

THE CONDITION OF HEMATO AND LIQUOR-ENCEPHALIC BARRIERS OF THE HUMAN BRAIN IN ACUTE BLOOD LOSS ON THE BACKGROUND OF ALCOHOLEMIA AND DRUG INTOXICATION

By means of the histological methods, scanning and transmission electronic microscopy the cerebral cortex of the brain (field 6), the walls of III and IV ventricles of the brain of dead people who had died of acute anemia on the background of alcohol intoxication and drug intoxication have been studied. Thinning of the basal membrane of capillaries, the development of fissures in the places of contact of neighboring endothelial cells, between basal membrane and endothelial cells swelling of pericytes have been revealed. In the wall of the brain ventricles a marked polymorphism of the ependymal cells is noted, disturbance of the continuity of the layer. On the surface of ependyma accumulation of blood cells, thickening of detritus, crystal structures are revealed. Reported symptoms reflect the disturbance of permeability of hematoencephalic and liquoroencephalic barriers of the brain with a combination of traumatic blood loss with alcohol and drug intoxication.

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The steady increase in the number of poisonings with ethanol and drugs makes it necessary to carry on scientific research, including development of clinical, laboratory, sectional and histological signs supporting or rejecting the suggestion of toxic effect of each poison (Klevno et al., 2007). Forensic medical histology increasingly uses a large arsenal of methods developed by modern morphological science. In some cases only using of electronic microscopic method can help in establishing the brain damages caused by poison (Ishizova and Tumanov, 2003). Many electronic microscopic studies of the brain are devoted to the study of its hematoencephalic barrier (HEB) in different pathological conditions (Hankeldiev et al., 2004). Electronic microscopic studies revealed the presence of so-called tight junctions or complexes between the endothelial cells of the brain, preventing the passage of fluid from the lumen of the microcirculatory bloodstream and perivascular space through the intercellular fissures of endothelium.

Peculiarity of the structure of the brain capillaries with their continuous tight junctions, the presence of thick basal membrane of these capillaries with well-developed layer of pericytes adjacent to it, and also surrounding of basal membrane by astrocytes pedicles appear as reliable barrier to fluid filtration from the lumen of capillaries. It is established that the functioning of the cerebral circulation and liquorodynamics is closely related to each other (Moskalenko, 2002).

In addition to the HEB in the brain there is also liquoroencephalic barrier (LEB). Morphological substrate of LEB is the ependyma of the brain ventricles (Rubin and Farber, 2004). Disturbance of the integrity of this barrier may be one of the causes of the brain edema, accompanying craniocerebral trauma (Alimov, 1992). Swelling of the brain, as a nonspecific process develops in many poisonings. Intoxication with ethyl alcohol and drugs takes a leading place in the structure of all fatal poisoning. Manifestation of metabolic disorders typical in ethanol poisoning and disturbance of vascular permeability

are marked edema of the brain and the pia mater encephali (Babakhanian and Petrov, 2002).

Changes in the structure of protective barriers of the brain with a combination of acute blood loss with intoxication of alcohol and drugs have not been studied yet. The purpose of this study is to determine the morphology and permeability of the hemato and liquoroencephalic barriers of the brain in acute anemia on the background of poisoning with ethanol and drugs.

By means of histological methods, scanning and transmission electronic microscopy (SEM) the cortex hemispheres of the brain (box 6), the walls of III and IV ventricles of the brain of dead persons aged 18 to 60 years, died of acute anemia on the background of alcoholic intoxication and drug intoxication (5 cases) have been studied.

In all cases hemorrhage was due to stab and cut wounds of vessels, tissues and inner organs. Quantitative determination of ethanol in blood and urine were determined by gas and fluid chromatography method. Ethanol concentration in the blood of persons who died ranged from 0.8% to 4% on. The presence of drugs in blood, urine and internal organs was determined by thin layer chromatography plates "Silufof" and "Sorbfil". Drugs in all cases related mainly to the series of opioids.

