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DISTURBANCES IN THE PERMEABILITY OF THE CEREBRAL BLOOD VESSELS IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY *

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The purpose of this study was to determine the permeability of brain vessels in experimental hepatic encephalopathy. The experiments were carried out with 30 experimental and 21 control white Wistar rats.

In the experimental group liver cirrhosis was induced by subcutaneous injection of CCl_4 in liquid paraffin. The control group was treated with liquid paraffin. All animals were given 1·5% silver nitrate as marker of the permeability of brain vessels.

Fatty degeneration of the liver was observed after 2 months of experiment, liver cirrhosis after 4 and 6 months. In the animals of the 2-month group there were slight glial abnormalities in the brain characteristic of hepatic encephalopathy. Its full morphological picture was observed in animals killed after 4 and 6 months. In this period there also appeared lesions bearing evidence of increased permeability of the cerebral vessels for silver salts. The barrier remained impermeable for Evans blue-coupled albumin. These facts seem to indicate the selective damage of barrier mechanisms for silver salts. They remained normal for high molecular weight substances. The fact that glial alteration precedes the appearance of silver in the cerebral parenchyma suggests the role of degenerated glia in the production of the disturbances of barrier mechanisms. In turn, the increased vascular permeability can augment the gliopathy.

In spite of numerous, exhaustive investigations many elements of the pathomechanism of brain lesions related to hepatic encephalopathy due to various, nonspecific liver damage are still obscure.

The abnormalities of glia which prevail in the brain in cases of spontaneous and experimental hepatic encephalopathies (*Adams, Foley* 1949, *Bager* 1949, *Laphame* 1961, *Mossakowski* 1966a, 1966b) suggest that primarily we are dealing with a gliopathy which in turn results in injury of other elements of brain parenchyma. There exist, however, other essential elements of the morphology of hepatic encephalopathy, such as focal spongy degeneration of nervous tissue, particularly typical of portal systemic encephalopathy (*Sherlock et al.* 1954),

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and generalized cerebral edema which occurs in the majority of cases of spontaneous hepatic encephalopathy (*Mossakowski* 1966a), and in a considerable percentage of experimental cases (*Mossakowski* 1966b). The question thus arises whether the abnormalities of glia similar to those described by *Bignami et al.* (1965) and *Carnoga et al.* (1967) in cases of experimental ouabaine encephalopathy which runs with typical focal spongiform degeneration of nervous tissue are responsible for both groups of the mentioned lesions or whether yet another factor, namely the disturbed permeability of cerebral blood vessels plays a part here.

The purpose of this study was to determine the possible deviations in the permeability of cerebral blood vessels and their role in the pathomorphology of hepatic encephalopathy. Since the cerebral lesions in hepatic encephalopathy develop very slowly (*Lapham* 1961, *Mossakowski* 1966b), the use of a long-term method for the evaluation of vascular permeability seemed more justified than the application of routine barrier markers. In our opinion the intravital silver method described by *Wislocki* and *Leduc* (1952) fulfills best the requirements. These authors found that in normal conditions the silver is deposited only in the pia mater, stroma of choroid plexus, in the endothelium of blood vessels and in those regions of the nervous system which are lacking the barrier mechanisms: area postrema, epiphysis and hypophyseal stalk (*Wiśniewski, Olszewski* 1962). In his studies on the effect of hypothermia on the blood-brain barrier *Baldwin* (1969) confirmed the fitness of this method for the investigation of brain vessels' permeability.

MATERIAL AND METHODS

The studies were carried out with 51 white Wistar rats of both sexes, aged 2 months at the beginning of experiment. In order to induce liver cirrhosis carbon tetrachloride dissolved in liquid paraffin was subcutaneously injected to 30 experimental rats in the dose of 0·1 cc per 100 g of body weight, every second day according to *Georgijew et al.* (1968). Twenty one control rats were treated subcutaneously with liquid paraffin in the same amount per 100 g of body weight. According to the technique of Wislocki and Leduc (1952) both groups of animals received for drinking silver nitrate solution.

