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Cerebral Hypoxia and Ischemia: The Forensic Point of View: A Review

ABSTRACT: In cases with suspected brain anoxia/ischemia and hypoxia/hypoxemia a neuropathological investigation should give additional information to elucidate the cause of death and its pathophysiological mechanisms. Primary ischemic brain damage is associated with morphological and biochemical alterations. While acute ischemic neuronal injury reveals axon sparing and selective neuronal lesions due to the release of large quantities of glutamate, late neuronal death is associated with antiapoptotic growth factors, and decreased expression of microtubule-associated proteins and tubulin. On the morphological level ischemia can be detected by necrosis of neurons, proliferation of microglia, and astrocytes in vulnerable regions of the brain. In cases of permanent ischemia the so-called pale nervous cell injury is observed, in cases of partial perfusion the so-called dark nerve cell injury and apoptosis are detectable. In spite of the considerable advantages of recent research, presently there is no reliable qualitative marker to ascertain death due to acute hypoxic or ischemic events.

KEYWORDS: forensic science, brain ischemia, brain hypoxia, neuronal injury, morphology

Primary or secondary injury of the brain and spinal cord is found in more than 50% of all forensic autopsies. Death due to functional disturbances of the brain is suggested in a great number of these cases, i.e., in about 25–30% of all autopsies. In one up to two thirds of these cases cerebral hypoxia and/or ischemia explains the functional failure of the central nervous system (CNS). Therefore, in 10–20% of all autopsy cases a forensic pathologist is called for an expert's report on the pathophysiology and morphology of the CNS under hypoxic/ischemic conditions (own experiences). Giving a short summary we review the state-of-art of recent scientific discussions and give theoretical and practical advice to forensic pathologists.

Pathophysiological Background

Hypoxia and Ischemia

Ischemia and hypoxia have often been considered to be of similar nature, especially regarding the hemodynamic level. But, while ischemia is characterized by a reduction or stop of the general or regional cerebral blood flow resulting in irreversible neuronal destruction, hypoxia leads to an increase in cerebral blood flow (1) preponderantly resulting in a reversible breakdown of neuronal functions. If the airways become obstructed (as in: bronchial asthma, aspiration) or atmospheric oxygen is cut off (as in: drowning or plastic bag on the head), cerebral perfusion continues, but blood gas values undergo an alteration by increasing pCO_2 and secondary arterial dilatation. Because of the continuous blood flow, waste products as lactate, acidosis, etc., do not increase. In case of a transient hypoxic exposition the breakdown of neuronal function will be reversible. On the other hand, mechanical occlusion of the cervical vessels (as in: hanging) induces not only a total stop of intracerebral circulation, but also a change in the blood gas values and pH within the brain associated with acidosis and increase of lactate. As a result of both these processes energy depletion occurs within a few seconds to minutes, the K^+/Na^+ pump is disturbed with sequela of an early cytotoxic edema. In case of reperfusion the primary cytotoxic edema will be overlapped by a (delayed) vasogenic edema within 10–20 min. The hemodynamics is crucial to the clinical outcome and reversibility because of the metabolic and molecular mechanisms, which are of secondary importance (2).

The above-mentioned models for the hemodynamic alterations of asphyxiation and the described biochemical differences between "ischemia" and "hypoxia" are associated with different functional and morphological sequelae in the CNS. While in hypoxia the intracerebral vascular system always reacts *functionally* to a drop in pO_2 and pH—and increase in both pCO_2 and extracellular K^+—by lowering the vascular tone, the CNS reacts to ischemia with a variety of *structural* alterations. Further, the extent and speed of these changes depend entirely on the severity of the acidosis (3).

