ELECTRON-HISTOCHEMICAL INVESTIGATION OF CAPILLARY PERMEABILITY OF THE CENTRAL NERVOUS SYSTEM IN BURN SHOCK

V. P. Tumanov

UDC 617-001.36-02:617-001.17-092.9-07:616.16-008.6-076

The permeability of capillary walls in the CNS in burn shock was studied in experiments on animals. During the period of burn shock, a marker (peroxidase) was introduced into the blood stream of the animals. Electron-microscopic investigation showed that the capillary wall in the CNS is permeable to substances with a molecular weight of 40,000, such as peroxidase, in an animal in the stage of burn shock.
KEY WORDS: capillaries of the CNS; capillary permeability; burn shock.

The CNS plays an important role in the pathogenesis of burn shock [1]. It was accordingly decided to study the morphological changes in the capillary wall and neurons after extensive burns.

Previous investigations [2, 3] showed that deep thermal burns affecting a considerable part of the body surface are accompanied by marked changes in the nervous system and, in particular, in the synaptic apparatus. Disturbances of permeability of the capillary wall also arise in burn shock.

In this investigation an attempt was made by electron histochemical methods to study the fine mechanisms of membrane permeability, i.e., to detect disturbances of the microcirculation in the brain in burn shock.

Substances can be transported through the cytoplasm of endothelial cells in several ways: through the intercellular spaces, by means of the micropinocytotic vesicles, by diffusion through micropores in the plasmalemma, and through membrane fenestration [4].

Karnovsky [5] studied the permeability of blood vessels in intact animals by the use of peroxidase and lanthanum as markers. He found that the brain capillaries are in fact impermeable to these markers, for the zone of obliteration of the capillary is part of the morphological substrate of the blood–brain barrier. In other internal organs the interendothelial spaces are not closed by zones of obliteration but from what are known as obliteration spots 4-5 nm in diameter (except in the CNS). Karnovsky considers that the intercellular spaces of capillaries are permeable to substances with a molecular weight not exceeding 40,000. The basal layer of the capillary, according to most workers, plays a secondary role in the process of permeability [5, 6].

EXPERIMENTAL METHOD

Experiments were carried out on 15 albino rats. A burn was applied to the shaved surface of the skin by means of a special electric heater (12 animals). The area of the burn was 20% of the body surface and its depth corresponded to the IIIB degree, which was verified histologically. The animals were killed 15, 30, and 45 min after one-stage infliction of the burn and intravenous injection of a solution of peroxidase (type IV, in

Laboratory of Histochemistry and Autoradiography, Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 6, pp. 758-759, June, 1977. Original article submitted November 30, 1976.

