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A histological study of the development of the penis of wild-type and androgen-insensitive mice

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INTRODUCTION

The penis of the rat and the mouse possesses skeletal and erectile tissues chiefly comprising the os penis and corpus cavernosum penis (Glucksmann, Ooka-Souda, Miura-Yasugi & Mizuno, 1976; Beresford & Clayton, 1977; Murakami & Mizuno, 1984*a*). The os penis of rats and mice consists of the proximal segment (p-segment) and distal segment (d-segment). The p-segment is formed by fusion of a hyaline growth cartilage in its proximal half and a membrane bone in its distal half. The d-segment is formed initially as fibrocartilage, and, in the case of rats, becomes gradually ossified. Two developmental stages have been demonstrated in the os penis of rats: the first is the mesenchymal condensation which is independent of androgens and observed in both male and female fetuses; the second is the phenotypic differentiation of chondrocytes and osteocytes which is caused by androgens and occurs only in males and androgen-treated females (Glucksmann & Cherry, 1972; Murakami & Mizuno, 1984*a*, *b*; Murakami, 1986). In the clitoris of female mice, however, a membrane bone homologous to the distal half of the p-segment of the os penis is formed (Glucksmann *et al.* 1976). It is not yet known whether the membrane bone in the clitoris of female mice is induced by a low level of androgens in female mice (Pang & Tang, 1984) or whether its differentiation is independent of androgens.

Testicular feminisation (*Tfm*) in the mouse is an X-linked mutation (Lyon & Hawkes, 1970) which results in insensitivity to androgens due to the absence of androgen receptors (Ohno & Lyon, 1970; Attardi & Ohno, 1974). The *Tfm* mice have been used in many studies to demonstrate androgen-target tissues (Kratochwil & Schwartz, 1976; Cunha & Lung, 1978; Lasnitzki & Mizuno, 1980). The present study was undertaken to demonstrate the tissues whose development is regulated by androgens in the penis of mice. These appear to be the os penis, corpus cavernosum penis, corpus cavernosum glandis, corpus cavernosum urethrae, the urethra itself, and the spines on the surface of the glans penis.

MATERIALS AND METHODS

The mice carrying the *Tfm* mutation and *Tabby* (*Ta*) as a coat colour marker were kindly provided by Dr T. Mizuno of the University of Tokyo and Dr K. Moriwaki of the National Institute of Genetics in Mishima. These mice were originally provided for Dr Mizuno by Dr M. F. Lyon. Female mice, heterozygous for the *Tfm* and the *Ta* ($X^{Tfm,+}/X^{+,Ta}$), were mated with male mice ($X^{+,Ta}/Y$). Age of the newborn pups was designated as 0 days old on the day of birth. 28 *Tfm* mice ($X^{Tfm,+}/Y$) and 19 normal male mice ($X^{+,Ta}/Y$) were examined at corresponding ages ranging from 0 to 180 days (Table 1). Male and female mice of the C3H/Tw and C57BL/10SnSlc

Table 1. *Numbers of penes examined at each age*

Age (days)	0	1	3	4	6	7	12	14	21	28	40	56	180
$X^{Tfm,+}/Y$	2	2	3	1	1	2	1	2	3	2	5	1	3
$X^{+,Ta}/Y$	2	2	2	2	2	1	—	2	2	—	3	—	1

