Blood Volume, Hematocrit and Pressure Relationships in the Isolated Perfused Dog Brain

BY VICTOR A. LEVIN, M.D.,* AND DAVID D. GILBOE, PH.D.†

Abstract: Blood Volume, Hematocrit and Pressure Relationships in the Isolated Perfused Dog Brain

The intracranial blood volume and hematocrit of the isolated perfused dog brain preparation were determined with Cr$^{51}$-labeled erythrocytes. The intracranial blood volume was determined to be 8.6 ml/100 gm brain (SE ± 1.2 ml). Over the range of perfusing hematocrits studied, the relationship of perfusing hematocrit (Hct$_{PB}$) to intracranial hematocrit (Hct$_{IC}$) was found to be a linear function (Hct$_{IC}$ = 5.44 + 0.702 Hct$_{PB}$). At a peripheral hematocrit of 20% the Hct$_{IC}$/Hct$_{PB}$ = 1.0, whereas at hematocrits of 50 to 55% this ratio dropped to 0.87.

With increasing perfusing hematocrits the carotid artery pressure rose markedly and there was flattening of the EEG. Since the isolated dog brain is a closed system void of extracranial anastomoses, high viscosity blood cannot circumvent the cerebral circulation but must follow the high resistance networks of the deep cerebral vasculature until sludging of the erythrocytes becomes sufficient to cause anoxia and loss of autoregulation. In the intact erythrocythemic animal, however, increases in arteriolar pressure can be reflected downstream to the large cerebral and meningeal vessels with extracranial shunting of high hematocrit (high viscosity) blood from the brain.

ADDITIONAL KEY WORDS erythrocythemia isotope-labeled erythrocytes extracranial shunts increased carotid artery pressure

Introduction

This study was undertaken to determine the approximate intracranial blood volume of the isolated perfused "dog brain" and to discover if, in the absence of extracranial vessels, the ratio of intracranial hematocrit (Hct$_{IC}$) to perfusion hematocrit (Hct$_{PB}$) was influenced by changing the perfusing hematocrit. Previous experiments with intact animals indicated that Hct$_{IC}$/Hct$_{PB}$ decreased significantly with increasing hematocrit of the perfusing blood. This conclusion was at variance with previous studies which suggested that the Hct$_{IC}$/Hct$_{PB}$ was a constant ratio.8 To further support our hypothesis, we recalculated the experimental data of Larsen and Lassen2 and Oldendorf et al.8 and found that regression analyses of their data produced results similar to our own.1

The third goal of these experiments was to study the relationship between early perfusion deaths in the isolated brain preparation and high hematocrit blood. In particular, we were interested in determining if this phenomenon was secondary to elevated arterial pressure and increased cerebral vascular resistance and, if so, whether it was reversible under conditions of high arterial Pco$_2$ or by returning to low hematocrit perfusing blood.
In the past, brain blood volume and hematocrit have usually been determined from the study of isolated pieces of brain tissue. For such studies, erythrocytes labeled with Fe\(^{59}\) or Cr\(^{51}\) and albumin bound to T-1824 dye, Cr\(^{61}\), I\(^{125}\), or I\(^{151}\) were injected into the intact animal; after an appropriate equilibration period, the animal was sacrificed and pieces of brain were removed, weighed, and assayed for isotope activity or dye concentration. Using the same isotopes, the blood volume and intracranial hematocrit have been calculated in vivo, either from the differences between carotid artery and jugular vein concentrations or by direct external counting of gamma radiation over the skull.

The blood volume and hematocrit determinations by tissue assay are based on samples obtained from a limited number of brain areas and emphasize values from the minute vessels of the brain, while measurements involving the carotid artery and jugular vein include a far greater area than the intracranial contents by virtue of the many extracranial anastomoses. This last fact complicates interpretation of data obtained by arteriovenous differences. If one could control the volume and hematocrit of the extracranial blood and be certain that only the cranial contents were being perfused by this blood, a more precise correlation could be made between the perfusing hematocrit and the intravascular plasma and red cell volumes. The surgically isolated and perfused dog brain approximates these conditions and permits study of the brain's intracranial vasculature.

