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# Albumin Macroaggregates for Brain Scanning: Experimental Basis and Safety in Primates

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The dual purpose of this study is to determine the possible application of radioalbumin macroaggregates for brain hemisphere scanning and to evaluate the potential danger of cerebral microembolism associated with lung scanning.

Radioisotope brain scanning is becoming a cardinal procedure along with electroencephalography, pneumoencephalography, and angiography in the evaluation of intracranial disease. Large space-occupying lesions (gliomas, meningiomas, metastatic tumors and hematomas) can be diagnosed with a high degree of accuracy by these procedures. It is still difficult to detect small lesions in the cortex, particularly those of vascular origin. In lung scanning with macroaggregated radioalbumin, the lung image represents the arterial flow pattern, and areas of decreased or increased radioactivity indicate relative regional ischemia, or hyperemia, respectively (1-5). A diagnostic method is needed which will demonstrate cerebrovascular abnormalities in the smaller arteries not consistently well visualized in the angiogram.

Preliminary hemisphere scans and toxicity studies in 12 dogs showed that the cerebral hemispheres could be visualized by scanning, but indicated a much smaller margin of safety for the brain than for the lung scanning procedure (6). The cerebral circulation in the dog differs greatly from man, therefore toxicity studies were extended to the monkey and baboon, who have a cerebral blood supply very similar to man. Investigations with these species are described in this paper along with studies of the *in vitro* human brain and of mechanisms by which macroaggregates are trapped temporarily but subsequently released from the arteriolar capillary bed.

## EQUIPMENT

A Picker Magnascanner II with a 3 inch sodium iodide crystal was used with 19 and 31 hole collimators. The latter gave better cerebral hemisphere delineation. Electroencephalographic recordings were obtained bilaterally with the scalp leads connected to a four-channel Offner type R Dynograph. A modified "C" clamp was placed between the hard palate and the infraorbital ridge to hold the animal's head securely with the vertex facing the scanning crystal (Fig. 1). Fundoscopic examinations were carried out with a May type ophthalmoscope. Photomicroscopic studies were done using a modified dissecting microscope with magnification of 50 and 120 ×. Ektachrome commercial type 7255 color film was used in the cine Kodak special 16 mm camera attached to the microscope.

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## MATERIALS

Eight adult male rhesus monkeys and two baboons were used. Phencyclidine[1], 3 mg/kg body weight was given intramuscularly for analgesia and light anesthesia.

[1]"Sernyl", obtained from Parke, Davis and Co., Detroit, Michigan.

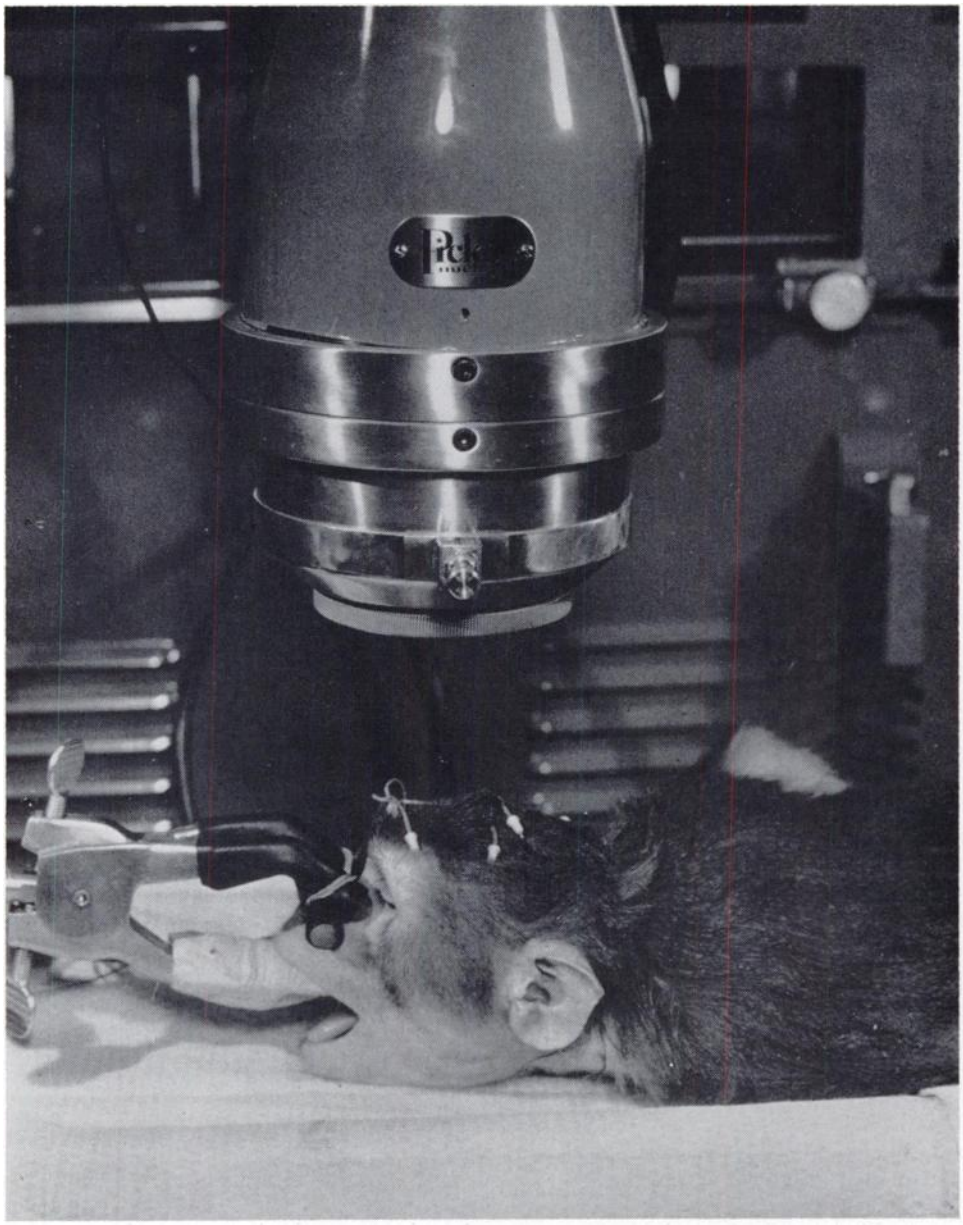

**Fig. 1. Position of monkey for vertex hemisphere scanning and EEG**

Eleven nonfixed cadaver brains were obtained shortly after autopsy for *in vitro* human brain scanning.

