

# EFFECT OF ADMINISTRATION OF SYNESTROL ON THE EPITHELIUM OF THE UTERUS AND VAGINA OF RABBITS AND RATS\*

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It has been shown by the work of V. G. Garshin [1] and his collaborators on inflammatory proliferation of epithelium, by the proliferative response of epithelium to hormones [2, 6, and others], and by the epithelial changes taking place in certain avitaminoses, that changes in the conditions of life of tissues may cause stable changes in the morphology and function of cells.

Data on changes in the uterine epithelium are scanty and often contradictory. We found no significant changes in the uterine epithelium under conditions of experimentally produced inflammation and artificial ectropion. It is known, however, that considerable changes may be caused in endocrine disorders.

The multilayered structures of the uterus have been regarded by many workers [4, 8] as originating from metaplasia of the uterine epithelium. N. I. Kazanskaya [2], who introduced hormones into an isolated uterine horn, showed that stratified uterine epithelium is of vaginal origin, migrated into the uterus, and this view is also held by E. L. Prigozhina [6], O. G. Prokofeva [7], and others.

Migration of vaginal epithelium into the endometrium has also been noted in inflammatory conditions by Ya. S. Klenitsky [3] in mice, and by E. A. Popova [5] in rats.

The present paper gives the results of a study of changes in the uterine and vaginal epithelium in endocrine disorders.

## EXPERIMENTAL METHODS

Experiments were performed on 50 rats\*\* and 22 rabbits 1½-2 months old. After preliminary castration and section of one uterine horn, the animals were given subcutaneous injections of 2% solutions of Synestrol in oil, 3 times weekly, as follows: 0.1-0.2 ml per dose for rats, and 0.4 ml for rabbits. Since this dosage caused necrosis of the uterine mucous membrane of rabbits it was reduced 40-fold for 2 rabbits 3 months after the beginning of the experiment.

The rats were killed at various times, from one week to 4 months after the beginning of the experiment, and the rabbits from one week to 6 months.

Transverse and longitudinal sections of various parts of the uterus and vagina were prepared; in some cases serial sections were made. The sections were stained with hematoxylin-eosin, picrofuchsin, and Heidenhain's hematoxylin.

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\*\* Part of the material was supplied by T. B. Zhuravleva.

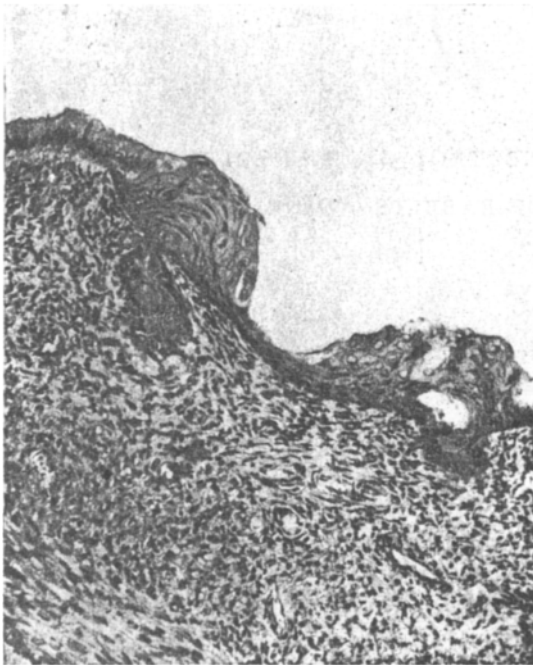


Fig. 1. An isolated rat uterine horn. Fungiform outgrowths of stratified epithelium from uterine glands, developing in response to administration of Synestrol, for one month.



Fig. 2. Compact macroalveolar carcinoma of the uterus of a rabbit, developed after administering Synestrol for 4 months.

#### EXPERIMENTAL RESULTS

On laparotomy, the uterine horns were seen to be enlarged in all animals, to a maximum at the end of the experiment. Pyometra was found in 5 rats, with perforation and resulting peritonitis.

##### Experiments on rats

The vaginal mucous membrane of the rats was lined with stratified cornified epithelium, consisting of 6-12 layers (normally 2-3 layers). The proliferating stratified epithelium forms numerous papillae growing into the mucous membrane, and numerous mitoses are evident in its germinative layer. The vaginal epithelium grows in a continuous sheet into the cervical canal and into the intact horn of the uterus. When it encounters uterine epithelium the vaginal epithelium undermines it, and separates it from the mucosa, and in entering the endometrium it either occludes the openings of the glands or grows into their lumen. In 5 rats the mucosa of the intact uterine horn was lined with stratified cornified squamous epithelium of vaginal origin, but in the great majority of cases the longitudinal sections showed that only part of the endometrium was lined with vaginal epithelium, above which was columnar uterine epithelium with only sporadic patches of stratified vaginal cells. It was possible in serial sections from such animals to establish continuity of the stratified flat celled endometrial epithelium with that of the vagina. In other cases stratified squamous epithelium was found in the distal parts of the uterine horn, and its continuity with vaginal epithelium could not be established from the serial sections; this was found in 7 animals.

