Evaluation of a New Cuvette Densitometer for Determination of Cardiac Output

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Quantitation of cardiac output by the Stewart-Hamilton method will have extended usefulness if the accuracy of measuring the concentration of Evans blue dye in whole blood is established. Cardiac outputs by this method have been compared in dogs with outputs by the rotameter and the Fick method. The constant-flow photometer (Friedlich) was used initially, but certain difficulties in its use led to the development of a new cuvette densitometer. Comparisons using both these instruments gave average variations of plus 7.0 per cent and minus 5.4 per cent from the reference methods. With proper caution cardiac outputs in dogs can be accurately obtained from dye dilution curves in whole blood.

The Stewart method for quantitating cardiac output as modified by Hamilton and co-workers for vital dyes is laborious and time consuming. Recently Friedlich, Heimbecker and Bing have described an instrument which allows a continuous record to be taken of the arterial dilution curve of Evans blue dye in whole blood. In their study, nine comparisons of cardiac output were made with the Fick method and, of these, three differed by more than 25 per cent. Beard and Wood have also compared dye dilution outputs recorded with the cuvette oximeter with Fick outputs in humans and found a similar degree of scatter. In the Fick technic as ordinarily used, changing rates of blood flow due to respiratory variations, sinus arrhythmia, or an unsteady state might be smoothed out in a three- to five-minute period, but these variations could markedly influence a 20-second dye dilution curve. Since it is important to know whether quantitative data can be obtained from a continuous record of the dye dilution curve, simultaneous comparisons were made of cardiac outputs determined from such curves, and from those obtained with the rotameter, which measures blood flow directly. Comparisons were also made with the Fick method in situations in which the aforementioned variables which might affect the two methods differently were largely removed. The work was begun using a modification of the Friedlich constant-flow photometer; however, instrumental difficulties were encountered which precluded its routine use. Accordingly, a new cuvette densitometer was developed which eliminated the major undesirable features of the Friedlich photometer. The results of the comparisons using both instruments are reported here.

Apparatus

The densitometer consists of a power-amplifier and photoelectric pick-up unit connected by a cable. The physical separation of the two units provides convenience, flexibility, and close approximation of the photoelectric unit to the experimental animal. The latter unit is shown schematically in figure 1.

The optical system of the pick-up unit consists of a housing for a voltage stabilized 3 candlepower incandescent lamp, collimating lenses and a Corning sharp cut filter, a 630 µ interference filter, a cuvette with Luer-Lok connectors through which the blood flows, and a housing for the photomultiplier tube. A filter wheel at the top of the photomultiplier tube housing controls the position of six optical density wedges. These wedges serve as absolute calibrating standards and allow the instrument to be tested for stability at various gain settings.

Either a direct writing or mirror type gal-
The galvanometer can be used to record the photomultiplier tube output; however, it has been found convenient to use a fluid damped galvanometer with photographic recording.\* As used in the determination of cardiac output the background optical density of blood flowing through the cuvette is suppressed to a convenient level moving the galvanometer trace to the lower edge of the photographic paper. Then, as blood with an increasing concentration of dye passes through the cuvette, the upright deflection of the galvanometer beam is a linear function of the dye concentration because the response of the electronic circuit has been made logarithmic. Thus the response of the instrument to equal increments in dye concentration remains constant at each gain setting regardless of background optical density. This logarithmic response makes it possible to transcribe the dye dilution curve from the photographic paper directly to a semilogarithmic plot of optical density against time, decreasing the work necessary in calculating the cardiac output.

The gain setting used is predetermined by the amount of dye to be injected, the sensitivity of the galvanometer, and the gross level of cardiac output. For ease in reading the photographic records the gain setting is adjusted to give a maximum deflection of 7 to 15 cm. With a little experience this setting can be made and left untouched unless very large variations in cardiac output are expected.

Figure 2 illustrates the calculation of cardiac output with the densitometer. The dye dilution curve (fig. 2a) is plotted at one-second intervals as the logarithm of deflection against time (fig. 2b), and the straight line downslope is extrapolated to 1.0 mm. deflection. The average height of this plot, calculated by area per unit of time, is then converted to an average concentration of dye in milligrams per cubic centimeter by means of the calibration curve (fig. 2c), and this value is used in Hamilton's formula for calculating the cardiac output.\* Three dye concentration points have been used to establish each calibration curve. With few exceptions the calibration curve with the densitometer (fig. 2c) is essentially linear and approximates zero deflection at zero dye concentration.

