ELECTRON-MICROSCOPIC INVESTIGATION OF REMOVAL OF AUTOGENOUS RED BLOOD CELLS FROM THE SUBDURAL SPACE

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The inner layer of the dura and the arachnoid mater of the dog brain were studied in the electron microscope after injection of 0.2-0.5 ml autogenous blood into the subdural space. The arachnoid of the excretory canals and the structural elements of the inner layer of the dura (cells of the meningeal layer, the layer of collagen fibrils and microfibrils, the wall of the blood capillaries of the internal capillary network) form the morphological substrate of the CSF-blood barrier-I between the CSF and blood in the capillaries of the internal capillary network of the dura. Red blood cells were found to penetrate from the subdural space into the substance of the dura, where they concentrated around the capillaries of the internal capillary network but did not actually penetrate into the arachnoid.

KEY WORDS: subdural space; excretory canals; outer arachnoid endothelial layer of the arachnoid mater; CSF-blood barrier-I.

The main path for CSF drainage from the subarachnoid space is through the arachnoid mater in the region of the excretory canals of the leptomeninges into the subdural space, from which the CSF (subdural fluid) enters the blood capillaries of the inner capillary network of the dura mater [1-6]. Autogenous red blood cells, injected experimentally into the subarachnoid space, penetrate with the outflowing CSF into the blood capillaries of the inner capillary network of the dura mater [8-10]. However, the mechanism whereby red blood cells penetrate from the subdural space into the dura mater is not quite clear.

The object of this investigation was to study the ultrastructure of formations bounding the subdural space on the outside (the inner layer of the dura) and inside (the arachnoid) when autogenous blood is injected into the subdural space. Since the ultrastructure of the blood capillaries of the inner capillary network has not been adequately described in the literature [12, 13], their normal ultrastructure also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 15 mongrel dogs weighing 6-10 kg under morphine-hexobarbital anesthesia (0.3 ml 1% morphine/kg body weight, 0.05 g hexobarbital/kg body weight). The skull was trephined unilaterally in the parietotemporal region, the dura was punctured carefully with a fine needle, and 0.3-0.5 ml autogenous blood taken from a peripheral vein was injected slowly into the subdural space. At various times (15, 30, and 60 min) after injection of the blood the outer surface of the dura was irrigated with 2.5% glutaraldehyde solution in phosphate buffer. The dogs were then killed by electrocution and pieces of the dura and arachnoid measuring 2×2 mm^2 were removed and immediately immersed in 2.5% glutaraldehyde solution for 1 h, and then postfixed in 1% OsO_4 solution buffered by Millonig's method for 1.5 h. The specimens were dehydrated and embedded in the usual way for electron-microscopic examination. Sections were cut on a Reichert ultramicrotome.

Series of sections were attached to blind-grids and stained with uranyl acetate solution and by Reynolds' method. Electron micrographs were obtained on electron microscopes of the JEM-5 and Jeol-100B types with magnifications of between 3000 and 40,000.

