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Development of the os penis in genital tubercles cultured beneath the renal capsule of adult rats

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INTRODUCTION

Marked sexual dimorphisms exist in the mammalian secondary sex organs and many non-genital organs. Concerning the mechanism of sexual differentiation, Jost (1965) demonstrated in mammals that hormonal secretion of the testis causes development of the male phenotype and that the female-like phenotype develops in the absence of gonads, an observation that has been confirmed repeatedly. In some of the secondary sex organs, such as the mammary gland (Kratochwil & Schwartz, 1976) and prostate (Cunha & Lung, 1978; Lasnitzki & Mizuno, 1980), mesenchymal cells play an essential role in the morphogenesis facilitated by androgenic hormones. However, even in these organs, the developmental fate of the mesenchymal cells has not been fully analysed, probably because the mesenchymal cells have few distinctive histological characteristics.

The os penis of the rat is a mesenchymal tissue complex whose differentiation is dependent on androgens, and the cells composing the os penis, i.e. the chondrocytes and osteocytes, have distinct histological characteristics (Glucksmann & Cherry, 1972; Glucksmann, Ooka-Souda, Miura-Yasugi & Mizuno, 1976; Yoshida, Kadota & Fukunishi, 1980; Vilmann & Vilmann, 1983; Murakami & Mizuno, 1984*a*, *b*). Therefore, the os penis is a useful system for the study of androgen-dependent development of mesenchymal cells. In previous papers (Murakami & Mizuno, 1984*a*, *b*), it was proposed that the rudiments of the os penis can be formed without androgens, and that the overt differentiation of chondrocytes and osteocytes of the os penis is caused by androgens. To confirm this proposition and to analyse the factors regulating the development of the os penis, the genital tubercles were cultured beneath the renal capsule of adult male rats. It was found that a culture system involving the intracerebral transplantation of genital tubercles, performed by Beresford & Clayton (1977), was not sufficient for the development of the os penis. *In vitro* organ culture of the genital tubercles was also inadequate for the differentiation of the bones of the os penis, though cartilages of both segments of the os penis were well differentiated (Murakami & Mizuno, 1984*b*). In the present study, the development of the os penis in the genital tubercles of fetal rats cultured beneath the renal capsule of adult male rats or of castrated hosts treated with either androgens, oestrogen, or anti-androgen has been examined. The androgen dependency of the development of the corpus cavernosum penis, which lies proximally to the os penis, has also been investigated.

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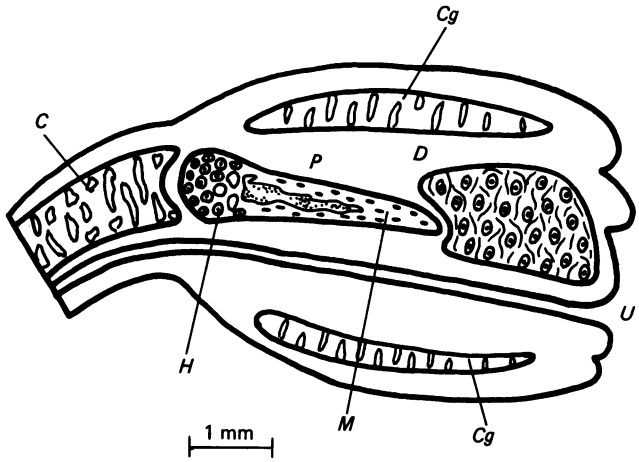


Fig. 1. Schematic illustration of a longitudinal section of the penis of a 4 weeks old male rat. The distal end of the organ is on the right of the picture. *C*, corpus cavernosum penis; *Cg*, corpus cavernosum glandis; *D*, distal segment of os penis; *H*, hyaline cartilage of the p-segment; *M*, membrane bone of the p-segment; *P*, p-segment of os penis; *U*, urethra.

MATERIALS AND METHODS

Animals

Inbred Wistar/Tw rats and inbred Wistar Lewis rats were used for the transplantation experiments. Wistar Imamichi rats were also used for examination of the normal histology of the os penis which is similar to that of Wistar/Tw and Wistar Lewis strains. The animals were mated during the night and copulation was confirmed by the presence of spermatozoa in the vaginal smear next morning. The conceptus was designated to be 0·5 days old at noon of that day.

