MEASUREMENT OF BLOOD FLOW AND VOLUME IN THE FOREARM OF MAN; WITH NOTES ON THE THEORY OF INDICATOR-DILUTION AND ON PRODUCTION OF TURBULENCE, HEMOLYSIS, AND VASO-DILATATION BY INTRA-VASCULAR INJECTION [1]

By R. ANDRES,[2] K. L. ZIERLER, H. M. ANDERSON, W. N. STAINSBY, G. CADER, A. S. GHRAYYIB, AND J. L. LILIENTHAL, JR.

(From the Departments of Medicine and Environmental Medicine, The Johns Hopkins University and Hospital, Baltimore, Md.)

(Submitted for publication September 2, 1953; accepted November 12, 1953)

In the course of a series of studies on neuromuscular function it became desirable to investigate in man the metabolism of skeletal muscle *in situ*. For this purpose it was decided to apply the Zuntz-Fick principle to determine the exchange of metabolites between muscle and blood in the forearm with circulation occluded at the wrist. The selection of this segment was guided by the following considerations: (a) Convenience; (b) the large muscular volume relative to the volume of skin (1); (c) the predominance of blood flow through muscle relative to skin (2, 3); and (d) the relative avascularity of other structures suggesting that no less than 80 per cent of total forearm blood flow is muscular in distribution.

In addition to the estimation of arterio-venous concentration differences in substances of interest, the principle requires a measurement of blood flow through the segment under study. Of methods in current use, only venous-occlusion plethysmography provides an absolute numerical value for blood flow through a human extremity. Despite the wealth of observations recorded by this method it was deemed desirable to develop another approach which might yield continuous recording of blood flow as well as serial samples of arterial and venous blood. Furthermore, the sources of error of the plethysmographic method have been studied carefully but not overcome completely (4), and, to our knowledge, no verification of the method by an independent technic has been made in man.

It appeared that blood flow might be measured in a fashion convenient for these proposed studies by application of the indicator-dilution technic which would make possible the simultaneous estimation of the volume of distribution. Indeed, it was conceivable that by proper selection of indicators, injected simultaneously, it might be possible to assess in the forearm its vascular volume as well as the volumes of total and of extracellular water, and from these values to estimate the volume of intracellular water. Metabolic exchanges, then, could be referred to a unit volume of intracellular water, which in the forearm represents largely the muscular moiety.

The possibility of determining blood flow by measuring the dilution of an indicator was appreciated by Hering (5), and Stewart (6) described the theory and technic of this method for measuring cardiac output. Only W. F. Hamilton, in an unpublished experiment, has explored the applicability of the indicator-dilution principle to measurement of blood flow in an extremity, although the principle has been applied to the measurement of other regional flows (7, 8). It was against this background that indicator-dilution has been examined as a means of measuring segmental blood flow. Of the two forms of the indicator-dilution method, single, nearly-instantaneous, or continuous constant rate injection, the latter form was selected to answer the need for prolonged measurements of blood flow. The indicator chosen was Evans blue dye, T-1824, owing to the wealth of information available concerning its behavior within the vascular tree (see, for example, Gregersen [9], Gibson and Evans [10] and Dow, Hahn, and Hamilton [11]). It was planned, therefore, to inject a solution of T-1824 at a constant rate into the brachial artery at the elbow and to sample

[1] Aided by a contract between the Office of Naval Research, Department of the Navy, and The Johns Hopkins University (NR 113-241), and a grant (H-1327) from the National Institutes of Health, Department of Health, Education, and Welfare.

[2] Public Health Service Postdoctoral Research Fellow of the National Heart Institute.

continuously from an ipsilateral antecubital vein by means of an indwelling catheter.

Application of the dye-dilution principle proved more complicated than had been anticipated, and it became necessary to reconsider the principle in some detail so that inherent assumptions might be stated explicitly. It is the purpose of this report to consider the dye-dilution principle, to describe some complications arising in its application in the human forearm, and to report the results obtained in its use in normal subjects. Preliminary reports of some of these observations have appeared elsewhere (12, 13). The basic considerations of the particular problem presented herein appear directly applicable also to study of other flow systems.

The theory by which constant injection of an indicator permits measurement of flow is considered in detail in Appendix I. It is basic to the theory that, when indicator is injected continuously at constant rate, ultimately a steady state is reached in which the total quantity of indicator leaving the local vascular bed during unit time equals the quantity introduced during that time. Thus, if indicator is introduced at a rate of I mg. per min., and if there is no recirculation of indicator, then during the steady state the rate at which indicator leaves the local bed is also I mg. per min. But this rate is also the product of the concentration of indicator in plasma leaving the system during the steady state, C mg. per ml., and the rate of plasma flow, F ml. per min., or $I = CF$. Solving for flow, $F = I/C$.

When the concentration of indicator in venous return from a vascular bed is plotted against time during and after constant injection of indicator into the main artery supplying the bed, in theory, the curve shown in Figure 1A is obtained if no indicator recirculates. After a brief delay, representing the most rapid transit from the site of injection to the site of sampling, there is an inflow transient rising asymptotically toward a constant concentration. In the intact subject, however, indicator, injected into a peripheral artery, enters the general circulation and returns to the artery after dilution.

In practice, the contribution of recirculating dye to the total dye delivered to the forearm is slight because recirculated dye is diluted approximately

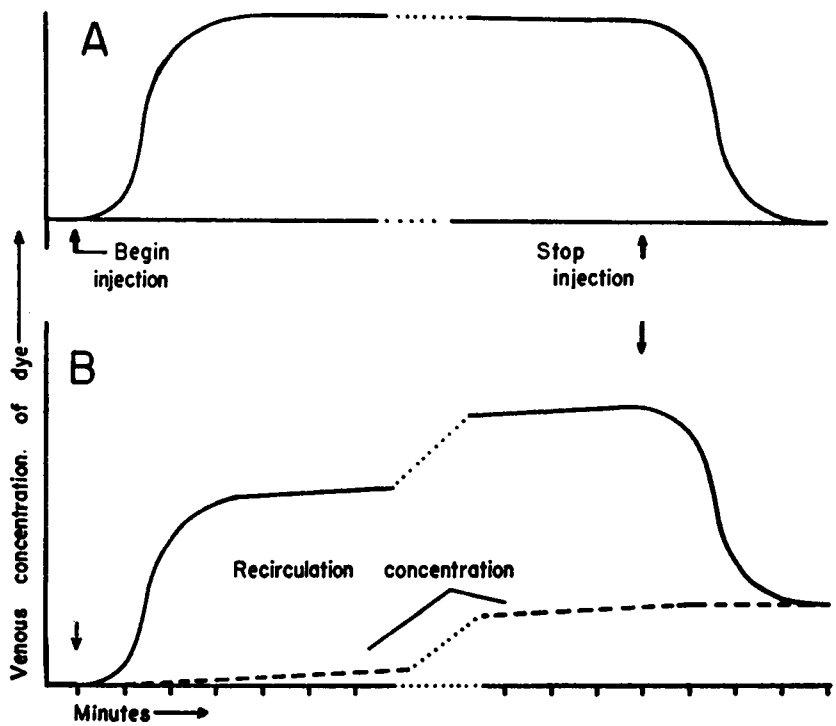

FIG. 1. IDEALIZED CONCENTRATION-TIME PLOT DURING CONSTANT INJECTION
A. No recirculation. B. Recirculation.

100-fold in total plasma volume of the body. Despite this dilution, if injection of dye is prolonged for a matter of minutes, dye accumulates sufficiently in recirculating arterial plasma to demand consideration.

The concentration of indicator in recirculating plasma is the resultant of the rate of injection of indicator, the rate of loss of indicator from the plasma, and the rate of mixing in total plasma pool. In practice, these factors result in a recirculating concentration which rises linearly and slowly with time. When the concentration of indicator in venous plasma from the local vascular bed is plotted against time, the idealized curve shown in Figure 1B is obtained. When injection stops there is a slight delay representing the most rapid transit time from the site of injection to the site of sampling, following which there is a curvilinear decline in concentration to an approximately constant value. This approximately constant value represents the concentration of recirculating dye. If flow is to be calculated from the sort of curve illustrated in Figure 1B, correction for recirculating indicator is necessary. Plasma flow is then defined by,

$$F = \frac{I}{C_0 - C_r} \qquad (1)$$

where C_0 is the concentration of indicator observed in venous plasma draining the vascular bed into which indicator is injected at constant rate, I, and C_r is the concentration of indicator in recirculating plasma. Several methods for estimating the concentration of indicator in recirculating plasma are presented in Appendix II.

The rate of flow of whole blood can be calculated from plasma flow and the hematocrit,

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

As has been indicated, the theory upon which equation (*1*) is based requires that a steady state be reached in which the quantity of indicator leaving the bed equals the quantity introduced during the same interval of time. A corollary requires that during this steady state the concentration of indicator be the same in all veins draining the local bed; that is, if blood from two veins draining the forearm is sampled simultaneously, in the steady state the concentration of indicator should be the same in the two veins. This corollary provides a means for testing the validity of the indicator-dilution method for measuring forearm blood flow.

In order to put the matter to a test it became necessary to estimate how rapidly the binding of T-1824 occurred, for if injected T-1824 were not bound to plasma proteins by the time it reached the first capillary net, an indeterminate amount of dye might escape from the vascular bed. It was determined *in vitro* that T-1824 was bound almost instantaneously to serum proteins. Aliquots of human serum were incubated in tubes in a water bath at 37° C. An aqueous solution of T-1824 was added to each tube to yield a final concentration of 150 mg. per liter of serum, at least five times the maximum concentration anticipated to occur in the *in vivo* experiments. At intervals varying from five seconds to zero seconds after addition of dye, trichloracetic acid was added to a final concentration of 10 per cent. The mixture was centrifuged and the supernant examined for T-1824. No dye was present in the supernants even when T-1824 and trichloracetic acid were added simultaneously. That binding had occurred in the brief instant *before* the proteins were denatured by trichloracetic acid was demonstrated by the observation that dye was incompletely bound to serum proteins when the proteins had been denatured by prior treatment with trichloracetic acid. With this evidence for rapid binding of T-1824 and protein, injection of dye into the human brachial artery was undertaken.

In pilot experiments, T-1824 was injected through a 20-gauge needle at constant rate (0.1 to 0.15 ml. per min. of a 0.25 per cent aqueous solution) into a brachial artery in normal subjects at rest, and venous blood was sampled simultaneously from two veins draining the injected forearm. The concentration of dye in one vein differed from that in the other vein; that is, dye had not been distributed uniformly throughout the forearm vascular bed.

There are several possible explanations for the fact that dye injected into the presumed single arterial inflow was found to be distributed unevenly in the various venous outflows of the forearm vascular bed. These explanations might be one or several of the following: (a) *Collateral arteries about the elbow.* If injected dye is distributed uniformly over the cross-sectional diameter of the brachial artery in the vicinity of the injection site,

then, when dye-laden blood arrives at the first branching of the brachial artery, which is the bifurcation, the concentration of dye in blood perfusing the radial artery will be identical with that perfusing the ulnar artery. Under these conditions the concentration of dye throughout the forearm vascular bed ultimately must become uniform, and the concentration of dye in all veins draining the forearm must become equal unless dyed blood is diluted or escapes from the flowing blood stream in some uneven fashion. Dyed blood might be diluted by blood from collateral arteries arising from the brachial artery proximal to the site of injection and joining the radial and ulnar systems distal to the injection level. If the total contribution of collateral arteries were no more than a few per cent of brachial artery flow there could be no measurable distortion of dye concentration on this basis. Even if the contribution of collateral arteries were considerable the concentration of dye in the two veins should remain essentially equal if the relative contributions to the radial and ulnar arteries were equivalent. Thus, if dye is distributed uniformly prior to the reentry of collaterals, then a subsequent lack of uniformity in dye concentration of significant degree could occur only if the collateral flow were relatively large and, in addition, if there were an appreciable disproportion in radial and ulnar collateral dilution. Although exact information concerning the contribution of collateral arteries is lacking, available roentgenographic evidence (14) of the comparative size of collateral arteries about the elbow suggests that it is most unlikely that they contribute more than a few per cent of total forearm arterial flow in the absence of major arterial obstruction. It is probable, therefore, that the role of collaterals in dilution of dyed blood can be neglected as an explanation for uneven venous concentrations except in unusual circumstances. (b) *Uneven escape of dye from vascular channels.* The possibility that dye might escape disproportionately from one or another part of the vascular bed and thus contribute to maldistribution was deemed unlikely because the quantity of albumin, to which the dye is bound so tightly, leaving *via* the lymphatics is negligible compared to that leaving *via* the veins, if evidence from the dog's limb may be applied to man (15). (c) *Anomalous bifurcation of the brachial artery.* Bifurcation of the brachial artery above the site of injection provides a special case which will be treated under Discussion. (d) *Incomplete mixing of dye and blood in the brachial artery.* It was suspected that maldistribution of dye observed in pilot experiments was the result of faulty mixing of dye and blood in the brachial artery so that radial and ulnar arterial blood may have carried differing concentrations of dye. As will be shown, it became evident that, although injected dye and blood probably did not mix thoroughly prior to the bifurcation of the brachial artery, in most cases there appeared to be sufficient intermingling of blood originating from the radial and the ulnar arteries to yield essentially uniform dilution of dye by the time it entered the major venous trunks leaving the forearm. Nevertheless, it remained true in theory that if mixing of dye and blood could be achieved prior to the bifurcation of the brachial artery, then radial and ulnar arterial blood must contain equal concentrations of dye and dye must be distributed uniformly over the forearm vascular bed, providing the role of collaterals could be neglected. Viewed in this light, the problem appeared to be one of achieving complete mixing of dye and arterial blood as near the site of injection as possible. In physical terms this is a problem of transforming momentarily the laminar flow of blood into turbulent flow. It is the purpose of this portion of this report to describe a method for accomplishing that end.

