Cerebral Ischemia and Reactive Hyperemia
STUDIES OF CORTICAL BLOOD FLOW AND MICROCIRCULATION
BEFORE, DURING, AND AFTER TEMPORARY OCCLUSION
OF MIDDLE CEREBRAL ARTERY OF SQUIRREL MONKEYS
By Thoralf M. Sundt, Jr., and Arthur G. Waltz

ABSTRACT
Correlative studies of cortical blood flow measured by the $^{85}$Kr washout technique and observations of the cortical blood vessels of 10 squirrel monkeys subjected to temporary occlusion of the middle cerebral artery are described. During occlusion, cortical blood flow in core areas of ischemia decreased to 0.12 to 0.90 ml/g/min (20 to 50% of preocclusion values) and became pressure dependent with failure of autoregulation. After release of the occluding clip, cortical blood flow was restored. Correlation between degree of vascular reaction judged by observation of the cortex and degree of hyperemia as determined by cortical blood flow was poor. There was incomplete correlation between the degree of hyperemia and the degree of preceding ischemia. Hyperemia, or "luxury perfusion," manifested by red venous blood, appears to be related to failure of cerebral tissue to utilize available oxygen as well as to "reactive" hyperemia, or supernormal blood flow, in regions previously ischemic. There was no demonstrable gradual failure of collateral circulation during occlusion. Cerebral edema was progressive and even progressed after restoration of cortical blood flow; it was incompletely correlated with the degree of ischemia and degree of hyperemia.

KEY WORDS
cerebral edema
cerebral autoregulation
luxury perfusion
red venous blood
cerebral infarction
krypton
cerebral blood flow

The concept of hyperemia or "luxury perfusion" (1-13) in relation to cerebral ischemia is generally accepted, but as yet there has been no documentation of the degree or extent of such a phenomenon after restoration of blood flow to an area of brain subjected to a known degree and period of ischemia. We chose the squirrel monkey for a study of such flow changes because of our extensive experience (180 animals) with a laboratory model of cerebral infarction in this species. It was therefore possible to correlate the changes of cortical blood flow recorded in this study with results of previous acute and chronic preparations in which the alterations of the microcirculation were described and photographed and in which areas of infarction and edema were studied temporally and anatomically (2, 8).

It has been shown that cerebral infarction does not develop in the squirrel monkey consistently unless the middle cerebral artery has been occluded for 3 to 6 hours (8). Therefore, these studies were based on this known reversible period of ischemia and were conducted over the first 2 to 3 hours after occlusion. We sought to determine: (1) the degree of hyperemia, (2) the relationship of the hyperemia to the degree of preceding ischemia, (3) the dependency of cortical blood flow in areas of ischemia on blood pressure during the period of major vessel occlusion (5, 6, 10), (4) the relationship between the appearance of the cortical edema and the degree of hyperemia.
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microcirculation and cortical blood flow with special reference to the color of venous blood (7, 8), (5) the presence or absence of a gradual failure of collateral circulation during the period of occlusion, and (6) the correlation of cerebral edema with the degree of ischemia or, after restoration of cortical blood flow, the degree of hyperemia.

Methods

Ten squirrel monkeys (Saimiri sciureus), average weight 750 g, were anesthetized with 0.25 ml of sodium pentobarbital (Nembutal, 50 mg/ml) injected into the intraperitoneal space with a 21-gauge hypodermic needle. A tracheostomy was then done and the animal was positioned in a head holder.

Under the operating microscope, the bifurcation of the right common carotid artery was exposed and the common, internal, and external carotid arteries were ligated temporarily by applying traction on 3-0 silk pull-through sutures. A polyethylene catheter (P 10), filled with heparinized saline and inspected for the absence of residual air bubbles, was then inserted into the external carotid artery and that vessel was ligated distal to the catheter site. This catheter was then connected to an automatic injector for the intermittent 125Kr injections to follow. In this manner, all fluid injected through the catheter would enter an isolated internal carotid artery system without impairment of or encroachment upon the integrity of that system. The temporary ligatures were removed from the major vessels and the catheter was sutured in place with a 4-0 silk suture. Blood loss for this procedure never exceeded 1 ml. The wound was closed with a /8-inch, 25-gauge hypodermic needle. A venous catheter was then placed in the animal’s right femoral vein and an arterial catheter in the right femoral artery, also utilizing the operating microscope. The arterial catheter was connected via a heparinized polyethylene tube to a strain gauge which was in turn connected to a Grass polygraph. There was no blood loss in this portion of the procedure.

