CHANGES IN THE ACCESSORY SEX GLANDS OF THE MALE RABBIT DUE TO SYNESTROL

A. G. Bobkov

From the Department of Pathological Anatomy (Head – Prof. M. A. Zakhar'evskaya) of the I. P. Pavlov First Leningrad Medical Institute (Director – Docent A. I. Ivanov)

(Received July 3, 1958. Presented by Academician N. N. Anichkov)

A considerable volume of research has been published in the literature, devoted to the effect of the estrogens on the male accessory sex glands [4-9, 11, 13 and others]. In spite of reports of dissimilar changes in these glands in response to the injection of estrogens to animals of various species [6, 7], no experiments have been carried out on rabbits. Furthermore, having observed inflammatory changes in the accessory sex glands from the action of hormones, the majority of authors have not devoted sufficient attention to them, nor have they considered the question of the possible effect of inflammation on the changes in the glands caused by administration of the estrogen. É. Z. Yusfina alone [10] points out the combined stimulating action of the estrogen (folliculin) and of inflammation on the growth of the tissue cells of the prostate gland of a rabbit, implanted by F. M. Lazarenko's method. Another estrogen – synestrol (hexestrol) – in É. Z. Yusfina's experiments inhibited the inflammatory growth of the epithelium, but this contradiction must have been due to the very large dose of synestrol which led to rapid proliferation of the connective tissue.

Having obtained some information on the inflammatory changes in the epithelium of the accessory sex glands of the male rabbit [1], we nominated the next step in the research to be comparison of these with the hormonal changes.

EXPERIMENTAL METHOD

Experiments were carried out on 22 young male rabbits, aged about 5 months and weighing from 1400 to 2200 g. Nine of these rabbits were subjected to preliminary castration. Every other day the rabbits were regularly injected with synestrol subcutaneously in 2% oily solution. For a period of 81 days the dose injected was 0.3 ml (0.006 g of pure synestrol), after which the sessional dose was increased to 0.45 ml (0.009 g). The times of the experiments were as follows: 4, 8, 12, 16, 22, 28, 36, 37, 60, 75, 97, 109, 122, 129, 143, 156, 159, 173, 179, 186, 198 and 211 days. The material was fixed in formalin and embedded in celloidin and paraffin wax. Histological sections were stained by Van Gieson's method and with hematoxylin-eosin, and some sections were also stained by Gram's method and mucicarmine.

EXPERIMENTAL RESULTS

The administration of considerable doses of synestrol caused wasting of the rabbits. The external appearance of the accessory sex glands was only slightly altered. Usually at first the glands were slightly reduced in size, and in the later periods of the experiment (4-5 months) they increased in size.

In early periods of the experiment (4-8 days) an unevenly marked hypermia and edema of the stroma developed, which increased in intensity until the 8th day, and then gradually diminished. On the 8th day, leucocytes accumulated in the vessels, and then migrated into the surrounding tissue. Meanwhile an increase took place in the numbers of histiocytes and lymphoid cells in the connective tissue. Infiltrative phenomena were found

primarily on the mucous membrane of the urethra and in the prostate gland, and then in the vesiculiform* and other glands. The leucocytes penetrated into the epithelium and accumulated in the lumen of the cavities of the glands. In the urethra, the leucocytic infiltration of the mucosa was observed at the level of the vesiculiform gland and more distally; in the more proximal segments it was absent. In the epithelium of the glands secretion was diminished and came to an end. Swelling of the cells of the basal row took place, so that they became enlarged and rounded. In some cells a vacuolar dystrophy was observed. At the same time, even at these stages a weak proliferation of the epithelium could be detected, as a result of which, on the 9th day of the experiment, the epithelial cells formed, in places, 3 or 4 rows. The proliferation of the epithelium had a focal character and usually was independent of the degree of expression of the infiltrative changes. In the castrated animals proliferation was observed sooner and developed at a more rapid rate, although considerable individual variations were observed in the rate of its development.