**Methods**

The brains were isolated from 12 (12-18 kg) mongrel dogs following a procedure described previously which involved the removal of the mandible, snout, and all extracranial soft tissues, leaving only the brain case intact. All animals received 60 mg of morphine 45 minutes before administration of 20 mg/kg of sodium pentobarbital. Anesthesia was maintained throughout the procedure with supplementary doses containing 60 mg of sodium pentobarbital. The isolated brain preparation was supplied by the internal carotid arteries and the anastomotic branches of the internal maxillary segment of the external carotid arteries. Venous blood was returned through a threaded Luer connector placed through the bone covering the ventral occipital sinuses.

The perfusion blood was oxygenated with a small disc oxygenator which was primed with about 300 ml of fresh, compatible, heparinized donor blood, and was equilibrated with 95% O\(_2\)/5% CO\(_2\). Arterial flow was provided by a variable speed occlusive-type pump which was calibrated in milliliters per minute. Pump speed was adjusted by hand to maintain an arterial pressure of 140 to 150 mm Hg which resulted in blood flow rates of approximately 50 ml/100 gm of brain per minute. The perfusion system was equipped with a 100-mesh stainless steel filter screen to remove small fibrin particles which could cause vascular occlusion and unwanted increases in arterial pressure. A sigmamotor peristaltic pump aided return of venous blood.
from the ventral occipital sinus to the oxygenator. A ten-tube Mayo heat exchanger was used to adjust the perfusion blood to the proper temperature to maintain the cortex at 37° C. Brain temperature was measured with a 22-gauge needle thermistor placed in the olfactory bulb. Arterial pump pressure, EEG, and brain temperature were recorded continuously on a five-channel polygraph. If the brain showed relatively normal electrical activity after isolation, the experiment was continued.

Cr\textsuperscript{51}-labeled erythrocytes were used to determine blood volume and hematocrit. Approximately 10 to 15 μC Cr\textsuperscript{51} were equilibrated with 10 ml of whole blood for one hour. Following this, the blood was centrifuged at 2,500 rpm (R.C.F. = 1,400) for five minutes, the plasma was removed, and the red cells were then added to and equilibrated with the blood in the second pump-oxygenator system for 45 minutes.

The two pump-oxygenator systems used in these experiments are shown schematically in figure 1. Initially, the brain was perfused with blood from one system until arterial pH, Pco\textsubscript{2}, and EEG activity were determined to be normal. Prior to changing from the first pump-oxygenator system to the second, the exact blood volume (PBV) of the second system was determined gravimetrically. Two blood samples were taken directly from the disc-oxygenator, one just prior to perfusion with the second pump oxygenator, triplicate 2 ml aliquots of each sample were counted for gamma emission in a well-type scintillation counter to a counting error of less than 2%. Microhematocrits were determined in each sample in duplicate and were corrected for 2.4% trapped plasma in the erythrocyte fraction.\textsuperscript{1}

After the experiment, the residual blood volume (RBV) in the tubes between the animal and the second system was determined. At the end of each experiment, the brain and upper cervical cord were removed and weighed.

The formula used to calculate the intracranial blood volume (BV) was derived from the following general formula (conservation of mass):

\[
(CPM)_n (Vol_1) = (CPM) (Vol_2)
\]

where CPM = gamma counts per minute per milliliter of blood at the time specified (t).

\[
Vol_1 = \text{pump blood volume in milliliters (PBV)}
\]

\[
Vol_2 = Vol_1 + \text{residual blood volume in tubes (RBV) + BV}
\]

Rearranging this general formula into a more workable form,

\[
BV = \left(\frac{CPM_n}{CPM_{10}} - 1\right) (PBV) - (RBV)
\]

The formula for intracranial hematocrit (Hct\textsubscript{10}) was also derived from the general formula:

\[
Hct_{10} = \left(\frac{CPM_n}{CPM_{10}} - 1\right) (PBV)
\]

and another after ten minutes of perfusion with the second pump oxygenator system. Triplicate 2 ml aliquots of each sample were counted for gamma emission in a well-type scintillation counter to a counting error of less than 2%. Microhematocrits were determined in each sample in duplicate and were corrected for 2.4% trapped plasma in the erythrocyte fraction.\textsuperscript{1}

The relationship between perfusing hematocrit and intracranial hematocrit is fairly consistent if one compares man, rat and the isolated dog brain. We commented previously\textsuperscript{1} that data on the cranial hematocrit in man obtained by other investigators\textsuperscript{2,8} could be plotted in similar manner to our data. A plot of

