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MORPHOLOGICAL CHANGES AND QUANTITATIVE TOPOGRAPHY OF COPPER IN THE BRAIN OF PATIENTS WITH HEPATIC COMA DUE TO ACUTE LIVER IMPAIRMENT *)

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The increase of copper content in the brain constitutes a permanent pathological feature in hepato-lenticular degeneration (Cummings, Kremer, 1959). Copper accumulation in the brain in this condition is supposed to play an essential role of as pathogenic factor leading to nerve tissue impairment, characteristic for this pathological process (Vogel, Evans, 1961). This inference has found confirmation in respect to glial changes in our previous studies in conditions of tissue culture (Mossakowski et al., 1970).

In cases of hepatogenic encephalopathy of various types, the copper content in the brain has been reported to be either normal or only slightly increased (Porter, Adams, 1956; Gubler et al., 1957; Wiśniewski et al., 1967), with the exception of the case reported by Mozai et al. (1962), where a significant increase of copper level was described. Wender and Kozik (1973) observed a significant accumulation of copper in various structures of the brain in a series of cases of clinically and morphologically verified portal-systemic encephalopathy.

This prompted us to study quantitatively the copper content in the brains of patients who died in hepatic coma due to acute liver damage.

MATERIAL AND METHODS

The material examined included 19 cases of acute liver impairment, therefrom 17 with verified viral hepatitis. Two cases represented toxic

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liver damage. Four cases from the group of viral hepatitis concerned patients with previous history of neurological and psychiatric disease, as well as other pathological processes related with long-term drug application. All patients were treated in the Department of Infectious Diseases, Medical School in Warsaw (Director: prof. dr med. B. Kassur).

General autopsy and histopathology of liver were performed at the Department of Morbid Anatomy, City Hospital for Infectious Diseases, Warsaw (Head: dr med. Z. Afek-Kamińska).

Brains taken at general autopsy, done 12—28 hrs following the patients'death, were fixed in neutral formalin. Neuropathological examination was carried out on sections from representative regions of the brain hemispheres, brain stem and cerebellum, stained with hematoxylin-eosin, cresyl violet, PAS, PAS-dimedon and according to the methods of van Gieson, Heidenhain, Kanzler-Arendt and Cajal.

Quantitative determination of copper was carried out by the Eden and Green method (1940) with sodium diethyldithiocarbamate on material previously fixed in formalin. In each case, and in two additional control cases of healthy subjects, who died due to street accidents, the copper content was determined in the following 12 brain structures — cortex and white matter of frontal and occipital lobes, caudate nucleus, putamen, globus pallidus, thalamus, pons, medulla, and cerebellar cortex and white matter.

CLINICAL AND PATHOLOGICAL CHARACTERISTICS OF THE MATERIAL

Clinical data

The material included 12 female and 7 male patients, aged from 12 to 70 years. The majority represented the 2nd, 3rd and 4th life decades. Disease duration, from first symptoms of icterus to patients' death, ranged from 7 to 148 days, with a mean period of 34 days. In 15 patients

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Fig. 1. Hypertrophied astrocytes from subcortical white matter, showing fragmentation of their processes. Cajal's impregn. × 100.