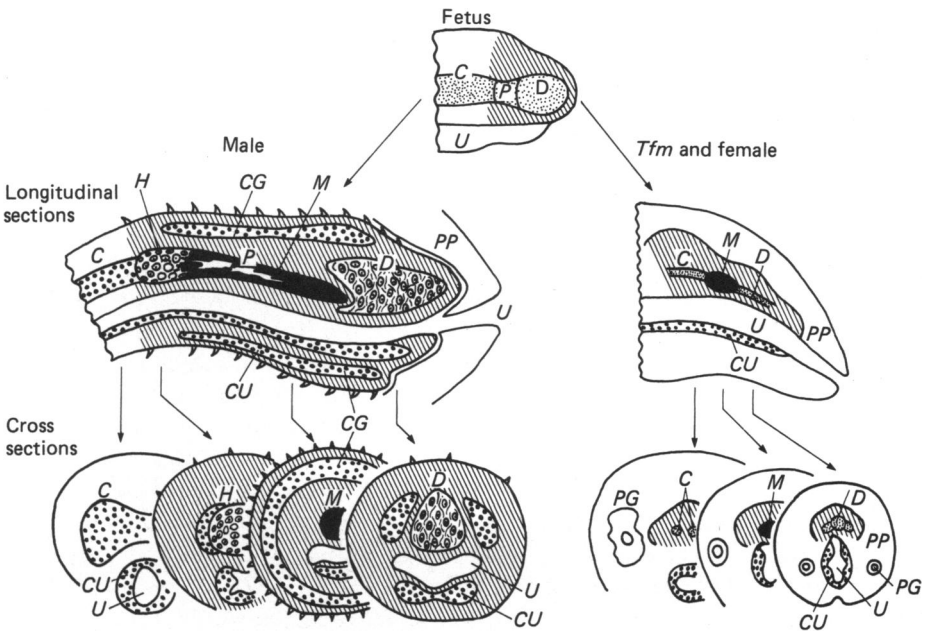


Fig. 1. Schematic illustration of the development of the penis of normal and *Tfm* mice. Large dots, erectile tissue; small dots, condensed mesenchymal cells; cross-hatching, glans penis or clitoridis; *C*, corpus cavernosum penis; *CG*, corpus cavernosum glandis; *CU*, corpus cavernosum urethrae; *D*, distal segment of the os penis; *H*, hyaline cartilage; *M*, membrane bone; *P*, proximal segment of the os penis; *PG*, preputial gland; *PP*, prepuce; *U*, urethra.

