Dispersion of Indicator Measured from Microvessels of Cat Mesentery

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ABSTRACT

The indicator-dilution method was used to investigate blood flow in the microvascular network of the mesenteric membrane of the cat by replacing the normal blood flow into the mesenteric artery with a dextran-saline solution for a finite period of time. The dilution of the dextran-saline bolus as it flowed downstream was measured in a selected arteriole and its adjacent venule with a microphotometric system. Based on an in vitro calibration study done on glass capillary tubes, the measured optical density was converted to the hematocrit. The dilution curve for a finite injection and for a step injection, the mean transit time (MTT), and the appearance time for the arteriole and the venule were computed. The dispersion found in the arterial system, a network of diverging branches, was reasonably well simulated by the dispersion in a parabolic flow system. However, the dispersion found in the venules, where the irregular capillary blood flow converges, was considerably skewed from that in a parabolic system. A wide distribution of MTT was found for the arterioles and the venules, and there was considerable overlap in the MTT distribution for these two groups. This finding cannot be simulated by a network with a parallel arrangement. The difference in MTT for pairs of arterioles and venules was distributed over a narrow range, probably indicating shunt flow in the mesenteric microvascular network.

KEY WORDS

arterioles indicator-dilution technique mean transit time venules appearance time dextran

The indicator-dilution technique has been used successfully to measure the blood volume and flow and the dispersion of indicator in vascular systems. This paper reports a method which employs the principle of the dilution technique to monitor the dispersion of indicator as it flows along the microvascular network of the mesenteric bed of the cat. The mesenteric bed was separated into several subunits so that more information on the dispersion of indicator in the bed could be extracted from the procedure.

After a bolus of indicator had been injected into the mesenteric artery, the dilution of this bolus as it flowed along an arteriole and its adjacent venule was monitored simultaneously by a microscopic, photometric system. From these measurements, dilution curves for step injections, appearance times, and mean transit times (MTT) were computed. A similar experiment was then performed on randomly selected microvessels of the mesenteric bed. In this way, the important characteristics of dispersion of the indicator in the arterial and capillary system could be identified; these characteristics could then be used to interpret the indicator-dilution curves obtained from the entire bed. In addition, we hoped that our results would determine the efficacy with which some models such as Sheppard's random walk model (1) or a parallel network model assuming parabolic flow (2) simulate the mesenteric vascular bed.

Conventional microvascular studies measure diameter, velocity, and pressure at a selected location in the vascular bed. Our method measures what has happened to a bolus that was injected at the mesenteric artery as it reaches a selected microvessel. Thus, information pertinent to upstream microvessels is contained in our dilution curves. Therefore, combination of our method with conventional measurements might allow the development of an improved approach for monitoring the regulation of flow in a complex vascular network. Since the dilution curve obtained from an arteriole can be regarded as the input time-concentration curve for the capillaries downstream, our measurements also provide needed data for a theoretical analysis of the transcapillary exchange of substances in a vascular bed.

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Methods

EXPERIMENTAL PROCEDURE AND APPARATUS

Fasted cats (2–3 kg) were used in this microcirculatory study. They were anesthetized with sodium phenobarbital (~ 40 mg/kg, iv) until no flexor withdrawal reflex could be evoked. During the experiment, additional anesthesia was given as needed.

A midline incision was made to exteriorize the intestines, which were then wrapped with saline-soaked gauze. After a 2-cm section of the superior mesenteric artery had been exposed, the vessel was tied midway along the exposed length. Then one end of a catheter loop filled with heparinized saline was advanced from this tie toward the aorta. With the other end advanced 1–5 cm into the mesenteric artery distal to the occlusion, the flow into the mesenteric artery was restored through the loop. The time period between occlusion of the artery and restoration of blood flow ranged from 5 to 10 minutes. Heparin (1000 units/kg body weight) was infused into the artery.

The cat was transferred to an animal holder attached to the stage of a Microstar 20 microscope (American Optical Co.). A portion of the mesenteric membrane was placed on a pedestal raised 0.5 cm higher than the surface of the stage. Through a series of holes drilled around the circumference of this pedestal, a vacuum of about 10 cm H2O was employed to hold the membrane in place. In this way, the movement of the membrane induced by intestine motion and respiration was significantly minimized.

