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Arteriolar, Capillary, and Venular FITC–Dextran Time–Concentration Curves and Plasma Flow Velocities (40555)

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Wayland and Johnson (1) reported that it is possible to determine blood flow velocity in the microvasculature from the measurement of red cell velocity. Several laboratories (2, 3) including our own (4, 5), report that plasma velocity is less than red cell velocity as measured from the mean transit time of indicators through whole organs or tissues. An independent measurement of plasma velocity in microvessels would be necessary to study the characteristics of plasma flow in the microvasculature.

Nellis and Lee (6), Starr and Frasher (7, 8), Gaehtgens *et al.* (9), and Riva *et al.* (10) report indicator dilution techniques to monitor the dispersion of an injected indicator as it flows along individual vessels of the mesenteric microvascular network for the determination of plasma velocities using the mean transit time in cat mesenteric microvessels. Rosenblum (11) used the indicator appearance time for accessing plasma velocity in cerebral microvessels. None of these reports demonstrate the ability to analyze time–concentration curves simultaneously in numerous parallel- and series-coupled vessels from a single injection.

A method has been developed for recording indicator dilution curves in a number of series- and parallel-connected arterioles, venules, and capillaries downstream from the injection site of a single impulse of indicator. The curves may also be recorded from two or more sites in the same vessel for calculation of plasma velocity and volume flow from the mean transit times and vessel dimensions. Determination of the indicator dispersion from these curves, as a function of the flow through these series- and parallel-coupled vessels, should provide a means of assessing parameters of microvascular flow.

Methods. Cats (0.6 to 1.0 kg) were pretreated with atropine (0.075 mg/kg) to reduce salivation. The animals were anesthetized with Dial–Urethane (20 mg/kg Dial; 80 mg/kg Urethane) intravenously and supplemented as necessary. A femoral artery was cannulated for monitoring arterial pressure and a femoral vein for injections. An endotracheal tube was also inserted.

The animal was positioned on its back and an incision made to the right of the midline by thermocautery. A loop of terminal ileum was exteriorized and placed on a plastic sheet. The exposed tissues were immediately superfused with a warmed gelatin–Ringer's solution. A branch of the mesenteric artery was cannulated with PE-10 tubing for injections of FITC (fluorescein isothiocyanate)-labeled dextran (70,000 MW) (Fig. 1). The flow to the area to be studied was not interrupted as the cannula tip was positioned at the bifurcation of the arteries.

The cat was mounted on its side on a specially constructed stage of an AO microstar 20 microscope. The exposed mesentery was constantly superfused with the gelatin–Ringer's solution and maintained at 37°. Mercury light sources were used with appropriate filters for fluorescence microscopy. A closed-circuit television camera with a silicon tube was mounted on the microscope and the images recorded on a video tape recorder (VTR). Time in minutes, seconds, and tenths and hundredths of seconds was recorded from a time inserter (TI) with the VTR and monitor. The hundredth-second switch was activated by the indicator injection and recorded on the VTR. Either 10× or 15× oculars and a 10× objective were used on the microscope.

A Vista Electronics video sampler (V.S.) was connected in series with the VTR and the monitor. The V.S. had two intensity-sensitive windows, each one placed over the centerline of a microvessel on the monitor. The output of the silicon tube is linear over the range of intensities in this study. The output of the video sampler windows were also linear with changes in intensity. A pho-

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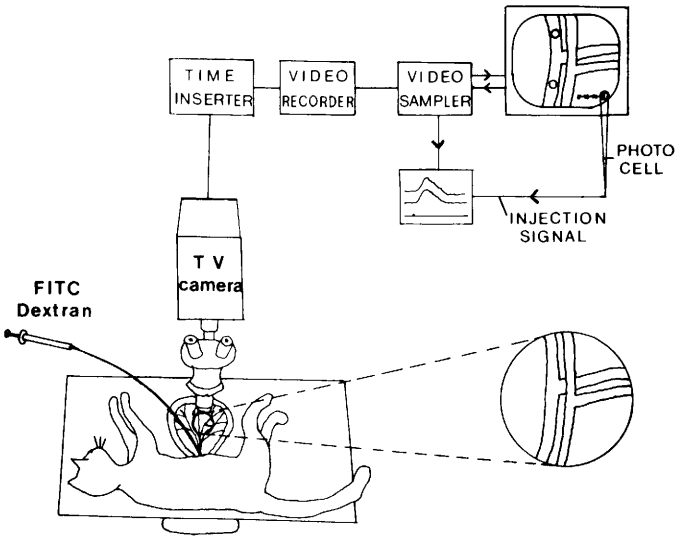

