Can Plasma Skimming or Inconstancy of Regional Hematocrit Introduce Serious Errors in Regional Cerebral Blood Flow Measurements or Their Interpretation?

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Abstract:
Can Plasma Skimming or Inconstancy of Regional Hematocrit Introduce Serious Errors in Regional Cerebral Blood Flow Measurements or Their Interpretation?

Recent reports indicate that in cases of elevated plasma or blood viscosity, red cell velocity through the cerebral microcirculation is unimpaired while plasma transit is retarded. One explanation may be that plasma skimming is increased, with increased shunting of plasma into longer, less direct flow paths, while an increasing proportion of red cells take shorter paths. Such inhomogeneities of flow on a microvascular level can be directly demonstrated in cases of endothelial damage or anoxia, and may produce an altered regional hematocrit.

Measurements of regional cerebral blood flow are dependent upon regional hematocrit because this affects the tissue:blood partition coefficient ($\lambda$) of diffusible indicators. Hence changes in regional HCT may produce errors in the calculation of regional blood flow unless the value of $\lambda$ is corrected to reflect altered HCT. Moreover, no matter what kind of indicator is used, our functional interpretation of regional blood flow measurements is dependent upon our assumption that the 'blood' in the region maintains a constant capacity to carry nutrients like oxygen. Thus the lack of a constant relationship between plasma flow and red cell flow may produce errors either in the measurement or interpretation of regional flow measurements. These errors will increase in importance as blood flow is measured in smaller and smaller volumes of brain.

Additional Key Words
- viscosity
- erythrocytes
- microcirculation
- intracerebral arterioles and venules
- WBC
- cellular flow
- hemoglobin

Introduction

Recently, we reported a series of studies\textsuperscript{1-8} in which the viscosity of either plasma or whole blood was elevated, and in which red blood cell (RBC) velocity through the cerebral microcirculation appeared unimpaired while plasma transit was retarded. There are several possible explanations\textsuperscript{1-8} for the apparent differential effect of altered viscosity on the behavior of RBC and plasma. In presenting our work before various groups of rheologists and bioengineers, we find that one of these explanations appears favored. This explanation suggests that plasma skimming is increased; there is an increased shunting of plasma into side branches of the microvasculature with a relative increase in the proportion of erythrocytes taking shorter, more direct paths with a
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lower total resistance. Whatever the explanation for our results, however, the principle of a differential behavior of red cells and plasma is well established within the microcirculation.\textsuperscript{4} The existence of high hematocrit (HCT) and low HCT vessels is well documented in microvascular beds which can be directly observed. Various indirect methods of studying flow suggest the existence of vessels from which red cells may be temporarily excluded,\textsuperscript{5} and the phenomenon of plasma skimming is of considerable importance in the kidney where a variety of physiological phenomena can be explained on this basis.\textsuperscript{6-8} Moreover, even within a single arteriole or venule the HCT can be altered merely by altering the diameter of the vessel, an apparent explanation for the Fahraeus-Lindqvist effect, wherein red cell acceleration is observed as vessel diameter is reduced.\textsuperscript{9, 10} Recent observations of surface vessels in experimental cerebral infarction have demonstrated red cell sludging in the microvasculature,\textsuperscript{11} a phenomenon which could certainly lead to regional alteration in the HCT of blood perfusing the ischemic zone. Our own studies\textsuperscript{12} have demonstrated that an injured vascular endothelium can lead to virtual exclusion of erythrocytes from a cerebral vessel, while the vessel continues to be perfused by plasma. A similar exclusion of erythrocytes from plasma-filled channels can be observed in anoxic animals with slow flow (fig. 1). The sum of these observations, both our own and those in the literature, has led us to a consideration of the errors that might be introduced into measurements of regional cerebral blood flow (rCBF) by alterations in regional HCT. To our surprise we have found that those measuring rCBF appear to implicitly assume that plasma and red cells are affected in a parallel manner during alterations in regional perfusion or adjustments of vessel diameter. It is our intention to review the theoretical basis for an effect on rCBF of a change in regional HCT. We will also point out the difficulties in interpreting the functional significance of an rCBF value when the regional HCT is unknown. Once these points are made clear, the importance of implicitly assuming that regional HCT remains unchanged in health and disease will also be clear. We do not believe that such an assumption can be made without proof in each disease state or experimental condition. In view of our own data and the literature cited above, we doubt that the assumption is valid. We hope that this review will encourage those using techniques which measure rCBF to recognize and test the assumption.