Other variants are also to be found. Thus in Rat No. 35, examined 3 months after the beginning of the experiment, stratified epithelium was found in the endometrium, without marked vertical anisomorphism of the layer, the margin of which had an eroded appearance. The layer contained mucus vacuoles, and the cytoplasm of the cells of all its layers contain numerous mucin granules.

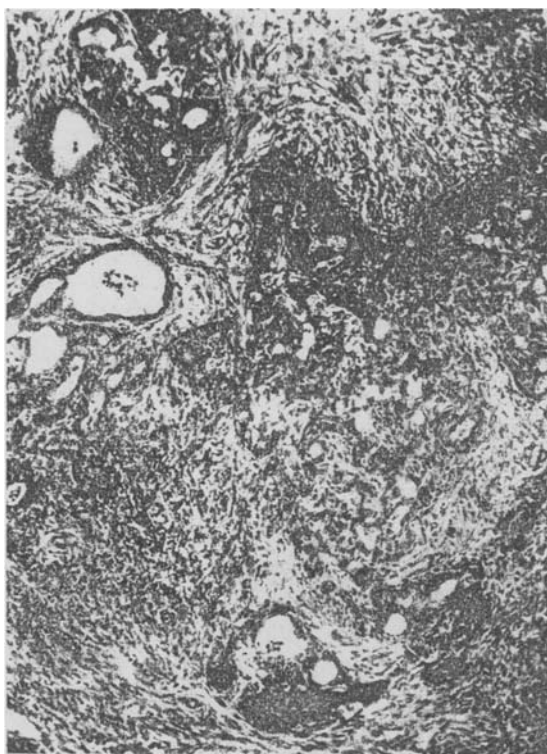


Fig. 3. Section through part of an adenocarcinoma of the uterus of a rabbit, developed after administration of Synestrol for 4 months.

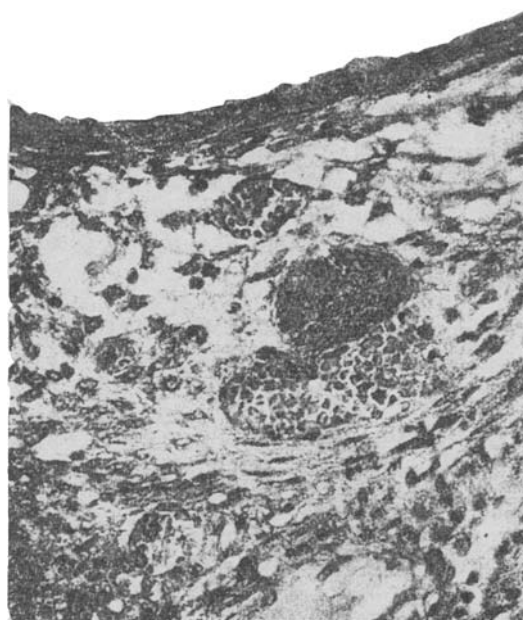


Fig. 4. Cancer cells in a lymph vessel of a rabbit uterus, after administration of Synestrol for 4 months.

In view of the discovery of stratified epithelium not connected with the vaginal epithelium it became necessary to make a detailed study of serial sections of the isolated uterine horn. This revealed islets of stratified epithelium in 3 rats, consisting of polygonal cells, arranged in 4-5 layers, and flattened at their free surfaces. These islets are connected with endometrial glands, in which proliferation of cells was observed, with formation of nests of small undifferentiated cells completely filling the lumen of the gland.

In many cases the groups of undifferentiated cells protruded through the duct of the gland into the uterine horn, and in such cases the upper layer consists of flattened cells, giving the appearance of stratified squamous epithelium (Fig. 1). Less frequently, the undifferentiated epithelium of the uterine glands is seen beneath a layer of columnar epithelium as rows of subepithelially distributed cells. Where the number of such cells is large they are differentiated into stratified epithelium having cells containing scarcely any mucin inclusions.

In the early stages of the experiment the columnar epithelium of the uterine horns is mostly in a single layer. The cells are high, with spherical or irregular nuclei, which are poor in chromatin, and are located at different levels. The upper surface of the cells is frequently convex, and is surmounted by a barely perceptible covering of mucus. Vacuoles not containing mucin are to be found in the cytoplasm of these cells.

In the distal parts of the uterus the epithelium forms wedge-shaped and fan-shaped papillae, arranged in a number of rows. The upper part of the cells seems somewhat drawn out, and extrusion of cytoplasm is sometimes visible. Some groups of cells are ciliated.

In other parts of the uterus the epithelium contains large amounts of mucus, and the nuclei are situated basally (resemblance to cervical epithelium).

Inflammatory processes are frequently evident in the wall of the uterus. Leucocytes are to be seen in the intact horn of the uterus, together with necrotic cells, mucus, and keratinous masses.

Considerable dystrophic changes in the columnar epithelium of the uterus are present in the latter stages of the experiment. The cells are vacuolated, and do not contain mucin. The nuclei are of irregular shape, and are sometimes lobulated, and often pycnotic or karyorhectic. The uterine glands are small in number, and are lined mostly with clear columnar epithelium, the cells of which are better preserved.

