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ELECTRON MICROSCOPY OF HEPATOGENIC ENCEPHALOPATHY IN RATS INDUCED BY THIOACETAMIDE INTOXICATION

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Chemical liver impairment by the use of various hepatotoxic substances, alongside with surgically performed portal-systemic shunt, constitutes a most common model for experimental studies on the pathogenesis of hepatic encephalopathy (Diemer 1978; Mossakowski 1981). The hepatotoxic action of most chemical compounds used is accompanied by numerous disadvantageous factors. On one hand they consist in a fulminant action of some of them on the liver, reducing the time necessary for development of the exponents of nervous system involvement, on the other, in the necessity of their prolonged application of many months, leading to the appearance of some additional, even incidental pathogenic factors. The possibility of simultaneous action on both liver and brain parenchyma has also to be taken into consideration, thus limiting the distinction between brain lesions resulting from liver impairment and those due to the direct influence of the toxic substance on the nerve tissue.

In recent years, in the search for the possibly best hepatotoxic substance free of the above mentioned inconveniences, thioacetamide, known for a long time in experimental liver pathology (Gupta et al. 1956; Vorbrodt et al. 1966) has been successfully applied.

Thioacetamide intoxication leads within a short period of time to severe liver damage with typical biochemical exponents of its acute failure and both morphological and biochemical abnormalities characteristic of hepatogenic encephalopathy (Hilgier 1983a; Hilgier et al. 1983). Moreover, comparative studies carried out on nerve tissue cultures, showed that features of hepatogenic gliopathy *in vitro* apeared only in cultures grown in nutrient media containing blood serum of rats with previously thioacetamide-induced liver impairment. In the cultures which were run with an exogenous compound added directly to their nutrient

medium, non-specific degenerative changes were the only feature (Kraśnicka et al. 1983). The hepatotoxic action of thioacetamide given in a single or double dose was found to be short-lasting, as far as biochemical exponents of acute liver failure are concerned. This raised the question of either persistence or reversibility of morphological abnormalities in the brain parenchyma, prompting us to perform a series of electron microscopic studies on the central nervous system of rats intoxicated with thioacetamide at the time when the abnormalities in blood biochemistry indicating liver failure were disappearing.

MATERIAL AND METHODS

The studies were performed on albinotic female rats, weighing 180—200 g, which were given in an interval of 24 h two intraperitoneal injections of thioacetamide in a dose of 250 mg/kg body weight. The control animals received intraperitoneal injections of buffered physiological salt solution.

The animals were sacrificed in groups of three (2 — experimental ones and 1 control) by intracardiac perfusion with 2 percent glutaraldehyde in cacodylate buffer, pH 7.2, on the 14th and 21st days after the second injection. Brains removed from the skull were left in the same fixative for the next 24 h at 4°C. Then they were cut coronally into slices 1 mm thick. For electron microscopy, tissue samples, 1 mm^3 in diameter were taken from the frontal cortex, striatum and subcortical white matter. They were postfixed for 1 h in 2 percent osmium tetroxide, dehydrated in graded ethanol solutions, embedded in Epon 812 and then cut on a ultramicrotome. The ultrathin sections were counterstained on grids with uranyl acetate and lead citrate and examined under an electron microscope JEM 7A.

RESULTS

Ultrastructural abnormalities were present in all the examined regions of the brain. They were of the same nature and they did not differ essentially in animals sacrificed 14 and 21 days after intoxication.

The great majority of cortical and striatal nerve cells were normal (Fig. 1). In both structures some "dark neurons" were seen incidentally. They revealed an increased density of the cytoplasm and marked distention of channels and cisternae of the rough endoplasmic reticulum. In the cerebral cortex some nerve cells with features of progressing disintegration were found. This took the form of reduction and/or disappearance of rough endoplasmic reticulum and accumulation of small, irregular aggregates of floccular substances in their cytoplasm (Fig. 2). Neuropil elements such as dendrites, nerve terminals and myelinated and nonmyelinated axons were apparently normal. So were oligodendrocy-