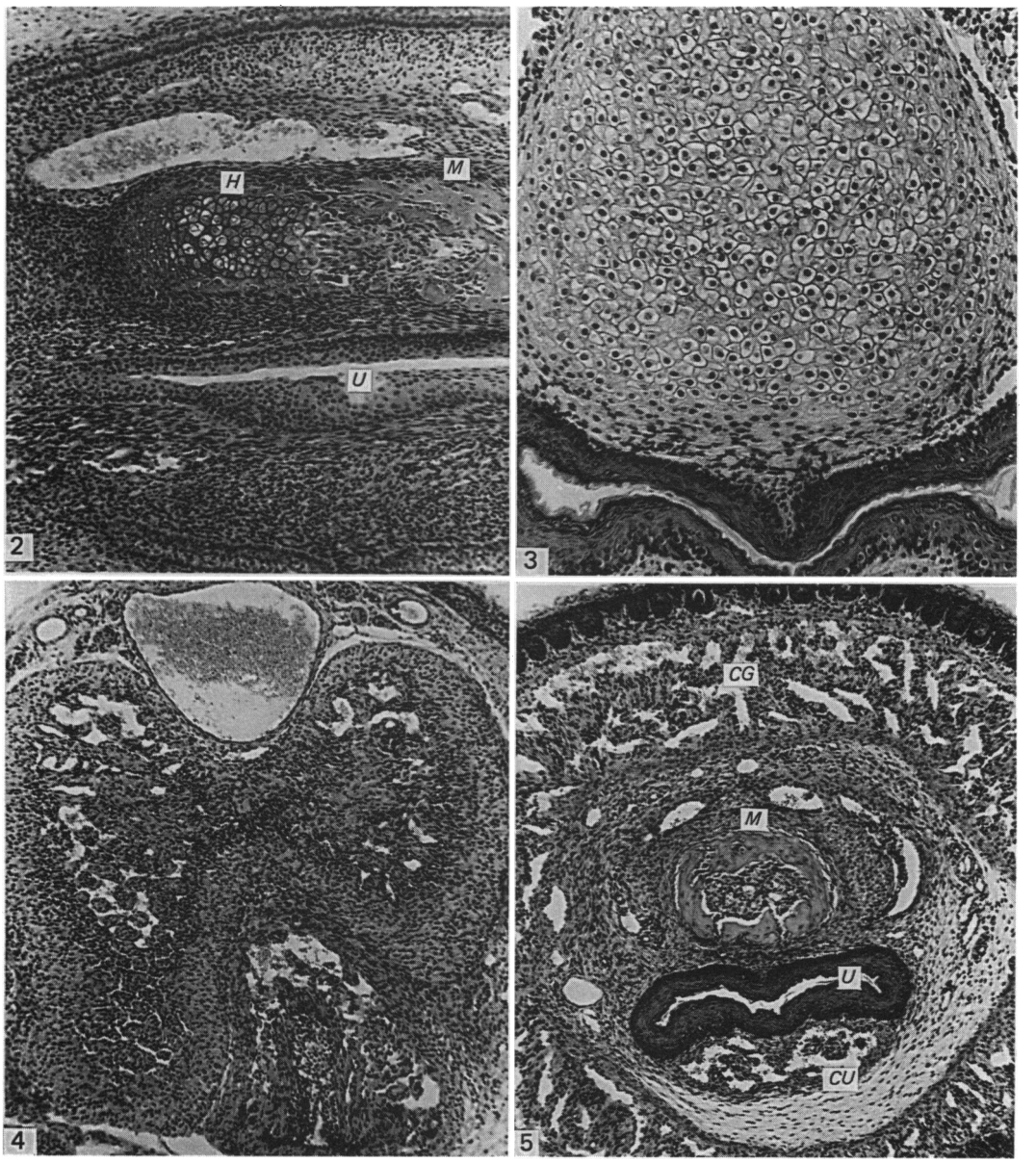
strains were also examined. The animals were anaesthetised with ether and killed by cervical dislocation. The penes were excised and fixed with Bouin's fluid, 10% formalin, or 95% ethanol. Heavily calcified samples were decalcified after the formalin fixation. The fixed penes were embedded in paraffin, sectioned, and stained with alcian blue (pH 1·0) and haematoxylin and eosin.

RESULTS

In this paper, the upper surface of the penis which is near to the abdomen is called 'dorsal side' or 'upper side'. This surface was referred to as the 'ventral side' in a previous paper (Murakami & Mizuno, 1984*a*).

Skeletal and erectile tissues in the penis

An outline of the development of the penis of normal male mice ($X^{+,Ta}/Y$) and *Tfm* mice is schematised in Figure 1. Development of the penis of the C3H and C57BL strains was similar to that of the male carrying the *Ta* except that the latter lacked the preputial glands due to the hemizygous *Ta* (Drews, 1975). The skeletal and erectile