FIG. 1. Schematic diagram of the experimental arrangement.

tocell was also placed over the injection marker on the face of the monitor. The outputs of each of these were recorded. Additional vessels could be sampled by repositioning the windows with subsequent replaying of the video tape. The curves could then be compared on the same time base because the injection signals were common to all recordings. The heights of the recorded curves were not comparable one to another because of the variability in the depth of the vessels in the tissue. Vessel dimensions (internal diameters and distance between recording sites) were measured as the calibrated distance between the two windows of the video sampler. Reproducibility of the measurement of vessel diameter is represented by 30 attempts at determining this parameter in an approximately 27 μM vessel yielding a mean value of 26.90 μM, SD = 0.96, SE = 0.17.

The mean transit time ($\bar{t}$) is the time required for an average particle to flow from the point of injection to the recording site and was calculated for each curve from the following (18):

$$\bar{t} = \int_0^\infty t\, h(t)\, dt.$$

The distance between two recording sites (A and B) in a given microvessel was determined as the calibrated distance between the two windows of the video sampler. The distance (mm) divided by the difference in the mean transit times of the curves ($\bar{t}_{B-A}$) recorded at the sites gives the mean plasma velocity ($\bar{v}$) (mm/sec) in this vessel.

The volume flow (Q) as ml/sec through a vessel was calculated as follows when r is the vessel radius: $Q = \bar{v}\, \pi\, r^2$.

The vascular volume (V) of a microvascular segment was calculated as follows:

$$V = Q \times \bar{t}_{B-A}.$$

The dispersion of indicator through a microvascular network was assessed as follows. The size of the injection bolus was constant for a given experiment (0.05 to 0.2 ml). The size was determined by the quality (maximum scene contrast) of the images on the CCTV monitor, thus the better the quality, the smaller the size of the bolus. At least 80% of the experiments received the smallest bolus size. The duration of the injection was constant, 0.5 sec, and controlled by a spring-loaded syringe. Therefore, dispersion of indicator which occurred throughout the vascular network was a function of the flow patterns through the vessels. The FITC–dextran solution was 3.5 g%. Red cell aggregation was not observed subsequent to the injections.

Changes in the following parameters were used in assessing indicator dispersion. The time for the fastest-flowing indicator to arrive at the recording site from the point of injection is t_a. The closer the ratio $t_a/\bar{t}$ is to 1 the less would be the dispersion. The time for the

peak or maximum concentration of indicator to reach the recording site is t_p. The amount of dispersion occurring in the early part of the time–concentration curve is t_p-t_a and would be an index of the rate of rise of the curve. The duration of the entire curve is t_E. The dispersion of indicator during the period of the curve when concentration is decreasing is $t_E - t_p$. Arteriolar, capillary, and venular $\bar{t}$ were compared. The ratio $t_p/\bar{t}$ if less than 1 would indicate that the average particle of indicator flows more slowly than the particles at the peak indicator concentration.

Time–concentration curves and plasma velocities were determined in arterioles, capillaries, and venules of a mesenteric module from a single injection of FITC–dextran. The modular structure was described by Frasher and Wayland (12).

The effects of vasodilation were also determined in mesenteric arterioles, capillaries, and venules by the above procedures. Control plasma velocities, vessel dimensions, and dispersion indices were determined as the average of at least two successive injections of FITC–dextran. The superfusate solution was then stopped, isoproterenol (1:5000) was topically applied, and the injections of FITC–dextran were then repeated.

Related values were statistically compared and reported as ± SEM. Means were compared, using a paired t test. Means were considered significantly different if the probability was 0.05 or less.