The animals were sacrificed in groups, 2, 4 and 6 months after the beginning of injections of carbon tetrachloride. In order to avoid possible differences related to the period of silver nitrate administration and age of the animals, the experiment was organized in such a manner that all the animals drank silver nitrate for 7 months and were sacrificed in the 9th month of life. Each experimental group comprised ten animals, and each control one — seven.

Material from three standard brain areas (cerebral hemispheres at the level of the infundibulum and thalamic region, brain stem and cerebellum at the

level of transition of the pons into the bulbus), as well as from the liver was taken for histological examination. The material was fixed in 10% neutral formalin and embedded in paraffin. The brain sections were stained with hematoxylin and eosin, after *Kanzler-Arendt* and *Heidenhain* and after *Gridley*. The frozen brain sections were impregnated with gold salts after *Cajal*. The liver sections were stained only with hematoxylin and eosin. Unstained sections of both organs were studied in the dark field in order to localize the silver deposits. In order to evaluate the damage to the blood-brain barrier three rats of each group received an intravenous injection of Evans blue in 7% solution of bovine albumin 2 hours before being killed. The distribution of the marker in the tissue was evaluated grossly, and then in the sections under fluorescent microscope with a HBO 200 lamp.

RESULTS

Clinical observations. Starting from the 4th month of experiment the majority of rats showed signs of ascites. The animals became apathetic, sluggish, without appetite, they lost their hair. The skin and mucosae became gray. Four animals of the 6-month and two of the 4-month group succumbed before the end of experiment.

Morphological observations. In animals of the control group there were no deviations from normal except the gray discoloration of internal organs, cerebral dura mater and the hypophyseal stalk.

In sections stained histologically no morphological abnormalities were found either in the brain or in the liver. The brain sections examined in the dark field showed the presence of abundant deposits of silver in the parenchyma and vessels of the pineal body (Fig. 1), in the vessels of the infundibulum and neurohypophysis (Fig. 2), and in the stroma of the choroid plexus (Fig. 3). The silver deposits were less abundant in the pia mater and in the walls of intra- and extracerebral vessels (Fig. 4). The cerebral parenchyma was, however, free of metallic deposits. The hepatic connective tissue was heavily impregnated. Cerebral and hepatic morphology of experimental animals of the 2-month group showed essential differences when compared with animals treated with carbon tetrachloride for 4 and 6 months. This justifies the separate description of all these groups.

In the animals of the 2-month group the liver showed fatty degeneration of variable degree of the parenchymal cells without any proliferation of connective tissue. The brain of these animals exhibited hyperplasia and hypertrophy of astrocytes, particularly on the cortico-subcortical junction. These changes were accompanied by astrocytic degeneration, mainly in the form of what is called clasmatodendrosis, and a few type II Alzheimer cells. There

