EXPERIMENTAL NEUROLOGY 1, 130-154 (1959)

Brain Damage in the Monkey, *Macaca mulatta*, by Asphyxia Neonatorum

JAMES B. RANCK, JR. AND WILLIAM F. WINDLE[1]

Laboratory of Neuroanatomical Sciences, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare; and Department of Anatomy, School of Medicine, University of Puerto Rico

Received January 12, 1959

Asphyxia neonatorum was induced in monkeys near term by detaching the placenta at hysterotomy under local anesthesia, keeping the fetal membranes intact. Eleven to 16 minutes later the fetuses were delivered from their membranes and resuscitated by pulmonary insufflation with oxygen. The infant monkeys showed neurologic deficits during life. Five were killed by perfusion-fixation at 2 to 9 days of age. Brains of these and of 2 which were not asphyxiated were studied. A common pattern of structural alteration was encountered in the nervous system of the asphyxiated monkeys. Nuclei were symmetrically affected; those most consistently and severely damaged were the nucleus of the inferior colliculus, gracile and medial cuneate nuclei, roof nuclei of cerebellum, ventral posterior group of thalamic nuclei, globus pallidus, putamen, and vestibular nuclei. The cerebral cortex was severely damaged in only one monkey. Lesions began with primary nerve cell and, less frequently, neuroglia cell lysis and loss. Secondary damage of myelin sheaths, and reactions of astrocytes, endothelial cells, vascular adventitial cells, and phagocytes were noted. A relation of lesions to vascular distribution was not apparent. Hemorrhages were seldom encountered.

Introduction

The neuropathology of asphyxia neonatorum is poorly understood. Our knowledge of it in human infants has been summarized by Cammer-

[1] Many people took part in the experiments on which this article is based, but the authors conducted the histologic study and prepared the manuscript. Those participating in operations and physiologic studies were: Drs. C. J. Bailey, Marisa I. R. Ramirez de Arellano, C. A. Pfeiffer, J. G. Frontera, W. Stiehl, L. Guth, Irene Miale, and S. A. Altmann. Dr. J. Cammermeyer acted as consultant in neuropathology. Assistance was given by Mrs. Armonia Stiehl, Mrs. Elizabeth Dorrill, Mr. J. O. Smart, and Mr. J. Torres. Histologic preparation was largely the responsibility of Mrs. Elena Baker, photography, Mr. Kuritzky. Two preliminary reports have been published (9, 14). Dr. Ranck's present address is: Department of Physiology and Biophysics, University of Washington, Seattle, Washington.

meyer (1), and Corner and Anderson (2). Many neuropathologic conditions encountered in human infancy are thought to be attributable to asphyxia before or during birth, but rarely has the relationship been clearly established. The neuropathology of asphyxia neonatorum in other species has been studied extensively only in the guinea pig (15). This is the first experimental study in a primate and is confined to consideration of the first 5 monkeys that were asphyxiated by an intra-amniotic method and studied histologically. Other asphyxiated monkeys, some of which required no resuscitation and now show little or no residual deficits, have not been killed for study.

The present report emphasizes histologic changes in the brain. The physiologic aspects of asphyxia neonatorum in monkeys, including neurologic examinations, electroencephalographic studies, and results of psychologic tests, will be considered at a later time when more experiments will have been completed.

Material and Methods

Mature monkeys (*Macaca mulatta*) were obtained commercially. Some were mated in the laboratory; others were pregnant on arrival. The latter were X-rayed to estimate gestational age by visualizing the fetal skeleton.[2] Hysterotomy was performed under local anesthesia late in gestation. The uterine contents were removed through a mid-line incision by quickly detaching the placenta, thus retaining the fetus within its intact membranes. Asphyxiation was timed from the moment of placental separation until the membranes were opened, the fetus removed from the amnion, and resuscitation initiated by pulmonary insufflation through a tracheotomy. Resuscitation by positive pressure oxygen or 95 per cent oxygen and 5 per cent carbon dioxide through the tracheotomy was continued until the infant's own breathing was well established. Details concerning the 7 animals in this series are listed in Table 1. Because of the unknown effect of the uterine handling on the oxygen concentration in the fetal blood before placental separation, the times of asphyxiation cannot be interpreted exactly. Motion picture photography was employed during

[2] It is a pleasure to acknowledge cooperation of Dr. Gertrude van Wagenen and Mr. Joseph Negri in determining stages of development from the X rays. The mean time of spontaneous delivery in Dr. van Wagenen's monkey colony has been 168 days (10) with an average birth weight for females and males of 467 and 481 gm (11). Fully mature infants have been born in our colony as early as 154 days.

TABLE 1
CONDENSED DATA FROM EXPERIMENTS[a]

Monkey	Protocol no.	Sex	Gestation (days)	Delivery	Weight: Birth (gm)	Weight: Death (gm)	Asphyxiation (min) (sec)	First gasp (min) (sec)	Respiration established (min) (sec)	Age killed (days)
A	M-090657W	M	unknown	V	412	442	— —	— —	— —	4
B	M-082857W	F	148	V	200	236	— —	— —	— —	4
C	M_b-112057B	F	157 ± 1	H	403	450	15:43	43:43	67:43	9
D	M-041558B	M	158	H	498	474	15:50	25:50	46:00	8
E	M-062457W	M	unknown	H	459	unknown	14:00	22:45	48:45	4
F	M-090357B	M	164	H	400	407	11:11	17:30	73:30	3
G	M-050657W	M	unknown	H	449	434	13:14	22:09	39:14	2

[a] Monkey B was a premature infant supplied by Dr. George W. Corner, Jr. For explanation of gestational ages, see footnote 2. Spontaneous vaginal births are indicated by V; hysterotomies by H. Times run continuously from the moment of placental separation.

all experiments. Similar records were made of activities of full-term and premature control infants. The motion pictures proved useful for review and comparison.

The infant monkeys were killed by the perfusion-fixation method (4) after lightly anesthetizing them with pentobarbital sodium. Autopsy was performed immediately thereafter and portions of all viscera placed in appropriate fixing solutions. The brain was removed within 2 hours after the perfusion and placed in a large volume of a 10 per cent solution of formalin containing 0.9 per cent sodium chloride. The time during which the brain was in fixing fluid was less than 7 days. The brain stem was separated from the cerebrum by an incision at the upper end of the midbrain. The cerebrum was subdivided by frontal sectioning in all but one specimen. Similarly, the brain stem, with or without the cerebellum intact, was subdivided. The principal pieces were embedded in paraffin and sections cut 10 microns thick. A few pieces were saved for frozen sections. Each block of the cerebrum and brain stem of animals A and C was serially sectioned and mounted; the cerebrum and brain stem of animal D were sectioned serially without subdivision. Representative sections of the blocks of other brains were mounted. Five regions of the spinal cord were prepared histologically in all animals except monkeys B and D.

Every tenth slide from the brains of monkeys A and C was stained with thionine buffered at pH 4.7, and adjacent slides were stained by the Woelcke myelin sheath technique. Selected sections were stained by Bodian's silver Protargol method, phosphotungstic acid hematoxylin (PTAH), Gomori's method for iron, and frozen sections were treated with Sudan black B for fat. Sections from each block of the other brains were stained with gallocyanine and thionine.

