

# MORPHOLOGY AND PATHOMORPHOLOGY

## GENESIS OF STRATIFIED SQUAMOUS EPITHELIUM IN THE UTERUS

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Adenocystic hyperplasia of the endometrium was produced in mice experimentally by prolonged intravaginal administration of dihydrostilbestrol. Preliminary division of the uterine cornua in these mice showed that stratified squamous epithelium appears in the uterus, not as a result of metaplasia of the endometrium, but following invasion of epithelium of distal parts of the genital tract.

The role of inflammatory changes in the uterus in the pathogenesis of processes leading to the appearance of stratified squamous epithelium in the endometrium has been demonstrated experimentally [2]. In experiments on mice with division of the uterine cornua [4], and also after special histological treatment of the material, including preparation of frontal histological sections passing through the uterus and vagina, which were then embedded en bloc in paraffin wax [1, 2], it was shown that inflammation is not associated with metaplasia of the endometrial epithelium, but with invasion of the cavity of the body of the uterus by stratified squamous epithelium from more distal parts of the genital tract. However, the results of these experiments are insufficient to explain the genesis of the stratified squamous epithelium found in the endometrium when affected not by inflammatory, but by other pathological changes. In particular, this phenomenon is observed in some cases of adenocystic hyperplasia of the endometrium in women.

Despite the connection observed with hyperestrinism, much regarding the genesis of adenocystic hyperplasia still remains unexplained. The study of this condition, as well as the elucidation of the possible genesis of the stratified squamous epithelium found in the mucous membrane of the body of the uterus in this disease, can perhaps be facilitated by the creation of suitable experimental models.

### EXPERIMENTAL METHOD

The object of this investigation was to reproduce adenocystic hyperplasia of the endometrium experimentally and to study the genesis of the stratified squamous epithelium which participates, along with the endometrial prismatic epithelium, in the formation of the pathological structures characteristic of this disease.

Experiments were carried out on 107 virgin female CC-57 (Wt) mice. Initially the animals were aged 1.5-2 months. Throughout the experiment the mice received dihydrostilbestrol systematically by intravaginal administration as a 0.2, 0.05, or 0.02% suspension in physiological saline. A polyurethane sponge was used as the vehicle for introducing the suspension into the vagina and preventing it from escaping therefrom prematurely. Pieces of sponge weighing about 2 mg were soaked in the dihydrostilbestrol suspension immediately before insertion. The animals were sacrificed on the 17th, 120th, and 273rd days in different experiments. In the relatively short experiments (17 days), dihydrostilbestrol was administered

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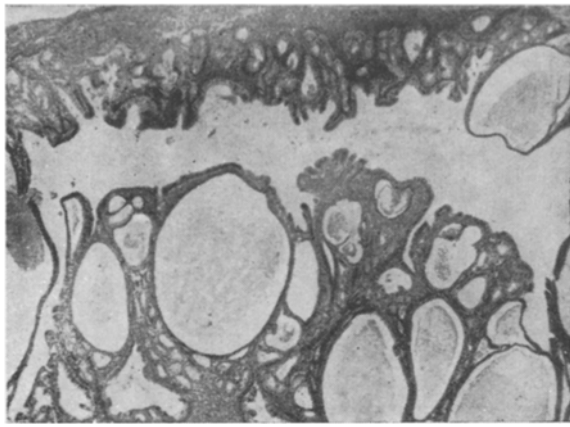


Fig. 1. Area of endometrium of mouse after systematic intravaginal administration of suspension of dihydrostilbestrol (total dose 0.735 mg) in physiological saline on polyurethane sponges, 273rd day of experiment. Signs of adenocystic hyperplasia. Hematoxylin-mucicarmine, 8 × (loupe).