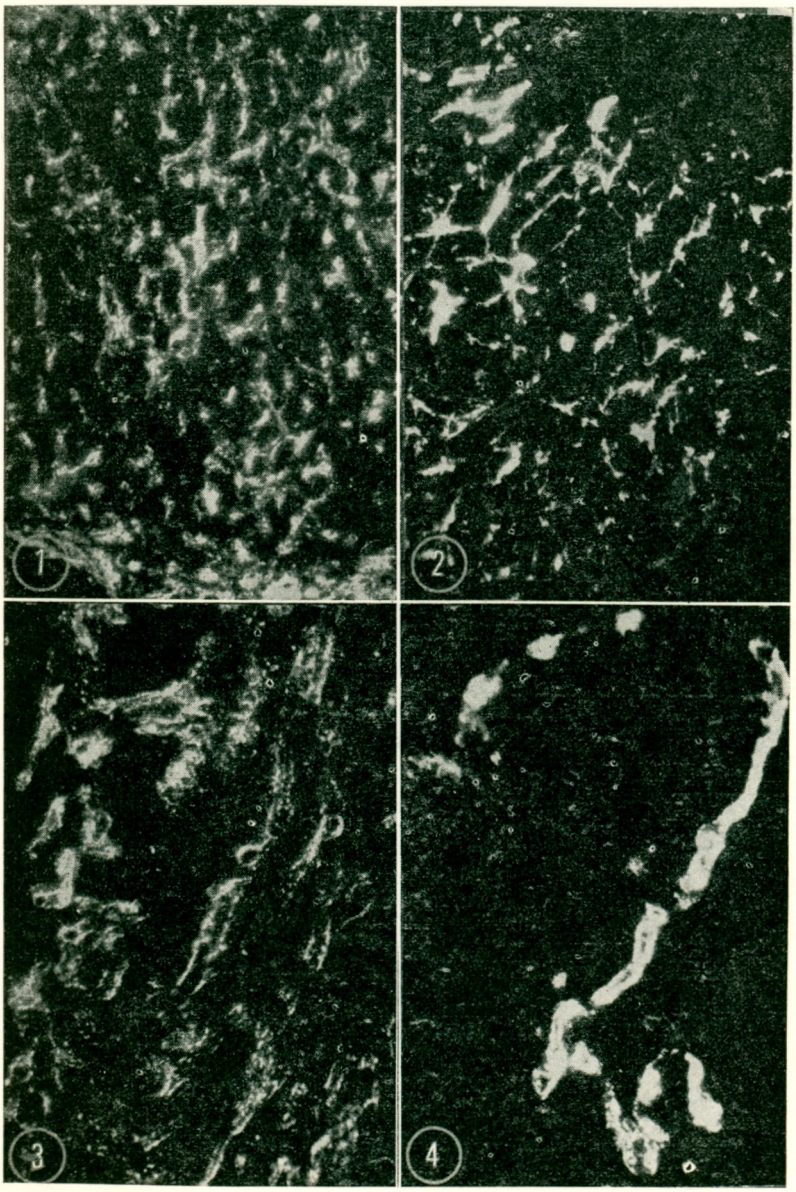

Fig. 1. Silver-impregnated pineal body vascular net. Abundant granular metallic deposits outside the vascular stroma. Dark field, unstained section. × 200.
Fig. 2. Abundant silver deposits in posterior lobe of hypophysis, situated in the vascular wall and in the stroma. Dark field, unstained section. × 200.
Fig. 3. Silver-impregnated connective tissue stroma of the choroid plexus of the lateral ventricle. Dark field, unstained section. × 200.
Fig. 4. Silver-impregnated vessels and pia mater of the brain base. Dark field, unstained section. × 150.

