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EARLY ELECTRON-MICROSCOPIC CHANGES IN HEPATOGENIC ENCEPHALOPATHY INDUCED BY THIOACETAMIDE INTOXICATION IN RATS

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Although primary astrocytic alterations in both spontaneous and experimentally induced hepatogenic encephalopathy are commonly accepted, there exist considerable differences of opinion as far as their electron-microscopic nature is concerned. Descriptions of ultrastructural changes of astrocytes in natural hepatic encephalopathies in man, based on biopsy material are rare and as a rule concern advanced stages of the pathological process (Martinez, 1968; Foncin, Nicolaides 1970). They indicate the appearance of non-specific degeneration of astrocytes, involving both perikarya and processes. In descriptions of animal material, including various experimental models and different stages of hepatogenic encephalopathies, two types of astrocytic abnormalities can be distinguished. The first is represented by an increased cytoplasmic volume concomitant with an enhanced content of cytoplasmic organelles, mostly mitochondria and rough endoplasmic reticulum. On the contrary, the second type consists in remarkable reduction of cellular organelles in watery looking astrocytic cytoplasm, this being accompanied by an increased content of dense bodies and glycogen accumulation (Zamora et al. 1973; Norenberg, Lapham 1974; Ostenda et al. 1976). Norenberg (1981) considers the first group of changes as exponents of metabolic and functional activization of astrocytes, typical for early stages of experimental hepatogenic encephalopathy, while the others are supposed to represent a late degenerative stage of the pathological process. However, this concept does not find confirmation in other observations. In experimental portal-caval encephalopathy Zamora et al. (1973) had noted an entirely opposite sequence of events, namely, swelling of cytoplasm and reduction of subcellular elements in early stages of the process, and reactive changes including proliferation of gliofilaments in

its later phases. Norenberg (1977) observed ultrastructural evidence of astrocytic activation in advaned stages of experimental hepatogenic encephalopathy.

The above mentioned discrepancies inclined us to performe electron-microscopic analysis of the early phase of thioacetamide-induced hepatogenic encephalopathy in rats. This experimental model is characterized by rapid severe liver damage accompanied by biochemical evidence of its insufficiency and morphological and metabolic features typical for hepatogenic encephalopathy (Hilgier, 1983, Hilgier et al. 1983). Our previous studies revealed that electron-microscopic alterations of the brain in the 2nd and 3rd weeks of its development consisted in generalized astrocytic degeneration leading to the formation of typical Alzheimer cells, type II (Mossakowski et al. 1984).

MATERIAL AND METHODS

Experiments were carried out on female albinotic Wistar rats, weighing 180—200 g, given two intraperitoneal injections of thioacetamide in doses of 250 mg/kg b. weight, in the interval of 24 h. Control animals received intraperitoneal injections of physiological salt solution. The animals were sacrificed in groups of 3 (2 experimental and 1 control) after 24 h and 7 days following the second injection, by transcardiac perfusion with 2 percent glutaraldehyde in cacodylate buffer, pH 7.2. The brains taken out of the skull were additionally fixed overnight in the same solution at 4°C. Then they were cut coronally into slices 1 mm thick. For electron microscopy 1 mm^3 tissue blocks were taken from the frontal cortex, cortico-subcortical junction of the same region and from the putamen. They were postfixed in 2% osmium tetroxide and processed in a routine way to embedding in Epon 812. Ultrathin sections, counterstained with uranyl acetate and lead citrate were examined in a JEM 7A electron microscope.

RESULTS

The same electron-microscopic abnormalities were present in all examined structures. Although the nature of tissue alteration in animals sacrificed 24 h and 7 days after intoxication was essentially similar, there existed, however, differences in their general pattern which justify their separate description.

Twenty four hours after intoxication neurocytes in both grey formations were well preserved. However, in most of them mitochondria were swollen with damaged or disappearing cristae (Fig. 1). In no case were so-called dark neurons present. Profiles of neuropil, mostly dendrites and both myelinated and unmyelinated axon cylinders showed no

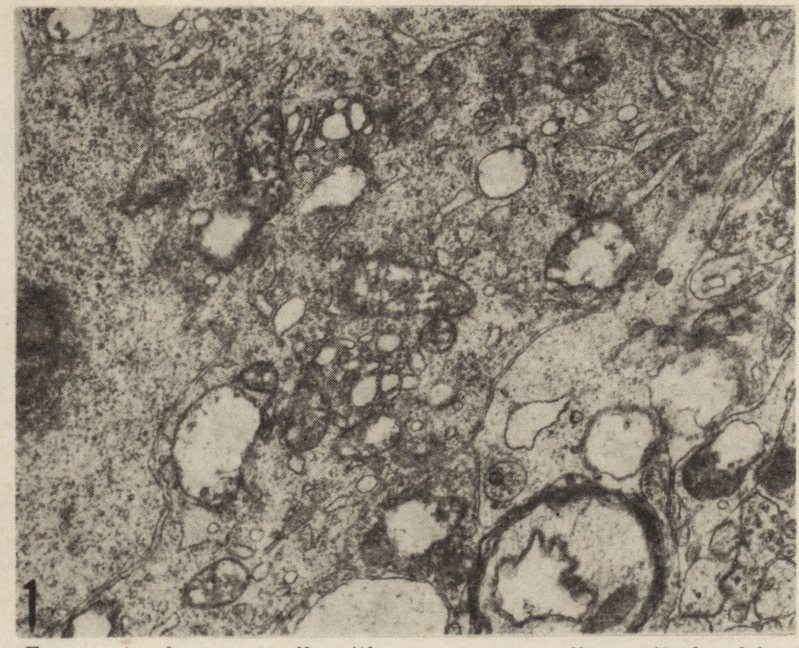

Fig. 1. Fragment of nerve cell with numerous swollen mitochondria and slightly dilated cisterns of Golgi apparatus. E.M. × 10 125

