ULTRASTRUCTURAL CHANGES IN SPERMATOGENIC EPITHELIUM AND CHIEF COMPONENTS OF BLOOD – TESTIS BARRIER IN SEXUALLY MATURE RATS AFTER REPEATED INJECTION OF ESTRADIOL DIPROPIONATE

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An electron-microscopic investigation was made of the testes of Wistar rats receiving repeated injections of 0.1% estradiol dipropionate solution in doses of 0.3 mg once a week for 10 weeks. Administration of the hormone led to an initial focal (after 2-4 weeks) and subsequent diffuse inhibition of spermatogenesis (after 6 weeks), accompanied by the development of destructive changes in the cells of the spermatogenic epithelium, which was reversible in character, so that spermatogenesis was restored 2 months later. The development of pathological changes in the spermatogenic epithelium was accompanied by disturbance of the ultrastructure of the chief components of the blood–testis barrier.

KEY WORDS: blood–testis barrier; estradiol dipropionate; spermatogenic epithelium.

Reversible disturbance of spermatogenesis takes place in sexually mature rats after repeated injections of estrogens as a result of inhibition of the secretion of pituitary gonadotropic hormones [2, 3, 8, 9]. There is evidence in the literature of the development of pathological changes in the testes of "estrogenized" animals that are accompanied by a disturbance of permeability of the blood–testis barrier (BTB) [1-3]. The chief structural components of the BTB are the Sertoli cells and myoid cells of the tunica propria of the seminiferous tubules [4].

No data could be found in the literature from which an idea could be obtained of the ultrastructural changes in components of the BTB and spermatogenic epithelium arising during disturbances of spermatogenesis in animals receiving estrogens and its recovery after the cessation of injections of the hormone. The present investigation was carried out to obtain such data.

EXPERIMENTAL METHOD

Experiments were carried out on 56 male Wistar rats weighing 190-240 g. Fifty animals each received injections of 0.3 mg of a 0.1% solution of estradiol dipropionate once a week for a total of 10 injections. The rats were killed every 2 weeks after the beginning of the experiment, and 2 months after the last injection. The weight of the animals and of their spleen, pituitary, prostate, and seminal vesicles was determined. For light microscopy the testes were fixed in Carnoy's or Bouin's fluid and embedded in paraffin wax. The sections were stained with hematoxylin–eosin. Pieces of the seminiferous tubules 1-1.5-mm long were excised from the removed testes for electron microscopy and placed in a 2.5% solution of glutaraldehyde in cacodylate buffer for 1.5 h at 4° C. After dehydration and treatment in propylene oxide the material was embedded in Epon–Araldite mixture. Sections were cut on the KB ultratome and stained with uranyl acetate and lead solution by Millonig's method [6]. The preparations were examined in the Tesla