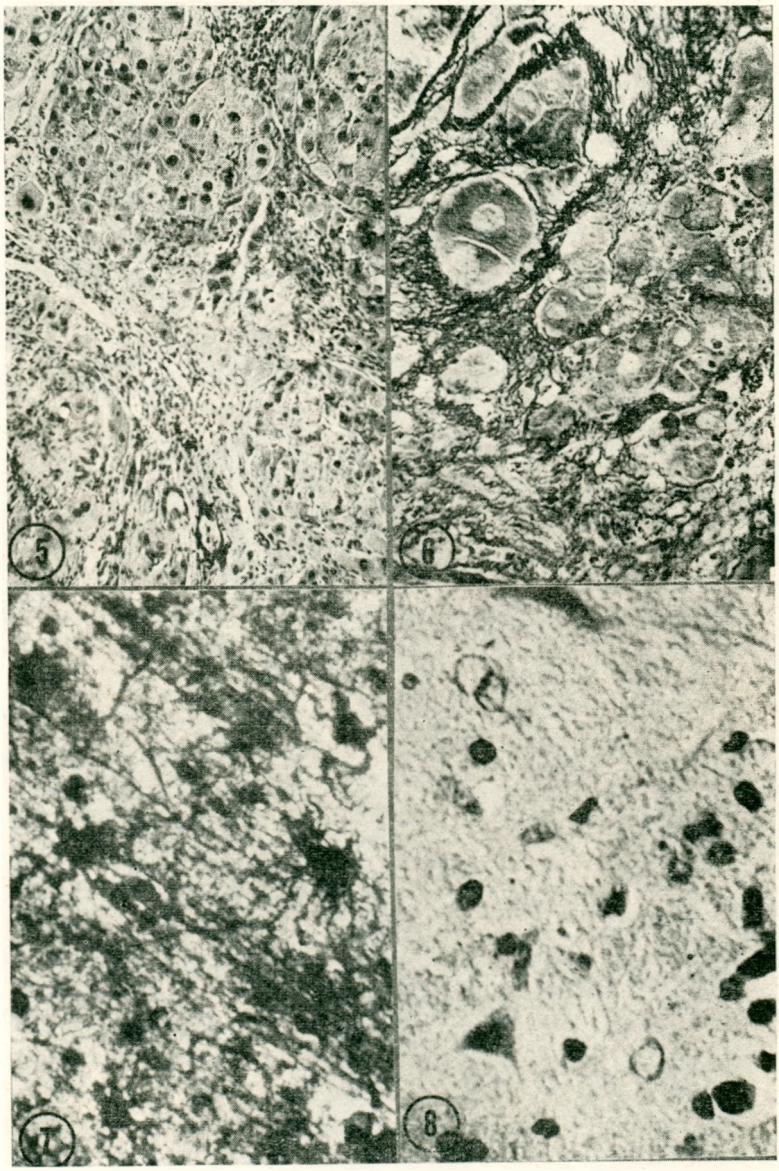

Fig. 5. Rat after 6-month experiment. Typical experimental liver cirrhosis. Foci of preserved liver parenchyma, regenerative nodules and marked proliferation of fibrous connective tissue. H. E., × 200.
Fig. 6. Rat after 6-month experiment. Abundant argyrophil fibers between the foci of preserved liver parenchymal cells. Gridley, × 400.
Fig. 7. Rat after 4-month experiment. Astrocytes with fragmented processes. Cajal, × 300.
Fig. 8. Rat after 6-month experiment. Numerous type II Alzheimer cells. H. E., × 200.

