Brain Damage in the Fetus and Newborn from Hypoxia or Asphyxia

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*Early Effects of Perinatal Asphyxia on the Brain**

DR. IGOR KLATZO: It can be assumed that the histopathologic changes in perinatal asphyxia described[1-3] with a characteristic pattern of distribution represent mainly the end result of an acute oxygen deprivation. To understand the mechanisms responsible for the brain pathology produced by asphyxia, the elucidation of early metabolic and structural alterations occurring in the affected tissue is of considerable importance.

The present observations were carried out at the Laboratory of Perinatal Physiology on 16 term monkeys delivered by cesarean section under local anesthesia and asphyxiated for approximately 14 minutes at birth. Following resuscitation the monkeys were sacrificed in groups of two or three at 1 hour, 12 hours, 24 hours, 4- and 7-day intervals after asphyxia. Glycogen, as well as glycogen-metabolizing, respiratory, hydrolytic and proteolytic enzymes were studied histochemically in the brain tissue. Ultrastructural localization of glycogen was assessed by electron microscopy. Changes in vascular permeability were determined by intravenous administration of Evans blue followed by gross and microscopic tracing of the dye indicator.

The first and most conspicuous finding from these investigations was of dense glycogen accumulations in the cytoplasm and extensions of neuroglial cells; these were already very pronounced by 12 hours of age. The neuroglial cells with the abnormal glycogen deposits were predominantly of astrocytic type. They were conspicuous in both the gray and white matter (Figs. 5A and 5B). In the white matter, occasional oligodendrocytes also contained dense glycogen granules. The abnormal glial glycogen continued to be at high levels at 24 hours postasphyxia but ceased to be evident in animals sacrificed at four days or later, with the exception of one monkey killed after seven days in which reactive astrocytes in the white matter still contained glycogen-positive material.

The earliest histochemical changes demonstrable one hour after asphyxia consisted of markedly increased activity of the phosphorylase, UDFG-glycogen transferase and aminopeptidase enzymes. The histochemical staining for phosphorylase and UDPG-glycogen trans-

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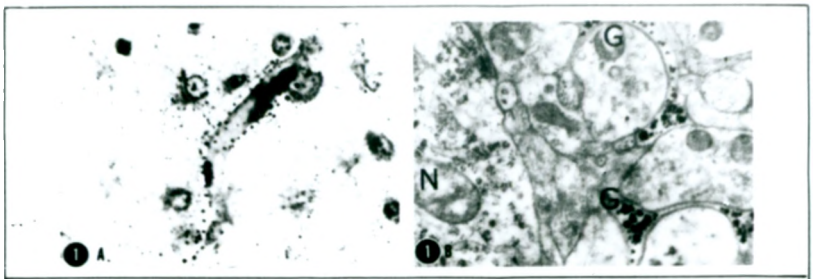

Figure 5—**A. White matter of a hemisphere: 12 hours after asphyxia. Glycogen droplets conspicuous in perivascular astroglia. PAS stain: X 780. B. Lower layers of the cerebral cortex: 24 hours after asphyxia. N—neuron. G—glial processes containing dark clumps of glycogen granules. Electronmicrograph stained with lead acetate: X 14,200**

ferase was very intense in astrocytes of both gray and white matter, as well as the immediate vicinity of blood vessels, whereas in brains of normal control animals these enzymes in corresponding locations were either undetectable or exhibited very low enzymatic activity. The activation of glycogen-metabolizing enzymes persisted during the glycogen accumulation period and then returned to normal approximately when the glycogen glial deposits were disappearing.

The increased activity of aminopeptidase was evident at one hour as a conspicuous extension of the positive staining from the blood vessels into the surrounding tissue and by the appearance of the positive reaction in the cytoplasm of certain neuronal groups.

The behavior of respiratory enzymes such as DPN and TPM diaphorase, succinic, lactic, glucose-6-phosphate dehydrogenases, cytochrome oxidase, as well as alkaline and acid phosphatases showed features similar to those previously described in conditions of anoxia and ischemia.[4,5] The changes concerning these enzymes in our material were also predominantly focal in character and appeared in areas which revealed severe morphologic alterations.

Alterations in blood-brain barrier function were found in five of seven tested animals. Only in two were these changes apparent grossly. The most striking feature of extravascular Evans blue penetration microscopically was the selective localization of the dye in individual neurons or astrocytes, without evidence of perivascular exudation such as observed in direct vascular injury[6] or of diffuse spreading of the tracer characteristic for vasogenic type of edema.[7] Both grossly and microscopically evident blood-brain barrier changes were restricted to predilection areas such as posterior ventral thalamus or vermis of cerebellum.

