Flow Estimation by Indicator Dilution (Bolus Injection): Reduction of Errors Due to Time-Averaged Sampling During Unsteady Flow

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Flow Estimation by Indicator Dilution (Bolus Injection)

REDUCTION OF ERRORS DUE TO TIME-AVERAGED SAMPLING DURING UNSTEADY FLOW

By James B. Bassingthwaighte, M.D., Ph.D., Thomas J. Knopp, B.A. and Dennis U. Anderson, B.A.

ABSTRACT

Indicator dilution techniques used for the estimation of flow (F), mean transit time ($\bar{t}$), dispersion (σ), and mean transit time volume (V) in the circulation are subject to error when (1) flow is not steady and (2) concentrations are obtained by sampling at a constant rate (time averaging) rather than at rates proportional to the instantaneous flow past the sampling site (volume averaging).

Using a simple descriptive model for indicator transport, the effects of simulated aortic flow or of sinusoidal flow of widely variable frequency were assessed. Errors in estimates of F, $\bar{t}$, σ, and V are greater with bolus injections than with constant-rate injections. Errors are roughly proportional to the amplitude of variation in flow. They are maximal when the period of flow fluctuation is similar to the passage time of the dilution curve, which, for the human central circulation, is about the time for one respiratory cycle. With sinusoidal flow between 50% and 150% of the mean flow, errors were at worst up to 60% in F, 30% in $\bar{t}$, 50% in σ, and 70% in V, with a bimodal distribution. Errors are minimal at cardiac frequencies. The troublesome lower frequencies can be avoided. Preliminary tests of a method for converting time- to volume-averaged concentrations gave encouraging results

ADDITIONAL KEY WORDS blood flow dye curves cardiac output circulatory dispersion mathematical model circulation model computer simulation nonstationary differential operators

■ Flow in the circulation fluctuates continuously, but the classical approaches to the uses of indicator dilution techniques for the estimation of blood flow (F), mean transit time ($\bar{t}$), and dispersion (σ) in the circulation are based on the assumption that flow is steady (1, 2). It is therefore important to estimate the magnitudes of errors involved in the calculation of F, $\bar{t}$, and σ. The indicator dilution method is theoretically accurate if the flow stream can be sampled by obtaining volumes in proportion to the instantaneous flow past the sampling point. This is volume-averaged sampling (3). Ordinarily, we sample the flow stream at a constant rate or observe its average concentration in a cross section continuously; this is time-averaged sampling. Although some indication of the magnitudes of the errors resulting from time-averaged sampling has been given for constant-rate dye injections (4) and for the Fick technique (5-7), no quantitative estimates of the errors have been made for the technique of sudden bolus injection. It is the purpose of this paper to describe the sources and magnitudes of such errors and to provide some insight into mechanisms for reducing them.

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Theoretical Approach

Previous studies on the transport functions of material in the arterial system of the human leg (8), in the aorta (9), and in the pulmonary circulation (10) of the dog have shown that the relative dispersion, $\sigma/\bar{t}$ (the ratio of the standard deviation of transport function, σ, to its mean transit time, $\bar{t}$, is approximately constant over a wide range of flows. It has been deduced that, even over a 10-fold range of flows with Reynolds numbers ranging from 300 to 3,000, the spatial dispersion of indicator is apparently independent of the flow and dependent solely on the distance traveled along the vascular system (8).

On this basis it appears reasonable to examine the effects on slug-injection dye curves of continuously variable flow, $F(t)$, in a conservative, constant-volume system. To obtain a wide range of frequencies and amplitudes of variation in flow and to have injections made during different phases of cyclic variation in flow, a model system was used; in doing this a mathematical model has some advantages over a hydraulic model.

A linear differential operator with flow-proportional frequency coefficients and with other coefficients constant satisfies the requirement of constant relative dispersion, and the coefficients can be chosen so that the impulse response is similar to a dye dilution curve in the circulation.

A fairly high-order operator is required to produce an impulse response (transport function, or probability density function of transit times), $h(t)$, with an appropriate delay and $\sigma/\bar{t}$. A twelfth-order operator was used in this study; this order is not high enough to provide a perfect description of $h(t)$ in the circulation but is a compromise used to avoid overly long solution times. (A continuously variable delay operator in series with a low-order operator was found to be descriptive but was numerically less reliable at high frequencies.) The model's $h(t)$ is a skewed unimodal density function similar in shape to others (11-13) which could have been used for this study but which cannot be computed as quickly.

The operator may be represented by the family of six differential equations, consisting of three identical pairs of second-order operators:

$$\left.\begin{aligned} \frac{d^2y_i}{dt^2} + 2\zeta_1\omega_1\frac{dy_i}{dt} + \omega_1^2 y_i &= \omega_1^2 y_{i-1} \\ \frac{d^2y_{i+1}}{dt^2} + 2\zeta_2\omega_2\frac{dy_{i+1}}{dt} + \omega_2^2 y_{i+1} &= \omega_2^2 y_i \end{aligned}\right\} \quad (1a)$$

with $i = 1,3,5$

in which

$$\omega_2 = \beta\omega_1 \quad (1b)$$

and

$$\omega_1 = 2\pi\alpha\frac{F(t)}{V}. \quad (1c)$$

Here, y_i is the output of the ith component of the model, y_0 is the concentration-time curve of indicator at the input to the segment of the circulation, y_6 is the output concentration-time curve $C'(t)$.

The model parameters, ζ_1 and ζ_2, must be set so that $h(t)$ has no negative values (that is, the response is overdamped). By varying ζ_1, ζ_2, and β, widely different vascular segments may be simulated with flow constant or variable at any positive rate. α is a normalizing, dimensionless constant, which has a value such that $\bar{t}$ of $h(t)$ equals V/F during steady flow.

A segment of the circulation acts as a low pass filter, causing delay in transit and damping of the higher frequency fluctuations in concentration. In a linear system, when the input is sinusoidal at frequency ω, so also is the output. The cutoff frequency, ω_c, is defined as the frequency of sinusoidal variation in concentration at which the ratio of output amplitude to input amplitude is 0.707, or $1/\sqrt{2}$. When a system has constant volume and its $h(t)$ has constant relative dispersion then ω_c, like ω_1 and ω_2, is proportional to F. Thus, increasing the flow enables higher frequency information to be transmitted along a segment of the circulation, while reducing the flow reduces the input frequencies discernible at the downstream or output end of the segment. When flow is continuously variable, any input signal, y_0, is frequency modulated in

a transient fashion by changes in flow, and the high-frequency content of y_0 is filtered to an extent reciprocally related to the flow.

