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ULTRASTRUCTURAL STUDIES ON EXPERIMENTAL HEPATOGENIC ENCEPHALOPATHY

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Numerous morphological studies on hepatogenic encephalopathy, performed both on human and experimental animal material (Adams, Foley, 1949; Lapham, 1961; Mossakowski, 1966a, 1966b; Shiraki, 1968) point to the primary impairment of astroglia as underlaying this pathological process. Mossakowski et al. (1970) have shown, that in experimental hepatogenic encephalopathy, glial changes are accompanied by an increased permeability of brain vessels to silver salts, with unchanged barrier for conventional protein markers.

Insofar, the rich and diversified picture of morphological changes, observed in the light microscope, found but few and incomplete manifestations in the electron microscopic picture. This prompted us to undertake electron-microscopic studies of hepatogenic encephalopathy with the use of an already tested and reproducible experimental model of a known pathomorphological picture and dynamics. In the line with previous observations (Mossakowski et al., 1970) particular attention was devoted to the picture of the blood vessels and glia.

MATERIAL AND METHODS

The studies were carried out on 24 female Wistar rats, 3-months old at the beginning of the experiment. To produce liver cirrhosis, the experimental animals in the number of 16 were given subcutaneously injections of 50% carbon tetrachloride solution in liquid paraffin in a dosis of 0.5 ml CCl_4 per 100 g of body weight. The injections were

given 3 times a week for a period of 2, 4 and 6 months. The 8 control animals received injections of pure liquid paraffin with the same frequency and for the same period. To avoid possible age-dependent differences in the ultrastructural picture of the brain, the experiment was arranged so as to sacrifice all the animals at the age of 9 months.

After decapitation the brain was removed from the skull as quick as possible. Small tissue blocks were taken from the cortico-subcortical area of the fronto-parietal region and fixed by immersion in 3% glutaraldehyde in Millonig buffer, pH 7.2, for 2 hrs, and thereafter for 1 hr in 2% osmium tetroxide. The samples were dehydrated in ethanol of increasing concentration and embedded in Epon 812. Blocks were cut in a Tessla ultramicrotome. The ultrathin sections were contrasted with uranyl acetate and lead citrate. The observations were made in a JEM 7A electron microscope.

Light microscopic studies of brain and liver tissue were done on formalin-fixed material. Paraffin sections were stained with hematoxylin and eosine, van Gieson's and Gridley's methods.

Fig. 1. Hepatogenic encephalopathy of 6-month duration. Endothelial cells of cortical capillary vessel contain numerous pinocytic vesicles. Interendothelial junctions form long, tortuous canals. x 4 900.

Ryc. 1. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Śródbłonki korowego naczynia włosowatego zawierają liczne pęcherzyki pinocytarne i wodniczki. Zespolenia międzyśródbłonkowe tworzą wydłużone, kręte kanały. Pow. 4 900 x.

Fig. 2. Hepatogenic encephalopathy of 6-month duration. Endothelium of capillary vessel contains numerous pinocytic vesicles located at different depth of the cell. x 15 000.

Ryc. 2. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Śródbłonki naczynia włosowatego z licznymi pęcherzykami pinocytarnymi położonymi na różnej głębokości komórki. Pow. 15 000 x.

Fig. 3. Hepatogenic encephalopathy of 6-month duration. Interendothelial junction of the capillary vessel in the form of widened electron-lucide slit. x 14 800.

Ryc. 3. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Zespolenie międzyśródbłonkowe naczynia włosowatego występuje w postaci poszerzonej optycznie pustej szczeliny. Pow. 14 800 x.

