Experimental Regional Cerebral Ischemia in the Middle Cerebral Artery Territory in Primates

Part 1: Angio-Anatomy and Description of an Experimental Model With Selective Embolization of the Internal Carotid Artery Bifurcation

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SUMMARY Selective embolization of the internal carotid artery bifurcation (ICA bifurcation) was performed in monkeys (Macaca mulatta) to study acute regional cerebral ischemia in the middle cerebral artery (MCA) territory with minimum surgical intervention in the neck under sedated conditions.

The anthropomorphic similarity in angio-anatomy of the carotid system of monkeys and the use of silastic spheres, as artificial emboli, of the critical diameter of 1.2 to 1.4 mm resulted in the overall success rate of 87% in localizing the site of embolization to the ICA bifurcation, producing ischemia in the whole middle cerebral artery territory.

All the animals with ICA-bifurcation embolization had contralateral deep motor weakness and conjugate eye deviation with nystagmus toward the site of embolization. Simultaneous EEG recording showed flattening of the basic background activities over the affected MCA area and cerebral arteriograms showed definite retrograde filling of the proximally occluded MCA. Clinical recovery was observed in a few animals within two to five hours of embolization. Gross ischemic swelling in the affected MCA territory, particularly in the gray matter, became obvious in six of eight animals which were exposed to four to five hours of ischemia.

The angio-anatomical study of the carotid system of this experimental animal as a background for this MCA stroke model confirmed the previous observations of other investigators that the extremely abundant leptomeningeal anastomoses would be one of the major factors leading to the variability in the clinicopathological pictures seen in the models of proximal MCA occlusion. In addition, the pre-parenchymal anastomoses in the base of brain between the medial striate arteries from the proximal anterior cerebral (ACA) and lateral lenticulostriate arteries from the MCA were observed and described as a possible functional collateral to the basal ganglia in case of proximal MCA occlusion.

Introduction

SURGICAL OCCLUSION of the intracranial artery of an experimental animal is now a widely used method to produce an experimental stroke model of focal cerebral ischemia. However, this method is not free from artifactual surgical damage to the brain parenchyma and intracranial vessels even with microsurgical techniques. Moreover, the loss of CSF during the preparation and anesthesia needed for the meticulous surgery may alter the physiological conditions of the brain at least in the acute phase.

These possible artifacts may be avoided by the embolization method which requires only mild sedation of the animal and minor surgical intervention to the cervical carotid. However, the major disadvantage of the embolic method has been how to localize the site of embolization in one major intracranial artery.

We have developed an MCA stroke model in primates (Macaca mulatta) in which the ICA bifurcation was selectively embolized with commercially available silastic emboli introduced into the carotid system from the neck leaving the entire head intact.

The purpose of this report is to present the details of the selective embolization of the ICA bifurcation in monkeys and its feasibility as an MCA stroke model. The other aspect of this communication is the angio-anatomical study of the carotid system of subhuman primates as a background for this MCA stroke model.

Methods

Angio-Anatomical Study of the ICA System

The ICA system in the base of the brain was dissected and examined under a dissecting microscope in monkey brains (Macaca mulatta) which had been used in multiple experiments and fixed in 10% formalin solution. Attention was paid to the small branches of the terminal ICA, ICA bifurcation, and proximal anterior cerebral artery (ACA) and MCA, which may be involved in the occluded segment in this stroke model. Distance from the center of the ICA bifurcation to the origins of the major branches from the ICA and MCA were measured under the dissecting microscope. Similarly, the number of the perforators, which also may be involved in the occluded segment, was counted.

The angio-anatomical findings were studied further by intravascular dye injection and microangiography in six baboon brains (Papio cynocephalus). Carbonblack-micropaque solution was manually injected into the cannulated ICAs of the freshly removed brains or perfused from the cervical carotid, after pre-washing with saline and 10% for-
Embolic material. Dynamic steps of the filling patterns of the injected dye were photographed. After adequate fixation in 10% formalin, microangiograms were obtained by soft tissue x-ray technique to evaluate the intraparenchymal distribution of the vessels.

Selective Embolization of the ICA Bifurcation

General Preparation

Seventeen healthy adult monkeys (Macaca mulatta), weighing 3.3 to 3.5 kg, were used in this experiment. Sedation of the animals was achieved with Sernylan (phenylcyclidine hydrochloride). Initially the animal was given Sernylan (1.0 mg per kilogram of body weight, i.m.) and atropine sulphate (0.1 mg, i.m.) and was maintained with additional Sernylan (2 to 4 mg, i.m., every 50 to 70 minutes).