Transplantation of genital tubercles

Pregnant mothers were killed by cervical dislocation at 16·5, 18·5 and 20·5 days of gestation, and fetuses were removed from the uterus. The genital tubercles were excised from male and female fetuses. The prepuce was removed mechanically with forceps from genital tubercles of 20·5 days old fetuses to prevent cell degeneration caused by anoxia during the culture. The isolated genital tubercles were transplanted beneath the renal capsules of syngenic adult male rats aged 3–8 months. The operation was performed under anaesthesia using 25 mg Nembutal (Abbot Laboratories, Ill., U.S.A.)/kg body weight. The hosts were killed by cervical dislocation at 5–14 days after the transplantation, and the transplants were fixed with 4% neutral formalin, decalcified with formic acid (Morse, 1945), embedded in paraffin, sectioned, and stained with Alcian blue, pH 1·0, and haematoxylin and eosin.

Castration and treatment with hormones

Adult male rats aged 3–8 months were castrated under ether anaesthesia. At 1–2 weeks after castration, genital tubercles of 18·5 days old male and female fetuses were transplanted beneath the renal capsule of the castrated rats. The host animals were injected subcutaneously with various doses of testosterone (Koch-Light Laboratories, England): 25, 50, 250 or 500 μg/kg body weight per day (suspended in 300–500 μl of saline or dissolved in 100 μl of sesame oil) or with dihydrotestosterone (Kasei Chemicals, Tokyo, Japan): 500 or 1000 μg/kg body weight per day

(suspended in 300–500 μl of saline) or with oestradiol benzoate (Schering Co., F.R.G.): 50 μg/kg body weight per day (suspended in 300–500 μl of saline) from the day of transplantation. The hosts were killed by cervical dislocation at 2 weeks after the transplantation, and the transplants were examined as described above.

Genital tubercles of 14·5 and 15·5 days old male and female fetuses in which the rudiments of the os penis had not yet formed were also transplanted into the castrated males and cultured for 10 days. Some of these host animals were treated every day with cyproterone acetate (kindly presented by Dr F. Neumann of Schering A.G., Berlin, to Dr I. Lasnitzki of Strangeways Research Laboratory, Cambridge, U.K.) at a dose of 10 mg/kg body weight (suspended in 150–200 μl of sesame oil) to prevent the androgenic effects which might be caused by non-gonadal steroids. The transplants were fixed in 4% neutral formalin and examined by ordinary histological procedures.

RESULTS

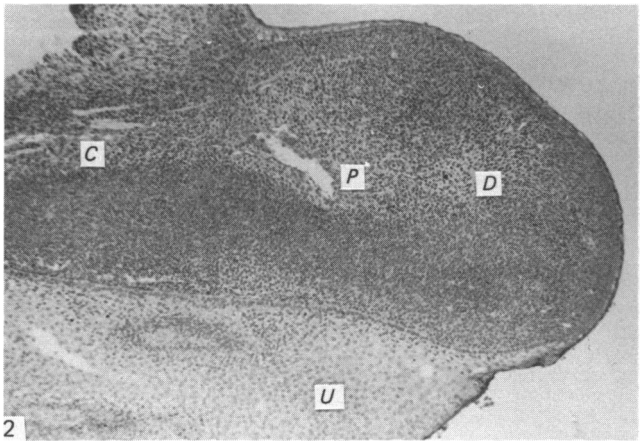
Normal histology of the skeletal tissues in the penis of rats