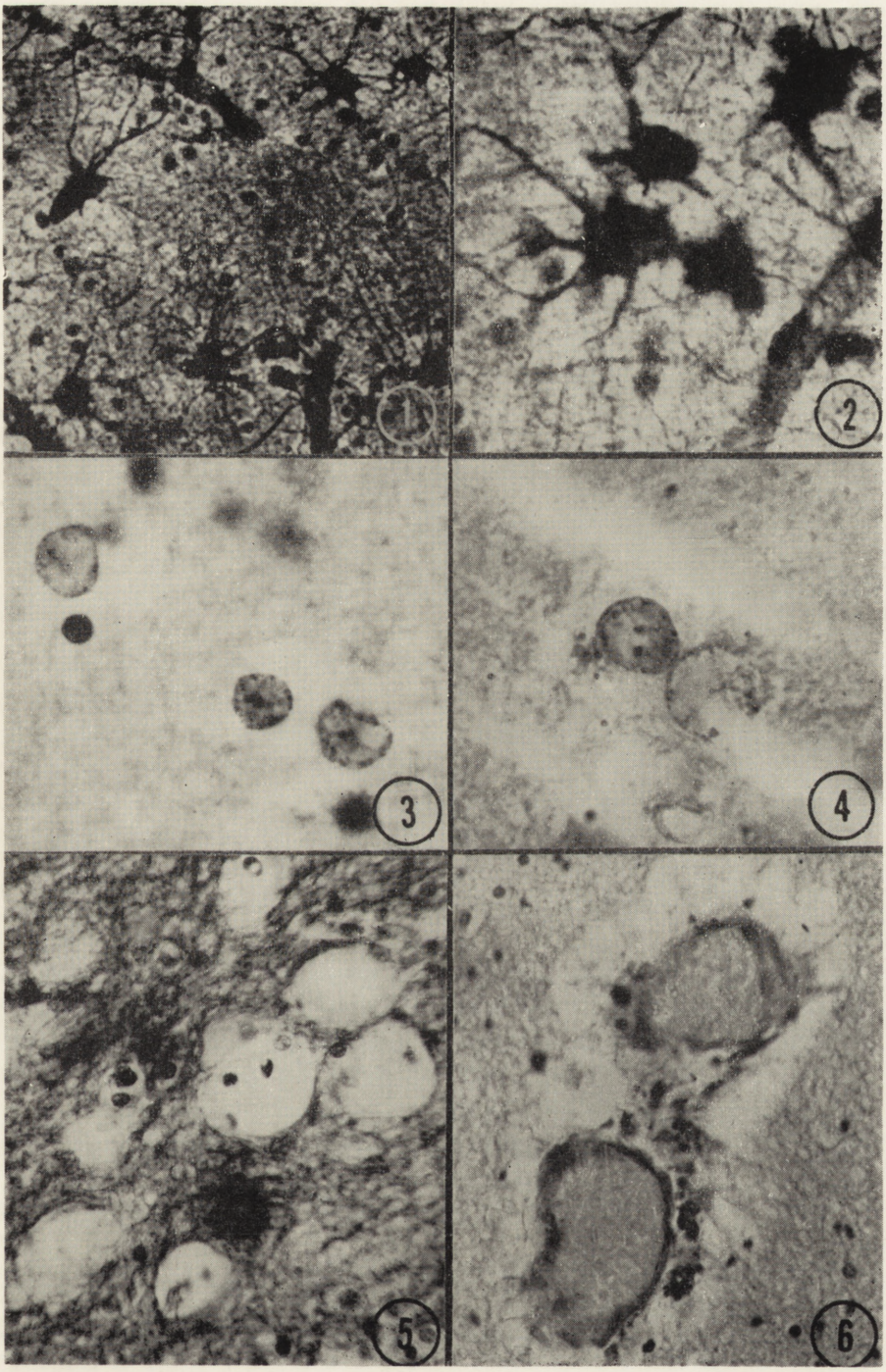
Fig. 2. Klasmatodendrosis of grey matter astrocytes. Cajal's impreg. × 400.

Fig. 3. Abnormal astrocytic nuclei of Stadler and Alzheimer type. Cerebral cortex. H-E. × 1 000.

Fig. 4. Small intranuclear glycogen inclusion within Alzheimer cell type II. PAS-dimedon. × 1 000.

Fig. 5. Spongy degeneration of subcortical white matter. Note the normal appearance of myelin. Heidenhain meth. × 400.

