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In spite of an impressive volume of studies devoted to the effects of oxygen deprivation on the brain the basic pathogenic mechanisms involved remain poorly understood. To a large extent this can be attributed to divergence in methodology applied, and particularly to the lack of closer correlation between observations of morphological changes on the one hand and physiological biochemical data on the other. In experimental investigations the confusion has been further aggravated by heterogeneity with regard to the type of oxygen deficit produced in a given experimental model (anoxic, ischemic, cytotoxic hypoxia, *etc.*), as well as by differences in chronological stages studied (mostly late sequelae in morphological observations; mostly acute changes in physiological and biochemical evaluations).

One type of oxygen deficit of special clinical importance is the perinatal asphyxia which significantly contributes to the incidence of birth injuries. Numerous clinical and experimental observations on this condition revealed characteristic topographical patterns in distribution of morphological changes which were interpreted by some authors as examples of pathoclisis (33). However, it can be assumed that these findings refer primarily to the end stages of the process, and it is obvious that to interpret the mechanisms responsible for the final sequelae of asphyxia, the elucidation of *early* metabolic and structural alterations occurring in the affected brain tissue is of considerable importance.

In the previous biochemical investigations of oxygen deprivation in the brain, the histochemical studies were related mostly to the situation where the cells were irreversibly damaged (3, 4, 5, 27, 35), whereas the quantitative bio-

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chemical assays were carried out primarily on tissue homogenates which did not permit any cytostructural correlations (14, 18, 28).

The present study is concerned with histochemical observations on the perinatal asphyxia in the newborn monkey, with special attention to the early stages. Since the role of the vascular component has been frequently implied in pathogenesis of post-asphyxic changes, these observations were supplemented by assessment of the vascular permeability using Evans blue as the conventional blood-brain barrier (BBB) tracer.

MATERIAL AND METHODS

Experiments were carried out on 15 newborn monkeys (Macaca mulatta) delivered by caesarean section at 153 to 163 days of gestation (normal gestation period approximately 158 to 163 days).

Hysterotomy was performed under local anesthesia. Immediately after the uterus was opened the umbilical cord was clamped and a rubber balloon containing physiological saline solution was slipped onto the head of delivered newborn animals. Asphyxiation was carried out at 37°C for 8.5 to 14 minutes, in the majority of the animals lasting 12.5 minutes. Shorter asphyxiation time was applied when the animal's condition was poor. Asphyxiated newborn monkeys were resuscitated by pulmonary insufflation with a positive pressure of gas mixture composed of 95 per cent oxygen and 5 per cent carbon dioxide given through an intratracheal catheter. Resuscitation continued until spontaneous respiration was reestablished, varying in time from 22 to 66 minutes (average: 38 minutes, 50 seconds). After resuscitation the animals were placed in an incubator at 37°C. The few most severely damaged monkeys were supplied further with 95 per cent oxygen/5 per cent CO_2 mixture. Blood samples for pO_2, pCO_2, pH, and lactate levels were taken at 3 minute intervals both during asphyxiation and resuscitation. In all cases samples of maternal blood and of that from the umbilical artery were obtained prior to asphyxiation.

Out of 15 experimental animals 3 were sacrificed at 1 hour following asphyxiation, 2 at 10 to 12 hours, 5 at 24 hours, 2 after 4 days, and 3 after 7 days. Two control animals, 1 from a normal vaginal delivery and 1 delivered by caesarean section were sacrificed without asphyxiation at 24 hours and 7 days respectively.

Brain and spinal cord were removed as quickly as possible and sectioned into standard blocks which were either placed into appropriate fixatives according to the method used or quickly frozen on tissue holders. The blocks included motor and sensory cortex, ventrolateral thalamic nuclei, inferior colliculus, medulla at the level of vestibular nuclei, *i.e.*, regions which according to previous studies (2, 21) proved to be specially susceptible to asphyxic damage.

For routine histology the tissues were fixed by immersion in 10 per cent neutral formalin, embedded in paraffin, and stained with hematoxylin-eosin and Luxol blue-cresyl violet. For observations on glycogen, tissue was fixed by immersion in Rossman's fluid, embedded in paraffin and stained according to the cold Schiff's method, preceded by 1 hour of incubation in 5 per cent Dimedon* solution (6). Following this procedure only glycogen material stains red. In some sections the presence of glycogen was determined by specific enzymatic digestions prior to PAS stainings.

Ultrastructural localization of glycogen was assessed in 3 asphyxiated animals (1 hour, 10 hours, 24 hours) and 1 control animal. Fixation for electron microscopy was carried out by transcardiac perfusion with 2.5 per cent glutaraldehyde in PO_4 buffer (pH 7.4) postfixed in osmium, and embedded in Epon 812. Lead tartrate or citrate staining was utilized routinely, while uranyl acetate was employed as a differential stain.

* 5,5-Dimethyl-1, 3-cyclohexadion.

Histochemical assays for various enzymes were carried out on frozen, cryostat cut tissue. The microchamber method of Penar (24) was applied in all enzymatic studies. Incubation media with no substrate was used in control tests. The methods were as follows:

1. Glycogen-phosphorylase *a* and total glycogen-phosphorylase activities were demonstrated by Takeuchi and Kuriaki (31, 32) methods as modified by Godlewski (12).