Ryc. 1. Fragment komórki nerwowej z licznymi obrzmiałymi mitochondriami i nieznacznie poszerzonymi zbiornikami aparatu Golgiego. M.E. Pow. 10 125 ×

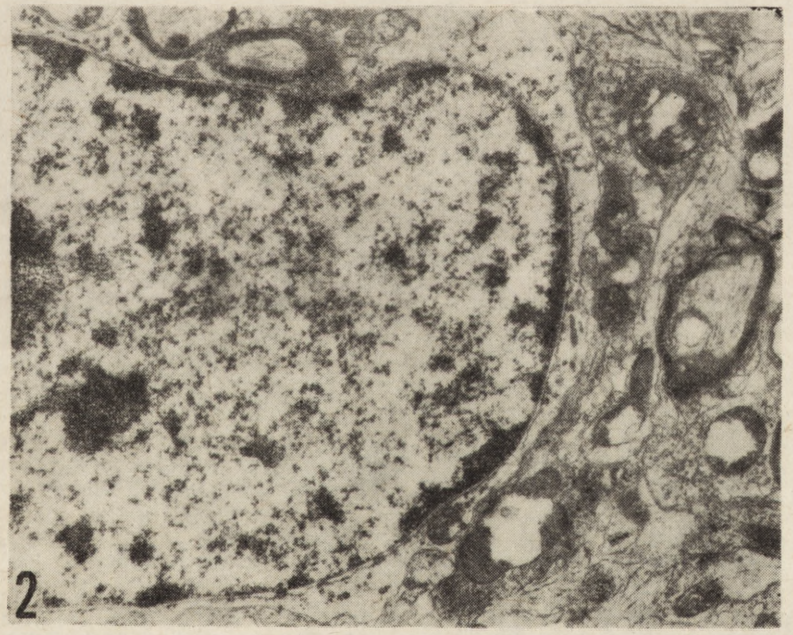

Fig. 2. Fragment of oligodendrocyte with apparently normal ultrastructural features. Neighbouring neuronal processes and axonal terminals with swollen mitochondria. E.M. × 10 125

Ryc. 2. Fragment oligodendrocytu o prawidłowym obrazie ultrastrukturalnym. W otoczeniu widoczne obrzmiałe mitochondria wypustek nerwowych i zakończeń synaptycznych. M.E. Pow. 10 125 ×

Fig. 3. Fragment of astrocyte with abundant cytoplasm containing swollen mitochondria, dilated channels of endoplasmic reticulum (arrows) and ribosomal aggregations. E.M. × 11 850

Ryc. 3. Fragment astrocytu z obfitą cytoplazmą zawierającą obrzmiałe mitochondria, poszerzone kanały siateczki śródplazmatycznej (strzałki) i obfite skupienia rybosomów. M.E. Pow. 11 850 ×

Fig. 4. Fragments of two astrocytes. The right one with remarkably developed cytoplasmic structures. The cell on the left side with with apparently normal cytoplasmic features and slightly dilated perinuclear space. (arrow). M — mitochondria, G — Golgi apparatus. E.M. × 28 800

Ryc. 4. Fragmenty dwóch astrocytów. Komórka po prawej stronie zawiera znacznie rozwinięte struktury cytoplazmatyczne. Komórka po stronie lewej z cytoplazmą o typowym obrazie, nieznaczne poszerzenie przestrzeni okołojądrowej (strzałka). M — mitochondrium, G — aparat Golgiego. M.E. Pow. 28 800 ×

