THE EFFECT OF THE INTRA-CAROTID INJECTION OF CERAMIC MICROSPHERES ON THE ELECTROENCEPHALOGRAM OF THE DOG

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By


* * * * * *

The Ohio State University
1969

Approved by

Adviser

Department of Veterinary Physiology and Pharmacology
ACKNOWLEDGMENTS

The sacrifices of my wife Peggy can be read between the lines of this dissertation. Her devotion and encouragement were my support.

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Major Field: Veterinary Physiology

# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>PURPOSE</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>3</td>
</tr>
<tr>
<td>Animals</td>
<td></td>
</tr>
<tr>
<td>Microspheres</td>
<td></td>
</tr>
<tr>
<td>Preparation of microspheres for intra-carotid injection</td>
<td></td>
</tr>
<tr>
<td>Intracarotid injection technique</td>
<td></td>
</tr>
<tr>
<td>Recording apparatus</td>
<td></td>
</tr>
<tr>
<td>Collection of tissues</td>
<td></td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>20</td>
</tr>
<tr>
<td>Cerebrovascular occlusion</td>
<td></td>
</tr>
<tr>
<td>Studies utilizing carbonized microspheres</td>
<td></td>
</tr>
<tr>
<td>RESULTS</td>
<td>51</td>
</tr>
<tr>
<td>80μ microspheres treated group</td>
<td></td>
</tr>
<tr>
<td>50μ microspheres treated group</td>
<td></td>
</tr>
<tr>
<td>15μ microspheres treated group</td>
<td></td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>80</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>85</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>87</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>89</td>
</tr>
<tr>
<td>Table</td>
<td>Description of animals used in dissertation and size and approximate number of microspheres injected into the internal carotid artery</td>
</tr>
<tr>
<td>-------</td>
<td>---------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Description of animals used in dissertation and size and approximate number of microspheres injected into the internal carotid artery</td>
</tr>
<tr>
<td>2.</td>
<td>Standard sizes of microspheres used and approximate number per milligram</td>
</tr>
<tr>
<td>3.</td>
<td>Summarization of EEG findings and neurologic observations in all animals</td>
</tr>
</tbody>
</table>

v
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Photomicrographic comparison of a human hair and carbonized ceramic microspheres. Courtesy 3M Company, St. Paul Minnesota</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Relationship of size of microsphere and number of microspheres per milligram</td>
<td>9</td>
</tr>
<tr>
<td>3.</td>
<td>Location of placement of surface electrodes for recording the electroencephalogram</td>
<td>13</td>
</tr>
<tr>
<td>4.</td>
<td>The electroencephalogram of a normal, alert dog.</td>
<td>17</td>
</tr>
<tr>
<td>5.</td>
<td>The electroencephalogram of a normal, asleep dog.</td>
<td>19</td>
</tr>
<tr>
<td>6.</td>
<td>Representation of the approximate size of the internal carotid circulation of the cerebrum</td>
<td>26</td>
</tr>
<tr>
<td>7.</td>
<td>Blood supply to the cerebrum via pial vessels and their branches. Anastamotic branches through which collateral blood flow may occur are indicated by arrows. Roman numerals indicate layers of the cortex.</td>
<td>28</td>
</tr>
<tr>
<td>8.</td>
<td>The EEG of a dog immediately following the injection of 80μ microspheres into the internal carotid artery demonstrating a marked reduction in amplitude of the EEG wave form for periods as long as two and one-half seconds.</td>
<td>53</td>
</tr>
<tr>
<td>9.</td>
<td>The EEG of a dog seventy-two hours after the injection of 80μ microspheres into the internal carotid artery. The EEG has a diffuse, bilateral reduction in amplitude.</td>
<td>55</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>The EEG of a dog seventy-two hours following the injection of 80μ microspheres into the right internal carotid artery. The EEG had a marked reduction of amplitude initially but subsequently developed a diffuse slow wave pattern of 50μV, 5 Hz.</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Photomicrograph of 80μ microspheres lodged in a pial artery. X 70.</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>The EEG of dog number nine one hour postoperative illustrating diffuse bursts of 50μV, 5 Hz activity which persisted for one second periods.</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>The EEG of dog number nine demonstrating persistent bilateral reduction in amplitude in the occipital leads.</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>The EEG of a dog seventy-two hours following the injection of 50μ microspheres. Indications of spindle activity of 25μV amplitude occur.</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Photomicrograph of 50μ microspheres lodged in a parenchymal artery in the cerebral cortex. X 70.</td>
<td></td>
</tr>
</tbody>
</table>
PURPOSE

Evaluation, diagnosis and treatment of internal neoplasms has been facilitated by the availability of carbonized microspheres which can be labelled with radioactive nuclides. The basis for their use is to also provide the medical and biomedical researcher with a new tool for scintillation scanning of organs and for studying various physiological processes such as blood flow and distribution, capillary size measurement, clearance mechanisms in the lung and lymphatic flow.

Studies on the use of microspheres in man and experimental animals have been largely restricted to the lung, liver, spleen and prostate with only two reports on a very limited number of cases concerning the effects of their introduction into the cerebral circulation in sub-human primates.

A study of the effects of the introduction of microspheres into the cerebral circulation of the dog was instituted using the electroencephalogram (EEG) for evaluation of cerebral cortical function. The electroencephalogram is accepted as a sensitive instrument for evaluation of cerebral cortical function.
The basis for the utilization of the labelled microspheres is that they will completely lodge and remain in the perfused organ. The brain, especially the cerebral cortex, is highly susceptible to ischemia produced by thrombus or embolism and the introduction of the microspheres into the cerebral circulation has the potential of producing microinfarcts. It was hypothesized that injection of non-labelled microspheres into the internal carotid artery would be a method of producing cerebral microinfarcts in dogs for study of the effects on the EEG. In addition the feasibility and safety of perfusion of the dogs brain with microspheres could be concurrently evaluated.
MATERIALS AND METHODS

Animals

Intracarotid injection

The subjects used were 17 (seventeen) normal healthy dogs of mixed-breeds varying ages, sex and size (Table 1, dogs 1-17).

Microspheres

Microspheres are small spherical ceramic particles (Fig. 1) capable of being labelled with a variety of isotopes. They are available in varying size. Those utilized in this study were 80μ ± 20μ, 50μ ± 10μ, and 15μ ± 5μ as listed in Table 2 which shows the milligram.

The microspheres are black in appearance and consist of carbon, hydrogen and oxygen. They have an absolute density of 1.3 grams/cc and resist temperatures up to 400°C. They are insoluble in all organic or inorganic solvents at room temperature.