2. UDPG-glycogen transferase activity was studied by means of the Takeuchi and Glenner reaction (30). In all 3 reactions postincubation staining with Gram's iodine solution and PAS was used.

3. Respiratory enzymes: NADH-tetrazolium reductase (DPN-diaphorase), NADPH-tetrazolium reductase (TPN-diaphorase), succinic (SDH), lactic (LDH), glucose-6-phosphate (G-6PDH) dehydrogenases were demonstrated by Nitro-BT methods (14, 25). Sections heated at 60°C prior to incubation served as controls.

4. Cytochrome oxidase (COX) activity was studied according to Burstone's p-Aminodiphenylamine method (9). In addition to p-Aminodiphenylamine the incubation medium contained 1 drop/50 ml of 8-amino-1,2,3,4-tetraquinoline. The reaction was performed at pH 7.4. Cytochrome C in final concentration of 5 mg *per* 50 ml of incubation medium was added; control reactions were performed by admixing sodium cyanate (10^{-3}M final concentration) to the incubation medium.

5. Aminopeptidase activity was assessed with the Azo-dye method of Gomori (13) modified by Burstone and Fold (10). L-Leucyl-B-napththylamide (5 mg/50 ml) was used as a substrate.

6. Alkaline and acid nonspecific phosphatase activities were assessed by Burstone's naphthol AS-phosphate methods (7, 8). In the observations on alkaline monophosphatase activity, napththol AS-TR served as a substrate, while naphthol AS-B1 was used in acid phosphatase assay. In both reactions Red violet LB was applied. The reaction for alkaline phosphatase activity was performed at pH 8.5, whereas that for acid phosphatase was carried out at pH 5. Sections for alkaline and acid monophosphatase activity, as well as those for aminopeptidase were prefixed in cold acetone.

For assessment of vascular permeability 7 animals were given 2 ml of 2 per cent Evans blue intravenously 1 to 2 hours prior to sacrifice, and the distribution of indicator was studied grossly and under the fluorescence microscope.

RESULTS

1. Clinical Observations

The effect of asphyxia on clinical conditions varied greatly in individual cases. Some animals evidenced an excellent recovery without any signs of functional deficits, whereas the others were severely damaged, showing poor recovery with depression of vital functions and presence of abnormal neurological signs. Generally, the clinical symptomatology was that described previously by Myers (21). The biochemical parameters such as pH, pO_2, pCO_2, and lactate reached their most extreme values usually 10 to 20 minutes after asphyxia, and generally the extent of biochemical deviations paralleled the severity of clinical condition. It is worth mentioning that only one of the experimental animals showed seizures in the form of recurrent status epilepticus.

2. Conventional Histopathology

No abnormalities were seen in animals sacrificed 1 hour after asphyxia. The 10 hour animal showed swelling of capillary endothelium, accompanied by

slight accumulation of neutrophil leukocytes, and pronounced tigrolysis in several areas of the ventrolateral portion of thalamus, inferior colliculi and anterior spinal horns. On the other hand, in monkey sacrificed after 12 hours the changes consisted only in slight tigrolysis noticeable in inferior colliculi and anterior spinal horns.

In animals with longer post-asphyxiation survival time the histopathological changes were pronounced and were present usually in characteristic pattern of distribution. The most common sites of asphyxic damage were thalamus (especially the ventrolateral nucleus), subthalamic nucleus, lateral geniculate body, inferior colliculi, vestibular nuclei, dentate nucleus, anterior spinal horns, and motor cortex.

Histopathological abnormalities in the areas mentioned consisted primarily in degeneration and loss of neurons. Astrocytic and microglial response was pronounced in areas of severe damage. Swelling and proliferation of capillary endothelium were common. Occasionally, in severely injured areas there was a slight perivascular lymphocytic infiltration. Frank necrotic foci with typical tissue reactions were observed only rarely and in the most severely damaged animals.

3. Histochemical Observations

a. Glycogen: The normal control animals showed glycogen in the following locations: choroid plexus, ependyma with the adjacent subependymal zones, superficial layers of the cerebral and cerebellar cortex, as well as the Purkinje cell layer in the cerebellum. In these regions glycogen was recognizable histochemically by the presence of discrete PAS-positive granules in perivascular tissues and in astrocytes. The only neurons constantly containing glycogen material were those of the mesencephalic nucleus of the trigeminal nerve and some nerve cells of the pontine nuclei and of the reticular formation. Occasionally, glycogen-positive neurons could be seen in the oculomotor system and in the anterior horns of the spinal cord.

No abnormal change in glycogen was observed in animals sacrificed 1 hour after asphyxia.

A conspicuous increase in histochemically demonstrable glycogen was evident in animals killed after 10 and 12 hours. Examination of these monkeys revealed the presence of abnormal glycogen deposits throughout the gray matter with accentuation of glycogen granule density in regions which were shown histopathologically to be specially susceptible to asphyxia. However, the observation relating the presence of more conspicuous amounts of abnormal glycogen to these susceptible regions was valid only up to a certain degree of intensity of asphyxic injury, since severely damaged areas of the ventrolateral thalamus and inferior colliculus in 10 hour animals were completely devoid of recognizable glycogen.