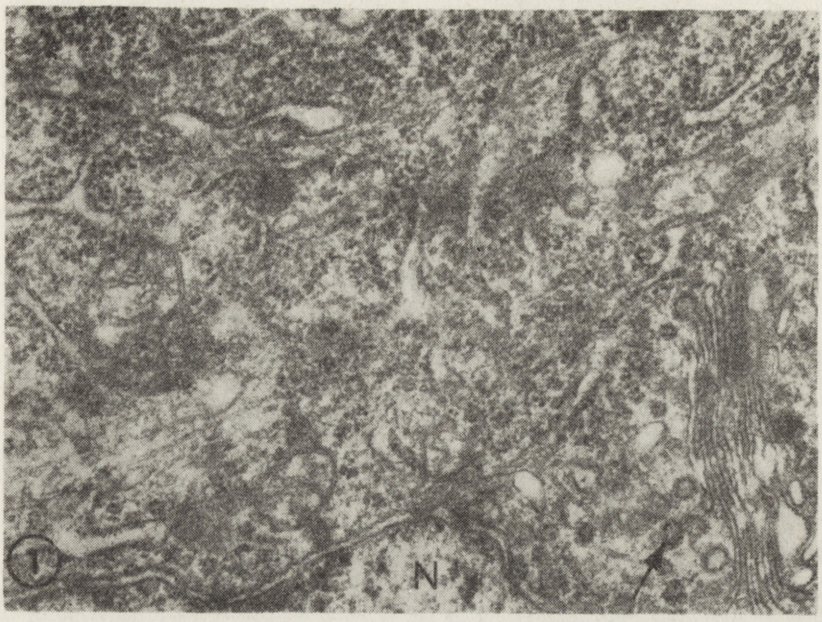

Fig. 1. Animal surviving 14 days after intoxication. Fragment of cortical neuron. In the vicinity of the nucleus (N) channels of rough endoplasmic reticulum, some mitochondria, aggregates of ribosomes, and Golgi apparatus with a few coated vesicles (arrow) are visible. × 37 500

Ryc. 1. Zwierzę z 14-dniowym przeżyciem po zatruciu. Fragment komórki nerwowej kory. W pobliżu jądra (N) widoczne są kanały siateczki śródplazmatycznej szorstkiej, pojedyncze mitochondria, skupienia rybosomów oraz fragment układu Golgiego z pojedynczymi pęcherzykami opłaszczonymi (strzałka). Pow. 37 500 ×

tes both in the grey and white matter (Fig. 3). Astrocytes on the contrary, revealed pathological changes, varying in intensity. Numerous astrocytes with evident features of degeneration were present in all the examined structures alongside with those, which did not show any ultrastructural abnormality. The light cytoplasm of affected astroglial cells was greatly impoverished in all subcellular organelles (Fig. 4). In extreme situations, enlarged, sharply delineated nuclei with delicate, evenly distributed chromatin were situated against the background of amorphous substances, containing some remnants of severely damaged cytoplasmic organelles, mostly shrunken mitochondria (Figs 5, 6). Astrocytic processes either lying free in the neuropil or surrounding capillaries were changed in a similar manner. Perivascular end feet were severely swollen. Many of them were filled with delicate amorphous masses with some residual, impaired subcellular structures, mostly dark, condensed mitochondria (Figs 7, 8).

This type of changes was present in both experimental groups. It seemed, however, that they were more common and advanced in animals surviving 21 days following thioacetamide intoxication. Alongside with capillaries surrounded by abnormal astrocytic processes, numerous blood vessels with entirely normal tissue surroundings were also present (Fig. 9).