No nuclide was incorporated into the microspheres used in this study, however the microspheres are routinely available with $^{169}$Yb, $^{51}$Cr, $^{85}$Sr and $^{141}$Ce with an activity range of 1-10 mc/gm.
<table>
<thead>
<tr>
<th>Dog</th>
<th>Age</th>
<th>Weight in KG</th>
<th>Sex</th>
<th>Size and Number of Microspheres Injected (Approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 mo.</td>
<td>8.2</td>
<td>Female</td>
<td>80(\mu)-100,000</td>
</tr>
<tr>
<td>2</td>
<td>12 mo.</td>
<td>10.9</td>
<td>Female</td>
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</tr>
<tr>
<td>3</td>
<td>9 mo.</td>
<td>9.1</td>
<td>Female</td>
<td>80(\mu)-100,000</td>
</tr>
<tr>
<td>4</td>
<td>6 mo.</td>
<td>9.1</td>
<td>Male</td>
<td>80(\mu)-100,000</td>
</tr>
<tr>
<td>5</td>
<td>12 mo.</td>
<td>10.0</td>
<td>Male</td>
<td>80(\mu)-100,000</td>
</tr>
<tr>
<td>6</td>
<td>8 mo.</td>
<td>9.1</td>
<td>Female</td>
<td>50(\mu)-100,000</td>
</tr>
<tr>
<td>7</td>
<td>18 mo.</td>
<td>12.7</td>
<td>Female</td>
<td>50(\mu)-100,000</td>
</tr>
<tr>
<td>8</td>
<td>36 mo.</td>
<td>6.8</td>
<td>Female</td>
<td>50(\mu)-100,000</td>
</tr>
<tr>
<td>9</td>
<td>8 mo.</td>
<td>9.1</td>
<td>Male</td>
<td>50(\mu)-100,000</td>
</tr>
<tr>
<td>10</td>
<td>24 mo.</td>
<td>8.2</td>
<td>Female</td>
<td>50(\mu)-100,000</td>
</tr>
<tr>
<td>11</td>
<td>8 mo.</td>
<td>9.1</td>
<td>Female</td>
<td>15(\mu)-100,000</td>
</tr>
<tr>
<td>12</td>
<td>15 mo.</td>
<td>9.1</td>
<td>Male</td>
<td>15(\mu)-100,000</td>
</tr>
<tr>
<td>13</td>
<td>24 mo.</td>
<td>6.8</td>
<td>Female</td>
<td>15(\mu)-100,000</td>
</tr>
<tr>
<td>14</td>
<td>12 mo.</td>
<td>6.8</td>
<td>Male</td>
<td>Sham operated</td>
</tr>
<tr>
<td>15</td>
<td>15 mo.</td>
<td>9.1</td>
<td>Male</td>
<td>80(\mu)-100,000</td>
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<tr>
<td>16</td>
<td>36 mo.</td>
<td>9.2</td>
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<tr>
<td>17</td>
<td>24 mo.</td>
<td>9.1</td>
<td>Male</td>
<td>50(\mu)-100,000</td>
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Figure 1.—Photomicrographic comparison of a human hair and carbonized ceramic microspheres. Courtesy 3M Company, St. Paul, Minnesota.
### TABLE 2

<table>
<thead>
<tr>
<th>Standard Size ( )</th>
<th>Number of Microspheres Per mgm (approximate)</th>
<th>Weight of Sample Injected (mgm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 + 5</td>
<td>440,000</td>
<td>2.5</td>
</tr>
<tr>
<td>50 + 10</td>
<td>12,000</td>
<td>10</td>
</tr>
<tr>
<td>80 + 20</td>
<td>3,000</td>
<td>40</td>
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</tbody>
</table>

### Preparation of microspheres for intracarotid injection

Microspheres were weighed to attain approximately 100,000 particles (Fig. 2) for each injection. The microspheres were suspended in 3ml of 6% dextran. Clumps of spheres in the dextran were dispersed by exposure to ultrasonic vibration as described by Blanchard\(^9,10\). The mixture was sterilized by boiling the container for 15 minutes.
Figure 2.—Relationship of size of microspheres and number of microspheres per milligram.
NUMBER OF MICROSPHERES PER mg

MICROSPHERE DIAMETER IN MICRONS

$10^5$

$10^4$

$10^3$
Intracarotid injection technique

Dogs were maintained in general surgical plane anesthesia with an endotracheally administered methoxyflurane oxygen mixture from a standard gas anesthesia machine. The neck of the dog was prepared for aseptic surgery by clipping, shaving and scrubbing.

The bifurcation of the right common carotid into the internal carotid and external carotid was dissected free from surrounding tissues in a technique described for cerebral angiography by Hoerlein. A three inch incision in the area of the wing of the atlas facilitates a lateral approach. The carotid sinus was anesthetized with a sterile topical 4% solution of lidocaine HCl to prevent possible reflex alterations in cerebral blood flow due to the surgical or injection procedure. The external carotid artery was occluded and a bulldog clamp placed temporarily on the common carotid artery. A sterile 3 way I.V. stopcock with attached 33 inch extension tube with an internal volume of 3.6 ml was attached to a 26 guage needle placed in the common carotid artery rostrad to the bulldog clamp. The microspheres suspended in dextran were forced into the carotid artery and a flush of 5cc of 6% dextran followed. Sterile plastic syringes were used as the microspheres lodged between the barrel and plunger of
glass syringes preventing injection. Following the injection the bulldog clamps were removed and routine surgical closure was made.

**Recording apparatus**

Serial electroencephalograms were recorded with an Offner Type T electroencephalograph. This instrument is a stable eight channel transistorized apparatus consisting of amplifiers, preamplifiers and pen writing galvanometers. A paper speed of 2.5 cm/sec was utilized for this investigation and the eight channels were balanced so that 7 mm were equal to 50 uv. The time constant on all channels was set at .03 seconds. The Offner Type T electroencephalogram is capable of faithfully recording electrical potentials in the range of 0 c.p.s. to 150 c.p.s. The EEG tracings were made according to the technique described by Redding$^{55,56}$. The EEG tracings are obtained by attaching cadmium plated alligator clip electrodes to the scalp over the cranium. Local anesthesia, to prevent pain from the clips, was obtained by subcutaneous infiltration of 0.5 per cent lidocaine HCl. Electrode placement consists of two frontoparietal electrodes, one vertex electrode, two occipital electrodes and one electrode placed over the external occipital protuberance as a ground (Fig. 3).
Figure 3.--Location of electrodes for recording the electroencephalogram of the dog.
Using this placement technique it was possible to simultaneously record (1) the four areas of the cerebral cortex against the vertex as a common reference, (2) left hemispheric leads, (3) right hemispheric leads.

Serial recordings were performed in a quiet, darkened, temperature controlled room. Serial tracings were obtained on the subject prior to induction of general anesthesia during the injection procedure and on an every third day schedule for 2 weeks post-injection.

Certain nomenclature is applied to the graphic elements of the EEG. The basic unit is the wave which is measured from its base to its peak and then to the base of the next wave which is different than the interpretation in physics of the measuring of a sinusoidal fluctuation from one intersection of a baseline to the next. Wave forms may be sinusoidal or bizarre with a short steep rise and a long undulating fall or appear as spikes or bursts of specific activity. Waves are counted per unit time and assigned to divisions of frequency range as follows:

- **Beta waves**: $14-30\text{Hz}$
- **Alpha waves**: $8-13\text{Hz}$
- **Theta waves**: $4-7\text{Hz}$
- **Delta waves**: $0.5-3\text{Hz}$
The amplitude is variable but generally increases with the lower range of frequencies.

The normal EEG for dogs has been described\textsuperscript{55, 56} and is illustrated in Figures 4 and 5.

**Collection of tissues**

Previous studies\textsuperscript{33-35} had shown that two weeks was sufficient time for malacia to develop as a result of any infarction of cerebral tissue.

Dogs were anesthetized with pentobarbital sodium (30 mgm/kg). The carotid arteries and jugular veins were exposed bilaterally and cannulated. 2cc of a 2% Trypan blue solution was injected into the right common carotid artery to stain for loss of integrity of the blood brain barrier\textsuperscript{51}. The brain was perfused first with normal saline and then with 10% formalin. The brain was then removed intact and placed in 10% formalin until sectioned.
Figure 4.—The electroencephalogram of a normal, alert dog.
Figure 5.—The electroencephalogram of a normal, asleep dog.
Asleep

LO-V
RO-V
LF-V
RF-V
LO-LF
RO-RF
LO-RO
LF-RF

1 sec.  50μV
LITERATURE REVIEW

Cerebrovascular occlusion

Anthony et al.\textsuperscript{1} occluded one middle cerebral artery (M.C.A.) in dogs. Electrocorticograms exhibited either suppression or slowing. Dogs permanently occluded developed neurological impairment varying from circling and hemiparesis to hemiplegia and inability to stand. Dogs temporarily occluded (15 min.-3 hrs.) showed no deficit or had neurological impairment varying from mild involvement to inability to stand. The electrical changes were attributed to membrane depolarization of the cells and axons in the area rendered ischemic by the occlusion. The type of change observed by Anthony et al.\textsuperscript{1} is similar to the ones produced by asphyxial tracheal obstruction\textsuperscript{18} and anoxia (nitrogen inhalation)\textsuperscript{17}. Variability of the electroencephalogram was attributed to the fact that the source of injury was found to be principally in the central white matter of the hemisphere and not the surface. The variable effect of collateral circulation in protecting parts of the cerebral area from maximum ischemic effects
probably alters the position of surface zones of high
potential with respect to the surface electrodes which
are placed invariably with respect to the same anatomical
landmarks. This factor Anthony et al.\textsuperscript{1} felt explained
the variability in EEG pattern between dogs. They noted
that occlusion of the middle cerebral artery in the dogs
was not attended by cardiovascular or blood pressure
changes.