The material for transmission electron microscopy (TEM) was fixed in 2.5% solution of glutaraldehyde to 0.1M phosphate buffer (pH 7.2), postfixated in 1% solution of osmium tetroxide. After dehydration in an alcohol-acetone the material was poured into a mixture of Epon and araldite. Semithin and ultrathin sections were made on ultra microtome "Reichert - Jung" (Reichert, Austria). Semithin sections were stained respectively with methylene blue and basic fix and examined by photo optical method. Ultrathin sections were contrasted with uranyl acetate and lead citrate in Ultrastainer LKB. Ultrathin sections were examined with an electron microscope H-600 (Hitachi, Japan). For scanning electron microscopy (SEM) the material was fixed with glutaraldehyde on phosphate buffer followed by complete fixation in 1% solution of osmium tetroxide in the same buffer, subjected to dehydration in alcohol, acetone, then dried by critical point in the apparatus of the NDS-2 and sputtered with gold in the apparatus IB-2. The samples were studied in a scanning electron microscope SEM 405A (Hitachi, Japan).

In TEM study of the autopsy material, obtained after death from hemorrhage on a background of combined poisoning with ethanol and drugs, in the cerebral cortex of the brain significant changes in the ultra structure of the capillaries have been revealed. They appear in the thinning of the basal membrane of capillaries. Pericytes have extremely electronic light cytoplasm and swollen nuclei with a thin layer of heterochromatin under karyolemma. Between the basal membrane and endotheliocyte large vacuoles are determined. The cytoplasm of endotheliocyte is also differed by extreme enlightenment; there are few polyribosomes in it. Intercellular contacts lose their density, it is noted the appearance of fissures in the places of contact of neighboring endothelial cells (Figure 1). In the lumen of the capillaries the swollen red blood cells and elongated cells with oval are present, which are apparently desquamated endothelial cells. Around the capillary neuropil is vacuolated. Some of the vacuoles in the neuropil correspond to the diameter of the capillary in size.

In those who died from hemorrhage on the background of combined poisoning with ethanol and drugs not only vacuolization of neuropil, but also marked as changes in neurons takes place. Many pyramidal neurons are wrinkled, their cytoplasm is condensed, and as a result of this nucleus and the majority of intracellular organelles are not revealed. However, it identifies the swollen mitochondria, the matrix of which has very low electron density, as well as dense lipofuscin bodies. In other pyramidal cells exposed to wrinkling, the extracellular structure and intracellular vacuoles are distinctly different, both in the cell body and in its processes. The cells in the condition of marked hydropic disturbances are noted along with the shrunken neurons. These disturbances are manifested in full enlightenment of matrix of the cytoplasm, absence of organelles, swollen nuclei and indistinct borders due to disturbances of the integrity of the plasma membrane. Numerous

axons and dendrites are swollen, their matrix is enlightened. The structure of the cerebral ventricles is subjected to significant changes which enclose marked polymorphism of ependyma cells, disturbances of their continuity of their layer at considerable length. Ventricular surface looks rough, small elevation of its tiny and deepening are marked. Ependyma in the ventricles, along with deprived areas of ependy covering, has the areas of accumulation of polymorphic cells with false multilayer (Figure 2). Lumina of microvessels in subependymal layer are sharply expanded, desquamation of endothelial cells is marked over a large distance. In the lumen of small vessels there are microthrombi, emboli and discolored red blood cells. Studies by SEM showed not only the roughness of the microrelief ependyma, but and substantial hollowness in the neuropil of various sizes (Figure 3). There are a lot of red blood cells on the surface of ependymocytes; most of them are pathological forms. These include stomatocytes, red blood cells with a comb, there are also other irreversible pathological forms of erythrocytes (Figure 4). Besides, on the inner surface of the ventricles not only the accumulation of blood cells is revealed, but also the layers of detritus. In addition, the formation, having a form of crystalline structures often occurs.

FIGURE 1. DISTURBANCE TIGHT JUNCTIONS OF THE CAPILLARY ENDOTHELIUM OF THE PYRAMIDAL LAYER OF THE CORTEX, LARGE VACUOLES IN THE NEUROPILO IN HAEMORRHAGE ON THE BACKGROUND OF COMBINED POISONING WITH ETHANOL AND DRUGS. TEM, x 20000