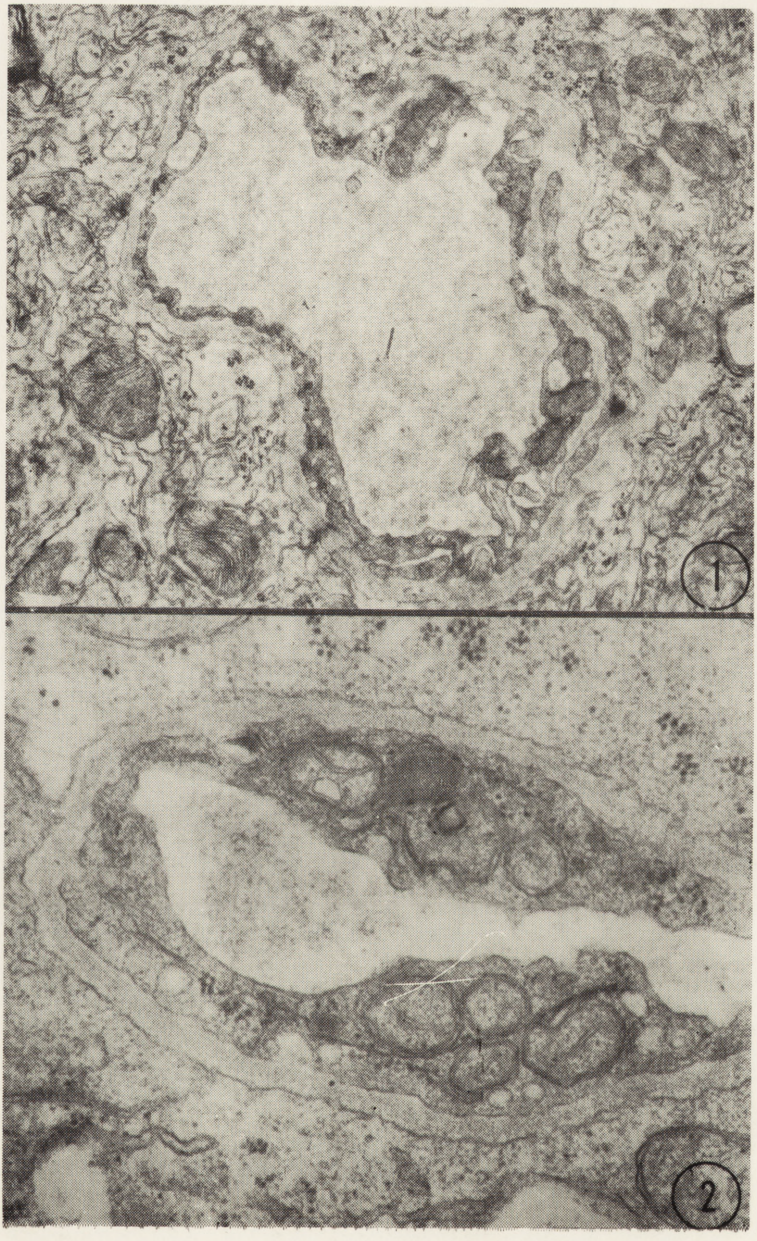
Fig. 4. Hepatogenic encephalopathy of 6-month duration. Cortical capillary vessel surrounded by entirely disintegrated perivascular astrocytic process. x 7 200.