Resume of Observations during Life

Monkeys A and B. The behavior of the normal, newborn (monkey A) was representative of that described in other newborn monkeys by Hines (3). Seven other nonasphyxiated monkeys delivered by hysterotomy and 3 born vaginally in our laboratory appeared to be similar to monkey A. A normal newborn monkey characteristically exhibits the following: It has a strong grasp response of hands and feet; rights itself readily when placed on its back or side; progresses by coordinated movements of the extremities while lying in a sprawled position; reacts to loud sounds but does not respond with mass movements; sucks vigorously; and swallows without difficulty.

The premature control animal (monkey B) exhibited behavior that was qualitatively no different from that of full-term monkeys except that it was unable to carry out the sprawling type of locomotion. However, its movements were less frequent, slower and weaker than those in monkey A. Monkey B was less active at 4 days of age than any full-term infant monkey in our colony.

Monkey C. The infant became bilaterally rigid 90 minutes after delivery. Alternating flaccidity and rigidity were present throughout the rest of its life. It was poikilothermic, body temperature being maintained by application of heat.

When it was 2 days old, funduscopic examinations revealed many small, flame-shaped hemorrhages as well as blurred and slightly elevated optic disks. Papilledema could not be observed on subsequent days.[3] The eyes did not move when the head was turned and the pupils failed to react to light. A corneal reflex was present only on the seventh day.

A grasp reflex was occasionally present in the hands only, but with little strength or duration. The monkey was able to suck and swallow weakly at 4 to 6 hours of age, but soon thereafter lost these functions. It never righted itself, nor did it appear to try to do so. It lay on its side or back and rarely moved. Occasionally mass movements, lasting 2 to 20 seconds, occurred spontaneously or, on the first day, could be initiated by loud sound, pin prick, or vigorous handling; thereafter it was unresponsive to stimulation for long periods of time. Its posture was symmetrical, with neck and trunk flexed, arms rigidly adducted and crossed ventrally or dorsally, fingers flexed in fists, legs retracted at the hips, and knees flexed or extended. It appeared to be in deep coma throughout life.

The infant was fed by gastric tube throughout its life and additional fluid was given subcutaneously. Jaundice was never seen. Hematologic examination on the day of death revealed 16.5 gm of hemoglobin per 100 ml, and 3,845,000 red blood cells and 9,460 white blood cells per cubic millimeter of blood—values within the normal range for infant monkeys.

Symptoms of pneumonitis were noted when the animal was 7 days old. During the last 2 days of its life it was often limp, inactive, and pale, but never cyanotic. It was killed when it was 9 days old rather than risk further brain damage secondary to the pneumonia.

[3] Fontanels and sutures are closed in newborn *Macaca mulatta*.

Monkey D. This infant recovered rapidly from acute effects of asphyxia neonatorum, but could not right itself and showed little spontaneous activity 3 or 4 hours later. When it was 4 days old it was quite inactive and hyporesponsive to stimulation. It showed no obvious abnormalities except an inability to suck, which persisted until it was 6 days old. It had no tremors nor abnormalities in muscle tone and was able to walk at 6 days, but its motor ability, strength, and agility were less than expected of a normal animal. It seemed to be unable to localize sound.

Funduscopic examinations revealed numerous petechial hemorrhages but no papilledema. Jaundice was never observed. At no time were symptoms of pneumonitis encountered. In fact, its general physical condition was excellent and there is little doubt that, had we not killed this animal at 8 days of age to obtain a specimen of age similar to monkey C, it could have survived.

Monkey E. Acute signs of distress disappeared several hours after asphyxiation. The monkey could not suck until it was 3 days old and then it sucked poorly. It was usually inactive, vocalized seldom, and had a tendency to sleep through feeding periods. It was dormant, apathetic, and showed little spontaneous motion.

Jaundice was not observed. The animal was in good health at the time it was killed at 4 days to obtain a specimen of this age. There is little doubt that it could have survived.

Monkey F. The infant's respiration was established with difficulty after asphyxiation and resuscitation. It experienced intervals during which breathing became infrequent, and pulmonary insufflation was restarted on several occasions during the first 3 hours of life.

Its movements were random and massive and appeared to be poorly coordinated. It could not right itself and lay on its side. It was hyperirritable and reacted to loud sounds with a marked startle during the first 12 hours. At 24 hours, spontaneous activity and reactivity to sound decreased to below normal levels. At 36 hours and again at 52 hours of age the infant exhibited convulsive seizures recurring every minute. The electroencephalogram showed a spike and wave pattern during these. Status epilepticus was controlled by administering pentobarbital sodium in doses of 1 mg. There was little spontaneous activity and the animal was quite unresponsive to stimulation at 48 hours. It could never suck and swallowed irregularly.

Jaundice was not seen. Funduscopic examinations were not made. The animal remained in critical condition and began to exhibit periods of apnea at the end of 3 days. It seemed to be in coma and, in view of the likelihood that it would die, it was killed by perfusion-fixation at 3 days of age.

Monkey G. The infant recovered rapidly from acute effects of asphyxiation. It was hyperactive and hyperirritable during the first 12 hours and rarely could right itself on the first day. It usually lay on its side and executed random, poorly coordinated movements. Vigorous motor activity followed loud and sharp sounds. The level of activity and reactivity declined after the first day. At no time could the infant suck. Fibrillations of tongue muscles were seen at 48 hours.

The animal was never jaundiced. It was killed at the end of 2 days, at which time symptoms of pneumonitis were observed, its general condition was deteriorating, and prolonged survival seemed unlikely.

Autopsy

Congenital abnormalities were observed in 2 of the 7 monkeys. Monkey G had a deformity of the right upper extremity involving partial agenesis of the hand. The humerus was unaffected but radius and ulna were only three-fourths the length of those on the normal side. Monkey C had a minor abnormality at the tip of the tail where there was a hairless nodule connected with the rest of the tail by a thin strand of tissue. There were no congenital abnormalities of the nervous system.

Three monkeys (C, F, and G) were found to have scattered foci of pneumonitis. No foci were seen in monkey D, but polymorphonuclear leucocytes were present in an intact bronchus. Sections of the viscera revealed subserosal petechial hemorrhages in the adrenal glands and kidneys of monkey F. Intracellular pigment granules were numerous in the splenic pulp of this animal. There appeared to be an unusually large number of polymorphonuclear leucocytes among red corpuscles in the splenic sinuses of monkey D.

Results of Neuroanatomic Examinations

Monkeys A and B. The brain of monkey B differed very little from that of monkey A even though the animal may have been born 20 days prematurely. The principal differences between the brains of these 2 control monkeys were that all neurons in B were slightly smaller and more closely packed than those in A. The neuroglia cells in the brains of these 2 monkeys appeared to have attained about the same stage of development and, except in a few regions, no indication of "normal astrocytic hyperplasia" was encountered. All abnormalities in other specimens were checked against both monkeys A and B to be sure that maturational differences were not confused with pathologic changes.

Monkey C. The brain of monkey C was studied in greatest detail because this animal showed the most severe damage during life and was kept alive for the longest time. Neuropathology of the remaining animals will be described in reference to the findings in monkey C.

No gross abnormalities were observed except suggestive widening of sulci and possibly slight enlargement of lateral and third ventricles.

