An Animal Model of Cerebral Infarction
Homologous Blood Clot Emboli in Rats

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SUMMARY An experimental model of cerebral infarction is created in rats by intracarotid injection of their homologous blood clots. This is the first small animal model in which embolization was achieved by homologous blood clots. The infarcts were produced predominantly in the territory of the middle cerebral artery. The low mortality rate and excellent reproduction rate make possible the correlative study of morphological, biochemical, and metabolic parameters at selected points in time to reconstruct the pathogenesis and natural history of focal cerebral ischemia and its relation to blood elements.

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HILL ET AL' WERE THE FIRST to use the injection method of homologous blood clots into the carotid artery as an experimental model for cerebral infarction in dogs. This model proved to be very similar to human cerebral infarction but it was difficult to assess the extent and outcome of different parameters. Furthermore, the cost is prohibitive to use dogs in large scale studies. We therefore used rats as an experimental animal and understood that the injection of homologous blood clots into the carotid artery was by far the best method to produce reliable and reproducible infarcts in rat brain in our experimental design. Our results are described to demonstrate its usefulness in the study of cerebral infarction.

Material and Method
Preparation of Blood Clot Emboli
Sixty Wistar strain male rats, weighing between 200 and 300 gm, were used. The animals were anesthetized with ether, and 0.1 ml of blood was obtained by cardiac puncture with a tuberculin syringe and stored at room temperature for 48 hours for clot formation. The clot was separated from the serum and was fragmented by injecting it through a 26 gauge needle into normal saline. The latter step was repeated three times. A 0.2 ml clot suspension with fragments of varying sizes but no more than 100 µ used for the embolization.

Procedures of Embolization
The animals were again anesthetized with ether. The bifurcation of the left common carotid artery, and the internal and external arteries were surgically exposed. The vagus nerve and sympathetic plexus remained intact. A temporary clip was applied around the common carotid artery about 15 mm below the bifurcation. Following a smooth and gentle injection, the needle was drawn out and the site of injection was sealed off with surgical adhesive. The clip of the external carotid artery was removed. The thread around the common carotid artery was removed and the flow was established. The surgical site was closed. For control studies, a sham operation was performed in ten animals consisting of either a permanent ligation of the left common carotid artery or an injection of 0.2 ml physiologic saline with no emboli.

Results
Clinical Observation
No operative death of animals was present. At injection of the emboli, a mild tonic deviation of the head to the side of embolization and facial twitching were observed in virtually all animals. All but some severely damaged animals took fluid and food spontaneously soon after recovery from anesthesia. Various clinical manifestations such as a narrowing of eye fissure and pallor of the eyeball on the affected side, licking, biting, circling, tilting of the head, limping, or decreased spontaneous activity appeared singly or in any combination. Their severity varied from animal to animal. Fourteen of 60 animals (23%) suffered from intermittent seizures within two hours after embolization and they were all associated with prolonged recovery, respiratory distress, obtundation, or coma. Nine out of 14 such severely damaged animals died within the first two days and the remaining five animals showed a rapid improvement. The clinical signs were much improved in most animals by the 5th post embolic day, but complete disappearance of initial clinical signs was rare. Instead, easy excitability or loss of weight that was not found at the initial insult, was newly noted in ten animals. Control animals that underwent sham operation showed no comparable clinical signs and remained asymptomatic. The animals surviving the first 48 hours were killed with a conventional transcardiac perfusion on 4, 7, 14, 28, and 60 days after embolization.

Pathology
In the majority of the animals, 75% (45 of 60 animals), edema, necrosis, or cystic cavity was observed dependent on the age of lesions externally or on cut surfaces. The remaining 15 animals were also invari-
ably involved by scattered microscopic sized infarcts. The clinical signs certainly depended upon the extent of the lesions. The animals dying within the first 48 hours suffered from massive ipsilateral hemispheric edema with some midline shift toward the opposite hemisphere.

