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EXPERIMENTAL STUDIES ON THE PATHOGENESIS OF HEPATOGENIC ENCEPHALOPATHY

STUDI SPERIMENTALI SULLA PATOGENESI DELL'ENCEFALOPATIA EPATOGENICA

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RIASSUNTO. *Sono state svolte ricerche istopatologiche sui cervelletti di ratti neonati coltivati in medium contenenti siero di pazienti con malattia di Wilson e di altri con coma epatico di tipo iperammonemico oppure sieri di controlli normali ai quali venivano aggiunti rame ed ammonio esogeni.*

Gli esperimenti provarono che sia il rame che l'ammonio sono responsabili delle alterazioni gliali tipiche della malattia di Wilson e dell'encefalopatia epatogena. Per di più l'applicazione di sodio malonato, un inibitore della succinato deidrogenase, nella cultura dei tessuti portò alla formazione di alterazioni gliali identiche a quelle ottenute col rame e l'ammonio esogeni. Il loro sviluppo poté essere completamente evitato con l'applicazione simultanea di α-oxoglutarato.

SUMMARY. *Hystopathological studies have been carried out on newborn rats cerebellum cultured in media containing blood serum: 1) from patients with Wilson's disease; 2) with hyperammonemic form of hepatic coma; 3) from healthy subjects; exogenous copper and ammonia were added.*

The experiments indicated that both copper and ammonia may be responsible for glial abnormalities typical for both Wilson's disease and hepatogenic encephalopathy. Moreover application of sodium malonate, an inhibitor succinate dehydrogenase into the tissue culture resulted in formation of glial changes which were identical with those obtained with both exogenous copper and ammonia. Their development could be completely prevented by simultaneous application of α-oxoglutarate.

Neurological complications of both chronic and acute liver insufficiency are well known from a vast clinical literature (1, 6, 9, 10, 13, 19, 21, 26, 27). They are taking the form of a progressive neurological disease, characterized by periodically occurring disturbances of consciousness, including comatous states, which are accompanied by focal neurological disorders, combining features of pyramidal, extrapyramidal and cerebellar syndromes. In some cases, mostly those with acute liver failure, comatous state is the only neurological complication.

Great proportion of the above mentioned clinical abnormalities is accompanied by typical pathological changes in the

