10. Persistent Changes in Uterus and Vagina in Rats Given Injections of Estrogen for the First Thirty Postnatal Days

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In this laboratory, different kinds of experiments have been carried out with persistent-diestrous rats secured by daily injections of increasing doses of estrogen for the first 30 days of the postnatal life. A majority of females receiving the injections exhibit vaginal smears of the diestrous type continuously after they attain puberty, vaginal cornification never taking place (Takasugi, 1956). Their ovaries are similar in structure to those of weanling rats, neither follicles beyond the stage of antrum formation nor corpora lutea being encountered in them.

However, some rats given similar injections of estrogen show prolonged or persistent vaginal cornification seemingly like that in persistent-estrous rats obtained by different technics. It was recently found that changes in the uteri and vaginae of such persistently estrous rats were not abolished by ovariectomy, or hypophysectomy followed by either ovariectomy or ovariectomy plus adrenalectomy.

Materials and Methods. Female rats of the Wistar strain or the inbred strain maintained in this laboratory were used. Rats were given subcutaneous injections of estradiol for 30 consecutive days starting on the day of birth. Daily doses were 20 μg in 0.02 ml of sesame oil for the first 15 days and 100 μg in 0.02 ml oil for the second 15 days, or 20 μg in 0.02 ml of oil for the first 10 days, 40 μg in 0.04 ml of oil for the middle 10 days and 80 μg in 0.08 ml of oil for the last 10 days. When the animals reached 60 days of age, daily examination of vaginal smear was begun and continued for periods varying from 78 to 170 days.

Nine rats which had shown prolonged or persistent vaginal cornification during the observation periods were hypophysectomized by the parapharyngeal route. In 8 out of the 9 rats, vaginal smears were followed for 21–24 days longer, at which time the animals were subjected to ovariectomy (2 cases) or ovariectomy plus adrenalectomy (6 cases). The remaining one rat died 10 days after hypophysectomy. The ovaries and adrenals of all the 9 rats were weighed and fixed in Bouin's solution. In the 9th animal, the uterus and vagina were also fixed. Adrenalectomized rats were given a 1% solution of NaCl as drinking water after the operation.
The 6 hypophysectomized-ovariectomized-adrenalectomized rats were sacrificed 7–10 days after the second operation, whereas the 2 hypophysectomized-ovariectomized rats, 22 and 23 days, respectively.

In addition to these, 3 rats which had received 20 and 200 µg estradiol for the first and the second 15 days of the postnatal life, respectively, were ovariectomized at 85 days of age. The animals were placed under intermittent observations until sacrifice performed at 235 days of age, i.e. 150 days after ovariectomy. These animals were given to the writer by Dr. Y. Arai, some 50 days prior to killing.

At sacrifice, uterus, vagina and adrenals, if they were present, were taken out from each animal, freed from extraneous tissues, weighed and fixed in Bouin's solution. The sella turcica of hypophysectomized rats was carefully examined to check the completeness of the operation. Materials for histological studies were sectioned in paraffin and stained with Delafield's hematoxylin and eosin.

**Observations.** In 8 of the 9 hypophysectomized rats, hypophysesctomies were complete. All these completely hypophysectomized rats except for one which died 10 days after which exhibited atrophic ovaries (5–10 mg in weight) and adrenals (11–17 mg) at the time of the second operation or autopsy (in hypophysectomized-ovariectomized rats), performed 21–44 days after hypophysectomy. In the one rat which died earlier, the ovaries were as small as, but the adrenals were larger than those of the other animals, weighing 9 and 27 mg, respectively. The adrenals of the one animal retaining a fragment of adenohypophysis and of 3 ovariectomized rats with their hypophyses intact were of normal size (38–66 mg).

**Vaginal smears.** In all but one completely hypophysectomized rats and the one retaining the hypophyseal fragment, vaginal smears consisted mainly of cornified cells throughout the observation periods, even after removal of the ovaries or the ovaries and adrenals, although some leucocytes and nucleated epithelial cells occasionally appeared in the smears. Vaginal smears of the remaining one completely hypophysectomized rat were also of the estrous type for 5 days after hypophysectomy but a good many leucocytes made their appearance from the 6th day on, and for about a week preceding ovariectomy plus adrenalectomy performed 22 days after hypophysectomy, vaginal smears contained a large number of leucocytes in addition to cornified cells and epithelial cells. The second operation induced no change in the characteristics of vaginal smears until the rat was killed 7 days later.

Vaginal smears of 3 ovariectomized rats given to the writer by Dr. Arai were examined for 30 days preceding sacrifice performed 150 days after ovariectomy. During the period of observation, the
3 animals exhibited continued vaginal cornification with occasional occurrence of a small number of leucocytes.

