CHANGES IN THE TESTIS AND EPIDIDYMIS OF ALBINO RATS IN AVITAMINOSIS A

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The information given in the literature about changes in the testicular glands and the seminiferous tubules in avitaminosis A is inadequate and often contradictory. Most authors [6, 11, 14, 18] have paid special attention to the study of the convoluted tubules of the testis, and have described atrophy in them in albino rats, with disappearance of the germ cells. Bouin [7], however, considers that the spermatogenic cells are resistent to avitaminosis A. E. Ya. Gertsenberg and co-workers [3] found no changes in the testes in some rats, while in others they observed degeneration of the testicular epithelium and depression of spermatogenesis.

In the interstitial tissue of the testis of albino rats some authors have found edema [18], others diminution of the secretion of the Leydig's cells [7], and a 3rd group report an increase in the number of these cells [13].

Atrophy and, rarely, focal epidermoid metaplasia have been described in the epididymis [18]. No account has been taken of the anatomical and histological differences between the various parts of the epididymis. Six zones are distinguished in the epididymis [15], 3 of which are situated in the head, the 4th and part of the 5th form the body, and the remaining part of the 5th together with the 6th form the tail of the epididymis. In addition, the passage of the semen is not the only function of the epididymis. The epithelium of the tubules also performs a secretory [10, 15] and a resorptive [16] function.

The object of the present investigation was to study the changes in the testis and various parts of the epididymis in animals of different ages (before and after sexual maturation) with avitaminosis A.

EXPERIMENTAL METHOD

Experiments were carried out on 58 experimental and 19 control male rats aged about 2 months (weight 70-80 g) and 3 months (weight 110-130 g) at the beginning of the experiments. The experimental animals were kept on a

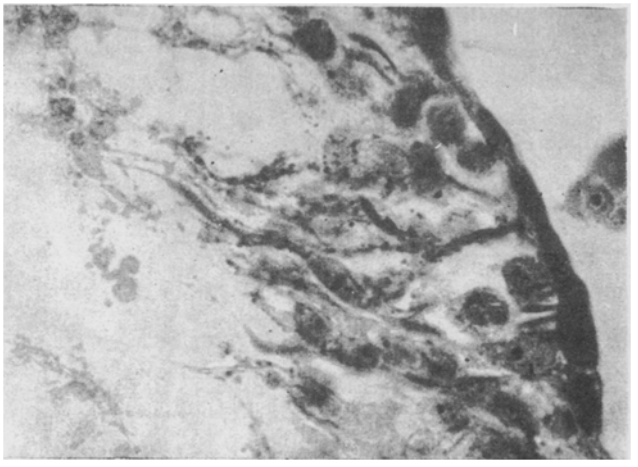

Fig. 1. Degeneration of spermatogenic cells. Distintegration of the heads of the spermatozoa into granules. Rat aged 3.5 months, kept on a diet deficient in vitamin A for 1.5 months. Heidenhain's iron-hematoxylin. Objective 90x, ocular 10x.

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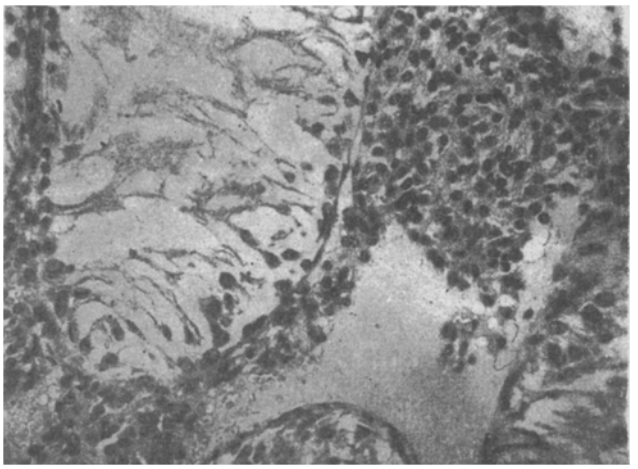

Fig. 2. Sertoli syncytium and a few spermatogonia in a convoluted tubule of the testis. Hyperplasia and atrophy of the Leydig's cells. Edema of the stroma. Rat aged 5 months kept on a diet deficient in vitamin A for 3 months. Van Gieson. Objective 20x, ocular 15 x.