Results. Mesenteric module. Figure 2 is a schematic drawing of a typical mesenteric module with the multiple recording sites. The inner loop is arteriolar (21 μm average diameter) and the outer loop is venular (30 μm average diameter). The inflow of indicator is from the upper right corner and the lower right corner. Venous outflow of indicator is at the lower right corner and lower left corner. Also, arteriolar outflow is at the upper left corner. The direction of flow through arterioles, capillaries, and venules is denoted by arrows. The contour of the curves change (i) along an arteriole, capillary or venule; and

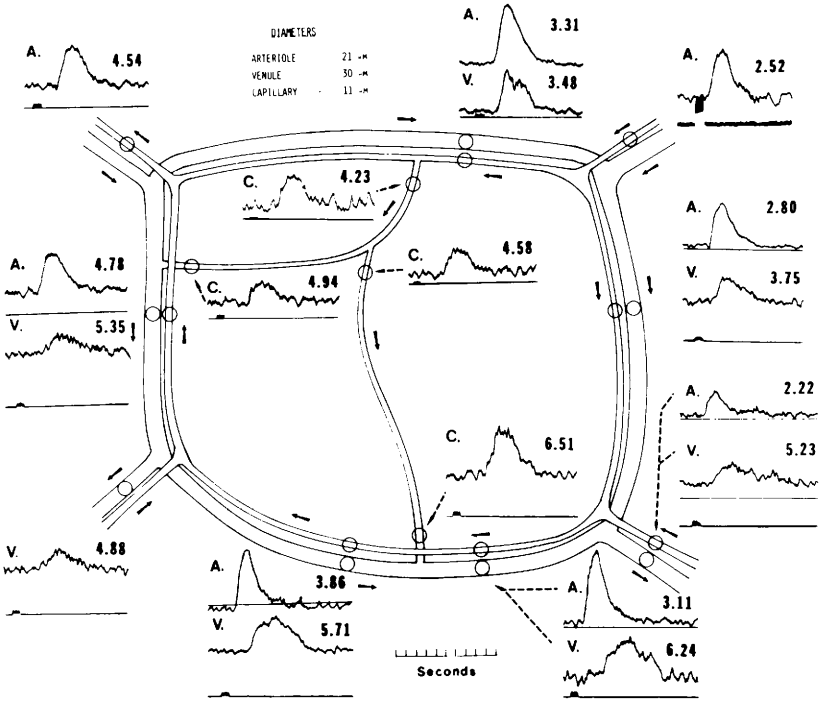

FIG. 2. Schematic drawing of a mesenteric module. Circles indicate the sites of recording of time–concentration curves. Solid arrows indicate direction of flow in the microvessels. Broken arrows relate curves to recording site. A. = Arteriole, V. = Venule, C. = Capillary. The mean transit time (sec) is noted for each curve. Final magnification 26,000×.

(ii) from arteriole to capillary to venule. Venous and capillary recordings show many small variations presumably due to uneven red cell flow through these vessels. The mean transit time for each curve is also on Fig. 2. These values show progressive increases as the indicator flows through the arteriolar ring, through the capillaries, into the surrounding venules and finally leaves the module. Plasma velocities averaged 0.49 mm/sec for the arterioles, 0.34 mm/sec for the venules, and 0.29 mm/sec for the capillaries. The velocities are computed between two sites on a vessel where there is no branching.

Arterior plasma flow velocities. In a separate group of three cats the mean plasma velocities ($\bar{v}$) obtained with two successive injections of FITC–dextran measured in 20 arteries, averaged 2.69 ± 0.41 mm/sec and 2.79 ± 0.42 mm/sec, respectively; $\bar{t}$ averaged 2.09 ± 0.18 sec and 2.16 ± 0.19, respectively. The average vessel diameter was 48.6 ± 5.8 μm and the range was 14 to 106 μm. There were no significant differences in the $\bar{t}$ or in plasma velocity between the two successive injections. There was considerable variation in velocities between vessels as represented by the large standard errors. There was also some variation in plasma velocity between injections even though the mean values were similar. One would expect this variability with the irregular nature of microvascular flow.

