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CHANGES IN THE ULTRASTRUCTURE OF COMPONENTS OF THE BLOOD – TESTIS BARRIER IN CIRCULATORY HYPOXIA

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Changes in the ultrastructure of the blood–testis barrier in rats 30 and 60 min and 1 and 30 days after ligation of the testicular artery were studied by electron microscopy. The results showed that blocking of the blood flow to the testis causes rapidly progressive changes in all components of the blood–testis barrier. Micropinocytosis and destructive changes increase in the cytoplasm of the endotheliocytes of the capillaries, ending in microclasmatosis. The tunica propria of the seminiferous tubules is highly sensitive to ischemia. It becomes thickened, the nuclei and cytoplasmic organoids of its cellular components are deformed, and folding and infiltration of the basement membrane increase. Vacuolation of the cytoplasm of the sustentocytes is accompanied by destruction of the cell membrane and by separation of the sustentocytes from the tunica propria of the tubules.

KEY WORDS: blood–testis barrier; ischemia; ultrastructure.

The blood–testis barrier is formed by the wall of the blood and lymphatic capillaries, the tunica propria of the seminiferous tubules, sustentocytes, and interstitial tissue [1, 2, 5, 6, 11]. It was shown previously [4, 10, 11, 14] that this endothelial lining of the blood capillaries of the testis is continuous and contains microvilli. According to some observations [7-9, 12, 13], the tunica propria of the seminiferous tubules in rats consists of two cellular (myoid cells and fibroblasts) and two noncellular (inner and outer) layers of complex construction. Sustentocytes, which form specialized connections by their cell membranes [14], are organ-specific components of this barrier.

Light-optical data on the harmful influence of ischemia of the testis on the state of its generative components [5] are present in the special literature, but ultrastructural changes in the components of the blood–testis barrier after disturbance of the blood supply to the testis have been inadequately studied.

The object of this investigation was to study the character of the submicroscopic changes in the components of this barrier in the testes of rats under conditions of circulatory hypoxia produced by ligation of the testicular artery at the point where it arises from the abdominal aorta.

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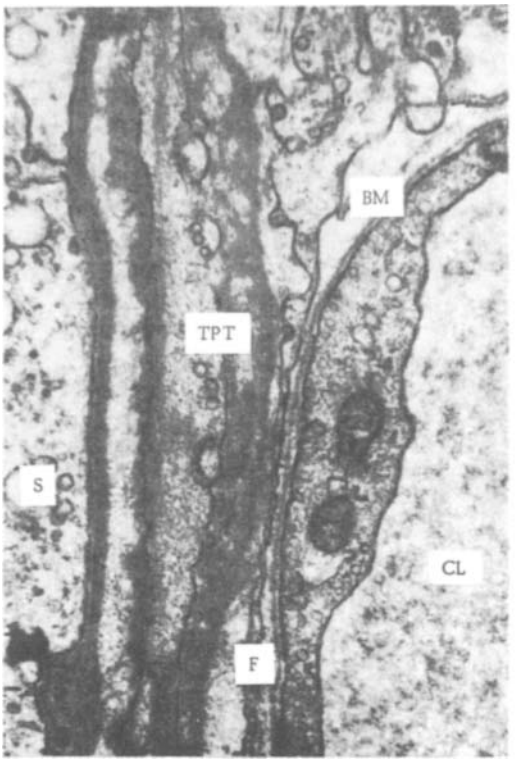

