A Classification of Experimental Models of Brain Ischemia

G. F. Molinari, M.D.* and J. P. Laurent, M.D.

SUMMARY A system is presented for the classification of experimental models of cerebral ischemia by a mechanism of induction and distribution of the cerebral insult. Application of the proposed system to the scientific literature may help to resolve apparent conflicts in experimental findings and establish a frame of reference upon which to estimate the relevance of specific animal data to clinical phenomena. Experimental ischemic lesions and pathophysiological states must be considered the net product of variables in etiology and distribution of ischemia in the species chosen under the specific anesthesia selected.

AS METHODS improve for the quantitative analysis of biochemical and physiological data, more sophisticated investigations of cerebral blood flow, metabolism and electrocortical activity have been undertaken. While many of the current techniques are not yet adapted to routine use in humans, potentially relevant information is being derived from a wide variety of animal models in diverse species. Since many flow techniques are invasive, posing risk to patients, and because biochemical analysis, ultramicroscopy and autoradiography require fresh, specially prepared postmortem tissue at fixed time intervals rarely available from clinical sources, many basic as well as clinical hypotheses are appropriately tested in animal models.

Historically, experimental methods have improved with each technical advance, and, coupled with increased awareness of veterinary literature, various techniques and experimental species have proved innovative. Many methods have temporarily prospered only to fall into disuse or disrepute. Nowhere is this more evident than in the field of experimental cerebrovascular disease. Recent symposia illustrate the wide variety of surgical induction methods, species variations, monitoring and data processing techniques currently in use in the effort to develop a physiological approach to the management of acute cerebral ischemia.

One would hope that the models queried are representative of pathological states which occur in nature in species which have anatomical, chemical, and physiological resemblance to man.

In order to assist in the interpretation of data derived from diverse sources, to readily identify information of potential clinical utility, and to foster the development of collaborative studies and compatible data pools, a classification system for models of cerebral ischemia is proposed in figure 1.

Definition of Ischemia

The classification proposed in figure 1 addresses only models of ischemia, "the disease processes whereby healthy blood does not circulate properly through the blood vessels." Conditions referred to as anemic anoxia and anoxic anoxia are relatively rare phenomena in nature, and, while they may be superimposed upon ischemic conditions, these pathophysiological variants are not addressed in the proposed classification. Nor should data derived from experimental models of these conditions be confused with observations made in ischemia.

Species Variables

While anthropomorphism in the anatomy of cerebral circulation is highly desirable and readily perceived, some of the more popular experimental models capitalize on specific anatomical variants from the human prototype. These anatomical variations seem to violate the general principle of Shellshes that anatomical patterns expressed early in phylogeny persist in later and higher species in the phylogenetic hierarchy. This nostrum promulgated in 1927 is widely accepted and, in fact, is implicit in the rationale of all scientific investigations based on animal models. Another basic tenet essential to the credibility of animal modeling is the presumed parallelism and independence among physiological, biochemical and anatomical variations. Among these variables, morphology is the most readily perceived and, in practice, becomes the most useful basis for selection of type animals. It is somewhat illogical, however, to assume that species which are anatomically diverse from each other and from man should function in physiological and biochemical patterns similar to man.

*Section on Head Injury and Stroke, Applied Neurologic Research Branch, Collaborative and Field Research, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Building 36, Room 4A03, Bethesda, Maryland 20014.
CLASSIFICATION OF EXPERIMENTAL MODELS OF BRAIN ISCHEMIA/Molinari et al. 15

TABLE I Distribution of Ischemia

<table>
<thead>
<tr>
<th>Distribution of Ischemia</th>
<th>Extravascular</th>
<th>Intravascular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brachiocephalic-A. Ligation (+)</td>
<td>Hypotension, (+)</td>
<td>Cardiac Arrest (+)</td>
</tr>
<tr>
<td>Hemispheral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int. Carotid A. Ligation (+) with:</td>
<td>Intracerebral Perfusion with-Wax, Polyvinyl Acetate</td>
<td></td>
</tr>
<tr>
<td>Incomplete, or Artiﬁcial Anatomic Circle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multifocal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embolism with-Microspheres, Autologous Blood Clot (+)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIGURE 1 A schematic representation of a classiﬁcation of experimental models of ischemia by etiology and distribution of the circulatory impairment. Clinical counterpart n man refers to the relevance of specific models to common spontaneous or surgically induced cerebrovascular insults. While multifocal extravascular occlusions could theoretically occur in man during desperate attempts to arrest intraoperative subarachnoid hemorrhage, such situations are rare and have not been widely chosen as targets for experimental modeling. Therefore, there is no entry in that category in the classiﬁcation system.

