Morphological Responses of Cerebral Tissues to Temporary Ischemia

RONALD F. DODSON, YUKIO TAGASHIRA, YASUO KAWAMURA AND LENA WAI-FONG CHU

SUMMARY: *The ultrastructural responses of cerebral tissue following temporary periods (½, 1, 2, 3, or 4 hour) of right, middle cerebral artery, (MCA) occlusion were studied acutely after a 3 day or 7 day interval following the removal of the MCA clip. Cortical and basal ganglia tissues for each ischemic duration were compared at 3 post-occlusive periods (acute, 3 days, 7 days).*

With the short periods of ischemic insult (½, 1, 2, 3, and 4 hour), the temporal and insular cortex contained no greater changes in the 7 day group than in the 3 day group.

The basal ganglia were more susceptible to MCA occlusion as indicated by more marked cytological changes and/or necrosis in all intervals of ischemia.

RÉSUMÉ: *Les réponses ultrastructurales du tissu cérébral suivant des périodes variables (½, 1, 2, 3 ou 4 heures) d'occlusion de l'artère cérébrale centrale droite (ACC) furent étudiées intensément après un intervalle de 3 ou 7 jours suivant le retrait de la pince. Les tissus du cortex et des noyaux gris centraux pour chaque période ischémique étaient comparés à 3 périodes post-occlusives (aigue, 3 jours, 7 jours).*

Avec de courtes périodes d'insulte ischémique (½, 1, 2, 3 et 4 heures), le cortex temporal et insulaire ne contenait pas de changements plus importants dans le groupe de 7 jours que dans le groupe de 3 jours.

Les noyaux gris centraux étaient plus susceptibles à l'occlusion ACC comme l'indiquaient des changements cytologiques plus marqués et/ou une nécrose.

From the Departments of Neurology and Pathology, Baylor College of Medicine and the Baylor-Methodist Center for Cerebrovascular Research, Houston, Texas 77025.

This work was supported by grant NSO9287 from the National Institute of Neurological Diseases and Stroke, and grant RR 00350 from the General Clinical Research Centers Branch, Division of Research Resources, NIH, Bethesda, Md. 20014.

Reprint requests to: Dr. Ronald F. Dodson, Department of Neurology, Baylor College of Medicine, 1200 Moursund Avenue, Houston, Texas 77025.

INTRODUCTION

The vulnerability of the central nervous system to brief periods of anoxia-ischemia is well recognized, and definition of the ultrastructural response of tissue to various intervals should be considered of the highest importance since they contribute to understanding the pathogenesis and treatment of patients with vascular/hemodynamic complications. These changes are best determined by the use of animal models to study cerebral ischemia since the parameters can be readily controlled (Plum, 1973). The question of morphological and functional restoration of integrity after temporary periods of ischemia has been the subject of several recent reports (Hossmann and Kleihues, 1973; Drewes, Gilboe and Betz, 1975; Sundt, Grant and Garcia, 1969; Miller and Myers, 1972; Hossmann, 1972; Lanner, Lechner and Ott, 1972; Hossmann and Zimmermann, 1974). These reports contain varied concepts as to relationships between duration of ischemia and the degree of recoverability. However, it is likely that variation in the animal species and the models for producing ischemia account for many differences.

The present study employs the well-established model of transorbital middle cerebral artery (MCA) occlusion in the squirrel monkey from which information has been learned regarding ultrastructural changes in cerebral infarction (Garcia, Cox and Hudgins, 1971; Hudgins and Garcia, 1970; Dodson, Kawamura, Aoyagi, Hartmann, and Cheung, 1973; Garcia and Kamijyo, 1974). Because the ultrastructural response of cerebral tissue subjected to temporary periods of ischemia followed by restoration of flow (Sundt, et al, 1969) has received limited attention to date, the present work reports a more extensive investigation of this subject.

MATERIALS AND METHODS

The right middle cerebral artery was occluded via the transorbital approach in lightly anesthetized (sodium pentobarbital) squirrel monkeys (Saimiri scirreus) by the method described earlier (Hudgins et al, 1970; Dodson, et al, 1973). For purposes of comparison with acute infarction studies already reported (Dodson et al, 1973) animals were divided into groups based on their respective periods of occlusive insult (½ hr., 1 hr., 2 hr., 3 hr., or 4 hr.). After the period of vascular occlusion, the clip was released and 2 animals from each group were maintained under post-operative care for either a 3 day or 1 week period. The animals were perfused by the intracardiac technique with a 3% glutaraldehyde solution in 0.1 M phosphate buffer prior to ultrastructural studies. The brains were removed and sectioned coronally so samples could be obtained from comparable areas involved, (right) and contralateral, (left) hemispheres. Areas selected for study included temporal, insular, and parietal cortex, as well as putamen, globus pallidus, and caudate nucleus.