**FIGURE 1**

Panel A: Vessels on the surface of the mouse brain, as seen with a tungsten light source after mouse received sodium fluorescein, i.v. Red cells appear black in the photo, and in the smaller branches of the venule (v) and arteriole (a) the blood stream appears granular because of abnormally slow flow. Arrows point to apparently empty or nearly empty vessel segments. Calibration bar at top equals 80 μ.

Panel B: Same field as above, but viewed with mercury lamp and appropriate filters for demonstrating plasma which is fluorescent because it contains sodium fluorescein. Note that fluorescein has not yet reached main venular channel. The empty or nearly empty arterioles and venules in Panel A (arrows) are shown in B to be widely patent and filled with fluorescent plasma. In other words these are low HCT channels from which cells have been largely excluded.
Effect of Hematocrit on Measurement of rCBF

The measurement of rCBF is generally carried out by measuring the washout, clearance, or exchange of a diffusible indicator from the brain.\textsuperscript{13-17} The formula for rCBF may be written in a variety of mathematically equivalent forms,\textsuperscript{16} one of which is:

\[ r\text{CBF} = \lambda \times \frac{100}{t}, \]

where \( t \) is the mean transit time of the indicator moving through the volume of tissue under consideration. It can be seen that the flow value obtained is proportional to \( \lambda \) (lambda), the tissue: blood partition coefficient of the indicator, and represents the distribution of the indicator between the perfused tissue and the perfusing blood. The value of \( \lambda \) will depend upon the degree to which the indicator is distributed between erythrocytes and plasma in the blood itself.\textsuperscript{13, 15, 16} If the indicator is not uniformly distributed between cells and plasma, then \( \lambda \) will be a function of HCT, and this is the case for three of the indicators, each of which are more soluble in RBC than in plasma. These indicators are Xenon and krypton\textsuperscript{13, 16} (both commonly used), and trifluoriodomethane (less commonly used).\textsuperscript{15}

Thus, for \( ^{85}\text{Kr} \), \( \lambda \) ranges from 1.06 to 1.17 as HCT is reduced from 50 to 25\textsuperscript{18, 18}; for \( ^{133}\text{Xe} \), \( \lambda \) is increased from 0.8 to 0.95 in gray matter and from 1.5 to 1.8 in white matter as blood hemoglobin is decreased from 16 to 7 gm/100 ml.\textsuperscript{18} Similarly for trifluoriodomethane, \( \lambda \) shifts from 0.6 to 0.85 as HCT is altered.\textsuperscript{15}

Only for antipyrine\textsuperscript{14C} can we find no reference to an effect of HCT on \( \lambda \), perhaps because antipyrine is supposedly distributed equally throughout the body-water.\textsuperscript{17} However, because of their high hemoglobin content red cells are only 67\% water by volume.\textsuperscript{18} Thus, antipyrine ought to be distributed more in plasma than in cells. Moreover, there must be something wrong with the assumption that the \( \lambda \) for antipyrine is dependent only upon tissue water content,\textsuperscript{17} since \( \lambda \) for this indicator is essentially equal in both white and gray matter even though the water content of gray matter is about 21\% greater than that of white matter.\textsuperscript{19, 20} Until these questions concerning the distribution of antipyrine are resolved, we would prefer to see data on the relationship of HCT to \( \lambda \) for this indicator before assuming no effect of HCT on \( \lambda \).