Methods

The equations were solved numerically on a digital computer using a simulation control system (14) providing automatic or manual means of changing parameters and allowing choice of variables for on-line display of solutions.

Specific Model Parameters.—For this study, a value of 0.28 was chosen for $\sigma/\bar{t}$. To obtain this, ζ_1 was set to 0.95, ζ_2 to 0.8, and $\beta = 1.82$. α was 1.32. This is still quite general because $\sigma/\bar{t}$ has a rather narrow range in the circulation—from 0.2 in peripheral vessels to about 0.45 for the pulmonary circulation (8-10).

Input, $y_0(t)$.—For a pulse injection, the total mass of indicator injected is m. The rate of entry of indicator at the upper end of the circulatory segment is $\dot{m}(t)$ or dm/dt. If the injection is made at the entrance,

$$\left.\begin{aligned} y_0(t) &= \frac{\dot{m}(t)}{\bar{F}(t)} \text{ for } 0 < t \leq T_{inj} \\ y_0(t) &= 0 \quad \text{for } t < 0 \\ &\qquad\quad t > T_{inj} \end{aligned}\right\}, \qquad (2)$$

in which $\dot{m}(t)$ mg/sec is the indicator injection rate and T_{inj} is the duration of injection. Therefore m is $\int_0^{T_{inj}} \dot{m}(t)dt$. For consistency of approach, $F(t)$ should be considered to be the instantaneous average flow at a cross section of the vessel, the injection to be flow labeling, and, therefore, the concentration, $y_0(t)$, to be an instantaneous flow-weighted average across the cross section. Similarly the output concentration, $y_6(t)$, is considered to be the average concentration in the cross section (3). The transport function or unit impulse response of the model, $h(t)$, is $y_6(t)$ when the input function, $y_0(t)$, is an impulse or very short pulse of high amplitude and unit area. In this study, the injection rate was constant at 8 mg/sec for 0.5 seconds, the total amount of indicator injected being 4 mg.

Flow, F(t).—For the simulation the flow was sinusoidal:

$$F(t) = \bar{F}[1 + K\sin(\omega t + \phi], \qquad (3)$$

in which $\bar{F}$ is the mean flow over one cycle, K = amplitude of sinusoidal variation, ω = frequency of oscillation in flow (radians/sec). $F(t)$ was limited to positive values when K was greater than 1.0. ϕ was defined to be zero when the midpoint of the injection coincided with $F(t) = \bar{F}$ during the part of the cycle when $F(t)$ was increasing. Another reference flow used for the analysis was $\bar{F}_u$, defined as the mean flow from the time of injection until the time at which the concentration had diminished to 1% of its peak value.

Estimation of $\bar{F}$, $\bar{t}$, and σ.—Estimates were obtained from the curves with unsteady flow by using the output concentration-time curve, $C'(t)$, with the assumption of steady flow and a linear system:

$$F_u = m \Big/ \int_0^\infty C'(t)dt. \qquad (4a)$$

$$\bar{t}_u = \left(\int_0^\infty t \cdot C'(t)dt \Big/ \int_0^\infty C'(t)dt\right) - t_{inj}. \qquad (4b)$$

$$V_u = F_u \bar{t}_u. \qquad (4c)$$

$$\sigma_u = \left[\left(\int_0^\infty C'(t)(t - \bar{t}_u)^2 dt \Big/ \int_0^\infty C'(t)dt\right) - \sigma^2_{inj}\right]^{\frac{1}{2}}. \qquad (4d)$$

As indicated by equations 4b and 4d, the mean transit time $\bar{t}_u$ and standard deviation σ_u are corrected for delay and dispersion of the input function at the injection site. No extrapolation of the downslope was necessary, since recirculation was not simulated.

Results

Adequacy of the Model.—In Figure 1 are shown the output of the model, $y_6(t)$ or $C'(t)$, superimposed on a dye dilution curve, $C(t)$, recorded using a sampling system with a 0.1-second mean transit time from the

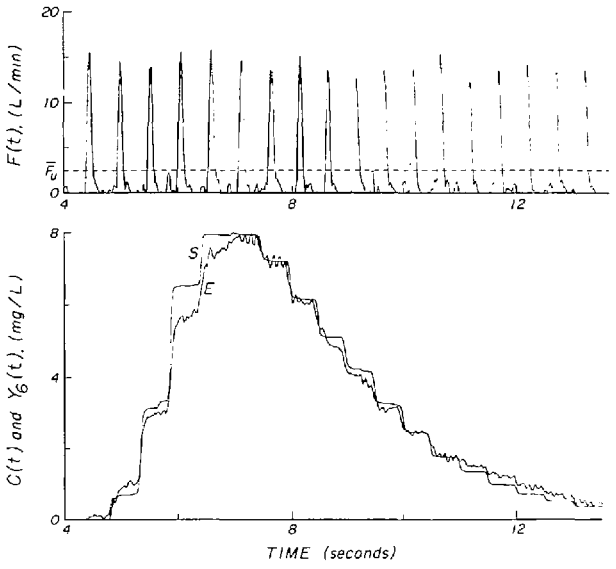

FIGURE 1

Comparison of model output, $y_G(t)$, labeled S with an experimental dye curve from the ascending aorta, C(t), labeled E when the model was driven by the recorded flowmeter signal F(t). The parameters of the model were V = 250 ml, $\bar{F}$ = 45 ml/sec, $\zeta_1 = 1.1$, $\zeta_2 = 0.8$, $\omega_1/\omega_2 = 7.0$. The input pulse to the model was 1 second in duration and was delayed 1.6 seconds compared to the actual injection.

ascending aorta of a dog after a slug injection of 1.25 mg of indocyanine green into the pulmonary artery. The signal obtained from the flowmeter (Carolina Medical Electronics, Winston-Salem, N. C.) on the ascending aorta was used as F(t) in equation 1c. In view of the fact that the animal's system between injection and sampling points is a variable-volume system and the model is a constant-volume system, the model is considered to give a remarkably good fit to the data.

Errors in Estimation of Flow from Dye Curves Obtained from Time-Averaged Sampling.—Simulated dye dilution curves have been obtained at 250 different frequencies of sinusoidal flow (ω/ω_c ranging from 0.002 to 2.0) and with six different phases of injection in order to suggest the envelope of error, and with K = 0.5. The 1,500 results therefore encompass the range of situations existing experimentally.

