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Intracerebral transplantation of the genital tubercle in the rat: the fate of the penile bone and cartilages

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INTRODUCTION

The mammalian penis resembles a limb in protruding from the trunk, being covered with epidermis, and in most species, being stiffened with bone, and sometimes cartilage as well (Burt, 1960). However, unlike limb buds, the genital tubercle has not been transplanted with the object of testing factors influencing skeletal development (cf. Fell, 1956; Felts, 1961; Reynolds, 1972). Crouse (1956) grafted limb buds into the brains of young rats, and the mesenchyme differentiated into cartilage which was partly replaced by endochondral bone.

The genital tubercle of the newborn male rat contains several blastemata, including one for the penile bone that appears a day or two later (Ruth, 1934), and a more distal one for a separate fibrocartilaginous anterior process which itself undergoes partial ossification after 2 or 3 months (Beresford, 1970). At birth, the female rat tubercle is of similar size, but bone and fibrocartilage do not develop in the clitoris (Wiesner, 1934) unless the newborn pup is given testosterone (Glucksmann & Cherry, 1972).

The transplantation of genital tubercles from newborn male and female rats into the brains of infant rats described in the present paper hoped to answer the following questions. Will a penis of normal form grow from a transplanted male tubercle? If bone forms, will it acquire the secondary, adventitious (Murray, 1957) hyaline growth cartilage that provides for its further longitudinal growth (since movement is apparently needed for such secondary cartilage to form, at any rate in relation to avian membrane bones (Hall, 1968; Murray & Drachman, 1969) while the grafted tubercle should be relatively immobile in the brain)? Will the fibrocartilaginous anterior process differentiate, even if the bone does not? If it did it would be an exception to the proximo-distal sequence noted in the differentiation of skeletal elements (e.g. in avian limbs, Wilde, 1950), and would go against the tendency for progenitor cells on the outside of established bones to become osteoblasts rather than chondroblasts when transplanted to less 'functional' environments such as the brain (Meikle, 1973, 1975).

MATERIALS AND METHODS

Sprague-Dawley rats were taken from the mother within 24 hours of birth and killed by decapitation after cooling to immobility in a freezer. The region of the genital tubercle was swabbed with 70% ethyl alcohol. Instruments and glassware

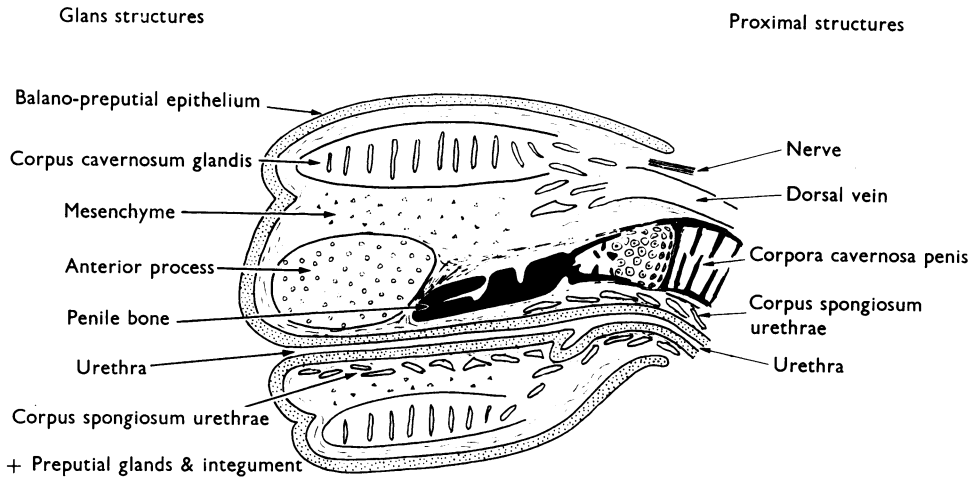

Fig. 1. This diagram depicts the structures within, and proximal to, the glans penis, sectioned longitudinally in the midline. Rat 7–10 days old.

were boiled. The manner of excision of the tubercle was changed during the experiment, and the findings for the resulting two groups will be given separately. In the first group the tubercle was grasped with forceps, pulled away from the body, and cut across close to the body wall without removing the skin. The tubercle was left sitting on a wound until ready for transplantation.

In the second group, first of all most of the skin was cut from the tubercle, and then the denuded tubercle was held while the dense, fused corpora cavernosa penis (corpus fibrosum) (Fig. 1) were cut through near the pubic symphysis. The skinned tubercle was placed in a boiled 0·9 % solution of sodium chloride containing ampicillin and some suspended methyl testosterone. Methyl testosterone has a low solubility in water, but its very fine particles adhered to the transplant during the 5–10 minutes needed to prepare the host. The sex of the donor was gauged from the distance of the tubercle from the anus, and confirmed by examination of the gonads and internal genitalia.

Each host was a suckling rat, aged 1–8 days, sexed by inspection of its tubercle–anus distance, and anaesthetized by cooling. The scalp was swabbed with alcohol and cut in the midline. In the left parietal bone two parallel sagittal cuts were made, 5 mm long and 2 mm apart, with the tip of a scalpel blade. A third cut was made across the anterior ends of the previous cuts, making a flap of bone which could be bent upwards and later pushed back into place again after thrusting the tubercle deeply downwards and laterally into the brain with forceps. The skin wound was closed with one or two metal clips. In seven hosts the transplants were placed instead under the skin of the back in the lumbar region. All female and some male hosts were injected once, subcutaneously between the scapulae, with 0·05 ml of a solution of 1 g testosterone propionate in 20 ml corn oil (dose = 2·5 mg).

