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THIOACETAMIDE-INDUCED HEPATIC ENCEPHALOPATHY IN THE RAT

II. CYTOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON ASTROCYTES CULTURED *IN VITRO*

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The use of hepatotoxic agents to produce liver damage has become a routine procedure in studies on experimental hepatic encephalopathy (for a review see Diemer 1978). In the preceding paper we have emphasized some advantages of using thioacetamide (TAA *) as a hepatotoxic compound which rapidly and reproducibly induced changes in blood parameters and brain metabolism resembling those observed both in other experimental models and in human subjects suffering from hepatic failure (Hilgier et al. 1983). In general, however, the results obtained with the use of hepatotoxic models cannot be interpreted unanimously. The major problem is the difficulty to distinguish between the events related to liver damage and the direct neurotoxic effects caused by the agent or its metabolites. The present work was an attempt to evaluate separately the hepatogenic and direct neurotoxic aspects of the thioacetamide model, with the emphasis on the morphology and ultrastructure of the astrocytes as the primary target cell in hepatic encephalopathy. To this end, we have compared the effect of TAA itself and of the serum obtained from rats treated with TAA according to the previously established protocol (Hilgier et al. 1983), on astrocytes grown in tissue culture *in vitro*.

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* Abbreviations used: TAA — thioacetamide, GDH — glutamic acid dehydrogenase, SDH — succinic acid dehydrogenase, G6PDH — glucose-6-phosphate dehydrogenase.

MATERIAL AND METHODS

The studies were performed on 3-week organotypic cultures of the newborn rat cerebellum, carried out in a routine way (Kraśnicka, Mossakowski 1965). Cultures designed as experimental group I were grown for 3 days in the media containing the sera derived from rats given two intraperitoneal injections of TAA in the dose of 250 mg per kg body weight at 24 h intervals (Hilgier et al. 1983). The animals were exsanguinated 48 h after the second TAA injection. The sera of TAA-treated animals composed 50 percent of the total volume of culture medium. In the experimental group II the toxic agent was given directly to the culture medium in final concentration of either 25 or 50 mg%. The exposure to TAA in cultures with its lesser concentration lasted 2, 4 and 6 days, while in those with higher concentration of the toxic agent only 2 days. Control cultures were grown in the routine medium for the time periods corresponding to those in experimental groups I and II.

For light microscopic observations the cultures were stained according to the Nissl and PAS methods. Histochemical reactions disclosing the GDH, SDH and G6PDH activities were performed as described previously (Mossakowski et al. 1970).

The material for electron microscopic studies was routinely fixed and embedded in Epon 812. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined under the JEM 7A electron miscroscope.

RESULTS

Group I

Light microscopic observations of cultures grown in the sera derived from TAA-treated rats revealed the presence in all of them of single or grouped hypertrophied glial cells (Fig. 1). These astrocytes aquired oval shape, were mostly depleted of processes and contained one or more of hyperchromatic nuclei on the cell periphery. Their cytoplasm was filled with coarse, PAS-positive granules (Fig. 2). The GDH and SDH activities were decreased as compared with the control cultures in all the glial cells, including the pathological forms described above, while the G6PDH reaction was enhanced in the hypertrophied cells lacking processes or with rudimentary processes, but unchanged in normal astroglia.

Electron microscopic examination of this group disclosed likewise typical astrocytic changes. The majority of astroglial cells contained abundant cytoplasm and light nuclei with uniformly distributed chromatin, except for some condensations adjacent to the nuclear envelope.

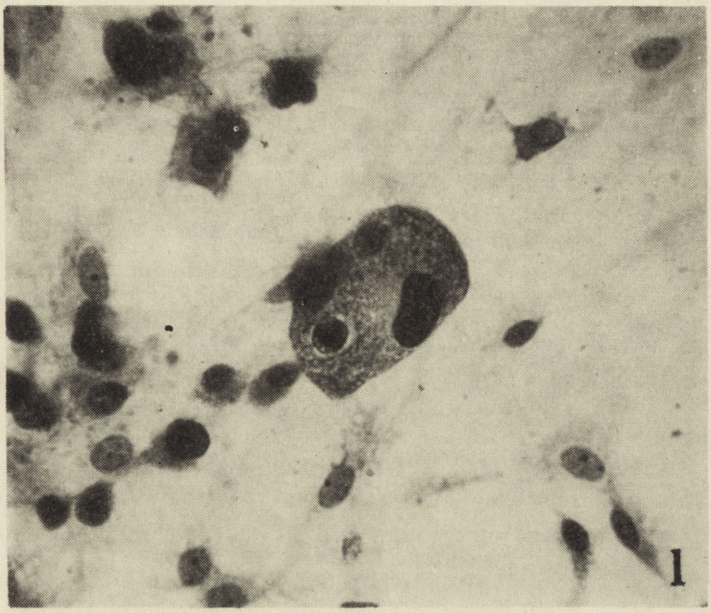

Fig. 1. Group I. Polynuclear Opalski cell free of processes. Nissl. × 400
Ryc. 1. Grupa I. Wielojądrzasta, bezwypustkowa komórka Opalskiego. Nissl. Pow. 400 ×

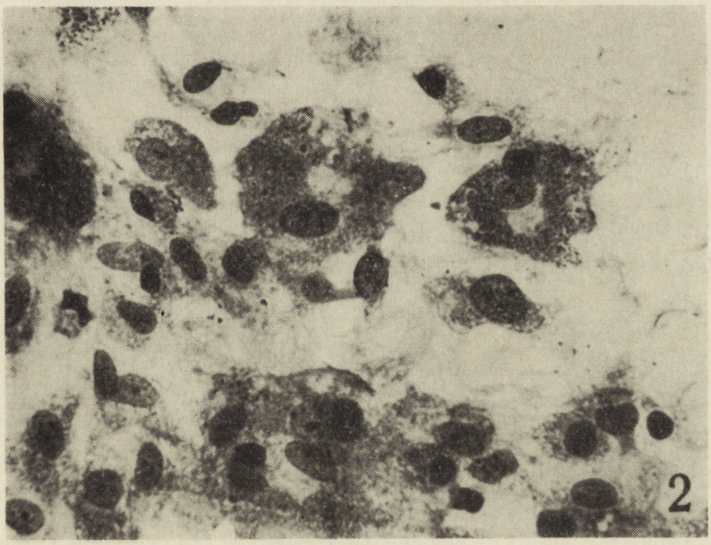

Fig. 2. Group I. Numerous hypertrophied astrocytes (Opalski-like cells) with coarse PAS-positive granules in the cytoplasm. PAS. × 400
Ryc. 2. Grupa I. Liczne hipertroficzne astrocyty, podobne do komórek Opalskiego z gruboziarnistym PAS-dodatnim odczynem w cytoplazmie. PAS. Pow. 400 ×

