Effects of Reduced Hematocrit on Erythrocyte Velocity and Fluorescein Transit Time in the Cerebral Microcirculation of the Mouse

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ABSTRACT
The red blood cell velocity and the arteriole-to-venule transit time of sodium fluorescein were measured in the pial microcirculation of 15 mice immediately before and after phlebotomy and hemodilution. The acute anemic state produced by the phlebotomy and hemodilution was accompanied by an increase in the velocity of the red blood cells and of the fluorescent plasma. Such increments in velocity must be attributable to either an increased blood pressure or a fall in cerebrovascular resistance. Increases in pressure were generally absent, as was vasodilation, one cause of a decreased resistance. On the other hand, decreased blood viscosity accompanies a fall in hematocrit and could therefore account for a decreased vascular resistance in every animal. These data are consonant with earlier reports in animals and man of an increased cerebral blood flow accompanying anemia. Moreover, the data support those workers who attributed this increase in flow at least partly to a decrease in blood viscosity.

KEY WORDS
rheology anemia blood viscosity plasma flow plasma velocity cerebral blood flow

In 1951 Heyman et al. reported that cerebral metabolism in human subjects was adversely affected by anemia (1), and they noted that cerebral blood flow was significantly increased in anemic patients and cerebral vascular resistance was significantly reduced. Since their original paper, there has been little work on the relation of cerebral blood flow to reduced hematocrit levels. The increased flow in anemia has been confirmed in animals and man (2–4); however, the mechanism of this increase has been called into question. Haggendal et al. found that increased flow was related not to the reduced number of erythrocytes per se but to a reduction in the oxygen-carrying capacity of the blood (3). Low levels of oxygen in arterial blood or tissue result in cerebral vasodilation and a fall in cerebrovascular resistance (5). However, according to Heyman et al. (1), reduced oxygen levels with consequent vasodilation cannot completely explain the increased cerebral blood flow found in anemic patients, since breathing an atmosphere of 100% oxygen would reduce but would not abolish the increase in flow. For this reason, Heyman et al. concluded that decreased cerebrovascular resistance was produced both by a vasodilation from low cerebral oxygen tensions and by a decrease in blood viscosity caused by the reduced hematocrit.

In the studies referred to above, measurements of cerebral blood flow were based on flow through large volumes of brain tissue in which the size and nature of the blood vessels vary widely. It would also seem desirable to study flow in single vessels in the microcirculation supplying the brain, and to separately investigate the movement of erythrocytes and the movement of plasma. The value of such procedures is emphasized by studies indicating that the predicted in vivo effects of
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viscosity changes measured in vitro may be dramatically modified by microcirculatory events (6), and by previous publications showing that RBC and plasma may be differentially affected by alterations in blood viscosity (7, 8). In the present study we have reexamined the relation between reduced hematocrit and cerebral blood flow by studying corpuscular velocity and the velocity of fluorescein, a plasma label, within the pial microcirculation.

Methods

Experimental Animals and Observations of Pial Vessels.—Male Swiss mice were anesthetized with pentobarbital sodium. Previously described surgical procedures (8—10) included tracheotomy, craniotomy, and stripping of the dura to expose the underlying “pial vessels,” which lie under the transparent arachnoid membrane in the subarachnoid space on the surface of the brain. Here they are bathed in their own cerebrospinal fluid, and their branches form the principal blood supply for the underlying cerebral cortex. Pial arterioles and venules 10—65 μ i.d. were observed during these experiments, using a Leitz Ultropak microscope and a 22 X objective. In addition to direct visual observation through the microscope, the vessels were photographed and data collected as indicated below.

Measurements of Erythrocyte Velocity and Plasma Velocity.—Erythrocyte velocity was measured by high speed micrcocinematography at framing rates as high as 1200/sec. Details are described in a previous publication (10). When the film is projected at normal speeds or as single frames, individual erythrocytes can be followed as they move across the screen in slow motion. The cellular velocity was calculated from the framing rate, the magnification of the film projected on the screen, and the number of frames taken by a cell or group of cells in traversing a measured distance along the vessel. Measurements of erythrocyte velocity were made in the same mouse before and after abrupt reduction of hematocrit (see below). Repeated examination of the same film footage gives velocity values within 10% of each other. There is great variability in velocity values from animal to animal, depending on blood pressure, vessel size, the place in the vascular bed, and other factors including the portion of the cardiac cycle in which the measurement is made. A velocity pulse is produced by the cardiac pulse, but with the present technique is evident in only some of the vessels. Velocity was measured as closely as possible to the minimal (nadir velocity) and maximal (peak velocity) points in a given pulsatile cycle. For vessels in which a velocity pulse was not clearly defined, velocity was measured at a randomly selected point on the film. As indicated in our earlier publication (10) and by Bloch (11), the density of the optical image makes it much more difficult to measure velocity at the center of the vessel than at the wall. Therefore, only nine measurements were made of velocity at the vessel center. These were accompanied by measurements at the vessel wall, over exactly the same film footage. In 12 other vessels, velocity was measured only of erythrocytes at the vessel wall.