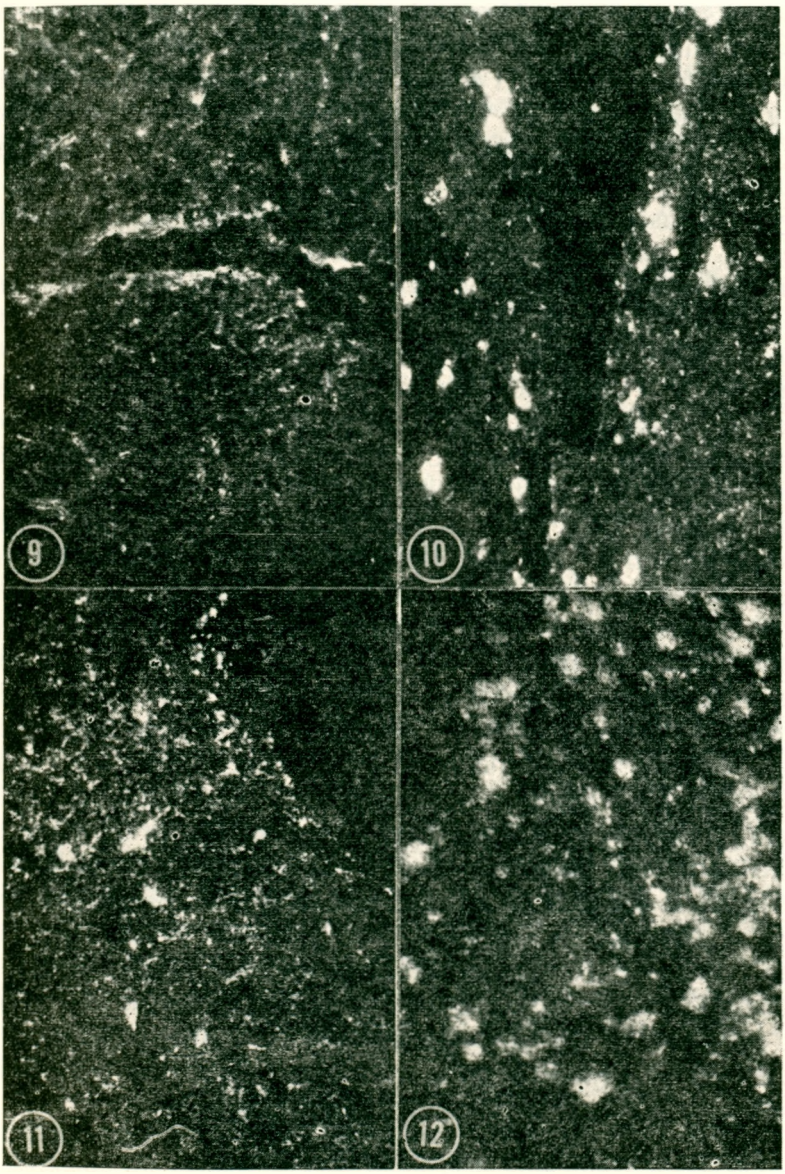

Fig. 9. Rat after 6-month experiment. Numerous silver granules around a vessel of the basal nuclei. Dark field, unstained section. × 300.
Fig. 10. Rat after 4-month experiment. Numerous silver-filed astrocytes in the subependymal area of the 3rd ventricle. Dark field, unstained section. × 240.
Fig. 11. Rat after 4-month experiment. The glial cells of cerebral fornix filled with bright metallic deposits. Dark field, unstained section. × 200.
Fig. 12. Rat after 6-month experiment. Basal pontine nuclei. The majority of nerve and glial cells are loaded with metallic granules. Dark fie'd, unstained section. × 200.

were no noticeable features of brain edema. In the dark field the brain morphology did not differ from that observed in control animals.

In the animals sacrificed after 4 and 6 months of the experiment liver cirrhosis was fully developed (Fig. 5, 6). The brain of these animals exhibited all characteristic features of hepatic encephalopathy. The essential pattern of changes was identical in both groups, in spite of slight quantitative differences in the intensity of lesions. Generalized proliferation of glial cells was the most striking phenomenon. It concerned all cerebral structures with a pronounced predominance of lesions at the cortico-subcortical junction, and in the cerebellar molecular layer. This was associated with hypertrophy of single astrocytes and their degeneration resulting in fragmentation and total breakdown of the processes (Fig. 7). A number of Alzheimer cells, type II (Fig. 8), as well as what is called transitional cells occurred in all formation of the gray matter. The Alzheimer cells, type I, and Opalski cells were absent in all cases. Moreover, numerous nerve cells in all structures of gray matter, in the first place in the brain cortex, basal ganglia and in the cerebellar cortex showed generalized features of nonspecific degeneration. No abnormalities were found in the structure of brain blood vessels. In two animals of the 6-month group small foci of spongy degeneration were found in the brain stem. Observation in the dark field revealed more abundant silver deposits in the typical brain regions described above than in the control animals. Besides, tiny, granular metallic deposits were present in the neuropil of brain cortex and of other gray structures. They were more numerous around the blood vessels (Fig. 8). Numerous glial cells, mostly astrocytes, were filled with silver granules. They occurred in all parts of the central nervous system and were most numerous in the white matter. The greatest number of silver-bearing astrocytes was found in the paraventricular region (Fig. 10), as well as in the submeningeal areas of the brain and brain stem. The metallic deposits were also found in the glial cells of the corpus callosum and brain fornix (Fig. 11). The nerve cells of the basal ganglia, pontine nuclei, *cornu Ammonis*, and to a lesser degree Purkinye cells contained intracytoplasmic metallic granules (Fig. 12). In comparison with the control group there was no increase in the silver deposits in the vascular endothelium. In no group of animals did Evans blue penetrate beyond the vascular bed.