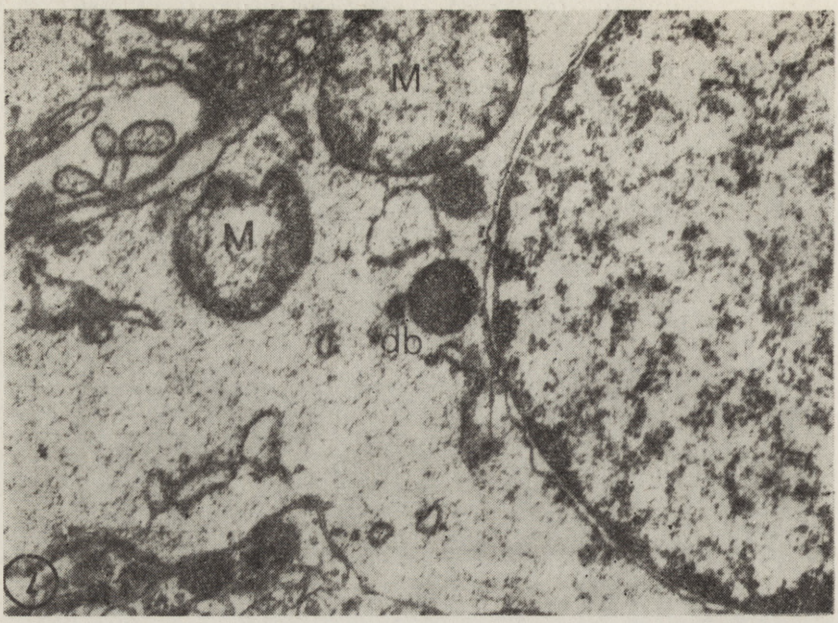

Fig. 2. Animal surviving 21 days after intoxication. Fragment of cortical neuron with almost complete lack of rough endoplasmic reticulum. Among irregular floccular substance aggregates single dense bodies (db) and degenerating mitochondria (M) are visible. × 40 000

Ryc. 2. Zwierzę z 21-dniowym przeżyciem po zatruciu. Fragment komórki nerwowej (stożek aksonalny) kory. Prawie całkowity brak struktur siateczki śródplazmatycznej. Wśród nieregularnej substancji kłaczkowatej pojedyncze ciała gęste (db) i wyrodniejące mitochondria (M). Pow. 40 000 ×

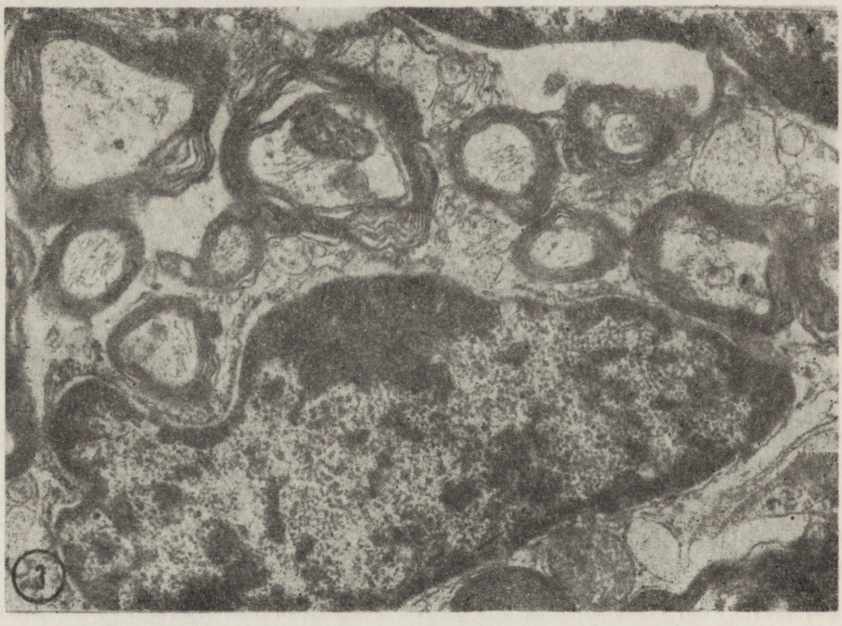

Fig. 3. Animal surviving 21 days after intoxication. Fragment of subcortical white matter with unchanged oligodendrocyte among myelinated nerve fibers. × 12 500

Ryc. 3. Zwierzę z 21-dniowym przeżyciem po zatruciu. Podkorowa istota biała. Wśród licznych zmielinizowanych aksonów widoczna prawidłowa komórka oligodendrogleju. Pow. 12 500 ×