As pointed out by Bloch (11), high-speed micrcocinematography reveals that the plasma gap between erythrocytes and the vessel wall is not of uniform width, in spite of a uniform appearance when photographed at slower speeds. In agreement with Bloch, we found that the cell column had an irregular width, with many erythrocytes in the most peripheral layer of the axial stream apparently touching the vessel wall or remaining 1—2 μ away from it, and retaining their position as they moved downstream for distances of as much as 75 μ (11). In the present study, these cells, closest to the vessel wall, in the most peripheral layer of the axial stream, at the widest part of the stream, were used for measurements of velocity “at” the vessel wall. A single portion of such a cell (leading edge, trailing edge, or center) was selected for tracking. Since this cell remained at the edge of the axial stream throughout the tracking period and since the stream maintained its configuration throughout that period, the distance of the cell from the center of the stream remained relatively constant during tracking. In most vessels the axial stream did not change width after the onset of anemia. In such vessels, not only were the tracked cells always the cells nearest to the wall and farthest from the center, but their absolute distance from the center remained relatively constant. In all vessels, the distance from the portion of the cell being tracked to the vessel wall remained relatively constant at 3 ± 1 μ, both before and after production of anemia. This distance from the wall was obtained from actual measurements where the walls were clearly visible, and is consistent with the width of the peripheral plasma layer measured by comparing the radius of the axial stream photographed at 500—1200 frames/sec with the radius of the plasma column labeled with fluorescein and photographed at 40 frames/sec (see below). The latter comparison showed that the average width of the peripheral plasma layer is 1.9 ± 0.5 μ, a value in agreement with the width of 2—3 μ shown by Bloch (11) for rat mesenteric vessels 10—40 μ in diameter. The width of the plasma layer when
estimated in this manner is somewhat smaller than the distance from the vessel wall to the axis of the cell nearest the wall, because the plasma layer when measured with low speed techniques is not a true plasma layer but contains portions of the cells nearest the wall (11).

Plasma velocity was measured by using sodium fluorescein as a plasma marker (8, 11). The fluorescent dye (0.2 ml of a 2% solution) was injected into the tail vein, and its first passage from a pial arteriole, through the underlying cortex, to a pial venule, was filmed with appropriate excitation and barrier filters, as described in earlier publications (8, 12). Only arterioles and venules 11–50 μ in diameter were used in this portion of the study. Filming was done at 40 frames/sec, and the arteriole-venule transit time was determined by counting the number of movie frames between first appearance of the dye in a pial arteriole of appropriate size and first appearance in a venule also falling within the prescribed size range (8, 12). Because of interpretive problems arising from recirculation of dye and residual fluorescence, each mouse did not serve as its own control. Rather, the values in anemic mice were compared with those from normal controls obtained in earlier studies (8, 12).

Production of Acute Anemia.—An acute reduction of hematocrit was obtained by removing approximately 0.3 ml of blood from the jugular vein or the retro-orbital plexus and replacing it immediately with an equal volume of homologous plasma injected into the tail vein. The purpose of the plasma injection was to restore a normal blood volume and minimize the fall in blood pressure that might otherwise follow the large phlebotomy. In practice, however, the phlebotomy and replacement injection were followed by quite variable changes in blood pressure (see Results).

Measurements of Blood Pressure.—Systolic blood pressure was measured with a miniature blood pressure cuff and a pulse transducer placed on the mouse's tail (13, 14).

Measurements of Hematocrit and Blood Gases.—Microhematocrit determinations were performed on each mouse before and after production of anemia. Arterial CO₂, O₂, and pH levels were obtained on all anemic animals and in control mice treated in an identical manner except for the absence of phlebotomy. An ultramicro blood gas analyzer was used (Instrumentation Laboratories).

