obtained from the patient with that derived from a small calibration system built into the sampling line. In order to determine the accuracy of their method we constructed a model so that the "calculated" flow using their method of dynamic calibration could be compared with the actual flow through the system.

THEORY

The area of a dilution curve is controlled by two factors, the quantity of dye injected and the flow through the system. If we assume that the areas of the dilution curve from both patient and calibration system are the same, then the flow rate in the patient (Q) is related to the flow rate in the calibration system (Qc) as the quantity of dye injected into the patient (I) is related to the quantity of dye injected into the calibration system (Ic), i.e. Q:Qc as I:Ic, or $Q = \frac{I \times Qc}{Ic}$.

The areas of the dilution curves from the patient (A) and calibration system (Ac) are seldom identical; correction for this difference must therefore be made.

$$Q = \frac{I \times Qc}{Ic} \times \frac{Ac}{A}, \text{ or } Q = \frac{I \times Qc \times Ac}{Ic \times A}.$$

This equation can be solved for Q, as I, Ic, A, Ac, and Qc can all be measured.

METHOD

A diagram of the model used is shown in Fig. 1. A double roller pump (A) was set to deliver an arbitrary flow through the system and a cuvette withdrawal pump (B), capable of maintaining a constant sampling rate, was used to withdraw fluid through the cuvette (C) (Norman, 1959) from the sampling site (D). A known quantity of dye was injected into the calibration loop (E) and the dilution curve obtained (calibration curve) was recorded on a Honeywell Brown Recorder. With all aspects of the recording and measuring system unchanged this procedure was followed by the injection of a known quantity of the same dye solution at the injection site (F) and a dilution curve was obtained from the model system.

The flow through the model, including that withdrawn through the cuvette, was collected and measured,

FIG. 1.—Diagram of model system. A, double roller pump; B, cuvette withdrawal pump; C, cuvette; D, sampling site; E, calibration loop; F, injection site; G, aspirators; H, 5 ml. injection syringe; and K, micro-injection syringe.

and the minute volume calculated (measured flow). The area of the two dilution curves was measured by planimetry and the flow through the model system calculated from the formula already described.

TABLE I
COMPARISON OF MEASURED FLOW AND CALCULATED FLOW IN 25 CONSECUTIVE EXPERIMENTS

Experiment number	Measured flow (l./min.)	Calculated flow* (l./min.)	Percentage error
1	1.69	1.61	-4.7
2	2.80	2.76	-1.4
3	3.95	4.31	+9.3
4	6.39	6.02	-5.8
5	6.12	6.40	+4.6
6	5.71	5.52	-3.2
7	3.33	3.25	-2.4
8	4.26	4.42	+3.7
9	4.47	4.24	-5.2
10	4.91	5.17	+5.3
11	5.22	4.99	-4.4
12	2.57	2.60	+1.2
13	6.35	5.57	-12.3
14	3.53	3.46	-2.0
15	5.73	5.78	+0.9
16	3.66	3.70	+1.1
17	3.23	3.05	-5.6
18	3.04	2.94	-3.3
19	4.02	3.48	-13.4
20	3.08	2.90	-5.9
21	2.12	2.08	-1.9
22	2.11	1.91	-9.5
23	2.95	2.82	-4.4
24	2.24	2.14	-4.5
25	2.99	2.82	-5.7

* Using Sparling *et al.*'s (1960) method of calibration.

RESULTS

In 25 consecutive experiments the measured flow through the model system was compared with the calculated flow using Sparling *et al.*'s (1960) dynamic method of calibration. In these experiments the measured flow was varied from 1.69 l./min. to 6.39 l./min. The results are shown in Table I, and Fig. 2. The percentage error ranged from -13.4 to $+9.3$, with a mean error of -2.78 , (standard deviation 5.2%). There was a 95 per cent probability that the calculated flow was within -13.0 to $+7.4$ per cent of the measured flow.

FIG. 2.—Twenty-five consecutive comparisons of the calculated flow in ml./min. using Sparling *et al.*'s (1960) method of calibration and the measured flow in ml./min. For statistical analysis see text.

DISCUSSION

Results from a model system show that Sparling *et al.*'s (1960) dynamic method of calibration offers a 95 per cent probability that the calculated flow will fall within -13.0 to $+7.4$ per cent of the measured flow. There was, however, a tendency to underestimate the flow but this error was small and independent of flow rate.