Microscopic study revealed extensive damage throughout the central nervous system. The involvement of the gray matter was most striking. It was slight in some places, notably the cerebral cortex and cerebellar cortex, and a few regions appeared to be unaffected. Diencephalic and brain-stem nuclei, when affected, were usually damaged throughout their entire extent. The changes ended abruptly at their borders. Lesions were confluent throughout the whole reticular formation as well as in the ventral posterior parts of the thalamus. They were bilaterally symmetrical everywhere except possibly the putamen.[4]

Alteration of nerve cell bodies was prevalent, some cells being destroyed beyond the point of identification and others showing various degrees of cytolysis. It was estimated that roughly half of all subcortical neurons showed some involvement. Astrocytes and oligodendrocytes were likewise affected in some of the regions of neuronal loss. The loss of astrocytes and the atrophic changes seen in them in thionine-stained sections was borne out in adjacent sections stained with PTAH.

Astrocytic hyperplasia occurred in some of the nuclei. This appeared as an increase in the number of astrocytes and with hypertrophy of individual cells. Sections stained with PTAH showed the early proliferation of glial fibers.

No hemorrhages nor vascular thromboses were found in sections of the nervous system except in the retina where petechial hemorrhages were seen funduscopically. Enlarged or dilated blood vessels were encountered in some regions, and proliferation of cells from the adventitia of these

[4] Two atlases were used for localization of structures in the cerebrum (7, 12). Since there is no detailed atlas of the monkey brain stem, that of Olszewski and Baxter (8) on the human brain stem was employed. The classifications of these atlases will be used, but the terminology will be anglicized except in footnotes.

vessels was observed (Figs. 3, 5). There, also, the endothelial cells were hypertrophic. Sections stained by Gomori's method revealed no iron in the nervous system.

Phagocytosis was not well developed, although many areas contained numerous small cells, presumed to be early macrophages. None of these

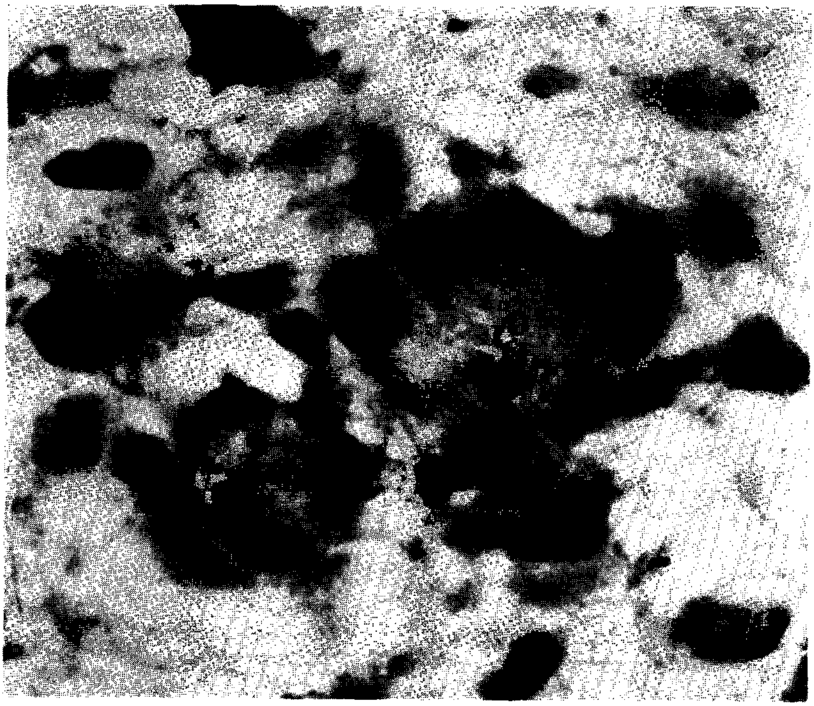


FIG. 1. Two degenerating neurons (n) surrounded by phagocytes with darkly staining nuclei in the ventral posterior thalamus of the 9-day asphyxiated monkey. Photomicrograph of a thionine-stained section; 1725 ×.

cells had reached the stage of compound granular corpuscles. Sections stained with Sudan black B revealed many such cells with small fat inclusions. Nerve cells of certain symmetrical regions of the thalamus, usually in advanced stages of cytolysis, were surrounded by numerous phagocytes, some resembling microglia cells (Fig. 1).

Although myelination of fiber tracts in the newborn monkey is quite incomplete and some tracts are unmyelinated, it was possible to observe degeneration in sections stained by Woelcke's method. Globules of degenerated myelin were found scattered in tracts and often arranged in rows parallel to the axis cylinders comprising the tract (Fig. 2). The distribution of degenerating myelin sheaths was similar to the distribution of damage to axis cylinders observed in sections stained with Bodian's silver Protargol method. It was about what would have been predicted, assuming the degeneration to be secondary to a primary destruction of nerve

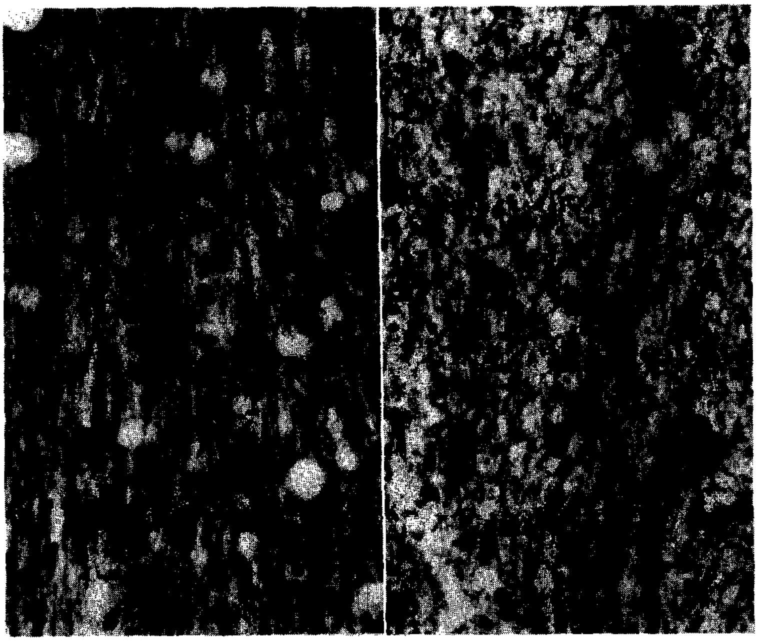


FIG. 2. Internal capsule of the full-term control monkey in A, and of the 9-day asphyxiated monkey in B. A few normal myelinated fibers (f) remain in B. Nuclei of oligodendrocytes may be seen (g). Photomicrographs of sections stained by Woelcke's myelin sheath method; 276 X.

cell bodies. The oligodendrocytes in degenerating white matter had a more ragged appearance than those in the control brain and their orderly arrangement in rows was disrupted. No astrocytic or vascular changes were observed in the white matter.

We have placed lesions of nuclei of monkey C brain in 5 categories in respect to the type of histopathology observed.