Fig. 6. Enlarged perivascular space in oedematous white matter. H-E. × 100.



disease lasted less than 4 weeks. All patients developed deep coma, lasting from 1 to 15 days (in 17 patients shorter than 10 days). Neurological symptoms occurred in 15 patients. In all of them a remarkable increase of bilirubin level in blood serum (maximal values 7—49 mg%) was found, while that of ammonia, examined in 13 cases, was elevated only in 8 of them, being normal in the five remaining ones. All patients received conventional treatment, in three of them total blood perfusion through swine liver was performed.

Histopathology of liver

In 15 cases diffuse liver necosis was noted, this being focal in nature in one case. Two cases represented acute necrotizing hepatitis, while in one subacute hepatitis was diagnosed in a biopsy specimen. In some cases slight, insignificant features of recent liver cirrhosis were present.

Neuropathological data*)

Pathological changes in the central nervous system were present in all cases, differing both in their nature and intensity. Two types of changes could be distinguished. The first type of abnormalities consisted of changes typical for hepatogenic encephalopathy. They included diffuse proliferation of astroglia, involving mostly grey matter structures and subcortical white matter, hypertrophy of astrocytes (Fig. 1) concomitant with their degeneration in the form of klasmatodendrosis (Fig. 2), transitory glial cells of Stadler type, Alzheimer cells type II (Fig. 3), intranuclear glycogen inclusions (Fig. 4), perinuclear PAS-positive deposits and typical foci of spongy degeneration (Fig. 5). The abnormalities of second type were typical for an acute, unspecific toxic encephalopathy and consisted of severe oedema (Fig. 6) and hyperaemia of the brain, widespread nerve cell degeneration and diffuse or focal neuronal loss, most advanced in the cerebral and cerebellar cortex. Changes of both types were superimposed, varying in their intensity from case to case. However, the more or less intensive features of hepatogenic encephalopathy were present in all cases. The full pathological picture of hepatogenic encephalopathy was present in 4 cases. Three of them represented Inose type (1952) of encephalopathy, one an ischemic variant (Shiraki, 1968). In seven further cases also Inose type of hepatogenic encephalopathy was revealed, however, in five of them no glycogen inclu-

*) A detailed neuropathological picture of cases has been included in the paper by M. J. Mossakowski, Z. Kraśnicka, B. Kassur, Z. Olejnik „Patomorfologia ośrodkowego układu nerwowego w ostrych uszkodzeniach wątroby" — Neuropat. Pol. 1974, 12, 51—62.

sions were found, while in two others foci of spongy tissue degeneration were absent. In the remaining 8 cases the pathological features of hepatogenic encephalopathy, although present, were much less complete and intensive.

Chemical data

In all the brains examined the copper level was higher than in control material. This increase, varying from one structure to another, reached values more than 6-fold higher relative to the control values (Diagram 1). The highest copper increase was noticed in cases of toxic liver impairment and in those cases of viral hepatitis which concerned patients with preceding disease processes. In these cases the increased