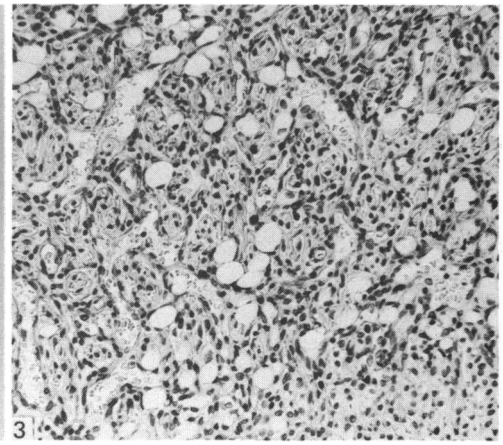
The normal development of the os penis in the rat has been reported in detail in previous papers (Murakami & Mizuno, 1984*a*; Murakami & Mizuno, 1986). In the penis of the rat, the corpus cavernosum penis, the proximal segment (p-segment) of the os penis and the distal segment (d-segment) of the os penis are situated proximodistally in that order (Fig. 1). The rudiments of the os penis and corpus cavernosum penis of the rat are formed as dense mesenchymal cell masses in the genital tubercles of male and female fetuses at 16·5–18·5 gestation days (Fig. 2). These rudiments differentiate into cartilages, bones, and erectile tissue in the penis of the male rat after birth, while in the female, these tissues do not differentiate phenotypically unless the animal is treated with androgens. The rudiment of the corpus cavernosum penis begins to differentiate into an erectile tissue possessing lacunae and trabeculae (Fig. 3) at about one week after birth. The p-segment of the os penis is made of Haversian bone with a hyaline growth cartilage at its proximal end (Fig. 4). The p-segment is formed by the fusion of a membrane bone and a hyaline cartilage within one week after birth, and grows by endochondral ossification. The d-segment of the os penis is a fibrocartilage (Fig. 5) which is formed at about 4–6 weeks after birth and ossifies gradually from 10 weeks after birth.

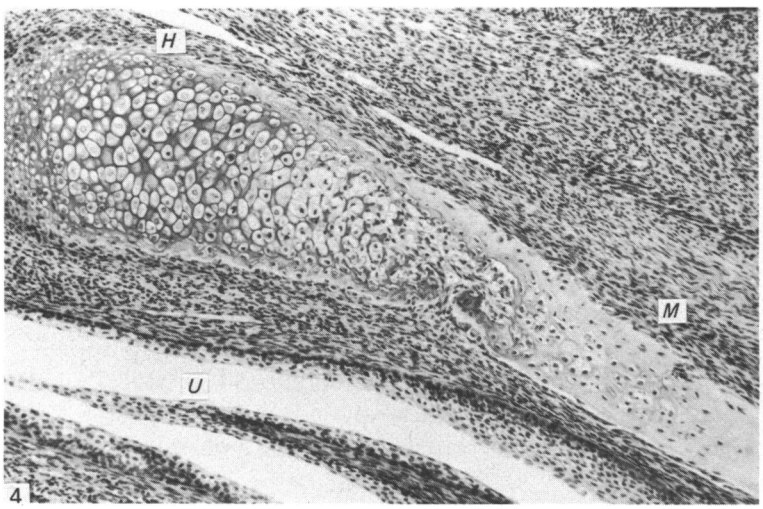
Transplants beneath the renal capsule of adult male rats

The following description does not distinguish the sex of the donors because the developmental fate of the transplants taken from male and female fetuses is almost the same. The terms 'os penis' and 'corpus cavernosum penis' are also used for the homologous tissues which develop in the female transplants unless especially indicated.

In the genital tubercles of 18·5 days old male and female fetuses, the rudiments of the p- and d-segments of the os penis and the rudiment of the corpus cavernosum penis were recognisable as dense mesenchymal cell masses (Fig. 2). When the genital tubercles were cultured in normal adult male hosts, a membrane bone and a hyaline cartilage were formed in the rudiment of the p-segment of the os penis at 8–11 days after transplantation (Fig. 6), and the rudiment cells of the d-segment began to secrete extracellular matrix stainable with Alcian blue, a characteristic sign of chondrogenesis, at 8–11 days (Fig. 7). The rudiment of the corpus cavernosum







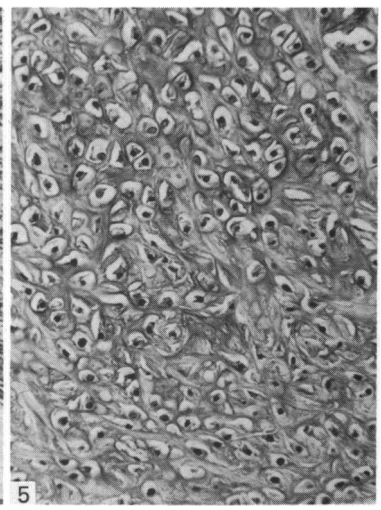


Fig. 2. Longitudinal section of the genital tubercle of a male fetus at 18·5 days gestation. The distal end of the organ is on the right. The rudiments of corpus cavernosum penis (*C*), p-segment of os penis (*P*), and d-segment of os penis (*D*) are recognised as dense mesenchymal cell masses. *U*, urethra. × 66.