As a result of the proliferation a thickening of the epithelial layer took place, with the formation of structures of transitional and stratified squamous epithelium. We first observed structures resembling transitional epithelium on the 16th day of the experiment in a castrated rabbit, in the ducts of the vesiculiform, prostate and upper Cowper's glands. This epithelium consisted of 2 or 3 rows of light, imbricate cells. At the base of the epithelium was differentiated a layer of smaller cells, and on its surface there was a layer of cubical or flattened cells, which were the cells of the superficial layer of the pre-existing two-layered epithelium. Stratified squamous epithelium with keratinization and desquamation of keratinized layers was first observed on the 37th day of the experiment in solitary acini of the vesiculiform gland, together with insignificant signs of proliferation

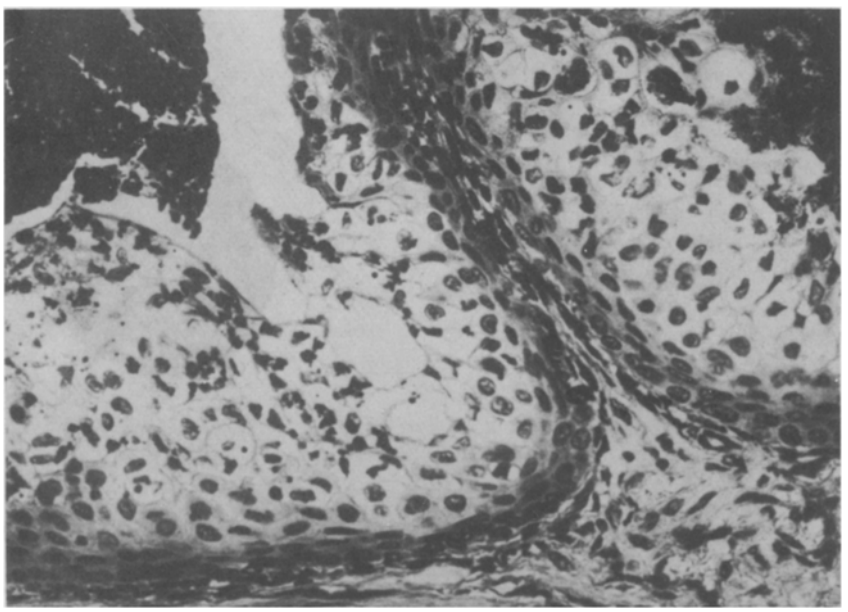

Fig. 1. Experiment at 122 days (0.426 g synestrol). Differentiation of stratified squamous epithelium at the base of a layer of degenerating transitional epithelium in the vesiculiform gland. Van Gieson's stain.

of the epithelium in other cavities. The formation of transitional and stratified squamous epithelium became a constant sign only later – 2 months and more from the onset of administration of synestrol.

In the vesiculiform and prostate glands transitional epithelium was formed in the majority of the acini of the glands. Areas of thickened epithelium which had not attained the degree of differentiation of the transitional, and areas of two-layered epithelium could, however, always be found. In places, at the base of the layers of transitional epithelium, differentiation of stratified squamous epithelium was observed. At such places the upper zone of the epithelial layer consisted of large, light imbricate cells, corresponding to transitional epithelium, and the lower was composed of compact, polygonal or flattened cells with a sharply outlined basal layer of small cubical cells (Fig. 1). In these layers, the transitional epithelium had completely desquamated in places, and was replaced by stratified squamous nonkeratinizing epithelium.

* The glandula vesicularis found at the neck of the bladder. – Publisher.

Proliferation of the epithelium of the upper Cowper's gland began in the major ducts. Gradually the process spread to involve the epithelium of the smaller ducts. The terminal divisions remained intact for a long time, but at 2 months their epithelium had also begun to proliferate. In these ducts, transitional and stratified squamous epithelium developed at the same times of the experiment and in the same combinations with each other as in the vesiculiform and prostate glands. The layers of stratified squamous epithelium in the upper Cowper's gland were highly developed, and in places consisted of 20 or more layers of cells.

In the seminal vesicle the distal divisions in normal conditions are often lined with transitional epithelium too. For this reason the designation of structures composed of transitional epithelium here as new formations resulting from the action of synestrol must be made with care. Nevertheless, under experimental conditions, transitional epithelium was found in the seminal vesicle more often and at a higher level, sometimes throughout its whole extent, which was not observed without the action of synestrol. The frequently found mitoses were further evidence of proliferation of the epithelium of the vesicle. Sometimes two sharply delineated zones could be distinguished in the transitional epithelium of the seminal vesicle: a superficial consisting of large, light cells, and a deep, formed of several rows of far smaller and darker cells. On the basis of comparison with the two-layered structures of transitional and stratified squamous epithelium of the other glands described above, these appearances in the seminal vesicle must evidently be regarded as a tendency toward the formation of stratified squamous epithelium. However, the latter was found very rarely in the seminal vesicle. Only in one