Since anatomical anthropomorphism is the principal guide to selection of animals for physiological investigation, then unquestionably subhuman primates best meet this criterion. Therefore, while speciﬁc scientiﬁc hypotheses may possess this variation, the question of relevance is moot. The experimental neuropathologist, therefore, assumes that lesions which appear morphologically similar to those occurring in man are caused by pathogenetic processes similar to those which pertain to humans. The experimental neuropathologist, therefore, assumes that induction methods which cause lesions with gross and microscopic characteristics of clinical infarctions produce physiological and biochemical changes which replicate the pathogenesis of ischemia in humans. These various induction methods are the subject of the proposed classiﬁcation system based upon the distribution and etiology of the ischemic process.

Pathological Anthropomorphism

Spontaneous cerebrovascular diseases, including arteriosclerosis, are known to occur in various species of animals phylogenetically inferior to man. Ischemic cerebral insults are rare events, however, except in aging swine. The comparative neuropathology of cerebrovascular disease has been extensively reviewed and reported by Frankhauser, Luginbühl and McGrath. Their exhaustive work supports the hypothesis that lesions which appear morphologically similar to those occurring in man are caused by pathogenetic processes similar to those which pertain to humans. The experimental neuropathologist, therefore, assumes that induction methods which cause lesions with gross and microscopic characteristics of clinical infarctions produce physiological and biochemical changes which replicate the pathogenesis of ischemia in humans. These various induction methods are the subject of the proposed classiﬁcation system based upon the distribution and etiology of the ischemic process.

Variables Introduced by Anesthesia

The proposed classiﬁcation does not take into account the choice of anesthesia used in modeling experiments. Barbiturates are reputed to have a protective effect in experimental models of global ischemia and focal ischemia; while ketamine, considered by some to be a metabolic stimulant, increases cerebral blood flow and may intensify substrate demand, thereby introducing metabolic or physiological artifacts. Similarly nitrous oxide, known to produce anoxia, when used in conjunction with experimental ischemia, may produce lesions of combined ischemic-anoxic etiology.

Bases for Classiﬁcation

ETIOLOGY OR MECHANISM OF ISCHEMIA

Two major approaches are available for disruption of the circulation of healthy blood to the brain: (a) extravascular arterial compression by extrinsic ligation, and (b) manipulation of the intravascular hemodynamics by embolism or hypotension. Both have distinct advantages and disadvantages.

Extravascular occlusions occur in humans during surgical treatment; in experimental animals extravascular compression produces acute ischemia in the vascular distribution distal to the clip or ligature. Small spring clips may be applied under direct visual control so that the precise site of vascular occlusion is known. Furthermore, clips may be removed after known time intervals and therefore may be used in modeling temporary or transient ischemia. However, intracranial arterial occlusion requires craniectomy, and the direct disruption of the autonomic nerve supply to the vascular bed seems unavoidable and may introduce physiological and biochemical artifacts.

Intravascular methods spare the arterial nerve supply, do not require craniectomy, and, while usually irreversible, may be used for pathophysiological studies spanning acute and chronic phases. However, the site of vascular occlusion is controlled only by the size and physical characteristics of the embolic substance used. Embolic material must pass through a rete mirabile in all laboratory species other than dogs and primates in order to occlude intracranial vessels.

DISTRIBUTION OF ISCHEMIA

Brachiocephalic-vertebral arterial ligation in species possessing this anatomical variant have been used as highly efﬁcient models of global ischemia. Since man does not possess this variation, the question of relevance is moot. While extrinsic transcutaneous compression of the neck with an inﬂatable cuff is used as an easily reversible model of time-graded global ischemia, this manipulation mimics an exceedingly rare event, namely, strangulation. Of unique pathophysiological signiﬁcance in both strangulation and this popular model is the fact that arteries and veins are simultaneously compressed. Furthermore, cross clamps on major vessels, even proximal to the carotid sinus, modify with the stimulus-response patterns known to be mediated by the carotid body and its innervation.

Most of the events occurring in nature which result in global ischemia originate endogenously in the cardiovascular system, namely, hypotension caused by myocardial infarction, ventricular ﬁbrillation, etc. While such episodes are relatively common in man and warrant experimental investigation, extrinsic compression of major vessels to the brain does not serve as a satisfactory model of this condition.