Figure 3 shows the general relationship between plasma velocity and arteriolar diameter. The velocity is least in the 10- to 20-μm arterioles, increases rapidly with diameter until the 51- to 75-μm range of arterioles, and then changes little up to the 90- to 110-μm arterioles. The average velocity of this latter

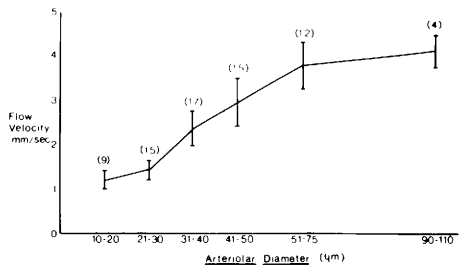

FIG. 3. Relationship between plasma flow velocity and arteriolar diameter in cat mesentery. (Brackets indicate SEM.)

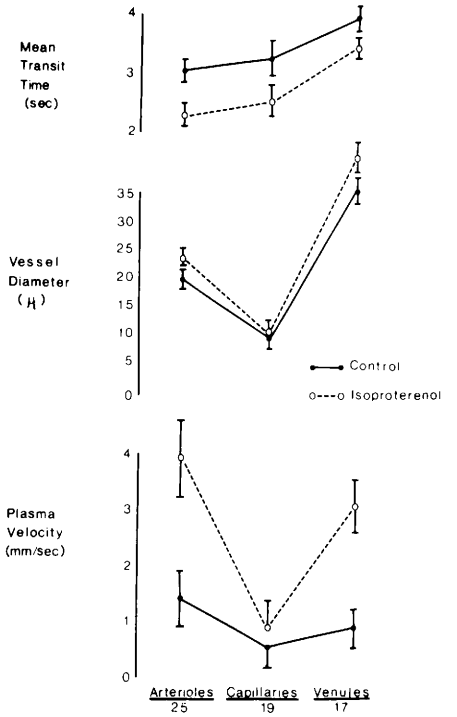

FIG. 4. Effects of isoproterenol on mean transit time, microvessel diameter, and plasma velocity in arterioles, capillaries, and venules. (Brackets indicate SEM.)

group may be comparatively low due to the small sample. The wide variation in flows at each vessel size is indicated by the large SD which was 0.67, 0.88, 1.48, 2.05, 1.84, and 0.74, respectively for the six groups of vessels in Fig. 3.

Effects of isoproterenol. The data are presented in Fig. 4. Twenty-five arterioles, 19 capillaries, and 17 venules are included in this study. The mean femoral arterial pressure of these kittens averaged 103 ± 10 mm Hg. The topical application of the drug did not significantly change the femoral arterial pressure. Therefore, changes in $\bar{t}$ and calculated flow velocity were the result of changes in flow resistance.

From point of injection to the arterioles, capillaries and venules $\bar{t}$ increased from one series-connected vascular segment to the next during the control measurements. Isoproterenol vasodilation caused a significant decrease in $\bar{t}$ to each of these segments. Capillary diameter did not significantly increase but capillary flow velocity did increase by a small but significant amount. Arteriolar and

venular diameter and velocity also increased significantly.

Table I presents indices of indicator dispersion during control and with isoproterenol from the site of injection through the segments of the microcirculation. The control ratio $t_a/\bar{t}$ averaged 0.51 ± 0.03 in the arterioles 0.45 ± 0.04 in the capillaries and 0.39 ± 0.03 in the venules. Isoproterenol did not significantly change these values. The average $t_p - t_a$ values were 0.93 ± 0.04 sec for arterioles, 1.15 ± 0.15 sec for capillaries, and 1.94 ± 0.18 sec for venules. Isoproterenol did not significantly change the arteriolar or capillary values but significantly decreased the venular values to 1.59 ± 0.13 sec. The duration of the entire curve from time of injection until the final particles of FITC–dextran pass the recording site (t_E) increased slightly from the arteriole to the capillary segment. However, the venular t_E time was significantly increased from the arteriolar and capillary values. Isoproterenol decreased these values significantly from the control in all three vessel segments. The venular values were still significantly greater than the arteriolar and capillary values; $t_E - t_p$ decreased from the arteriole to the capillary segment but the venular values increased to nearly double the capillary times. Isoproterenol significantly reduced all of these values but did not change the relationship between vascular segments.

