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A Dynamic Method for the Calibration of Dye Dilution Curves in a Physiological System

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Sparling *et al.* (1960) reported a dye dilution technique for the measurement of the cardiac output, which avoided any form of chemical or spectrophotometric analysis. The method involved the comparison of the area of two dilution curves, one obtained from the subject and the other from a small calibration system built into the sampling line. An evaluation of this dynamic method in a model has already been reported (Emanuel and Norman, 1963).

The experiments described here were designed to determine the accuracy of this technique *in vivo*, by comparing it with an established method. The results of simultaneous cardiac output estimations were compared using the dynamic method described by Sparling *et al.* (1960) and Hamilton's method of multiple arterial samples (Hamilton *et al.*, 1928).

Subjects and Method

Observations were made on a series of heparinized greyhounds initially anæsthetized with thiopentone and intubated with a cuffed endotracheal tube. During the experiments the animals were maintained on oxygen and halothane. The right atrium was catheterized via the external jugular vein. The femoral arteries were exposed and polyethylene catheters introduced into the distal part of the abdominal aorta. Multiple timed arterial samples were collected from the right femoral catheter (Fig. 1). These samples were centrifuged at 3,000 r.p.m. for 15 minutes, and the optical density of the plasma was determined using a "Unicam" spectrophotometer at 620 millimicrons. The packed red cells were assumed to include 6 per cent trapped plasma (Gregersen and Schiro, 1938). Blood was taken from the dog at the beginning of the experiment so that the arterial samples could be standardized against serum from the same animal after known quantities of dye had been added. The cardiac output was then calculated as described by Hamilton *et al.* (1928).

The left femoral catheter was connected to a cuvette (Norman, 1959) and a sampling pump with a constant known withdrawal rate. Dilution curves were recorded on a Honeywell Controls Ltd. potentiometric recorder. Between the arterial sampling site and the cuvette a calibration loop was built into the sampling line (Fig. 2). The loop consisted of a polyvinyl chloride tube 16·0 cm. long and 6·0 mm. internal diameter, filled with silicone glass beads with an average diameter of 3·0 mm., leaving a fluid volume of 2·6 ml. The calibration system was arranged with a three-way tap at each end, so that it could either be included or excluded from the main sampling line. When dye was injected for calibration from the micro-syringe (B, Fig. 2) the taps were turned so that the blood passed through the calibration loop, but when the dilution curve from the animal was recorded the calibration loop was excluded from the sampling line. Calibration injections consisted of 50 micro-litres of dye injected from a standard 50 micro-litre syringe. Injections were made through a short piece of thick-walled rubber tubing fixed between the arterial sampling site and calibration loop (Fig. 2). The dilution curve from the animal was preceded and followed by dilution curves from the calibration loop. The mean of the areas of these two calibration curves was used in calculating the cardiac output from the following formula (Emanuel and Norman, 1963):

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

where Q was the cardiac output of the animal; I, the

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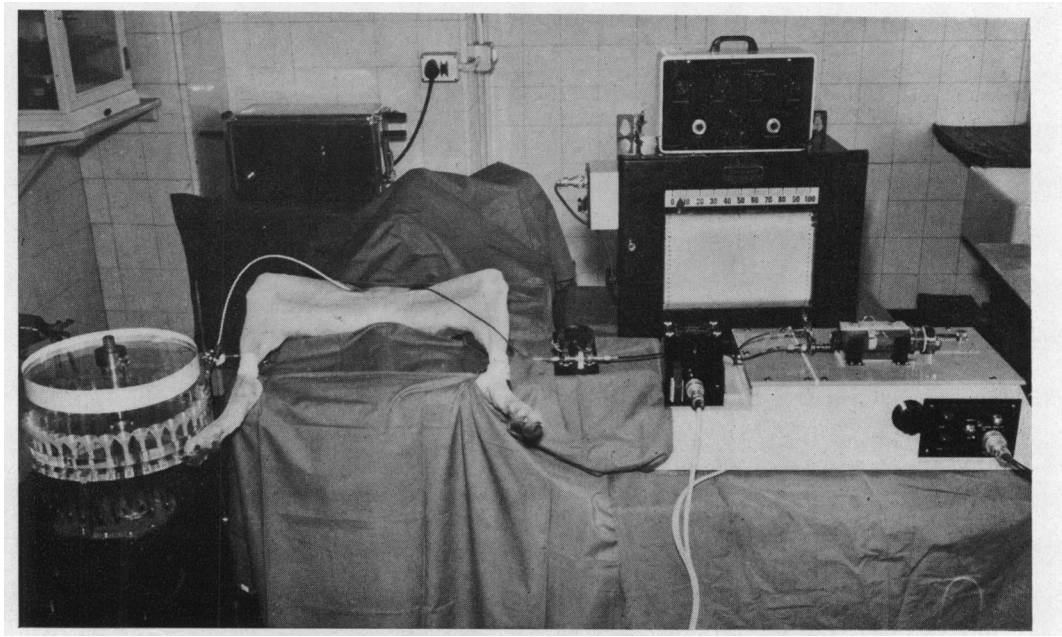