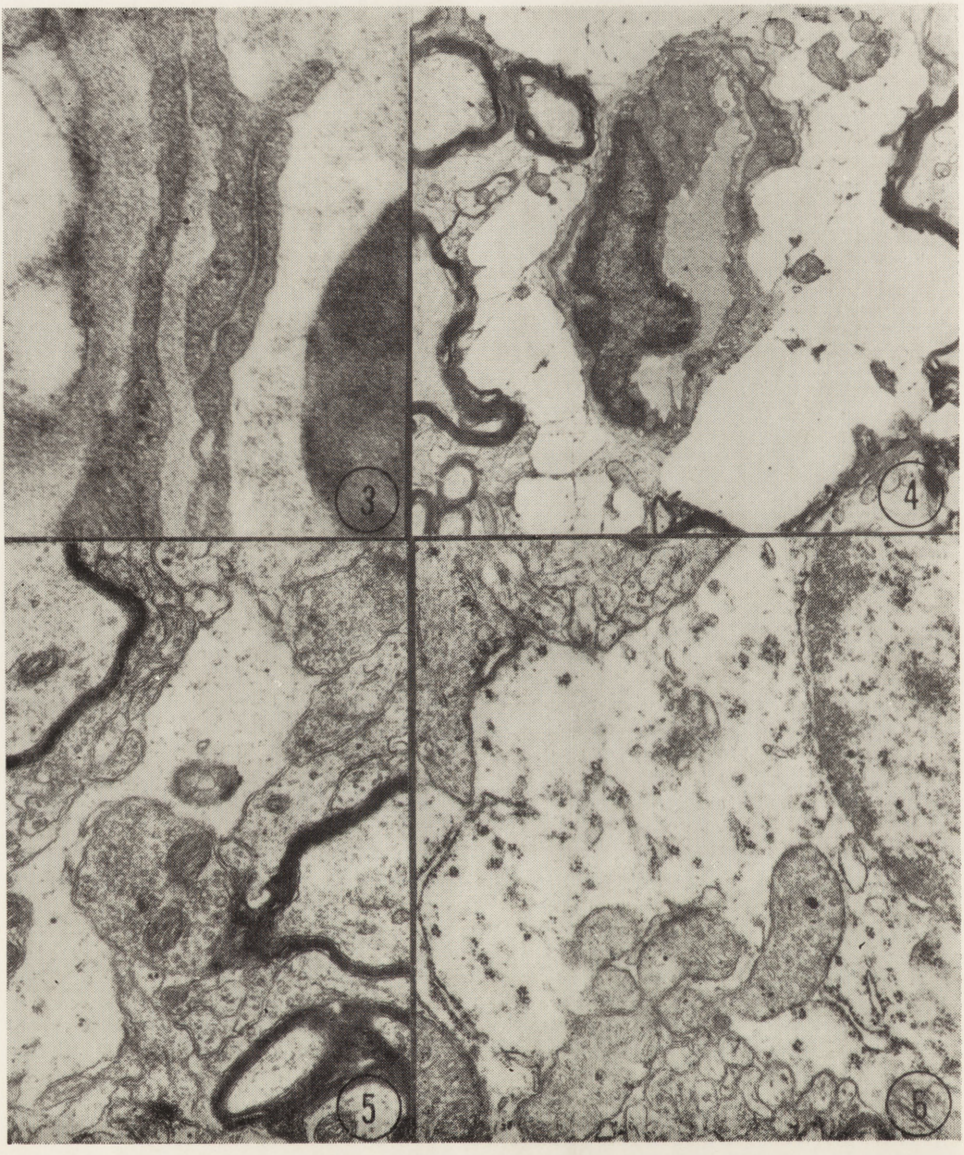
Ryc. 4. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Korowe naczynie włosowate otoczone ciężko uszkodzonymi okołonaczyniowymi wypustkami astrogleju. Pow. 7 200 x.