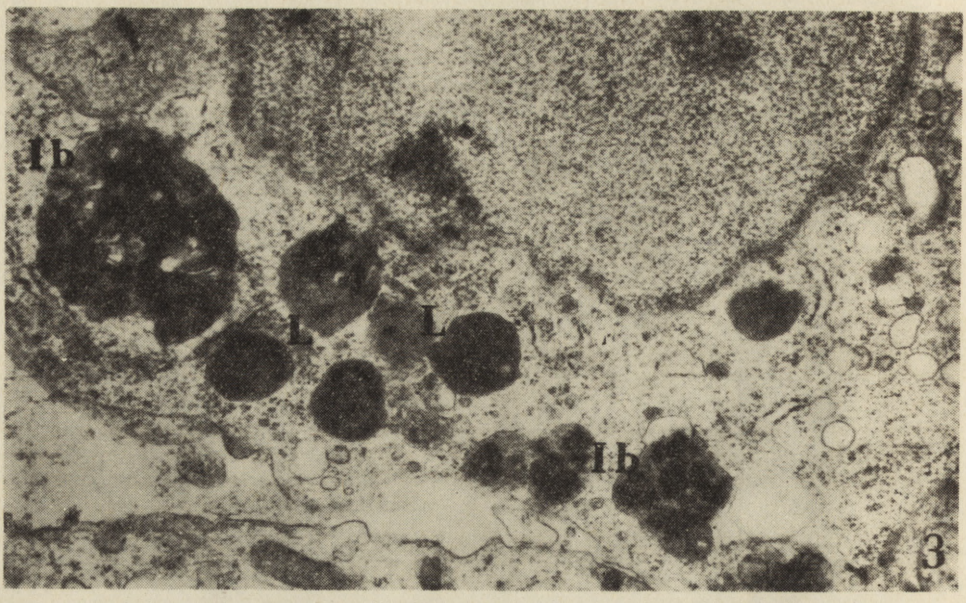

Fig. 3. Group I. Fragment of Opalski cell. In the cytoplasm numerous lysosome-like bodies (L), large membrane-bounded inclusion bodies (Ib), containing granular and amorphous material and small mitochondria. × 21 700

Ryc. 3. Grupa I. Fragment komórki Opalskiego. W cytoplazmie liczne ciała lizosomo-podobne (L), duże obłonione ciała wtrętowe (Ib), zawierające ziarnisty i bezpostaciowy materiał oraz drobne mitochondria. Pow. 21 700 ×

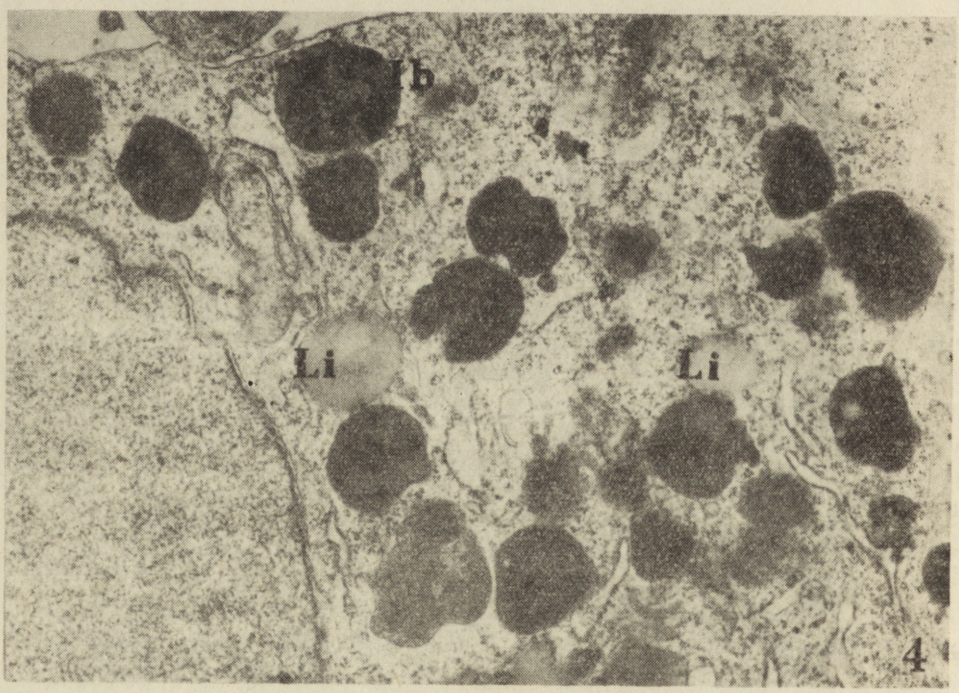

Fig. 4. Group I. Astrocyte fragment. In the cytoplasm inclusion body (Ib), numerous lysosome-like structures and lipid droplets (Li). × 21 700

Ryc. 4. Grupa I. Fragment astrocyta. W cytoplazmie ciało wtrętowe (Ib) oraz liczne struktury lizosomo-podobne i krople lipidów (Li). Pow. 21 700 ×

The mitochondria were small or elongated and less numerous than in the control astrocytes. The endoplasmic reticulum appeared in the form of short, slightly dilated channels covered with randomly distributed ribosomes. The Golgi apparatus was poorly developed and formed