The stratified epithelium (of both uterine and vaginal type) of the uterine mucosa is also in good condition.

#### Experiments on rabbits

Naked eye examination of the rabbit uterus reveals two different types of epithelium. The lower parts of the vagina are lined with pale, dense glistening epithelium, while in the upper parts it is of looser texture, and of a pale pink color. When stained by Zilliacus' method the vagina presents a motley picture, due to alternation of yellow staining stratified epithelium with greenish brown columnar epithelium. At the later stages of the experiment the yellow staining epithelium covers most of the surface of the vagina, stopping short about 2-3 cm below the cervix.

Histological examination shows that the mucosa of the lower part of the vagina is lined with stratified squamous epithelium, which gradually thins out to be replaced by a single layer of tall, clear, columnar cells containing large amounts of mucin.

Erosion of the cervix is evident in sporadic cases. The mucosa is raised, due to formation of loose edematous tissue containing numerous thin-walled blood vessels. The cells of the unaffected parts of the cervical epithelium are tightly packed, and are tall and slender. The nuclei are elongated, or are of irregular form, and stain irregularly. Vacuoles not containing mucin are frequent in the cytoplasm. The cilia are retained, but are frequently stuck together. Necrobiotic changes are evident in scattered groups of cells.

During the early stages of the experiment the uterine mucosa is lined with tall clear columnar epithelium, which is sometimes arranged in two layers. Most of the cells are ciliated (resemblance to cervical epithelium). At later stages necrotic changes are more frequently encountered, and by the end of the third month of the experiment the uterine epithelium has practically all perished.

The glandular epithelium is in a better state of preservation, and mitotic proliferation of cells is to be seen in it.

The following changes take place when administration of Synestrol is continued in small dosage. The uterine horn of rabbits killed after 4 to 6 months consists of an agglomerate of cysts, 4 x 5 cm large. The epithelium of the cysts and glands is variegated. Side by side with columnar uterine and cervical epithelium we see, sometimes in one and the same gland, patches of stratified epithelium, which either spread laterally or grow downwards. In the former variant the nest of stratified epithelium completely occludes the lumen of the gland, while in the latter the cells of the basal layer are less differentiated, and stain more deeply; they invade the muscular and serous layers of the uterus. Here the epithelial proliferations appear as large clumps of polygonal cells, often atypical and exhibiting marked polymorphism, with frequent mitoses (Fig. 2); in other places they are in the form of narrow strands of small, deeply staining cells, forming fine looped structures or mixed gland-solid tissue (Fig. 3). Sporadic groups of cells forming these strands show small mucin inclusions. Atypical epithelial cells are to be seen in the uterine lymph vessels (Fig. 4).

These changes, viz., polymorphism, atypical forms of cells, destructive growth, and local metastases, are evidence of cancer. It is peculiar in showing simultaneous presence of various stages of differentiation. The above description of the proliferations corresponds with macro- or micro-alveolar solid tumor, adenocarcinoma, or adenomatous secondary solid carcinoma. Its development was preceded by protracted destructive epithelial metaplasia, secondary regeneration, cyst formation, and conversion of uterine epithelium into stratified epithelium.

Uterine cancer has been produced in rabbits by N. G. Soboleva, by painting the cervix with coal tar or mustard oil, in mice by Ya. S. Klenitsky [3] by the application of carcinogens and estrogens, and in rats by E. L. Prigozhina by administering Synestrol. The earliest work on the effect of hormones on proliferation of the uterine epithelium of rabbits was done by Pierson [9], who found polypoid-cystic adenomatous proliferation and transformation of the uterine epithelium into a stratified squamous type. We have not been able to find any references in the literature to hormonal causation of uterine cancer in rabbits. We have been able to produce it in one of our experiments.

The experiments show that in animals in which there is a sharp boundary between the uterine epithelium and the stratified vaginal epithelium in the region of the external os, as in women, it is possible to observe, in the course of endocrine disturbances, stratified epithelium in the uterus of different origins, i.e., stratified squamous epithelium from the vagina, and stratified uterine epithelium.

The presence of stratified epithelium in the uterus of rabbits and rats, and especially in isolated horns of the latter animals, the connection between this epithelium and proliferation of indifferent cells of the uterine glands, and the retention, to some extent, of their mucus-producing function, are evidence of the capacity of uterine epithelium to undergo conversion into stratified squamous epithelium under conditions of hormonal imbalance.

The presence of mucus in stratified epithelium cells can in many cases be ascribed to its aspiration from the lumen of the uterus. However, accumulations of mucin granules in the cytoplasm of the cells of all layers of the stratified epithelium are evidence of mucus formation within them, the more so inasmuch as mucogenesis is retained in rabbit uterine cancer cells.

Our experiments thus show that under conditions of hormonal imbalance the single-layered uterine columnar epithelium may undergo conversion into cervical-type epithelium and into stratified squamous epithelium, and into cancerous tissue in rabbits.

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