THE DESIGN AND USE OF A DYE-DILUTION TECHNIQUE FOR CONTINUOUS MEASUREMENT OF BLOOD FLOW IN THE FOREARM AND HAND OF MAN

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ABSTRACT

THE DESIGN AND USE OF A DYE-DILUTION TECHNIQUE FOR CONTINUOUS MEASUREMENT OF BLOOD FLOW IN THE FOREARM AND HAND OF MAN

by Roger Allen Wolthuis

The continuous-infusion indicator-dilution technique has been used previously to measure blood flow in the forearm and hand of man. During brachial arterial indicator infusion, blood samples were taken from downstream veins and a contralateral artery. Samples were later analyzed for indicator concentration in order to calculate blood flow. Thus, with previous methods, measurements were discontinuous, and blood flow values were not immediately available. The present study sought to improve the technique by developing a detection system which provides continuous and immediate blood flow measurements. A Gilson cuvette densitometer, modified for improved stability, was used to measure venous indocyanine green dye concentrations in the dog forelimb and human forearm during intrabrachial arterial dye infusion (.42 mg/min). Of special concern was the use and accuracy of this instrument during very low cuvette blood withdrawal rates. In a series of eight dogs, dye was infused into the

brachial artery of the isolated, pump-perfused forelimb while downstream dye-concentration plateaus were continuously recorded by withdrawing venous blood through the cuvette at 1.5 ml/min. Forelimb flow rates were varied over the range 25 to 225 ml/min. Actual forelimb flow was measured by the timed collection of total venous outflow in graduated cylinders. In a series of six dogs, dye and RISA were simultaneously infused into the brachial artery of the intact, naturally perfused forelimb while downstream dye-concentration plateaus were continuously recorded by withdrawing venous blood through the cuvette at 1.5 or 2.4 ml/min. Brachial artery inflow was measured independently by a rotameter; discontinuous RISA-dilution flows were calculated by the technique outlined by Overbeck (1966). Forelimb blood flow was varied over the range 25 to 200 ml/min by adding either nor-epinephrine or acetylcholine to the infusion of indicator. Finally, in the forearms of eight men, dye and RISA were simultaneously infused into the brachial artery and downstream dye concentrations were continuously recorded for periods up to two hours by withdrawing forearm venous blood through the cuvette at 1.5 or 2.4 ml/min. Blood flow was again varied over the range 13 to 345 ml/min by adding either nor-epinephrine or acetylcholine to the indicator infusion, or by having the subject exercise his hand. Indicator infusion followed the technique

outlined by Overbeck (1966). In addition, quantitative measurements were made of systemic recirculating dye during continuous dye infusion.

In 69 paired blood flow measurements from the isolated dog forelimb series, dye calculated flow differed from actual forelimb flow by a mean percent difference of $-.5 \pm 10.3$ (S.D.). In the naturally perfused dog forelimb, both indicator-dilution techniques over-estimated brachial arterial inflow, probably because of the presence of un-dyed forelimb collateral blood flow. However, there was good correlation ($r = .97$) between dye and RISA-dilution flow measurements at the higher cuvette blood withdrawal rate (2.4 ml/min). In the human forearm studies, the correlation coefficient between dye-dilution and RISA-dilution calculated blood flow was .99 at a cuvette blood withdrawal rate of 2.4 ml/min. In addition, during continuous dye infusion for up to three hours, recirculating dye concentrations were both low ($< .5$ mg/L) and quite constant.

The present study has demonstrated, for the first time, the practicality of using a cuvette densitometer at low cuvette blood withdrawal rates for continuously measuring forearm venous dye concentrations, and thus blood flow, during continuous dye infusion. This system provided measurements which were in close agreement with

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

SURVEY OF LITERATURE

Introduction

This chapter introduces theoretical and historical information pertinent to the present study. The section on theory, presented first, is designed to orient the reader and to make the succeeding historical review more meaningful. Theory of indicator-dilution techniques has been extensively reviewed elsewhere by Stephenous, 1948; Andres et al., 1954; Meier et al., 1954; Zierler, 1958, 1962; Kupic et al., 1966; Cropp et al., 1966. Relevant theoretical considerations are summarized below without reference to these individual authors.

Theory of Indicator (Dye) Dilution Technique

A single-circuit, open flow model with a constant fluid flow is shown in Figure 1. An indicator, continuously infused at the injection site, begins to appear at the outflow after a delay known as the traversal time. Continuous sampling for this indicator at the outflow site would produce the curve shown in Figure 2. The shape of the upslope portion of this curve is

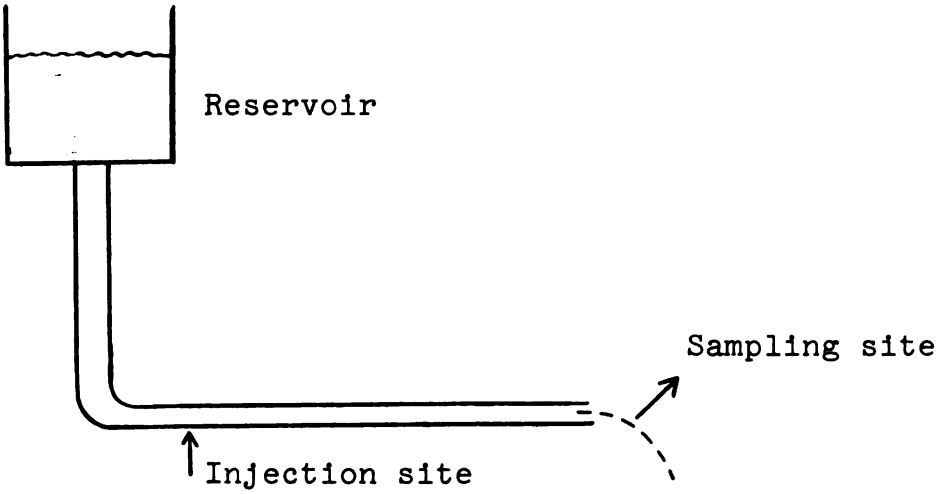


Figure 1.--Single-circuit, open flow model.

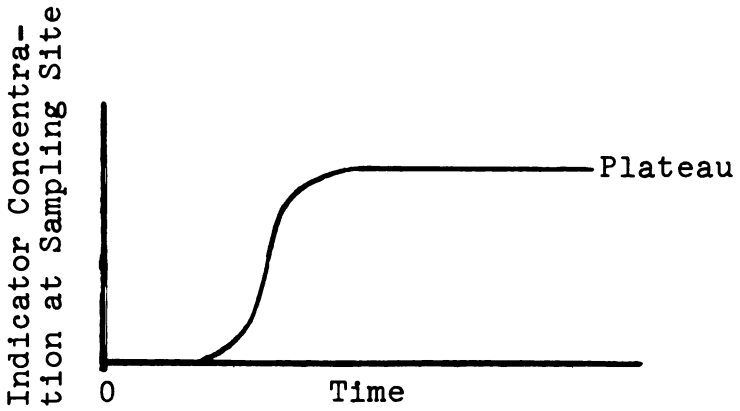


Figure 2.--Constant infusion, indicator-dilution curve obtained in the single-circuit, open flow model.

primarily a function of three variables: (1) the degree of mixing of indicator with the fluid, (2) the linear velocity of the fluid, and (3) the volume of the system between the points of indicator infusion and sampling. The outflow indicator concentration eventually reaches a steady state described by the flat concentration plateau. This steady state represents an equilibrium

in which the rate of indicator infusion is equal to the rate at which indicator is leaving at the outflow site. The relationship between indicator leaving at the outflow site, indicator concentration at the outflow site, and fluid flow is given by equation 1:

$$\text{RATE OF INDICATOR LEAVING OUTFLOW SITE (mg/min)} = \text{FLOW (ml/min) X CONCENTRATION OF INDICATOR AT OUTFLOW SITE (mg/ml)} \quad (1)$$

With indicator-infusion and fluid-flow rates constant, equation 1 may be re-written as follows:

$$\text{RATE OF INDICATOR INFUSION (mg/min)} = \text{FLOW (ml/min) X CONCENTRATION OF INDICATOR AT OUTFLOW SITE (mg/ml)} \quad (2)$$

Rearranging terms leads to the equation for flow:

$$\text{FLOW (ml/min)} = \frac{\text{RATE OF INDICATOR INFUSION (mg/min)}}{\text{CONCENTRATION OF INDICATOR AT OUTFLOW SITE (mg/ml)}} \quad (3)$$

Instead of continuous indicator infusion, an indicator could be injected into the flowing fluid as a bolus. With reference to the model system in Figure 1, a bolus injection of indicator will describe a time concentration curve at the sampling site similar to that shown in Figure 3. The character of this curve would be subject to the same variables already listed in connection with the constant infusion technique. If the amount of injected indicator were known, one could determine the

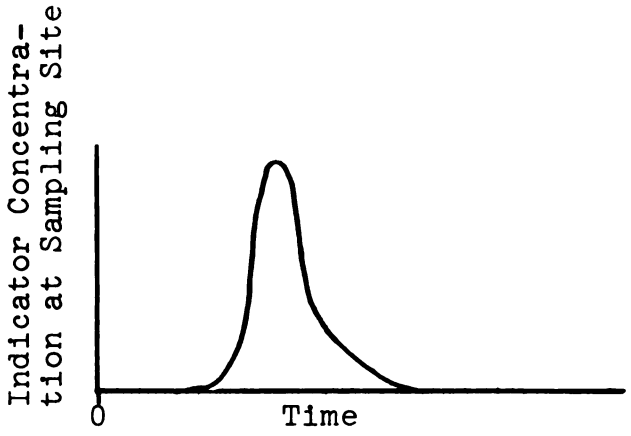


Figure 3.--Bolus injection, indicator-dilution curve obtained in the single-circuit, open flow model.

mean indicator concentration under the curve and calculate flow from the following relationship:

$$\text{FLOW (ml/min)} = \frac{\text{AMOUNT OF INDICATOR INJECTED (mg)}}{\text{MEAN CONCENTRATION OF INDICATOR UNDER CURVE (mg/ml) X TIME UNDER CURVE (min)}} \quad (4)$$

The mean, time-dependent indicator-concentration obtained from a bolus injection is comparable to the steady state indicator concentration plateau observed with the constant infusion method.

A multiple circuit, open flow model is shown in Figure 4. Indicator may now travel by more than one route to arrive at the outflow site. The average time it takes all indicator particles to arrive at the outflow site (mean transit time) may be greater than the mean transit time for a single-circuit, open flow model. Hence, in the multiple circuit, open flow model, both

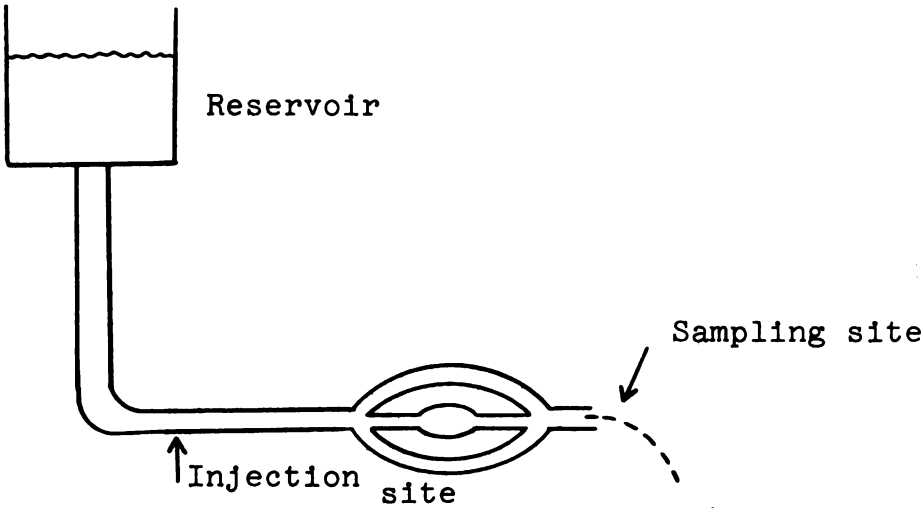


Figure 4.--Multiple circuit, open flow model.

the constant infusion and bolus injection indicator-dilution curves may differ in character from those obtained with the single-circuit, open flow model. This change in character would be evident in the shape of the upslope portion of the constant infusion curve, and, in the time interval observed under the bolus injection curve. The relationship between mean transit time, fluid flow, and volume of the model is given in equation 5:

$$\text{VOLUME (ml)} = \text{FLOW (ml/min) X MEAN TRANSIT TIME (min)} \quad (5)$$

A third model, shown in Figure 5, differs from those considered above in that the fluid and indicator are allowed to recirculate. Recirculating indicator modifies the slope of a constant infusion indicator dilution curve as illustrated in Figure 6. It may be seen that the initial portion of this curve may be

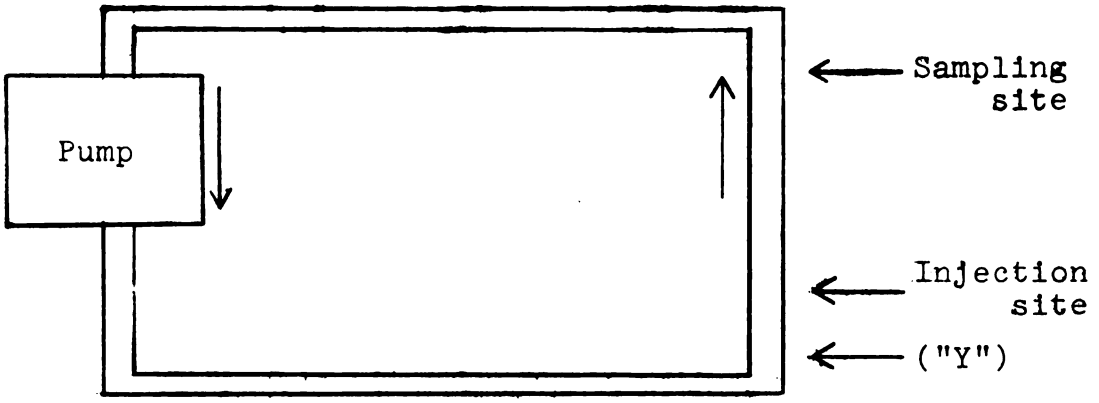


Figure 5.--Single-circuit, closed flow model with recirculation.

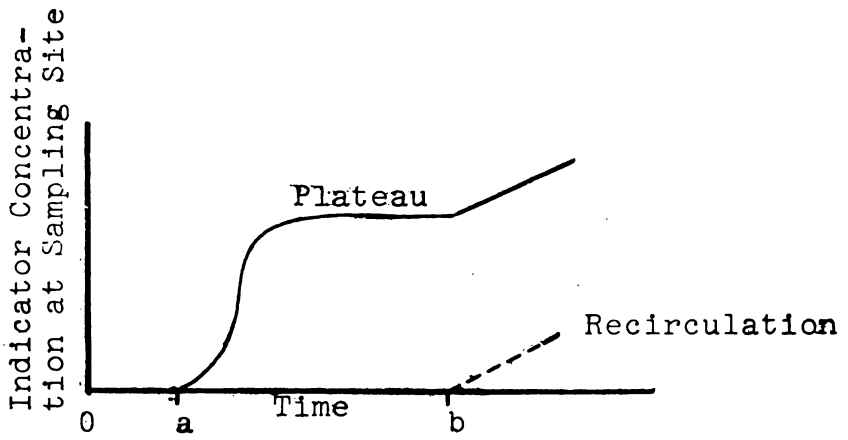


Figure 6.--Constant infusion, indicator-dilution curve obtained in the single-circuit, closed flow model with recirculation (dashed line).

identical to that obtained in the open circuit models previously discussed. However, after an interval of time (a-b) in which indicator circulates once around the model, recirculating indicator is added to the indicator-concentration plateau, and a constant rise in the curve is recorded. The dashed line in Figure 6 represents

the actual concentration of recirculating indicator in this example. Flow in this model may be estimated with equation 6, derived from equation 3.

$$\text{FLOW (ml/min)} = \frac{\text{RATE OF INDICATOR INFUSION (mg/min)}}{\text{CONCENTRATION OF INDICATOR AT SAMPLING SITE MINUS CONCENTRATION OF RECIRCULATING INDICATOR (mg/ml)}} \qquad (6)$$

The amount of recirculating indicator can be determined by sampling the circulating fluid upstream to the indicator infusion site (e.g., point "Y" in Fig. 5). The interval a-b is independent of the placement of infusion and sampling sites in the single-circuit, closed flow model. However, in the multiple circuit, closed flow model to be discussed later, the interval a-b may be a function of the position of and distance separating the infusion and sampling sites.

Figure 7 shows a bolus injection indicator-dilution curve obtained in the single-circuit, closed flow model

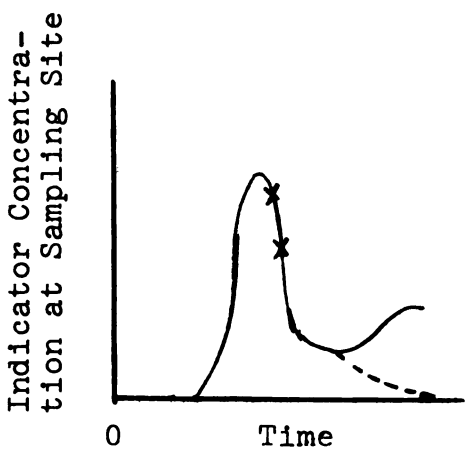


Figure 7.--Bolus injection, indicator-dilution curve obtained with the single-circuit, closed flow model with recirculation.