For convenience in making a practical interpretation of the results, we considered a working example similar to the pulmonary circulation in a dog: V = 250 ml, and $\bar{F}$ = 40 ml/sec. For these values, with steady flow, $\bar{t}$ = 6.25 seconds, σ = 1.73 seconds, and ω_c = 0.51 cycles/sec.

Either of the two correct average flows, $\bar{F}$ or $\bar{F}_u$, defined after equation 3, may be desired by the investigator, and therefore both will be considered. F_u is the estimate obtained by equation 4a, and $F_u/\bar{F}$ is plotted versus relative frequency of flow variation in the upper panel of Figure 2. At high frequencies the error is negligible. At lower frequencies

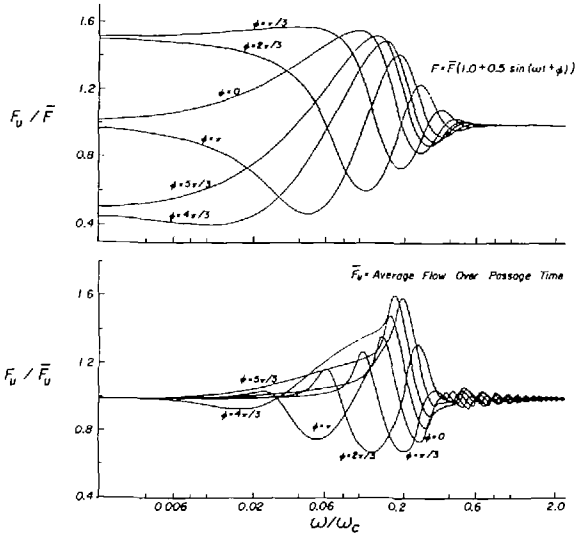

FIGURE 2

Effect of frequency and phase of sinusoidal flow on ratio of calculated flow to mean flow. Actual flow is F, flow calculated by equation 4a is F_u, and $\bar{F}$ and $\bar{F}_u$ are mean flows.

there is broadening of the envelope of error, a hypothetical pair of lines enclosing all of the lines at various phases. But at both very low and very high frequencies there is almost no error in $F_u/\bar{F}_u$ (lower panel of Figure 2) $\bar{F}_u$ being the average flow during the passage of the dye curve. The largest errors in F_u occur in the middle range of frequencies when the highest concentrations pass by the sampling site at a time when the flow is either fastest or slowest, causing the area under the curve to be under- or overestimated. Since the maximum error in area with various phases is almost symmetrical about the correct value, the error in F_u is skewed toward overestimation.

The frequency at which maximal errors occur is an expression of the fundamental frequency of the dilution curve and is related to the spatial dispersion of the bolus in the flowing fluid. The maximal error occurs at $\omega/\omega_c = 0.15$. This broad generalization is independent of the particular parameters of the model and indeed of the model itself. σ/t and ω_c have a roughly reciprocal relation, and the data of Figure 2 would be little affected by a shift in σ/t. Only if the form of $h(t)$ were dramatically different in different segments of the circulation would this generalization not hold, but dramatic differences have not been seen.

Effect of Amplitude of Variation in Flow on Areas of Dye Curves.—The envelope of error in Figure 2 would be broader if K were larger than 0.5. But the relationship between the amplitude of the fluctuation in flow and the magnitude of the error produced is not simply linear (Fig. 3). A_u and $A_{\bar{F}_u}$ are, respectively, the areas of the dilution curves with flow unsteady and with flow steady at $F(t) = \bar{F}_u$. Figure 3 presents a plane of $A_u/A_{\bar{F}_u}$ versus K at $\omega/\omega_c = 0.15$. The difference between A_u and $A_{\bar{F}_u}$ usually increases with increasing amplitude, but is clearly not linear.

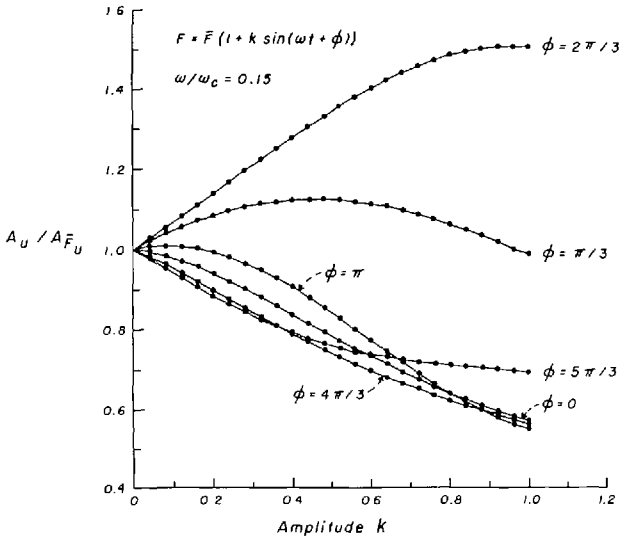

FIGURE 3

Effect of amplitude of flow variation on area of dilution curve.

There is also an asymmetrical distribution of the error at different phases. The area is dependent on the length of time each portion of the bolus in the blood stream remains at the sampling site. If the portion of the bolus with the highest concentration passes the sampling site when flow is at a low point in the cycle, the area is overly large (for example, at $\phi = 2\pi/3$) compared to that at steady flow. When the bolus is not very broad relative to the distance traveled along the stream in one cycle, it is most likely that the peak will pass when flow is fast and therefore that the resultant areas will be small ($\phi = \pi$, $4\pi/3$, $5\pi/3$, or 0).

Errors in Estimation of $\bar{t}$ from Dye Curves Obtained by Time-Averaged Sampling.—If flow were steady, $\bar{t}$ would be V/F. In the upper panel of Figure 4 is plotted the ratio $\bar{t}_u/V/\bar{F}$. The errors are maximal at low frequencies, particularly when injection was made when the flow was near its minimum ($\phi = 4\pi/3$ or $\phi = 5\pi/3$). At respiratory frequencies (around $\omega/\omega_c = 0.2$ in our working example), the errors are small relative to those at the lowest frequency. At high frequencies there is essentially no error in $\bar{t}_u$.