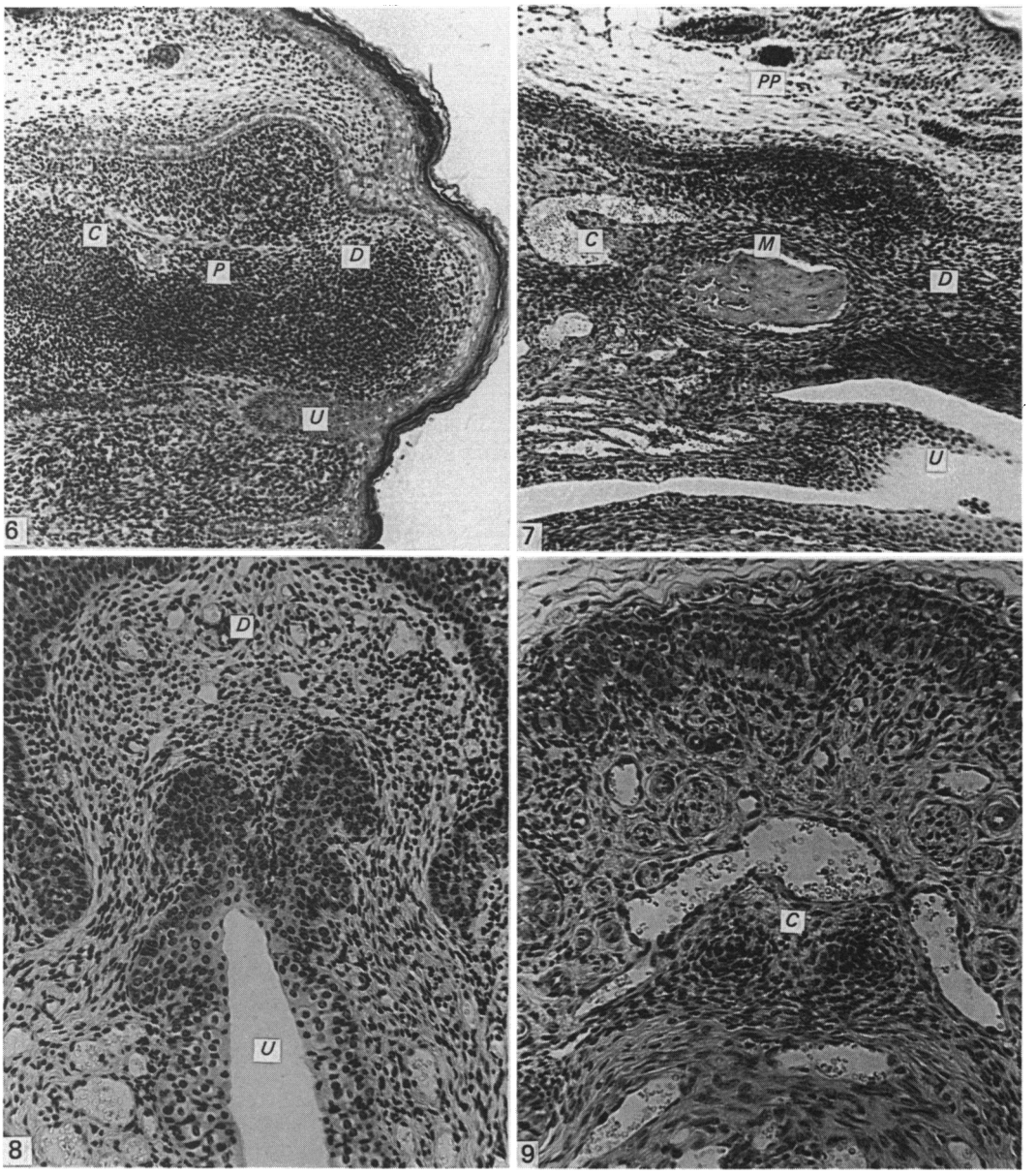
Figs. 2–5. Histological sections of skeletal and erectile tissues in the penis of normal male mice.

Fig. 2. Longitudinal section of the p-segment of the os penis at 4 days of age. The proximal end of the organ is on the left of the Figure. *H*, Hyaline cartilage; *M*, membrane bone; *U*, urethra. × 100.

Fig. 3. Cross section of the d-segment of the os penis at 3 weeks of age. Fibrocartilage has begun to develop. × 160.

Fig. 4. Cross section of the corpus cavernosum penis at 2 weeks of age. × 80.

Fig. 5. Cross section of the glans penis at 2 weeks of age. *CG*, Corpus cavernosum glandis; *CU*, corpus cavernosum urethrae; *M*, membrane bone of the p-segment of the os penis; *U*, urethra. × 80.



Figs. 6–9. Histological sections of skeletal and erectile tissues in the penis of *Tfm* mice.

Fig. 6. Longitudinal section of the penis at 0 days of age. The distal end of the organ is on the right. Rudiments of the p- (*P*) and d-segments (*D*) of the os penis and the rudiment of the corpus cavernosum penis (*C*) are recognisable as condensations of mesenchymal cells. *U*, Urethra. × 100.