Again the flow was momentarily interrupted while the catheter was connected to an indicator-injecting device that consisted of a Masterflex pump, a four-way valve (Hamilton Co.), a reservoir loop, and a rotary solenoid (S8217-26, Lendex Inc.) (Fig. 1). Since the speed of the pump was adjusted until the pressure drop across the injection system was zero, it was necessary to monitor the pressures at the distal and proximal ends of the mesenteric cannula with a pair of Statham pressure gauges. The mesenteric arterial flow was 15–25 ml/min.

Red blood cells were tagged by removing them at the intestine and replacing them with a dextran-saline solution. The four-way valve was positioned by the rotary solenoid. The normal position of the valve, shown in the lower left corner of Figure 1, ensured a direct connection between the distal and proximal ends of the arterial catheter. Thus, blood from the aorta flowed directly into the mesenteroy. When the solenoid was activated, it took 0.05 seconds to rotate the four-way valve 90°. The resulting configuration of the valve and the flow path is sketched in the insert at the bottom of Figure 1. With this valve setting, blood flowed into the reservoir filled with a dextran-saline solution. Thus, the solution flowed into the distal end of the catheter and then into the mesenteric bed. When the solenoid was deactivated, the valve returned to its original position, and the system was perfused with blood again. In this way, a bolus of saline could be injected into the vascular bed for a controlled period of time. We defined this injection procedure as a finite injection, in contrast to the conventionally known step injection or impulse injection.

By adjusting the solenoid control circuit (3), the period of saline injection could be varied. Usually an injection of 0.5 ml of saline was sufficient to provide a well-defined curve recording from a pair of microvessels. The volume of the reservoir loop was about 5 ml. The loop was gradually filled with blood as the experiment proceeded. Occasionally, we flushed the loop with dextran-saline solution to clear it of blood. The mesenteric membrane was suffused with warm saline and kept warm by an infrared lamp.

The microscope’s objective and condenser were Leitz 20x long-working-distance objectives. The numerical aperture of each was 0.3. The image of a selected portion of the microvascular bed was projected onto a 6-inch frosted glass viewing screen. The total magnification on the screen was 110x. After the injection of the dextran-saline bolus, a decrease in the hematocrit in the arterioles occurred and was followed by a significantly smaller decrease in the hematocrit in the venules.

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A reduction in the hematocrit in the microvessels was indicated by an increase in the light transmitted through two fiber optic bundles (Dow Corning Glass Co.) mounted on the screen. They were positioned over the center of two selected microvessel images (Fig. 1). The diameter of the fiber optic bundles was 0.24 cm, which corresponds to the image of a 25 μm microvessel in this system. These fiber optic bundles transmitted light to RCA 1P-28A photomultiplier modules powered by two Heath EU-703-20 photomultiplier modules. Signals from these photomultipliers and a signal indicating the position of the four-way valve were recorded on magnetic tape, using a Vetter FM multiplexer and a Sony home-entertainment tape recorder. The valve position signal was used to compute the beginning and the end of the injection.

The microscope light source was a 100-w halogen-quartz lamp. Without control, the drift of the light level from the lamp created a significant base-line shift in the recorded signal. Therefore, light output was monitored by a photodetector, and a feedback circuit was used to regulate lamp voltage (3).

**DATA ANALYSIS**

A recording of rough data is presented in Figure 2. The light intensity collected by a fiber optic bundle from the kth microvessel is I_k, and I* is some preset reference level. The light intensity transported through a light guide centered over an arteriole yielded the top log I_k/I* vs. t curve in the figure. The middle curve was obtained from the venule adjacent to this arteriole. The bottom curve in Figure 2 indicates the position of the valve. Time zero is the time at which the saline bolus began to flow into the mesenteric artery. For this particular experiment, the period of injection, t, was 2.3 seconds.

These analog data were sampled by an Ambilog computer at a rate of 50 samples/sec and recorded on a digital tape. The digital data was smoothed by averaging sets of five consecutive samples. Our final analysis was done on curves represented by 10 samples/sec. The analysis program was written for a CDC 6400 computer.