In a translation from Russian of a study of the
collateral circulation to the brain Klosovskii\textsuperscript{37} noted
the relationship of the pia-arachnoid vessels to those of
the brain. He stated that his studies and others he
reviewed revealed a complex network of vascular anastamoses
of different sizes. Arterial occlusion resulted in
shifting of large amounts of blood from one region of
distribution of an artery to another. The anatomical
structure of the pial and intracerebral capillary beds
account for collateral blood supply in the area of an
occluded artery.

Occlusion by thrombosis or embolization of an
artery arising from the circle of Willis or one of its
main branches running into the pia-arachnoid membrane
usually results in softening of the nervous tissue in the
area supplied by the occluded artery. This is presumptive
evidence that cerebral arteries in certain conditions
function as "end arteries."
Other studies cited by Klosovskii\(^3\) have demonstrated that in some instances cerebral arteries may be occluded without causing softening of cerebral tissues. Kosmarskaya\(^3\) performed a series of experiments in dogs ligating the anterior or middle cerebral arteries on one or both sides. He noted that bilateral ligation of the anterior cerebral arteries in dogs did not result in softening or hemorrhage and concluded that the areas receiving blood from these two vessels could switch over completely to a supply from the collateral vessels from the middle and posterior cerebral arteries.

Occlusion of the middle cerebral artery in adult dogs could be accomplished bilaterally without brain softening if one artery was ligated some time after the first one. Occlusion of the middle cerebral artery in puppies under 6 months either resulted in death within a few hours or softening of the area supplied.

Vasodilatation of cortical and white matter capillaries supplied by other ipsilateral branches of the circle of Willis was observed by Kosmarskaya\(^3\) immediately after occlusion. Capillaries in marginal zones showed maximal dilatation in comparison with the diameter of those in regional distribution of branches. In the border region between the anterior and middle cerebral arteries the intracerebral capillary network serves to equalize
blood pressure in the area of the cerebral artery at the moment of its occlusion and also provide compensatory flow. Collateral blood supply is not always adequate to ensure normal function of nerve tissues in the area of the occluded vessel.

The greatest possibility for effective collateral blood supply is thought to be the pia-arachnoid network. This is best developed in man, dogs, cats and rabbits in which peripheral branches of the main cerebral arteries join with each other through anastomoses.

Klosovskii\textsuperscript{37} states that in a way similar to man the anterior, middle and posterior cerebral arteries are seen to anastomose over the cerebral surface. Areas of marginal blood supply simultaneously receive blood from branches of the anterior and middle and posterior cerebral arteries which are usually located on the lateral sides of marginal convolutions but some branches do penetrate the internal part of the cerebral hemispheres. The structure of the pial arterial network in the region of collateral blood supply in the dog follows the same basic principles as seen in man. In the areas of collateral blood supply are concentrated a large number of major anastomoses between branches of the same artery.

Vessels of 160\(\mu\) anastomose by means of a vessel 120\(\mu\) microns in diameter. Larger branches do not enter
into direct anastomosis. Smaller branches measuring 40, 60 or 80$\mu$m were shown to anastomose and then give off radial arteries (Fig's. 6 and 7).

The cerebral blood supply is classified as follows:

A. Two General Types
   1. Superficial or conducting arteries
   2. Penetrating or nutrient arterioles

B. Classification by Size

<table>
<thead>
<tr>
<th>Diameter</th>
<th>Order</th>
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</thead>
<tbody>
<tr>
<td>Internal carotid arteries</td>
<td>3-2mm</td>
</tr>
<tr>
<td>Vertebral arteries</td>
<td></td>
</tr>
<tr>
<td>Circle of Willis</td>
<td>2-1.5mm</td>
</tr>
<tr>
<td>Branches of Circle of Willis over the cerebral cortex</td>
<td>1.5mm-100$\mu$m</td>
</tr>
<tr>
<td>characteristic anastamosis</td>
<td></td>
</tr>
<tr>
<td>no pial capillaries</td>
<td></td>
</tr>
<tr>
<td>Nutrient arterioles</td>
<td>51-16$\mu$m</td>
</tr>
<tr>
<td>Arise from large and small pial arteries and penetrate cortex to supply six layers of cortical mantle and white matter.</td>
<td></td>
</tr>
<tr>
<td>Nutrient arterioles</td>
<td>25-12$\mu$m</td>
</tr>
<tr>
<td>Continuation of 4.</td>
<td></td>
</tr>
<tr>
<td>Precapillary segment of nutrient arterioles</td>
<td>12-8$\mu$m</td>
</tr>
<tr>
<td>No anastamosis until they connect with another penetrating arteriole. Function as &quot;end arteries.&quot;</td>
<td></td>
</tr>
<tr>
<td>Capillaries</td>
<td>6$\mu$m</td>
</tr>
</tbody>
</table>

A characteristic of the pial network in dogs is that the end arteries are connected through anastomoses of 10-20$\mu$m size. These anastomoses connect the "blind end"
Figure 6.—Representation of the approximate size of the internal carotid circulation of the cerebrum.
PIAL (0.5mm-100μ)

NUTRIENT ARTERIOLE (51-16μ)

CAPILLARIES (8μ)

PRECAPILLARY (25-12 μ)

MIDDLE CEREBRAL (1.5-0.5mm)

CIRCLE OF WILLIS (2-1.5mm)

INTERNAL CAROTID (3-2mm)
Figure 7.—Blood supply to the cerebrum via pial vessels and their branches. Anastamotic branches through which collateral blood flow may occur are indicated by arrows. Roman numerals indicate layers of the cortex.
of arteries in the pia-arachnoid from which rise radial arteries which penetrate into the brain substance.

Klosovskii's\textsuperscript{37} work showed that the general principle of structure of pial arterial network is the same in adult man and dog but that the network of the dog is less differentiated, and contains a larger number of anastomoses.

Jones and Bagchi\textsuperscript{30} concluded that in their studies in humans it was possible to lateralize and localize the pathological area produced by cerebral thrombosis by use of the EEG. In their series thrombosis of the middle cerebral artery was evidenced by slow wave activity with mid-temporal, anterior temporal, lateral premotor and lateral parietal areas being the most commonly involved areas. Slow wave activity, dimunition in amplitude of alpha activity and occasional spikes were observed.

Harvey and Rasmussen\textsuperscript{23} occluded the right middle cerebral artery in nineteen monkeys. Nine animals had permanent occlusions and ten animals were occluded for periods of ten to fifty minutes. A decrease in electrical potential was observed within one minute following occlusion in all but five animals. The decrease was not found to be proportional to the size of the infarct which developed.

In nine of the ten animals temporarily occluded there was some return of normal EEG within 30 minutes after
termination of occlusion. In five of these cases the EEG returned to a preoperative pattern. In two-thirds of the post-operative recordings of all animals permanently occluded there was a persistent decrease in amplitude over the entire right hemisphere. Abnormal slow waves appeared in these animals but clinical seizures were only observed in three cases and involved the contralateral arm and face.

According to Strauss and Greenstein\textsuperscript{67} in 70\% of humans with cerebrovascular disease no delta slow wave activity was observed.