diet deficient in vitamin A suggested by the Leningrad Vitamin Institute [5]. The duration of the experimental and control investigations was between 4 and 140 days. In the younger rats, 2 months old, external signs of avitaminosis A, which have been adequately described [3, 18], appeared 1.5 months from the beginning of the experiment, and in the 3-month old rats, they appeared after 2.5 months. Despite individual variations, the gain in weight of the experimental rats was less than that of the controls, and sometimes their weight fell below its initial level. A parellel trend was observed between the changes in the weight of the testis, which was weighed together with the epididymis, and the body weight.

EXPERIMENTAL RESULTS

Immature spermatogenic cells were present in the testis of the rats at the age of 2 and 2.5 months, but the tubules of the testis and epididymis had a narrow lumen and contained no spermatozoa. By the age of 3 months the rats attained sexual maturity, and the testis and epididymis showed signs of organ-specific differentiation characteristic of the adult animal. They were not visibly different in the control rats and in those kept on a diet deficient n in vitamin A.

The changes in the testis and epididymis of the rats 2 months old at the beginning of the experiments as a result of vitaminosis A appeared after 1.5 months, whereas in the animals 3 months old initially they appeared after 2.5 months. By this time spermotogenesis in the experimental animals was depressed, the convoluted tubules were smaller in diameter, the number of spermatozoa was reduced, and fewer spermatids and spermatocytes could be seen. The heads of the spermatozoa were fragmented into small granules (Fig. 1). The nuclei of the spermatids and spermatocytes showed signs of vacuolation and lysis. Among the spermatids, large spherical involutive forms with two or several nuclei could sometimes be seen. The appearance of such forms after exposure of the testis to various unfavorable factors has been reported by other authors [4, 8, 11]. In the lumen of many of the tubules, desquamated spermatogenic cells, some of them fragmented into granules, and a basophilic fluid were found. As a result, some of the tubules were empty and contained only Sertoli cells and a few spermatogonia. Sometimes calcium salts were deposited in the empty tubules. However, besides death of the spermatogenic cells at these periods of the experiment, foci of proliferation could still be seen, with mitoses at the level of the spermatogonia and spermatocytes. An albuminous fluid accumulated in the stroma of the testis and the number of Leydig's cells increased.

If the avitaminosis was severe, spermatogenesis stopped completely. The convoluted tubules of the testis were greatly narrowed, and contained only a Sertoli syncytium and a few spermotogonia (Fig. 2), in agreement with earlier reports [11] of the possibility of repair. No mitoses were present. Numerous fat droplets and tiny PAS-positive granules accumulated in the cytoplasm of the Sertoli syncytium. Several authors [9, 17] regard the lipid inclusions in the Sertoli cells as the result of phagocytosis of accessory bodies. Deposition of fat in the Sertoli syncytium has also been observed during degeneration of the germ cells under the influence of x-ray irradiation and hyperestrinization [9]. In consequence of these observations, the accumulation of lipids in the Sertoli syncytium in avitaminosis

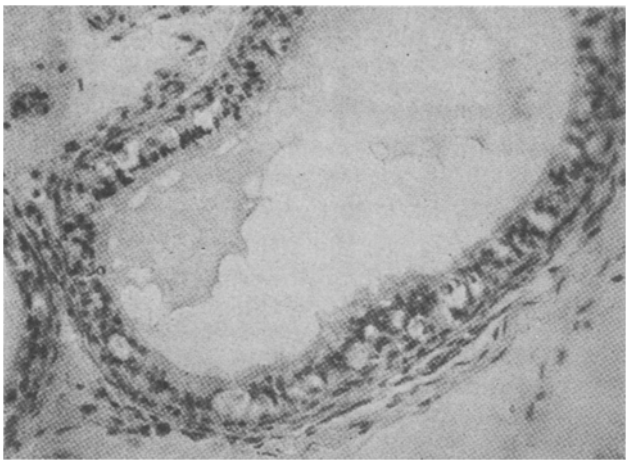

Fig. 3. Necrosis of the principal cells of the epithelium of a duct of the epididymis in the 3rd zone with the formation of intraepithelial cysts. Rat aged 5 months kept on a diet deficient in vitamin A for 3 months. Hematoxylin-eosin. Objective 20×, ocular 15 ×.

may be regarded either as the result of increased phagocytosis of the disintegrating spermatogenic cells or as the result of depression of the utilization of the phagocytosed substances.

