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Proximal–distal sequence of development of the skeletal tissues in the penis of rat and the inductive effect of epithelium

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SUMMARY

The penis of adult rats comprises the corpus cavernosum penis and the proximal and distal segments of os penis which are situated proximal–distally in this order. Androgens are necessary for the phenotypic differentiation of these tissues. In the present study, genital tubercular mesenchyme of foetal rats was recombined with or without homologous or heterologous epithelia and transplanted beneath the kidney capsule of syngeneic adult male rats, and the development of the corpus cavernosum penis and the os penis in the transplants was examined. Only in the presence of epithelia can the genital tubercular mesenchyme acquire the capacity for differentiation into the corpus cavernosum penis, the proximal segment of os penis, and the distal segment of os penis in this chronological order. The inductive effect of epithelium is a permissive step in the differentiation of the os penis of rat. The epithelium seems to be necessary for the process of rudiment formation of the os penis and the corpus cavernosum penis.

INTRODUCTION

The penis of the rat possesses a skeletal system chiefly comprising the corpus cavernosum penis and the os penis. Mesenchymal cells in the genital tubercle of rat foetuses form the rudiment of the corpus cavernosum penis, the rudiment of the proximal segment (p-segment) of os penis, and the rudiment of the distal segment (d-segment) of os penis in proximal–distal arrangement. In the male rat, the rudiment of the corpus cavernosum penis develops into an erectile tissue possessing lacunae and trabeculae, the rudiment of the p-segment into a membrane bone in its *distal half* and a hyaline cartilage in its *proximal half*, and the rudiment of the d-segment into a fibrocartilage responding to androgens after birth (Fig. 1) (Glucksmann & Cherry, 1972; Beresford & Burkart, 1977; Beresford & Clayton, 1977; Yoshida, Kadota & Fukunishi, 1980; Murakami & Mizuno, 1984*a*,*b*). The chondrogenesis and osteogenesis in the os penis and the erectile tissue formation in the corpus cavernosum penis of rats are dependent on androgens, while the rudiments of these tissues can be formed without androgens (Murakami, in preparation).

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Key words: os penis, epithelial–mesenchymal interaction, chondrogenesis, osteogenesis, androgens, genital tubercle.

Mesenchymal cells in various organs in vertebrate embryos need inductive interaction with the epithelium to differentiate into cartilages and bones when cultured *in situ, in vivo*, or *in vitro*. The inductive effects of epithelium have been reported in the development of the cartilages in limb bud (Saunders, 1948; Gumpel-Pinot, 1972), scleral cartilage (Newsome, 1972), and membrane bone in mandible (Tyler & Hall, 1977). Especially in the limb bud, the progenitor cells of cartilages need the apical ectodermal ridge (AER) to acquire the potency of differentiation, and the chondrogenic potency is acquired first by the progenitor cells of the most proximal part of the future limb, then by the progenitor cells of the more distal parts, that is, in proximal–distal sequence (Saunders, 1948; Summerbell, 1974). In order to understand the processes of skeletal development and the role of epithelial–mesenchymal interactions in this process, it is necessary to determine whether or not development of the skeletal tissues in the penis of rat proceeds in proximal–distal sequence, and whether or not the epithelial–mesenchymal interaction is necessary for these tissues to acquire the potency of differentiation. Male and female genital tubercles transplanted beneath the kidney capsule of adult male rats can form the corpus cavernosum penis, the p- and d-segments of os penis preserving their normal arrangement (Murakami, in preparation). In the present study, the genital tubercular mesenchyme of foetal rats was recombined with or without epithelia and transplanted beneath the kidney capsule of adult male rats, and development of the corpus cavernosum penis and the os penis in the transplants was examined.

