Effects of Blood Pressure and Blood Viscosity on Fluorescein Transit Time in the Cerebral Microcirculation in the Mouse

By William I. Rosenblum, M.D.

ABSTRACT

Fluorescein transit time from pial arterioles to pial venules was measured in the mouse by analyzing motion pictures taken at 40 frames/sec. Increased blood pressure reduced the transit time. Increased blood viscosity lengthened the transit time. Viscosity was increased by rendering the animals polycythemic, or by infusions of high molecular weight dextran. Two different dextrans were used. The one with the higher molecular weight (500,000) produced a higher viscosity and a more prolonged transit time than the one with the lower molecular weight (250,000). These dextrans produced a prolongation of the transit time equal to, or greater than that, observed in the polycythemic animals, even though whole blood viscosity in polycythemia exceeded that of the dextran treated mice over a wide range of physiologically pertinent shear rates applied in vitro in a rotational viscometer. Results indicate that increases of blood viscosity produce decreases in the velocity of the plasma, and that viscosity increments brought about by raising the hematocrit, may be less effective than viscosity increments brought about by raising the viscosity of the plasma.

ADDITIONAL KEY WORDS: microcirculation, blood viscosity, pial vessel, fluorescein transit time, plasma flow, hematocrit, polycythemia, blood pressure, plasma viscosity, high molecular weight dextran, cinematography.

Fluorescein (sodium fluorescein) has been used for a number of years in the analysis of retinal circulation (1-3). More recently the cerebral vasculature has been studied with this technique of labeling the plasma with a fluorescent dye (4-6). With respect to the retina there have been many studies in which the pattern of fluorescence has been used to identify morphologic abnormalities in the vasculature (1) and there has also been some attempt to characterize retinal blood flow in health and disease by measuring the speed with which the fluoresceinated plasma travels from the retinal arteries to retinal veins (1-3). In the brain, observations have been restricted to vessels on the cerebral surface (the pial vessels) and we are unaware of attempts to do more than characterize the pattern of vascular filling, or the structural details of the vascular pattern (4-6). We are not aware of systematic experimental studies designed to examine factors that affect fluorescein transit time through either the retinal or cerebral microcirculations. The cerebral microcirculation is the major interest of our laboratory, and it is the purpose of this communication to analyze several factors affecting fluorescein transit time through the cerebral vasculature. We are particularly interested in the effect of alterations in blood viscosity, and the interaction of viscosity changes with changes in blood pressure.

Methods

ANIMALS, AND SURGICAL, MICROSCOPIC TECHNIQUES

Male Swiss mice were used weighing 20 to 35 grams. These animals were prepared as in our previous studies of cerebral microcirculation (7).
Cranialotomy was performed after tracheotomy, in animals anesthetized with sodium pentobarbital (7.5 mg/100 g). The dura was stripped, revealing the underlying, transparent arachnoid membrane beneath which lay the pial vasculature in the subarachnoid space. Thus, these vessels were constantly bathed by their own cerebrospinal fluid. In addition, the transparent arachnoid membrane was kept moist with isotonic saline. Most physiological solutions were not needed because the observations lasted only a few minutes, and because vascular reactivity was not one of the variables under investigation. Rather, what was being measured was the transit time of the fluorescein through vessels of a certain range of sizes, following manipulation of blood pressure and viscosity. That both the surgical procedure and the observational techniques were relatively atraumatic was attested to by the absence of leaking dye from the pial vessels. The vessels themselves were observed through a Leitz Ultraphot microscope at magnification of 135x. They ranged from 10 to 70 μ in diameter, with most being 50 to 70 μ. There were no significant differences between the mean vessel diameters of any experimental group and its control.