Five rabbits under pentobarbital sodium anesthesia were used for photomicroscopic studies of blood flow in the exteriorized omentum.

Albumin $^{125}I$ and $^{131}I$ macroaggregates (10-100 $\mu$ in diameter) were used as test agents.

## PREPARATION OF $^{125}I$ AND $^{131}I$ MACROAGGREGATES

### *Albumin $^{125}I$ Aggregates:*

1. Prepare a 0.2 percent radioalbumin solution 50-100 $\mu$C/mg by diluting the 1% $^{125}I$ Albumatope[2] with physiological saline in a sterile multiple injection type rubber stoppered bottle.
2. The pH adjusted to 10 $\pm$ 0.5 by adding sterile 0.2 N NAOH.
3. The solution is heated in a water bath at 79° C for 20 minutes and agitated continuously. It is then cooled below room temperature by immersion in cold water.
4. The pH is reduced from 10 to 5.5 by adding 0.2 N HCl.
5. The suspension is heated at 79° C for another 5 minutes as in step 1 to increase the size range to 10-100 $\mu$.
6. Particle size is measured with a filar micrometer and the number of aggregates/mm$^3$ is determined by counting the stained particles (rose bengal, pH 5.5) in a hemocytometer.
7. Test for sterility and store at 5° C.

### *High Specific Activity Albumin $^{131}I$ Suspensions:*

Suspensions in the 10-100 $\mu$ size range containing up to 1500 $\mu$C/mg albumin $^{131}I$ are now available commercially for investigative purposes.[3] They are produced by adjusting the pH of the albumin solution (0.1%) to 5.5 and heating it for 20 minutes or longer at 79° C.

### *Particles Size Measurement and Counting of Macro Radioalbumin Aggregates:*

The suspended aggregates are stained with Rose Bengal (pH 5.5) and viewed microscopically at 430 $\times$ magnification in a hemocytometer. Particle size is estimated with a filar micrometer. The number of aggregates larger than 10 $\mu$ per cubic millimeter is determined as in counting red blood cells. Rose Bengal is used for staining and dilution purposes (pH 5.5).

## PROCEDURES

### *Monkey and Baboon Studies:*

Following light anesthesia, six EEG leads were placed in the scalp of the monkey and a preinjection recording was made for 10 minutes as a baseline control. Then under sterile conditions, the *right* anterior neck region was incised

[2] "Albumatope", obtained from E. R. Squibb and Sons, Radiopharmaceutical Division.

[3] E. R. Squibb and Sons, Radiopharmaceutical Division.

obliquely from a point just below the angle of the right mandible toward the midline. Dissection was carried down to the carotid sheath. Procaine 1% (1 ml) was instilled into the operative area before freeing the common and internal carotid arteries from surrounding structures to reduce the possibility of carotid body stimulation and internal carotid artery constriction during subsequent dissection. An umbilical tape was placed around the right common carotid artery for stabilization during puncture of the carotid bulb with a No. 26 short bevelled needle and injection of test material. Injection time averaged 20-30 seconds. Volume injected never exceeded 1.5 ml. Gentle tampanade over the injection site for 2-3 minutes stopped the bleeding. Four michel clips were used to close the skin edges temporarily to permit immediate scanning and EEG recording.

The area of highest count rate was located over the right hemisphere and marked. Both hemispheres were scanned, the left for control purposes. Serial scanning was continued until at least a 50 percent reduction in maximum count rate was noted. EEG recordings were made every 15 minutes for two or more hours depending upon evidence of abnormal activity.

The operative site was then sutured with 3-0 black silk following completion of these procedures. The animals were returned to their cages and observed for behavioral changes and tested for evidence of neurological disturbances following recovery from anesthesia.

Intervals of two weeks or more were maintained between carotid injections to allow for wound healing and adequate observation. Three animals were sacrificed at 2, 3 and 5 months for histological study of the brain and leptomeninges with H and E and Luxol blue stains to determine early cellular and vascular changes. The remaining animals are to be sacrificed after 12 months for evidence of chronic cytoarchitectural alterations.

*Phantom and In Vitro Human Brain Scanning:*

Phantom brain scans were done in anteroposterior, lateral and vertex projections following insertion of a balloon into the right half of the skull, which was then filled with a solution of $^{125}I$ or $^{131}I$. *In vitro* human brain scans were done soon after obtaining the unfixed cadavar brain. The blood vessels were immediately flushed with saline through catheters tied into the internal carotid arteries until return fluid was clear. The brains were from senile patients and showed evidence of moderate to severe atherosclerotic changes in the blood vessels. These abnormalities interfered with the profusion and subsequent distribution of the labeled particles. The anterior and posterior communicating arteries were tied to permit injection of only one side. The integrity of the vascular bed was tested following instillation of 1 ml sky blue dye into the tubing. Small leaking vessels were clipped. Suspensions of $^{125}I$ or $^{131}I$ macroaggregates were then injected and saline irrigation was continued for 10 minutes to simulate the normal circulation. The brain was placed in a skull through a dorsal opening and then positioned for scanning.

