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Induction of Estrogen-Independent Persistent Vaginal Cornification in Cyproterone Acetate (CA)-Induced Feminized Male Mice

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Summary. Pregnant ICR/JCL mice were treated with 6 mg of cyproterone acetate (CA) from days 14 to 20 of pregnancy to feminize male offspring. Feminized males delivered on day 20 of pregnancy by cesarean section were castrated the same day, injected with estradiol-17β(E_2) or sesame oil from the day of delivery (=day 1) to day 10 and sacrificed on day 60. In oil-injected feminized males, the vaginal epithelium was atrophic and did not show cornification. In feminized males given 20 μg E_2 neonatally, the vaginal epithelium exhibited well-differentiated stratified squamous organization, but was not cornified in seven out of the nine mice of this group. In the mice treated with 50 μg E_2, persistent cornification was recognized most frequently in the posterior two-thirds of the vaginal epithelium which is considered to originate from the urogenital sinus. However, the incidence of cornification in the anterior one-third which may contain the epithelial cells of müllerian duct was low. These results provide supporting evidence for the possible participation of epithelial cells which come from the urogenital sinus in the development of estrogen-independent persistent vaginal cornification in neonatally estrogenized mice.

Key words: Feminized male mice – Antiandrogen – Neonatal estrogen treatment – Estrogen-independent persistent vaginal cornification.

Introduction

Perinatal treatment of female mice with estrogen induces irresversible proliferation and cornification of the vaginal epithelium, which are not abolished by the removal of ovaries and adrenals – this is referred to as estrogen-independent persistent vaginal cornification (EIPVC) (Takasugi *et al.*, 1970; Kimura, 1975; Takasugi, 1976). If these estrogenized animals were kept alive for a long period, the vaginal epithelium was quite hyperplastic and frequently of a precancerous nature, and could occasionally be transformed into neoplasia (Dunn and Green, 1963; Takasugi and Bern, 1964; Kimura and Nandi, 1967; Forsberg, 1975). Recently, a possible association between intrauterine exposure to diethylstilbestrol (DES) and cancer development of the vagina and cervix in human offspring has been suggested (Herbst and Scully, 1970; Herbst *et al.*, 1971; Tsukada *et al.*, 1972). These evidences

indicate that profound interference with normal epithelial differentiation by exogenous estrogen at the perinatal period may result in irreversible cellular alterations in the female reproductive tracts.

It has been reported that administration of cyproterone acetate (CA), an antiandrogen, to pregnant rats (Neumann *et al.*, 1966; Forsberg *et al.*, 1968) or mice (Suzuki, 1976) inhibits the differentiation of male reproductive tracts and induces the formation of a vaginal anlage in male offspring. In these feminized males, most of the vagina is considered to develop from the urogenital sinus, while only a small anterior part arises from the müllerian epithelium (Forsberg *et al.*, 1968; Suzuki, 1976). This type of vagina appears to be useful in analyzing the embryologic origin of cell populations of the epithelium showing EIPVC as mentioned above. Therefore we attempted to examine whether the vaginal epithelium of CA-induced feminized male mice could show EIPVC following neonatal estrogen treatment as described in neonatally estrogenized females (Takasugi *et al.*, 1970; Takasugi, 1976).

Materials and Methods

Adult females of ICR/JCL mice were mated with males of the same strain. The day on which the vaginal plug was found was designated as day 1 of pregnancy. Pregnant females were injected with 6 mg of CA in 0.05 ml of a mixture of benzyl benzoate and castor oil from days 14 to 20 of pregnancy. Fetuses were delivered on day 20 of pregnancy by cesarean section and reared with the help of foster mothers. Feminized males were castrated on the day of delivery and divided into 3 groups. They were treated once daily for the first 10 days of life with 0.02 ml sesame oil (Group 1), 20 μg estradiol-17β(E_2) in 0.02 ml oil (Group 2), or 50 μg E_2 in oil (Group 3). The animals were sacrificed at 60 days of age. Genitourinary tracts were fixed in Bouin's solution and serial paraffin sections were stained with H & E.

Results

The appearance of the external genitalia of CA-induced feminized males resembled that of normal females. However, the vagina did not have its own outlet and opened jointly with the urethra at the base of the undeveloped phallus. Histologic examination revealed a short vagina developed dorsal to the urethra, which ended blindly in the cranial portion. The separation of the vagina and urethra was distinct in the cranial one-third of the vagina, but, in most cases, it was less distinct in the caudal two-thirds. Because of insufficient development of the urethro-vaginal septum, the vagina opened to or contacted with the urethra at the level of its anterior two-fifths (Fig. 1). However, the epithelia of the vagina and urethra were easily distinguishable by their histological appearance. To examine the effect of neonatal estrogen treatment on the vaginal histology, the vagina in CA-induced feminized males was tentatively divided into three parts; an anterior part – the cranial vagina from the blind end to the level of contact with the urethra, a posterior part – from the level of the anterior end of the bulbourethral glands to the perineum, and a middle part – between the anterior and posterior parts (Fig. 1). The results are summarized in Table 1. In oil-injected feminized males (Group 1), the vaginal epithelium was atrophic, showing 2–3 layers of cells (Fig. 2). Cornification did not take place except at the perineal end of the posterior part. In estrogenized groups, well-differentiated stratified squamous vaginal epithelium developed in all animals of the groups. In two out of 15 feminized males treated with 50 μg E_2 neonatally (Group 3), persistent vaginal cornification occurred in all three parts of the vagina, when the animals were sacrificed 50 days after the cessation of estrogen injections (Fig. 3). In the other 13