Fig. 7. Longitudinal section of the penis at 2 weeks of age. The proximal end of the organ is on the left. *C*, Rudiment of the corpus cavernosum penis; *D*, rudiment of the d-segment of the os penis; *M*, membrane bone of the p-segment of the os penis; *PP*, prepuce; *U*, urethra. × 100.

Fig. 8. Cross section of the penis at the level of the d-segment of the os penis at 2 weeks of age. *D*, Rudiment of the d-segment of the os penis; *U*, urethra. × 210.

Fig. 9. Cross section of the penis at the level of the corpus cavernosum penis. *C*, Rudiment of the corpus cavernosum penis. × 210.

tissues in the penis of normal male mice comprise the os penis, corpus cavernosum penis, corpus cavernosum glandis, and corpus cavernosum urethrae and their development has been partially described by Glucksmann *et al.* (1976); it is similar to that of rats (Murakami & Mizuno, 1984*a*). In the present study of the normal male mouse, the rudiment of the corpus cavernosum penis and the rudiments of the p- and d-segments of the os penis were formed as condensed mesenchymal cell masses in the fetal period. At 1–3 days of age, a membrane bone was formed in the distal half of the rudiment of the p-segment, and a hyaline cartilage was formed in its proximal half. These two components of the p-segment fused immediately and successively grew by endochondral ossification (Fig. 2). The rudiment of the d-segment in the normal male developed into fibrocartilage at 2–3 weeks (Fig. 3). A well developed corpus cavernosum penis (Fig. 4), the corpus cavernosum glandis, and the corpus cavernosum urethrae were formed in the penis of normal males at 1–2 weeks after birth (Fig. 5).

In the *Tfm* mice, the rudiments of the p- and d-segments of the os penis and the rudiment of the corpus cavernosum penis were formed as in the normal male (Fig. 6). At 1–3 days of age, a membrane bone was formed in the rudiment of the p-segment, but the hyaline cartilage in the p-segment and the fibrocartilage in the d-segment of the os penis were not yet formed (Fig. 7), and the cells in these tissues remained undifferentiated as in the fetus (Fig. 8) and could not be recognised in the adult. The cells of the rudiment of the corpus cavernosum penis in the *Tfm* mice remained undifferentiated (Fig. 9), and the corpus cavernosum glandis and the corpus cavernosum urethrae were poorly developed, although blood vessels were abundant in the appropriate regions (Fig. 8).

Urethra, spines on the glans penis, and preputial gland

The urethral epithelium in male and female fetuses was connected to the surface epithelium of the genital tubercle at its ventral (lower) side. In the normal male, the urethral epithelium began to separate from the surface epithelium of the tubercle and its orifice was restricted to the distal end concomitantly with the growth of the glans penis and the closure of the developing prepuce on the ventral side of the penis in the perinatal period (Fig. 10). Pycnotic cell debris which may have resulted from cell death was often observed in the separating region of the urethra (Fig. 11). Morphogenesis of the urethra of the *Tfm* mice was almost similar to that in females but very different from that in the normal males. In the *Tfm* mice, the urethral epithelium remained open on the ventral side of the penis until about 4 days after birth (Fig. 12) and then began to close, accompanying the fusion of the prepuce (Fig. 13). This morphogenetic change in the urethra was not accompanied by growth of the glans, which remained as small as that of the clitoris. Cell death was rarely observed in the separating region of the urethra.

On the skin of the glans penis of the normal males, spines whose appearance resembled the filiform papillae on the tongue began to be formed thickly at about 10 days after birth. These structures consisted of highly keratinised epidermis and mesenchymal cells possessing long cell processes (Figs. 14, 15). The fine structure of the spines is currently under investigation. Such spines were scarcely formed on the glans penis of the *Tfm* mice (Fig. 16) although a few immature spines were sometimes observed.