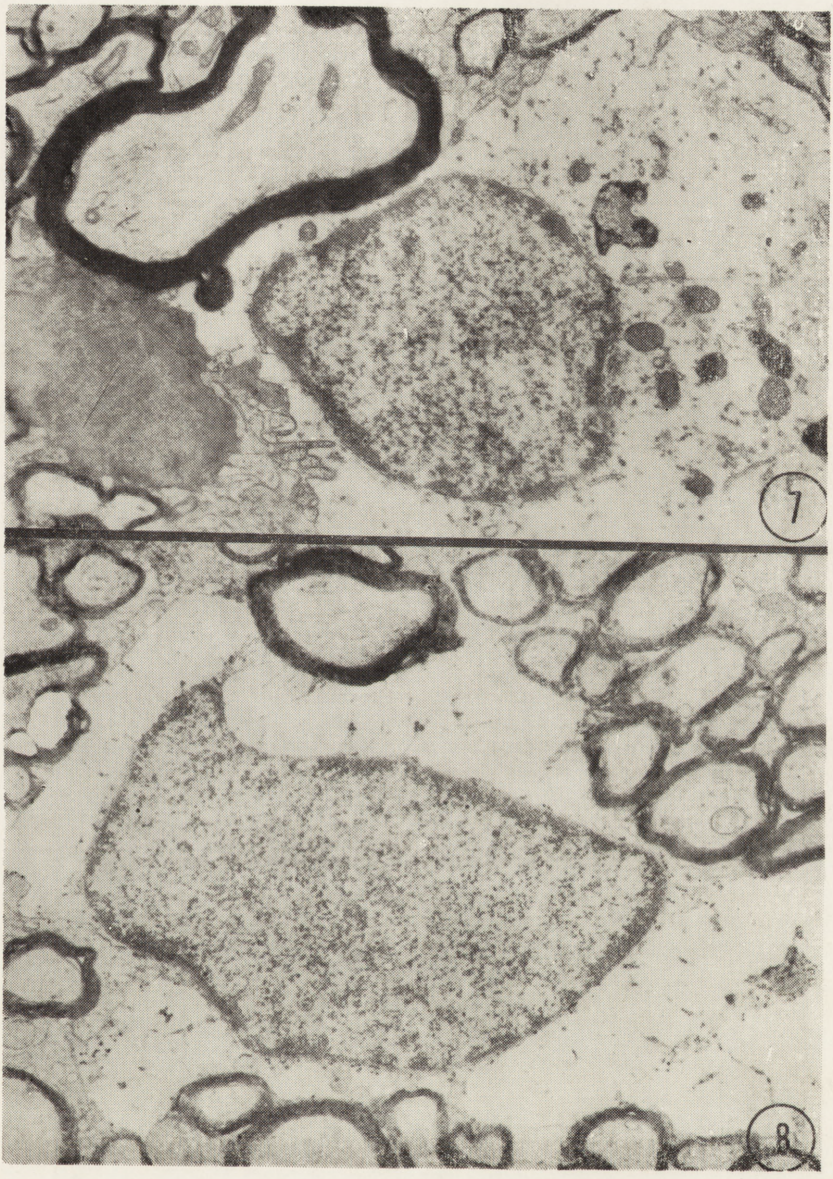
Fig. 5. Hepatogenic encephalopathy of 6-month duration. Swollen, organelle-free astrocytic process situated among other well preserved structural elements of neuropil. x 13 000.

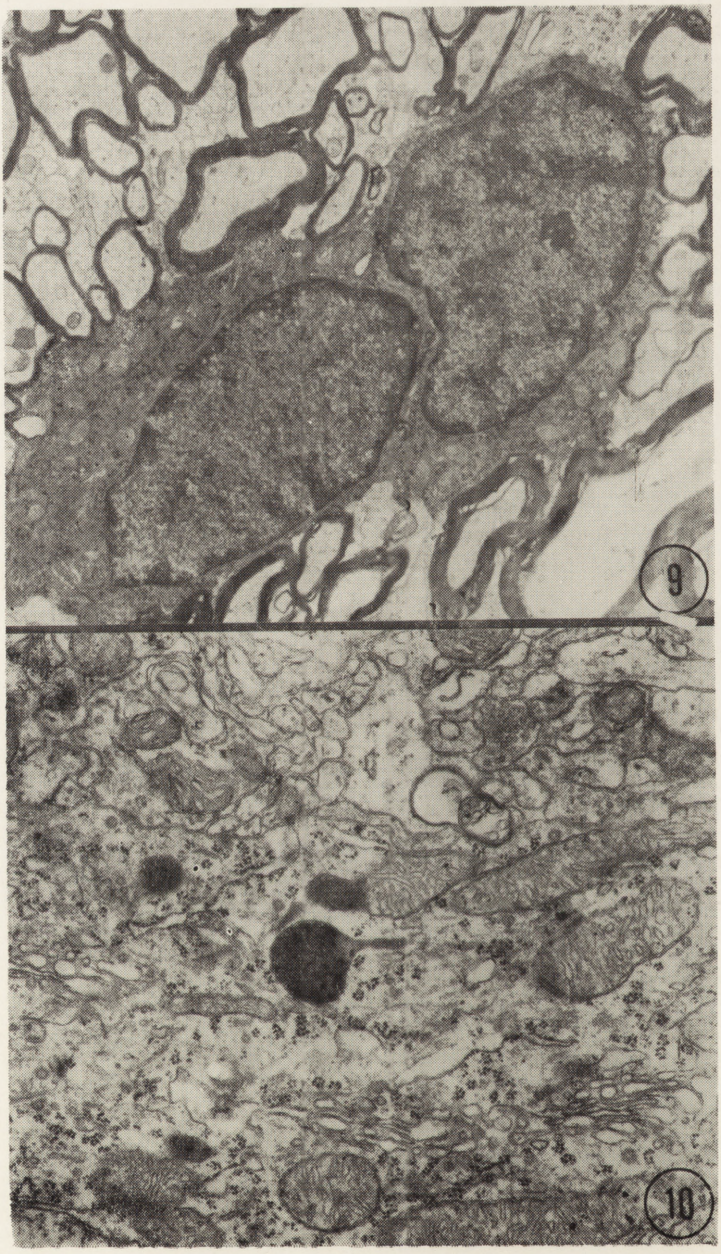
Ryc. 5. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Obrzmiała, pozbawiona organelli subkomórkowych wypustka astrocytarna, położona wśród prawidłowo zachowanych elementów neuropilu. Pow. 13 000 x.