*Dynamic Studies of Omental Vessels:*

Following light anesthesia, a two inch incision was made in the midline abdominal wall of the rabbit below the xiphoid process. A segment of the omentum was exteriorized and covered with plastic film. A polyethylene tube (P.E. No. 90) was threaded up the femoral artery into the aorta to about the level of the superior mesenteric artery for injection of the aggregates stained with blue dye. The omental vessels were viewed microscopically at a magnification of 100 ×. The entry and removal processes were studied following repeated injections of the stained particles (10-100 $\mu$). The physical mechanisms of particle entrapment and release from the arteriolar bed were recorded dynamically on 16 mm color movie film.

## RESULTS AND INTERPRETATION

*Cerebral Hemisphere Scans:*

Forty internal carotid injections were made in eight adult monkeys and two baboons. Four of the ten animals were injected on six separate occasions, two had four and the remaining four animals had only two intracarotid injections. Ten separate lots of radioalbumin macroaggregates were used. However, it was shown that the size distribution within this range, varied considerably from one lot to another. Scans with the same lot of macroaggregates showed similar distribution of radioactivity and cerebral hemisphere delineation. Time for the first 50 percent reduction in the maximum cerebral count rate was de-

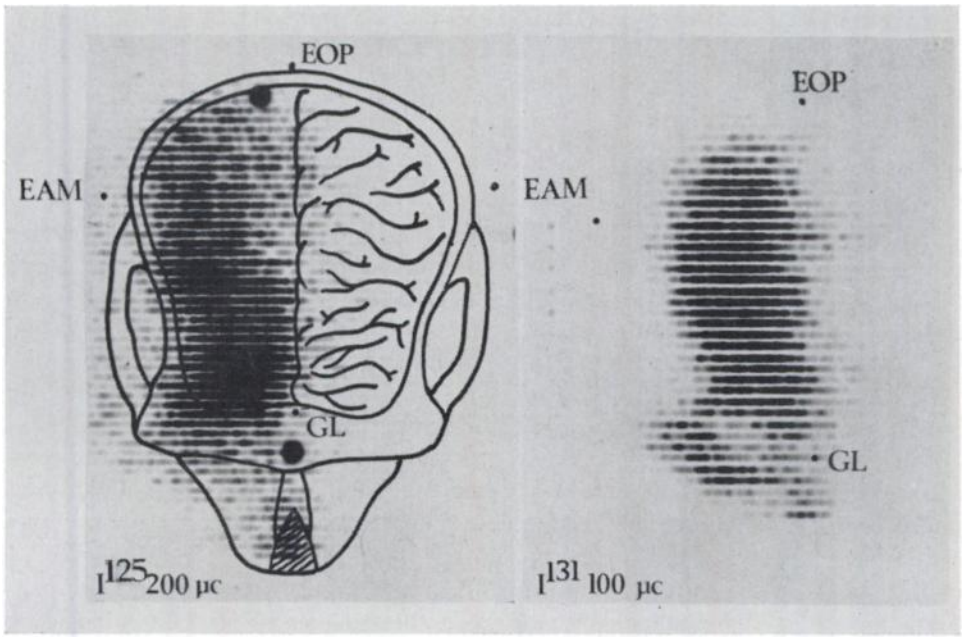


**Fig. 2. Monkey hemisphere scans with $^{125}I$ and $^{131}I$ albumin macroaggregates. Gl—glabella; EAM—external auditory meatus; EOP—external occipital protuberance. See text.**

pendent on the number and size range of the carrier albumin particles. Aggregates of 40-60 $\mu$ (mean diameter) allowed sufficient time (80 min) to scan the hemisphere and gave good scan images. The scans revealed no evidence of the tracer entering the left side (Fig. 2). The right olfactory bulb, retro-orbital and supra-orbital regions were well outlined, particularly in the baboons. This observation prompted careful fundoscopic examination. No evidence of retinal embolization was noted in any of the animals.

*$^{125}I$ Vertex Scans:*

Twenty-three intracarotid injections were made using $^{125}I$ labeled aggregates 80-220 $\mu$C and 0.8-1.3 mg of carrier albumin. Approximately one and one-half times more $^{125}I$ than $^{131}I$ was needed to produce scans of equal radiodensity. The $^{125}I$ scans showed quite uniform distribution of radioactivity within the right hemisphere except for the posterior parietal region (Fig. 2). The greater skull thickness in this region in adult males caused this decrease in radiodensity. Scans of female monkeys with less variation in bone thickness, showed more uniform distribution of radioactivity.

*$^{131}I$ Vertex Scans:*

Seventeen intracarotid injections were made using $^{131}I$ labeled aggregates in doses ranging from 60-180 $\mu$C and 1.5 to 11 mg of carrier albumin. The hemisphere scan images were much less influenced by variations in bone thickness and showed a more even distribution of radioactivity (Fig. 2).

Four scans were done following *common* carotid artery injections. Hemisphere delineation was distorted in the lateral and posterior areas by radioactivity in the overlying temporal and posterior nuchal muscles supplied by the external carotid artery.

*Electroencephalography:*

The EEG showed transient ($\sim$ 15 min) changes in the alpha pattern and some spiking in only the two monkeys who received 10 and 11 mg of albumin particles, respectively. All other EEG examinations were interpreted as within normal limits. However, because of the limited number of scalp leads used in this study, discrete cortical lesions could have been masked by recording between such widespread areas.

*Cerebral Angiography:*

Two monkeys had cerebral angiography following three and five internal carotid injections, respectively. The angiograms showed no evidence of vascular obstruction in either hemisphere nor evidence of contralateral arterial filling. There was no shift of the anterior cerebral artery in the anterioposterior projection.