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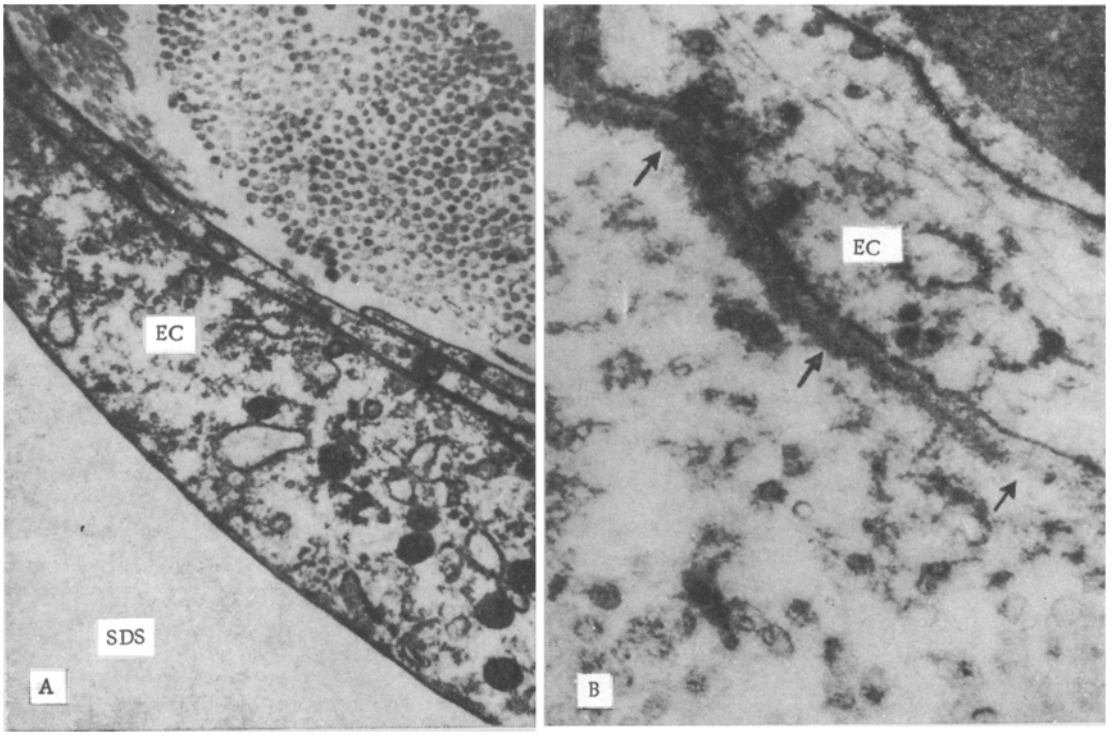

Fig. 1. Normal ultrastructure of dura and blood capillary of inner network. A) Endothelial cells (EC) of inner layer of dura lining the subdural space (SDS). Well developed bundles of collagen fibrils above (15,000×). B) Area of EC of blood capillary. Concentration of microfibrils can be seen externally to it, in the form of an osmiophilic line running parallel to the plasma membrane of the cell (arrows). Collagen fibrils and bundles of microfibrils below. Red blood cell in lumen of capillary in top right corner (40,000×).

EXPERIMENTAL RESULTS

Collagen fibrils and fibrocytes were found in the dura. On the side of the subdural space the dura was lined by flattened cells [12, 13].

The surface of the dura facing the subdural space was found to be lined by a layer of flattened endothelial cells arranged in one or two rows. The cell bodies at the periphery changed into gradually tapering processes. Bundles of collagen fibrils arranged in the plane of cross-section through the dura at an angle to each other bordered directly on this layer of cells (Fig. 1A). Bundles of microfibrils, concentrations of elastin, and fibroblasts were seen among the collagen fibrils.

The walls of blood capillaries, of different diameters, in the inner capillary network consist of endothelial cells arranged in one row. The cells are flattened and their nuclei irregularly oval in shape. The cell bodies gradually taper toward the periphery. Intercellular junctions consist of spots and zones of obliteration. Small mitochondria, structures of the smooth and rough endoplasmic reticulum, and filaments are present in the cytoplasm [9, 11]. Externally to the endothelial layer the ground substance contains concentrations of microfibrils in the form of an osmiophilic line up to 25 nm thick, which runs parallel to the plasma membranes of the endothelial cells and at a distance from them of 20-25 nm (the subendothelial zone), and it borders on bundles of collagen fibrils and fibroblasts (Fig. 1B).

According to some workers [1, 2, 4, 5] resorption of subdural fluid from the subdural space takes place by the blood capillaries of the inner capillary network of the dura. The ultrastructural features of the blood capillaries of the dura described above must therefore be considered, along with the tissue structures of the dura, to be the morphological equivalent of the apparatus concerned with resorption of subdural fluid initially into the capillary, and later into the venous system of the dura.