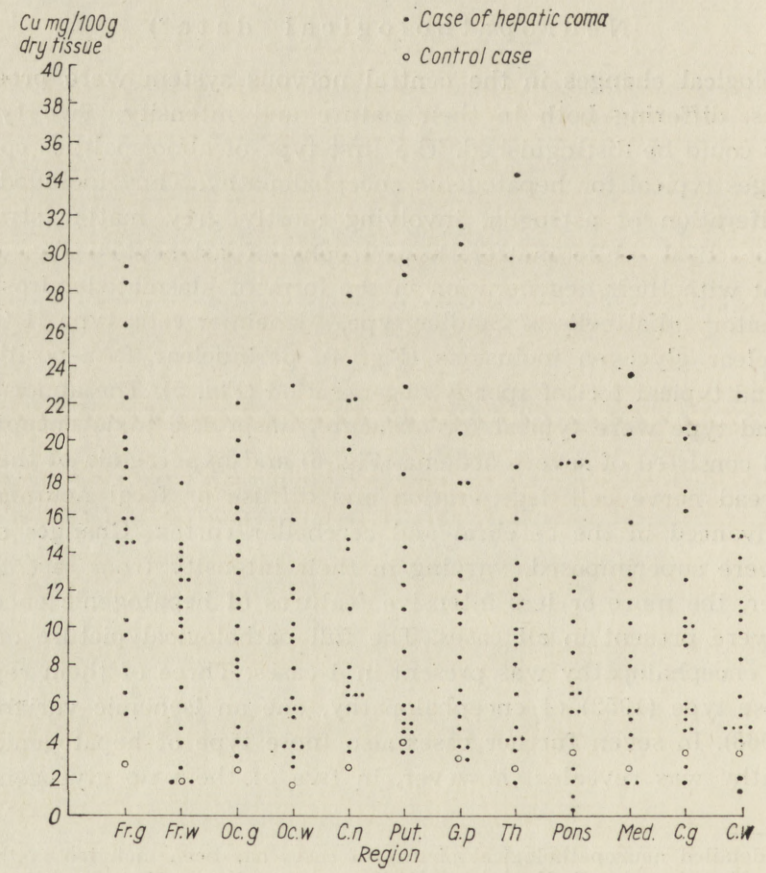


Diagram 1. Copper content in selected regions of the brain in cases of hepatic coma due to acute liver impairment.

copper content concerned equally all the brain structures, while a significant topographic variability was typical for the group of noncomplicated cases of viral hepatitis.

As regards the copper level in the brain, our series of cases can be divided into 3 groups: a) with highest relative copper increase, represented by 4 cases, in which the mean copper content, derived from the 12 brain structures examined was about 6 times higher as compared with control brains (17.04 mg/100 g dry tissue against 2.84 mg/100 g dry tissue in the norm); b) with moderate relative increase, including 11 cases, in which the rise of the copper level corresponded to the mean increase determined in all the examined cases, (10,84 mg/100 g dry tissue, i.e. a 4-fold increase respective to normal values); c) with a slight increase, represented by 4 cases, in which the copper level did not exceed more than 2 times the control values (5.20 mg/100 g dry tissue).

Table 1. Quantitative topography of copper content increase in brains of patients, who died in hepatic coma due to acute liver impairment

Brain structure	Number of cases			
	degree of increase 6	4	2	0
Grey matter of frontal lobe	13	2	4	—
White matter of frontal lobe	9	3	4	3
Grey matter of occipital lobe	8	7	3	1
White matter of occipital lobe	8	6	4	1
Medulla	8	3	6	2
Caudate nucleus	7	3	8	1
Globus pallidus	5	2	7	5
Thalamus	4	7	6	2
Cerebellar cortex	3	3	11	2
Pons	2	5	8	4
Putamen	2	2	9	6
Cerebellar white matter	1	3	13	2

The topography of copper content increase is illustrated in Table 1. As may be seen, the highest copper level, exceeding 6 times the control values was determined in the cerebral hemispheres, mostly in the cortex and white matter of the frontal lobes. Second as regards increase of copper were the same structures of the occipital lobe, medulla and caudate nuclei. In the remaining structures, such an increase of copper level was noted only in few cases, being mostly within the range of 4- and 2- fold increase as compared to the control cases. In individual

cases no copper content increase was found in these anatomical areas. Notworthy is the lack of copper content increase in the putamen in as many as 6 cases from our series.

Comparison of the intensity of brain tissue impairment, as expressed by the presence of pathological features of hepatogenic encephalopathy, with the degree of copper accumulation did not show their complete parallellism (Diagram 2). It should be mentioned, however, that the group with either full morphological picture of hepatogenic encepha-