Fig. 3. Section of corpus cavernosum penis of a 2 weeks old male rat. × 170.

Fig. 4. Longitudinal section of the p-segment of the os penis of a 1 week old male rat. The proximal end of the organ is on the left. *H*, hyaline cartilage; *M*, membrane bone; *U*, urethra. × 95.

Fig. 5. Section of the d-segment of the os penis of a 6 weeks old male rat. The fibrocartilage has differentiated. × 170.

penis began to form an erectile tissue possessing trabeculae and lacunae at 11 days (Fig. 6). At 14 days after transplantation, endochondral ossification began in the hyaline cartilage of the p-segment (Fig. 8) and a fibrocartilage was formed in the d-segment (Fig. 9); the rudiment of the corpus cavernosum penis developed into erectile tissue (Fig. 8). The proximodistal arrangement of the corpus cavernosum penis and of the p- and d-segments of the os penis seen in the penis of normal male rats was preserved also in the transplants, even though the shape of the transplants became deformed during culture (Fig. 10). The time course of chondrogenesis and osteogenesis in the p- and d-segments of the os penis in the 18·5 days old genital tubercles cultured in normal males is summarised in Table 1. The development of the os penis in 16·5

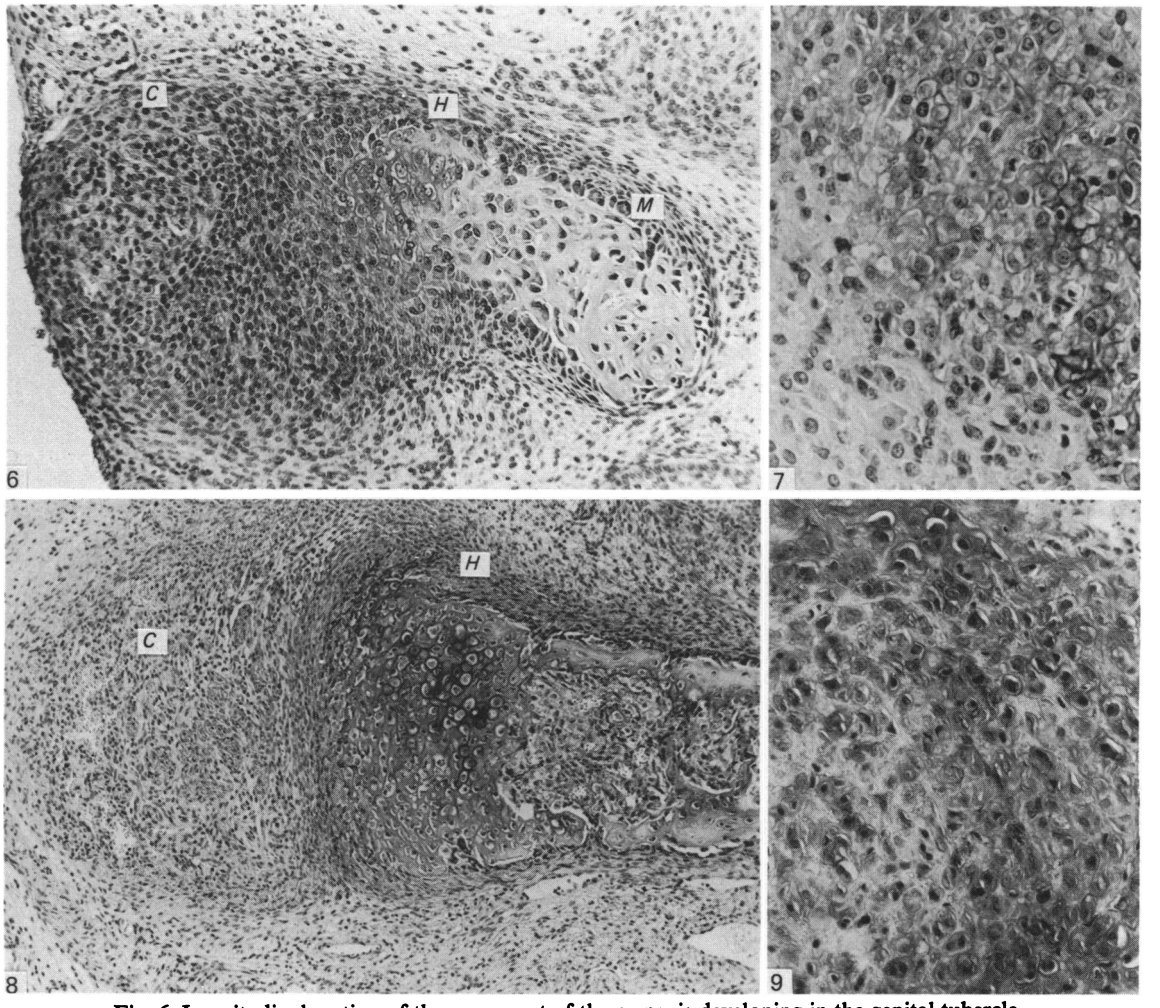