When $\bar{F}_u$ is considered to be the correct reference flow (Fig. 4, bottom), the differences between $\bar{t}_u$ and $t_{\bar{F}_u}$ which equals $V/\bar{F}_u$, and is the mean transit time which would have existed had the flow been steady at $\bar{F}_u$) are small at very low and at high frequencies; maximal errors occur at the mid range of frequencies. However, the largest errors in $t_u/t_{\bar{F}_u}$ occur not at $\omega/\omega_c = 0.15$ to 0.2 as did errors for F_u but at $\omega/\omega_c = 0.1$ and are less than for $F_u/\bar{F}_u$.

Errors in Estimation of σ from Dye Curves Obtained by Time-Averaged Sampling.—In Figure 5 is plotted the relative dispersion $\sigma_u/\bar{t}_u$ as a ratio to that which would have been obtained at the steady flow, $\sigma_s/\bar{t}_s$ (which is identical to $\sigma_{\bar{F}_u}/\bar{t}_{\bar{F}_u}$). As with $\bar{t}_u$, maximum error occurs around $\omega/\omega_c = 0.1$. The usefulness of the ratio $\sigma/\bar{t}$ is emphasized

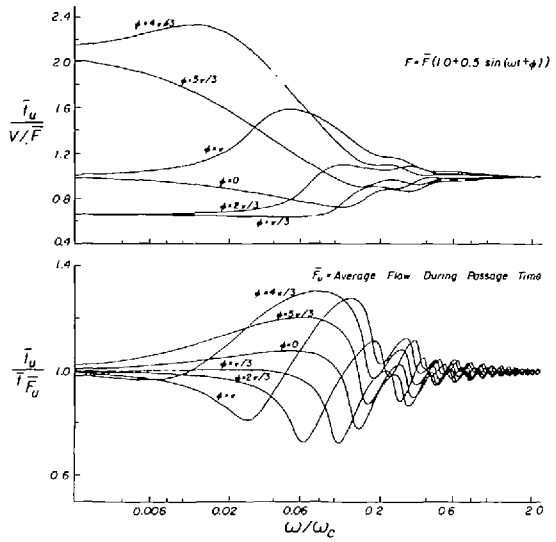

FIGURE 4

Effect of frequency and phase of sinusoidal variation in flow on estimated mean transit time. Ordinate is scaled twice as large on lower panel as on upper panel.

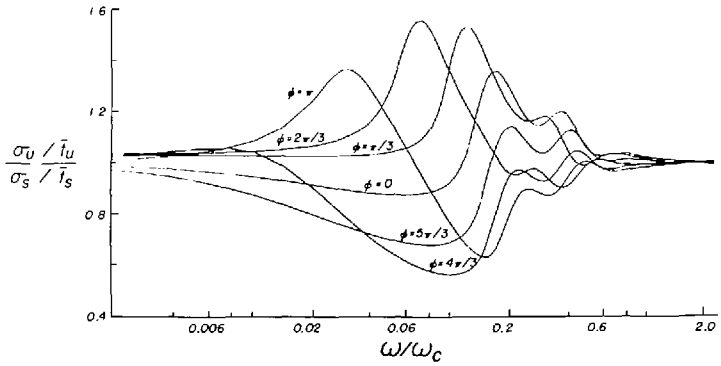

FIGURE 5

Effect of frequency and phase of sinusoidal variation in flow on relative dispersion of dye dilution curve. In this hypothetical situation described by the model, spatial dispersion of indicator is entirely independent of flow, and therefore frequency of variation in flow has no influence on actual spread of bolus with respect to distance along segment of circulation.

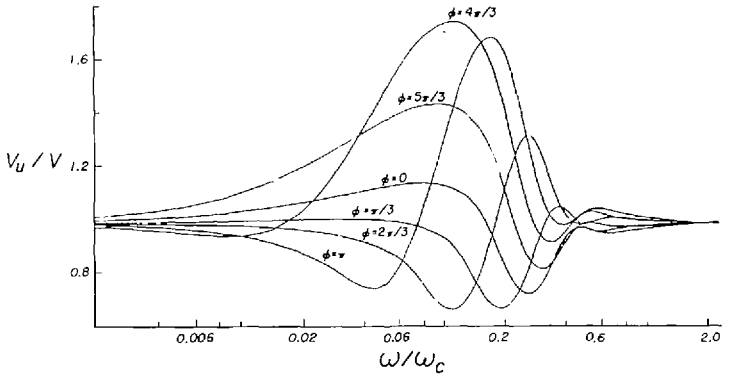

FIGURE 6

Effects of phase of sinusoidal variation in flow on estimates of mean transit time volumes. $V_u = F_u \cdot \bar{t}_u$.

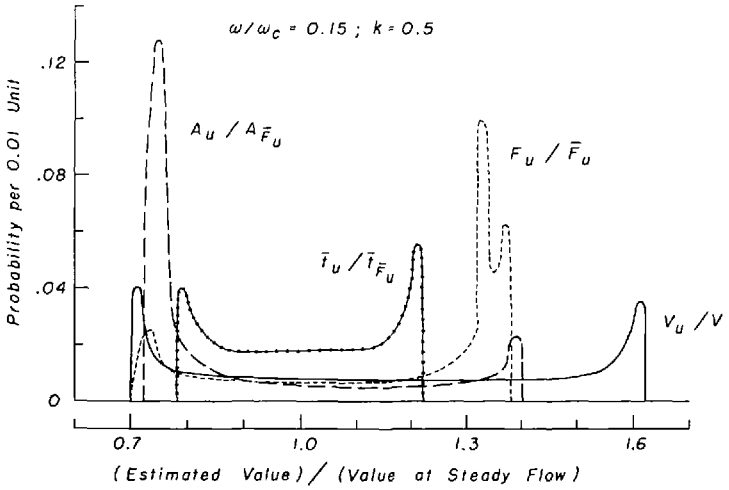

FIGURE 7

Probability density functions of errors in estimating A, F, $\bar{t}$, and V from dye dilution curves during unsteady flow. P is probability of occurrence per 0.01 unit, so total area of each curve is 1.0. For these curves, $\omega/\omega_c = 0.15$, $K = 0.5$, *and injections were made at all* ϕ *with equal probability. (Data obtained from 200 solutions with* ϕ *at steps of* 0.01π *from 0 to* 2π.)

because the error in relative dispersion is much less than that for the actual dispersion σ.