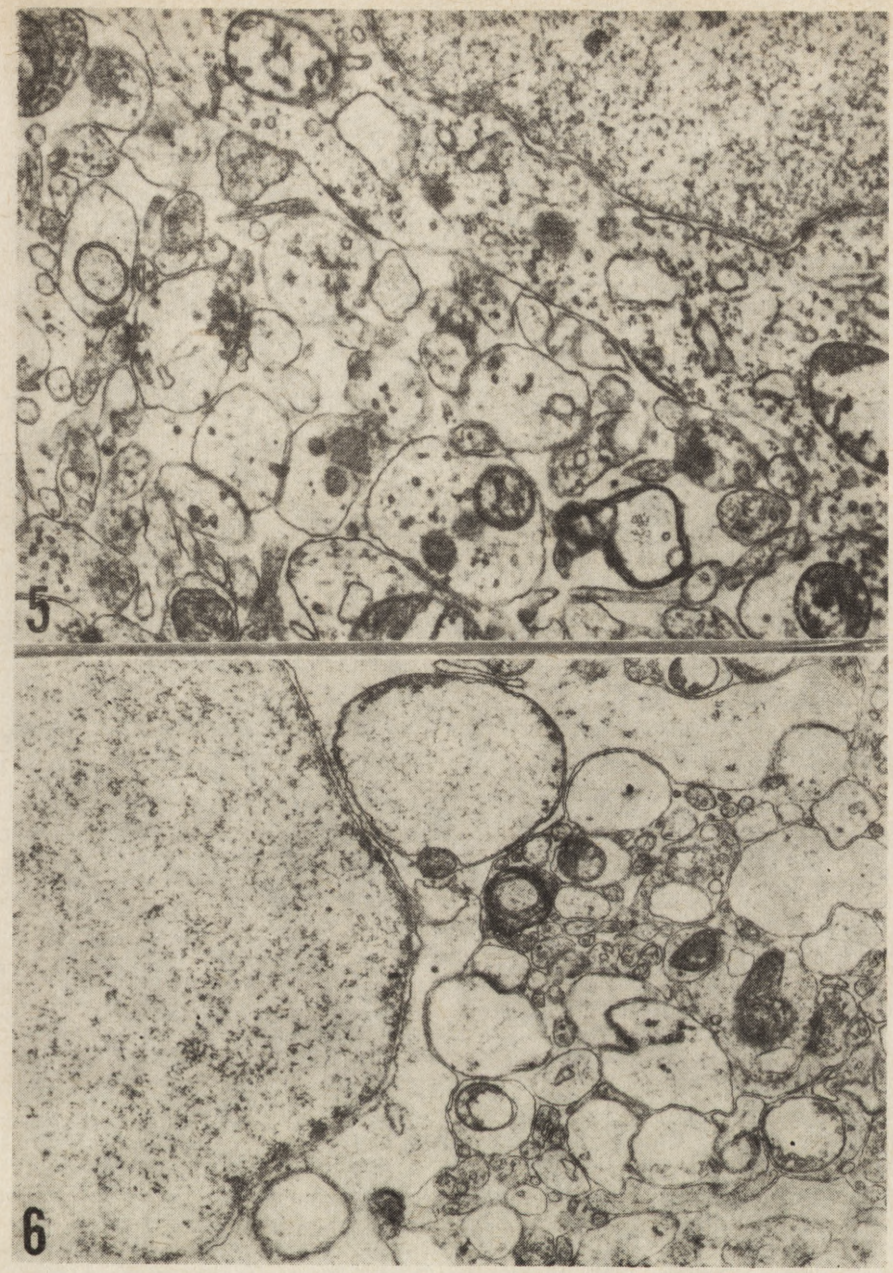

Fig. 5. Fragment of astrocyte with abundant cytoplasm rich in subcellular organelles. Dilated channels of endoplasmic reticulum and small dense bodies are visible. E.M. × 11 850

Ryc. 5. Fragment astrocytu z obfitą cytoplazmą, bogatą w organelle subkomórkowe. Widoczne poszerzone kanały siateczki śródplazmatycznej oraz pojedyncze ciała gęste. M.E. Pow. 11 850 ×

Fig. 6. Fragment of astrocyte with remarkably swollen cytoplasm, containing single abnormal mitochondria and small ribosomal aggregates. E.M. × 12 150

Ryc. 6. Fragment astrocytu z obrzmiałą cytoplazmą perykarionalną i wypustkową zawierającą jedynie pojedyncze obrzmiałe mitochondria i drobne skupienia rybosomalne. M.E. Pow. 12 150 ×