Fig. 6. Longitudinal section of the p-segment of the os penis developing in the genital tubercle of an 18·5 days old fetus cultured in an adult male for 11 days. *C*, corpus cavernosum penis; *H*, hyaline cartilage; *M*, membrane bone. × 150.

Fig. 7. Section of the d-segment of the os penis developing in the genital tubercle of an 18·5 days old fetus cultured in an adult male for 11 days. The mesenchymal cells have begun to secrete Alcian blue-positive extracellular material. × 280.

Fig. 8. Longitudinal section of the p-segment of the os penis developing in the genital tubercle of an 18·5 days old fetus cultured in an adult male for 14 days. *C*, corpus cavernosum penis; *H*, hyaline cartilage of the p-segment. × 100.

Fig. 9. Section of the d-segment of the os penis developing in the genital tubercle of an 18·5 days old fetus cultured in an adult male for 14 days. The fibrocartilage has differentiated. × 210.

and 20·5 days old genital tubercles cultured in adult male hosts was similar to that in cultured 18·5 days old genital tubercles except that mature chondrocytes of the d-segment differentiated in the transplants of 20·5 days old genital tubercles at 11 days after transplantation (Table 1). Since fibrocartilage appears at 4–6 weeks after birth in the d-segment during the normal development of the os penis, the results indicate that the differentiation of the fibrocartilage of the d-segment is accelerated in culture.