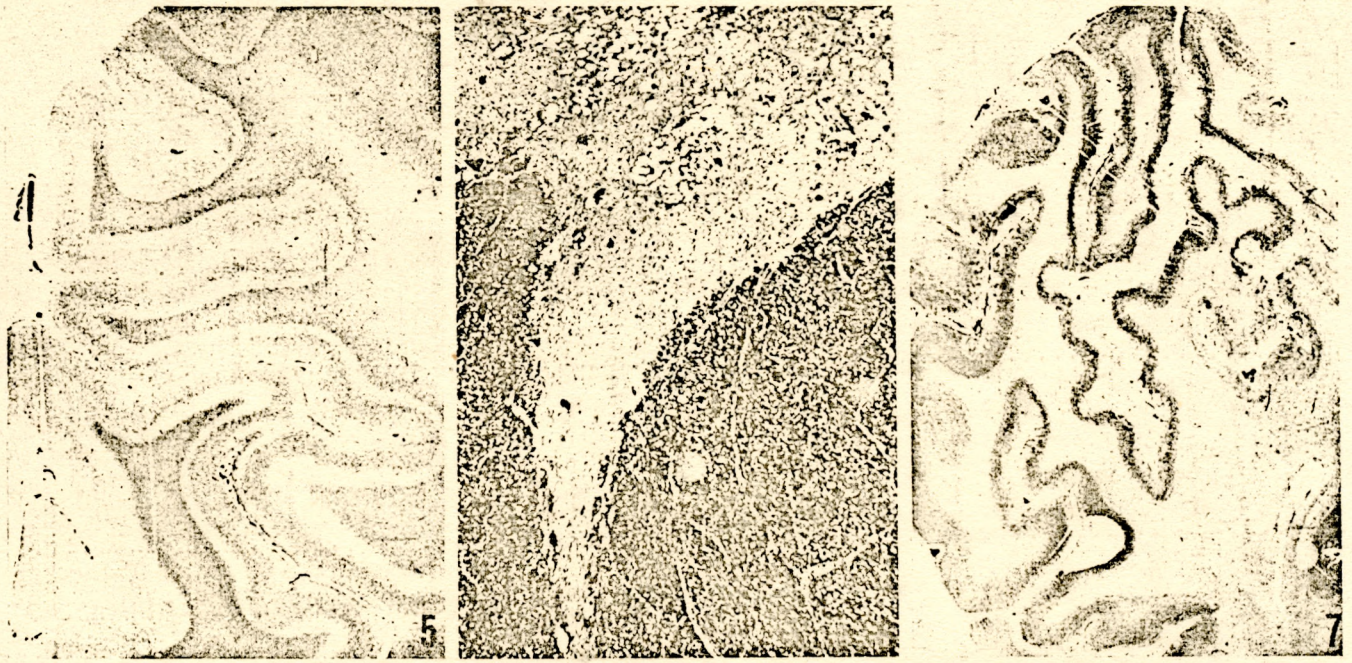


FIG. 5 - Laminar spongiosis in the cortico-subcortical junction in a case of acute hepatogenic encephalopathy. Heidenhain. Magn. glass.
FIG. 6 - Spongy degeneration of the basal ganglia in a case of portal-caval encephalopathy. Heidenhain. Magn. glass.
FIG. 7 - Superficial, laminar necrosis of the occipital cortex in a case of acute hepatogenic encephalopathy. H-E. Mang. glass.

brain. In our own material comprizing 74 cases of chronic liver damage, pathological changes typical for hepatogenic encephalopathy were present in 66 cases (9, 10). Similar abnormalities, although less advanced, were found in 24 of total 26 cases with acute liver insufficiency (13).

The most common pathological feature found in the brain in cases of liver disease are astrocytic abnormalities, consisting in the concommitance of their progressive and regressive reaction.

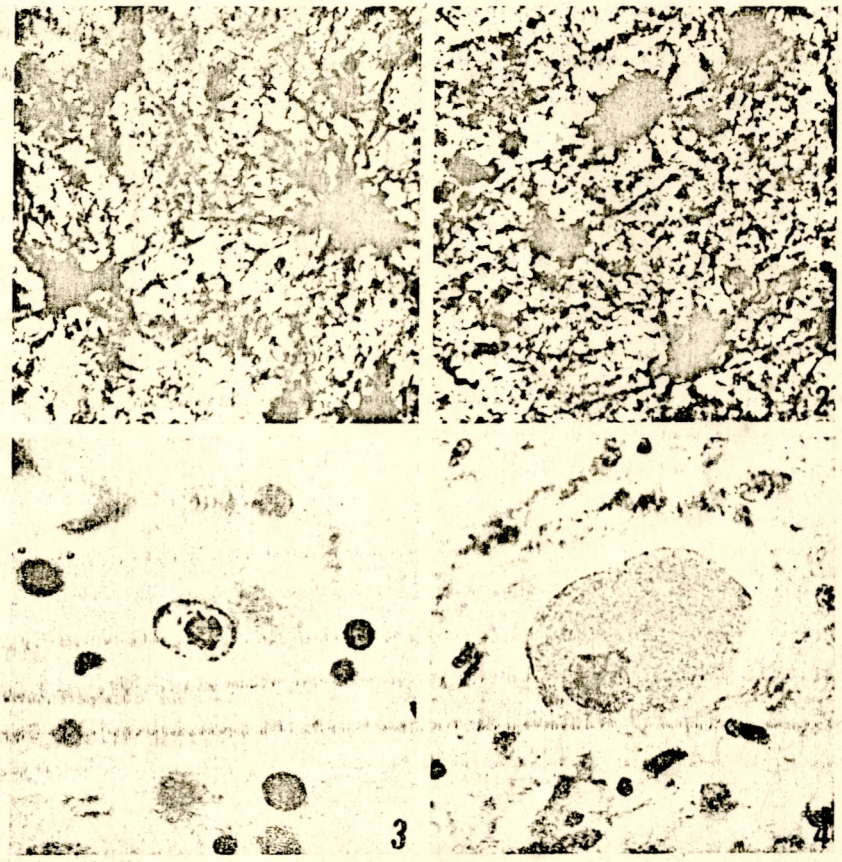


FIG. 1 - Hypertrophied astrocytes in the cerebral cortex in a case of chronic hepatogenic encephalopathy. Cajal's gold, x 450.

