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The Temporal Evolution of Hypoglycemic Brain Damage

II. Light- and Electron-microscopic Findings in the Hippocampal Gyrus and Subiculum of the Rat*

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Summary. Part I of this paper has documented the evolution of dark neurons into acidophilic neurons in the superficial laminae as well as the reversion of dark neurons to normal neurons in the deep laminae of the cerebral cortex in hypoglycemic brain damage. The present study describes the temporal evolution of hypoglycemic brain damage in the hippocampus.

The evolution of dark neurons to acidophilic neurons was confirmed in this brain region. Four additional problems were addressed: Firstly, delayed neuronal death was looked for, and was found to occur in areas of CA1 undergoing mild damage. However, it was not preceded by a morphological free interval, had ultrastructural characteristics distinct from delayed neuronal death in ischemia, and hence should be considered a distinct phenomenon.

Secondly, the gradient in the density of neuronal necrosis in the rat hippocampal pyramidal cell band was exploited to test the hypothesis that a more severe insult causes a more rapid evolution of neuronal changes. This was found to be the case, with a temporal spectrum in the timing of neuronal death: Necrosis occurred already after 2 h medially in the subiculum, and was delayed by up to several weeks laterally in CA1.

Thirdly, the almost universal sparing of CA3 pyramidal neurons after 30 min hypoglycemic isoelectricity was exploited to address the question of whether reactive changes, which could with certainty be deemed reversible, occur in CA3. Mitochondrial injury was seen in these cells, and was found to be recoverable. No reactive changes of the type previously described following ischemic insults were observed.

Fourthly, the astrocytic and vascular response of the tissue was studied. A sequence of astrocytic changes representing structural and probably metabolic activation of astrocytes was seen, consisting of morphological indices of increased turnover of cellular components. Capillaries demonstrated endothelial pits, vesicles, and prominent microvilli hours to days after recovery.

The results demonstrate that, in the hippocampal gyrus as in other brain regions, hypoglycemic brain damage is distinct from ischemic brain damage and likely has a different pathogenesis.

Key words: Hypoglycemia – Hippocampus – Neuronal necrosis – Mitochondria – Astrocyte – Endothelial microvilli

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Introduction

It is well known that the hippocampal pyramidal cellls, especially those of the CA1 zone, are vulnerable to both ischemic and hypoglycemic insults [8, 13, 21–24, 29, 34, 35, 40]. In the gerbil, recent studies of reversible ischemia of short duration have shown that the different hippocampal neuronal types react differently to an ischemic insult [9, 23, 35]. Thus, whereas a certain proportion of CA4 cells die within the first 8–12 h of recovery, massive necrosis of CA1 neurons occurs first after a free interval of 1–3 days ("delayed neuronal death") [21–24]. The CA3 cells, in contrast, exhibit alterations resembling Nissl's re-

active cell change, or central chromatolysis, alterations which are largely reversible [9, 23]. Slow "maturation" of ischemic neuronal damage has been observed in the rat as well, and was shown to encompass both hippocampal and neocortical neurons [24, 29, 31].

As mentioned in the previous article [4], it has often been stated that ischemic and hypoglycemic brain damage are similar [6–8, 11, 33, 39]. Our results have shown, however, that this is not the case. Thus, when recovery was instituted for 1 week following isoelectric hypoglycemia of 10–60 min duration, localization of neuronal damage differed from that following ischemia [2, 3]. This also applies to the hippocampus. Neuronal necrosis in the dentate gyrus was conspicuous, with a novel localization to the crest of the gyrus. In the hippocampal gyrus proper, the CA1 and subicular neurons were found not to be equally susceptible along the pyramidal cell band, damage being mild to absent laterally near the border with CA3, progressing to more severe damage medially into the subiculum. Damage was virtually absent from CA3 after 30 min of isoelectricity.

The purpose of the present study was to assess the evolution of neuronal and tissue damage in the hippocampal pyramidal ribbon. The first question posed was whether delayed neuronal death, preceded by a free interval of hours or days, occurs following hypoglycemia as well as ischemia. Our second question concerned the rate of evolution of irreversible neuronal damage. The hypothesis has been advanced that the rate of evolution is proportional to the severity of the insult [17]. In studying this, we exploited the unique gradient of hypoglycemic damage along the subiculum – CA1 band, assuming that regional variations in the density of cell necrosis reflected corresponding differences in the severity of the insult. The third question studied was whether hypoglycemia, like ischemia, leads to transient changes of the central chromatolytic type or whether the CA3 cells fail to react to the insult both initially and following long-term recovery. Finally, given these regional differences in the density of neuronal necrosis, we posed the question whether reactive changes occurred in glial and vascular elements of the tissue and whether they relate to the density of neuronal necrosis.

To provide answers to all these questions, we examined tissue of the hippocampal gyrus and subiculum by light- and electron-microscopic techniques, using samples obtained during 10–60 min of hypoglycemic isoelectricity, and with recovery of a few minutes to weeks and months. The results provide additional evidence that hypoglycemic brain damage is unique, being distinctly different from that incurred following ischemia.

Material and Methods

Animal Model

A previous publication describes more completely the model used [2]. The first publication in this series [4] summarizes the procedures used in the present experiments, and the material presented here is derived from the same animals.

Forty-six rats were killed after delta wave EEG slowing only (control), and after 5, 10, 30, and 60 min of isoelectricity without recovery with glucose. After recovery with glucose, survival intervals were 5, 10, 30, and 60 min; 2, 3, 4, 6, 8, 12, 18, and 24 h; 2, 3, 5, 7, 14, 21, and 42 days; and 3, 6, and 12 months of recovery following either 30 or 60 min of cerebral isoelectricity. One or two rats were studied at each time interval.

Light- and Electron-microsopic Procedure

Perfusion fixation and histological processing was performed as reported in the previous article [4]. Brains were sectioned coronally into 1.8 mm thick slices, and one half of the brain was used for light microscopy in soft plastic polymer embedding medium (EFL-67, Serva Feinbiochemica, Heidelberg, FRG). Sections were cut at 1.5 μm and were double-stained with acid fuchsin and cresyl violet.

The other half of the brain was used for electron microsopy, the sampled areas being indicated in Fig. 1. The hippocampus was dissected, and five samples each of the medial subiculum and lateral CA1 cell band were taken. Five samples of the CA3 pyramidal cell band were taken roughly halfway between the hilus of the dentate gyrus and the border with CA1. The stratum radiatum of the subiculum was sampled as well. A detailed study of the dentate gyrus will be reported separately [5]. After processing in ascending concentrations of ethanol and embedding in Epon 812, thick sections were prepared from each area at each time interval, and stained with toluidine blue. After thin sectioning and staining with uranyl acetate and lead citrate, sections were viewed on a JEOL JEM 100C electron microscope.

Results

Animal Model

Results pertaining to the animal model have been published in detail previously [2] and are summarized in the previous article [4].

Subiculum

After 30 min of isoelectricity, light and dark cells were present, as described previously in the cerebral cortex [1, 4, 20]. Swollen mitochondria with fractured cristae were seen in both dark neurons and neurons of normal electron density. Because of the previous suggestion of a CSF-borne neurotoxic substance mediating neuronal necrosis due to hypoglycemic isoelectricity, the apical dendrites of the stratum radiatum subjacent to the pia mater were examined from the area indicated in Fig. 1. The dendrites were segmentally swollen (Fig. 2). Already after 10 min of isoelectricity, damaged mitochondria were seen in the dendritic processes between the pial surface and the perikaryon.