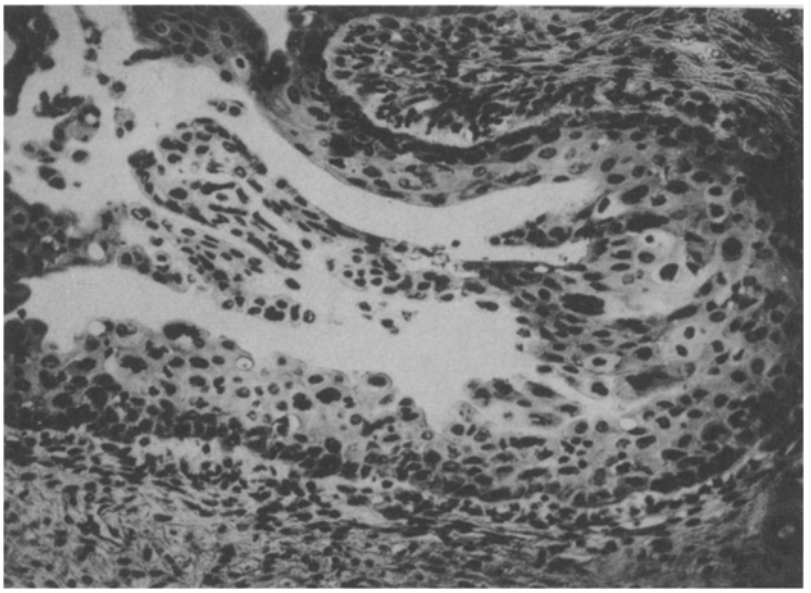

Fig. 2. Experiment at 60 days (0.18 g synestrol). Atypia and polymorphism of the cells in areas of proliferation of the epithelium of the seminal vesicle. Multinuclear epithelial cells. Van Gieson's stain.

case was it possible to observe typical stratified squamous nonkeratinizing epithelium in those areas of the seminal vesicle where subacute productive inflammation was well marked underneath the epithelium. Adjoining these areas destruction of the epithelial lining of the vesicle by the inflammatory process was observed.

Sometimes the proliferation of the epithelium of the seminal vesicle led to the formation of solid epithelial outgrowths, projecting into the lumen. These outgrowths were characterized by gross atypia and polymorphism, and consisted of cells and nuclei of a variety of shapes and sizes, of nuclei with different staining properties and of multinuclear epithelial cells. These tissues were reminiscent of carcinoma, but no infiltrative growth was observed (Fig. 2).

In the lower Cowper's gland transitional, and sometimes stratified squamous nonkeratinizing epithelium, formed the normal lining of the distal divisions of the ducts. In the experiments the layers were observed to be

much thicker, and the structures mentioned were formed in the medium-sized ducts. No proliferation of the epithelium of the small ducts and terminal divisions was observed.

In the ampullae of the seminiferous tubules the epithelium preserved its two-layered structure. In only one case, when in the membrane itself obvious inflammation was observed, was the epithelium thickened and permeated with leucocytes, consisting of 3 or 4 rows of lightly stained cells.

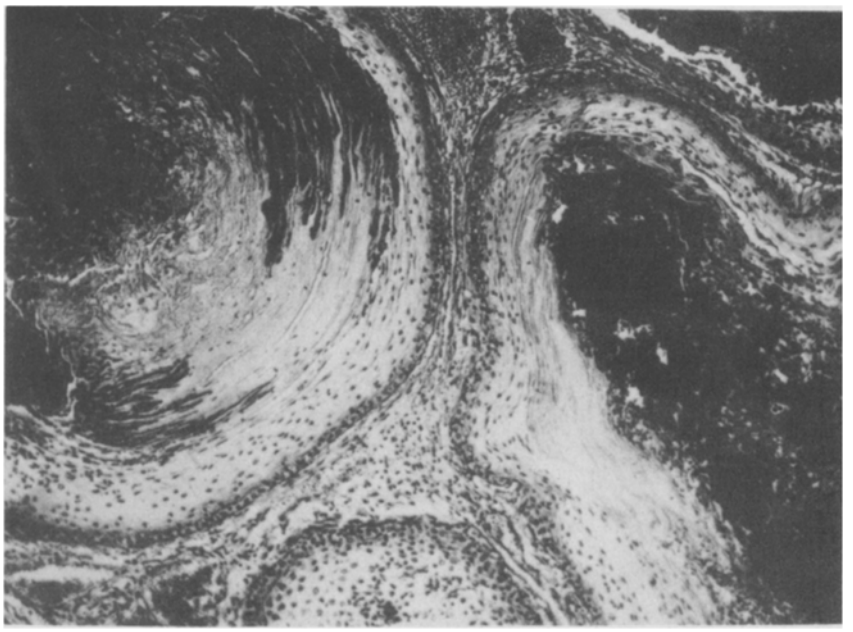

Fig. 3. Experiment at 186 days (0.705 g synestrol). Stratified squamous epithelium with marked keratinization in the vesiculiform gland. Van Gieson's stain.

