Cerebral Arteriovenous Shunts Re-examined

BY DAVID W. ROWED, M.D., VIOLET J. STARK, B.A., PAUL B. HOFFER, M.D., AND SEAN MULLAN, M.D.

Abstract:
Cerebral arteriovenous (A-V) shunts have been described in some histological studies, but their existence has been denied in others. Previous attempts to detect the presence and amount of A-V shunting using radioactive microspheres have suggested that large amounts of blood could bypass cerebral capillaries in this fashion. A reappraisal seemed indicated.

The present study, using the radioactive microsphere technique, confined the injected spheres specifically to the cerebral circulation of a nonhuman primate. The conclusion of this study is that structural, noncapillary A-V shunting definitely occurs, but is very small and variable in amount.

The implications of this finding in relation to previous studies and to the clinical phenomena of red cerebral veins and early venous filling are discussed.

Additional Key Words
"thoroughfare channels" radioactive plastic microspheres Rhesus monkey

Noncapillary blood vessels connecting arteries and veins are present in many vascular beds, but the question of their existence in the cerebral circulation is still a matter of considerable controversy and some potential importance.

Histological and injection studies of human brain have suggested three types of arteriovenous (A-V) shunts differing with respect to size and site: (1) pial A-V anastomoses, 20 to 160 μ in diameter, (2) precapillary "thoroughfare channels" present in large numbers in both gray and white matter, 8 to 12 μ in diameter, and (3) "arteriovenous bridges," rare, and found in subcortical white matter only, 14 to 25 μ in diameter. The first type was observed in human brain, and the latter two were described in both humans and dogs.

Cerebral capillary diameters are said to vary little in vertebrates, and in the human have an average diameter of 5.8 μ, with a range of 4 to 8 μ.

Unfortunately, other elegant histological and microangiographical studies have failed to confirm the presence of these latter "thoroughfare channels" and A-V bridges, and suggest that vessels with such an appearance have, on close scrutiny, proved to be illusory.

Another approach to the study of arteriovenous shunting employs radioactive microspheres. In the case of cerebral A-V shunting, virtually all of the spheres bypassing cerebral capillaries, if of appropriate size, will be arrested in the pulmonary capillaries. Thus the amount of such shunting can be easily quantified.

Cerebral A-V shunting has been previously studied by this technique, the microspheres being injected into the neck vessels of dogs. The results showed large amounts of radioactivity in the lungs, but were not conclusive because the microspheres injected were not confined to the cerebral circulation.

The purpose of the present study is to...
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establish the magnitude of intracerebral arteriovenous shunting in a nonhuman primate, using radioactive microspheres. The essential prerequisite for obtaining valid data, in our opinion, is the confinement of microspheres to the cerebral blood vessels.

Material
The microspheres used in this experiment are made of "carbonized" plastic, with an incorporated label of $^{141}$Ce (principal gamma emission—145 kev). (Microspheres were obtained from 3M Company, St. Paul, Minnesota.)

The spheres were suspended in isotonic saline solution, with a small amount of polysorbate 80 added to retard aggregation. Initial specific activity was approximately 10 mCi/gm.

The size range was purported to be $15 \pm 5 \mu m$, but actual sizes were determined by measuring aliquots of the spheres using a microscope fitted with a filar micrometer eyepiece. The size range in our samples of microspheres was 12.5 to 20 $\mu m$, with a distribution as shown in figure 1.

Methods
The Rhesus monkey (Macaca mulatta) was chosen as an experimental subject because its cranial circulation is comparable to that of the human with respect to communications between the intracranial and extracranial circulations.12 Sixteen healthy adult Rhesus monkeys of mixed sex and weighing 3.5 to 4.8 kg were used in this study.

The animals were anesthetized with intraperitoneal sodium pentobarbital, 35 mg/kg initially,
followed by small intravenous increments, if required. Endotracheal intubation was carried out, and the animals were allowed to breathe spontaneously. Polyethylene catheters were introduced into the femoral artery and vein, and advanced well centrally. Normovolemia was maintained with small intravenous infusions of isotonic saline solution. Systemic arterial blood pressure was monitored by connecting the arterial catheter to a manometrically calibrated Statham strain gauge and a Beckman Type R Dynagraph. Arterial blood gases were monitored to ensure that $P_{O_2}$ and $P_{CO_2}$ remained within normal limits.