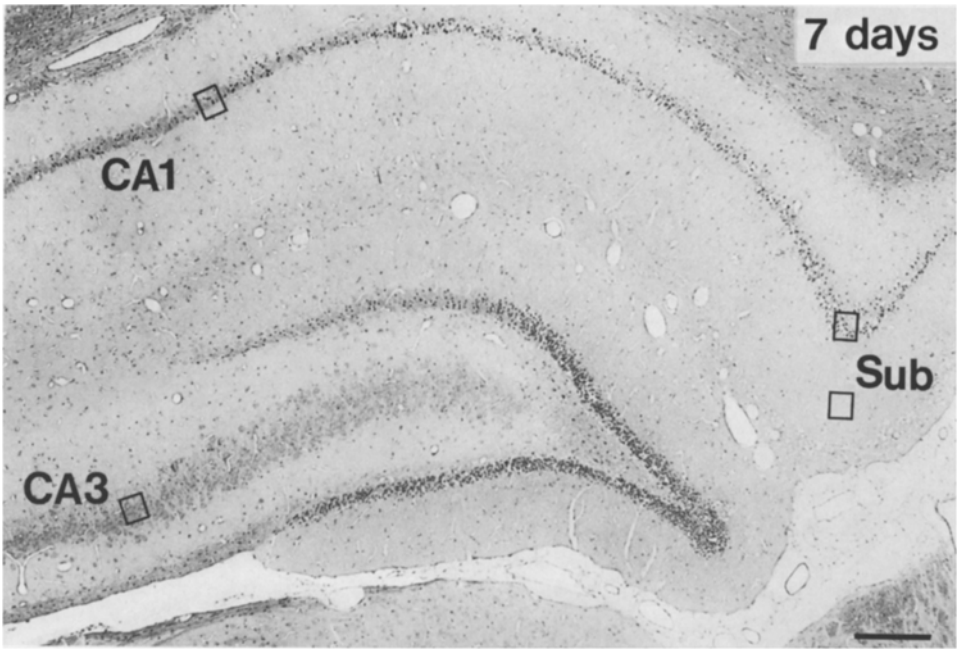

Fig. 1. Overview of the hippocampus 1 week after 60 min of hypoglycemic isoelectricity, with the areas taken for electron microscopy indicated *in boxes* (□). There is dense neuronal necrosis in the medial portion of the subiculum, with a tapering density of CA1 neuronal necrosis laterally. No CA3 damage is apparent. Acid fuchsin/Cresyl violet. Bar: 200 μm

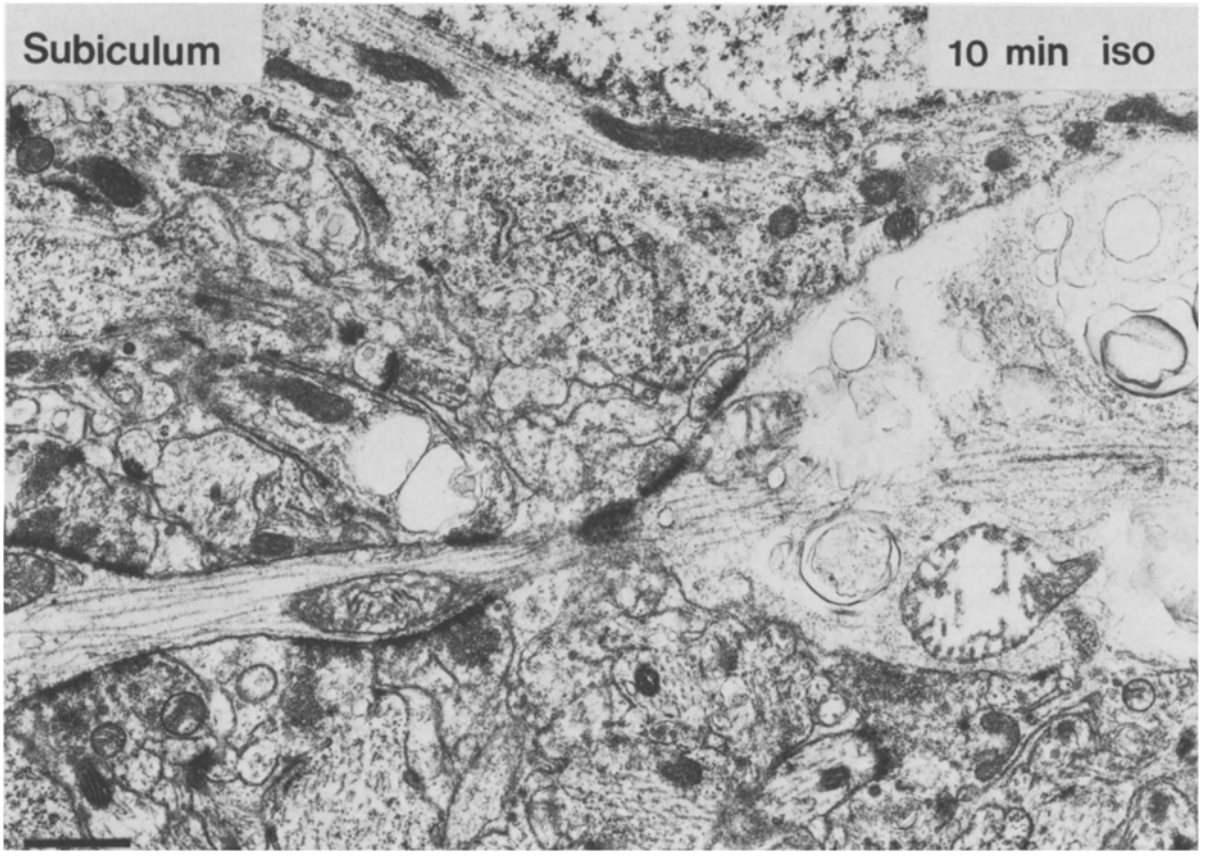

Fig. 2. Subiculum after 10 min of isoelectricity. Dendrites of the stratum radiatum are segmentally swollen and contain mitochondria with disordered cristae and varying degrees of swelling. The neuronal perikarya have not yet undergone dark cell transformation at this time. Bar: 1 μm