Results

Table 1 shows hematocrit, erythrocyte velocity, and vascular diameter for each arteriole or venule in which velocity was measured both before and after phlebotomy.

Reduction of Hematocrit and Increase of Erythrocyte and Plasma Velocities.—Fifteen mice were bled. Due to laboratory error only 14 pairs of hematocrit determinations were performed. Naturally, phlebotomy with replacement of plasma resulted in a decreased hematocrit in every case. The mean hematocrit of the 14 mice before phlebotomy was 44 ± 1 SE and after, 37 ± 1. The average reduction in hematocrit was 15% ± 1.3% (P < 0.01, Wilcoxon test [15]).

Twelve of the 15 mice were used for studies of erythrocyte velocity in arterioles, venules, or both. Three mice were used only for measurements of fluorescein transit time (arteriole-venule transit time), and two were used for measurements of both the velocity and transit time. The velocity was measured in 13 arterioles (12 mice) before and after phlebotomy. Velocities depended on whether they were measured at a random point in the velocity pulse or at the peak (P) or nadir (N) of the pulse cycle. For erythrocytes closest to the vessel wall, these velocities varied from 2.7 to 10.3 mm/sec before anemia, and from 4.1 to 13.8 mm/sec after anemia. In animal 7, velocity was also measured in the center of the axial stream, where it was found to be faster (0.5 mm/sec before and 4.0 mm/sec after anemia), than near the wall. In 10 of the 12 mice (11 of the 13 arterioles), all velocity measurements showed an increase after phlebotomy. These mice were scored as showing an increased arteriolar erythrocyte velocity after induction of anemia. The increase ranged from 6% to 213% (median 52%). In one mouse, velocity increased (40%) at the nadir but did not change at the peak of the pulse cycle. This mouse was conservatively scored as showing no change. Only one of the 12 mice showed a diminution in the arteriolar velocities (1–5%). The finding of an increased arteriolar velocity in 10 of the 12 mice was one which would be highly unexpected on the basis of chance alone (P = 0.012, sign test [15]). In the venules, velocity was also significantly increased with the onset of anemia. Eight venules were examined in eight...
### TABLE 1
Effects of Phlebotomy and Hemodilution on Hematocrit, Erythrocyte Velocity, Systolic Blood Pressure, and Arteriole and Venule Diameters

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Erythrocyte velocity "at wall" was always measured from cells nearest the vessel wall, and in all venules and one arteriole (see text), center-line velocities were also recorded. In vessels with demonstrable pulse, peak (P) and nadir (N) velocities are given. Velocities not labeled P or N are from vessels in which a pulse could not be defined. Two arterioles were examined in mouse 4. Values are before (B) and after (A) phlebotomy.
mice. In each case velocity was measured for erythrocytes nearest the wall and for those at the center of the stream. Before reduction of hematocrit, the velocities ranged from 0.7 to 2.0 mm/sec at the wall, and from 0.9 to 3.0 mm/sec at the center. At the wall, every value but one increased with anemia (P < 0.02, Wilcoxon test). At the center of the venules every value increased with anemia (P < 0.01 Wilcoxon test). These increments in velocity in venules following anemia, ranged from 32% to 232% (median 105%).

In the mice in which fluorescein arteriole venule transit time was measured, the reduction of hematocrit was followed by acceleration of the plasma marker. Thus the mean transit time was 0.35 ± 0.03 seconds (SE) in the anemic group (n = 5), compared with 0.65 ± 0.03 seconds for a control group (n = 11) similarly anesthetized and operated upon. The difference between the two groups is highly significant (P < 0.01, t-test). The anemic and control groups had virtually identical blood pressures, 87 ± 7 for the anemic and 91 ± 3 for the control animals.

Effects of Phlebotomy on Blood Pressure and Blood Gases.—Systolic pressure was successfully measured before and after phlebotomy in 15 mice. Mean pressure before phlebotomy was 84 ± 4.0 and after phlebotomy, 82 ± 5.3, indicating the absence of a systematic change in the pressure. In seven of the mice, the pressure change was 10 mm Hg or less. In five of the remaining eight animals, pressure diminished.

The arterial CO₂ was determined in 15 mice, 5 of which had received fluorescein. The data for the latter were the same as those for the mice that did not receive fluorescein, and the two groups have been combined. The mean PaCO₂ was 64 ± 4 mm Hg after anemia, compared with 49 ± 4 mm Hg in a group of control animals with identical anesthesia and surgical treatment. The anemic mice had a significantly higher PaCO₂ (P < 0.02, t-test).