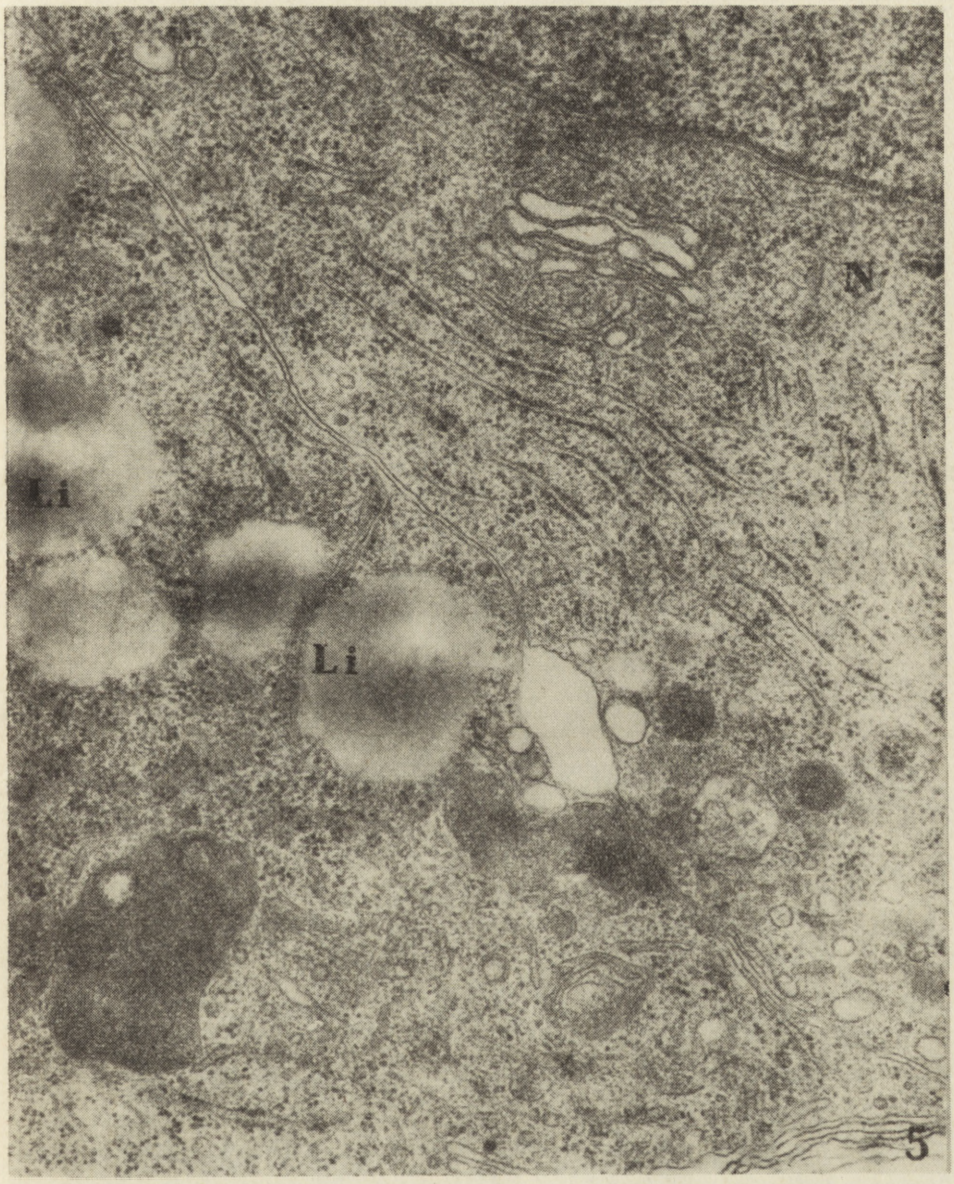

Fig. 5. Group I. Fragment of neuron and oligodendrocyte. Apparently normal ultrastructure of nerve cell (N) and numerous lipid droplets (Li) in the oligodendrocyte cytoplasm. × 28 200

Ryc. 5. Grupa I. Fragment komórki nerwowej i oligodendrocyta. Prawidłowa struktura komórki nerwowej (N) i liczne krople lipidowe (Li) w cytoplazmie oligodendrocyta. Pow. 28 200 ×

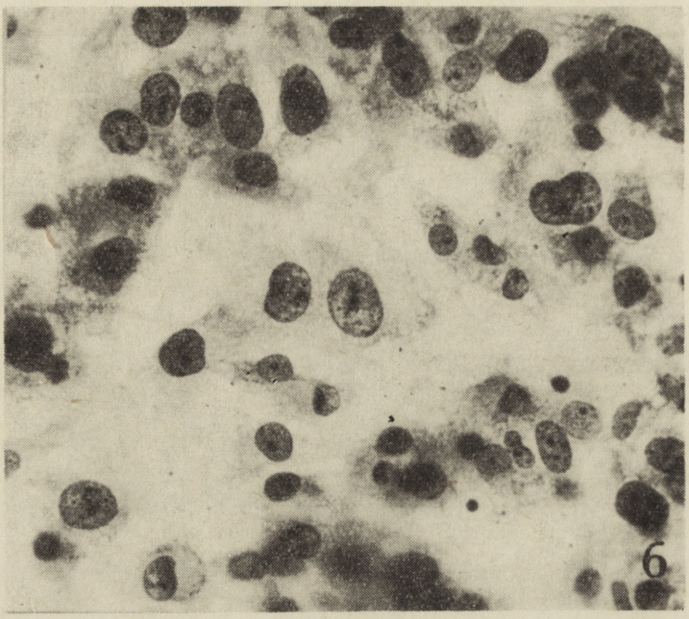

Fig. 6. Group II (TAA 25 mg%, 4 days). Swelling and vacuolization of astrocytic cytoplasm. Polymorphous astrocytic nuclei. Nissl. × 400

Ryc. 6. Grupa II (TAA 25 mg%, 4 dni). Obrzmienie i wakuolizacja cytoplazmy astrocytów oraz znaczny polimorfizm ich jąder. Nissl. Pow. 400 ×

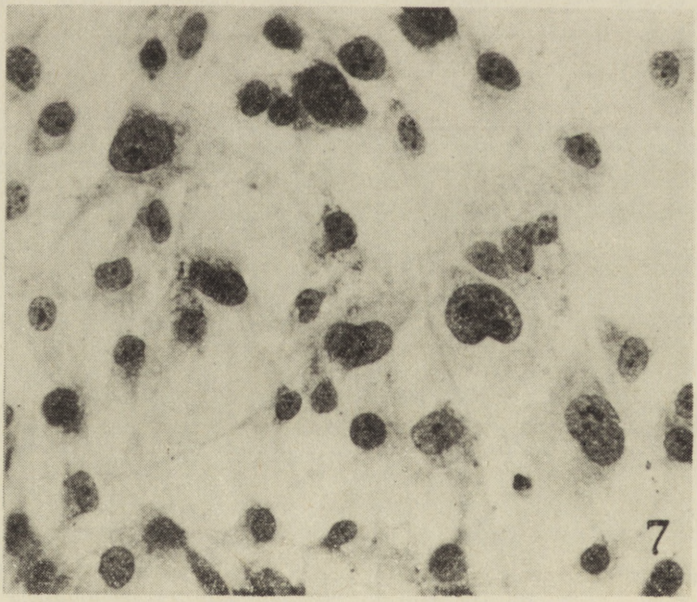

Fig. 7. Group II (TAA 25 mg%, 6 days). Significant destruction of the astrocytic cytoplasm. Glial cell nuclei of different size and shape. Nissl. × 400