(Fig. 5), and illustrates the effect produced by recirculating indicator. Provided the volume of the flow model is small, the downslope of the bolus injection indicator-dilution curve does not return to the baseline. Instead, a secondary rise occurs concurrent with the appearance of indicator recirculation. Hamilton (1932) showed that the initial portion of the dilution curve downslope becomes a straight line when replotted on semilogarithmic coordinates (Fig. 8). Assuming that the

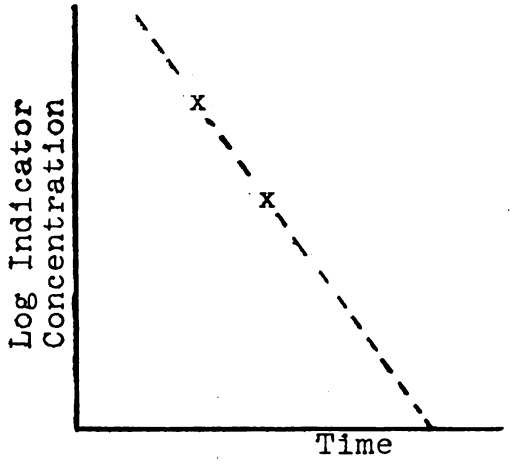


Figure 8.--Semilogarithmic plot of downslope from bolus injection curve.

initial logarithmic relationship between time and concentration holds for the final portion of the downslope as well, this technique disposes of the recirculating effect. Flow is then calculated by equation 4.

Finally, a multiple circuit, closed flow model is presented in Figure 9. This model approximates the cardiovascular system of mammals. Indicator infusion

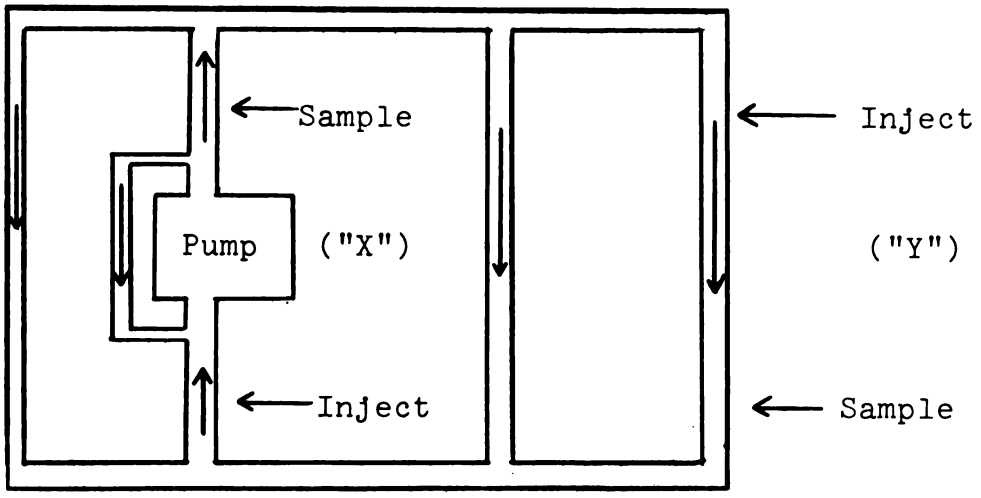


Figure 9.--Multiple circuit, closed flow model with recirculation.

and sampling sites may be variously located to measure either pump flow ("X") or flow through a single-circuit of the model ("Y"). The constant infusion and bolus injection indicator-dilution curves are similar to those previously shown in Figures 6 and 7, respectively. However, in this multiple circuit, closed flow system, the infusion and sampling sites must be carefully chosen to minimize the recirculation effect. For example, the measurement of flow at site ("X") would be difficult because of the quick circulation of indicator through the adjacent shunt. This shunt would cause recirculating indicator to appear earlier at the sampling site. On the other hand, the measurement of flow in a single-circuit ("Y") would be less affected by recirculating indicator because indicator is not shunted in this instance and must then travel the entire circuit before reappearing.

There are several basic assumptions underlying the use of the indicator-dilution technique. In the case of a non-diffusible indicator: (1) it is assumed that the distribution of transit times for indicator particles is the same as that for blood flowing through the given system; (2) it is assumed that complete mixing of indicator with blood occurs upstream to the sampling site. Complete mixing only occurs in the presence of turbulent flow. In the measurement of cardiac output, indicator is infused at or near the right heart and mixing of indicator and blood is caused by turbulent blood flow within the ventricles. Regional arterial blood flow, on the other hand, is normally laminar, not turbulent, flow. Thus, extraordinary measures must be used to produce mixing of indicator with the arterial blood in order to measure regional blood flow accurately; (3) it is assumed that infused indicator remains in the blood between the injection and sampling sites (non-diffusible indicator); (4) it is assumed that total flow through the system remains constant during the period between injection and sampling of indicator; (5) it is assumed that the volume of the system does not change during the experimental period; (6) it is assumed that the distribution of transit times of indicator particles does not change during the measurement of flow; and (7) it is assumed that recirculation either does not occur or that it can be measured.

Blood flow in the mammal is pulsatile rather than constant. Cropp and Burton (1966) have studied the effect of varying or pulsatile flow on estimates of blood flow made by constant-infusion, indicator-dilution methods. They have shown that a

> . . . sampling site some distance down a flow stream may be exposed to a low indicator concentration (generated by systole) for a much longer time than the actual duration of systole. This is so because the segment of low concentration may move past the sampling site at low diastolic velocities. As a result, the indicator detector at the distant sampling site may report a low indicator concentration for too long a time and a high indicator concentration for too short a time. This results in an over-estimate of the total flow by indicator-dilution techniques.

These authors have called this effect the "distance distortion error." This type of error would occur if the sampling site were located in the same vessel as the injection site, i.e., downstream in the same artery during measurement of regional blood flow.

According to Cropp and Burton (1966), the distance distortion error is largely removed and flow is measured with great accuracy by the constant infusion indicator-dilution method where

> . . . flow is pulsatile but of constant stroke volume and rate with a mixing region between infusion and sampling points. This mixing region acts like a low pass filter--attenuating the high frequency oscillations in the concentration time curve produced by the heart without attenuating significantly those oscillations produced by gradual changes in flow. Use of such a mixing region precludes the possibility of obtaining information about instantaneous flow by indicator-dilution methods.

Thus, if the capillary bed acts as a satisfactory mixing region, sampling downstream to the capillaries of a vascular bed during continuous indicator infusion into the artery supplying the bed would probably eliminate the distance distortion error, but would only allow measurement of mean, not instantaneous, regional blood flow.

Historical Development of Indicator-Dilution Methods

In 1829, Hering reported a technique for the measurement of blood circulation time. He injected potassium ferricyanide into one jugular vein and noted the elapsed time before this indicator could first be detected in the contralateral vein. Sixty years later, as an improvement on Hering's technique, Stewart (1897) detected an intravenously infused salt solution by the change in electrical conductivity of arterial blood. Quantitative determination of the dilution of the salt comprised the original Stewart measure of cardiac output:

$$\text{Cardiac Output (ml/min)} = \frac{\text{Rate of salt infusion (ml/min)}}{\text{Concentration of salt at sampling site (ml/ml)}}$$

Henriques (1913) used injections of thiocyanate as his indicator. Thiocyanate was easily measured chemically, but Henriques found that it remained in the blood sufficiently long to produce a recirculation effect.

Henriques demonstrated that the indicator did not travel in blood as a square wave, as envisioned by Stewart, but rather in a dispersed form. This dispersion was reflected at the sampling site by a slow rise and fall in the indicator concentration.

Hamilton and his colleagues developed the bolus injection indicator-dilution technique with a classical series of papers beginning in 1928 (1928a, 1928b, 1930, 1932). Their problem was twofold: (1) a solution of the curvilinear response to a bolus indicator injection, and (2) the separation of recirculating indicator from the primary dilution curve. In model systems simulating the mammalian circulation, in heart-lung preparations, and in intact animals, they were able to show that an intravenous bolus injection of indicator (Vital Red or Evans Blue dyes), and semilogarithmic extrapolation of the disappearance curve in arterial blood provides a measure of cardiac output by the following formula:

$$\text{Cardiac Output} = \frac{60I}{\bar{c}t}$$

I is the amount of indicator injected, $\bar{c}$ the average concentration of indicator under the corrected curve (summation of concentration values at one-second intervals divided by the duration of the curve in seconds), and t the duration of the dilution curve.

The multiplicity of present day techniques are an outgrowth of this early work. They have been reviewed elsewhere (Cournand, 1945; Hamilton, 1953; Dow, 1956; Schlenk, 1966).

Development of the Constant Infusion Indicator-Dilution Technique

Holt (1944) reported the use of a constant infusion indicator-dilution method for estimating cardiac output in anesthetized dogs. He infused Evans Blue dye at a constant rate into the right atrium for 13 seconds and sampled femoral artery blood 8-23 seconds after dye infusion was begun. Reasonable indicator concentration plateaus were obtained at the sampling site, and recirculating dye appeared no earlier than the 16th second.

Hamilton and Remington (1947) reviewed the original work of Stewart and suggested that his constant infusion method was valid so long as recirculation of indicator did not occur. In a series of experiments with a model and in the dog, Hamilton and Remington showed that the slowest moving infused indicator particles appeared at the sampling site chosen by Hamilton and Remington much later than the fastest moving particles, and well after the appearance of recirculating indicator. Hence, the recirculation effect distorted the critical portions of the constant infusion, indicator-concentration plateau as obtained by these workers. They concluded that the

constant infusion technique could not accurately measure blood flow due to the presence of indicator recirculation.

Howard, Hamilton and Dow (1953) studied indicator dilution curves made simultaneously by the bolus injection and the constant infusion techniques in a model and in the dog. Once again, their conclusion was that both techniques were valid only in the absence of recirculation. However, in contrast, Shepherd, Bowers and Wood (1954, 1955) simultaneously determined cardiac output by three methods: direct Fick, bolus injection, and constant infusion indicator-dilution techniques. All three techniques gave the same cardiac output estimate. In 20 of 22 trials with the constant infusion technique, there was a 2-7 second indicator concentration plateau prior to the onset of indicator recirculation.

The work of Shepherd et al. (1954, 1955) led to the eventual acceptance of the constant infusion indicator-dilution technique. In addition, these authors demonstrated that the achievement of a constant infusion plateau, prior to recirculation, was dependent on the location of the infusion and sampling sites. Similarly, Peterson et al. (1954) infused indicator at a constant rate near the root of the aorta and sampled downstream aortic blood. They obtained 7-10 second indicator concentration plateaus prior to onset of recirculation and reported values in close agreement with direct Fick measurements.

Constant Infusion Indicator-Dilution and Forearm Blood Flow

The successful development of a continuous-infusion indicator-dilution technique for measurement of cardiac output soon yielded new applications. This technique was first used to measure regional blood flow by Andres *et al.* (1954). They infused Evans Blue dye into brachial artery and sampled the effluent blood from a deep and a superficial forearm vein, calculating forearm blood flow by the following equations:

$$\text{Forearm Plasma Flow (L/min)} = \frac{\text{Rate of indicator infusion (mg/min)}}{\text{Mean concentration of dye in venous plasma minus concentration of indicator in recirculating plasma (mg/L)}}$$

$$\text{Forearm Blood Flow} = \frac{\text{Plasma Flow}}{1 - \text{Hematocrit}}$$

If complete mixing of infused dye and forearm blood had occurred, effluent blood in all forearm veins would have contained equal concentrations of the dye. However, Andres *et al.* (1954), found that indicator concentrations in their forearm veins differed significantly.

These authors suggested several explanations for their venous indicator concentration differences. Undyed blood from collateral arteries about the elbow might have disproportionately diluted the brachial arterial blood-dye mixture downstream in the forearm vascular tree. This explanation was discarded because the authors

felt that total collateral arterial blood flow in the forearm was too low to explain the differences in indicator concentration. A second possible explanation might be an unequal escape of indicator between the infusion and the sampling sites. However, Evans Blue dye was shown to be rapidly bound by serum albumin which is essentially non-diffusible. High bifurcation of the brachial artery might have led to inadvertent dying of radial or ulnar arterial blood exclusively, causing significant differences in venous indicator concentrations. It had been shown previously (Quain, 1854) that the incidence of anomalous brachial artery bifurcation in the human was 20 percent. Thus, 20 percent of the cases of inadequate mixing could be explained on this basis. Finally, incomplete arterial mixing of indicator might have occurred, resulting in an unequal distribution of the indicator to downstream vascular beds. This was a real possibility, and these authors attempted to improve intra-arterial indicator mixing by creating arterial turbulence with jet injection of indicator.

In 63 forearm experiments on 27 subjects, Evans Blue dye was infused at a constant rate into brachial arterial blood while two forearm veins were sampled simultaneously. Dye was infused via either a standard arterial needle or a "jet injector" needle. Despite theoretical indications and favorable data from model

and dog studies, the jet injection did not improve indicator mixing in the forearm. In addition, the jet injector produced erythrocyte hemolysis and limb vasodilation.

In a final group of 10 subjects, resting forearm blood flow was repeatedly measured during dye infusion through a standard needle. Statistical analysis of the data allowed the authors to conclude that sufficient mixing of indicator upstream to the sampling site (mainly in the capillary-venous portion of the vascular bed) was obtained in four-fifths of their subjects to permit the accurate measurement of forearm blood flow.

Wahren (1965, 1966) also used the continuous infusion indicator-dilution technique to measure forearm blood flow. He infused indocyanine green dye (0.6-1.5 mg/min) at a high volume rate (34 ml/min) into the brachial artery for one-minute intervals, relying on mixing of dye and blood by a volume effect. Blood was sampled discontinuously in the radial artery and a deep vein. The degree of mixing of dye with blood was determined by comparing dye concentrations in the radial artery with those from the deep vein. The recirculation dye level was interpolated from dye concentrations obtained immediately before and 15 seconds after the dye infusion period.

There was a large difference between radial artery and deep vein dye concentrations in 17 percent of the forearm flow determinations. These cases were attributed to undetected, high bifurcation of the brachial artery with consequent dying of radial or ulnar arterial blood exclusively. Equal dye concentrations at the two sampling sites were found in 83 percent of the experiments, suggesting satisfactory mixing. In addition, simultaneous flow estimates by venous occlusion plethysmography agreed closely with flow estimates obtained by the indicator-dilution technique.

Wahren (1966) reported that the high volume rate of indicator infusion apparently did not have an effect on pre-infusion flow patterns in the forearm. In pilot studies, deep and superficial vein oxygen saturation remained unchanged after infusion of dye began. Furthermore, the decrease in venous hemoglobin concentrations, following onset of indicator infusion, supported the conclusion that infused dye was simply added to the initial forearm blood flow.

Overbeck (1966) measured forearm and hand blood flow with the continuous infusion indicator-dilution technique, using I^{131} labeled human serum albumin (RISA) as his indicator. He developed and used an arterial jet injector needle which distributed indicator laterally into the stream of arterial blood. The kinetic energy

of infusate was 4500 g. cm^2 sec^{-2}, substantially below the level reported by Andres to cause hemolysis. By comparing the effectiveness of this jet needle against standard needles in a model, in the isolated dog forelimb, and in the human forearm, Overbeck was able to show statistically significant improvement in indicator mixing with his jet injector system.

All of the techniques described above for measuring forearm and hand blood flow provided discontinuous, not continuous, measurements of blood flow. In addition, flow measurements were available only after completion of the experimental procedure, because blood had to be processed before indicator concentrations could be determined.

Indocyanine Green: Properties

Evans Blue dye (T-1824) and reduced hemoglobin both absorb light maximally at 640 millimicrons. Hence, a change in blood reduced hemoglobin concentration will interfere with the measurement of this dye by the *in-vivo*, whole blood cuvette densitometry technique.

Fox *et al.* (1957), in conjunction with Heseltine and Brooker, reported the use of indocyanine green, a dye which maximally absorbs light in the infrared region (800 millimicrons). At this wavelength, light transmission by oxyhemoglobin and reduced hemoglobin is

identical. Indocyanine green dye (ICG) is marketed under the trade name Cardio-Green (Hynson, Westcott and Dunning, Baltimore, Maryland).

The structural formula of ICG is given in Figure 10. It has a molecular weight of 775. Its light absorption in plasma closely follows Beers' law; there is a linear increase in optical density with increasing concentrations of homogeneous solutions of the dye. However, in non-homogeneous solutions (i.e., blood), ICG

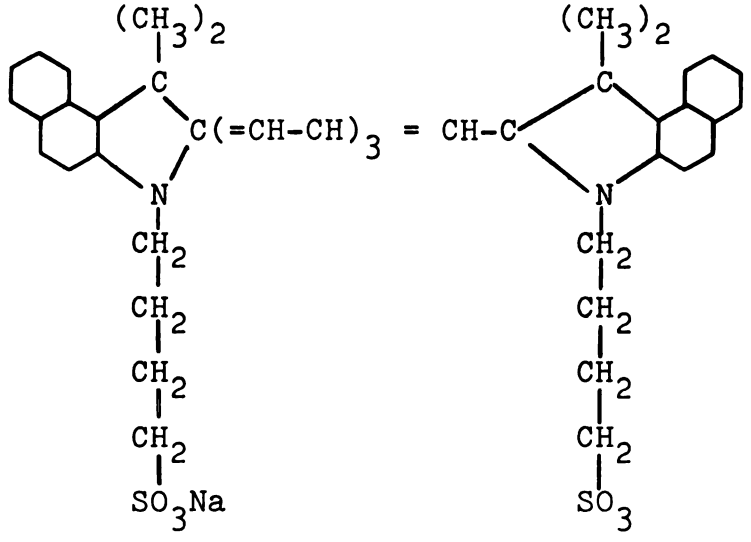


Figure 10.--Structural formula for indocyanine green (Fox et al., 1957).

absorption does not closely follow Beers' law. Therefore, devices for detecting ICG in whole blood (densitometers) usually contain a linearizing circuit.

