The age-dependent difference in the response of the vagina to the neonatal administration of testosterone propionate in Long-Evans female rats

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Summary. The ability of the vaginal epithelium to respond to testosterone propionate by producing keratin was established when female rats were 3 days of age, whereas the vaginal stroma from rats 2 days old or less was capable of responding to the androgen and might destroy the vaginal epithelium.

It has been shown that in female rats given injections of testosterone propionate (TP) for a short period from the day of birth, the vagina ends blindly under the perineal skin or has a common opening with the urethra[1-3], although precocious opening of the vaginal orifice occurs in female rats given injections of TP at 3 days of age[4-6]. In this investigation, in order to clarify the cause of the age-dependent difference in the effects of the androgen on the developement of vagina, early changes in the vagina in female rats with neonatal treatments of TP and 17β-estradiol (E_2) were examined histopathologically.

New-born female Long-Evans rats were divided into the following groups: I) neonatally intact control rats, II) rats treated with 10 µg of E_2 within 24 h after birth, III) rats treated with 1.25 mg of TP at 0 (IIIa), 1 (IIIb), 2 (IIIc), 3 (IIId) and 5 (IIIe) days of age. A single s.c. injection of each steroid in 0.05 ml of sesame oil was given to each neonatal rat. The perineal regions of all the animals were examined once daily for the existence of the vaginal orifice until they were 100 days old. The pelvic parts of neonatal rats in groups I, II, IIIa and IIId were removed and immediately fixed in Bouin's solution for conventional histological examination and in acetone (4 °C) for alkaline phosphatase staining[7-9], and examined microscopically.

The effects of neonatal administration of sex steroids on the vagina are shown in the table. Although vaginal opening was extremely accelerated in rats with an injection of E_2 at 0 days of age and in rats with an injection of TP at 3 or 5 days of age, the formation of the vaginal orifice was suppressed in rats with an injection of TP at 0, 1 or 2 days of age, compared with neonatal intact control rats. The histologic examination of rats with an injection of E_2 at 0 days of age or TP at 3 days of age revealed the appearence of cells with keratohyalin granules at 3 days, the production of keratin at the center zone of the solid distal part of the vagina at 5 days, and the establishment of the vaginal canal by the fall of the keratinous substance 7 days after the administration of each steroid. On the other hand, keratinization and canalization were not observed in the vaginas of rats with an injection of TP at 0 days of age when they were examined at 0, 1, 3, 5 and 7 days of age. In 7-day-old female rats treated with E_2 at 0 days of age, there was a high activity of alkaline phosphatase in the vaginal epithelium but no activity in the vaginal stroma (fig. 1), while in 7-day-old rats treated with TP at 0 days of age there was a slight activity in the vaginal epithelium, towards the urethra, although the stroma was extremely positive (fig. 2). In 7-day-old rats treated with TP at 3 days of age, there was a high activity in both the epithelium and the stroma (fig. 3). In 7-day-old neonatal intact control rats, there was no activity in the epithelium and the stroma of the solid distal part of vagina.

The results of this investigation clearly show that the vaginal canalization is induced by keratinization of vaginal epithelium in rats treated with TP at 3 days of age as well as in rats treated with E_2 at 0 days of age. According to our findings and other studies[10,11] showing the induction of precocious opening of the vaginal orifice in ovariectomized rats by injections of TP and the incapacity of androgens to

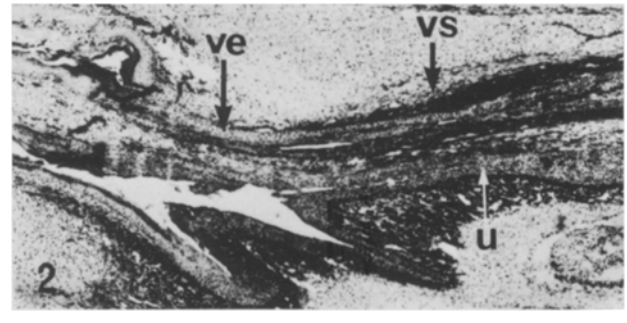

Figure 2. A slight activity in the vaginal epithelium (ve) shifted toward the urethra (u) and a high activity in the vaginal stroma (vs) in 7-day-old female rats treated with TP at 0 days of age. ×40.

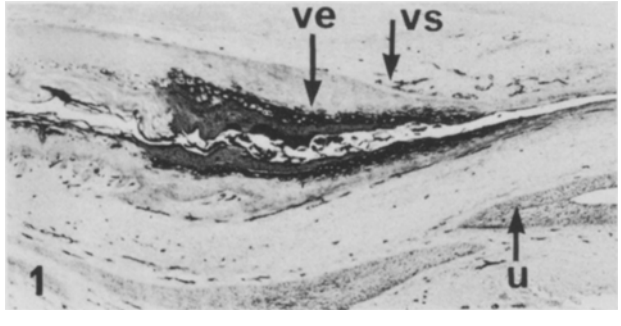

Figure 1. A high activity of alkaline phosphatases in the vaginal epithelium (ve) but no activity in the vaginal stroma (vs) in 7-day-old female rats treated with E_2 at 0 days of age. ×40.