A clinical study was conducted in humans by Feiring and Sussman\textsuperscript{16} to differentiate thrombosis of the middle cerebral artery and infarction resulting from a lesion affecting its branches by angiography and electroencephalography. They found that suppression of voltage and almost complete absence of alpha activity occurred over the affected hemisphere. This was accompanied by occasional diffuse bilateral slow wave activity of higher amplitude on the side of the lesion.

A 1951 study\textsuperscript{64} stated that in vascular lesions of the brain, such as cerebral thrombosis, 80 per cent of the human patients examined have normal EEG's four to five days after the episode. The majority of patients had mild focal disturbance immediately following the
episode but this EEG abnormality resolved itself even if no return of lost motor function occurred.

Cohn et al.\(^ {13}\) reported that few studies have been made of serial EEG's of patients suffering from diseases of the cerebral vessels. They found that infarctions almost uniformly gave rise to slow waves which appeared either as generalized activity or over the area of the primary lesion. Acute lesions gave rise to unilateral slow waves from the frontal region or as a general part of the hemispheric phenomenon. Superficial lesions gave rise to much more slow activity, focal or general, than deep subcortical lesions. Lesions of the cerebellum and brain stem which did not interfere with the ventricular system, did not give rise to focal or general slow activity. The focal or general slow activity tended to resolve itself rapidly with stabilization of the clinical signs. Early electric activity "recovery" took place even with destruction of large areas because the infarcted area acted as a good conductor of the potentials of adjacent intact neurons. Other lesions which continued to show slow activity were felt to be those which failed to stabilize or that the generators of alpha rhythm were destroyed.

Twenty-nine dogs were used to examine the EEG patterns produced by experimental occlusion of one middle cerebral artery (M.C.A.)\(^ {14}\). Massive lesions were found in
the cortex in the fronto-temporoparietal region, the straitum, the internal capsule, the anterior thalamus and rarely the hypothalamus with the majority of the lesions confined to the internal capsule and the caudate nucleus. Abnormal neurologic signs in these dogs consisted of a contralateral spastic hemiparasis, loss of placing reaction and of position sense. Circling movements occurred toward the side of the lesion. Changes in the EEG varied with the size of the lesion with slow frequencies being most common over the involved hemisphere. Flattening was evident over the frontal and temporal regions with flattening over an entire hemisphere only occurring in massive involvement of the cortex. Recordings became symmetrical and more nearly normal after two or three weeks.

Chusid and de Gutierrez-Mahoney\textsuperscript{12} examined the effect of ligation of one internal carotid artery on the electroencephalogram of humans suffering from intracranial vascular disease. They report a decrease in alpha activity immediately post-operative. Eight days post-operative there appeared a difference between contralateral hemispheres with trains of irregular, low voltage slow waves occurring over the operated side.

Yeager and Walsh\textsuperscript{72} in unilateral ligation studies of the internal carotid in humans also report a reduction
in the alpha frequency and a replacement of alpha by slow, irregular waves of 1-1/2 to 6Hz for a period of nine days post-operatively with a gradual return of normal alpha.

In 1945 Roseman et al.\textsuperscript{58} also described a decrease in alpha frequency and bursts of delta activity in humans with unilateral internal carotid ligations.

Rosenblum and Zweifach\textsuperscript{61} in studies on cerebral circulation of the dog state that there exists extensive anastomoses between arteries of the brain and those supplying extra-cerebral portions of the head. Rosenblum\textsuperscript{60} further states that anastomoses between vessels of the brain substance are rare or nonexistent until the precapillary portion of the vascular bed is reached. Therefore the question of whether the anastomoses are arterial, venous or arteriovenous is avoided.

It was suggested by Grashchenkov\textsuperscript{19} that the absence of major anastomoses within the brain increases the importance of anastomoses on the surface and postulated that the arterial network on the pial surface serves to equalize blood pressure over the cerebral surface.

Rosenblum\textsuperscript{60} stated that the lack of anastomoses between perforating arteries means that if such a vessel is occluded the tissue it supplies can receive blood only by way of capillary anastomosis originating from the terminus of another perforating artery. He pointed out
that although the extensive capillary or precapillary anastomosis found in the brain of most mammalian species prevent their classification anatomically as true end arteries they can physiologically be classified as end arteries because the anastomotic capillary net is inadequate to supply sufficient blood to a specific cellular locus when the perforating artery to the locus is occluded in some manner.

Batson\(^8\), Ask-Upmark\(^2\) and Jewell\(^29\) describing arterial and venous intercommunications in the dog, cat, monkey and man cite the presence of a "rete mirabile" or diffuse arterial communication network over the surface of the brain which is seen in the dog and cat but not man and the monkey. These authors describe intercommunications between the external and internal carotid circulations in the dog and cat but on the basis of dye studies Batson\(^8\) concluded that these communications were not important in man. No significant communication between the extracranial circulation and the vertebral arteries distal to their passage through the dura was demonstrated.

Hill and Parr\(^{25}\) found that local cerebral thromboses were usually detectable for a short period after occurrence and if severe enough were evidenced by a residual low amplitude delta wave focus.
Roseman et al.\textsuperscript{58} reporting on humans made a distinction between superficial and deep lesions in stating that in lesions at or near the surface electroencephalographically accessible slow wave activity was observed. In small lesions the EEG focus was small, sharp and limited to the vicinity of one or two electrodes. In large lesions involving thromboses of a major vessel such as the middle cerebral artery the slow wave activity was greater and involved correspondingly more leads. In deep lesions, those involving the internal capsule or brainstem they found little to no evidence of slow wave activity with the only notable finding being amplitude asymmetry. EEG abnormalities were found to persist for varying periods of time depending on extend of the lesion, edema and vasospasm.

An experimental study of the electroencephalographic patterns in experimental vascular occlusion in dogs observed changes that varied with the size of the lesion, time lapse since occlusion and recording technique\textsuperscript{14}. The most pronounced EEG abnormality appeared as a flattening of electrical activity most evident in the frontal and temporal leads. This flat activity was replaced in a few weeks by slow wave high voltage activity of a diffuse nature.
In 1955 Ralston et al. permanently occluded the middle cerebral artery in sixteen monkeys. Five operated monkeys were kept as normotensive controls. Six operated monkeys were made hypotensive by bleeding until blood pressure fell to 60-80 mm Hg over a 10-20 minute period. The blood pressure was then returned to a normal 100 mm Hg by transfusion. Levels of mean blood pressure as low as 60 mm Hg did not cause any definite change in the EEG of most animals. The level of anesthesia produced a complicating factor in the interpretation of the EEG's during the anemic hypotensive studies. Ralston et al. did hold the impression that hypovolemic hypotension did increase the severity of brain damage produced by occlusion of the middle cerebral artery. EEG's recorded failed to show a correlation between severity of EEG change and extent of infarction.

Potter and Taylor examined the EEG in thirty-four patients before, during and after carotid artery ligation in the neck. They agreed with Wise et al. that slight to marked abnormalities may appear following carotid ligation without clinical evidence of cerebral damage. Both Potter and Taylor and Wise et al. noted focal changes consisting of homologous difference of potential, wave form, frequency and synchrony. Potter and Taylor differed with Wise et al. as to the
ultimate value of the EEG to predict changes produced by carotid clamping. Potter and Taylor\textsuperscript{53} stated that in their opinion the only contraindication to carotid ligation was when gross hemiplegic signs developed during a thirty minute trial period of occlusion prior to permanently occluding the artery.

EEG's in thirty-six human patients with clinical symptoms of cerebrovascular disease were examined by Meyer et al.\textsuperscript{46}. These patients with carotid artery insufficiency consistently developed EEG slowing.

