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Development of Human Fetal Prostate in Culture

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Summary. The direct influence of androgens on prostatic development was studied by culturing explants of urogenital sinuses of human embryos and fetuses aged 6 to 13 weeks in the presence of either 10^{-7} mol/l testosterone or dihydrotestosterone for 6 days. The differentiation of prostatic epithelial cells started both in control and androgen-treated explants in the ninth week, and androgens accelerated the differentiation of the secretory pathway organelles in the epithelial cells. The amount of granular endoplasmic reticulum and the number of Golgi complexes increased in the presence of androgens, especially in the older fetuses. Incipient secretory activity appeared in some cells during the culture and the differentiation occurred sooner in the androgen-treated explants than in the controls or in vivo. The differentiation and maintenance of the urogenital mesenchyme was also better in the presence of the hormone. The direct epitheliomesenchymal cell contacts seen earlier in vivo were also seen during the differentiation in vitro. These contacts may indicate an inductive role of the mesenchyme in the glandular morphogenesis. Androgens were not able to induce the epithelial differentiation in vitro before the first signs of mesenchymal differentiation were evident in vivo. The results suggest either that the mesenchymal changes are not solely determined by androgens or that the early explants may require additional factors for proper differentiation.

Key words: Human, Prostatic, Differentiation, Androgens, Interaction, Ultrastructural

INTRODUCTION

Differentiation and maintenance of the human prostate depend on the supply of testicular hormones, especially testosterone (17, 18, 28). The active androgen in prostate has been shown to be 5 α-dihydrotestosterone in both in vivo and in vitro experiments (13). Much attention has been paid to the prostatic epithelium as the primary target of androgens. Recently, the importance of mesenchyme as a possible target tissue of androgen in the regulation of differentiation and homeostasis of accessory sex organs has been shown by several investigators (4, 5, 7, 8, 19, 20).

The mesenchyme surrounding the epithelium of the urogenital sinus at the level of the mesonephric openings begins to differentiate before the epithelium in the human fetal prostate (17). After mesenchymal differentiation the epithelium of the urogenital sinus begins to form solid epithelial outgrowths into the mesenchyme followed by the lumen formation (18). Epithelio-mesenchymal interaction in the form of direct cellular contacts occurs during the polarization of the epithelial cells and the establishment of the secretory system in the fetal prostate. Incipient secretory activity in the fetal prostate occurs simultaneously with maximal testosterone levels in fetal testis and amniotic fluid (6, 15).

In the present study we investigated the direct effects of androgens on the differentiation of human fetal prostatic primordium in organ culture at the ultrastructural level. Special attention was paid to possible signs of epithelio-mesenchymal interaction during the differentiation.

MATERIAL AND METHODS

Embryos and Fetuses

The embryos and fetuses were collected from legal abortions and transported in culture medium from the hospital. The ovulation ages determined

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according to Streeter (25) from the foot lengths (4-17 mm) or crown-rump lengths (23-100 mm) were 6-13 weeks. The sex of embryos and fetuses was determined by histological examination of gonads, or by X chromatin staining und Y chromatin fluorescence (21).

Organ Culture

The prostatic part of the urethra was immediately removed under a stereomicroscope in aseptic conditions and the organ culture method of Trowell (26) was followed with minor modifications. The tissue piece (about 2 mm^3 in size) was mounted onto a strip of moistened lens paper on a grid of stainless steel. The grid was placed in a Petri dish with 10 ml Medium 199 (Flow, Irvine) containing 10% of newborn calf serum, Sodium G-Penicillin (50 IU/ml) and Streptomycin (50 µg/ml). The cultures were maintained in a chamber with saturated humidity at 37°C and flushed every day for 5 min with a mixture of 95% oxygen and 5% carbon dioxide.

Culture times of two days to 3 weeks were tested and 6 days was selected for the final experiments, because the cellular ultrastructure was well maintained for that period. The culture medium was changed every second day.

Testosterone (17 β-hydroxy-4-androsten-3-one; Merck, Darmstadt) and dihydrotestosterone (17 β-hydroxy-5 α-androstan-3-one; Ikapharm, Ramat-Gan) dissolved in propylene glycol were added to the culture media, to give a final concentration of 10^{-7} mol/l. The same volume of propylene glycol was added to the control cultures.