Errors in Estimation of V.—The ratio of V_u (calculated by equation 4c) to V is shown in

Figure 6. Errors in V_u are very large when the errors in $\bar{t}$ and in F_u are both in the same direction—for example, with $\phi = \pi$ at $\omega/\omega_c = 0.15$. Whereas the largest errors occur between $\omega/\omega_c = 0.02$ and 0.20 and are mainly overestimates, at other frequencies the errors in F_u and $\bar{t}_u$ or in $F_u/\bar{F}$ and $\bar{t}_u/V/\bar{F}$ are in opposite directions, which reduces the error in V_u. This is particularly apparent at very low frequencies.

Reliability of Mean Values.—The data on area and flow, as exemplified by Figures 2 and 3, illustrate that in the presence of unsteady flow randomization of the phase of injection results in several estimates whose mean may differ from the true mean. Figure 2 shows that when ω/ω_c is between 0.02 and 0.20 the mean flow will be significantly overestimated. At $\omega/\omega_c = 0.15$, the mean of the six estimates is 1.15. When ω/ω_c is less than 0.02, the average F_u is $\bar{F}$. Figure 3 shows that the magnitude of the biasing of the mean can only be approximately linearly related to the amplitude K. That biasing toward lower-than-correct mean estimates can also occur is seen for $F_u/\bar{F}_u$ at $\omega/\omega_c = 0.3$ (Fig. 2), where the mean of the six estimates is about 0.97.

However, the mean ratio of $F_u/\bar{F}_u$ or $A_u/A_{\bar{F}_u}$ is not very informative because the distribution of errors at a given frequency is bimodal or trimodal, depending on the frequency at which the distribution is examined and on the nature of the flow. In Figure 7 are shown the distributions of error in $A_u/A_{\bar{F}_u}$, $F_u/\bar{F}_u$, $\bar{t}_u/t_{\bar{F}_u}$, and V_u/V at $\omega/\omega_c = 0.15$ with all ϕ equally likely. The peaks of the probability density function mainly occur immediately adjacent to the envelope of error and occur when the peak concentration of the bolus is passing by the sampling site at either maximal or minimal flow. This occurs most obviously at $\omega/\omega_c = 0.15$, since the sampling site is one-half wavelength downstream, at which point the magnitude of the errors is maximal. A similar result was obtained by Cropp and Burton (4) for constant-rate injections. (A wavelength is considered to be the mean distance traveled by the blood and indicator during one cycle.) At $\omega/\omega_c = 0.15$, the probability of obtaining estimates within $\pm 10\%$ of the correct value was 13.5% for A_u, 12.6% for F_u, 35% for $\bar{t}_u$, and 18% for V_u. The probability of overestimating the mean was 30% for A, 70% for F_u, 52% for $\bar{t}_u$, and 62.5% for V_u.

Discussion

General.—A sinusoidal $F(t)$ was used in this study because its general form is close to a wide variety of flow patterns and also because, even when $F(t)$ in nature is not sinusoidal, the fundamental frequency of $F(t)$ is the important source of error rather than the higher-frequency harmonic content.

The prominent sources of variation in flow occur with cardiac contraction and with pulmonary ventilation, and thus the frequencies of these sources of error are readily determined. At any given frequency, the importance of the flow variation depends on its amplitude. In general, cardiac frequencies are sufficiently high that even large amplitude fluctuations are of little consequence, but respiratory variations in flow, although of relatively low amplitude, are quite important because of their lower frequency.

Fluctuations at Cardiac Frequencies.—The flow at the root of the aorta during the cardiac cycle varies from zero to almost five times the mean flow (15). In a peripheral artery the range is usually less (0.2 to 2.0 times the mean) but is very dependent on the physiologic state of the subject. The errors are exacerbated when there is reversal of flow as can occur in th abdominal aorta during diastole when splanchnic flow is high (16), a situation which we could not simulate using the methods described. At normal heart rates, systole is shorter than diastole, a situation which could produce larger errors than sinusoidal flow and which can be demonstrated by using aortic flow as $F(t)$, as shown in Figure 1.

Nevertheless, in general, flow variation at cardiac frequencies produces little error; Figures 2,6 show this, for the estimates are very nearly constant when ω/ω_c is greater than 1.0 or when the cycle length is less than a quarter of the dye curve passage time. Ac-

cordingly, when using dye dilution curves in two hydraulic circulation models with varying degrees of valvular regurgitation, Hoffman and Rowe (17, 18) observed no significant differences between estimates obtained during steady flow and those obtained with flow pulsating at even the rather low rates of 40 to 50/min.

Fluctuations at Low Frequencies.—Respiratory fluctuations have lower amplitude but are more important because the frequencies are lower. Hoffman and co-workers (19) observed the maximal stroke volume during inspiration in awake dogs to be "sometimes 50% greater than the minimal stroke volume in expiration, but as a rule the difference was the order of 10-20%." In humans, Vermeire and Butler (20) observed pulmonary capillary flows during inspiration as much as four times the flow during expiration. From their Table 1 we calculate that the amplitude of respiratory variation in flow (the difference between maximum and minimum, divided by two times the average) averaged 24% in their 15 subjects and ranged up to 62%. Gabe and co-workers (15) observed 25% fluctuations in stroke volume in the pulmonary artery of humans with deep respirations and 20% in the aorta.

These remarks are made to emphasize that the theoretical effects of variations in flow with the respiratory cycle are in fact seen in reality. Of equal importance in animals in poor condition or in humans in shock are the flow variations that occur with Mayer waves. Killip (21) observed up to twofold fluctuations in aortic flow over cycles lasting 15 to 40 seconds. Knowing that low cardiac output accompanies such states, we may predict with surety that these fluctuations would interfere with accurate estimation of flow, because the relative frequency, ω/ω_c, would be in the range 0.05 to 0.2.

Experimental Estimates of Errors with Unsteady Flow.—The results of this study provide a theoretical background for considering the variation in estimates of area or flow in human and animal studies, and permit us to estimate the concentration that time-averaged sampling in the presence of unsteady flow makes to the total variability in the estimates obtained by the dye dilution method.

For example, in a previous study, dilution curves were obtained simultaneously from pairs of sampling sites in the dog (22) while the ventilation rate was 40/min. The standard deviation of the difference between the areas of the paired curves was about 6%. Injections were random with respect to respiratory phase. Mean transit times from central venous injection sites to aortic sampling sites were 4 to 10 seconds (corrected for the sampling-system transit) at different flow rates. By equation 6, using $\sigma/\bar{t} = 0.45$ (10), ω_c is 0.50 to 1.20 cycles/sec. Therefore, ω/ω_c for respiratory variation was 0.6 to 1.4, and it is reasonable to conclude that respiration produced only minor errors in the results of that study, and that the standard deviations of 5 to 7% were due mainly to spontaneous variations in flow at lower frequency. Injection upstream from the lung reduces errors due to nonmixing, and streaming at the aortic sampling site is probably of little importance when the bolus takes many heart beats to pass.