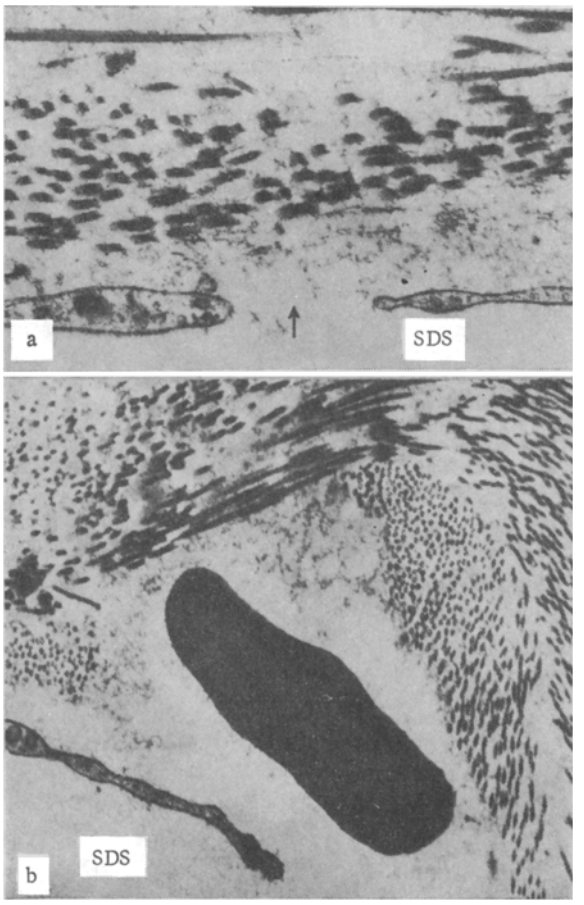

Fig. 2. Inner endothelial layer of dura 30 min after injection of autogenous blood into subdural space. A) Widened intercellular space (arrow) on boundary of peripheral areas of neighboring endothelial cells. Bundles of collagen fibrils and microfibrils loosely arranged (15,000×); B) red blood cell at moment of penetration into inner layer of dura. Part of collagen fibrils and microfibrils (10,000×). SDS) Subdural space.

The arachnoid in the region of the excretory canals, cells of the inner endothelial layer of the dura, bands of collagen fibrils and microfibrils, and the walls of the blood capillaries of the inner capillary network of the dura, taken as a whole, are the morphological substrate of the CSF-blood barrier (CSF BB-I) between the CSF and blood in the capillaries of the inner capillary network [8].

Autogenous red blood cells injected into the subdural space are found near the endothelial cells of the inner layer of the dura. Deformed red blood cells are also found on the boundary with the peripheral parts of the endothelial cells, the spaces between which are frequently widened (Fig. 2A); the bundles of collagen fibrils and microfibrils of the inner layer of the dura are loosely arranged (Fig. 2B).

Red blood cells are distributed in the substance of the inner layer of the dura among bundles of collagen fibrils and microfibrils and they border on fibroblasts and endothelial cells of the blood capillaries. Red blood cells surround the latter on all sides and are in close contact with the outer surface of the endothelial cells. Microfibrils lying next to the endothelial cells on the outer side are more loosely arranged than normally and occur only as isolated groups (Fig. 3A).

After injection of autogenous blood into the subdural space, no red cells are seen in the