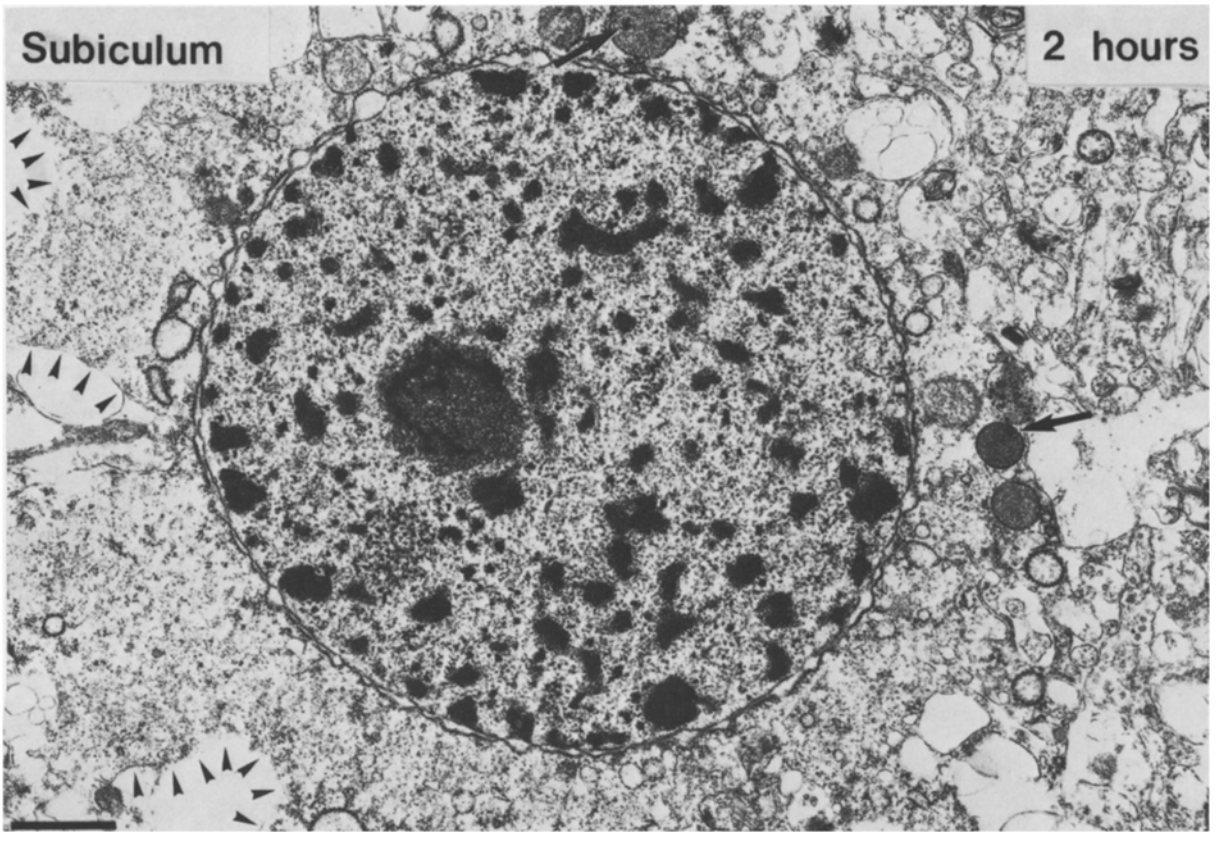

Fig. 3. Subiculum 2 h after 60 min of isoelectricity. The characteristic coarse nuclear stippling of chromatin has appeared, along with flocculent densities in the mitochondria and rupture of the plasma membrane. These features together indicate neuronal necrosis. Bar: 1 μm

These mitochondria showed fractured cristae and were often swollen (Fig. 2).

Already after 2 h, a transition to acidophilic neurons had occurred. By electron microscopy, these showed the ultrastructural features of cell death, consisting of mitochondrial dense deposits, amorphous cytoplasm, and rupture of the cell membrane, or cytorrhexis (Fig. 3). After survival intervals longer than 2 h, little further change was seen in the light- or electron-microscopic appearance of the acidophilic neurons, until their removal from the tissue by simple cytolysis or through engulfment by macrophages. Neuronal cytolysis in the subiculum was well underway at 1 week, in contrast to the events seen concurrently in the CA1 neurons (Fig. 4).

CA1

A slower evolution of events was seen in the CA1 pyramidal cells as compared to the subiculum, especially in the more lateral samples near the border with CA3. Medially, nearer the subiculum, the density and time course of neuronal necrosis in CA1 resembled those in the subiculum: During isoelectricity, dark and light neurons were present, the former transforming into acidophilic neurons between 3 h and 1 day of recovery.

Between 1 and 3 weeks, however, neurons were still present in the lateral protion of CA1 which showed preserved cellular outlines with tinctorial changes (Fig. 4). Neurons here showed an affinity for both acid and basic dyes, imparting to them a purple hue. These amphophilic neurons had preserved cellular outlines for 1 to 2 weeks (Fig. 4), but at 3 weeks they appeared shrunken and homogeneous.

Electron microscopy of neurons from these lateral locations revealed neuronal forms which did not resemble the electron-microscopic appearance of the acidophilic neurons. Thus, rather than the coarse stippled chromatin, amorphous cytoplasm, mitochondrial dense deposits, and ruptured cell membrane of the acidophilic neurons, these amphophilic neurons demonstrated homogeneous nuclei, cytoplasm containing recognizable organelles, mitochondria without dense deposits, and preserved cell membranes (Fig. 5, top). The endoplasmic reticulum of these cells tended