I. Mixing of dye with blood in the measurement of forearm blood flow

Before the question of mixing is considered in detail, in order to place this phase of the report in proper perspective, it must be emphasized that the technic designed to produce rapid mixing of injectate and arterial blood frequently resulted in the destruction of erythrocytes with subsequent increase in blood flow (see Section II). Nevertheless, experience with this technic has led to observations which are of independent interest, and the technic remains applicable to those problems in which complete mixing of injectate and flowing blood must be achieved *at the site of injection* and in which some hemolysis and consequent increase in blood flow may be tolerated.

The ratio of the forces tending to drive fluid particles apart (inertial) to the forces tending to hold them together (viscous) can be stated for any

specific flowing system as a dimensionless expression called the Reynolds number. When the former forces are sufficiently dominant, flow is turbulent; otherwise flow is laminar. At the transition between laminar and turbulent flow, the ratio defines the *critical* Reynolds number.

The critical Reynolds number for blood has been determined *in vitro* by Coulter and Pappenheimer (16) and estimated *in vivo* by Reynolds, Light, Ardran, and Prichard (17). *In vitro,* it was of the order of 1000, similar to that of water; *in vivo,* the estimate was about twice as high. It can be calculated that the Reynolds number of blood flowing in the human brachial artery is no greater than one-tenth of the critical Reynolds number and safely within the range of laminar flow. The magnitude of the difference between the Reynolds number in the brachial artery and the critical Reynolds number for blood implies that laminar flow is so stable in the brachial artery that ordinary methods of constant injection are unlikely to provoke turbulence and consequent mixing of dye and blood. Early attempts to produce *in vitro* mixing based on imposing wire loop barriers or simultaneous injection of large numbers of small bubbles of CO_2 were unsuccessful.

It does not matter whether turbulence originates by alteration of Reynolds number of blood or of injectate; in the vicinity of the site of injection the character of flow is a property of a new fluid system composed of both blood and injectate. Random intermingling of dye-laden injectate and blood will occur if the Reynolds number of the injectate is sufficiently great to provoke a disturbance over the entire cross-section of the brachial artery.

Of the variables defining the Reynolds number, that which can be modified best by as much as ten-fold in a system composed of injectate and blood flowing in an artery is the linear velocity of the injectate. The linear velocity of the injectate is determined by the volume injected per unit time divided by the cross-sectional area of the orifice of the injector. Thus, linear velocity varies inversely as the cross-sectional area or as the square of the radius of the injector. An enormous increase in Reynolds number can be produced, therefore, by decreasing the orifice of the injector. Further advantage accrues by directing the injection upstream; theory of counter-injection, however, is incomplete, and it is not yet possible to predict this advantage quantitatively.

Dr. Stanley Corrsin, Department of Aeronautics, The Johns Hopkins University, whose assistance in this aspect of the problem was invaluable, calculated that under conditions obtaining in human experiments turbulence should be achieved if dye were injected at a rate of 1 ml. per min. through a jet orifice of 25 μ (0.001 inch) diameter. The production of jet injectors with orifices of this dimension is described in Appendix II. Jet injectors were tested in (a) a flow model, (b) the femoral artery of the dog, and (c) the forearm of man.

(a) *Production of local turbulence and mixing in a flow model.* A length of glass tubing, 4 mm. I.D., approximately the diameter of the human brachial artery, was connected by rubber tubing to a water reservoir so that water flowed through the tubing at constant rate and with low Reynolds number. A jet injector, roughly 25 μ diameter at the orifice, connected to the constant-injection apparatus, was inserted into the flow model, so that the tip of the injector and the injected dye stream were visible through the glass tubing. Dye solution was injected at varying rates against the water

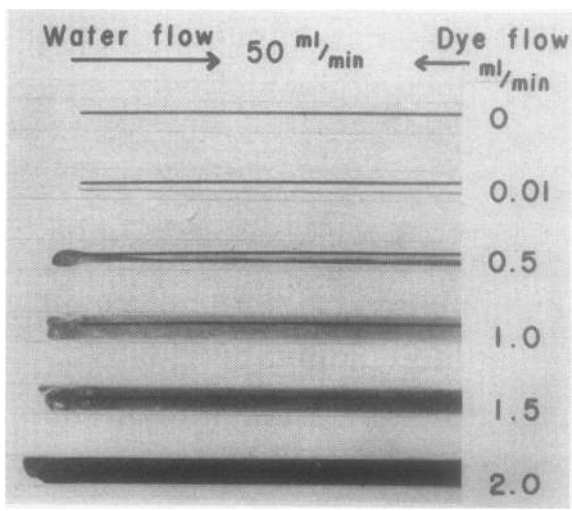

FIG. 2. TRANSITION FROM LAMINAR TO TURBULENT FLOW IN THE MODEL

Six serial photographs were taken at increasing dye injection rates. Rate of water flow remained constant. The walls of the glass tube appear as six pairs of thin black lines. The heavier black line in the center of each glass tube is the steel needle with modified jet tip. At a flow of 0.01 ml. per min. a filament of dye is seen issuing from the needle tip and coursing down the tube below the steel injector.

stream. The results appear in Figure 2. At low rates of injection (low linear velocity) the fundamental laminar flow of the system was undisturbed, and dye traveled downstream as a single filament. As the rate of injection was increased (higher linear velocity) local turbulence was created, and the dye was distributed uniformly over the onflowing water. That this turbulence was only local was demonstrated by the simultaneous injection of two solutions into the water stream; through the proximal injector, dye solution was injected with sufficient linear velocity to produce turbulence; through the second injector, 2 cm. distal to the first, dye solution was injected at low velocity and was seen to travel downstream as a single filament. Laminar flow, therefore, was reestablished quickly.

Effectiveness of mixing was tested further in a model, with rubber substituted for glass tubing. Two collecting needles were inserted at various well-separated distances downstream from the injector and at different depths into the lumen. Mixing was considered established when dye concentrations of simultaneous samples from the two collecting needles were identical. The effect of various constant rates of dye injection on the degree of mixing as measured by the ratio of dye concentrations in simultaneous samples from the two collectors is illustrated in Figure 3. By these studies it was possible to determine for injectors

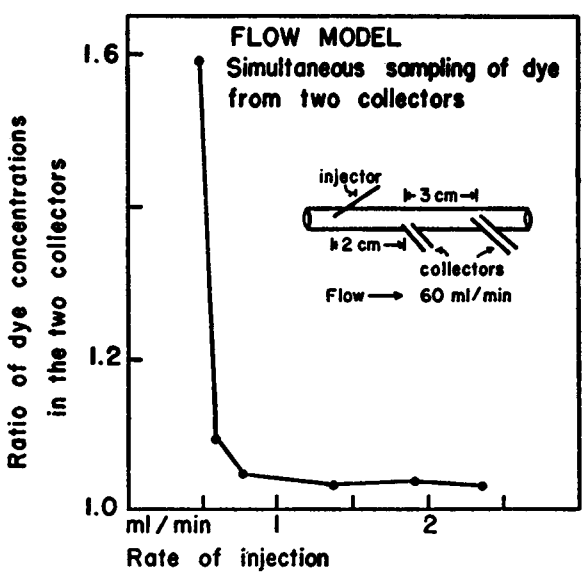

FIG. 3. TEST OF EFFICIENCY OF JET INJECTOR IN FLOW MODEL

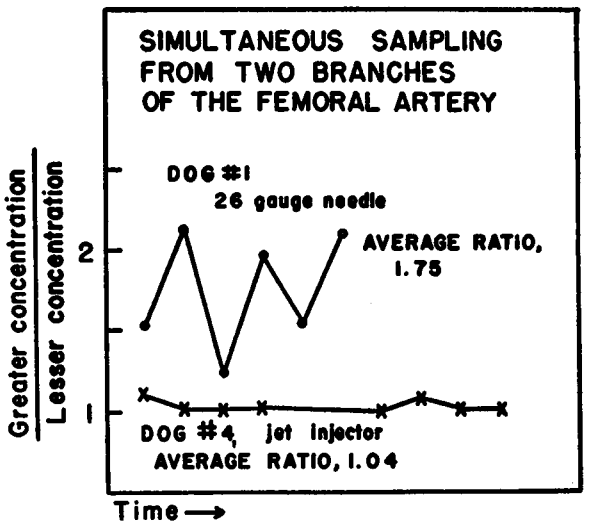

FIG. 4. COMPARISON OF JET AND NON-JET INJECTORS IN THE DOG

The characteristic lack of mixing in arterial blood with non-jet injector is compared with thorough mixing achieved by a jet injector.

of various known diameters the minimum dye injection rates necessary to produce mixing within 2 centimeters of the site of injection at rates of water flow approximating resting blood flow in the brachial artery.

(b) *Mixing of dye in the femoral artery of the dog.* In three dogs the femoral artery was isolated and all but two of its branches ligated. The free branches were cannulated and from them blood was sampled for determination of dye concentration. A solution of T-1824 was injected at constant rate against the direction of flowing blood two to three centimeters upstream from the proximal collector. Results are illustrated in Figure 4. When injection was made through a 26-gauge needle there was inadequate mixing, as demonstrated by the widely differing concentrations of dye in the two collectors. When injection at the same volume rate was performed through the jet injector the ratios of dye concentrations were nearly unity, indicating that the dye had been distributed uniformly within a distance of several centimeters. These experiments suggest that, in any situation in which a branchless segment of blood vessel several centimeters in length is available, indicator might be injected and blood collected through one double-lumen needle, and provided mixing had been accomplished in the available segment, the determina-

tion of flow through a blood vessel could be accomplished with only a single needle puncture.

(c) *Mixing of dye in the arm of man.* In 63 experiments in 27 subjects T-1824 was injected at constant rate into a brachial artery at the elbow, and blood was sampled continuously and simultaneously from two veins draining the injected arm. In nearly every case one of the veins was deep and the other superficial, and usually they were on opposite sides of the forearm. The hand was excluded from the circulation by a pressure cuff in all but nine subjects. Dye was introduced either through a jet injector or directly through the arterial needle to determine whether or not more satisfactory distribution of dye might occur with the use of jets. In two subjects dye was injected only through a 20- or an 18-gauge arterial needle. In 16 subjects dye was injected only through jet injectors; nine of these were steel needles, and seven were polyethylene tubes (Appendix II). In nine subjects injection through the arterial needle and through the polyethylene jet injector was used in sequence without changing either the site of injection or the location of collecting catheters; in six of these, injection directly through the arterial needle preceded that through the jet injector; in the remainder the reverse was true.

In most subjects several trials were carried out; each trial consisted of injection at constant rate for a period sufficient to reach and maintain steady-state concentration for five to ten minutes. Multiple trials were performed in order to study effects of various rates of injection and to observe the characteristics of the inflow and washout transients.