Acute Ischemic Lesions of the Neuron

Primary ischemic brain damage as a result of brain perfusion interruption is followed by secondary phenomena like cytotoxic brain edema within seconds or minutes, concomitant with a shift of the K^+ ions into extracellular space and Na^+ ions and water from extracellular space into the cytoplasm (4,5), being a result of oxygen depletion and consecutive reduction of the Na–K ATPase activity. Employing histochemical methods in animal experiments different authors were able to demonstrate the drop of intracellular K^+ following unilateral ischemia (5,6) as well as a dependence on the duration of the ischemia (3). In case of reperfusion usually the cytotoxic edema is accompanied by an increase in brain volume (brain swelling) with consequent vascular compression, causing a decline of cerebral perfusion and a secondary disturbance of the blood brain barrier (7). The sequela will be an overlapping vasogenic edema, which in most cases masks the primary (cytotoxic) edema. The edema itself aggravates the anoxic damage, thus giving rise to a vicious cycle. Simultaneous leukocyte–endothelial

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cell interaction within 10–20 min is accompanied by a release of toxic substances, especially the cytokines interleukin-1β, interleukin 6, tumor necrosis factor α, tumor necrosis factor β, and free radicals, leading to an emigration of leukocytes (8). The injury also triggers the development of a gliosis within 12–24 h, with a proliferation of astrocytes and their transformation into reactive astrocytes with abundant GFAP-positive cytoplasm (9).

On the subcellular level, acute ischemic neuronal injury is characterized by a mitochondrial alteration. Opening of the mitochondrial permeability transition pore (MPTP) can be triggered in postischemic neurons by a variety of molecular events occurring like ATP depletion, intracellular Ca^{2+} overload, and accumulation of free radicals. All of them are consequences of impairment of mitochondrial function and energy homeostasis of the neuron (10). Under ischemic conditions mitochondria lose their capacity for oxidative phosphorylation. The resulting loss of energy leads to the above-described electrolyte disturbances developing a cellular edema, i.e., a swelling of mitochondria. The sudden increase in extracellular K^+ is associated with a rapid increase of intracellular Ca^{2+}. Changes of intracellular Ca^{2+} play a crucial role in the destructive events of neurons (11), because abnormal levels of intramitochondrial Ca^{2+} alter the activity of the electron transport chain (ETC) complexes leading to an impairment of oxidative phosphorylation with reduced ATP levels. High levels of intramitochondrial Ca^{2+} can promote the release of cytochrome C, which can trigger the apoptotic cascade as well as the generation of free radicals, which can damage the proteins, lipids, and DNA of the cell (12).

Additionally, ischemic neuronal injury induces a release of large quantities of glutamate into the extracellular space of the brain (13,14), thus axon-sparing as well as selective dendritic lesions occur in neurons later destined to die in ischemia. It seems reasonable to conclude that a component of ischemic neuronal death is excitotoxic, with glutamate being the chief excitant. Moreover, global ischemia is an important stress factor of the brain and therefore the genes coding for heat shock proteins, such as hsp70 (15–17) and the immediate early gene response (c-fos, c-jun, junA, or junB) are switched on (18).

Delayed Neuronal Lesion

Relatively independent of this acute form of neuronal damage a delayed type of neuronal death is observed as not all neurons die immediately after a 5–10 min period of global ischemia, but within hours and days thereafter. Only after the primary ischemic insult itself has ceased, delayed neuronal death is set off due to a deleterious secondary process within the so-called recovery period. The phenomenon is associated with antiapoptotic growth factors, including insulin-like growth factor (19), vulnerability of microtubule-associated protein (MAP), and tubulin (20). Both those proteins are downregulated in neurons in the postischemic period possibly due to enhanced sensitivity of postischemic neurons to afferent stimuli, mitochondrial dysfunction, as well as postischemic alterations in calcium and glutamate homeostasis. Therefore, delayed neuronal death is the result of complex biochemical and molecular interactions.

Experiments give further evidence of a delayed neuronal damage, which can be observed especially in the CA1 sector of the hippocampus (Fig. 1) (1,17). A substantial number of hippocampal neurons is initially recovered more rapidly than any other structure of the brain, but the protein synthesis is secondarily suppressed after 12–24 h of recirculation. This may be explained by the fact of metabolic suppression being preceded by a phase of neuronal hyperexcitability (21).