Besides the conspicuous increase of glycogen deposits in the gray matter, abnormal accumulation of this compound was evident also in the white matter. Glycogen distribution in the white matter was very widespread, but it also

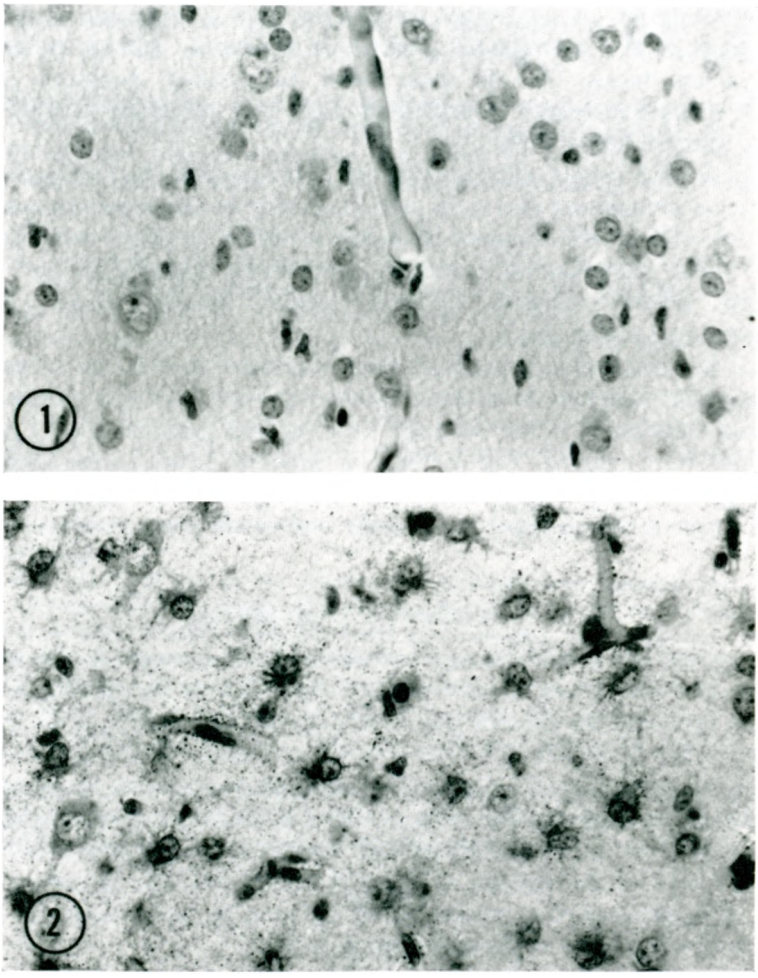


FIG. 1. Control animal sacrificed 24 hours following vaginal delivery. Subcortical white matter in the region of the centrum semiovale. Histochemical staining is entirely negative for glycogen. Dimedon-PAS-Hematoxylin stain; × 340.

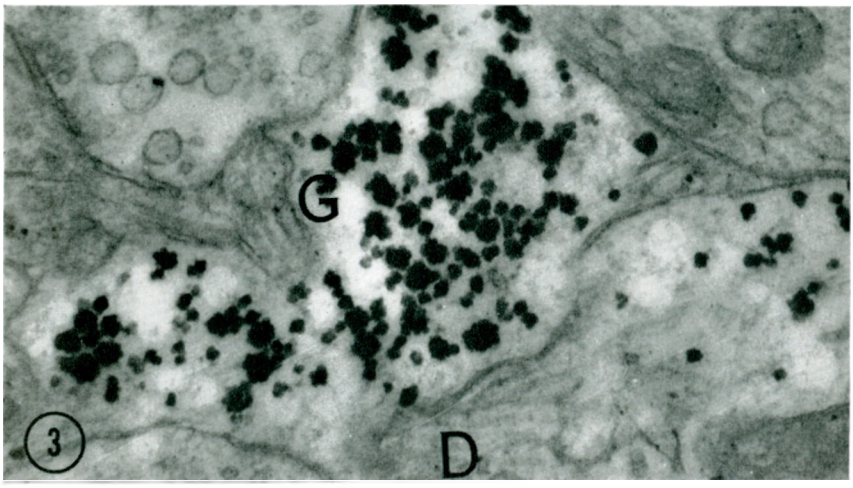
FIG. 2. Similar area as in Fig. 1 in monkey sacrificed 10 hours after asphyxia. Numerous astrocytes show abundant glycogen both stained diffusely in the cytoplasm and in form of granules scattered in the neuropil but especially conspicuous along the astrocytic processes and around the capillaries. Dimedon-PAS-Hematoxylin stain; × 300.

appeared to be in definite relationship to the stages of myelination. As a rule, abnormal glycogen was sparse in heavily myelinated white matter, whereas it was very prominent in unmyelinated areas, and particularly, in tracts undergoing active process of myelination.

Cytostructurally, glycogen was found primarily in astroglial cytoplasm. Both in gray and white matter, glycogen was demonstrable either by diffuse staining of astrocytic cytoplasm or by the presence of discrete granules outlining glial cell body and processes (figs. 2, 9). Glycogen granules were particularly prominent in perivascular location, and electron microscopic observa-

tions proved them to be distributed within the astrocytic vascular processes. Occasionally, glycogen material was evident within the oligodendroglial processes. In the gray matter the relation of glycogen to cellular structures was sometimes unclear and it could be vaguely localized as being within the neuropil. In no instance was abnormal accumulation of glycogen observed in the nerve cells.