Adequacy of mixing of injected dye and arm blood was examined by comparison of the concentrations of dye in blood from the two veins sampled simultaneously. In this analysis of the data, only concentrations during the steady state were considered. All concentrations were corrected for recirculation by the interpolation method described in Appendix I.

The answers to two questions were sought from the experimental data: 1) To what extent did the concentrations of dye in deep and in superficial veins differ from each other; and 2) What effect was produced on these differences by jet injection as compared to the usual low-velocity injection. The analysis to be presented in somewhat more detail below indicated that in one-half of the experiments the samples from the two veins had arisen from the same statistical population, in two-thirds of the experiments the concentration in either vein differed from the mean by less than 10 per cent, and in four-fifths of the measurements the difference was less than 20 per cent. In addition, it became clear that despite theoretical indications and the favorable data developed in experiments in the model and the femoral artery of the dog, no reduction in differences between veins had been achieved by jet injection in the forearm of man.

For convenience of analysis the following symbols have been introduced:

C_D, a concentration of dye in the deep vein of the pair sampled.

C_S, the concentration of dye in the superficial vein, occurring simultaneously with C_D.

n, the number of pairs of samples in a single experiment.

$C_M = \frac{C_D + C_S}{2}$, the mean concentration of a pair of samples.

$\bar{C}_D = \frac{\sum C_D}{n}$, the mean concentration of dye observed in the deep vein during a single experiment under constant conditions.

$\bar{C}_S = \frac{\sum C_S}{n}$.

$\bar{C}_M = \frac{\sum (C_D + C_S)}{2n} = \frac{\sum C_M}{n}$.

$\text{r.d.} = \frac{C_D - C_S}{C_D + C_S} 100$, the relative difference, the per cent by which a concentration of dye in either vein differs from their mean concentration, C_M.

$\text{m.r.d.} = \frac{\sum \text{r.d.}}{n}$, the mean relative difference in a single experiment.

The analysis consisted first of subjecting the differences of $\bar{C}_D$ and $\bar{C}_S$ to the t test. The difference between the means exceeded chance at the one per cent level in 50 per cent of the subjects and exceeded chance at the five per cent level in 60 per cent no matter which type of injector was used. Similarly the mean concentrations from either vein, $\bar{C}_D$ or C_S, were examined in terms of the per cent by which each mean concentration differed from the overall mean, $\bar{C}_M$, in a single experiment. In

TABLE I

Constancy of flow

Coefficient of variation* about mean flow			<5	<10	<15	<20	<25
Cumulative per cent of subjects	Jet	Forearm (16)	24	51	83	89	94
		Plus hand (8)	6	46	71	71	83
	Non-jet	Forearm (9)	11	78	78	89	89

* Coefficient of variation = $\frac{\sigma}{\bar{C}_M}$ 100, where σ is the standard error of $\bar{C}_M$, and $\bar{C}_M$ is the mean dye concentration obtained from all paired samples during an experiment. About two-thirds of observations in a series will differ from their mean by the per cent described by the coefficient of variation. Where multiple experiments were performed on a single subject from a single pair of sampling sites the tabulation has been adjusted to prevent overweighting by any subject. Figures in parentheses represent numbers of subjects.

some 80 per cent of subjects, $\bar{C}_D$ or $\bar{C}_S$ differed from $\bar{C}_M$ by no more than 20 per cent, and in two-thirds of the subjects the disagreement did not exceed 10 per cent. Again no advantage was attributable to injection by jet.

An implicit assumption for the analysis just described is the relative constancy of the rate of blood flow. This factor was examined by calculating the coefficient of variation of $\bar{C}_M$, the overall mean concentration of dye. This tests not only constancy of flow but inseparably also the reproducibility of the whole analytical technic. Results appear in Table I. In most subjects forearm blood flow was remarkably constant. For example, in 90 per cent of subjects the coefficient of variation was less than $\pm$ 20 per cent; that is, during about two-thirds of the period of observation in each of these subjects, flow did not vary from its mean by more than 20 per cent. Table I suggests also that flow may have been more variable when the hand was not excluded from the circulation, although statistical test of the data, by Chi-squared, did not demonstrate a difference.

If dye were distributed uniformly, the mean relative difference in dye concentration, m.r.d., should not differ significantly from zero. The m.r.d. was examined by the *t* test. The appropriateness of this analysis is independent of real alterations in blood flow from one sample pair to the next, and the test is discriminating if the rapidity with which alteration in concentration of dye reflected alteration in blood flow was the same in the two venous samples (*e.g.*, Figure 5, Subject Co). Again the results failed to demonstrate superiority of jet injection; in about 60 per cent of subjects, with either type of injector, it was highly probable (probability of chance occurrence less than 1 per cent) that the m.r.d. represented a real difference in dye concentration.

The results of these tests are interpreted to indicate that in approximately half the subjects the concentrations of dye in the two veins sampled were in the same statistical population and that dye was, therefore, distributed uniformly over venous blood draining the forearm. Examples of statistical agreement appear in Figure 5 (Subject S and Subject G).

In the remaining half of subjects, in whom the concentrations of dye in the deep vein were in a different statistical population from those in the superficial vein, *thorough* mixing of blood and dye had not occurred. Despite this, in most instances, by either method of analysis the differences between paired samples were relatively small. In Figure 5 (Subject C and Subject B) are examples of types of dye-concentration patterns in which small differences between the two veins were interpreted to be significant as tested by these methods. It is clear, however, that many of these experiments could be used for measurement of blood flow. The greatest disagreement between concentrations in two veins is illustrated in Figure 5, Subject E. In this case the possible error in measurement of flow is large.

If all blood flowing from all the veins of the forearm could be pooled and a sample then taken for determination of dye concentration, this value would determine correctly the true flow rate. This theoretical dye concentration will be referred to as the *true* mean concentration of dye. Estimation of the error involved in calculating flow from the mean dye concentration obtained by sampling only two

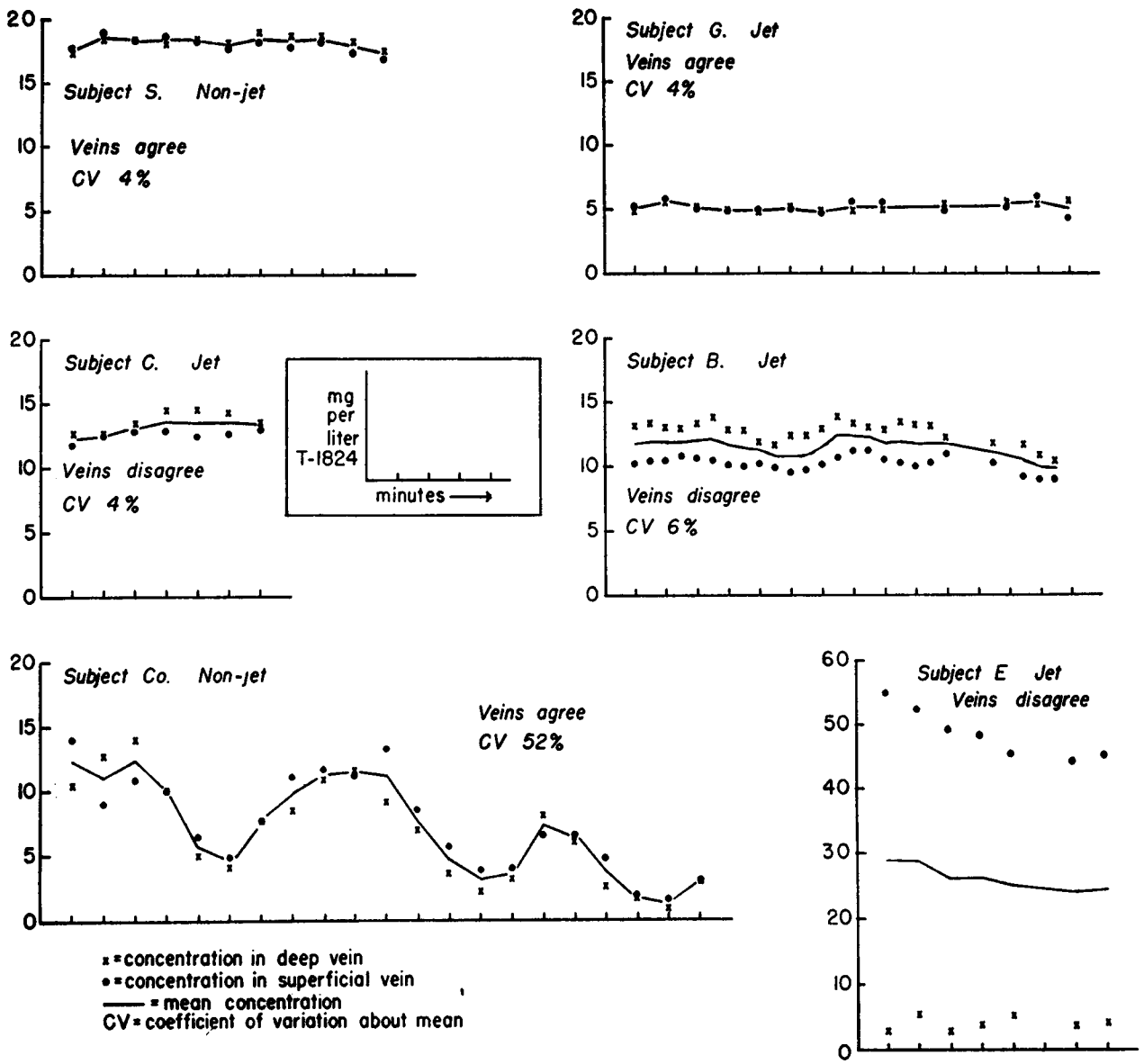

FIG. 5. THE CONCENTRATION OF T-1824 IN PLASMA SAMPLED FROM A DEEP AND FROM A SUPERFICIAL VEIN DURING CONSTANT INJECTION

Illustrations of congruence and disparity observed with both jet and non-jet injectors. When analysis indicated that the samples from both veins had their origin in the same statistical population the chart is labelled "veins agree" (see Text).

of the veins draining the forearm can be made. If the blood in the two large veins sampled represented a very large portion of total forearm blood, it is probable that departures in dye concentration elsewhere in the forearm would not cause the true mean concentration of dye in all blood flowing from the forearm to lie outside the range established by the paired samples. If this assumption be true, then the mean concentration of dye, calculated from all paired samples, provides a measure of blood flow with known error. Accordingly, the subjects studied have been classified in terms of the mean relative difference between paired samples in Table II. On this basis, in 80 per cent of subjects, the calculated mean dye concentration, $\bar{C}_M$, did not differ from the *true* mean dye concentration

TABLE II

Mean relative difference between pairs of dye concentrations in two veins simultaneously sampled

Mean relative difference, %*			<5	<10	<15	<20	<25
Cumulative per cent of subjects	Jet	Forearm (17)	26	48	59	67	67
		Plus hand (9)	44	74	78	78	100
	Non-jet	Forearm (10)	40	70	80	80	80

* Relative difference, %, $= \frac{C_D - C_S}{C_D + C_S} 100$, where C_D and C_S are the concentrations of dye in blood obtained simultaneously from a deep vein and a superficial vein, respectively. Mean relative difference is the algebraic mean of all per cent differences observed in a single experiment. Where multiple experiments were performed on a single subject from a single pair of sampling sites the calculations have been adjusted to prevent overweighting by any subject. Figures in parentheses represent numbers of subjects.

by more than ± 20 per cent. This analysis also failed to demonstrate superiority of the jet injector.

In Table II, and in similar calculations of differences between means, the data suggest that dye may have been distributed more uniformly when the hand was included in the circulation than when it was excluded. The abundant communications between branches of radial and ulnar arteries occurring in the hand suggest that improved distribution of dye might be expected when the circulation passes through the hand. However, analysis by Chi-squared of results with the hand included and excluded did not reveal significant difference.