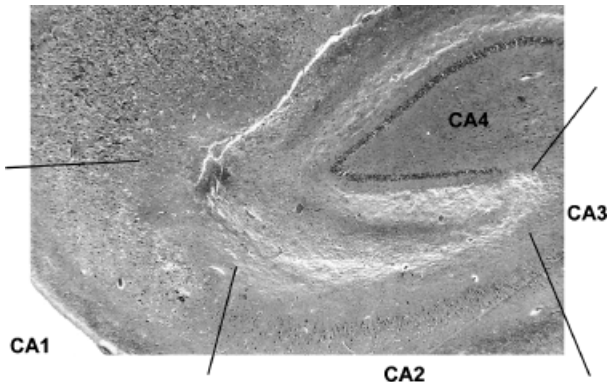

FIG. 1—*CA sectors (CA1–CA4) of the hippocampal area demonstrating a lack of neurons in the CA1 segment as a consequence of cardiac arrest and reperfusion for 6 days (MAP2, magnification × 50).*

Reperfusion

Considering molecular aspects in the reperfusion period a burst of free radicals occurs and causes oxidative damage to proteins, lipids, and DNA. The oxidative damage of the mitochondrial membranes triggers the release of cytochrome C and caspase 9, which leads to an activation of caspase 3, the main executioner of apoptosis. Among numerous rearrangements of DNA, especially of mitochondrial DNA (mtDNA), an accumulation of the 4977 bp deletion (common deletion) is observed in specific regions of the brain under conditions associated with ischemia (22,23). In a sophisticated study on single cells it has been observed that deletions of mtDNA were associated with focal ETC abnormalities. These may in part be explained by a 4977 bp deletion or other deleterious events which remove coding sequences of mtDNA including cytochrome oxidase (COX), ATPase, or complex I (22,24–26).

Time Dependency

Clinical experience has shown that reactivation of parts of the CNS function—or of parts of the brain—is only possible during the first 10 min of total cerebral ischemia under normothermic conditions, because nerve cells are extremely sensitive to any reduction in oxygen supply. Complete interruption of the blood and/or oxygen supply leads to time-dependent changes shown in Table 1 (27). Even though animal studies could demonstrate the reversibility of ischemic nerve cell damage, in normothermic humans irreversible brain death regularly occurs within 10 min of continuous anoxia. Only under hypothermic conditions (e.g., cardiac arrest after being buried by avalanches) as well as under pharmacological influences (e.g., intoxication by barbiturates) a neuro-

TABLE 1—*Clinical results of a cerebral oxygen deficit in dependence on the duration of the disruption of the oxygen supply (according to Grote (27)).*

5 s	Marked functional impairment of the CNS
8–12 s	Total loss of function of the CNS (loss of consciousness)
20–30 s	Formation of a flat EEG
8–10 min	Resuscitation time for the brain
4 min	Resuscitation time for the entire organism with asystolia

CNS, central nervous system.

protection occurs, which results in a reduction of brain metabolism and an increase of resuscitation time having been evaluated by Walpoth and colleagues (28,29) to be 141 ± 50 min in 15 patients after accidental deep hypothermia.

On the other hand, it has been shown in animal experiments that under normothermic conditions—even in primates—the neurons can be reactivated after 60 min of complete interruption of the brain circulation, i.e., a total ischemia (21,30). Recovery of neuronal function after total ischemia could be achieved by inducing an acute rise in blood pressure at the start of reperfusion and by balancing electrolytes, the high pH (alkalized blood), and blood gas values during reperfusion. Among the criteria confirming reactivation are incorporation of amino acids into nerve cells as well as the electrical activity of the cortex. These findings suggest that nerve cells themselves do not play the decisive role but rather other factors such as vessel wall lesions and/or a disturbance of the pH, electrolytes, and metabolites (3). Although this has not succeeded in humans up to now, it does give rise to hope for successful treatment in the future.

Brain Morphology

Transient Ischemia

Ischemic injuries are expressed in different brain areas depending on the type and extent of arterial supply (Fig. 2). They occur mainly in the marginal zones of arterial supply areas (end arteries = triple watershed zone; 1, 16). If ischemic damage is suspected, the fissure between the first and second gyrus of the frontal lobe, the CA1 region of the hippocampus, the Purkinje cell layer at the cerebellum, as well as corpus striatum or, specifically, the globus pallidus should be examined carefully. The distribution of the hypoxic damage can vary with the type of damage (31).