The control $t_P/\bar{t}$ ratio averaged 0.82 ± 0.06, 0.88 ± 0.05, and 0.80 ± 0.06 in the arteriolar, capillary, and venular segments, respectively. It was not significantly changed by the isoproterenol in the arterioles and capillaries but was increased significantly in the capillaries following isoproterenol.

Discussion. There are several reports (6–10) in the literature concerning the determination of plasma flow velocity in the microvasculature. The methods as described are limited by recording from only a single vessel (9), the necessity of multiple injections of indicator to study series- and parallel-coupled vessels (6) or the ability to determine plasma velocity only from a large arteriole to a large venule (10). The simultaneous measurement of plasma velocity in several series- and parallel-coupled vessels in a microvascular area, subsequent to a single bolus injection of FITC–dextran, is described in this report.

The indicator time–concentration curves obtained in this study are typical of those conventionally recorded consequent to a bolus injection of an indicator. The arteriolar curves increase rapidly in concentration, reaching a peak. The indicator concentration then decreases, at first rapidly and then somewhat more slowly. The curves exhibit a longer t_a and delayed t_p and t_E as the plasma flow velocity decreases. The reverse occurs with increasing flow velocity. Capillary curve time parameters are greater than the arteriolar values. Venular curves are more complicated. They not only have extended time parameters but are not always smooth curves. They often have more than one peak as a result of indicator reaching the venule from capillaries with widely different flow velocities and lengths. There appears to be no consistent change in the contour of the curves as the indicator traverses a single vessel provided the upstream and downstream recordings are made in a segment between vascular branches.

Variations in light intensity with alteration

TABLE 1. INDICES OF FITC-DEXTRAN DISPERSION BEFORE AND DURING ISOPROTERENOL VASODILATION

	Arteriole	Capillary	Venule
Control			
$t_a/\bar{t}$	0.51 ± 0.03	0.45 ± 0.04	0.39 ± 0.03
$t_p - t_a$ (sec)	0.93 ± 0.04	1.15 ± 0.15	1.94 ± 0.18
t_E (sec)	6.23 ± 0.90	6.32 ± 0.97	9.44 ± 1.26
$t_E - t_p$ (sec)	3.79 ± 0.32	3.23 ± 0.24	6.21 ± 0.42
$t_p/\bar{t}$	0.82 ± 0.06	0.88 ± 0.05	0.80 ± 0.06
Isoproterenol			
$t_a/\bar{t}$	0.56 ± 0.04	0.44 ± 0.05	0.42 ± 0.04
$t_p - t_a$ (sec)	0.93 ± 0.05	1.04 ± 0.14	1.59 ± 0.13
t_E (sec)	5.05 ± 0.54	5.46 ± 0.61	7.41 ± 0.59
$t_E - t_p$ (sec)	3.09 ± 0.21	2.52 ± 0.20	4.09 ± 0.36
$t_p/\bar{t}$	0.83 ± 0.06	0.92 ± 0.08	0.85 ± 0.05

in hematocrit or vessel size may be a subject of concern. Riva *et al.* (10) report that FITC–dextran concentration is linearly related to the light intensity measured in the microvessel. However, Jendrucko and Lee (19) report that there is a dependency on hematocrit and tube diameter in determining optical density in a study utilizing glass capillary tubes. It would seem, however, from their curves that even if a bolus of indicator is injected into the arterial stream large enough to decrease the hematocrit by 10%, this would cause a change in optical density of less than 2%. Assuming the hematocrit remained the same as the indicator passed through the series-connected microvessels the time–concentration curves should be directly related since the hematocrit would be a constant for a given vessel diameter and the time–concentration curves would then be a function of the indicator passage.

Mean plasma velocities calculated from two successive injections of indicator were insignificantly different. There was some variability between determinations as would be expected since microvasculature blood flow is quite variable over short time periods. It, therefore, is often necessary to make several control velocity measurements as well as several following hemodynamic alteration to make certain that average changes are valid.