**TABLE 1**

Summary of Experimental Blood Volume Corrected to 100 gm of Brain Weight and the Corresponding Hct\textsubscript{10} and Hct\textsubscript{20}

<table>
<thead>
<tr>
<th>Blood volume (ml 100 gm)</th>
<th>Hematocrit, perfusing (%)</th>
<th>Hematocrit, Intracranial (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.9</td>
<td>20.0</td>
<td>23.5</td>
</tr>
<tr>
<td>3.6</td>
<td>24.9</td>
<td>20.9</td>
</tr>
<tr>
<td>12.8</td>
<td>26.6</td>
<td>22.3</td>
</tr>
<tr>
<td>9.9</td>
<td>34.2</td>
<td>24.1</td>
</tr>
<tr>
<td>2.2</td>
<td>34.4</td>
<td>26.7</td>
</tr>
<tr>
<td>13.3</td>
<td>37.6</td>
<td>29.2</td>
</tr>
<tr>
<td>9.4</td>
<td>38.1</td>
<td>36.7</td>
</tr>
<tr>
<td>2.9</td>
<td>39.2</td>
<td>33.1</td>
</tr>
<tr>
<td>6.6</td>
<td>42.0</td>
<td>35.5</td>
</tr>
<tr>
<td>7.8</td>
<td>42.0</td>
<td>43.1</td>
</tr>
<tr>
<td>9.6</td>
<td>44.9</td>
<td>35.0</td>
</tr>
<tr>
<td>11.1</td>
<td>45.9</td>
<td>36.0</td>
</tr>
</tbody>
</table>

8.6 ± 1.2 (SE)
our data together with the values reported by others is seen in figure 3. The tests for equality of the intercepts, slopes, and standard error of the estimate for the regression lines demonstrated no statistically significant difference between our data and that of Oldendorf et al.\textsuperscript{3}

The relation of elevated perfusing hematocrit to carotid artery pressure for a typical experiment is shown in figure 4. Initially, this animal was perfused with blood of hematocrit 17%; during that period a carotid pressure of 130 mm Hg was maintained. When blood with a hematocrit of 45% was pumped through the same brain, the pressure rose markedly to 310 mm Hg. Even with a PCO\textsubscript{2} of 64 mm Hg, a PCO\textsubscript{2} normally sufficient to cause vasodilatation and reduction in pressure,\textsuperscript{5} the carotid pressure did not fall significantly. Reduction of the perfusing hematocrit, however, did cause a significant drop in pressure to 220 mm Hg before the experiment was terminated.

**Discussion**

**BLOOD VOLUME**

The blood volume of brain tissue has been determined to be 1.2 to 3.3 ml/100 gm of brain.\textsuperscript{6,7} In those studies, an isotope or dye was injected into a peripheral vein and the animal was sacrificed after a short equilibration time. The tissues were in some cases drained of blood;\textsuperscript{6,7} consequently, the blood volumes were “residual” brain blood volumes. In a carefully conducted series of experiments, Everett et al.\textsuperscript{8} froze the entire animal in liquid nitrogen, rapidly removed the brain, and
determined the blood volumes in various areas of the brain as well as other organs. Brain blood volume varied from 2.2 to 4.0 ml/100 gm brain depending on the area studied.

Nylin et al. calculated the intracranial blood pool of man to be 77 ml. Assuming an average adult brain weight of 1,300 gm, their data would yield an intracranial blood volume of 5.9 ml/100 gm of brain.

Rosomoff reported that the contribution of blood to the intracranial contents was 2.3% in the dog. Since the time required to completely freeze the dog brain was prolonged in these experiments, it is difficult to correlate these brain blood volume determinations with values obtained by other investigators.

A major criticism of our study of intracranial blood volume in the dog is the wide range of values and the relatively high SE. This may be partially explained by the fact that the 8.6 ml/100 gm brain reported includes not only the blood present in the brain but also the blood in the cranial sinuses. Visual inspection of the cranium at the time of brain removal indicated sizable differences in the sinus capacity of the various skulls. Since these studies were performed on mongrel dogs of varying size and differing skull conformations, we feel that a portion of this variability must be tolerated and accepted as individual animal variation. The near “normal” distribution of the points around the mean lends further support to this contention. An additional factor accounting for the relatively large BV could be that the cerebral vessels in our preparation were maximally dilated. Our BV, therefore, would represent the maximum rather than the average value.