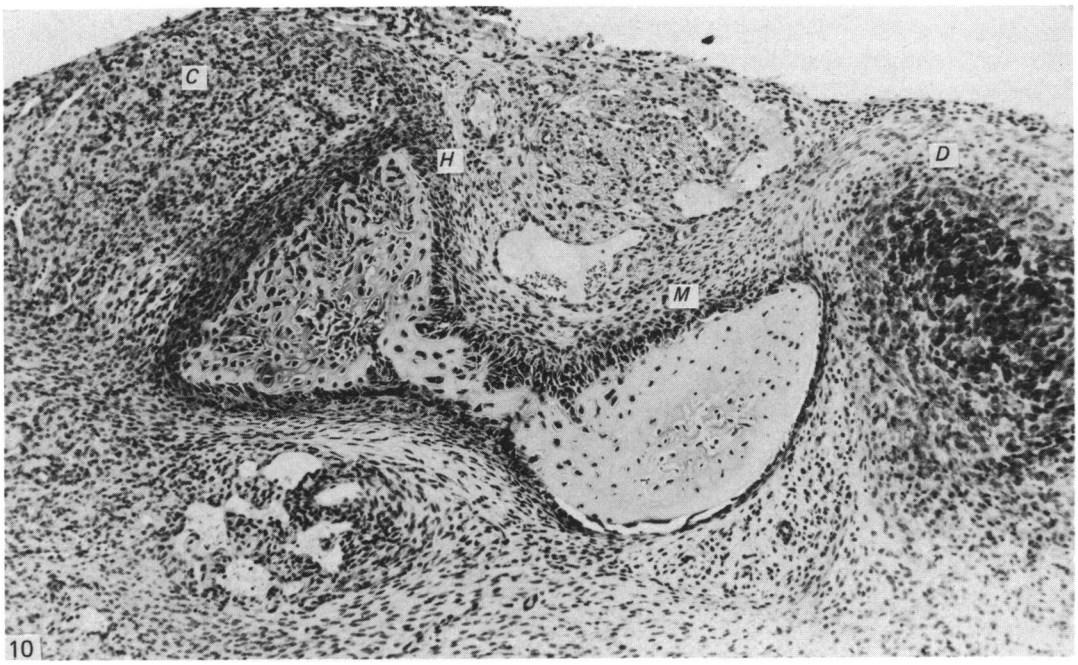


Fig. 10. Longitudinal section of the genital tubercle of an 18·5 days old fetus cultured in an adult male for 14 days. The corpus cavernosum penis (*C*), the ossifying hyaline cartilage (*H*) and membrane bone (*M*) of the p-segment, and the fibrocartilage of the d-segment (*D*) have formed, preserving the proximodistal arrangement. × 110.

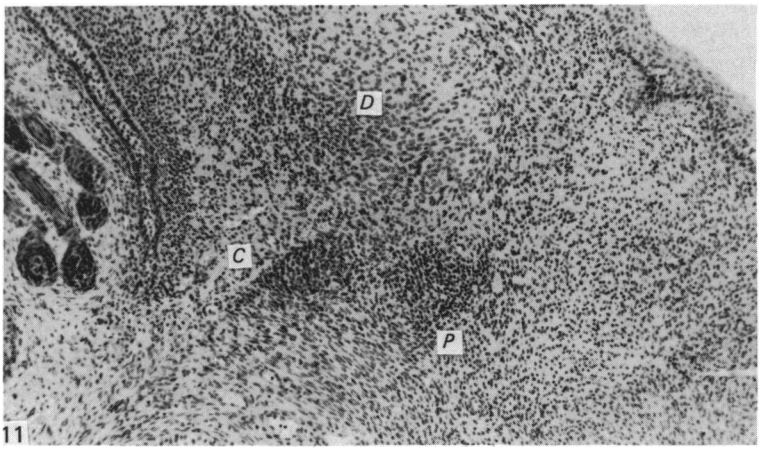
Table 1. *Chondrogenesis and osteogenesis in genital tubercles cultured in adult males*

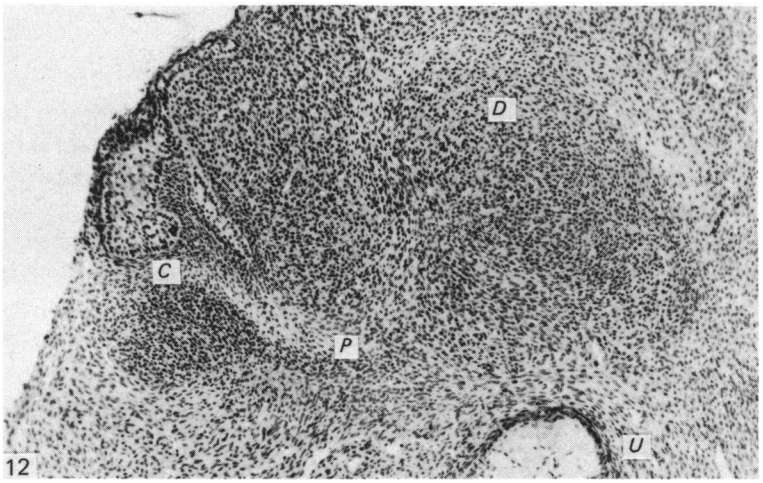
Fetal age of donors (days)	Days after transplantation	Number of transplants	% of the transplants having cartilages and bone		
			p-segment of os penis		d-segment of os penis fibrocartilage
			hyaline cartilage	membrane bone	
16·5	5	6	0	0	0
	8	3	33	67	0
	11	7	29	57	0*
	14	4	75	100	75
18·5	5	4	0	0	0
	8	6	17	67	0
	11	6	83	83	0*
	14	8	88	100	100
20·5	8	7	14	43	0
	11	6	50	100	100

* The rudiment cells began to secrete Alcian blue-positive material.