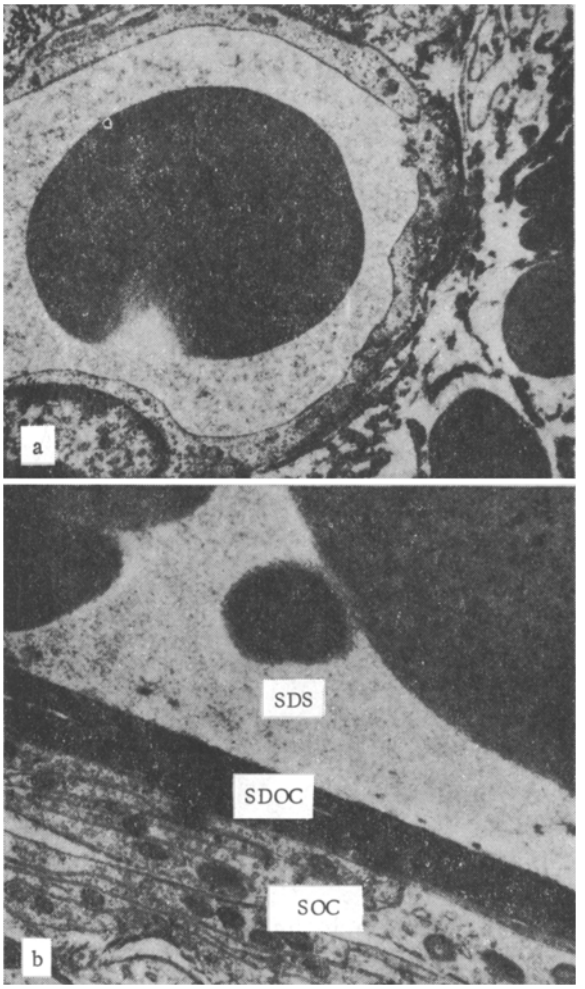

Fig. 3. Blood capillary of inner capillary network of dura and arachnoid of excretory canal 40 min after injection of autogenous blood into subdural space (10,000×). A) Red blood cells lie outside endothelial cells of capillary (right). Bundles of microfibrils and collagen fibrils surrounding capillary are loosely arranged. Lumen of capillary occupied by red blood cell; B) subdural space (SDS) packed with red cells and loose masses of delicately osmiophilic material. Bottom part of figure shows outer arachnoid endothelial layer of arachnoid mater: sublayer of desquamated osmiophilic cells (SDOC) and sublayer of osmiophobic cells (SOC).

substance of the arachnoid, by contrast with what is observed after injection of autogenous blood into the subarachnoid space of experimental animals [7, 8]. The cells in the sublayer of desquamated osmiophilic cells of the outer arachnoid endothelial layer of the arachnoid mater are in close contact with one another, whereas under normal conditions the intercellular spaces are of considerable size [6] (Fig. 3B).

After injection of autogenous blood into the subdural space the red blood cells thus penetrate into the inner layer of the dura, where they are found in large numbers around the blood capillaries of the inner capillary network; red cells from the subdural space do not penetrate into the arachnoid or into the subarachnoid space.

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STRUCTURAL AND IMMUNOMORPHOLOGICAL CHARACTERISTICS OF THE HUMAN THYMUS DURING EMBRYONIC DEVELOPMENT

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The thymus of 100 human fetuses was studied between the 4th and 34th weeks of intrauterine development by means of histological, histochemical, immunomorphological, and electron-microscopic methods. Development of the organ from the standpoint of development of the functional system is described. The anlage of the thymus can be detected at the 5th week of fetal development; it reflects the properties of the epithelium of a foregut organ. By the 7th-8th week differentiation of the reticuloendothelium and population of the organ with lymphocytes are beginning to take place and antigenic specificity is found on the surface of the lymphocytes. The zone of growth of the reticuloendothelium of the thymus, the significance of Hassall's corpuscles, the appearance of two subpopulations of T lymphocytes, and their quantitative changes are described. In the period from the 11th until the 34th week of fetal development the number of T lymphocytes forming rosettes with sheep's red blood cells virtually does not change (70-90%), whereas the number of T lymphocytes forming rosettes with autogenous red cells increases during this period from 23 to 70%.

KEY WORDS: human fetal thymus; lymphocyte; rosette-forming cells.

After the discovery of cellular immunity and identification of the T system of cells among lymphocytes [14] and determination of the role of the thymus, the central organ of lymphopoiesis, in immunologic reactions [2, 7, 12], the morphology and character of changes in the epithelial basis of the thymus during prenatal human development still remain inadequately studied. Since the work of Galustyan [1], the basic question of the nature of this

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