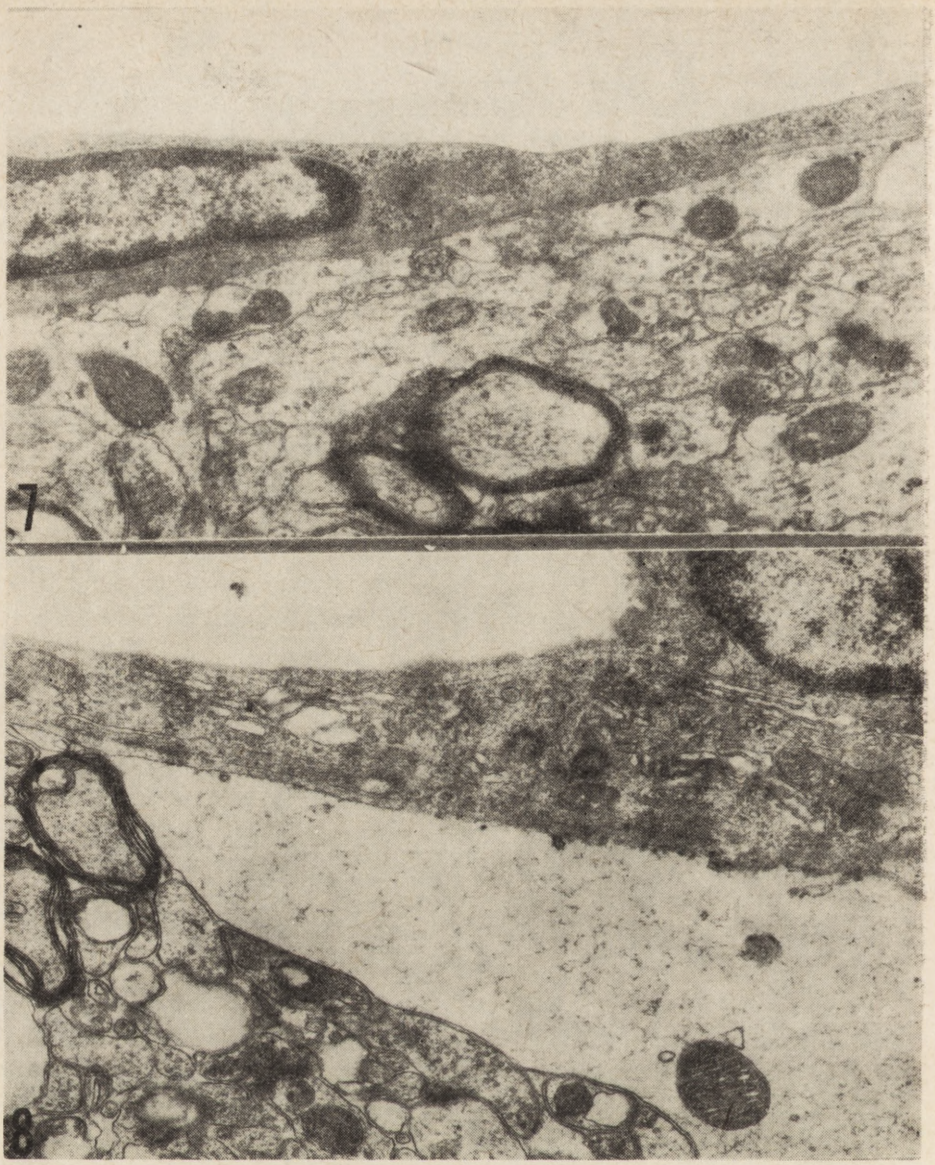

Fig. 7. Fragment of capillary vessel with normal ultrastructure of the wall and unchanged neuropil surroundings. E.M. × 10 125

Ryc. 7. Fragment naczynia włosowatego o prawidłowej strukturze ścian i niezmienionym otoczeniu. M.E. Pow. 10 125 ×

Fig. 8. Fragment of capillary vessel with adjacent swollen astrocytic process, containing amorphous floccular material and a single dark mitochondrium. E.M. × 12 150

Ryc. 8. Fragment naczynia włosowatego z przylegającą znacznie obrzmiałą wypustką astrocytu, zawierającą wyłącznie bezpostaciowy materiał kłaczkowaty i pojedyncze ciemne mitochondrium. M.E. Pow. 12 150 ×