Long-term-survived ischemia with consecutive reperfusion is detectable by microscopic examination, when necrosis of neurons, patches of demyelinization, parenchymal loss, and a proliferation of microglia and astrocytes become visible. As indicated in Table 2 these changes follow a regular time course (17) (Fig. 3).

The common cytologic marker of an ischemic-injured neuron in case of reperfusion is the acidophilic/eosinophilic alteration of the cytoplasm and the shrunken nucleus, which can be demonstrated using hematoxylin and eosin (H&E) stain (Fig. 4*a*). When applying Nissl stain a swelling of mitochondria with cytoplasmic condensation occurs (Fig. 4*b*).

In recent years, an additional set of antibodies gives rise to the possibility of detecting the functional loss of nerve cells under microscopic examination. Among others, failure of immunolabeling of MAPs (see [20]), neuron-specific enolase (NSE), and α-tubulin (33,34), the stress proteins, and the immediate early genes as well as upregulation of TGF and its receptor will aid to detect hypoxic nerve cell injury as soon as possible (35,36).

Permanent Ischemia

If the entire blood supply to the brain is irreversibly stopped (lack of reperfusion—type: "hanging"), simultaneous respiratory arrest occurs. If there is no resuscitation, a mild increase in brain weight, congestive hyperemia (78%), edema equivalents (61%), and perivascular bleeding (50%) on 204 brains of hanging victims can be encountered (37).

Anoxia without brain reperfusion in cases that survived, i.e., cases with respirator, is more difficult to detect by morphological criteria. Prolonged intracranial circulatory arrest and/or increased intracranial pressure aggravated by vasogenic edema, finally lead to the clinical and morphological criteria of brain death. Within the first 24 h it can only be diagnosed by a massive increase in brain volume. During this period a demarcation of the optical nerve, the pituitary gland, and the cervical part of the medulla or cervical spinal cord can be discerned (38). Ultimately, signs of necrosis develop with a loss of nuclear staining (the so-called

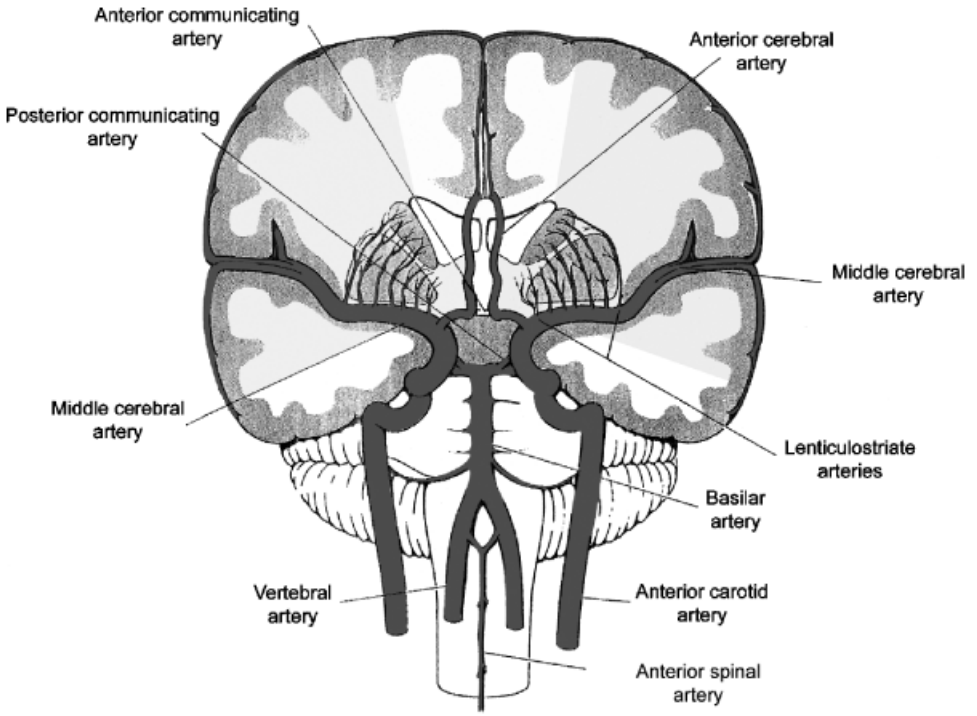

FIG. 2—*Arterial supply of the brain as graphically demonstrated on a cross-section of the cerebrum (according to Powers (32), modified).*