When the hosts had warmed up and were moving about, they were returned to the mother. After 2–24 days each host was killed by inhalation of chloroform. A cut was

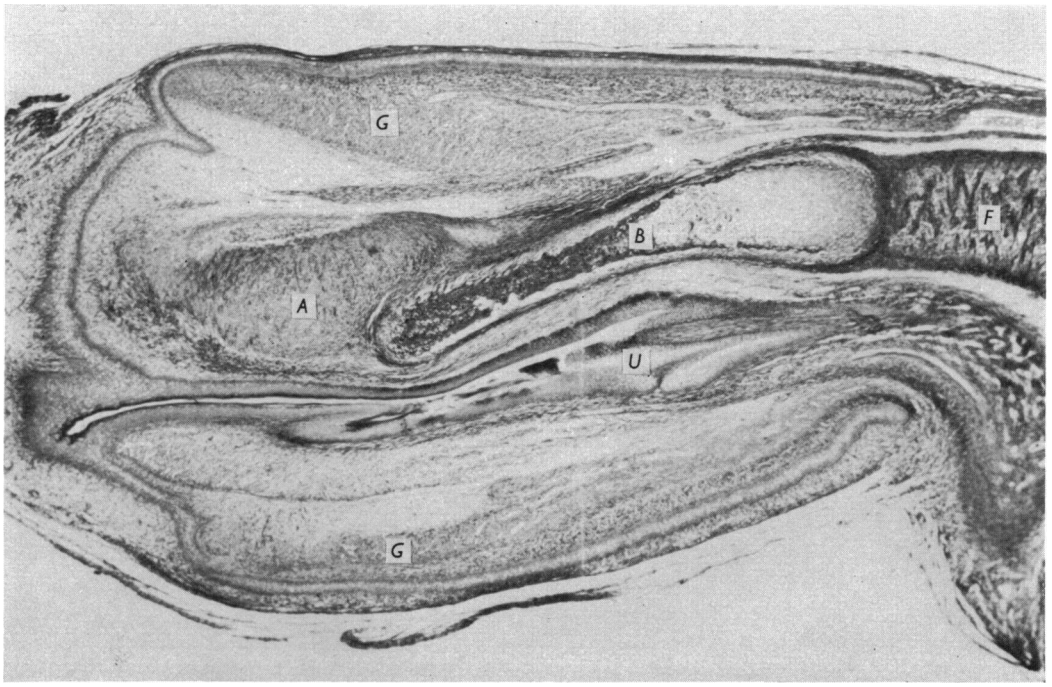

Fig. 2. The glans penis of an 8 day old rat sectioned longitudinally and stained with Lison's method to show the structures represented in Fig. 1. The lettering used here, and in subsequent figures, is: *U*, urethra; *B*, bone or its blastema; *A*, anterior process; *G*, corpus cavernosum glandis; *F*, corpus fibrosum. × 39.

made around the top of the skull, and it was removed as a cap. The brain was carefully lifted out whole, and the inside of the skull was examined. Note was made as to whether or not the implant was present, attached to the skull, and free of infection. The implant, along with any attached skull, was fixed in Heidenhain's Susa for 1 day followed by decalcification in a mixture of 3 % nitric acid, 5 % formic acid and 10 % formaldehyde. The sex of the host was confirmed from its gonads.

The decalcified tissues were embedded in paraffin and cut at 10 μm. Sections were mounted serially and stained either with haematoxylin and eosin or by Lison's (1954) method which uses chlorantine red for bone and alcian blue for cartilage (Hall, 1967).

Controls were needed to show how normal and hormone-treated untransplanted male and female genital tubercles develop. Phalluses, therefore, were taken for histology from 40 normal male and female rats aged from birth to 1 year, and over 100 rats given testosterone at birth.

These studies extend the findings of Ruth (1934), Wiesner (1934, 1935) and Glucksmann & Cherry (1972). Phallic development in the normal male and virilized female rat is based on preliminary studies already published (Beresford, 1973, 1975).

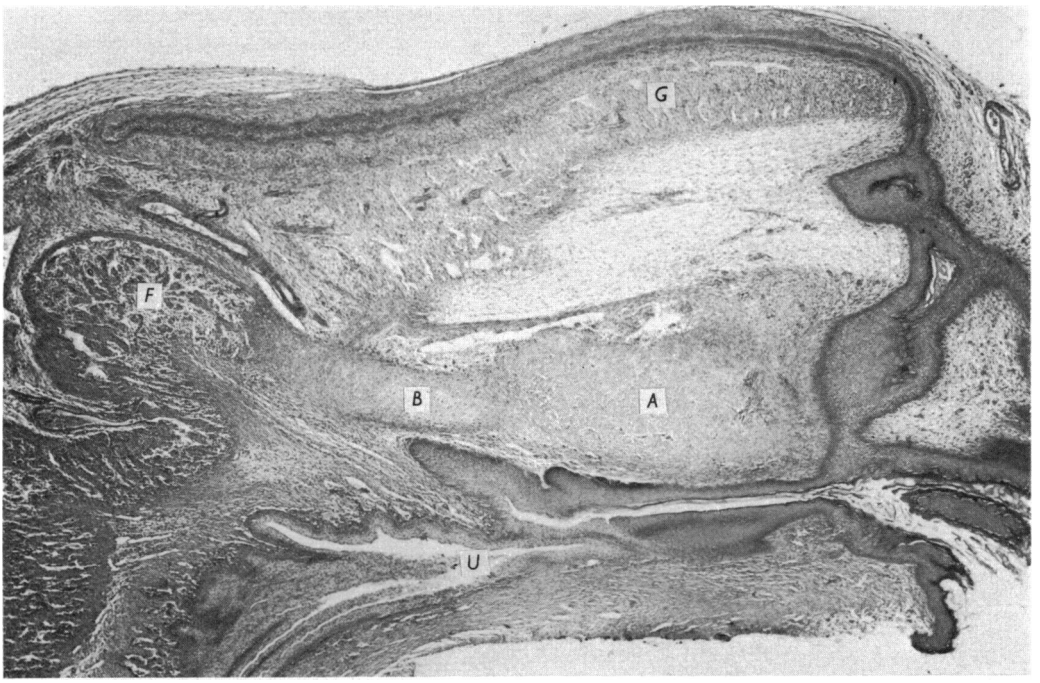

Fig. 3. The hypertrophied clitoris, of an 18 day old female rat given male hormone at 3 days of age, sectioned longitudinally and stained with H & E. × 39.