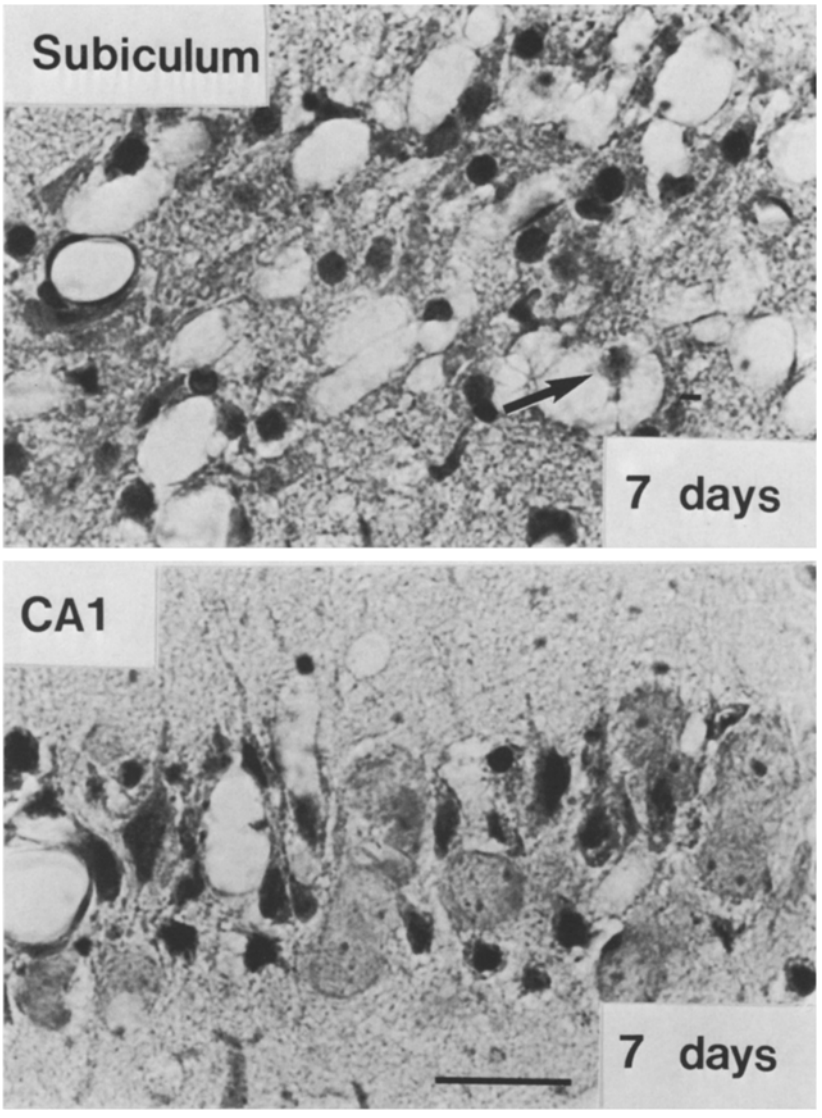

Fig. 4. Comparison of the rate of neuronal cytolysis in the subiculum and CA1 at 1 week. Cytolysis of acidophilic neurons is well advanced in the subiculum, leaving vacuoles in place of perikarya undergoing dissolution (*arrow*). In the lateral portion of CA1, however, many cells still show preserved nuclear and cell outlines (*arrow*) and have an affinity for both acid and basic dyes (amphophilia). Acid fuchsin/ Cresyl violet. Bar: 50 μm

to be dilated, as did the Golgi apparatus, with a general increase in electron density. Cell membrane breaks finally did appear, heralding cytorrhexis (Fig. 5, bottom).

CA3

The pyramidal cells of CA3 revealed little evidence of even evanescent damage by light microscopy.

By electron microscopy, low-power examination revealed an apparent paucity of findings in the CA3 neurons, with only dark degeneration seen in the occasional intervening mossy fiber axons of the dentate gyrus (Fig. 6, top). However, closer examination of the cytoplasm of CA3 pyramidal cells revealed mitochondrial damage (Fig. 6, bottom), with fractured cristae and various degrees of swelling. These changes disappeared by 1 week.

Astrocytic Changes

The sequential changes in the astrocytes of CA1 resembled those of the cerebral cortex [4]. At early times, e. g., following 12 h recovery, astrocytes showed watery cytoplasm and numerous mitochondria (Fig. 7, top).

By 1 week, densely fibrillated astrocytes were seen without intervening cell membranes between nuclei (Fig. 7, middle). Numerous degenerating mitochondria were seen with a homogeneous interior, containing only remnants of cristae. Multiple Golgi complexes with irregular dilatation of the cisternae were seen (Fig. 7, middle).

At 3 weeks, there were numerous effete mitochondria and residual bodies in an enlarged cytoplasm. Endoplasmic reticulum and glial fibrils were prominent (Fig. 7, bottom).

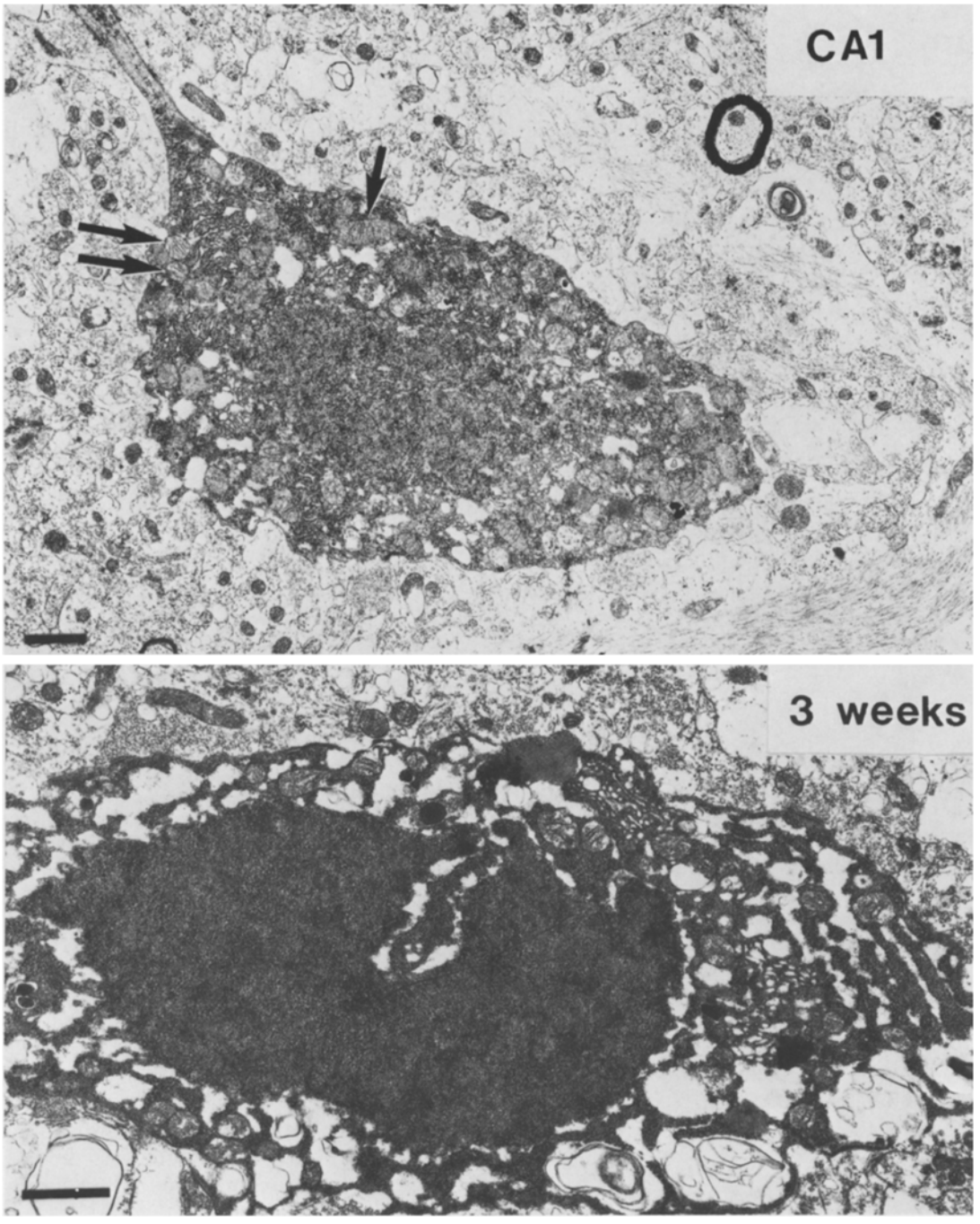

Fig. 5. Laterally placed CA1 neurons, 3 weeks after 30 min of isoelectricity. There is a generalized increase in electron density of the cells, and nuclear changes exceed cytochplasmic changes. The nuclei are amorphous, and completely devoid of any chromatin pattern. The upper neuron is remarkably well preserved. There is mild dilatation of the endoplasmic reticulum, but mitochondria contain discernible cristae and do not contain flocculent densities (*arrows*). An adjacent neuron at a slightly more advanced stage of cell breakdown (lower) shows greater dilatation of the endoplasmic reticulum, but flocculent densities are notably still absent from mitochondria. Cytorrhexis is beginning at the lower right. Bar: 2 μm

Blood Vessels

The capillaries of the CA1 zone showed changes resembling those seen in the cerebral cortex [4], consisting of prominent endothelial pits and vesicles in the cytoplasm. Numerous long and branching microvilli were seen, often projecting a considerable distance into the lumen of endothelial cells (Fig. 8). This appearance persisted for the first few days after the

