EFFECT OF VITAMIN A ON THE TESTES

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KEY WORDS: spermatogenesis; vitamin A; cryptorchidism.

Vitamin A (retinol) and its synthetic preparations are widely used in medical and agricultural practice. Retinol is essential for the metabolism, growth, and development of the growing organism, it supports the activity of the organ of vision, the normal state of function of the epithelial tissues, and the formation of the bony skeleton, and it also increases the resistance of the organism to diseases [1, 2, 8].

Retinol plays a particularly important role in reproductive function. The gonads require several times more vitamin A than other organs [6, 7, 9, 10, 12]. At the same time, prolonged administration of retinol can cause poisoning in the form of hypervitaminosis A [3-5]. The creation of new and less toxic vitamin A preparations and the study of the effects on the body are thus urgent tasks.

Preparations of this kind include water-soluble retinoic acid. The writers showed previously that administration of retinoic acid causes changes in proliferative activity of the spermatogonia of the convoluted seminiferous tubules of the testis in animals and changes in the histophysiological state of the interstitial cells.

The aim of this investigation was to study the effect of water-soluble retinoic acid on the testes during the development of pathological changes in them as a result of exposure to unfavorable conditions due to experimental cryptorchidism.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 3-4 months (n = 21). The animals were divided into three groups, with 7 in each group. Unilateral cryptorchidism was produced in all the animals by transposition of the testis into the peritoneal cavity, where it was sutured to the peritoneum [11]. Animals of group 1 served as controls and were given injections of physiological saline (the solvent for retinoic acid) in a total dose of 0.3 ml. The experimental animals received a 1% solution of water-soluble retinoic acid: in group 2 in a total dose of 0.1 ml, in group 3 in a dose of 0.3 ml. The preparation was injected intraperitoneally. On the 8th day after the operation the testes were removed from the animals, weighed, and fixed in Bouin's fluid and 10% buffered formalin, and then embedded in paraffin wax. Sections 6 μ thick were stained with hematoxylin and eosin. The number of spermatocytes and spermatogonia and the number of them in a state of division, were counted in the seminiferous tubules in the sections, and the number of interstitial cells was counted between the tubules. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

After transplantation of the testes into the peritoneal cavity their weight decreased in the course of the experiment (by 6.8%). The microscopic picture of the structure of the convoluted seminiferous tubules changed considerably: Their wall became thin and their lumen wide. Atrophy of the spermatogenic epithelium took place in the tubules on account of degeneration of its cells. Mature sex cells disappeared completely from the tubules, indicating loss of the spermatogenic function of the testis. In most tubules there were no spermatids. Nevertheless, large round structures ("sperm balls"), with many, often pycnotic, nuclei or fragments of them, and with intensely stained cytoplasm, could be seen constantly in the tubules. They could be seen to be formed by fusion of spermatids in the spermatogenic epithelium followed by desquamation into the lumen of the tubules (Fig. 1).

Spermatocytes were not found in all tubules. Their total number in the testis was 27% lower than in intact animals. Degenerating spermatocytes were observed, with homogeneous, overstained cytoplasm. Having

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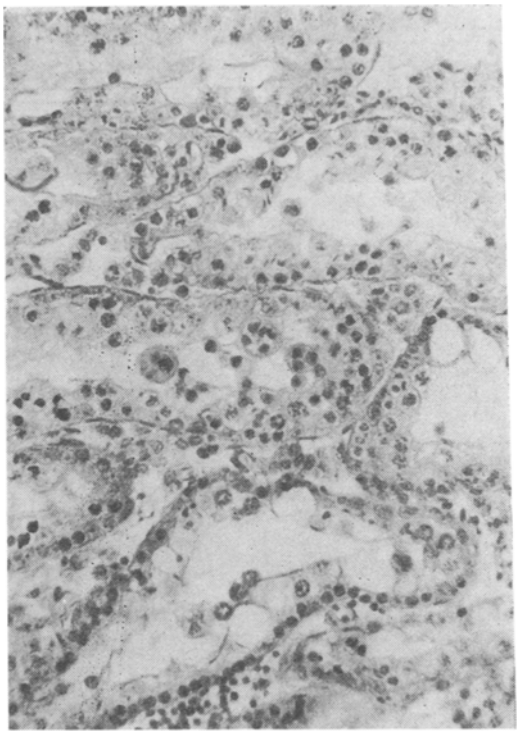

Fig. 1. Degeneration of spermatogenic epithelial cells of seminiferous tubules. Formation of sperm balls. Here and in Fig. 2: hematoxylin and eosin, magnification 6.3 × 25.

lost their connection with the supporting cells they fell into the lumen of the tubules, where their nuclear apparatus disappeared through lysis. Round spaces were left in the epithelium in place of the dying spermatocytes.