The stratified squamous epithelium of the vesiculiform, prostate and upper Cowper's glands was affected by keratinization (Fig. 3). The keratin scales desquamated into the lumen. The accumulation in the lumen of desquamated epithelial cells, masses of keratin and disintegrating leucocytes led to gross dilation of the acini of the glands, which sometimes reached a diameter of 0.3-0.5 cm. Dilatation of the cavities caused enlargement of the particular glands.

In late stages of the experiment (5-6 months) sclerosis of the stroma of the accessory sex glands developed, with atrophy of muscle bundles and epithelium. Evidently in the conditions of these experiments acellular sclerosis was predominant, since no significant proliferation of fibroblasts was observed.

Signs of degeneration became more intense in the epithelial layers. Many cells became infiltrated with fat and were converted into pseudoxanthomatous cells with a very lightly stained, scanty protoplasm. The cells of the stratified squamous epithelium which did not undergo keratinization were desquamated in large numbers; their nuclei showed pyknosis and rhexis, but alongside degeneration of some cells mitoses were found in others, demonstrating the continuing growth.

The dissimilar changes in the epithelium of the different glands under the influence of synestrol were evidence of specific features in the morphological and functional differentiation of the epithelium in various parts of the reproductive system.

After administration of synestrol, dystrophic, exudative-infiltrative and proliferative phenomena were combined in the glands, and hence, from its formal signs, the process as a whole could be described as inflammation [12]. Proliferation of epithelium could not, however, be equated with ordinary inflammatory growth as distinguished by V. G. Garshin [3], since no correspondence was usually observed between the proliferation of the epithelium and the degree of expression of the inflammatory changes in the underlying tissue. Furthermore, under the influence of synestrol, the formation of stratified squamous epithelium and keratinization of this

epithelium were expressed far more intensively than in inflammation. A hormonal origin must therefore be accepted for the epithelial proliferation studied in this research. In spite of the fact that hormonal growth of epithelium is an independent form of growth, independent of inflammation, the latter may, nevertheless, affect hormonal growth. This must account for the formation of stratified squamous epithelium in the seminal vesicle only in the presence of inflammation, whereas in the absence of inflammation no squamous cell structures developed in this gland.

In the conditions of these experiments no transformation of hormonal epithelial growth into blastomatous was observed. This was in agreement with the findings in the literature [2].

SUMMARY

Epithelial proliferation associated with dystrophic changes, inflammatory infiltration and sclerosis of the stroma (at the late periods of experiment) are observed in the accessory sexual glands of male rabbits as the result of subcutaneous injections of synestrol. Epithelial proliferation is frequently associated with its metaplasia into transitional and stratified squamous types with casual cornification. The growth of the epithelium induced by synestrol injection is independent of the inflammation. However, the latter may influence this growth.

LITERATURE CITED

[1] A. G. Bobkov, Byull. Éksptl. Biol. i Med. , 39, No. 4, 68-72 (1955).

[2] V. Gardner, Progress in the Study of Cancer, vol. 1, pp. 122-192, Moscow, 1955 [Russian translation].

[3] V. G. Garshin, Inflammatory Proliferation of Epithelium, Its Biological Importance and Relation to the Problem of Cancer, Moscow-Leningrad, 1939 [In Russian].

[4] B. V. Klyucharev, Arkh. Patol., 9, No. 2, 75-83 (1947).

[5] B. V. Klyucharev, Arkh. Patol., 10, No. 2, 44-47 (1948).

[6] B. V. Klyucharev, Problems of Oncology, 7, pp. 29-35, Moscow, 1954 [In Russian].

[7] V. B. Klyucharev, Experimental Neoplasms of the Prostate Gland and Their Hormone Therapy, Leningrad, 1954 [In Russian].

[8] V. P. Konoplev, Problems of Oncology, 7, pp. 20-28, Moscow, 1954 [In Russian].

[9] B. I. Monastyrskaya, Problems of Oncology, vol. 1, 1, pp. 158-162, Moscow, 1949 [In Russian].

[10] É. Z. Yusfina, Arkh. Anat. Gistol. i Émbriol., 33, 4, 55-60 (1956).

[11] H. Burrows, Biological Actions of Sex Hormones. Cambridge, 1949.

[12] O. Lubarsch, In the book: Pathologische Anatomie. Hrsg. von L. Aschoff. Jena, 1928, Bd. I, S. 540.

[13] G. H. Twombly and G. T. Pach, Endocrinology of Neoplastic Diseases. Oxford, New York, 1947.