Animals sacrificed at 24 hours showed abnormal deposition of glycogen similar in distribution and intensity. Areas of severely damaged tissue were usually glycogen-free and only the surrounding zones showed accumulations of this material (fig. 4).



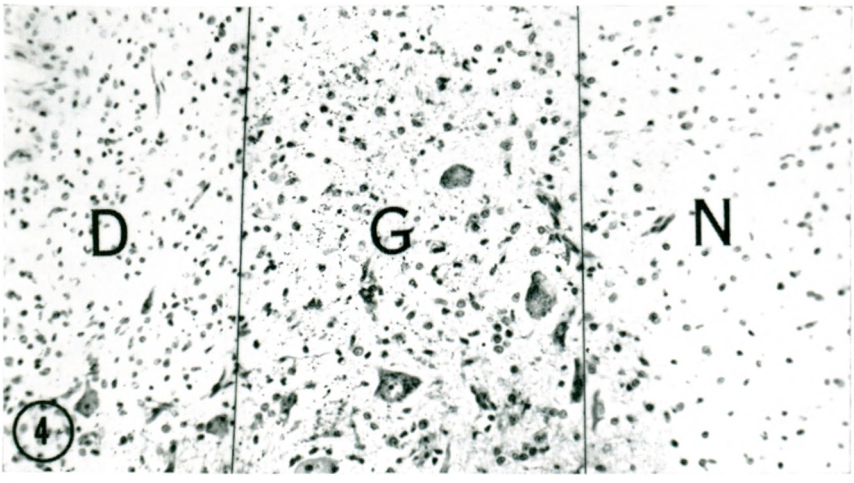


FIG. 3. Monkey sacrificed 24 hours after asphyxia. Subcortical region from the frontal gyrus. Typical glycogen rosettes (G) are seen within an astrocytic process. A dendrite (D) also contains a few granules of glycogen; × 24,600.

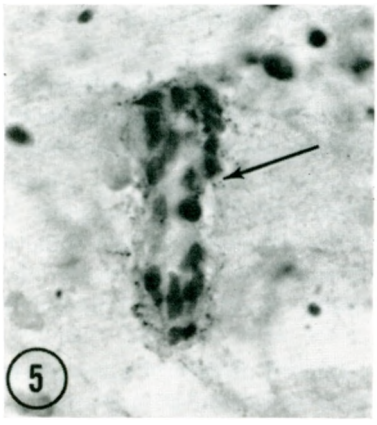
FIG. 4. Spinal cord of the monkey sacrificed 24 hours after asphyxia. Areas of normal tissue (N) and of severe destruction (D), both free of glycogen, are separated by an intermediate zone (G) showing abundant glycogen granules. Dimedon-PAS-Hematoxylin stain; × 120.

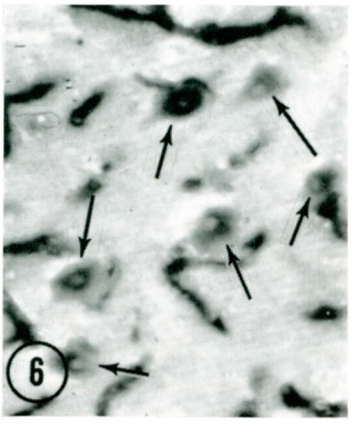
Animals sacrificed during the subsequent days showed, with the exception of one 7-day monkey, a marked reduction in abnormal, histochemically demonstrable glycogen. The glycogen pattern either reverted to normal or the alteration was confined to a very slight increase in scattered glial glycogen inclusions throughout the gray and white matter. Denser accumulations of glycogen could be seen occasionally only in the immediate vicinity of severely damaged, necrotic foci. In the 7-day monkey wide areas of the white matter presented large, glycogen-containing astrocytes. This was not, however, a generalized finding, but accumulation of glycogen in the white matter could be spatially related to an extensive focus of asphyxic injury in the thalamus.

b. Glycogen-Metabolizing Enzymes: With regard to phosphorylases in normal controls the unmyelinated white matter showed practically no activity. On the other hand, a diffuse, positive reaction was conspicuous in the heavily myelinated tracts of the brain stem, such as the lemniscus medialis and lateralis, fasciculus longitudinalis medialis, and the white matter of the cerebellar folia. The neuropil of the gray matter in general was slightly positive. More distinct staining was observed in the inferior colliculi, subthalamic nucleus, globus pallidus, and ventrolateral nucleus of the thalamus. Activity of the cerebral and cerebellar cortex, putamen, and medial nuclei of the thalamus was low, and appeared only in total phosphorylase preparations. Cytologically, neurons, glia, and blood vessels showed no activity with the following exceptions. Phosphorylase-positive neurons were present in the nucleus mesencephalicus of the trigeminal nerve, n. subthalamicus, globus pallidus, and some motor neurons of the brain stem. Astrocytes with phosphorylase activity were seen in the fornix, subependymal zones, and in subpial regions of the brain stem. Slight phosphorylase-positive activity was evident around some of the blood vessels in the molecular layer of the cerebral cortex. As a rule, in normal animals activity of phosphorylase-*a* was markedly lower than that of total phosphorylase.