The preputial glands were formed in normal males of C3H and C57BL strains, but not in males carrying the *Ta* mutation ($X^{+,Ta}/Y$) as described by Drews (1975). In the *Tfm* mice, preputial glands which were smaller than those of normal males were formed.

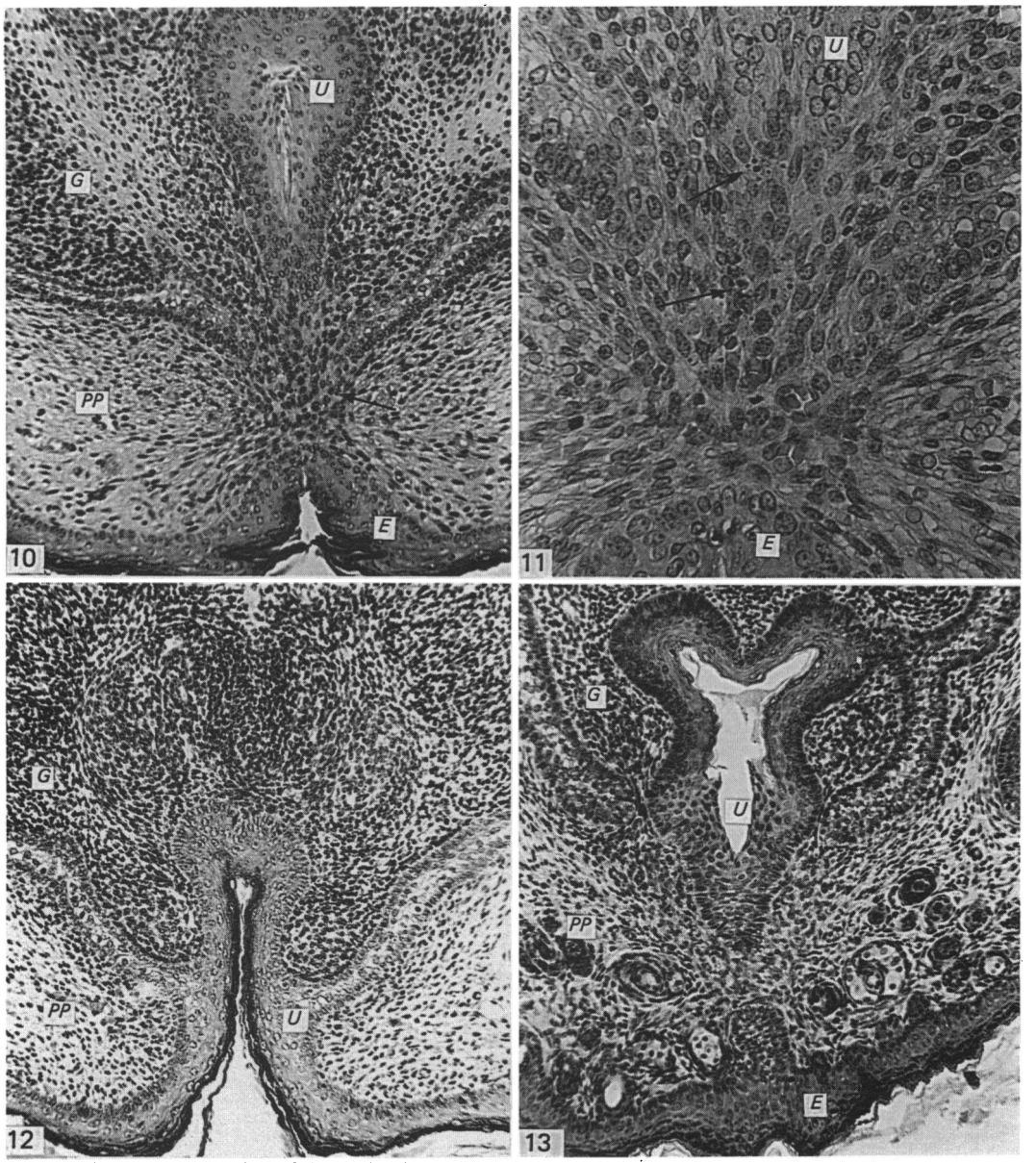


Fig. 10. Cross section of the penis of a normal male at 0 days of age. The urethral epithelium (*U*) is separating from the surface epithelium (*E*) of the penis (arrow). *G*, Glans penis; *PP*, prepuce. × 160.