FIG. 2 - Klasmatodendrosis leading to complete lack of astrocytic processes. Cerebral cortex in a case of chronic hepatogenic encephalopathy Cajal's gold, x 450.

FIG. 3 - Alzheimer cell, type II with an intranuclear glycogen inclusion. Cerebral cortex in a case of chronic hepatogenic encephalopathy Best's carmine, x 900.

FIG. 4 - Opalski cell in the cerebral cortex in a case of chronic hepatogenic encephalopathy. H-E, x 600.

There is widespread, generalized proliferation of astrocytic nuclei, with a significant hypertrophy of individual astrocytes (fig. 1). These are superimposed by dominating regressive changes taking the form of less or more advanced klasmatodendrosis (fig. 2). Numerous astrocytes lacking processes and their ameboid forms are seen. Alzheimer cells, type II in all their forms as described by Stadler (28) are present in practically all gray structures and in the subcortical white matter. In a great majority of cases the intranuclear glycogen inclusions are to be found both in naked nuclei and in those of unchanged astrocytic nuclei (fig. 3). Opalski cells (fig. 4) occur in a great proportion of chronic cases, but they are absent in all acute ones. Their cytoplasm is filled with neutral and acid mucopolysaccharides, as indicated by PAS and Alcian blue stainings.

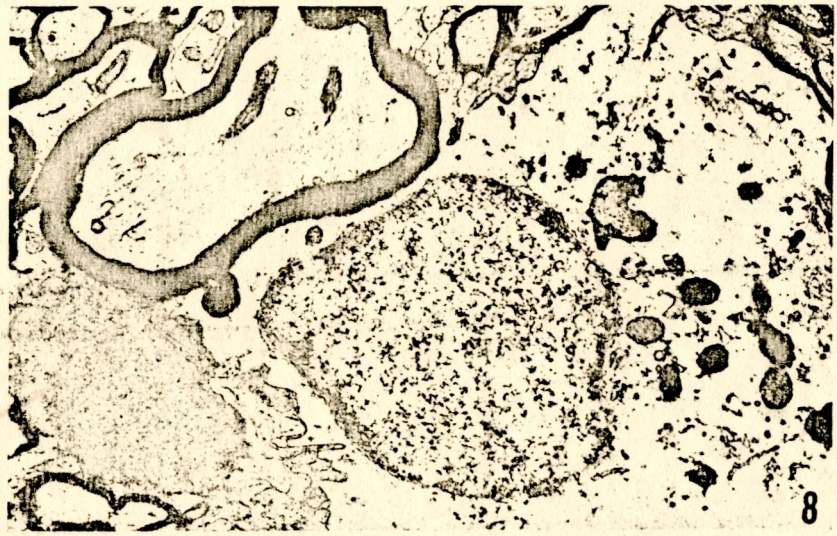


FIG. 8 - Astrocyte with severe disintegration of cytoplasm. Electron lucent cytoplasm contains fragments of rough endoplasmic reticulum, some small mitochondria with a dark matrix and heterogenous dark bodies. Case of experimental hepatogenic encephalopathy. x 15.000.

The second feature common to most cases of hepatogenic encephalopathy is spongy degeneration of the brain tissue. Cortico-subcortical junction (fig. 5), cerebral cortex, basal ganglia (fig. 6) and cerebellar dentate nuclei are the sites of predilection for spongiotic changes. Relatively good preservation of myelin and neurons, as well as lack of either glial or mesenchymal reaction are fairly typical features of spongy degeneration observed in hepatogenic encephalopathy. Rare cortical necroses