Type 1 lesions were the most severe. A central core of greatest damage was seen, in which few neurons, astrocytes, or oligodendrocytes were

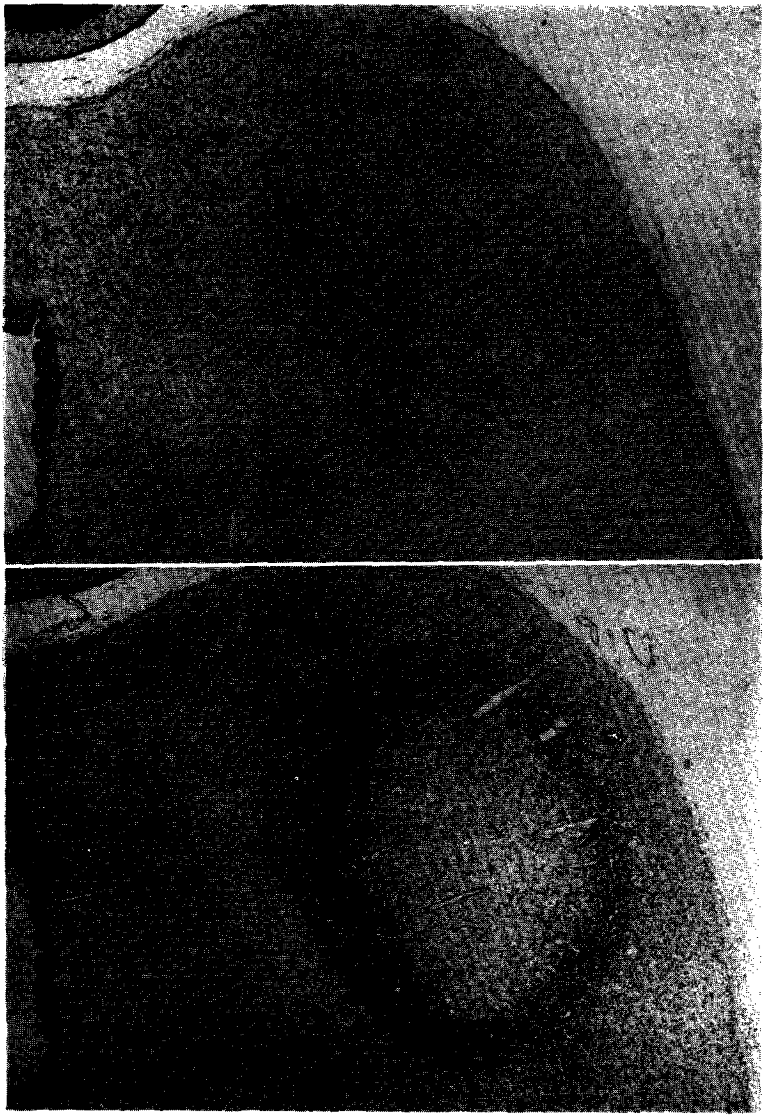


FIG. 3. Inferior colliculus of the full-term control monkey in A, and of the 9-day asphyxiated monkey in B. The lesion is largely confined to the nucleus of the inferior colliculus. Photomicrographs of thionine-stained section; 18 ×.

identifiable (Figs. 3B, 4B). Around this central core there were many cells resembling fibroblasts proliferating from the adventitia of enlarged blood vessels, and many hypertrophic endothelial cells (Fig. 4A). Macrophages, in early stages of development, and hyperplastic astrocytes were present peripherally.

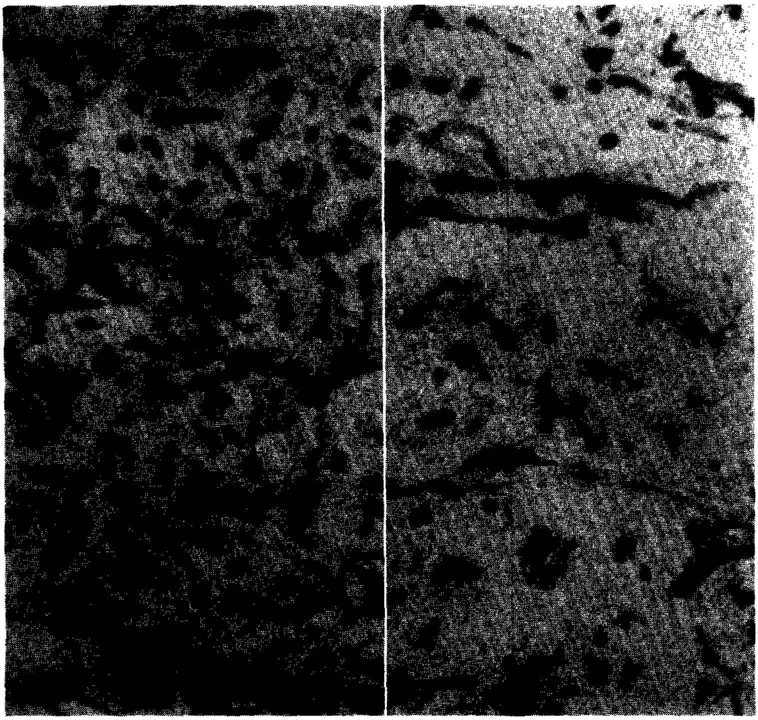


FIG. 4. Nucleus of the inferior colliculus of the 9-day asphyxiated monkey. Both A and B are from the section shown in Fig. 3B; the dark outer rim is shown in A and the light central core in B. Photomicrographs; 276 ×.

In some of the lesions (subtype 1a), all neurons were damaged, and most were unidentifiable. The nucleus of the inferior colliculus is an example.[5] It was the most severely affected of all (Figs. 3, 4). A central clear area in each nucleus contained only blood vessels, cells resembling fibroblasts, a few early macrophages, and cell remnants. A peripheral

[5] The other regions involved in type 1a lesions are: nu. olivaris sup. and a small but symmetrical spot in each nu. vent. post. lat. pars caud. of the thalamus.

ring was more vascular and cellular than the central area and contained more early macrophages and cells in less advanced stages of degeneration.

Other lesions (subtype 1b) contained some normal neurons, usually in the periphery, although a few occurred also in the central core of

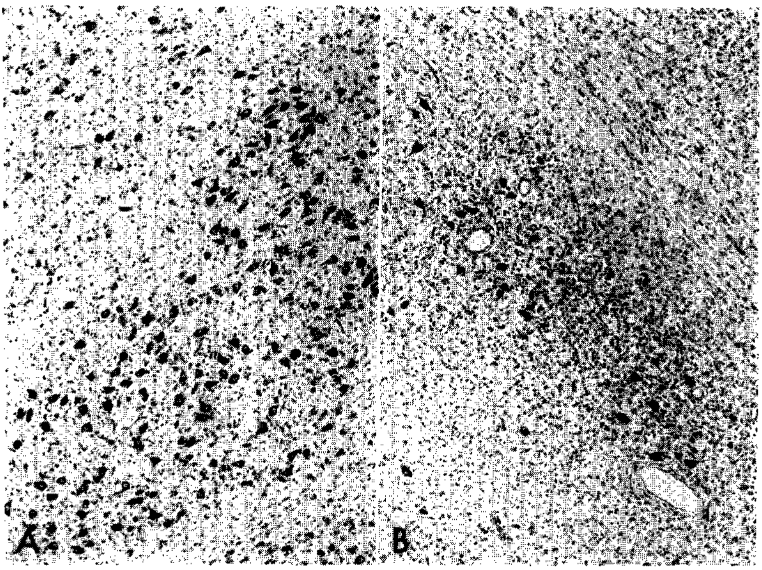


FIG. 5. Motor nucleus of the trigeminal nerve of the full-term control in A, and of the 9-day asphyxiated monkey in B. The left side is represented in A; the right side, in B; the apparent size difference is of no significance. Photomicrographs of thionine-stained sections; 42 ×.

greatest damage and reaction. The motor nucleus of the trigeminal nerve is an example of this type of lesion (Figs. 5, 6).[6]