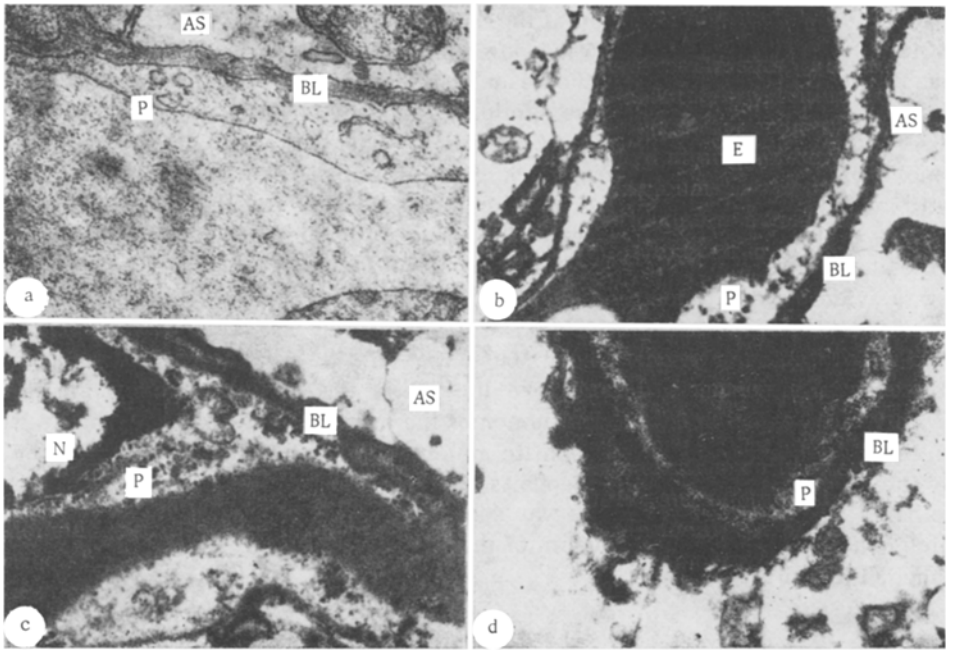

Fig. 1. Ultrastructural changes in capillary wall in cerebral cortex after thermal burn and injection of peroxidase: a) dilatation of pinocytotic vesicles of endothelial cell and swelling of basal layer. Karnovsky's reaction for peroxidase. Control of peroxidase activity with inactivation by sodium azide, 25,000 ×; b) intense staining of erythrocyte in lumen of capillary, reaction product contained in pinocytotic vesicles located in central part of endothelial cell. Osmiophilia of basal layer of capillary wall, 15 min after injection of peroxidase and burning, 20,000 ×; c) homogeneous electron-dense material in capillary lumen, in pinocytotic vesicles, moderate staining of basal layer, 30 min after injection of peroxidase and burning, 25,000 ×; d) intensive deposition of osmiophilic material in erythrocyte, in lumen of capillary, in cytoplasm of endothelial cell and, in particular, in basal layer, which actively branches and penetrates into cytoplasm of astrocyte surrounding capillary, 45 min after injection of peroxidase and burning, 30,000 ×. BL) Basal layer; N) nucleus; AS) astrocyte; P) pinocytotic vesicles; E) erythrocyte.

a dose of 10 mg/100 g body weight). Physiological saline with peroxidase was injected into three control animals, which were not burned.

The substrate for ultrahistochemical detection of peroxidase was 3,3-diaminobenzidine [7]. This dye gives a homogeneous reaction product of high contrast, and the precipitate formed does not react with the embedding medium or solution of contrasting substances. As a control of enzyme activity, material inactivated by heating was incubated and sodium azide was used, for it completely inhibits peroxidase activity [8] (Fig. 1a). Pieces of brain (cerebral cortex, area PA^{m} of the sensomotor cortex, layer V) were fixed in acrolein solution at pH 7.2. Frozen cryostat sections were cut, incubated, and postfixed in osmium solution, and then embedded in Araldite. Ultrathin sections were stained with lead and examined in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

In the control animals at various times after injection of peroxidase the reaction product could be observed in the lumen of the vessels. The blood cells were intensely stained. Inclusions of the reaction product for peroxidase appeared in the pinocytotic vesicles in the cytoplasm of the endothelial cells closer to the lumen of the capillary only when 30 min had elapsed after injection, whereas in the initial portions of the intercellular spaces it appeared after 45 min. The basal layer remained free. The cytoplasm of the pyramidal neurons contained a moderate number of Nissl's bodies and the endoplasmic reticulum was well developed. Solitary lysosomes and lysosome-like bodies were seen around the interphase nuclei. Axosomatic synapses were unchanged and the astrocytes and oligodendrocytes showed no special features.

On electron-microscopic investigation of the capillaries of the cerebral cortex of the animals in burn shock the following changes were found: 15 min after one-stage burning and injection of peroxidase the reaction product was deposited in the lumen of the vessels in the form of a dark homogeneous mass and granules. The pinocytotic vesicles also contained dense osmiophilic granules. The presence of dark osmiophilic material also was observed in the intercellular spaces and in the basal layer (Fig. 1b). The most characteristic feature of the morphological picture of burn shock after 30 min was the further and more intensive penetration of the reaction product (for peroxidase) in the cytoplasm of the endothelial cells, the arrangement of the pinocytotic vesicles containing dark, dense granules, around the nucleus, and their intrusion directly into the basal layer of the capillary. It must be emphasized that the lumen of most capillaries was no longer intensely stained. This was evidently due to removal of the reaction product from the blood stream and its entry directly not only into the components of the capillary, but also into tissues adjacent to it (Fig. 1c). The permeability of the capillary wall was increased 45 min after burning. This was shown by an abundance of pinocytotic vesicles filled with osmiophilic material, which were distributed throughout the cytoplasm of the endothelial cells; the reaction product could be seen both in the lumen of the intercellular space and in the basal part of the spaces between the endothelial cells. Osmiophilic material occupying the basal part of the spaces sometimes passed directly into the basal layer; dense deposits of reaction product could also be seen in the subendothelial space. The basal layer of the capillaries was somewhat widened and had the appearance of branched processes, which penetrated into the cytoplasm of the adjacent astrocytes and oligodendrocytes for a considerable distance (Fig. 1d).

LITERATURE CITED

1. A. A. Vishnevskii, M. I. Shraiber, Yu. D. Volynskii, et al., Abstracts of Proceedings of the First All-Union Conference on Thermal Burns [in Russian], Moscow (1972), pp. 48-52.
2. D. S. Sarkisov, B. V. Vtyurin, and V. P. Tumanov, in: Abstracts of Proceedings of the First All-Union Conference on Thermal Burns [in Russian], Moscow (1972), pp. 64-66.
3. V. P. Tumanov, Arkh. Pat., No. 11, 62 (1973).
4. V. A. Shakhlamov, Capillaries [in Russian], Moscow (1971), p. 62.
5. M. J. Karnovsky, J. Cell Biol., 35, 213 (1967).
6. H. W. Florey, Brit. Med. J., 2, 487 (1966).
7. R. C. Graham and M. J. Karnovsky, J. Histochem. Cytochem., 14, 291 (1966).
8. Y. Czaninski and A. M. Catesson, in: VII Congrés International de Microscopie Electronique, Vol. 1, Grenoble (1970), pp. 569-570.