The PaO₂ in the anemic mice was not significantly different from that in the control animals (96 ± 7 mm Hg vs. 95 ± 5 mm Hg).

Discussion

The data clearly indicate that erythrocyte velocity increases after abrupt reduction in hematocrit. These increases occurred in 28 of the 31 measurements made in 13 arterioles and 8 venules. All but one of the arteriolar measurements were at the vessel wall. Whether these measurements are indicative of changes in average linear velocity across the entire vessel can be questioned. Should the velocity profile (16) change to a blunter form because of a reduced hematocrit, the average velocity might not qualitatively follow velocity changes at the wall. Whether it did so would actually depend on the velocity of cells closer to the center of the vessel. However, reductions in hematocrit do not result in blunter profiles; in fact, the profiles become more parabolic (16); consequently, velocity changes at the wall in these experiments would not be expected to be accompanied by a decrement in velocities elsewhere in the velocity profiles. Our data support this contention because, whenever center-line velocities were measured (one arteriole and all eight venules), anemia was accompanied by an increment in these velocities as well as those near the wall. Thus we can be reasonably confident that the velocity increments observed in these experiments do represent increases in average velocity across the entire vessel.

In addition to increments in erythrocyte velocity, the transit time of fluorescein from cerebral arteriole to venule decreases significantly, indicating that the velocity of plasma
also increased after the reduction in hematocrit. If significant vasoconstriction does not occur, increases in erythrocyte or plasma velocity should be reflected by an increased cerebral blood flow, whether the latter was measured by a technique dependent on a red cell marker or a plasma marker. Since in our experiments most vessels were unchanged in diameter after phlebotomy, our data would appear to confirm earlier studies in which cerebral blood flow was increased both after chronic anemia in man (1, 2) and acute anemia in animals (3, 4).

Of further interest was our ability to detect a pulsatile change in the velocity of erythrocytes within many arterioles (10). This change represents the cardiac cycle and is a manifestation of the peripheral pulse at the microcirculatory level (10). In many vessels we were able to detect both a peak velocity and a minimal or nadir velocity (10), both before and after induction of anemia. In so doing, we were able to show that both the peak and nadir velocities were increased when the hematocrit fell. In a few arterioles and all but one venule, no pulse was detected, and the velocity value was a random value obtained from an undefined part of the pulse cycle. In all of these cases the velocity increased when the hematocrit fell.

There are two possible explanations for increases in the velocity of erythrocytes in both arterioles and venules for the increased velocity of plasma. One explanation is an increase in perfusion pressure (10, 12). Since half the mice in this study display insignificant alterations in systemic systolic pressure, and since systolic pressure actually diminished in five other animals, an increased pressure cannot be implicated as the cause of the postphlebotomy velocity increments.

A second possible explanation for increased velocities is a decrease in vascular resistance. Such a decrease can be caused either by vasodilation or by a decrease in blood viscosity. The absence of vasodilation in the arterioles has been briefly discussed above. Although there were several factors in this study which might have caused dilation of the cerebral vessels, the fact is that most arterioles and venules (13 out of 21) failed to demonstrate an alteration in diameter when postphlebotomy velocities were measured. Of course one cannot rule out the presence of dilation at some point in the cerebral vascular bed not under observation, but there is not evidence to support such an assumption. However, because of difficulties in determining dilations on the order of 2μ, we cannot rule out the possibility that changes of 10% or less in diameter did occur during this study. It is of interest that where dilation was observed (Table 1) it represented a change in diameter of 10% or greater.

Among the factors that might be expected to cause cerebral vasodilation, is the decreased tissue oxygen tension that may accompany anemia (1, 3, 5). However, in our study the degree of anemia was rather modest (15% reduction in hematocrit), and one wonders whether tissue O2 tension would diminish sufficiently to cause vasodilation, especially if erythrocyte and plasma flow is accelerated, as was the case in this study (17).

A fall in blood pressure might also have been expected to produce vasodilation due to autoregulation in the cerebral vascular bed (13). However, autoregulation serves to maintain flow at its initial level; it does not increase flow. Vasodilation without an increase in flow can only result in a decrease in the linear velocity of cells and plasma. Consequently, vasodilation as an autoregulatory response to a fall in pressure, cannot account for the increased velocities observed in the present study. Nevertheless, brief comment on autoregulation in these animals seems in order. In mice the most pronounced passive changes in vessels of the small magnitude studied here usually subside in a few minutes, to be followed by complete or partial autoregulation with maintenance at least of control diameter (12). In the presence of a drop in blood pressure one would expect passive narrowing of the vessels; hence even maintenance of normal diameter indicates a change in tone, and an autoregulatory response which acts to minimize, though it does...
not prevent, decreases in volume flow. Since several minutes elapsed between phlebotomy and further examination of pial vessels, at least a partial autoregulatory response was to be expected, and arteriolar diameter changes were consistent with such response, showing dilation or no change in diameter in all mice with a fall in blood pressure.