Ryc. 7. Grupa II (TAA 25 mg%, 6 dni). Znaczne uszkodzenie cytoplazmy astrocytów oraz zróżnicowanie kształtów i wielkości ich jąder. Nissl. Pow. 400 ×

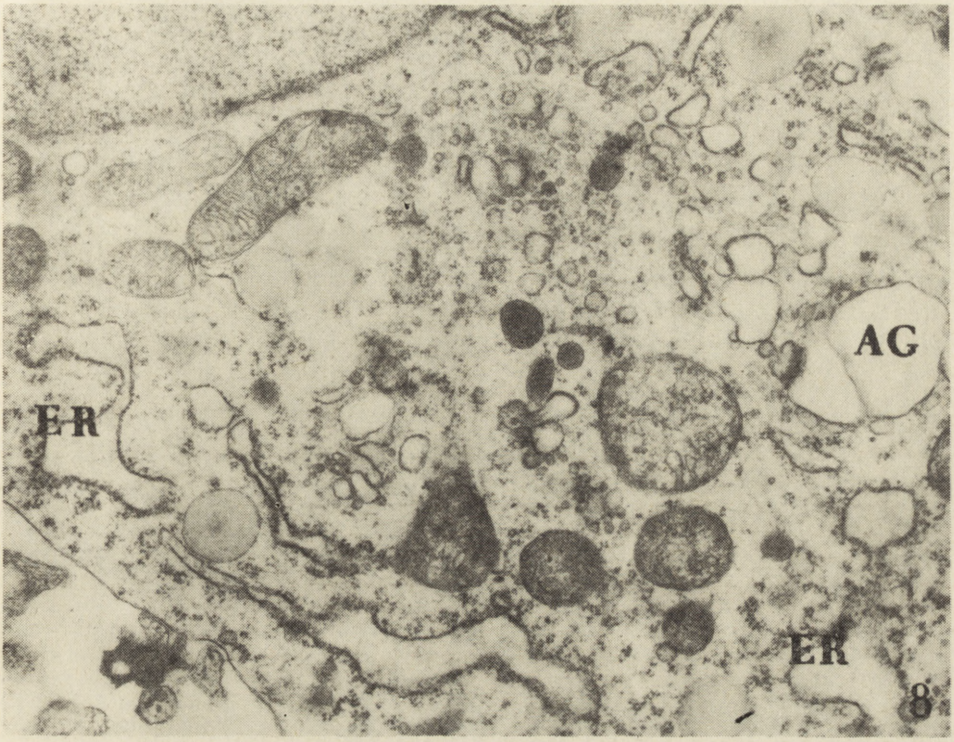

Fig. 8. Group II (TAA 25 mg%, 2 days). Astrocyte fragment. Dilated endoplasmic reticulum channels (ER), containing electron-lucid material. Some channels of Golgi apparatus are also distended (AG). × 28 200

Ryc. 8. Grupa II (TAA 25 mg%, 2 dni). Fragment astrocyta z poszerzonymi kanałami siateczki śródplazmatycznej (ER), zawierającymi elektronowo-jasny materiał. Niektóre kanały aparatu Golgiego (AG) znacznie poszerzone. Pow. 28 200 ×

typical grouping of channels and vesicles. Some of the astroglial cells with abundant cytoplasm contained membrane-bounded inclusion bodies of irregular shape and different sizes composed in part of aggregated electron-dense material (Fig. 3). Beside, the cells were characterized by the presence of numerous lysosome-like structures (Figs 3, 4). No significant structural changes of nerve cells or myelin were observed in this group. The oligodendrocytes contained numerous lysosomes and lipid droplets (Fig. 5).

Group II

The cultures grown with 25 mg% TAA for 2 days showed no light microscopic changes. Prolonged TAA treatment (for 4 or 6 days) induced distinct morphological alterations in the whole cell population in the growth region. The glial cells were characterized by swollen cytoplasm with numerous vacuoles (Fig. 6) and contained enlarged, in a few instances kidney-shaped nuclei (Fig. 7). Pronounced degenerative changes hampered the distinction of the particular cell types. Glial cell

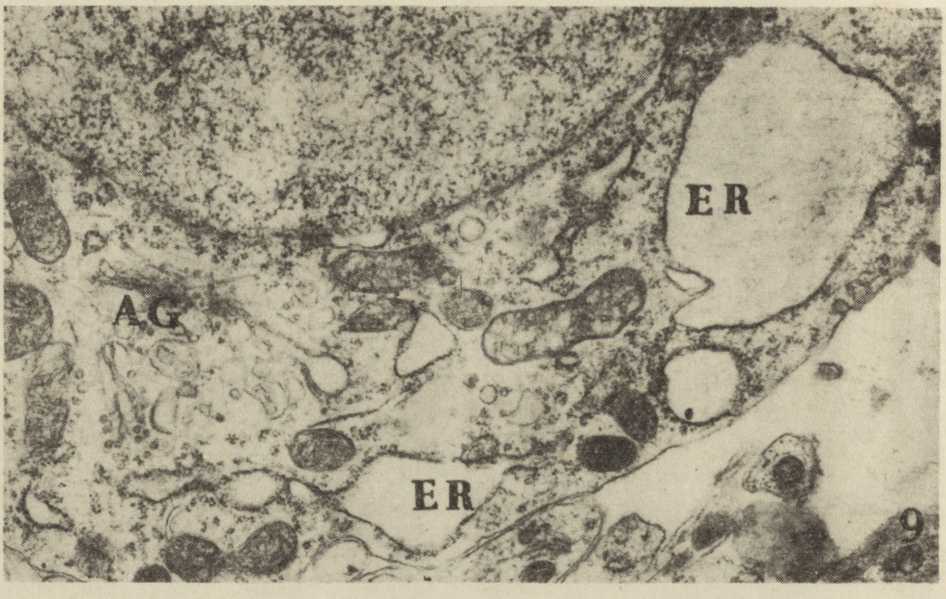

Fig. 9. Group II (TAA 25 mg%, 2 days). Astrocyte fragment. Cytoplasm with markedly dilated rough endoplasmic reticulum channels (ER) poor in ribosomes. Small mitochondria and very poorly developed Golgi apparatus (AG). × 21 700