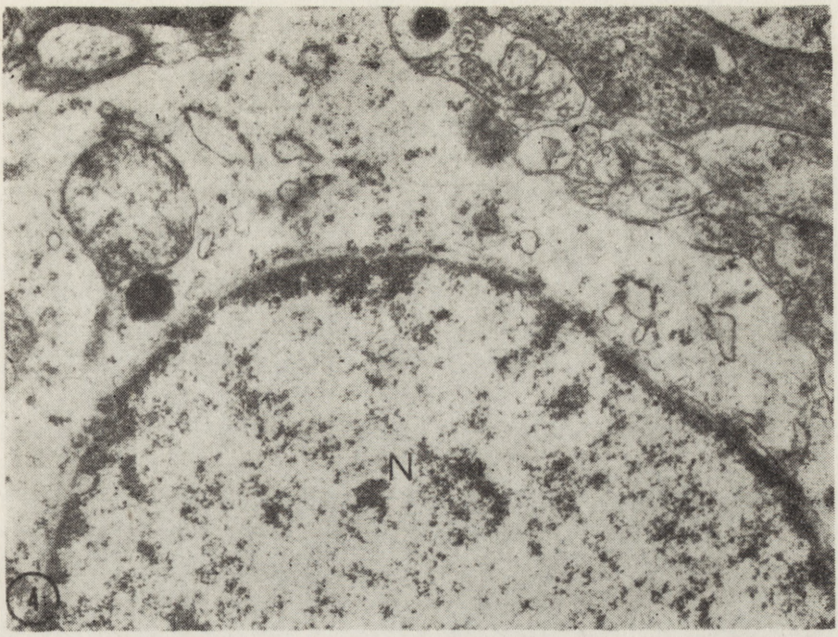

Fig. 4. Animal surviving 14 days after intoxication. Fragment of astrocyte from cerebral cortex. Nucleus (N) with irregular aggregations of coarse chromatin and light nucleoplasm. Light cytoplasm with rudimentary channels of rough endoplasmic reticulum, and small aggregates of ribosomes. Single mitochondrion and dense body are visible. × 26 000

Ryc. 4. Zwierzę z 14-dniowym przeżyciem po zatruciu. Fragment astrocytu kory. W jądrze (N) nieregularne skupienia chromatyny oraz przejaśnienie nukleoplazmy. Cytoplazma jasna ze szczątkowo zachowanymi kanałami siateczki śródplazmatycznej oraz drobnymi skupieniami rybosomów. Mitochondrium i ciałko gęste. Pow. 26 000 ×

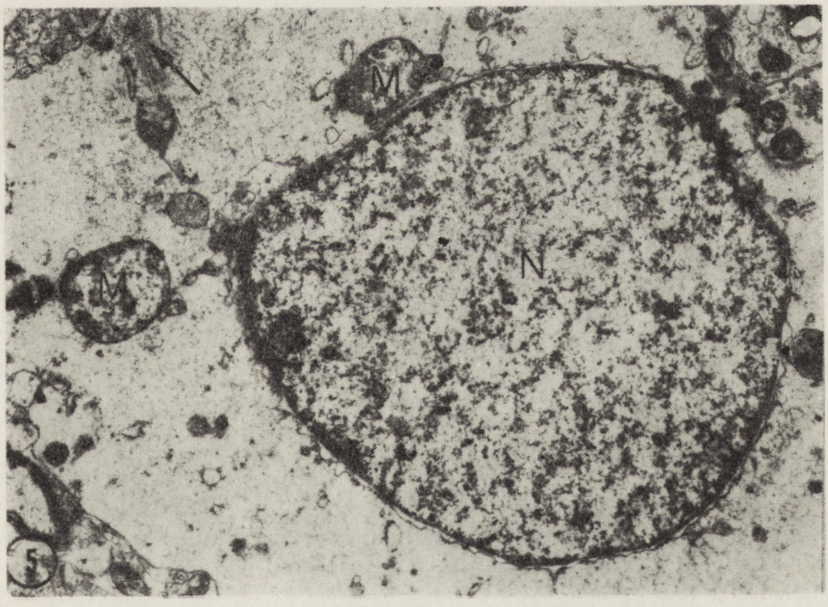

Fig. 5. Animal surviving 21 days after intoxication. Cortical astrocyte with well preserved nucleus (N). Cytoplasm filled with dispersed amorphic substance. A few degenerating mitochondria (M) and delicate bundle of gliofilaments (arrow) are visible. × 21 000

Ryc. 5. Zwierzę z 21-dniowym przeżyciem po zatruciu. Astrocyt kory mózgu. Dobrze zachowane jądro komórkowe (N). W miejscu cytoplazmy rozproszona substancja amorficzna z pojedynczymi wyrodniejącymi mitochondriami (M), oraz delikatnym pęczkiem włókienek glejowych (strzałka). Pow. 21 000 ×