The effect of plasma protein binding, pH, temperature and chemical additives on ICG optical activity have

been studied. Fox et al. (1960) reported a change in peak absorption of ICG (775 to 800 mu) as an aqueous solution of this dye is added to blood. Bassingthworghte et al. (1960) were able to show that this change in absorption is due to binding of the dye to a plasma protein (primarily albumin). Stabilization of plasma ICG absorption at 800 mu occurs very rapidly.

Because the dye is prepared initially in an aqueous solution, the effect of pH on optical activity was studied. Peak absorption of the dye remained unchanged over a range of pH 6.0 to 9.0. Varying the temperature of an aqueous dye solution also failed to change ICG absorption characteristics. ICG itself failed to effect the pH of triple distilled water (pH 5.5) in concentrations up to one milligram per milliliter.

ICG is highly soluble in distilled water (Fox et al., 1960), but poorly soluble in saline solutions. Once ICG is dissolved in distilled water, however, NaCl may be added up to 9 g percent without causing precipitation of ICG. Optical density of ICG decreases with increasing salinity, but this effect disappears on addition of dye and saline to plasma. Varying the pH of plasma (7.0 to 7.8), the plasma protein concentrations (10 to 100 percent of normal) and the plasma NaCl level by the addition of .3 to 1.5 g percent all fail to influence the optical density of ICG in plasma.

While ICG was found to be stable in blood and plasma for up to eight days, Fox et al. (1960) reported varying instability of ICG in aqueous solutions. They found dilute aqueous solutions of ICG increased in optical density initially, thereafter decreasing in activity. More concentrated solutions (1 mg/ml) exhibited lesser changes. Barbier and DeWeerdt (1964) reported a high rate of optical deterioration of dilute ICG solutions (2.5 mg percent). Their findings are illustrated in Figure 11. They also verified earlier

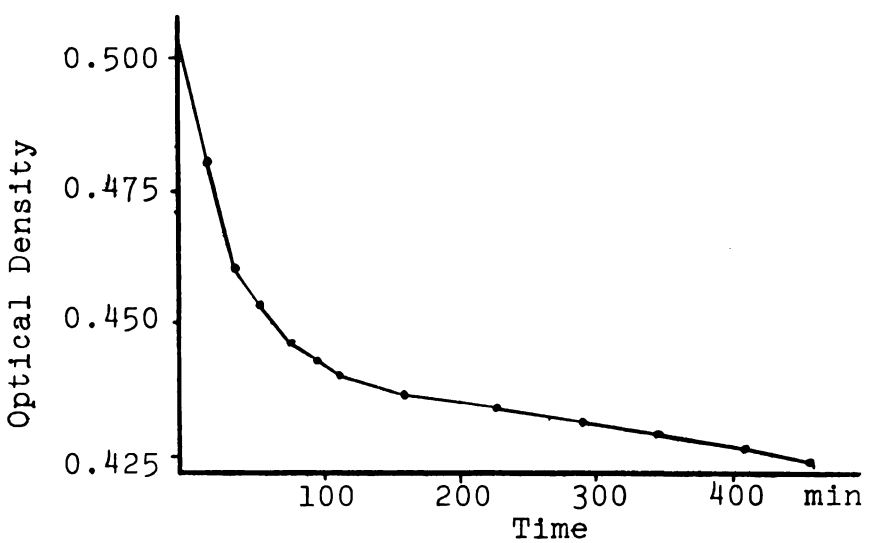


Figure 11.--Rate of optical deterioration of dilute ICG solution (Barbier and DeWeerdt, 1964).

reports that ICG is stable in plasma. Chromatographic work by these workers showed that the optical deterioration of ICG in aqueous solution is due to the formation

of a ring structure at the SO_3^- group, with concommitant saturation of the unstable C=N group, loss of the SO_3^- function and appearance of a C=O character in infrared spectra. The probable formula for the new compound is given in Figure 12.

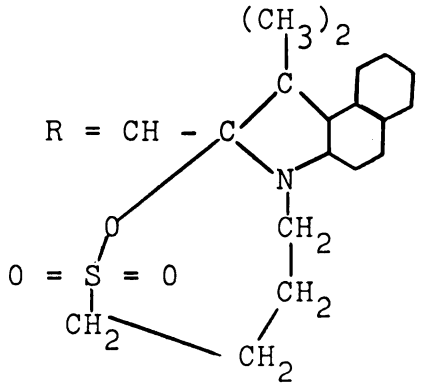


Figure 12.--Structural formula of ICG following deterioration in aqueous solution.

Cobb (1965) has recently shown that various reducing agents markedly affect the spectrophotometric characteristics of ICG in both plasma and aqueous solutions. For example, heparin preparations containing sodium bisulfite lowered the optical density of ICG (1 mg/L) by 72 percent. Sodium metabisulfite, sodium borohydride and potassium borohydride also markedly alter the optical density of ICG at 800 mu. Other heparin preparations with different preservatives have no apparent effect on ICG optical activity.

Finally, ICG has been found to be non-toxic in both acute and chronic toxicity studies in animals. No

untoward effects attributable to the use of this dye in man have been reported at blood concentrations as high as 5 mg/kg (Fox et al., 1960).

Indocyanine Green and Hepatic Removal

Since the observation by Fox et al. (1957) that ICG is excreted into the bile, there has been considerable interest regarding the role of the liver in removing this dye from the blood. Table 1 presents data on hepatic removal of ICG when given in single dose injections. Hunton et al. (1960) found that an increase in the loading dose of ICG decreased the plasma disappearance rate

TABLE 1.--Hepatic removal of injected indocyanine green dye in dogs and humans.

Worker	Species	ICG Injected	Disappearance Rate
Hunton (1960)	dog	1 mg/kg	average 7.6%/min
	"	9 mg/kg	" 3.8%/min
Ketterer (1960)	"	0.5 mg/kg	" 5.4%/min
Wheeler (1958)	"	1 mg/kg	" 9.6%/min
Hunton (1960)	human	.25 mg/kg	" 25.9%/min
Reemtsma (1960)	"	?	" 23.2%/min
Cherrick (1960)	"	.5 mg/kg	" 18.5%/min

of the dye, but noted that the total quantity of dye being removed per unit time was increased. It was noted

by others that the disappearance rate varied substantially among subjects with apparently normal liver function.

Hepatic removal appears to be the sole route for clearance of ICG from the blood. It has been shown that arterial concentrations do not differ from venous concentrations of ICG across limb vascular beds (see Table 2), and excretion of ICG by the kidneys does not occur (Cherrick et al., 1960). Various workers (Wheeler et al., 1958; Hunton et al., 1960; Ketterer et al., 1960) have reported recovering 92 to 97.5 percent of an injected ICG dose from the bile. There is little, if any, recycling of bile-excreted ICG via intestinal absorption (Wheeler et al., 1958).

Hepatic ICG extraction is a function of blood flow through the liver, and the ICG concentration in this perfusing blood. As long as the extraction mechanism is not saturated and hepatic blood flow remains relatively constant, the rate of hepatic ICG extraction becomes a function of the systemic blood ICG concentration. An increase in systemic ICG concentration leads to an increase in the hepatic ICG extraction rate (Hunton et al., 1960). Thus, with continuous ICG infusion (i.e., with the continuous-infusion indicator-dilution technique), one should obtain a steady state systemic ICG concentration (recirculating ICG) during which ICG infusion equals hepatic ICG extraction. Cherrick et al. (1960)

primed one patient with 10 mg of ICG and followed this with a constant ICG infusion (0.5 mg/min) for 50 minutes. His plasma ICG levels during the infusion period are presented in Table 2.

TABLE 2.--Arterial and venous ICG levels during constant ICG infusion (Cherrick et al., 1960).

Following Start of ICG Infusion	Brachial Arterial ICG	Antecubitol Venous ICG
minutes	mg/L	mg/L
5	1.68	1.66
10	1.64	1.64
15	1.54	1.56
20	1.47	1.43
25	1.38	1.42
30	1.28	1.26
35	1.33	1.37
40	1.36	1.34
45	1.40	1.39
50	1.52	1.54

Cherrick et al. (1960), concluded that there was no detectable arterial-venous ICG concentration difference, and "detectable peripheral tissue uptake of ICG did not occur." It is apparent, in addition, that the concentration of circulating ICG remained quite constant during this infusion period.

Cuvette Densitometry

One of the earliest cuvette densitometer systems was reported by Friedlich, Heimbeck and Bing (1950). Their system consisted of a flow-through blood optical

chamber interposed between a filtered light source and a multiplier phototube. Following indicator injection, arterial blood was withdrawn from the subject through the cuvette, and indicator concentration changes were directly and continuously recorded.

Dow (1956) has reviewed the history and use of cuvette densitometers elsewhere. The introduction of cuvette densitometry was of immediate value in the measurement of cardiac output by the dye-dilution technique. While cuvette blood withdrawal rates employed in this technique were relatively high (20 to 40 ml/min); inscription of the arterial dye-dilution curve took less than a minute and blood loss to the subject was minimal. The literature, on the other hand, contains little information about the use of cuvettes at relatively low blood withdrawal rates. Fox et al. (1957), noted a relationship between lower cuvette blood withdrawal rates in the range 9 to 26 ml/min and decreasing cuvette frequency response. Sekelj et al. (1967) reported the design of a cuvette insensitive to blood flow in the range 10 to 40 ml/min. Below this range, however, there was a sharp decrease in recorded optical density with flow as shown in Figure 13.

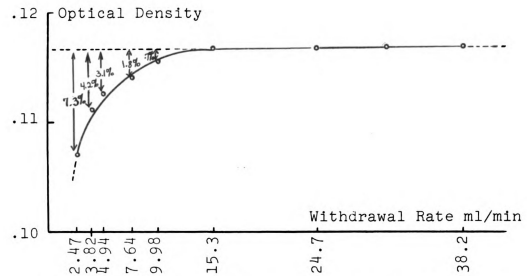


Figure 13.--Change in optical density of the same blood-dye mixture at various withdrawal rates (Sekelj et al., 1967).

CHAPTER II

INSTRUMENTATION

Introduction

The Gilson cuvette densitometer (model DTL, Gilson Medical Electronics, Middleton, Wisconsin) was used in the present study. The performance of this instrument was evaluated in terms of the four characteristics Sekelj et al. (1967), considered optimum in the operation of a cuvette densitometer system. These are: (1) the instrument should be "stable and free of drift," (2) it should be "relatively insensitive to changes in the rate of blood withdrawal through the cuvette," (3) it should be "linear in response to changes in optical density over the range of dye concentrations employed," and (4) it should be "insensitive to changes in blood hematocrit." The following four sections treat each of these criteria individually.

Stability of the Gilson Cuvette Densitometer

It was apparent early in the present study that the cuvette densitometer was electronically unstable, particularly at low cuvette blood withdrawal rates (see

following section). For example, cuvette output drifted excessively during the withdrawal of undyed blood through the cuvette, producing an unstable baseline and hampering the accurate measurement of dye-concentration plateaus. Sensitivity of the cuvette also changed with time as evidenced by the difference between pre- and post-experiment cuvette calibration curves; this change in sensitivity introduced error into calculated blood flow measurements. Finally, the optical chamber nylon interfacing was found to adsorb dye from perfusing blood-dye mixtures, resulting in an apparent increase in recorded optical densities with time.

The manufacturer of this instrument was notified of these findings and a modification program begun. In the densitometer, along with basic circuitry design changes, temperature sensitive components were carefully shielded from heat-producing elements. In the cuvette, the filtered light source was moved away from the heat-sensitive, light-dependent resistor (e.g., photocell). The optical chamber nylon interfacing was replaced with a non-adsorbing Teflon material.

Because of the continuing modification of the cuvette densitometer during this study, quantitative measurements of instrumental stability were not deemed appropriate. A qualitative estimate of sensitivity stability may, however, be made for the system in its

present state by comparing the pre- and post-experiment cuvette calibration curves obtained in later experiments. In experiments 107, 108, and 109 (Chapter IV), there was a mean cuvette sensitivity shift of -2.2 percent (range -9 percent to +12 percent) during the four-hour interval between cuvette calibrations. This can be compared with a mean sensitivity shift of +6 percent per four-hour interval observed in the earlier experiments. Further improvement in instrumental stability is expected from the continuing modification program.

Sensitivity of the Gilson Cuvette to Changes in Blood Withdrawal Rates

According to the manufacturer, the Gilson cuvette is relatively insensitive to changes in blood withdrawal rates in the range 20 to 40 ml/min. Withdrawal rates within this range are suitable for discontinuous indicator-dilution measurement of cardiac output, as the inscription of the arterial dye-dilution curve takes less than a minute and blood loss to the subject is thus minimal. However, the present study required continuous cuvette sampling of venous dye concentrations over periods of up to 120 minutes. This could be accomplished only by using a substantially lower cuvette blood withdrawal rate, both because of limited venous blood flow in the vessel being sampled, and the necessity for minimizing blood loss in the subject.

At low blood withdrawal rates the Gilson cuvette displayed a sensitivity to rate of blood flow through the cuvette similar to that described by Sekelj et al. (1967) (see Chapter I, Figure 13). During the sampling of blood containing 12 mg/L dye concentration, a change in blood withdrawal rate from 3.8 to 1.5 ml/min caused a 3 percent decrease in recorded optical density. A change in withdrawal rate from 1.5 to .76 ml/min produced a 10 percent drop in recorded optical density. The mechanism of this flow dependency is not clear. It may be related to changes in erythrocyte orientation within the cuvette optical chamber at low blood withdrawal rates. These changes in orientation could affect the apparent optical density of the perfusing blood. Cuvette flow dependency could also be due to variation in axial streaming at the lower withdrawal rates. In any case, with the use of this cuvette at withdrawal rates which make the cuvette flow-dependent, care must be exercised to maintain a constant blood withdrawal rate; variations in the recorded dye-concentration plateau might be an artifact induced by a change in the cuvette blood withdrawal rate.

A cuvette blood withdrawal rate of 1.5 ml/min was used initially in the present study. The withdrawal rate was increased to 2.4 ml/min in later experiments in an effort to bring dye-dilution flow measurements

into agreement with flow measurements made by an independent technique. These studies are presented in subsequent chapters.

Response of the Gilson Cuvette Densitometer to Changes in Optical Density

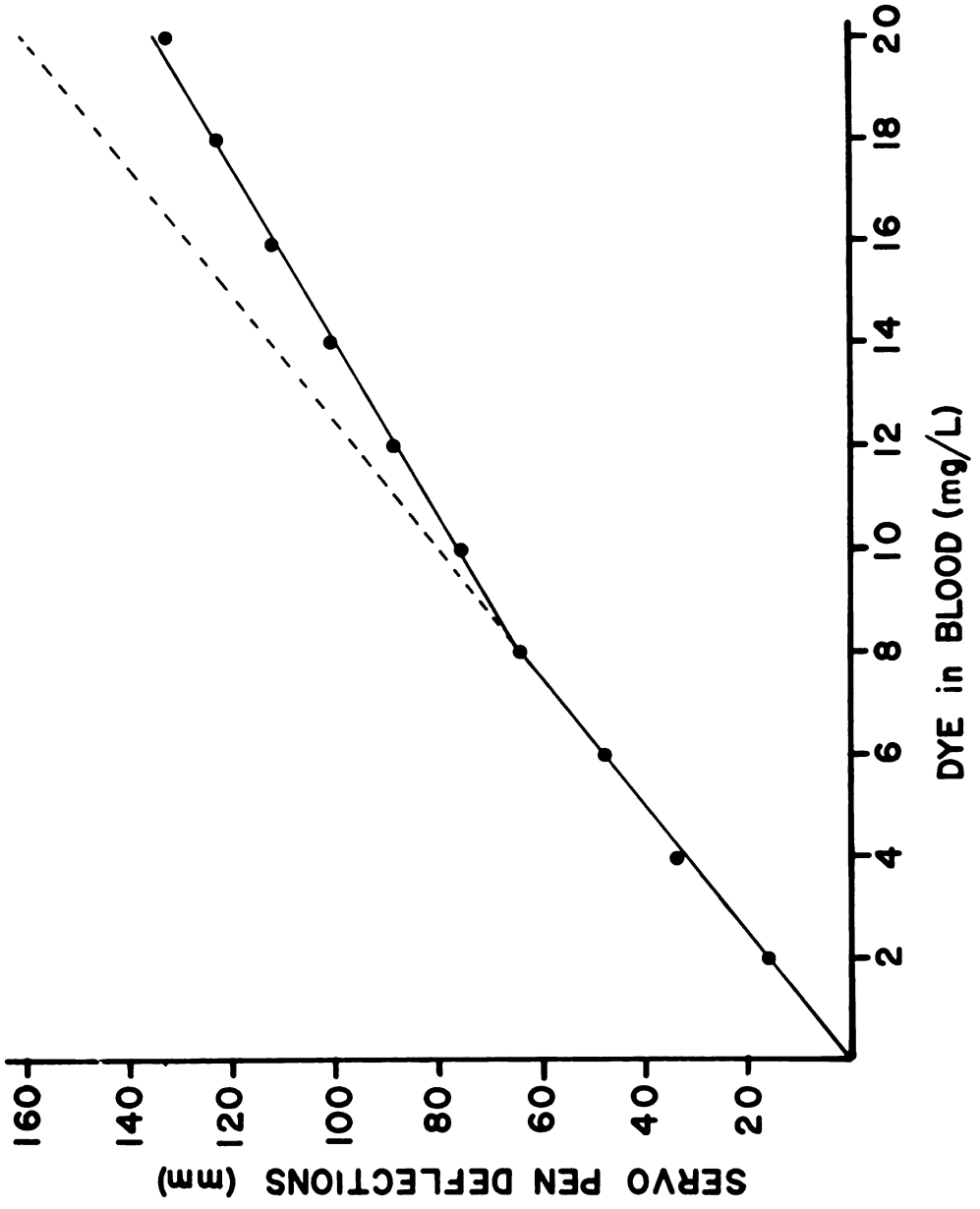
Cuvette Linearity

Eleven experiments were conducted in which aliquots of outdated, citrated human blood were dyed to give known blood-dye mixtures over the range 0 to 20 mg/L. These mixtures were withdrawn (1.5 or 2.4 ml/min) through the cuvette and the response recorded. Figure 14 is a plot of the cuvette response from one of these experiments. The dotted line is an extension of the first four plotted points drawn to illustrate the shift in linearity. This shift in linearity occurred in all eleven experiments, and is apparently a characteristic of the instrumentation. The linearity shifted at a dye concentration between 8 and 10 mg/L. The presence of a non-linear cuvette output requires a more rigorous calibration technique.