Spermatogonia of types A and B still remained in all tubules, and many of them were in a state of mitotic division. However, the number of dividing spermatogonia in this case was 14% less than in intact testes. The presence of spermatogonia in the tubules, of which type A is represented by stem cells in the spermatogenic epithelium, indicates the possibility that spermatogenesis may recover in the testis under suitable conditions. Supporting cells (sustentacular cells) also were preserved in the tubules. They lost some of their cytoplasm, which was shed into the lumen of the tubules with degenerating spermatids, to form a membrane around the sperm balls thus formed. Meanwhile, the residual parts of the spermatogenic epithelium, including spermatogonia and spermatocytes, remained covered by cytoplasm of the sustentacular cells, a matter of great importance because of their barrier function.

Interstitial cells (glandulocytes) were present between the convoluted seminiferous tubules in small groups. Their number and structure were normal. It can accordingly be considered that the testes were continuing to perform their endocrine function. After injection of a 1% solution of water-soluble retinoic acid into the animals in a dose of 0.1 ml, changes similar to those in the control were observed in testes transplanted into the peritoneal cavity. Spermatogenesis ceased in the convuluted seminiferous tubules due to atrophy of the spermatogenic epithelium: Spermatozoa and spermatids disappeared from it, and the number of spermatocytes was somewhat reduced. The spermatogonia were preserved and in many tubules were in a stage of division. Interstitial cells, with the usual structure, were seen between the tubules. It was also found that the weight of the testis and the number of dividing spermatogonia in its tubules were 4 and 7% greater respectively in animals receiving retinoic acid than in the controls.

In animals receiving water-soluble retinoic acid in a dose of 0.3 ml the signs of hypervitaminosis were observed: wasting, loss of appetite, falling out of the hair, inertia. The weight of the transplanted testes were reduced, more so than in the control (by 8%). The spermatogenic epithelium in the convoluted tubules underwent total or partial destruction (Fig. 2). Only flattened sustentacular cells were left in some tubules. In other tubules spermatogonia were still being produced and a much reduced number of spermatocytes was found: by

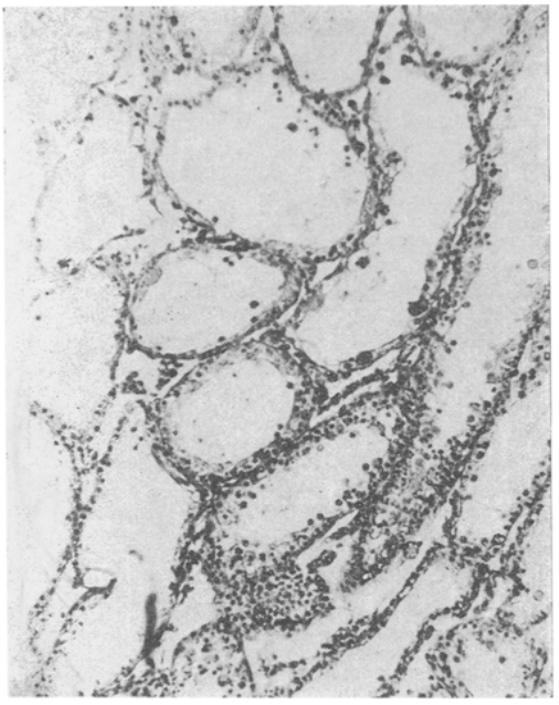

Fig. 2. Atrophy of spermatogenic epithelium of seminiferous tubules.