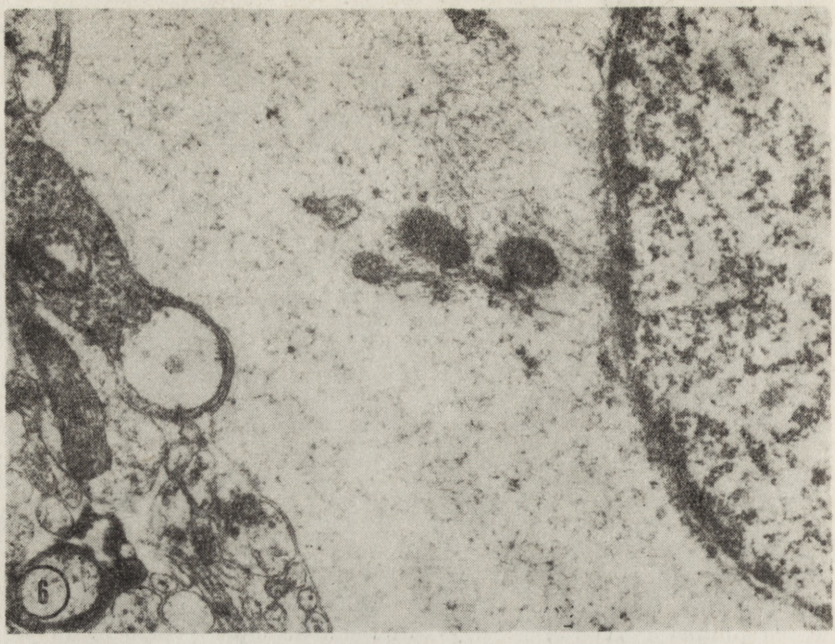

Fig. 6. Animal surviving 21 days after intoxication. Fragment of striatal astrocyte. Total disintegration of cytoplasm with well preserved nucleus. × 21 500

Ryc. 6. Zwierzę z 21-dniowym przeżyciem po zatruciu. Fragment astrocytu ze zwojów podstawy. Widoczna całkowita dezintegracja cytoplazmy, przy zachowanym jądrze komórkowym. Pow. 21 500 ×

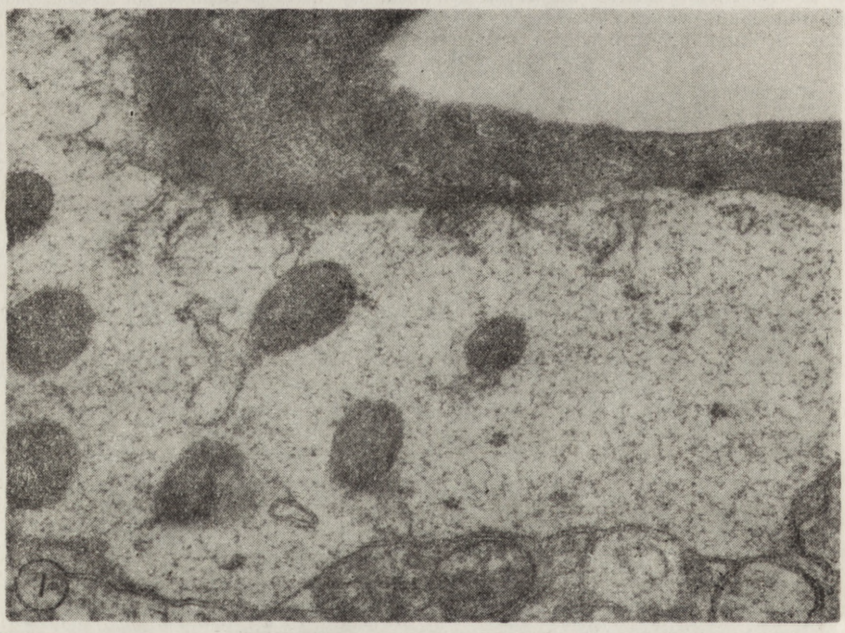

Fig. 7. Animal surviving 21 days after intoxication. Fragment of striatal capillary vessel. Neighbouring astrocytic process with a few rudiments of rough endoplasmic reticulum and single mitochondria. × 37 500