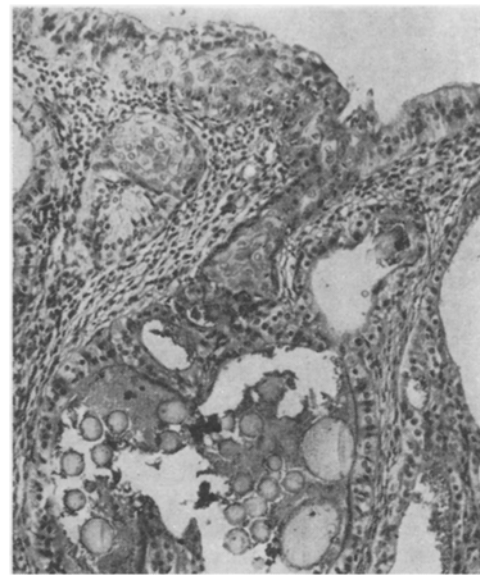


Fig. 2. Same experiment, same time. Area of endometrium of another mouse. Fragments of stratified squamous epithelium, replacing prismatic epithelium of endometrium. Hematoxylin-mucicarmine, 230 ×.

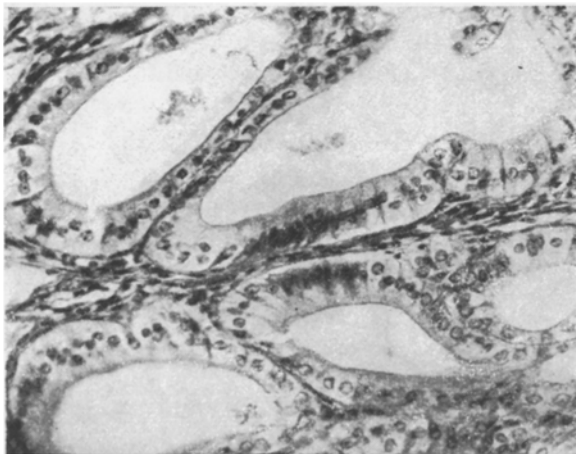


Fig. 3. Same experiment, 120th day. Focus of adenogenic proliferation of endometrium. Hematoxylin-mucicarmine, 440 ×.

five times a week (12 insertions in the course of the experiment), and in the other experiments (120 days – 30 insertions, and 273 days – 60 insertions) it was given once or twice a week. The dose of dihydrostilbestrol each time varied from between 0.025 and 0.01 mg to 0.001 mg per mouse; the total dose given in the experiments ending on the 17th day was 0.24 mg per mouse, in those ending on the 120th day it was 0.46 mg per mouse, and in those ending on the 273rd day, it was 0.735 mg per mouse. The methods used to introduce the sponge into the vagina of the mice, of obtaining and fixing the pathological material, and of cutting and staining the histological sections were described previously [1, 3, 6]. In most of the animals, the uterine cornua were divided as a preliminary measure 1-2 weeks before intravaginal

administration of dihydrostilbestrol began. The division was performed under ether anesthesia, between two ligatures, in about the middle third of the cornu. Access to the cornua was obtained through an incision along the linea alba of the abdomen. The wound was closed with silk sutures. The animals were killed with chloroform on the day after the final administration of dihydrostilbestrol.

## RESULTS

The results are given in Table 1. In experiments lasting 120 and 273 days, the animals developed papillomas of the vagina. On the 273rd day papillomas were found in all 20 animals, and on the 120th day in nine of the 17 mice.\*

\*Endophytic changes in the vaginal epithelium, classed as initial [1], were found in the remaining mice on the 120th day; on the 17th day the vaginal epithelium differed from normal only in its somewhat increased proliferative activity.

TABLE 1. Pathohistological Changes in the Genital Tract of Female Mice after Systematic Intravaginal Administration of 0.025--0.001% Suspensions of Dihydrostilbestrol in Physiological Saline on Polyurethane Sponges

Group of animals	No. of animals	Duration of experiment (in days)	Papillomas of the vagina	Attached part of body of uterus				Dedifferentiation of endometrial epithelium of isolated part of body of uterus	
				inflammation	fragments of stratified squamous epithelium	phenomena of adenocystic hyperplasia			
						total	with sclerosis of stroma		with foci of adenogenic proliferation
Without division of cornua . . .	15	17	—	12	8	—	—	—	
With division of cornua . . . . .	55	17	—	52	39	—	—	47	
The same . . . . .	17	120	9	4	14	15	2	17	
The same . . . . .	20	273	20	—	19	20	13	20	

Inflammatory changes in the attached part of the body of the uterus were absent in the longest experiments (273 days); they were found in only four of the 17 mice on the 120th day of the experiment, but in 64 of the 70 mice in the short experiments ending on the 17th day.