Pampigloine and Waterson\textsuperscript{50} observed the EEG changes that occurred in children 3 weeks to 12 years of age when subjected to partial or complete occlusion of cerebral blood flow during various surgical procedures involving the heart or major vessels. Spontaneous cardiac arrest produced a rapid increase in slow waves followed by bilateral isoelectric cortical silence. Electrical activity gradually returned to normal following re-establishment of heart beat requiring several minutes to one hour. No change in the EEG was produced in five out of five cases in which the inferior vena cavae was occluded. Obstruction of venous outflow from the brain produced by clamping both superior vena cavae for thirty seconds was followed in thirty seconds by progressively increasing slow waves which regressed to a normal pattern in one
minute in eight out of ten cases. Occlusion of the pulmonary artery and aorta one-half minute after clamping the vena cavae produced no added change in the EEG but occasional muscle action potentials superimposed on the flat EEG suggested a mild tonic seizure in the anesthetized curarized patient. Complete flattening of the EEG after total occlusion of the blood flow to and from the brain always outlasted the time of the occlusion. In two children the flattening persisted for twenty minutes. All patients fully recovered their intellectual, motor and sensory function.

Kempinsky\(^{32}\) studied changes in steady potential gradients produced by focal cortical ischemia which followed occlusion of one middle cerebral artery in the cat. He found that when a steady potential is recorded from each of several loci in the cortex simultaneously the magnitude of the shift decremented toward the periphery of the ischemic region. These changes attained an amplitude of 5 to 11 mV and could be transient or continue for an hour. He suggested that the origin of steady potential gradients resulting from focal cerebral injury was explained as the algebraic summation of the effects of injury of individual anatomic units and is observed in the surfact EEG as a spreading depression or normal rhythm.
The cerebrovascular anatomy and disease processes in pigs and dogs in comparison to man was studied by Luginbuhl\textsuperscript{42} in 1966. He stated that it may be generalized that the pattern of the circle of Willis is basically similar in the dog and man. Luginbuhl\textsuperscript{42} pointed out that the exact field of supply of the branches of the middle and anterior cerebral arteries and others involved in disease processes is not known in the dog. Flow patterns in the components of the circle of Willis have not been studied in the dog and are based on macro appearance. A major difference is the fact that the dog is a macrosomatic species which implies differences in the flow through the anterior cerebral arteries as well as the possibility of an increased collateral supply to this area from the collateral branches of the external carotid. Differences in the cerebral circulation are undoubtedly produced by the different body position of the dog. Luginbuhl\textsuperscript{42} concluded that despite the differences and because of the similarities in the general mode of blood supply to the brain and the anastomotic system of vessels, the dog shared enough common features with the cerebral arterial system of man that comparisons could safely be made not only of cerebrovascular lesions, but also of changes in nervous tissue.
Roseman et al.\textsuperscript{58} advocated the use of serial EEG recordings in evaluating cerebral vascular lesions in humans. Emphasis was placed on correlating serial EEG's with clinical signs in localization, differential diagnosis and prognosis. They stated that initial EEG changes varied with the area insulted and the resulting vasospasm and edema. Superficial lesions produced prominent delta activity which was not observed in deep subcortical lesions. In cerebral edema secondary to thrombotic lesions there was delta activity which generally began to disappear in ten to fourteen days. Rosenblum et al.\textsuperscript{61} indicated that the presence of delta activity in a regressive lesion indicated possible improvement while absence of delta activity in the presence of neurologic deficit was suggestive of a deep lesion involving the internal capsule or that maximal recovery had already occurred and that in either instance a poor prognosis for further functional recovery was indicated.

Temporary occlusion of the common carotid artery below the carotid sinus by digital compression was performed by Skillcorn and Aird\textsuperscript{66} during which EEG recordings were performed in sixty unselected human cases and ten normal controls. Fifteen (25\%) of the patients showed marked EEG abnormalities predominantly over the ipsilateral hemisphere. Five patients who had internal
carotid thrombosis as diagnosed by cerebral arteriograms showed abrupt appearance of 2-5 Hz slow waves associated with irregularity of form and bilateral asynchrony. The majority of patients so affected displayed EEG changes within 10-20 seconds following digital compression and in those cases in which loss of consciousness or other neurologic symptoms occurred the EEG changes invariably preceded the clinical manifestations by a few seconds.

Frankhauser et al. discussed thrombosis and embolism of cerebral vessels in dogs. They stated that the development of infarction depends on caliber of the vessel involved, proportion of the lumen obstructed, rate of development of thrombus allowing time for collateral circulation to become functional and vulnerability of the area involved to deprivation of oxygen and nutrition.

They reported the most common form of embolus found in dogs to be a bacterial embolus originating from some infectious process with emboli from neoplasms being the next most frequently observed. Constant sites of infarction included the basal half of the external capsule, areas of the putamen and septum pellucidum, caput of the caudate nucleus and adjacent portions of the internal capsule and pallidum. Occasional lesions were also found in the inferior portions of the hippocampal formation. Recent lesions appeared greyish and discolored with
cerebral tissues being ill-defined and having a soft consistency. In lesions of longer duration there was a shrinking or cribiform appearance of the area infarcted. The most obvious gross changes were cavities were from 0.1 to 1 cm in diameter with a predilection for the external capsule, striate body, septum pellucidum and hippocampus.

Hill et al. produced cerebral infarcts in twenty-five dogs by injecting 0.2 cc of homologus clot into one internal carotid artery in a technique described by Moyes et al. They divided the animals into groups depending on the severity of neurologic impairment. Mild signs consisted of circling, limping, and paresis of one extremity. Moderate hemiparesis and visual disturbances were considered to be moderate signs. Severe hemiplegia and coma were considered to be severe signs. Twenty dogs (80%) showed infarction of some portion of the brain; nineteen (76%) showed neurologic deficit before death and four (16%) died within twenty hours post-operatively. Neurologic deficit was generally present at time of recovery from anesthesia but occasional animals subsequently developed severe neurological signs and died. Five dogs (25%) had infarcts confined to the ipsilateral thalamostriate area. Seven dogs (35%) had infarction to the thalamostriate area and cortical grey matter. Seven animals (35%) had infarctions of the ipsilateral
thalamostriate area and cortical gray matter. The structure most commonly involved was the anterior part of the caudate nucleus where infarction was found in nineteen of twenty cases (95%). In all cases the infarct involved the distribution of either part or all of the middle cerebral artery and in one case the contralateral caudate nucleus. The infarcts were all of a hemorrhagic appearance with the thalamostriate area appearing most hemorrhagic, cortical gray matter less hemorrhagic and cortical white matter least hemorrhagic. The size of the area infarcted had no relation to the severity of the neurological signs. The authors noted the difficulty in assessing neurological deficit in dogs and made no attempt to correlate any electrophysiological parameters with neurological deficit. in dogs and made no attempt to correlate any electrophysiological parameters with neurological deficit.

Cantu and Ames described the distribution of lesions produced by perfusing the brains of fifty ischemic rabbits with a suspension of carbon particles. The lesions were not reproducible or symmetrical but the most frequent pattern observed was a wedged shaped filling defect beginning in the subcortical white matter and terminating in the intermediate layers of the cortex. Cortical lesions were observed in the watershed zones between the
anterior, middle and posterior cerebral arteries where vascular supply is less sufficient.

In cerebral ischemia produced by various vascular diseases in man the EEG was characterized in these disorders in man as falling into three patterns. Frequently observed was a delta rhythm associated with a localized lesion. Slow waves of an epileptiform nature, spike and spike and wave, were also observed. The most characteristic observation they made and correlated with cerebral vascular disease was an asymmetry between contralateral leads and the absence or diminution of alpha rhythm.

Sundt and Waltz occluded one middle cerebral artery in sixteen cats. There was clinical evidence of cerebral infarction in all animals evidenced as weakness and spasticity of the extremities contralateral to the occluded artery. Cats circled toward the side of the occlusion and had a reduction of alertness. The degree of impairment was related to the degree of involvement of the internal capsule rather than to the size of the infarct. The infarcts occurred in watershed areas in which perfusion by collateral vessels was least adequate. No attempt at EEG evaluation was made.
Studies utilizing carbonized microspheres

Microspheres have been utilized in studies on regional blood flow to various organs, capillary size measurement, particulate clearance from the lung, fetal circulation, changes in regional blood flow in hemorrhagic shock, lymphatic flow, and adjuvant cancer therapy.