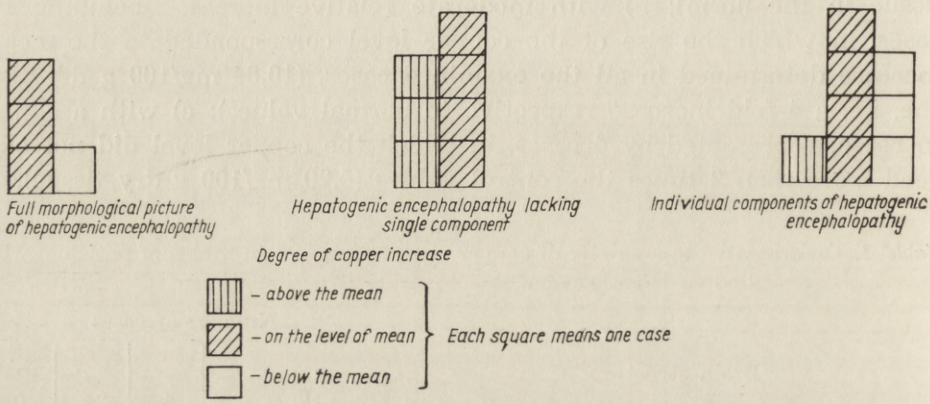


Diagram 2. Copper content and intensity of pathological changes in the brains of patients with acute liver impairment.

lopathy or that lacking only single component of this pathological syndrome, included cases in which the copper content increase in the brain was either within the range or exceeded the calculated mean. In the only case from this group with low copper content although presenting a full morphological picture of Inose type hepatogenic encephalopathy, the intensity of these changes was much weaker than in other ones.

In the group in which the pathological components of hepatogenic encephalopathy, although present, were weak and incomplete cases with copper content increase in the range of the mean value predominated. The group included also 3 of 4 cases with a slight rise of copper level. The only case with high copper values represented toxic liver impairment, in which as has been noticed before, the copper increase was higher and more generalized than in patients with viral hepatitis.

DISCUSSION

The presented results show a significant copper accumulation in the brain to take place in cases of hepatic coma due to acute liver impairment. This copper content increase sometimes reaches values as high as

those found in Wilson's disease. However, the topography of copper deposition in our material differs from that in hepato-lenticular degeneration. The most pronounced differences concern the putamen, which is the site of predilective metal accumulation in this disease (Okinaka et al., 1961). However, these differences in the distribution of excess copper accumulations in the tissues in cases of Wilson's disease and in hepatogenic encephalopathy, due to acute liver damage, as well as between cases of toxic liver impairment and those of viral hepatitis found in our series remain unexplained. The question also arises as to whether an increase of copper content in brain tissues occurs in all cases of hepatic coma, or if it is limited to those due to acute liver pathology. The literature data concerning this question in cases of hepatogenic encephalopathy, accompanying longlasting nonspecific liver diseases are so far not univocal.

The phenomenon of excessive copper accumulation in the brain in consequence of acute liver necrosis or necrotizing hepatitis is complicated as to its origin. In normal conditions copper in the human organism is mostly deposited in the serum ceruloplasmin, liver hepatocupreins and in brain cerebrocupreins (Warren et al., 1960). In case of massive liver impairment , not only a reduced binding of copper by the liver proteins takes place, but also its release from disintegrated liver cells. Reduced synthesis of serum proteins, and thus of ceruloplasmin, as well as stasis of bile and resorption of its components into blood constitute additional aggravating factors. Considering the high affinity of brain proteins to copper, the above mentioned phenomena may be factors facilitating deposition of this metal in the tissues of the central nervous system. The passage of copper from blood to brain may be promoted by disturbances of the blood-brain barrier, which find their expression in a marked brain oedema. Our previous studies (Mossakowski et al., 1970b) have provided evidence for an increased permeability of cerebral blood vessels for low molecular weight compounds (metals) in the condition of experimental hepatogenic encephalopathy. Furthermore Wiśniewski et al. (1966) found an increase of copper content in experimental brain oedema.