Also of interest is the absence of marked vasodilation in the presence of significant elevations in PaCO$_2$. The latter is the most potent natural stimulant of cerebral vasodilation (5). In our study, after phlebotomy, eight mice had a PaCO$_2$ greater than 65 mm Hg, yet only five exhibited dilation of one or more vessels under observation. Moreover, in three cases the dilation did not exceed 10% of the control diameter. As indicated above, changes of less than 10% may not be readily detectable with our techniques. Under these circumstances, we must admit that small changes in diameter may have occurred and may have reduced vascular resistance in animals with elevated CO$_2$ levels. However, CO$_2$ measurements were made only at the end of the experiments; hence CO$_2$ levels could have been elevated from the very beginning of the experiment and remained constant throughout, rather than increasing only after phlebotomy. In such cases, dilation due to the high CO$_2$ level would have been present before phlebotomy and could not have accounted for further reduction in vascular resistance after phlebotomy. Moreover, increased velocities were found even in mice with normal or nearly normal PaCO$_2$. For example, in animals with a PaCO$_2$ of 40 and 45 mm Hg, the RBC velocity increased 30% and 20% respectively in spite of a drop in systolic pressure of 30 mm Hg in the first mouse and 20 mm Hg in the second.

It is apparent from the preceding paragraphs that the experimental data fail to provide evidence implicating vasodilation as the factor reducing the vascular resistance each time there was an increase in erythrocytes or plasma velocity. On most occasions, vasodilation was not observed, while its possible causes would either have resulted in diminished linear velocity or were not always present when increased velocity was observed. In contrast, one factor which reduces vascular resistance was always present. That factor was a reduced blood viscosity.

The relation between hematocrit and viscosity is well known (18-20), and our previous studies have shown that the dependence of viscosity on hematocrit extends to the mouse (8, 12, 20). Thus, although pre- and postphlebotomy viscosity measurements were precluded by the size of the sample required for such measurements, we can state with assurance that viscosity was reduced in every mouse with a reduced hematocrit. Since a decreased viscosity is the only resistance-lowering factor known to be present in every mouse in this study, it is reasonable to assert that reduced viscosity played a role in producing the accelerated erythrocyte and plasma velocities found after phlebotomy. Indeed, in the absence of demonstrable, pronounced vasodilation in most of these animals, reduced viscosity may be the sole cause of the increased velocities, particularly in the seven mice in which these velocity increments were accompanied by a fall in blood pressure. Thus our data supports the contention of Heyman et al. (1) that decreased viscosity is at least a partial cause of increased cerebral blood flow in anemia.

Since alterations in viscosity were probably responsible for the velocity changes observed in this study, it is appropriate to inquire into the possible effects on viscosity of variables other than hematocrit. Rand et al. (21) have shown that increased CO$_2$ levels and severe respiratory acidosis have little or no effect on viscosity. When pH falls below 7, a very slight rise in viscosity may occur (21). In the present study, the pH after phlebotomy was 7.17 ± 0.03 in 15 mice. Moreover, since the effect of increased CO$_2$ or reduced pH is an increased viscosity, such an effect could not have produced the reduced cerebrovascular resistance which must have accompanied anemia in our investigation.

The exact relation between in vitro viscosity data and in vivo "apparent viscosity" is
unknown. Among the few studies of this question, there is one which states that large changes in vitro are reflected by quite small changes in "apparent viscosity" in vivo (6). If this is so, it indicates simply that factors determining viscosity levels in vitro are modified by other factors playing a role in the microcirculation. Moreover, theoretical analysis and examination of blood flowing through glass tubes (16, 22-24) have indicated that as one approaches true capillaries, effects of hematocrit are minimized and "apparent viscosity" in vivo approaches that of plasma alone. Thus our in vivo observations are particularly important since they clearly indicate that in the mouse the cerebral microcirculation is affected by reductions in hematocrit.

**Acknowledgment**

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**References**


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