Cerebral Microangiography After Hypocarbia and Hypercarbia

DEMONSTRATION OF DEEP VASCULAR CHANGES PRODUCED BY PaCO2 VARIATIONS IN THE NORMAL AND ISCHEMIC BRAIN OF THE RHESUS MONKEY

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Abstract:
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Ischemic infarction was obtained in the basal ganglia and internal capsule of the Rhesus monkey by clipping the middle cerebral artery at its origin through a transorbital approach.

The postmortem microvasculature was demonstrated using microtized barium (Micropaque) and soft x-ray technology. The effects on this microvasculature induced by antemortem variations in PaCO2 in the normal and the acutely infarcted animal were studied. Microangiography was shown to be useful in the study of dynamic vascular changes in the deep structures of the ischemic brain.

Additional Key Words cerebral infarction microvasculature

Following the introduction of the concepts of luxury perfusion and intracerebral steal, the vascular bed in and around areas of brain ischemia has been subjected to considerable scrutiny and attempted modifications. Direct studies are carried out by observation of exposed superficial cerebral vessels, autoradiography and fluorescence techniques. Indirect observations using isotopic flow measurements and polarographical depth electrodes have also proved to be informative.

Radiographical angiography in vita does not yet permit consistent and adequate observation of vessels less than 200 μ, i.e., in that vascular district where autoregulation is said to take place. In recent years there has been a reassessment of the value of postmortem microangiography with fine barium suspensions in the study of brain and spinal cord vascularization. With this methodology one can evaluate the vascular architecture in depth as well as the various patterns of collateral circulation. The microangiographical observations will also permit a correlation between anatomical measurement and information obtained from other methods of study.

The present report deals with a group of microangiographical observations in the normal brain and in experimental deep cerebral infarction of the Rhesus monkey. The thrust of our research has been directed toward developing a methodology enabling us to demonstrate dynamic changes occurring in the microvasculature under different chemical, metabolic, and blood pressure changes. The initial emphasis of our investigation has been directed toward the effects of different arterial concentrations of carbon dioxide.

Methods
Twenty-nine Rhesus monkeys weighing 2.7 to 3.8 kg were used (table 1). Nine animals were uninfarcted controls. In 20 monkeys occlusion of the middle cerebral artery at its origin was carried out through a transorbital approach using an operating microscope* and a small Scoville clip.

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TABLE 1
Classification of Experimental Animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Number in series</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>Normal control</td>
</tr>
<tr>
<td>B 1</td>
<td>3</td>
<td>No infarct with hyperventilation for one hour with 100% O₂</td>
</tr>
<tr>
<td>B 2</td>
<td>3</td>
<td>No infarct with ventilation for one hour with 5% CO₂, 95% O₂</td>
</tr>
<tr>
<td>C 1</td>
<td>4</td>
<td>Infarct control; sacrificed four hours</td>
</tr>
<tr>
<td>C 2</td>
<td>5</td>
<td>Infarct control; sacrificed five days</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>Infarct; sacrificed four hours following one-hour hyperventilation with 100% O₂</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>Infarct; sacrificed four hours following one-hour ventilation with 5% CO₂, 95% O₂ mixture</td>
</tr>
</tbody>
</table>

Anesthesia consisted of phencyclidine hydrochloride (Sernylan), 5 mg per kg, pentobarbital 25 mg per kg, and gallamine triethiodide (Flaxedil) 1.0 mg per kg. Blood pressure was monitored with a Statham strain gauge via a femoral cutdown. Arterial blood gases were analyzed at 20-minute intervals on a Corning Digital 160 Blood Gas Analyzer. During the procedure the monkeys received an average of 150 cc of D5W solution and rectal temperature was maintained above 35°C.

FIGURE 1

Angiographical demonstration of occlusion of right middle cerebral artery at its origin in living monkey.

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At first all animals not acutely sacrificed were subjected to cerebral arteriography at two or three days postocclusion. Later angiography was performed only on random animals as a control. Figure 1 shows a typical angiogram done three hours after occlusion of the middle cerebral artery in the Rhesus. At autopsy the position of the clip was verified in each case.