In our laboratory, Jendrucko and Lee (4) used the same microscopic, photometric system to study the light transmission of blood flowing in glass tubes with diameters ranging from 37 to 116 μ. The tube was placed between a feed reservoir and a collecting reservoir. The standard microcentrifuge method was used to measure the hematocrit of a blood sample from the feed reservoir. In this in vitro experiment with glass capillary tubes, the light intensity, I_k, at zero hematocrit in the tube was first measured. Then I*, the intensity when the hematocrit in the reservoir feeding the tube was Ht*, was measured. The following correlation was found to provide a reasonable fit with the experimental data obtained by Jendrucko and Lee (3):

\[ 1 - I_k/I* = b_k(Ht^* + a_1Ht^* + a_2Ht^*), \]  

(1)

for 0 < Ht* < 45%. The constants are a_2 = -3.3 and a_1 = 3.8. (A 30% hematocrit means Ht* = 0.3 in Eq. 1.) The value of b_k is 2.8, 3.9, or 5.5 for a tube with a diameter of 37, 46, or 73 μ, respectively.

Since an extremely large bolus was needed to reduce the hematocrit to zero at the microvessel, the value of I_k was not measured. To analyze our data, it was necessary to assume that the hematocrit of the blood collected from the microvessels was equal to the hematocrit, Ht, in the mesenteric artery. This value was measured after every five successive injections. The light intensity I_k* is the light intensity transmitted by the vessel at normal hematocrit, Ht. Thus, we obtain

\[ 1 - I_k*/I* = b_k(Ht + a_1Ht + a_2Ht), \]  

(2)

The value of I_k* was found by averaging I_k before the bolus injection. Combining Eqs. 1 and 2 leads to the following equation.

\[ \frac{I_k(t)}{I_k*} = \frac{1 - b_k(Ht_k + a_1Ht_k + a_2Ht_k)}{1 - b_k(Ht + a_1Ht + a_2Ht)}. \]  

(3)

For a given b_k, the value of Ht_k(t) (the hematocrit at the kth microvessel, which is a function of time because of the bolus injection) was computed using Eq. 3 from the recorded data and the equation log (I_k/I_k*) = log (I_k*/I*) - log (I_k/I*). The quantity Ht_k(t) was calculated by Eq. 3 from the measurement of light intensity; it was assumed to equal the hematocrit that would be found if the blood were collected from the microvessel. This assumption has been validated in vitro for glass tubes larger than 59 μ in diameter (5). However, the tube hematocrit obtained by centrifuging the blood trapped in the tube differs from the collecting hematocrit (4, 5). In the terminology of Gonzales-Femandez (6), the tube hematocrit can be considered to be the mean cross-sect-

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**FIGURE 2**

Basic form of recorded signals. Top: From an arteriole. Middle: From the adjacent venule. Bottom: Indicates the beginning and the end of plasma injection. The injection period is 2.3 seconds.
tional concentration and the collecting hematocrit the mean flow concentration. The latter was used as the hematocrit in this study.

Because of the difficulty involved in measuring the diameter of a microvessel accurately and the possible dissimilarity between the in vivo system and the in vitro arrangement, we chose $b_k$ so that the computed dilution curve would satisfy the identity to be described next. If $Q$ is the flow through the mesenteric artery and $q_k$ is the flow through the $k$th microvessel, then after the injection an amount of red blood cells equal to $Q/H_t \cdot \tau$ is depleted from the flow into the mesenteric artery. The amount of red blood cells depleted from the flow passing through the $k$th microvessel is given by

$$f_0^\infty q_k[H_t - H(t)]dt.$$  

(4)

If the bolus injection does not alter the distribution of red blood cells in the microvascular bed, then the radio of total flow in the $k$th vessel to total flow equals the ratio of total flow in the $k$th vessel to total flow in the mesenteric artery. In mathematical form, it is

$$f_0^\infty q_k[H(t) - H_k]/(Q - H_t \cdot \tau) = q_k/Q.$$  

(5)

which can be simplified to

$$f_0^\infty (1 - H(t)/H_t)dt = \tau.$$  

(6)

An iterative scheme was used to select $b_k$ such that the computed hematocrit curve $H(t)$ satisfied Eq. 6. The value of $b_k$ so determined did not deviate by more than 30% from the value derived from our in vitro experiments on a glass tube of similar size.