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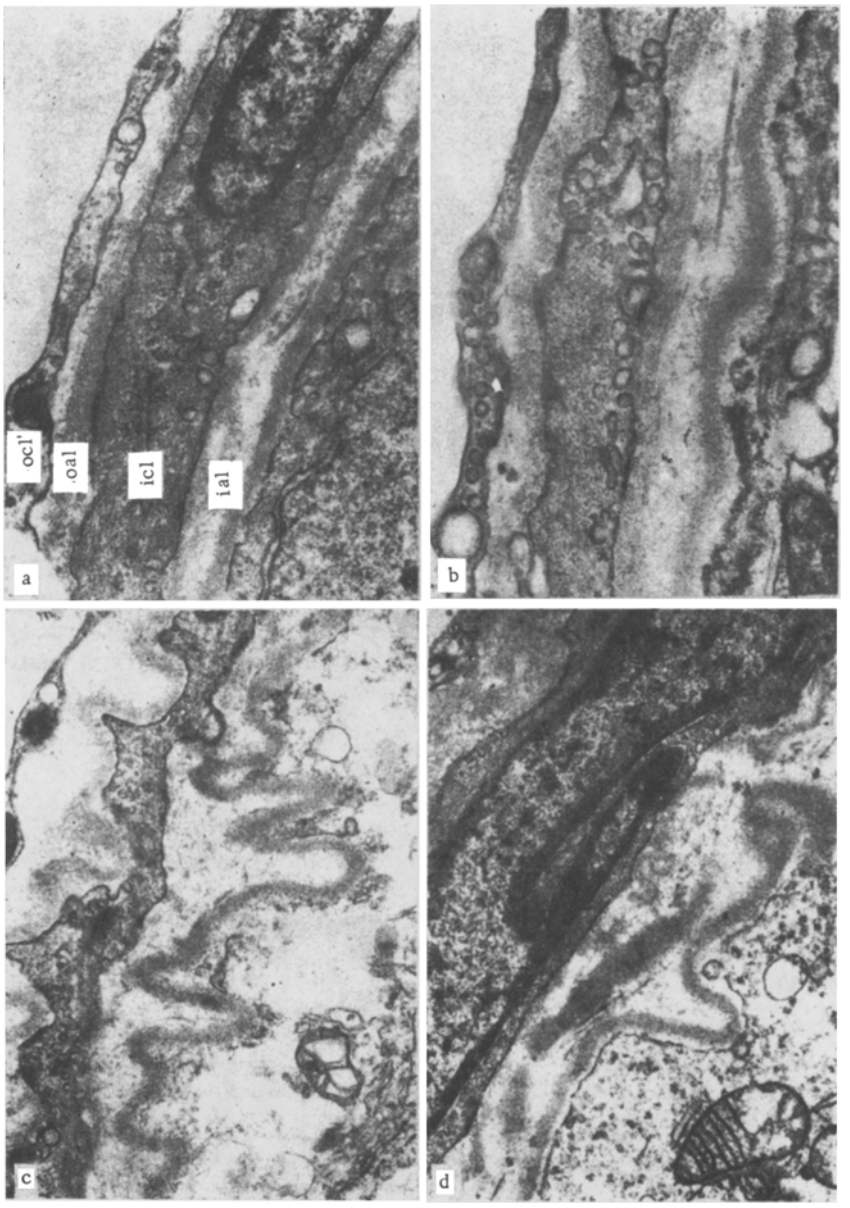

Fig. 1. Tunica propria of seminiferous tubules of intact rats and of rats receiving repeated injections of estradiol dipropionate: a) tunica propria of seminiferous tubule of intact rat (ocl – outer cell layer; oal – outer acellular layer; icl – inner cell or myoid layer; ial – inner acellular layer) (22,000 ×); b) slight folding of basement membranes of acellular layers of tunica propria of seminiferous tubules after administration of 0.6-1.2 mg hormone to animals (22,000 ×); c) hillock-like outgrowth of cytoplasm of inner cell layer, considerable folding of basement membranes of acellular layers of tunica propria of seminiferous tubules following administration of 1.8 mg hormone (30,000 ×); d) altered shape of nucleus of acellular layer of tunica propria of seminiferous tubule after injection of 2.4-3 mg hormone into animals (22,000 ×).

BS-516 microscope.

EXPERIMENTAL RESULTS

In rats receiving 10 injections of estradiol dipropionate (3 mg of the hormone) the body weight was reduced (179 g); the weight of the testes (187 mg), prostate (18 mg), and seminal vesicles (80 mg) was considerably reduced; the weight of the pituitary gland (17 mg) was increased compared with the control animals (217 g, 1350, 254, 1008, and 9 mg, respectively). The gradual decrease in weight of the testes was

accompanied by changes in their morphological structure and by an increase in the severity of the morphological changes in the tubules.

In most seminiferous tubules no considerable morphological changes were observed 2-4 weeks after the beginning of the experiment (0.6-1.2 mg estradiol dipropionate), although pycnosis of the cell nuclei of the spermatogenic epithelium was found in some tubules, empty spaces were formed in the seminiferous tubules, and the number of layers of the germinal epithelium was reduced. The interstitial cells of these animals lost their specific structure.

Six weeks after administration of estradiol dipropionate (1.8 mg hormone), the focal destruction was replaced by a diffuse disturbance of spermatogenesis in all tubules: the tubules became star-shaped or elongated, disorganization of the spermatogenic epithelium with desquamation of the sex cells and the formation of polynuclear spermatids was observed, and foci of total destruction of the tubules were seen.

Eight weeks after the beginning of the experiment (2.4 mg hormone) the tubules remained modified in shape, many degenerating cells were observed, and extremely few spermatids were present.

After 10 weeks spermatogenesis was halted at the stage of secondary spermatocytes.

Two months after the end of injections of the hormone the body weight of the animals was increased (251 g); the weight of the testes (1700 mg), prostate (200 mg), and seminal vesicles (1000 mg) was increased; the weight of the pituitary (10 mg) was reduced; the normal structure of the testes and spermatogenesis were restored.