Ryc. 7. Zwierzę z 21-dniowym przeżyciem po zatruciu. Fragment naczynia włosowatego zwojów podstawy. Do ściany naczynia przylega wypustka astrocytu, w której widoczne są jedynie resztki siateczki śródplazmatycznej i pojedyncze mitochondria. Pow. 37 500 ×

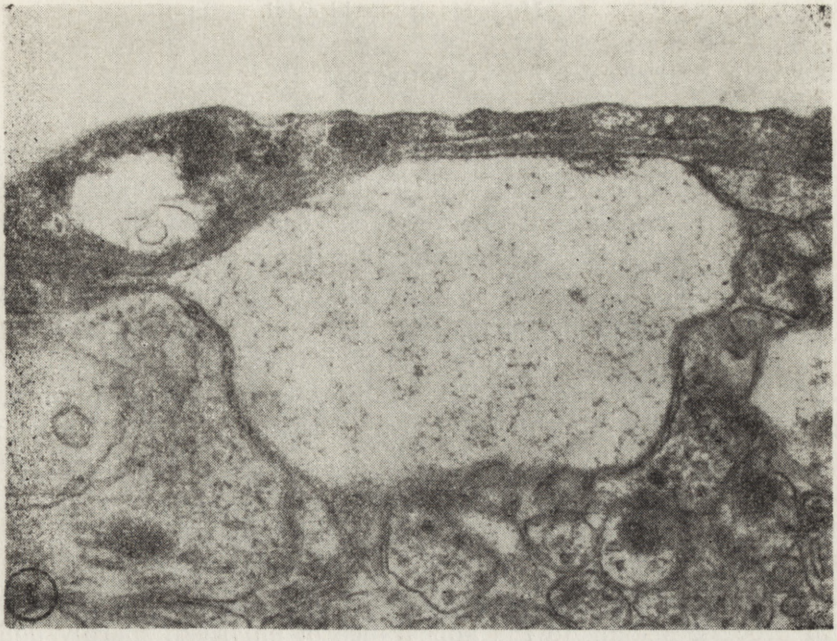

Fig. 8. Animal surviving 21 days after intoxication. Fragment of cortical capillary. Neighbouring astrocytic process totally devoid of any subcellular structures. × 37 500

Ryc. 8. Zwierzę z 21-dniowym przeżyciem po zatruciu. Fragment naczynia włosowatego kory mózgu z przylegającą wypustką astrocytu pozbawioną wszelkich struktur. Pow. 37 500 ×

Fig. 9. Animal surviving 21 days after intoxication. Fragment of striatal capillary vessel with entirely normal structure of the tissue surroundings. × 33 000

Ryc. 9. Zwierzę z 21-dniowym przeżyciem po zatruciu. Fragment naczynia włosowatego zwojów podstawy. Obraz prawidłowy, bez zmian patologicznych. Pow. 33 000 ×

DISCUSSION

Ultrastructural abnormalities found in the brains of rats intoxicated with thioacetamide concerned exclusively the astrocytic population, with almost entirely unchanged other cellular elements of the nerve tissue. Impaired neurons were so sporadic that they could be considered as either incidental or secondary findings, this being in full agreement with the opinion considering astrocyte lesion as a primary feature in hepatogenic encephalopathy (Mossakowski 1966; Seitelberger 1970).

The electron microscopic abnormalities of astrocytes resembled to a great extent those described by Ostenda et al. (1976) in experimental hepatic encephalopathy induced by prolonged carbon tetrachloride intoxication and by Norenberg et al. (1974) in late stages of portal-systemic encephalopathy. They were also similar to lesions found in some cases of human hepato-cerebral pathology (Martinez 1968; Foncin 1970; Foncin, Nicolaidis 1970). They revealed a typical evolution of the pathological process, leading to the appearance of enlarged, sharply delineated astrocytic nuclei, surrounded by remnants of highly disintegrated cytoplasm. This picture seemed to represent an electron microscopic equivalent of Alzheimer cells, type II. Abnormalities involving astrocytic processes, as confronted with light microscopic observations, could correspond to the features of klasmatodendrosis. Therefore, astrocytic lesions varying in intensity can be considered as subsequent stages of Alzheimer cells, type II formation. In the light of the accepted role of astrocytes in transport phenomena in the brain, severe alteration of their perivascular processes may lead to profound disturbances in the function of the blood-brain interphase.