We were concerned that microspheres might enter the extracranial circulation via the ophthalmic artery or the intracavernous branches if they were simply injected into the extracranial internal carotid artery (ICA). Accordingly, the intracranial bifurcation of the ICA was exposed by a modification of the retro-orbital approach of Sundt and Waltz. The arachnoid was dissected off the proximal portion of the middle cerebral artery (MCA) just sufficiently to allow application of a small aneurysm clip across the origin of that vessel. The MCA was clipped immediately prior to the injection of microspheres to prevent reflux into the ICA. (Magnification of vision facilitates, but is not essential for, this part of the operative procedure.)

A specially constructed 30-gauge needle, shown in figure 2, was then introduced into the lumen of the proximal MCA, and the microspheres were injected slowly and gently, and their passage was followed under direct vision. (Hollow steel tubing for needle construction was obtained from C. A. Roberts Company, Franklin Park, Illinois.) One to 3 ml of isotonic saline was then injected to remove residual spheres from the polyethylene tubing. The needle was then withdrawn and a pledget of absorbable gelatin sponge was placed over the injection site. The aneurysm clip was removed immediately and gentle pressure applied over the sponge. The number of spheres in the injection syringe varied between $2.2 \times 10^6$ (Animal No. 10) and $7.3 \times 10^6$ (Animal No. 9).

**FIGURE 2**

Method of injection. The tip of the needle is shown within the lumen of the proximal middle cerebral artery (MCA) which has been occluded with a small aneurysm clip. ACA—anterior cerebral artery. ICA—internal carotid artery.
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The animal was allowed to survive for a period of not less than 30 minutes and was then killed instantly with an intravenous bolus of sodium pentobarbital. The brain and lungs were removed and placed in covered plastic containers.

Brain and lungs were counted by being placed 20 cm from the face of a two-inch thallium-activated sodium iodide crystal scintillation probe. Orientation of all brains was identical, the center of the detector being placed over the midpoint of the Sylvian fissure of the injected hemisphere. Twenty-minute counts were obtained for each organ.

The brain and lungs were fixed in neutral buffered formalin for a minimum of ten days, and multiple tissue blocks were then taken and sectioned at several levels for light microscopic study.

Results

The count rates for lungs and brains and their ratio is shown in table 1. Note that the ratio of lungs/brain uptake did not consistently increase when greater numbers of microspheres were injected.

The mean value of the ratio of lungs/brain counts per minute (cpm) for the series is 0.25%, and there is obviously a considerable amount of variation, despite apparently uniform experimental conditions. Great variability in the amount of A-V shunting has been observed consistently in other vascular beds,

and does not invalidate the experimental results. The point that requires emphasis here is that the standard deviations given in table 1 do not pertain to the overall variability of the experimental results in different animals. Rather, they are concerned with the reproducibility of the individual count rates. In summary, the amount of shunting is always very small, but variable.

We believe that, with the method employed in these experiments, the ratios expressed in table 1 are the best index of the amount of A-V shunting. Use of the radioactivity of the microspheres in the injection syringe as a denominator for comparing the lung activities is unsatisfactory because a few microspheres may be lost at the injection site and there is always some residual activity in the injection syringe and needle.

There may be slight attenuation of count rates using this method, but most of the results reported here were checked by counting the organs in a well-counter with a five-inch sodium iodide crystal. When this was done the ratio of lungs/brain cpm remained unchanged, although the absolute count rates were increased by the more sensitive well-counter. Selected carcasses, following removal of the brain and lungs, were counted using the gamma camera and no significant activity was found.

<table>
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<tr>
<th>Animal no.</th>
<th>Net counts per min — brain</th>
<th>Net counts per min — lungs</th>
<th>Ratio lungs/brain counts per min (%)</th>
<th>Standard deviation (%)</th>
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Note that standard deviations do not pertain to the overall variability of the experimental results, but only to the reproducibility of the individual count rates.