Cuvette Calibration

Fresh ICG dye was prepared for each experiment. A standard technique for calibrating the cuvette densitometer was used for all *in vivo* blood flow experiments. A 70 ml sample of well-heparinized blood was obtained; 30 ml were set aside as a blank-blood sample. The

Figure 14.--Gilson cuvette response to blood-dye concentrations in the range 0-20 mg/L.

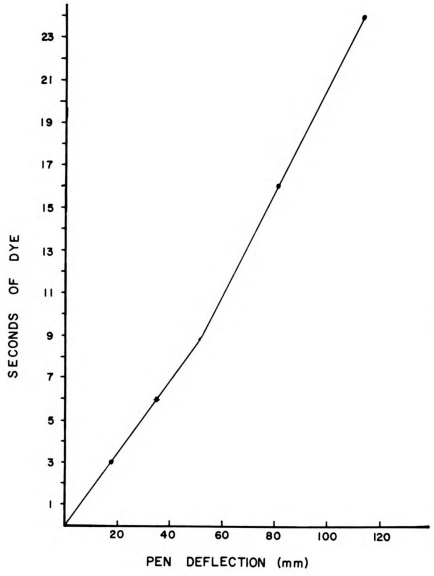

SERVO PEN DEFLECTIONS (mm)
160
140
120
100
80
60
40
20
2
4
6
8
10
12
14
16
18
20
DYE in BLOOD (mg/L)

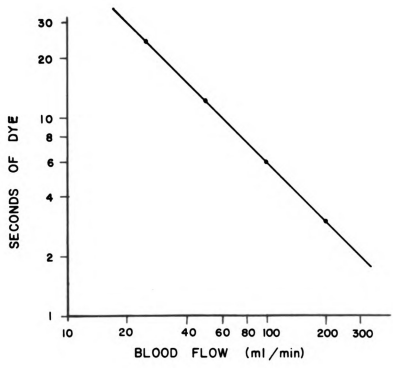
remaining blood was divided into four 10 ml aliquots. Dye was pumped ("infused") into these aliquots for 3, 6, 16, and 24 seconds respectively. The rate of dye infusion was the same as that used during subsequent brachial arterial dye infusion (.42 mg/min). The calibration aliquots were then drawn through the cuvette in sequence. The recorder response in millimeters of pen deflection was plotted on the abscissa. On the ordinate, concentration of dye was plotted in terms of "seconds of dye" according to the method used above in making the calibration aliquots. A typical cuvette-recorder calibration curve is shown in Figure 15. The non-linear cuvette response is quantitatively similar to that described in the previous section.

A second standard curve was used to convert the seconds of dye value to actual blood flow (ml/min). Since 3 seconds of dye in 10 ml of blood is equivalent to 60 seconds of dye in 200 ml of blood, a 3 second dye concentration represented the concentration which would be present in blood flowing at 200 ml/min. In a similar manner 6, 12, and 24 seconds of dye represented 100, 50, and 25 ml/min blood flow, respectively. These points were found to fall on a straight line when plotted on 2-cycle log-log paper with blood flow on the horizontal axis and seconds of dye on the vertical axis, as shown in Figure 16.

Figure 15.--Typical calibration curve from the Gilson cuvette.

Figure 16.--Standard curve for converting "seconds of dye" to blood flow (ml/min).


SECONDS OF DYE
23
21
19
17
15
13
11
9
7
5
3
1
20
40
60
80
100
120
PEN DEFLECTION (mm)


SECONDS OF DYE
30
20
10
8
6
4
2
1
10
20
40
60
80
100
200
300
BLOOD FLOW (ml/min)

Calibration Error

Two experiments were performed to determine the amount of error associated with the cuvette calibration procedure. Both citrated human and heparinized dog blood were used. In each experiment, a carefully prepared series of 50 and 5 ml blood aliquots were infused with dye (.42 mg/min) for 120 and 12 seconds respectively. The blood-dye samples were well mixed, and withdrawn through the cuvette in sequence. A mean response was determined for the various samples in each group, and the percent deviation from this mean was averaged to give mean percent error. Data from these studies are presented in Table 3.

TABLE 3.--Data showing the relationship between size of calibration blood aliquot and their reproductability.

Aliquot size	50 ml blood + 120 sec dye	5 ml blood + 12 sec dye
Human blood	2.3 mean % error of 6 samples	3.2 mean % error of 6 samples
Dog blood	3.4 mean % error of 7 samples	4.6 mean % error of 7 samples

It is apparent that there was a small reduction in error associated with the use of larger blood-dye calibration aliquots. Since the use of smaller blood calibration aliquots in man helped reduce overall experimental blood loss, a compromise calibration blood

aliquot size of 10 ml was selected. It is apparent that there was a slightly higher mean percent error associated with calibrations using dog blood. This increased error may be related to the generally higher erythrocyte sedimentation rate of dog blood; at the low cuvette blood withdrawal rates used in this study, changes in the homogeneity of blood may be more readily detected by the cuvette.

Blood Temperature

Two *in vivo* experiments were conducted to determine whether or not variations in blood temperature were critical during the cuvette calibration procedure. Undyed blood was obtained from a dog and added to two prewarmed beakers (37° C.), one containing dye. As soon as possible, these two blood samples were withdrawn in turn through the cuvette, and the responses recorded. Both the undyed and dyed blood aliquots were again withdrawn through the cuvette some time later, after they had reached room temperature (24° C.). The above procedure was repeated in each of two dogs; there was no apparent difference in cuvette response to the blood samples read immediately (approximately 33° C.) and the same samples read at room temperature. It was concluded that calibration of the cuvette could be carried out with blood at room temperatures.

Sensitivity of the Gilson Cuvette Densitometer to Changes in Blood Hematocrit

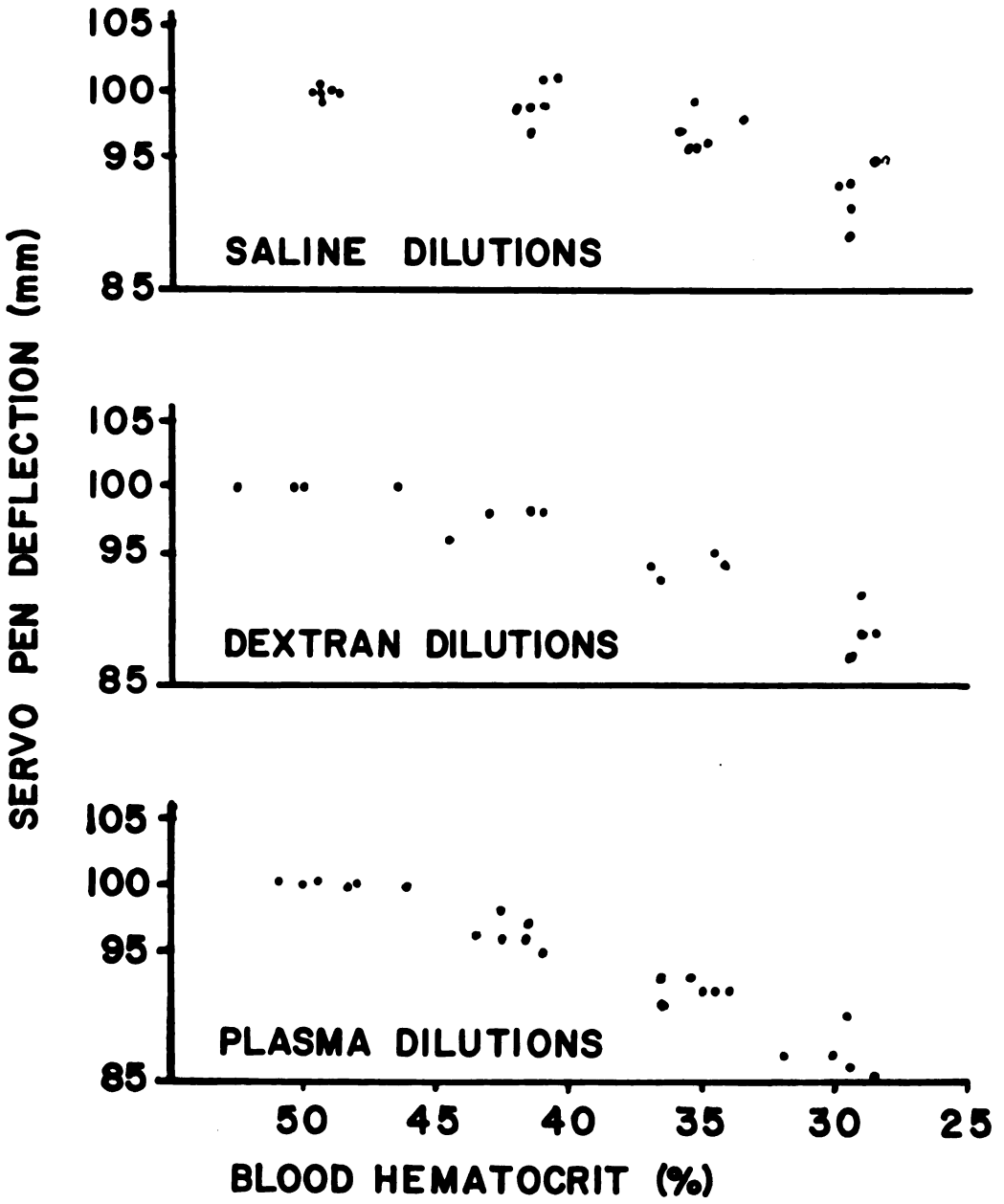
Sixteen *in vitro* experiments were conducted to determine the effect of changing blood hematocrit on cuvette sensitivity. Multiple aliquots of outdated, human citrated blood were carefully diluted with either human plasma, dextran, or isotonic saline, within the hematocrit range 50 percent to 30 percent. Dye was added to each aliquot (17 mg/L) and the various aliquots then withdrawn through the cuvette. The response of the cuvette to varying blood hematocrits is shown in Figure 17. The same data are presented in a somewhat different form in Table 4.

TABLE 4.--Mean percent change in optical density (pen deflection) per 1 percent decrease in blood hematocrit.

	Range of Hematocrit Changes			
	50-45	45-40	40-35	35-30
Saline	-.12	-.19	-.37	-.68
Dextran	-.54	-.57	-.59	-.61
Plasma	-.61	-.62	-.65	-.67

It is apparent that saline dilution of blood causes a smaller decrease in optical density over the hematocrit range 50 percent to 35 percent than do dextran and plasma dilutions. The decrease in blood optical density with decrease in hematocrit becomes greater at the lower

Figure 17.--Change in optical density (pen deflection) of the same blood-dye mixture at various blood hematocrits.


SERVO PEN DEFLECTION (mm)
105
100
95
85
SALINE DILUTIONS
DEXTRAN DILUTIONS
PLASMA DILUTIONS
50
45
40
35
30
25
BLOOD HEMATOCRIT (%)

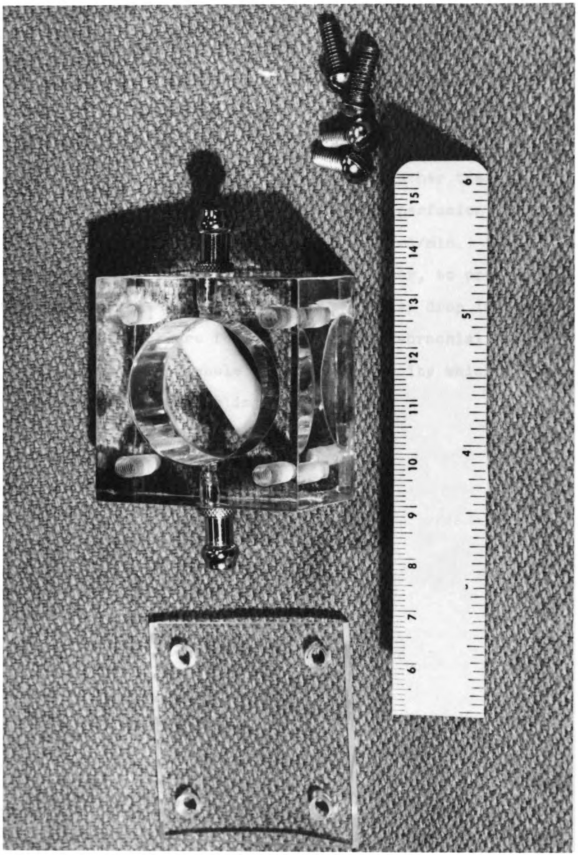
hematocrits, and is quite similar in magnitude for all three diluents over the hematocrit range 35 percent to 30 percent.

Sekelj et al. (1967) found, with their cuvette system, a slight linear increase in optical density as the hematocrit increased from 24 percent to 59 percent. Since they used their own cuvette and did not explain their technique, it is not possible to compare their results with the present findings. Apparently this hematocrit phenomenon is independent of the instrumentation used.

Design and Use of a Blood Mixing Chamber

The studies to be described in Chapters IV and V required an arterial blood mixing chamber to insure adequate mixing of arterially infused dye with the blood. A mixing chamber was designed and built to serve this purpose. The chamber consisted of a leucite plastic block with a cylindrical chamber into which was placed a Teflon coated magnetic stirring bar. Chrome fittings conducted blood into and out of the stirring chamber. Figure 18 is a photograph of the disassembled mixing chamber. In operation, the mixing chamber was inserted in the arterial cannula upstream to the brachial artery. The chamber was placed above a magnetic stirrer. Dye was infused upstream to the chamber which insured the complete mixing of dye with blood prior to entry into the brachial artery.

Figure 18.--Photograph of the disassembled blood mixing chamber.



In vivo tests were conducted to determine the hemolyzing effect, if any, of the mixing chamber on perfusing blood. The mixing chamber was interposed upstream to a pump in the pump-perfused forelimb of a dog and pump perfusion pressure was recorded. There was no change in pump perfusion pressure whether the mixing chamber was turned on or off. Pump perfusion flows were varied over the range 25 to 200 ml/min. The limb vascular bed was responsive, however, to products of hemolysis as demonstrated by a sharp drop in pump perfusion pressure following the intra-brachial arterial injection of whole blood at a velocity which produced hemolysis of the injected blood.

