An appropriate surgical technique for the production of cerebral infarction must fulfill, among others, the following criteria in order to be suitable for electron microscopy (EM) studies: (1) the method of arterial occlusion should yield a high percentage of infarcts with predictable average size; (2) there must be avoidance of surgical manipulation (i.e., retraction) of the cerebral tissues or exposure of the same to the atmosphere; and (3) the method for occluding the artery must be one that permits fixation by perfusion of the ischemic and nonischemic brain.

Modifications to a previously devised method for induction of cerebral infarct are herein described. This new surgical approach has made it possible to conduct detailed and sequential ultrastructural analysis of experimental cerebral infarctions.

**ADDITIONAL KEY WORDS**
- focal cerebral ischemia
- experimental arterial occlusion
- electron microscope

**Introduction**

The successful development of an adequate model for the study of experimental cerebral infarction by electron microscopy (EM) techniques has constituted an elusive pursuit. This can be largely explained by the fact that ligation of extracranial arteries in many animal species is seldom followed by the development of cerebral infarct. On the other hand, several reported methods of occluding intracranial vessels such as the middle cerebral artery (MCA) necessitate retraction of the cerebral tissue and exposure of the same to atmospheric conditions. Either of these factors would be sufficient to induce ultrastructural abnormalities of the nerve cells or of the glia.

A technique was described in 1966 in which surgical occlusion of the MCA in the squirrel monkey was accomplished through a retro-orbital route. When tested in our laboratory, the procedures of this surgical maneuver were found to result in alterations of the blood-brain-barrier (BBB) permeability even before clipping of the arterial vessel was effected. As reported elsewhere, it was determined that extravasation of BBB indicators, such as Evans blue and colloidal carbon, occurred in areas where the brain was merely traumatized by retractors as well as in cerebral...
The animal has been anesthetized and a tube has been placed in the trachea. Head holder and position for microsurgery are shown as well as the periorbital incision and the sutured eyelids. The latter prevents excessive cerebrospinal fluid leakage.

Modifications of the retro-orbital surgical approach have permitted exposure and dissection of the arachnoid from around the initial segment of the MCA in squirrel monkeys.

Introduction of these modifications has eliminated changes in BBB permeability to Evans blue. Furthermore, the occlusion of the MCA performed according to our method has consistently resulted in a predictable syndrome which includes the development of contralateral hemiparesis. At the time of writing this article 15 of 16 animals had survived the surgical intervention.

One final condition for the successful in vivo fixation of the ischemic portion of brain presupposed reopening of the MCA distal to the point where it has been previously clipped. The fact that the artery reopens in all cases had already been determined in another group of animals, similarly operated, and in whom direct visualization of the distal branches of the MCA had been accomplished through a skull window.

A detailed report of the histological and ultrastructural abnormalities that occur in the cerebral microvasculature of those animals operated via the transorbital route is currently being prepared.

**Methods**

Squirrel monkeys (Saimiri sciurea) having an average body weight of 0.8 kg were anesthetized...
with an intrapleural injection of sodium pentobarbital using a dose equivalent to 20 mg/kg of body weight. Once they were sedated and approximately 45 minutes before occlusion of the MCA a total amount of 0.5 ml of a 2% solution of Evans blue in saline was administered through a leg vein. The animals were then intubated and placed in a head holder as shown in figure 1.

The scalp incision and all dissection procedures are done with sharp instruments. Hemostasis is obtained only with the Malis bipolar electrocautery. Orbital exenteration is facilitated by incising and collapsing the globe. The orbital contents are then dissected subperiosteally, with care taken not to enter the nasopharynx. A curved hemostat is placed about the apex of the intraorbital muscular cone and the optic nerve as well as the orbital contents are excised. The remaining “stump” of muscular cone and optic nerve shrinks out of the way with the help of the Malis cautery and can be excised further if necessary. With the help of the Zeiss binocular operating microscope and a small pneumatic drill, the optic foramen is enlarged superiorly and laterally. This permits visualization of the intracranial dura; upon incising the latter, the suprachiasmatic portion of the internal carotid artery and the proximal segment of the anterior cerebral artery as well as the MCA can easily be identified (figure 2). No retraction of cerebral tissues is necessary at any time. Manipulation of the microscope and of the head holder facilitates dissection of the MCA. Utilizing a higher magnification, the underlying arachnoid can be teased away from the vessels with a No. 11 scalpel blade, freeing them for the application of a small Mayfield clip as shown in figure 3. The dural opening is then covered with moist gelfoam, the wound is closed and the eyelids are sutured together.