Hematocrit

Earlier work has shown that the relationship of brain hematocrit to venous hematocrit is linear approaching unity at hematocrits of 20 to 25% while falling between 0.8 and 0.9 at hematocrits of 55%. The HctIC/HctTR of the dog follows that of man and the rat quite closely, being 0.95 at a perfusing hematocrit of 20% and 0.82 at a perfusing hematocrit of 50%. There can be little question that the ratio of HctIC/HctTR is not a constant and decreases with increased peripheral hematocrit values. One explanation for this phenomenon, in addition to the Fahraeus-Lindquist effect, could be that, in vitro, as blood of high hematocrit (high sedimentation) passes through branching glass tubes of varying sizes, the hematocrit is lower in the blood of the smaller diameter side branches. This lower erythrocyte to plasma ratio was consistently found in glass tubes of 20 μ or less. Since cerebral arterioles are 12 to 50 μ, there would be some vessels which could follow a similar pattern of erythrocyte/plasma inequality, thus having a lower hematocrit in the smaller vessels than in the larger cerebral vessels. Our experiments with the rat in vivo support the concept of a preponderance of large vessels in the cortex and small vessels in the subcortex: a conclusion reached because the hematocrit of the subcortex compared to the cortex was lower (p = 0.001).

The fact that the HctIC/HctTR at high perfusing hematocrits is less than at low hematocrits could be related to accelerated erythrocyte transit through the brain with the higher hematocrit blood. These phenomena, reflecting the flow of blood through “small tubes,” could maintain the volume flow of blood per unit time without increasing the volume of the blood vessels appreciably, while at the same time compensating for the increased viscosity and cerebral resistance. However, studies on polycythemic patients suggest that the majority have significantly reduced cerebral blood flows (CBF) with some of the lowest CBF ever reported for humans being recorded in polycythemic humans (22 cc/100 gm/min). It seems unlikely, therefore, that acceleration of erythrocyte transit time through the brain could be responsible for the phenomenon of decreasing HctIC/HctTR with increasing perfusing hematocrit in the intact animal.

Alternatively, the inability of the isolated cranial circulation to adjust to increasing hematocrits may be simply the inability of smaller vessels to increase their erythrocyte content beyond a certain maximum determined by vascular distensibility, blood viscosity and shear rates. This is reasonable since the smaller vessels of the brain will be the first to manifest increased cerebrovascular resistance as a consequence of increased blood viscosity. In addition, no vasodilatation at high hematocrit was noted at a Pco2 of 64 mm Hg, suggesting that the vessels were unlikely to distend further or were partly occluded by fibrin, platelets, or erythrocyte aggregates. Postmortem pathologi-
BRAIN BLOOD VOLUME, HEMATOCRIT AND PRESSURE RELATIONSHIPS

cal studies did not demonstrate occlusion of vessels.

Normally, the volume of blood flow, corrected for the weight of cortex (gray matter) and subcortex (white matter), is 3.4 to 4.3-fold greater for the cortex than the subcortex. In some pathological states preferential augmentation of cortical blood flow is reported with concomitant diminution of subcortical blood flow. Although there are insufficient data on cerebral flow patterns in polycythemias, it seems probable that their cortical blood volume and flow rate would increase while their subcortical blood volume and flow rate would decrease. If the flow in the external carotid and other extracranial vessels was determined simultaneously in the intact animal, it is likely that one would find relatively augmented cranial blood flow rates with increasing peripheral hematocrits while overall cerebral blood flow declined. The pathological condition of polycythemia vera with the clinically manifest plethoric facies and purplish mucus membranes of the head and neck may be explained by hypoxic ischemia, or loss of autonomic regulation. The reasons for the transient inability to reverse phenomena account for the pressure changes. This was also substantiated by the absence of fixed pathological vascular obstruction at postmortem examination. The reasons for the transient inability of the vessels to respond to elevated Pco₂ and thus the cerebral vessels increase by 20%, the corresponding viscosity factor (pressure/viscosity/radius to the 4th power) would be 4.9 times greater than its value at a pressure of 130 mm Hg. The most a cerebral arteriole has been seen to increase in diameter is 50%; in this case a viscosity increase of 12 times could be balanced without exceeding a carotid pressure of 310 mm Hg. We might postulate that the majority of cerebral vessels in the isolated perfused dog brain cannot dilate more than 20% and that blood viscosity probably increases no more than threefold in the period of one hour to account for a pressure of only 210 mm Hg. Since the pressure actually rises to 310 mm Hg we assumed that no further cerebral vasodilatation was possible in the isolated system. In an attempt to answer this question we perfused animals at low hematocrit (15 to 20%) and followed this with high hematocrit blood (40 to 45%). The consequence of this is seen in figure 4. The pressure rises precipitously and even elevation of the Pco₂ to 64 mm Hg fails to lower the pressure significantly, suggesting that the cerebral vessels were nonreactive to changes of Pco₂. When the perfusing hematocrit is then lowered to 15 to 20% the pressure slowly falls, indicating that reversible phenomena account for the pressure changes. This was also substantiated by the absence of fixed pathological vascular obstruction at postmortem examination. The reasons for the transient inability of the vessels to respond to elevated Pco₂ could be explained by hypoxic ischemia, overdistention, or loss of autonomic regulation.