After the value of $b_k$ had been derived, we calculated the dilution curve for the finite injection, $P_1$, as

$$P_1(t) = 1 - H(t) - H_k.$$  

(7)

If $\tau$ approaches $\infty$, then the finite injection response, $P_1(t)$, becomes the step response, $H(t)$, the dilution function for a step injection. To compute $H(t)$, we superposed a sequence of finite responses, each of them shifted by a time $\tau$ from the previous one to yield:

$$H(t) = \sum_j^{\infty} P_1 (t - n\tau) = \sum_j^{\infty} (1 - H_k (t - n\tau)/H_t).$$  

(8)

Subsequently, the mean transit time, $\overline{\tau}$, was computed according to

$$\overline{\tau} = f_0^\infty \{1 - H(t)\}dt.$$  

(9)

The computer program found the maximum value of the record during the 5 seconds preceding the bolus injection. The appearance time, $t_a$, was defined as the time when the average value of five samples before and five samples after was greater than this maximum value.

A 10-20% change in the values of $q_k$ and $q_k^*$ did not alter the computed value of the MTT by more than 0.1 seconds. The noise in the recorded light intensity could relate to fluctuations in the hematocrit at the measurement site.

One major difficulty of the experiment was to prevent unexpected shifts in the light intensity record; these shifts were, in almost all cases, due to movement of the microvessel away from the fiber optic bundle. In Figure 2, a fluctuation in light intensity is apparent just before $t = 0$. If a similar fluctuation had occurred at or before the real appearance time, then the computed $t_a$ would have been in error and would not have been used in the data analysis. Such a fluctuation, however, affects the computation of MTT only slightly. Thus, MTT could have been calculated from this curve, but the appearance time could not have been determined.

An example of a computed arteriole dilution curve for a finite injection is sketched in Figure 3. For the purpose of clarity, only five data points per second are presented.

**CHARACTERISTICS OF THE DILUTION CURVE AND THE EFFECT OF BOLUS SIZE ON DILUTION**

For the experimental procedure developed in this paper, we replaced red blood cells with a dextran-saline solution and used the absence of red blood cells in a microvessel to measure the dilution curve. Since this area of low hematocrit flows in the same manner as all red blood cells, it is likely that the dilution monitored is the dilution for red blood cells.

When 5–10 ml of saline was injected into the mesenteric artery, we observed a complete clearing of red blood cells in the arterioles of certain areas. This observation demonstrates that the blood flow to the arterioles in these areas is derived solely from the cannulated mesenteric artery. Similar clearing could be demonstrated for the venules when an extremely large bolus was used; however, if flow stopped in the vessels of interest after flow in the mesenteric artery had been interrupted, it was usually assumed that no collateral circulation existed. Our measurements were made on vessels in these areas. Therefore, the microvascular network under study can be considered to be a system with one input and many outputs.

In this microvascular study, we measured the change in light transmission in an extremely small cube; its size, when magnified by the microscope and displayed on the viewing screen, is equivalent to the size of the fiber optic bundle. The volume of the cube is about $0.015 \times 10^{-4}$ cm$^3$, which is about $10^{-7}$ times the volume of the sample used in a densitometer. Therefore, to enhance the signal-to-noise ratio, our set-up was designed to provide

**FIGURE 3**

Computed response, $P_1$, for finite injection. Zero is the time a 2.3-second bolus begins to flow into the mesenteric artery. The power law (Eq. 12) and the exponential function (Eq. 14) were used to fit these data points. The value of $n$ is calculated from the time ratio, $t_a/H_t$, computed from the experimental record. Note that the data are better fitted by the exponential function.
a high incident light and a large change in light absorption by the indicator in the microvessel. The former was achieved by the condenser which focused the light onto a small portion of the mesenteric membrane. The latter was accomplished by selecting red blood cells as our indicator: red blood cells provided a larger light intensity change per concentration change than did any of the other indicators that we tested.

When we used methylene blue as an indicator, the light absorption at the microvessel was barely detectable even at very high indicator concentrations. The sensitivity of our instrumentation precluded the use of methylene blue and other dyes of similar nature. Because of the sedimentation of red blood cells in the reservoir loop, we did not use blood of various hematocrits to demonstrate the linearity of the dilution response.