TABLE 2—*Sequence of microscopic changes in brain infarcts (according to Kalimo et al. (17)).*

>1 h	Microvacuoles within neurons (swollen mitochondria) followed by perineuronal vacuolation (swollen astrocytic processes)
4–12 h	Neuronal cytoplasm becomes increasingly eosinophilic, Nissl bodies disappear, nucleus becomes pyknotic and nucleoli are no longer visible. Blood–brain barrier begins to leak
15–24 h	Neutrophil leukocyte infiltration begins
2–3 days	Macrophages (foam cells) appear. In larger infarcts these cells may be present for months
5 days	Neutrophil leukocyte infiltration ceases
Around 1 week	Proliferation of astrocytes around the core infarct begins

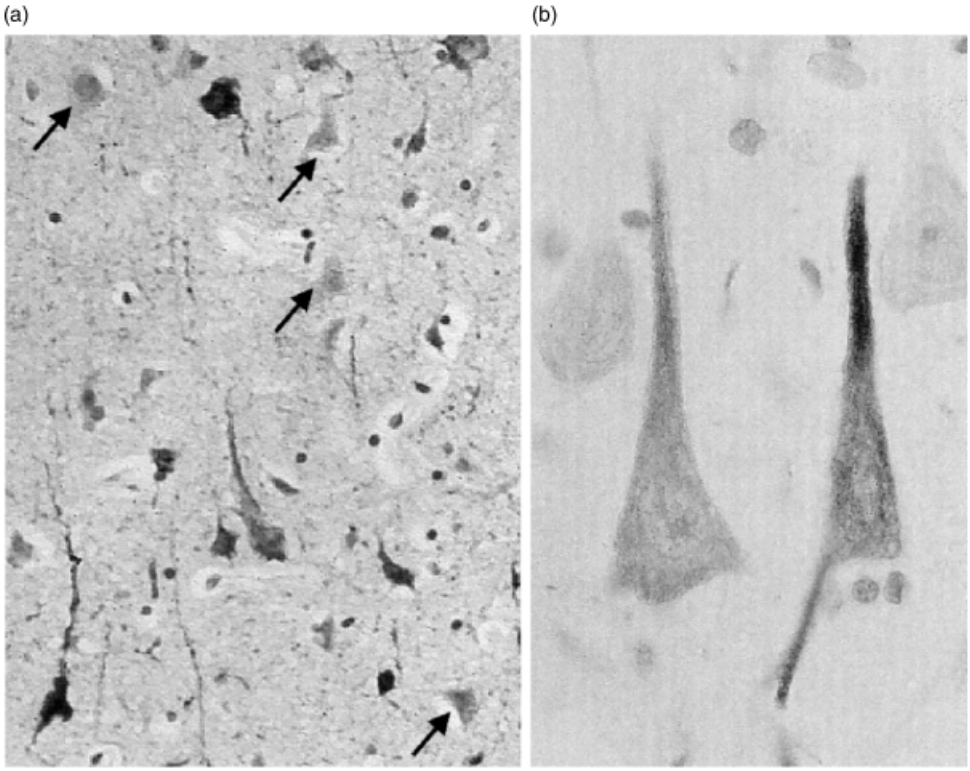

FIG. 3—*Pale type of neuronal injury as a result of permanent ischemia, which is characterized by a downregulation of microtubule-associated protein 2 (MAP2): beside MAP2 reactive neurons single MAP negative neurons (arrows) are seen in the frontal cortex of the brain (magnification (a) × 300; (b) × 1000).*