Rudolph and Heyman\textsuperscript{63} injected labelled microspheres suspended in 6\% dextran or whole blood into the umbilical arteries of the lamb to measure blood flow to the placenta. They noted no immediate physiological effect on peripheral circulation as indicated by their observation that the distribution pattern of subsequent injections did not vary from the first.

In 1967 Phibbs et al.\textsuperscript{52} described the flow of blood through a segment of medium sized arteries in rabbits by examining the distribution of labelled microspheres. They found no evidence of sedimentation of microspheres and showed that flow of microspheres from medium sized arteries into smaller branches was proportional to blood flow.

Intestinal blood flow studies with labelled microspheres has been described by Grimm and Lindseth\textsuperscript{21}. Their work was inconclusive while VanHeerden et al.\textsuperscript{69} perfusing the jejunum of dogs with hypertonic glucose and labelled microspheres showed that blood flow to the
gastrointestinal tract increased only in the part being perfused with hypertonic glucose.

The distribution of blood flow to the canine heart was described using labelled microspheres. This group found a uniform distribution of spheres in the normal dog heart and that arteriovenous shunting was responsible for 2% to 4% of total flow to the myocardium and occurred in vessels larger than 50 microns in diameter. Jaffe et al. using labelled microspheres in dogs with ligated coronary arteries observed that coronary collateral circulation normally occurred in coronary arteries of less than fifteen microns in diameter but that following ligation these vessels enlarged to between thirty and fifty microns in diameter. Hamlin et al. injected fifty micron labelled microspheres into the jugular vein of dogs to assay blood distribution to the lungs via the pulmonary artery. Concentration of radioactivity was nearly equal for all lobes of the lung suggesting that perfusion rates of blood was identical for all lobes of the dog lung.

Rabbits and dogs were used to evaluate the usefulness of labelled microspheres in diagnosing pulmonary embolism. Fifty micron microspheres were found to be uniformly distributed throughout all areas of the lung except the occluded area. Haynie et al. used fifty micron labelled microspheres to evaluate entrapment of
to the fact that extensive areas of the cortex had diminished blood flow caused by blockade of pial arteries of the eighty and fifty micron size. The smaller twenty-five micron spheres were trapped entirely in the penetrating arterioles where possible shunting could occur. Disruption of the blood brain barrier was examined by intravenous injection of 2 ml of 2% Trypan blue. A dissecting microscope was used to examine for the presence of the black microspheres which were lodged as described in the previous statements. The uninjected hemisphere served as a control.

In studies of uterine blood flow in pregnant sheep it was established that the degree of arteriovenous shunting in the uterus in vessels greater than twenty-five microns is negligible. These facts were based on the distribution of three different mean diameter labelled microspheres. In another study Makowski et al. measured umbilical blood flow in sheep feti varying from ninety to one hundred and fifty days of gestation.

Neutze et al. and Kaihara et al. studied changes in regional blood flow distribution to the heart, brain and liver and intestine and extremities during hemorrhagic shock. Distribution of labelled microspheres injected into the left heart showed that there was a fractional increase in cardiac output to the heart, brain and liver
while that fraction to the extremities and splanchnic capillary bed was decreased.

LaFave et al.\textsuperscript{39,40} evaluated labelled microspheres in the dog by splenic artery injection. They found them to be of experimental value in the study of treatment of leukemia, homograft rejection and the life cycle and function of dog lymphocytes. The entrapment of the labelled microspheres in the spleen produced a prompt and sustained lymphopenia while other cellular elements of the blood remained unaffected. LaFave et al.\textsuperscript{40} and Ariel\textsuperscript{3,7} also found that labelled microspheres exerted a pronounced effect in preventing take of tumor transplants in the lungs and liver of rabbits.

Madden et al.\textsuperscript{43} studied the function of limiting vascular diameters in lung, kidney and muscle of rats by use of various size of labelled microspheres in an attempt to explain lodging of metastatic tumor cells in those tissues. Ya et al.\textsuperscript{71} used labelled microspheres in dogs to check localization of isotope in kidney, lung, liver and intestine. They demonstrated that they produced little damage by embolism except in the kidney where they produced severe cortical necrosis.

Murphy et al.\textsuperscript{48} attempted to delineate the cerebral vasculature in rabbits with albumin microaggregates rather than microspheres. Microaggregates ranging in size of
from 25 to 50 microns in the same injection were introduced into the right common carotid artery of 25 rabbits. The microaggregates were found to lodge in both the gray and white matter of one or both cerebral hemispheres. The procedure produced no clinical, gross pathologic or microscopic alterations in the experimental animals.

Rosenthal\textsuperscript{62} injected labelled albumen microaggregates ranging in size of from five to thirty microns into a common carotid artery of forty-three human patients with cerebral vascular disease. EEG's were monitored in only three cases but they noted no significant changes. Kennady and Taplin\textsuperscript{33,35} used labelled albumen microaggregates to evaluate the danger of cerebral microembolism associated with lung scanning. Batch size of the microaggregates ranged from 10-100 microns. This work was performed in monkeys and baboons. EEG recordings showed transient changes in alpha patterns and some spiking but no evidence of marked abnormality was observed. One baboon developed a left hemiparesis which improved slowly over a two and one-half week period.
RESULTS

80μ Microsphere Treated Group

Each dog in this group had a prolonged post-operative recovery period from anesthesia.

Two dogs died post-operatively. Dog number three died nine hours post-operatively while dog number fifteen died twenty minutes after removal from the anesthetic machine.

The EEG of each dog in this group displayed a marked reduction of amplitude within 10 seconds after the administration of the microspheres in dextran. These periods of reduced potential persisted for varying lengths of time. Initially the periods were as short as two and one-half seconds (Fig. 8). In three of the animals in this group a flat EEG persisted for seventy-two hours post-operatively (Fig. 9). Following this the EEG in two dogs did develop diffuse, synchronous slow wave activity of 50 μV, 5Hz (Fig. 10).

Neurologic evaluation

Dog number three remained dazed and semicomatose post-operatively. There was a left torticollis, bilateral
Figure 8.—The EEG of a dog immediately following the injection of 80μ carbonized ceramic microspheres into the internal carotid artery demonstrating a marked reduction in amplitude of the EEG wave form for periods as long as two and one-half seconds.
Figure 9.—The EEG of a dog prior to seventy-two hours after the injection of 80/μ microspheres into the internal carotid artery. The EEG pattern has a diffuse bilateral reduction in amplitude.
Figure 10.—The EEG of a dog seventy-two hours following the injection of 80μm microspheres into the right internal carotid artery. The EEG had a marked reduction of amplitude initially but subsequently developed a diffuse slow wave pattern of 50μv, 5 Hz.
extensor rigidity of the pectoral limbs, loss of placing and righting reflexes. Flexor reflexes could be elicited with a strong toe pinch stimulus. These signs persisted until the dog expired nine hours post-operatively.

Dog number fifteen appeared to be in a normal post-operative recovery state. Respirations were thoracic in nature and regular. Cardiac rate and character, pulse rate and character were within normal limits. Color of mucus membranes was good. Flexor reflex and palpebral reflex were observed to be normal. There was increased jaw tone developing and the endotracheal catheter had been left in place. Death occurred suddenly without any warning. Unfortunately EEG electrodes had been removed prior to returning the dog to its cage for recovery.

Histology

Macroscopic examination of the brains showed no abnormalities in the dura or lepto-meninges and no evidence of recent or old hemorrhage or encephalomalacia of the parenchyma.