*$^{197}Hg$ Chlormerodrin Scans:*

Four monkeys who had multiple intracarotid injections of macroaggregates during a 6-8 months period were then given $^{197}Hg$ chlormerodrin (10-20 $\mu$C/kg

body weight) intravenously and scanned four hours later. Radioactivity was only slightly higher than background over both hemispheres and no discrete areas of increased radiodensity were found. The negative results indicate that the multiple injections produced no alterations in the blood brain barrier. However, reestablishment of this barrier could have taken place during the long time intervals (2-3 months) since the last injection. Therefore, other monkeys are to be scanned 24-48 hours after macroaggregate injections to explore the possibility of early but reversible injury.

*Complications:*

One baboon given 10.0 mg of albumin particles developed a left hemiparesis which slowly improved over a 2½ week period. This complication may have been caused in part by traction on the internal carotid artery. During this time, the injection technique was being developed. The remaining nine monkeys and baboons had no behavioral disturbances detectable by observing their eating habits and attention seeking mannerisms or manifestations of motor weakness.

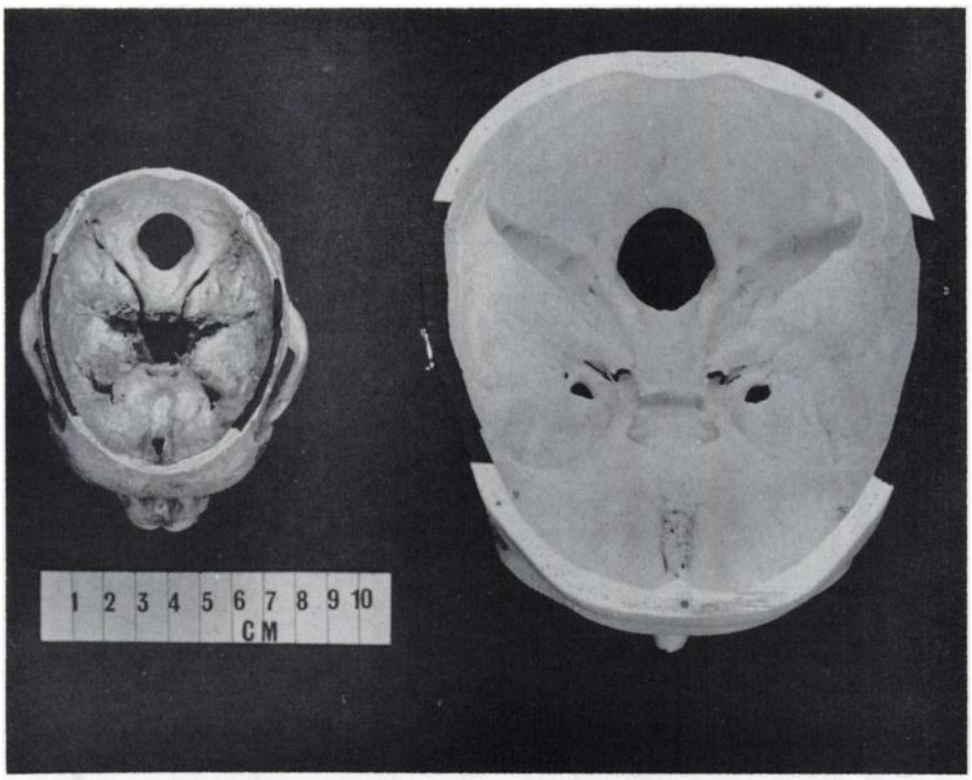


**Fig. 3. Comparison of the monkey and human skull in size and thickness. Black tape outlines the temperoparietal bone thickness. Note greater variation in man.**

*Histology:*

Macroscopic examination of the brains in three monkeys showed no abnormalities in the dura or leptomeninges and no evidence of recent or old hemorrhage. The brain slices likewise appeared grossly normal. Microscopic examination of brain sections from the frontal and parietal regions revealed no distinct differences between the injected and control hemisphere. No evidence of vascular occlusion, alterations in the cortical cytoarchitectural pattern or areas of infarction were noted. One monkey had slight thickening of the leptomeninges with increased vascularity but this was observed over the whole brain section.

PHANTOM AND IN VITRO HUMAN BRAIN SCANS

The difference in skull thickness between monkey and man is illustrated in Fig. 3. The effect of bone thickness in the human skull on the phantom scan pattern was quite evident in the AP and lateral projections, particularly with $^{125}I$ (Fig. 4). The thin temporal and orbital bones produced alterations (increased radioactivity) in the scan image.

Similar information was obtained from unfixed cadaver brains (7).

*Comparison of $^{125}I$ and $^{131}I$ Hemisphere Scans:*

Scans were done in the three projections. The effect of bone thickness was again quite evident on the AP and lateral scans, particularly with $^{125}I$. The thick posterior parietal bone produced decreased radiodensity in both the $^{125}I$ and $^{131}I$ vertex scans, as illustrated in Fig. 5. It was impossible to compare these particular scans in respect to the scan images of the hemisphere because the same brain could not be injected with both $^{131}I$ and $^{125}I$ aggregates. The brains