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Examination of the brains revealed that the microspheres were confined mainly to the MCA territory on the injected side. In many cases, there was some spilling over into the adjacent anterior cerebral (ACA) and posterior cerebral (PCA) arterial territories. This presumably occurred by way of either the pial arterial plexus or precapillary arterial anastomoses. In microscopic sections, the microspheres were distributed in the penetrating arterioles and their branches, mainly in the deeper layers of the cerebral cortex and, to a lesser extent, in the white matter. A representative area of cerebral cortex is shown in figure 3.

Most of the spheres are lodged singly in arterioles at the plane of the section, although a pair of microspheres is seen in the lower central part of the field in figure 3. The apparent variation in size of the microspheres is due, of course, to the fact that the plane of the section does not pass through the greatest diameter of all the spheres.

A single higher power view of a microsphere trapped in an arteriole is shown in figure 4. The arteriole is shrunken and collapsed on either side of the sphere.

Occasionally, several microspheres were "stacked up" in an artery and this, we believe, was due to total blockage of the distal arterial bed in the territory supplied by such vessels.

In all of the animals reported in this series, the presence of microspheres in the lungs was confirmed in microscopic sections (even in animal No. 20). They occurred infrequently in the pulmonary sections, lodged singly in precapillary vessels, as shown in figure 5.

The question of "leaching" of isotope is inevitably raised in the evaluation of quantitative studies of radioactive microsphere distribution. The tracer isotope in the microspheres employed in this study is incorporated as an integral part of the sphere during manufacture, and assays of the suspending medium show no
significant activity even over prolonged periods of time. The short duration of these experiments allows little time for in vivo "leaching." Furthermore, the large numbers of microspheres seen in the brain correlate well with the fact that virtually all the detectable radioactivity in the animal carcass was confined to brain. The small number of microspheres that did not lodge in brain arterioles became trapped predictably in pulmonary vessels, where their presence was detectable by scintillation detector and with the microscope. For all these reasons, it is believed that negligible "leaching" of isotope occurs.

**Discussion**

The purpose of this study was to apply the radioactive microsphere technique to study noncapillary arteriovenous shunting in the brain of a nonhuman primate. We believe the method described above satisfactorily confines the injected microspheres to a portion of the cerebral circulation insofar as this is possible in vivo.

The inescapable conclusion of the study is that noncapillary A-V shunting occurs in the MCA distribution of the Rhesus monkey, but that the amount of such shunting, relative to total blood flow in the same vascular bed, is very small under the experimental conditions noted above.

The percentage of A-V shunting observed is of a much smaller order than that observed by Benda and Brownell in the dog. In the dog, however, there are abundant anastomoses between the internal and the external carotid arteries. The discrepancy, therefore, between the degree of shunting observed by these authors and that seen in this study would appear to be due to passage of the microspheres into the extracerebral tissues where,
apparently, a large amount of A-V shunting occurred.

Much larger amounts of arteriovenous shunting than that seen in the present study have been observed in other vascular beds, notably in the hind limb of the dog.\(^2\)\(^,\)\(^8\) Even by comparison with vascular beds where very little A-V shunting occurs, e.g., the superior mesenteric arterial circulation,\(^8\) the amount of shunting seen in the present study is strikingly low.

Three types of structural A-V shunts have been described in the cerebral circulation as outlined in the introduction.\(^3\)\(^,\)\(^4\) It is difficult to be certain whether all of these were utilized by the microspheres in their passage from artery to vein.

There is no doubt that the pial A-V anastomoses described by Rowbotham and Little,\(^3\) if present, would be exposed to the spheres since, with rare exceptions, all of the microspheres passed beyond the pial plexus to lodge in penetrating arterioles. The larger arteriovenous bridges described by Hasegawa et al.\(^4\) would also be exposed to any microspheres passing into subcortical white matter. Whether the smaller "thoroughfare channels" described by Hasegawa et al.\(^4\) served to transport microspheres from arteries to veins is less certain. Neither of the latter types of A-V shunts has, to our knowledge, been described in the Rhesus monkey, but if, indeed, they exist in both dog and human, their presence in the nonhuman primate is likely.