The femoral artery and vein were then cannulated for infusion of maintenance Ringer-lactate solution (0.365 ml per minute) and for sampling arterial blood for periodical monitoring of arterial pH, PaO2, PaCO2, plasma electrolytes, osmolality and microhematocrit. Similarly a catheter (PE 190) was inserted into the other femoral artery for periodical monitoring of arterial pressure. Spontaneous respirations were permitted through an endotracheal tube. Temperature was monitored with a rectal probe and an infant NG-tube was used as a bladder catheter.

Embolization Technique

After the general preparation, the animal was fixed on a headholder in a prone position with the head rotated 45° to the right. The left carotid artery bifurcation was then exposed. First, the external carotid artery (ECA) and its branches were ligated. Vascular clamps were utilized to occlude the common carotid artery (CCA) below and the internal carotid artery (ICA) above. An incision was made in the proximal ECA. Two silastic spheres, 1.2 to 1.4 mm in diameter and 1.5 to 1.7 mg in weight, were introduced at one to two emboli at a time will be discussed later. A Medicut intravascular cannula (18 gauge) attached to a syringe filled with, approximately one hour after the embolization, a PE 160 catheter (PE 190) was inserted into the other femoral artery to the length of 30 cm for monitoring of arterial blood pressure. Spontaneous respirations were permitted through an endotracheal tube. Temperature was monitored with a rectal probe and an infant NG-tube was used as a bladder catheter.

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Prompt backflow down the cervical carotid was a good indicator of successful intracranial passage of the emboli. Absence of a prompt backflow, presumably indicating the emboli at the large collaterals, the ophthalmic and the posterior communicating artery, required additional flushes of saline to advance the emboli distally into the ICA bifurcation. However, the most reliable indicator of a successful embolization of the ICA bifurcation was the clinical evidence of acute MCA occlusion which followed shortly after, i.e., contralateral flaccid paralysis and ipsilateral conjugate eye deviation with nystagmus in the horizontal plane. These clinical signs were well correlated with the EEG and cerebral arteriograms. In one animal, a sham operation was carried out to evaluate the effect of flushings with saline four times without introducing the artificial emboli using EEG monitoring.

Finally the proximal ECA was ligated, the vascular clamps were removed to resume the carotid flow, and the incision site was closed. The time for the procedure was less than 30 minutes and the surgical hemorrhage was minimal.

Clinical Observation of the Animal

The animals were observed for two to five hours after the embolization, the head being fixed on the headholder in a prone position. Additional doses of Sernylan were given to obtain minimal sedation (enough to keep the animals on the experimental table). Vital signs and vital functions were monitored periodically. Contralateral motor weakness in the forelimb following embolization was graded as follows: Grade 0 = no evidence of weakness of the contralateral forelimb (the animal exhibited free spontaneous movement with or without noxious stimulation), Grade 1 = any slight movement or grasp response on stimulation only, Grade 2 = some evidence of motor tone, but without movement even after stimulation, and Grade 3 = complete flaccid paralysis. The conjugate eye deviation and nystagmus in the horizontal plane toward the site of embolization were graded simply from (−) to (+ +).

Simultaneous EEG recordings were done in some animals to evaluate the clinical signs of acute MCA occlusion. EEG needle electrodes were placed bilaterally in the skull overlying the distribution of the MCA and control areas. Cerebral angiograms also were obtained in some animals to evaluate the site of embolization and/or ischemic area. Approximately one hour after the embolization, a PE 160 catheter was placed in the right CCA via the ECA and 2 ml of 60% Renografin were injected manually and a series of arteriograms were taken. Plain skull films also were taken to evaluate the site of the intracranial emboli.

At the end of the experimental period, the animals were killed quickly and the whole brains were removed. After the location of emboli in the base of the brains was confirmed, the brains were cut coronally at the level of the anterior commissure for gross pathological study of the sections. As this experiment was originally designed for the study of acute regional cerebral ischemia on brain water and electrolytes, which is the subject of a subsequent article, an exhaustive pathological study is not presented.

Results

Angio-Anatomy of the ICA System

In monkeys the ICA is more developed than the ECA and the branching pattern of the cervical carotid is almost iden-
FIGURES 1A AND B. Embolization technique in the cervical carotid artery.

critical to that of man. The ICA gives off the ophthalmic artery just after the ICA pierces the dura of the cavernous sinus to appear intracranially. The ophthalmic artery proceeds anteriorly under the optic nerve into the orbital cavity, where it has rich collateral anastomoses with the branches of the ECA. When the carbonblack-micropaque solution was perfused from the common carotid after ligation of the ICA, complete filling of the intracranial ICA system could be observed through these ECA ophthalmic anastomoses. Other ECA-intracranial artery anastomoses were observed at the most anterior part of the frontal fossa, where the external ethmoidal artery has fine anastomoses with the olfactory bulb and falx branches of the ACA. However, these ECA-ICA anastomoses are not so well developed in subhuman primates as they are in the lower species such as cats and dogs. Moreover, the maxillocarotid anastomotic arteries, which are prominent in cats and dogs,² could not be clearly demonstrated in monkeys and baboons.