Fig. 11. Separating region of the urethra in Figure 10. Pycnotic cell debris is present (arrows). *U*, Urethral epithelium; *E*, surface epithelium of the penis. × 410.

Fig. 12. Cross section of the penis of a *Tfm* mouse at 3 days of age. The urethra (*U*) is still open on the ventral (lower) side of the penis. *G*, Glans penis; *PP*, prepuce. × 160.

Fig. 13. Cross section of the penis of a *Tfm* mouse at 1 week of age. The urethral epithelium (*U*) is separating from the surface epithelium (*E*) of the penis. *PP*, Prepuce; *G*, glans penis. × 160.

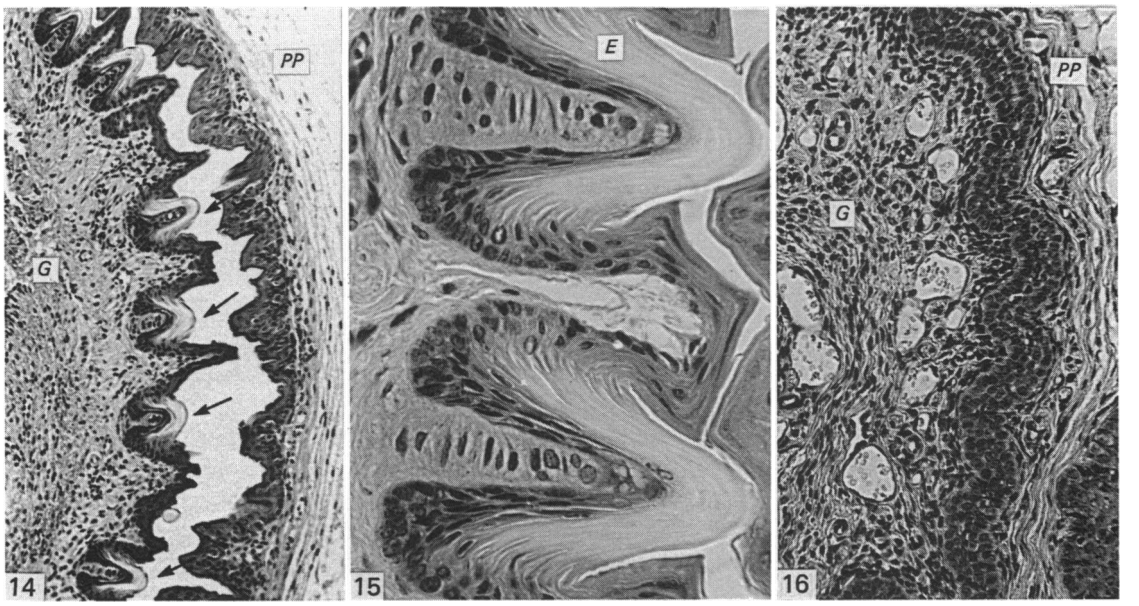


Fig. 14. Skin of the glans penis of a normal male at 40 days of age. Numerous spines (arrows) are formed. *G*, Glans penis; *PP*, prepuce. × 160.

Fig. 15. Spines of the glans penis of a normal male at 6 months of age. *E*, Keratinised epidermis. × 410.

Fig. 16. Skin of the glans penis of a *Tfm* mouse at 40 days of age. The epidermis of the glans penis and the prepuce are still adherent. *G*, Glans penis; *PP*, prepuce. × 210.

