METHODS

Modification of Injection Mapping of Vascular-Capillary Bed in Cerebral Nervous Tissue

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A simple original method of simultaneous staining of the cell elements on cerebral cortex and its vascular bed is developed. It is based on perfusion of polyvinyl acetate emulsion prepared on 2% paraformaldehyde solution with phosphate buffer at pH 7.4.

Key Words: *nervous tissue; blood vessel; brain*

The problem of blood supply to the brain under normal and pathological conditions is intensively studied, since the state of the cerebral vascular bed plays an important role in humoral and trophic regulation of CNS and in the development of ischemic or hypoxic damage to cerebral tissue [1-3]. However, in comparison with other vascular regions, the cerebral vascular-capillary system (CVCS) is poorly studied, which can be explained by its structural complexity and limited power of the routine methods [3,4,7].

Both injection and non-injection techniques are employed to study the cerebral hemocirculatory bed and organization of its circulatory system. The non-injection methods are based on impregnation of metal salts onto the vascular structures [4]. To a greater extent, the potency of these methods depend on strict composition of the histological reagents, use of only fresh samples, and individual experience of a researcher. Histochemical methods locate various subdivisions of the vascular bed according to activity of their enzymes: alkaline phosphatase (EC 3.1.3.1), Na^+,K^+-ATPase (EC 3.6.1.4), acetylcholinesterase (EC 3.1.1.7), butyrylcholinesterase (EC 3.1.1.8), and transferase (EC 2.3.1.6). The corresponding reactions produce optically dense deposit. Despite attraction of the histochemical methods, they have some inherent limitations. Among them are the limitation in the choice of fixatives and time of exposure (the samples should be fresh and treated immediately after isolation). The histochemical reactions are highly sensitive to incubation conditions. They need the control tests for specificity, which assess sensitivity to the specific and non-specific activators and inhibitors of enzymatic reactions. Finally, simultaneous use of alternative staining methods in one histological preparation is impossible. Moreover, the choice of investigation object and its species differences are critical for successful application of impregnation and other routine histochemical methods. The change of animal specie (rat, rabbit, cat, *etc*.) inevitably results in radical changes in the conditions needed to carry out histochemical reactions, the composition of reagents, and incubation protocol. These are the main reasons impeding wide use of these methods [4,5,8].

Injection methods based on filling of CVCS with dense optical agents lack the disadvantages of the non-injection technique. Therefore, we can say that further elaboration of the injections methods will be useful in the development of novel ideas on the structure of cerebral vascular system.

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Our original method of vascular bed mapping in the brain is based on filling of vasculature with water emulsion of polyvinyl acetate dye, which cannot penetrate the vascular wall.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino rats weighing 200-250 g (n=8). The rats were narcotized with nembutal (30-40 mg/kg) and fixed on a surgical table. The thoracic and abdominal cavities were open. Initially, the animals were perfused with 0.9% NaCl via the left ventricle for complete removal of the blood completely from the circulation system. Then perfusion was continued with polyvinyl acetate emulsion prepared on 2% solution of paraformaldehyde and phosphate buffer at 7.4. After perfusion, the rats were decapitated; the brain was isolated and postfixed in Carnoy fluid for 2 h at room temperature. The samples were embedded in paraffin [6]. Sections (10-20 μ) were mounted on glass, deparaffinized, and stained with