Fig. 1

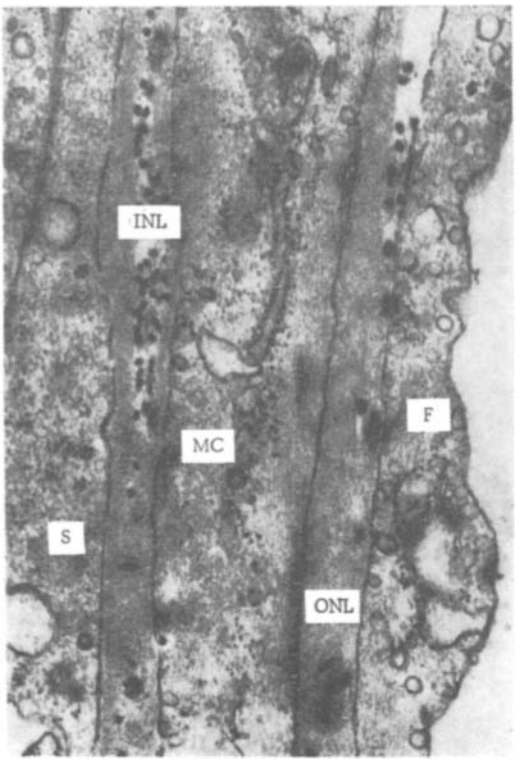

Fig. 2

Fig. 1. Components of normal blood–testis barrier of a rat. CL) Capillary lumen; BM) basement membrane of capillary; F) fibroblast; TPT) tunica propria of tubule; S) sustentocyte. 10,000×.

Fig. 2. Ultrastructure of tubular component of blood–testis barrier after 30 min of ischemia in a rat. F) Fibroblast; ONL) outer noncellular layer; MC) myoid cell; INL) inner noncellular layer; S) sustentocyte. 20,000×.

EXPERIMENTAL METHODS

Experiments were carried out on 12 rats. Pieces of the testis were fixed in 1% osmium tetroxide solution, dehydrated in a series of alcohols, and embedded in a mixture of Epon and Araldite. Ultrathin sections cut on the Tesla BS-490A and UMTP-2 microtomes were stained by Reynolds' method and examined in the UEMV-100V electron microscope.

EXPERIMENTAL RESULTS

Swelling of the nuclei and cytoplasmic matrix of the endotheliocytes of the blood capillaries of the testis took place 30-60 min after ligation of the testicular artery. Numerous microvilli, the pinhead expansions of which projected freely into the lumen of the vessel, appeared on the inner cell membrane of the endothelial cells. The mitochondria of the endotheliocytes were increased in volume, their fine-grained matrix was vacuolated, and the orderly arrangement of the cristae was disturbed in places. The tubules of the cytoplasmic reticulum were dilated and their cross sections contained many free ribosomes. The lamellar complex was represented mainly by large vesicles with pale contents. The basement membrane of the blood capillaries was reduced in thickness in some places.

The nuclei of the myoid cells of the tunica propria of the seminiferous tubules were irregular in shape by this time. Diffuse, small osmiophilic granules appeared in the nucleoplasm. The swollen cytoplasmic structures of the perikaryon of these cells lost the clarity of their outlines, and multivesicular bodies formed by fusion of microvesicles appeared among them. The matrix of the mitochondria became homogenized, the integrity of their outer membrane was disturbed in places, and their cristae were in a state of fragmentation. The Golgi lamellar complex was widened and its vesicles merged into large sacs with translucent contents.

Similar changes were observed at this stage of the experiment in the cytoplasm of the sustentocytes. The integrity of the connections between them, like those between the myoid cells, was undisturbed.

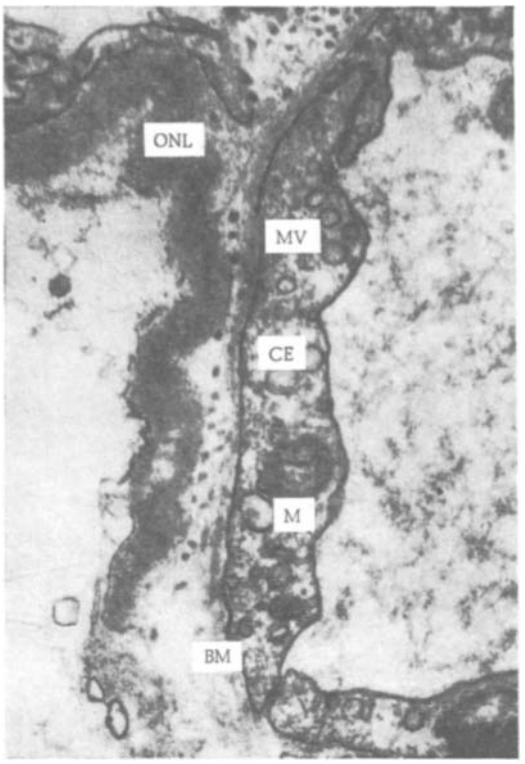

Fig. 3. State of components of blood–testis barrier of a rat on 30th day of acute ischemia. CE) Cytoplasm of endotheliocyte; MV) micropinocytotic vesicles; M) mitochondrion; BM) basement membrane of capillary; ONL) outer noncellular layer of tunica propria of seminiferous tubule. 18,000×.