Electron Microscopy

The cultured tissue pieces were fixed with purified 0.24 mol/l glutardialdehyde (Glutaraldehyde EM, Leiras, Finland) and 4.1 mmol/l $CaCl_2$ in 0.1 mol/l sodium cacodylate-HCl buffer pH 7.4 for 3 at +4°C as tested and described in detail in a previous study (17). After fixation the tissue samples were washed overnight in 0.23 mol/l sucrose and postfixed with 39 mmol/l osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 mol/l s-collidine-HCl buffer, pH 7.4 (9). After dehydration the specimens were orientated under a stereomicroscope and embedded in Epon 812 (Merck, Darmstadt). Transverse 1 µm sections of the whole tissue piece were cut with an LKB Huxley ultramicrotome and stained with toluidine blue for light microscopy. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate and examined in a Jeol JEM T8 or Jeol JEM 100C electron microscope.

RESULTS

Horizontal sections were cut at several levels from the whole explant for light microscopic orientation, and thin sections of a smaller area for electron microscopy. Serial light microscopic sections were prepared from a few explants. After sex identification, only explants from male embryos and fetuses were included. Explants of the urogenital sinus under the age of seven weeks degenerated in culture with or without hormones to such an extent that they could not be included in this study. The epithelial cells of the embryos had large lobular nuclei and their cytoplasm contained varying amounts of lysosomes and lipid droplets. More degenerative changes occurred in the untreated explants, especially in the mesenchyme.

Ages from Seven to Ten Weeks

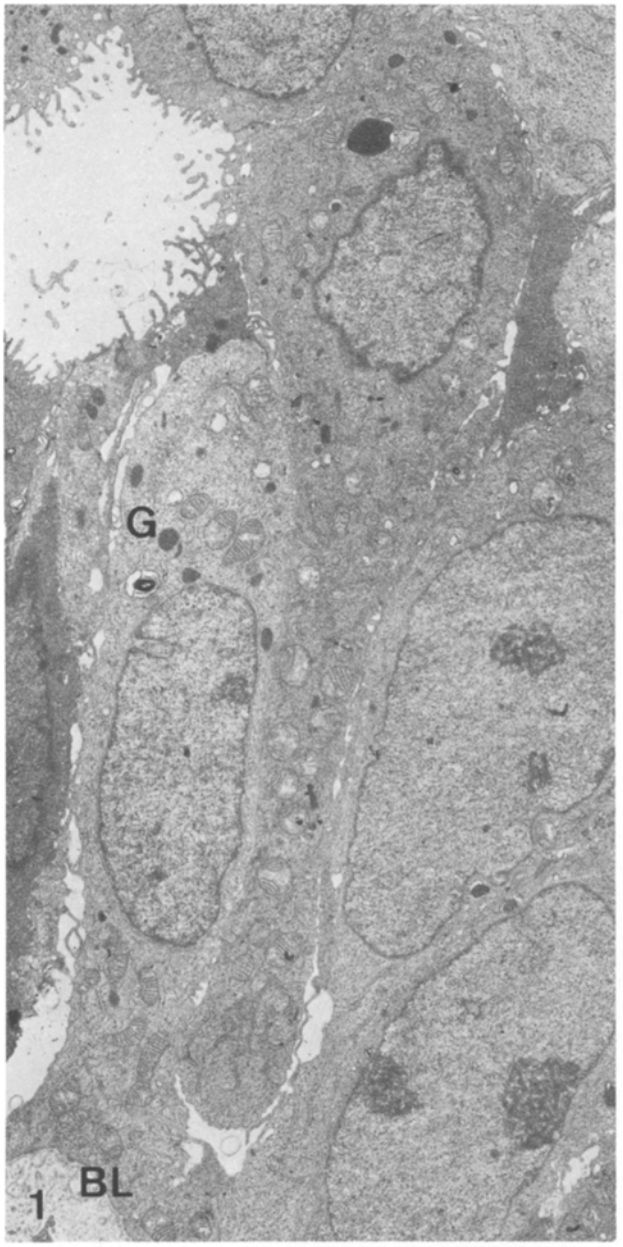
The early epithelium in the control culture was composed of several layers of primitive cells with some luminal microvilli at the age of seven weeks. The typical junctional complexes were present throughout the study. At the age of eight weeks cuboidal epithelial cells began to grow into the mesenchyme, and simultaneously the mesenchymal cells sent processes toward the epithelial basal lamina. Lumen formation occurred in the epithelium in the ninth week and the epithelium consisted mainly of three to four cell layers. The apical cells began to polarize and a few cells contained luminal electron dense granules or empty vesicles (Figs. 1 and 2).