In some trials with the jet injector it was observed that blood dripping from the venous catheters became bright red. When blood flow was estimated from dye dilution in these cases it was found to have been increased, sometimes as much as ten-fold. This increase in blood flow proved to be related to hemolysis produced by the impact of jet injection and is discussed further in the next section. With this knowledge it became desirable to examine the possibility of producing mixing of dye at a rate of injection too low to produce hemolysis. To this end dye was injected at several rates through the jet injector in each of a series of subjects. It was possible by this means to discover a rate of injection which did not appear to disturb resting blood flow. When mixing was tested by methods previously cited, there was no evidence that the dye was distributed more uniformly during resting flow than during accelerated flow.

II. Vasodilatation produced by forceful intra-arterial injection; mechanical fragility of erythrocytes

The possibility that turbulent flow created by high velocity injection might destroy erythrocytes was suggested (18) in studies on the vasodilating properties of hemolyzed blood in the perfused coronary circulation of the dog.[3] The observation arose from the discovery that rapid injection of any liquid through a 27-gauge needle produced vasodilatation which was accompanied by destruction of erythrocytes. These investigators and, independently, Binet and Burstein (19) confirmed previous studies of Fleisch (20) indicating that the vasodilating property of hemolyzed blood was dependent on adenosine triphosphate, and perhaps related substances, released from erythrocytes.

An exact measurement has been made of the shearing stresses which rupture erythrocytes exposed to turbulent flow. The results will be reported in detail elsewhere (21). Briefly, it was found that mechanical destruction of erythrocytes was related to the kinetic energy per unit time of the injection. By jet injection of solutions of isotonic NaCl *in vitro* into a pool of citrated human blood it was determined that hemolysis became detectable when the kinetic energy per second of injection reached 10,000 to 20,000 $g.cm.^2sec.^{-2}$. From this range of values it was predicted that, when injection into the human brachial artery was performed, hemolysis and consequent vasodilatation should begin to occur at injection rates of 0.020 to 0.025 ml. per sec. in the case of injectors 50 μ in diameter, 0.014 to 0.017 ml. per sec. for 38 μ injectors, and 0.008 to 0.010 ml. per sec. for 25 μ injectors. These values, predicted from *in vitro* experiments, agree closely with those at which vasodilatation did occur in man, as illustrated in Table III. A rate of 0.85 to 1.07 ml. per sec. is needed to achieve the critical energy range when

[3] We are indebted to Dr. Robert L. Post, Vanderbilt University School of Medicine, for calling our attention to these observations.

TABLE III

Production of hemolysis and vasodilatation by jet injection

Diameter of injector	Range of critical injection rates to produce hemolysis *in vitro*	Range of critical kinetic energy/sec. of injection	
		To produce hemolysis *in vitro*	To produce vasodilatation*
μ	*ml./sec.*	*g. cm.² sec.⁻²*	
25	0.008–0.010	10,000–20,000	9,000–18,000
37.5	0.014–0.017	10,000–20,000	12,000–34,000
50	0.020–0.025	10,000–20,000	10,000–24,000

* The range was determined from an analysis of all jet injection experiments. The lower figure of critical kinetic energy for each size injector is defined by the lowest value at which blood flow exceeded the normal resting limits established by non-jet injection; the higher figure similarly represents the highest value at which flow was still within the normal limits.

injection is carried out through an 18-gauge needle (I.D. 838 μ). This is at least 20 times the maximal injection rate employed in the "non-jet" experiments reported here.

The remarkable vasodilating potency of hemolyzed blood was shown by an experiment on the hind limb of the dog. Flow through the gastrocnemius muscle *in situ* was determined volumetrically by collection of total venous outflow. Retrograde jet injection of normal saline was made into the arterial supply of the muscle. When injecting conditions were such that only 0.09 ml. of blood was being hemolyzed each minute (kinetic energy per second of 86,000 g.cm.²sec.⁻²), there was a seven-fold increase in total blood flow over the pre-injection resting value (3.3 to 23.5 ml. per min.).

The interrelationships between kinetic energy of injection, the hemolysis which it produces, and the consequent vasodilatation were observed in man. The amount of hemolysis was estimated approximately by a rearrangement of the Gibson and Evans formula (10) so that hemoglobin in plasma might be measured in the face of contamination with T-1824. The results in one subject are illustrated in Figure 6A. As the kinetic energy per second of injection was raised stepwise from 33,000 to 179,000 g.cm.²sec.⁻² hemolysis was increased approximately four-fold, and plasma flow through the forearm and hand rose from 5.7 to 15.0 ml. per min. per 100 ml. Figure 6B illustrates the results obtained in 26 experiments in 12 subjects in which the KE/sec. of injection ranged from 500 to 179,000 g.cm.²sec.⁻² It may be noted that as injection energy increased, plasma flows rose progressively to ten-fold those of resting values.

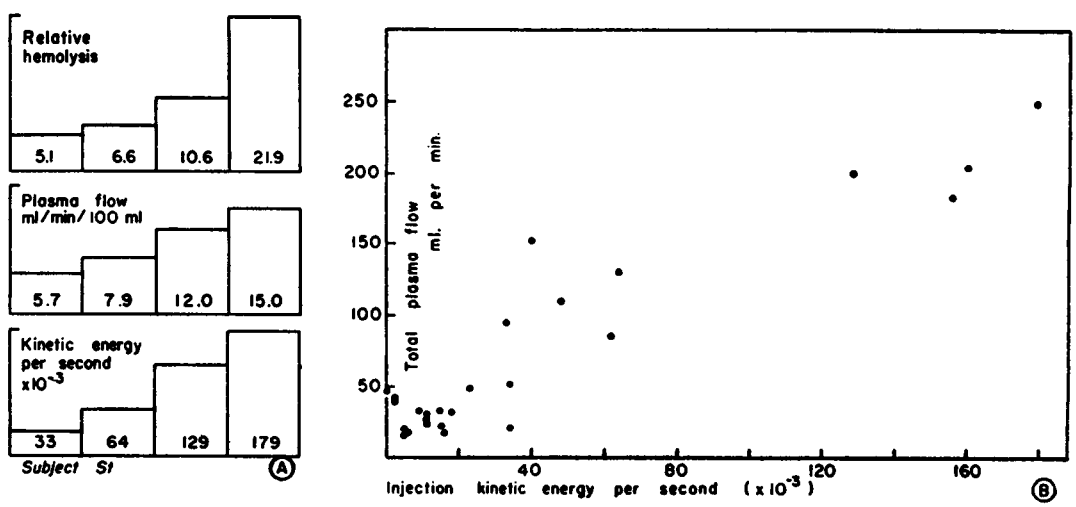

FIG. 6. THE INTERRELATIONS OF KINETIC ENERGY OF INJECTION, DESTRUCTION OF ERYTHROCYTES, AND VASODILATATION

A. Results in one subject with increasing injection rates. The mean kinetic energy, plasma flow, and relative hemolysis for each injection period is given. The relative hemolysis was calculated from the product of 1) the optical density of the plasma samples read against blank plasma at 574 mμ and corrected for contaminating T-1824, and 2) the plasma flow per min. per 100 ml. arm.

B. Illustrates the relation between kinetic energy of injection and plasma flow. At relatively high injection energy the flow increases over ten-fold.

III. Resting blood flow in the forearm

Resting blood flow in the forearm was determined in ten subjects by means of non-jet injectors. As has been indicated in the previous section, the kinetic energy of injection was too low in these experiments to produce hemolysis and consequent vasodilatation. Plasma flow was calculated from the expression I/C, where C was $\bar{C}_M$, the over-all mean concentration of dye in all samples from two veins corrected for recirculating concentration of dye by the method of interpolation (Appendix II). Blood flow was calculated from plasma flow and the hematocrit was corrected arbitrarily on the assumption that 6 per cent of it represented trapped plasma (22).

Results are listed in Table IV. Mean resting plasma flow was 21.8 ml. per min. or 2.67 ml. per min. per 100 ml. of forearm, with a range of 16.0 to 28.8 ml. per min. and of 1.7 to 3.8 ml. per min. per 100 ml. of forearm. Excluded from Table IV are data obtained in three of the ten subjects studied. In two of these the mean relative difference in dye concentration in the two veins exceeded 20 per cent, and it was considered that in them there was reason to doubt adequacy of mixing of dye and blood. In the third subject (Figure 5, Subject Co), although the mean relative difference was small, there were large phasic variations in plasma concentration; this coefficient of variation about the mean plasma concentration was 52 per cent.

TABLE IV

Resting flow in the forearm in man, measured with non-jet injection

Subject	m.r.d.*	Total plasma flow	Plasma flow	Blood flow
	%	*ml./min.*	*ml./min./ 100 ml. forearm*	*ml./min./ 100 ml. forearm*
Mc	4.7	16.2	1.66	2.70
C	1.5	16.0	1.78	2.98
S	0.9	20.8	2.14	3.71
R	13.3	28.5	2.48	4.36
L	5.3	19.8	3.29	5.70
Bz	8.1	22.4	3.45	6.18
G	8.1	28.8	3.84	7.05
Mean		21.8	2.67	4.67

* m.r.d., the mean relative difference in per cent, $= \frac{100}{n} \Sigma \frac{C_D - C_S}{C_D + C_S}$, where C_D and C_S are the concentrations of dye in paired samples from, respectively, a deep vein and a superficial vein, and n is the number of paired samples.

TABLE V

Resting flow in the forearm in man, measured with jet injection

Subject	Kinetic energy/sec. of injection	m.r.d.	Total plasma flow	Plasma flow	Blood flow
	g. cm.² sec.⁻²	%	*ml./min.*	*ml./min./ 100 ml. forearm*	*ml./min./ 100 ml. forearm*
M	9,200	6.3	33.7	5.61	9.00
Ce	500	10.5	47.4	4.25	6.81
	2,400	5.1	42.4	3.81	6.10
	2,400	0.5	39.2	3.52	5.65
	18,300	0.8	33.3	2.98	4.79
Gr	4,800	5.9	21.4	2.14	3.77
C	5,100	9.2	16.4	1.83	3.33
	15,700	4.1	17.6	1.96	3.58
Mc	14,900	5.7	23.1	2.37	3.86
Bz	14,900	6.0	34.3	5.27	9.45
S	6,000	19.1	27.2	2.79	4.84
R	11,000	12.9	27.9	2.42	4.27
G	11,400	0.9	31.7	4.23	7.46
B	11,400	10.4	25.4	1.97	3.36
Mean*			28.2	3.23	5.53

* In the calculation of the over-all means, the mean values for the multiple experiments in subjects Ce and C were used.

Therefore, in these three subjects there is reason to suspect that the mean concentration of dye was an improper measure of resting flow.

In 10 of the 23 experiments in which blood flow was measured during injection of dye through polyethylene jet injectors, the kinetic energy of injection was less than that required to produce significant hemolysis. The data (Table V) in these 10 provide a measure of blood flow which probably is very nearly that of the resting state, although comparison of Tables IV and V demonstrates that even these low-energy injections produced a slight but significant increase in blood flow.

IV. Volume of plasma in the forearm in man

There is derived in Appendix I an expression to estimate the volume of plasma in the forearm from consideration of those areas of the dye concentration-time curve which relate to transient changes in dye concentration preceding achievement of the steady-state (buildup) and following cessation of injection (washout). The relation of flow through the vein sampled to volume of plasma in the bed drained by the vein is assumed to be the same as the relation of total flow from all veins draining the forearm to the total volume of plasma in the forearm. An additional assumption underlying

the development of this expression is that the concentration of dye in the vein sampled is representative of that in all venous exits from the forearm. In theory it is unlikely that this situation is precisely the case. The shape of the buildup and washout transients of the concentration-time curve and the areas beneath them are functions of a linear velocity-path length relationship for the aggregate of dye particles in each venous sample. Since this relationship must vary greatly in the forearm vascular bed (*i.e.*, there are many possible paths and many possible velocities), the concentration of dye in any single vein need not be representative of dye concentration in all venous exits *except* during the steady state.

However, the possibility remains that vascular interlacing introduces sufficient interchange of dye particles to make the assumption valid as a first approximation. To examine this possibility, the apparent volume of plasma in the forearm was determined by sampling simultaneously from two veins draining the forearm in six normal subjects, five males and one female. Excluded from this calculation are those subjects in whom resting rates of flow did not prevail, as well as those in whom the mean relative difference in concentration of dye during the steady state was more than 20 per cent. Thus, apparent plasma volume was determined from data obtained under conditions in which blood flow was reasonably constant with the subject in the resting state, and in which dye presumably was distributed ultimately in a uniform fashion throughout the venous vascular net.