RESULTS

Control rats

Figure 1 shows diagrammatically, and Figure 2 is a photomicrograph of, the distal penis of an unoperated 8 day rat, omitting the skin and laterally situated preputial glands lying outside the balano-preputial epithelium. The structures that normally develop within the glans, and which might be expected to grow in a transplanted male tubercle, are the peripheral sheath of erectile tissue, the corpus cavernosum glandis (c.c. glandis), separated by mesenchyme from the anterior process, penile bone, small corpus spongiosum urethrae and the urethra. At later times the significant changes are: size increases; the growth cartilage comes to occupy proportionately less of the length of the penile bone; and the anterior process has larger chondrocytes with capsules staining with alcian blue and a matrix with more collagen. Much later than the time of the end of this experiment (26 days), the balano-preputial epithelium separates to allow the glans to protrude from the prepuce.

When the newborn female rat is given excess testosterone a phallus forms (Fig. 3) that resembles and differs from a penis in the following ways. The erectile c.c. glandis develops well, but is deficient below the urethra, where the sub-urethral tissues fail to match the growth of those dorsal to the urethra. The corpus fibrosum enlarges and develops thick internal trabeculae, but is contorted into a sinuous form. An anterior process of fibrocartilage forms. However, the clitoral bone that also appears

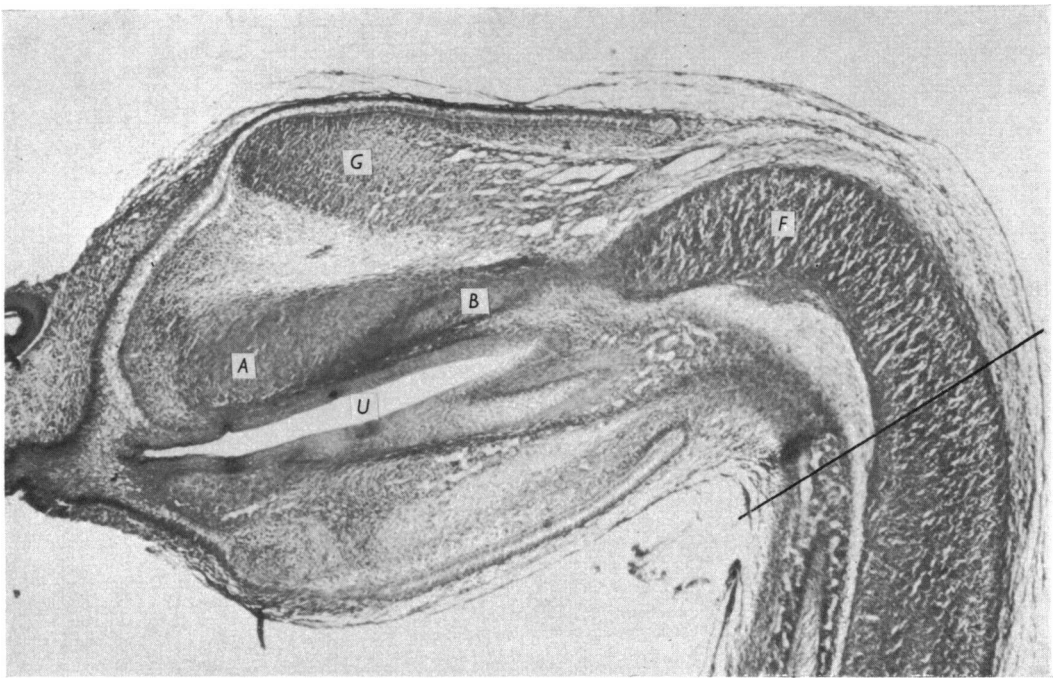

Fig. 4. The genital tubercle of a 2 day old rat male in longitudinal section, stained with Lison. The line indicates the level of trans-section from the donor. × 49.

fuses with the process and is only a short stub, lacking the secondary growth cartilage. Furthermore, the bone is slower to form than the penile bone, not being seen before 1 week.

The male genital tubercle 2 days after birth is shown in Figure 4 with the balano-preputial and urethral epithelia present. Condensations of cells – blastemata – for the c.c. glandis, anterior process and penile bone are recognizable. The c. fibrosum already has a capsule, trabeculae and lacunae. Similar structures are present, but are a little smaller and less well defined in the newborn male (Fig. 5) and female tubercles that were cut through at the level indicated (Fig. 4) for grafting. The transplants included the primordia for the preputial glands.

Group 1 *rats*

Table 1 gives the sexes of the donors and the sexes, ages, survival times, and hormone treatments of the 36 hosts. Four hosts died and one was killed early because of sickness. In 28 instances the transplant was found, but in only 13 did the transplant contain a living, uninfected glans. In some animals the graft area showed dense accumulations of polymorphonuclear leukocytes surrounding and infiltrating degenerate tissue. Other grafts were alive, but had leukocytes clustered in the lumen of a keratinizing cyst.

In transplants with minor or no infection the same tissues survived and grew irrespective of the sex of host and donor. The preputial glands enlarged and formed