To test the validity of our experimental procedure, it is desirable to show that the bolus does not disturb the transport of the remaining red blood cells through the system and that the response calculated for a constant injection is relatively independent of bolus size. To show the effect of bolus size, a sequence of dilution curves for a venule obtained with injection periods of 0.7, 1.0, and 1.8 seconds is presented in Figure 4 (top). The curves are the smoothed versions of the computed results. As shown, for a longer finite injection, the dilution curve has a larger change and reaches its maximum at a later stage. To compare these curves, the responses, $H$, for a constant injection were calculated from each of the curves and are presented in the bottom of Figure 4. The $H$ curves computed for the injection periods of 1.0 and 0.7 seconds are identical, but a small deviation is seen for the longest period (1.8 seconds). However, the values of MTT calculated from the three curves are not significantly different. Only when the bolus duration is longer than 2–3 seconds does the $H$ curve move significantly toward the ordinate. In summary, if the injection duration remains below 2 seconds, then the calculated values of MTT and the calculated $H$ curves are independent of injection duration.

**Results**

**DISPERSION OF THE INJECTED BOLUS**

Before discussing the dispersion of indicator in a complex vascular network, let us review the dispersion in a straight tube, the Poiseuille (parabolic) flow system (6). The responses at a downstream section of the system for a sudden injection at a certain upstream section, $h$, and those for a constant injection, $H$, are given by

\[
\begin{align*}
    h &= 0, \\
    H &= 0, \\
    t < t_a \\
    h &= 2t_a^2/t^3, \\
    H &= 1 - t_a^2/t^2, \\
    t &> t_a
\end{align*}
\]

where the appearance time $t_a$ is one half of MTT. For a finite period of injection, $\tau$, the response, $P_T$, is given by

\[
P_T(t) = H(t) - H(t - \tau).
\]

The curve described by Eq. 11 rises sharply after the appearance time. At time $t_a + \tau$, the curve reaches its maximum and then reverses its trend with a sudden change in slope.

The time it takes for the fastest moving indicator to travel from the injection site to the site of measurement is $t_a$; the MTT, $\bar{t}$, is the time it takes an average particle to travel the same distance. The ratio of these two times, $t_a/\bar{t}$, can be used to define the dispersion of the indicator while it travels from the mesenteric artery to the microvessel. For example, the same $\bar{t}$ but a larger $t_a$ will imply that the fastest moving particles are moving at a speed closer to that of the average ones, i.e., a smaller dispersion.

For the 22 arterioles studied, the value of $t_a/\bar{t}$ ranged from 0.46 to 0.73 with an average value of 0.59. The distribution of the ratio is shown in the top of Figure 5. If the dispersion of indicator in the arterial system is similar to that in a straight tube, then $t_a/\bar{t}$ will equal 0.5. The distribution given in Figure 5 shows that 20% of the arterioles had a dispersion characteristic similar to that of a straight tube. However, for most arterioles, the ratio was higher than 0.5, and the dispersion was less than that in a straight tube.
The data presented in Figure 3 illustrate the similarity between the response found at an arteriole and the finite response calculated for a straight tube. Let $t_m$ be the time for the experimentally measured $P$ to reach its maximum. For a straight tube, $t_m = t_a + r$ or $(t_m - t_a)/r = 1$. For 20 arterioles, the ratio $(t_m - t_a)/r$ was computed and found to be $1.02 \pm 0.04$ (SD). A sudden change in slope at time $t_m$ was universal among the arterioles studied. These observations suggest that the $h$ function for these arterioles was similar to that for a parabolic flow system.

To find the functions $h$ and $H$ to fit our experimental results, we employed the following power law as a first trial:

\[
\begin{align*}
  h &= 0, \\
  H &= 0, \\
  n &= (n_t - t_a)^{-1}, & n > n_t \\
  n &= 1 - (n_t - t_a)^n & n < n_t
\end{align*}
\]

Note that the dispersion in a straight tube is specified by $n = 2$. The ratio $t_a/t$ is related to the power $n$ by

\[
t_{a/T} = 1 - 1/n. \tag{13}
\]

As a second trial, we assumed an exponentially decay function for $h$ as was used by Hamilton et al. (7) in their theory of indicator-dilution techniques. It is given by

\[
H = 0, \quad t \leq t_a \\
H = 1 - \exp[n(1 - t/t_a)], \quad t > t_a \tag{14}
\]

The time ratio relates to the decay factor $n$ by

\[
t_{a/T} = n/(n + 1). \tag{15}
\]

The function $h$ in Eq. 12 or Eq. 14 increases from 0 to $n/t_a$ at $t = t_a$. Since $d(t_a/t)/dn$ is always positive, a higher $n$ indicates a larger $t_a/t$, a sharper decay, and less dispersion. Note that the functions in Eqs. 12 and 14 are defined when $t_a$ and $t$ are specified.