Glycogen-synthetase reaction (UDPG-transferase) resembled that of phosphorylase-*a* being generally even weaker. There was no activity in astrocytes with the exception of subependymal areas. The only neurons showing a positive reaction were those of the mesencephalic tract of the trigeminal nerve and in anterior horns of the spinal cord.

A distinct alteration in glycogen-metabolizing enzymes was apparent in animals sacrificed 1 hour following asphyxia. With regard to phosphorylases there was a conspicuous shift concerning relative proportions of total and *a* phosphorylases. Whereas in normal animals total phosphorylase activity was considerably higher than that of form *a*, in 1-hour animals the intensity of phosphorylase-*a* reaction was approaching that seen in preparations demonstrating the total phosphorylase. Another change consisted in the appearance of phosphorylase activity and that of UDPG-glycogen transferase in locations where they were absent in normal controls. This refers to demonstration of activity of these enzymes around the blood vessels (fig. 5), as well





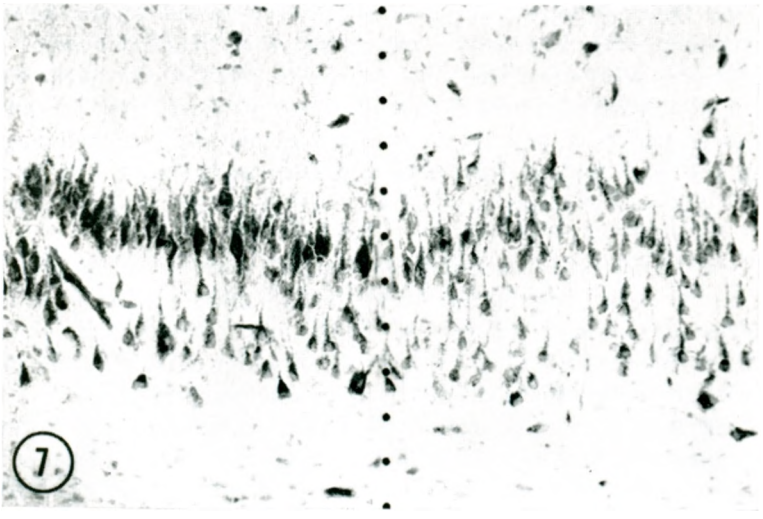


FIG. 5. Subcortical region of the frontal gyrus in the animal sacrificed 1 hour after asphyxia. Phosphorylase-*a* activity is demonstrated by enzymatically formed polysaccharide granules visible around a blood vessel (arrow); × 280.

FIG. 6. Aminopeptidase activity in the anterior spinal horn of the animal sacrficed 10 hours after asphyxia. Enzyme activity is seen in the walls of the blood vesels and in the large neurons (arrows); × 240.

FIG. 7. Acid phosphatase (Burstone) activity in the Ammon's horn of the monkey sacrificed 12 hours after asphyxia. To the right from the dotted line is the Sommer sector which shows definite reduction in the enzyme activity; × 180.

as in the astrocytes, predominantly in areas of the white matter (fig. 8) either unmyelinated or undergoing active myelination, *i.e.*, in regions which in later stages showed a remarkable accumulation of glycogen.

In animals sacrificed 10 and 12 hours after asphyxia there was a further increase in activity of all glycogen-metabolizing enzymes, and it was particularly conspicuous with regard to astrocytes and immediate perivascular regions. In the 10-hour animal glycogen-free areas of severe damage in ventro-

lateral thalamic nuclei and inferior colliculi were devoid of any enzymatic activity.

A new pattern of enzymatic changes was demonstrable in animals killed 24 hours after asphyxia. There was generally increased total phosphorylase activity while phosphorylase *a* became markedly reduced. Independently, there was also an obvious reduction in UDPG-glycogen transferase. There was a widespread general reduction in glycogen-metabolizing enzyme activity around small veins. In the cerebral cortex the reduction showed some laminar pattern. The severely damaged asphyxia-susceptible areas were devoid of enzymatic activity, which, on the other hand, was increased in the immediately adjacent zones.

In animals sacrificed at later stages, the general picture of glycogen-metabolizing enzymes returned to normal with the exception of enzymatically hyperactive zones about numerous severely damaged foci which were free of any activity. In these areas the prominent glycogen-phosphorylase activity in the hypertrophic reactive astrocytes was related to the inactive *b* form.

c. Respiratory Enzymes: The first changes in oxidative enzymes were demonstrable in animals sacrificed 1 hour after asphyxia and they consisted in a widespread reduction of activity in neuropil immediately surrounding blood vessels in the gray matter. This was especially pronounced with regard to SDH and COX and was least noticeable in DPN-diaphorase and LDH reactions.

At 10 and 12 hours a decreased perivascular activity of SDH was still marked; on the other hand, astrocytes applied to blood vessels of the cerebral cortex revealed increased activity of LDH and DPN-diaphorase. In 24-hour animals this increase of activity in perivascular astrocytes became generalized with regard to other oxidative enzymes including SDH.