Ryc. 9. Grupa II (TAA 25 mg%, 2 dni). Fragment astrocyta z cytoplazmą zawierającą znacznie poszerzone kanały szorstkiej siateczki śródplazmatycznej (ER) ubogie w rybosomy. Drobne mitochondria i bardzo słabo rozwinięty aparat Golgiego (AG). Pow. 21 700 ×

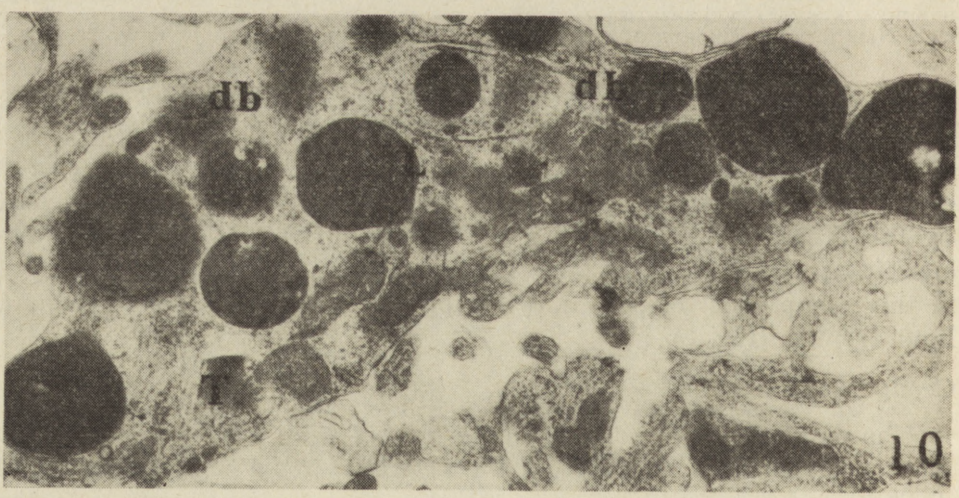

Fig. 10. Group II (TAA 25 mg%, 2 days). Oligodendrocyte fragment, containing numerous lisosome-like structures (L), dense bodies (db), microtubules (T) and small shrunken mitochondria with dark matrix. × 28 200

Ryc. 10. Grupa II (TAA 25 mg%, 2 dni). Fragment oligodendrocyta, którego cytoplazma zawiera liczne struktury lizosomo-podobne (L), ciała gęste (db), mikrotubule (T) oraz małe obkurczone mitochondria z ciemną macierzą. Pow. 28 200 ×

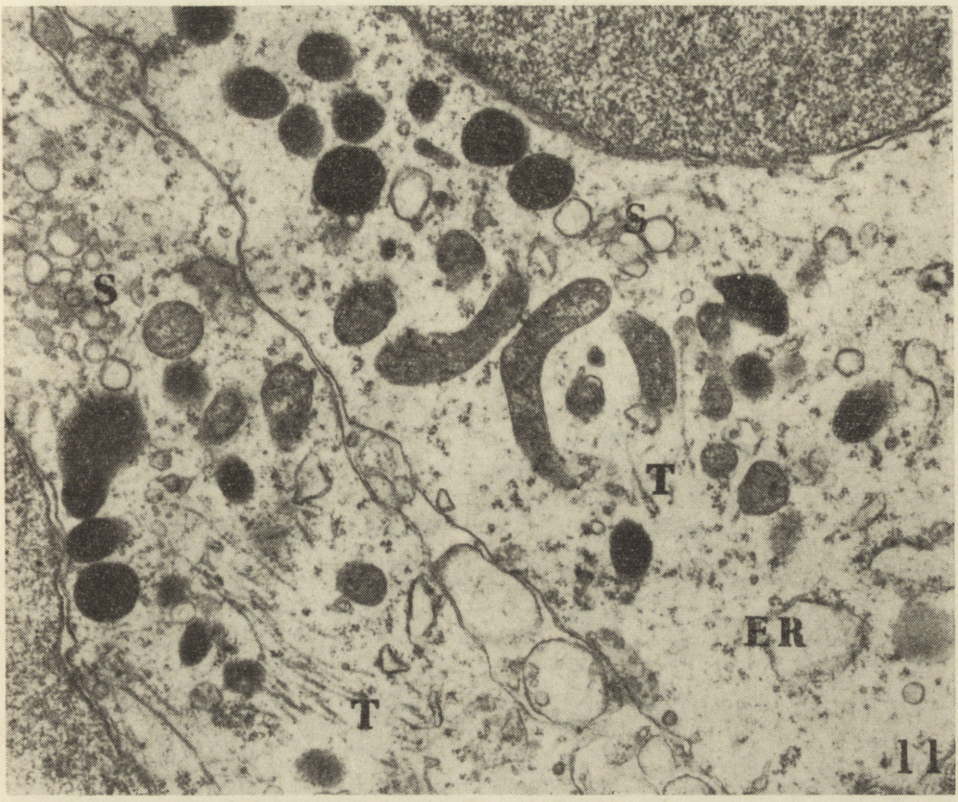

Fig. 11. Group II (TAA 25 mg%, 6 days). Fragment of astrocyte with pale cytoplasm containing short segments of dilated rough (ER) and smooth (S) endoplasmic reticulum channels, numerous lysosome-like structures and microtubules (T). × 21 700

Ryc. 11. Grupa II (TAA 25 mg%, 6 dni). Fragment astrocyta z jasną cytoplazmą zawierającą krótkie odcinki kanałów szorstkiej (ER) i gładkiej (S) siateczki śródplazmatycznej, liczne struktury lizosomo-podobne i mikrotubule (T). Pow. 21 700 ×

cytoplasm contained fine PAS-positive granules. The histoenzymatic reactions revealed decreased activities of all the three dehydrogenases examined.

