# Histochemical Differentiation of the Vaginal Anlage in Cyproterone Acetate-Induced Feminized Male Mice

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It is now accepted that the vaginal anlage of newborn female rats and mice consists of two components. The cranial part is derived from the Müllerian duct (Müllerian vagina), and the caudal part is the vaginal plate which arises from the urogenital sinus (sinus vagina)[1,2,3]. In newborn male rats and mice exposed to cyproterone acetate (CA) prenatally, the formation of the vaginal anlage and feminization of their urogenital tracts have been reported by several investigators[4,5,6]. In these feminized males, the vaginal anlage is also made of two parts which show the structural differences. A short blind tube whose lumen is covered by columnar epithelium occurs cranially to the solid cell cord, which is protruded from the dorsal wall of the urogenital sinus. The former structure is considered to be homologous to the prostatic utricle in the males. It has been stated that glycogen content and distribution of activities of some enzymes such as acid and alkaline phosphatases are different in the cranial and caudal parts of the vaginal anlage in females, depending on their embryonic origin[1,7,8,9]. In the present study, as one step to verify the embryonic origin of the vaginal anlage of CA-induced feminized male mice, glycogen content and enzyme activities of acid and alkaline phosphatase and $\beta$-glucuronidase in the vaginal anlagen of newborn female and feminized male mice were examined histochemically.

## MATERIALS AND METHODS

Mice of the ICR strain were used in the present experiments. Injection of 6 mg of cyproterone acetate (CA) in 0.05 ml mixture of benzyle benzoate and caster oil (1:10) were given subcutaneously to the pregnant mice from days 14 to 20 of pregnancy*. On the day of birth, the offspring delivered by caesarian section. The pelvic parts were removed and transfered immediately to the following histochemical procedures.

*PAS-reaction:* The materials were fixed in Carnoy's solution, neutral formalin and Bouin's solution. Sections cut at 10 $\mu$m in palaplast were stained with the periodic-acid-Schiff (PAS).

*Alkaline phosphatase:* The materials were fixed in chilled acetone for 24 hours. After fixation, the materials were dehydrated, embedded in soft paraffin and sectioned at 10 $\mu$m. The enzyme activity was demonstrated according to the Ca-Co method of Gomori[10]. The sections were incubated for 30 minutes or 1 hour at 37°C.

*Acid phosphatase and $\beta$-glucuronidase:* The materials were fixed in chilled 10% formol-calcium (pH 7.4) solution for 24 hours and then transfered to Holt's hyper gum-sucrose solution for 24–48 hours[11]. For demonstration of acid phosphatase and $\beta$-glucuronidase, the methods of Barka and Anderson[12] and Hayashi *et al.*[13] were employed, respectively. The sections were incubated for 3 to 6 hours at 37°C.

## RESULTS AND DISCUSSION

The distribution of PAS-positive substance and activities of alkaline phosphatase, acid phosphatase and $\beta$-glucuronidase in the vaginal anlage of newborn females and feminized males were summerized in Table 1.

*PAS-reaction:* Since the PAS-positive substance in the epithelium of the

* Insemination was confirmed in the next morning by finding either spermatozoa in the vaginal smears or vaginal plug. The day of which spermatozoa or vaginal plug were found was designated day 1 of the pregnancy.

**Table 1.** Histochemical reaction of the epithelia at the junctinal region of the vaginal anlage in female and CA-induced feminized male mice.

| | Female vagina | | Feminized male vagina | |
|---|---|---|---|---|
| | Müllerian vagina | Sinus vagina | Cranial vagina | Caudal vagina |
| PAS | −(8)* | ++(8) | −(12) | +++(12) |
| Alkaline phosphatase | +(8) | −(8) | +(10) | −(10) |
| Acid phosphatase | ++(8) | +−±(8) | ++(5) | +−±(5) |
| β-glucuronidase | ++(8) | +−±(8) | ++(5) | +−±(5) |

*: No. of animals.

−: Negative reaction, ±: Uncertain reaction, +: Weak reaction, ++: Moderate reaction, +++: Strong reaction.

vaginal anlage decreased remarkably after the saliva-test, it was considered to be chiefly glycogen. In females, a strong PAS-reaction was observed in the vaginal plate (sinus vagina). In the proximal region of the Müllerian vagina close to the vaginal plate, the reaction was almost negative. Thus, a noticeable difference in PAS-reaction was found to form a sharp boundary between the Müllerian and sinus vagina (Fig. 3). In feminized males, the PAS-reaction of the solid cell cord (Fig. 2) of the vaginal anlage which protruded from the dorsal wall of the urogenital sinus was stronger than that of females. In contrast, the epithelium of the cranial part of the vaginal anlage which is homologous to the prostatic utricle was totally negative. This difference of PAS-reaction between the cranial and caudal parts of the vaginal anlage provided a distinct boundary where two different epithelial cells contacted each other (Figs. 1 and 4).

*Alkaline phosphatase:* In females, this enzyme activity was demonstrated weak to moderate in the epithelium of the Müllerian vagina, while it was absent in the sinus vagina. In the feminized males, the distribution pattern of the enzyme activity was similar to that of females. The enzyme activity was also present in the columnar epithelium of the cranial part of the vaginal anlage, and no enzyme activity was found in the solid cell cord (Figs. 5

and 6).

*Acid phosphatase and β-glucuronidase:* In females, these enzyme activities were weak to moderate in the most part of the epithelium of Müllerian vagina, and weak or uncertain in the solid cell cord of sinus vagina (vaginal plate). However, a strong enzyme activity was demonstrated in the epithelial cells in the region where Müllerian and sinus components met each other. In feminized males, these enzyme activities showed a similar distribution pattern to that of females except the eithelium of the cranial part of the vaginal anlage showed more intense than that of Müllerian vagina of females.