**Histology of ovaries, uteri and vaginae.** The ovaries of completely hypophysectomized rats contained small solid follicles and those at the beginning of antrum formation, but neither maturing follicles nor corpora lutea. The uteri of these rats varied from 75 to 127 mg in weight.

If normal female rats are ovariectomized, both the uteri and vaginae undergo a rapid atrophy. The uterine epithelium consists of a single layer of cubical cells and the vaginal epithelium of about 2 layers of cells. In the present series of hypophysectomized rats, even in the absence of the ovaries and adrenals, the uterine epithelium was much thickened. Moreover, in 5 cases, it exhibited stratified areas surrounded by hyperplastic cylindrical epithelium (Fig. 1) or had undergone an overall stratification (Fig. 2). The epithelium lining the caudal portion of uterine horns was invariably stratified and in the one rat which died 10 days after hypophysectomy there was a

Fig. 1. Insular metaplasia of the uterine epithelium in the rat 37 days after hypophysectomy and 15 days after ovariectomy. ×400.

Fig. 2. Metaplasia of the uterine epithelium in the rat 31 days after hypophysectomy and 10 days after ovariectomy-adrenalectomy. Many leucocytes in the uterine lumen. ×400.

Fig. 3. Squamous metaplasia of the uterine epithelium in the rat 150 days after ovariectomy. ×400.
squamous epithelium with cornified superficial layers.

The uterine epithelium occasionally showed mitotic figures. On the other hand, infiltration of leucocytes and signs of cystic degeneration were frequently observed in the epithelium. In a majority of the rats, the uterine cavity was almost empty, while in a few animals it contained a large number of leucocytes together with some epithelial-like cells (Fig. 2).

The vaginal epithelium was thickened and stratified in all animals. Moreover, in 5 rats, superficial layers of the epithelium were cornified (Fig. 6) or had undergone parakeratosis (Fig. 5). In such cases, a typical granular layer was found beneath the cornified layers. In one rat, there were some areas covered with mucified cells in the vaginal epithelium. Leucocyte infiltration into the epithelium had occurred in some animals. The epithelium occasionally showed some down-growths (Fig. 6).

In 3 non-hypophysectomized rats sacrificed 150 days after ovariectomy, the vaginal epithelium was strongly cornified. The epithelium of the caudal portions of uterine horns was also stratified and cor-
nified. In one of the 3 rats, the epithelium of the middle portion of a uterine horn had undergone squamous metaplasia showing well developed cornified layers on its surface (Fig. 3). The other horn of the same animal was slightly distended, being filled with leucocytes (pyometra). The epithelium of this horn was flattened and degenerating. In the second rat, one uterine horn was provided with a hyperplastic epithelium, while the other horn was totally devoid of the cavity. In the last animal the cavities were obliterated in both uterine horns, the epithelium being no longer observable. Examination of cross sections of the uterine horns revealed that the area where there had been the cavity was occupied by loosened stromal cells containing some brownish masses of degenerating cells. The uteri of these 3 rats weighed 383, 182, and 244 mg, respectively.

Discussion. The proliferative changes in the uteri and vaginae as observed in the rats which had received daily injections of large doses of estradiol for the first 30 postnatal days were seemingly similar to those caused in rats and mice by continued administration of estrogen for a prolonged period. (For reviews of previous literature, see Allen, Hisaw and Gardner, 1939; Lipschutz, 1950; Burrows and Horning, 1952.)

However, the rats were given no injections of estrogen since the 31st day of life and the changes in the uteri and vaginae were found several months after the last injection. They were not abolished by later removal of the hypophysis, ovaries and adrenals. Even 150 days after ovariectomy, 205 days after the last injection of estradiol, the vaginal epithelium was strongly cornified and, in one such rat, the uterine epithelium had undergone a squamous metaplasia. It seems evident that the proliferation of the epithelial cells of the uterus and vagina in these animals was of atypical nature, independent of estrogen stimulation. The epithelial cells appear to have been affected irreversibly and permanently by estrogen treatment during early postnatal days.

In mice, Gardner (1959), Takasugi, Bern, and DeOme (1962), and Takasugi (1963) induced persistent vaginal cornification by injections of androgen or estrogen for a brief period in early postnatal life which was not abolished by later ovariectomy, adrenalectomy or hypophysectomy. Unfortunately, these workers have not studied the uteri of the mice. The rats and mice appear to respond to early postnatal estrogen treatment in a similar manner, although the mice are more susceptible than the rats.

Summary. In some of rats given injections of estradiol for the first 30 postnatal days, persistent epithelial proliferation, culminating in stratification and cornification, was induced in the uteri and vaginae.
The changes were not abolished by later ovariectomy, or hypophysectomy followed by ovariectomy or ovariectomy plus adrenalectomy. Therefore, they were independent of estrogen stimulation and irreversible.

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**References**


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