On the basis of the dimensions given for these "thoroughfare channels" (outside diameter 8 to 10 \(\mu\) in humans, and 6 to 8 \(\mu\) in dogs), one might be tempted to assume that the microspheres used in these experiments would not pass through them. Indeed, it would be difficult to distinguish with certainty between shunt vessels of this order of size and true...
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capillaries using the microsphere method. Upon reflection, however, we believe that one need not accept the absolute sizes stated, even if the method of measurement was precise. All of these observations were made on fixed tissues, albeit cut with a cryostat. In such preparations, there may be significant shrinkage and collapse of the smaller blood vessels.10, 17 (Red blood cells in the same preparation were said to measure 4 μ in diameter.)

Moreover, there is the further possibility that such vessels are capable of dilatation or constriction in vivo. Hasegawa et al.4 do not describe the histological features of their “thoroughfare channels” in detail, but certainly contractile elements are described in similar vessels elsewhere.1 Earlier studies suggested that vasoactive nerves did not extend to the smaller intracerebral arteries,18 but more recent work has traced adrenergic fibers down to the level of arterioles 15 μ or less in diameter.10, 20 Pharmacological effects on the cerebral circulation, including the influence of respiratory gases, are well known.21

It is very likely then, though not certain, that the microspheres used in this study would have reached the “thoroughfare channels” if they were present. Since the amount of A-V shunting detected is very small, and the “thoroughfare channels” described by Hasegawa et al.4 were said to be frequent, it appears, therefore, that our microspheres did not pass through them. We are inclined to agree with Saunders and Bell4 in doubting the existence of “thoroughfare channels” in the brain.

The existence and the magnitude of structural A-V shunting are matters of clinical, as well as theoretical, importance, particularly where ischemia is present.

Red cerebral veins are a well-known clinical and experimental phenomenon.22-24 We will not attempt to catalogue all of the situations in which red venous blood (RVB) is seen in the cerebral circulation. In summary, though, RVB could occur in three general circumstances: (1) in the presence of structural A-V shunts, (2) with greatly increased blood flow in excess of tissue requirements, and (3) if there is decreased metabolism, or death of tissue, so that oxygen is not utilized.

Structural A-V shunting occurs in arteriovenous malformations (AVMs) and vascular neoplasms.22, 24 Confirmation of this phenomenon is given by regional 133Xe clearance studies.25

RVB in most other situations has been attributed to decreased brain metabolism or death of tissue, e.g., in association with hypoglycemia or ischemia.23 It seems unlikely that increased blood flow alone, without concomitant structural A-V shunts, or decrease in metabolism will produce RVB.

In addition to RVB, there is another clinical situation which appears to relate to structural A-V shunting. There is good angiographical evidence for early venous filling in cases of cerebral ischemia26 and trauma.27 It seems unlikely that such rapid transit of contrast medium from arteries to veins can occur solely via capillaries unless the latter are capable of incredible dilatation.

Conclusions

Our study demonstrates that, under normal physiological conditions, a distinctly small percentage of the blood flow in the MCA territory passes through A-V shunts 12.5 μ or more in diameter. Smaller-sized structural arteriovenous shunts may exist, though their potential importance in disease states associated with RVB or early venous filling seems questionable.

It is possible that structural A-V shunts can “open up” or dilate in pathological states. This can be tested by applying this experimental model to other experimental conditions. In preliminary studies we are not convinced that hypercapnia, topical hypothermia, or phenoxybenzamine have any influence on the amount of A-V shunting,28 but other factors may be significant.

We believe that it is more likely, however, that the noncapillary arteriovenous shunts which we have detected in the Rhesus monkey brain are relatively few and are variable in number. They may be confined to the pial circulation. It is probable, therefore, that structural channels do not play an important part in the pathogenesis of RVB exclusive of their role in neoplasms and AVMs. The question of the genesis of early venous filling cannot be clearly resolved at this time.

On the basis of our data, we think it likely that the “shunting” associated with RVB, in most situations, is on a metabolic basis.
Until now, reliable quantitative data on the amount of structural shunting in normal brains have been lacking.

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References
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