In any case, for \( ^{85}\text{Kr} \), \( ^{133}\text{Xe} \), and trifluoriodomethane, \( \lambda \) is definitely affected by HCT, and since rCBF is proportional to \( \lambda \),\textsuperscript{10} rCBF also will be affected by HCT. As indicated in an earlier paragraph, \( \lambda \), and hence rCBF, for these indicators may decrease by 10\% to 40\% of their initial value, as HCT increased from 25 to 50. Thus, a failure to recognize a regional shift in HCT might result in a sizable error in the calculation of rCBF because of a failure to change the value of \( \lambda \) used in the calculation. The magnitude of this error would depend upon which indicator was used, and upon the magnitude of the shift in HCT.

The question arises as to whether or not large changes in HCT could occur in the brain. First of all, we must reiterate that unless one is aware of their possible significance, no attempt will be made to look for regional or temporal shifts in HCT. In other organs, large regional shifts in HCT have been postulated and, in skeletal muscle and kidney,\textsuperscript{5-8} much experimental evidence has been gathered to support this concept. The evidence for significant and variable alterations in regional renal HCT began with the observation of an extremely low HCT in vessels within the kidney relative to that in the renal artery or renal vein. This HCT ratio might be as low as 50\%.\textsuperscript{4} It was ascribed to a routing of plasma over long routes through the kidney in low HCT channels while erythrocytes took shorter, high HCT paths.\textsuperscript{6} This was called "plasma skimming." Other workers had confirmed great variations in regional HCT within the kidney by direct observation of individual glomeruli in vivo.\textsuperscript{21}

The degree of skimming depended on the status of a number of physiological variables, such as overall HCT and blood pressure.\textsuperscript{5-8} In the brain, as in the kidney, the mean HCT is lower than the HCT in the arteries or veins supplying or draining that organ.\textsuperscript{22-25} Moreover, regional differences in HCT within the brain have been reported. Thus, in the rat, the white matter HCT is significantly lower than the gray matter HCT.\textsuperscript{22} Within the parenchyma of the brain, there are abundant capillary and precapillary channels connecting intracerebral arterioles with intracerebral venules.\textsuperscript{26, 27} Consequently, an anatomical substrate for plasma skimming does exist. One consequence of
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Skimming in the kidney is a more rapid transit time for RBC than for plasma, through that organ. A similar phenomenon exists for brain. In our own studies on increased plasma viscosity and increased blood viscosity, the normal difference between plasma and RBC velocity appears significantly accentuated, a phenomenon which could be explained by increased plasma skimming. Finally, as indicated in figure 1, a separation of plasma from cells has been directly observed at least in abnormal states on the surface of the brain, and increased sludging which would lead to such separation has been observed in surface vessels over ischemic areas.

From the preceding data it would seem that regional differences in HCT may exist in the brain and that the regional HCT may not be constant from one physiological or pathological state to another. We have explained how this might result in an erroneous value for rCBF because of an effect on the partition coefficient (λ) of the indicator. Whether such errors, in fact, do occur will depend not only on the magnitude of the shift in HCT but also on the size of the volume through which rCBF is measured. Our techniques for measuring rCBF often depend on the average λ in the volume. The smaller the volume in question the more likely it becomes that regional changes in HCT or λ will affect our results. Obviously, much work is necessary to determine the magnitude of changes in regional HCT as well as the size of the tissue volume in which such changes can occur. It may be that many such changes fall within the relatively large standard deviations found for most methods of determining rCBF. However, we have thus far concerned ourselves only with the values calculated for rCBF. Of equal importance is the interpretation of these values. Errors in interpretation also may arise because of the failure to appreciate regional variations in HCT which changes from time to time and state to state. These errors may be more important than the error in numerical value assigned to rCBF. This second kind of error is discussed below.