The main advantage of this method of calibration over those previously described is that no sample of blood is required for calibration, thus eliminating all chemical and spectrophotometric analysis. In addition, perfect linearity of the measuring and recording system is unimportant if the quantities of dye injected are adjusted so that the peak deflection of the dye curve from the patient and the calibration curve are approximately the same. In common with the method described by Emanuel *et al.* (1957) a dye that disappears rapidly from the circulation, can be used as it only has to remain within the vascular bed during the initial circulation from the injection site to the sampling site.

The accuracy of this method depends on a constant known sampling rate, exact measurement of the amount of dye injected, and the stability of the measuring and recording system for the time required to obtain the two dye curves. Adequate stability is not difficult to achieve as the interval between the two injections can be reduced to a few seconds if the calibration curve is recorded first, so that the second injection is not delayed on account of recirculation.

These experiments show that Sparling's dynamic method of calibration is sufficiently accurate in a model system to justify its physiological application.

SUMMARY

In 25 consecutive experiments with a model system, the calculated flow using Sparling *et al.*'s

(1960) dynamic method of calibration had a 95 per cent probability of being within -13.0 to $+7.4$ per cent of the actual flow.

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APPENDIX

Experimental Details

A Double Roller Pump (A) whose rotation speed had been slightly increased was used throughout this work.

The Model System was similar to that described by Newman *et al.* (1951) and consisted of three small aspirators (G) of different volumes arranged in series, to represent the right heart, lungs, and left heart respectively. Complete mixing in each aspirator was obtained by a small magnetically rotated stirrer.

Injection and Sampling Site. The injection site (F) was between the roller pump and the first (right heart) aspirator and consisted of an 18 gauge needle fixed rigidly into the system, with its lumen facing downstream. The sampling site (D) was a similar needle about 60 cm. beyond the last (left heart) aspirator with its lumen facing upstream.

Flow Measurement. The water leaving the model system was collected and measured to the nearest 5 ml. The volume of water withdrawn through the cuvette was added to this and the minute volume of the pump calculated.

Model System Injection. A 5-ml. all-glass syringe (H) mounted in a holder with a fixed back stop was connected to one limb of a small Y piece, the other limb was attached to a syringe filled with 10 ml. water. The Y piece had a minimal dead space and was designed to ensure that all dye was easily and completely washed into the system. The dye was injected rapidly, and flushed into the system by 10 ml. of water from the second syringe.

The accuracy of this method was confirmed by weighing the volume delivered through the dry Y piece. Twelve measurements showed a maximum difference of 0.005 ml. when the volume was 2.0 ml. (0.25%). After repeated use these syringes delivered slightly larger volumes, the increase was approximately 0.010 ml. in 2.0 ml. (0.5%). The injection syringes were therefore recalibrated after every six injections.

The Cuvette Withdrawal Pump (B) was a modification (J. Norman, 1962, personal communication) of that originally described by Emanuel *et al.* (1957). 50-ml. all-glass syringes were used for sampling. The pump withdrawal rate was checked by continuous sampling from a burette and was found to vary maximally by 0.1 ml./min. in 20 ml./min. a variation of 0.5 per cent. This variation did not increase if the system from which the sample was drawn was at a pressure of 100 mm. Hg.

The Calibration Loop (E) was a polyvinyl chloride tube 16.0 cm. long, 6.0 mm. internal diameter, filled with siliconed glass beads with an average diameter of 3.0 mm. leaving a fluid volume of 2.6 ml. Injections of dye for calibration were made through a short piece of thick-walled rubber tubing fixed between the loop (E) and the sampling site (D).

Calibration Injection. A standard 50 μ l. (0.050 ml.) syringe (K) was used. The calibration injection consisted of 10 μ l. dye solution in the lower part, and 40 μ l. water in the upper part of the syringe. The accuracy of these syringes when new was extremely good; after 100 injections, however, the volume delivered had fallen by 2 μ l., i.e. 4 per cent of the total volume. The micro-syringes were therefore replaced after every 15 injections.

The Dye. This was Evans Blue (T. 1824). As the spectral absorption of this dye was found to be unstable in the presence of minute traces of metallic salts, all water pumped through the model system was previously passed through an ion exchange plant.

Areas of Dilution Curves. These were measured by polar planimetry. Each area was measured three times; the maximum difference between the readings for any one curve was 2 per cent.

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