In order to estimate the magnitude of variation in the method, separate calculations of apparent plasma volume were made using dye concentrations, C_D and C_S, obtained from the two veins, and using C_M, the mean of these dye concentrations. Three apparent volumes were defined as

$$V_D = \frac{I}{\bar{C}_D} \cdot \frac{Area_D}{\bar{C}_D}$$

$$V_S = \frac{I}{\bar{C}_S} \cdot \frac{Area_S}{\bar{C}_S}$$

$$V = \frac{I}{\bar{C}_M} \cdot \frac{Area_M}{\bar{C}_M},$$

where I is the rate of injection and where the subscripts refer to measurements from samples from the deep or superficial vein or from the mean of the two. The areas are those beneath the curve described by the concentration of dye during the washout following cessation of injection or the equivalent area above the curve during buildup to the steady-state concentration of dye (Figure 8, Appendix I). All concentrations of dye were corrected for recirculating dye.

TABLE VI

Forearm plasma volume *

Subject	Sex	Volume of forearm	V_S	V_D	V	Per cent error	Per cent of forearm volume
		ml.	*ml.*	*ml.*	*ml.*		
B	M	1,200	51.3	42.3	46.4	10.6	3.9
R	M	1,150	65.2	47.2	54.9	18.8	4.8
S	M	975	52.5	54.8	53.7	2.2	5.5
G	M	750	31.4	57.7	42.7	35.1	5.7
Bz	M	650	62.8	38.5	47.5	32.2	7.3
L	F	600	29.2	29.4	29.3	0.3	5.2
Mean			48.7	45.0	45.8		5.4

* V_S and V_D = plasma volumes calculated from $\bar{C}_S$ and $\bar{C}_D$, respectively. V = plasma volume calculated from $\bar{C}_M$ and, therefore, is not necessarily the arithmetic mean of V_S and V_D.

It can be seen from the formulae above that precise establishment of a steady-state concentration is crucial for accurate estimation of volume. Not only does the square of the steady-state concentration appear as a factor in the denominator of these formulae, but the area factor in the numerator is sensitive to the experimentally determined value of the steady-state concentration. If the steady-state concentration is over-estimated, for example, both numerator and denominator will be falsely high. While these errors will tend to cancel out, a small error in estimation of steady-state concentration may lead to a larger error in estimation of volume. Similarly, a small difference between $\bar{C}_D$ and $\bar{C}_S$, the steady-state concentrations in the two veins, may lead to a larger difference in estimate of volume. It should be pointed out that the steady-state concentration can be located only by inspection of the experimental concentration-time curve and that, in practice, the graphic selection of the steady-state concentration may be difficult. Finally, it should be noted that estimate of volume, V, from mean concentrations becomes less and less meaningful as the difference between V_D and V_S expands.

Results appear in Table VI. On the assumption that the real plasma volume of the forearm lay be-

tween V_D and V_S, the limit of error of the estimate of V is the per cent of V by which V differed from either V_D or V_S, whichever difference was greater. The mean volume of plasma in the forearm was 45.8 ml., with a mean limit of error of 17 per cent. Despite variation within pairs of estimates for a given subject, the mean of apparent volumes for the series calculated from data obtained from all deep veins agreed closely with that obtained from all superficial veins; mean V_D was 45; mean V_S was 48.7.

If only the mean value for forearm plasma volume for an individual subject is considered, it is of interest that, despite a two-fold variation in total forearm volume, determined by displacement of water, the forearm plasma volume was virtually constant among the five male subjects, ranging only from 43 to 55 ml. of plasma. The value in the single female subject was only about half that of the males. The per cent of forearm volume which was occupied by plasma varied from 3.9 to 7.3 per cent.

DISCUSSION

Fundamental to application of the indicator-dilution principle, in which indicator is introduced at constant rate, is the assumption that indicator is distributed uniformly over some cross-section of the vascular bed prior to the point of sampling. In theory the simplest way to ensure uniform distribution is to produce thorough mixing of injected dye and blood at the site of injection. For this purpose there was designed a jet injector which had theoretical advantage, and which was effective in flow models and in the isolated femoral artery of the dog. However, the jet injector failed to improve the chances of mixing dye uniformly throughout the forearm vascular bed in man. Furthermore, its use introduced the hazard of distorting blood flow by producing hemolysis and consequent vasodilatation. For these reasons, and because use of the jet injector introduces technical difficulties beyond those attendant on the use of non-jet (18- or 20-gauge needle) injectors, it does not appear profitable to continue to use the jet injector for measurement of *resting* blood flow.

Two questions arise concerning the observed distribution of dye in man. Why did the jet injector fail to produce better mixing than the non-jet injector? Why was there mixing when the non-jet injector was used?

In theory, if dye and blood mix thoroughly during a given interval of time at one rate of blood flow, they will mix during approximately the same time at another rate of blood flow, other conditions remaining constant, but during that time incompletely mixed blood will travel a different distance. When blood flow is increased five-fold, if this increase in velocity is inadequate in itself to elevate the Reynolds number to its critical value, then the distance required for mixing is increased approximately five times. In such a case, it is probable that incompletely mixed blood would sweep beyond the bifurcation of the brachial artery, and the concentration of dye in the radial and ulnar arteries would agree only by chance. However, if blood flow is accelerated sufficiently, the Reynolds number of the flowing stream may reach the critical Reynolds number and turbulence will result. Since Reynolds number of resting flow in brachial artery is probably less than one-tenth of the critical Reynolds number, a ten-fold or greater increase in blood flow would be required to provoke turbulence and immediate mixing of dye with blood. This much increase was seldom reached in the observations reported here; it is of interest that in the four experiments with the highest blood flows (35 to 49 ml. per min. per 100 ml. of arm, approximately ten times the mean resting blood flow), the mean relative differences in dye concentration between the two veins were never very large: 2, 5, 5, and 16 per cent. These considerations leave unexplained those cases in which resting flow obtained and in which jet injection failed to produce mixing. In some cases, in order to inject at kinetic energy too low to produce hemolysis, failure may have resulted from the use of a rate of injection too low to provoke turbulence. Further explanation may lie, in part, in anomalous arterial supply. Usually the brachial artery terminates several centimeters distal to the humeral intercondylar line. However, Quain (23) demonstrated in a dissection of 481 human arms that, in 20 per cent, bifurcation of the brachial artery occurred above the elbow. Detection of high division of the brachial artery by palpation of two distinct arterial pulsations in the antecubital space is rarely successful; one or the other division often lies deep to muscular or tendinous tissue, leaving

only one arterial pulse to be felt. In other instances it is likely that the two arteries are so juxtaposed that detection of separate pulsations is impossible. It may be expected, therefore, that in approximately 20 per cent of subjects, dye was injected into either the radial or the ulnar, rather than into the brachial artery so that perfusion of the entire forearm bed by dye-laden blood was incomplete. In fact, the percentage of failures to achieve adequate mixing of dye and blood did not differ from that to be anticipated from anatomical considerations.

There remains to be explained the effectiveness of the non-jet injector, which, in theory, failed to provoke turbulence prior to the bifurcation of the normal brachial artery. An explanation may be found in the rich interlacing of venous channels. Blood from forearm muscles generally collects in *venae comitantes* of the arteries. A portion subsequently leaves the forearm with the *comitantes* of the brachial artery, but much of it joins the superficial system through a large communicating branch at the antecubital space, the profunda vein or the "black vein" of Holling (24). This was the deep vein catheterized in the subjects studied herein. This penetrating vein undoubtedly contains a mixture of unknown proportions of blood which originated from both major terminals of the brachial artery. Any intermingling of venous blood will have the effect of reducing differences in concentration of dye, approaching that concentration which would have existed if dye had been distributed uniformly by arterial perfusion. For example, if mixing were incomplete in the brachial artery so that the concentration of dye in radial arterial blood were twice that in ulnar arterial blood, interchange of only one-third of the blood between two veins, one draining exclusively ulnar, the other exclusively radial arterial blood, would yield concentrations of dye in the two veins which differed from their mean by only ± 11 per cent.

A probable mechanism responsible for the experimental observations may be summarized as follows: In most cases dye was injected into the brachial artery. When the non-jet injector was used, mixing was incomplete in the brachial artery; radial arterial dye concentration differed from ulnar arterial dye concentration. When the jet injector was used and when blood flow was accelerated, mixing was similarly incomplete in the brachial artery, provided this acceleration was inadequate in itself to cause mixing in the limited distance available prior to bifurcation. Under these conditions, when blood was sampled simultaneously from two veins, the concentration of dye in one vein differed significantly, by statistical analysis, from the concentration of dye in the second vein; there were two discrete dye-concentration populations in about half the cases. Nevertheless, there was sufficient intermingling of venous blood so that the concentrations of dye tended to approximate one another. The mean concentration of dye was probably a valid measure of blood flow in about 80 per cent of subjects. In the remainder there are four possible explanations available for discrepant concentrations. (a) Owing to anomalous high bifurcation of the brachial artery, dye was injected entirely into either radial or ulnar artery and venous intermingling was inadequate to produce randomization of dye concentration when one major artery was completely free of dyed blood. (b) The rate of injection was so low (as in the case of the first pilot experiments in which dye was injected through a 20-gauge needle at rates of the order of one-tenth those used subsequently in definitive experiments) that a thin filament of dye-laden blood flowed almost entirely into either radial or ulnar artery, simulating condition (a) (See Figure 2). (c) In some individuals intermingling of venous blood was inadequate to produce randomization of dye concentration under conditions of injection in which adequate distribution of dye occurred in most subjects. (d) There was asymmetric distribution of collaterals which carried a major portion of forearm blood flow. So long as blood carried by collaterals dilutes blood originating from the radial and from the ulnar arteries to an equivalent degree, the contribution of collaterals will not cause maldistribution of dye. Rather, the dilution of dye effected by addition of collateral blood to the forearm will be reflected accurately in an appropriate concentration of dye in venous blood, which will then be a measure of total forearm blood flow, brachial artery plus collateral arteries about the elbow.

Values for resting blood flow, reported herein, are compared in Table VII to those reported by others who used plethysmography. Forearm blood flow varies with ambient temperature, as indicated in the table, and, in the case of plethysmography in

TABLE VII

Forearm blood flow determined by plethysmography and by dye-dilution

Investigator	Water bath temp.	Ambient temp.	Blood flow		Method
			Mean	Range	
	(*Cent.*)	(*Cent.*)	*ml./min./100 ml.*		
Barcroft and Edholm (26)		18.5	3.1	2.6–3.6	Air plethysmograph
Slaughter, Brown, and Wakim (27)		27–29	4.9	1.7–7.3	Air plethysmograph
Barcroft and Edholm (25, 26)	33	15–20	2.7	1.9–3.8	Water plethysmograph
Barcroft and Edholm (25, 26)	35	15–20	4.2	1.5–7.0	Water plethysmograph
Present study		25–27	4.7	2.7–7.1	Dye-dilution

which the part is contained in a water bath, with the temperature of the water bath. Results obtained by the dye-dilution method agree well with those obtained by air plethysmography. They agree also with those obtained by water plethysmography when the temperature of the water bath was 33 to 35° C., temperatures considered by Barcroft and Edholm (25) to ensure the most constant blood flow. In a few of our subjects, venous occlusion plethysmography was done simultaneously with the dye-dilution procedure in a cooperative study with Dr. Brian McArdle. The results are inadequate for detailed comparison, but, in general, flow per 100 ml. forearm measured by dye-dilution was 50 per cent greater than that measured by plethysmography (water bath temperature was 34°). This difference may reflect real differences in the methods or it may be owing in part to the fact that the simultaneous measurement required the plethysmograph to be placed more distally than usual in order to provide room for the arterial injector, so that the plethysmograph may have enclosed a volume of forearm with relatively less vascular supply. Furthermore, these preliminary simultaneous studies were done with high energy jet injections with resulting vasodilatation and high blood flows, a condition in which the plethysmographic technique may tend to yield lower than actual values.