**Fluorescein Transit Time.**—Sodium fluorescein was injected into the tail vein, as a 2% solution in a 0.2-ml volume. The final concentration of dye in the blood stream is approximately that required to give maximal fluorescence as calculated in vitro (2). In a preliminary series of experiments 0.04 ml of a 10% solution was used. There was no significant difference in the fluorescein transit time associated with this smaller volume; however, it was found that the degree of fluorescence was highly variable and often too faint to be useful. Therefore, the larger volume was selected for experimental use. A Hycam motion picture camera was used to film the passage of fluorescein from arterioles to venules. The arrangement of lenses and the 200 watt mercury lamp light source have already been described (8). Filming was done at 40 frames/sec using high speed Ektachrome forced processed to an ASA equivalent of 1,000. Accurate framing rates were assured by simultaneously recording, on the edge of the film, the signal from a timing light source have already been described (2). In a preliminary series of experiments 0.04 ml of a 10% solution was used. There was no significant difference in the fluorescein transit time associated with this smaller volume; however, it was found that the degree of fluorescence was highly variable and often too faint to be useful. Therefore, the larger volume was selected for experimental use. A Hycam motion picture camera was used to film the passage of fluorescein from arterioles to venules. The arrangement of lenses and the 200 watt mercury lamp light source have already been described (8). Filming was done at 40 frames/sec using high speed Ektachrome forced processed to an ASA equivalent of 1,000. Accurate framing rates were assured by simultaneously recording, on the edge of the film, the signal from a timing light generator. A BG 12 excitation filter and a barrier filter with a transmission peak at 510 mn used were to detect the green fluorescence emitted by the fluorescein. The film was projected with a model LM film analyzer, and the frame was noted at which fluorescence first appeared in the field in any arteriole less than 50 μ in size. The dye passed through the arterioles into the underlying brain, where it produced a fluorescent "blush." The dye then emerged from the underlying brain via venules which empty into the pial vessels. The number of frames was counted between first appearance of dye in the arteriole, and first appearance in any venule less than 50 μ in size. By keeping arbitrary size limits of 50 μ or less for the diameter of vessels used in analysis, one could maintain relative homogeneity of the size variable for all experimental and control groups. Since the framing rate was known, the time taken for dye to travel from arteriole to venule could be calculated from the number of frames elapsing during this passage.

**Measurements of Blood Viscosity.**—The measurements were performed on a range of shear rates utilizing either a Couette viscometer (GDM, manufactured by Allied Research Associates, Concord, Mass.), or a Wells-Brookfield cone plate viscometer (model 1/2 LVT, Brookfield manufacturing Corp.). Blood for measurement was anticoagulated with either ACD solution or heparin. Microhematocrit determinations were performed on all samples.

Measurements of viscosity required 1.8 ml of whole blood, hence exsanguination of the mice was required. Fluorescein may alter the viscosity, at least by producing hemodilution and reduction of hematocrit. Since it is not possible to obtain a sample of blood at the moment fluorescein passes from arterioles to venules, and since it is not possible to obtain an adequate sample from the region of the fluorescein "front" as it passes from arteriole to venule, it is not possible to measure the viscosity of the microvolume of blood actually making this transit. There is no way to determine whether the viscosity in the fluorescein front is closer to that of control blood (blood without fluorescein) or to that of blood from mice in which fluorescein has mixed completely with the circulation. We elected to demonstrate the effects of dextran or of polycythemia on viscosity by making our viscosity measurements with blood from animals that had not received fluorescein. Our purpose is only to show the general effect of the procedures on blood viscosity and the relative changes of in vivo viscosity at the fluoresceinated front. Similar assumptions underlay all work attempting to relate in vitro viscosity values to apparent viscosity in the microcirculation. These data merely confirm in the mouse what was already well known from studies of other mammals. We assume that these measurements are reflected, in a quantitative manner, by changes of in vivo viscosity at the fluoresceinated front. Similar assumptions underlay all work attempting to relate in vitro viscosity values to apparent viscosity in the microcirculation.

**Alterations of Viscosity.**—Blood viscosity was altered in one of several ways. First, viscosity was altered by raising the viscosity of plasma. This was accomplished by injecting solutions of high molecular weight dextran into the tail vein. Two solutions were used, one containing dextran with an average molecular weight of 250,000 (D-250)
and one with average molecular weight of 500,000 (D-500). These dextrans were dissolved in distilled water to make a 30% solution. Solutions of greater concentration were too difficult to inject. At these concentrations, 0.3 ml had to be injected to achieve the desired level of blood viscosity, in part because the dextran acts as a plasma expander, thereby reducing hematocrit, an effect which tends, in itself, to reduce the viscosity of whole blood (9).

In addition to raising blood viscosity by increasing the viscosity of blood plasma, we have also raised blood viscosity by increasing the hematocrit. This was accomplished by daily intraperitoneal injection of packed red blood cells (10).

**ELEVATION AND MEASUREMENT OF BLOOD PRESSURE**

Each of the procedures used to elevate blood viscosity, also elevated the blood pressure of the animals involved (see below). In addition, experiments were carried out in which blood pressure was elevated by injecting norepinephrine (1 to 10 μg) subcutaneously. These doses do not produce constriction of pial vessels when directly applied to the cerebral surface (7). Systolic pressure was measured by a tail cuff and pulse transducer (11, 12).