In experiments with the constant flow photometer\* the flow curves obtained were of the same order of magnitude as those described for the densitometer and could be analyzed with equal accuracy. At times, however, erratic responses occurred which could only partly be explained on the basis of photomultiplier tube fatigue or battery decay. In many experiments a straight line drawn through a semilogarithmic plot of the three calibrating points failed to pass through zero concentration at 100 per cent transmission, and cardiac outputs calculated from these calibration curves failed to check well with rotameter flows. Therefore, no experiments were accepted as reliable unless the straight line drawn through the three calibrating points approximated zero concentration at 100 per cent transmission.

**METHODS**

Comparisons of blood flow as measured by the dye dilution technic with flow measured by the rotameter were obtained in both an artificial pump system and open chest dogs. Comparisons of cardiac output were also made between the dye dilution and the Fick methods in closed chest dogs.

The in vitro comparisons were made in the pump system diagrammatically shown in figure

\* Type OE-1 galvanometer, sensitivity 600 mm./ma./M., natural frequency 90 cycles/sec., in a S-14C oscillograph. Hathaway Instrument Co., Denver, Colo.
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3 (left of the dotted line). Blood was forced through the rotameter by means of a Dale-Schuster pump. Approximately 1 liter of well-oxygenated blood was required. The dye was injected into the input side of the rotameter, and a continuous sample of mixed blood and dye was obtained from the rotameter drainage tube.

In the open chest preparation comparisons were made in mongrel dogs weighing 10 to 15 Kg. anesthetized with sodium pentobarbital, or morphine sulfate (3 mg. per kilogram) subcutaneously followed by a 1:1 mixture of Dialurethane and pentobarbital intravenously in one-half hour. Artificial respiration was maintained by positive pressure ventilation using either oxygen or compressed air with oxygen supplement. The chest was entered by removing portions of the third through seventh left ribs; heparin (10 mg. per kilogram) was injected initially and one-half this dose repeated every 30 minutes. Before each cardiac output determination by the dye method an arterial sample was withdrawn for calibration. A constant blood flow through the cuvette was maintained by a suction device previously described. The volume of injected dye was measured by weighing dye syringes before and after injection. Each experimental animal was bled at the end of the experiment, the blood filtered, and the rotameter calibrated with this

Fig. 2. Calculation of cardiac output in a typical experiment using the densitometer. (a) Photographic record of dye dilution curve in whole blood; (b) semilogarithmic plot of dye dilution curve; (c) calibration curve of three known concentrations of Evans blue dye in whole blood.

Fig. 3. Schematic diagram showing pump system and its use in evaluating the dye dilution technic in vitro and in vivo. Discussion in text.
blood. The area under the rotameter flow curve bracketing each dye dilution curve was integrated, the average height calculated and the mean flow during this time read from the rotameter calibration.

In one open chest group the rotameter was tied into the pulmonary circuit by a modification of the technic of Seeley and Gregg as illustrated schematically in figure 3. Blood drained from a cannula in the right auricle into a reservoir and was pumped by a Dale-Schuster pump into the two pulmonary arteries, the right ventricle being completely by-passed. Dye was injected into the input side of the rotameter and arterial blood sampled through an aortic sound. Some difficulty in maintaining a constant arterial oxygen saturation was encountered in this preparation. Changes in this variable affect the optical density of blood at 630 m\(\mu\), since this is not an isobestic point for reduced and oxidized hemoglobin. Furthermore, the lungs of these animals became edematous soon after the preparation had been made, which could result in dye being lost from the circulation on its initial passage through the lungs. For these reasons only a small number of satisfactory comparisons were obtained in this preparation.

In the second open chest group comparisons were made with the rotameter connected to a special cannula tied in the descending segment of the thoracic aorta. All aortic branches between the coronary ostia and the cannula were ligated and the right carotid artery perfused from the output side of the rotameter. Dye was injected through a catheter whose tip lay in the superior vena cava, and arterial blood withdrawn from the aorta through a sound passed down the left carotid artery. The measured rotameter flow was corrected for coronary flow estimated to be 5 per cent of the total cardiac output, except in two comparisons with massive adrenaline injection, in which a 10 per cent* correction was used.