DISCUSSION

Androgen-dependency of the development of the skeletal and erectile tissues in the penis

Skeletal and erectile tissues in the penis of rats and mice comprise the os penis, corpus cavernosum penis, corpus cavernosum glandis, and corpus cavernosum urethrae. I have previously demonstrated that the formation of the rudiments of the os penis and corpus cavernosum penis in rats is independent of androgens, and that the phenotypic differentiation of the chondrocytes, osteocytes and erectile tissues in these rudiments is caused by androgens (Murakami, 1986). I also showed that almost all the mesenchymal cells in the genital tubercles of fetal rats possess androgen-binding capacity (Murakami, 1987). The os penis of the normal male mice consists of hyaline cartilage and a membrane bone in the p-segment and fibrocartilage in the d-segment. In this investigation, only the membrane bone developed in the penis of the *Tfm* mice although the formation of the rudiment of the os penis was similar to that in normal males. The membrane bone is also formed in the clitoris of female mice (Glucksmann *et al.* 1976). These results suggest that the formation of rudiment of the os penis in mice is independent of androgens, as in rats, and that the membrane bone of the p-segment of male and female mice can develop independently of androgens in contrast to that of rats. The development of the hyaline cartilage of the p-segment and the fibrocartilage of the d-segment of mice is caused by androgens as in rats. The mechanisms causing the difference in the androgen-dependency of the membrane bone of the p-segment between mice and rats are as yet unknown. These tissues would be useful for a study of the mechanism by which androgens regulate phenotypic differentiation in certain tissues.

The corpus cavernosum penis, corpus cavernosum glandis and corpus cavernosum urethrae were poorly developed in the *Tfm* mice, suggesting that the development of these tissues is also dependent on androgens.

Androgen-dependent morphogenesis of the urethra and skin of the glans

Morphogenesis of the urethra is very different in male and female mice. In normal males, the urethral epithelium separated from the surface epithelium of the genital tubercle at its ventral side in the perinatal period. In androgen-insensitive *Tfm* mice, the urethral epithelium began to separate only after 4–6 days of age. The process of separation of the urethral epithelium from the surface epithelium of the genital tubercle may be accelerated by androgens. Autoradiographic study has demonstrated that the mesenchymal cells around the urethral epithelium are intensely labelled with ^{3}H-androgens in the genital tubercle of rats whose urethral morphogenesis is identical to that of mice (Murakami, 1987). Mesenchymal cells may play an important role in the separation of the urethral epithelium in the same manner as the androgen-dependent regression of the epithelium of the mammary glands in mice (Kratochwil & Schwartz, 1976).

Hairy spines were thickly formed on the skin of the glans penis in normal male mice. Development of these spines is also thought to be dependent on androgens because they were scarcely present in the *Tfm* mice. Similar spines are also formed in male rats, and a preliminary study has shown that such spines can be induced in female rats by neonatal treatment with androgens (In preparation). The spines consist of a highly keratinised epidermis and mesenchymal cells possessing long cell processes. The identity of androgen-target cells in these structures is still unknown. The mesenchymal cells may possibly be the target cells since androgen-binding capacity is demonstrated in almost all the mesenchymal cells in the glans penis but not in the epidermis in rat fetuses (Murakami, 1987). Considering their shape and location in the penis, the spines possibly play some role in mechanoreception in the glans penis.

SUMMARY

Development of the penis of wild-type and androgen-insensitive (*Tfm*) mice was compared histologically to demonstrate possible androgen-dependent histogenesis in this organ. The os penis of the normal males consists of a hyaline cartilage and a membrane bone in the proximal segment and a fibrocartilage in the distal segment. Only the membrane bone of the proximal segment developed in the *Tfm* mice. The corpus cavernosum penis, corpus cavernosum glandis, and corpus cavernosum urethrae developed only to a small degree in the penis of the *Tfm* mice. It is also suggested that the morphogenesis of the urethra and formation of spines on the skin of the glans penis are dependent on androgens.

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