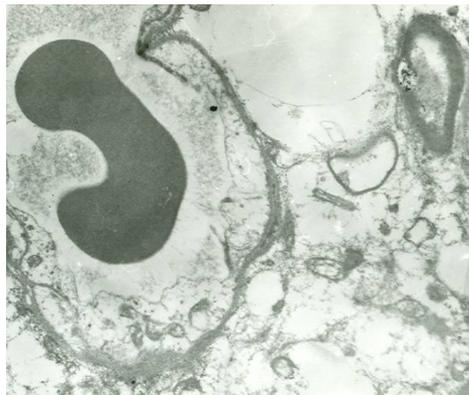


FIGURE 2. DISTURBANCE OF THE STRUCTURE OF EPENDYMA OF VENTRICLE OF THE BRAIN VENTRICLE AND VACUOLIZATION OF NEUROPILO IN HAEMORRHAGE ON THE BACKGROUND OF COMBINED POISONING WITH ETHANOL AND DRUGS. HEMATOXYLIN AND EOSIN STAINING. TEM, x 100

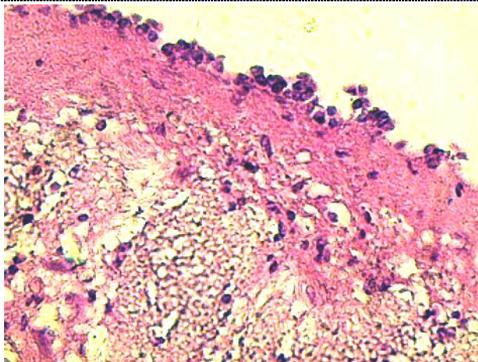
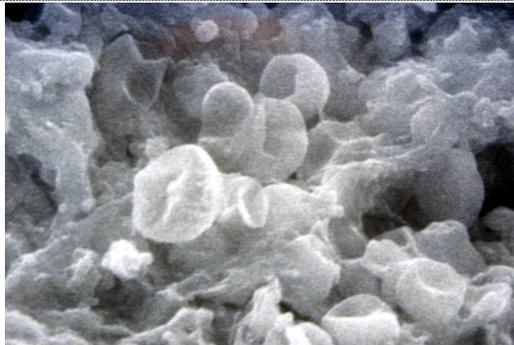


FIGURE 3. ROUGH MICRORELIEF AND "EMPTINESS" IN THE NEUROPILO OF THE BRAIN VENTRICLE WITH HEMORRHAGE ON THE BACKGROUND OF COMBINED POISONING WITH ETHANOL AND DRUGS. SEM, x 500



FIGURE 4. NUMEROUS PATHOLOGICAL FORMS OF RED BLOOD CELLS ON THE SURFACE OF THE BRAIN VENTRICLE WITH HEMORRHAGE ON THE BACKGROUND OF COMBINED POISONING WITH ETHANOL AND DRUGS. SEM x 3000



Our studies with employment of TEM and SEM made it possible to reveal disturbances of the structural base of permeability, both the HEB and LEB. Ultra structural study of gray substance of the brain revealed disturbances of all structures of the walls of capillaries that make up the blood-brain barrier: endothelial cells, basal membrane pericytes. Vacuolization of the neuropil, its swelling and hydropic disturbances of neurons reflect the disturbances of the permeability of microcirculation blood stream, hence the permeability of HEB in general. The presence of vacuoles between endothelial and basal membrane shows the possibility of endothelial cell desquamation and severe damage to the HEB. Pyknosis and loss of neurons, especially of pyramidal cells may be related, apparently, with the action of ethanol and drugs. SEM study made it possible to reveal a disturbance of liquorodynamics in the brain with acute anemia in the background of poisoning with ethanol and drugs. Presence of pathological forms of erythrocytes on the surface of ependymocytes suggests that they can also be in the fluid of ventricles. Roughness of the surface relief of the ventricles is apparently due to irregular swelling of subependymal layer. Swelling of this layer is manifested by the presence of hollowness of different size voids. Complicated change of the surface relief of the ventricle can also be caused by the fact that different parts of the brain, located in its wall, have varying degrees of edema. Our research results show a close relationship of ependyma condition with the

degree of hydration of ventricular wall and the presence of hemorrhage within it. Intake of red blood cells in cerebrospinal fluid of ventricles can be seen as adaptive phenomena causing elimination of excess fluid and red blood cells from brain tissue. The appearance of crystalloid structures on the surface of the ventricles may be due to removal of toxic substances or their metabolites from brain tissue in the cerebrospinal fluid.

Thus, acute anemia on the background of alcohol and drug intoxication is, accompanied by a disturbance of both the blood-brain permeability and liquororoenephalic barriers.

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