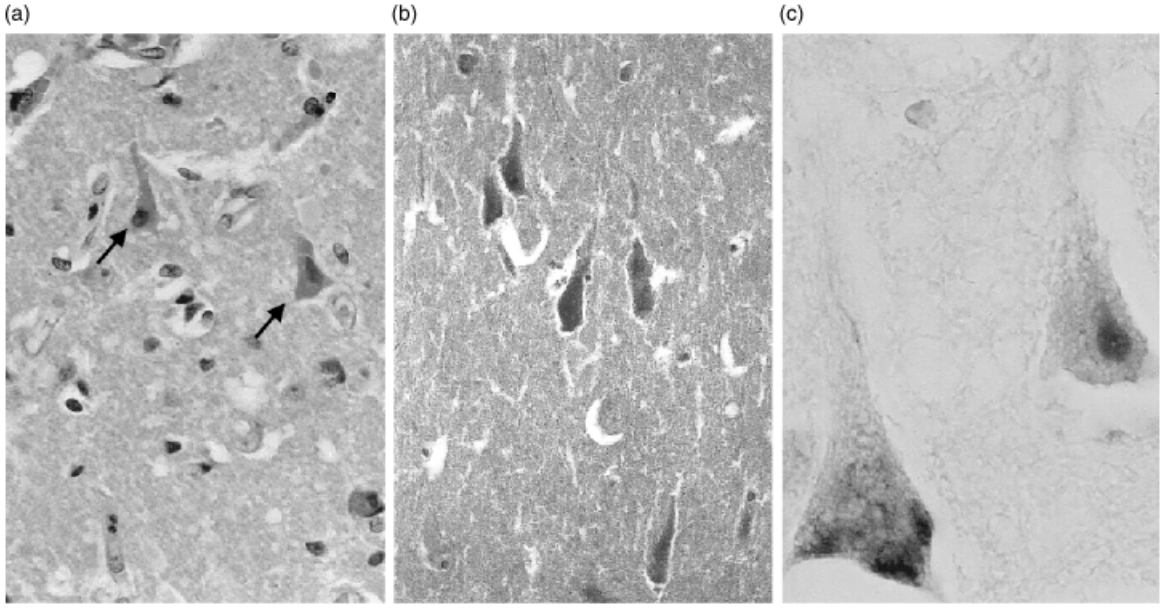

FIG. 4—*So-called dark type of ischemic neuronal alteration, as demonstrated (a) by hematoxylin and eosin stain (distinct eosinophilic color of the cytoplasm), (b) by Luxol fast blue (dark staining), and (c) by Nissl stain (microvacuolation of the cytoplasm) (magnification: (a, b) × 300; (c) × 1000).*

TABLE 3—*Neuronal alterations following different types of ischemia (sources: Kalimo et al. (39), Lipton 1999 (40)).*

Cytologic Alteration	Neuronal Changes			
	No Reperfusion: Pale Type	Reperfusion: Dark Type	Homogenizing Cell Change: Ghost Cells	Apoptosis
Neuronal structure	Round, swollen	Triangular	Triangular	Round
Nucleus	Slightly coarse chromatin	Marked condensed, irregular clumped chromatin	Irregular chromatin, clumping, somewhat shrunken	Smoothly contoured, spherical—or lunar crescent—shaped masses of uniformly condensed chromatin, apoptotic bodies
Cytoplasm	Mircotubules and other filamentous structures are absent	Markedly condensed, acidophilic; peripheral chromatolysis	Extremely pale, slightly shrunken with small vesicles	Dark and pyknotic, some vacuolation, not eosinophilic
Astrocytic edema	—	Perineuronal vacuoles, spongy neuropil	—	—
Expression time	Within 10–30 min (42) or within 3–4 h (43)	Within 30 min to 1 h (44) or within hours (45)	Within 24 hours (46)	Within 1 h (47) or 24 h (48) or days (49)
Cytological visualization	Downregulation of MAP2	H&E stain: eosinophilic	Nissl stain: loss of nisslbodies	Upregulation of caspase 3

H&E, hematoxylin and eosin.

"washed-out phenomenon"). The intravital autolysis of the brain is characterized by massive cerebral edema, a macroscopic dusky brown discoloration of all gray areas of the brain, and a loss of brain tissue consistency. Microscopy reveals congested vessels partly associated with intravascular precipitation of fibrin and subsequent loss of cellular staining in the absence of reactive changes.