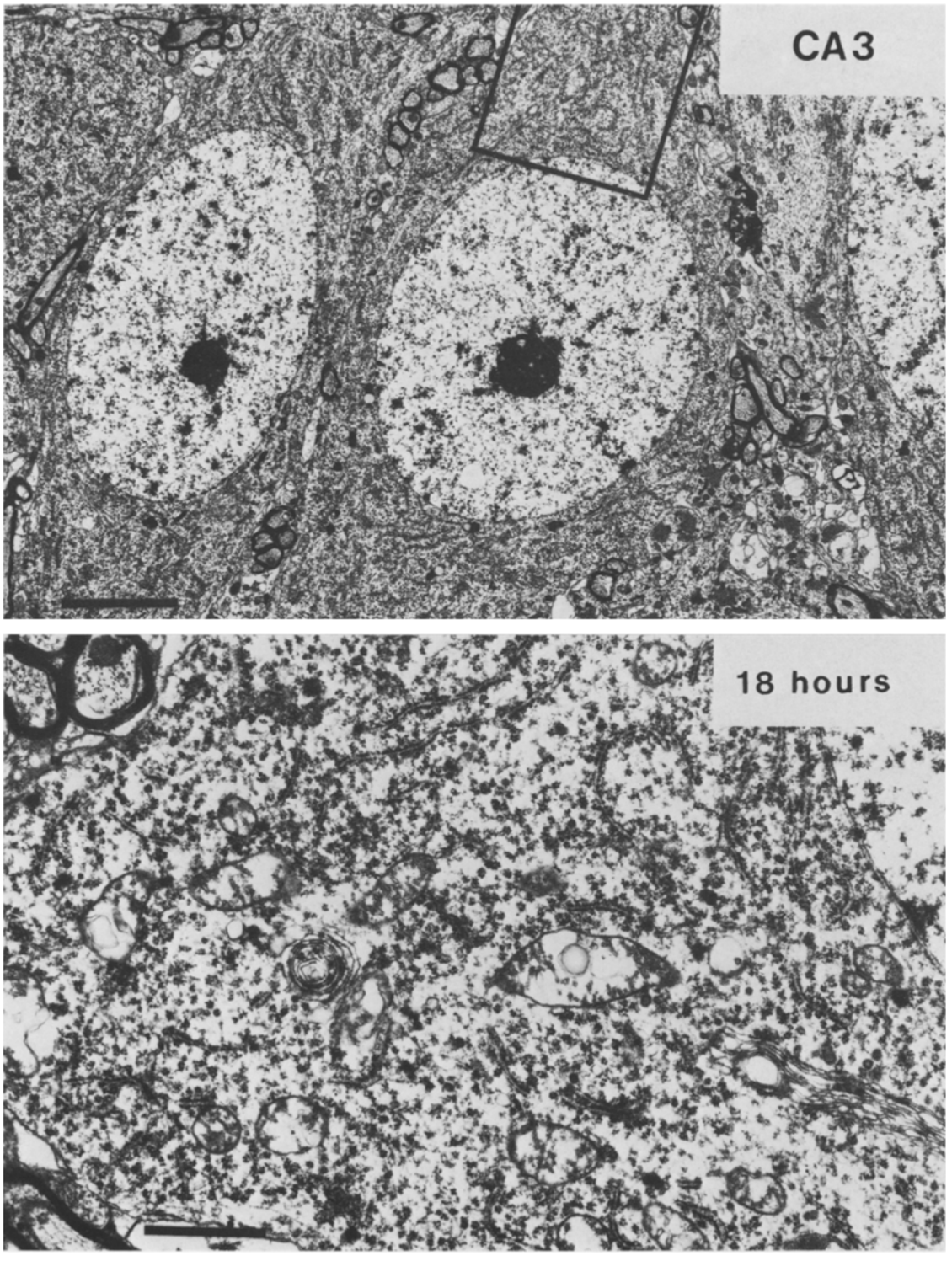

Fig. 6. A row of CA3 pyramidal neurons, 18 h after 30 min of isoelectricity, show apparently little change at low magnification, although mossy fiber axons undergoing dark degeneration are seen (*upper*). A higher magnification (*lower*) of the area of neuronal cytoplasm indicated *in box* (□), however, reveals grossly disturbed mitochondrial architecture, with fractured cristae and varying degrees of swelling. Bars: 4 μm (*upper*), and 1 μm (*lower*)