Electron-microscopic investigation of the testes of the control rats revealed a four-layered structure of the membrane of the seminiferous tubules, all the layers of which were strictly parallel to one another (Fig. 1a). The acellular layers consisted of basement membranes and regularly arranged collagen fibrils. Cells of the inner cell layer or myeloid layer were epithelioidin arrangement, and open or closed (more commonly) communications could be seen between them. The nuclei of these cells were elongated, and contractile filaments were present at the periphery of the cytoplasm. The mitochondria were found or oval and two types of endoplasmic reticulum were seen. The outer cell layer consisted of typical fibroblasts with no contractile filaments in their cytoplasm.

The nuclei of the Sertoli cells located in the tunica propria of the seminiferous tubules had characteristic indentations and electron-dense nucleoli. The cytoplasm of the Sertoli cells contained round, oblong, or oval mitochondria of various sizes, with tubular cristae and numerous ribosomes and polysomes; residual particles and particles of inclusions could be seen.

The spermatogonia also were located on the basement membrane. Their nuclei were oval and their mitochondria had parallel cristae.

Spermatocytes were characterized by synaptonemal complexes or lateral bands of future synaptonemal complexes.

The spermatids were easily distinguishable from the remaining cells of the spermatogenic epithelium by the presence of an acrosome and axial complex.

After injection of 0.6-1.2 mg estradiol dipropionate into the rats slight folding of the acellular layers of the tunica propria was found in the tubules, and in some cases the nuclei of the Sertoli cells were seen to be fragmented (Fig. 1b). After injection of 1.8 mg hormone into the animals marked changes were observed both in the tunica propria of the tubules and in the spermatogenic epithelium and Sertoli cells (Fig. 1c). Disorganization of collagen fibrils was seen in the tunica propria of the seminiferous tubules, and folding of the basement membranes of the acellular layers was observed. The cells of the spermatogenic epithelium were separated from the basement membrane by an amorphous space. Destroyed axial complexes of the tails were found in the newly formed spermatids.

Injection of 2.4-3 mg estradiol dipropionate into the animals led to further changes in the cell layers of the tunica propria of the tubules and their nuclei became triangular or lobate (Fig. 1d). The inner cell layer in certain tubules consisted of two rows of cells. Deep invaginations were found in the spermatogenic epithelium and Sertoli cells, and the formation of binuclear spermatids with a single acrosome also was observed.

Two months after the end of injections of the hormone the folding of the basement membranes disappeared, the cell layers acquired their typical form, and the spermatogenic epithelium and Sertoli cells had a normal ultrastructure.

Injection of estradiol dipropionate into experimental animals in large doses thus caused thickening of the basement membranes, together with a change in shape of the nuclei of the cell layers, disorganization of the collagen fibers, and destruction of the germinal epithelium and Sertoli cells.

Changes of this sort, namely, the appearance of folding of the basement membranes and separation of the germinal epithelium from the membrane of the tubules, were observed by Sabo et al. [5] during the investigation of cryptorchid testes. In the opinion of these workers, folding of the acellular layers of the membrane is the result of compression of the germinal epithelium, in which destructive changes were observed.

The functions of the BTB in estrogenized animals were studied by Davydova [1, 3], who showed that injection of estradiol dipropionate into rats leads to increased permeability of the BTB to intravenously injected rivanol, and that an increase in the dose of injected hormone correlates with changes in the permeability of the BTB. Analysis of these findings led to the suggestion that the BTB participates in the maintenance of hormonal homeostasis.

The ability of exogenous estrogens to evoke inhibition of spermatogenesis by inhibiting the gonadotropic activity of the pituitary has been established [8].

At the same time it has been shown that administration of estrogen starting from the first day after birth does not prevent, but merely delays the formation of the BTB [9]. Johnson [5] also found that absence of gonadotropins in adult rats as a result of hypophysectomy does not alter the permeability of BTB to intratesticularly injected acriflavine.

The results of the present investigation, showing that substantial but reversible ultrastructural changes take place in the components of the BTB in estrogenized animals to a degree that correlates with the destructive changes in the spermatogenic spithelium, can be regarded as confirming Davydova's data on changes in BTB function in animals with disturbance of the hormonal status; they support the hypothesis that the barrier mechanism of the testis participates in the regulation of hormonal homeostasis [3].

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