**Results**

**Effects of Blood Pressure on Fluorescein Transit Time.**—When the systolic pressure was raised by injection of norepinephrine, the fluorescein transit time was significantly shortened as indicated in Table 1. Table 1 compares results from two groups of mice, those receiving the pressor drug and those which did not. Animals were not used as their own controls, in part because of residual fluorescence that often remains after a single injection of the dye, and alters the background on which the second measurement would have to be made.

**Effects of High Molecular Weight Dextran or Polycythemia on Blood Viscosity.**—Figure 1 shows the data for representative samples of blood anticoagulated with heparin. The rise in viscosity with decreasing shear rate is clearly shown. The shear rates utilized are all within the physiologic range for the vessels in question. This can be ascertained by computation from measured cellular velocities near the wall of these vessels (8). Qualitatively similar data have been obtained for large numbers of mice, with blood anticoagulated by either heparin or ACD solution. The mice utilized in constructing Figure 1 came from groups with varying hematocrits. Thus normal animals had hematocrits ranging from 26 to 43 (M = 39, n = 5), polycythemic mice had values of 64 to 73 (M = 68, n = 9), mice treated with D-250 had hematocrits of 36 to 43 (M = 39, n = 5) and those treated with D-500 had values of 32 to 37 (M = 34, n = 5). Unless the hematocrits of identically treated animals are identical, the viscosities may differ, since viscosity is partially dependent upon hematocrit. This can be observed in Figure 1 which illustrates the elevated viscosity of polycythemic blood and the lower, normal viscosity of blood with a normal hematocrit. Figure 1 also shows the viscosity of blood from animals treated with high molecular weight dextran. This blood has a reduced hematocrit due to the hemodilution produced by the dextran. Nevertheless, as the Figure indicates, the blood containing dextran has an elevated viscosity, higher than that of normal blood, in spite of the latter's higher hematocrit. Finally, Figure 1 shows that of the two types of dextran, D-500 induces the greater elevation of blood viscosity. This is due, at least in part, to the higher viscosity of plasma containing D-500 as compared with plasma containing D-250, the molecule with the lower molecular weight. The viscosity of

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>P.T.T.*</th>
<th>P.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 20)</td>
<td>70 ± 5</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Norepinephrine (n = 29)</td>
<td>134 ± 10</td>
<td>0.46 ± 0.03</td>
</tr>
</tbody>
</table>

*Fluorescein transit time (mean seconds ± se).
plasma from animals treated with D-500 was approximately 3.3 cp, (N = 3, range 3.32 to 3.36) compared with 2.7 cp (N = 3, range 2.59 to 2.88) for plasma containing D-250, and 0.99 cp for plasma from normal mice (N = 3, range 0.95 to 1.02). The measurements of plasma viscosity were performed at 60, 30, 15, and 6 sec⁻¹, and values remained constant over this range.

Effects of High Molecular Weight Dextran on Fluorescein Transit Time.—Table 2 shows that both D-250 and D-500 significantly prolonged the arteriovenous passage of sodium fluorescein. The control values shown were obtained from a large group of mice not injected with any material. A smaller group of 10 mice received intravenous saline in the same volume as the dextran given to the

| TABLE 2 |
|-----------------|-----------------|-----------------|
| Effect of High Molecular Weight Dextran on Fluorescein Transit Time |
| Control (n = 20) | Dextan-250 (n = 10) | Dextan-500 (n = 31) |
| t < .05 | t < .01 | t < .01 |
| 0.00 ± .03 | 0.50 ± .88 | 0.60 ± .91 |
| 1.01 ± .08 | 0.00 ± .88 | 0.00 ± .88 |

*Two-tailed t-test; †Mean seconds ± se; ‡One-tailed t-test.
TABLE 3
Effect of High Molecular Weight Dextran on Systolic Blood Pressure

<table>
<thead>
<tr>
<th></th>
<th>Control* (n = 20)</th>
<th>Dextran-250 (n = 21)</th>
<th>Dextran-500 (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>70 ± 5</td>
<td>97 ± 11</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>P</td>
<td>&lt; .01</td>
<td>P &lt; .01</td>
<td></td>
</tr>
</tbody>
</table>

*Anesthetized with pentobarbital (7.5 mg/100 g), tracheotomy and craniotomy performed.
† Mean (mm Hg) ± SE.

TABLE 4
Effect of Polycythemia on Blood Pressure and Fluorescein Transit Time

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 20)</th>
<th>Polycythemic (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>79 ± 5</td>
<td>93 ± 10</td>
</tr>
<tr>
<td>P</td>
<td>&lt; .05*</td>
<td>P &lt; .05</td>
</tr>
<tr>
<td>P.T.T.*</td>
<td>0.68 ± 0.07</td>
<td>0.87 ± 0.10</td>
</tr>
</tbody>
</table>

* Two-tailed t-test; † Fluorescein transit time (mean seconds ± SE); ‡ One-tailed t-test.