The changes of focal character were related predominantly to the susceptible regions and were especially prominent in the inferior colliculi. In this location, 1-hour animals showed reduction in G-6PDH activity. At later stages the reduction in activity applied also to other respiratory enzymes but it remained most pronounced with regard to G-6PDH. As a rule there was an increased activity in all respiratory enzymes in the zones surrounding severely damaged regions.

d. Aminopeptidase: In normal animals, histochemically assessed activity of the proteolytic enzyme leucine-aminopeptidase was strictly confined to vascular walls, leptomeninges, and stroma of the choroid plexus.

Generally, the changes in this enzyme were related only to areas susceptible to asphyxia.

In animals sacrificed at 1 hour there was a noticeable increase of enzymatic activity in and around the blood vessels involving the regions of lateroventral thalamic nuclei, inferior colliculi, *etc.*

In animals sacrificed at later stages the neurons also showed positive reaction in their cytoplasm (fig. 6). This change, however, was present only prior to any histological evidence of asphyxic damage to the neurons. As a rule,

there was no activity in the severely affected foci, while the surrounding zones showed hyperactive reactions in blood vessels and neurons.

e. Nonspecific Acid Monophosphatase: In animals sacrificed at 1 hour the only change in activity of this enzyme consisted in an increased reaction demonstrable in pericytes of capillaries and small arteries within the cerebral cortex.

At 10 and 12 hours this was more evident and, at the same time, certain neuronal groupings such as the bipyramidal cells in Sommer's sector of the Ammon's horn began to show reduced activity (fig. 7).

In later stages with foci of severe damage there was a pattern similar to that previously described with other enzymes. The severely damaged regions were depleted of enzymatic activity, whereas the surrounding zones were hyperactive. In the latter locations many neurons revealed strongly positive cytoplasmic granules, similar to those described by Novikoff (22) as cytosomes.

f. Nonspecific Alkaline Monophosphatase: The earliest changes in nonspecific alkaline monophosphatase appeared only after 24 hours and consisted of focal reduction of neuropil activity in the most severely damaged areas. The blood vessels even in those regions showed no abnormality.

4. Blood-Brain Barrier Observations

Two animals sacrificed at 1 hour after asphyxia revealed no abnormal changes. The disturbance in vascular permeability was found, however, in the remaining 5 animals sacrificed at different later stages.

Grossly, the damage to the blood-brain barrier was rather inconspicuous and was manifested by the presence of rare small foci of blue discoloration found in various regions of the gray matter. An exception was a totally different pattern observed in 1 animal sacrificed 12 hours after asphyxia which revealed a diffuse blue staining involving wide areas of the white matter in the cerebral hemispheres.

Microscopically, the areas of abnormal vascular permeability in the gray matter showed a characteristic picture. The blood vessels themselves usually appeared normal, and only very exceptionally did they show evidence of vascular wall penetration by the tracer. The striking feature was the intense red fluorescence of Evans blue-albumin complex in the individual neurons (fig. 10). Both cytoplasm and cell nuclei showed presence of the tracer, frequently the intensely affected cells being intermingled with those in which the presence of tracer fluorescence was minimal or absent. In a few rare instances, the oligodendrocytes as well as the neurons contained the indicator. The blue-stained areas of the white matter in the 12-hour animal showed a striking red fluorescence of astrocytes and occasionally oligodendrocytes, microscopically. The blood vessels did not reveal obvious pictures of leakage, neither was there evidence of a diffuse "mottled" spread of indicator through the white matter as has been previously described in experimental edema (16).

5. Ultrastructural Observations

In the normal animal the presence of glycogen was very inconspicuous. It was observed only in small amounts as particulate aggregates or individual rosettes in the cytoplasm of occasional astrocytes.

No increase in glycogen was observed in the monkey sacrificed 1 hour after asphyxia. On the other hand, moderate astrocyte and dendritic swelling was frequently observed. There was no evidence of enlargement of extracellular spaces.

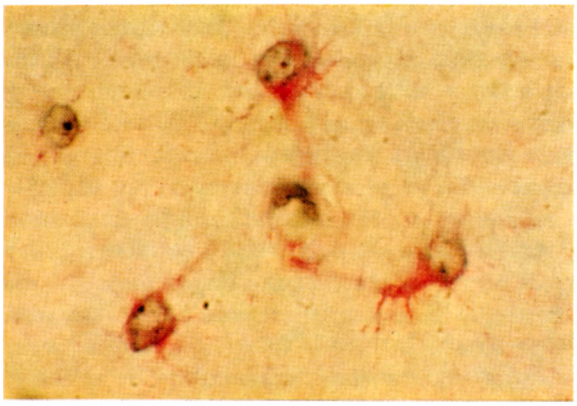
A strikingly abundant glycogen was demonstrable in animals sacrificed 10 and 24 hours after asphyxia. In various structures of the gray matter there was an ubiquitous appearance of abundant glycogen deposits in astrocytic cell bodies and processes (fig. 3). This was particularly marked in the perivascular regions. Oligodendroglia showed a small amount of cytoplasmic glycogen. Occasionally, glycogen granules occurred in some neuronal processes.

In the white matter astrocytes demonstrated large quantities of glycogen in unmyelinated or actively myelinating regions. There was little glycogen in areas with advanced myelination. Oligodendrocytes in the white matter contained much more glycogen than did those in the gray matter.