In comparisons done in closed chest animals the techniques used for the Fick determination were as described by Seeley, Nerlich, and Gregg for the open chest dog. Blood samples and oxygen consumptions were taken over a three-minute period, and the dye dilution output was measured during the middle of the Fick determination. By the combination of constant artificial respiration, morphine-barbital anesthesia, and procaine amide infusion a steady state was maintained for the three-minute period as evidenced by the stability of the oxygen usage curve, tidal volume, heart rate, pulse pressure, and blood pressure level. Small rhythmic variations in blood pressure were associated with artificial respiration, but the effect of such changes was minimized by the use of a respiratory rate of 24 per minute.

**RESULTS**

Figure 4 is a graph of rotameter or Fick flow as the reference method plotted against dye dilution flow. The 43 technically satisfactory comparisons shown in this graph were made in a variety of hemodynamic states. Flows ranged from 477 to 2299 cc. per minute, hematocrit from 38.0 to 54.0 per cent, heart rate from 72 to 222 beats per minute, and mean blood pressure from 48 to 194 mm. Hg. In any one experimental animal the maximum change in cardiac output between comparisons was from 726 to 2274 cc. per minute.

With the constant flow photometer, 17 comparisons were made in the artificial pump system, but only five were technically satisfactory. Thirteen comparisons were obtained in six dogs with the rotameter tied into the pulmonary circuit, but only four comparisons in two dogs were acceptable. All the photometer experiments with the rotameter in the aorta were acceptable (13 comparisons in four dogs). In 21 comparisons using the densitometer (five in the pump system, nine with the rotameter in the aorta, and seven with the Fick method), no experiments had to be discarded because of technical difficulties with the instrument.

In one experiment five determinations of cardiac output as measured by the densitometer were grossly different from the aortic rotameter.
flow. The standard deviation of the error of these five comparisons is three times greater than the standard deviation of the error of the other comparisons, and for this reason they are not included in the graph. A likely explanation for these discrepancies is that the total cardiac output as measured by the dye method was not passing through the rotameter because of an unligated aortic branch proximal to the rotameter. Unfortunately, an autopsy was not done in this experiment, so that this remains a matter of speculation.

Excluding these five comparisons the maximum difference between the dye dilution cardiac output using the constant flow photometer and the rotameter was plus 29 per cent and minus 10 per cent, with average variations of plus 7.5 per cent and minus 4.6 per cent. In the comparisons of the densitometer with either the rotameter or the Fick as the reference method maximum variations were plus 20 per cent and minus 10 per cent, the averages being plus 7.0 per cent and minus 5.4 per cent. The equation of the regression line for all 43 comparisons is $Y = 1.05x - 26.3$. The degree of correlation did not seem to be influenced by the level of cardiac output, arterial hematocrit, heart rate, or blood pressure. It should be
stressed that all comparisons were done under conditions of full arterial oxygen saturation.*

These findings are believed to validate the use of the cuvette densitometer as a quantitative indicator of cardiac output in dogs. The method has been used to investigate the relationship between end diastolic pressure and stroke volume in the anesthetized dog. Its possible application to human subjects is now being investigated.

**Summary**

Forty-one comparisons of cardiac output were made between a rotameter and the dye dilution technic as modified for photoelectric instruments which measure the concentration of Evans blue dye in whole blood. Seven comparisons were also made between the dye method and the Fick method for cardiac output during a three-minute period in which a steady state was maintained. The work was begun using the constant flow photometer of Friedlich and colleagues, but because of instrumental difficulties, a new cuvette densitometer was developed and its use is described in the text. Although satisfactory experiments were more difficult to obtain with the constant flow photometer, the results with the two instruments are comparable, with maximum differences from rotameter and Fick values of plus 29 to minus 10 per cent, and average differences of plus 8 to minus 5 per cent. It is concluded that the dye dilution method as applied to whole blood when used as described in this report accurately measures the cardiac output in dogs.

* The accuracy of the densitometer method for cardiac output under varying degrees of reduced arterial saturation is being tested using an anerobic calibration technic.

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