MATERIALS AND METHODS

Inbred Wistar Lewis rats and inbred Wistar/Tw rats were used for the transplantation experiments, and Wistar Imamichi rats were also used for study of the normal histology of the

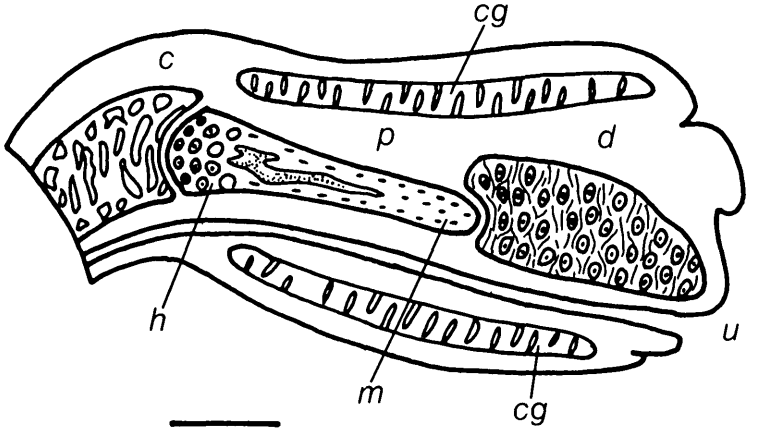


Fig. 1. Schematic illustration of the longitudinal section of the skeletal tissues in the penis of the 4-week-old male rat. The distal end of the penis is on the right, and the proximal part is on the left of the picture. *c*, corpus cavernosum penis; *cg*, corpus cavernosum glandis; *d*, distal segment of os penis; *h*, hyaline cartilage; *m*, membrane bone; *p*, proximal segment of os penis; *u*, urethra.

penis, which was similar to that of Wistar Lewis and Wistar/ Tw 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 as 0·5 days old at 12:00 of this day. Genital tubercles were excised from male and female foetuses at 14·5–18·5 days of gestation, and treated with 0·02% collagenase (Worthington Biochemicals Co., Code CLS) dissolved in saline for 30–50 min at room temperature. Urethral epithelium and surface epithelium of genital tubercles were removed with forceps, and the isolated mesenchyme was washed with 50% foetal bovine serum in saline for 30–50 min at room temperature. For control explants, the surface epithelium was not removed, or was removed and recombined with isolated mesenchyme of the genital tubercle. These two kinds of control explants brought about the same results. The recombined explants were attached to a Millipore filter (pore size 1·2 μm), placed on a stainless steel grid in a small glass dish, incubated in BGJb medium (Biggers, Gwatkin & Heyner, 1961) supplemented with 20% foetal bovine serum and 100 μg ascorbic acid ml^{-1}, and cultured at 37°C for 1 day to ensure adhesion between mesenchyme and epithelium. Explants with or without epithelium were transplanted beneath kidney capsule of syngeneic adult male rats (ages 3–8 months) under anaesthesia of the hosts with 25 mg Nembutal (Abbot Laboratories, Ill., USA) per kg b.w. For examination of the specificity of the epithelial effect, dorsal epidermis and urinary bladder epithelium of 16·5-day foetuses and urinary bladder epithelium of adult rats were isolated by the collagenase treatment and washed with 50% foetal bovine serum in saline and recombined with the isolated genital tubercular mesenchyme of 16·5-day foetuses. The heterologous recombination with the mesenchymes at earlier stages could not be performed because of the technical difficulties. The recombinates were cultured *in vitro* for 1 day, and transplanted in adult males. 2 or 3 weeks after transplantation, the transplants were fixed with neutral formalin, decalcified with formic acid (Morse, 1945), and embedded in paraffin. The sections of the transplants were stained with Alcian blue (pH 1·0)–haematoxylin–eosin. Normal histology of the skeletal tissues in the penis was also studied by the same procedure.