Fig. 6. Hepatogenic encephalopathy of 4-month duration. Astrocyte with swollen, electron lucent cytoplasm containing normal mitochondria, fragments of rough endoplasmic reticulum and scanty free ribosomes. x 9 000.









RESULTS

On the light microscopy the brains of animals sacrificed after 4 and 6 months of experimental treatment revealed the features of hepatogenic encephalopathy, which were similar in their nature and intensity to those described in our previous paper (Mossakowski et al., 1971).

The electron microscopic picture of the brain of the animals decapitated after 2 months of the experiment did not significantly differ from that of the control animals. It should be emphasized that at the time, no features of liver cirrhosis were observed in the experimental animals, but only generalized changes of the character of hepatocytes' steatosis.

In the experimental animals, both after 4 and 6 months of the experiment significant abnormalities of the electron-microscopic picture were noted as compared with control material; the fundamental pattern of tissue lesions being identical in both groups, the only difference appearing in their intensity and range. At that time features of liver cirrhosis were found, much more intensive in animals with 6 months of experimental treatment.

Ryc. 6. Encefalopatia wątrobowa w 4 miesiącu doświadczenia. Astrocyt o obrzmiałej, optycznie przejrzystej cytoplazmie, zawierający prawidłowe mitochondria, fragmenty szorstkiej siatki śródplazmatycznej i skąpe wolne rybosomy. Pow. 9 000 x.

Fig. 7. Hepatogenic encephalopathy of 6-month duration. Astrocyte with advanced disintegration of cytoplasm. Electron lucent cytoplasm of the cell contains fragments of rough endoplasmic reticulum, some small mitochondria with a dark matrix and heterogenous dense bodies. x 15 000.

Ryc. 7. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Astrocyt z zaawansowaną dezintegracją cytoplazmy, zawierającej nieliczne mitochondria o ciemnej macierzy, fragmenty szorstkiej siatki śródplazmatycznej i heterogenne ciała gęste. Pow. 15 000 x.

Fig. 8. Hepatogenic encephalopathy of 6-month duration. Astrocyte with completely disintegrated cytoplasm. Large nucleus with diffusely spread, scanty chromatin seems to be located in entirely empty space. x 4 550.

Ryc. 8. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Astrocyt z całkowicie dezintegrowaną cytoplazmą. Duże jądro ze skąpą chromatyną sprawia wrażenie położonego w pustej przestrzeni. Pow. 4 550 x.