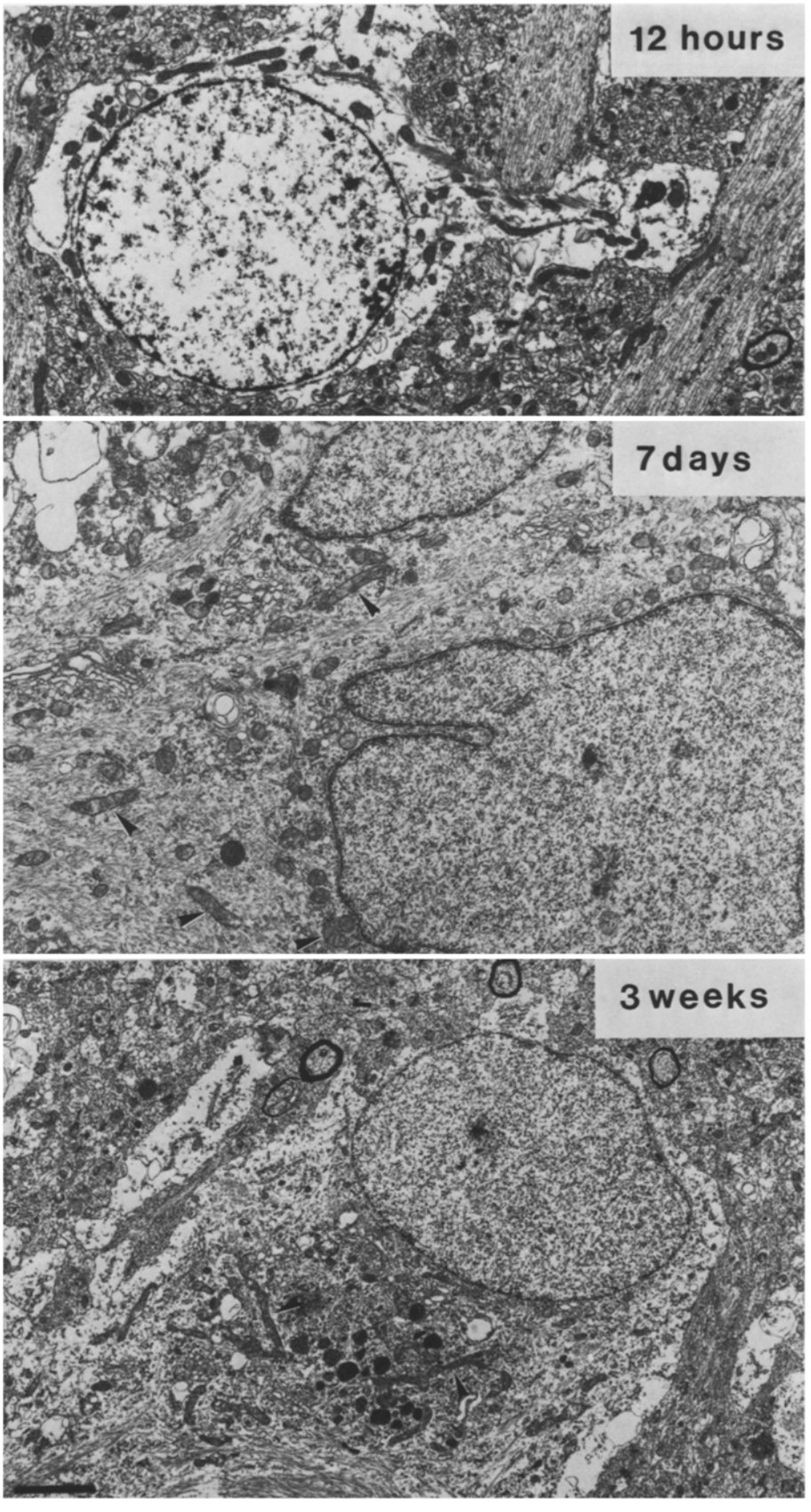

Fig. 7

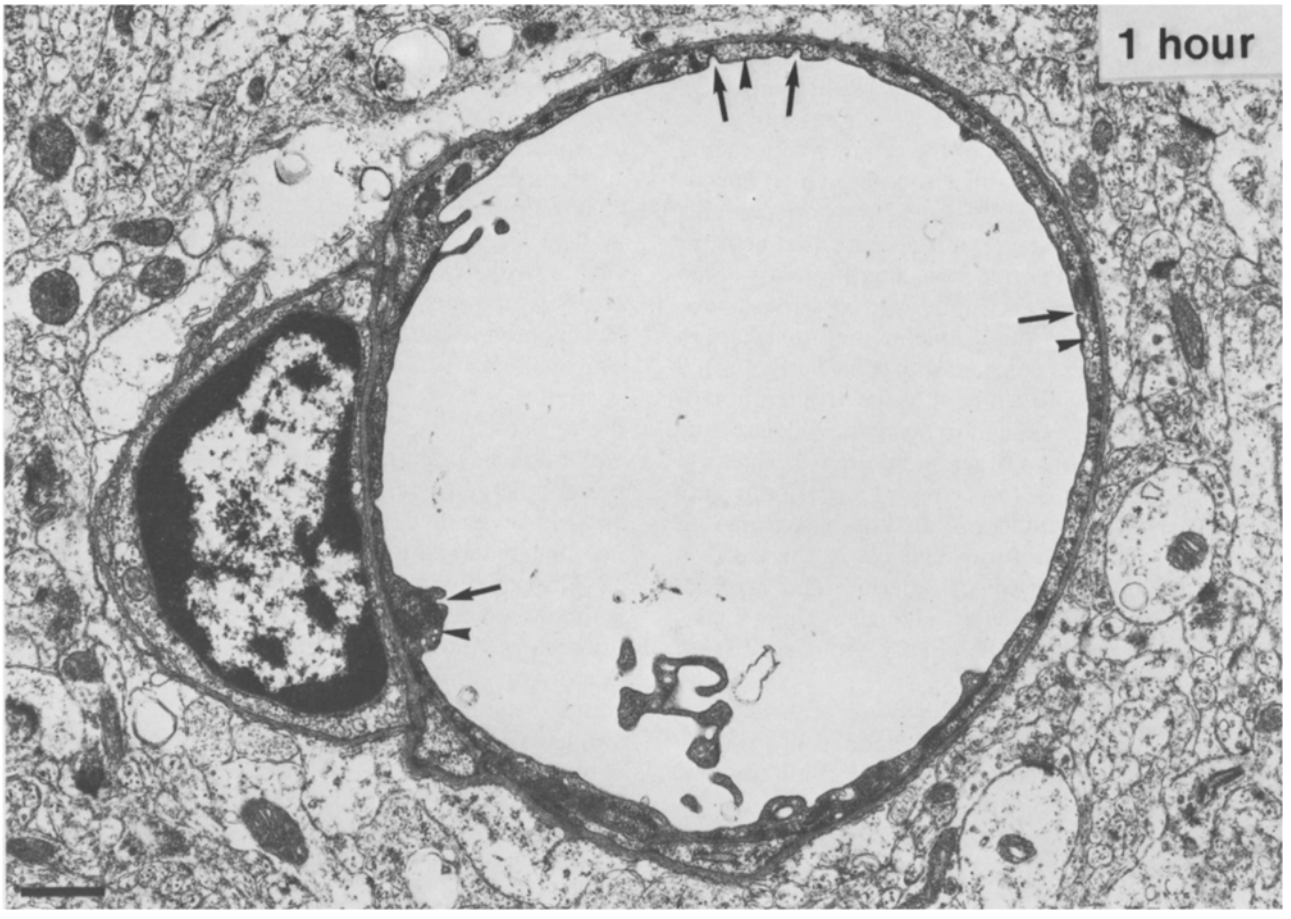

Fig. 8. After 1 h of recovery, hippocampal and neocortical capillaries demonstrate numerous microvilli, which often branch and many of which project a considerable distance into the lumen. Endothelial pits (*arrows*) and vesicles (*arrowheads*) are prominent. Bar: 1 μm

insult, and was more prominent after 60 min isoelectricity than after 30 min isoelectricity.

Discussion

The present findings confirm those of the previous paper that acidophilic neurons evolve through time via a transformation from dark neurons [4]. This evolution, as seen most clearly in the medial CA1 and subicular neurons, represents the transformation of a potentially reversible cellular injury into an irreversible one. Discussion of this topic can be found in the previous paper [4].

We shall discuss the results presented above in relation to the questions outlined in the Introduction.

Delayed Neuronal Death

In the gerbil, it has now been well established that following short periods of ischemia, from 3 to 5 min, neuronal death in the CA1 pyramidal cells occurs following an interval where morphological abnormalities are minimal to absent [21–24, 35, 36]. The neuronal cytolysis occurs abruptly after this morphological "free interval", with rapid removal of neuronal perikarya from the neuropil [21, 22].

Fig. 7. Sequence of astrocytic changes seen in both the neocortex and CA1 zone of hippocampus. After 12 h recovery following 30 min of isoelectricity, the astrocytes show numerous elongated mitochondria in an expanded watery cytoplasm. At 1 week of recovery, astrocytes with no intervening cell membranes are seen. There is cytoplasmic hypertrophy with fibril accumulation and an increase in cellular organelles. Numerous dilated cisternae of Golgi apparatus are seen. Effete mitochondria are numerous (*arrowheads*). After 3 weeks of recovery, residual bodies and lysosomes are now more numerous in the central cytoplasm of this hypertrophic astrocyte. Glial fibrils abound in the peripheral cytoplasm. Bar: 4 μm

The disappearance of CA1 neuronal perikarya from the neuropil in lateral locations near the border with CA3 was found to be delayed in the present study. Thus, cytolysis occurred after 1–6 weeks in these locations, and the outlines of the cell membranes and cellular organelles were still visible in many neurons after 3 weeks. However, we found no evidence of a morphological free interval in the sense that neurons with configurational or tinctorial alterations appeared during recovery. Furthermore, the electron-microscopic appearance of these neurons reported here in hypoglycemia differs considerably from that reported in ischemia [22, 23]. In contrast to the proliferation of rough endoplasmic reticulum seen in ischemia, the present results in hypoglycemia showed the greatest change in the nucleus, consisting of amorphous gray nucleoplasm with total loss of discernable chromatin, accompanied by a uniform increase in the electron density of nucleus and cytoplasm. This distinct appearance, with the greatest change in the nucleus, may indicate a nuclear rather than a cytoplasmic death in these cells.