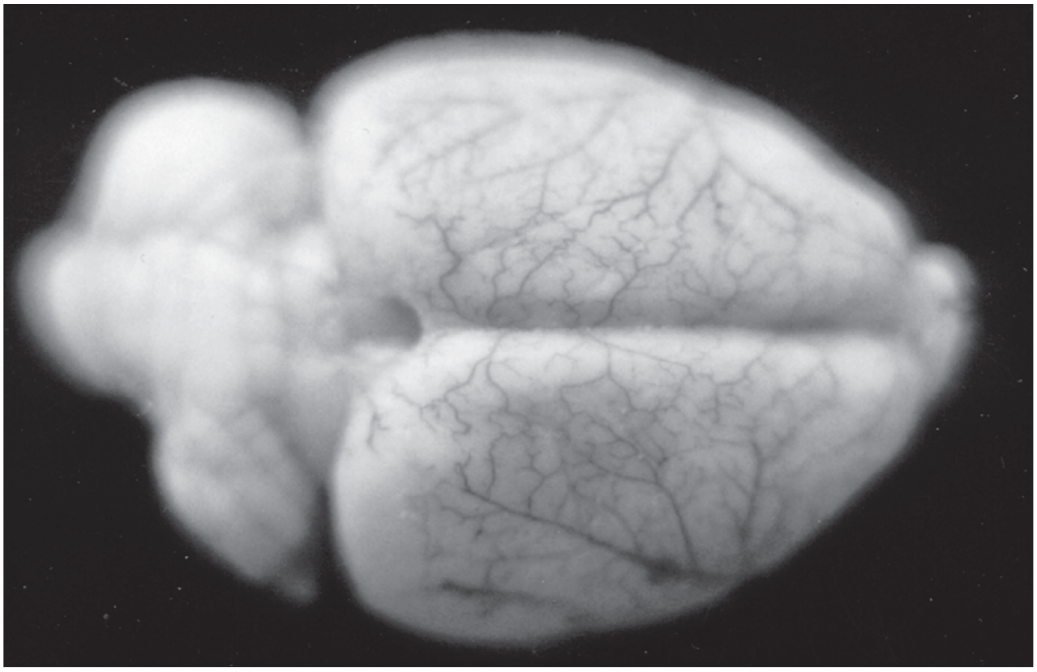

Fig. 1. Surface of rat brain after isolation from the cranium. The dark vascular bed is seen against the background of light cortex, ×4.

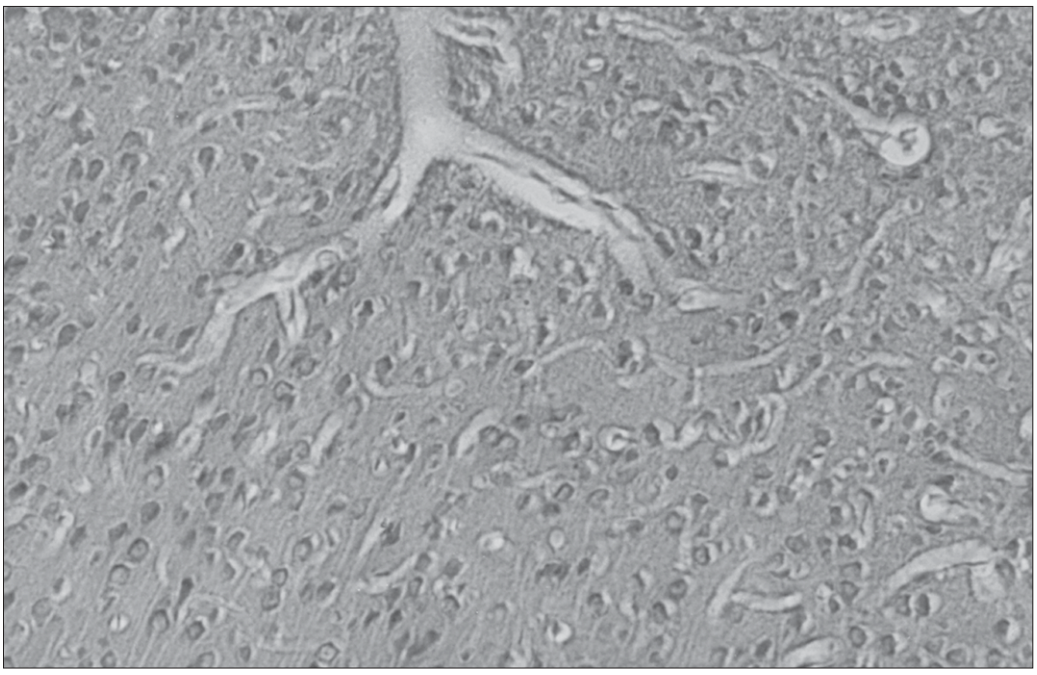

Fig. 2. Neurons and vascular-capillary network in rat brain, ×125.

a mixture of 2% water solutions of methyl green and pyronine, and then with 2% solution of bromocresol green prepared on phosphate buffer at pH 4.05. Then the preparations were dehydrated and embedded in Canada balsam.

RESULTS

After perfusion and fixation, blood vessels stained in dark color were clearly visible on the surface of isolated brain (Fig. 1). After deparaffinization and staining, blue-violet nuclei of neurons encompassed by a border of light cytoplasm and green neuropile and vessels of different calibers, whose walls had more dense color than their lumens, were seen on cerebral sections (Fig. 2). If the pia matter remained on cerebral sections, the arteries could be seen located in parallel to the brain surface, where the arterioles originated and penetrated the cortex at some aslant direction to its surface.

The proposed method was developed to reveal the vascular bed in cerebral nervous tissue. In addition, it can reveal and assess the state of various elements of the vascular system and the cell elements in the cortex, which is useful in the studies of pathological states of the brain related to the disturbances of cerebral circulation during hypertension crisis and stroke.

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