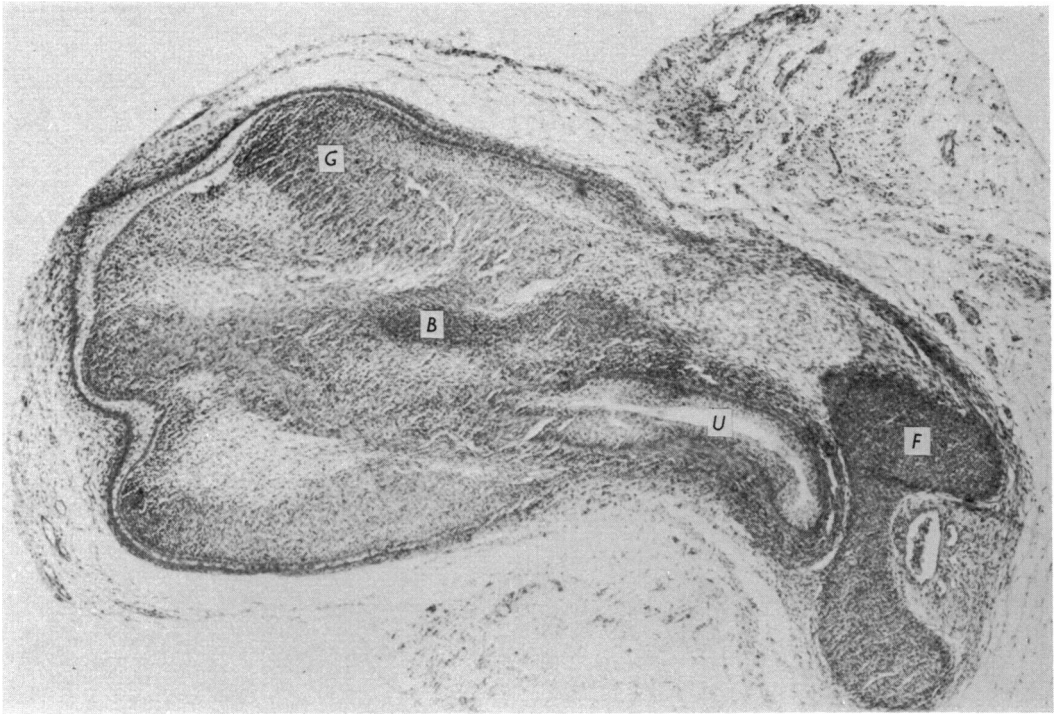

Fig. 5. The genital tubercle of a newborn male rat sectioned in an almost longitudinal plane, stained with Lison. × 39.

Table 1. *Intracerebral transplantation of genital tubercle. Group* 1 *rats: sex, treatment results*

Donor sex	Host: Sex	Host: Hormone-treated	Host: Age (in days) at transplantation	Survival time (in days)	No. of animals	Healthy glans found
M	M	No	2–8	2–24	29	9*
F	M	No	1–3	12–13	3	2
M	F	Yes	3	11–15	2	1*
F	F	Yes	3	18–21	2	1

* One glans in each division has erectile tissue and fibrocartilage.

secretion-filled cysts (Fig. 6). At the tip of the phallus, where the skin joins the balanal epithelium, a cyst formed with a lumen partly filled with keratin and continuous with the urethra. The balano-preputial epithelium survived to demarcate the glans in the 13 uninfected specimens. Except in two animals, the non-epithelial tissues of the glans took the form of a fibrous tissue, denser than mesenchyme, but staining weakly with eosin and with the stains of Lison's method. In some there was a central area of granular, basophil degeneration (Fig. 7) around which phagocytic giant cells were

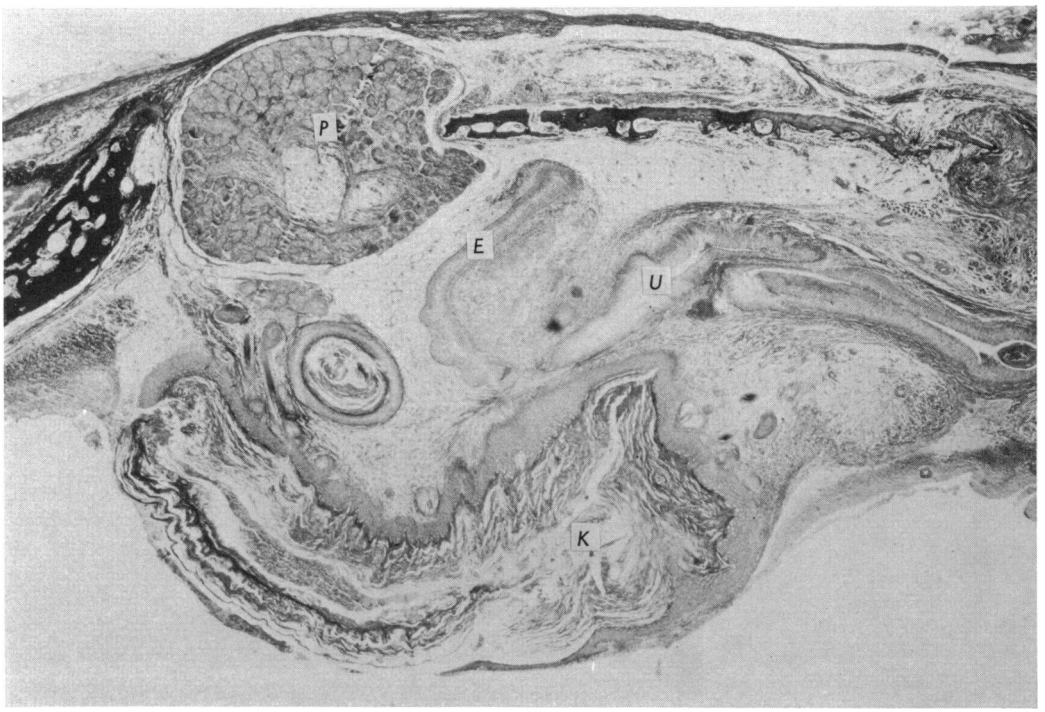

Fig. 6. A female genital tubercle, transplanted to the brain of a 7 day female host, given hormone and surviving for 9 days, is attached to the skull. A well-developed preputial gland (*P*) lies in a gap in the skull. The balano-preputial epithelium (*E*) partially demarcates the undeveloped glans. A large keratinizing cyst (*K*) is close to the urethra. Lison's stain. × 39.

clustered (Fig. 10). In two tubercles, surviving 11 and 15 days, and marked by asterisks in Table 1, some differentiation had taken place within the glans. Erectile tissue lay in the peripheral position typical of the c. c. glandis, and an anterior process of fibrocartilage (Fig. 8) had developed. However, the penile bone was absent. Also, the corpus spongiosum urethrae was not seen.

One conclusion reached was that a more antiseptic technique avoiding transplantation of skin was needed. Another was that ectodermal tissues fared better than mesodermal ones. Thirdly, despite the growth of the preputial glands, it was possible that the glans was not developing normally because of a failure of male hormone to reach the transplant early enough, or in adequate amount. Accordingly, the technique was modified, as described previously, for the second group.