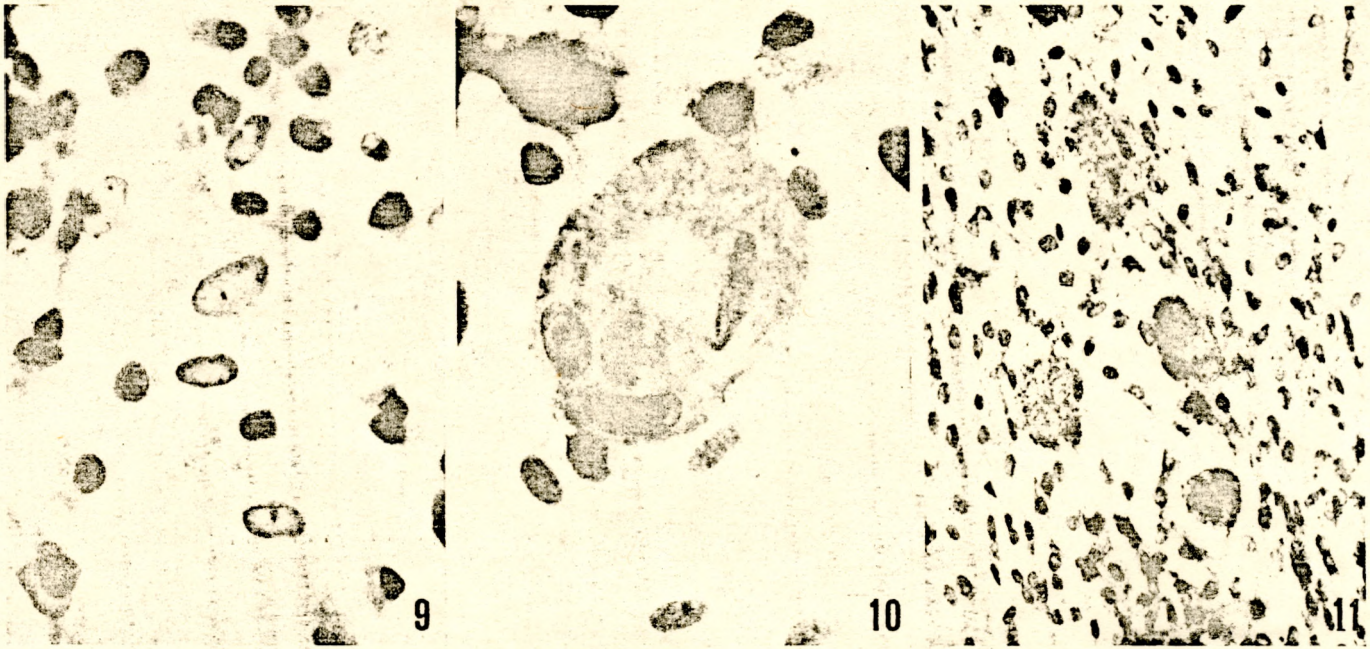


FIG. 9 - Naked glial nuclei in organotypic culture of newborn rat cerebellum maintained in a medium containing serum from a patient with hepatic coma. Cresyl violet, x 600.
FIG. 10 - Multinucleate Opalski cell in organotypic culture of newborn rat cerebellum maintained in a medium containing serum from a patient with hepatic coma. Cresyl violet, x 600.
FIG. 11 - Group of Opalski cells with cytoplasm containing PAS-positive deposits in organotypic culture of newborn rat cerebellum maintained in a medium containing serum from patient with hepatic coma. PAS, x 400.

(fig. 7), neuronal abnormalities, taking the form of either non-specific degeneration or patchy neuronal loss, features of brain edema and hyperemia complete the pathomorphology of hepatogenic encephalopathy. Observations carried out on human material (1, 6, 9, 10, 13, 19, 27), confirmed by experimental studies on animals with chemical (16, 18, 20) and/or diatory (13) liver damage as well as those with portal-caval shunt performed operativelly (3, 7, 15) are indicative that glial pathology is the primary and most fundamental element of brain damage in cases of liver insufficiency (fig. 8). Spongy degeneration of brain tissue and neuronal abnormalities seem to be the secondary phenomena. On the other hand, the gliopathy observed in hepatogenic encephalopathy is in its general pattern similar if not identical to that occurring in hepato-lenticular degeneration, in which brain lesions are considered to be due to the excess accumulation of copper ions in the brain tissue.