The samples were fixed an additional 12 hours, rinsed in two buffer washes, and post-fixed for 4 hours in a 1% osmium tetroxide/phosphate buffered solution. Following ethanol dehydration, Spurr embedding procedures were utilized (Spurr, 1969). Light microscopy was carried out on

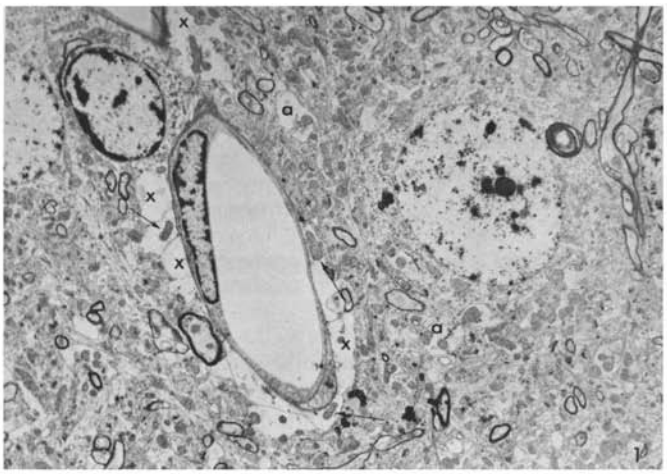


Figure 1—Early changes in right putamen consist of swollen perivascular foot processes (x) and a few involved astrocytic fibers (a). Mitochondria (arrows) are recognizable within the astrocytic processes and are not appreciably altered at this stage. 30 min. occlusion-perfused immediately. x4,300.

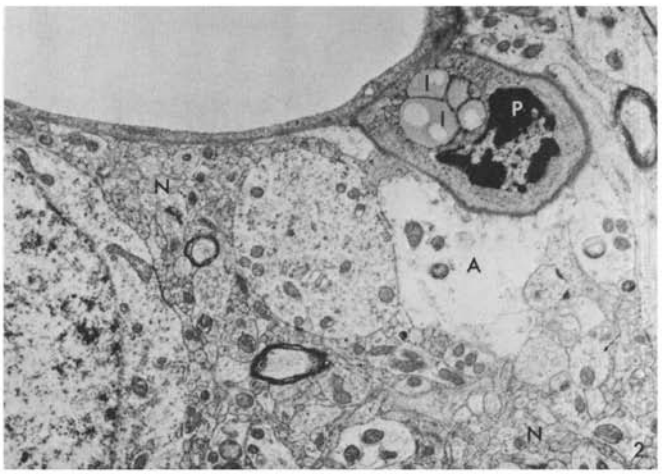


Figure 2—A swollen astrocytic foot process (A) lies adjacent to normal appearing capillary. A large lipid (1) type structure occupies a considerable area within the cytoplasm of the pericyte (P). Neuropil (N) structures around the swollen foot process are morphologically normal. Right putamen; 2 hr. occlusion-perfused 3 days later. x9,706.

semi-thin plastic sections of each block which were stained with the polychromatic procedure of Ghidoni (Ghidoni, Campbell, Adams, Thomas and Ramos, 1968). A sufficient number of blocks were obtained from each region to assure that the sampling technique did not miss the main areas of infarction. The areas which contained the greatest alterations from each region, were then thin sectioned and these preparations were studied with an RCA EMU-4 electron microscope.

RESULTS

The severity of acute tissue response following cerebral ischemia or trauma is usually referable to the extent of brain edema (Shaw, Alvord, Jr., and Berry, 1959; Ng and Nimmannitya, 1970; Plum, 1964; Klatzo, 1967). In the model used in this experiment to produce ischemia, perivascular edema was evident as early as 30 min after occlusion. Ultrastructural changes within grey matter are initially intracellular and are characterized by astrocytic foot swelling (Dodson et al, 1973). As reported earlier, these changes follow a sequential pattern of involvement which progresses from the perivascular areas into the astrocytic perikaryon and finally into all parenchymal elements. The regions within the territory of the MCA which are most sensitive to

Figure 3—Perivascular (x) as well as intra-parenchymal areas (y) of edema are evident in this field. A morphologically normal capillary (C) and pericyte (P) are shown. A perivascular oligodendrocyte (O) contains numerous "lysosomal-like" structures (arrows). Right caudate nucleus, 30 min. occlusion-perfused 3 days later. x5,882.