Group 2 rats

The modifications greatly reduced the incidence of infection. The proportion of transplants recovered was 36 out of 41. Table 2 gives information similar to Table 1 for the donors and hosts. In the transplants as a whole some findings were similar to those of group 1, viz. good growth of the preputial glands, formation of a keratinizing cyst at the opening of the urethra (Fig. 9), and survival of the balanal and urethral

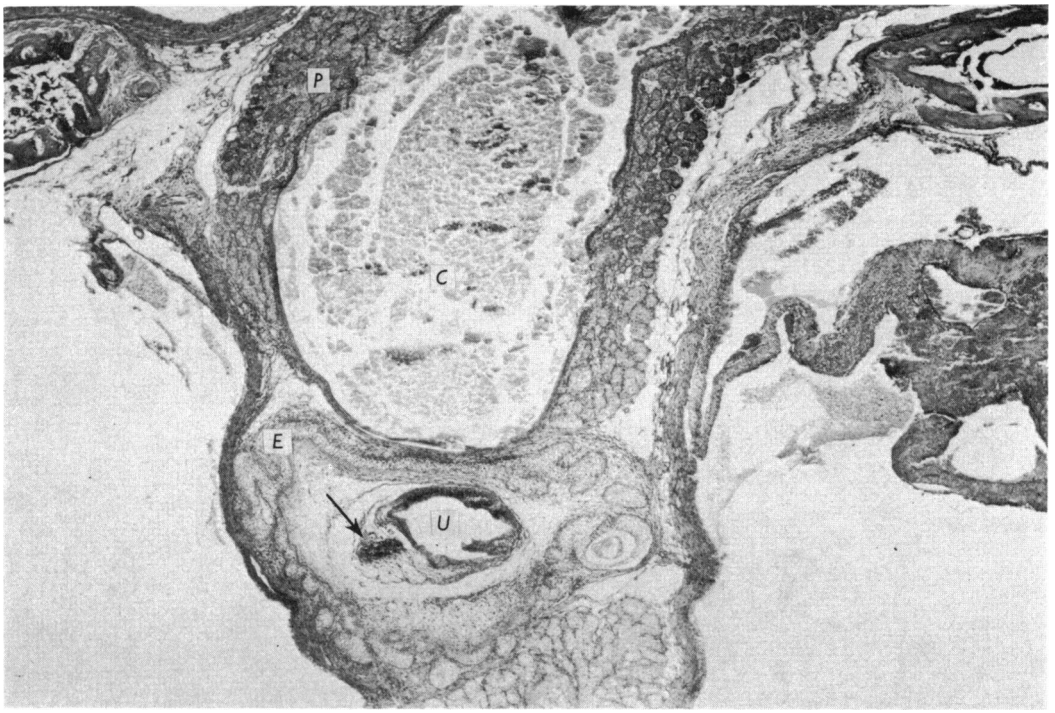

Fig. 7. A male tubercle, transplanted to the brain of a 3 day male host and surviving for 21 days, is attached to the skull. There is a large preputial gland, with a cyst (*C*). An arrow marks the area of degeneration beside the urethra in the undeveloped glans. H & E stain. ×98.

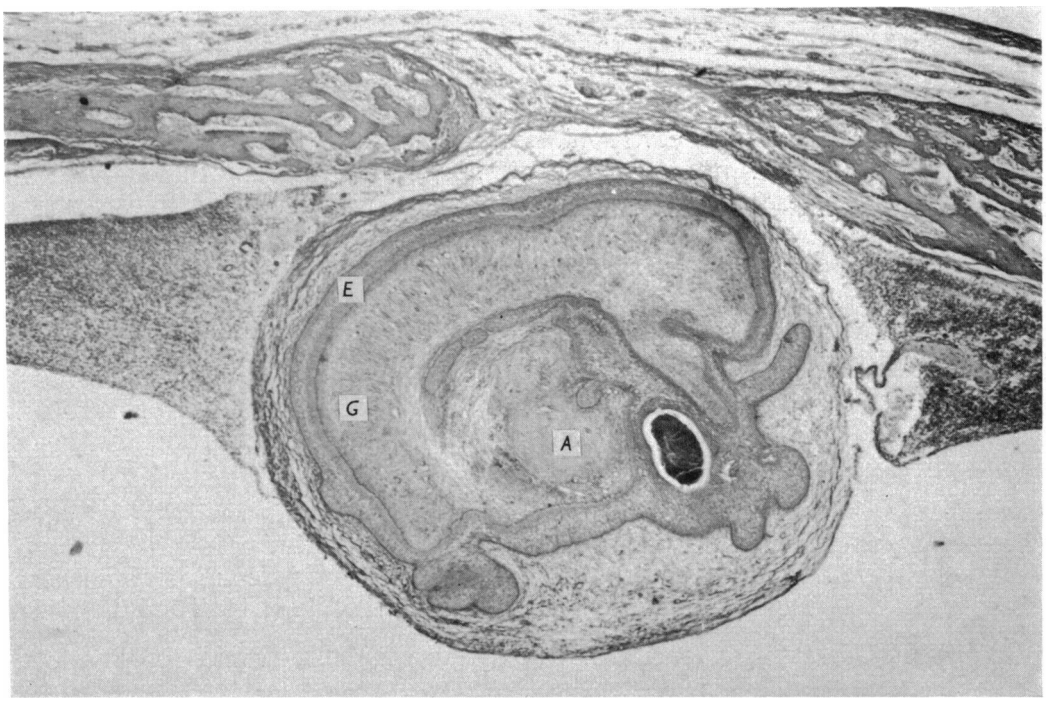

Fig. 8. A male genital tubercle, transplanted at 3 days, to a male host and surviving for 11 days is attached to the skull. The glans with its enclosing epithelium has developed a small anterior process as well as erectile tissue. H & E. ×59.