Fig. 1. The control epithelium of a 10-week old fetus. The apical cells have begun to polarize and their cytoplasm contains some electron dense granules (G). BL: basal lamina. x 5000

Fig. 2. A higher magnification of the apical part of the epithelium with some electron dense granules (G) and empty vesicles (arrow). A 10-week old control fetus. x 15.000

Fig. 3. Granules containing membranous material in the apical epithelial cells of a testosterone-treated 9-week old fetus. GR: granular endoplasmic reticulum; M: mitochondrion; N: nucleus; arrow: plasma membrane interdigitation. x 6000

Fig. 4. Apical part of epithelial cells in a 10-week old fetus cultured in the presence of dihydrotestosterone. The cells have several Golgi complexes (GC). The arrow indicates plasma membrane interdigitations. N: nucleus. x 15.000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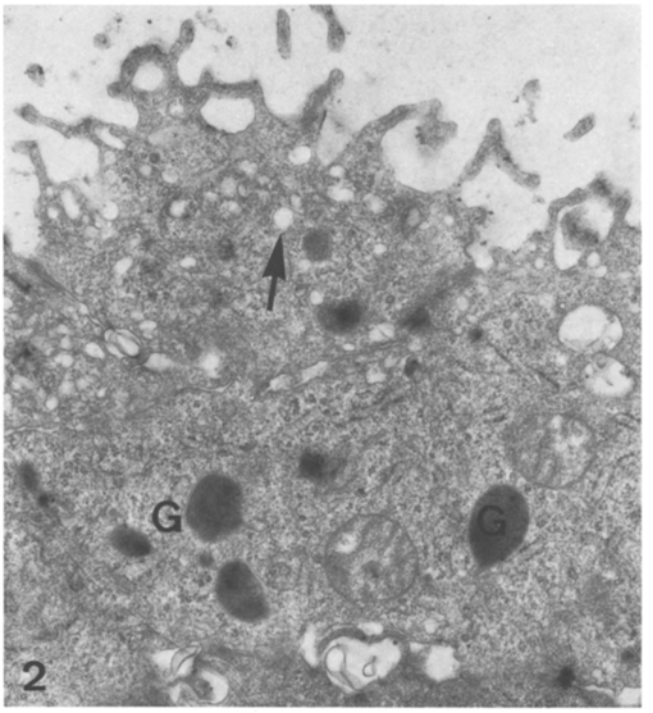
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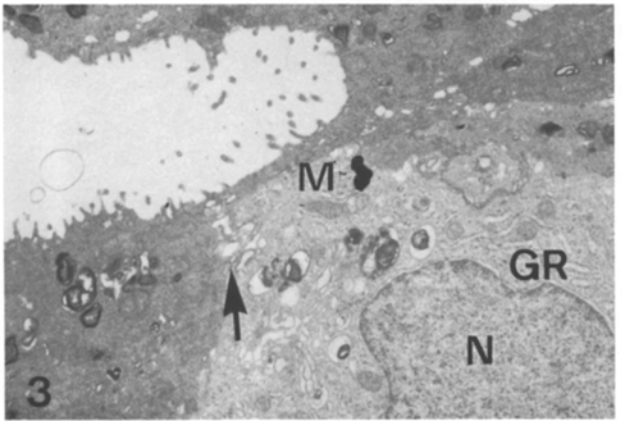
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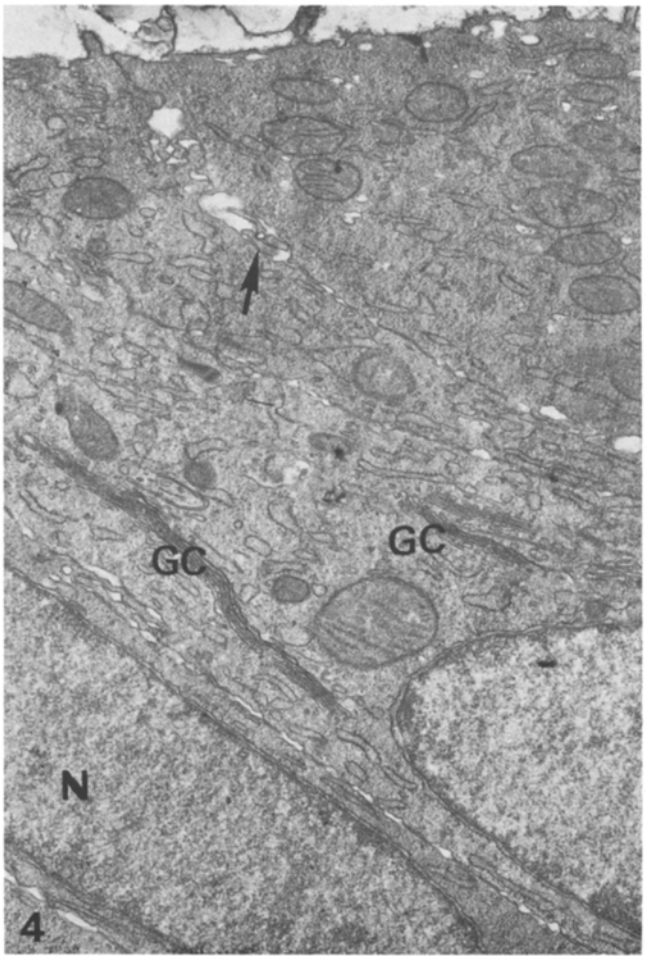
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The cultured mesenchyme resembled that in our previous in vivo study (17). The amount of granular endoplasmic reticulum in the mesenchymal cells adjacent to the basal lamina increased gradually and from the ninth week onwards some of them differentiated into fibroblasts. Collagen fibres appeared concomitantly in the intercellular spaces (Fig. 5).