Effect of Hematocrit on Interpretation of rCBF Values

The essence of our point is that blood is not a material of constant composition, and that its functions are variably dependent upon different blood constituents. In order for our measurements of rCBF to have meaning they must reliably reflect the parameter we are interested in. If we are interested in O₂ delivery, then rCBF must reliably reflect RBC flow. If we are interested in glucose delivery or acid clearance, then we may be more interested in whether rCBF reliably reflects plasma flow. A measure which fails to distinguish between the flow of RBC and the flow of plasma may give us a misleading idea about the flow of the component of interest, unless we assume that the flow of cells always parallels that of plasma. From our earlier remarks it will be apparent that such an assumption is not supported by a general knowledge of microcirculatory phenomena and is called into question by specific experimental facts concerning cerebral microcirculation in particular.

Among current techniques for measuring rCBF are clearance measurements of ⁸⁵Kr or ³⁵Xe. Since both of these agents are distributed to a greater extent in RBC than in plasma, it follows that, at equilibrium, they will reflect the flow of RBC to a greater extent than the flow of plasma. This is especially true for Xe, whose tissue to blood partition coefficient λ is more than twice as dependent upon HCT than is the λ of ⁸⁵Kr. However, with either agent marked exclusion of cells from the volume in question might result in an rCBF which, to a larger extent than usual, depended on the flow of plasma. Thus, it is conceivable that over an infarct with RBC sludging, a "blood" flow measurement might be predominantly a measure of plasma flow and give a quite misleading idea of tissue oxygenation in the infarcted volume. Indeed, the discrepancies sometimes observed between rCBF and the histological findings in an infarcted area may possibly be ascribed, at least in part, to an inability to distinguish between plasma flow and erythrocyte flow in the infarcted volume of tissue.

Even for the cortex as a whole, increased plasma skimming might lead to erroneous conclusions from relatively accurate measurements of "blood" flow, if enhanced skimming led to increased RBC flow (through short channels) and decreased plasma flow (through longer or higher resistance paths). For example, in polycythemia, "blood" flow measured with ⁸⁵Kr is not altered between HCT of 30 and 75. The flow increased rapidly at HCT...
below 30. This has been interpreted as indicating a minimal effect of viscosity on CBF, and a dependence of CBF on tissue O₂, which would diminish when the HCT fell below 30, leading to vasodilatation and increased flow. However, our studies indicate that possibly due to enhanced plasma skimming, RBC velocity may be accelerated or remain constant in polycythemia while plasma transit is retarded. Since °Kr is distributed more in RBC than in plasma, the measurement with °Kr may give a picture of flow weighted in favor of the movement of cells and obscuring a decline in plasma velocity through the same volume of brain. A value weighted in favor of red cell flow is of interest if one is concerned with oxygen delivery, but even here a misleading impression may be given by a measurement of rCBF which reflects the average flow of RBC in the volume of tissue being monitored. Since increased skimming redistributes cells and plasma among the vessels within the tissue volume, skimming might produce inhomogeneity of oxygenation among neurons within that volume. Thus, the rCBF might show a normal “blood” flow while concealing uneven perfusion due to the plasma skimming. Of course, problems of uneven perfusion are often considered in discussion of rCBF validity, but such discussions generally consider perfusion in terms of vessels carrying blood and vessels not carrying blood. We are pointing out a different kind of uneven perfusion, namely, marked differences in HCT of adjacent vessels or groups of vessels.

If local HCT were sufficiently reduced by plasma skimming, one might expect a decline in CMRO₂. The same study of polycythemia which failed to detect any effect of increased HCT on cortical rCBF also failed to recognize a decline in CMRO₂ in the presence of increased HCT. However, the data on CMRO₂ shows only four experiments with HCT greater than 60. Moreover, these points appear to be at or below the mean for the CMRO₂ values obtained in experiments at lower HCT, rather than being symmetrically distributed around the mean. It would appear that there is insufficient evidence upon which to base a conclusion of normal CMRO₂ in uncomplicated polycythemia; hence, the possibility of increased plasma skimming and consequent inhomogeneous oxygen perfusion remains open.