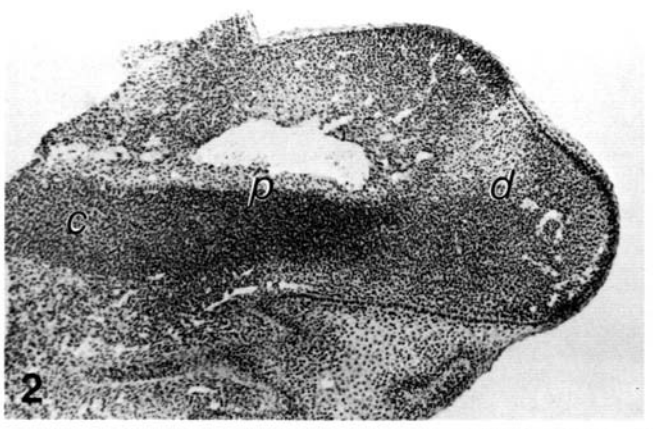
RESULTS

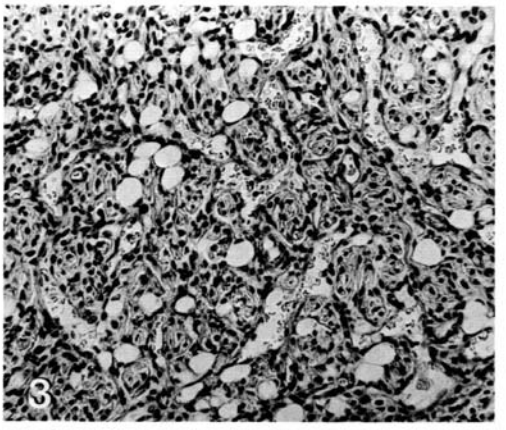
Normal histology of the skeletal tissues in the penis

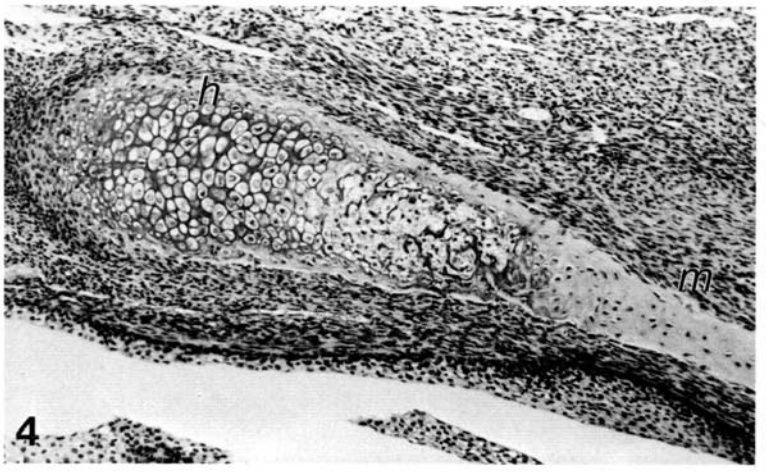
Normal development of the os penis of the rat was described in detail in the previous paper (Murakami & Mizuno, 1984*a*). Briefly, the penis of the rat has the corpus cavernosum penis, the p-segment of the os penis, and the d-segment of the os penis, which are situated proximal–distally in this order (Fig. 1). The rudiments of these tissues are formed as dense mesenchymal cell masses in the genital tubercles of male and female foetuses at 16·5–18·5 days of gestation (Fig. 2). The corpus cavernosum penis is an erectile tissue possessing lacunae and trabeculae (Fig. 3) and begins to be formed at about 1 week after birth. The p-segment of the os penis is a Haversian bone with a hyaline cartilage at its proximal end (Fig. 4). The p-segment is formed by fusion of a membrane bone and a hyaline cartilage within 1 week after birth, and grows successively by endochondral ossification. The d-segment consists of a fibrocartilage (Fig. 5) which is formed at about 4 weeks after birth and ossified gradually from about 10 weeks after birth.

Development of genital tubercular mesenchyme cultivated in recombination with homologous epithelium

We used genital tubercles at five developmental stages: 14·5, 15·5, 16·5, 17·5 and 18·5 days of gestation. At 14·5 and 15·5 days of gestation, neither the rudiment of the corpus cavernosum penis nor that of the os penis was yet formed. At 16·5 days, mesenchymal condensation began in the ventral side of the urethra. At 17·5







Figs 2–5. Normal histology of the skeletal tissues in the penis of the rat.