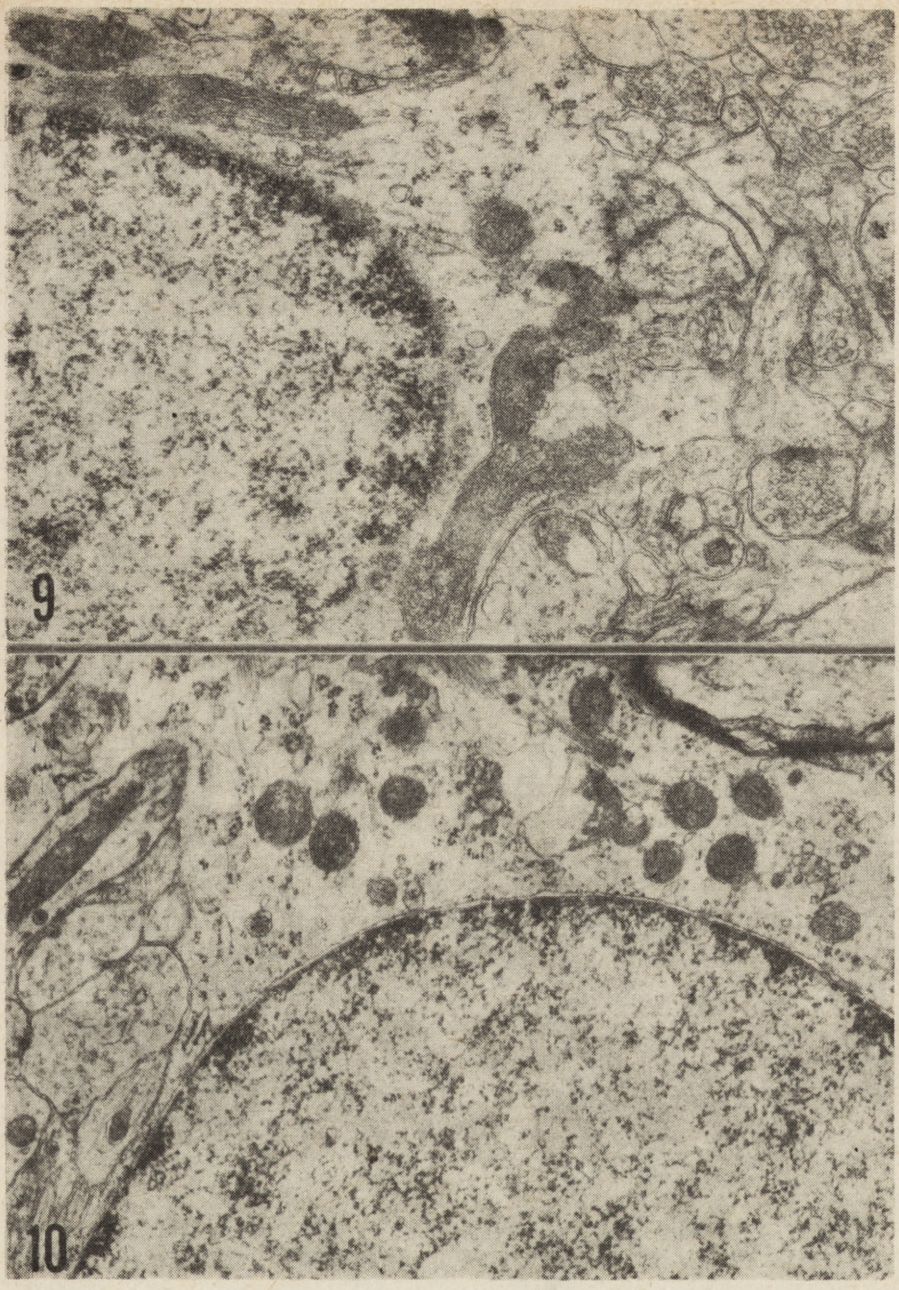

Fig. 9. Fragment of astrocyte. In the cytoplasm numerous elongated or branched mitochondria and scarce endoplasmic reticulum in the form of round and elongated cisterns are seen. Less abundant ribosomal aggregates. E.M. × 18 000

Ryc. 9. Fragment astrocytu. W cytoplazmie widoczne liczne, wydłużone lub rozgałęzione mitochondria oraz siateczka śródplazmatyczna w postaci okrągłych i wydłużonych zbiorników. Mniej obfite skupienia rybozomalne. M.E. Pow. 18 000 ×

Fig. 10. Fragment of astrocyte. Normal nucleus. Light cytoplasm contains numerous small mitochondria, searce fragments of endoplasmic reticulum, small irregularly distributed ribosomal aggregates and some neurofilaments. E.M. × 12 150

Ryc. 10. Fragment astrocytu z prawidłowym jądrem i przejaśnioną cytoplazmą zawierającą liczne drobne mitochondria, skąpe fragmenty siateczki śródplazmatycznej, małe nieregularnie rozrzucone skupienia rybosomalne i pojedyncze neurofilamenty. M.E. Pow. 12 150 ×