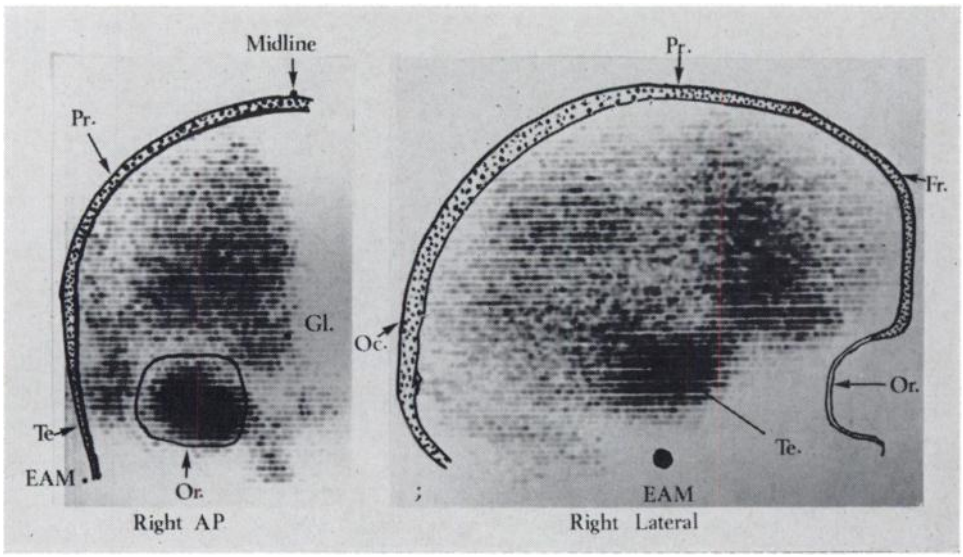


**Fig. 4. Human phantom scans in AP and lateral projections using an inserted balloon filled with a solution of $Na^{125}I$.**

were obtained from individuals 55-75 years of age and the associated atherosclerotic changes in the small arteries might well have altered the distribution of macroaggregates from the pattern found in the living brain.

*Transverse Section Hemisphere Scans:*

To determine the distribution differences of the macroaggregates in the cortical gray and white matter, the brain was quick frozen (dry ice and acetone) and a transverse section (1 cm thick) was made through the posterior frontal area. Scans of this section showed the highest levels of radioactivity over the midline frontal and temporal areas and the region of the lateral Sylvian fissure (Fig. 6). The surface cortical gray matter had much more radioactivity than the underlying white matter. This finding correlates well with the known greater vascularity of the gray matter as illustrated diagrammatically in Fig. 6. The significance of this observation will be considered in greater detail in the discussion.

*Observations of Stained Aggregates in the Pial Vessels:*

In two brains, the $^{131}I$ macroaggregates were stained with Pontamine sky blue dye before injection. The largest particles (50-100 $\mu$) could be identified macroscopically in extremely small pial vessels of the anterior and middle cerebral artery beds (Fig. 7). Following the initial scan, saline irrigation (1 liter) was continued for one hour. The number of visible particles were definitely reduced and on rescanning the count rate was less than 50 percent of that in the same area of the initial scan.

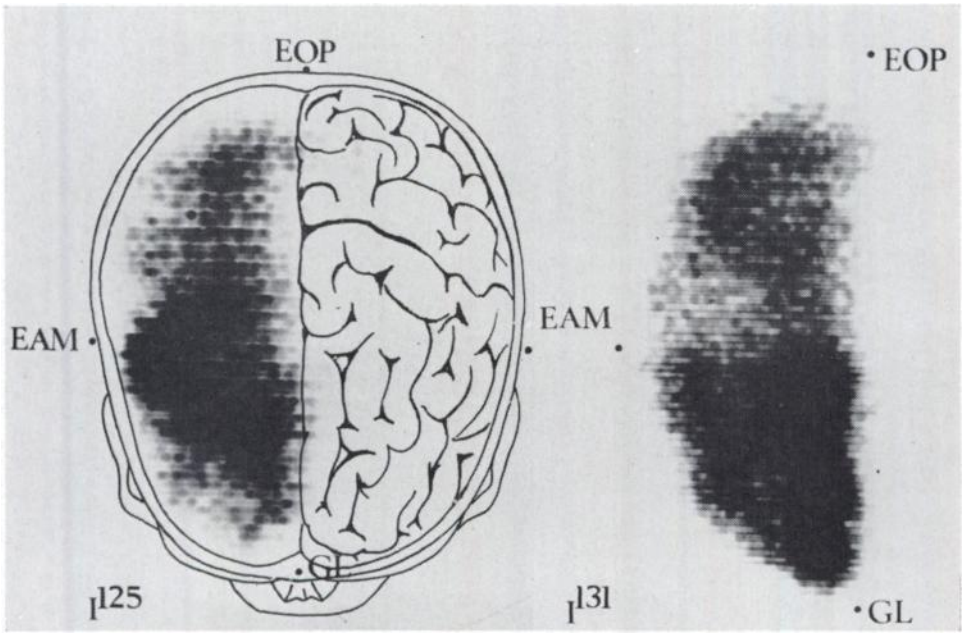


**Fig. 5.** ***In vitro*** **human brain scans with $^{125}I$ and $^{131}I$ albumin macroaggregates. Note the greater variation of radiodensity in the $^{125}I$ scan as compared with $^{131}I$. See text.**