Fig. 2. Longitudinal section of the genital tubercle of an 18·5-day male foetus. The proximal end is on the left, and the distal end is on the right. The rudiments of the corpus cavernosum penis (*c*), the proximal (*p*), and the distal (*d*) segments of os penis were recognized as dense mesenchymal cell masses. ×64.

Fig. 3. Section of the corpus cavernosum penis of a 2-week-old male. ×170.

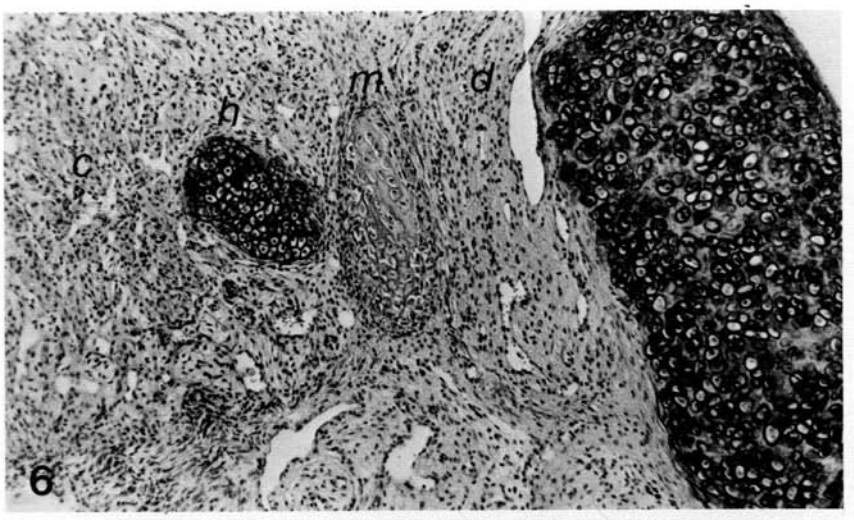
Fig. 4. Longitudinal section of the p-segment of os penis of an 1-week-old male. The proximal end is on the left. The hyaline cartilage (*h*) and the membrane bone (*m*) fused together. Note the beginning of the endochondral ossification. ×94.

Fig. 5. Section of the fibrocartilage of the d-segment of os penis of an 8-week-old male. ×170.

Fig. 6. Longitudinal section of the genital tubercular mesenchyme of a 17·5-day foetus recombined with the homologous epithelium, transplanted in an adult male rat, and cultivated for 3 weeks. The corpus cavernosum penis (*c*), the hyaline cartilage (*h*) and the membrane bone (*m*) of the p-segment, and the fibrocartilage of the d-segment (*d*) of os penis differentiated preserving the arrangement seen in normal males *in situ*. ×110.

Fig. 7. Section of the genital tubercular mesenchyme of a 14·5-day foetus transplanted in an adult male rat and cultivated for 3 weeks. A small erectile tissue is assumed to be a part of corpus cavernosum penis. ×170.