For the data presented in Figure 3, the time ratio $t_a/t$ was 0.575. From Eq. 13 we computed $n = 2.4$ for the power law, and from Eq. 15 we computed $n = 1.4$ for the exponential function. The responses for finite injection predicted by Eqs. 12 and 14 are shown in Figure 3. The solid line was calculated from Eq. 12 and the broken line from Eq. 14. A reasonable fit with the exponential function is indicated. For ten sets of data fitted in this fashion, seven were best represented by the exponential function, whereas the remaining three were best represented by the power law.

The ratio $t_a/t$ for the 30 venules studied was examined in the same fashion as was that for the arterioles. The ratios ranged from 0.38 to 0.74 with an average of 0.54. Their distribution is sketched in the bottom of Figure 5.

Figures 2, 4, and 5 show that the dilution curves for venules differed from those for arterioles in three respects. First, the dilution curve recorded from a venule did not exhibit a sudden change in slope at time $t_m$. Next, the distribution of $t_a/t$ included smaller values for the venules, indicating a wider dispersion. Finally, the ratio $(t_m - t_a)/r$ was much larger than unity for the venules. Thus, the function $h$ given in Eq. 12 or Eq. 14 could not be used to define the dispersion of indicator from the mesenteric artery to the venules.

Figure 6 shows the distribution of $t_a$ among the arterioles and venules studied. The $t_a$ distribution of the arterioles was considerably skewed from that of the venules.
DISTRIBUTION OF MEAN TRANSIT TIME

Because MTT is closely related to the vascular volume of the system under study, we examined how MTT was distributed in the mesenteric network.

Dilution curves at 41 arterioles were measured. The values of MTT ranged from 2 to 7.5 seconds and the average MTT was 3.8 seconds. The distribution of MTT is shown in the top of Figure 7. The arterioles were further divided into two groups according to their size. One group consisted of arterioles with diameters larger than 35μ. The new distribution in MTT is sketched in the middle of Figure 7. The arterioles with diameters smaller than 35μ were included in the second group, and their distribution is plotted in the bottom of Figure 7. A diameter of 35μ was selected as the dividing point so that the number of vessels in each group would be approximately equal. Note that the distribution for the larger arterioles was somewhat narrower than that for the smaller arterioles. This narrow distribution possibly results because the larger arterioles are in closer proximity to the terminal arteries which form a more regular system. The wide distribution of smaller arterioles is another indication of the irregular arrangement and flow among these microvessels.

A similar analysis was also performed on the distribution of MTT for the 49 venules studied. The distributions for the entire population, for venules larger than 50μ, and for those smaller than 50μ are sketched in Figure 8. Although the blood in the smaller venules was collected by the larger venules, we surprisingly found that the values of MTT for the larger venules were smaller. This observation is probably due to shunt flow through the thoroughfare channels.

For a parallel network, MTT increases monotonically from the large arterioles to the small arterioles, the capillaries, the small venules, and the large venules. There are no overlaps in MTT distribution among any of these groups. Our results suggest that the mesenteric network cannot be modeled by a parallel network.

Although the signals from an arteriole and its adjacent venule were recorded, occasionally results from one channel were too noisy for data reduction. Considering the vessels studied in Figures 7 and 8, we could form 30 arteriole-venule pairs. The distribution of venous MTT minus arterial MTT for these vessel pairs was examined and is presented in Figure 9. The distribution of these values was much less than the distribution of the values of MTT found at arterioles or venules. When these 30 pairs were separated into two groups according to the diameter of the arterioles, the distribution was similar.