Table 2. *Transplantation of genital tubercle. Group 2 rats: sex, treatment, results*

Donor sex	Host: Sex	Host: Methyl-testosterone treated	Host: Age (in days) at transplantation	Survival time (in days)	No. of animals	Differentiated glans found
			Intracerebral			
M	M	No	3–7	9–21	14	4
M	M	Yes	3–5	10–21	7	2
F	F	Yes	4–7	9–21	11	4
M	F	Yes	6	10	2	0
			Subcutaneous			
M	M	No	7	10–14	3	0
F	F	Yes	7	10–14	4	1

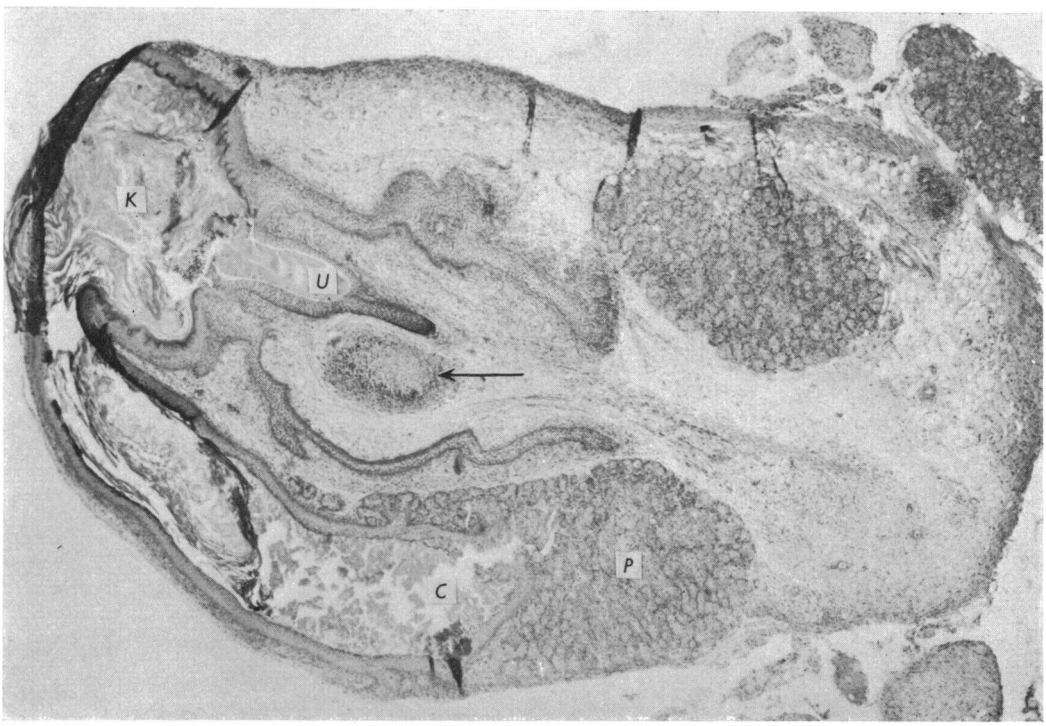

Fig. 9. A male genital tubercle, transplanted at 3 days to a male host and surviving for 15 days, is lying free in the brain. The preputial gland of one side has a cyst communicating with the keratinizing cyst (*K*) into which the urethra opens. An area of degeneration (arrowed) lies in the fibrous tissue of the undeveloped glans. H & E. × 39.

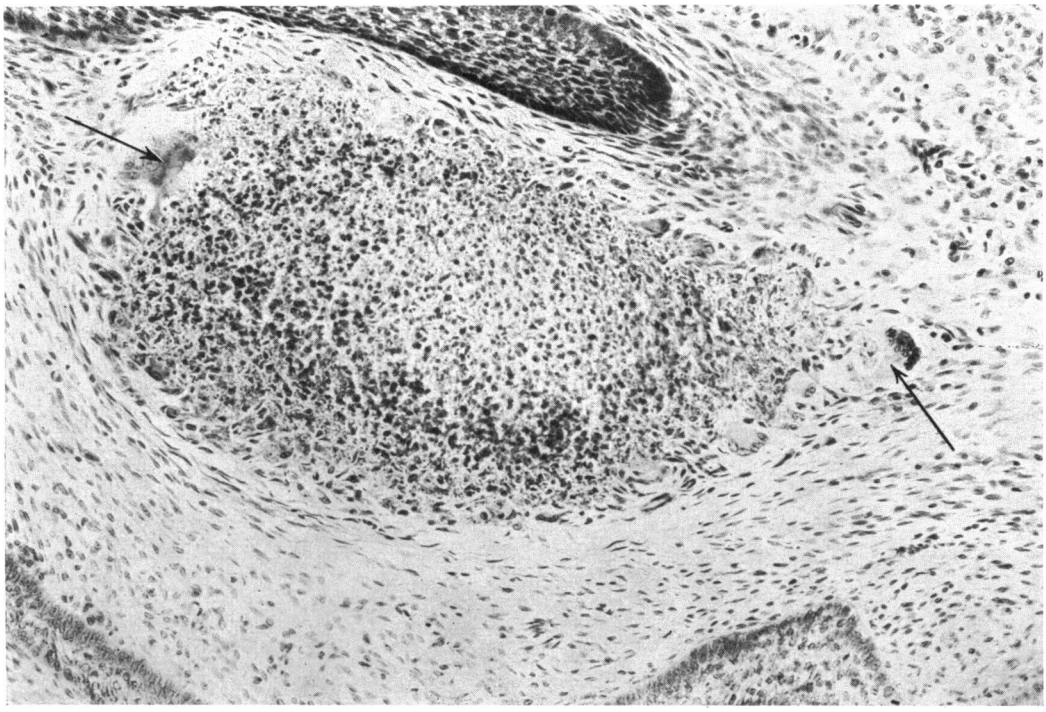

Fig. 10. The area of degeneration in Fig. 9 has giant cells (arrowed) at its periphery. × 200.