The layers of stroma between the convoluted tubules appeared thickened on account of proliferation of the Leydig's cells, which evidently was a compensatory reaction to the cessation of the spermatogenic function of the tubules (Fig. 2). At this time, however, the Leydig's cells had lost their lipid inclusions and were atrophied. Some of them were elongated in shape and were hardly distinguishable from fibroblasts. It is natural to assume that the secretion of androgens was depressed following the atrophy of the Leydig's cells, and visible evidence of this was given by the atrophy of the epididymis and of the accessory sex glands. The changes described in the Leydig's cells are in good agreement with reports in the literature [12, 13] of stimulation of the growth of the secondary sex organs following administration of testosterone and gonadotropin to animals with avitaminosis A.

In the seminiferous passages during the development of avitaminosis the diameter of the tubules diminished and spermatozoa disappeared from their lumen, where they were undergoing phagocytosis by macrophages. The epithelial cells of the rete testis were so flattened that they resembled the vascular endothelium. The epithelium in the efferent ducts of the testis was atrophied. The nuclei were more closely and irregularly packed, evidently because of the decrease in the diameter of the ducts. In the principal cells the tiny vacuoles in the supranuclear zone of the cytoplasm were less obvious. Here and there a few principal cells showed degeneration. The epithelium of the duct of the epididymis also showed signs of atrophy, but the height of the epithelial cells, which depends on the degree of stretching of the tubules, showed little change when these were constricted. In the 4th zone binucleated cells with unusually large nuclei were sometimes seen. The signs of resorptive and secretory activity of the epithelium were less marked. The stereocilia were usually intact, although in some places they were not clearly distinguishable. The clear zone and the vacuoles in the cytoplasm of the principal cells were almost invisible or had disappeared. Among the clear cells smaller forms with fewer granules were predominant, but in some cases these cells could not be found.

In the epithelium of the 3rd and 4th, and less frequently the 2nd and 5th zones of the duct of the epididymis, degeneration and necrosis of some of the principal cells were observed. Single cells, or less commonly small groups of them, were round in shape, their cytoplasm was dissolved or fragmented into granules, and their nucleus has ruptured and showed signs of lysis. As a result, many tiny intraepithelial cavities were formed (Fig. 3). In contrast to the clear cells, producing a holocrine secretion, the degenerating cells contained no lipids staining with Sudan III, and were PAS-negative. Only occasionally could a few palely stained granules be detected in them by the PAS reaction. The contents of the cavities were not stained by mucicarmine. In some cases a swollen cell was converted into an anuclear sphere of hyaline type, staining brightly by the PAS reaction. In isolated cases the epithelium of the duct of the epididymis showed vacuolar degeneration. Single large vacuoles with traces of palely stained contents occupied the supranuclear zone of the cytoplasm.

As reported also by other authors [3], no metaplasia into stratified squamous epithelium was observed in the seminiferous passages, despite the fact that in the larynx, trachea, urethra, and the ducts of the prostate and salivary glands, squamous-cell metaplasia and keratinization have often been described. Some investigators [18], although describing keratinization in the seminiferous passages, consider that it is rare. At the same time, squamous-cell metaplasia is possible in the epithelium of the duct of the epididymis in other conditions, for example in inflammation [1] or hyperstrinization [2]. In the latter case it develops in the period of administration of an excess of female sex hormone. After administration of this hormone has stopped, the layer of squamous cells which has formed becomes detached, and atrophy of the epithelium of the seminiferous passages becomes the predominant feature, associated with atrophy of the testis.

It may be concluded from these observations that the action of avitaminosis A, causing metaplasia of the epithelium, is combined with depression of the secretion of androgens, causing atrophy of the seminiferous passages, and the action of the hormonal factor on the latter is stronger than that of the avitaminosis.

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