Microspheres were observed macroscopically in the pial branches (Fig. 11) of the cerebral arteries over both hemispheres but predominantly in the right hemisphere and parenchyma (Fig. 12).

The dura was well perfused with the trypan blue but there was no macroscopic evidence of the dye in the brain parenchyma.
Figure 11.—Photomicrograph of 80 μm microspheres lodged in a pial artery. X 70.
Figure 12.—Photomicrograph of 80μ microspheres lodged in a parenchymal artery in the cerebral cortex. X 70.
Summary

A summary of the EEG and neurologic observations is found in Table 3.

50\textmu m microsphere treated group

Five dogs in this group had a prolonged post-operative recovery period from anesthesia. Dog number seventeen recovered normally in fifteen minutes after administration of anesthesia ceased.

Each dog in this group displayed an immediate post-injection reduction of amplitude in the EEG as did the 80\textmu m treated group. One hour post-operatively the EEG of dog number nine had diffuse bursts of 50/\textmu v, 5 Hz activity which persisted for one second periods (Fig. 13). During the two week observation period the EEG of dog number nine remained bilaterally flat in the occipital leads (Fig. 14). In dog number ten the EEG had random spindles of 25/\textmu v amplitude (Fig. 15).

Neurologic evaluation

Dog number nine had a left torticollis, peddling, loss of righting and placing reflexes and depressed spinal reflexes for 48 hours post-operatively. This condition gradually regressed until the dog could maintain its balance and posture normally. A loss of vision was suspected and examination revealed the dog had a central
### TABLE 3
SUMMARIZATION OF EEG FINDINGS AND NEUROLOGIC ABNORMALITIES OBSERVED IN ALL ANIMALS

<table>
<thead>
<tr>
<th>Dog</th>
<th>Microsphere size</th>
<th>EEG</th>
<th>Neurologic Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80μ</td>
<td>Flattened at injection</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>80μ</td>
<td>Flattened at injection-50μv, 5 Hz slowing began in 72 hours</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>80μ</td>
<td>Flattened at injection-Persistent flattening until expiration.</td>
<td>Left Torticollis-Left Circling Pectoral limb extensor rigidity Loss of placing and righting reflexes Depressed flexor reflex Died 9 hrs. post-operatively</td>
</tr>
<tr>
<td>4</td>
<td>80μ</td>
<td>Flattening for 2-1/2 sec. at injection Low amplitude normal for two weeks</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>80μ</td>
<td>Flattening at injection for ninety seconds Low amplitude normal for two weeks</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>50μ</td>
<td>Right occipital lead Horners Syndrome showed increased amplitude, slow wave activity, days 50-100μv, 3-5 Hz, at injection</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>Microsphere size</td>
<td>EEG</td>
<td>Neurologic Abnormalities</td>
</tr>
<tr>
<td>-----</td>
<td>------------------</td>
<td>-----</td>
<td>-------------------------</td>
</tr>
<tr>
<td>7</td>
<td>50µ</td>
<td>Less pronounced flattening on injection, normal amplitude and frequency post-operatively for two weeks</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>50µ</td>
<td>Post-injection flattening</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>50µ</td>
<td>Post-injection flattening, 1 hr. post-operative diffuse bursts of 50µV, 5 Hz activity for 1 sec. periods, bilaterally flat occipital leads</td>
<td>Left tonicollis, pedaling, depressed spinal reflexes, persisted two days post-operative, Central blindness and abnormal gait persisted until dog terminated</td>
</tr>
<tr>
<td>10</td>
<td>50µ</td>
<td>Post-injection flattening, occasional spindling of 25µV amplitude</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>15µ</td>
<td>Post-injection flattening, normal until euthanasia</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>15µ</td>
<td>Little or no flattening on injection, normal for 2 week period</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>15µ</td>
<td>Post-injection flattening, normal for 2 week period</td>
<td>None</td>
</tr>
<tr>
<td>Dog</td>
<td>Microsphere size</td>
<td>EEG</td>
<td>Neurologic Abnormalities</td>
</tr>
<tr>
<td>-----</td>
<td>------------------</td>
<td>-----</td>
<td>-------------------------</td>
</tr>
<tr>
<td>14</td>
<td>Sham Operated</td>
<td>Flattening at injection (10 seconds)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No other abnormalities</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>80μ</td>
<td>Prolonged flattening (13 minutes)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow, low amplitude, disorganized 50μ, 5 Hz</td>
<td>Died 20 minutes post injection</td>
</tr>
<tr>
<td>16</td>
<td>80μ</td>
<td>Normal preanesthetic injection record</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post injection flattening</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 minute post-injection hemispheric flattening</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Persistent reduction in amplitude in all leads</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>50μ</td>
<td>Post-injection flattening</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Persistent reduction in amplitude in all leads</td>
<td></td>
</tr>
</tbody>
</table>
Figure 13.—The EEG of dog number nine one hour post-operative illustrating diffuse bursts of 50 μV, 5 Hz activity which persisted for one second periods.
Figure 14.—The EEG of dog number nine demonstrating persistent bilateral reduction in amplitude in the occipital leads.
Figure 15.--The EEG of a dog seventy-two hours following the injection of 50μ microspheres into the internal carotid artery. Indications of random spindle activity of 25μv amplitude occur.
blindness as the examination of the fundus disclosed no normalities. The dog was alert and active. The gait was abnormal with searching motion of the forelegs appearing as a lateral hypermetria. All other neurological parameters appeared within normal limits. The dog remained healthy in all other respects until euthanasia.

Dog number six developed a Hörn's Syndrome in the right eye associate with trauma to the vago-sympathetic trunk during the surgical procedure. The dog remained normal in other respects and the Hörn's Syndrome regressed in nine days post-operatively.

**Histology**

Macroscopic examination of the brains revealed findings similar to those of the 80μ treated group. (Figures 16 and 17). No gross evidence of infarction, hemorrhage or encephomalacia was found in the occipital cortex which was a disappointing observation and must require microscopic examination for final evaluation.

**Summary**

A summary of the EEG and neurologic observations is found in Table 3.

**15μ microsphere treated group**

Each dog in this group recovered rapidly as compared to the other two treatment groups.
Figure 16.—Photomicrograph of 50μ microspheres lodged in a pial artery. X 70.
Figure 17.—Photomicrograph of 50μ microspheres lodged in a parenchymal artery in the cerebral cortex. X 140.
The post-injection EEG of each dog in this group displayed reduction in amplitude. It was, however, not as prolonged as in the 80µ and 50µ treated groups. The EEG's rapidly returned to normal pre-treatment levels and remained within normal limits for the entire observation period.

**Neurologic evaluation**

There were no neurologic abnormalities developed in this group and dogs remained normal active and alert during the observation periods.

**Histology**

Macroscopic observations were similar to the other two groups with the exception that the small 15µ microspheres could not be observed in the blood vessels.

**Summary**

A summary of the EEG and neurologic observations is found in Table 3.

**Control**

The sham control was operated as were the treatment groups and 6% dextran was injected into the internal carotid circulation.

Recovery from anesthesia was considered within normal limits. The EEG had a reduction in amplitude similar to
the treatment groups but it persisted for only ten seconds and the record then returned to normal pre-injection activity. The dog continued to have a normal EEG for the observation period.

**Neurologic evaluation**

No neurologic abnormalities were observed.

**Histology**

No macroscopic abnormalities were observed in the control dog.
DISCUSSION

The prolonged recovery period from anesthesia following the injection of the $80\mu$ microspheres may be attributed to one or both of the following causes:

First, Kennady and Taplin\textsuperscript{33,35} found that 100% of the microspheres injected into monkeys remained trapped in the cerebral circulation. This blockage should result in decreased perfusion of neural tissue, the result of which would be a relative hypoxia, a relative hypoglycemia, a decreased metabolic rate, a decreased removal rate of metabolic by products and anesthetic agent and the net result could be a prolonged return to normal. The $15\mu$ microspheres may become lodged in the capillary circulation in which shunting and redistribution of blood flow would not lead to the aforementioned events with the result that the dogs recover in a normal time period.