Use of the dye-dilution principle, as applied herein, is technically more cumbersome than plethysmography. It remains crucial to test for uniformity of dye distribution in each subject by sampling simultaneously from two veins. However, the method permits continuous registration of blood flow with excellent reproducibility and is well-suited for study of local metabolism. The sensitivity of this method in detecting rapid changes in rate of blood flow was tested by continuous measurement before, during, and after a brief period of voluntary flexion of the wrist against resistance. The post-contraction rise and fall in flow through the forearm was a replica of that described by Grant who measured the phenomenon by venous occlusion plethysmography (28).[4]

The method has the further advantage of providing a measure of forearm plasma volume. Values obtained indicate that the relative volume of plasma in the forearm is similar to the relative volume of plasma in the entire body, approximately 5 per cent (29). It is of interest that, although the relative volume of plasma in the forearm varied over a two-fold range among the male subjects studied, the absolute volume of plasma was nearly constant, suggesting that variations in forearm volume were owing largely to differences in content of relatively avascular tissue. Preliminary experiments suggest that the principle and the method are suitable also for measurement of other fluid compartments in the forearm.

SUMMARY

By continuous injection of a solution of Evans blue dye, T-1824, at constant rate it has been possible to measure blood flow through the forearm of man. Reconsideration of the Stewart dye-dilution principle indicated the desirability of achieving mixing of injected dye and flowing blood at the site of injection, prior to the bifurcation of the brachial artery, if distribution of dye was to be uniform throughout the forearm vascular bed.

[4] In the situation in which the rate of flow changes, there is an attendant change in dye concentration. A delay in time occurs, however, between the change in flow and the change in concentration in venous blood, and this lag is inversely proportional to the new rate of flow. At high rates of flow the delay will be of the order of seconds; at very low flow rates the delay may be in minutes.

Theory and experience with mechanical models suggested that this might be accomplished by injecting T-1824 at a constant rate through an injector of very small orifice. However, this forceful injection produced sufficient destruction of onflowing erythrocytes to cause vasodilatation; the technique was not suitable, therefore, for measurement of *resting* blood flow.

When dye was injected with a kinetic energy insufficient to produce hemolysis and vasodilatation, mixing of dye and blood prior to bifurcation of the brachial artery was uncertain. However, in about ⅘ of subjects there appeared to be sufficient intermingling of blood originating from radial and ulnar arteries to produce a relatively uniform distribution of dye among the veins draining the forearm, thus permitting measurement of resting blood flow. In seven normal subjects resting blood flow varied from 2.7 to 7.0 ml. per min. per 100 ml. of forearm, with a mean of 4.4.

Consideration of either the inflow or the washout transients of the dye concentration-time curve provided estimates of the volume of plasma in the forearm. Among adult male subjects, despite a two-fold variation in the total volume of the forearm, the volume of plasma therein was constant, 43 to 55 ml. The proportion of forearm volume which was occupied by plasma volume varied from 3.9 to 7.3 per cent, with a mean of 5.5 per cent.

APPENDIX I

By K. L. Zierler

MATHEMATICAL CONSIDERATIONS

A. *Theory of continuous injection*

Although Stewart (6) proposed many years ago that constant injection of an indicator could be used for measurement of flow, except for the work of Hamilton and Remington (30) there has been no formal consideration of the problem. It has been accepted generally that when indicator is injected at constant rate into a vascular bed ultimately all blood free of indicator will be displaced from the system by indicator-laden blood and the concentration at exits from the system will become equal to the rate of injection (mass per unit time) divided by the constant rate of volume flow (volume per unit time) through the system, provided that there is no recirculation. The analysis which follows differs fundamentally from previous examinations of the problem, and, while it leads to an approximate solution for the case of the forearm vascular bed which agrees with intuitive concepts of the asymptotic nature of the concentration function, it demonstrates that in the general case the approximation need not hold and

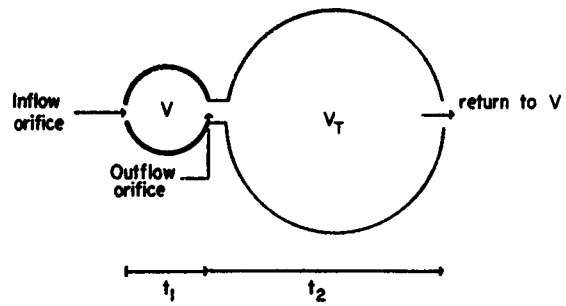

FIG. 7. A FLOW MODEL

Volume V is a portion of a larger system, $V + V_T$, through which fluid circulates continuously at constant rate F. Beginning at time zero, indicator is introduced at constant rate at the inflow orifice of V. Concentration of indicator is measured at the outflow orifice of V, identified in the text as r_o. At time t_1 some indicator arrives at r_o. The fastest traverse time from r_o through V_T and back to the inflow orifice of V is t_2.

it provides a correction term which can be evaluated experimentally.

Figure 7 represents a fixed volume, V, unrestricted as to shape or internal architecture. Through the container fluid flows, entering V at a fixed orifice and leaving V at another fixed orifice identified by r_0. Any point in V is identified by r. Once fluid has passed through V and out at r_0 it continues through some other volume, V_T, and returns to the entrance of V; *i.e.*, it recirculates.

The time-course of events is also illustrated in Figure 7. Indicator is injected continuously into the inflow orifice at constant rate, I units of indicator per unit time. Sometime after the start of injection, say at time t_1, indicator first appears at r_0. At some later time, say $t_1 + t_2 = t_0$, some indicator has completed the circuit back to the site of injection and recirculation begins. It has been found experimentally (see Appendix II) that under these conditions the concentration of recirculating indicator is represented reasonably well by a linear equation. Therefore, the rate, i(t), at which indicator enters the volume, V, at any time, t, may be represented by

$$i(t) = I + a(t)(t - t_0), \text{ where } a(t) = \begin{cases} 0 \text{ if } t < t_0 \\ a \text{ if } t \geq t_0 \end{cases} \quad (1)$$

and I and a are constants.

It is desired to describe the concentration at the outflow orifice, $C(r_0, t)$, as a function of time. It is assumed that indicator is so injected that it is distributed uniformly through the fluid entering V. Therefore, the concentration of indicator at the inflow orifice is precisely the rate of entry divided by flow, or $\frac{i(t)}{F}$. Since i(t) does not decrease with time and indicator can only be diluted during its passage through V, the concentration of indicator at any point within V must be less than or equal to the concentration at entrance; that is,

$$C(r, t) \leq \frac{i(t)}{F}. \quad (2)$$

It is important to note that instantaneous mixing within V is not assumed.

Now let $Q(t)$ represent the quantity of indicator in V at time t. Its rate of change, $Q'(t)$ equals the rate at which indicator enters V less the rate at which it is removed; that is,

$$Q'(t) = i(t) - F\,C(r_0, t). \tag{3}$$

The mean concentration of indicator in V is $\frac{Q}{V}$ and its rate of change is

$$\frac{Q'(t)}{V} = \frac{1}{V}[i(t) - F\,C(r_0, t)].$$

Under fixed conditions the rate of change of concentration at the exit, $C'(r_0, t)$, will bear some relation to the rate of change of mean concentration. Consideration of this relationship will assist in relating $C(r_0, t)$ to the other parameters of the system. To this end it is convenient to introduce the function

$$g(t) \equiv \frac{\text{rate of change of } C(r_0, t)}{\text{rate of change of mean concentration}} = \frac{C'(r_0, t)}{\frac{Q'(t)}{V}}$$

$$g(t) \equiv \frac{V\,C'(r_0, t)}{i(t) - F\,C(r_0, t)}. \tag{4}$$

When injection begins, $C(r_0, t) = 0$ and remains zero until t_1. At time t_1 a quantity of indicator, $t_1 I$, is contained in V; the mean concentration is then $\frac{Q(t_1)}{V} = \frac{t_1 I}{V}$. If, for the moment, recirculation is ignored, both $\frac{Q(t)}{V}$ and $C(r_0, t)$ must approach the concentration being introduced, $\frac{I}{F}$. Since, at t_1, $\frac{Q(t_1)}{V} > C(r_0, t) = 0$, it is evident that over some subsequent interval of time $C'(r_0, t)$ must be greater than $\frac{Q'(t)}{V}$. It appears reasonable to assume that $C'(r_0, t) > \frac{Q'(t)}{V}$ holds for all $t > t_1$, an assumption supported by experimental measurement. Despite some initial irregularity introduced by the appearance of recirculation, it is assumed that the inequality holds for all $t > t_1$. When recirculation is present, the rate of change of both $\frac{Q(t)}{V}$ and $C(r_0, t)$ must approach the rate of change of the concentration at entry, $\frac{i'(t)}{F} = \frac{a}{F}$, $(t > t_0)$.

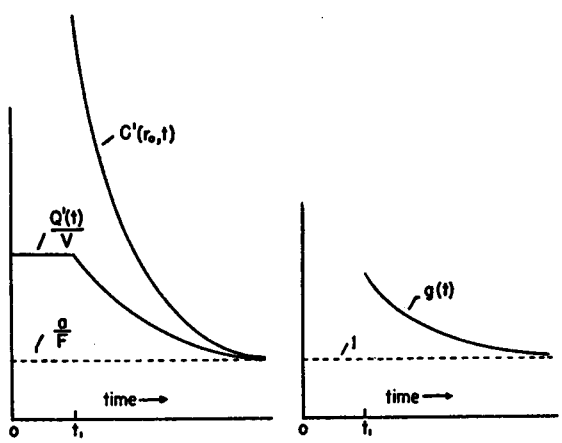

FIG. 8. FUNCTIONS WHICH FIT THE REQUIREMENTS OF $C'(r_0, t)$, $Q'(t)/V$, AND $g(t)$

Injection of indicator begins at time = 0 on the abscissa. Indicator first appears at the exit at time t_1. $C'(r_0, t)$ is zero until t_1. $C'(r_0, t)$ and $Q'(t)/V$ approach a/F, which is the constant rate of change of concentration of recirculating indicator. $g(t)$ is the ratio of the two derivatives, is greater than one at t_1, and approaches one.

In terms of the function $g(t)$, the assumptions reduce to

$$g(t) \geq 1 \text{ for } t > t_1$$

and when recirculation is present,

$$\lim_{t \to \infty} g(t) = 1.$$

Functions exhibiting these characteristics are shown in Figure 8.

Since $g(t)$ is basic to the formulation of $C(r_0, t)$ it is desirable to indicate something about its nature in physical terms. If mixing were instantaneous; for example, if an efficient stirrer were placed in V, the rates of change of concentration would be identical throughout V and $g(t) = 1$. If the indicator were dispersed only slightly in its passage through V, so that it traveled essentially along a single path almost as an intact slug, V would be nearly saturated before any indicator appeared at r_0. Then at time t_1 $C'(r_0, t)$ would be very large and $\frac{Q'(t)}{V}$ very small, making $g(t)$ very large. Between these two extremes may be considered to lie the real condition of dispersion of indicator through a vascular bed, and $g(t)$ may be expected to assume intermediate values greater than one, but approaching one as the vascular bed becomes saturated.

Equation (4) is a first order linear differential equation in $C(r_0, t)$. Rearranged in familiar form, it is

$$C'(r_0, t) + \frac{F}{V} g(t)\, C(r_0, t) = \frac{g(t)\, i(t)}{V}.$$

Its solution, by routine methods, is

$$C(r_0, t) = e^{-\frac{F}{V} G(t)} \int_0^t \frac{g(t)\, i(t)}{V} e^{\frac{F}{V} G(t)}\, dt,$$

where $G(t) = \int_0^t g(s)\, ds$, boundary conditions are met at $t = 0$, and s is a dummy variable substituted for t for convenience of integration.

Substitution for $i(t)$ from equation (1) yields

$$C(r_0, t) = \frac{I}{F}\left[1 - e^{-\frac{F}{V} G(t)}\right] + \frac{a(t)}{F}\left[(t - t_0) - \int_{t_0}^t e^{-\frac{F}{V}[G(t) - G(s)]}\, ds\right]. \tag{5}$$

For the case of constant injection without recirculation, $a(t) = 0$, and equation (5) becomes

$$C(r_0, t) = \frac{I}{F}\left[1 - e^{-\frac{F}{V}G(t)}\right], \qquad (6)$$

which approaches $\frac{I}{F}$ for large t since $G(t) \to \infty$.