In case of hepatogenic encephalopathy many factors have been claimed to be responsible for the brain abnormalities. The role of main pathogenic factor has been attributed to ammonia (2, 8). This opinion was supported by numerous experimental data (3, 7, 15). Contradictory to this is a great number of hepatogenic encephalopathies with normal ammonia level (21). Other metabolic products, such as for instance non-identified products of liver tissue desintegration (19), abnormal protein metabolites, mostly those of aromatic amino-acids (21) or bilirubin itself or products of its metabolism (4, 24) were ascribed the role of leading pathogenetic factor. Immunopathological mechanisms have been also taken into consideration (25).

Considering gliopathy as the primary pathological process underlaying brain abnormalities in both Wilson's disease and hepatogenic encephalopathy we have performed a series of experiments aiming to elucidate the influence of copper and ammonia on glia in the condition of tissue culture (11, 17).

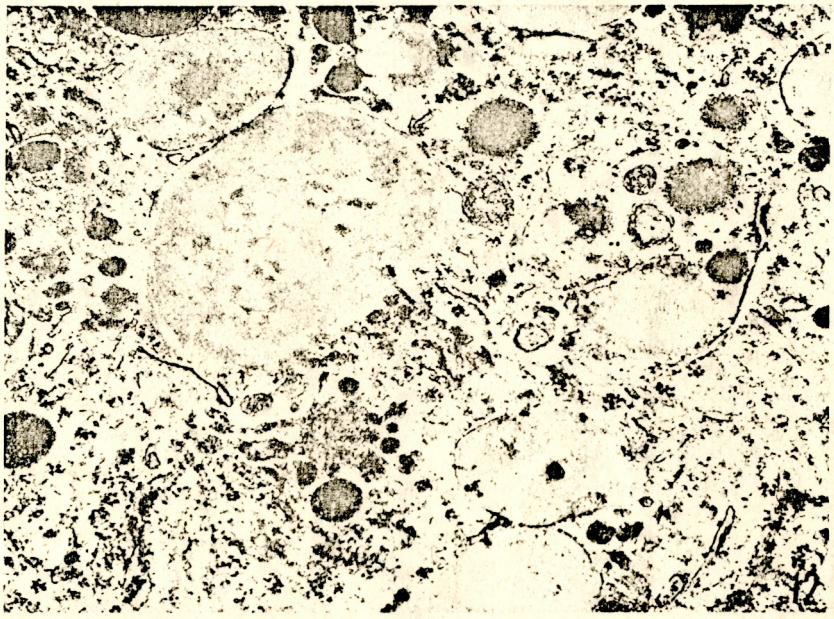
The studies were carried out on the newborn rats cerebella cultured in medium containing blood serum from patients with Wilson's disease and from those with hyperammonemic form of hepatic coma or sera of healthy subjects, to which exogenous copper and ammonia were added. The content of former corresponded to that found in the brain of patients with Wilson's disease, while the ammonia amount was identical with that present in blood serum of patients with hyperammonemic hepatic coma.

In all experimental groups typical glial abnormalities were found, consisting in non-specific astrocyte impairment with the

presence of cells showing features of Alzheimer cells type I and II (fig. 9) and Opalski cells (fig. 10). The histochemical study of Opalski cells disclosed their cytoplasm being filled with granular deposits of both neutral (fig. 11) and acid mucopolysaccharides, which on the ME level took the form of large, electron-light intracytoplasmatic inclusions (fig. 12). Histoenzymatic studies showed remarkable changes in the activity of several dehydrogenase activity was the most pronounced one. On the electron microscopic level great lisosomal accumulation of copper deposits in Opalski cell was found in all experimental groups (fig. 13), but that with exogenous ammonia. The experiments indicated that both copper and ammonia may be responsible for glial abnormalities typical for both Wilson's disease and hepatogenic encephalopathy.