Hemispheral ischemia can be conveniently induced in the Mongolian gerbil because the species lacks an anastomotic...
circle of Willis. This feature is shared by other mammals, notably ruminants in which the vertebral arteries supply the muscles of the neck, in fact, neck structures derive at least part of their blood supply from arteries originating intracranially. West and Matsen have deliberately modified the circle of Willis in primates to achieve control of hemispheric blood flow by manipulation of the internal carotid artery in the neck. These models are useful and popular, especially the gerbil model. However, they model a relatively unusual situation encountered in human disease, namely, surgical treatment of intracranial aneurysms by carotid ligation in persons with congenital or advanced arteriosclerotic malfunction of the circle of Willis. Furthermore, the gerbil has a disproportionate representation of blood supply to the interior or parenchyma compared to vessels supplying the rather rudimentary cortical mantle. This may be significant in the interpretation of physiological and biochemical findings in the light of current theories on the neurogenic-myogenic interactions in regulation of cerebral blood flow.

Intra-arterial perfusion of a cerebral hemisphere with wax and polyvinyl acetate usually causes ipsilateral cerebral infarction. Even if injection rates are empirically determined which favor selective ipsilateral perfusion without crossover, these substances produce long serpiginous occlusions of multiple adjacent vessels through their entire length. While these vascular occlusions are technically segmental and prohibit collateral circulation, nothing quite so definitive is known to occur in the natural history of cerebrovasculature.

Most popular for use in higher species, including primates, is the direct application of microsurgical spring clips to the middle cerebral artery. Ingenious surgical approaches have been developed to minimize the artifacts induced by surgical craniectomy and vascular manipulation. Nonetheless, single clips produce an exceedingly narrow segmental occlusion, barely greater than the width of the clip itself. Furthermore, the effect of the clip on the caliber of downstream branches of the middle cerebral artery and microcirculation has been well documented.

The recent report by Harper and his group suggests that the nerve supply to cerebral vessels adjusts the upper limits dependent upon the quantity and quality of meningocerebral anastomoses. The influence of anatomical variations in vessels supplying the rather rudimentary cortical mantle. This may be significant in the interpretation of physiological and biochemical findings in the light of current theories on the neurogenic-myogenic interactions in regulation of cerebral blood flow.

Multiple clips placed serially along a vessel eliminate collateral perfusion and effectively isolate the occluded segment. This method does not remove the possibility of a neurogenic artifact complicating physiological and biochemical data analyses. Moreover, the middle cerebral artery is frequently sectioned along with its nerve supply when surgically induced segmental occlusions are intended to be permanent. Certainly electrocautery, applied to the adventitial surface of cerebral arteries, induces vasospasm, effects downstream hemodynamics and almost certainly destroys the vascular nerve filaments.

Embolic methods can be accomplished which cause minimal disruption to the nerve supply traveling in the adventitial surface of cerebral arteries. An imaginative model of cerebral embolism was developed in 1955 at the Mayo Clinic, namely, the autologous blood clot injection method. This has been used with modifications by other investigators with variable degrees of success. However, clots must be aged extracorporeally for 48 hours to insure successful infarction; fragmentation of clots is unavoidable during injection. Because of fragmentation, final occlusions and infarctions are multifocal and cannot be precisely controlled. Distinction between embolic clots and postmortem intravascular clots is difficult even in acute experiments, and in chronic experiments, revascularization is both a possible and a probable event. Nonetheless, despite some undesirable features, the autologous blood clot method offers many distinct advantages and may be the preferred technique for certain studies despite its current lack of popularity.

Relatively large doses of precisely calibrated microspheres when injected into the carotid arterial system produce widespread precapillary occlusions in both large and small animals. While attractive because of convenience and highly imaginative when coupled with radio-nuclide labeling techniques to trace the fate of injected emboli, multiple microsphere embolism is usually fatal, restricted in usefulness to acute experiments, and has no counterpart in man.

Single microspheres and "macrospheres," such as steel ball bearings or plastic substances, may be used to induce intravascular point occlusions of the pial or larger cerebral vessels. While simple and straightforward, counterparts in nature are rarely observed. Vascular occlusions in human postmortem material are usually segmental, causing absolute ischemia in the distribution of multiple penetrating vessels originating from the occluded segment. The severity and duration of ischemia distal to the occluded segment are dependent upon the quantity and quality of meningocerebral anastomoses. The influence of anatomical variations in collateral supply on size and distribution of ischemic infarctions is well illustrated by Vander Eecken and Adams in their classical paper and later by Vander Eecken in his monograph.