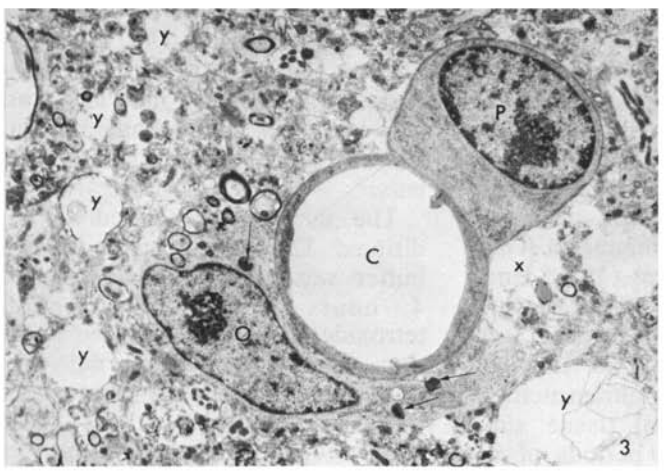
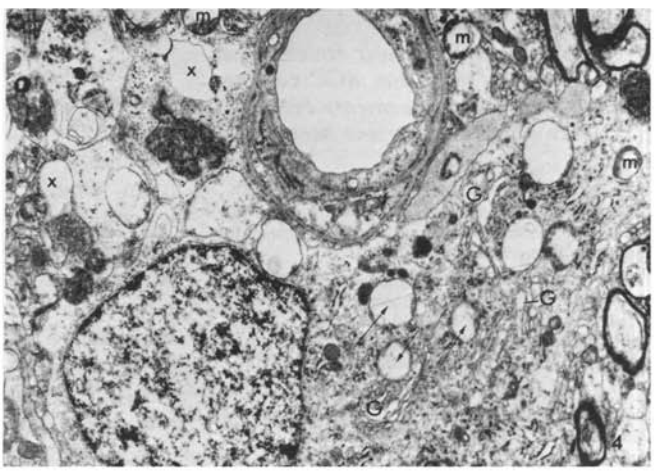


Figure 4—Numerous vacuolar structures are found in the different parenchymal cell types. Some vacuoles are adjacent to Golgi complexes (G) whereas others (x) are limited by either the plasmalemma, distended endoplasmic reticulum (arrows), or altered mitochondrial membranes (m). Right putamen, 2 hr. occlusion-perfused 1 week later. x5,546.



https://doi.org/10.1017/S0317167100020217 Published online by Cambridge University Press

changes following periods of ischemia are the basal ganglia and the temporal cortex (Dodson et al, 1973).

No animals were lost during the post-operative period. We have found these results to be consistent with the time intervals of MCA occlusion.

Since tissue changes resulting from ischemia are similar to those resulting from autolysis alone, the fields shown in this presentation are of sufficiently low magnification to allow an evaluation of microvascular clearing (indicative of good perfusion), preservation and quality of endothelial cells, (which are swollen in autolysis but not in acute ischemia of the degrees used in this experiment) and finally to allow an evaluation of organellar presentation in the adjacent parenchymal regions. The last point is most important since mitochondrial abnormalities are prevalent in an autolytic response but are not an early indicator of ischemic change.

Observations for each time period of occlusive insult will be reported in detail:

Thirty Minute Occlusion:

In animals subjected to 30 min. occlusion and immediately perfused, ultrastructural changes within the right putamen and caudate nucleus (Fig. 1) consisted predominantly of patches of perivascular swelling of astrocytic foot processes and presence of some edematous fibers. Those animals maintained for a 3 day period (following 30 min. occlusion) had more advanced involvement within the globus pallidus, with swollen astrocytic fibers and cell bodies seen frequently in the caudate nucleus (Fig. 3). Fluid accumulation in some areas had extended from the membrane-bound components into the extracellular areas, presumably by membrane rupture/degeneration. The cortical areas appeared morphologically intact.

In animals studied 1 week following 30 min. MCA occlusion, tissue changes were limited to the basal ganglia, comparable to those seen at 3 days. The cortical areas were free of involvement.