The animal was then repositioned and under the operating microscope, via the extradural approach, the animal’s right middle cerebral artery was exposed and prepared for later extradural clipping. One modification of the approach previously described was the aspiration of the global contents so that the approach was through the posterior orbit but still extradural. This minimized brain retraction and preserved the essential elements of the extradural approach. It was a modification of the approach described by Hudgins and Garcia (15). Later, the dura was removed separately and the hemisphere was covered with plastic film (Saran). Blood loss for this portion of the experiment averaged 2 to 3 ml.

The superficial microcirculation of the cerebral cortex was observed through the operating microscope. A Celgel tube with an end-window and a diameter of 6 mm was placed over the parietal region of the involved hemisphere for separate determinations of cortical blood flow (16, 17). 125Kr dissolved in physiologic saline to which was added a small amount of heparin was injected with an automatic injector in volumes of approximately 0.3 ml during 3 seconds into the catheter in the stump of the external carotid artery, delivering the entire bolus to the internal carotid circulation. The appearance and clearance of the beta activity of the diffusible radioactive indicator from the brain were recorded on magnetic tape and by rate meters and strip recorders (6, 9). Pulses were counted from the magnetic tape with a two-channel digital rate meter.

The kinetic analysis of Zierler (18) was used to calculate cortical blood flow (CBF) by using the equation CBF = k(H0 - Hlo /A lo ), with k adjusted for the hematocrit value of the animal (9, 16).

After base-line studies of cortical blood flow, the right middle cerebral artery of the animal was occluded with a miniaturized Mayfield clip. Flow studies were performed for approximately 2 hours, at which time the clip was removed and recording was continued for another 1 hour. In some animals this procedure was altered because of edema, and the clip was removed before 2 hours. In two animals the studies after removal of the clip were not made because of deterioration of the preparation.

Cortical blood flow was studied approximately every 15 minutes and was correlated with the appearance of the microcirculation of the cortex. Special attention was paid to the development or absence of cerebral edema and to the color of venous blood. In one animal these alterations were photographed by a technique previously described (2, 9). During and after occlusion, values of importance were recorded, including the mean arterial blood pressure, arterial PCO2, arterial pH, arterial Po2, and hematocrit.

On the basis of the stability of the blood pressure and hematocrit value, the animals were divided into two groups.

Group 1.—This group consisted of five animals
TABLE 1
Animals with Variable Blood Pressure or Hematocrit Value before, during, and after Occlusion of Middle Cerebral Artery

<table>
<thead>
<tr>
<th>Animal</th>
<th>Time</th>
<th>Cortical blood flow (ml/g/min)</th>
<th>Cortical vein color</th>
<th>Cerebral edema</th>
<th>MABP (mm Hg)</th>
<th>Po2 (torr)</th>
<th>pH</th>
<th>Po2 (torr)</th>
<th>Hct</th>
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<td>black</td>
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<td>85 – 125</td>
<td>37</td>
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<td>After</td>
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<td>1+</td>
<td>105</td>
<td>34</td>
<td>7.43</td>
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*Not studied after occlusion because of deterioration.

Results

Group 1.—In these five animals there was a sharp decrease of cortical blood flow after occlusion of the middle cerebral artery, in which either blood pressure or hematocrit value was variable (Table 1). In these animals there was persistent oozing of blood around the edges of the craniectomy and the temporalis muscle, resulting from the heparin in the irrigation solution of the blood pressure catheter and in the krypton solution. Blood loss was replaced with fluids, and although this would at times bring the blood pressure to normal levels, it remained labile and the animal became a fragile preparation with hemodilution and a decreasing hematocrit value.

Group 2.—This group consisted of five animals in which the blood pressure and hematocrit value were stable (Table 2). In these animals, less heparin was used in the catheters and there was no oozing around the edges of the surgical field; in fact, blood loss was uncommon. In animals in which there was blood loss from this source, the loss was replaced with blood drawn from donor monkeys so that there would be no alterations in the hematocrit value or blood volume.