Recently, we have demonstrated the feasibility of inducing segmental occlusions of the main stem of the MCA using a minimum of surgery and anesthesia procedure in dogs and rhesus monkeys. If the nerve supply to the occluded segment is in fact disrupted using these models, the effect is secondary and occurs over the same time axis as might be expected in spontaneous thromboses or emboli. Moreover, the lesions produced occur in vascular territories and parenchymal distributions commonly affected by cerebral ischemia in man, namely, the deep and surface branches of the MCA. Pathologically, the ischemic lesions have gross and microscopic characteristics similar to those encountered in the clinical laboratory.

As long as anthropomorphism is vascular and cerebral structures remain the basis for selection of models in which to study basic physiology and biochemistry, anthropo-
CLASSIFICATION OF EXPERIMENTAL MODELS OF BRAIN ISCHEMIA/Molinari et al. 17

morphism in pathology should be the most reliable guide to the study of pathogenesis and therapeutic trials.

Conclusions

Retrospective review of the vast literature on physiologic-

and biochemical changes after cerebral ischemia might be more rewarding if data were interpreted and segregated in the light of the proposed classification of models used in their generation. Indeed, many apparent paradoxes may dis-

appear, such as the conceptual conflict between the “no reflow phenomenon” caused by neck compression in rabbits and the phenomenon of red infarction caused by reflow into focal ischemic lesions in man and experimental primates. Models designed to explore the basic facts of physiology and biochemistry of the nervous system need be limited only by the imagination of the investigative scientist. However, the experimental pathologist is restricted by the fundamen-

tal and biochemical changes after cerebral ischemia might

References

1. Langfitt TW: Cerebral circulation and metabolism. Summary of Inter-
15. Takeshita H, Okeda Y, Sato A: Effects of ketamine on cerebral circula-
17. Chaavea A: Comparable Anatomy of the Domesticated Animal. Sec-
19. Garcia J, Kamijo Y, Kalimo H, et al: Cerebral ischemia: The early structural changes and correlation of these with known metabolic and
20. Snyder J, Lind B, Safar P: Cerebral variables following 15 minutes of circu-
24. West CR, Matsen FA III: Effects of experimental ischemia on electro-
pathetic nervous activity on cerebral blood flow. Arch Neurol 27: 1–6, 1970
26. Molinari GF: Unpublished data
28. Sandi TM Jr, Waltz AG: Experimental cerebral infarction: Retro-
29. HUDGINS WR, Garcia JH: Transorbital approach to the middle cerebral artery of the squirrel monkey: A technique for experimental cerebral in-
farction applicable to ultrastructural studies. Stroke 1: 107–111 (Mar-
Apr) 1970
31. HUDGINS WR, Garcia JH: The effect of electrocereotomy, atmospheric ex-
poure, and surgical retraction on the permeability of the blood-brain-
32. COHEN HP, Waltz AG, Jacobson KE: Catecholamine content of cerebral tissue after occlusion or manipulation of middle cerebral artery in cats. J Neurosurg 43: 32–36 (July) 1975
34. Harrington T, Di Chiro G: Effect of hypocarbia and hypercarbia in ex-
37. Harvey J, Rasmussen T: Occlusion of the middle cerebral artery: An ex-
40. Rumbaugh CL, Davis DO, Gilson JM: Experimental cerebral emboli-
42. Kogure K, Busto R, Scheinberg P, et al: Energy metabolites and water content in rat brain during the early stage of development of cerebral in-
43. Penny JK, Netsky MG: Experimental embolic occlusion of a single lepto-
45. VANDER ECKEN HM, Adams RD: The anatomy and functional sig-
46. VANDER ECKEN HM: The Anastomoses Between the Leptomeningeal Arteries of the Brain; Their Morphological, Pathological, and Clinical Significance. Springfield, Illinois, Charles C Thomas, 1959
47. Molinari GF: Experimental cerebral infarction. II. Selective segmental occlusion of intracranial arteries in the dog. Stroke 1: 224–231 (July-
Aug) 1970
A Classification of Experimental Models of Brain Ischemia
G. F. MOLINARI and J. P. LAURENT

Stroke. 1976;7:14-17
doi: 10.1161/01.STR.7.1.14

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/7/1/14

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/