For the case of constant injection with recirculation the term in $e^{-\frac{V}{E}G(t)}$ again drops out of equation (5) for large t and

$$C(r_0, t) \sim \frac{i(t)}{F} - \frac{a}{F}\int_{t_0}^{t} e^{-\frac{F}{V}[G(t)-G(s)]}\,ds. \qquad (7)$$

Thus, $C(r_0, t)$ is asymptotically somewhat less than $\frac{i(t)}{F}$. This is consistent with the intuitive concept, since the concentration at exit will lag behind that at entry due to the time required to traverse V. A bound on the correction is readily found, since for $t > t_0$, $G'(t) = g(t) \geq 1$, and, therefore, $G(t) - G(s) \geq t - s$, and

$$\frac{a}{F}\int_{t_0}^{t} e^{-\frac{F}{V}[G(t)-G(s)]}\,ds \leq \frac{a}{F}\int_{t_0}^{t} e^{-\frac{F}{V}(t-s)}\,ds$$

$$= \frac{aV}{F^2}\left[1 - e^{-\frac{F}{V}(t-t_0)}\right],$$

which approaches $\frac{aV}{F^2}$. Since $g(t)$ approaches one, $\frac{aV}{F^2}$ is a general asymptotic result and

$$C(r_0, t) \sim \frac{i(t)}{F} - \frac{aV}{F^2}. \qquad (8)$$

All members of the error term $\frac{aV}{F^2}$ can be estimated. For example, it can be estimated that if T-1824 were injected at constant rate into the central circulatory bed (heart, great vessels, and pulmonary vascular bed) and its concentration measured at a systemic arterial exit from that bed, the error term may be of the order of 20 to 40 per cent of the anticipated asymptote, $\frac{I}{F}$, corrected for recirculation. However, in the case of injection into the brachial artery it can be estimated that the error term is of the order of 1 or 2 per cent as follows:

The concentration of recirculating T-1824 at the inflow orifice is $\frac{at}{F}$, which is nearly identical with the total quantity of dye introduced into the body, It, distributed over a volume, V_T, which ultimately will be the total plasma volume; that is, $\frac{at}{F} = \frac{It}{V_T}$, and $a = \frac{IF}{V_T}$. With this value for a, $\frac{aV}{F^2} = \frac{IV}{FV_T}$. The ratio of $\frac{aV}{F^2}$ to $\frac{I}{F}$, therefore, is $\frac{V}{V_T}$. If recirculating dye were completely distributed in total plasma volume, $\frac{V}{V_T}$ would be of the order of $\frac{50}{5{,}000} = 0.01$. In the brief time available, dye is distributed incompletely and V_T is somewhat less than total plasma volume; if it is half as great, $\frac{V}{V_T} = 0.02$. In the central circulation, V is of the order of 1,000 and $\frac{V}{V_T}$ may be in the range of 0.2 to 0.4.

Since the error term is negligible for purposes of measurement of forearm flow, equation (8) can be replaced by the approximation,

$$C(r_0, t) \sim \frac{i(t)}{F} = \frac{I}{F} + \frac{a(t - t_0)}{F}, \qquad (9)$$

which is sufficiently true during the steady state. From equation (9), the expression which was used for calculation of forearm flow follows immediately.

$$F \approx \frac{I}{C(r_0, t) - \frac{a(t - t_0)}{F}}. \qquad (10)$$

Flow equals the rate of constant injection divided by the observed concentration at exit corrected by subtraction of the concentration of recirculating indicator, adjusted for its time intercept.

B. *Measurement of volume of fluid compartments*

It has been indicated in the main body of this report that a plot of indicator against time leads to an estimate of volume, V. Treatment, similar in part to that which follows, has been proposed independently by Lewis (31).

As was defined in equation (3), the rate of change of the quantity of indicator in V, is

$$Q'(t) = i(t) - F\,C(r_0, t).$$

In the case of injection at constant rate into the brachial artery, the buildup of $C(r_0, t)$ is so rapid that the contribution of recirculating indicator can be neglected during this period, and $i(t)$ may be assumed equal to I, the constant rate of injection. The equation for $Q'(t)$ can be integrated to yield

$$Q(t) = It - F\int_0^t C(r_0, t)\,dt; \qquad (11)$$

that is, the quantity of indicator which remains in V at time t is the difference between the quantity injected during t and the quantity which flowed from V during t.

It will be recalled that, from equation (6), $C(r_0, t)$ attains a maximum value, $\frac{I}{F}$. When this occurs, $Q'(t) = 0$, a steady state exists and $Q(t)$ is a maximum. At this time, the total quantity of indicator, Q, is distributed over V at concentration $\frac{I}{F}$; that is,

$$\frac{Q}{V} = \frac{I}{F}. \qquad (12)$$

The quantity Q of equation (12) is defined by assigning appropriate limits to equation (11),

$$Q = I\,t_s - F\int_0^{t_s} C(r_0, t)\,dt, \qquad (13)$$

where t_s is the time at which the steady state is attained and $C(r_0, t) = \frac{I}{F}$, as illustrated in Figure 9. Q is repre-

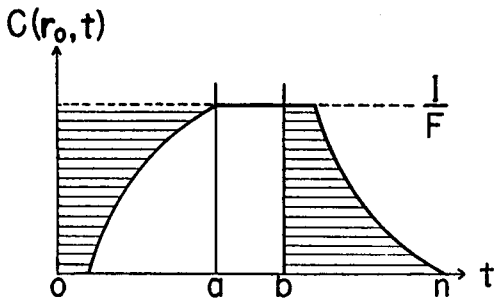

FIG. 9. CONCENTRATION OF INDICATOR ON THE ORDINATE AGAINST TIME ON THE ABSCISSA

Indicator injected at constant rate without recirculation. Steady-state concentration, I/F, reached at time t_a. Injection stopped at t_b and concentration returned to zero at time t_n. Hatched area above concentration on left is Area I. Hatched area below concentration on right is Area II.

sented by the left-hand shaded area in Figure 9 multiplied by F. This area will be called Area I.

When injection ceases, the quantity of indicator in V again changes as indicator is washed out. The rate at which indicator enters V is the rate at which indicator is recirculating, which may be considered nearly constant once injection has ceased. Therefore, the rate of change of the quantity of indicator in V may be described by

$$Q'(t) = at_b - F\,C(r_0, t), \qquad (14)$$

where t_b is the time at which injection ceased and a is the constant rate at which recirculating indicator had been increasing, defined in equation (*1*).

By an inversion of the argument upon which the description of the buildup of $C(r_0, t)$ was based and considering that indicator-poor fluid enters at constant rate, at_b, a volume saturated uniformly with a higher concentration of indicator, it can be stated that indicator will be washed out of V until the concentration throughout V is reduced to the concentration at entry, $Q'(t)$ again becomes zero, and a new steady state exists. Therefore, equation (*14*) can be integrated to yield

$$Q = at_b(t_n - t_b) - F\int_{t_b}^{t_n} C(r_0, t)\,dt, \qquad (15)$$

where t_n is the time at which $Q'(t)$ again equals zero and all indicator has been washed out of V. Q is thus the product of flow and the area under the washout transient corrected for constant recirculation, illustrated by the right-hand shaded area in Figure 9. This area will be called Area II.

Since Area I and Area II both equal $\frac{Q}{F}$, they are equal and either can be used for calculation of volume, which can now be defined from equation (*12*) as

$$V = \frac{F(\text{Area I})}{\frac{I}{F}} = \frac{F(\text{Area II})}{\frac{I}{F}}. \qquad (16)$$

All parameters are measurable. When $C(r_0, t)$, corrected for recirculation, is plotted against time, Areas I and II can be obtained by planimetry. However, since the buildup and washout transients are complicated and incompletely described, and since the intervals of collection of blood samples were long relative to the time t_a, in the case of measurement of forearm plasma volume in the experiments reported herein, the transients cannot yet be constructed accurately as smooth curves. The area can, however, be determined accurately as a sum rather than as an integral by the following method:

The integral of equation (*11*) can be integrated as a mean,

$$\int_0^t C(t)\,dt = t\,\bar{C}(t),$$

where $C(t)$ is the observed concentration corrected for recirculation. The concentration of indicator in any sample of plasma is the mean concentration, $\bar{C}(t_i)$, during the time of sampling, Δt, if the rate of sampling during Δt is essentially constant. Therefore, the area below the buildup transient,

$$\int_0^{t_a} C(t)\,dt = \Delta t \sum_{}^{t_a} \bar{C}(t_i),$$

and a similar sum describes the area below the washout transient. Therefore,

$$\text{Area I} = It_a - \Delta t \sum^{t_a} \bar{C}(t_i)$$

and

$$\text{Area II} = \Delta t \sum_{t_b}^{t_n} \bar{C}(t_i).$$

Finally, it may be noted that nothing in the assumptions restricts the indicator to plasma, and that the formal treatment holds for indicators distributed in other fluid compartments.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. Paul Meier, Department of Biostatistics, School of Hygiene and Public Health, The Johns Hopkins University, for his criticism and suggestions in the preparation of Appendix I.

APPENDIX II

1. *Motor-driven syringe* [1]

Injection of the dye solution at rates up to 2.5 ml. per min. through orifices of only 25 to 50 μ required of the syringe and its driving mechanism certain unusual characteristics of performance: constant rate of injection without leak at operating pressures of 150 p.s.i. Power was provided by a 6T60 Heller motor and controller [2] whose electronic regulation furnished a constant selected r.p.m.

[1] Mr. L. S. Reynolds constructed the apparatus, and he and Dr. S. A. Talbot aided in the design.

[2] Gerald K. Heller Co., 1540 Ridgely Avenue, Baltimore 30, Md.

despite varying load. The motor shaft speed was reduced 1000-to-1 by a gear train to yield less r.p.m. at higher torque. The shaft of the final gear had a long square broach into which was fitted the squared end of a threaded driving screw. The screw passed through a fixed nut to provide thrust surface and the other end bore on the plunger of the syringe whose barrel was fixed securely. The rate of advance of the screw and, thus, the volume displaced from the syringe were determined readily, after calibration, by measuring the r.p.m. of the motor shaft by means of a tachometer. After a warm-up period of 20 minutes, this apparatus delivered at a constant rate up to 2.5 ml. per min. with a variation never exceeding one per cent for periods of several hours duration.

The problem of manufacture of a leak-proof syringe to operate at high pressures without resorting to a tedious and easily-marred lap-fit of plunger and barrel was solved as follows. The barrel (50 ml. capacity) and plunger were machined from stainless steel to a loose fit. The internal end of the plunger was faced with a 2 cm. thick disc of teflon,[3] turned to a diameter which permitted ready introduction of the plunger into the barrel with snug fit when all parts were at normal room temperature. Advantage was taken then of the nine-fold difference in coefficients of thermal expansion between teflon and steel. When the syringe and its contents were warmed to 38° C. the facing teflon disc expanded enough to seize the barrel wall and no leak occurred.

The syringe tip was fitted with a Luer-Lok to which was attached a 27-gauge hypodermic needle. Polyethylene PE 10[4] tubing, ending in the jet injector tip was slipped over the needle and secured by ties of rubber bands.

The thermal "memory" of teflon precluded sterilization by heat; if heated to 100° C. the teflon facing expanded irreversibly and the fit was destroyed. Adequate sterilization was obtained by soaking the syringe, injectors, and catheters in 1:1,000 benzalkonium chloride, which was thoroughly rinsed away with sterile saline solution just prior to use.

2. *Collection apparatus and dye concentration determination*

Venous samples for estimation of the serum concentration of T-1824 were collected continuously through siliconed polyethylene catheters (Clay-Adams PE 90, 1.27 mm. O.D., 0.86 mm. I.D.) introduced through 16-gauge thin-wall or 15-gauge regular wall hollow needles into one or more antecubital veins. In most instances, blood from these catheters would drip for several hours without clotting. Serial samples of venous blood flowing from the collecting catheters were allowed to drip into siliconed (G. E. Dri-film, SC 87) 75 × 10 mm. test tubes during 15 to 60 second intervals. A minimum of about 0.3 ml. of serum was necessary for analysis; the 30 − 60 second samples averaged approximately 0.5 to 1.0 ml. whole blood per tube during periods of resting flow. Samples from two veins were collected simultaneously. The tubes were arranged in circular racks, and these were turned manually in response to a timer signal for each collection. The specimens were allowed to clot. Clotting and clot retraction were promoted by incubation at 37° C. Specimens were centrifuged for ten minutes at 900 g. Serum was transferred directly to a microcuvette for measurement of optical density at 620 mμ in the Beckman spectrophotometer. When necessary, correction for mild hemolysis was made by the method of Gibson and Evans (10); a few severely hemolyzed samples were discarded.