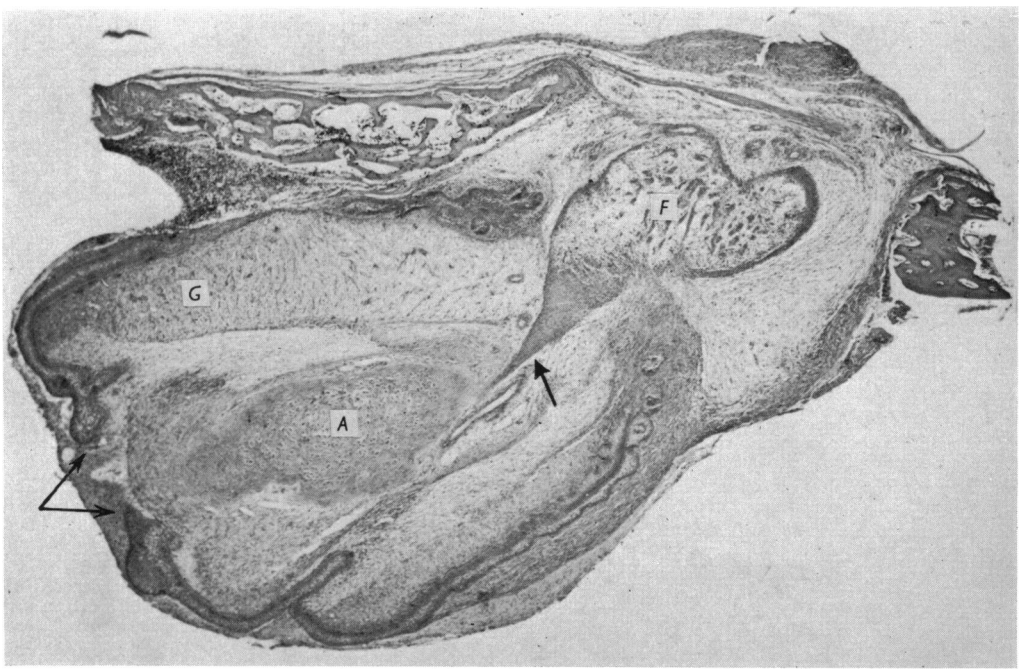

Fig. 11. A female tubercle, transplanted to a 5 day female host, and given male hormone and surviving for 14 days, is attached to the skull and sectioned sagittally, but missing the urethra. A tapering cord of cells (heavy arrow) joins the corpus fibrosum to the anterior process. There is a gap (arrowed) in the balano-preputial epithelium. H & E. × 39.

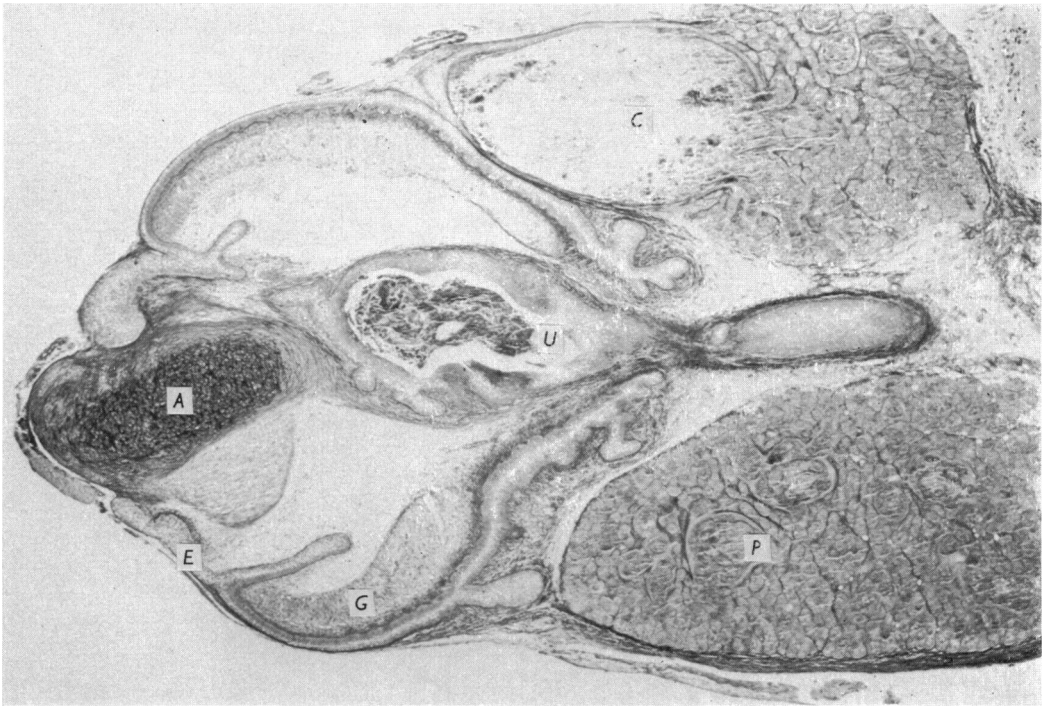

Fig. 12. A male tubercle, transplanted to a 5 day male host and surviving for 14 days, lies free in the brain, and has been sectioned longitudinally. Over half of the transplant is taken up by the preputial glands. The glans has reached the maximal development seen in any grafted tubercle. Lison's stain. × 39.

epithelia. However, there was marked variability in the fates of other transplanted glans tissues. In some grafts the glans was small, no differentiation had taken place, and an area of degeneration was noted (Figs. 9, 10). In other cases the glans was larger, with a small erectile body, and degeneration was absent. However, the best developed transplants did not differ significantly from those from the two rats of group 1 which formed recognizable differentiated structures within the glans. The 10 largest tubercles showed c.c. glandis tissue and the anterior process. In 4 grafts a cord of cells originated at the distal end of the corpus fibrosum and tapered to join the anterior process (Fig. 11). This cord resembles, and is in the same position as, the blastema of the penile bone in the 1 and 2 day tubercles. The corpus fibrosum (Fig. 11) was present, enclosed in its capsule, in most grafts, but had not grown significantly, and was less dense than when transplanted.

This state, where erectile tissue and an anterior process, but no bone, are present (Fig. 12) represents maximal differentiation. The degree of differentiation did not differ between male and female tubercles or between those with survival times of nine days and those which survived for 21 days. The last column of Table 2 gives the number of transplants that reached such a condition of maximal differentiation in each of the treatment groups. The anterior process formed in male and female tubercles, in male and female hosts, and without regard to whether the transplant was