Fig. 9. Hepatogenic encephalopathy of 6-month duration. Two oligodendrocytes localized in subcortical area, with entirely normal ultrastructural picture. x 4 550.

Ryc. 9. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Dwie komórki oligodendrogleju o całkowicie prawidłowym obrazie ultrastrukturalnym. Pow. 4 550 x.

Fig. 10. Hepatogenic encephalopathy of 6-month duration. Fragment of neuronal cytoplasm, containing numerous canals of Golgi complex, among which many coated vesicles are seen, and free ribosomes as well as some dense bodies. x 9 000.

Ryc. 10. Encefalopatia wątrobowa w 6 miesiącu doświadczenia. Fragment cytoplazmy komórki nerwowej, zawierającej liczne kanały zespołu Golgiego z położonymi wśród nich pęcherzykami obłonionymi, wolne rybosomy i nieliczne ciała gęste. Pow. 9 000 x.

The electron-microscopic tissue changes involved the structure of the capillary vessels and selectively the astrocytic glia. The remaining tissue elements were unchanged or showed only very slight ultrastructural abnormalities.

The ultrastructural changes of the capillary vessel walls found already in the 4-months group, were manifested first of all by a marked enhancement of the pinocytosis in the cells of endothelium (Fig. 1). The endothelial cells were abundantly filled with numerous pinocytic vesicles, found on various depth of their cytoplasm (Fig. 2). This phenomenon was particularly well marked in animals after 6 months of experiment. In the same vessels the myelin-like structures were noted in the endothelial cells, these structures being usually situated in the vicinity of the cell membranes, facing the vessel lumina. The intercellular junctions of endothelium showed a significant extension (Fig. 3). In addition irregularity and folding of the interendothelial junctions was seen.

Already in the 4-months group a significant swelling of perivascular astrocytic processes was observed. Their cytoplasm was characterized by a decrease of electron density and reduction of subcellular elements. At a higher intensity of changes, observed mostly in the 6-months group, the blood vessels were surrounded by wide electron-light spaces of astrocytic processes, which were almost completely deprived of cytoplasmic subcellular elements (Fig. 4). Occasionally, the narrowed lumina of the capillary vessels gave the impression of being compressed by the extended perivascular astrocytic processes. The reduction of subcellular elements was characteristic not only for perivascular astrocytic processes but also for those situated in the neuropil, contrasting with the remaining unchanged elements of nerve tissue (Fig. 5).

Analogical changes concerned the astrocyte perikarya. When their impairment was less extensive, the cell cytoplasm was characterized only by a marked swelling (Fig. 6). More intensive changes proceded with a significant disintegration of endoplasmic structures, first of all of the endoplasmic reticulum. In these cases ,in the electron-light cytoplasm only fragments of the rough and smooth endoplasmic reticulum, some aggregations of free ribosomes and vacuoles of various size, probably originating from the extended endoplasmic reticulum were found to occur (Fig. 7). In some of the cells an increased number of dense bodies and a slightly increased number of glycogn rosettes were observed. The latter appeared both in the perikarya and processes of the astrocytes. At the highest intensity of changes, the enlarged nuclei of astrocytes were situated in an empty, organelle—free cytoplasm (Fig. 8). The nuclei of astrocytes showed relatively slight changes, mostly concerning their

size and distribution of chromatine, being scarcer and more loosely spread out. In some of the astrocytic nuclei the accumulations of small electron-dense grain could be seen, the structure of which differed both from aggregations of the nuclear RNA and cytoplasmic glycogen grains.

Contrary to the massive involvement of astrocytes, oligodendroglia showed practically no ultrastructural changes (Fig. 9). The same was true for the nerve cells in which only occasionally an increased number of dense bodies and insignificant distention of endoplasmic reticulum channels were observed (Fig. 10).