The posterior communicating artery (PCA) arises from the posterior aspect of the ICA at a right angle and is no longer a continuation of the ICA in adult monkeys as is seen in cats and dogs.² ¹⁰ The origin of this vessel is located somewhere between 3.5 and 5.0 mm (average 4.0 mm) proximal to the center of the ICA bifurcation (table 1). A group of hypothalamic perforators branch off from this vessel and from its junction to the ICA.

The ICA then courses around the optic chiasm and gives off the anterior choroidal artery from its lateral aspect. The anterior choroidal artery may have one or more origins, located somewhere between 0.9 and 3.0 mm (average 2.1 mm) proximal to the center of the ICA bifurcation. This artery gives off branches to the amygdala and hippocampal area, and thus has cortical anastomoses with the branches of the MCA and PCA over the inferomedial part of the tip of the temporal lobe. The anterior choroidal artery also supplies the tail of the caudate nucleus, medial division of the globus pallidus, ventral nuclear group of the thalamus, part of the lateral geniculate body, and part of the posterior limb of the internal capsule, and terminates in the choroid plexus of the temporal horn of the lateral ventricle, where it

**TABLE 1** Distance from the Center of the ICA Bifurcation to the Origins of the Major Branches of the Terminal ICA and Proximal MCA

<table>
<thead>
<tr>
<th>PCA</th>
<th>Anterior choroidal artery</th>
<th>Anterior temporal artery</th>
<th>OF artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>3.5-4.5 (3.9)</td>
<td>0.9-3.0 (2.1)</td>
<td>0-7.2 (4.0)</td>
</tr>
<tr>
<td>Right</td>
<td>3.5-6.0 (4.0)</td>
<td>1.2-3.0 (2.2)</td>
<td>0-6.0 (3.2)</td>
</tr>
</tbody>
</table>

Nine monkey brains, fixed in 10% formalin, were used. Number in parentheses is arithmetic mean. In some animals the temporal poler artery was seen to originate from the terminal portion of the ICA. In 75% of the cases, the anterior temporal artery was seen as a first major branch from the proximal MCA proximal to the origin of the orbitofrontal artery. In general, the branching pattern and arterial caliber were quite variable between hemispheres as well as between the individual animals. PCA = posterior communicating artery, OF = orbitofrontal artery.

**TABLE 2** Number of Perforators from the Terminal ICA, ICA Bifurcation, Proximal ACA, Proximal MCA, Orbitofrontal and Distal MCA (Distal to the Orbitofrontal Artery) in Monkeys

<table>
<thead>
<tr>
<th>Terminal ICA</th>
<th>ICA bifurcation</th>
<th>Proximal ACA</th>
<th>Proximal MCA</th>
<th>OF artery</th>
<th>Distal MCA (distal to OF artery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>0-2</td>
<td>1-2</td>
<td>2-4</td>
<td>1-4</td>
<td>4-7</td>
</tr>
</tbody>
</table>

OF = orbitofrontal. Perforators from the proximal ACA mean recurrent arteries from the ACA (Heubner's artery in man). These arteries were large (> 20 μ in caliber) as were the lateral lenticulostriate arteries which originate mostly from the OF artery. These were rich pre-parenchymal anastomoses between these vessels over the olfactory trigone and the anterior perforating substance. These vessels also had anastomoses with the cortical branches of the ACA and MCA. Nine monkey brains were used.
FIGURE 2. Base view of a baboon brain which was freshly removed and injected with carbonblack-micropaque solution through the cannulated ICAs after pre-washing with saline and 10% formalin. The proximal segment of the right ACA and basilar artery were ligated before the dye injection. The supratentorial part of the brain was completely perfused with the dye, except for the segmentally occluded ACA. Note rich leptomeningeal anastomoses and variety in vascular pattern and caliber.

has rich collateral anastomoses with the branches of the posterior choroidal artery from the PCA.

From the terminal part of the ICA, a few fine perforators arise to supply the anterior hypothalamus and septal area (table 2).

The ICA then divides into the ACA and MCA, forming the ICA bifurcation. Both ACAs unite into a single pericallosal artery in subhuman primates to enter the interhemispheric fissure, where it gives off the cortical branches to both hemispheres. From the proximal part of the ACA, two to four recurrent arteries (Heubner's artery in man) arise. These perforators constitute a large portion of the medial striate arteries in subhuman primates and, in some of these animals, they have unusually rich anastomoses, over the olfactory trigone and anterior perforating substance, with the lateral lenticulostriate arteries from the MCA before penetrating into the parenchyma (figs. 2 to 5). A few fine perforators originating from the ICA bifurcation or the proximal MCA stem also contribute to the medial striate group.