CHAPTER III

CONTINUOUS DYE-DILUTION FLOW MEASUREMENTS IN THE ISOLATED, PUMP-PERFUSED DOG FORELIMB

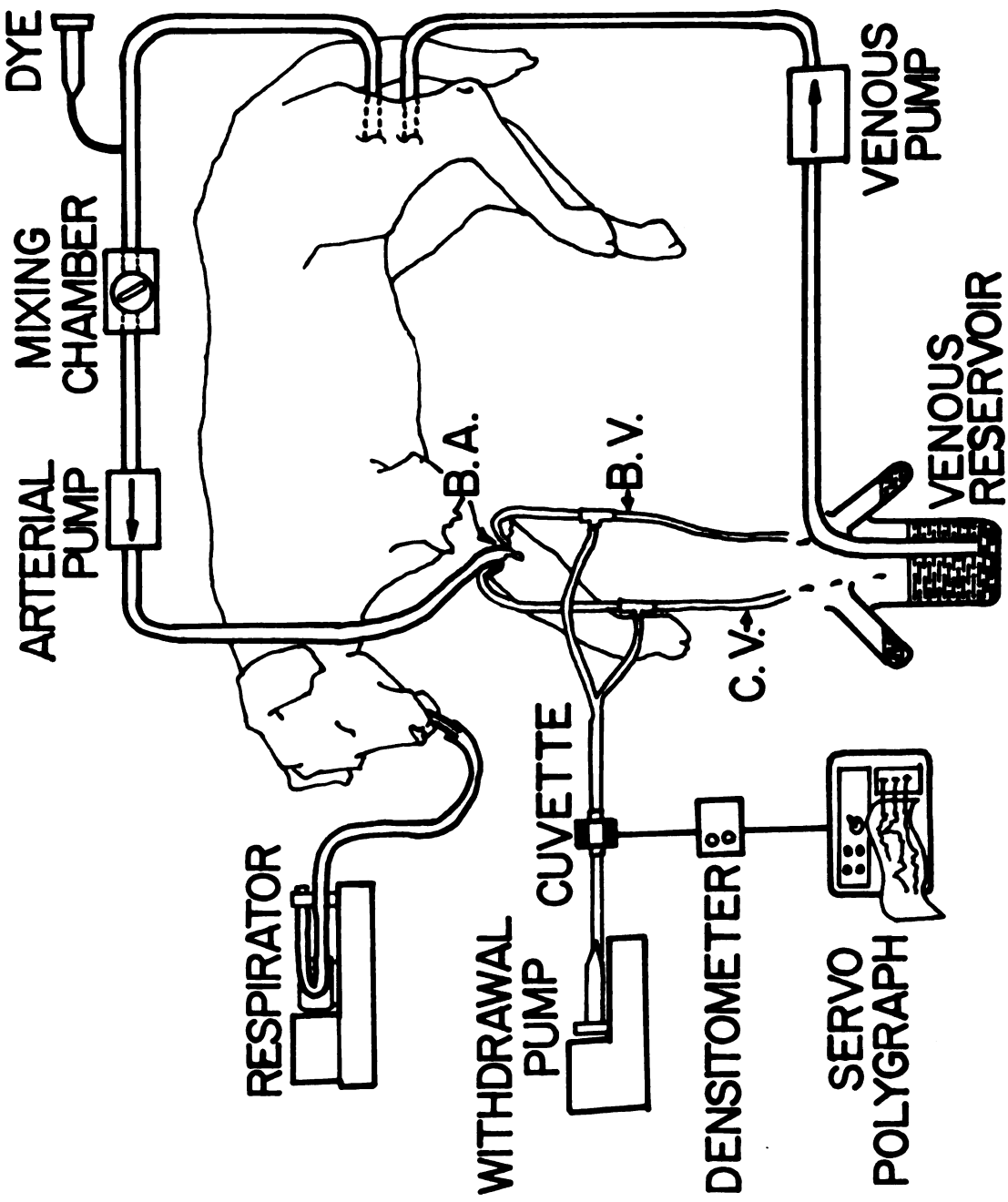
Purpose of Investigation

This section of the investigation was designed to provide a direct comparison of dye-dilution calculated blood flow with actual blood flow. The isolated, pump-perfused dog forelimb was a suitable model for these studies in that both the duration of steady-state flow and the range of flow rates in the forelimb could be readily controlled and measured. By comparing dye-dilution calculated and actual flow measurements an estimate of the accuracy of the dye-dilution system could be made.

Methods

Figure 19 is a diagram of the experimental preparation. Eight mongrel dogs (18-27 kg) were anesthetized with sodium pentobarbital (30 mg/kg), intubated and provided with external respiration. Except for the brachial artery and brachial and cephalic veins, the right forelimb was totally severed from the body at a

Figure 19.--The isolated, pump-perfused dog forelimb experimental preparation.


DYE
MIXING CHAMBER
ARTERIAL PUMP
B.A.
B.V.
VENOUS PUMP
VENOUS RESERVOIR
C.V.
RESPIRATOR
WITHDRAWAL PUMP
CUVETTE
DENSITOMETER
SERVO POLYGRAPH

point midway between the shoulder and elbow. Tourniquets were applied to the tissues of both stumps. The dog was systemically heparinized (10,000 U.S.P. units). The femoral artery was cannulated and the cannula connected to the blood mixing chamber (see Chapter II). A pressure-independent (0-300 mm Hg) Sigmamotor (Middleport, N. Y.) pulsatile blood pump was interposed between the mixing chamber and brachial artery so that the forelimb arterial inflow was solely supplied by pump. Pump flow was varied over the range 25-225 ml/min to simulate the expected range of blood flows in the resting human upper extremity.

Total venous outflow from the severed limb drained separately from the cephalic and brachial veins into a single reservoir. Polyethylene tubing connected limb venous catheters to the dye cuvette, and, the dye cuvette to a Harvard (Model 600-910, Harvard Apparatus Co., Dover, Mass.) withdrawal pump. The cuvette response to venous blood and blood-dye mixtures was recorded on a Gilson servo polygraph. Venous reservoir blood was returned to the femoral vein by a second Sigmamotor pump.

The cuvette was calibrated before and after each experiment, and a mean of the two calibrations was used for calculating dye-dilution blood flow. The calibration procedure followed the protocol outlined in Chapter II. Cardio-Green dye was freshly prepared for each experiment. The dye was diluted with distilled water and isotonic saline to a concentration of 0.55 mg/ml.

The measurement of forelimb flow by the continuous-infusion dye-dilution technique was conducted in the following manner (refer to Figure 20). The arterial blood pump was set at a desired flow rate. Venous blood was withdrawn through the cuvette (1.5 ml/min) until a stable baseline response was observed on the polygraph recording. Arterial dye infusion was then begun (0.42 mg/min, 0.76 ml/min) upstream to the mixing chamber, and blood from the two venous cannulae was withdrawn in sequence through the cuvette until a dye-concentration plateau was recorded for each vein. The dye infusion was then stopped and limb venous dye concentration was allowed to return toward the baseline. Limb blood flow was simultaneously measured by timed collection of total venous outflow in graduated cylinders.

The slope of the dye-concentration plateau(s) was taken as an indication of dye recirculation (see Figure 6, Chapter I). The baseline (dashed line) was drawn parallel to the plateau (Figure 20), in order to correct for this recirculation (see equation 6, Chapter I). The height of the dye-dilution plateau above the corrected baseline was used to calculate blood flow.

Results

A total of 69 paired blood flow measurements were made in eight dogs. The results are presented in Table 5. Overall, dye-dilution calculated forelimb flow

Figure 20.--A typical dye-concentration plateau from the isolated, pump-perfused dog forelimb series. [The baseline (dashed line) is drawn parallel to the dye-concentration plateaus to correct for recirculating dye.]

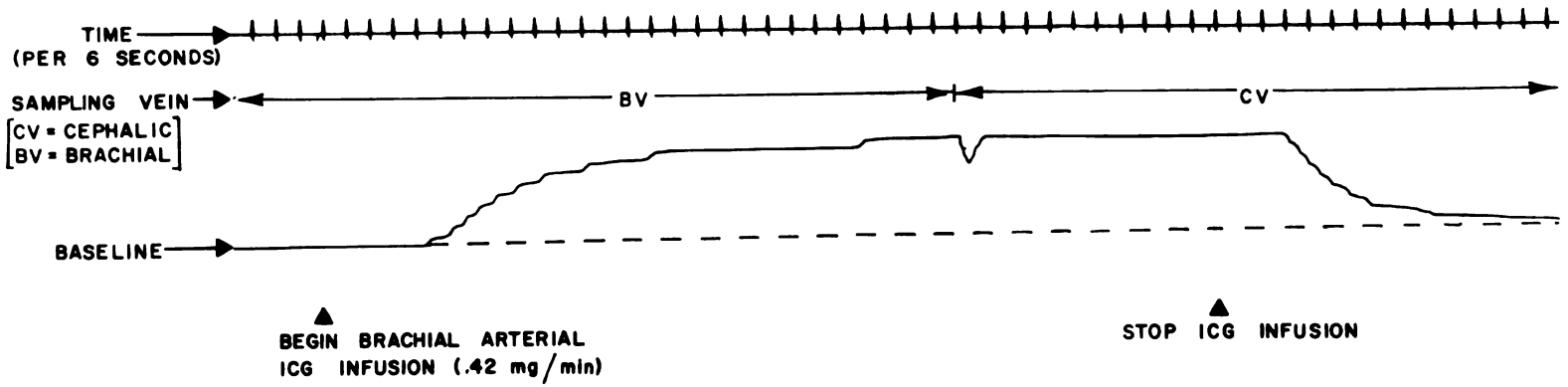
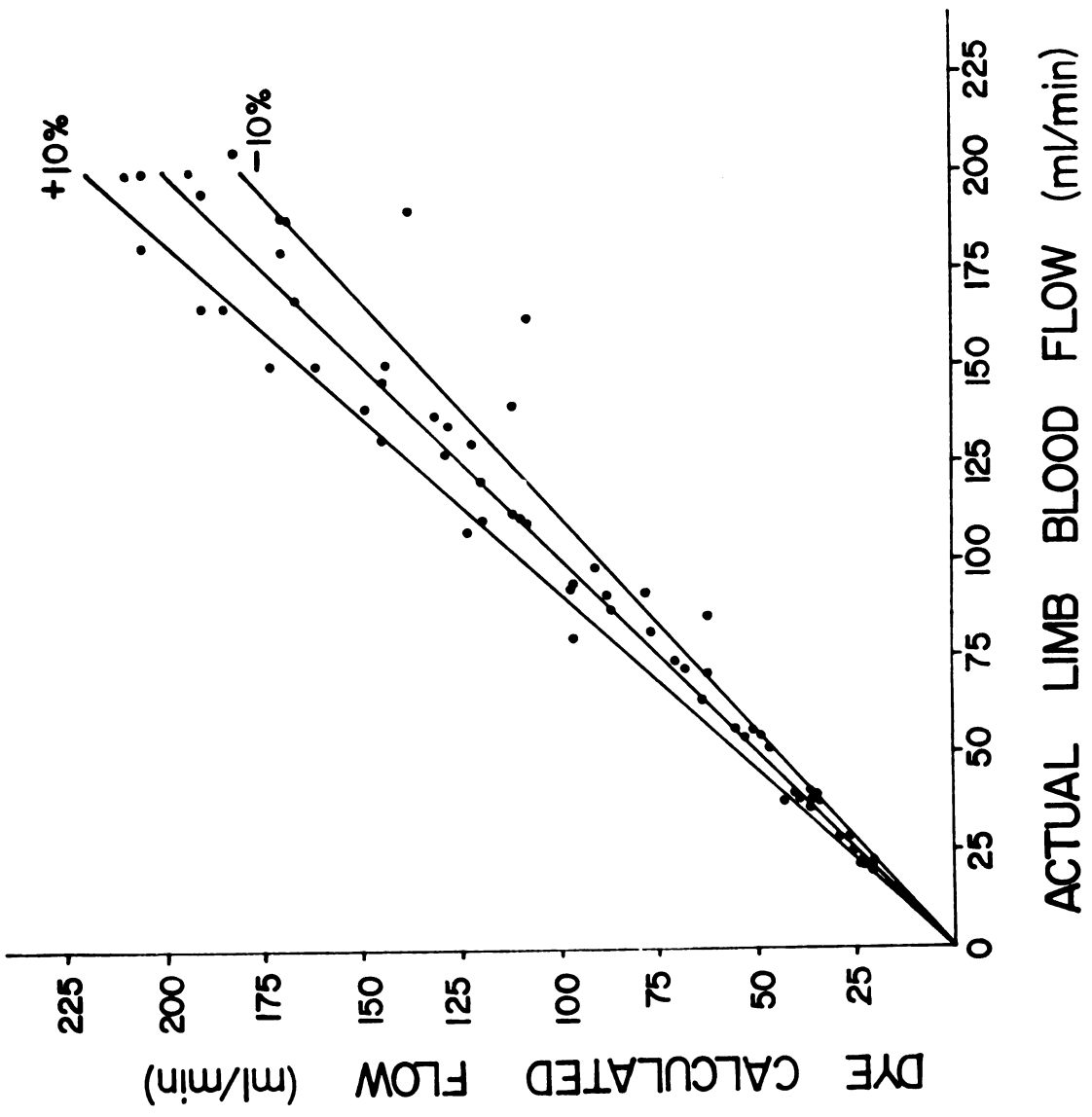

TIME
(PER 6 SECONDS)
SAMPLING VEIN
[CV = CEPHALIC
BV = BRACHIAL]
BV
CV
BASELINE
BEGIN BRACHIAL ARTERIAL
ICG INFUSION (.42 mg/min)
STOP ICG INFUSION

TABLE 5.--Comparison of actual and dye-dilution calculated blood flows in the isolated, pump-perfused dog forelimb series.

Dog No.	Actual Flow	Dye-Dilution Calculated Flow	$\left(\frac{\text{Dye - Actual}}{\text{Actual}} \times 100\right)$ % Difference
	ml/min	ml/min	
40	92	97.5	+ 5.9
	37.5	42.7	+13.8
	127	129	+ 1.5
	187.5	168	-10.4
	24.5	26	+ 6.1
41	93	97	+ 4.3
	134	128	- 4.4
	56.3	56.5	+ 0.3
	167	168	+ 0.5
	22	23	+ 4.5
	200	193	- 3.5
	39	41	+ 5.1
42	110	119	+ 8.1
	138	149	+ 7.6
	22.1	20.5	- 7.2
	91	78	-14.2
	162	108	-33.3
	56	51.5	- 8.0
	190	138	-27.3
	37.5	36	- 4.0
	129	122	- 5.4
	165	185	+12.1
	165	185	+12.1
	165	191	+15.7
43	89.7	88.5	- 1.3
	119.7	122	+ 1.9
	36.2	37.3	+ 3.0
	137	132	- 3.6
	179	170	- 5.0
44	109	108	- 0.9
	21.8	24	+10.0
	53.7	49	- 8.7
	199.2	209	+ 4.9
	73.2	71	- 3.0
	150.3	144	- 4.1
	39.3	37	- 5.8
	194	191	- 1.5
45	111	110	- .9
	148	145	- 2.0
	71.4	68	- 4.7
	39.3	35	-10.9
	188	170	- 9.5
	21.5	21.5	0
	87	87	0
	53.6	53	- 1.1
	210	180	-14.2
46	107	123	+14.9
	69.3	62.5	- 9.8
	180.9	206	+13.8
	37.9	35.3	- 6.8
	139.5	112	-19.7
	51	47	- 7.8
	84.7	62.5	-26.2
	19.4	21	+ 8.2
	79.1	97	+22.6
47	81.7	77	- 5.7
	97.9	91	- 7.0
	111.5	111	- 0.4
	131	145	+10.6
	21.3	22.5	+ 5.6
	199.5	206	+ 3.2
	38.4	39.5	+ 2.8
	63.4	64.5	+ 1.7
	150	162	+ 8.0
	150	173	+15.3
	150	162	+ 8.0
	28	29.7	+ 6.0
	28	26.8	- 4.2
	28	30	+ 7.1

differed from actual forelimb flow by a mean difference of -0.5% ± 10.2 (S.D.). The correlation coefficient is .97. The data are also presented graphically in Figure 21. In this plot, the center solid line is the line of identity; points on this line represent a dye-dilution calculated flow equal to actual flow. The two lines on either side of identity represent a 10 percent error for dye-dilution calculated flows. It is clear that there was greater variability of dye-dilution calculated flows at high forelimb flow rates. This variability at higher flow rates occurred primarily in measurements made in two dogs, numbers 42 and 46, experiments which were technically very satisfactory; no justification for discarding these two experiments was found. In general, the lower dye-concentration plateaus obtained during high blood flow rates may be less accurate than plateaus during low flows because of the lower instrumental signal to noise ratio.

Figure 21.--A plot of actual versus dye-dilution calculated blood flows in the isolated, pump-perfused dog forelimb.