The epithelium in the explants cultured in the presence of testosterone or dihydrotestosterone consisted of several cell layers at the age of seven weeks as in the controls and in vivo embryos. From the age of eight weeks the hormones induced epithelial growth into the surrounding mesenchyme. Lumen formation occurred in the epithelium, but the epithelium still consisted of several layers. Concomitantly, the amount of granular endoplasmic reticulum increased and the Golgi complexes grew in number and size more than in control explants (Fig. 4). Secretion granules appeared in the apical parts of the epithelial cells. The lumen formation occurred more rapidly in the presence of hormones and mitotic figures were also seen more often. Testosterone especially tended to induce formation of granules containing membranous material (Fig. 3).

In the mesenchymal cells the amount of granular endoplasmic reticulum increased in the presence of hormones (Fig. 6) and the epithelio-mesenchymal interaction in the form of direct contacts between the basal epithelial and mesenchymal cells had already occurred by the ninth week. No specialization of plasma membrane in these sites was noted (Fig. 7). Golgi complexes also increased in size and number and membrane-containing vesicles similar to those found in the epithelial cells were seen in some mesenchymal cells.

Ages from Eleven to Thirteen Weeks

The interdigitations increased in the control epithelial cells. The epithelium was mostly stratified at the age of thirteen weeks (Fig. 8). Some apical cells were polarized with luminal microvilli and cell debris was seen in the acinar lumen.

The basal lamina was folded (Figs. 8 and 12) and some direct epitheliomesenchymal contacts were then seen in the control cultures as well. The mesenchymal cells became fibroblastic (Figs. 8 and 12) and began to form a lamina propria around the acini.

The epithelial cells treated with testosterone or dihydrotestosterone contained more granular endoplasmic reticulum and Golgi complexes than the controls. This increase was more prominent at this age than in the earlier fetuses cultured in the presence of androgens. The luminal columnar cells were polarized and at the age of thirteen weeks the acinar epithelium in some glands consisted of two layers with electron dense apical granules in some cells (Figs. 9 and 10). Also a few basal cells with electron dense granules were present (Fig.. 11). The plasma membrane interdigitations continued and the same kind of granules containing membrane whorls as in the earlier ages were present abundantly in the explants cultured with testosterone (Fig. 15). Mitotic figures were also seen. Peri- or supranuclear intermediate (10 nm) cytoplasmic filament bundles were observed throughout the study (Fig. 15).

Direct epithelio-mesenchymal contacts were also seen during this period with androgen treatment, and the amount of granular endoplasmic reticulum and Golgi complexes as well as intercellular collagen fibres was increased when compared to controls of the same age (Figs. 13 and 14). Granules with membrane whorls were also seen in some mesenchymal cells.

DISCUSSION

The present experiments on human fetal prostate in culture demonstrate for the first time that androgens induce the differentiation of urogenital epithelial cells into secretory prostatic cells. The interdigitation of plasma membrane during the culture might also be a sign of androgen action. Plasma membrane interdigitation has been shown in canine prostate when 5α-androstane-3α, 17α-diol was present in the culture medium (23). Degenerative changes have been observed in human prostatic cultures without hormones (27). The fetal tissue used in this study may differ from that of the adult in growing capacity, and thus continue the differentiation in vitro, if the differentiation was initiated in vivo before the culture. In animal experiments the prostate, once induced by androgens, is maintained throughout life, although it would not develop into a secretory gland (16).