All measured concentrations of dye in serum were corrected for recirculation. The concentration of recirculated dye can be determined directly by sampling continuously or frequently from the contralateral brachial artery or from a contralateral vein. Alternatively, and more easily, recirculation may be measured by the following method of interpolation, the results of which agree closely with those obtained by direct determination of recirculating concentrations of dye. When injection of dye has ceased and dye has been washed out of the injected forearm, the concentration of dye in the forearm comes into equilibrium with that in the circulation as a whole. In theory, this final recirculation concentration of dye represents dilution in total body plasma of all dye injected. The quantity of dye ultimately in the general circulation is the sum of the dye which was already circulating at the moment injection ceased plus the dye which was in the forearm in concentration exceeding the concentration of recirculating dye. If the final recirculation concentration is extrapolated back to the time at which injection ceased, the extrapolation will give a falsely high estimate of recirculation concentration at that time, since it ignores the quantity of dye which was washed out of the forearm (see Figure 1). To correct the estimate for this quantity of dye, the final recirculation concentration is extrapolated back only to a time representing one minute after cessation of injection. The possible error of this arbitrary correction is less than the error of not correcting at all, although the magnitude of the difference in estimated concentration is quite small. From this extrapolated concentration-time point a straight line is drawn to a point representing zero concentration and time one minute after the start of constant injection. Concentrations of dye represented by this line are subtracted from the observed venous concentrations.

3. *Manufacture of jet injector*

The production of an intra-arterial injector of small caliber and minute orifice was accomplished by modification of the very tip of a 45 cm. length of polyethylene tubing (PE 10, 0.584 mm. O.D., 0.279 mm. I.D.). A 2 cm. length of wire with a diameter of 25, 38 or 50 μ (1, 1.5 or 2 mil) was inserted into one end of this PE 10 tubing, and then wire and polyethylene tubing were threaded together into a tapering glass capillary whose inside diameter would not permit passage of the PE 10 tubing past the mid-portion. Heat (from the surface of a hot plate at 175° C.) was applied directly to the glass capillary just distal to the end of the polyethylene tubing until the walls of the plastic tubing were seen to flow into intimate contact with

[3] Chemiseal, U. S. Gasket Co., Camden 2, N. J.

[4] Clay-Adams Co., Inc., New York, New York.

the wire over the distal millimeter or so of the PE 10 tubing. After brief cooling the plastic was withdrawn from the glass, and the wire pulled from its bed. This end segment of modified PE 10 tubing, approximately 1 mm. long with an I.D. of 25, 38, or 50 μ, provided the fine gauge injector tip for production of the jet stream. This simple method for manufacturing reproducible jet tips was devised by Dr. Brian McArdle. Jet injectors were initially made from steel needle tubing of 26–28 gauge by a process of spinning and rolling the tip of tubing with a length of fine wire in place in the tip of the tubing. The wire was withdrawn when a snug fit was obtained. Unlike the polyethylene injectors which had very precise, smooth jet segments, the steel injectors examined microscopically revealed an irregular orifice of uncertain size. This type was no longer used when the polyethylene technique was devised.

4. *Experimental procedure*

Of the subjects whose blood flow was determined, 12 were laboratory personnel. The other 15 were convalescent patients who had no muscular or peripheral vascular disease except mild degrees of asymptomatic peripheral arteriosclerosis commensurate with the age of some of the patients.

The tests were carried out with the subjects supine, and the forearm extended on a padded arm board at 60° to the long axis of the body. An effort was made to conduct the determinations with the subjects at rest; however, in some of the early determinations, while the technique was still in a developmental state, this was not always achieved. The temperature of the ambient air was 25 to 27° C. The syringe and jet injectors which had been sterilized in benzalkonium chloride (1 : 1,000) were rinsed with sterile saline which had been passed through a Seitz filter pad in order to eliminate any tiny particles which might occlude the jet injector. Similarly filtered saline was used to dilute the ampoule of Evans blue solution [5] for injection. The collecting catheters were rinsed with sterile saline. The syringe was filled with sterile diluted T-1824, approximately 0.5 mg. per ml. and warmed to, and maintained at , 38° C.

The order in which venous catheterization and arterial puncture were carried out was governed only by the anatomical relationships in the antecubital fossa and the ease with which the various maneuvers could be accomplished. The antecubital space was carefully examined in an attempt to discover palpable anomalies in arterial supply to the forearm. In several potential subjects the brachial artery was found to bifurcate proximal to the antecubital space, and these subjects were excluded from the study. Under local procaine anesthesia an 18-gauge Riley arterial needle was inserted counter to the direction of blood flow into the brachial artery in the antecubital space, and, again using local procaine, the antecubital vein or veins were entered with a 15- or 16-gauge thin-walled needle. The venepuncture was done "upstream" in contrast to the usual technique in order that the collecting catheters might be passed distally into the forearm. The PE 90 collecting catheters were passed a distance of several centimeters through the venepuncture needles, and the needles withdrawn. Occasionally a valve was encountered but gentle maneuvering often enabled the catheter to slip past. During periods when blood flow collections were not being made, a slow drip of sterile isotonic saline or 5 per cent glucose solution was allowed to run through the collecting catheters. Under such circumstances, at least 2 ml. of venous blood, representing at least ten times the volume of a catheter, was permitted to wash out the catheters before collection for determination of dye concentration was considered valid. Samples of either venous or arterial blood were collected for determination of hematocrit and of optical density of blank serum for use in determination of dye concentration. The jet injector, when used, was threaded past the tip of the 18-gauge arterial needle to, but not beyond, the intercondylar line at the elbow. A slow infusion of dye solution was maintained through the jet during its introduction and throughout the time it remained in the arterial needle even while blood flow was not actually being measured, in order to avoid back flow and clotting of blood in the injector catheter. A thin sleeve of fibrin clot quickly formed between the inner wall of the arterial needle and the outer wall of the injector; it was unnecessary to provide other means of fixing the injector. The hand was excluded from the forearm circulation by application of a 7 cm. sphygmomanometer wrist cuff inflated to 280 mm. of Hg. The motor controller was turned to the desired injection rate, and venous samples were collected serially as described above. At the close of the test run, after the injection had been discontinued for at least five minutes, a final sample for estimation of recirculating dye was collected.

Forearm volume was measured by water displacement using the upper border of the wrist cuff and the level of the medial epicondyle at the elbow as landmarks.

[5] Warner-Chilcott Laboratories, 113 West 18th Street, New York 11, New York.

REFERENCES

1. Abramson, D. I., and Ferris, E. B., Jr., Responses of blood vessels in the resting hand and forearm to various stimuli. Am. Heart J., 1940, **19**, 541.
2. Stewart, H. J., and Evans, W. F., The peripheral blood flow under basal conditions in normal male subjects in the third decade. Am. Heart J., 1943, **26**, 67.
3. Hertzman, A. B., and Randall, W. C., Regional differences in the basal and maximal rates of blood flow in the skin. J. Applied Physiol., 1948, **1**, 234.
4. Landowne, M., and Katz, L. N., A critique of the plethysmographic method of measuring blood flow in the extremities of man. Am. Heart J., 1942, **23**, 644.
5. Hering, E., Versuche über das Verhältniss zwischen der Zahl der Pulse und der Schnelligkeit des Blutumlaufs. Ztschr. f. Physiol., 1833, **5**, 58.

6. Stewart, G. N., Researches on the circulation time and on the influences which affect it. IV. The output of the heart. J. Physiol., 1897, **22**, 159.
7. Gibbs, F. A., Maxwell, H., and Gibbs, E. L., Volume flow of blood through the human brain. Arch. Neurol. & Psychiat., 1947, **57**, 137.
8. Shenkin, H. A., Harmel, M. H., and Kety, S. S., Dynamic anatomy of the cerebral circulation. Arch. Neurol. & Psychiat., 1948, **60**, 240.
9. Gregersen, M. I., A practical method for the determination of blood volume with the dye T-1824; a survey of the present basis of the dye-method and its clinical applications. J. Lab. & Clin. Med., 1944, **29**, 1266.
10. Gibson, J. G., II, and Evans, W. A., Jr., Clinical studies of the blood volume. I. Clinical application of a method employing the azo dye "Evans blue" and the spectrophotometer. J. Clin. Invest., 1937, **16**, 301.
11. Dow, P., Hahn, P. F., and Hamilton, W. F., The simultaneous transport of T-1824 and radioactive red cells through the heart and lungs. Am. J. Physiol., 1946, **147**, 493.
12. Zierler, K. L., Andres, R., and Lilienthal, J. L., Jr., Transfer rates across muscle cell membranes in man: measurement of blood flow and fluid compartments in skeletal muscle. J. Clin. Invest., 1951, **30**, 684.
13. Zierler, K. L., Andres, R., Anderson, H. M., Stainsby, W., and Lilienthal, J. L., Jr., Measurement of limb blood flow in man by constant infusion of T-1824. Federation Proc., 1952, **11**, 177.
14. Orrin, H. C., The X-ray Atlas of the Systemic Arteries of the Body. London, Bailliere, Tindall, and Cox, 1920.
15. Drinker, C. K., and Yaffey, J. M., Lymphatics, Lymph, and Lymphoid Tissue; Their Physiological and Clinical Significance. Cambridge, Mass., Harvard University Press, 1941.
16. Coulter, N. A., Jr., and Pappenheimer, J. R., Development of turbulence in flowing blood. Am. J. Physiol., 1949, **159**, 401.
17. Reynolds, S. R. M., Light, F. W., Jr., Ardran, G. M., and Prichard, M. M. L., The qualitative nature of pulsatile flow in umbilical blood vessels, with observations on flow in the aorta. Bull. Johns Hopkins Hosp., 1952, **91**, 83.
18. Chambliss, J. R., Demming, J., Wells, K., Cline, W. W., and Eckstein, R. W., Effects of hemolyzed blood on coronary blood flow. Am. J. Physiol., 1950, **163**, 545.
19. Binet, L., and Burstein, M., Sur les propriétés vasomotrices du sang. Présence d'une substance vasodilatatrice dans les globules rouges. Compt. rend. Soc. de biol., 1950, **144**, 796.
20. Fleisch, A., Une substance vasodilatatrice des érythrocytes. Schweiz. med. Wchnschr., 1938, **19**, 223.
21. Andres, R., Zierler, K. L., Ghrayyib, A. S., and Lilienthal, J. L., Jr., To be published.
22. Jackson, D. M., and Nutt, M. E., Intercellular plasma and its effect on absolute red blood cell volume determination. J. Physiol., 1951, **115**, 196.
23. Quain, R., The Anatomy of the Arteries of the Human Body and Its Applications to Pathology and Operative Surgery. London, Taylor and Walton, 1844.
24. Holling, H. E., Observations on the oxygen content of venous blood from the arm vein and on the oxygen consumption of resting human muscle. Clin. Sc., 1939, **4**, 103.
25. Barcroft, H., and Edholm, O. G., The effect of temperature on blood flow and deep temperature in the human forearm. J. Physiol., 1943, **102**, 5.
26. Barcroft, H., and Edholm, O. G., Temperature and blood flow in the human forearm. J. Physiol., 1946, **104**, 366.
27. Slaughter, O. L., Brown, H. S., and Wakim, K. G., Effects of tetraethyl-ammonium chloride on blood flow in the extremities of man. J. Lab. & Clin. Med., 1948, **33**, 743.
28. Grant, R. T., Observations on the blood circulation in voluntary muscle in man. Clin. Sc., 1938, **3**, 157.
29. Gamble, J. L., Chemical Anatomy, Physiology and Pathology of Extracellular Fluid; A Lecture Syllabus. Cambridge, Mass., Harvard Univ. Press, 1947.
30. Hamilton, W. F., and Remington, J. W., Comparison of the time concentration curves in arterial blood of diffusible and non-diffusible substances when injected at a constant rate and when injected instantaneously. Am. J. Physiol., 1947, **148**, 35.
31. Lewis, A. E., Measurement of thoracic visceral plasma volume. Am. J. Physiol., 1953, **172**, 195.