DISCUSSION

Selective impairment of astroglia, predominating in the electron-microscopic picture of the experimental hepatogenic encephalopathy, is consistent with a number of light-microscopic observations pointing to the primary gliopathic nature of the process. The abnormalities in the ultrastructure of astrocytes found by us resemble those described in humans in hepatogenic encephalopathy (Martinez, 1968), portal systemic encephalopathy (Foncin, Nicolaidis, 1970) and in Wilson's disease (Gruner, quoted by Foncin, 1970). These abnormalities show no specific electron microscopic features. They do not diverge from the ultrastructural picture of astrocytes in cases of severe brain edema (Hirano et al., 1967). These changes may manifest unspecific impairment of glia, predominating in the histological picture of hepatic encephalopathy and leading to disintegration of astrocytic cells. It seems, however, that the pictures of maximal swelling of astrocyte cytoplasm with complete disappearance of endoplasmic cell organelles found by us may constitute the ultrastructural manifestation of the glial changes described as Alzheimer cells, type II. Likewise, the glycogen accumulations correspond to the perinuclear deposits of this polysaccharide described by Shiraki (1967) in human pathology and by Mossakowski et al. (1970) in experimental animals. The character of the small, electron dense grains agglomerations present in the altered nuclei of astrocytes remains unclear. In view of their ultrastructural picture it does not seem possible to consider them as an electron microscopic manifestation of the intranuclear glycogen inclusions, observed in the light microscopy.

No structures corresponding to the Opalski cells were found in our material, this being consistent with the light microscope observations. Neither were ultrastructural manifestations of the progressive changes of astroglia, observed, which were described by Ma Hta Kyn and Cavanagh (1970) and Cavanagh and Ma Hta Kyn (1971) in cases of experimental porto-caval encephalopathy.

The described changes in electron microscopic picture of capillary vessels, particularly the features of intensive pinocytosis, as compared with their absence or insignificance in normal brain vessels (Brightman and Reese 1970) may be the expression of changes in the vessel permeability. They may form the morphological background of the increased blood vessel permeability described by Mossakowski et al. (1971) in cases of experimental hepatogenic encephalopathy.

Our observations do not answer the question concerning the mutual relations between vascular and cellular changes. Both are present in animals after a 4-month experimental period and become enhanced after 6 months of experimental treatment. They do not occur in rats treated for only 2 months. Presumably, studies on animals with an experimental period longer than 2 months and shorter than 4 months would give a picture of the differences in the dynamics of appearance and development of both type of changes.

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OBRAZ MIKROSKOPOWO-ELEKTRONOWY DOŚWIADCZALNEJ ENCEFALOPATII WĄTROBOWEJ

Streszczenie

Przeprowadzono badania mikroskopowo-elektronowe mózgów szczurów z encefalopatią pochodzenia wątrobowego, wywołaną przewlekłym stosowaniem czterochlorku węgla.

Najwcześniejsze nieprawidłowości ultrastrukturalne stwierdzono u zwierząt po 4-miesięcznym stosowaniu CCl_4. Osiągały one maksymalne nasilenie po upływie 6 miesięcy.

Zmiany w obrazie mikroskopowo-elektronowym dotyczyły ścian naczyń włosowatych i gleju astrocytarnego. Nieprawidłowości w obrazie naczyń wyrażały się wzmożeniem aktywności mikropinocytarnej śródbłonków i poszerzeniem złącz międzyśródbłonkowych, które uznano za podłoże zwiększonej przepuszczalności ścian naczyniowych. Zmiany patologiczne gleju astrocytarnego wyrażały się bardzo znacznym obrzmieniem cytoplazmy wypustek i perikariów komórkowych, prowadzącym do jej całkowitej dezintegracji strukturalnej. Powiększone jądra astrocytów, o rozrzedzonym układzie chromatyny, zawieszone w pozbawionej organelli cytoplazmie, uznano za mikroskopowo-elektronowy wykładnik komórek Alzheimera typu II.

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ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКАЯ КАРТИНА ЭКСПЕРИМЕНТАЛЬНОЙ ПЕЧЕНОЧНОЙ ЭНЦЕФАЛОПАТИИ

Резюме

Были проведены электронно-микроскопические исследования мозга крыс с печеночной энцефалопатией, вызванной применением четыреххлористого углерода.