Against the background of the presented electron microscope observations, compensatory features concerning ammonia metabolism in the brain noticed at this stage of the pathological process (Hilgier 1983a) seem to be connected with the function of the remaining, unchanged astrocytic population. Astrocyte proliferation, difficult for electron microscopic evaluation, is also a typical feature of hepatogenic encephalopathy both in humans and in experimental animals (Mossakowski 1978). Its exponents were found in early stages of hepatogenic encephalopathy in rats intoxicated with thioacetamide (Hilgier et al. 1983).

It seems worth mentioning that characteristic astroglial abnormalities in the experimental model used were present and progressing at that time, when biochemical exponents of acute liver failure and brain metabolic abnormalities were disappearing (Hilgier 1983a; Hilgier et al. 1983). This may have been connected with what is called maturation of the pathological process. The maturation phenomenon, described originally by Klatzo (1975) in the ischemic pathology of the brain, seems to be a feature of a more universal nature. On the other hand, it may

result from a protracted accumulation of toxic substance(s) in the brain. The astrocytic pathology in hepatogenic encephalopathy has been usually connected with the toxic action of ammonia, owing to its excess accumulation in the brain, resulting from its insufficient detoxication (Mossakowski 1978). However, in the applied experimental model and scheme of thioacetamide intoxication, excess accumulation of brain ammonia was not a feature at any period of observation, despite its highly increased content in the blood serum. The continuously noticed accumulation of brain glutamine was considered as an indication of a highly sufficient ammonia detoxication system.

Basing on the observations in other experimental models of hepatogenic encephalopathy, Hilgier (1983b) postulated the existence of an alternative mechanism of astrocytic damage, connected with the neurotoxic action of α-ketoglutaramate. This substance, being a direct product of glutamine metabolism, is known to accumulate in the brain in hepatogenic encephalopathy (Duffy et al. 1974). In the light of the data presented this mechanism seems to be highly probable in the case of hepatogenic encephalopathy induced by thioacetamide intoxication. However, this hypothesis requires biochemical confirmation.

MIKROSKOPOWO-ELEKTRONOWY OBRAZ ENCEFALOPATII WĄTROBOWEJ U SZCZURÓW W ZATRUCIU TIOACETAMIDEM

Streszczenie

Przedstawiono wyniki analizy mikroskopowo-elektronowej mózgu szczurów zatruwanych tioacetamidem w okresie cofania się wykładników biochemicznych niewydolności wątroby i zaburzeń metabolicznych w ośrodkowym układzie nerwowym. Stwierdzono, iż nieprawidłowości tkankowe w mózgu dotyczą wybiórczo astrocytów. Prześledzono kolejne fazy uszkodzeń komórek gwiaździstych, aż do uformowania komórek Alzheimera typu II.

Wobec braku nagromadzenia amoniaku w mózgu, przy wybitnym wzroście glutaminy, wysunięto przypuszczenie, że czynnikiem odpowiedzialnym za uszkodzenie astrocytów może być α-ketoglutaraminian, stanowiący neurotoksyczny produkt metabolizmu glutaminy.

ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКАЯ КАРТИНА ПЕЧЕНОЧНОЙ ЭНЦЕФАЛОПАТИИ У КРЫС ОТРАВЛЕННЫХ ТИОАЦЕТАМИДОМ

Резюме

Представлены результаты электронно-микроскопического анализа головного мозга у крыс отравляемых тиоацетамидом в периоде отступания биохимических показателей недостаточности печени и метаболических расстройств в центральной нервной системе. Констатировано, что тканевые аномалии в головном мозгу относятся селективно к астро-

цитам. Прослежено поочередно фазы повреждений астроцитов вплоть до образования клеток Альцгеймера II типа.

Из-за недостатка накопления аммиака в головном мозгу во время выдающегося роста глутамина сделано предположение, что фактором ответственным за повреждение астроцитов может быть α-кетоглутарамат, представляющий собой невротоксический продукт метаболизма глутамина.

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