The secretion granules in this study resemble those seen in vivo in fetal and adult human prostate and in animal prostate in vivo and in vitro (1, 18). However, all six different forms of secretory vacuoles found in the adult prostate (1) were not seen in vitro with or without hormones. The morphological characteristics of a secretory epithelium developed earlier with androgens induce the differentiation of urogenital contents, which increased in number with testosterone, may be residual bodies (2, 3). They could also have an active part in prostatic differentiation and not be the result of aging and regression, as suggested in a study of postnatal differentiation of rat prostate (10, 11). The lysosomes could be involved in the crinophagy of

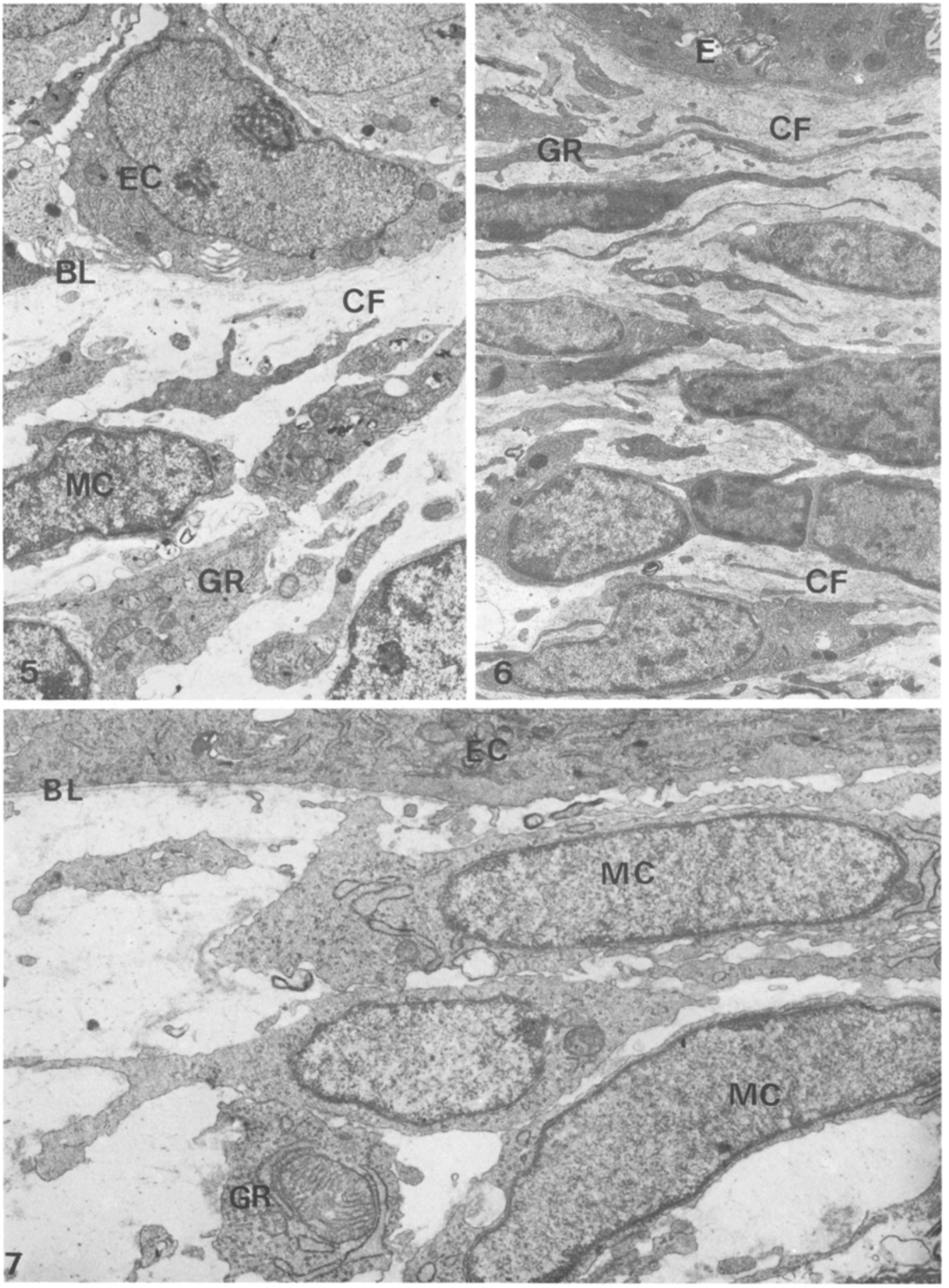
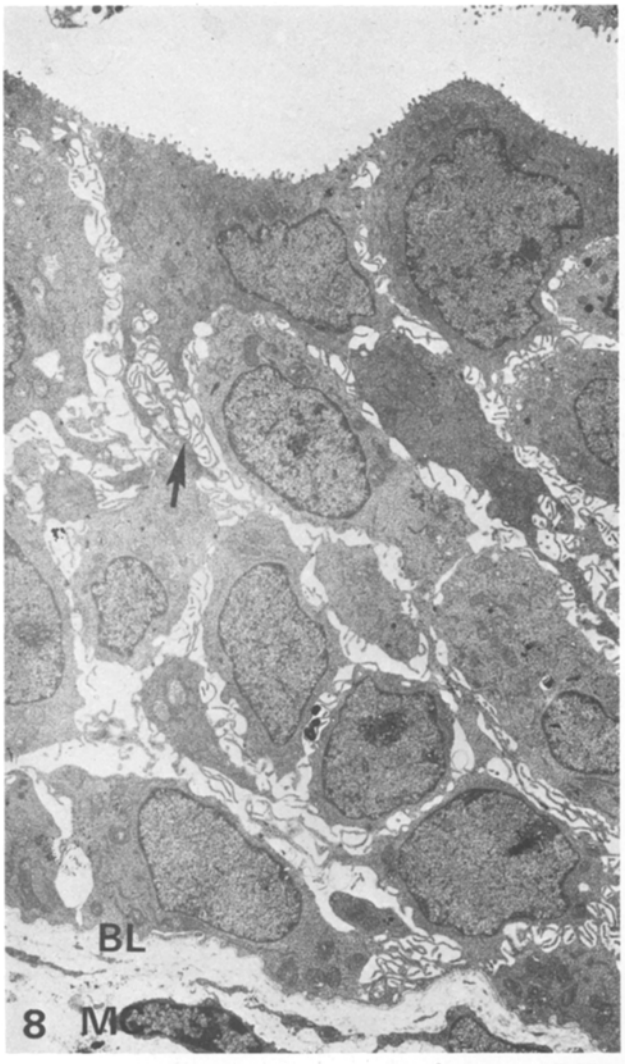