The temporal polar or anterior temporal artery may arise from the terminal ICA or ICA bifurcation. However, the origin of this vessel was rather widely distributed on the MCA stem as far as 7.2 mm distal to the ICA bifurcation. This vessel has collateral anastomoses with the branches of the anterior choroidal and PCA over the tip of the temporal lobe.

The orbitofrontal artery, which arises from the anterior aspect of the MCA stem somewhere between 4.2 and 6.7 mm (average 5.4 mm) distal to the ICA bifurcation, is a unique feature of the angio-anatomy in these animals (figs. 2 to 4). This artery arises from the MCA stem in a short (1 to 3 mm), thick (50% to 75% of the MCA diameter) trunk, which then divides into the cortical branches and a group of large lateral lenticulostriate arteries. The cortical branches supply most of the orbital surface of the frontal lobe and have rich leptomeningeal anastomoses with the branches of the ACA. The large lateral lenticulostriate arteries, four to seven in number, usually originate from the trunk of the orbitofrontal artery, then turn back medially, dividing several times before penetrating the parenchyma at the anterior perforating substance. Then they sweep back laterally in the parenchyma, in a large curve, concave mediodorsally, to supply the striatum, the lateral division of the globus pallidus, the claustrum, and the inner and outer capsule. In some animals there were a few small perforators arising directly from the MCA stem near the origin of the orbitofrontal artery, which join the lateral lenticulostriate group. In three monkey hemispheres, in which the origin of the orbitofrontal artery was located rather laterally, it was...
observed that one of the large lateral lenticulostriate arteries originated directly from the proximal MCA stem to join the remaining lateral lenticulostriate arteries from the orbitofrontal artery, forming rich pre-parenchymal anastomoses between them.

The MCA stem then divides into two or three candelabra branches in the orifice of the lateral sulcus. These cortical branches have rich leptomeningeal anastomoses with the ACA and PCA over the lateral aspect of the cerebral hemisphere.

When the carbonblack-micropaque solution was injected through the cannulated ICAs distal to the origin of the posterior communicating artery, after ligation of the MCA at its origin or after segmental ligation of the proximal ACA, the collateral filling of the medial striate or lateral lenticulostriate arteries through the pre-parenchymal anastomoses could be observed easily in baboon brains. However, no intraparenchymal anastomoses could be identified on microangiograms (fig. 5). The collateral anastomoses between the anterior and posterior choroidal arteries were observed in the choroid plexus, and via the anastomoses the proximal PCA could be filled with the dye when perfused into the ICA distal to the origin of the posterior communicating artery.

Despite the single pericallosal artery in monkeys and baboons, anthropomorphism of angio-anatomy of the ICA system in these animals appeared to be fairly well established. However, the branching patterns and the calibers of the vessels were quite variable between the individual hemispheres as well as between the individual animals. Moreover, in addition to the extremely abundant leptomeningeal anastomoses between the ACA, MCA and PCA, the pre-parenchymal anastomoses between the medial striate arteries from the proximal ACA and the lateral lenticulostriate arteries from the MCA possibly may serve as functional collaterals to the basal ganglia in these animals.

**Location of the Emboli in the Intracranial Vessels**

The site of emboli in the base of the brain could be identified easily on postmortem examination (fig. 6). The embolic spheres were usually located side by side in the vessel. Twelve of 16 animals had segmental occlusion two spheres in length (<2.8 mm), and two animals had occlusion three spheres in length (<4.2 mm). In 14 cases, the occlusion occurred exclusively at the ICA-MCA junction. However, two patterns of occlusion were seen, probably due to the variability in the orifice caliber of the MCA and possibly to the slight fluctuation in the size and shape of the silastic spheres.

The type of occlusion which we intended to produce was seen in 11 of the animals in which the distal embolus was
lodged in the ICA bifurcation and in which the proximal sphere was located in the terminal ICA distal to the origin of the posterior communicating artery (fig. 6). Thus, in addition to occlusion of the ICA bifurcation, this type of occlusion involved the origin of the anterior choroidal artery and a few fine perforators from the terminal ICA. The other type was observed in three animals in which the distal sphere was plugged just inside the orifice of the MCA and the proximal sphere was arrested in the ICA bifurcation. In this second type of occlusion, the anterior choroidal artery was not involved in the occluded segment. Two of the three animals with the latter type of occlusion had large MCA caliber. These 14 cases were considered to be successful in localizing the site of embolization in the ICA bifurcation and in producing acute ischemia in the MCA territory, resulting in the overall success rate of 87% (table 3).