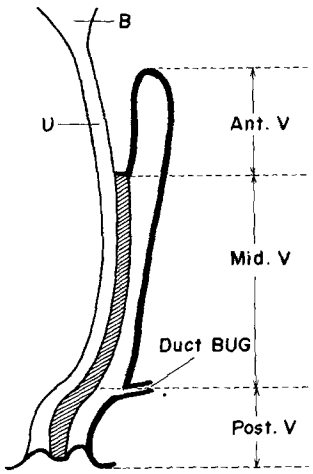
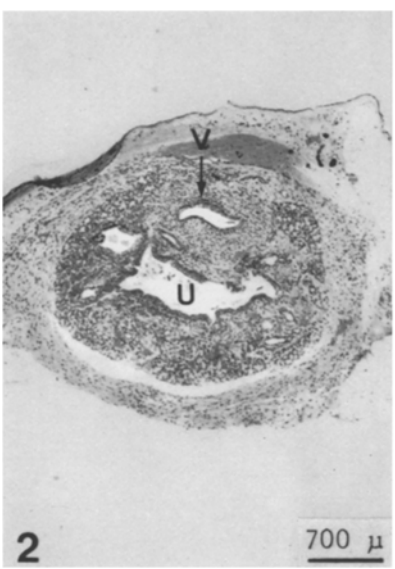


Fig. 1. A schematic drawing of the vagina of CA-induced feminized male mouse. *Ant. V* anterior part of vagina; *Mid. V* middle part of vagina; *Post V* posterior part of vagina; *B* bladder; *Duct BUG* duct of bulbourethral glands. The lined area shows the urethrovaginal septum. In most of CA-induced feminized males, separation between the urethra and vagina was not always complete, because of insufficient development of the urethrovaginal septum.

mice (Group 3), the epithelium of the anterior part showed hyperplasia, but was not cornified. In the middle part of the vagina, various degrees of cornification were recognized in 10 out of the 15 mice of Group 3 (Fig. 4). In these animals, cornification was seen continuously from the middle to the posterior parts of vagina. In three of the remaining five mice in which cornification did not occur in the anterior and middle parts of the vagina, cornification was observed in the posterior part of the



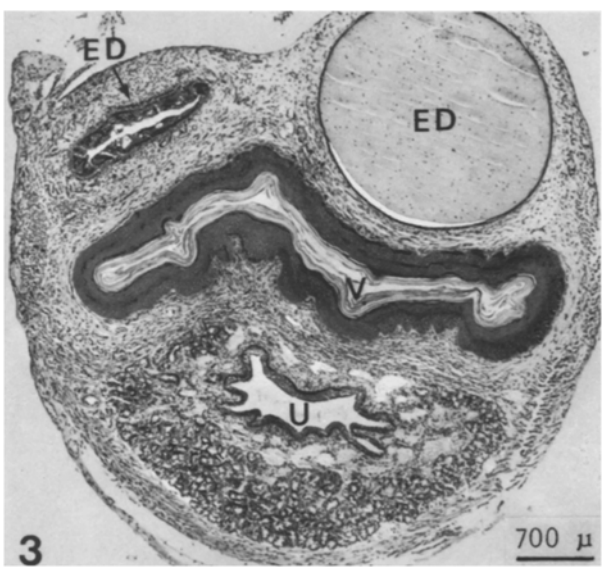


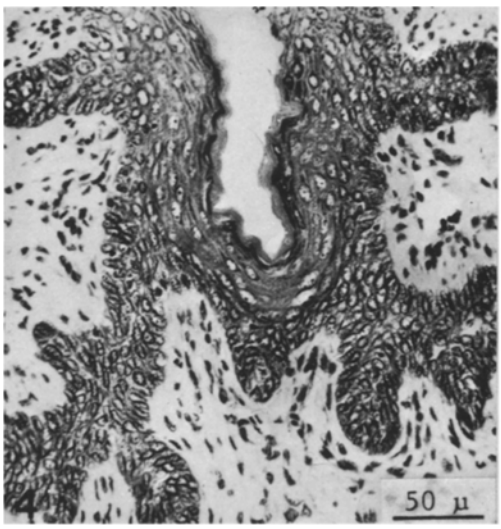
Fig. 2. Cross section of anterior vagina of an oil-treated feminized male. Note atrophic vaginal epithelium. *U* urethra; *V* vagina

Fig. 3. Cross section of anterior vagina of a feminized male treated with 50 μg E_2 neonatally. Note well-differentiated squamous stratification and cornification. *ED* ejaculatory duct or its remnant

Table 1. Effect of neonatal estrogen treatment on vaginal epithelium of CA-induced feminized male mice