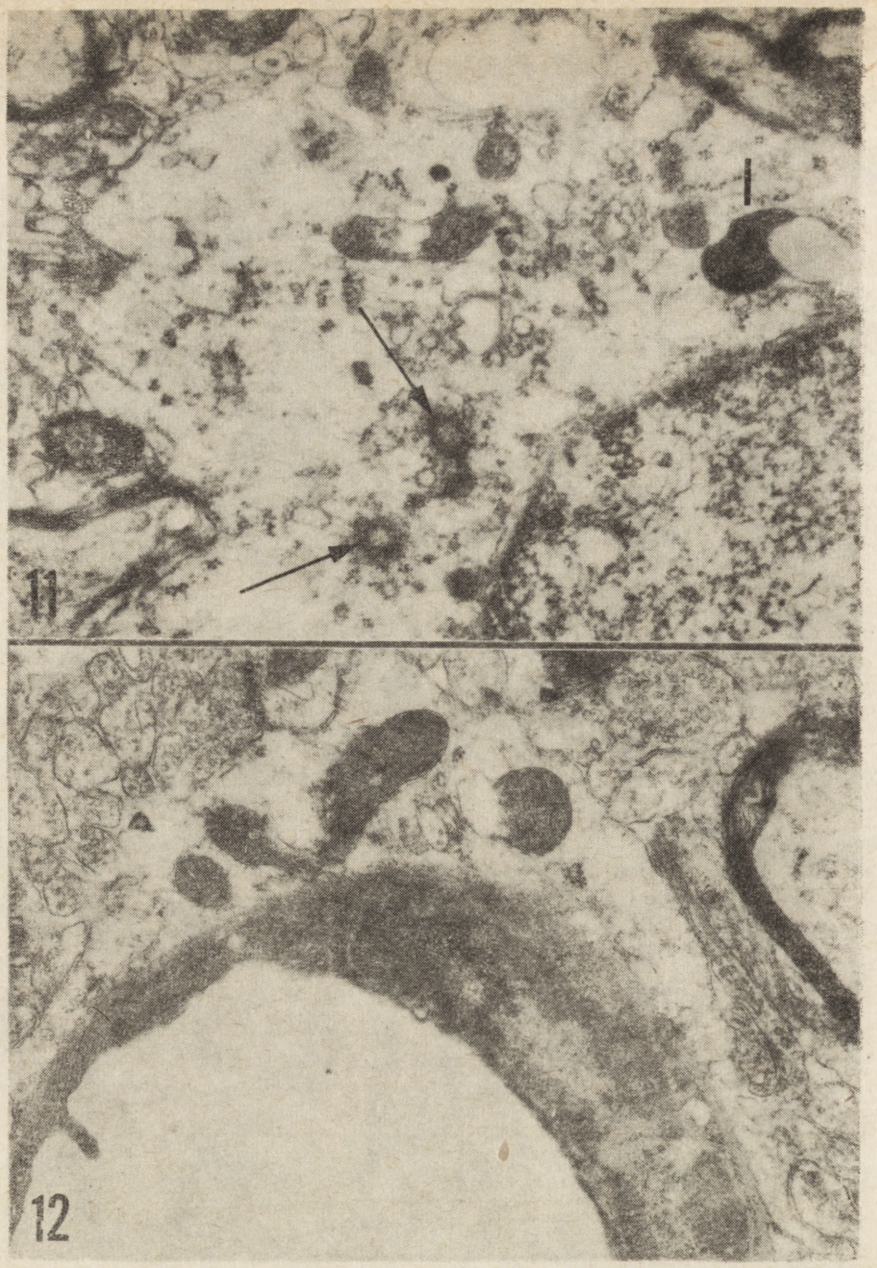

Fig. 11. Fragment of astrocyte with advanced disintegration of cytoplasmic structures. Large areas of cytoplasm are light and deprived of subcellular structures. Some swollen mitochondria and irregular accumulation of endoplasmic reticulum cisterns are seen. L — lysosome, C — centrioles (arrows). E.M. × 31 800

Ryc. 11. Fragment astrocytu z zaawansowaną dezintegracją struktur cytoplazmatycznych. Przejaśniona na dużych obszarach cytoplazma pozbawiona struktur subkomórkowych. Widoczne są nieliczne obrzmiałe mitochondria, nieregularne skupienia zbiorników siateczki śródplazmatycznej, lizosom (L) i centriole (C) (strzałki). M.E. Pow. 31 800 ×

Fig. 12. Fragment of capillary wall. Adjacent astrocytic process with mostly swollen mitochondria and accumulation of amorphous floccular and vesicular material. E.M. × 34 500

Ryc. 12. Fragment ściany naczynia włosowatego. Przylegająca wypustka astrocytu zawiera mitochondria, częściowo obrzmiałe, oraz bezpostaciowy kłaczkowaty i drobnopęcherzykowy materiał. M.E. Pow. 34 500 ×

pathological changes, except some mitochondrial swelling. Unchanged were oligodendrocytes, both in grey and white matter (Fig. 2). Alterations involved astrocytes in all examined structures. The nuclei revealed a typical ultrastructural pattern. They were light and contained a small amount of fine-granular chromatine evenly distributed with some peripheral condensation. The nuclear envelope was sharply outlined. The cytoplasm of most astrocytes was voluminous and much richer in subcellular organelles as compared with control material. Alongside with typical ribosomal aggregations, numerous large mitochondria, some of them swollen and abundant fragments of granular endoplasmic reticulum were present (Figs 3, 4, 5). Some channels of rough endoplasmic reticulum were distended forming vesicular structures. Sporadically small dense bodies were seen. Golgi apparatus was well developed. In addition to so changed astrocytes dominating the electron microscopic picture, there were cells with entirely normal ultrastructure. In some pictures severely swollen astrocytes were present. The cytoplasm of their perikarya and processes was watery in appearance and contained only a few swollen mitochondria and some small ribosomal aggregations (Fig. 6). The capillary network showed no abnormality. Both endothelial cells and neuropil structures surrounding capillaries, including astrocytic processes were unchanged, (Fig. 7). However, sporadically remarkably swollen perivascular astrocytic processes, containing delicate floccular material and scarce dense mitochondria were present (Fig. 8).

In animals sacrificed 7 days following intoxication ultrastructural abnormalities were limited to astrocytes. Neurons, oligodendrocytes, morphological elements of neuropil and capillary walls were unchanged. Similarly as in the previously described group, hypertrophied astrocytes were numerous. However, the amount of subcellular structures in their cytoplasm was undergoing reduction (Fig. 9). This concerned mostly channels of rough endoplasmic reticulum and free ribosome aggregates (Fig. 10). The next step of abnormalities consisted in cytoplasmic disintegration with total or partial loss of organelles (Fig. 11). The electron-microscopic picture was dominated by astrocytes with watery cytoplasm, containing some remnants of subcellular organelles. Most of the capillaries were surrounded by swollen astrocytic processes, containing a few organelles and both floccular and vesicular material (Fig. 12). Astrocytes revealing normal ultrastructure were very rare.

DISCUSSION

Ultrastructural abnormalities found in experimental material involved almost exclusively astrocytes. This confirms the commonly accepted opinion concerning primary astrocytic damage in hepatogenic encephalopathy (Mossakowski 1966; Seitelberger 1970; Norenberg 1981).