TABLE 5
Fluorescein Transit Time in Polycythemic and Control Mice with Matched Blood Pressures

<table>
<thead>
<tr>
<th></th>
<th>B.P.</th>
<th>P.T.T.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 12)</td>
<td>92 ± 10</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>Polycythemic (n = 12)</td>
<td>93 ± 10</td>
<td>0.93 ± 0.12</td>
</tr>
</tbody>
</table>

* Fluorescein transit time (mean seconds ± SE); † Two-tailed t-test.

These data indicate that fluorescein transit

dextran, polycythemia not only increases blood viscosity (Fig. 1), but also prolongs the transit of fluorescein through the cerebral microcirculation (Table 4). The polycythemic mice also had elevated blood pressures (Table 4) which undoubtedly reduced the effects of polycythemia on fluorescein transit time. This is indicated by a comparison of Tables 4 and 5. Table 5 compares polycythemic mice with normal mice whose blood pressures had been elevated by norepinephrine. Each polycythemic mouse was matched with a normal mouse having a blood pressure most closely resembling that of the polycythemic animal. With the blood pressures in the two groups equated, the prolongation of transit time in polycythemia becomes more apparent and the difference in transit times shown in Table 3 becomes a difference of greater magnitude and higher statistical significance, as indicated in Table 5.
time through the cerebral microcirculation can be affected by blood pressure, high molecular weight dextran and polycythemia. Although it is difficult to rule out some binding of fluorescein or fluorescent plasma to erythrocytes, we assume that fluorescein transit time represents plasma flow almost exclusively, because the fluorescence is much greater in zones rich in plasma compared to zones rich in cells but poor in plasma, and because independent measurement of erythrocyte velocity has demonstrated that after elevation of viscosity the flow of fluorescein (13) like the flow of plasma (9, 14) is retarded more than the flow of erythrocytes. Since fluorescein travels with the plasma and since it is reasonable to assume that increased pressure will accelerate plasma flow, it is understandable that increased pressure accelerated the transit time of fluorescein. This acceleration should not be construed as evidence against the presence of "autoregulation" or constancy of flow in the presence of altered pressure. Constancy of flow in autoregulation refers to the volume rate of flow, while our data and the terms "transit time" or "plasma flow" refer only to the velocity with which a given unit of plasma, represented by the fluorescein label, moves from point to point. The volume rate of flow would depend on the diameter of the vessels and cannot be calculated from transit time data unless some method is found for measuring the concentration of the plasma indicator. It is possible that a change in the volume of the vascular bed could ameliorate the effect of a change in plasma velocity or plasma transit time (see discussion on polycythemia); however, such effects should not obscure the important rheologic fact that the linear velocity of the plasma has been altered.

Although we are not dealing with volume flow, the diameter of the vessels becomes important in another context. We have taken care to confine our measurements to vessels between 10 and 70µ in diameter. This was done to minimize changes in the linear distance from arteriole to venule. However, if a systematic shift in diameter were to occur in one group of mice, dilatation for example, then a systematic decrease would be introduced in the distance from arteriole to venule, since a vessel within the prescribed size range would actually be smaller than it appeared and hence would be closer to the capillary bed. Such a change might actually reduce the apparent transit time. To eliminate concern on this point, several facts should be stated. First, as previously indicated in studies with angiotensin or angiotensin plus norepinephrine (12), acute pressure increases like those observed herein are only associated with vasodilatation of a few minutes duration, after which some degree of autoregulation is manifest and the vessels return to control dimensions or even constrict. The present observations, with norepinephrine or with dextran as the pressor agent, confirmed our earlier work and all measurements of transit time were performed after dilatation had ended. Thus, our results concerning blood pressure and A-V transit time cannot be accounted for on the basis of vasodilatation. Second, since CO2 is a potent determinant of vascular diameter we have also investigated arteriolar PCO2 in several groups of animals treated in the same manner as those in this study, with D-250 or D-500, and subjected to identical operative procedures. These measurements on Hood from the carotid arteries examined in an ultramicro blood gas analyzer revealed a PCO2 of 38 ± 3 (n = 10; mean ± SE) for the D-250 group and 41 ± 4 (n = 11) for the D-500 group. The mean values are close to 40, the usual mammalian norm, and are not significantly different from the value of 43 ± 6 (n = 11) found in our control mice, operated upon but not given high molecular weight dextran. Thus, changes in blood gases cannot account for our data, at least in dextran-treated animals. Finally, these considerations of vascular diameter are only pertinent to our conclusions because of a theoretical possibility that the location of point of measurement along arteriole or venule might bias our results, if varied inadvertently in a
In dextran-treated animals, the plasma viscosity was elevated; consequently, one might expect the flow of fluorescein to be retarded. Since D-500 raises the viscosity of plasma more than D-250, it seems reasonable that the former retarded plasma flow to a greater extent than did the latter. However, the increased viscosity of the plasma might act not only as a direct cause of reduced plasma velocity, but also as an indirect cause of prolonged fluorescein transit. For example, if increasing plasma viscosity in some way altered the distribution of erythrocytes across the lumen, the mean velocity of plasma or a plasma indicator might be altered. Normally, in the microcirculation, the red cells tend to move toward the center of the vessel, and the zone near the vessel wall becomes relatively rich in plasma (9). Possibly increasing the viscosity of plasma accentuates the movement of erythrocytes toward the center of the vessel with resultant displacement of additional plasma toward the vessel wall. Since flow is slowest near the vessel wall (9), such a displacement would increase the proportion of plasma located in the zone of slowest flow. Such an explanation for prolonged plasma transit time might also pertain to the data from polycythemic mice, if the pial vessels in these animals also had a widened plasmatic zone, perhaps because of displacement of plasma toward the periphery by the increased mass of centrally migrating erythrocytes.