One Hour Occlusion:

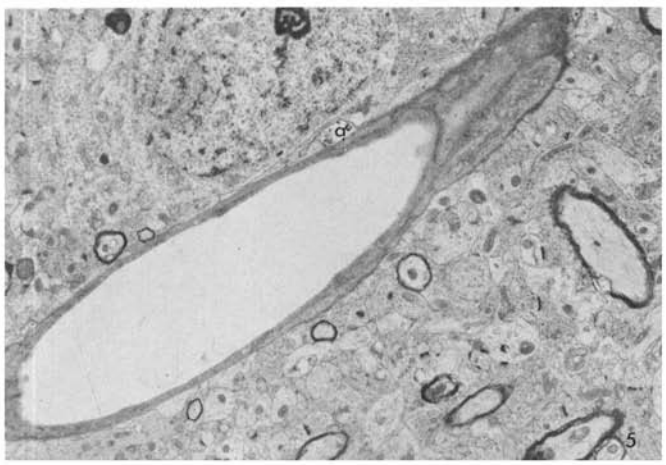
Animals fixed immediately following a 1 hour period of MCA occlusion contained small areas of perivascular swelling (intra-astrocytic) within the right putamen, globus pallidus, and caudate nucleus. The right cortical areas of brains studied 3 days following 1 hour occlusion contained swelling in the perivascular and perikaryon components of the astrocytic elements. In the putamen and caudate nucleus swelling extended into the neuronal and oligodendroglial cell types, as well as the astrocytic structures. Animals sacrificed a week following 1 hr. occlusion had scattered loci within the basal ganglia, comparable to the involvement of 3 day animals.

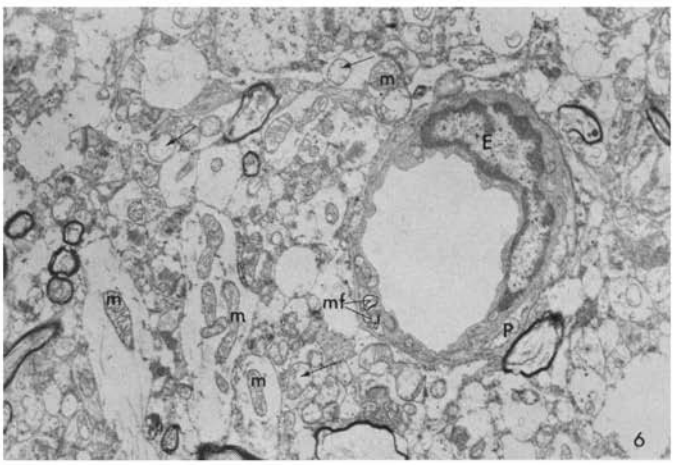
Two Hour Occlusion:

Acute tissue response in cortical areas (temporal and insular) was limited to slight pervascular swelling, whereas in the right caudate, putamen, and globus pallidus, astrocytic fibers and some astrocytic cell bodies were swollen. In the 3 day post-occluded group, changes were no further advanced than in the acute group (Fig. 2). However, in the post-occlusion group studied after a 1 week interval, cortical response was comparable to or less than that in the 3 day group. In the basal ganglia, changes were more advanced than in the earlier groupings, and swelling extended into all parenchymal cell types (Fig. 4). The intracellular response consisted of large numbers of vacuoles. These

Figure 5—Changes following 3 hr. acute occlusion of perivascular involvement of the astrocytic elements (a). Right globus pallidus, 3 hr. occlusion-perfused immediately. x20,266.

Figure 6—Extensive changes in parenchymal elements reflect an overall edematous response. Although some mitochondria are swollen (arrow), others (m) have clearly recognizable cristae. Several myelin figures (mf) can be seen in the endothelial cytoplasm (E) while the pericytic component (P) of the capillary has a reduced cytoplasmic density and a decrease of formed structures. Right globus pallidus, 3 hr. occlusion-perfused 1 week later. x9,484.





https://doi.org/10.1017/S0317167100020217 Published online by Cambridge University Press

membrane-bound structures were derived from metabolically important elements of the Golgi complex, endoplasmic reticulum, plasmalemma, and occasionally from a mitochondrion. Pericytic changes consisting of reduced cytoplasmic density were seen frequently.

Three Hour Occlusion:

Morphological responses in the acute animal included intra-astrocytic perivascular swelling in the temporal and insular cortex, and more advanced astrocytic swelling in the fibers and perikaryon of the basal ganglia (Fig. 5).