In the interpretation of early changes in nervous tissue after perinatal asphyxia several findings are of interest. The striking phenomenon of glycogen accumulation of glial cells at 12-24 hours after asphyxia must be related to some disturbance in glucose metabolism. A reduced utilization of glucose has been shown to occur following ischemic-anoxic brain insult,[8] and it appears likely that a similar deficiency is operative after perinatal asphyxia. Such an assumption is further supported by studies[9] in anoxia demonstrating a reduced protein and nucleotide synthesis largely dependent on glucose supply.

The early activation of glycogen-metabolizing enzymes would be in line with the assumption of a progressive accumulation of unused glucose in these cells and would be against the possibility of a block in enzymatic glycogen breakdown mechanisms. As in radiation injury[10] glycogen accumulation obviously represents a sensitive and reversible alteration which could be useful in the study of anoxic effects on brain tissue below the threshold of histologic injury demonstrable with conventional techniques.

Activation of aminopeptidase at one hour in neurons which presumably later undergo necrosis represents most likely an increased proteolytic activity in these irreversibly damaged cells.

In the interpretation of the changes in blood-brain barrier function it might be of significance that the penetration of the indicator was observed mostly in animals in which asphyxia was associated with a severe acidosis. In this connection, Bakay and Bendixen[11] have shown in hypoxemia that the blood-brain barrier damage was directly related to the degree of concurrent hypercapnia. The characteristic pattern of tracer localization, confined to individual neurons or astrocytes without pictures of diffuse penetration and deposition, implies that the degree of blood-brain barrier injury must be slight, allowing only minimal escape of the dye which is then selectively absorbed by the damaged individual cells.

Discussion

DR. KOENIG: This preparation elegantly demonstrates the induction of new enzymes in glia very shortly after an anoxic insult. The earliest morphologic change seems to be a nuclear hypertrophy or enlargement.[12] Anoxia seems to have the capacity for inducing other enzymes in reactive glia, e.g., glutamate dehydrogenase and glycerophosphate dehydrogenase.[13,14]

DR. KLATZO: Within an hour the glia, especially the astrocytes, are very intensely involved.

DR. ELLIOTT: I do not understand how the glycogen content can increase during asphyxia.

DR. CLAUDE A. VILLEE: This is probably a time relationship; the findings relate to the recovery period following asphyxia.

DR. SAMUEL P. BESSMAN: Did you verify the glycogen by any chemical method?

DR. KLATZO: We are planning to undertake quantitative biochemical assays on glycogen. The histochemical reaction was strictly specific for this compound. In our previous study on radiation injury to the brain, similar histochemical appearance of glycogen in glia cells was paralleled by a marked quantitative increase as determined biochemically.

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*Lysosomes and Anoxic Brain Damage**

DR. DAVID BRANDES: Extensive information establishing a relation between lysosomes and cellular damage following injury has recently been accumulated.[1,2] On the basis of studies[1] in animals and from the observation of pathologic material, there is strong circumstantial evidence that points to the lysosomal hydrolytic enzymes as the main agents concerned with the process of chemical breakdown of injured tissues undergoing degradation. The involvement of lysosomes in pathologic changes following ischemia or anoxia, which are of interest to this symposium, has been shown to occur in the central nervous system and other organs. An increase of lysosomes in relation to radiation-induced damage of the central nervous system has also been shown in experimental studies.

Our experiments were designed to explore whether lysosomes play a significant role in tissue damage in newborn monkeys subjected to asphyxia. Observations of tissues from the central nervous system were made at 2, 6, 24 and 48 hours after the episode of asphyxia. The brains were initially fixed by perfusion with 3 percent or 6 percent glutaraldehyde in 0.1m cacodylate buffer, pH 7.4. Portions of the inferior colliculus, posterior ventral nucleus of thalamus, lateral nucleus of thalamus, and spinal cord were removed and fixation was completed in the same mixture for two hours. Processing of tissues for electron microscopy, including sections prepared for the demonstration of acid phosphatase at the ultrastructural level, has been described elsewhere in detail.[3]

Our results have shown that in normal newborn monkeys acid phosphatase activity in neurons is predominantly localized in the Golgi apparatus (Fig. 6) which is believed to be involved in the formation of lysosomes. A more widespread distribution of acid phosphatase activity in the Golgi elements occurs in some of the neurons from asphyxiated monkeys (Fig. 7), and lysosomes are encountered more frequently in these cells. Widespread phosphatase activity and numerous lysosomes were also observed in altered cells identified by light microscopy as degenerating neuronal elements. Phosphatase activity in myelinated nerve fibers has also been found in these asphyxiated monkeys. The preliminary nature of these observations does not as yet permit conclusions as to whether lyso-

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