Comparison of the topographic distribution of copper increase in the brain structures, with that of tissue morphological changes, the latter concerning mostly astrocytes, indicates their distinct convergence. Most aboundant copper deposition occurs in those regions, which show the greatest advancement of morphological abnormality. This topographic correlation as well as the fact that copper deposition generally procedes development of tissue pathology, suggest the role of copper as a factor underlying glial tissue impairment, as has been previously shown by

Wiśniewski et al. (1966) on animal material and Mossakowski et al. (1970a) in tissue culture conditions. Obviously this does not exclude the possibility of pathogenic action of other factors, such as for instance ammonia (Bessman, Bessman, 1955; Mossakowski et al., 1972), the level of which was markedly elevated in one half of the cases examined, or bilirubin, which was remarkably increased in all cases, and which was shown by a number of authors to exert a pathogenic effect on the nervous tissue (Schutta, 1967; Hai-Chen et al., 1969).

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OBRAZ PATOMORFOLOGICZNY A TOPOGRAFIA ILOŚCIOWA MIEDZI W MÓZGACH CHORYCH ZE ŚPIĄCZKĄ WĄTROBOWĄ W OSTRYCH USZKODZENIACH WĄTROBY

Streszczenie

Przeprowadzono porównawczą analizę obrazu patologicznego i topografii ilościowej miedzi w mózgach 19 chorych, zmarłych w śpiączce wątrobowej, która rozwinęła się w przebiegu wirusowego zapalenia wątroby, bądź jej toksycznego uszkodzenia. Wiek chorych zamykał się w granicach 12—70 lat, czas trwania choroby 7—148 dni, czas trwania śpiączki 1—15 dni.

We wszystkich przypadkach stwierdzono cechy morfologicznego uszkodzenia mózgu o typie encefalopatii wątrobowej o bardzo znacznym zróżnicowaniu ich natężenia, na które nakładały się zmiany charakterystyczne dla ostrej, nieswoistej encefalopatii toksycznej. W 4 przypadkach obserwowano pełny zespół morfologiczny encefalopatii wątrobowej, w 7 dalszych do pełnego zespołu tego typu encefalopatii brakowało pojedynczych elementów, takich jak wtręty glikogenowe lub ogniskowe zwyrodnienie gąbczaste, w pozostałych 8 występowały pojedyncze elementy morfologicznego zespołu encefalopatii.

We wszystkich przypadkach stwierdzono również znaczny wzrost zawartości miedzi w mózgu, charakteryzujący się istotnym zróżnicowaniem topograficznym, odmiennym od opisywanego w przypadkach choroby Wilsona. W 4 przypadkach obserwowano bardzo znaczny wzrost poziomu miedzi, przekraczający 6-krotnie zawartości materiału kontrolnego, w 11 — umiarkowany, odpowiadający 4-krotnemu wzrostowi w stosunku do normy, a w 8 — niski, przewyższający dwukrotnie poziom prawidłowy.

Zestawienie obrazu patomorfologicznego mózgu z poziomem przyrostu zawartości miedzi nie wykazało całkowitej korelacji, jednakże większość przypadków z pełnym lub prawie pełnym zespołem morfologicznym encefalopatii wątrobowej reprezentowały grupę z wysokim i umiarkowanym przyrostem miedzi, podczas gdy przypadki z niepełnym i słabo wyrażonym zespołem encefalopatii — zamykały się w grupie z umiarkowanym i niskim wzrostem poziomu metalu w mózgu.

Największe i najczęstsze przyrosty zawartości miedzi dotyczyły kory mózgu okolicy czołowej i potylicznej oraz istoty białej tych płatów, to jest obszarów mózgu wykazujących najbardziej zaawansowane zmiany morfologiczne. W dalszej

kolejności występowało gromadzenie się metalu w jądrze ogoniastym i opuszce. Zwracała uwagę niska zawartość miedzi w skorupie.

W oparciu o analizę badanego materiału autorzy wysuwają przypuszczenie o patogenetycznej roli miedzi w kształtowaniu obrazu encefalopatii wątrobowej w przebiegu ostrych uszkodzeń wątroby.

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