Uncontrolled localization of the emboli was encountered in two animals. In one animal (No. 12), one of the emboli was lodged in the ICA bifurcation, but the other was in the distal MCA stem at the origin of the orbitofrontal artery. In the other animal (No. 7), two spheres were located in the distal MCA stem at the origin of the orbitofrontal artery and a third sphere, which was introduced in this animal, was found arrested in the ICA bifurcation, but the other was in the proximal MCA stem at the origin of the orbitofrontal artery and a few fine perforators from the terminal ICA and ICA bifurcation could be visualized with the dye injection method via the preparenchymal anastomosis with the lateral lenticulostriate arteries and vice versa.

### Table 3: Animals, Saline Flushing, Number and Location of Emboli, Neurological Deficits, and Postmortem Gross Ischemic Changes in the Brain

<table>
<thead>
<tr>
<th>Animals</th>
<th>Wt. (kg)</th>
<th>No. emboli</th>
<th>No. saline flushing</th>
<th>Location of emboli</th>
<th>Duration of exp. (hrs.)</th>
<th>Neurological deficits</th>
<th>Gross ischemic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Distal</td>
<td>Proximal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.4</td>
<td>2+1</td>
<td>2+1</td>
<td>ICA-B(1)</td>
<td>ICA-T(2)</td>
<td>2</td>
<td>3 3 + + + + None</td>
</tr>
<tr>
<td>2</td>
<td>4.4</td>
<td>2</td>
<td>1</td>
<td>ICA-B</td>
<td>ICA-T</td>
<td>2</td>
<td>3 3 + + None</td>
</tr>
<tr>
<td>3</td>
<td>3.8</td>
<td>2</td>
<td>1</td>
<td>ICA-B</td>
<td>ICA-T</td>
<td>2</td>
<td>3 1 + + None</td>
</tr>
<tr>
<td>4*</td>
<td>3.3</td>
<td>2</td>
<td>1</td>
<td>MCA-O</td>
<td>ICA-B</td>
<td>3</td>
<td>3 1 + + + Putamen?</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>2</td>
<td>2</td>
<td>ICA-B</td>
<td>ICA-T</td>
<td>3</td>
<td>3 3 + + + + + + None</td>
</tr>
<tr>
<td>6*</td>
<td>3.9</td>
<td>2</td>
<td>2</td>
<td>MCA-O</td>
<td>ICA-B</td>
<td>3</td>
<td>3 3 + + + + Putamen?</td>
</tr>
<tr>
<td>7*</td>
<td>4.9</td>
<td>2+1</td>
<td>2+1</td>
<td>MCA-D(2)</td>
<td>ACA-P(1)</td>
<td>3</td>
<td>1 1 + + + + None</td>
</tr>
<tr>
<td>8</td>
<td>3.8</td>
<td>2+1</td>
<td>2+2</td>
<td>ICA-B(1)</td>
<td>ICA-T(2)</td>
<td>4</td>
<td>3 2 + + + + + + None</td>
</tr>
<tr>
<td>9</td>
<td>5.1</td>
<td>2</td>
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<td>ICA-T</td>
<td>4</td>
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<tr>
<td>10</td>
<td>3.9</td>
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<td>2</td>
<td>ICA-B</td>
<td>ICA-T</td>
<td>4</td>
<td>3 1 + + + + Sylvian convolution, putamen</td>
</tr>
<tr>
<td>11</td>
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<td>ICA-B</td>
<td>ICA-T</td>
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<td>3 3 + + + + + + None</td>
</tr>
<tr>
<td>12</td>
<td>3.8</td>
<td>2</td>
<td>1</td>
<td>MCA-D</td>
<td>ICA-B</td>
<td>4</td>
<td>3 3 + + + + Putamen</td>
</tr>
<tr>
<td>13</td>
<td>5.3</td>
<td>2</td>
<td>2</td>
<td>ICA-B</td>
<td>ICA-T</td>
<td>5</td>
<td>3 3 + + + + + + + + Sylvian convolution, putamen</td>
</tr>
<tr>
<td>14</td>
<td>4.3</td>
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<td>1</td>
<td>ICA-B</td>
<td>ICA-T</td>
<td>5</td>
<td>3 3 + + + + + + Sylvian convolution, putamen</td>
</tr>
<tr>
<td>15</td>
<td>4.6</td>
<td>2</td>
<td>1</td>
<td>MCA-O</td>
<td>ICA-B</td>
<td>5</td>
<td>3 3 + + + + + + + + Sylvian convolution, putamen</td>
</tr>
<tr>
<td>16</td>
<td>4.6</td>
<td>2</td>
<td>1</td>
<td>ICA-B</td>
<td>ICA-T</td>
<td>5</td>
<td>3 3 + + + + + + + + Sylvian convolution, putamen</td>
</tr>
</tbody>
</table>

*Animals which had unusually large-caliber MCA. Number in parentheses is the number of spheres in each part of the vessel.
in the proximal ACA. This animal also had a large-caliber MCA.