Application of TAA in the higher dose (50 mg%) led to significant changes already within 2 days after administration. The changes mostly involved the astrocytes. The astrocytic nuclei were enlarged, pale, with a distinct nuclear membrane and dark nucleolus. The cytoplasm did not stain and its residual fragments accumulated around nuclei. Some of the cells showed swollen cytoplasm and the presence of small vacuoles. Most of the glial cells contained PAS-positive substance in the form of fine granular deposits. The enzymatic reactions disclosed only traces of GDH and SDH activities and a somewhat higher G6PDH activity.

Electron microscopic observations of the cultures subjected to a 25 mg% TAA dose for 2 days revealed ultrastructural changes mostly

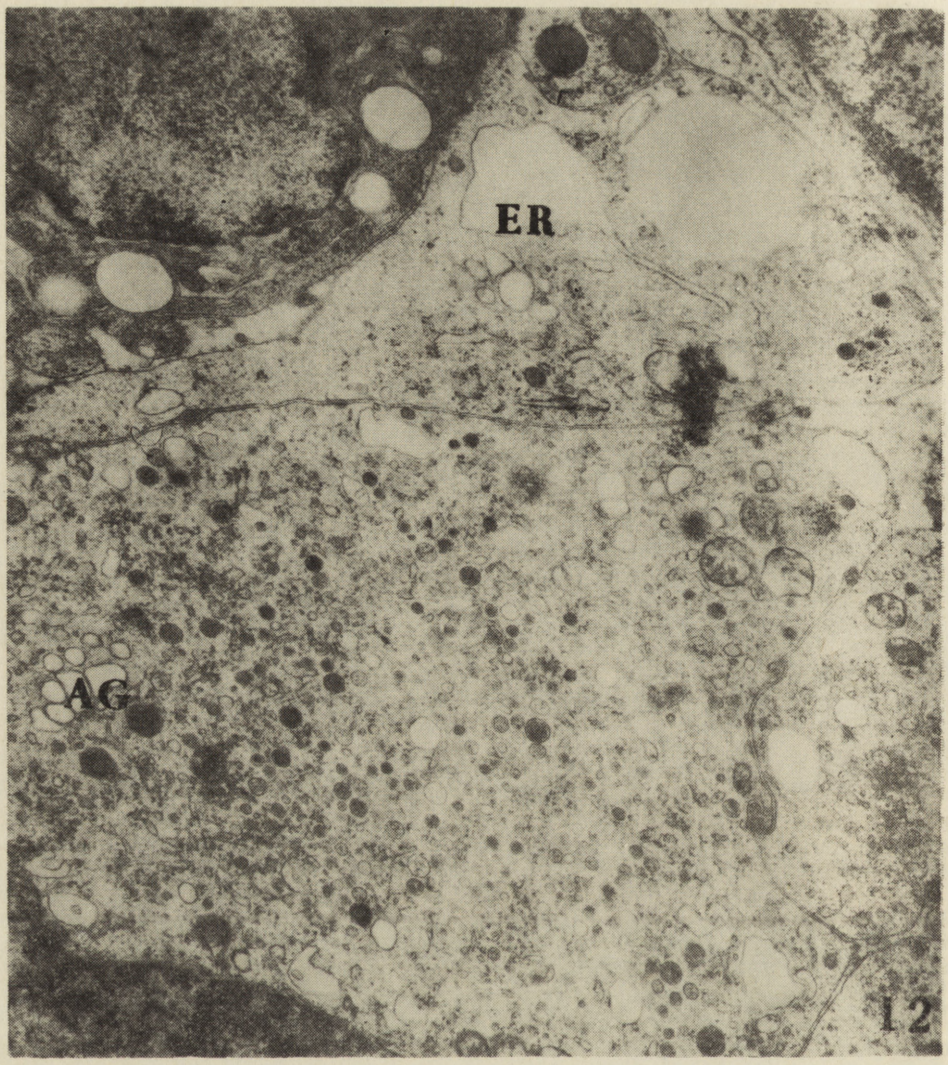

Fig. 12. Group II (TAA 50 mg%, 2 days). Fragments of some glial cells. In the upper part a cell with baloon-like dilatation of the rough endoplasmic reticulum channels (ER). Adjacent cell with numerous small lysosomes and vesicular structure of the smooth endoplasmic reticulum. AG — Golgi apparatus. In the upper left part fragment of oligodendrocyte. × 28 200

Ryc. 12. Grupa II (TAA 50 mg%, 2 dni). Fragmenty kilku komórek glejowych. W górnej części komórka z balonowato rozdętymi kanałami szorstkiej siateczki śródplazmatycznej (ER). Przylegająca komórka zawiera liczne drobne lizosomy i pęcherzykowe struktury siateczki gładkiej. AG — aparat Golgiego. W górnej lewej części fragment oligodendrocyta. Pow. 28 200×

involving astrocytes. Astrocytic nuclei were chromatin-poor, with evenly distributed chromatin granules. Some of them revealed seggregation of the nucleolar material. Most of the astrocytes had dilated rough endoplasmic reticulum channels filled with electron-lucid material (Figs 8, 9). In a number of cells slight cytoplasm swelling with a significant deple-

tion of cellular organellae were observed. The majority of mitochondria were small, but ultrastructurally normal. Golgi apparatus was moderately developed with some channels dilated. Oligodendrocytes contained in the cytoplasm increased number of dense bodies, lisosome-like structures, lipid droplets, glial tubules and small and shrunk mitochondria with a dark matrix (Fig. 10).

After 4 days of TAA treatment the ultrastructural changes in the glial cells became more pronounced. The cytoplasm of astrocytes contained an increased number of lysosomes and abundance of glial tubules and glial filaments. Some of the cells were characterized by the proliferation of smooth endoplasmic reticulum in the form of small vesicles.

In the cultures subjected to TAA for 6 days a further progression of ultrastructural changes was observed. The dilated rough endoplasmic reticulum transformed into vesicular structures, mostly free of ribosomes. In some of the cells regions of cytoplasm free of organelles or their fragments were visible. In other cells proliferation of smooth endoplasmic reticulum was observed to appear in the form of numerous vesicles (Fig. 11). The astrocytic processes were rich in glial filaments. The most significant changes in the ultrastructure of astrocytes became apparent in the cultures subjected to a 2-day TAA treatment in a 50 mg% dose. The cell nuclei contained chromatin aggregates under the envelope and electron dense nucleoli with a distinct granular part. Mitochondria were markedly swollen and enlarged, with an electron transparent matrix and rudimentary cristae. The rough endoplasmic reticulum channels were very dilated in most of the cells, and in some of them proliferation of the smooth endoplasmic reticulum was observed. The Golgi apparatus was poorly developed and forming vesicular structures (Fig. 12).