Hence, although neuronal cytolysis occurred after a delay in the lattermost CA1 pyramidal neurons, both the time of occurrence and the elelctron-microscopic appearance of the cell damage were sufficiently different to distinguish these two forms of delayed neuronal death.

Temporal Evolution of Neuronal Damage

In ischemia, the hypothesis has been advanced that the rate of evolution of neuronal damage is proportional to the severity of the insult [17]. Subsequent results obtained thus far have borne out the validity of this hypothesis in ischemia in the CA1 sector of the gerbil [21, 22]. In hypoglycemia in the rat, hippocampal damage in the CA1 pyramidal cell band is more severe medially, marked by denser selective neuronal necrosis in the subiculum and adjacent CA1. There is a lessening gradient of neuronal necrosis toward the border with CA3. The present model of hypoglycemia thus offered a situation which could be exploited to test this hypothesis in this same neuroanatomic area in the rat in hypoglycemia. This variation in the density of neuronal damage and in the time course of neuronal death along the hippocampal pyramidal ribbon cannot be explained by the neurochemical organization of this structure [38].

The present results have demonstrated that indeed, in the medial subiculum, especially the portion exposed to the subarachnoid space, evolution of dark neurons to acidophilic neurons occurs extremely rapidly, within 2 h. The electron-microscopic appearance of the neurons at 2 h fulfils previously established criteria for neuronal death, consisting of flocculent densities in the mitochondria [14, 18, 19, 37], rupture of the nuclear and cytoplasmic membranes, and a characteristic coarse stippling of the nuclear chromatin [4]. This electron-microscopic appearance corresponds to the acidophilic neurons of light microscopy [4].

The predominantly nuclear changes outlined above, in the neurons undergoing delayed neuronal cytolysis, contrast with the preponderance of cytoplasmic abnormalities seen in the classic acidophilic neuronal necrosis seen more medially. Hence, the possibility is raised that the lethal injury may occur in the nucleus in the delayed form of neuronal death, and in the cytoplasm or cell membrane in classic acidophilic neuronal necrosis. Cell membranes were notably intact even at 3 weeks in the lateral CA1 neurons undergoing delayed degeneration.

The examination of the portion of the subpial subiculum was undertaken specifically with the previouly outlined hypothesis in mind of a CSF-borne neurotoxin causing cell damage in hypoglycemia accompanied by isoelectricity [3]. The segmentally swollen dendrites, containing swollen mitochondria with disrupted cristae were absent in control material. These dendritic abnormalities appeared in the stratum radiatum already after 10 min of cerebral isoelectricity. The mitochondria seen resembled those seen in the perikarya of cerebral cortical and hippocampal pyramidal neurons during and after hypoglycemic isoelectricity, and represent, as discussed in the previous article, a non-specific response to injury [4].

Reactive Changes in CA3

Here, the changes observed after 30 min of isoelectricity can be safely regarded as reversible, since neuronal necrosis is virtually absent in the present model after this time interval [2]. Even after 60 min, only CA3 pyramidal cells near the ventricles in animals with hydrocephalus are damaged [3].

In confirmation of this, no neuronal necrosis was seen in these cells in the present study. However, mitochondrial injury was present in the cytoplasm of numerous CA3 cells, even after only 30 min isoelectricity. The absence of neuronal necrosis in this region of the brain after this dose of insult, and the failure of these changes to evolve into neuronal necrosis in the present study, indicate the reversibility of this degree of mitochondrial injury in these neurones. The non-lethal nature of such mitochondrial neuronal damage has been previously established for CA3 neurons in ischemia [29], and in neuronal types other than CA3 pyramidal cells in both ischemia [28] and hypoglycemia [4].

Astrocytes

The sequence of alterations in the astrocytes of CA1 was identical to those previously alluded to in the superficial cerebral cortex [4]. A similar astrocytic response has been reported in ischemia [27]. The proliferation of astrocytic mitochondria after several hours suggests a reactive response of the astrocyte requiring increased energy production. It was not due to neuronal necrosis, since such astrocytes appeared in areas devoid of dead or dying neurons.

By 1 week, numerous degenerate mitochondria were seen, with a homogeneous interior lacking the normal cristae, or showing only remnants of cristae. These effete mitochondria were interpreted to be derived from the numerous mitochondria seen after several hours of recovery, and could be seen evolving from them at intermediate time intervals. The Golgi apparatus became more prominent and numerous than usual, accompanied by synthesis of cytoplasmic fibrils.

By 3 weeks, there were considerable aggregations of residual bodies in the cytoplasm, with some effete mitochondria and intermediate forms remaining.

In view of the evolution of these events in the astrocytic cytoplasm, this sequence is interpreted as a response to demands for increased energy production in the astrocyte, associated with a reactive process causing increased mitochondrial turnover. Cultured astrocytes have been shown to degrade ingested mitochondria into cytoplasmic residual bodies by 2 weeks [10]. Indeed, the cytoplasmic appearance of the astrocytes at 3 weeks is quite similar to that pictured in reactive neuronal cytoplasm of CA3 neurons, 3 weeks following ischemia [9].

Blood Vessels

The endothelial response in the CA1 zone was similar to that seen in the cerebral cortex in this model [4], with the formation of endothelial pits, vesicles, and branching microvilli. Such changes were confined to the capillary portion of the microcirculation, and were not seen in arterioles or venules.

Cerebral capillary endothelial cells normally have few vesicles or microvilli [12, 25, 26, 32], these structures perhaps indicating increased vascular permeability [25]. Cerebral endothelial microvilli have also been reported after ischemia [12, 16], in hypertension [15], and in traumatic brain injury [30], and hence must be considered a nonspecific response to injury. Still, the results demonstrate that hypoglycemia accompanied by isoelectricity leads to alterations of glial cells and capillaries as well as neurons.