Group	Treatment	No. of mice	Histology of vaginal epithelium								
			Anterior part			Middle part			Posterior part		
			A–NC[a]	H–NC[b]	H–C[c]	A–NC	H–NC	H–C	A–NC	H–NC	H–C
1	Neonatal castration + oil days 1–10	5	5	0	0	5	0	0	5	0	0
2	Neonatal castration + 20 μg E_2 days 1–10	9	0	9	0	0	9	0	0	7	2
3	Neonatal castration + 50 μg E_2 days 1–10	15	0	13	2	0	5	10	0	2	13

[a] A–NC = atrophic stratified squamous epithelium, not cornified.
[b] H–NC = hypertrophic stratified squamous epithelium, not cornified.
[c] H–C = hypertrophic stratified squamous epithelium, cornified.



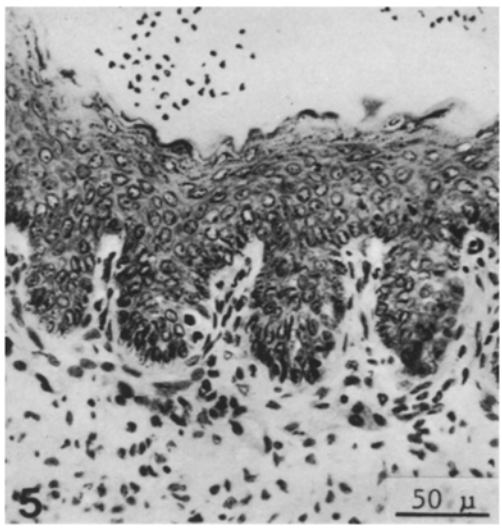


Fig. 4. Vaginal epithelium of middle part of vagina of a feminized male receiving neonatal treatment with 50 μg E_2. Note cornification and epithelial downgrowths
Fig. 5. Vaginal epithelium of middle part of vagina of another feminized male injected with 50 μg E_2 neonatally. Note epithelial downgrowths

vagina. Regardless of the occurrence of cornification, the vaginal epithelium of all Group 3 mice consisted of 7–16 cell layers and showed downgrowths into the stroma (Fig. 5). In the animals injected with 20 μg E_2 neonatally (Group 2), the vaginal epithelium showed considerable squamous stratification consisting of 7–12 layers of cells. However, cornification restricted in the posterior vagina was observed only in two out of nine mice of this group.

Discussion

The results of the present study show that the development of the vaginal anlage induced by prenatal exposure of the male mice to CA was markedly stimulated by neonatal estrogen treatment. Since our feminized males were castrated on the day of birth and autopsied 50 days after estrogen treatment had been stopped, persistent vaginal cornification observed in the mice of Group 3 seems to be estrogen-independent and of a similar nature to that in the females treated with high doses of estrogen during the neonatal days, which show vaginal cornification permanently after ovariectomy and adrenalectomy (Takasugi 1963; Kimura and Nandi, 1967; Mori, 1969). For induction of EIPVC, some strain differences in the response of the vaginal epithelium to estrogen given neonatally have been reported: daily injections of 5 to 25 μg E_2 for 3 to 10 days starting within 3 days after birth were found to produce this condition among several strains (Takasugi *et al.*, 1970). Since 20 μg E_2 for the first 10 days of life was enough to induce irreversible vaginal cornification in ICR female mice (Kimura, personal communication), the vaginal threshold for induction of EIPVC appeared to be higher in CA-induced feminized ICR males.

It has recently been suggested that the embryonic origin of DES-induced vaginal adenocarcinoma in human females is müllerian duct tissue (Herbst *et al.*, 1972; Forsberg, 1972). These adenocarcinomas have often been described as coexisting with vaginal adenosis (Herbst and Scully, 1970; Hill, 1973; Gilson *et al.*, 1973). The condition morphologically similar to adenosis in the human vagina has been reported in the fornicocervical region of mice given DES neonatally (Forsberg, 1975). As for EIPVC, Takasugi (1971; 1976) postulated that the occurrence of a particular cell population (B cells) in the fornicocervical region and the junctional portion of the müllerian and sinus vaginae contributed to the development of EIPVC. These cells showed nodular hyperplasia and squamous metaplastic transformation in response to estrogen given neonatally. Although the occurrence of B cells in the histogenesis of the vaginal epithelium was not examined in our CA-induced feminized males, the fact that persistent cornification occurred in the anterior vagina of two mice of Group 3 could not rule out this possibility, because the anterior part of the vagina is thought to contain epithelial cells of the müllerian duct. (Forsberg *et al.*, 1968; Suzuki, 1976.) However, a high incidence of EIPVC was found restricted to the middle and posterior portions of the vagina in most of the estrogenized mice (Group 3). Therefore, the participation of the urogenital sinus epithelial cells in induction of EIPVC must also be considered, since the epithelial cells of these portions are thought to come entirely from the urogenital sinus (Forsberg *et al.*, 1968; Suzuki, 1976). Further long-term observations will be needed to examine whether these epithelial cells actually contribute to the development of hyperplastic epithelial lesions or tumoral changes in estrogenized feminized males as found in the estrogenized females.

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