The basement membranes of the inner and outer noncellular layers of the tunica propria of the seminiferous tubules were considerably swollen compared with normal (Fig. 1), the clarity of their outlines was disturbed, and their density reduced (Fig. 2).

The nucleoplasm of the endotheliocytes of the testicular blood capillaries 24 h after interruption of the blood flow along the testicular artery had low electron density, focal accumulation of chromatin granules was observed in some places, and the nucleolus was not differentiated. The cytoplasm of the endotheliocytes showed intensive hydrophilic swelling, pallor, and vacuolation, and the number and size of the micropinocytotic vesicles were increased. The structure of the mitochondria was considerably disturbed, with diffuse homogenization of the matrix and fragmentation of the cristae. Destruction of the membranes of the components of the lamellar complex was observed. On the surface of the endotheliocytes facing the lumen evaginations of cytoplasm of different shapes and sizes were formed; they had a wide base or they projected into the narrow lumen of the capillaries on thin pedicles. In other capillaries small areas of cytoplasm of the endotheliocytes were detached from the basement membrane, which was left bare on the side of the lumen.

The cytoplasm of the myoid cells by this time had become vacuolated. Many mitochondria had lost their integrated structure. The integrity of the cytoplasmic membrane of these cells lining the lumen was disturbed in places, with the appearance of invaginations on it, alternating with swellings.

The basement membranes of the tunica propria of the tubules were partially fragmented and the arrangement of the collagen fibrils in them was haphazard. The cytoplasmic matrix of the sustentocytes was clear and the number of cisterns in it was reduced, but they were appreciably widened. The plasma membranes at the specialized junctions between adjacent cells were parallel in arrangement and the thread-like filaments in the peripheral zones of the cytoplasm were reduced.

The blood capillaries 30 days after ligation of the testicular artery were deformed and their lumens narrowed. The nuclei of the endotheliocytes had a very pale karyoplasm. They were shrunken, irregular in shape, and their chromatin was distributed at the periphery. The inner plasmalemma of the endotheliocytes contained deep invaginations. The number of micropinocytotic vesicles was increased and they were concentrated chiefly near the inner and outer cell membranes. The vesicles were fused in some places to form multivesicular bodies. The basement membrane of the blood capillaries was partly fragmented, its fibrillary structure was disturbed, and the layers were not differentiated. By this stage of the experiment, only the outer noncellular layer remained of the tunica propria in some seminiferous tubules. It was twice as wide as normally, and twisted into loops or fragmented in some places (Fig. 3). Components of the inner noncellular layer – the basement membrane of the spermatogenic epithelium – and also the myoid cells showed similar changes. The cytoplasm of the latter was pale and vacuolated, its organelles showed destructive changes, and they were consequently difficult to identify.

In these experiments acute arterial insufficiency of the testis led to progressive ultrastructural changes in the components of its blood–testis barrier. In particular, micropinocytosis increased in the cytoplasm of the capillary endotheliocytes and the microvilli became more numerous, thus compensating to some extent for the disturbed transcapillary transport. The pores and fenestrations characteristic of blood capillaries of endocrine organs were not found in the rat testis either normally or after obstruction to the blood flow [1]. Compared with the capillaries, the tunica propria of the convoluted seminiferous tubules, which is a complex and stratified structure, was more sensitive to ischemia. This was shown by its thickening, the deformation of the nuclei and cytoplasmic organoids of the cellular components, and the folding and infiltration of the basement membranes. Similar electron-microscopic changes have also been found in the substrate of the blood–testis barrier of rats with posttraumatic orchitis [2].

Besides vacuolation of the cytoplasm and accumulation of lipid drops, dilatation and fragmentation of the cisterns of the endoplasmic reticulum, and destruction of the cell membranes, the sustentocytes, which perform a supporting function and are indirectly responsible for maintaining metabolism of the spermatogenic epithelium [9] and, at the same time, protecting it against immunocompetent blood cells and the action of antibodies [5, 6], were separated from the tunica propria of the tubules.

In previous investigations at the light-optical level the writers showed that the spermatogenic epithelium is exceptionally highly sensitive to harmful factors such as circulatory hypoxia [3, 5]. The profound changes which arise as a result of such an injury in the structures of the blood–testis barrier and revealed by electron-microscopic study are described in this paper.

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