Based on the concept of repeating modular organization (8), we constructed a simplified mesenteric microvascular network and employed the principle of indicator conservation at various arterial bifurcations and venous junctions to investigate the significance of this narrow distribution (9). This theoretical analysis suggested that the narrow distribution was probably due to a higher shunt flow through the capillaries or the thoroughfare channels more proximal to the supplying arterioles of the repeating module.

In Figure 10, the major arterioles and venules in a microvascular network of 8 × 15 mm is sketched. Their arrangement is similar to the repeating modular organization found by Frasher and Wayland (8). The size of the vessels is drawn to physical scale. Because of their small dimensions the numerous capillaries and possibly some thoroughfare channels in the network were not mapped in our experiment, and they are not shown in Figure 10. The blood supplied to this network was derived from the arterioles situated at three locations.
indicated by A, B, and C. The direction of flow is shown by the arrows. At the lower left corner of the figure, the flow occasionally reversed its direction as indicated by bidirectional arrows.

The measured values of MTT are given in the figure next to the microvessel in which the measurement was made. To avoid confusion at certain locations, a line was used to connect MTT with its measurement site. The entire dilution experiment took 1.5 hours.

From either A or B, the value of MTT usually increased downstream along the arterial network. The distribution of MTT was different for the venous network. The value of MTT for the upstream venules was either close to or larger than the value of MTT for the downstream venule at B. Results presented in this figure are consistent with those presented in Figures 7-9, i.e., there is a wide distribution in the values of MTT for the arterioles and the venules but a narrow distribution in the values of the MTT difference.

**Discussion**

The injection system developed for this investigation switched the flow at the mesenteric artery from blood (the D cannula in Fig. 1) to a dextran-saline infusion (the G cannula) at time zero and back at some later time r. If, under these circumstances, the indicator was introduced so as to achieve a uniform concentration over the cross

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**FIGURE 7**

*Distribution of the mean transit time (MTT) for the arterioles (top). Since some arterioles are upstream of others, they were separated into two groups, those having a diameter larger than 35μ and those having a diameter equal to or smaller than 35μ. The resultant redistributions in MTT for these two groups are presented (middle and bottom, respectively). Note that a narrower distribution was found for the group with the larger size.*

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Figure 8

Distribution of MTT for the venules (top). The vessels were divided into two groups according to their sizes. A narrower distribution for the larger vessels (middle) similar to that seen for the larger arterioles is present. The plot for the smaller vessels is also shown (bottom).

section of the injection site, from which it was carried with a velocity profile identical to that of the blood that had flowed through the system previously, then the fluid could be considered to have been flow tagged as defined by Gonzalez-Fernandez (6).

Our injecting method was more elaborate than the conventional method which delivers a bolus of indicator over a short period through a syringe needle inserted at the injection site. Because of our rapid switching of the flow and the achievement of a uniform time-concentration curve, we probably demonstrated the sudden jump in the dilution function, $h$, for impulse injection in all of the arterioles that we studied. Since the conventional results can be considered to be a distribution of impulse injections, the correspondent dilution at various microvessels can be derived by appropriate summation of the dilution function $h$.

At the measuring site, the fiber optic bundle was centered over the image of a microvessel. The recorded light intensity was then transformed to the collecting hematocrit through a formula derived from an in vitro study using glass tubes. If the distribution of the red blood cells and the velocity inside a microvessel were identical to those in glass tubes of the same size, then the hematocrit $H_{tz}$ could be considered to be a measure of mean flow concentration as defined by Gonzalez-Fernandez (6).

The satisfaction of these two conditions, the tagging of flow and the measurement of the mean
flow concentration, is essential for the establishment of a valid indicator-dilution technique (6, 10). For parabolic flow, the indicator-dilution curve measured from the mean cross-sectional concentration does not yield a finite MTT, since \[ t \int_0^\infty h \cdot dt \to \infty \] (h is defined by Eq. 12 with \( n = 1 \)).

Our results indicate that certain characteristics found in parabolic flow are also found in the arterial system, a diverging network. Less dispersion was observed in the real system than was predicted for parabolic flow, indicating a blunt velocity profile in the system. A blunt velocity profile could result from either a blunt velocity profile in the microvessels, the absence of the no-slip condition present in parabolic flow, or both. These two factors tend to make all particles travel nearer the same speeds and hence result in a larger ratio of \( t_0 / t \).