If the residual variation in a carefully controlled study in anesthetized animals was 7%, then it is not surprising that, in studies done on awake humans to compare the Fick and dye techniques, the combination of spontaneous variation in flow and errors due to time-averaged sampling should result in standard deviations of 10 to 15% (23-25). A similar standard deviation was found by Hamilton and associates (26) in a comparison of the dye technique with readings from an electromagnetic flowmeter.

The extensive observations of Opdyke (27) can probably be explained in the light of our model experiments. He observed what he considered to be an extraordinarily large variance in the ratios of the values for cardiac output calculated from dye curves sampled from the descending aorta or from the left atrium to those values calculated from dye curves obtained simultaneously from the pulmonary artery. His Figure 2 shows non-Gaussian, highly platykurtotic distributions of

ratios, which are comparable to those in our Figure 7 except that the theoretical bimodality with the peaks at the extremes of the distribution is not apparent. This is not surprising in view of the presence of more than one source of variation and the fact that he was comparing flow estimates from two sampling sites rather than an estimated flow with a true mean flow. It is particularly interesting that he noted that the ratios of flow estimates obtained when the injection was made 0.1 second after the onset of inspiration (at 10 to 12/min) averaged 1.08 (LA sampling) and 1.23 (descending aorta), and when the injection was made 4.0 seconds after the onset of inspiration averaged 0.94 (LA) and 0.89 (aorta). These observations substantiate the theoretical results of our Figure 2: at ω/ω_c of about 0.2 and with the injection early in the inspiratory phase (i.e., $\phi = 0$, $\pi/3$ or $\pi/2$) then the cardiac output, F_{PA}, calculated from the PA curve, would be underestimated. The corresponding estimate, F_{ao}, calculated from the relatively dispersed dye curve sampled simultaneously from the descending aorta, for which ω/ω_c is likely less than 0.1, would have less error, i.e., from our Figure 1 the values for $F_v/\bar{F}_v - 1.0$ would be smaller. Therefore Opdyke's ratio, F_{ao}/F_{PA}, would be predicted to be greater than 1.0 with injection in this phase. With injection 4 seconds after the onset of inspiration ($\phi = \pi$ or $4/3\ \pi$ in our Fig. 2), F_{PA} would be markedly overestimated, resulting in the low values of F_{ao}/F_{PA} that he observed.

Practical Applications of the Theory.—For different segments of the circulatory system, the skewness and kurtosis vary little, but the relative dispersion, $\sigma/\bar{t}$, differs from region to region. But the important fact is that for a given segment $\sigma/\bar{t}$ remains almost independent of flow (8-10). This permits us to estimate ω/ω_c and to devise means for reducing the errors.

On the graphs showing error in estimates, ω/ω_c has been used as the abscissa. When ω_c can be obtained in an experimental situation, then the possible importance of an interfering frequency, ω, can be estimated from the graphs in Figures 2 to 7. If the amplitude of flow variation can be estimated, then an approximate estimate of the probable error can be obtained and its accuracy improved by estimating ϕ. When a system has transport functions at different steady flows which have constant shape (constant $\sigma/\bar{t}$, skewness, etc.) then ω_c is reciprocally related to $\bar{t}$:

$$\omega_c = R'/\bar{t} \quad \text{(radians/sec)}. \tag{5}$$

For the model parameters used in this study ($\sigma/\bar{t} = 0.28$ for example), R' is 20 radians. This type of relationship can be generalized somewhat approximately for a family of systems having right-skewed transport functions and a range of relative dispersions:

$$\omega_c \simeq \frac{R(\bar{t}/\sigma)}{\bar{t}} = \frac{R}{\sigma}, \tag{6}$$

where R is about 5.6 radians. Using this, one can obtain ω_c from σ above or from $\bar{t}$ and $\sigma/\bar{t}$. Equation 6 is an extrapolation from equation 5 and is applicable over the narrow range of $\sigma/\bar{t}$'s observed in the circulation.

The $\bar{t}$ to use in equation 6 is the mean transit time through the segment of the system in which flow is unsteady, that is, the $\bar{t}$ of the recorded curve minus that of the sampling system. In experimental situations, other corrections may be important. For example, when sampling from the radial artery after dye injection into the pulmonary artery, the subclavian and brachial arteries may be considered as an extension of the sampling system. If the flow in these peripheral arteries is constant, then their $\bar{t}$ also should be subtracted in order to find $\bar{t}$ through the central circulation only. If the peripheral arterial flow is a constant fraction of aortic flow, thus providing ideal flow proportional sampling (3), then no such correction should be made. Ordinarily one cannot ascertain the steadiness of peripheral arterial flow and therefore advancing the sampling catheter to the ascending aorta eliminates the uncertainty. The principle is that, to obtain the pertinent $\bar{t}$, sampling should be from a segment through which all of the injected indicator passes—or better still, from the most downstream point

of the artery in which flow is proportional to aortic flow.

Reduction of Error.—When it is recognized that flow is varying and that ω/ω_c is 0.05 to 0.30 or when the cycle length is one-half to three times the pertinent mean transit time, a number of methods are available for reducing this source of error. These are (1) increase the interfering frequency, (2) decrease the interfering frequency, (3) reduce the amplitude at the interfering frequency, (4) move the injection and sampling sites closer together to increase ω_c (effectively decreasing the interfering frequency toward $\omega/\omega_c = 0.06$), (5) increase the distance between the injection and sampling sites (decreasing ω_c and effectively increasing the interfering frequency toward $\omega/\omega_c = 0.6$), (6) prolong the duration of injection, and (7) average several estimates.

The correct choice of maneuver is important, for a wrong choice can have drastic effects on the error. For example, if the interfering frequency were at $\omega/\omega_c = 0.30$, a shortening of the distance between injection and sampling sites which doubled ω_c would result in the interfering frequency occurring at $\omega/\omega_c = 0.15$, the position of maximal error, and would produce a quadrupling of the magnitude of the envelope of error compared to that at ω/ω_c of 0.30.