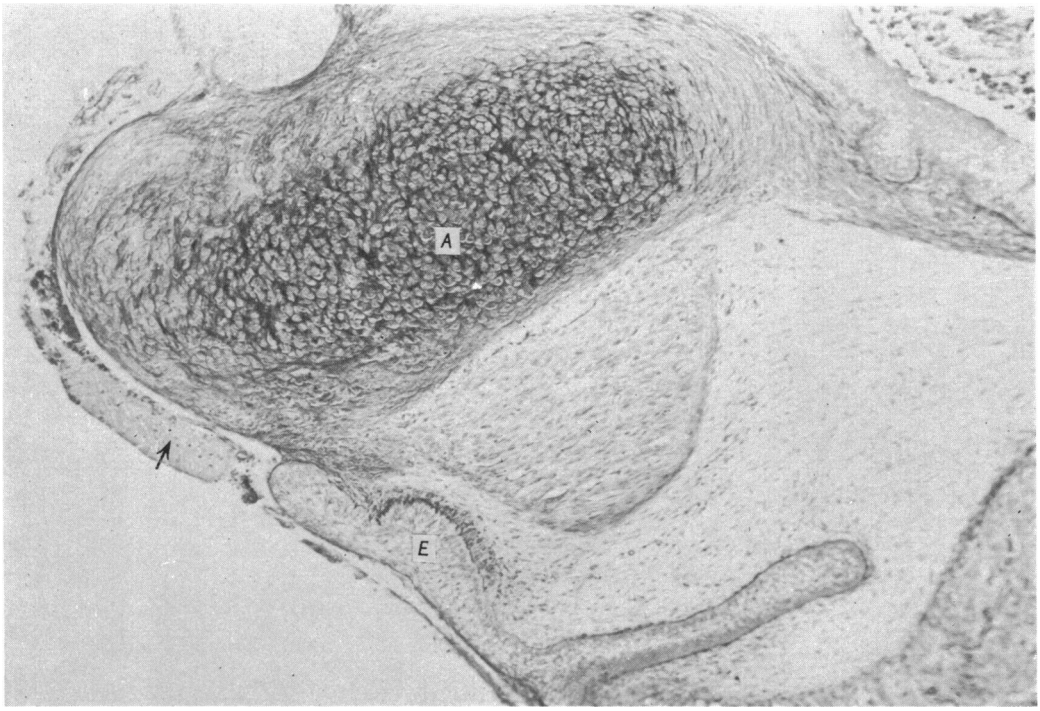

Fig. 13. The distal end of the glans penis of Fig. 12 has a well-developed fibro-cartilaginous anterior process. The balano-preputial epithelium is interrupted, exposing the interior of the glans to the brain (arrowed). Lison's stain. ×98.

intracerebral or subcutaneous, whether or not male hosts received exogenous hormone, and, in the cranium, whether the graft was free in the brain or attached to the dura. In some, but not all, intracerebral grafts the balano-preputial epithelium was interrupted in its distal region (Fig. 13) and no longer separated the anterior process from cerebral tissue.

Maximal differentiation was noted in roughly one third of the intracerebral transplants, but only in one of seven subcutaneous ones. As was expected in the immunologically less privileged subcutaneous situation, all the grafts were infiltrated to some degree by lymphocytes. Nevertheless, the glans was present in five, and had grown in four, of which one had an anterior process.

DISCUSSION

One unfulfilled hope was that the grafted male tubercle would develop into a structure with a bone that could later be tested for its susceptibility to the withdrawal of androgen (Burkart & Beresford, 1972), avoiding the factor of altered sexual use changing the mechanical stresses on the organ, which is inevitable *in situ*. Because neither the bone nor its secondary cartilage formed, no light was shed on the 'secondary cartilage-resulting-from-movement' hypothesis. The partial, and some-

times total failure, of the mesodermal tissues of the glans to develop may be because of inadequate revascularization, or poor timing – grafting coming only 1 or 2 days before the expected appearance of penile osteoblasts; Meikle (1973) saw cell division cease for one or more days after intracerebral grafting of mandibular condyles, and proliferation may be needed for differentiation (Vonderhaar & Topper, 1974). Another factor could be loss of innervation: penile development in marine molluscs (Le Gall & Streiff, 1974), and the differentiation of cartilage and bone in amphibian limb regeneration (Singer, 1952), for example, are influenced by nerves.

Genital tubercles of male and female ducks have been grown in tissue culture (Wolff & Wolff, 1952), and Dufaure (1964) successfully grafted genital tubercles of viviparous lizard embryos to the allantois of other embryos. However, the mammalian tubercle is more complex in that it generally has skeletal primordia so, perhaps not surprisingly, only parts of the tubercle prospered after transplantation. The preputial glands grew well in both male and female transplants, accumulating secretion in cysts while keratin formed at the urethral opening. Evidently the testosterone reached the glands because it is known that testosterone is needed to stimulate copious secretion in female preputial glands (Salmon, 1938; Marois & Marois, 1974). Evidently also the hormone penetrated deeper, reaching the interior of the glans, because the anterior process developed in some grafted female tubercles. This fibrocartilaginous anterior process fails to form in male rats castrated at birth (Wiesner, 1934) and develops in the clitoris only when testosterone is given (Wiesner, 1935; Glucksman & Cherry, 1972). The grafted female cells that formed an anterior process could not have been determined for cartilage formation at the time they were transplanted shortly after birth, but must have differentiated into chondroblasts under the influence of testosterone in the brain.

Certain conclusions follow. Ectodermal tissues of the tubercle fare better than mesodermal ones. A deficiency of androgen is not a factor in the poor mesodermal performance. For its differentiation into a skeletal element, the grafted primordial anterior process is unusual in needing male hormone, being independent of the more proximal penile bone, and unaffected by the absence of mechanical stimuli associated with cleaning by the mother and crawling.

SUMMARY

Genital tubercles of 70 newborn male and female rats were transplanted into the brains of unrelated infant rats. Seven other tubercles were placed subcutaneously. All female, and some male, hosts were injected with testosterone propionate. After surviving from 2–24 days, histological study of 49 successful grafts showed survival of the urethral and balano-preputial epithelia and growth of the preputial glands, which formed secretion-filled cysts and became the major component of the graft. The fate of the mesodermal tissues within the glans varied between remaining in an undeveloped state, with only pale fibrous tissue and an area of granular degeneration and giant cells, and achieving an incompletely differentiated state in which erectile tissue and the anterior process of fibrocartilage had formed and the glans had grown but the penile bone and its secondary growth cartilage failed to appear. Grafts could reach this degree of differentiation of the glans irrespective of transplantation site,

attachment to the host dura, the sex of donor or host, and whether or not male hosts were given exogenous hormone.

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