The copper accumulation in abnormal glial cells obtained in cultures with sera from patients with hepatic coma turned our attention to its possible role in pathogenesis of brain lesions in non-specific liver damage (29). The chemical studies of various portions of the brain of 19 cases with acute liver insufficiency, due mostly to viral hepatitis revealed significant increase of copper content in the brains of all cases; this varying remarkably both from case to case and from one brain structure to another. The greatest increase of copper content more than 6-fold with regard to the metal level in brains of healthy subjects was found in brain hemispheres, mostly cerebral cortex and subcortical white matter. Second with regard to increase of copper content were medulla and caudate nuclei. In the remaining structures such an increase was noted only in few cases, usually ranging between 4- and 2-fold. Comparison of the topography of copper deposition with that of morphological tissue alteration showed full convergence of both phenomena. Most abundant copper accumulation occurred in regions with the greatest advancement of morphological abnormalities, expressed first of all by glial changes. Similarly, increased copper accumulation in the brain was found in rats, in which liver cirrhosis was produced by carbon tetrachloride administration (5). An increased copper content was also observed in brains of patients with porto-caval encephalopathy by W e n d e r and K o z i k (30). Its penetration to the brain in cases of liver insufficiency may be facilitated by changed blood vessel permeability (fig. 14, 15) showed by us in other series of experiments (16, 18, 20) and by abnormal binding with serum proteins.

Reduction in the activity of oxido-reductive enzymes, noted in abnormal glial cells prompted us to apply various chemical



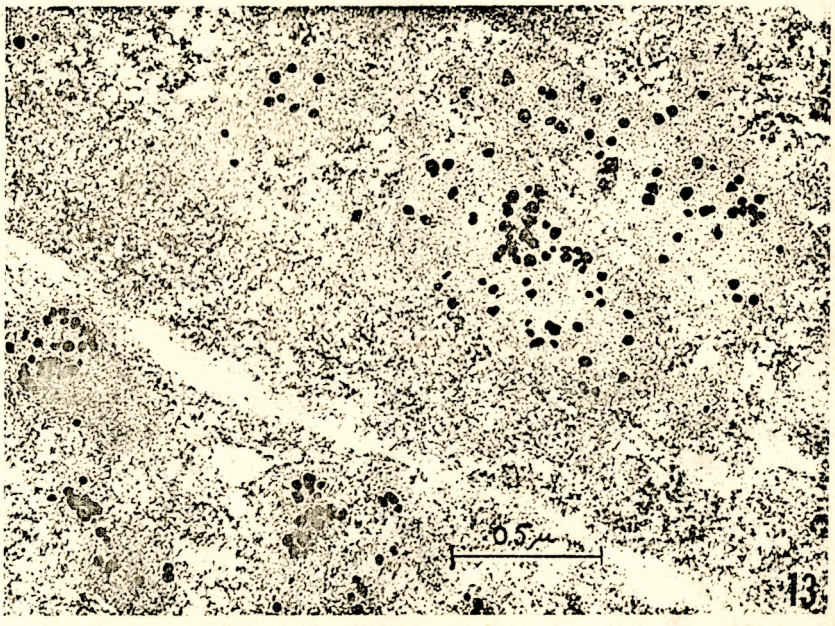


FIG. 12 - Fragment of Opalski cell from organotypic culture of newborn rat cerebellum maintained in medium containing serum from a patient with hepatic coma. Cytoplasm of the cell contains numerous dense bodies and large electron-lucent spherical bodies (mucopolysaccharides). x 26.000.
FIG. 13 - Fragment of Opalski cell from organotypic culture of newborn rat cerebellum, maintained in medium containing serum from a patient with hepatic coma. Abundant lysosomal copper deposits are present. Method of Schener et al. (23). x 40.000.