Clinical Observation and Gross Ischemic Changes of the Brain

The animals sedated with Sernylan and fixed on a head-holder in a prone position exhibited fairly free movement and/or grasp response of the forelimbs. Restrainment of the un-affected limbs was needed throughout the experimental period. The eyes were open and spontaneous vertical nystagmus was almost always observed prior to embolization. However, nystagmus in the horizontal plane was never seen before successful embolization of the ICA bifurcation. Corneal, pupillary and gag reflexes were preserved. Horner’s pupil was not uncommon (69%) on the side of the carotid surgery. Vital signs and vital functions before and during the experimental period are shown in table 4. Two animals had tachypnea (60 to 70 per minute) and a moderate depression of the PCO2 (28.8 to 34.4 mm Hg) after the initial administration of Sernylan, which persisted through the experimental period. The tachypnea was thought to be a side effect of Sernylan. Slightly high blood pressure in this series may have been due to Sernylan and/or mild sedation of the animal. There was no significant effect of embolization on vital signs and vital functions.

All 14 animals with occlusion of the ICA bifurcation had complete flaccid paralysis of the contralateral forelimb (Grade 3) and ipsilateral conjugate eye deviation with nystagmus in the horizontal plane shortly after embolization (table 3). The EEG showed simultaneous flattening of the basic background activities over the affected MCA area (fig. 6) and cerebral arteriograms showed definite retrograde filling of the affected MCA to the occluded site (fig. 7). Skull x-rays were not successful in visualizing this small sphere on the film.

Three of 14 animals (Nos. 3, 4 and 10) with occlusion of the ICA bifurcation, however, showed apparent clinical recovery from the initial deep motor weakness at 30, 80 and 140 minutes, respectively. One of them had a large-caliber MCA with a very well-developed orbitofrontal artery, which might serve as a significant source of collaterals from the ACA.

Gross ischemic change in the brains of these 14 animals was as follows: No gross ischemic change was observed in the brains which were exposed to two hours of ischemia. Two of three brains which were exposed to three hours of ischemia showed equivocal ischemic discoloration only in the basal ganglia in the affected MCA territory. Six of eight brains which were exposed to four to five hours of ischemia showed apparent gross ischemic changes in the affected MCA territory such as ischemic discoloration and widening of the gyri along the sylvian fissure, loss of distinct junction between the gray and white matter, and discoloration and increase in the surface area of the basal ganglia as well as the midline shift to the opposite side in the more severely affected cases (see Part 2, fig. 21). No gross ischemic changes in the white matter, such as the juicy and bulky appearance of the cut surface, could be identified clearly. No gross hemorrhagic infarct was encountered in this series. The contralateral hemispheres appeared normal.

The animal (No. 12) in which the proximal MCA stem was trapped with two embolic spheres had deep neuronal deficit, but showed mild gross ischemic change only in the basal ganglia of the affected side after four hours of ischemia. The animal (No. 7) with two emboli in the distal MCA stem at the origin of the orbitofrontal artery and another embolus in the proximal ACA did not have a significantly deep motor deficit (Grade 1). The animal remained in the same condition throughout the three hours of the experiment. No gross ischemic change was observed in this brain.

The animal in which the sham operation was performed exhibited no neurological deficit, no significant change in EEG, and no gross change in the brain after four hours of observation.

Discussion

In cats and dogs, the extracranial part of the ICA is only poorly developed and the blood supply to the forebrain is largely dependent on the ECA system through the very well-developed maxillocarotid anastomoses and on the vertebral-basilar system through the posterior communicating artery, which is more of a continuation than a branch of the intracranial ICA even in the adult animal.10 Conversely in the subhuman primates, the ICA is very well developed and the maxillocarotid anastomoses could hardly be identified in this study. The pattern of the arterial branching and the relative sizes of the ophthalmic and posterior communicating arteries to the ICA are similar to those of the human brain.

This well-developed ICA system and the greater branching angle and larger orifice caliber of the MCA than that of the ACA at the ICA bifurcation facilitated the migration of the artificial emboli to the ICA-MCA system.9 In addition, careful selection of the silastic spheres, 1.2 to 1.4 mm in diameter, made it possible, with the aid of saline flushing, to selectively localize the site of embolization to the ICA bifurcation.