1. Increase the frequency. Increasing respiratory rate is practical because not only does the envelope of error fall off quickly at high frequencies but also the tidal volume and the amplitude of variation in cardiac output diminish at higher rates. This improves the estimation of $\bar{F}$ and also of $\bar{F}_u$. This, therefore, is the recommended mode of reducing the respiratory influence on the amplitude of variation in cardiac output and in the error of estimation of flow by the standard slug-injection dye dilution technique.

2. Decrease the frequency. If the interfering frequency is respiratory, one may ask the patient to breathe more slowly or, if the patient is anesthetized, the ventilation rate may be reduced. If tidal volume were also reduced this would help, but the method may not be very effective if, in order to maintain the respiratory minute volume, the tidal volume must also be increased. In general this method is not very useful because, even though it improves the accuracy of the estimation of $\bar{F}_u$, it decreases the accuracy of estimation of $\bar{F}$.

3. Reduce the amplitude. This is very useful in those patients undergoing cardiac catheterization who frequently tend to have sighing respiration or to hyperventilate and who, on request, will breathe quietly and shallowly during the period of recording of the dilution curve. However, one cannot go to the extreme of asking the subject to stop breathing during the recording of the curve, for he may then unwittingly perform a Valsalva or a Mueller maneuver and change the flow. Also, if continuous sampling densitometry is used, the densitometer base line will shift as CO_2 accumulates, changing erythrocyte size and blood optical density.

4. Move injection and sampling sites closer together (reduce intravascular transit time). This has the disadvantage that, as ω_c is increased to avoid respiratory fluctuations in flow, fluctuations at cardiac frequencies gain importance. Despite this, the maneuver may still be useful because, in shortening the segment length, the absolute dispersion (not the relative dispersion) is reduced and the separation of the dye passing the sampling site for the first time from that recirculating can be made with greater precision. Therefore, in some cases more accuracy may be gained than lost. However, as with method 2, the accuracy of estimation of $\bar{F}_u$ is improved more than that of $\bar{F}$.

5. Increase the distance between injection and sampling sites (increase intravascular transit time). By shifting ω_c to a lower value, the interfering respiratory and cardiac cycles both are shifted to the right toward $\omega/\omega_c = 0.6$ or beyond, and the errors are reduced. The risk is that the accuracy of extrapolation to exclude recirculation may be diminished.

6. Prolong duration of injection. When injection and sampling sites are close together and ω_c is high, some reduction in errors is

obtained by prolonging the injection. This is most effective in situations in which recirculation does not obscure the downslope of the curve. For example, with $K = 0.5$ at $\omega/\omega_c = 0.2$ (*the region of maximal error*), the minimum to maximum range of $F_u/\bar{F}$ at $T_{inj} = t/16$, $t/4$, t, and $4t$ was 0.6 to 1.6, 0.7 to 1.5, 0.8 to 1.4, and 0.9 to 1.3, respectively. (Figures 2 through 7 were obtained with $T_{inj} = t/12.5$.)

7. Averaging of estimates. One might attempt to improve the estimates of F, A, $\bar{t}$, and V by making several observations with injections at different phases and averaging them. With distributions such as those in Figure 7 it is apparent that the mean will have a systematic bias and a large standard error. Biasing of the average cannot be avoided in the midrange (0.02 to 0.20) of interfering frequencies; but as Katz and Shinnar have pointed out (S. Katz and R. Shinnar, personal communication), it is minimized by averaging the variable itself rather than its components, i.e., the best estimate of V is $\Sigma V_u/N$, where N is the number of observations, rather than the average $(\Sigma F_u/N) \cdot (\Sigma \bar{t}_u/N)$. Similarly, when dye curves have been obtained by sampling two or more sites simultaneously, dividing the amount injected by the average of the areas of the dilution curves is useful, since the sampling sites will be at different distances or wavelengths downstream.

Approximation to Volume-Averaged Sampling.—Errors due to unsteady flow cannot be completely avoided with the techniques suggested above, and it is therefore highly desirable to obtain a volume-averaged sample, one in which the dye-blood mixture is sampled in proportion to the flow in each part of the cross section of the vessel. One method for converting time-averaged sampling to approximate volume-averaged sampling is to use a velocity signal from the flow stream near the sampling site. The equation of conservation of the injected material passing by the sampling site for the first time is

$$m = \int_0^\infty F(t) \cdot C(t)dt. \qquad (7)$$

Dividing both sides by the mean flow, $\bar{F}_u$, taking the reciprocals, and multiplying by the injected dose, m, leads to

$$\bar{F}_u = \frac{m}{\int_0^\infty [F(t)/\bar{F}_u] \cdot C(t)dt} \qquad (8)$$

The ratio, $F(t)/\bar{F}_u$, is a weighting factor for the instantaneous concentrations, $C(t)$; it is large when $F(t)$ is large and zero when the flow is stopped. Since this ratio is defined as having a mean value of 1.0 over the passage time of the dilution curve, it can be obtained from a linearly flow-proportional signal which need not be calibrated in absolute terms. This can be derived from the pressure difference between two orifices of a double-lumen catheter (22) or from a velocity transducer (28). Equations similar to equation 8 can be used for $\bar{t}$ and σ. While such a method can be expected to provide a vast improvement in accuracy, the problems of cross-sectional averaging of flow and of concentration remain to plague the perfectionist.

When this technique is applied to the dye curves in Figure 1, the estimates of $\bar{F}_u$, $\bar{t}$, $\sigma/\bar{t}$, and V were 99.6%, 99.0%, 99.2%, and 98.6% of those given by the classical equations. For this curve, ω_c was calculated by equation 6 to be 0.4/sec. The heart rate and respiratory rates were 2.08/sec and 0.66/sec, giving values of ω/ω_c of 5.2 and 1.65 at these frequencies. At these values of ω/ω_c the errors in the estimates should theoretically be small, as the above observed values suggest.

Determination of Flow-Dependent Transport Function.—The assumptions that ω_c is proportional to $F(t)$ and that other shaping parameters of $h(t)$ do not change are reasonable for fairly wide ranges of estimates of flow in large vessels and for the pulmonary and renal circulations. However, dispersion, skewness, and kurtosis of $h(t)$ would vary whenever a change in flow results in a change of velocity profile in large vessels or in a change of the distribution of regional flows in a capillary network. The approximation can be useful in obtaining a description of the

transport function when $F(t)/\bar{F}_u$ is known. General treatments have been given by Stephenson (1) and Sherman (29), and a closer approach to the problem was provided by Zadeh (30). His treatment can be simplified considerably when the injection is an impulse, $\delta(t)$, and when concentration and flow are the same across the cross section of the vessel.