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References

1. Agardh C-D, Kalimo H, Olsson Y, Siesjö BK (1980) Hypoglycemic brain injury. I. Metabolic and light-microscopic findings in rat cerebral cortex during profound insulin-induced hypoglycemia and in the recovery period following glucose administration. Acta Neuropathol (Berl) 50:31–41
2. Auer RN, Olsson Y, Siesjö BK (1984) Hypoglycemic brain injury in the rat: Correlation of density of brain damage with the EEG isoelectric time. A quantitative study. Diabetes 33:1090–1098
3. Auer RN, Wieloch T, Olsson Y, Siesjö BK (1984) The distribution of hypoglycemic brain damage. Acta Neuropathol (Berl) 64:177–191
4. Auer RN, Kalimo H, Olsson Y, Siesjö BK (1985) The temporal evolution of hypoglycemic brain damage. I. Light- and electron-microscopic findings in the rat cerebral cortex. Acta Neuropathol (Berl) 67:13–24
5. Auer RN, Kalimo H, Olsson Y, Wieloch T (1985) The dentate gyrus in hypoglycemia. Pathology implicating excitotoxin mediated neuronal necrosis. Acta Neuropathol (Berl) (submitted)
6. Brierley JB, Brown AW, Meldrum BS (1971) The nature and time course of the neuronal alterations resulting from oligaemia and hypoglycemia in the brain of *Macaca mulatta*. Brain Res 25:483–499
7. Brierley JB, Meldrum BS, Brown AW (1973) The threshold and neuropathology of cerebral "anoxic-ischemic" cell change. Arch Neurol 29:367–374
8. Brierley JB (1976) Cerebral Hypoxia. In: Greenfields Neuropathology, 3rd edn, chapt 2. Arnold, London, pp 43–85
9. Bubis JJ, Fujimoto T, Ito U, Mrsulja B, Spatz M, Klatzo I (1976) Experimental cerebral ischemia in Mongolian gerbils. V. Ultrastructural changes in H3 sector of the hippocampus. Acta Neuropathol (Berl) 36:285–294
10. Collins VP, Arborgh B, Brunk U, Schellens JPM (1980) Phagocytosis and degradation of rat liver mitochondria by cultivated human glial cells. Lab Invest 42:209–216
11. Courville CB (1957) Late cerebral changes incident to severe hypoglycemia (insulin shock). Their relation to cerebral anoxia. Arch Neurol Psychiatry 78:1–14
12. Dietrich WD, Busto RS, Ginsberg MD (1984) Cerebral endothelial microvilli: Formation following global forebrain ischemia. J Neuropathol Exp Neurol 43:72–83
13. Finley KH, Brenner C (1941) Histologic evidence of damage to the brain in monkeys treated with Metrazol and insulin. Arch Neurol Psychiat 45:403–438
14. Garcia JH, Kamijyo Y, Kalimo H, Tanaka J, Viloria JE, Trump BF (1975) Cerebral ischemia. The early structural changes and correlation of these abnormalitites with known metabolic and dynamic abnormalities. In: Whisnant J, Sandok BA (eds) Ninth conference, cerebral vascular diseases. Grune & Stratton, New York, pp 313–323
15. Hazama F, Ozaki T, Amano S (1979) Scanning electron-microscopic study of endothelial cells of cerebral arteries from spontaneously hypertensive rats. Stroke 10:245–252
16. Hills CP (1964) Ultrastructural changes in the capillary bed of the rat cerebral cortex in anoxic-ischemic brain lesions. Am J Pathol 44:531–543

17. Ito U, Spatz M, Walker JT, Jr, Klatzo I (1975) Experimental cerebral ischemia in Mongolian gerbils. I. Light-microscopic observations. Acta Neuropathol (Berl) 32:209–223
18. Jennings RB, Shen AC, Hill ML, Ganote CE, Herdson PB (1978) Mitochondrial matrix densities in myocardial ischemia and autolysis. Exp Mol Pathol 29:55–65
19. Kalimo H, Garcia JH, Kamijyo Y, Tanaka J, Trump BF (1977) The ultrastructure of brain death. II. Electron microscopy of feline cortex after complete ischemia. Virchows Arch [Cell Pathol] 25:207–220
20. Kalimo H, Agardh C-D, Olsson Y, Siesjö BK (1980) Hypoglycemic brain injury. II. Electron-microscopic findings in rat cerebral cortical neurons during profound insulin-induced hypoglycemia and in the recovery period following glucose administration. Acta Neuropathol (Berl) 50:43–52
21. Kirino T, Sano K (1984) Selective vulnerability in the gerbil hippocampus following transient ischemia. Acta Neuropathol (Berl) 62:201–208
22. Kirino T, Sano K (1984) Fine structural nature of delayed neuronal death following ischemia in the gerbil hippocampus. Acta Neuropathol (Berl) 62:209–218
23. Kirino T (1982) Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 239:57–69
24. Kirino T, Tamura A, Sano K (1984) Delayed neuronal death in the rat hippocampus following transient forebrain ischemia. Acta Neuropathol (Berl) 64:139–147
25. Palade GE, Simionescu M, Simionescu N (1979) Structural aspects of the permeability of the microvascular endothelium. Acta Physiol Scand [Suppl] 463:11–32
26. Peters A, Palay SL, Webster H de F (1976) The fine structure of the nervous system. The neurons and supporting cells. Saunders, Philadelphia London Toronto, pp 296–302
27. Petito CK, Babiak T (1982) Early proliferative changes in astrocytes in postischemic noninfarcted brain. Ann Neurol 11:510–518
28. Petito CK, Pulsinelli WA (1984) Sequential development of reversible and irreversible neuronal damage following cerebral ischemia. J Neuropathol Exp Neurol 43:141–153
29. Petito CK, Pulsinelli WA (1984) Delayed neuronal recovery and neuronal death in rat hippocampus following severe cerebral ischemia: possible relationship to abnormalities in neuronal processes. J Cerebr Blood Flow Metabol 4:194–205
30. Povlishock JT, Becker DP, Sullivan HG, Miller JD (1978) Vascular permeability alterations to horseradish peroxidase in experimental brain injury. Brain Res 153:223–239
31. Pulsinelli WA, Brierley JB, Plum F (1982) Temperal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol 11:491–498
32. Reese TS, Karnovsky MJ (1967) Fine structural localization of a blood-brain barrier to exogenous peroxidase. J Cell Biol 34:207–217
33. Richardson JC, Chambers RA, Heywood PM (1959) Encephalopathies of anoxia and hypoglycemia. Arch Neurol 1:178–190
34. Stief S, Tokay L (1932) Beitrag zur Histopathologie der experimentellen Insulinvergiftung. Z Ges Neurol Psychiat 139:434–461
35. Suzuki R, Yamaguchi T, Kirino T, Orzi R, Klatzo I (1983) The effects of 5-minute ischemia in Mongolian gerbils. I. Blood-brain barrier, cerebral blood flow, and local cerebral glucose utilization changes. Acta Neuropathol (Berl) 60:207–216
36. Suzuki R, Yamaguchi T, Li C-L, Klatzo I (1983) The effects of 5 minute ischemia in Mongolian gerbils. II. Changes of spontaneous neuronal activity in the cerebral cortex and CA1 sector of the hippocampus. Acta Neuropathol (Berl) 60:217–222
37. Trump BF, McDowell EM, Arstila AU (1980) Cellular reaction to injury. In: Hill RB, LaVia MF (eds) Priniciples of pathobiology, 3rd edn, chapt 2. Oxford University Press, New York Oxford, pp 20–111
38. Walaas I (1983) The hippocampus. In: Emson PC (ed) Chemical neuroanatomy. Raven Press, New York, pp 377–358
39. Webster H de F, Ames A III (1965) Reversible and irreversible changes in the fine structure of nervous tissue during oxygen and glucose deprivation. J Cell Biol 26:885–910
40. Weil A, Liebert E, Heilbrunn G (1938) Histopathologic changes in the brain in experimental hyperinsulinism. Arch Neurol Psychiatry 39:467–481

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