Type 2 lesions were those in which there were chromatolytic neurons, astrocytic hyperplasia, and early macrophages. There were no fibroblast-like cells and no glial atrophy or loss. In some of these lesions (type 2a) there were no normal neurons remaining, although most neurons were identifiable, and the lesion was uniform throughout the nucleus without

[6] The other regions involved in type 1b lesions are: nu. pallidus (med. segment); nu. subthalamicus; nu. habenularis lat. pars magnocellularis; all parts of nu. geniculatus med.; nu. suprageniculatus; nu. interstitialis (Cajal); nu. oculomotorius principalis; nu. nervi trochlearis; nu. nervi trigemini sensibilis principalis; nu. tr. spinalis trigemini oralis and interpolaris; nu. vestibularis med.; nu. gracilis; nu. cuneatus med; and roof nuclei of the cerebellum.

a central area of greater damage. An example of type 2a is the lateral segment of the globus pallidus on either side.[7]

In other lesions (subtype 2b), there were some normal neurons, usually scattered throughout the nucleus, but sometimes more numerous peripher-

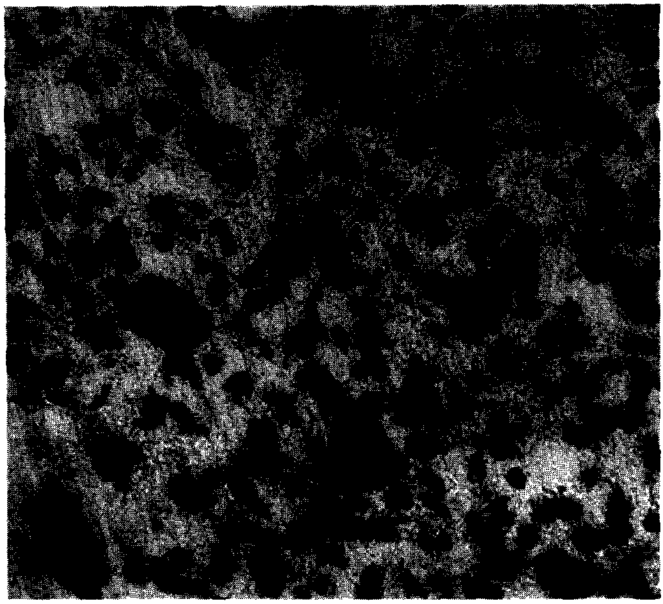


FIG. 6. Motor nucleus of the trigeminal nerve; same section as Fig. 5B. Note normal neurons in the midst of damaged neurons and the phagocytic and astrocytic reactions; a vascular fibroblastic reaction was present elsewhere in this nucleus. Photomicrograph; 276 ×.

ally. The brain-stem reticular formation of each side was involved in one large coalescent lesion of this subtype.[8] The putamen, another

[7] The other regions involved in type 2a lesions are: nu. mamillaris med.; several individual thalamic nuclei (ant. med., centrum medianum, limitans, centralis lat.); a single coalescent thalamic lesion (nu. vent. lat. pars oralis, nu. vent. post. lat. pars oralis and caudalis, nu. vent. post. med., nu. vent. post. med. pars parvocellularis); nu. tr. spinalis trigemini caudalis, subnucleus magnocellularis; nu. proprius cornu dorsalis of cervical, lumbar, and sacral spinal cord.

[8] The other regions involved in type 2b lesions are: most of nu. putamen; several thalamic nuclei (nu. vent. lat. pars postrema, lat. post., lat. dors., vent. post. inf., med. dors., pulvinaris, reticularis, centralis latocellularis, centralis inf. reuniens); both parts of nu. ruber; nucleus of Darkschewitsch; griseum centrale mesencephali; nucleus of Perlia; colliculus super.; nu. intercollicularis; substantiae nigrae; nu.

example, had some special features. This was the only nuclear lesion which may not have exhibited perfect symmetry inasmuch as it consisted of many small, sharply circumscribed areas of damage scattered irregularly throughout the caudal part. About half of each putamen was involved. All of the small neurons in the damaged areas showed severe cytolysis, but only about a third of the large neurons were affected.

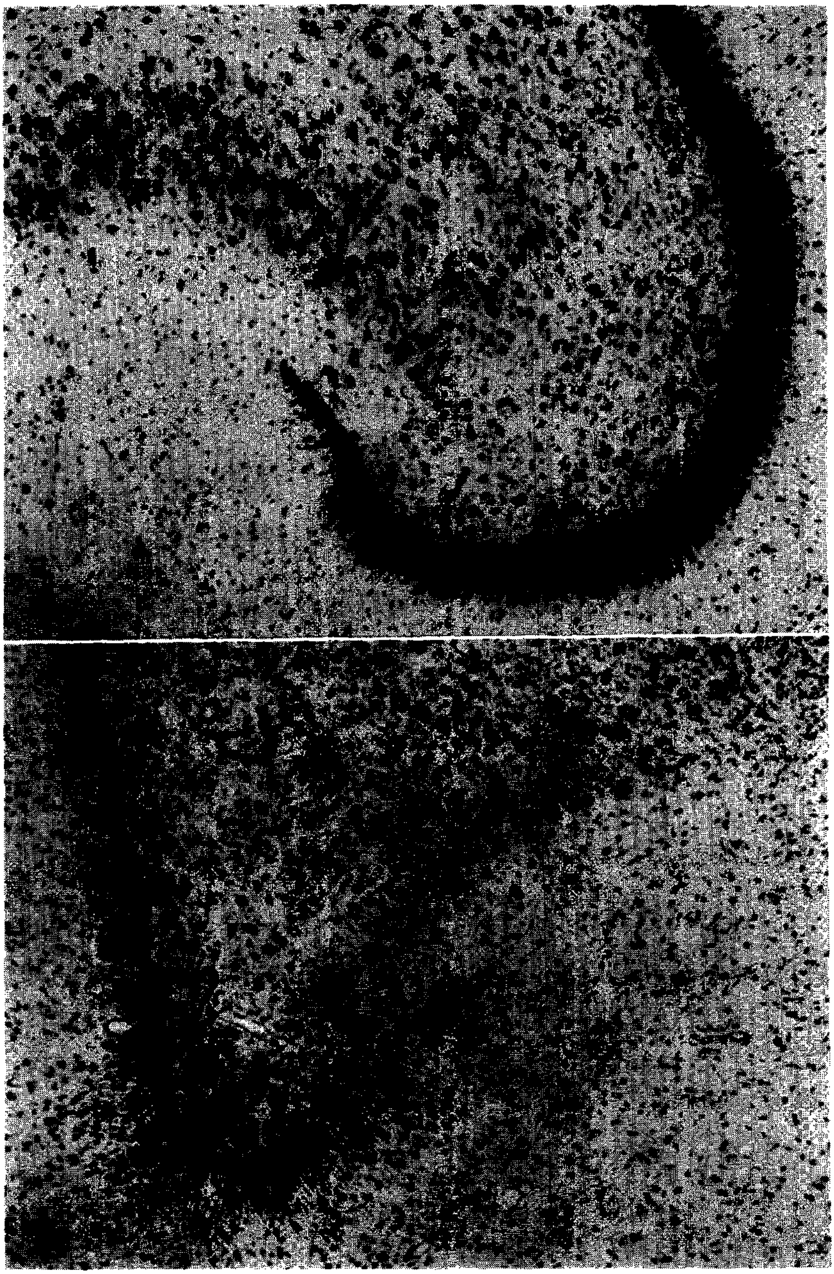
Type 3 lesions contained normal neurons, neurons with moderate cytolysis, and astrocytic hyperplasia. Neuroglia cells appeared to have suffered no loss, lysis, or atrophy. There were no macrophages, cells resembling fibroblasts, nor vascular changes to be seen. The hippocampal formation is an example of this type (Fig. 7).[9] Most neurons in the ventral third of the granular layer of the dentate gyrus were affected and astrocytic hyperplasia was encountered in the adjacent molecular layer. Other parts of the dentate gyrus and hippocampus appeared to be normal, except for slight cytolysis and astrocytic hyperplasia in the third of the hippocampus nearest the dentate gyrus.

