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Changes in the Blood-Brain Barrier During Experimental Cirrhosis of the Liver

V. A. Mishchenko, O. A. Goryukhina, and R. D. Ilyuk

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The state of the blood-brain barrier (BBB) is essential for nervous system functioning and for vital activity as a whole. According to current notions, the barrier is represented by perivascular processes (connecting piece), capillaries including the endothelium, and a basal membrane with cells closely knit with it - pericytes and mast cells. The basal membrane consists of two components: an acellular component, characterized by a fibrillar structure and amorphous substance adjoining the endotheliocytes, and a cellular component, represented by pericytes composing a duplicate of the basal membrane [9]. Numerous pericyte processes envelop the capillary, some of them penetrating through the acellular component of the basal membrane and terminating on endotheliocytes. Pericytes are believed to contribute to the capillary "motor innervation" and to transfer to capillaries information on changes in the metabolic environment. As a result, brain capillary endotheliocytes develop a response to biologically active substances such as histamine, serotonin, etc. [13].

Other authorities [8] believe that pericytes also produce an intermediate substance and act as a barrier.

The processes of the plasmalemma laminar surface (glycocalyx) contribute to metabolite capture and transfer from the capillary bed.

Recently tissue basophils (mast cells) with organ specificities typical of the nervous system were referred to the BBB [5]. These cells are considered to be mediators regulating metabolic processes by secreting and absorbing from adjacent tissues bioactive substances (histamine, serotonin, heparin, catecholamines, and some proteolytic enzymes).

The brain's microcirculation is 85% controlled by astrocyte perivascular processes [1,7]. These cells provide not only support and transport of substances to neurocytes, but an oxygen reserve as well, which is indispensable for them, particularly so in extreme situations.t Such a conclusion was drawn in a study of the animal brain's reaction to hypoxia [5]. Neurocytes directly adjacent to the capillary wall in the hypothalamic area were found to be the first to react to hypoxia. Besides, astro-

Department of Nervous Diseases, St. Petersburg State Medical Institute of Sanitation and Hygiene. (Presented by I. P. Ashmarin, Member of the Russian Academy of Medical Sciences)

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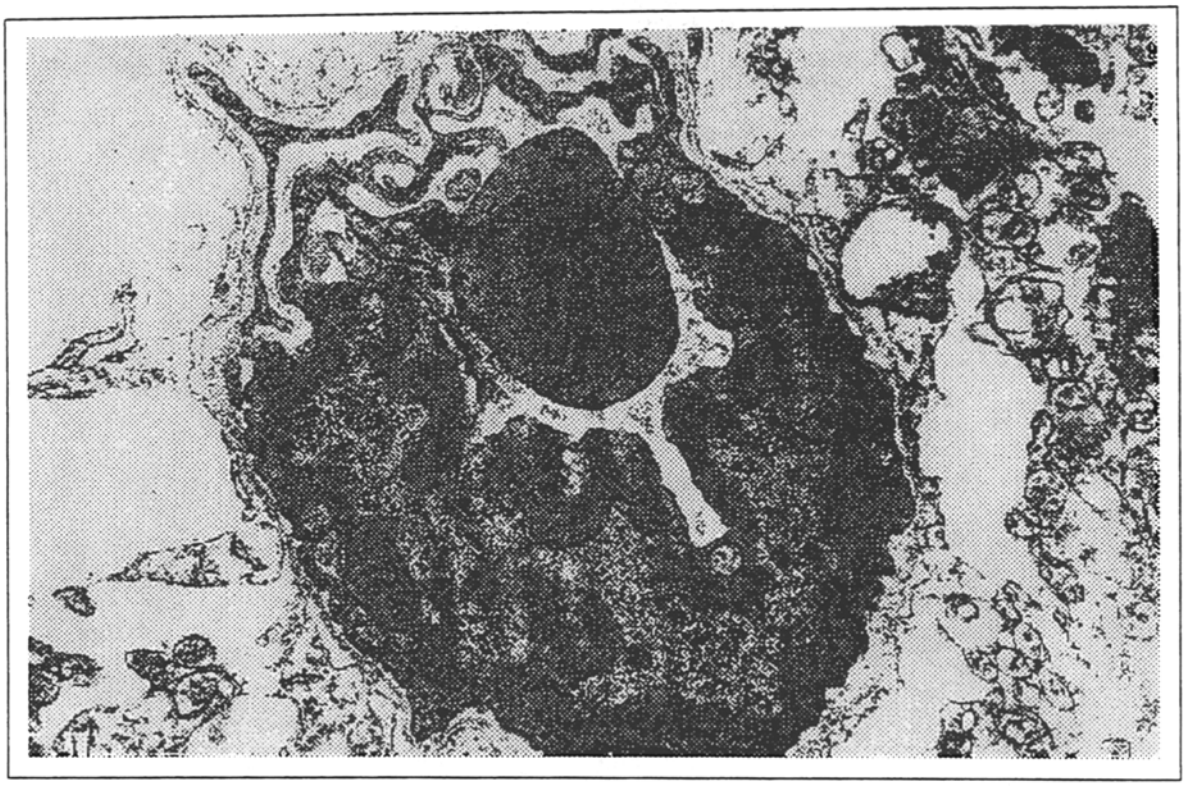

Fig. 1. Brain (cerebellar) tissue of a rat with cirrhosis of the liver. Pericapillary edema. Drastic enlargement of endotheliocyte. ×25,000.

cytes may possess a protective function, even by phagocytosis; one more possible function of these cells is maintenance of neurocyte trophism and of dence contacts in the endothelium [5].