Neuronal Alterations (Table 3)

Using Nissl stain a local or generalized *permanent ischemia without reperfusion* results in a so-called "pale nerve cell injury" (39,40), marked by intraneuronal and astrocyte edema, dilation of nuclear chromatin, and slight swelling of mitochondria and endoplasmatic reticulum as well as loss of expression of microtubule-associated protein (MAP see [20,41]) (Fig. 3). These cytological phenomena may be seen earliest after 10–30 min (41,42) or within 3–4 h (43) of survival time, in general only after several hours.

In cases of *global or only partial reperfusion* "dark neuronal damage" is detectable. This so-called "dark nerve cell injury" can be detected by H&E stain (eosinophilic alteration of the cytoplasm) as well as by Nissl staining due to condensation of cytoplasm and karyoplasm, excessive distension of mitochondria and swelling of perineural and perivascular astrocytic processes (Fig. 2), which is seen within 30 min up to 60 min (44), or within some hours (45). The differential diagnosis must consider so-called "dark neurons" (46,47), which can be caused, among other things, by postmortem mechanical lesions of the brain and are characterized by condensation of the chromatin and a reddish tinge of the cytoplasm in H&E-stained slides.

As a consequence of *reperfusion* likewise in cases of *permanent focal ischemia* homogenizing cell changes, so-called ghost neurons (Fig. 5), appear marked by an irregular chromatin clumping, a shrunken cytoplasm which may be fragmented with small vesicles and dense bodies (48,49). This type of cell change will arise about 24 h after the ischemic event (48) and 6–12 h after permanent ischemia (49).

Another phenomenon characteristic for reperfusion as well as for permanent focal ischemia is the *programed neuronal death*, i.e., *apoptosis*. This type of neuronal degeneration will appear in the penumbra of permanent lesions after one to several hours (50), 24 h after 2 h temporal occlusion (51), and several days after very short temporary lesions (52). Morphologically the cell change is characterized by uniformly condensed chromatin, apoptotic bodies, and darkened, pyknotic cytoplasm, as well as an activation of caspase 3 (53) (Fig. 6).

The extent of neuronal damage depends on the time course and the duration of the injury and reperfusion as well as the extent of pO_2 reduction, glucose and lactate levels, acidosis, brain temperature, and blood pressure. For forensic purposes, it is crucial to know the duration of the interval before ischemic damage described by Spielmeyer (54) can first be detected. The literature reports a wide variation, ranging from 7.5 min (55) to 3 h (54) up to a maximum of 15–25 h (31). These differences may be due—at least in part—to the factors mentioned above, which do indeed vary from case to case. With survival times amounting to hours, reactive and destructive changes will be observed in addition to

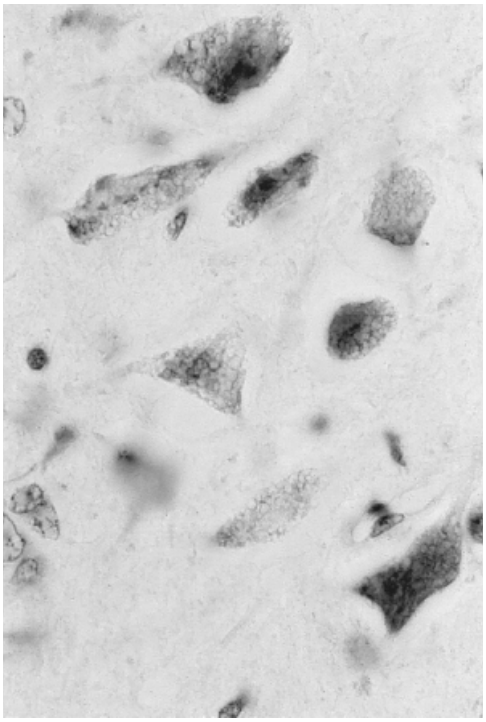

FIG. 5—*Ghost cells that are seen in case of reperfusion and permanent ischemia (Nissl stain, magnification* × *500).*