Besides changes concerning glycogen, the animals sacrificed at 10 and 24 hours frequently showed neuronal changes: mitochondrial enlargement, nuclear indentation, dispersion or swelling of ergastoplasm. Astroglial swelling was common. There was no enlargement of extracellular spaces.

DISCUSSION

The most interesting finding in the present study is the striking reaction of glycogen and glycogen-metabolizing enzymes. Undoubtedly, this reaction is related to a disturbance in normal glucose metabolism which must follow oxygen deprivation in asphyxia, and in this respect a reduced utilization of glucose in anoxic-ischemic rat brain has been demonstrated by Atkinson and Spector (1). Neurons being the main "consumers" of glucose are apt to suffer first, and this assumption is supported by abundant evidence in the literature emphasizing the vulnerability of neurons in hypoxia, as well as by our present BBB observations demonstrating selective uptake of the tracer by the affected nerve cells. Curiously enough, in perinatal asphyxia glycogen does not, however, accumulate in neurons, but appears in glia and predominantly in astrocytes. The essential rôle of astroglia in glucose metabolism and in regulating transport of glucose from blood vessels to neurons has been stressed by Friede (11). If this assumption is correct, it is possible to envisage a situation in which damaged neurons are unable to properly utilize the glucose which then begins to pile up in astrocytes and is converted, at the same time, into glycogen for storage. This would require an intensified activity of glycogen-synthesizing enzymes. Our observations, showing a conspicuous increase in activity of phosphorylases and UDPG-glycogen transferase in astrocytes 1 hour after asphyxia, support this contention, indicating that increased glycogen synthesis precedes histochemical and ultrastructural accumulation of the com-



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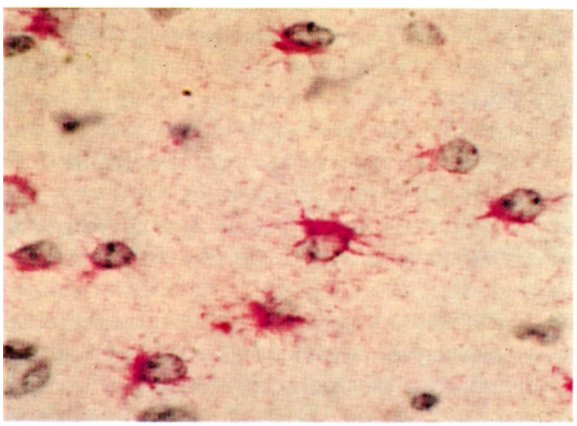

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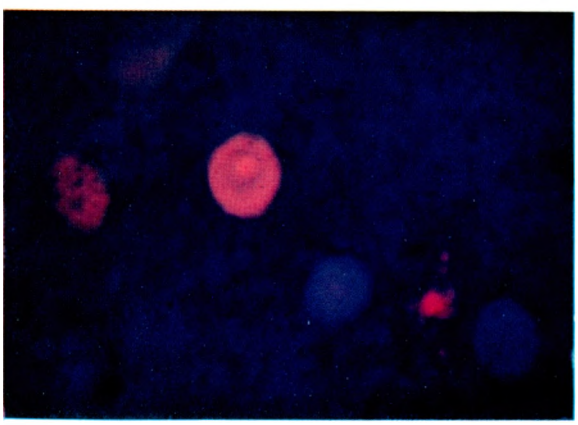

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FIG. 8. Subcortical white matter in the monkey sacrificed 1 hour after asphyxia. Astrocytes show UDPG-glycogen transferase activity in their cytoplasm; × 760.

FIG. 9. Subcortical white matter in the animal sacrificed 10 hours after asphyxia. Astrocytes showing abundant glycogen in their cytoplasm; Dimedon-PAS-Hematoxylin stain; × 680.

FIG. 10. Cerebellum of the asphyxiated monkey injected intravenously with Evans blue and sacrificed after 12 hours. Individual Purkinje cells display intense red fluorescence of the Evans blue tracer. Unstained frozen section under fluorescence optics; × 560.

pound itself for some time. It can be assumed that, when the asphyxic injury reaches above a certain threshold of intensity, it will also abolish glycogen synthesizing activity in astrocytes and, therefore, one should not expect to see glycogen in severely damaged areas. This may account for a "zonal" effect observed frequently in the present investigation (fig. 4), and which is particularly clearly outlined in a previous study on the effect of alpha particle radiation in the brain (17) in which the accumulation of glycogen in astrocytes was within a sharply delineated zone above the Bragg-peak band of severe injury. It appears then that the histochemical demonstration of abnormal glycogen in glia is greater in regions where the intensity of damage is within the range in which there is interference with the utilization of glucose in neurons and a simultaneous "stimulatory" effect on enzymatic activities in astrocytes.

Accumulation of glycogen in astroglia has been previously reported in the vicinity of brain tumors (23), stab wounds (11, 23, 26), and particularly as an effect of radiation (17, 19). In these reports the abnormal glycogen was localized primarily in the gray matter, which would be consistent with the present interpretation implying decreased glucose utilization in neurons and its increased storage in astroglia. However, in the present observations on asphyxia we have demonstrated for the first time massive and widespread accumulation of glycogen in astrocytes of the white matter. Of significance here may be the fact that the glycogen increase was selectively confined to areas of the white matter which were either unmyelinated or undergoing myelination, *i.e.*, tissue very active metabolically and with high glucose utilization. The high demand for glucose in myelinating white matter was demonstrated by Murray *et al.* (20) in their studies on myelination *in vitro*. It is interesting that in our observations abnormal glycogen in the white matter was conspicuous also in oligodendrocytes, *i.e.*, cells which are presumed to be intimately involved in myelin formation.