The relationship of high perfusing hematocrit and elevated carotid artery pressure is relevant to other organ perfusion systems as well, since the causal relationship between early organ mortality and high hematocrit may be directly related to the phenomenon of increased pressure secondary to increased cerebral vascular resistance. When flow is constant in our system, pressure is proportional to viscosity/radius⁴ of vessel. It is established that viscosity increases with hematocrit and that this is accentuated at low shear rates. Given the hypothetical situation that the carotid artery pressure increases from 130 mm Hg to 310 mm Hg (fig. 4) and that the radii of the cerebral vessels increase by 20%, the corresponding viscosity factor (pressure/viscosity/radius to the 4th power) would be 4.9 times greater than its value at a pressure of 130 mm Hg. The most a cerebral arteriole has been seen to increase in diameter is 50%; in this case a viscosity increase of 12 times could be balanced without exceeding a carotid pressure of 310 mm Hg. We might postulate that the majority of cerebral vessels in the isolated perfused dog brain cannot dilate more than 20% and that blood viscosity probably increases no more than threefold in the period of one hour to account for a pressure of only 210 mm Hg. Since the pressure actually rises to 310 mm Hg we assumed that no further cerebral vasodilatation was possible in the isolated system. In an attempt to answer this question we perfused animals at low hematocrit (15 to 20%) and followed this with high hematocrit blood (40 to 45%). The consequence of this is seen in figure 4. The pressure rises precipitously and even elevation of the Pco₂ to 64 mm Hg fails to lower the pressure significantly, suggesting that the cerebral vessels were nonreactive to changes of Pco₂. When the perfusing hematocrit is then lowered to 15 to 20% the pressure slowly falls, indicating that reversible phenomena account for the pressure changes. This was also substantiated by the absence of fixed pathological vascular obstruction at postmortem examination. The reasons for the transient inability of the vessels to respond to elevated Pco₂ could be explained by hypoxic ischemia, overdistention, or loss of autonomic regulation.

The question of high hematocrit blood, per se, accounting for the high pressure was further investigated when it was suggested that rouleaux formation, resulting from the mechani-
ical agitation of pumping, might contribute to increased viscosity at high perfusing hematocrits. On several occasions, blood drawn from the manifold (F in figure 1) was Wright-stained and examined for rouleaux formation. Since no rouleaux were observed at the manifold and no vessels were occluded in the brain postmortem, it was concluded that their existence could not be a significant influence on the perfusion pressures.

From our experiments we have concluded that during perfusion of the isolated dog brain with high hematocrit blood maximal dilatation of the cerebral arterioles occurs and that the pressure phenomena recorded are a direct manifestation of high viscosity blood. In past experiments we have shown that cerebral vessels of the isolated brain preparation are responsive to alterations in arterial Pco$_2$. High hematocrit blood may block the autoregulatory response of the cerebral arterioles, but whether this effect is mediated chemically or by pressure directly is moot at this point.

These experiments can only suggest a relationship between extracranial shunts in polycythemia. Absolute proof would require in vivo studies of cerebral blood flow and extracranial blood flow, and the determination of erythrocyte transit times through the brain at varying peripheral hematocrit levels.

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