The prompt backflow down the cervical carotid artery after saline flushing was a good indicator of the successful intracranial passage of the emboli beyond the major collateral branches, the ophthalmic and posterior com-

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**Table 4. Vital Signs, Arterial Blood Gases, Plasma Electrolytes and Osmolality, Hematocrit and Urine Output Before and After Embolization**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mm Hg)</td>
<td>141 ± 15</td>
<td>140 ± 20</td>
</tr>
<tr>
<td>Pulse rate (per min)</td>
<td>137 ± 20</td>
<td>156 ± 16</td>
</tr>
<tr>
<td>Res pirations (per min)</td>
<td>38 ± 11</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.0 ± 0.8</td>
<td>38.6 ± 0.6</td>
</tr>
<tr>
<td>Arterial blood gases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.402 ± 0.028</td>
<td>7.401 ± 0.032</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>43.9 ± 4.2</td>
<td>40.8 ± 2.9</td>
</tr>
<tr>
<td>Po2 (mm Hg)</td>
<td>85.2 ± 8.5</td>
<td>85.9 ± 7.3</td>
</tr>
<tr>
<td>Plasma electrolytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>146.2 ± 3.8</td>
<td>145.1 ± 4.5</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>3.1 ± 0.3</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>106.3 ± 4.8</td>
<td>108.0 ± 4.4</td>
</tr>
<tr>
<td>Plasma osmolality</td>
<td>300.4 ± 0.9</td>
<td>297.2 ± 5.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40 ± 3</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Urine output (ml/min)</td>
<td>—</td>
<td>0.135 ± 0.063</td>
</tr>
</tbody>
</table>

*Mean and SD in 14 animals. MABP = mean arterial blood pressure.*
municating arteries. The simultaneous development of contralateral deep motor weakness and ipsilateral conjugate eye deviation with horizontal nystagmus was also a reliable indicator of successful occlusion of the ICA bifurcation, thus producing acute ischemia in the MCA territory. Furthermore, these neurological signs correlated well with the simultaneous EEG recording which showed flattening of the basic background activities over the affected MCA area and with the cerebral arteriograms which showed nonfilling of the occluded MCA and a definite retrograde filling to the affected MCA territory via leptomeningeal collaterals. Plain skull films, however, were not successful in visualizing these tiny spheres, although they are impregnated with barium.

Molinari et al. reported that a cylindric embolus made of a silicone compound, 1.5 mm in diameter, would engage the lumen of the proximal MCA in monkeys. However, in our experience with the silastic spheres, the sphere (1.5 mm in diameter) was only exceptionally located in the ICA bifurcation or beyond. It was usually arrested in the clinoid or suprachlomoid portion of the ICA below the bifurcation, in which case the animal did not have a significant degree of motor weakness or showed complete recovery soon after embolization.

Too vigorous and forceful flushings of saline may be harmful to the brain in terms of producing artificial hypertension and this should be avoided. In one sham operation, a bolus of saline was flushed four times without introduction of spheres. The animal had no neurological deficit, no detectable change in the EEG, and no pathological change in the brain after four hours of observation.

Faced with the varieties of the vessel caliber we decided to introduce multiple spheres, two spheres at a time and an additional one when needed, which were slightly different in size, in hope of localizing at least one of the spheres in the ICA bifurcation. Moreover, it was assumed that the proximal sphere might prevent the distal one from later distal migration by eliminating the transmission of the pulsatile pumping effect of arterial pressure and by attenuating the vascular waves transmitted murally to the distal sphere.

In fact, primary proximal occlusion and later distal migration of an embolus have been suggested experimentally by Molinari in dogs, using a cylindric silicone embolus. Later distal migration of an embolus may occur with the development of vasoparalysis or resorption of initial vascular spasm distal to the primary site of occlusion. It also may be true that an embolus arrested in an arterial division may stay at its primary site of lodging, while an embolus in a tubular part of an artery easily may be carried more distally.
from the primary site of lodging, because of the sudden reduction of the vessel caliber and the defective muscular layer in the former part of the artery.

Therefore, the multiple-spheres method which was adopted in this study was started more or less with a trial and error method at the beginning. However, based on clinical observation and postmortem examination of the location of the emboli, the overall success rate of 87% was achieved in this attempt to localize the site of occlusion at the ICA-MCA junction and to produce ischemia in the occluded segment. In three animals, however, the two emboli were arrested one behind the other, with the distal embolus just inside the orifice of the MCA and the proximal one in the ICA bifurcation. This type of embolization did not involve the origin of the anterior choroidal artery in the occluded segment.