Type 4 lesions contained some neurons exhibiting slight changes, but there were no changes in other elements. The cerebral cortex, cerebellar cortex and spinal ganglia were the only structures involved in lesions of this type. Although neurons of the cerebral neocortex and pyriform cortex vary in size, the average size in monkey C (Fig. 8B). was less than in the controls (Fig. 8A). They usually stained more lightly than in the controls and many had ragged cytoplasmic borders. A few large pyramidal cells, but no others, were lysed. None appeared to have been lost, although counts were not made. There were no changes in the cortex

parabrachialis med.; nu. lemnisci lat., dors. and vent.; nu. abducentis; nu. vestibularis sup.; nu. cochlearis dors. and vent.; and some of the gray matter of the spinal cord. The reticular formation of the whole brain stem was involved in a single coalescent lesion along with: regio praetectalis; nu. tegmentalis ant.; nu. peripeduncularis; nu. interstitialis of post. commissure; zona incerta; and the tegmental fields of Forel. The nuclei of the raphae and other medial nuclei of the brain stem were not coalescent with the reticular formation lesion.

[9] Other regions involved in type 3 lesions are: several thalamic nuclei (nu. vent. lat. pars med., parafascicularis, paracentralis, centralis sup. lat.) ; nucleus of Edinger Westphal; nu. trapezoidalis; and nu. vestibularis lat.

FIG. 7. Ventral half of the dentate gyrus and the adjacent hippocampus of the right side of the full-term control monkey in A, and the left side of the 9-day asphyxiated monkey in B. Photomicrographs of thionine-stained sections; 105 ×.



of the cerebellar hemispheres, but cytolysis and loss of Purkinje cells were encountered in a few folia of the vermis.

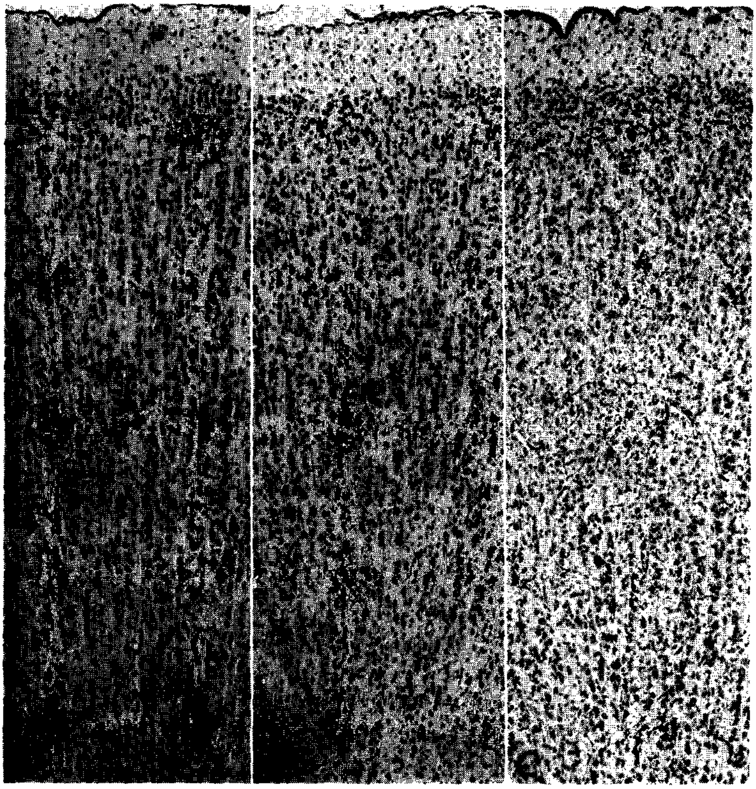


FIG. 8. Identical regions from the medial surface of the primary motor area (FA) of the cerebral cortex of the full-term control monkey in A, the 9-day asphyxiated monkey in B, and the 3-day asphyxiated monkey which had convulsive seizures in C. Photomicrographs of thionine (A, B) and gallocyanine-stained (C) sections; 42 ×.

Type 5 lesions showed no changes in neurons, but astrocytic hyperplasia occurred uniformly throughout the nuclei involved. The posterior hypothalamus is an example.[10]

[10] Some of the other regions involved in type 4 lesions are: many thalamic nuclei; nu. geniculatus lat.; griseum pontis; many nuclei of the raphae and other medial nuclei of the brain stem; nu. nervi facialis; nu. ambiguus; nu. nervi hypoglossi; nu. tractus spinalis trigemini subnu. zonalis and gelatinosus; zona spongiosa and substantia gelatinosa of the spinal cord.

Not all structures in the nervous system were affected in monkey C. There were apparently unaffected areas scattered throughout the cerebrum and brain stem, including about one-fifth of all the subcortical gray matter. Some of the normal appearing structures were the olfactory, amygdaloid and caudate nuclei, anterior hypothalamus, a few thalamic nuclei, and all parts of the inferior olivary nuclei.

Monkey D. The only gross pathology encountered was epidural hemorrhage. Beneath the right parietal bone there were two organizing masses of blood, 1.5 $\times$ 2 cm, and 2 mm thick, which did not compress the brain visibly.

Microscopically the brain of monkey D was similar to that of monkey C. Nuclei and regions most severely damaged in the latter were likewise damaged symmetrically in the brain of this animal, often with a less severe reaction. However, the same types of histopathology observed in monkey C were seen in this animal. It was estimated that about one-third as many neurons were involved as in the brain of monkey C. The only structure in which there was greater damage in monkey D than in monkey C was the putamen which was involved totally on both sides. Small neurons were destroyed beyond recognition and, as in monkey C, large neurons were either unaffected or showed only moderate degrees of cytolysis. Furthermore, the putamen of monkey D exhibited some loss and atrophy of neuroglia cells. Many early macrophages were present.

The nucleus of the inferior colliculus was less severely damaged than in monkey C, inasmuch as the center still contained a few recognizable neurons and a few neuroglia cells; however, all the neurons were damaged. Cells resembling phagocytes were seen around the blood vessels of this nucleus.

Some regions showing pathologic changes in monkey C, appeared to be unaffected in the brain of monkey D. For example, the hippocampal formation, cerebellar cortex, superior colliculus, some of the thalamic nuclei, and the reticular formation (down to and including the cervical spinal cord) appeared to be normal in the brain of monkey D. A large coalescent lesion was again seen in the ventral posterior thalamus.

Monkey E. A small amount of recently extruded blood was seen at autopsy over the sacral spinal cord. No other gross abnormalities were encountered.