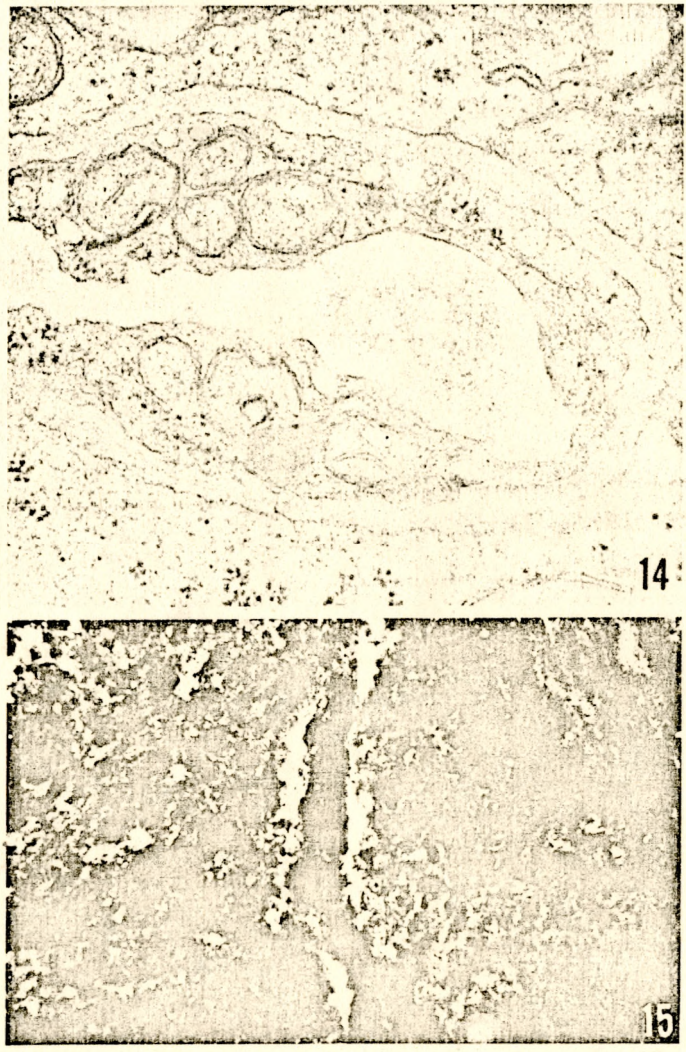


FIG. 14 - Capillary vessel from a case of experimental hepatogenic encephalopathy. Increased number of pinocytic vesicles, located at different depth of the endothelial cells. x 15.000.
FIG. 15 - Bright silver grains surrounding the blood vessel wall and deposited loosely in basal ganglia neuropil of a rat with experimentally induced hepatogenic encephalopathy. Method of Wislocky and Leduc (31). Dark field, unstained section, x 250.

inhibitors of this enzymatic system to obtain gliopathy typical for hepatogenic encephalopathy. Application of sodium malonate an inhibitor of succinate dehydrogenase into the tissue culture resulted in formation of glial changes which were morphologi-

cally, histochemically and ultrastructurally identical with those obtained with both exogenous copper and ammonia (22). Their development could be completely prevented by simultaneous application of α-oxoglutarate, which is involved in the first step in ammonia detoxication towards glutamine. Other chemical compounds such as sodium glutamate or d-penicillamine revealed either partial or complete preventive effect limited to some experimental groups (12, 14).

Basing on the above data the following conclusion seems to be justified.

Regardless the nature of the noxious factors involved the gliopathy, being the primary and fundamental pathomorphological feature of the hepatogenic encephalopathy, eventually results from disturbed ammonia detoxication within the central nervous system due to absolute or relative insufficiency of α-oxoglutarate, which is involved in the first step of basic pathway of ammonia detoxication. It seems plausible that in cases of hyperamonemia due to liver failure the normal intracellular content of α-ovoglutarate is not sufficient to detoxicate excess amount of ammonia penetrating free to the central nervous system. Excess accumulation of copper in the brain (perhaps also some other substances), disturbing metabolism in Krebs cycle, results in reduced intracellular production of α-oxoglutarate. This in turn leads to insufficient detoxication of ammonia occurring even in its normal amount. This hypothesis offers an uniform explanation of identity of glial abnormalities occurring both in Wilson's disease and in non-specific liver damage, those due to the action of copper and ammonia. Primary astrocytic involvement may find its explanation in transport function of astrocytes, their role in ammonia metabolism in the central nervous system and their relatively low oxidative metabolism.

Considering the difficulties in direct transposition of experimental observations, based mostly on tissue culture experiments to the conditions of human pathology, a new series of experiments have been performed (15). Rats with hepatogenic encephalopathy produced by porto-caval shunting were given intraperitoneal injections of α-oxoglutarate in a dosis 14.1 mg/100 g body weight for a period of 7 weeks. The preliminary results indicate significant reduction in number of abnormal glial cell occurring in various portions of the central nervous system. Their number amounting to around 50% of glial population in non-treated animals was lowered to 8-10% in the treated ones. These promising results can sofar be considered as preliminary and need further confirmation.

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