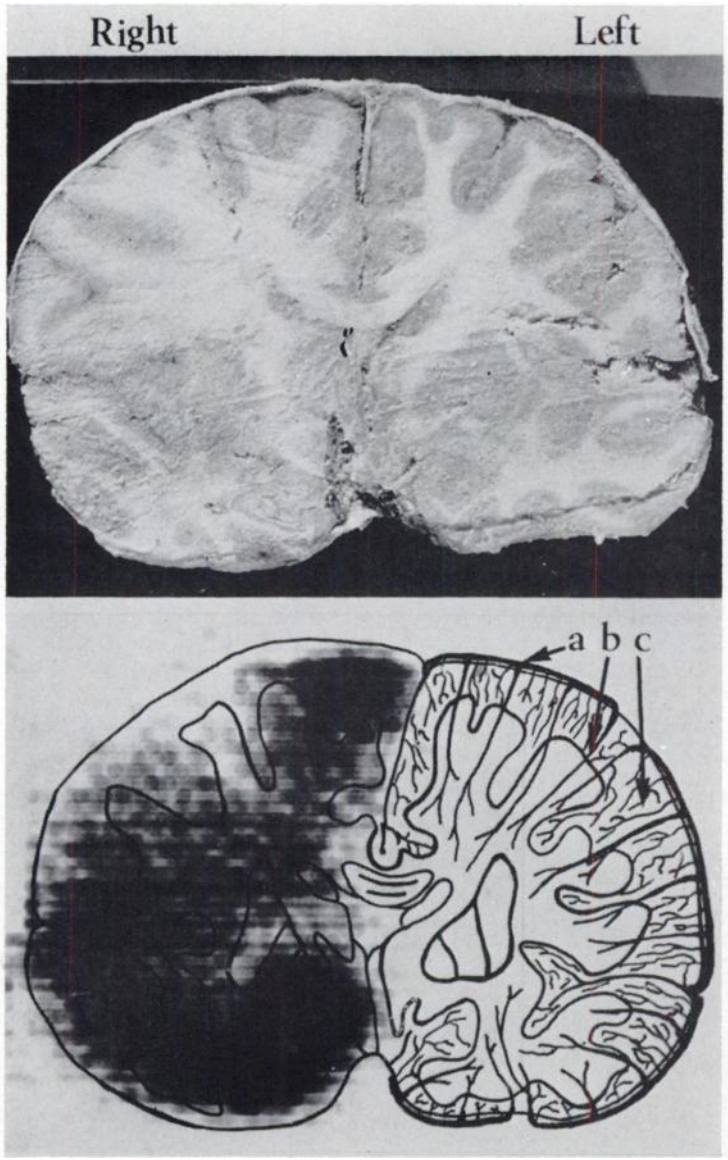


**Fig. 6. Above—frozen transverse section of the human brain. Dura intact over dorsal surface.**

**Below—$^{131}$I albumin macroaggregate scan of brain section (same brain as shown in Fig. 5).**

**Diagramatically illustrated on the left side: (a) the small arteries of the pia mater; (b) giving off larger and longer nutrient arterioles to the white matter; and (c) more numerous and smaller nutrient arterioles to the gray matter.**

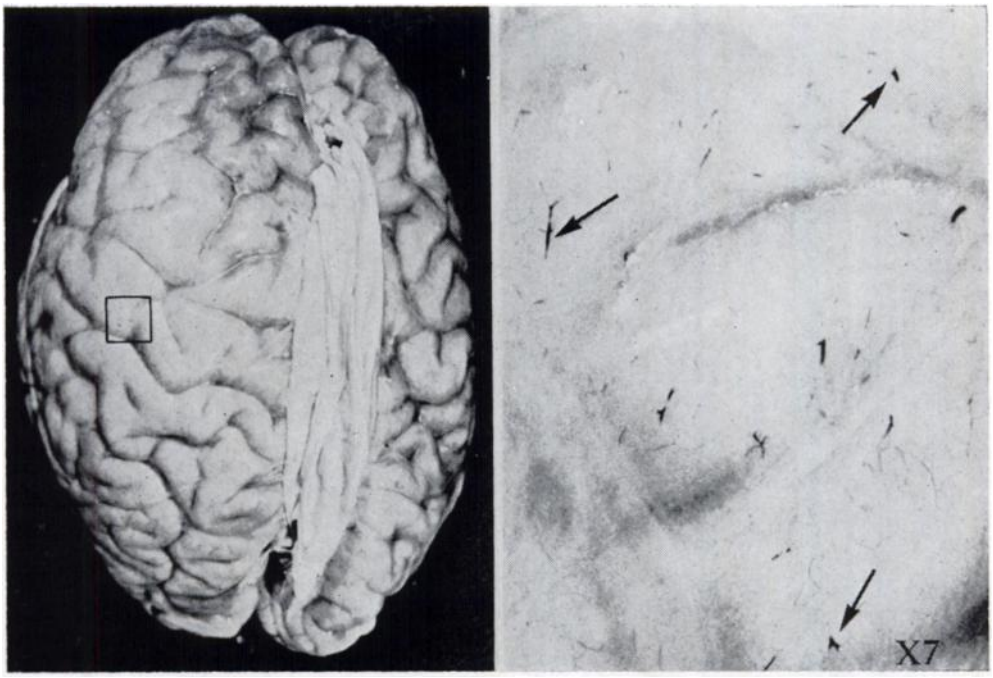


**Fig. 7.** *In vitro* **human brain following injection of stained albumin macroaggregates (10-100 $\mu$ size). Square outline shows region of enlargement (x 7) on the right side. Arrows show clumps of macroaggregates in the pial vessels.**