TABLE 2
Animals with Stable Blood Pressure and Hematocrit Value before, during, and after Occlusion of Middle Cerebral Artery

<table>
<thead>
<tr>
<th>Animal</th>
<th>Time</th>
<th>Cortical blood flow (ml/g/min)</th>
<th>Cortical vein color</th>
<th>Cerebral edema</th>
<th>MABP (mm Hg)</th>
<th>Po2 (torr)</th>
<th>pH</th>
<th>Po2 (torr)</th>
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<tr>
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<td>4+</td>
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<td>33.5</td>
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<td>87</td>
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<td>7.49</td>
<td>97</td>
<td>47</td>
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<tr>
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occlusion to 0.20 to 0.90 ml/g/min, or a value approximately 20 to 50% of the preocclusion flow (Fig. 1, Table 1). Cortical blood flow during the period of occlusion was directly related to the peripheral blood pressure: when there was a decrease or increase in the blood pressure, there was a secondary decrease or increase of the flow (animals 2, 4, and 6). Also, there was some recovery from the initial shock of occlusion in most animals in that the greatest decrease in flow occurred in the first 15 minutes, with some recovery occurring as flow through collaterals compensated in part for the initial ischemia. When the clip was removed from the middle cerebral artery, there was prompt increase of cortical blood flow in three animals. In two of these, the flow returned to the preocclusion value; in one animal, hyperemia resulted in a flow value 200% of the preocclusion value.

Visual observations of the cortex before, during, and after the period of occlusion revealed essentially the same features previously reported and discussed below. The bright redness of the cortical venous blood after restoration of flow was of special interest. There was seemingly no direct or obvious correlation in this group of animals between cerebral edema and the degree of ischemia during the vessel occlusion, but in one animal in which moderate edema was developing during the period of occlusion (animal 6), the degree of hyperemia following the release of the occluding clip was much greater than average and could have represented failure of autoregulation. In this animal, edema continued to progress after removal of the clip.

Group 2.—In these five animals there also was a sharp decrease in cortical blood flow after occlusion to 0.12 to 0.82 ml/g/min, or a value approximately 20 to 50% of the preocclusion flow (Fig. 2, Table 2). During the period of occlusion cortical blood flow remained stable, in contrast to the previous group, and there was no indication of a gradual failure of collateral circulation during the period of occlusion. In two animals (8 and 11) in which there was some compensatory flow through collateral channels with increasing cortical blood flow rather than failure of flow during the time of occlusion, there was an associated
early development of edema. However, in one other animal (5) in which edema developed there was no such response, so this was not a consistent observation.

During the period of occlusion, the venous blood remained black in four animals but in one animal (8) it took on a reddish blue appearance. This was an animal with severe cerebral edema and increasing collateral blood flow prior to removal of the occluding clip and with dramatic increase in cortical blood flow after removal of the clip.

After removal of the clip, cortical blood flow increased promptly in all five animals, with hyperemia developing in three to values of flow as high as 150% of that before occlusion. The degree of cortical vascular reaction on visual observation was misleading; in all instances it appeared that there was marked vascular dilatation and all veins became bright red, yet the flow changes did not always reflect the dramatic appearance of the cortical color. Figure 3 shows the changes in animal 11, in which the clip was removed prior to the end of the 2-hour period of occlusion because of the development of early cerebral edema. Although the black and white photographs do not depict the changes in the color of the cortex, from a golden hue to a bright pink, or in the color of the venous blood, one is able to observe the alterations in the caliber of the arterioles (arrow) sequentially throughout this period of photographic recording. It was noted that edema beginning during ischemia progressed after restoration of blood flow (animals 5, 8, and 11).

Discussion

These measurements and observations were made in areas of ischemia in the squirrel monkey brain which are known to develop infarction after permanent occlusion of the middle cerebral artery (2, 19) but with shorter periods of occlusion have been shown to be areas of potential recovery (8). The technique that was used measures blood flow in cortex only from the surface of the brain to a maximal depth of 0.5 to 0.75 mm (16). Therefore, it was of considerable interest and importance to us to know that cortical blood flow in the core areas of potential massive infarction in this animal can decrease to a low of 0.12 ml/g/min (20% of the preocclusion flow) in some animals and to about 0.60 ml/g/min (approximately 40 or 50%) in other animals. These values are similar to those found for deeper regions of an ischemic cerebral hemisphere by autoradiographic techniques (11, 12) and to values obtained in humans when an internal carotid artery is occluded for endarterectomy (20, 21). Forty percent or even 20% of normal values for cortical blood flow (as measured