In analyzing the shape of an indicator-dilution curve, Sheppard (1) employed a random walk model to simulate the network of real vascular beds. His one-dimensional random walk model and the probability function for the dilution curve can be derived from a one-dimensional plug flow with a dispersion in that dimension (dispersed plug flow, ref. 11). This kind of dispersion represents mass diffusion. This model does not simulate the results found in the present study of the mesenteric network. The impulse dilution curve, \( h \), in the arterioles exhibited a jump followed by a decay. This dispersion was primarily due to a nonuniform velocity profile.

In the venous system, the flow in the capillaries converges to a venule. Therefore, the dilution curve in the venule can be obtained by appropriately summing the dilution curves of the capillaries. Two

![Figure 9](http://circres.ahajournals.org/)

Distribution of the arteriole-venule MTT difference ( arteriole + venule - arteriole ) for 30 pairs. Note the narrow distributions for the population of all pairs, those with large arterioles, and those with small arterioles.

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This similarity was not evident in our records. Thus, the increase in the dispersion of the indicator as it travels from the arteriole to the venule is primarily due to the irregular pathways that converge into the venules.

These findings can be applied to other one-input microvascular beds. Only the degree of irregularities will differ from one bed to another. For example, the narrow distribution in the arteriole-venule MTT difference could be unique to the mesenteric bed.

Theoretically, more parameters can be extracted from an indicator-dilution curve to provide a better quantification of the dispersion. For example, the second moment of the dilution curve \( \left\langle \tau^2 \right\rangle \) can be calculated and used as an index defining the skewness of the curve (12). However, in the present investigation, the noise in the signal, amplified further by the factor \( \tau^4 \), resulted in an unrealizable computation of second moment. The noise due to random fluctuations in hematocrit could be reduced by improving the fiber optic system to achieve a cleaner signal.

This indicator-dilution technique measures the effect that all microvessels upstream from a selected microvessel have on an indicator. It complements existing techniques which measure local microvascular parameters. For example, the product of the measured flow and MTT can be used as a volumetric index of the microvessels upstream from the measurement site. This product might be changed by humoral and neural effects on upstream vessels. Thus, the present approach, combined with local measurements, might become a useful tool for investigating humoral and neural mechanisms such as those responsible for local regulation.

Our results are also useful to the study of transcapillary exchange of substances. The dilution curve measured in the arterioles can be regarded as the input time-concentration function to the capillaries, the sites of exchange. Most analyses assume that the function for impulse injection, \( h \), rises slowly to its maximum and then falls by an exponential decay. The input function found in the present study (Eq. 12 or Eq. 14) shows a sudden jump in concentration at the appearance time. Lee and Fronek (13) have shown that the back diffusion time is significantly influenced by how fast the concentration rises to its maximum. How do our results affect our understanding of the process of back diffusion? How will the irregularities in the flow modify the interpretation of a double indica-

kinds of dispersion are evident from this summation procedure. One is the dispersion from the mesenteric artery to the capillary. Since the capillary is still part of the diverging network in a vascular bed, its dilution function is probably similar to that of the arterioles. The other dispersion is due to the irregular pathway between the artery and the capillaries. If all of the pathways were identical, then the dilution curve for the venule would be similar to that for the arteriole.

FIGURE 10

Map of MTT distribution in a mesenteric microvascular network. The size of the vessels is drawn to scale. Arrows indicate the direction of blood flow. This network, similar to those found by Frasier and Wayland (8), derived its blood from the arterioles situated at A, B, and C. The value of MTT is given next to the vessel where the measurement was made. At several sites, a line connects the MTT value to the site to avoid confusion. The distribution is consistent with those given in Figures 7-9, a wide MTT distribution for the arterioles or venules, but a narrow one for the MTT difference.
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dilution curve? In addition, what important information on the distribution of blood flow and volume can be extracted from a thorough mapping of MTT in a vascular bed? The results obtained in the present investigation can serve as the basis for a more fundamental analysis of these questions.

References
7. Hamilton WF, Moore JW, Kinsman JM, Spurling RG: Simultaneous determination of the greater and lesser circulation time, of the mean velocity of blood flow through the heart and lungs, of the cardiac output and an approximation of the amount of blood actively circulating in the heart and lungs. Am J Physiol 85:377-389, 1928
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