The anterior choroidal artery is known to have collateral anastomoses with the cortical branches of the MCA and PCA over the tip of the temporal lobe, and with the posterior choroidal artery in the choroid plexus of the temporal horn of the lateral ventricle. These findings also were confirmed in the present study. The origin of the anterior choroidal artery is located at the terminal ICA below the bifurcation, but it may originate from the bifurcation itself or from the most proximal part of the MCA stem. Therefore, there is a high possibility that the anterior choroidal artery is involved in the occluded segment of this sort of embolization of the ICA-MCA junction. A few fine perforators from the ICA bifurcation also can be involved in the occluded segment. In some animals, particularly in baboons, the temporal polar artery was seen to originate from the terminal ICA (figs. 3 and 4).

The embolus in the ICA bifurcation obliterates the origins of both the ACA and MCA. However, the blood flow to the proximal part of the ipsilateral ACA can be guaranteed from the opposite side via the single pericallosal artery formed by junction of both ACAs in subhuman primates.

When carbonblack-micropaque solution was injected into the ICAs after ligation of the proximal ACA, the medial striate arteries originating from this obliterated segment could be filled retrogradely through the anastomoses with the lateral lenticulostriate arteries from the MCA over the olfactory trigonum. Likewise, when the MCA was ligated at its origin, the collateral filling of the lateral lenticulostriate arteries could be possible through the same anastomoses. However, the intraparenchymal anastomosis between the medial striate and lateral lenticulostriate arteries has been questioned and also could not be identified in this study.

The orbitofrontal artery provides a unique angio-anatomical feature of the monkey brain, as has been observed by Molinari et al. This artery originates from the anterior aspect of the MCA stem in a fairly constant manner, and most of the orbital surface of the frontal lobe is supplied by its cortical branches, which have rich leptomeningeal anastomoses with the ACA. Furthermore, this artery gives off a group of large lateral lenticulostriate arteries from its proximal trunk, except in a few cases when the origin of the orbitofrontal artery is more laterally located. The development of this artery in many of the hemispheres examined was so remarkable that, in case of proximal MCA occlusion, the collateral backflow to the large lateral lenticulostriate arteries via the anastomoses between the orbitofrontal artery and ACA seemed to be possible as well as the retrograde backflow from the distal MCA proper. Kaplan has noticed the anastomoses between the recurrent artery from the proximal ACA and the lateral lenticulostriate arteries and cortical branches of the ACA and MCA in the base of the human brain. These anastomoses in human brain, illustrated in his article, appear to be much less developed than they are in monkey and baboon brains. Crowell et al. have stated in their microangiographic study in monkeys that in several animals it was possible to trace the medial striate vessels back to the
ACA or to trace the lateral lenticulostriate vessels back to the MCA or both. Moreover, the leptomeningeal collaterals of monkey brains are so abundant that the complete retrograde filling of the proximally occluded MCA is quite common by cerebral angiography (fig. 7).

These angio-anatomical features, together with the wide variation between the individual animals, may explain the different consequences of experimental MCA occlusions — the wide variability in the extent and degree of the ischemic lesions and the poor correlation with the clinical deficits produced. It is also clear that the more extensive the ischemic area involved, the more severe will be the pathology. Symon et al. recently suggested in their MCA stroke model in baboons that when severe brain swelling is desired in the model, simultaneous occlusion of the anterior choroidal and middle cerebral arteries or the MCA and terminal carotid would appear to be necessary.

In this study, vital signs and vital functions were carefully monitored throughout the experimental period. All the animals with occlusion of the ICA bifurcation had deep neurological deficits shortly after embolization. Three of them, however, showed apparent clinical recovery from the initial deep motor weakness after 50 to 140 minutes. Gross ischemic changes of the brains became obvious particularly in the gray matter of the affected MCA territory after four to five hours of ischemia. No clear-cut correlation could be observed between the involvement of the anterior choroidal artery or terminal carotid in the occluded segment and clinicopathological pictures which followed. Neither gross hemorrhagic infarct nor animal death occurred. The development of the ischemic brain swelling appeared to be related to the duration of ischemia and the extent of the brain involved, which is apparently dependent on the amount of collaterals in individual animals. Details of the ischemic cerebral swelling and edema will be the subject of a subsequent article (see Part 2).

In two animals we failed to localize the site of embolization in the ICA bifurcation, presumably due to the variable orifice caliber of the MCA and the slight fluctuation in size and shape of the emboli used. However, these failures can be utilized as models of other types of MCA embolization — Animal No. 7 as a model of distal MCA occlusion, and Animal No. 12 as a model of a trapped MCA stem.

Due to the similarity in the chemical structure and clinical effects of Sernylan to ketamine, Sernylan may have similar effects on cerebral blood flow, metabolism, and physiological status of the brain. However, the dissociated anesthesia obtained with this drug appeared to be quite satisfactory for observation of the development of acute MCA stroke in monkeys.

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