Fig. 5. Developing mesenchyme of a 10-week old control fetus. BL: basal lamina; CF: collagen fibres; EC: epithelial cell; GR: granular endoplasmic reticulum; MC: mesenchymal cell. x 6000

Fig. 6. The mesenchymal cells forming a lamina propria around the developing epithelium (E). CF: collagen fibres; GR: granular endoplasmic reticulum. A 10-week old fetus cultured with dihydrotestosterone. x 6000

Fig. 7. The epithelial basal lamina (BL) has partly disappeared and an epithelial cell (EC) and a mesenchymal cell (MC) are in close contact with each other in a 10-week old hormone-treated explant. GR: granular endoplasmic reticulum. x 12.000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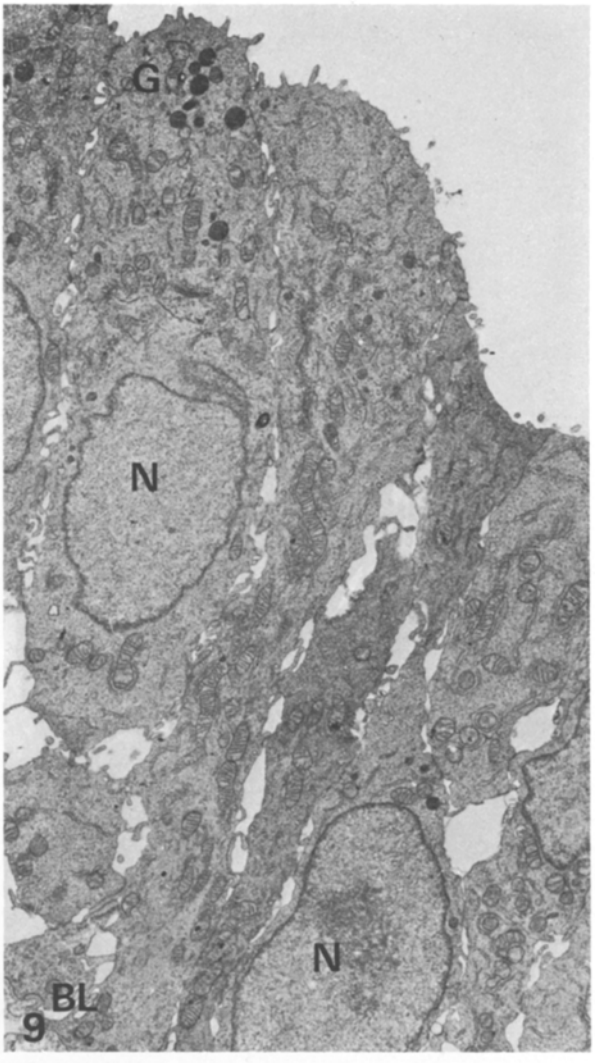
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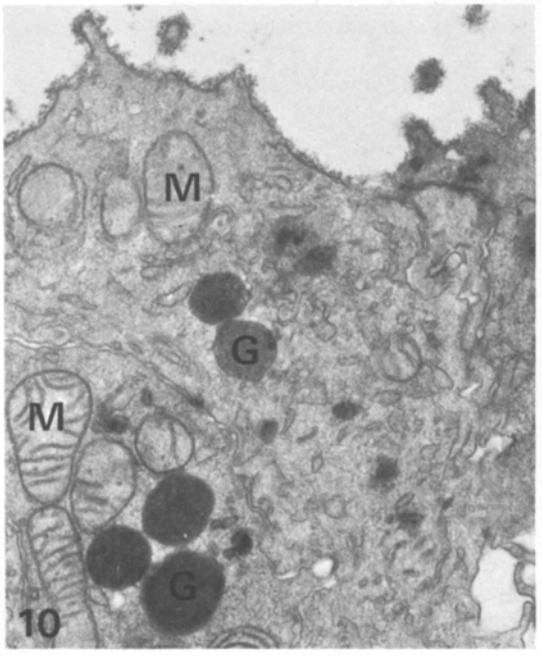
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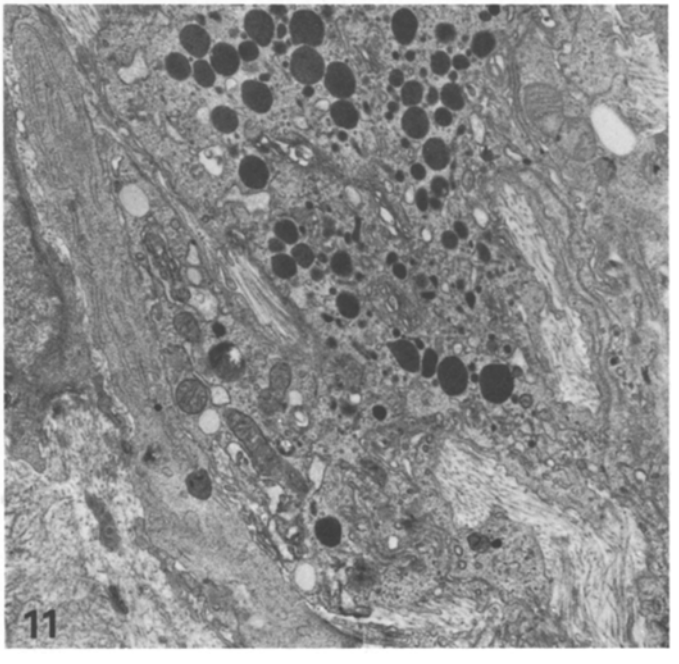
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excess secretory material as in the case of rat pituitary gland (24) and the rat mammary gland (14). The rare basal cells containing electron dense granules resemble enterochromaffin cells seen in human urethral and prostatic epithelium, and the functional significance of these cells is unknown (1).

Direct epithelio-mesenchymal cell contacts were seen in this study as in our in vivo study (18), and these types of contacts were more usual in the androgen treated explants, which might be a sign of the possible inductive role of mesenchyme in differentiation. This is in agreement with studies of sex-reversed mice heterozygous for testicular feminization, which suggest that the androgen responsiveness