Mild cytolysis was seen in a few nerve cells scattered throughout all regions of the nervous system. Areas of severe neuronal cytolysis were the nucleus of the inferior colliculus, superior olivary, superior and medial vestibular, medial cuneate and gracile nuclei, and roof nuclei of the cerebellum. The nucleus of the inferior colliculus exhibited especially severe cytolysis of all neurons, although they were still identifiable. These changes were found in some of the same groups of nuclei that were severely involved in monkey C. The spinal cord was unaffected.

Although some mild cytolysis was found in the cerebral cortex and diencephalon, major lesions were confined to nuclei of both sides of the brain stem. The damage observed in the reticular formation of monkey C was not observed in monkey E.

Nowhere were altered neuroglia cells observed in the brain of monkey E. No reaction of cells from the adventitia of blood vessels had appeared at this time.

No petechial hemorrhages were encountered in any sections of the brain, but intracellular pigment granules were present in the outer rim of the right inferior colliculus. These were encountered nowhere else in the nervous system in this or any other monkey.

Monkey F. No unequivocal gross changes were observed. Division of the brain into blocks revealed no gross pathology on the interior. Some blood was present in veins on the surface as well as in the interior of the brain, perhaps indicating that it had been incompletely washed out before the perfusion of fixing fluid.

Microscopically, there was found to be mild cytolysis of neurons scattered throughout all parts of the central nervous system. Neuroglia cells were lost or lysed in the putamen, medial segment of the globus pallidus, and nucleus of the inferior colliculus.

Histopathologic changes were distributed in a bilaterally symmetrical pattern almost exactly like that in the brain of monkey C. The same nuclei of the cerebrum, brain stem, and spinal cord were involved, except that the coalescent lesion of the reticular formation was confined to the midbrain. The hippocampal formation appeared to be less involved but the cerebral cortex and corpus striatum exhibited greater changes than these structures in the brain of monkey C.

No proliferative glial nor adventitial cell changes were encountered at this early stage. There appeared to be some hypertrophy of endothelial cells in the regions of greatest severity.

The cerebral cortex of monkey F was severely damaged (Fig. 8C), many neurons showing cytolysis, so that the change could be seen in a stained section with the naked eye. All neurons were identifiable but some had assumed the form of ghost cells. The intensity of the cytolysis of neurons in the cerebral cortex varied greatly from area to area, but was bilaterally symmetrical. The distribution of the severe damage did not follow closely any cytoarchitectural pattern. There appeared to be greater damage in the depths of some sulci on the dorsal surface and in the primary motor and sensory areas (FA, PB, and PC of von Bonin and Bailey) than elsewhere. The pyriform cortex was least affected.

Small neurons of the putamen were damaged beyond the point of identification but, as in monkey C, large neurons showed only moderate or slight cytolysis. The whole putamen was involved symmetrically along with part of the caudate nucleus.

Small extravascular collections of intact red blood corpuscles were scattered throughout the brain, but their distribution did not follow that of neuronal damage.

Monkey G. Grossly the brain of monkey G showed no abnormalities. The number of histologic sections examined was less than in the other specimens. Nevertheless, the histopathology was similar and followed the same bilaterally symmetrical pattern as in the other monkeys. Neuronal lysis was found to be scattered throughout the central nervous system, although it was less intense and fewer neurons were involved than in the brain of monkey C. Only in the central core of the nucleus of the inferior colliculus were neuroglia cells as well as neurons destroyed. In this monkey, killed only 48 hours after asphyxiation, the center of this nucleus closely resembled the central clear area in the 9 day old monkey C. Other severe neuronal damage was seen in a region in the ventral posterior thalamus, medial geniculate body, superior colliculus, principal oculomotor, superior olivary, medial cuneate and gracile nuclei, oral part of the spinal trigeminal nucleus, and in the roof nuclei in the cerebellum. The reticular formation and spinal cord were spared. No vascular changes nor proliferation of cells resembling fibroblasts were observed.

A few petechial hemorrhages were present in the medial cuneate and gracile nuclei, and in the spinal trigeminal nucleus. Perivascular cuffing with granular leucocytes was encountered around several blood vessels in a small area of the right subcortical white mater.

Discussion

Agonal changes, post-mortem artifacts, and alterations in deep structures caused by slow penetration of large pieces of tissue immersed in fixing agents (5) tend to obscure early specific effects of asphyxia neonatorum. The perfusion-fixation technique produced instantaneous death of cells in all parts reached by the vasculature, and a high degree of uniformity was attained in histologic preparations. For these reasons, the experiments in the monkey yielded more precise results than could be expected from human brain material obtained after death, and more subtle criteria of abnormality could be used.

Several findings in the brains of the present series were probably coincidental. One asphyxiated monkey (G) with pneumonitis had a few foci of embolic encephalitis. These foci resembled those in an additional brain of a nonasphyxiated monkey with focal embolic encephalitis. The scattered fresh red blood corpuscles encountered in two of the brains may have been unrelated or only secondarily related to asphyxiation. It is possible that the initial perfusion pressure caused weakened capillary walls to give way. The epidural hemorrhages in monkey D and the fresh hemorrhage over the sacral cord of monkey E appear to have been unrelated to the damage in the rest of the nervous system.

In other respects the 5 brains of asphyxiated infant monkeys presented a single neuropathologic entity, differing one from another mainly in gradations of intensity and time. They were similar in respect to symmetry of nuclear distributions of damage, with certain nuclei consistently involved. And they were similar in presenting evidence of a primary effect on nerve cells—to a lesser extent on neuroglia cells from which many of the later reactions developed.

The destruction of nuclei of both inferior colliculi in all the brains regardless of age, was most striking. Equally constant but less dramatic were the lesions in gracile and medial cuneate nuclei and in the roof nuclei of the cerebellum. Furthermore, most of the asphyxiated animals exhibited severe damage of the putamen, globus pallidus, ventral posterior thalamus, principal oculomotor nucleus, superior and medial vestibular, superior olivary, principal sensory and spinal trigeminal tract nuclei of both sides. Many of these lesions were seen at 2 days just as well as at 9 days after asphyxiation.

The cerebral isocortex and the putamen varied most in respect to sensitivity to the damaging factors, inasmuch as the degree of injury did not follow that in other parts of the brain.

That the primary effect of asphyxia was manifested as cytolysis of neurons and neuroglia, becomes clear by comparing specimens of different ages. There was no astrocytic hyperplasia nor phagocytic activity in the brains of monkeys which were 2 to 4 days old when killed, but nerve cells and some neuroglia cells of the gray matter were clearly affected, often having reached advanced stages in cytolysis, and some had already disappeared. The brains of the two oldest monkeys showed similar cytolysis and loss, but in addition there were marked reactive changes in astrocytes, microglia cells (or other phagocytic cells), and walls of blood vessels. A relationship was noted between degree of primary cell damage and intensity of the phagocytic reaction; both processes were marked in the gray matter and neither was prominent in the white matter. The myelin and oligodendrocytic changes in the latter may have been secondary to nerve cell damage.

The lesion of the inferior colliculus of monkey C can be considered as coagulative or global necrosis, and in this animal it appears to be in a different category from all other lesions. However, in each of the other animals, although the inferior colliculus was the most severely affected region, the damage (e.g., in D and E) was no worse than in many of the other parts of the brain of monkey C. It is thought to have been part of the same process and not basically different from the other lesions.