The preceding discussion of polycythemia is meant only as an example of the possible effects of plasma skimming on the interpretation of, or the functional meaning of, rCBF values. Other hypothetical instances could have been selected to illustrate our point. We recognize that increased plasma skimming is not the only possible explanation for findings of a normal rCBF in polycythemia. We selected polycythemia as an example because our findings of retarded plasma transit time with unimpaired RBC velocity and the suggestion of enhanced plasma skimming might offer at least a partial explanation for the normal rCBF observed in the experiments of others. We also selected polycythemia as our example because in the kidney, where significant and fluctuating levels of plasma skimming seem well established, such skimming has been used as the basis for an explanation of a flow-HCT relationship resembling that reported in the °Kr studies of brain.

Finally, our remarks concerning the meaning or functional interpretation of rCBF values need not be confined to situations in which diffusible indicators have been used. Nor should they be confined to cases where the indicator is unequally distributed between cells and plasma. No matter what the nature or distribution of the indicator, an interpretation of rCBF cannot be made without first making some assumption concerning the relative movements and relative distributions of plasma and RBC within the volume being monitored. The validity of the interpretation will then depend upon the validity of that assumption. It has been a major purpose of this review to make explicit what appears to be an implicit assumption underlying most studies; namely, that the flow of RBC and the flow of plasma change in parallel with one another as we move from one physiological or pathological state to the next. It would seem that this assumption is in need of validation, and that this problem of validation will become increasingly important as techniques are developed for monitoring flow in smaller and smaller cerebral volumes, which could be increasingly characterized by differences in regional HCT and by fluctuations in their regional HCT.

Summary

Measurements of regional cerebral blood flow (rCBF) are dependent upon regional HCT
because this affects the tissue-blood partition coefficient ($\lambda$) of many diffusible indicators in current use. If there are significant changes in regional HCT from one pathological state to another, or if regional HCT changes as the status of the microcirculation is altered, then errors in the measurement of rCBF may arise, unless the value of $\lambda$ is corrected to reflect the altered HCT. Even when a correct value for rCBF is obtained, the meaning of this value will be dependent upon regional HCT. Indicators distributed more in plasma than in erythrocytes reflect a flow which is weighted in favor of the flow of the plasma. Indicators distributed more in erythrocytes than in plasma reflect a flow which is weighted in favor of the flow of the erythrocytes. We can utilize rCBF as a measure which accurately reflects the flow of both components of blood only if we assume a constant relationship between the flow of plasma and the flow of cells. A similar proviso must be given whether we are working with diffusible or nondiffusible indicators or with indicators that are distributed solely in plasma or solely in erythrocytes.

Most studies of rCBF appear to implicitly assume that when the physiological state of the circulation is altered or a pathological state occurs, regional HCT and the relationship of plasma flow to erythrocyte flow remain constant. These assumptions may be incorrect. To the extent that they are incorrect, errors will arise in the measurement of rCBF because of a shifting tissue-blood partition coefficient in the case of diffusible indicators, and errors will arise in the functional interpretation of rCBF, irrespective of the nature of the indicator used in making the measurement. Data are available which suggest that these assumptions concerning HCT and the relationship of cellular flow to the flow of plasma may require validation. For example, because of plasma skimming, regional cerebral HCT and the relationship of cellular flow to plasma flow may be altered in cerebral infarction, in conditions characterized by endothelial damage, and during hyperviscosity states. It would seem necessary to assess the degree of plasma skimming, or the regional variability in HCT, in each condition to which regional blood flow measurements are applied. Validation of the assumptions concerning HCT and a constant relationship between plasma flow and the flow of red blood cells will become especially important as rCBF is measured in smaller and smaller volumes of brain.

References

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