Those animals studied 3 days following the 3 hr. occlusion contained no greater involvement of ultrastructural changes than the acute animals. After 1 week (post 3 hr. occlusion), cortical areas contained the same type of response as reported in the acute and 3 day group but there was further degradation of parenchymal elements in the basal ganglia. In the caudate nucleus, globus pallidus, and putamen, patches of tissue fragmentation indicated involvement of all cell types, including oligodendroglia, neurons, and astrocytes (Fig. 6). Capillary structures were not broken but endothelial cells contained some myelin figures. Adjacent pericytes often had swollen cytoplasms.

Four Hour Occlusion:

No appreciable alterations were observed in acute animals when compared with the 3 hr. group. Alterations varied from swollen astrocytic foot processes adjacent to capillaries in the insular and temporal cortex to more extensive astrocytic swelling in the globus pallidus, putamen and caudate nucleus.

In the temporal and insular cortex, edematous changes at three days (Fig. 7) were comparable to those described in acute animals, but at 1 week showed a reduced level of involvement (Fig. 8). However, in the basal ganglia areas, involvement was more extensive at 1 week than at 3 days. These changes involved all cell types, including neurons, pericytes, and oligodendroglia, whereas in the acute 4 hr. occluded group and 3 day group, involvement was limited to the astrocytic compartment. Capillary structures were not morphologically changed in these animals.

DISCUSSION

It is well established that in any experimental investigation dealing with brain edema, intensity and characteristics of change vary according to the model used, as well as between species studied with the same model (Klatzo et al, 1967). The concepts of cerebral recoverability or tolerance to periods of ischemia were supported by the works of Hossmann and Zimmermann, who reported resuscitation of the monkey brain after one hour of complete ischemia and by Sundt et al, (1969) who reported a number of their squirrel monkeys survived middle cerebral artery occlusion for three hours without development of an infarction.

In the present study, morphological changes within the territory of the MCA following selected intervals of MCA occlusion/reflow reveal the following conclusions: The response to the reflow condition in the more susceptible cortical tissue areas (temporal and insular) at 3 days is not different from the acute stage. In those animals studied after 1 week post-occlusion/reflow, cortical involvement was comparable to that in the acute animals of the same time interval, or there was less edematous influence.

The basal ganglia (globus pallidus, putamen, and caudate nucleus) were more sensitive to the temporary periods of occlusive insult than the cortical areas. After 3 days or 1 week the changes observed in these regions were either the same or slightly more advanced than those seen in shorter time periods.

For a period of study up to 1 week post-occlusion, cortical areas show an early involvement followed

Figure 7—Edematous changes in this field are of the intracellular-perivascular type (X). Right insular cortex, 4 hr. occlusion-perfusion 3 days later. x5,780.

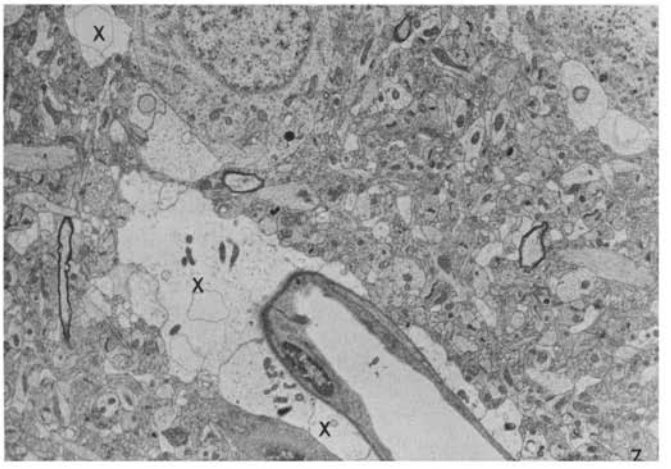
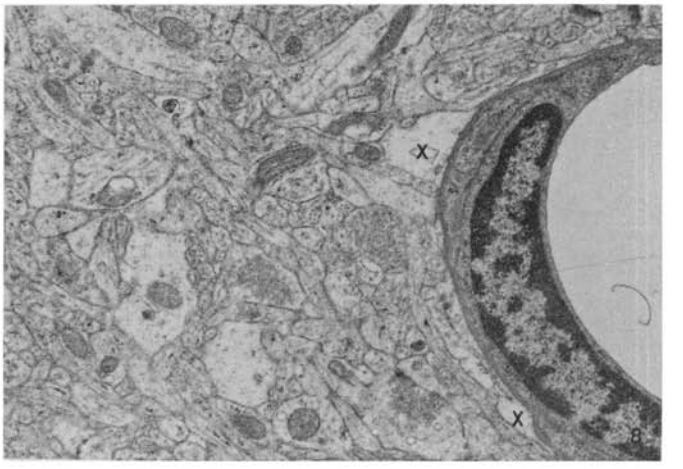


Figure 8—Changes at 1 week following a 4 hr. period of occlusion are limited to slight perivascular areas of swelling (X). Right insular cortex, 4 hr. occlusion-perfused 1 week later. x8,765.



by morphological stability. Components of basal ganglia, on the other hand, are in a more progressively degenerative state.