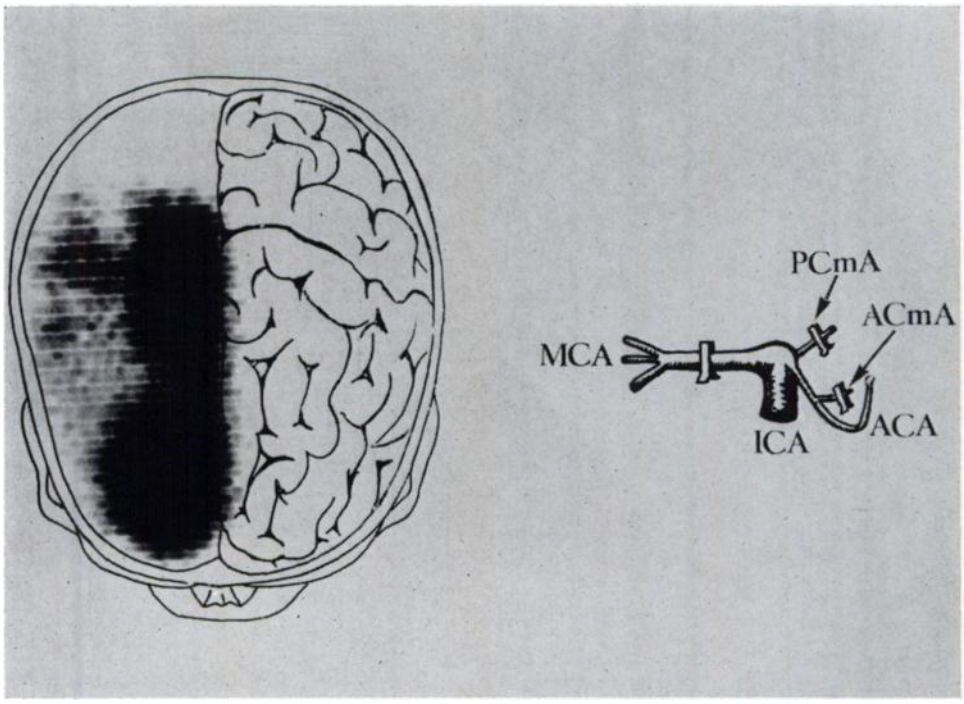


**Fig. 8.** *In vitro* **human brain scan after clipping the middle cerebral artery (MCA), the anterior and posterior communicating arteries (ACmA and PCmA). Only the anterior cerebral artery (ACA) is completely open to the internal carotid artery (ICA). The MCA was found later to be subtotally occluded.**

*Simulated Cerebral Artery Thrombosis:*

Cerebral artery thrombosis was simulated by placing a clip near the origin of the anterior or middle cerebral artery in the cadavar brain. As illustrated in Fig. 8, the clips on the communicating arteries prevented the test material from entering the left hemisphere and the area supplied by the right posterior cerebral artery. These areas were radionegative in the scan. The clip on the middle cerebral artery was not completely closed and some radioactivity appeared in the area supplied by this vessel. The area of increased radiodensity in this scan is in the region supplied by the anterior cerebral artery which was not clipped.

## DISCUSSION

The studies just described indicate that the potential danger of cerebral microembolization from *lung scanning* with radioalbumin macroaggregates is extremely remote. Also, the same test material can be given safely by intra-arterial injection in monkeys to visualize the cerebral hemispheres, provided the amount of particulate material does not exceed 2 mg or 0.04 mg/gm of brain tissue. These findings *suggest* that the safe dose for hemisphere scanning in man may be as large as 28 mg for (10-100 $\mu$) albumin aggregates. However, these statements require much further consideration.

*Lung Scanning and Cerebral Microemboli:*

During the development of lung scanning with radioalbumin macroaggregates, toxicity studies in rabbits and dogs indicated an extremely large margin of safety regarding pulmonary toxicity. The minimum amount of particulate material needed to scan human lungs (5-20 $\mu$g/kg) is at least 1000 times less than the minimum toxic dose in dogs (10-20 mg/kg). Also, the pulmonary capillary bed is shown to be a highly efficient filter for particles larger than 10 $\mu$. However, in patients with right to left cardiac shunts a considerable fraction of the dose, could enter the general circulation and about one-tenth of this is channeled to the brain. Therefore, a potential hazard from cerebral microemboli exists. It was this possibility that prompted our investigation of the cerebral toxicity of this material.

*Preliminary Cerebral Toxicity Observations:*

During the initial studies in dogs low specific activity test material was used (5-10$\mu$C$^{131}$I mg albumin). For the past six months, much higher specific activity albumin aggregates of the same size range were used (1000-1500 $\mu$C $^{131}$I/mg albumin). To our surprise, three monkeys were able to tolerate 7.5-11 mg doses of albumin particles by internal carotid artery injection without demonstrable central nervous system impairment. No monkeys given doses of 2 mg or less had detectable adverse neurological sequelae even when the procedure was repeated several times at intervals of 2-4 weeks. This apparent tolerance of the brain to temporary vascular obstruction is much more difficult to explain than that of the lungs.

*Relative Pulmonary Toxicity:*

Temporary occlusion of as many as 50 percent of the lung's arteriolar vessels can be produced by macroaggregate injection in normal dogs and complete recovery usually takes place within a few days (5). Blood is shunted immediately through the remaining unobstructed vessels and development of collateral circulation with the bronchial vessels prevents lung necrosis while the pulmonary arterial system regains its normal functional status (5). With aggregates in the 10-50 $\mu$ size range, 80-95 percent are initially trapped and are retained for several hours ($T_{\frac{1}{2}}$ in the lung = 2-4 hours) both in animals and man. Initial entrapment is most likely from mechanical obstruction of the arterioles by particles which are larger than the vascular lumina. Removal of these friable aggregates, which are composed of myriads of submicron sized particles, is not so readily explained.

*Removal Mechanisms as Observed in Vivo:*

Direct microscopic observations of the capillary bed in the rabbit's exteriorized omentum, following arterial injection of stained aggregates, shed some light on the mechanisms involved. Large aggregates (50-100 $\mu$) are first trapped in small arterioles. The particles are moved progressively forward by cellular bombardment. The vessel slowly dilates, blood cells and plasma move around the aggregate, reducing its size by displacement of small fragments. The aggregate is then carried further down the vessel and in some instances this process is repeated. In other vessels flow in the arteriole suddenly reverses its direction and the aggregate is carried into other vessels possibly as a result of changing intra-arteriolar pressure. Similar phenomena are observed in smaller vessels down to capillary size (5-10 $\mu$). Thus, it appears that the passage of these malleable aggregates through the arterioles is accomplished by cellular bombardment and fragmentation and by continuous forward and backward movements within the arterioles until the aggregates are converted into smaller sizes and elongated forms capable of traversing the capillary lumina. Phagocytic processes were not observed either *in vivo* or in microscopic examinations of rabbit or dog lung sections.

*Theoretical Possibilities in Macroaggregate Removal:*

The process(es) initiating macroaggregate removal from the arteriole in which it is mechanically trapped may be on the basis of several factors occurring concomitantly or in a short temporal span. These factors may be mechanical agitation, pH and electrical potential changes and local accumulation of $CO_2$ and lactic acid.