However, the most early electron-microscopic changes of astroglia differ essentially from those described in human material (Martinez 1968; Foncin, Nicolaides 1970) and in most cases of experimental pathology (Zamora et al. 1973, Norenberg, Lapham 1974, Ostenda et al. 1976). They are also different from the ultrastructural abnormalities found in later stages of the same experimental model (Mossakowski, Borowicz 1984). The most essential difference consists in the fact that a great part of astrocytic population in early stage of hepatogenic encephalopathy reveals features of functional and metabolic activization, with only sligh participation of degenerative changes. The dominating astrocytic alterations were similar to those described by Norenberg (1979) as well as Laursen and Diemer (1979) in early stages of hepatogenic encephalopathy induced by hyperamonemia accompanying urease application and portal-caval shunt. It is worth mentioning that thioacetamide-induced experimental hepatic lesions are also characterized by a remarkable increase of ammonia content in the blood, with its totaly normal level in brain tissue in the stage under study (Albrecht, Hilgier 1984). As astrocytes form the basic cellular compartment of the central nervous system, in which detoxification of ammonia to glutamine takes place (Norenberg 1981), it seems plausible to suggest that their activization evidences compensatory processes, which in turn condition a lack of ammonia level increase in the brain in the course of the pathological process, accompanied by its striking enhancement in the blood. The problem to what extend an enhanced activity of glutamate dehydrogenase and increased glutamate sythetase synthesis, mentioned by Norenberg (1981) in early stages of hepatogenic encephalopathy, be connected with the increased number of astrocytic mitochondria and their enlargement, and activization of rough endoplasmic reticulum observed in our material, remains open owing to the lack of parallel biochemical studies. Nevertheless, this hypothesis suggested by Norenberg (1981) seems both probable and attractive.

A feature, worth mentioning is the fact, that already in the earliest period of experimental hepatogenic encephalopathy with dominating evidence of astrocytic activation, degenerative changes appear in them. In this respect our observations differ from those of Norenberg (1977), who stressed the lack of astrocytic degeneration in early phases of hepatogenic encephalopathy. Basing on the here presented and earlier observations (Mossakowski, Borowicz 1984) it seem possible to suggest a sequence of astrocytic abnormalities in the evolution of experimental hepatogenic encephalopathy. The earliest process is characterized by progressive astrocytic changes, expressed as generalized proliferation and hypertrophy of astroglia. During evolution of the pathological process they are overlapped by astrocytic degeneration. As shown in animals with 7-day survival following intoxication, features of degeneration

appear in previously activated cells. Such a sequence of changes finds confirmation in light-microscopic neuropathology of natural hepatogenic encephalopathy in man and experimental animals, characterized by concomitance of both categories of cellular abnormalities. The question whether subsequent changes are due to the same factor or to additional pathogenic ones remains to be answered.

A distinct feature in the electron microscopic picture of the earliest phase of hepatogenic encephalopathy induced by thioacetamide intoxication as compared to its more advanced stages consists in appearance of neuronal abnormalities, taking the form of mitochondrial swelling and distention of cisterns of the Golgi apparatus. Nerve cell alterations do not belong to the morphological characteristies of hepatogenic encephalopathy. They have not been described in its other experimental models (Mossakowski 1981). Those, found in the presented series of experiments seem to be connected with the direct neurotoxic action of thioacetamide, although its permeability across unchanged cerebral vessels remains unknown. This mechanism seems to be even more probable as neuronal abnormalities occur exclusively in the earliest stage of the pathological process, being absent in the subsequent phase. It has been shown in the experiments performed on organotypic cerebellar cultures, that thioacetamide introduced into the nutrient media, without leading to features of hepatogenic gliopathy, resulted in nonspecific neuronal damage with predominat mitochondrial impairment (Kraśnicka et al 1983).

WCZESNE ZMIANY MIKROSKOPOWO-ELEKTRONOWE W ENCEFALOPATII WĄTROBOWEJ WYWOŁANEJ ZATRUCIEM TIOACETAMIDEM

Streszczenie

Oceniono obraz mikroskopowo-elektronowy mózgów szczurów poddanych dwukrotnemu zatruciu tioacetamidem, podawanym dootrzewnowo w dawce 240 mg/kg masy ciała w odstępach 24-godzinnych. Zwierzęta zabijano aldehydem glutarowym przez przezsercową perfuzję po upływie 24 godzin i 7 dni po drugim podaniu środka toksycznego.

W obrazie mikroskopowo-elektronowym dominowały nieprawidłowości astrogleju, przy minimalnym nieswoistym zwyrodnieniu neuronów, występującym tylko we wcześniejszej grupie zwierząt i całkowicie niezmienionych pozostałych elementach komórkowych ośrodkowego układu nerwowego. Zmiany astrocytów występowały bądź pod postacią ich aktywizacji, wyrażającej się zwiększeniem objętości cytoplazmy i wzrostem zawartości organelli cytoplazmatycznych, takich jak mitochondria i szorstka siateczka śródplazmatyczna, bądź też jako wykładniki ich zwyrodnienia: zubożenie organelli cytoplazmatycznych i wodnisty wygląd cytoplazmy. Zmiany typu pierwszego dominowały we wczesnym okresie po zatruciu. Po upływie 7 dni przeważały zmiany typu drugiego, bardzo nieliczne u zwierząt grupy wcześniejszej.

Uzyskane wyniki wskazują, iż najwcześniejszą fazę encefalopatii wątrobowej charakteryżuje aktywacja metaboliczna astrocytów, z którą wiązać zapewne należy brak wzrostu poziomu amoniaku w mózgu, mimo jego bardzo znacznego zwiększenia we krwi. W dalszej fazie procesu ewoluują one w kierunku zwyrodnienia astrocytów, stanowiącego charakterystyczny element obrazu neuropatologicznego zaawansowanej encefalopatii wątrobowej.