Fig. 8. Section of the genital tubercular mesenchyme of a 15·5-day foetus transplanted in an adult male rat and cultivated for 3 weeks. Only a large erectile tissue of corpus cavernosum penis differentiated. ×230.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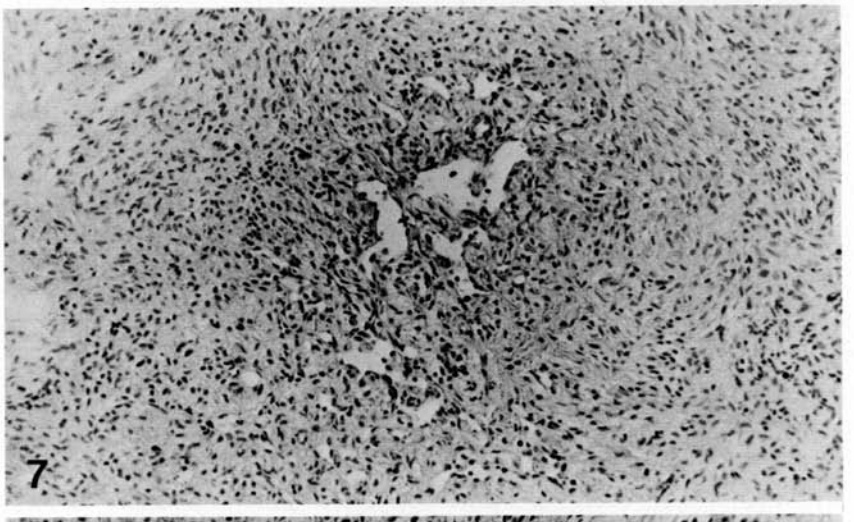
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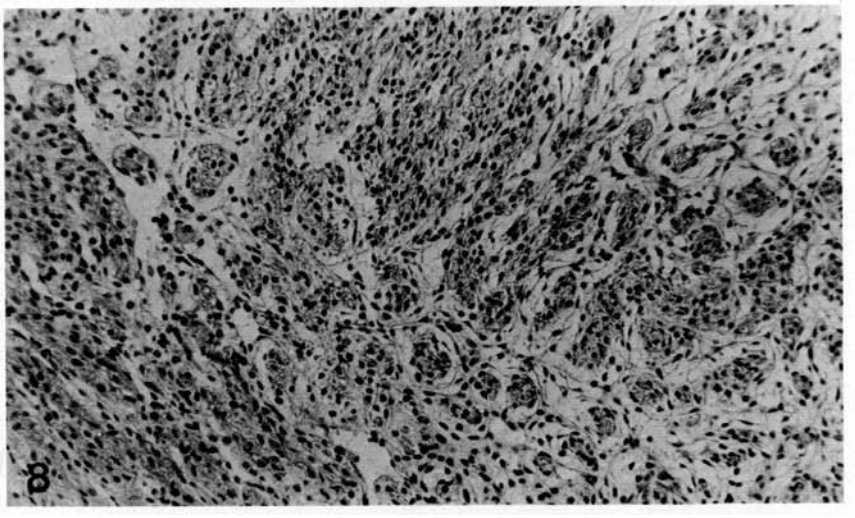
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and 18·5 days, the rudiment of the corpus cavernosum penis and the rudiments of the p- and d-segments of os penis were recognized as dense mesenchymal cell masses (Fig. 2). Since the developmental fate of transplants of both sexes was similar, the following results are described without distinguishing the sex of donors.

The transplants of mesenchyme recombined with epithelium became much larger than those of mesenchyme alone during *in vivo* cultivation. Within 2 or 3 weeks after transplantation, mesenchymes of recombinates taken from all the stages examined formed corpus cavernosum penis, a hyaline cartilage and a membrane bone of the p-segment of os penis, and a fibrocartilage of the d-segment of os penis in the same arrangement as that in normal males (Fig. 6, compare with Figs 1, 2–5). In some transplants, hyaline cartilage and membrane bone of the p-segment fused immediately after differentiation, as seen in normal males, while in other transplants, neither component of the p-segment fused, at least during the course of the experiments.

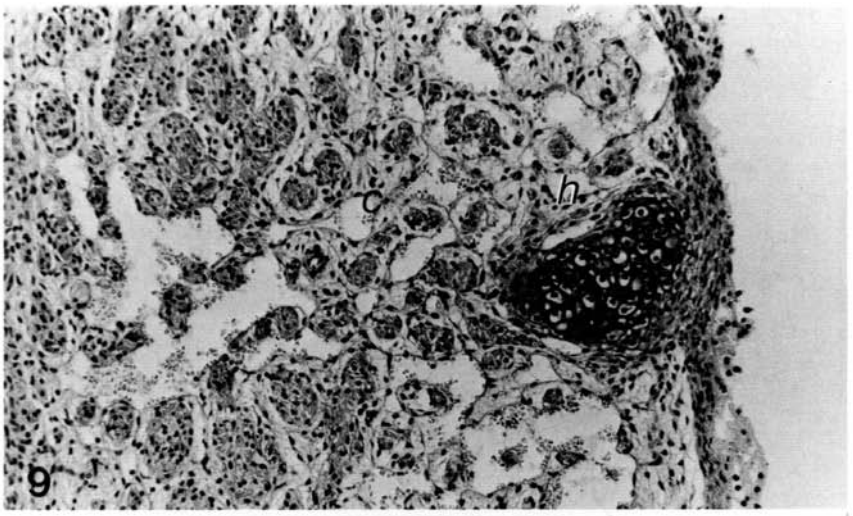
Development of genital tubercular mesenchyme cultured in the absence of the epithelium