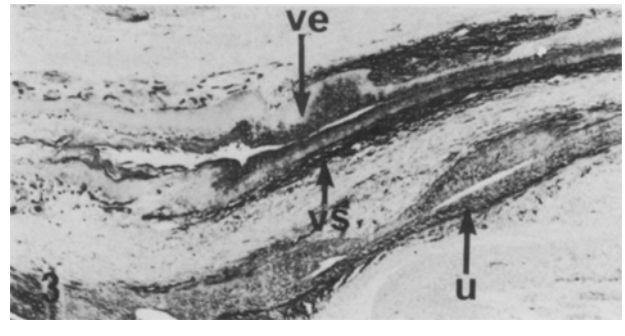

Figure 3. A high activity in both epithelium (ve) and stroma (vs) in 7-day-old rats treated with TP at 3 days of age. ×40.

Effects of neonatal administration of sex steroids on development of vagina in female rats

Groups and treatment		Age (days)	No. of rats in group	No. of rats with vaginal orifice	Age in days at visible vaginal opening (mean ± SD)
Ia	Intact control		17	17 (100.0%)	43.3 ± 4.2
IIa	E_2	0	16	16 (100.0%)	9.1 ± 2.0
IIIa	TP	0	28	0 (0%)[a]	-
b	TP	1	12	0 (0%)[a]	-
c	TP	2	16	2 (12.5%)[a]	36,40
d	TP	3	17	17 (100.0%)	12.9 ± 3.8[a]
e	TP	5	26	26 (100.0%)	18.7 ± 9.8[a]

E_2, 17β-estradiol (10 µg), TP, testosterone propionate (1.25 mg). [a] Differs from I; $p < 0.001$.

produce keratinization, it is reasonable to presume that the cytodifferentiation concerning the converting system of TP to E_2 might be established in vaginal epithelium when females are 3 days of age. The results obtained in this investigation by histochemical methods also show that the stromal cells around the vagina in 0-day rats are capable of responding to TP. On the basis of the general principle that mesenchyme largely controls morphogenesis[12,13] and the finding of an other study on mammary morphogenesis, showing that testosterone-activated mesenchymal cells condense mammary gland epithelium and cause the eventual destruction of mammary epithelium[14], it may be that TP-activated stromal cells around the vagina play an important role in the induction of the atresia of the vagina. However, the detailed process is not clarified. Thus, further studies are necessary.

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Effect of the photoperiod on corpus allatum activity in vitro in the beetle, *Pterostichus nigrita* F.

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Summary. In the carabid beetle *Pterostichus nigrita* reproduction is controlled by photoperiods and the corpus allatum hormone. Corpora allata were incubated in vitro and the release of juvenile hormone was quantified. Short-day conditions induced low activity of the corpora allata; long days, after short-day treatment, stimulate high corpus allatum activity, while long days alone have no effect.

In the carabid beetle *Pterostichus nigrita* sexual maturation is controlled by short-day (SD) and long-day (LD) photoperiods[1,2]. If female beetles are placed in LD conditions immediately after eclosion, the ovaries remain pupal. If the beetles are placed in SD, however, their ovaries develop previtellogenic oocytes but no yolk deposition can be observed. SD periods with less than 15 h of light induce such previtellogenesis (PVG) in more than 50% of the individuals. This stage of PVG can be overcome, when the female beetles are transfered from SD photoperiods into LD. Now, the 2nd step of maturation, vitellogenesis (VG) begins, the oocytes start to grow and the eggs are oviposited. Days with more than 13 h of light induce VG in more than 50% of the SD females. In this way the changing photoperiods from autumn until spring result in an exact timing of reproduction in springtime in Central Europe[2]. However, in this species the photoperiodic reaction is flexible and varies with geographical latitude as demonstrated for a subarctic population[3]. The species used for the reported experiments belongs to the β-form of the 2 biospecies known for *P. nigrita*[4].

The present experiments were designed to analyze the relationship between photoperiodic control and corpus allatum (CA) activity during maturation in *P. nigrita*. In a number of recent experiments, the hormonal basis of this 2-step photoperiodically controlled reproduction has been analyzed[2]. In view of these results the following hypothetical model was tested: Short days bring about low activity of the CA resulting in a low titer of JH in the hemolymph. As a result of this weak hormonal stimulation PVG occurs. Long days following the short-day treatment increase the JH secretion of the CA and eggs are produced. Long days alone do not stimulate the endocrine system.

To demonstrate this 2-step CA-activity, excised glands (CA