Comment has already been made on the bombardment of these friable aggregates by the circulating blood elements resulting in fragmentation into smaller aggregates. It is known from the extensive work of Sawyer and Pate (8) and Schwartz (9) on the electrical environment of blood vessels that the blood elements and the intima are relatively negative with respect to the adventitia.

In thrombosis (from mechanical, chemical or thermal injury) they have shown a reversal of this polarity which means the intima is positively charged with apparent attraction of the negatively charged blood cells (electrophoretic attraction phenomenon). Whether or not this plays a role in the entrapment or dislodgement of the radioalbumin macroaggregates, composed of thousands of positively and negatively charged submicron sized particles with a preponderant negative charge above the isoelectric point of albumin, is unknown. The possibility of progressive disintegration of the macroaggregates at the pH of plasma and agitation in the circulation was studied *in vitro*. No apparent changes in the appearance of these aggregates were observed during a 24 hour period.

Carbon dioxide is known to be a very effective vasodilator having both a central and peripheral action. Inhalation in 5 and 10 percent concentrations is often prescribed to patients with "small strokes" to enhance collateral blood flow. Arteries tied off have been observed to dilate distal to the ligature, presumably on the basis of vascular stasis with diminished $O_2$ carrying hemoglobin, accumulation of $CO_2$ and lactic acid. We have observed vascular stasis and dilation of the arteriole distal to the entrapped macroaggregate in the rabbit omentum. This dilation together with a proximal arteriolar pressure increase may release the aggregate into the dilated segment by a "cork popping" mechanism.

*Hemisphere Scanning and Anatomic Factors:*

To gain insight regarding the basis of brain hemisphere scanning with radioalbumin macroaggregates (10-100 $\mu$) an understanding of the arterial supply beyond the larger branches of the main cerebral arteries is important. The pia mater which covers the brain has no capillaries but has a fine network of arterioles interconnecting larger arterial vessels. From this network blood flows downward into many fine nutrient arterioles which supply the surface gray and underlying white matter. These vessels range from 15-50 $\mu$ in diameter. The larger ones nourish the deep white matter, the more numerous smaller vessels the gray matter. It is in these nutrient arterioles that the macroaggregates are initially trapped. The unfixed cadaver brain scan results support this location which is well demonstrated in the transverse brain section (Fig. 6). The capillary networks of the nutrient arterioles are not considered to be capable of providing adequate collateral circulation. According to oxygen consumption data, total interruption of the hemisphere circulation cannot be tolerated for more than five minutes at normal body temperature. Furthermore, the closer to the circle of Willis a cerebral artery is occluded, the more profound the regional ischemia of the brain tissue. One would expect that temporary occlusion (hours) of nutrient arterioles by the albumin aggregates should produce multiple areas of ischemic necrosis throughout the injected hemisphere. No such lesions were found during careful histological examination of the brains of three monkeys who had received several doses of macroaggregates by intracarotid artery injection. This apparent tolerance of brain tissue to relatively prolonged vascular obstruction of thousands of small vessels casts some doubt

on the validity of current concepts. It would appear that the brain's circulation has much more capacity to develop collateral circulation than was previously believed. On the other hand, it is possible that the obstruction produced by the albumin aggregates may not remain complete for more than a few minutes, at any one site, during the process of their fragmentation, transformation and eventual complete removal from the vascular tree. During serial hemisphere scans, the area of maximum count rate has been repeatedly demonstrated to change position. This observation suggests the migration of this particulate material and the dynamic nature of its passage through the cerebral vasculature. However, much more extensive histological study is needed to verify these preliminary findings.

*Visual Hazard:*

There are two reasons why the macroaggregates have not been observed on fundoscopic examination. The branches of the central retinal artery measure approximately 60-80 $\mu$ in diameter at the optic disc, calibers sufficient for passage of the macroaggregates. The retinal region where these arteries become smaller is difficult to visualize. Secondly, the central retinal artery is one of the *smaller* branches from the ophthalmic artery. *Larger* branches go to the lacrimal, anterior nasal and supraorbital areas. The tangential origin of the small central retinal artery does not place it in the direct pathway of blood flow, thereby the aggregates may largely by-pass the central retinal artery on their way through the larger frontal and supraorbital branches.

*Future Hemispheric Scanning in Man?*

Before cerebral hemisphere scans are performed in man with macroaggregated albumin, the toxicity of the agent must be re-evaluated when administered after radiographic doses of 50 percent sodium diatrizoate in primates. Furthermore, the investigations must include long term observations to detect minimum changes in behavior, motor performance and intelligence as well as more extensive histological examination of brain sections.

## SUMMARY AND CONCLUSIONS

Ten monkeys were given repeated internal carotid artery injections of radioalbumin aggregates (10-100 $\mu$) in doses of 0.8 to 11.0 mg. Doses below 2.0 mg produced no detectable central nervous system abnormalities. The following conclusions can be made at this time:

1. These central nervous system studies in primates indicate that if the entire carrier dose (0.2-0.6 mg) of radioalbumin macroaggregates injected for lung scanning in man were shunted into the general circulation the likelihood of cerebral microembolization is extremely remote.

2. The cerebral toxicity of macroaggregates must be re-evaluated after prior injection of 50 percent sodium diatrizoate in the monkey, inasmuch as the brain hemisphere scan may be performed in conjunction with angiography in man.

3. Initial arteriolar entrapment of macroaggregated albumin appears to be a purely physical mechanism. However, further study is needed to elucidate the mechanisms by which these friable particles are removed.

4. Before brain hemisphere scans are performed in man, long term studies in primates are needed to evaluate changes in behavior, intelligence and motor performance as well as detailed histological examination of brain sections.

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