The transplanted genital tubercular mesenchyme of 14·5-day foetuses formed only a small erectile tissue assumed to be a part of the corpus cavernosum penis and/or the connective tissue possessing no specialized structure (Fig. 7). The mesenchyme of 15·5-day foetuses formed a well-developed corpus cavernosum penis, while neither the os penis nor its rudiments were formed in transplants (Fig. 8). Mesenchyme of 16·5-day foetuses developed into corpus cavernosum penis and hyaline cartilage of the p-segment of os penis (Fig. 9), and, with a low frequency, membrane bone of the p-segment (Fig. 10), while neither the d-segment of os penis nor its rudiment were formed (Figs 9, 10). These transplants had a histological appearance as if they were truncated at the level of the p-segment of os penis (Figs 9, 10). The developmental fate of transplanted mesenchyme of 17·5- and 18·5-day foetuses was similar to that of mesenchyme recombined with epithelium, that is, corpus cavernosum penis, hyaline cartilage and membrane bone of the p-segment, and fibrocartilage of the d-segment of os penis were formed in the same arrangement as in normal males (Fig. 11). The

Fig. 9. Longitudinal section of the genital tubercular mesenchyme of a 16·5-day foetus transplanted in an adult male rat and cultivated for 3 weeks. Corpus cavernosum penis (*c*) and the hyaline cartilage (*h*) of the p-segment of os penis differentiated. ×170.

Fig. 10. Longitudinal section of another genital tubercular mesenchyme of a 16·5-day foetus transplanted in an adult male rat and cultivated for 3 weeks. Corpus cavernosum penis (*c*), the hyaline cartilage (*h*) and the membrane bone (*m*) of the p-segment differentiated. ×110.

Fig. 11. Longitudinal section of the genital tubercular mesenchyme of a 17·5-day foetus transplanted in an adult male rat and cultivated for 3 weeks. Corpus cavernosum penis (*c*), the hyaline cartilage (*h*) and membrane bone (*m*) of the p-segment, and the fibrocartilage of the d-segment (*d*) of os penis differentiated preserving the arrangement seen in normal males *in situ*. ×110.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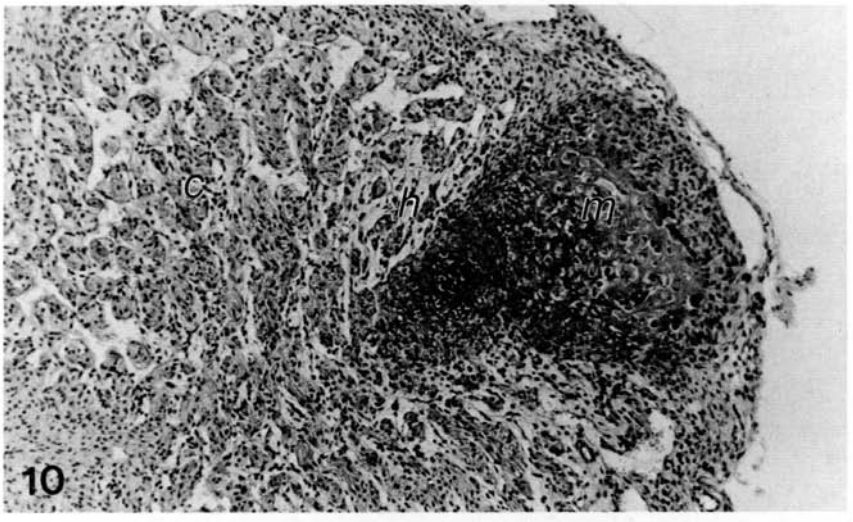
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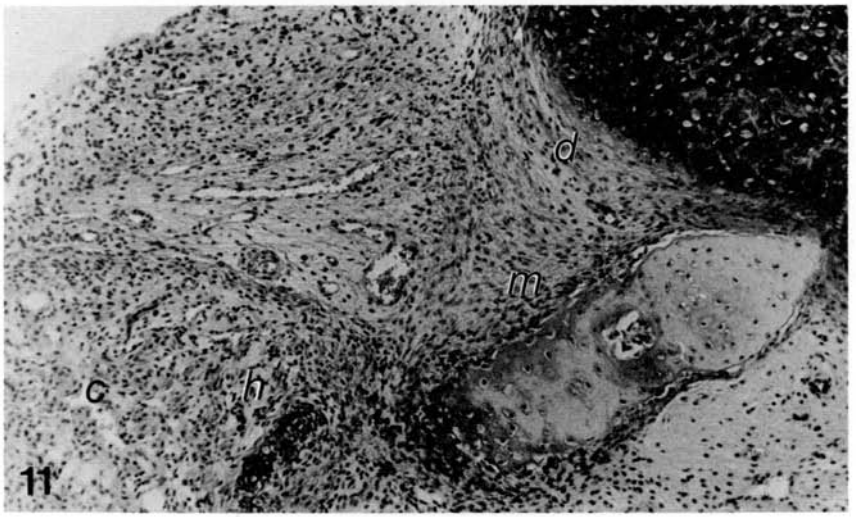
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Table 1. *Development of corpus cavernosum penis (c) and os penis in the genital tubercular mesenchyme recombined with or without homologous epithelium*