Fragments of stratified squamous epithelium in the mucous membrane of the body of the uterus were found in all groups of experiments in about half of the animals or more (8 of 15, 39 of 55, 14 of 17, and 19 of 20 animals, respectively). It must be emphasized that on the 273rd day of the experiment no sign of inflammation was present in any of the 19 mice in which fragments of stratified squamous epithelium were found. In all the long experiments, in which 37 mice were studied, signs of inflammation were present in four animals and fragments of stratified squamous epithelium in the mucous membrane of the body of the uterus in 33 mice.

In the overwhelming majority of mice (35 of 37 animals) adenocystic hyperplasia of the endometrium developed in the long experiments. On the 273rd day of the experiment in 13 of the 20 animals, signs of sclerosis of the connective tissue of the organ were present. Separate foci of particularly active proliferation of glandular epithelial structures (foci of adenogenic proliferation) were found in 10 mice at these times.

On the 17th day the epithelium of the attached part of the body of the uterus showed intensive secretory and proliferative activity. In some parts the prismatic epithelium lining the uterus appeared to consist of two or more rows, and some structures were found which bore a considerable resemblance to stratified epithelium (this in fact resulted from the chaotic accumulation of slightly flattened cells [1]). The epithelium of the glands still remained definitely simple in type, either prismatic or cubical.

At the late stages (120th and 273rd days), glands showing cystic changes were found in practically all fields of vision of the microscope under low power in the endometrium of the stump of the uterine cornu. Many cysts were of considerable size and compressed the surrounding glands and stroma. Remains of secretion could be seen within the cavity of the cysts. This secretion contained little mucus; occasional desquamated cells were present. The epithelium lining the uterus, the epithelium of the glands, and the epithelium lining the cysts had similar morphological features, namely irregular secretory activity and irregular proliferative activity. In different parts of it, some cells were pale, and possibly swollen, while others were dark and stretched in a vertical direction, as if compressed from the sides.

The isolated part of the body of the uterus by the 17th day was converted into one large thin-walled cyst filled with secretion (evidently, a serous secretion with traces of mucus) and with a few desquamated cells. Usually the epithelium lining the cyst cavity remained simple in character, with dark cytoplasm and a dark nucleus deeply stained with hematoxylin. In none of the 92 cases with division of the uterine cornu that were studied could fragments of stratified squamous epithelium be found in their isolated part.

Some of the features distinguishing the endometrium in the long experiments are illustrated in Figs. 1-3.

The ovaries also were studied in 25 animals. Signs of hyperplasia of the granulosa tissue were found (in five animals on the 120th and in five on the 273rd day) and in some cases tumors were present (theca-cell tumors of the ovary; in three animals on the 120th and in 12 on the 273rd day).

On the basis of the results of these experiments and of data in the literature concerning the possible genesis of stratified squamous epithelium present in the uterus under experimental conditions [2-4, 6], it can be taken as conclusively proved that this epithelium appears, not through metaplasia of the endometrial epithelium, but through invasion of epithelium of distal parts of the genital tract into the uterine mucous membrane. Metritis plays a leading role in the pathogenesis of this process. During prolonged and persistent hyperestrinism, however, metritis was an early, but gradually diminishing reaction. Meanwhile, the results provide clear evidence also of the leading role of chronic hyperestrinism in the pathogenesis of experimental adenocystic hyperplasia of the endometrium.

This experimental model of adenocystic hyperplasia, when studied morphologically, corresponds closely to its original [5, 7]. The discovery of fragments of stratified squamous epithelium in the mucous membrane of the body of the uterus in women in some cases of adenocystic hyperplasia evidently reflects the same features of the pathogenesis of this condition in women.

The results of this study of reproduction of adenocystic hyperplasia experimentally demonstrate that the pathological epithelium and connective-tissue structures involved in its formation are derived from the endometrium; the fragments of stratified squamous epithelium found in the endometrium are one of the factors which merely accompany the pathogenesis of adenocystic hyperplasia, possibly with some influence on the details of its pathogenesis, but not a direct component of it.

In the experiments described, just as under clinical conditions, the phenomena of adenocystic hyperplasia of the endometrium are definitely connected with the occurrence of hormonally active ovarian tumors. The extent to which these phenomena mutually influence each other is still not clear. In these experiments, both the changes in the uterus and changes in the ovaries developed in the course of chronic hyperestrinism.

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