+10%
−10%
DYE CALCULATED FLOW (ml/min)
ACTUAL LIMB BLOOD FLOW (ml/min)
0
25
50
75
100
125
150
175
200
225

CHAPTER IV

CONTINUOUS DYE-DILUTION FLOW MEASUREMENTS IN THE INTACT, NATURALLY PERFUSED DOG FORELIMB

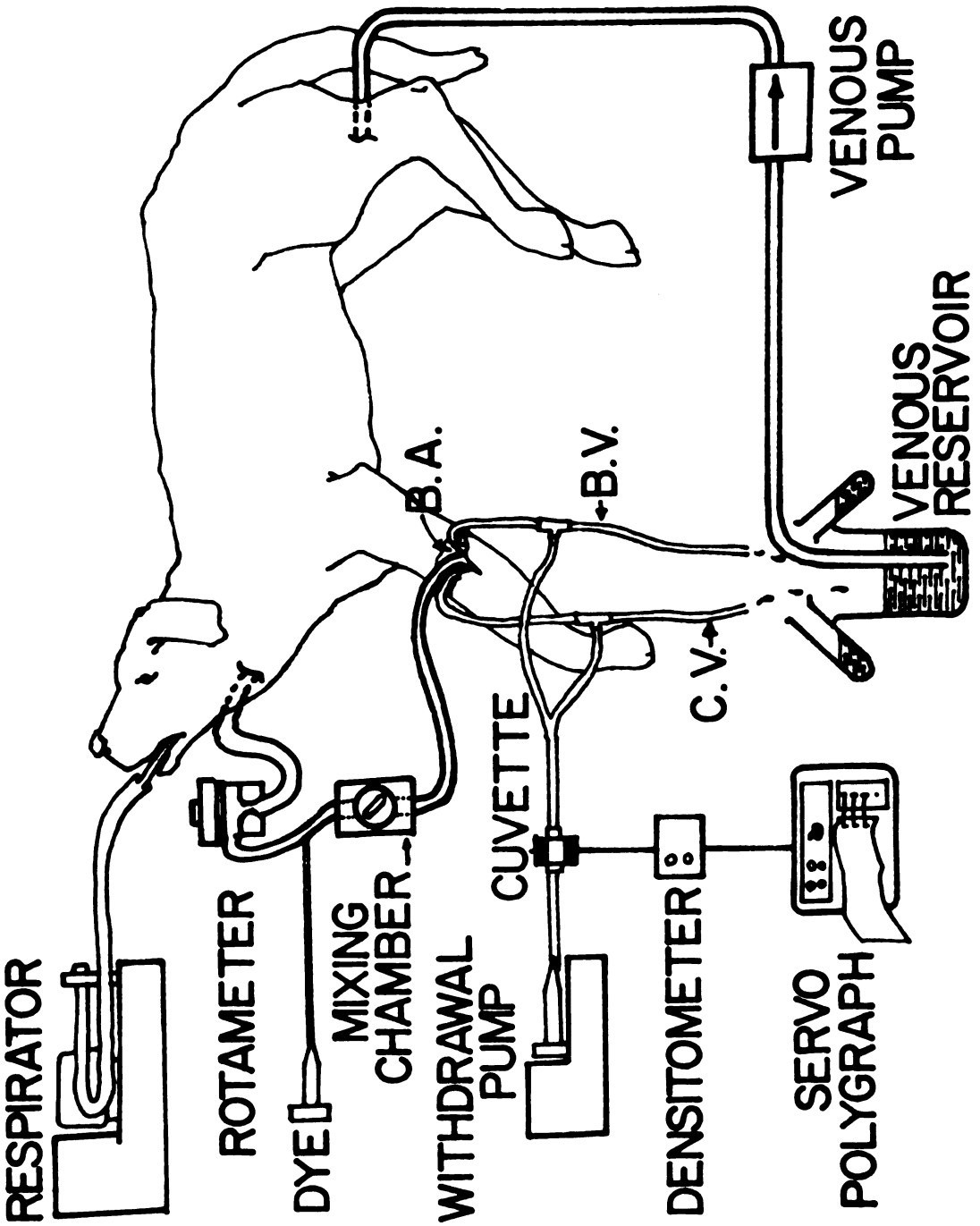
Purpose of Investigation

This section of the investigation was designed to test whether or not the addition of indicator to brachial arterial blood and the sampling of downstream indicator concentrations allowed accurate measurement of brachial arterial blood flow in the intact dog forelimb. The intact dog forelimb is more similar to the forearm and hand of man than is the isolated dog forelimb. Thus, inferences derived from this natural preparation may be more rationally extended to man. In addition, by infusing two indicators simultaneously (ICG and RISA) it was possible to compare the technically more difficult dye-dilution measurement system against the technically simpler RISA-dilution measurement system. Thus, the RISA-dilution system was used as an internal standard.

Methods

Figure 22 is a diagram of the experimental preparation. Six mongrel dogs (18-27 kg) were anesthetized with sodium pentobarbital (30 mg/kg), intubated and

Figure 22.--The intact, naturally perfused dog forelimb experimental preparation.


RESPIRATOR
ROTAMETER
DYE
MIXING CHAMBER
WITHDRAWAL PUMP
CUVETTE
DENSITOMETER
SERVO POLYGRAPH
B.A.
B.V.
C.V.
VENOUS RESERVOIR
VENOUS PUMP

provided with external respiration. The dog was systemically heparinized (10,000 U.S.P. units). The carotid artery was cannulated, and in turn connected to a Wilson-Shipley rotameter. Blood flowed from the rotameter through the arterial mixing chamber and into the forelimb brachial artery. A bypass circuit (not shown) allowed calibration and periodic flushing of the rotameter. Varying concentrations of nor-epinephrine (Levophed-Winthrop Laboratories) and acetylcholine (acetylcholine chloride--Merck) were infused into the brachial artery to alter forelimb blood flow over the range 25-200 ml/min.

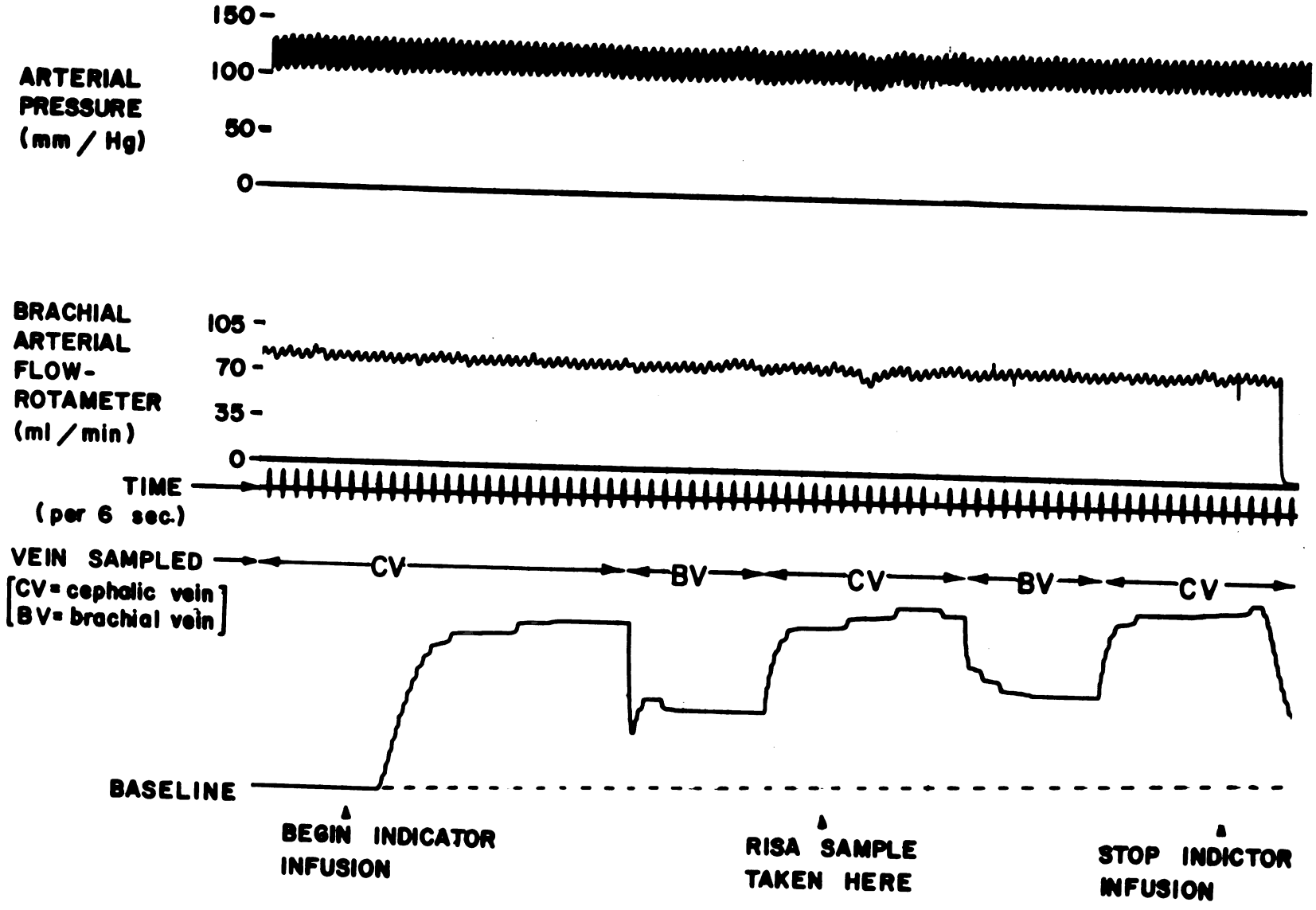
The cephalic and brachial veins drained separately into a single venous reservoir. Polyethylene tubing connected limb venous cannulae to the dye cuvette, and, the dye cuvette to the Harvard withdrawal pump. The cuvette response to blood and blood-dye mixtures was recorded on a Gilson servo polygraph. Venous reservoir blood was returned to the femoral vein by a Sigmamotor pump.

The cuvette was calibrated before and after each experiment and a mean of the two calibrations was used for calculating dye-dilution blood flow. The calibration procedure followed the protocol outlined in Chapter II. For rotameter calibration, a Sigmamotor pump was interposed between the carotid artery and rotameter, and the rotameter response to various known pump flow rates was recorded on the polygraph. Cardio-Green dye was freshly

prepared for each experiment. The dye was diluted with distilled water and isotonic saline to a concentration of 0.55 mg/ml. Serum was added (1 ml per 50 mg dye) to stabilize the optical activity of the dye in aqueous solution. RISA (Squibb-Albuminotope) was added to the dye solution.

The measurement of flow by the continuous-infusion indicator-dilution technique was conducted in the following manner (refer to Figure 23). A steady state brachial arterial blood flow was detected by observing rotameter flow. Every effort was made to prevent rotameter artifact due to fibrin collection. Venous blood was withdrawn through the cuvette (1.5 or 2.4 ml/min) until a stable baseline response was observed on the polygraph recording. Indicator was then infused upstream to the mixing chamber (0.42 mg/min, 0.76 ml/min), and blood from the two venous cannulae was withdrawn in sequence through the cuvette until a dye-concentration plateau was apparent. At this point, 2 ml aliquots of blood were taken from the brachial and cephalic venous outflows and from the arterial tubing upstream to the site of indicator infusion; these samples were used in calculating RISA-dilution blood flow. Indication infusion was stopped and the venous dye concentration returned toward the baseline.

Figure 23.--A typical dye-concentration plateau from the intact, naturally perfused dog forelimb series. [The baseline (dashed line) is drawn parallel to the dye concentration plateaus to correct for recirculating dye].


150
100
50
0
ARTERIAL PRESSURE (mm / Hg)
105
70
35
0
BRACHIAL ARTERIAL FLOW-ROTAMETER (ml / min)
TIME (per 6 sec.)
VEIN SAMPLED
[CV = cephalic vein
BV = brachial vein]
CV
BV
CV
BV
CV
BASELINE
BEGIN INDICATOR INFUSION
RISA SAMPLE TAKEN HERE
STOP INDICTOR INFUSION

Cephalic and brachial venous outflow were measured by timed collection of blood in graduated cylinders. Brachial arterial inflow was obtained directly from the rotameter recording. The slope of the dye-concentration plateau(s) was taken as in indication of indicator recirculation (see Figure 6, Chapter I). The baseline (dashed line, see Figure 23) was drawn parallel to the plateaus to correct for this recirculation (see equation 6, Chapter I). The height of the indicator-concentration plateau above the corrected baseline was used to calculate blood flow. The equation for RISA-dilution calculated flow is given as follows:

$$\text{Flow (ml/min)} = \frac{\text{CPM RISA infused per minute into brachial artery}}{\text{CPM/ml venous blood - CPM/ml arterial blood}}$$

In the case of dissimilar indicator-concentration plateaus in the two outflow veins, flow was calculated independently for each plateau, and a mean of these two flow rates recorded as calculated flow.

Results

A total of 33 multiple flow measurements was obtained from six dogs, and the results are presented in Table 6.

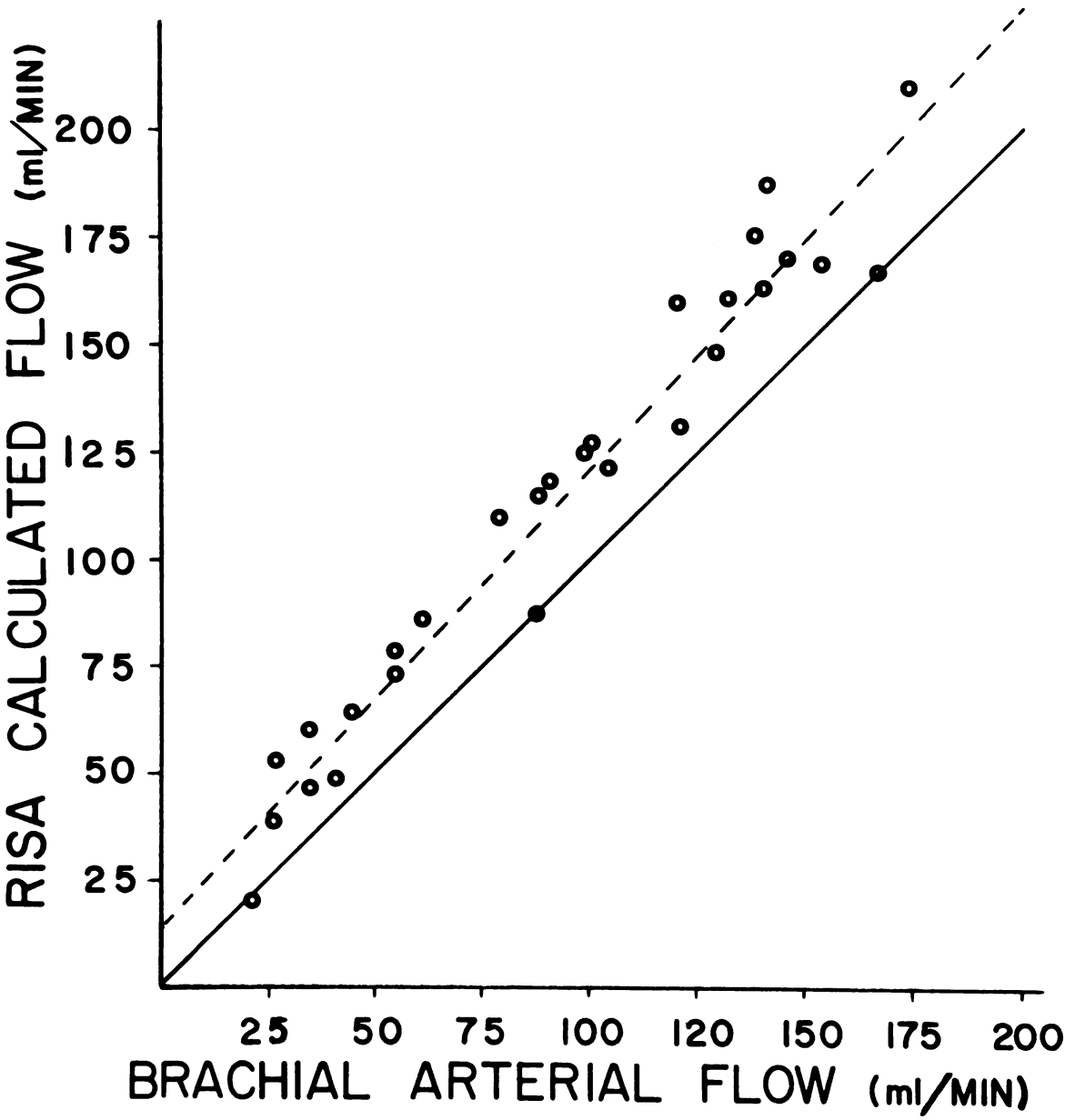
Figure 24 shows the relationship between RISA-dilution calculated flow and brachial arterial flow. The slope of the regression line (shown dashed) for RISA

TABLE 6.--Measured blood-flow values from the intact, naturally perfused dog forelimb series.

Dog No.	RISA-Dilution Calculated Flow	Dye-Dilution Calculated Flow	Brachial Arterial Flow (Rotameter)	Total Forelimb Venous Outflow
	ml/min	ml/min	ml/min	ml/min
104	130	145	---	---
	132	149	121	127
	168	178	167	164
	87	96	87	86
	48	51	42	46
	20	22	22	20
105		162	113	122
		188	138	140
		171	77	68
		96	28	37
106	79	109	54	72
	122	174	104	108
	170	200	145	150
	38	72	27	37
	65	92	45	58
107	127	143	100	115
	149	227	129	140
	169	285	153	175
	47	53	35	43
	115	134	87	105
	60	58	35	43
108	163	140	140	158
	210	200	173	192
	176	150	138	159
	125	126	98	114
	87	95	62	80
	73	71	54	64
	159	160	132	140
109	119	101	91	112
	188	163	140	157
	160	157	120	136
	110	119	78	99
	53	53	27	45

Figure 24.--A plot of brachial arterial (rotameter) versus RISA-dilution blood flows in the intact, naturally perfused dog forelimb.

The solid line is the line of identity; the dashed line is the calculated regression line for RISA dilution flow values.