A second hypothesis for prolonged recovery from anesthesia is the redistribution of microspheres from the cerebral circulation into the pulmonary circulation. Previous studies by Hamlin\textsuperscript{22} and others\textsuperscript{3,7,24} have examined the redistribution of microspheres from the pulmonary circulation into the cerebral circulation but no
investigator has evaluated if microspheres might enter the pulmonary circulation reducing perfusion of the lung and thereby interfering with gaseous exchange which would be essential to normal recovery from inhalation anesthesia.

EEG changes must be attributed to a decrease in perfusion of parenchyma with a resultant decrease in electrical activity occurring when there is a decrease in oxygen and energy substrate to the affected area(s). This hypothesis has been made in numerous publications on the EEG in cerebral vascular disease and ligation of the internal carotid artery of its branches\(^1,12,14,16,23,25,46\).

This study found as did numerous others\(^12,14,16,32,53,57,58,70,72\) that the EEG changes noted in vascular occlusion of the cerebral blood flow is observed to be a loss of amplitude of background alpha activity or the appearance of delta slow wave activity in leads over the affected area.

This study also agreed with other reports\(^14,16,23,53,66\) of lateralization of delta slow wave activity following the initial decrease in background alpha activity. Absence of delta activity in some dogs may indicate that any occlusion and subsequent impairment could be deep seated and not accessible for recording.

Certain technical problems are encountered in recording EEG's in dogs that are not found in similar
studies in man. Anatomically the dog brain is less accessible than man. The temporal area is protected by the very large mass of the masseter and temporalis muscles preventing recording of electrical activity from this area. The calvarium is smaller so that fewer electrodes can be utilized than in man.

Electrodes are always positioned according to anatomical landmarks. Previous reports have indicated that lesions due to embolus or thrombus usually occur in deep areas. Thus, electrode positions might be situated away from a high amplitude focus and record a smaller potential or show no alteration in activity.

Collateral circulation could lessen the size of the ischemic zone. This would result in fewer damaged neurons and a decrease in recorded abnormal activity at the surface electrodes.

Our macroscopic observations verified those of Kennady and Taplin. The finding of 80 μm and 50 μm microspheres entrapped within pial and nutrient vessels was noted by them in their primate study. There was no evidence of gross infarction or hemorrhage as noted in other studies. Serial microscopic examination may reveal microinfarcts similar to those noted by Kennady and Taplin. The absence of hemorrhage is possibly due to the small size of the microspheres and the presence of a vast collateral circulation in the dog.
The neurologic deficits noted in this study are similar to those noted in other experiments\textsuperscript{25,33,35} and clinical studies\textsuperscript{1,26} of cerebral vascular occlusion but did show a more definite impairment of neurological function than did that of Kennady and Taplin\textsuperscript{33,35}. The death of the two dogs in the 80\textmu treated group needs further examination as no gross abnormalities could be identified although there were definite neurologic and EEG changes which would indicate some pathological process. As these two dogs did not survive for long periods it is possible that microscopic examination of the brain may disclose a cause for the changes in neurologic function and EEG.

The one dog in the 50\textmu group displayed definite neurologic signs of loss of functional integrity for 48 hours post-operatively. Upon regression of these abnormal signs the cause of the blindness and abnormal gait should have produced some macroscopic evidence of change in the optic tracts or optic cortex, however, none was observed and it is hoped that microscopic examination will disclose a cause for the observed neurologic and EEG changes in this dog.

The dog is a difficult animal to evaluate neurologically as has been noted by others investigating cerebral disease\textsuperscript{25}. The investigator does not have the advantage
of questioning for subjective feeling of the patient. Evaluation of speech or of fine graded movements are impossible and the neurologist must rely on gross changes in neurologic function to signal significant alterations in cerebral continuity.

Perhaps the dog, because of its collateral circulation and lesser degree of differentiation of cerebral circulation than man, is not really a suitable subject for evaluating the EEG in cerebral vascular disease. The dog also is less dependent on the cerebral cortex and pyramidal or cortico-spinal tracts for normal function than is man and thus interruption in the integrity of this system in the dog may be less an embarrassment than in man.

However, the results of this study clearly show that the microspheres are capable of producing both neurologic and EEG changes and that the apparent impairment of cerebral function in the dog can be evaluated with the EEG.

Further evaluation of microspheres needs to be made utilizing greater numbers of dogs and labelled microspheres in order that localization, identification and quantification of microspheres may be positively made, as well as, the suggestion that redistribution of microspheres from the cerebral circulation to the lung and other tissues could be examined.
SUMMARY

The effect of the intracarotid injection of carbonized microspheres on the electroencephalogram of the dog was evaluated in seventeen dogs.

Microspheres suspended in 6% dextran were injected in the right internal carotid artery at the rate of approximately 100,000 per dose in 80\(\mu\), 50\(\mu\) and 15\(\mu\) sizes. One dog was sham operated.

Seven dogs were treated with 80\(\mu\) microspheres. Two dogs died post-operatively. All dogs in this group displayed a pronounced reduction in amplitude of the EEG immediately following injection of the microspheres. Two dogs developed low amplitude 50\(\mu\)v, 5 Hz slow wave activity after 72 hours.

Neurologic abnormalities consisted of left torticollis, left circling, pectoral limb extensor rigidity, depressed flexor reflex, loss of placing and righting reflexes in one dog and failure to recover from surgery in another.

Macroscopic observation of the brains of the 80\(\mu\) microsphere treated group disclosed numerous microspheres...
lodged in pial and nutrient vessels but no evidence of infarction, hemorrhage or encephalomalacia.

Six dogs were treated with the 50\(\mu\) microspheres. EEG's of this group appeared similar to those in the 80\(\mu\) microsphere treated group demonstrating a reduction in amplitude at time of injection and abnormal spindle and slow wave activity in the two week observation period.

One dog developed a central blindness following treatment with the 50\(\mu\) microspheres. Neurologic abnormalities in this dog consisted of a left torticollis, peddling, loss of righting and placing reflexes and depressed spinal reflexes for 72 hours after injection. A searching motion in the gait was observed following regression of the more severe neurologic signs.

Macroscopic observations of the brain were similar to those of the 80\(\mu\) treated group.

The 15\(\mu\) microsphere treated group showed only transient reduction in amplitude of the EEG following injection of the microspheres. No neurologic abnormalities were observed and the 15\(\mu\) microspheres were too small to be observed in the blood vessels.

The sham operated dog had a short 10 second reduction in amplitude of the EEG following the injection of 3 ml of 6\% dextran. Recovery was uneventful as was the observation period. No macroscopic abnormalities were seen in the brain.
CONCLUSIONS

The injection of 80 and 50 carbonized ceramic microspheres into the internal carotid artery of the dog was accompanied by demonstrable changes in the EEG and neurologic abnormalities.

EEG changes were an immediate reduction in amplitude following injection, and the development of low amplitude slow wave activity after seventy-two hours or the persistence of the reduction in amplitude of the EEG.

Neurologic abnormalities consisted of torticollis, loss of placing and righting reflexes, depression of spinal reflexes, blindness and abnormal gait.

The 80μ and 50μ microspheres can readily be observed in the blood vessels on the surface and within the parenchyma of the cerebral cortex.

The 15μ microspheres do not appear to produce any observable EEG or neurologic changes in the dog and are difficult to visualize macroscopically.

Collateral circulation to the cerebral cortex in the dog would appear to be sufficient to prevent macroscopic evidence of infarction, hemorrhage or encephalomalacia. Micro-infarction may be discovered in histologic examinations of the tissue.
BIBLIOGRAPHY


38. Kosmarskaya, E. N.: Occlusion of the Middle Cerebral Artery. cited in the above, 1951.