The present model can be used moderately well even if $F(t)/\bar{F}_u$ is not completely described. If respiratory and cardiac frequencies are known or recorded along with concentration-time curves from the input and output ends of the segment, then $F(t)$ may be considered as the sum of two periodic functions of unknown amplitude but of approximately known form. With the upstream curve used as input, $y_0(t)$, the parameters of the differential operator may be varied to obtain optimal matching of $y_\theta(t)$ with the curve recorded at the downstream point. Even with imperfect estimates of $F(t)$, this should provide much more accurate estimations of the circulatory transport function than could be obtained by assuming that $F(t) = \bar{F}$.

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References

1. Stephenson, J. L.: Theory of the measurement of blood flow by the dilution of an indicator. Bull Math Biophys 10: 117, 1948.
2. Meier, P., and Zierler, K. L.: On the theory of the indicator-dilution method for measurement of blood flow and volume. J Appl Physiol 6: 731, 1954.
3. González-Fernández, J. M.: Theory of the measurement of the dispersion of an indicator in indicator-dilution studies. Circ Res 10: 409, 1962.
4. Cropp, G. J. A., and Burton, A. C.: Theoretical considerations and model experiments on the validity of indicator dilution methods for measurements of variable flow. Circ Res 18: 26, 1966.
5. Stow, R. W.: Systematic errors in flow determinations by the Fick method. Minn Med 37: 30, 1954.
6. Visscher, M. B., and Johnson, J. A.: The Fick principle: Analysis of potential errors in its conventional application. J Appl Physiol 5: 635, 1953.
7. Zierler, K. L.: Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady states. J Clin Invest 40: 2111, 1961.
8. Bassingthwaighte, J. B.: Plasma indicator dispersion in arteries of the human leg. Circ Res 19: 332, 1966.
9. Bassingthwaighte, J. B., and Ackerman, F. H.: Mathematical linearity of circulatory transport. J Appl Physiol 22: 879, 1967.
10. Knopp, T. J., and Bassingthwaighte, J. B.: Effect of flow on transpulmonary circulatory transport functions. J Appl Physiol 27: 36, 1969.
11. Bassingthwaighte, J. B., Ackerman, F. H., and Wood, E. H.: Applications of the lagged normal density curve as a model for arterial dilution curves. Circ Res 18: 398, 1966.
12. Sheppard, C. W.: Mathematical considerations of indicator dilution techniques. Minn Med 37: 93, 1954.
13. Thompson, H. K., Jr., Starmer, C. F., Whalen, R. E., and McIntosh, H. D.: Indicator transit time considered as a gamma variate. Circ Res 14: 502, 1964.
14. Knopp, T. J., Anderson, D. U., and Bassingthwaighte, J. B.: SIMCON: Simulation control to optimize man-machine interaction. Simulation 14: 81, 1970.
15. Cabe, I. T., Gault, J. H., Ross, J., Jr., Mason, D. T., Mills, C. J., Shillingford, J. P., and Braunwald, E: Measurement of instantaneous blood flow velocity and pressure in conscious man with a catheter-tip velocity probe. Circulation 40: 603, 1969.
16. McDonald, D. A.: Blood Flow in Arteries. Baltimore, Williams & Wilkins Co., 1960, 328 pp.
17. Hoffman, J. I. E., and Rowe, G. G.: Some factors affecting indicator dilution curves in the presence and absence of valvular incompetence. J Clin Invest 38: 138, 1959.
18. Hoffman, J. I. E.: Calculation of output, central volume and variance from indicator-dilution curves. J Appl Physiol 15: 535, 1960.
19. Hoffman, J. I. E., Guz, A., Charlier, A. A., and Wilcken, D. E. L.: Stroke volume in conscious dogs: Effect of respiration, posture and vascular occlusion. J Appl Physiol 20: 865, 1965.
20. Vermeire, P., and Butler, J.: Effect of respiration on pulmonary capillary blood flow in man. Circ Res 22: 299, 1968.

21. KILLIP, T., III: Oscillation of blood flow and vascular resistance during Mayer waves. Circ Res 11: 987, 1962.
22. BASSINGTHWAIGHTE, J. B., EDWARDS, A. W. T., AND WOOD, E. H.: Areas of dye-dilution curves sampled simultaneously from central and peripheral sites. J Appl Physiol 17: 91, 1962.
23. HAMILTON, W. F., RILEY, R L., ATTYAH, A. M., COURNAND, A., FOWELL, D. M., HIMMELSTEIN, A., NOBLE, R. P., REMINGTON, J. W., RICHARDS, D. W., JR., WHEELER, N. C., AND WITHAM, A. C.: Comparison of the Fick and dye injection methods of measuring the cardiac output in man. Amer J Physiol 153: 309, 1948.
24. MILLER, D. E., GLEASON, W. L., AND MCINTOSH, H. D.: Comparison of the cardiac output determination by the direct Fick method and the dye-dilution method using indocyanine green dye and a cuvette densitometer. J Lab Clin Med 59: 345, 1962.
25. WERKÖ, L., LAGERLÖF, H., BUCHT, H., WEHLE, B., AND HOLMGREN, A.: Comparison of the Fick and Hamilton methods for the determination of cardiac output in man. Scand J Clin Lab Invest 1: 109, 1949.
26. HAMILTON, F. N., MINZEL, J. C., AND SCHLOBOHM, R. M.: Measurement of cardiac output by two methods in dogs. J Appl Physiol 22: 362, 1967.
27. OPDYKE, D. F.: Agreement of cardiac outputs calculated from paired indicator-dilution curves. J Appl Physiol 20: 9, 1965.
28. FRY, D. L., GRIGGS, D. M., JR., AND GREENFIELD, J. C., JR.: In vivo studies of pulsatile blood flow: Relationship of the pressure gradient to the blood velocity. *In* Pulsatile Blood Flow, edited by E. O. Attinger. New York, McGraw-Hill Book Co., Inc., 1964, pp. 101-114.
29. SHERMAN, H.: On the theory of indicator-dilution methods under varying blood-flow conditions. Bull Math Biophys 22: 417, 1960.
30. ZADEH, L. A.: Frequency analysis of variable networks. Proc IRE 38: 291, 1950.