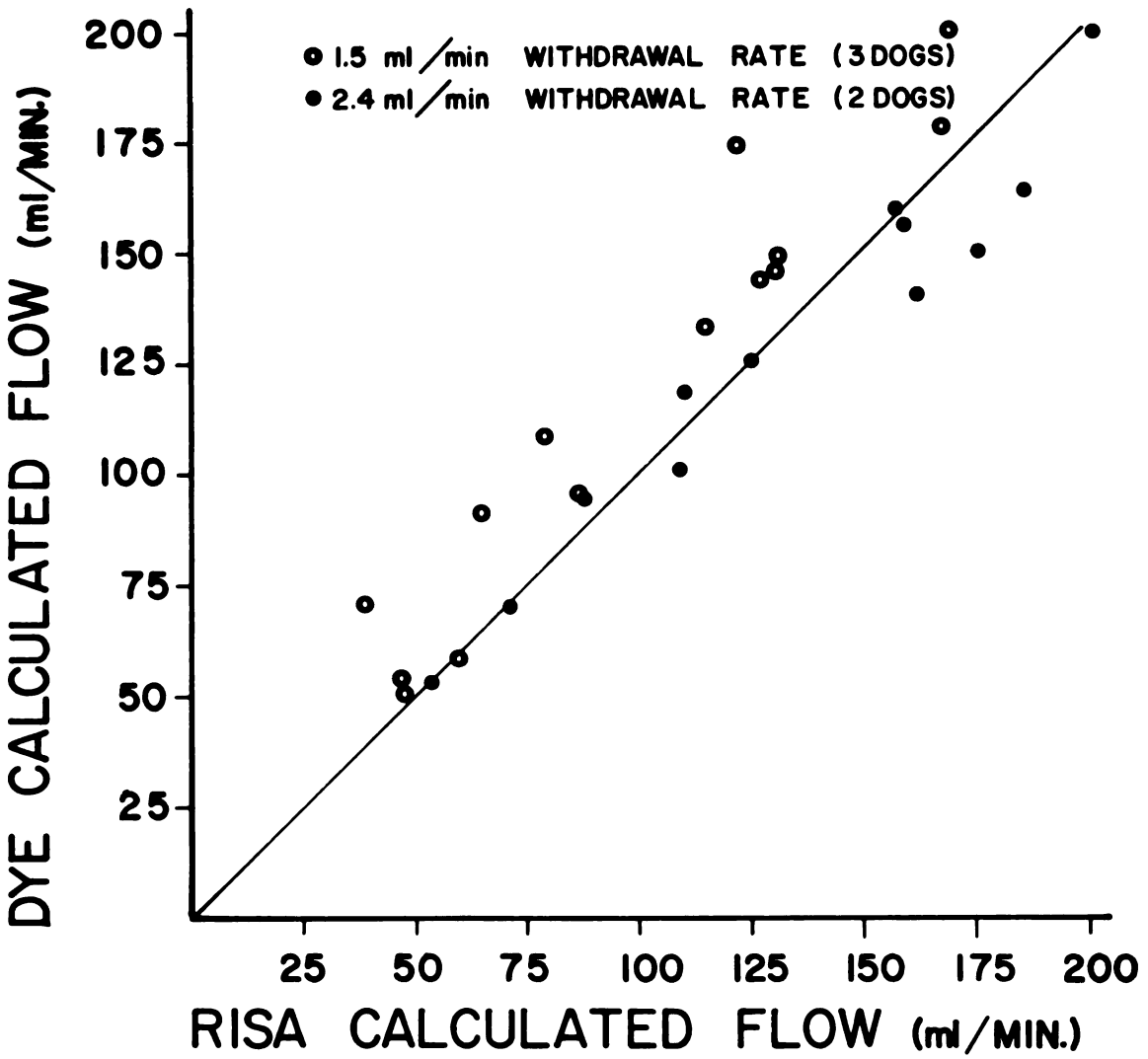

RISA CALCULATED FLOW (ml/MIN)
200
175
150
125
100
75
50
25
25
50
75
100
120
150
175
200
BRACHIAL ARTERIAL FLOW (ml/MIN)

is 1.08 and the y- intercept is +13.8 ml/min. For clarity, the dye-dilution calculated flows were omitted from this figure. It is apparent that, at least in the intact dog forelimb, the isotopic indicator-dilution method did not measure brachial artery blood flow, but rather some flow in excess of brachial artery flow.

Dye-dilution calculated flows in four dogs were obtained using a cuvette blood withdrawal rate of 1.5 ml/min. In two dogs (108 and 109) the cuvette was used at a withdrawal rate of 2.4 ml/min. Figure 25 shows the relationship between dye- and RISA-dilution calculated flows at the two cuvette blood withdrawal rates. A higher correlation was obtained between dye- and RISA-dilution calculated flows at the 2.4 ml/min withdrawal rate ($r = .97$) than for a similar comparison at the 1.5 ml/min rate ($r = .93$). However, statistically these correlations were not significantly different.

There are several indications for the presence of collateral arterial blood blow in this forelimb preparation. First, with few exceptions, dye- and RISA-dilution calculated flow were higher than measured brachial artery inflow, suggesting that the indicator in brachial artery blood was diluted by indicator-free collateral blood flow. Second, total cephalic and brachial limb venous outflow was almost always greater than measured brachial arterial inflow.

Figure 25.--A plot of dye- versus RISA-dilution calculated flow in the dog at two cuvette blood withdrawal rates.


1.5 ml/min WITHDRAWAL RATE (3 DOGS)
2.4 ml/min WITHDRAWAL RATE (2 DOGS)
DYE CALCULATED FLOW (ml/MIN.)
RISA CALCULATED FLOW (ml/MIN.)
200
175
150
125
100
75
50
25
25 50 75 100 125 150 175 200

CHAPTER V

CONTINUOUS DYE-DILUTION FLOW MEASUREMENTS IN THE HUMAN FOREARM AND HAND

Purpose of Investigation

This section of the investigation was designed to determine (1) the stability of systemic concentrations of ICG (i.e., recirculating ICG) during continuous intravascular ICG infusion in man, (2) the practicality of the present continuous-infusion dye-dilution technique for measuring blood flow in the human forearm and hand, and (3) the degree of agreement between blood flow in the human forearm and hand as measured simultaneously by the dye- and RISA-dilution techniques.

Methods

The experimental procedure was directly supervised by a licensed physician, H. W. Overbeck, M. D. All subjects participating in this study were selected by him and informed of the purposes, hazards, and procedures of the experiment. Written consent was obtained from all subjects. These healthy male volunteers, aged 21-30,

were studied in the resting, post-absorptive state at an ambient temperature maintained near 76° F. The subject was made comfortable in the supine position, and his arms supported at a 45 degree angle from the long axis of the body.

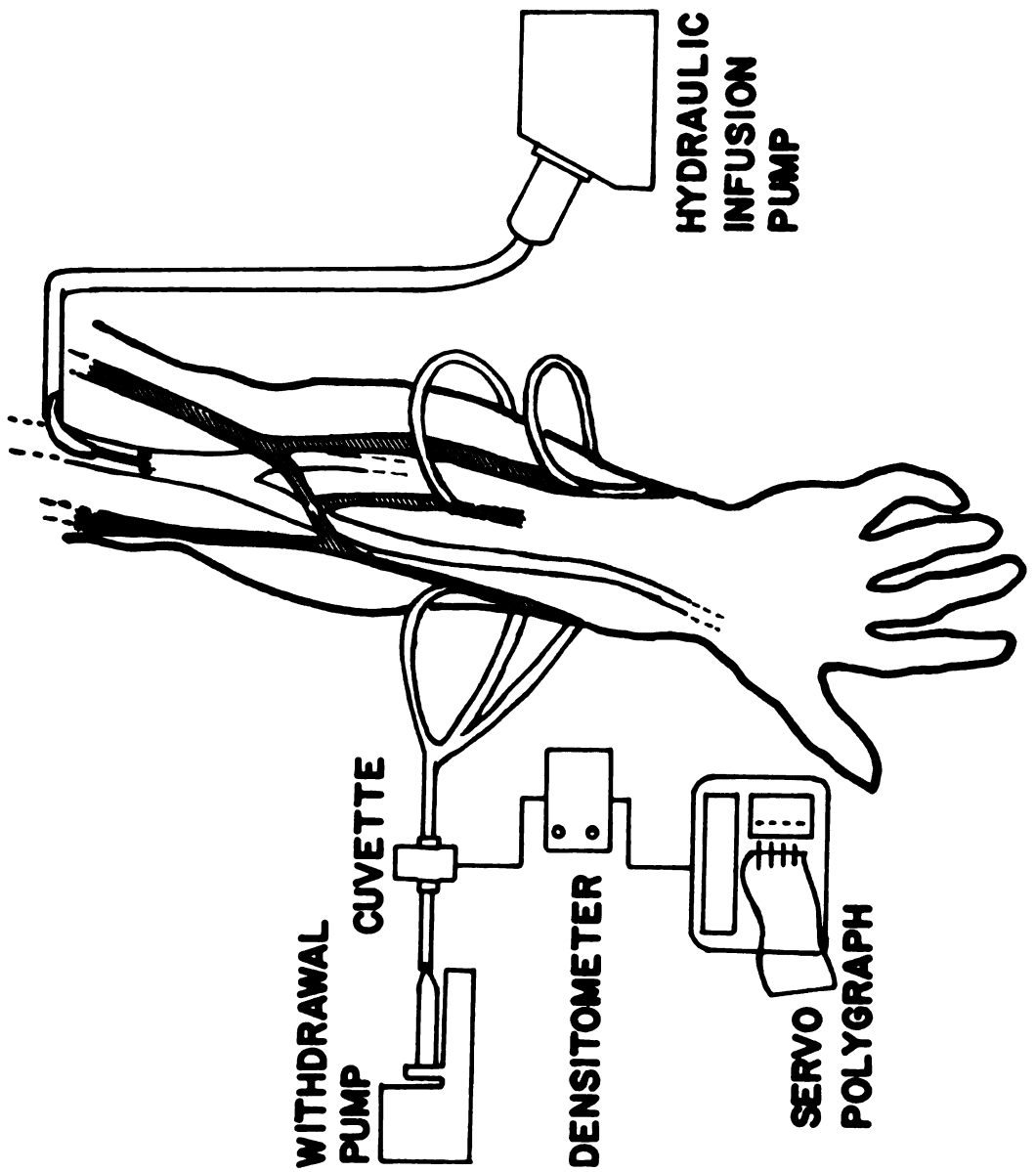
Recirculating dye concentrations were measured in eleven subjects during continuous ICG infusion. Dye was infused (0.42 mg/min) into either the antecubital vein or brachial artery of one forearm for varying lengths of time. In some instances a loading dose (.2 to .6 mg/L blood volume) of dye was given to the subject prior to the dye infusion period. Arterial or venous blood was sampled at varying intervals from the opposite forearm. Blood samples were heparinized and centrifuged. Plasma from each sample was carefully diluted (1:7) with saline. The optical density of each diluted sample was measured against an undyed plasma sample on a Beckman DU spectrophotometer. A calibration curve, obtained by measuring the optical density of known plasma-dye mixtures, allowed the conversion of sample optical density to its corresponding plasma-dye concentration (mg/L).

Forearm and hand blood flow was measured in eight subjects. Under sterile conditions, 18-gauge thin wall hypodermic needles were inserted in an upstream direction into the basilic, cephalic and/or antecubital veins of one forearm (designated ipsilateral forearm) distal to

the elbow. Thin wall, sterile Teflon cannulae were inserted through these needles, and the tips were positioned to insure an adequate venous blood supply to the cuvette. The 18-gauge needles were then withdrawn from the veins and the Teflon cannulae connected to the dye cuvette by sterile silastic tubing. Under local procaine (procaine hydrochloride, Cutter Laboratories) anesthesia, the brachial arteries of both forearms were cannulated in an upstream direction with 20-gauge Riley arterial needles. A jet injector needle (modified by Overbeck), requiring a volume flow rate of 2.7 ml/min, was inserted through the ipsilateral Riley needle until the tip of the jet needle lay within the lumen of the brachial artery. A blood reflux through the jet needle indicated that its tip lay within the arterial lumen. The ipsilateral Riley needle was then withdrawn. Venous cannulae and arterial needles were kept patent by intermittent flushing with a heparinized isotonic sodium chloride solution. The experimental preparation is shown in Figure 26.

Cardio-Green dye (50 mg) with RISA (50 uc/subject) was prepared in sterile isotonic solutions. Indicator in saline alone was infused intra-brachial arterially during resting blood flow measurements. Indicator in saline with either nor-epinephrine (Levophed-Winthrop Laboratories, 0.10 γ/min infusion rate) or acetylcholine

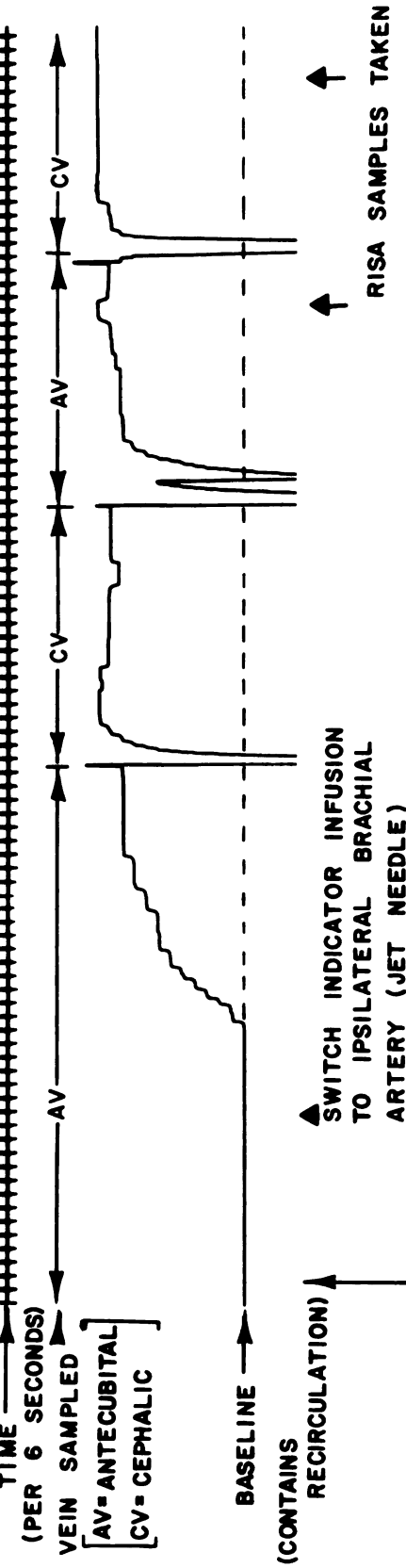
Figure 26.--Experimental preparation for continuous-infusion, indicator-dilution blood flow measurement in the human forearm and hand.


HYDRAULIC
INFUSION
PUMP
CUVETTE
WITHDRAWAL
PUMP
DENSITOMETER
SERVO
POLYGRAPH

(acetylcholine chloride, Merck, 6 γ/min infusion rate) was infused intra-brachial arterially to vary the resting blood flow rate. Forearm flow was also varied by having the subject exercise his hand. In every case, dye was infused at the rate of .42 mg/min.

The measurement of blood flow by the continuous-infusion indicator-dilution technique was conducted in the following manner (see Figure 27). Indicator infusion was begun into the contralateral arterial needle. Ipsilateral venous blood was withdrawn through the cuvette (1.5 or 2.4 ml/min) until a stable baseline response, indicating a constant level of recirculating dye, was observed on the polygraph recording. This usually required approximately 5 minutes. Indicator infusion was then changed to the ipsilateral arterial jet needle. Ipsilateral venous dye concentrations in the two downstream veins were recorded by withdrawing venous blood through the cuvette in sequence. (The sudden decrease in optical density, which is shown during switching from one vein to the other, was artifactual.) Two ml samples of venous and contralateral arterial blood were obtained during the recorder inscription of the dye-concentration plateau. These blood samples were used for calculating RISA-dilution blood flow. Indicator infusion was then returned to the contralateral artery.

Figure 27.--A typical dye-concentration plateau from the human forearm and hand. [The baseline (dashed line) is drawn horizontally because recirculation is constant in the human].


TIME
(PER 6 SECONDS)
VEIN SAMPLED
AV= ANTECUBITAL
CV= CEPHALIC
AV
CV
AV
CV
BASELINE
(CONTAINS
RECIRCULATION)
SWITCH INDICATOR INFUSION
TO IPSILATERAL BRACHIAL
ARTERY (JET NEEDLE)
RISA SAMPLES TAKEN
CONTINUOUS INDICATOR
INFUSION AT CONTRALATERAL
BRACHIAL ARTERY (.42 mg/mh)

The dye cuvette was calibrated after the experiment, following the protocol outlined in Chapter II. The height of the dye-concentration plateau above the baseline was used to calculate blood flow.