of mesenchymal cells may be more important than that of the epithelial cells (8, 20). Moreover, patients with Prune Belly Syndrome, in which disease there seems to be a defect in the mesenchymal differentiation, do not have a prostate (7). The recombination experiments of Cunha (4) have shown the regulative role of mesenchyme in prostatic differentiation. The regression of the mammary gland of the male mouse is also regulated by androgens via the mesenchyme (19).

The tissue culture technique has been used to study the effects of hormones in normal and abnormal prostate, in the study of prostatic carcinogenesis and in the study of epithelio-mesenchymal interaction (12). This technique offers the only possible way to study the direct action of hormones and other factors in the early differentiation of human fetal prostate. In this study the inability of androgens to induce prostatic differentiation before the first signs of mesenchymal differentiation were evident in vivo, might be due to the lack of factors other than androgens or due to the culture technique. Thus, the reason for no response with testosterone cannot be the lack of the enzyme 5α-reductase, because even dihydrotestosterone was not able to induce the differentiation before or at the age of seven weeks in this study. Moreover, Siiteri and Wilson (22) have demonstrated with incubation experiments that the embryonic urogenital sinus can form dihydrotestosterone from testosterone at this age. Our preliminary radioimmunoassay measurements from the culture medium after the addition of testosterone have also shown the presence of dihydrotestosterone in cultures from seven-and thirteen-week-old fetuses.