Самые ранние ультраструктурные нарушения были обнаружены у животных после 4-месячного применения CCl_4. Наивысшая их интенсивность достигалась по истечению 6 месяцев.

Изменения в электронно-микроскопической картине касались стенок капиллярных сосудов и астроцитной глии. Нарушения в картине сосудов проявлялись увеличением микропиноцитарной активности эпителия и расширением межэпителиальных соединений, которые расценивались как основа увеличенной проницаемости стенок сосудов. Патологические изменения астроцитов выражались значительным отеком цитоплазмы отростков и тела клеток, который приводил к общей структурной дезинтеграции. Увеличенные ядра астроцитов, отличающиеся разреженной системой хроматина, находящиеся в цитоплазме, лишенной органелл расценивались как электронно-микроскопический показатель клеток Альцхаймера II типа.

REFERENCES

1. Adams R. D., Foley J. M.: The neurological changes in the more common types of severe liver disease. Trans. Amer. Neurol. Soc., 1949, 74, 217—225.
2. Baker A. B.: The central nervous system in hepatic disease. J. Neuropath. exp. Neurol. 1949, 8, 283—294.
3. Brightman M. W., Reese T. S.: Tight junctions, gaps and fenestrae in the endothelium of normal and abnormal vertebrate brains. The Proc. Symp. on the Blood-Brain-Barrier. Tmiex. Oxford. 1970, 5—12.
4. Cavanagh J. B., Ma Hta Kyn: Type II Alzheimer change experimentally produced in astrocytes in the rat. J. Neurol. Sci. 1971, 12, 63—75.
5. Foncin J. F.: Pathologie ultrastructurale chez l'homme. Proc. VI. Internat. Congress Neuropath. Paris. Masson et Cie. 1970, 371—391.
6. Foncin J. F., Nicolaidis S.: Encephalopathie porto-cave: contribution à la pathologie ultrastructurale de la glie chez l'homme, Rev. Neurol. 1970, 123, 81—87.
7. Hirano A., Zimmerman H. M., Levine S.: Fine structure of cerebral fluid accumulation. In Brain Edema. ed. Klatzo I., Seitelberger F., Springer. Wien, New York. 1967. pp. 569—589.
8. Lapham L. W.: A study of protoplasmic astrocytes in experimentally produced hepatic encephalopathy. J. Neuropath. exp. Neurol. 1961, 20, 305—309.
9. Martinez A.: Electron microscopy in human hepatic encephalopathy. Acta neuropath. (Berl.) 1968, 11, 82—86.
10. Ma Hta Kyn, Cavanagh J. B.: Some effects of porto-caval anastomoses in the male rat. J. exp. Path. 1970, 51, 217—227.
11. Mossakowski M. J.: Some aspects of the morphology and histochemistry of the cerebral changes in hepatic coma. Proc. V. Intern. Congress Neuropath. Zürich. Elsevier, Amsterdam. 1966a, 981—986.
12. Mossakowski M. J.: Patomorfologia i histochemia spontanicznych i doświadczalnych encefalopatii pochodzenia wątrobowego. Neuropat. Pol. 1966b, 4, 231—304.
13. Mossakowski M. J., Śmiałek M., Pronaszko A.: Zaburzenia przepuszczalności naczyń krwionośnych w doświadczalnej encefalopatii wątrobowej. Neuropat. Pol. 1970, 8, 365—374.

14. Mossakowski M. J., Śmiałek M., Pronaszko A.: The blood-brain barrier abnormality in experimental hepatic encephalopathy. Berichte der I Donau Symposium für Neuropathologie, Wien, 1969. Wiener Med. Akademie 1971, 99—102.
15. Shiraki H.: The comparative study of various types of hepatocerebral diseases in Japan. In: The central nervous system. Some Experimental Models of Neurological Diseases. Ed. O. T. Bailey a. D. E. Smith. The Williams a. Wilkins Co. Baltimore, 1968, 252—272.

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