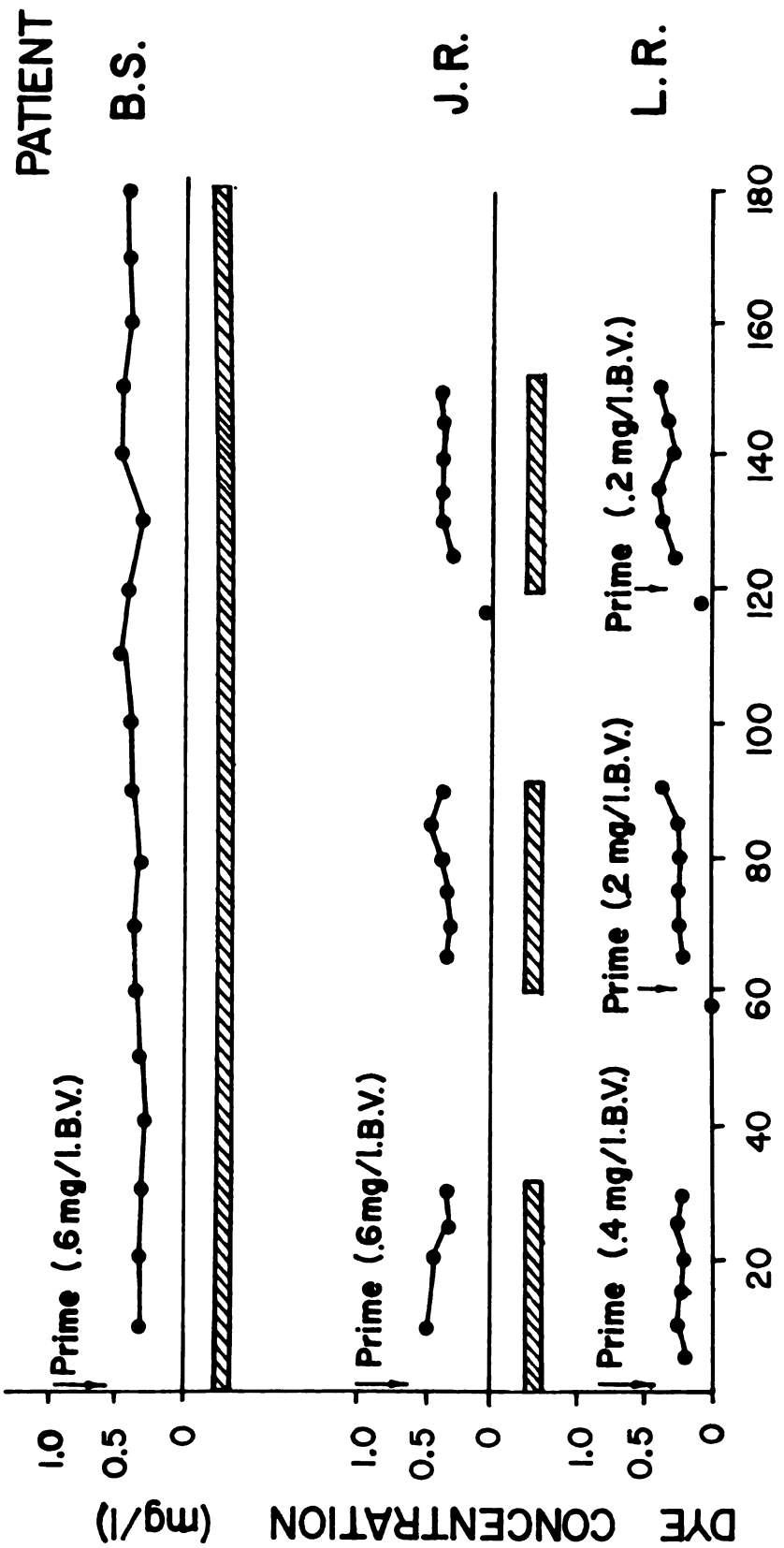
Results

Dye Recirculation

Figure 28 is a plot of recirculating ICG dye concentrations against time in three subjects. In subject B.S., blood-ICG concentrations varied between 0.32 and 0.52 mg/L during a continuous three-hour ICG infusion period (.42 mg/min). In subjects J.R. and L.R., ICG was infused for 30-minute periods with a 30-minute rest between infusions. Blood-ICG concentrations in these subjects were within the range 0-0.5 mg/L. Similarly, in eight other studies the level of recirculating ICG dye was low (< 0.5 mg/L) and quite constant with time. These data suggest that, under these conditions, a steady state is reached in which liver extraction equals dye infusion during the ICG infusion period. It is apparent that a steady state was rapidly achieved whether or not the subject was initially given a loading dose of dye.

The concentration of recirculating dye (< 0.5 mg/L) should be compared to the variations in dye concentration observed during dye-dilution blood flow measurements. These computed values range from 2.1 mg/L

Figure 28.--A graph of systemic ICG dye concentrations in man against time during continuous intravascular ICG dye infusion.


PATIENT
B.S.
J.R.
L.R.
Prime (.6 mg/l.B.V.)
Prime (.6mg/l.B.V.)
Prime (.4 mg/l.B.V.)
Prime (.2 mg/l.B.V.)
Prime (.2 mg/l.B.V.)
DYE CONCENTRATION (mg/l)
1.0
0.5
0
20
40
60
80
100
120
140
160
180
TIME (minutes)
DYE INFUSION (.42 mg/min)

for a flow of 200 ml/min to 16.8 mg/L for a flow of 25 ml/min. With recirculating dye concentrations varying, at most, in the range 0-0.5 mg/L, the maximum error introduced by changes in recirculating dye during the recording of dye-concentration plateaus is 23 percent ($\frac{0.5 \text{ mg/L}}{2.1 \text{ mg/L}} \times 100$) and 3 percent ($\frac{0.5 \text{ mg/L}}{16.8 \text{ mg/L}} \times 100$) for flows of 200 and 25 ml/min respectively.

Practicality of the Technique in Humans

Blood flow measurement was attempted in the forearms and hands of nine subjects. In one subject the forearm venous blood flow was inadequate for this technique and the experiment was terminated. In three other subjects, blood flow measurements were partially successful: in two subjects (203 and 204) by choice only one vein was sampled for indicator concentrations; in a third subject (214) only one vein would supply the required blood flow. In the remaining five subjects, two forearm veins were continuously and sequentially sampled for indicator concentrations during continuous indicator infusion. The most common technical problem encountered with the present technique was that of maintaining an adequate venous blood flow to the dye cuvette. Frequent manipulation of the venous cannulae was necessary to insure a continued blood supply. Blood clotting in the blood withdrawal tubing was usually prevented by

frequent flushing with isotonic saline. Total blood loss to the subject was less than 300 ml per experiment.

Comparison of Dye- and RISA-Dilution Flow Measurements

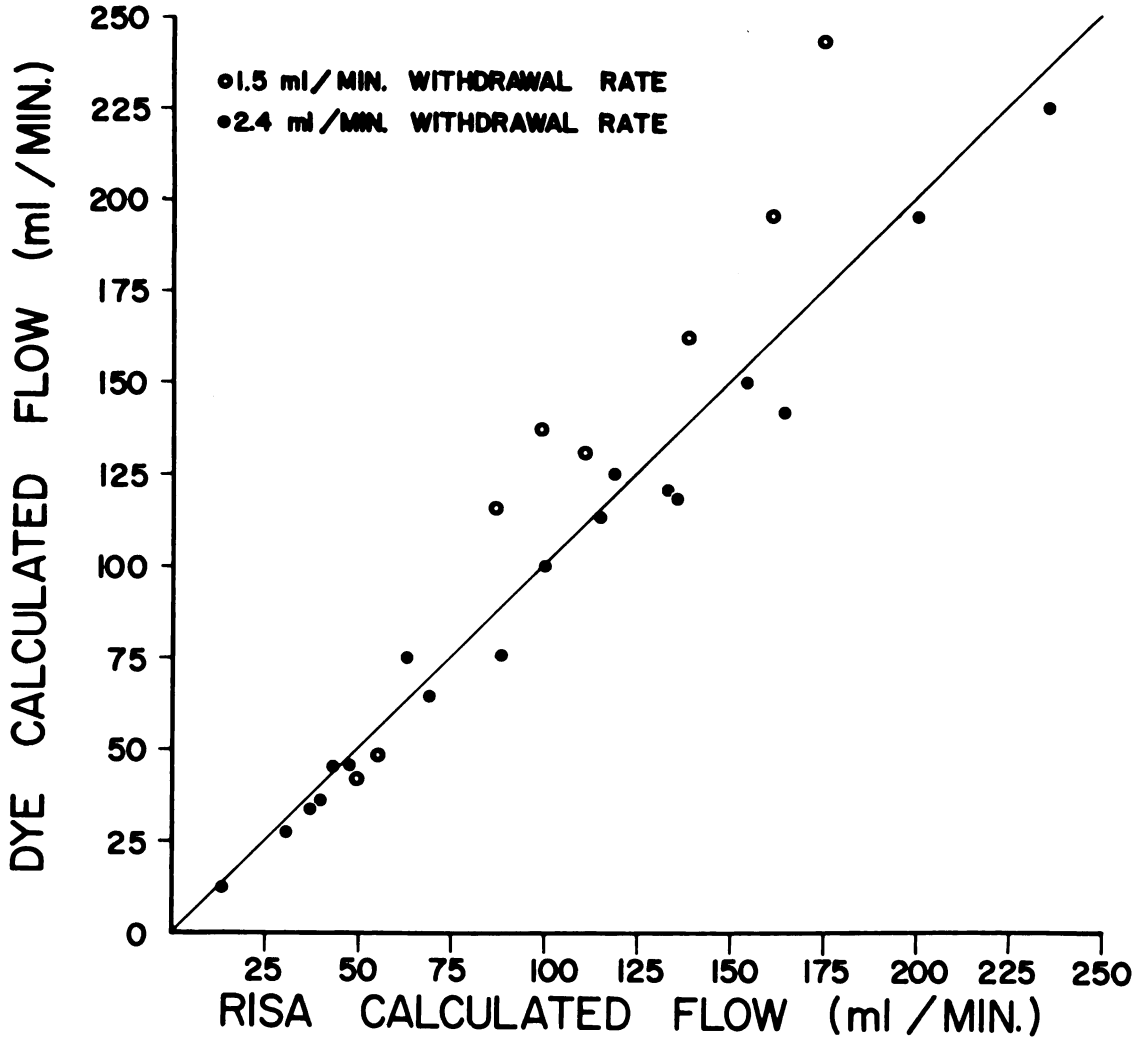
A total of 27 paired human forearm and hand blood flow measurements were made in eight subjects. The data are presented in Table 7. Dye-dilution calculated flows were obtained in subjects 203 and 204 using a cuvette blood withdrawal rate of 1.5 ml/min. In the remaining subjects the cuvette was used at a withdrawal rate of 2.4 ml/min. The relationship between dye- and RISA-dilution calculated flows, and cuvette blood withdrawal rate is shown in Figure 29. A higher correlation was obtained between dye- and RISA-dilution calculated flows at the 2.4 ml/min withdrawal rate ($r = .99$) than for the similar comparison at the 1.5 ml/min rate ($r = .97$). (Statistically, these correlations were not significantly different.) Apparently, dye-dilution calculated flows agreed better with RISA-dilution calculated flows at the higher cuvette blood withdrawal rate.

There is good evidence that subject 212 had an anomalous high brachial artery bifurcation, and that indicator was infused into the radial rather than the brachial artery. Thus basilic venous indicator concentrations were only slightly higher than recirculating indicator concentration--most of the indicator was

TABLE 7.--Comparison of dye- and RISA-dilution calculated flow measurements from the human forearm and hand.

Subject Number	Dye Calculated Flow	RISA Calculated Flow
	ml/min	ml/min
203	42	50
	48	55
	162	138
	194	161
204	131	111
	137	99
	243	175
	116	87
210	118	136
	150	154
	345	317
211	100	100
	195	200
	120	134
	114	115
212 (C.V. flow only)	36	38
	45	44
	27.5	31
	13.2	13.5
214 (B.V. flow only)	35	38
	75	63
215	126	118
	225	236
216	75	89
	142	165
	65	69
	46	48

Figure 29.--A plot of dye- versus RISA-dilution calculated flow in the human forearm and hand at two cuvette blood withdrawal rates.


o1.5 ml/MIN. WITHDRAWAL RATE
•2.4 ml/MIN. WITHDRAWAL RATE
DYE CALCULATED FLOW (ml/MIN.)
RISA CALCULATED FLOW (ml/MIN.)
0
25
50
75
100
125
150
175
200
225
250

distributed to the vascular bed drained by the cephelic vein. During nor-epinephrine infusion in this subject, only the radial portions of the forearm and hand visibly blanched, suggesting that there is a clear demarcation between the distribution of radial and ulnar arterial blood, at least in this subject.

CHAPTER VI

DISCUSSION AND CONCLUSIONS

Recirculating Dye

Constant-infusion indicator-dilution methods for studying blood flow have been complicated by the presence of changing concentrations of recirculating indicator. The accumulation of indicator in the blood necessitated an additional measurement and appropriate correction in the calculation of blood flow (refer to equation 6, page 7). The present study used a dye (ICG) which is rapidly extracted from the blood by the liver. It was hoped that use of a low dye infusion rate, coupled with good hepatic removal, would produce low and constant recirculating dye levels in the blood during constant-infusion dye-dilution blood-flow measurements. This was not the case in the dog forelimb studies presented in Chapters III and IV; dye extraction by the liver may have been impaired by anesthesia, shock, or undetected liver disease. However, dye recirculation was measured in the human during continuous dye infusion and was found to be both low in concentration and quite constant with time. During blood flow measurements in the human, therefore, recirculating dye was essentially

constant, adding a constant increment of optical density to the dye-concentration plateau. Thus, dye recirculation need be measured only at intervals and the measurement of regional blood flow in the human is greatly simplified. The constancy of recirculating ICG levels is therefore a major advantage of the measurement technique presented in this paper.

Actual Versus Calculated Blood Flow

The isolated dog forelimb series (Chapter III) was a preliminary study to determine if the cuvette densitometer dye-dilution method measured blood flow with reasonable accuracy. Dye-dilution calculated flows showed good agreement with actual flows--a significant finding in view of the fact that cuvette blood withdrawal rates were well below those ordinarily used. The reported accuracy was lower than that found by Overbeck (1966) in a similar series of experiments in the isolated, pump-perfused dog forelimb using RISA for discontinuous, indicator-dilution blood-flow measurements. In 30 paired measurements, he found that 77 percent of RISA-dilution calculated blood flow values were within $\pm$ 5 percent of actual forelimb flows. However, subsequent improvements in instrumentation have probably increased the accuracy of the cuvette densitometer dye-dilution method to a level equal to that of the RISA-dilution

method. This view is supported by data from the intact dog and human limbs comparing RISA-dilution and dye-dilution measurements.

Indicator Calculated Flow in the Intact Dog Forelimb

The isolated dog forelimb had only one arterial inflow, namely, that supplied by the blood pump. Such a preparation was quite dissimilar from the human forearm where it is possible that more than one artery supplies blood to the limb. Studies in the intact, naturally perfused dog forelimb, then, were designed to determine which blood flow parameter is measured during the infusion of indicator into the brachial artery of a naturally perfused limb; inferences drawn from these studies could be more rationally extended to measurements made in the human forearm. In the intact dog forelimb, dye-dilution measured a flow greater than brachial arterial flow. The over-estimation of brachial arterial flow probably indicates that undyed blood was entering the forelimb through collateral vessels, diluting dyed blood from the brachial artery. The presence of these collateral vessels has been confirmed anatomically. In addition, venous outflow from the limb usually exceeded brachial arterial inflow, also arguing for the presence of collateral blood flow. Interestingly, the RISA regression line was nearly parallel to the line of flow

identity (see Figure 24, Chapter IV), suggesting that the amount of collateral blood flow was almost constant over the range of limb blood flows studied (if the proportion of total collateral blood flow entering the sampled limb veins remained constant--an assumption which seems justified). The apparently constant rate of this collateral dilution would also seem to indicate that the anastomosis occurred on the venous, not arterial, side of the limb vascular bed. If anastomosis occurred on the arterial side, one might expect that dilution by collateral blood flow would increase during vasoconstriction and decrease during vasodilation, rather than remain fairly constant. It would seem logical to extend these results in the intact dog forelimb to the forearm and hand in man. It follows that indicator-dilution measurement of blood flow in the limb of man may represent measurement of brachial arterial blood flow plus a constant increment.

Dye- Versus RISA-Dilution Calculated Flows

Because of its relative technical simplicity, and thus potentially greater accuracy, the RISA-dilution technique was used as an internal standard for measuring blood flow in the present studies. Simultaneously measured dye-dilution blood flows were compared with RISA-dilution calculated flows in order to estimate further the accuracy of the present dye technique.

In both intact dog forelimb and human forearm, dye-dilution calculated flows tended to be higher than RISA-dilution calculated flows when a cuvette blood withdrawal rate of 1.5 ml/min was used. Increasing the cuvette blood withdrawal rate to 2.4 ml/min brought the two indicator-dilution measurements into better agreement, although this could not be statistically proven. An increase in the accuracy of dye-dilution calculated flows at a higher cuvette withdrawal rate is reasonable in view of the fact that the cuvette becomes less flow dependent, and thus more stable, at higher withdrawal rates. Overall, the good correlation between these two independent indicator-dilution techniques suggests that both techniques measure blood flow with equal accuracy in these applications.

Measuring Blood Flow in the Forearm and Hand of Man

Blood flow measurements in man are usually made by indirect techniques. Such techniques must provide reliable measurements without compromising the well-being of the subject. The present study has described a dye-dilution technique for measuring forearm and hand blood flow which meets both criteria. Less than 300 ml of blood are withdrawn from the subject during the procedure, and the principle complaint of subjects tested is the necessity for remaining supine and quiet

during the experiment. The constant level of recirculating dye eliminates the need for the sampling of contralateral arterial blood, and thus the necessity for contralateral arterial puncture. In addition, the present technique uniquely provides immediate and continuous blood flow measurements.

Errors in Technique

Instrumental Instability

Data on instrumental instability have been cited in Chapter II. While the present modification program has provided a reasonable degree of cuvette stability, further improvements are necessary. In addition, a cuvette densitometer system with a linear response would be highly desirable.

Stability of Flow Through the Cuvette

The sensitivity of the present cuvette densitometer system to changes in blood withdrawal rate has previously been described (see Chapter II). This phenomenon is a potential source of error in the present technique. Changes in cuvette blood withdrawal rate, caused by either a venous obstruction or a blood clot in the withdrawal cannula, are usually readily detected by a shift in the recording baseline during the withdrawal of undyed blood. However, a similar change in withdrawal rate during the recording of a dye concentration

plateau produces a change in the plateau which may be obscured by changes in forearm blood flow and vise versa. The sampling of blood from veins with a good blood supply and careful placement of the venous cannula both help to insure that blood flow through the cuvette is constant during the measurement of venous dye concentrations. Monitoring withdrawal pressures would also help to detect changes in cuvette withdrawal rate.

Blood Changes

The relationship between cuvette output and blood hematocrit has been discussed in Chapter II. In the studies in man, systemic blood hematocrit decreased less than 2 percent during the experimental measurement of forearm and hand blood flow thus adding insignificant error. Changes in other blood constituents and their effect on cuvette output have not been studied. Agents which change the shape of red cells, such as hypo- or hyper-tonic solutions, might alter the optical density of blood and interfere with flow measurements by this system.

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