РАННИЕ ЭЛЕКТРОННО-МИКРОСКОПНЫЕ ИЗМЕНЕНИЯ В ПЕЧЕНОЧНОЙ ЭНЦЕФАЛОПАТИИ ВЫЗВАННОЙ ОТРАВЛЕНИЕМ ТИОАЦЕТАМИДОМ

Резюме

Авторы оценили электронно-микроскопную картину головных мозгов крыс подверженных двукратному отравлению тиоацетамидом вводимым внутрибрюшинно в дозе 240 мг/кг массы тела в суточных промежутках. Животные были умерщвлены глутаровым альдегидом при помощи чрезсердечной перфузии после 24 часов и на 7 дню после второго введения токсического средства.

В электронно-микроскопной картине преобладали изменения астроглии при минимальной неспецифической дегенерации нейронов выступающих только в более ранней группе животных и при полностью неизмененных остальных клеточных элементах центральной нервной системы. Изменения астройитов выступали либо в форме их активизации выражающейся в увеличении объема цитоплазмы и в росте содержания цитоплазматических органелл таких как митохондрии и шероховатая внутриплазматическая сеточка либо в форме показателей их дегенерации: обеднение в цитоплазматических органеллах и водянистый вид цитоплазмы. Изменения первого типа преобладали в раннем периоде после отравления. По истечении 7 дней преобладали изменения второго типа очень немногочисленные у животных более ранней группы.

Полученные результаты указывают на то что самую раннюю фазу печеночной энцефалопатии характеризует метаболическая активация астроцитов с которой вероятно следует связывать отсутствие роста уровня аммиака в головном мозгу несмотря на его очень значительное увеличение в крови. В дальней фазе процесса они эволюционируют к дегенерации астроцитов составляющей характерный элемент невропатологической картины прогрессирующей печеночной энцефалопатии.

REFERENCES

1. Albrecht J., Hilgier W.: Brain carbonic anhydrase activity in rats in experimental hepatogenic encephalopathy Neuroscience Letters, 1984, 45, 7—10.
2. Foncin J. F., Nicolaidis S.: Encephalopatie porto-cave: contribution a la pathologie ultrastructurale de la glie chez l'homme. Rev. Neurol., 1970, 123, 81—87.
3. Hilgier W.: Encefalopatia wątrobowa wywołana podawaniem tioacetamidu. III. Zawartość amoniaku i substratów jego metabolizmu w mózgu. Neuropat. Pol., 1983, 21, 511—518.
4. Hilgier W., Albrecht J., Kraśnicka Z.: Thioacetamide-induced hepatic encephalopathy in rat. I. Preliminary morphological and biochemical observations. Neuropat. Pol., 1983, 21, 487—494.
5. Kraśnicka Z., Albrecht J., Gajkowska B., Mossakowski M. J.: Thioacetamide-induced hepatic encephalopathy in the rat. II. Cytochemical and ultrastructural studies on astrocytes *in vitro*. Neuropat. Pol., 1983, 21, 495—510.

6. Laursen H., Diemer N. H.: Morphometric studies of rat glial cell ultrastructure after urease-induced hyperammonaemia. Neuropath. Appl. Neurobiol., 1979, 5, 345—362.
7. Martinez A.: Electron microscopy in human hepatic encephalopathy. Acta neuropath. (Berl.), 1968, 11, 82—86.
8. Mossakowski M. J.: Patomorfologia i histochemia spontanicznych i doświadczalnych encefalopatii pochodzenia wątrobowego. Neuropat. Pol., 1966, 4, 231—304.
9. Mossakowski M. J.: Experimental modelling of hepatogenic encephalopathies. Neuropat. Pol., 1981, 19, 261—276.
10. Mossakowski M. J., Borowicz J. W., Hilgier W.: Electron microscopy of hepatogenic encephalopathy in rats induced by tioacetamide intoxication. Neuropat. Pol., 1984, 22, 185—195.
11. Norenberg M. D.: A light and electron microscopic study of experimental portal-systemic (ammonia) encephalopathy. Lab. Invest., 1977, 36, 618—627.
12. Norenberg M. D.: The astrocyte in liver disease. [In:] Advances in cellular neurobiology. Eds: S. Fedoroff, L. Hertz. Academic Press. New York, London, Toronto, Sydney, San Francisco 1981, 2, 303—352.
13. Norenberg M. D., Lapham L. W.: The astrocyte response in experimental portal-systemic encephalopathy: an electron microscopic study. J. Neuropath. exp. Neurol., 1974, 33, 422—435.
14. Ostenda M., Mossakowski M. J., Pronaszko-Kurczyńska A.: Ultrastructural studies on experimental hepatogenic encephalopathy. Neuropat. Pol., 1976, 14, 347—384.
15. Seitelberger F.: General neuropathology of central neuroglia: the concept of glial syndromes. Proc. VI Intern. Congress Neuropath. Masson, Paris 1970, 392—403.
16. Zamora A. J., Cavanagh J. B., Kyu M. H.: Ultrastructural responses of the astrocytes to portocaval anastomoses in the rat. J. Neurol. Sci., 1973, 18, 25—45.

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