The widespread, relatively early glial accumulation of glycogen in asphyxia appears to be a reversible and sensitive change, and as such it may serve as an indicator of asphyxic damage of a degree below the threshold of histopathological recognition. Its relationship to asphyxia-susceptible regions indicates that the same disturbance in glucose metabolism may be involved in severe irreversible tissue damage. In view of this, further elucidation of the biochemical mechanism of glycogen accumulation may contribute to a proper interpretation of characteristic patterns of selective vulnerability resulting from oxygen deprivation in the CNS.

With regard to the behavior of other enzymes studied, the changes were generally similar in type to those previously reported in various forms of oxygen deficiency (3, 4, 5, 27, 35). They consisted mainly in reduction and/or loss of activity in areas of severe damage and increased activity in the surrounding zones.

An interesting feature in the behavior of respiratory enzymes was the considerable difference in the degree of activity of individual enzymes, thus, *e.g.*,

the earliest and most pronounced reduction of activity was shown by G-6PDH, whereas SDH and COX activities appeared to be reduced after a conspicuous delay.

Concerning aminopeptidase activity, its early abnormal appearance in neurons in areas susceptible to severe focal injury most likely indicates an increased proteolytic activity in irreversibly injured nerve cells. This abnormal aminopeptidase activity preceding demonstrable reduction in respiratory enzymes suggests the possibility that both increased proteolytic activity (5, 35) and inhibition of protein synthesis (34) may underlie the damage of intracellular enzymatic systems essential to neuronal preservation and function.

With regard to lysosomal enzymes our observations have failed to reveal, even in susceptible areas, any increase in acid phosphatase which is supposedly indicative of elevated lysosomal activity (5). The only observed increase was present in perivascular infiltrates and pericytes which may indicate intensified resorption of tissue breakdown products. The sites in which Novikoff's cytolysomes (22) were found were large motor neurons in the anterior spinal horns located in the vicinity of necrotic foci. They were seen in the same neurons which demonstrated strong aminopeptidase activity.

In disturbances of vascular permeability an interesting feature was the selective localization of the fluorescent tracer in the cellular components of the nervous parenchyma (mostly neurons), which was especially striking with the dark background of neuropil seemingly free of the indicator (fig. 10) and with only rare and minimal evidence of direct leakage of Evans blue from the vessels themselves. Such a pattern of disturbance in vascular permeability, originally described in some of the chemical blood-brain barrier injuries (29), and frequently observed in experimental anoxic states (to be published), indicates concurrent existence of two conditions in the tissue. The first one suggests that the injury of the blood-brain barrier must be very slight since it allows the escape of the tracer from the affected blood vessels in such a minimal amount that its presence in the neuropil is practically undetectable under the fluorescence optics. The second condition is that there must be a selective injury of cellular elements which leads to an intense absorption of fluorescent tracer by these cells, presumably due to cell membrane alteration. With these two conditions present in certain areas, selective vulnerability of the neurons in preference to other cellular components was clearly demonstrated in our asphyxiated animals.

Some aspects of the behavior of the blood-brain barrier in perinatal asphyxia remain obscure, especially those concerning apparent lack of correlation of BBB disturbances with the intensity of histological tissue damage. Thus, *e.g.*, in some instances severely damaged foci in susceptible regions failed to show abnormal permeability to Evans blue, whereas the brightly fluorescent neurons were conspicuous in areas without appreciable histological damage. Equally puzzling remains a striking localization of the tracer in astrocytes of the white matter observed in 1 animal 12 hours after asphyxia.

SUMMARY

1. Histochemical observations were carried out on newborn monkeys delivered by caesarean section at 153 to 163 days of gestation and immediately asphyxiated for approximately 12 minutes.
2. The main histochemical finding consisted in widespread, abnormal accumulation of glycogen in glial cells (predominantly astrocytes) of both gray and white matter, which became conspicuous approximately 10 hours after asphyxia and tended to disappear after several days. Abnormal deposition of glycogen in glial cells was also demonstrated by electron microscopy.
3. Glycogen accumulation was preceded by a marked increase in phosphorylases and UDPG-glycogen transferase activities already demonstrable in animals sacrificed 1 hour after asphyxia.
4. Similarly early was the appearance of abnormal activity of aminopeptidase in neuronal groups which were shown to be especially susceptible to asphyxia and displayed evidence of histopathological damage at later stages.
5. The changes in respiratory enzymes were generally of a nature previously described, and they were characterized mainly by reduction in the activity of individual enzymes in regions of the gray matter which later showed evidence of histopathological damage.
6. Disturbances of the blood-brain barrier were demonstrable in animals sacrificed 10 hours and later after asphyxia. The main feature consisted in selective localization of the tracer in the cellular components of the parenchyma, mainly in the neurons. The changes were confined mostly to the gray matter without any clear correlation to the intensity of the histopathological damage.

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