Hence, the blood-brain barrier structures include the capillary endothelium, basal membrane, mast cells, and astrocytic perivascular peduncles.

Numerous data, including our own, confirming changes in the microcirculatory bed in patients with chronic liver disease called our attention to the status of the BBB. These were mainly light microscopy findings, and therefore we decided to undertake an electron microscopic study. This obliged us to refine the method of inducing cirrhosis of the liver in experimental animals.

Of the hepatotoxic agents used in experimental hepatology, tetrachloromethane is used most frequently [15].

The narcotic effect of this agent was discovered in the 18th century. Later, this drug was found to exert a marked toxic effect on the liver, and therefore it began to be used experimentally as a damaging agent [12].

The model of hepatitis and cirrhosis of the liver induced by tetrachloromethane injection to animals became the simplest one most frequently used by scientists. Numerous reviews of literature and original papers confirm the extraordinary effect of tetrachloromethane on the liver in chronic poisonong [2-4,6,10,11,14].

MATERIALS AND METHODS

Thirty female Wistar rats weighing 150-200 g were used in experiments. Chronic hepatitis and then cirrhosis of the liver were induced in them by oral administration of tetrachloromethane in a dose of 0.2 ml per 100 g weight every other day. The course of liver disease development during challenge was as follows: acute hepatitis manifested itself in 2 weeks, chronic hepatitis in 3 months, and cirrhosis of the liver in 6 months. The animals were then decapitated under ether anesthesia, and the brain and liver were removed for light and electron microscopic examination. Histological preparations for light microscopy were stained with hematoxylin-eosin after Nissl and Van Gieson; an Opton electron microscope was used.

RESULTS

Signs of cirrhosis in experimental rats were characterized by connective tissue growth in septa and lobules, by hepatocyte necrosis, the presence of cellular infiltrates in the connective tissue septum, and by fatty droplets.