In female fetuses and offspring, it has been reported that the activity of alkaline phosphatase is high in the epithelium of the cranial part of the vaginal anlage derived from the Müllerian duct (Müllerian vagina) and low or uncertain in that of the solid cell cord (vaginal plate) derived from the urogenital sinus[8], while, glycogen is abundant in the solid cell cord but almost absent in the Müllerian vaginal anlage[1,7,9]. In the present study, the distribution pattern of PAS-positive substance, alkaline phosphatase, acid phosphatase and β-glucuronidase in the vaginal anlage of feminized males were similar to those of females. Especially, for the distribution of PAS-positive substance and alkaline phosphatase, a clear difference was recognized between the epithelia of the two different embryonic origins in feminized males as well as in females. Therefore, although the size of the vaginal anlage, especially the cranial part, is much smaller in the feminized male mice, the vaginal anlage of feminized male mice appears to have the same origin as that of the females.

Although Neumann *et al.*[4] are of the opinion that the vaginal anlage of CA-induced feminized male rats is entirely of urogenital sinus in origin, present histochemical findings are well consistent with the histological findings of Forsberg *et al.*[5] and the present auther[6] that vaginal anlage of CA-induced feminized males is of dual origin, a small cranial part arising from the Müllerian duct, and a greater caudal part developing from the urogenital sinus.

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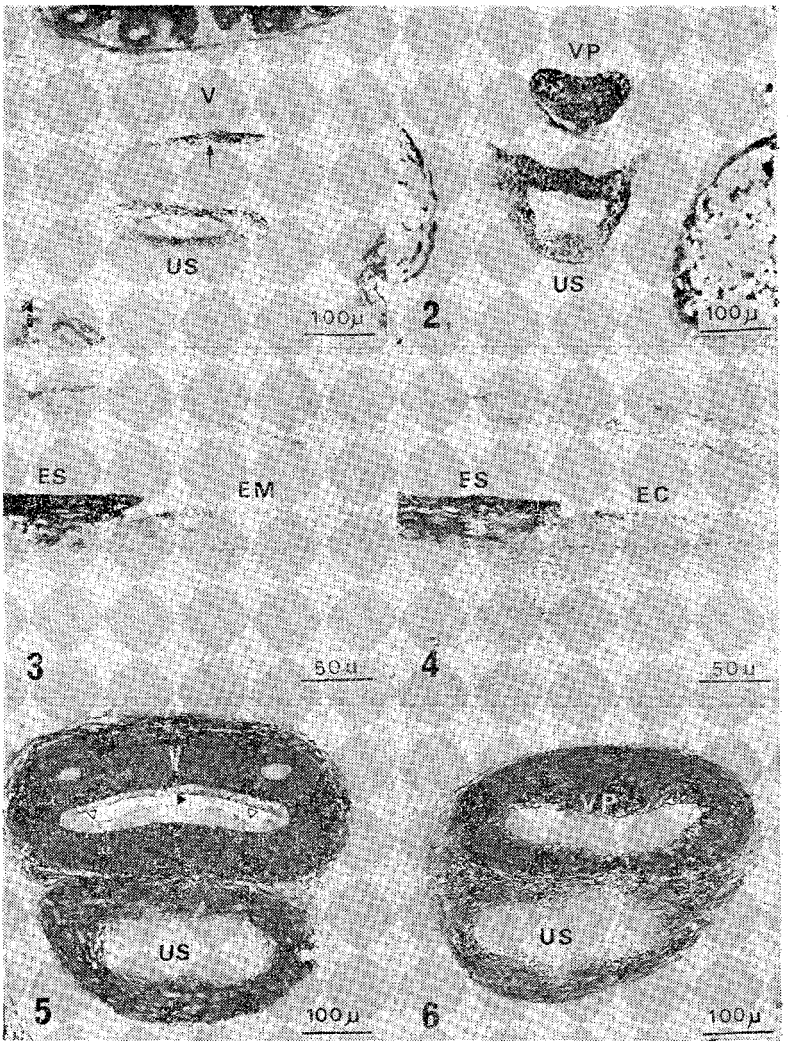
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**Figure legends**

Fig. 1. PAS-reaction in the junction between cranial and caudal parts of the vaginal anlage in a feminized male. PAS-positive region (arrow) corresponds to the caudal part (vaginal plate) of vaginal anlage, but in the cranial part PAS-reaction is negative. Urogenital sinus (US) is also PAS-positive. V; vagina.

Fig. 2. PAS-reaction in the caudal part of vaginal anlage in a feminized male. Vaginal plate (VP) and urogenital sinus (US) are PAS-positive.

Fig. 3. PAS-reaction in a female mouse at the comparable level of Fig. 1. Note PAS-positive reaction in the epithelium of sinus vagina (ES), negative in the Müllerian epithelium (EM).

Fig. 4. A part of the junctional region between the cranial and caudal vaginal anlagen in a feminized male. PAS-positive reaction is only observed in the epithelial cells of the caudal part (ES).

Fig. 5. Activity of alkaline phosphatase in the junctional region of the feminized male vaginal anlage. Strong activity of alkaline phosphatase is visible in the stroma. Note considerable activity in the cranial part (white arrow heads) and no activity in the caudal part (black arrow head). V; vagina.

Fig. 6. Activity of alkaline phosphatase in the vaginal anlage in a feminized male. Note negative alkaline phosphatase in the solid cell cord (vaginal plate, VP) and urogenital sinus (US).