Whatever the explanation for the prolonged fluorescein transit time in these hyperviscous animals, the effect of polycythemia would seem worth emphasizing since many workers have pointed out the relatively weak effect of hematocrit on blood flow within the microcirculation (9, 15-18). Of course, our own data can only be cautiously compared with data on blood flow because fluorescein serves principally to label only one component of the blood, namely the plasma; while as indicated earlier, the velocities of plasma and erythrocytes may be differentially affected by alterations in viscosity (9, 13, 14). Nevertheless, it seems relevant to point out that in small tubes blood flow appears dominated by the plasma viscosity rather than the hematocrit, with the apparent viscosity of blood approaching that of the plasma phase alone as the vessel diameter is diminished (16). However, in tubes of 20 to 60μ, the apparent blood viscosity may be 15 to 60% greater than plasma viscosity alone, and increases in hematocrit can blunt the viscosity profile in such tubes (16, 18). Thus, although other factors may be of greater importance, hematocrit can affect flow at least in vitro models of the microcirculation. Our data illustrate these points in vivo. The polycythemic mice did display a prolonged fluorescein transit time. However, the degree of prolongation was the same or less than that produced by infusion of
high molecular weight dextrans (Tables 2, 4, 5) even though the blood viscosity in polycythemic mice was considerably higher than the viscosity of blood from the mice given dextran (Fig. 1). In other words, increasing the hematocrit did reduce plasma flow, but not to the degree produced by an elevation in the viscosity of plasma.

Our data are also of interest because of their bearing on the general relationship of hyperviscosity to cerebral circulation. The effect on flow of changes in viscosity, in vitro, in tubes of fixed diameter, may be somewhat different than the effect in vivo, if the vasculature is capable of modifying its diameter to compensate for changes in viscosity. The increased viscosity in polycythemia has been thought to result in cerebral dysfunction because of a viscosity-dependent decrease in cerebral blood flow (19, 20). Such a decrease in cerebral blood flow has been demonstrated in some human patients (19, 20), however, such patients may also have a tendency toward thrombosis and may therefore present a picture of neurologic dysfunction due to hyperviscosity effects superimposed on an already abnormal vascular bed (21). Other workers have presented evidence of normal cerebral blood flow both in polycythemic animals and in animals given high molecular weight dextran (22, 23). They suggested that compensatory vasodilatation may have resulted in an unchanged cerebral blood flow during hyperviscous states. In our studies the increased plasma transit time (decreased velocity of plasma) could be accompanied by sufficient vasodilatation to produce a normal volume flow. Our observations indicate marked plethora in the pial microcirculation of polycythemic mice and offer some support for this suggestion. Studies of blood flow in the forearm have also suggested that the effects of changing blood viscosity may depend upon the initial vascular resistance or diameter (24).

The present findings serve to focus attention on the factors which can affect fluorescein transit time, or plasma velocity, at least in the cerebral microcirculation. Our animal models indicate that increases in blood pressure can shorten transit time, whereas increases in viscosity can prolong transit time. These experimental, in vivo, data may aid in the interpretation of data from clinical studies employing fluorescein for the analysis of cerebral circulation (5, 6). Our data may also be useful in the planning of mathematical models and in vitro models which will accurately reflect microcirculatory phenomena.

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References


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