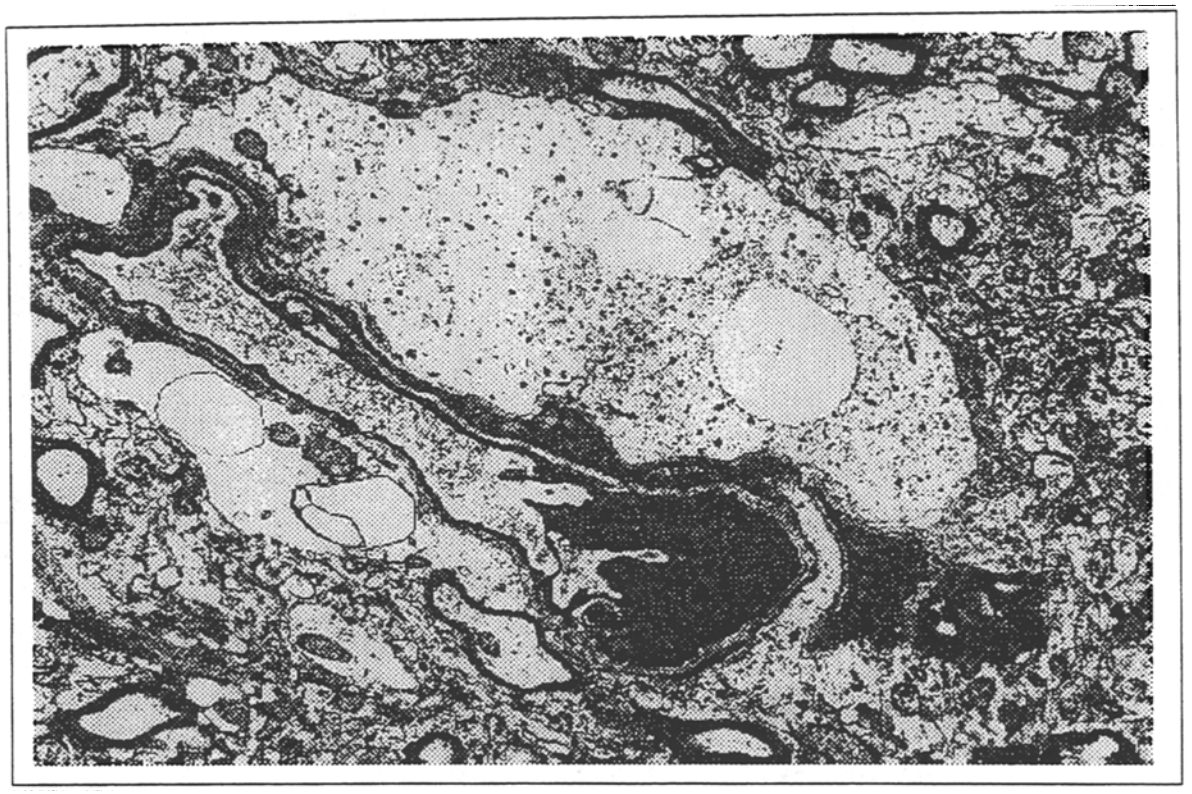

Fig. 2. Brain (frontal lobe) tissue of a rat with cirrhosis of the liver. Manifest edema of a pericapillary cell. Vacuole. ×10,125.

It is important to note that the changes were first detected in the liver and then in the brain; hence, vascular parenchymatous changes in brain tissue nay be regarded as secondary.

Histological shifts detected by light microscopy were characterized, first of all, by edema, particularly an interstitial edema found near vessels and in the pericelllular space. In addition to the perivascular edema, deformations and destructive changes of the vascular wall were found which were so grave that vascular integrity was impaired and perivascular hemorrhages with small and large foci were observed. Besides the vascular changes, the experimental animals developed signs of severe dystrophy of reticular cells of the pons, pericellular edema, and intermittent fibers. Cellular changes in other brain regions were characterized by swelling, vacuolation, the appearance of glial cells with large nuclei, the presence of shadow cells, and other signs of distrophy and death.

Tissue samples of the frontal and occipital lobes, upper portion of the brain stem, and cerebellum were examined under the electron microscope. Vascular changes presented as pericapillary edema, capillary wall deformation and destruction, and the "sludge" phenomenon; in other words, both peri- and intravascular shifts were discovered, namely extended spaces between endotheliocytes, pericyte edema, edema between a gliocyte and capillary, thinning of the endotheliocyte layer, and in some preparations enlarged endothelial cells. These changes often caused gross deformation of the capillaries.

Figure 1 shows white rat cerebellar tissue with a marked pericapillary edema and considerably enlarged endotheliocyte; Fig. 2 presents an electronogram of the frontal lobe tissue with pericapillary cell intensive edema and formation of a vacuole as well as gross deformation of a capillary.

Along with the capillary changes, edemas and destruction of the mitochondria in vascular peduncles of astrocytes, essential components of the blood-brain barrier, were noted. Moreover, extension of the endoplasmic reticulum and irregular distribution of ribosomes (accumulations thereof) were observed in the gliocytes, as well as membranous desquamation, mitochondrial dstruction and death in cell bodies, increased number of lysosomes, and cytoplasmic edema. Changes in gliocyte nuclei were characterized by nuclear membrane rupture, tortuous profiles of the nuclei, extended perinuclear space, nucleus flooding, and even the formation of an intranuclear vacuole.

Hence, electron microscopic examinations in rats with experimental cirrhosis of the liver showed structural changes in blood-brain barrier compo-

nents (capillary endothelium, basal membrane, astrocyte vascular peduncles) which reduced its protective function. Blood-brain barrier destabilization developing during chronic liver disease may affect the penetration of cerebrotoxins and other metabolic toxins that are produced in the course of digestion and are incompletely utilized by the liver, which fact may explain the development of a hepatic encephalopathy. These data necessitate a differentiated approach to therapy with due consideration of certain factors capable of potentiating the hepatic encephalopathy, particularly hepatotoxic drugs.

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Morphological Characteristics of the Brain in 20-21-Day-Old Rat Embryos and in 1-5-Day-Old Rats

B. Ya. Ryzhavskii and S. I. Biryukova

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The character of embryogeny in the brain and its state during the neonatal period have a marked effect on the further development of this organ [8,9,13]. This, as well as the high incidence of brain pathology in newborn infants and the difficulties of its correction [4,7], explain the high level of interest in such studies. The factors which determine the degree of development of the brain, such as its weight, the thickness of the cortex, the degree of neuronal differentiation, and so on have been analyzed [1,2,5,10,11]. It has been established that these parameters are affected by genetic and environmental factors, the level of different hormones in the blood, and the supply of oxygen and nutrients to the fetus [2,5,8]. At the same time, there is scant information on the role of different factors in the regulation of brain development, and so further study of the organ in embryos and in animals at the early stages of postnatal ontogenesis is called for.

Department of Histology, Khabarovsk Medical Institute. (Presented by V. A. Trufakin, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The brains of 49 20-day-old (5 litters) and 53 21-day-old (4 litters) embryos, and of 34 1-day-old

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