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Figure 3

Parietal cortex in squirrel monkey (animal 11). A: Before occlusion. B: Fifteen minutes after occlusion. Veins are darker, arteriolar caliber is normal, and cortical color is good. C: One hour after occlusion. Veins remain black, arteriolar caliber is decreased, and the vessel appears to be in spasm; cortex has areas of focal pallor (not clearly discernible in these black and white reproductions). D: Five minutes after removal of occluding clip. Changes of ische mia are reversing. E: Fifteen minutes after removal of clip. Arteriolar caliber is normal, veins are red, cortex has pink background; reactive hyperemia is maximal visually. F: Thirty minutes after removal of clip. Changes of reactive hyperemia are subsiding. Arrows point to cortical arterioles; other major vessels are veins.

During anesthesia) is vastly different from 0% like that in cardiac arrest, and therefore these observations of preserved (but decreased) cerebral circulation after occlusion of an artery supplying the brain provide further documentation that the 5-minute period cited for brain tolerances to ischemia in cardiac arrest cannot be extrapolated to the situation of occlusion of a single major cervical or cerebral vessel. The degree and duration of ischemia that the brain can tolerate without loss of neuronal function have not been determined with certainty but may be similar to those of these experiments.

"Absolute" hyperemia, defined as increases of cortical blood flow above preocclusion values (6, 11, 12), occurred after release of the clip occluding the middle cerebral artery in...
four of these animals, but in four others there was no significant increase in flow. Therefore, in one-half of the animals, "reactive" hyperemia (12) developed in response to preexisting ischemia. Arterial Pco2 was carefully controlled in all animals so, except for animal 3, alterations of Paco2 could not be a possible explanation for the reactive hyperemia. Similar hyperemia has been described by others using a different method for producing cerebral ischemia (13).

Even in those animals in which there was not a marked increase of cortical blood flow after removal of the clip, visual inspection of the cortex revealed "luxury perfusion" (1), manifested by bright red venous blood. The occurrence of red venous blood without an associated increase of cortical blood flow indicates a state of "relative" hyperemia (1, 6, 7, 11), perhaps caused by a failure of the cerebral tissue to utilize the available oxygen in the perfusing blood (7, 22). Impaired oxygen utilization could be due to ischemic damage to transport mechanisms, metabolic processes, or cellular organelles. Thus, the measurements of cortical blood flow indicate that "luxury perfusion" and bright red venous blood are not simply explained by reactive hyperemia or supernormal circulation to an area with ischemia (1, 3-7, 9-12, 22).

As reported in previous studies, cortical blood flow in areas of ischemia was shown to be dependent on the systemic blood pressure (4-6, 10, 23). This cannot be isolated from the blood volume because in those animals in which there was a decrease in blood pressure, this decrease was secondary to a decrease in blood volume. Thus blood flow in areas of ischemia is not only pressure-dependent but also volume-dependent, findings which correlate well with our unpublished observations made during clinical studies of cerebral circulation during carotid artery surgery.

These studies of cortical blood flow indicate that there is not a gradual failure of collateral circulation during the period of vascular occlusion if the animal has a stable blood pressure and blood volume and if cerebral edema has not developed to the point of representing a significant obstruction to flow.

Our chronic studies have indicated to us that cerebral edema is the chief cause of mortality in these animals after permanent or prolonged temporary occlusion of the middle cerebral artery (8, 19). Although the present studies were not carried over the period necessary to demonstrate cerebral edema consistently in all animals, it was already marked in six after only 1 to 2 hours of cerebral ischemia. It therefore appears that cerebral edema is a chief cause for the spread of a localized area of ischemia and that it can become a self-perpetuating phenomenon. Cerebral edema in areas of ischemia is at present poorly understood; most studies have been directed toward edema produced by techniques other than ischemia (24). It is an immensely complicated problem and beyond the scope of this report, although histochmical studies of the nature of this edema are presently in progress at this institution. One of the chief functions of cerebral metabolism is the maintenance of the integrity of the cell membrane and of the blood-brain barrier, so it would seem only natural that one of the first problems caused by cellular ischemia would be alterations of one or both of these functions, with the development of both intracellular and extracellular fluid accumulation.

This work points again to our relative ignorance regarding the histochemistry of cells in areas of ischemia and to the vital importance of investigating the cerebral edema of ischemia. Until this problem is attacked from a multidisciplinary approach, the possibility of definitive acute stroke management, medical or surgical, for the small or large vessel occlusion will remain dim indeed.

References

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