There was little evidence in any of these brains of vascular or hemorrhagic factors playing an important role in the development of the lesions. The distribution of lesions was unrelated to distribution of large vessels and the only changes seen in blood vessels were hypertrophy of endothelial cells, proliferation of cells from the adventitia, and dilatation of vascular lumens in areas of most severe damage of monkey C.

The petechial hemorrhages in the brains of monkeys F and G contained fresh blood cells and their distribution was somewhat different from that of the other changes. The hemorrhages in monkey E may have resulted from the convulsions. The pigment granules in monkey E, the retinal hemorrhages of monkey C and D, and the petechial hemorrhages and hematinlike pigment seen in the viscera of monkey F may have resulted from vascular leakage at or soon after the time of asphyxia, but appeared to have been unrelated otherwise to the major pathologic findings.

Asphyxia neonatorum sometimes resulted in fairly large hemorrhagic lesions in the guinea pig brain (15). More recent unpublished experiments in that species resulted in neuropathology with a lower incidence of hemorrhages than in the earlier study. As in the monkeys, lesions appeared to involve primary cytolysis of neurons and they were usually bilaterally symmetrical. Furthermore, the thalamus and brain-stem nuclei were more severely affected than the cerebral cortex. Neuroglia cells of the guinea pig differ considerably in appearance from those in the monkey brain; comparison of glial reactions in the two species was unsatisfactory during the first 9 days of life. However, glial scars were seen in older guinea pigs. There was much less cellular proliferation from blood vessels in guinea pig than in monkey brains. Lesions in guinea pig brains were prone to occur in the thalamus and geniculate bodies as well as in the brain-stem reticular formation, but were never seen in the colliculi. The thalamic lesions of the guinea pig almost invariably surrounded a prominent blood vessel which was usually dilated; focal hemorrhages occasionally occurred at these sites (13, Fig. 5).

Acute anoxia at the time of asphyxiation may not have been the only or even the primary cause of the damage. The associated hypercapnea, cardiovascular alterations, biochemical changes, singly or in combination, may have been other precipitating factors in the neurologic damage. Cardiovascular and biochemical changes were not determined during this study, and these factors cannot be evaluated at the present time. Nevertheless, it would seem that some regional variation in metabolic characteristics of nerve and neuroglia cells determined their susceptibility to asphyxia neonatorum. The occurrence of normal cells adjacent to extremely affected cells in many lesions, the symmetrical and comparatively uniform changes in nuclei, the symmetrical sparing of whole regions adjacent to utterly destroyed regions and the sparing of large cells in the putamen are difficult to explain except on the basis of such metabolic differences.

It is of interest to try to correlate pathology with observations on the reactions and behavior of the monkeys. Present data are inadequate to provide more than suggestions. The hyperreactivity to loud sound during the first 12 hours followed by hyporeactivity or complete absence of responses later on, may have been related to the massive destruction in the central acoustic pathways, notably the nucleus of the inferior colliculus, but also medial geniculate, superior olivary nuclei, and nucleus of the lateral lemniscus.

Much of the general hyporeactivity to handling, the spontaneous hypoactivity, and the incoordination of movements may have been related to massive loss in the major somatic afferent pathways, especially in sensory brain-stem nuclei and ventral posterior thalamic nuclei.

Inability to attain proper body righting or orientation, and other deficits in motor control may have been related to the destruction in vestibular as well as cerebellar nuclei. One of the monkeys exhibited convulsive seizures, and this was the only one in which the cerebral cortex was severely damaged. The only animals which appeared to be in coma were the two with lesions of the reticular formation. It is noteworthy that the lesions involved only the region of the reticular activating center of the midbrain (6) in one of these animals.

One would like to know also how the effects of asphyxia neonatorum in the monkey compare with those in human infants. Human material adequate for comparable cytological evaluation of acute changes has not been available. Post-mortem changes will undoubtedly mask many of the changes seen in the monkey. However, some of the damage was so severe in the monkey at 48 hours that it is reasonable to expect similar pathology to be encountered in human brains a few days after asphyxiation and resuscitation. More chronic experimental brains would be most useful for comparison with human material. Chronic asphyxiated monkey brains will be studied later.

The human neuropathologic entity most closely resembling the effects of asphyxia neonatorum in the monkey is kernicterus. There are similarities in the distribution and type of nerve cell changes in both conditions. Major differences between the findings in the monkey and those in human infants with kernicterus are absence in the former of the usual history of erythroblastosis fetalis, lack of clinical jaundice, lack of pigment in the lesions, frequent presence of neuroglia cell damage, and presence of marked astrocytic and phagocytic reactions.[11]

References

1. Cammermeyer, J., Neuropathology of asphyxia neonatorum. *In* "Neurological and Psychological Deficits of Asphyxia Neonatorum," W. F. Windle (ed.), Springfield, Illinois, Thomas, 1958 (ref. pp. 156-172).
2. Corner, G. W. Jr., and G. W. Anderson, Asphyxia of the human fetus in relation to brain damage. *In* "Neurological and Psychological Deficits of Asphyxia Neonatorum," W. F. Windle (ed.), Springfield, Illinois, Thomas, 1958 (ref. pp. 173-194).
3. Hines, M., The development and regression of reflexes, postures, and progression in the young macaque. *Contrib. Emb.* **30**: 153-209, 1942.
4. Koenig, H., R. A. Groat, and W. F. Windle, A physiological approach to perfusion-fixation of tissues with formalin. *Stain Techn.* **20**: 13-22, 1945.
5. Koenig, R., and H. Koenig, An experimental study of postmortem alterations in neurons of the central nervous system. *J. Neuropath.* **11**: 69-78 1952.
6. Magoun, H. W., "The Waking Brain," Springfield, Illinois, Thomas, 1958.
7. Olszewski, J., "The Thalamus of the *Macaca mulatta*," New York, Karger, 1952.

[11] The relation to kernicterus was discussed with Drs. A. Pentschew and C. Margoles of the Armed Forces Institute of Pathology who are preparing a report of 80 human cases of kernicterus.

8. Olszewski, J., and D. Baxter, "Cytoarchitecture of the Human Brain Stem," Philadelphia, Lippincott, 1954.
9. Ranck, J. B., Jr., and W. F. Windle, Asphyxia neonatorum in *Macaca mulatta*. *Physiologist* **1**: 63, 1958.
10. van Wagenen, G., Breeding and care of monkeys. *In* "Neurological and Psychological Deficits of Asphyxia Neonatorum," W. F. Windle (ed.), Springfield, Illinois, Thomas, 1958 (ref. p. 277).
11. van Wagenen, G., and H. R. Catchpole, Physical growth of the rhesus monkey (*Macaca mulatta*). *Am. J. Phys. Anthrop.* **14**: 245-273, 1956.
12. von Bonin, G., and P. Bailey, "The Neocortex of *Macaca mulatta*," Urbana, Illinois, Univ. Ill. Press, 1947.
13. Windle, W. F., Brain damage in the guinea pig after asphyxia neonatorum. *In* "Neurological and Psychological Deficits of Asphyxia Neonatorum," W. F. Windle (ed.), Springfield, Illinois, Thomas, 1958 (ref. pp. 31-43).
14. Windle, W. F., and C. J. Bailey, Neurological deficits of asphyxia neonatorum in the monkey (*Macaca mulatta*). *Anat. Rec.* **130**: 389-390, 1958.
15. Windle, W. F., R. F. Becker, and A. Weil, Alterations in brain structure after asphyxiation at birth. *J. Neuropath.* **3**: 224-238, 1944.