Transplants beneath the renal capsule of castrated male rats

When the genital tubercles of 18·5 days old fetuses were cultured in castrated hosts treated with or without saline injections, the rudiments of the corpus cavernosum penis and of the p- and d-segments of the os penis remained as undifferentiated mesenchymal cell masses in the transplants even after 14 days of transplantation





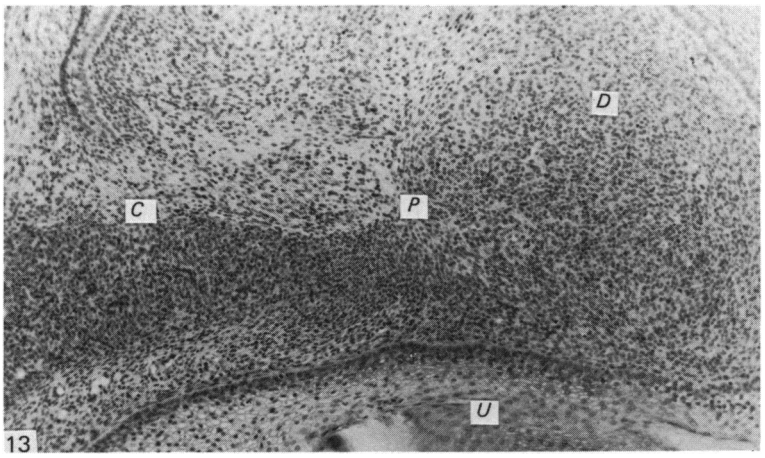


Fig. 11. Section of the genital tubercle of an 18·5 days old fetus cultured in a castrated male host for 14 days. The rudiments of the corpus cavernosum penis (*C*), p-segment of the os penis (*P*), and d-segment of the os penis (*D*) remain undifferentiated. × 100.

Fig. 12. Longitudinal section of the genital tubercle of a 14·5 days old fetus cultured in a castrated male treated with cyproterone acetate for 10 days. The rudiments of the corpus cavernosum penis (*C*), p-segment of the os penis (*P*), and d-segment of the os penis (*D*) have formed. *U*, urethra. × 100.

Fig. 13. Longitudinal section of the genital tubercle of a 15·5 days old fetus cultured in a castrated male treated with cyproterone acetate for 10 days. The rudiments of the corpus cavernosum penis (*C*), p-segment of the os penis (*P*), and d-segment of the os penis (*D*) have formed. *U*, urethra. × 100.

Table 2. *Chondrogenesis and osteogenesis in 18·5 days old fetal genital tubercles cultured in castrated males treated with androgens and oestradiol benzoate*

Hormones	Dose of hormones (μg/kg body weight per day)	Number of transplants	% of the transplants having cartilages and bone		
			p-segment of os penis		d-segment of os penis fibrocartilage
			hyaline cartilage	membrane bone	
None	—	24	0	0	0
Testosterone	25	11	9	55	0
	50	12	0	100	0
	250	6	17	67	0
	500	13	62	92	92
Dihydrotestosterone	500	5	100	100	100
	1000	3	100	100	100
Oestradiol benzoate	50	12	0	0	0

(Fig. 11). In some of the genital tubercles cultured in castrated hosts treated with 25, 50 or 250 μg testosterone/kg body weight per day, the membrane bone and, though with low frequencies, the hyaline cartilage of the p-segment were formed. However, the fibrocartilage of the d-segment did not differentiate. In most of the genital tubercles cultured in castrated hosts treated with a high dose of testosterone (500 μg/kg body weight per day), the hyaline cartilage and membrane bone of the p-segment and the fibrocartilage of the d-segment were formed, and the rudiment of the corpus cavernosum penis developed into erectile tissue. The development of the genital tubercles cultured in castrated hosts treated with high doses of dihydrotestosterone (500 or 1000 μg/kg body weight per day) was similar to that of transplants in hosts treated with a high dose of testosterone. The development of genital tubercles cultured in castrated hosts treated with oestradiol benzoate was similar to that of the transplants in hosts without hormone treatment. The incidence of chondrogenesis and osteogenesis of the os penis in 18·5 days old genital tubercles cultured in castrated hosts treated with hormones is summarised in Table 2.

In the genital tubercles of 14·5 and 15·5 days old fetuses, the rudiments of the os penis and the corpus cavernosum penis were not yet formed. When the tubercles were cultured in castrated hosts treated either with or without cyproterone acetate for 10 days, the rudiments of the os penis and the corpus cavernosum penis were formed in the transplants (Figs. 8, 9), though the os penis and the corpus cavernosum penis did not differentiate phenotypically.