Foetal age (days)	No. of transplants	Transplants with differentiated tissues (%)			
			P-segment of os penis		D-segment of os penis
		c	Hyaline cartilage	Membrane bone	Fibrocartilage
		With epithelium			
14·5	14	92	43	79	86
15·5	18	100	39	44	100
16·5	27	100	44	76	100
17·5	17	100	87	82	100
		Without epithelium			
14·5	14	55	0	0	0
15·5	20	100	0	0	0
16·5	31	100	55	10	0
17·5	15	100	93	73	100
18·5	5	100	100	100	100

c, corpus cavernosum penis.

Table 2. *Development of corpus cavernosum penis (c) and os penis in the genital tubercular mesenchyme of 16·5-day foetus recombined with heterologous epithelium*

Origin of epithelium	No. of transplants	Transplants with differentiated tissues (%)			
			P-segment of os penis		D-segment of os penis
		c	Hyaline cartilage	Membrane bone	Fibrocartilage
None	31	100	55	10	0
Genital tubercle of 16·5-day foetus	27	100	44	76	100
Dorsal skin of 16·5-day foetus	11	100	73	18	100
Urinary bladder of 16·5-day foetus	9	100	56	22	78
Urinary bladder of adult	5	100	60	0	100

c, corpus cavernosum penis.

frequency of the differentiation of corpus cavernosum penis, hyaline cartilage and membrane bone of the p-segment, and fibrocartilage of the d-segment of os penis in transplants is summarized in Table 1.

Development of genital tubercular mesenchyme cultivated in recombination with heterologous epithelia

Genital tubercular mesenchyme of 16·5-day foetuses was recombined with a fragment of heterologous epithelia and transplanted. We then examined whether

heterologous epithelia can induce fibrocartilage of the d-segment of os penis. Dorsal epidermis of 16·5-day foetuses, urinary bladder epithelium of 16·5-day foetuses, and urinary bladder epithelium of adult rats induced fibrocartilage of the d-segment with a high frequency. The development of corpus cavernosum penis, hyaline cartilage and membrane bone of the p-segment in these transplants was similar to that of transplants of mesenchyme of 16·5-day foetuses cultured in the absence of epithelium (Table 2).