For the PaO₂ modification experiments the normal and infarcted animals were ventilated with room air for three hours after clip placement and then used as hypocapnic, hypercapnic or control animals as indicated. A small animal respirator pump* was used in each case. When 60 minutes of ventilation were completed and gas values had reached appropriate levels (hypocarbia = PaO₂ < 22 mm Hg; hypercarbia = PaO₂ > 50 mm Hg), the animals were killed by decapitation. Initially the mixture was run into the carotids from a height of 50 cm or injected with an infusion pump.§ Later, hand injection was used. Although no difference was noted in the visualization of the larger vessels, considerable improvement in filling of vessels smaller than 100 μ was obtained with manual injection. The brains were then removed and placed in 10% formalin. After seven days, 1-cm thick coronal sections at the level of the internal carotid bifurcations were made using a measured brain-holding device. A Faxitron® Model 805 was used to record the microangiograms. Radiographs were then examined macroscopically and microscopically. For detailed analysis, each microangiogram was divided into 1-cm squares as diagrammed (fig. 2).

A dissecting microscope and a micrometer disk were used (magnification 10.5) to count and measure the vessels. In this experiment, vessels 90 μ or larger were analyzed in areas A-A'. Area A was chosen for counting because it represents the section with maximum ischemia in this preparation. A' is the control side. Only vessels crossing the lower grid mark of areas A-A' were tabulated, as this is the course of the perforating branches from the middle cerebral artery.

Results

Using this approach in the Rhesus monkey, the ischemia is confined to the anterior basal ganglia and internal capsule. After six to eight hours of occlusion, the infarction extends to an average of 1 cc in size. This will give a consistent neurological deficit of grade 2 to 3 based on the Crowell et al.¹¹ criteria for this species.

When the animals used in the acute experiments were killed after four hours of

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†Micropaque, Nicholas Laboratories Ltd., South (Bucks), United Kingdom.
‡Lux Detergent, Lever Brothers, New York, New York.
¶Field Emission Corp., McMinnville, Oregon.

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ischemia, an area of grossly visible softening was demonstrable just above and lateral to the clip. In acute specimens (fig. 3), the average size of this area was 0.3 to 0.5 cm. Animals sacrificed after five days of occlusion demonstrated grossly necrotic lesions averaging 1.0 by 1.2 cm in the same area (fig. 4).

The noninfarcted animals include groups A, B 1 and B 2. Group A consists of normal animals killed while breathing room air (fig. 5a). Monkeys included in the B 1 category are uninfarcted animals killed after one hour of hyperventilation with 100% oxygen (fig. 5b). The B 2 group (fig. 5c) consists of unoperated monkeys ventilated at a normal rate for one hour with a 5% CO₂, 95% O₂ mixture. Blood pressure remained in the normal range for all groups during the experiments.

A comparison of representative microangiograms from the three control situations (Groups A, B 1 and B 2) indicates that we have been able to demonstrate vascular changes in normal monkeys subjected to variations in P açO₂. These changes are parallel to those shown by others using different methodologies. In these groups, the animals were sacrificed when hypocarbic (P açO₂ < 22 mm Hg) showed a uniform narrowing of vessels, whereas the animals with increased P açO₂ (> 50 mm Hg) tended to demonstrate an increased number of large vessels. The total number of vessels 80 to 100 μ in diameter and their configuration are the same in all groups. The vessels smaller than 80 μ are uniformly filled. Microscopic counting of the six largest perforating branches of the middle cerebral artery in areas A from each group shows that in the normal monkey an average of four out of six are 90 μ or greater in diameter. Hypercarbic animals have six out of six in this range, whereas in low P açO₂ monkeys only two out of six perforators reach 90 μ.

In the infarcted series four animals were sacrificed at four hours after middle cerebral artery occlusion (Group C 1) and five at five days after occlusion (Group C 2). Microangiograms from the acute and chronically ischemic monkeys can be compared in figures 6a and 7. In these radiographs of the four-hour monkeys one can see that the area of eventual
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Figure 6

(A) Group C 1 four hours after right middle cerebral artery occlusion, BP 110/70; $P_{aCO_2}$ 35.8 mm Hg at sacrifice. (B) Group D, sacrificed four hours after occlusion. Hyperventilation 100% $O_2$ for one hour, BP 110/80; $P_{aCO_2}$ 20.8 mm Hg at sacrifice. (C) Group E sacrificed four hours after occlusion, ventilated 5% $CO_2$, 95% $O_2$ for one hour, BP 120/70; $P_{aCO_2}$ 50.3 mm Hg at sacrifice.

Necrosis has begun to delineate itself. Vessels of 90 $\mu$m in diameter or greater are preserved in the specimens. The loss of vascular filling seems to be entirely confined to the fine reticular vessels. This is compatible with the earliest changes in an area of infarction reported by others. The size of the nonfilling area approximates the grossly visible changes noted on fresh specimens (fig. 3) from the same time period. The five-day postocclusion animals (Group C 2) demonstrate a well-defined area of nonfilling in the ischemic area. A general decline in vascularity is noted.