DISCUSSION

Experimental liver cirrhosis, induced in rats with carbon tetrachloride, results in morphological lesions typical of hepatic encephalopathy. These lesions, although more pronounced, are identical in type and localization with those found in liver encephalopathy produced experimentally by a low-protein diet (*Lapham* 1961, *Mossakowski* 1966b). They show analogy with human hepatic encephalopathy (*Stadler* 1936, *Nikolajev* 1937, *Baker* 1949, *Mossakowski* 1966a)

and with lesions found in congenital hyperammonemia in children (*Braton et al.* 1970). It differs from portal systemic encephalopathy by the lack or minimal degree of spongy degeneration of the nervous tissue (*Sherlock et al.* 1954, *Summershall et al.* 1956, *Mossakowski, Szymchel-Paluszkiewicz* 1964). The fact that slight focal spongy degeneration was present only in animals submitted to the 6-month experiment, and absent in animals killed earlier, proves that the degeneration in question is a rather late phenomenon in hepatic encephalopathy.

Slight cerebral morphological lesions were observed already in the 2-month experimental group. In this period changes in the liver consisted only in generalized fatty degeneration of parenchymal cells, without any features of cirrhosis. This fact seems to support the opinion of *Nikolajev* (1937) that the products of breakdown and degeneration of liver parenchymal cells are responsible for the injury of the central nervous system.

The alteration of glia in the central nervous system coincides with the increased permeability of brain vessels for silver salts which appear in such components of brain parenchyma as the neuropil, astrocytes and some nerve cells. In cases with undisturbed vascular permeability the deposition of silver salts is strictly limited to the regions lacking the barrier mechanisms. This has been established both in experiment (*Wislocki, Leduc* 1952), and in human pathology (*Hill, Pittsbury* 1939). The increased vascular permeability found in our material is identical with the disturbances found by *Baldwin* (1969) in experimental hypothermia. We are dealing here probably with selective damage of the blood-brain barrier, since the same animals did not show any disturbances in permeability for the routine protein barrier marker such as Evans blue-bound albumin. This type of disturbances in permeability can play an essential role in human pathology since they can result in the penetration of the nervous system by harmful low-molecular weight chemical substances which in normal conditions fail to pass the vessel-brain barrier, or whose passing is greatly limited. Copper may belong to these substances. Its serum level considerably increases in cases of liver cirrhosis, as it has been shown by *Holmberg* and *Laurell* (1954) and *Mossakowski et al.* (1970).

On the basis of our observations it is difficult to determine unequivocally the interrelationship between the increased permeability of brain vessels for silver salts and the damage to glial tissue. The glial injury precedes in time the manifest disturbances of vascular permeability. It appears already after 2 months of experiment while the disturbances in permeability are noticeable only after 4 or 6 months, in other words in the period of fully developed encephalopathy. This does not settle, if course, the problem of the causative dependence of both phenomenona. The suggestion seems, however, justified that glial injury can play in this case an essential role in the disturbances of the barrier mechanism which, in turn, can influence the further development and progress of gliopathy.

This is indicated, among other things, by the accumulation of silver which is not without effect on the tissues and in the first place on the astrocytes. The fact should be also emphasized that the disorders in the barrier system are not associated in our material with any detectable abnormalities of the vascular structure.

CONCLUSIONS

1. Experimental CCl_4-induced liver cirrhosis results in the morphological pattern of hepatic encephalopathy with dominating damage of astroglia.
2. The glial alterations are associated with the increased permeability of the brain vessels for silver salts administered during the entire experiment.
3. Increased permeability for silver salts and the absence of permeability for protein barrier markers indicate the selective type of the barrier injury.
4. The early appearance of structural alterations of astroglia suggests their possible causative role in the disturbances of the barrier mechanisms observed in this experiment.

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