FIG. 1.—Anæsthetized dog showing femoral artery catheters in position, with arrangements for collecting multiple timed arterial samples from the right femoral catheter. The left femoral catheter is connected to the calibration loop, cuvette, and withdrawal pump. The Honeywell Controls Ltd. potentiometric recorder is seen in the background.

quantity of dye injected into the animal; Qc, the flow rate in the calibration system; Ac, the area of the dilution curve from the calibration loop; Ic, the quantity of dye injected into the calibration loop; and A, the area of the dilution curve from the animal.

Following a single injection of 10 ml. of indigo carmine into the right atrium, the cardiac output was measured simultaneously using Hamilton's method of multiple arterial samples from the right femoral catheter, and a continuously recorded dilution curve calibrated by Sparling's dynamic method from the left femoral catheter.

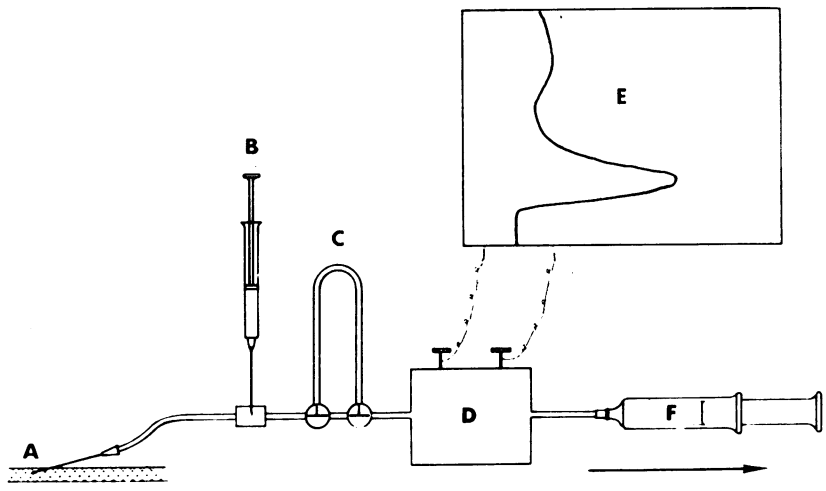

FIG. 2.—Diagram of the sampling line showing position of the micro-syringe (B) and calibration loop (C), with its three-way taps, in relation to the arterial sampling site (A) and the cuvette (D). E is the recorder and F is the withdrawal pump.

TABLE

COMPARISON OF HAMILTON TECHNIQUE AND DYNAMIC METHOD

CARDIAC OUTPUTS IN 41 CONSECUTIVE EXPERIMENTS

Experiment No.	Hamilton output (l./min.)	Dynamic output (l./min.)	Percentage error
1	4·24	4·55	+ 7·3
2	3·28	3·63	+10·7
3	2·70	2·60	− 3·7
4	2·66	2·58	− 3·0
5	3·52	3·76	+ 6·8
6	2·90	2·64	− 9·0
7	2·21	2·12	− 4·1
8	2·12	2·21	+ 4·2
9	4·16	4·31	+ 3·6
10	3·40	3·38	− 0·6
11	3·56	3·78	+ 6·2
12	3·19	3·24	+ 1·6
13	5·74	5·85	+ 1·9
14	3·94	3·51	−10·9
15	3·93	3·83	− 2·5
16	3·58	3·73	+ 4·2
17	3·20	3·12	− 2·5
18	3·0	3·10	+ 3·3
19	2·30	2·25	− 2·2
20	1·96	1·97	+ 0·5
21	4·48	4·23	− 5·6
22	3·24	2·96	− 8·6
23	2·26	2·21	− 2·2
24	6·52	7·23	+10·9
25	5·48	5·86	+ 6·9
26	4·93	4·71	− 4·5
27	3·59	3·75	+ 4·5
28	5·87	5·29	− 9·9
29	3·34	3·17	− 5·1
30	2·31	2·25	− 2·6
31	1·81	1·67	− 7·7
32	2.22	2.31	+ 4·0
33	2·30	2·38	+ 3·5
34	2·53	2·54	+ 0·4
35	2·79	2·49	−10·7
36	3·60	3·26	− 9·5
37	2·04	2·11	+ 3·4
38	5·7	5·19	− 8·9
39	5·0	4·71	+ 5·8
40	4·77	4·23	−11·3
41	4·07	4·07	0

Results

The cardiac output was measured simultaneously by the two methods in 41 experiments involving 11 dogs. The range of outputs during these experiments varied from 1·9 to 6·5 l./min. The results are given in the Table and Fig 3. The percentage error between the two methods varied from −11·0 to +11·0 per cent with a mean error of +1·2 per cent (standard deviation 6·1%). There was, therefore, a 95 per cent probability that the results were within −11·0 to +13·5 per cent of each other, indicating satisfactory agreement between the two techniques.