DISCUSSION

Since it is difficult to assume the existence of a selective hepatotoxic agent capable of inducing hepatogenic encephalopathy without interference of other pathological processes in the brain, less rigid criteria may be proposed favouring the selection of an agent to be applied in experimental studies. From the theoretical perspective, preference will be given to a compound which affects the liver: a) rapidly enough to ensure that, the onset of changes in the brain which are subsequent to the liver damage will precede both the manifestation of direct neurotoxic effects of the compound or its metabolites and the influence of disturbed function of other organs; b) strongly enough to render hepatogenic effects which will mask all the other effects. A number of earlier data have indirectly indicated that thioacetamide is the compound that might at least in part conform to such criteria. It was observed that intra-

peritoneal administration of this chemical into rats in doses similar to those applied in this study produced almost instantly degenerative changes in liver (Vorbrodt et al. 1966; Vorbrodt, Krzyżowska-Gruca 1970; Olason, Smuckler 1976). As to the neuropathological effects, this compound has not been thoroughly screened and the available data come, to our knowledge, from only few experimental approaches (Holm et al. 1977). Noteworthy in the context with this study is, however, that Holm and coworkers (1977) have noticed changes in EEG indicative of hepatic coma and morphological alterations in the glia of the cat brain following only two administrations of 50 mg per kg body weight of the compound in 24 h intervals. Considering that carbon tetrachloride, so far the most commonly used agent in such studies, produced changes of similar character and magnitude not earlier than after 4—6 months of continuous administration by the same route (Hilgier 1980; 1981a, b), thioacetamide appeared to be the compound of choice, if only for practical reasons.

Recent studies in this laboratory, in addition to confirming the rapid onset of degenerative changes in the liver following the administration of thioacetamide, have demonstrated that these changes are accompanied by the activation of enzymes involved in ammonia metabolism in the brain, which is the phenomenon typical for the early stages of hepatogenic encephalopathy (Hilgier et al. 1983). Although these results have rendered strong arguments in favor of the thioacetamide model, at least two essential questions related to the relevance of this model remained to be answered:

1) Do these biochemical changes in the brain reflect — or have the potential to develop to — the typical morphological and ultrastructural manifestations of hepatic encephalopathy?

2) What kind of changes are brought about by factors appearing in the blood subsequently to liver damage and what, in turn, is the contribution of thioacetamide itself?

Both questions directly pertain to the criteria to which a relevant hepatotoxic agent should conform. Beside, the second question is of importance in light of the possibility that thioacetamide or its metabolites may be transported across the blood-brain barrier, though a direct evidence is still missing.

The present study appears to give answers to these questions, which favour the selection of thioacetamide as a factor inducing hepatogenic encephalopathy. Incubation of astroglia cultures in the presence of sera derived from rats treated with thioacetamide long enough to produce liver damage resulted in the formation of pathological forms of astrocytes strikingly resembling those observed in other experimental hepatogenic gliopathies. The morphological features of these cells, such as large size, oval shape, lack of processes, granular cytoplasm and peri-

pherally situated nucleus, as well as their enzymatic properties including high G6PDH and low SDH and GDH activities, are virtually identical to those described for Opalski cells *in vitro* (Mossakowski et al. 1970). On the ultrastructural level, this similarity finds manifestation in the presence of inclusion bodies in the cytoplasm of astrocytes and changes in their normal cytoplasmic organelles (Mossakowski et al. 1971). Noteworthy, the other cell types of CNS present in the culture were much less affected by the sera, which agrees well with the picture observed as a rule in various forms of encephalopathy of hepatic origin (Mossakowski 1978). Thioacetamide added directly to the cultures of rat cerebellum tissue induced marked degenerative changes in the cells, which may be interpreted to reflect a generalized cytotoxic effect of the compound. These direct effect does not show any cellular selectivity. Moreover identical subcellular changes were previously observed to follow the administration of the compound to a variety of tissue ranging from rat liver parenchyma (Vorbrodt et al. 1966), to human fibroblasts and yeast cell cultures (Diala et al. 1980).

All the results suggest that, the gliopathy induced *in vitro* by the sera from thioacetamide-treated rats is causally related to the presence in these sera of factors which appear in the blood secondarily to liver impairment. The absence of direct effects of thioacetamide is not surprising, since the compound will most likely have disappeared from blood two days after administration, although this assumption remains to be confirmed experimentally. Hence, from the practical perspective, such a serum appears to be an excellent starting material for the fractionation of pathogenic factors involved in hepatogenic encephalopathy. Subsequently, this would allow to screen and classify the factors according to their pathogenic potential and to the character of morphological and biochemical changes they produce. The relevance of using such an experimental approach is underscored by the multitude of putative hepatogenic factors which have been suggested to be encephalopathic and which may be transported with the serum to the brain, but the role of which in inducing specific glial changes has not been conclusively established (for recent reviews see Fischer 1974; Mossakowski 1978; Zieve 1981). In the preceding paper we have demonstrated the increased levels of ammonia and phenols in sera of rats treated with thioacetamide according to the same protocol (Hilgier et al. 1983). While the role of the former in hepatogenic encephalopathy has been repeatedly confirmed in different experimental approaches, including direct tests in astrocytic cultures (Mossakowski et al. 1970), the pathogenic effects of phenolic compounds in the brain, though speculated upon, have not been studied in detail (Holm et al. 1977). Furthermore, in light of what has been said above, the two factors are unlikely exhausting the list of compounds present in the serum which may be pathogenic to the

brain. Further studies in this laboratory will be directed towards revealing the nature of these compounds.

The purity of the thioacetamide model needs to be further verified in studies *in vivo*. This verification is of particular importance in light of the possibility that the compound or its metabolites may penetrate the brain tissue and exert their neurotoxic action at early times after administration, before having been cleared from the body. In this connection, more information is needed about the permeability of the blood-brain barrier to thioacetamide and the metabolism of this compound.

ENCEFALOPATIA WĄTROBOWA U SZCZURA WYWOŁANA PODAWANIEM TIOACETAMIDU

II. Badania cytochemiczne i ultrastrukturalne astrocytów w hodowli *in vitro*

Streszczenie

Przeprowadzono ocenę wpływu tioacetamidu (TAA) — czynnika hepatotoksycznego prowadzącego w warunkach *in vivo* do rozwoju encefalopatii wątrobowej — na astrocyty w organotypowej hodowli móżdżku noworodków szczurzych.