It has been reported by Zweifach and Lipowsky (13) that there is a generally direct relationship between arteriolar diameter and red cell velocity. The data in this report describe a similar relationship between arteriolar diameter and plasma velocity although our values are considerably lower. It should be noted, however, that there is a large variation in plasma velocities in arterioles in any given diameter range. There is also a considerable overlap in plasma velocity values between arteriolar diameter groups.

A valid technique must be able to measure changes in plasma velocity resulting from altered hemodynamic conditions. The plasma velocity in arterioles, capillaries, and venules was determined during control conditions and during topical application of isoproterenol. The response to isoproterenol in the microvasculature seems to differ according to the tissue studied. In tenuissimus muscle, Fronek and Zwiefach (14) reported that intravenous administration of isoproterenol resulted in decreased arteriolar and venular pressures reflecting reduced systemic arterial and central venous pressures. Hutchins *et al.* (15) showed that in normal and SHR rats arterial injections of isoproterenol caused vasodilation in the cremaster muscle microvasculature. SHR vessels had lower thresholds but the normals showed a greater percentage increase in diameter. Miller and Harris (16) found no significant changes in the microvessels of the bat wing when exposed to isoproterenol. It has been reported by Altura and Zweifach (17) that topical application of isoproterenol caused a transient dilation of the rat mesenteric microvessels. The cat's mesenteric microvasculature responses to isoproterenol in the present report demonstrate a greater and much more sustained vasodilation of both arterioles and venules. The $\bar{t}$ of the FITC–dextran from point of injection to the capillary recording sites was, as expected, somewhat longer than the arteriolar time. However, the venular time was considerably extended over the capillary time. Isoproterenol caused a marked reduction in the $\bar{t}$ to the three types of series-connected microvessels. The local vasodilation caused by the topical application of the isoproterenol combined with the unchanged systemic arterial pressure resulted in marked increases in plasma velocity in both arterioles and venules with a lesser increase in velocity through the capillaries. The minimal increase in capillary plasma velocity is apparently due to the opening of many capillaries by the precapillary vasodilation, redistributing the flow through these vessels and thereby limiting the flow increase in a given vessel. One can calculate the mean plasma flow through each segment of the microvascular area from the average velocities and the calculated vessel cross-sectional area (assuming the vessel is cylindrical) from the average vessel dimensions of each type. Control calculated average arteriolar flow was 4.62×10^{-4} ml/sec and increased to 1.76×10^{-3} ml/sec with isoproterenol. Capillary flow increased from 5.50×10^{-5} to 8.24×10^{-5} ml/sec. Venular flow increased from 1.07×10^{-3} to 4.30×10^{-3} ml/sec.

The data also allows calculation of segmental vascular capacities if one uses the mean arteriolar flows as the input flows to the

microvascular segment and the $\bar{t}$ of the indicator between segments. The volume of the arteriolar-to-capillary segment averaged 9.24 $\times 10^{-5}$ ml and increased to 4.40 $\times 10^{-4}$ ml with isoproterenol. The volume of the arteriolar-to-venular vascular segment increased from 1.16 $\times 10^{-4}$ to 2.11 $\times 10^{-3}$ ml.