Discussion

These experiments show that the dynamic method of calibration (Sparling *et al.*, 1960) gives estimations of cardiac output comparable to those obtained by the Hamilton method of multiple arterial samples.

As previously reported (Emanuel and Norman, 1963), the accuracy of the dynamic method depends on a constant known sampling rate, exact measurement of the amount of dye injected into the subject and calibration loop, and the stability of the measuring and recording system for the time required to obtain the dilution curves. The stability of the system can be checked by comparing the areas of calibration curves recorded before and after the dilution curve from the subject.

The main advantage of the dynamic method of calibration is the absence of any chemical or spectrophotometric analysis, enabling the cardiac output to be calculated rapidly. Additional studies have shown that this technique can be simplified further by using a computer to calculate the areas of the dilution curves (Hamer *et al.*, 1966). Another advantage of the dynamic method is that a rapidly excreted dye such as indigo carmine can be used, as it has to remain in the vascular bed only during the initial circulation from injection site to sampling site.

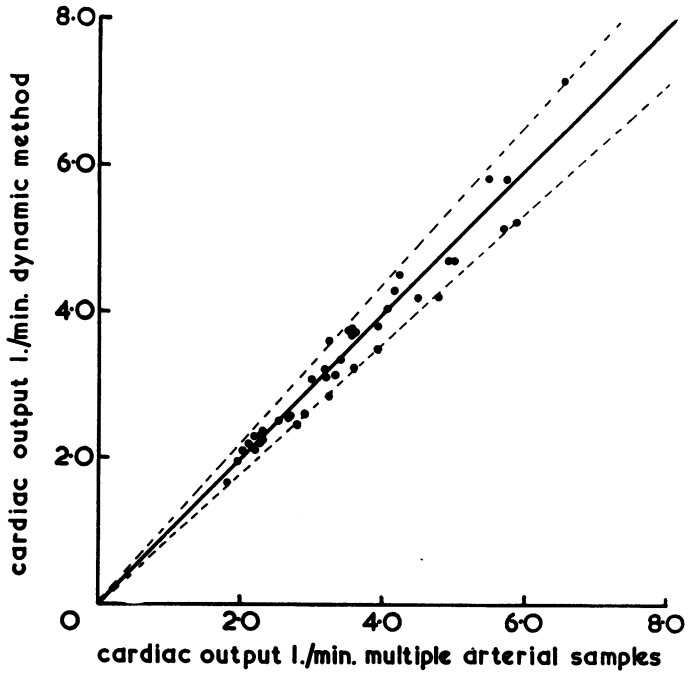

FIG. 3.—Forty-one comparisons of the cardiac output from 11 dogs measured simultaneously by Sparling's dynamic method and by Hamilton's method of multiple arterial samples. For statistical analysis see text.

SUMMARY

In 41 experiments involving 11 dogs the cardiac output was measured simultaneously by multiple arterial samples (Hamilton *et al.*, 1928) and from a continuously recorded dilution curve using the dynamic method of calibration (Sparling *et al.*, 1960). There was a 95 per cent probability that the results were within −11·0 to +13·5 per cent of each other, thus establishing the accuracy of this method of calibration in a physiological system.

REFERENCES

Emanuel, R., and Norman, J. (1963). Evaluation of a dynamic method for calibration of dye dilution curves. *Brit. Heart J.*, **25**, 308.

Gregersen, M. I., and Schiro, H. (1938). The behavior of the dye T-1824 with respect to its absorption by red blood cells and its fate in blood undergoing coagulation. *Amer. J. Physiol.*, **121**, 284.

Hamer, J., Emanuel, R., Norman, J., and Burgess, M. (1966). The use of a computer in the calibration of dye dilution curves by a dynamic method. *Brit. Heart J.*, **28**, 147.

Hamilton, W. F., Moore, J. W., Kinsman, J. M., and Spurling, R. G. (1928). Simultaneous determination of the greater and lesser circulation times, of the mean velocity of blood flow through the heart and lungs, of the cardiac output and an approximation of the amount of blood actively circulating in the heart and lungs. *Amer. J. Physiol.*, **85**, 377.

Norman, J. (1959). A stable cuvette of high sensitivity for indicator dilution curves. *Brit. Heart J.*, **21**, 107.

Sparling, C. M., Mook, G. A., Nieveen, J., Van der Slikke, L. B., and Zijlstra, W. G. (1960). Calibration of dye dilution curves for calculating cardiac output and central blood volume. In *C.R. 3e Congrès Européen de Cardiologie, Rome, Pars altera A*, p. 595, Excerpta Medica.