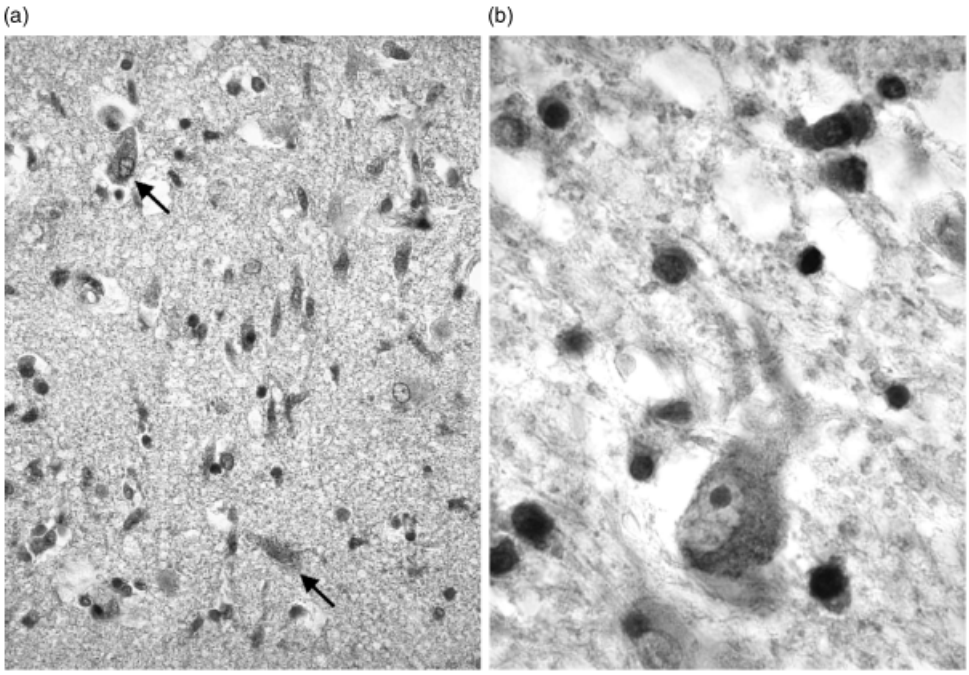

FIG. 6—*Apoptotic neuron as demonstrated by a distinct upregulation of caspase 3 (arrows; magnification: (a) × 300; (b) × 1000).*

ischemic neuronal changes. These changes allow among other phenomena a temporal classification of ischemic injury of unknown age for forensic purposes. Several authors have published tables including data on ischemic brain damage (56) and on trauma-induced cortical hemorrhages (57).

Morphological Sequelae

The cytological and histological consequences of global ischemia depend on the above-mentioned criteria as well as on the brain temperature and on pharmacological influences. The anatomical distribution of the neuronal injury is specific—as aforementioned—dependent on the vessel's anatomy, on the extent and duration of the perifocal edema and penumbra. If hypoxia/ischemia is prolonged, a selective parenchymal necrosis (selective neuronal necrosis) will develop, i.e., nerve cells die while the neuropil remains intact. In contrast, a strictly local ischemia, produces local tissue loss, a necrosis, and a so-called pseudocyst or cyst.

Regarding the specificity of ischemic nerve cell changes described here a similar characteristic morphological and topographical feature is also encountered in individuals with hypoglycemia or epilepsy (58). In contrast, however, hypoglycemia in animal experiments is not associated with neuronal degeneration in the cerebellar cortex, but the granule cells of the dentate gyrus develop necrosis and the neuronal necrosis in the cerebral cortex shows a superficial distribution (59). The reactive changes are nonspecific and can be observed in other types of brain injury.

Conclusion

To date there are only very few data on the very early neuropathological changes of acute anoxia/ischemia or hypoxia/hypoxemia. Microscopic findings on the brain, associated with death by hanging, are described by Jacob and colleagues (60–62); yet nowadays it is assumed that the observed nerve cell changes represent artifacts. These rare observations are opposed by an extensive literature (as well as own experiences) describing regularly structural neuronal changes, demonstrated by routine staining techniques, only after survival times of at least 3–7 h. From these data the conclusion can be drawn that early structural changes constitute only a questionable "proof" of anoxia when examining the vitality of nerve cells. The vitality and further morphological changes in the CNS depend on survival time and the course of the reactive alterations and may aid to classify the survival time of the event itself.

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