Leakage of cerebrospinal fluid from the orbit may occur, and in order to prevent microbial meningitis all animals are given Combiotic (veterinary), ½ cc intramuscularly b.i.d., beginning before surgery. With appropriate care, blood loss is limited to the amount necessary to wet one-fourth of a 4 X 4 in. surgical sponge. Blood pressure can be monitored by cannulating one of the anterior tibial arteries with a small needle. Practically all animals require warm towels postoperatively in order to combat hypothermia.

**Discussion**

Some of the advantages of the surgical technique for inducing regional cerebral ischemia that are described in this article reside in the fact that brain retraction and atmospheric exposure of the same are avoided. In addition, removal of the eye has resulted in less hemorrhage than that occurring when dissection is done through the temporalis muscle. Moreover, the resulting exposure of the MCA and of the adjoining vessels is
Coronal section of cerebral hemispheres from an animal killed by perfusion 72 hours following clip application to the middle cerebral artery. The area of histologically evident infarction is outlined. The lateral two thirds of the caudate nucleus and the entire putamen were grossly stained by Evans blue. (×5.)

surgically more advantageous than the one obtained through a more posterior approach.

Our observations have shown that extravasation of Evans blue takes place in: (1) brain traumatized by retractors, (2) brain exposed to atmosphere, and (3) cerebral parenchyma located beneath scalp and muscle incisions made with cutting cautery. This was interpreted as evidence of damage to the blood barrier which in some animals was associated with grossly visible edema. Our surgical technique, however, results in no staining of the brain parenchyma by Evans blue, and evidence of structural abnormalities by either light or electron microscopy examination in sham-operated animals has not been detected. The sham operation includes dissection of the arachnoid, as described in previous paragraphs, and placement of a small, open Mayfield clip around the origin of the MCA for a few seconds before the clip is withdrawn and the wound closed. These sham-operated animals have been noted to awake promptly, and no neurological deficit has been detected postoperatively.

After MCA occlusion the animals remain stuporous for 18 to 24 hours; they also develop easily observed contralateral hemiparesis and visual-field defects through the remaining eye. After approximately 24 hours they move about spontaneously and eat, but are much less combative than normal; marked hemiparesis persists in all animals. At autopsy, grossly visible staining of the brain with Evans blue and histological changes characteristic of ischemia were limited to areas located within the territory supplied by the MCA (fig. 4). No hypoxic nerve cell changes were seen elsewhere, particularly in the hippocampi of either side. In no instance did MCA occlusion fail to produce infarction. Only one animal has died in a group of 16 having the MCA occluded by
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the method described in this article; this occurred 72 hours postoperatively and apparently followed the development of brain edema secondary to extensive infarction.

Leakage of cerebrospinal fluid occurred in several animals, but none developed clinical or structural evidence of meningitis for up to one week. The small Mayfield clip is preferred because it is easily removed minutes before perfusion of the vascular system with glutaraldehyde, which is required in order to fix the brain adequately for EM. Surprisingly, the previously clipped artery reopens in almost every case, allowing adequate fixation of the ischemic portion of the brain. Although the clip is bulky, it lies in the orbit and does not come in contact with the brain.

There were only two operative deaths in 38 animals. One monkey, whose blood pressure was not monitored, bled from the scalp incision beneath the drapes. The second death took place about two hours following surgery; a fresh unilateral subdural hemorrhage was found at autopsy. Blood pressure was not monitored routinely because cannulation of the femoral artery very often was followed by gangrene of the distal part of the leg; capillary-flush pressure readings could not be reproduced consistently. However, histological and electron microscopic examination of the noninfarcted tissues obtained from 37 survivors revealed no detectable evidence of structural abnormalities indicative of hypoxemia.

References

Transorbital Approach to the Middle Cerebral Artery of the Squirrel Monkey: A Technique for Experimental Cerebral Infarction Applicable to Ultrastructural Studies

W. R. HUDGINS and JULIO H. GARCIA

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