157. **Effects of Progesterone plus Estradiol on Vaginal Epithelium Showing Estrogen-Independent Proliferation and Cornification in Neonatally Estrogenized and Androgenized Mice**

By Yasuhiko OHTA* and Taisen IGUCHI**


Female mice given neonatal injections of high doses of estrogen or androgen exhibit estrogen-independent persistent proliferation and cornification of the vaginal epithelium when adult (for review, see Takasugi, 1976). In those estrogenized neonatally, the response of vaginal epithelial cells to estrogen administration is permanently affected, as evidenced by a definite reduction in number of mitotic figures in the epithelium (Mori, 1975). Furthermore, perfect diestrous type vaginal smears do not occur during continued injections of progesterone in a majority of adult mice receiving estrogen or androgen neonatally (Kimura et al., 1967; Takasugi, 1976).

The present studies were undertaken to investigate vaginal response to progesterone plus estradiol in neonatally estrogenized or androgenized, ovariectomized adult mice showing estrogen-independent persistent alterations in the vaginal epithelium.

**Materials and methods.** Female mice of the C57BL/Tw strain were used in these experiments. Two groups of 20 mice were respectively given sc injections of 50 μg estradiol-17β (E) (Group II) and 100 μg 5α-dihydrotestosterone (DHT) (Group III), dissolved in 0.02 ml sesame oil, for 5 days from the day of birth. Twenty other females receiving neonatal injections of oil vehicle only served as controls (Group I). All mice were ovariectomized at 10 days of age. Ten of the mice given either E, DHT or oil neonatally were sacrificed at 60 days of age (Groups Ia, Ila and IIIa). The remaining 10 animals of each group were given daily sc injections of 2 mg progesterone (P) plus 0.01 μg E dissolved in 0.05 ml sesame oil for 5 consecutive days commencing at 60 days of age (Groups Ib, IIb and IIIb) and killed on the day following the last injection. The whole vagina was removed from each animal, fixed in Bouin's solution, and

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cut longitudinally in paraffin at 8 \mu m. The sections were stained by the PAS method for demonstration of mucus.

Results and discussion. In the 60-day-old, ovariectomized control mice, the vaginal epithelium was atrophic, consisting of 2–3 layers of cuboidal cells. By contrast, in mice given E or DHT neonatally (Groups IIa and IIIa) it was markedly proliferated. Superficial layers of the epithelium of the proximal and middle parts of vagina (proximal 1/5 and middle 2/5) were undergoing cornification or parakeratosis in all E-treated and 5 of the DHT-treated mice. The remaining 5 androgenized animals had both mucified and cornified areas on the surface of the epithelium. This is in good agreement with the findings that in mice given androgen neonatally, the vaginal epithelium was proliferated with mucified or cornified superficial layers, whereas, in neonatally estrogenized animals, the epithelium was always proliferated and cornified (Iguchi and Takasugi, 1976). In mice given DHT neonatally, the incidence of vaginal cornification appeared to vary with the age at ovariectomy, the presence of the ovaries for a longer period enhancing proliferation and cornification of the vaginal epithelium (Iguchi, 1976). The epithelium lining the fornico-cervical region was mucified to varying degrees in 4 of the 10 E- and 6 of the 10 DHT-treated mice (Table I). The rest of the animals showed a squamous metaplasia in the epithelium.

As shown in Table I, in the control mice sacrificed on the day following the last P-E injection (Group Ib), the epithelium was mucified throughout the vagina, except for the lower half of the distal vagina (lower 2/5) showing cornification. In E-treated mice killed after P-E injections (Group IIb), the epithelium lining the proximal

<table>
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<th>Group</th>
<th>Treatment</th>
<th>No. of</th>
<th>No. of mice showing mucification in the epithelium of</th>
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<tr>
<td></td>
<td>neonatal</td>
<td>adult</td>
<td>distal part</td>
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<tr>
<td>Ia</td>
<td>oil</td>
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<td>Ib</td>
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<td>P+E</td>
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<td>IIa</td>
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<tr>
<td>IIIa</td>
<td>DHT</td>
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<td>10</td>
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<tr>
<td>IIIb</td>
<td>DHT</td>
<td>P+E</td>
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\(^*\) Mucification took place in the upper half of this region in all mice.

\(^**\) Includes 4 mice showing mucification in only the lower half of this part.
and middle parts of vagina was not mucified in all but 2 animals with some mucified patches over the squamous epithelium in the proximal part, and with superficial cornification or parakeratosis in the middle part. By contrast, the fornico-cervical epithelium underwent mucification in 9 of the 10 E-mice of Group IIb (Table I). Analysis using Fisher's exact probability test revealed that the difference in number of mice showing fornico-cervical mucification between Group IIa and Group IIb mice was statistically significant (p=0.029). In 5 of the 10 DHT-mice sacrificed after P-E injections (Group IIIb), the epithelium of the proximal and middle parts of vagina was proliferated with superficial cornified and mucified patches, as in the 5 DHT-mice sacrificed without P-E injections (Group IIIa). In 4 other DHT-mice of this group, the epithelium lining the lower part of the middle vagina was mucified to varying degrees, the upper middle and proximal parts undergoing cornification or parakeratosis, often with small clusters of mucous cells. The last mouse of this group had fully cornified proximal and middle vaginas. The fornico-cervical epithelium was invariably mucified in all DHT-mice of Group IIIb (Table I). There was no significant difference in number of mice with mucified epithelium in the middle vagina between Groups IIIa and IIIb (p=0.070), while the number of mice showing fornico-cervical mucification was significantly larger in Group IIIb than in Group IIIa (p=0.043) (Table I). After P-E injections, the distal vaginal epithelium of mice given E or DHT neonatally was approximately the same as that in the E- or DHT-mice sacrificed at 60 days of age, showing proliferation with superficial cornification in the E-mice and proliferation without cornification or parakeratosis in the DHT-animals.

These findings appear to indicate that, except for the fornico-cervical region, the response of epithelial cells to P plus E in the vagina was markedly affected by neonatal injections of E or DHT. During or shortly after neonatal injections of E, the so-called B cells form a new epithelium capable of irreversible proliferation and cornification under the initial columnar epithelium in the fornico-cervical region as well as in the proximal and middle parts of vagina of E-mice (Takasugi, 1971; Iguchi et al., 1976). Iguchi et al. (1976) and Ohta and Iguchi, 1976) have pointed out that the distal vagina is not involved in the estrogen-independent vaginal changes induced by neonatal injections of E or DHT. It has not yet been worked out why the epithelium lining the distal vagina of neonatally estrogenized and androgenized mice fails to undergo mucification after P-E injections.

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