DISCUSSION

The corpus cavernosum penis, the p- and the d-segments of the os penis are situated proximodistally in the penis of the adult rat in that order. The present study demonstrates that the genital tubercles cultured beneath the renal capsule of adult males can form the corpus cavernosum penis, the p- and d-segments of the os penis preserving the normal arrangement, with a time course similar to that in normal males. Therefore, the culture method used in the present study will be useful for further studies on the development of the os penis. The clitoris of normal adult rats has neither bone nor cartilage, and the corpus cavernosum clitoridis is poorly

developed. However, the present investigation has revealed that the cartilage and bone homologous to the os penis and a well developed corpus cavernosum clitoridis can be formed in female genital tubercles when the tubercles are cultured in adult males. The results demonstrate that the potency of chondrogenesis, osteogenesis, and erectile tissue formation in the genital tubercles is equivalent in both sexes.

Overt differentiation of the corpus cavernosum penis and of the p- and d-segments of the os penis are observed in genital tubercles cultured in normal males and in castrated males treated with adequate doses of androgens, but not in castrated hosts without androgen treatment or which are given oestrogen treatment. The results clearly demonstrate that chondrogenesis and osteogenesis of the os penis and overt differentiation of the corpus cavernosum penis are caused by androgens.

The genital tubercles of 14·5 and 15·5 days old male and female fetuses can form the rudiments of the os penis and corpus cavernosum penis even when cultured in castrated hosts treated with cyproterone acetate. Preliminary experiments demonstrate that the progenitor cells of the os penis and corpus cavernosum penis have little androgen-binding capacity in normal fetuses at these stages (R. Murakami, in preparation). Since the testis of the rat secretes very little androgen at this time (Warren, Haltmeyer & Eik-Nes, 1973; Picon, 1976; Habert & Picon, 1984), the rudiments of the os penis and corpus cavernosum penis are thought to be formed independently of androgens.

In the normal development of the os penis, the p-segment is formed within one week after birth and the fibrocartilage of the d-segment begins to form at about 4 weeks after birth (Murakami & Mizuno, 1984*a*) when androgen secretion begins to increase (Corpéchot, Baulieu & Robel, 1981). In genital tubercles cultured in normal adult males or in castrated males treated with high doses of androgens, the p-segment and the fibrocartilage of the d-segment are formed 8–14 days after transplantation, while only the p-segment is formed in transplants cultured for 2 weeks in castrated host animals treated with low doses of testosterone. The results suggest that there is not equal susceptibility to androgens among progenitor cells of the p- and d-segments of the os penis or, at least, that chondrogenesis in the d-segment is accelerated by the high concentration of androgens in mature male host animals.

The levator ani muscle of rodents is another example of tissues of mesenchymal origin whose differentiation is dependent on androgens (Čihák, Gutmann & Hanzlíková, 1970). The levator ani muscle of the rat is present only in the male. The blastema of this muscle is present in the embryos of both sexes but the blastema in the female begins to regress after birth unless the pre- or perinatal females are treated with androgen (Čihák *et al.* 1970). This report on muscle and the results obtained in previous (Murakami & Mizuno, 1984*a*) and the present studies suggest that the processes which lead to sexual dimorphism in the os penis are similar to those in the levator ani muscle of the rat.

SUMMARY

Genital tubercles of male and female rats were cultured beneath the renal capsule of castrated and intact adult male rats treated with androgens, oestrogen, or anti-androgen, and the development of the os penis in the transplants was studied. When the genital tubercles were cultured in normal male hosts, a membrane bone and a hyaline cartilage of the proximal segment of the os penis were formed 8–11 days

after transplantation, and a fibrocartilage of the distal segment of the os penis at 11–14 days. In genital tubercles cultured in castrated males, the rudiments of both the proximal and distal segments remained as undifferentiated mesenchymal cell masses. However, similarly cultured genital tubercles were found to develop cartilages and bone when the hosts were treated with high doses of androgens. The potency of androgen-dependent chondrogenesis and osteogenesis was equivalent in the male and female genital tubercles. Chondrogenesis and osteogenesis of the os penis were caused by androgens, while the rudiments of the os penis were formed independently of androgens. The overt differentiation of the corpus cavernosum penis was also caused by androgens.

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