24% compared with the control, by 51% compared with intact animals. Only in a few tubules could dividing spermatogonia be observed. Multinuclear sperm balls were found relatively rarely in the lumen of the tubules and single degenerating spermatocytes were seen more frequently. Degenerating spermatogenic cells were shed from the wall of the tubule together with part of the cytoplasm of the sustentacular cells surrounding them. The sustentacular cells were preserved but lost a large part of their cytoplasm. As a result of this, in places where only a few spermatogonia remained from the spermatogenic epithelium, the sustentacular cells appeared flattened in shape, but despite this, they covered the surface of the spermatogonia. Flatter sustentacular cells lined areas of the tubules in which all the sex cells were destroyed. Congested blood vessels and glandulocytes with no visible pathological changes were observed in the interstitial tissue.

Under the extremal conditions associated with the production of experimental cryptorchidism, degeneration of the cells of the spermatogenic epithelium thus takes place in the testes. In the course of the experiment (8 days) mainly the most highly differentiated maturing sex cells (spermatozoa and spermatids) of the seminiferous tubules underwent degeneration. As a result the testes lost their spermatogenic function. Meanwhile the supporting (sustentacular) cells, which play an important barrier role in the seminiferous tubules, and also the interstitial cells (glandulocytes), responsible for the endocrine function of the testes, were preserved. Injection of a 1% solution of water-soluble retinoic acid in a dose of 0.1 ml into the animals in these cases did not increase the resistance of the spermatogenic epithelium to the unfavorable conditions, but maintained its possible capacity for regeneration. After injection of the acid in a dose of 0.3 ml a state of hypervitaminosis A developed, lowering the resistance of the spermatogenic epithelium to the action of unfavorable factors and depressing its regenerative potential. Meanwhile the barrier role of the sustentacular cells of the seminiferous tubules and the endocrine function of the interstitial cells of the testes were preserved.

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ELECTRON-MICROSCOPIC DEMONSTRATION OF CALCIUM IONS AT DIFFERENT STAGES OF SURFACTANT FORMATION IN NORMOTHERMIA AND HYPOTHERMIA

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KEY WORDS: calcium ions; surfactant; rabbit lung; cold.

Calcium participates in the integrative reactions of the body [2, 7, 11, 13, 14], including in phospholipid, protein, and carbohydrate metabolism [1, 5]. Calcium ions in lung tissues regulate the interstitial fluid pressure [9, 12]. The discovery of large numbers of calcium ions in washings from the bronchi and also in the amniotic fluid in the final stages of formation of the fetus [8, 15] probably indicates their active participation in surfactant formation.

The aim of this investigation was to study the localization and intensity of accumulation of calcium ions at the sites of formation of osmiophilic lamellar bodies (OLB) and their release from type II alveolocytes, under both normothermic and hypothermic conditions, because surfactant formation in the alveolocytes is intensified at low temperatures [3, 4].

EXPERIMENTAL METHOD

The lungs of 20 chinchilla rabbits were investigated. Ten rabbits constituted the control group. The remaining animals were cooled during a single session in a "Feurton-3101-01" climatic chamber for 3 h at − −30°C. Calcium ions were revealed in lung tissue (cardiac lobe) by the electron-histochemical method [6, 10], after preliminary perfusion fixation, with a control to verify absence of ammonium oxalate in the medium. Acid mucopolysaccharides were detected with ruthenium red, after immersion of the lung tissue in fixative [11]. A scanning cytophotometer, an improved version of the "Impulse analyzer" attachment for the UEM-100A electron microscope, was used for microscanning. The electrical signal during scanning (magnification (10,000) was led from the photomultiplier to a matching amplifier and integrator, where summation of the values of optical density took place, the result being proportional to the density of that part of the picture being analyzed under the electron microscope. The transformed analog signal was recorded on a "Konsul-254" printer. Eight gradations of density of the test object (from 0 to 7) were used in the investigation, the background of the object corresponding to "7" and the part of the specimen with the highest electron density corresponding to "0." To estimate activity of the histochemical reaction the overall density was calculated over the range from 0 to 4. For volume analysis of the OLB by the dot counting method, a Weibel's grid was used.

EXPERIMENTAL RESULTS

Histochemical reaction products were found in the form of small round electron-dense granules 12-15 nm in diameter, or of an area of dust-like granularity. Within the alveolar lining the granules of the histochemical reaction for calcium ions were found in small numbers in type I alveolocytes. The reaction products were rather more abundant in lung macrophages, especially in those regions of the cytoplasm where phagocytosis was in progress. The reaction for calcium ions took place most intensively in the cytoplasm of the type II alveolocytes (Fig. 1). The distribution of granules of the end products of the histochemical reaction for

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