Doświadczenia prowadzono w dwóch grupach. W pierwszej TAA podawano bezpośrednio do środowiska odżywczego hodowli w stężeniu 25 i 50 mg%, w grupie drugiej hodowle prowadzono w środowisku zawierającym surowicę zwierząt traktowanych uprzednio tym czynnikiem. Dodatek do hodowli surowicy zwierząt zatruwanych tioacetamidem wywoływał w astrocytach zmiany charakterystyczne dla gliopatii wątrobowej, znane z doświadczeń z innymi czynnikami patogennymi, a wyrażające się pojawieniem się przerosłych komórek gwiaździstych pozbawionych wypustek, których cytoplazma wypełniona była ziarnistymi złogami PAS-dodatnimi. Zmianom tym w obrazie mikroskopowo-elektronowym odpowiadały nieprawidłowości szorstkiej siateczki śródplazmatycznej i mitochondriów oraz obecność ciał wtrętowych w cytoplazmie. Sam TAA wywoływał nieswoiste zmiany zwyrodnieniowe astrocytów. Uzyskane wyniki wskazują, że patogenny czynnik (lub czynniki) prowadzący do rozwoju gliopatii wątrobowej znajduje się w surowicy zwierząt z uszkodzoną przez tioacetamid wątrobą. Nie jest nim natomiast sam czynnik hepatotoksyczny.

ПЕЧЕНОЧНАЯ ЭНЦЕФАЛОПАТИЯ У КРЫСЫ ВЫЗВАННАЯ ВВЕДЕНИЕМ ТИОАЦЕТАМИДА

II. Цитохимические и ультраструктурные исследования астроцитов в культуре

Резюме

Произведено оценку влияния тиоацетамида (ТАА) — гепатоксического фактора, ведущего в условиях in vivo к развитию печеночной энцефалопатии, на астроциты в органотипичной культуре мозжечка крысиновых новорожденных.

Эксперименты проводились в двух группах. В первой группе ТАА вводили непосредственно в жидкую среду культуры в концентрации 25 и 50 мг%, во второй группе культуру проводили в среде содержащей сыворотку животных подверженных предварительно действию этого фактора. Добавка к культуре сыворотки животных отравляемых тиоацетамидом вызывала в астроцитах изменения характерные для печеночной глиопатии, известные из экспериментов с другими патогенными факторами и выражающиеся в появлении переросших звездчатых клеток лишенных отростков, цитоплазма которых была выполнена гранулезными отложениями PAS положительными. Этим изменениям в электронно-микроскопической картине соответствовали аномалии шероховатой средиплазматической сети и митохондрий, а также наличие инклюзионных тел в цитоплазме. ТАА введенный в культуру вызывал неспецифические дегенеративные изменения астроцитов. Полученные результаты показывают, что патогенный фактор (или факторы) ведущий к развитию печеночной глиопатии находится в сыворотке животных с поврежденной тиоацетамидом печенью. Но сам гепатотоксический фактор не является патогенным фактором.

REFERENCES

1. Diala E., Mittwoch U., Wilkie D.: Antimitochondrial effects of thioacetamide and ethylenethiourea in human and yeast cell cultures. Br. J. Cancer, 1980, 42, 112—120.
2. Diemer N. H.: Glial and neuronal changes in experimental hepatic encephalopathy. Acta Neurol. Scand., 1978, suppl. 71, 58, 1—144.
3. Fischer J. E.: Hepatic coma in cirrhosis, portal hypertension, and following portocaval shunt. Arch. Surg., 1974, 108, 325—338.
4. Hilgier W.: Zawartość α-ketoglutaranu i glutaminianu w mózgu szczura w doświadczalnej encefalopatii wątrobowej. Neuropat. Pol., 1980, 18, 121—126.
5. Hilgier W.: Zawartość glutaminy oraz aktywność aminotransferazy glutaminowej w mózgu w doświadczalnej encefalopatii wątrobowej. Neuropat. Pol., 1981a, 19, 67—73.
6. Hilgier W.: Zawartość amoniaku oraz aktywność syntetazy glutaminowej i glutaminazy w mózgu w doświadczalnej encefalopatii wątrobowej. Neuropat. Pol., 1981b, 19, 351—358.
7. Hilgier W., Albrecht J., Kraśnicka Z.: Thioacetamide-induced hepatic encephalopathy in the rat. I. Preliminary morphological and biochemical observations. Neuropat. Pol., 1983, 21, 487—494.
8. Holm E., Striebel P., Munzenmaier R., Kaltermann R.: Pathogenese der hepatischen Enzephalopathie. Leber Magen Darm, 1977, 7, 241—254.
9. Kraśnicka Z., Mossakowski M. J.: Zagadnienie zmienności morfologicznej tkanki glejowej hodowanej *in vitro*. Neuropat. Pol., 1965, 3, 397—408.
10. Mossakowski M. J.: Experimental studies on the pathogenesis of hepatogenic encephalopathy. Riv. Patol. Nerv. Ment., 1978, 37, 25—36.
11. Mossakowski M. J., Borowicz J. W., Kraśnicka Z., Gajkowska B.: Ultrastructure of Opalski cells cultured *in vitro*. Acta neuropath., (Berl.) 1971, 19, 301—306.
12. Mossakowski M. J., Renkawek K., Kraśnicka Z., Śmiałek M., Pronaszko A.: Morphology and histochemistry of Wilsonian and hepatic gliopathy in tissue culture. Acta neuropath., (Berl.) 1970, 16, 1—16.
13. Olason D., Smuckler E. A.: Changes in hepatic nuclei induced by acetamide and thioacetamide. Arch. Pathol. Lab. Med., 1976, 100, 415—418.
14. Vorbrodt A., Krzyżowska-Gruca S.: The effect of thioacetamide on the embryonic rat liver and hepatoma cells. Folia Hist. Cytochem., 1970, 8, 11—22.

15. Vorbrodt A., Stęplewski Z., Krzyżowska-Gruca S.: The effect of thioacetamide on the rat liver parenchymal cells. Folia Hist. Cytochem., 1966, 4, 7—20.
16. Zieve L.: The mechanism of hepatic coma. Hepatology 1981, 1, 360—365.

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