The pallor of the eyeball, due probably to embolic occlusion of superior ocular artery, was a reliable parameter of the extent of the infarcts, since larger infarcts were more common in animals with the more severe pallor of the eyeball.

The territories of the left middle cerebral artery and anterior choroidal artery were invariably involved. There were also scattered, mostly microscopic sized lesions in the regions of anterior and/or posterior cerebral arteries in 15 animals. The anatomic structures most frequently involved were parietotemporal cerebral cortex 83% (50 of 60 animals), hippocampus 60% (36 animals), and thalamostriate areas 60% (35 animals). The contralateral hemisphere was involved with microscopic sized infarcts in 5 animals. No lesions were present in the cerebellum, brain stem, and spinal cord. In sham control animals there was no pathological lesion anywhere.

The lesions were basically pale, anemic, and complete, occasionally with microhemorrhage. The 6 hours old lesions were characterized grossly by cerebral swelling with obscuration of the grey and white matter border and microscopically by pallor of the tissue, eosinophilic neurons, and edema. By day 2 the affected areas become necrotic with histologic appearance of macrophages at the periphery, and the 4 days old infarcts were well delineated from the surrounding edematous areas and were evidenced by more foamy macrophages and scattered reactive glial cells (figs. 1 and 4). The 7 days old lesions showed more proliferation of glia and macrophages (fig. 2). By day 14, the lesions become cystic showing many foamy macrophages in the central areas and a newly formed glial meshwork at the periphery. Thereafter, progressive, more complete glial scar and fewer macrophages were noted in and about the infarcted areas (figs. 3 and 5).

Discussion

The use of homologous blood clots simulates better cerebral infarction in man than the heterologous products such as foreign bodies and makes possible systematic study of the roles of blood components, specifically platelets, in the evolution of regional cerebral ischemia based on different parameters of the experimentation. The morphological, biochemical, metabolic, and physiological evaluations of various modalities applicable to humans also become possible. Further, our model is inexpensive and fulfills the three fundamental requirements of Hudgins and Garcia for experimental cerebral infarction applicable to ultrastructural studies. They are 1) a high percentage of infarcts with predictable average size, 2) no surgical
manipulation of the cerebral tissue or exposure of the brain to the air, and 3) capability for in vivo perfusion fixation of the ischemic and nonischemic brain.

As to the general belief of an unpredictability of the location of lesions in embolic method, the present model produces rather predictable distribution of lesions by far more frequent in regions of the middle cerebral artery. This predictable distribution of the lesions might be related to the consistency of the size and physical characteristics of the blood clot emboli used in the study, and to the similarity of the cerebral vascular anatomy and hemodynamics in rats.

The procedure has a low mortality and excellent reproduction rate. An excess amount of emboli is related with a high mortality at the early stage of experiment due to rapid development of massive cerebral edema. Our experience shows that emboli made from 0.1 to 0.2 ml blood under conditions described here seem to be the most appropriate in amount and size in adult rats, 200 to 300 gm body weight for production.

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Figure 3. Coronal section of the brain 28 days after embolization shows cystic lesion of the infarcts.

Figure 4. Microphotograph of the infarcted area of 4 days old shows many foamy macrophages and reactive glial cells. (Paraffin embedded, hematoxylin eosin stain. 200 ×.)
of grossly visible lesion with no early loss of animals.

The infarcts in the present model are primarily of anemic type, being somewhat different in nature from those of humans in which hemorrhagic infarcts are more frequent. The reason for this is unclear at present. The histologic changes are similar to those described as a prototype of cerebral infarction in primates.8

It is our hope that the present model should form the basis for further progress in our understanding of various questions underlying cerebral infarction and other related vascular disorders. Incidentally, it is considered that carefully planned and executed ultrastructural studies with concurrent biochemical, metabolic, and physiological study provide the best ways for distinguishing reversible from irreversible cellular events due to ischemia.9, 10 Further, the present model will be applicable to the study of cerebral abscess using infected blood clot emboli.11

References
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