Any deviation in our findings from those classical concepts of developmental patterns in infarctions are explainable in that this model utilized normotensive animals, in which reflow is established following short periods of initial insult. Further studies of longer post-occlusion intervals using this model are in progresss at the present time to further clarify trends related to reversability.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Professor John Stirling Meyer, Department of Neurology, for his most helpful review of the final manuscript and assistance in the experimental design.

REFERENCES

DODSON, R. F., KAWAMURA, Y., AOYAGI, M. HARTMANN, A. and CHEUNG, L. (1973). A comparative evaluation of the ultrastructural changes following induced cerebral infarction in squirrel monkey and baboon. Cytobios, 8: 175-182.

DREWES, L. R., GILBOE, D. D. and BETZ, A. L. (1973). Metabolic alterations in brain during anoxic-anoxia and subsequent recovery. Arch. Neurol., 29: 385-390.

GARCIA, J. H., COX, J. V. and HUDGINS, W. R. (1971). Ultrastructure of the microvasculature in experimental cerebral infarction. Acta Neuropath., 18: 273-285.

GARCIA, J. H. and KAMIJYO, Y. (1974). Cerebral Infarction: Evolution of Histopathological changes after occlusion of a middle cerebral artery in primates. J. Neuropath. Exptl. Neurol., 33: 408-421.

GHIDONI, J. J., CAMPBELL, M. M., ADAMS, J. G., THOMAS, H. and RAMOS, E. E. (1968). A new multicolor staining procedure for one micron sections of epoxy embedment. Electron Microscopy Soc. Amer. Proc., 240-241.

HOSSMANN, K-A (1972). Recovery of the cat brain after transient ischemia. In Meyer, J.S., M. Reivich, H. Lechner and O. Erichhorn (eds): Research on the Cerebral Circulation, C. C. Thomas, Illinois, p. 103-111.

HOSSMANN, K-A and KLEIHUES, P. (1973). The recoverability of ischemic brain damage. Arch. Neurol., 29: 375-384.

HOSSMANN, K-A and ZIMMERMANN, V. (1974). Resuscitation of the monkey brain after 1 hr. complete ischemia 1. Physiological and morphological observations. Brain Res., 81: 59-74.

HUDGINS, W. R. and GARCIA, J. H. (1970). Transorbital approach to the middle cerebral artery of the squirrel monkey: a technique for experimental cerebral infarction applicable to ultrastructural studies. Stroke, 1: 107-111.

KLATZO, I. (1967). Neuropathological aspects of brain edema. J. Neuropath. Exptl. Neurol., 26: 1-14.

LANNER, G. LECHNER, H. and OTT, E. (1972). The isoelectric EEG-dependence on the duration of ischemia: An experimental contribution. In Meyer, J.S., M. Reivich, H. Lechner, and O. Echhorn (eds.): Research on Cerebral Circulation, C. C. Thomas, Illinois, p. 112-121.

MILLER, J. R. and MYERS, R. E. (1972). Neuropathology of systemic circulatory arrest in adult squirrel monkeys. Neurology 22: 888-904.

NG, L. K. Y. and NIMMANNITYA, J. (1970). Massive cerebral infarction with brain swelling: clinicopathological study. Stroke 1: 158-163.

PLUM, F. (1964). Brain swelling and edema in cerebral vascular disease. Proc. Assoc. Res. New. Ment. Dis., 41: 318-348.

PLUM, F. (1973). The clinical problem: How much anoxia-ischemia damages the brain? Arch. Neurol., 29: 359-360.

SHAW, C. M., ALVORD, E. C. Jr. and BERRY, R. G. (1959). Swelling of brain following ischemic infarction with arterial occlusion. Arch. Neurol. 1: 161-177.

SPURR, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26: 31-43.

SUNDT, T. M., GRANT, W. C. and GARCIA, J. H. (1969). Restoration of middle cerebral artery flow in experimental infarction. J. Neurosurg., 31: 311-322.