The series- and parallel-coupled vascular segments individually contributed to the dispersion of indicator. The ratio $t_a/\bar{t}$ decreased progressively from the arterioles to the capillaries to the venules indicating greater dispersion of indicator through the series-coupled segments. These values were not significantly altered by the isoproterenol. The values for these ratios are less than those of Nellis and Lee (6). This is probably due to the differences in shapes of our curves in contrast to their more rounded curves. This difference has been noted to be maintained even after hemorrhage (20). However, other measures of indicator dispersion were affected by the drug; $t_p - t_a$ control values increased progressively from arteriole to capillary to venule. The arteriolar and capillary values were not significantly affected by the isoproterenol, but the venular values were significantly reduced. This would indicate that the rate of rise of indicator concentration was more rapid in venules during isoproterenol than in control conditions. It could be concluded that the indicator traverses the capillaries more rapidly and uniformly following isoproterenol vasodilation than during the control period. The control $t_E - t_P$ values decreased from arteriole to capillary and then markedly increased in venules. Isoproterenol did significantly reduce these but did not change the relative relationships. These data would indicate dispersion was considerably less in the vasodilated mesenteric microvasculature than in the control normally partially constricted vasculature. The reduction in the duration of the downslope of the time–concentration curve from the arterioles to the capillaries could be explained on the basis of a change in relative parameters of the curve. Since t_E increased only slightly from arterioles to capillaries and $t_p - t_a$ increased, then the peak of the curve was closer to the mean time of the curve. This interpretation is further supported by the finding that the ratio $t_p/\bar{t}$ for capillaries was increased both during control and during isoproterenol. This indicates that the greatest number of particles are traversing the capillaries at a rate closer to the mean transit time. The venous values for $t_E - t_P$ and $t_P/\bar{t}$ were greatly increased over the arteriole and capillary values demonstrating the presence of many parallel capillary circuits having a wide range of transit times. A significant proportion of the capillaries therefore were of relatively long circuits and/or low plasma flow velocity. These data suggest, however, that there is considerably less indicator dispersion in the vasodilated mesenteric microvasculature than in the control normally partially constricted vasculature.

Summary. A method is described for determining plasma flow velocity in microvessels of the cat mesentery. Time–concentration curves of FITC–dextran were recorded from the video-tape-recorded images of fluorescent dye as it passes through the vessels. The plasma velocity in arterioles, capillaries, and venules were determined during control periods and during isoproterenol vasodilation. Vasodilation reduces the mean transit time of the indicator from the point of injection to the arterioles, capillaries, and venules. Plasma velocity is markedly increased by isoproterenol vasodilation. There is evidence of reduced dispersion of indicator by the microvessels with vasodilation.

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1. Wayland, H., and Johnson, P. C., J. Appl. Physiol. **22**, 333 (1967).
2. Groom, A. C., Morris, W. B., and Rowlands, S., J. Physiol. (London) **136**, 218 (1957).
3. Rapapart, E., Kuida, H., Haynes, F. W., Dexter, L., Amer. J. Physiol. **185**, 127 (1956).
4. Baker, C. H., and O'Brien, L. J., Amer. J. Physiol. **206**, 1291 (1964).
5. Baker, C. H., Amer. J. Physiol. **218**, 674 (1970).
6. Nellis, S. H., and Lee, J., Circ. Res. **35**, 580 (1974).
7. Starr, M. C., and Frasher, W. G., Microvasc. Res. **10**, 95 (1975).
8. Starr, M. C., and Frasher, W. G., Microvasc. Res. **10**, 102 (1975).
9. Gaehtgens, P., Benner, K. U., Schickendantz, S., and

Albrecht, K. H., Pflugers Arch. **361,** 191 (1976).
10. Riva, C. E., Feke, G. T., and Ben-Sira, I., Amer. J. Physiol. **234,** H315–H322 (1978).
11. Rosenblum, W. I., Microvasc. Res. **2,** 399 (1970).
12. Frasher, W. G., and Wayland, H., Microvasc. Res. **4,** 62 (1972).
13. Zweifach, B., and Lipowsky, H. H., Circ. Res. **41,** 380 (1977).
14. Fronek, K., and Zweifach, B. W., Amer. J. Physiol. **228,** 791 (1975).
15. Hutchins, P. M., Greene, A. W., and Rains, T. D., Microvasc. Res. **9,** 101 (1975).
16. Miller, F. N., and Harris, P. D., Microvasc. Res. **10,** 340 (1975).
17. Altura, B. M., and Zweifach, B. W., J. Pharmacol. Exp. Ther. **150,** 23 (1965).
18. Meir, P., and Zierler, K. L., J. Appl. Physiol. **6,** 731 (1954).
19. Jendrucko, R. J., and Lee, J. S., Microvasc. Res. **6,** 316–331 (1973).
20. Baker, C. H., Davis, D. L., and Sutton, E. T., Circ. Shock **6,** 61 (1979).

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