DISCUSSION

Mesenchymal cells of various organs in vertebrate embryos need inductive interaction with epithelia to differentiate into cartilages and bones when cultured *in situ, in vivo*, or *in vitro* (for a review, see Hall, 1983). The inductive effects of epithelium have been reported in the differentiation of cartilages in limb bud (Saunders, 1948; Gumpel-Pinot, 1972), scleral cartilage (Newsome, 1972), and membrane bone in the mandible (Tyler & Hall, 1977). In these studies, the inductive effects of epithelium were demonstrated by *in situ* (Saunders, 1948), *in vivo* (Newsome, 1972; Tyler & Hall, 1977), and *in vitro* organ-culture experiments (Newsome, 1976; Tyler & Hall, 1977; Gumpel-Pinot, 1980), suggesting that the epithelium also exercises an inductive action during normal development. In some of these experiments, heterologous epithelia are also active to induce cartilages or bones (Gumpel-Pinot, 1972; Hall, 1981). It has been suggested that the action of epithelium on progenitor cells of cartilage and bone is mediated by non-diffusible epithelial cell products (Newsome, 1976; Gumpel-Pinot, 1980; Hall & Van Exan, 1982; Hall, Van Exan & Brunt, 1983; Smith & Thorogood, 1983; Van Exan & Hall, 1984). In spite of these studies, the role of epithelium in normal development of cartilages and bones is not established yet, since in some culture conditions epithelium inhibits chondrogenesis of mesenchymal cells (Solursh, Singley & Reiter, 1981; Solursh *et al.* 1984).

In the present study, we demonstrated that progenitor cells of the corpus cavernosum penis and the os penis also need the presence of epithelium to acquire potency of differentiation. Epithelium is necessary only in the stages before 17·5 days of gestation. At about 17·5–18·5 days of gestation, all the rudiments of the corpus cavernosum penis, the p-segment of os penis, and the d-segment of os penis became clearly recognizable as dense mesenchymal cell masses (Murakami & Mizuno, 1984*a*). As described in Results, neither the cartilages and bone of the os penis nor even the rudiments of these tissues were formed when genital tubercular mesenchymes of the 14·5- to 16·5-day foetuses were cultured in the absence of epithelium. Androgens are necessary for phenotypic differentiation of the cartilages and bone of the os penis and the corpus cavernosum penis, while the rudiments of these tissues can be formed without androgens (Murakami, in preparation). These results strongly suggest that epithelium is necessary for the formation of the rudiments, which are recognizable as dense mesenchymal cell masses, that the rudiment formation is an indispensable step in the differentiation

of the os penis and the corpus cavernosum penis, and that androgens cause the phenotypic differentiation of the rudiment cells. The epithelium may also stimulate the proliferation of the mesenchymal cells, since mesenchymes cultured with epithelium became much larger than mesenchymes cultured without epithelium. The heterologous epithelia, such as foetal dorsal epidermis and urinary bladder epithelium of foetal and adult rats, could induce the d-segment of os penis, but they could not promote differentiation of the membrane bone of the p-segment as markedly as did homologous epithelium. The epithelial–mesenchymal interaction in heterologous recombinates might be incomplete. In any case, the inductive effect of epithelium on genital tubercular mesenchyme seems to be a permissive one, because all heterologous epithelia examined could induce differentiation of the d-segment of os penis in the mesenchyme.

In the development of chick limb bud, removal of AER at a certain stage causes truncation of the limb at a particular level: the later the removal, the more distal the truncation level (Saunders, 1948; Summerbell, 1974). From the results of AER-removal experiments and carbon-marking experiments, Saunders concluded that future wing parts are laid down in proximal–distal order and that AER is essential in this process. In the genital tubercular epithelium of rats, no structural resemblance to AER was found, and it is unknown yet how the progenitor cells of the corpus cavernosum penis and os penis are developed and arranged spatially. However, removal of the genital tubercular epithelium at a certain stage causes truncation of the skeletal tissues in the penis at a particular level, as described in Results: the later the epithelial removal, the more distal the truncation level. From the phenomenal similarity between the response of the limb bud and that of the genital tubercle to epithelial removal, a common process of development is supposed to exist in both the organs: the progenitor cells of the mesodermal tissues acquire differentiation potency in proximal–distal sequence, and epithelium is essential for progenitor cells to acquire this differentiation potency.

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