The results of application of this methodology to the experimental situation are seen in the series D and E animals (figs. 6b and 6c). Group D monkeys were begun on hyperventilation with 100% $O_2$, three hours after middle cerebral artery occlusion. After one hour of hyperventilation when the $P_{aCO_2}$ was less than 22 mm Hg, the animals were decapitated and Micropaque was injected. Group E monkeys were ventilated at a normal rate for one hour with a 5% $CO_2$, 95% $O_2$ mixture to $P_{aCO_2}$ values greater than 50 mm Hg. The serial $P_{aCO_2}$ values for these animals are shown in figure 8.

Figure 8

Range of $P_{aCO_2}$ values from animals in groups D and E.
The microangiograms for these acutely infarcting animals subjected to change in \( P_{\text{aCO}_2} \) reveal distinct changes from the control specimens. Preliminary tabulation of vessels also indicates that there is a significant difference between the hypocarbic and hypercarbic animals. A complete study is in preparation.

**Discussion**

We have found the transorbital approach to the middle cerebral artery a simple, reliable method of producing a stroke model. Operative mortality is small and one has excellent visualization of the clip position.

In our normal specimens, the perforating branches of the middle cerebral artery were approximately 90 \( \mu \) in diameter. We have focused our attention on vessels of this size which can be well delineated by the Micropaque and which can be accurately counted by the technique employed. In addition, the number of vessels with a diameter greater than 90 \( \mu \) appeared to parallel the number of 50 to 70 \( \mu \) vessels. In other words, paucity of vessels larger than 90 \( \mu \) was matched by scarcity of smaller vessels in most cases.

Several criticisms of our microangiographical technology are possible. An initial challenge was whether the time lapse between death and injection of Micropaque leads to change in vessel configuration.16 In an attempt to avoid such change, if it takes place, instant cessation of cerebral perfusion is attained by decapitation while ventilation is continued. Time lapse between death and injection is ordinarily a few minutes. Initially the heads were dropped into a freezing solution to prevent postmortem changes. This was discontinued when it was found that no difference in morphology could be demonstrated in the frozen and nonfrozen specimens. Some specimens were left unperfused for up to one hour after decapitation and these did not vary in vessel size and number from the immediately perfused animals. Again, some animals were perfused through nonocluded carotids while ventilation and heart beat continued. Vessel filling was poor, but the few vessels filled were of the same configuration and size as those prepared as usual. Our conclusion was that decapitation followed by rapid perfusion demonstrates antemortem vessel architecture.

A second question concerns the effects of the Micropaque itself. The barium-formaldehyde mixture is not inert and it may change the configuration of the vessels. An examination of the various microangiograms, however, does show variability among specimens. This is especially true of Groups B 1 and B 2 and C and D. Subtle differences are preserved even between infarcted and control sides in the same animal. We could only conclude that if the mixture has its own effect, it does not obliterate the morphology present at the time of sacrifice. Furthermore, all our observations regarding vessel number, size, and configuration are relative rather than absolute in nature, and have to be evaluated together with the control studies.

This brings us to the most vital criticism of the method. Does it, in fact, show the true configuration of the vessels subjected to variations in \( P_{\text{aCO}_2} \)? This question cannot be completely answered until in viva angiography of comparable resolution is attainable. In general, however, the effects seen in our specimens do indicate a constrictor effect with low \( P_{\text{aCO}_2} \) and a mild vasodilator effect with high \( P_{\text{aCO}_2} \). This is in keeping with the generally accepted effect of these gases. From this we feel we can conclude that physiological changes present in the vessels prior to sacrifice are demonstrated by our microangiograms.

**Conclusions and Summary**

1. The stroke model used gives a consistent area of infarction in deep cerebral layers with a minimal amount of brain exposure.

2. Microangiograms prepared in the manner described can fix at a point in time the dynamic changes taking place in the microvasculature.

3. The method allows us to duplicate in deeper structures some of the observations of others on the reaction of surface vessels.

4. We can demonstrate changes in vessel morphology following \( P_{\text{aCO}_2} \) changes in both normal and infarcted monkeys.

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Dr. Robert C. Brasch carried out the majority of the cerebral angiographical studies in the living monkeys. We are grateful for the technical assistance of Miss Ingeburg M. Walter and Mr. Clifford Saey.

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