A Classification of Experimental Models of Brain Ischemia

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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.

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 Shellshear 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 interdependence 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.
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**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.

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.

**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 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 primate in order to occlude intracranial vessels.

**DISTRIBUTION OF ISCHEMIA**

Brachiocephalic-vertebral arterial ligations 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 transcrotaneous 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 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.

Hemispheric 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 cerebrovascular disease.

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 window through which metabolic blood flow control mechanisms operate. The effect of the unavoidable crush injury to the middle cerebral artery (MCA) nerves along with chemical data analyses. Moreover, the middle cerebral collateral perfusion and effectively isolate the occluded artifacts inherent in the method.

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 radioisotope 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-
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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 physiologi-

cal 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 local 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 concepts and observations encountered in the natural history of human disease.

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