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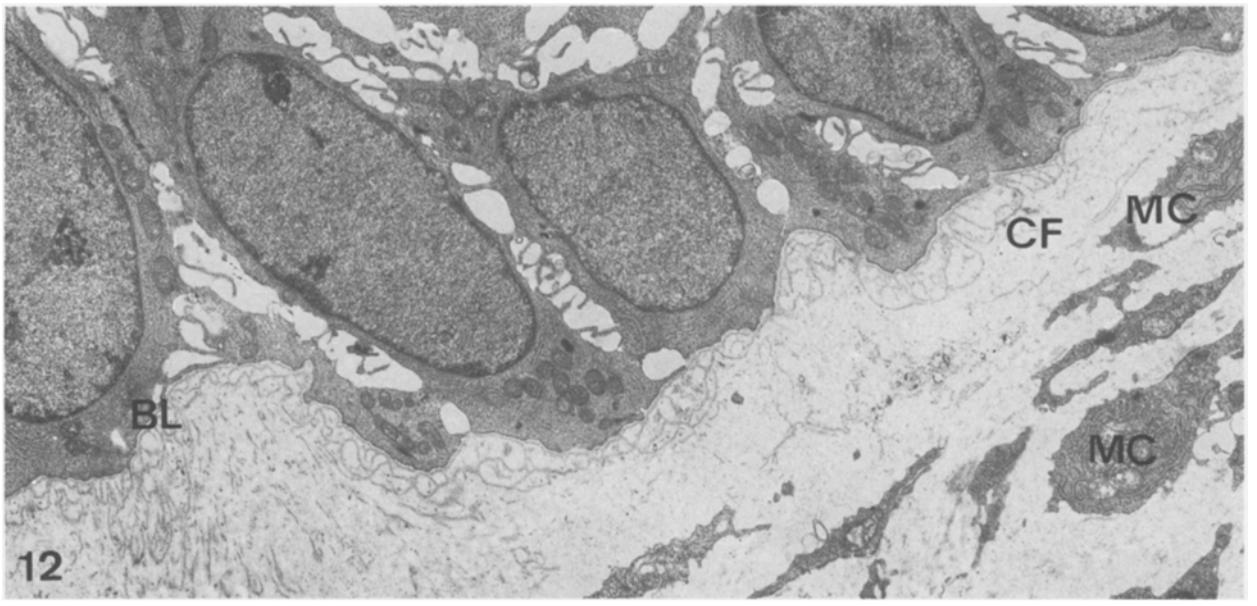
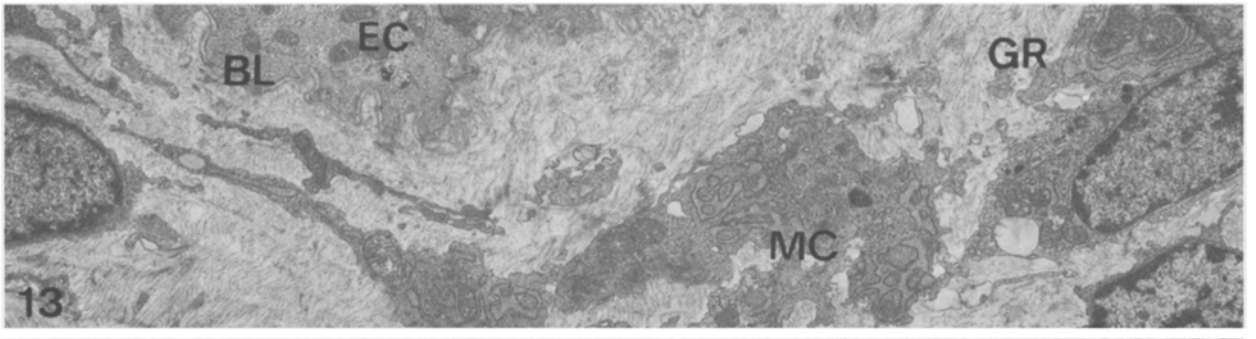
Fig. 8. Epithelium of a 13-week old control fetus. Slender cytoplasmic processes of epithelial cells project into the widened intercellular spaces (arrow). BL: basal lamina; MC: mesenchymal cells forming lamina propria around the acini. x 3000

Fig. 9. Well differentiated epithelium of a dihydrotestosterone-treated explant (13-week old fetus). The apical epithelial cells have polarized and some of them contain electron dense granules (G), BL: basal lamina; N: nucleus. x 7000

Fig. 10. A higher magnification of the apical granules (G) of the 13-week old hormone-treated fetus. M: mitochondrion. x 15.000

Fig. 11. Part of a basal epithelial cell filled with electron dense granules. A 13-week old fetus cultured in the presence of dihydrotestosterone. x 7000





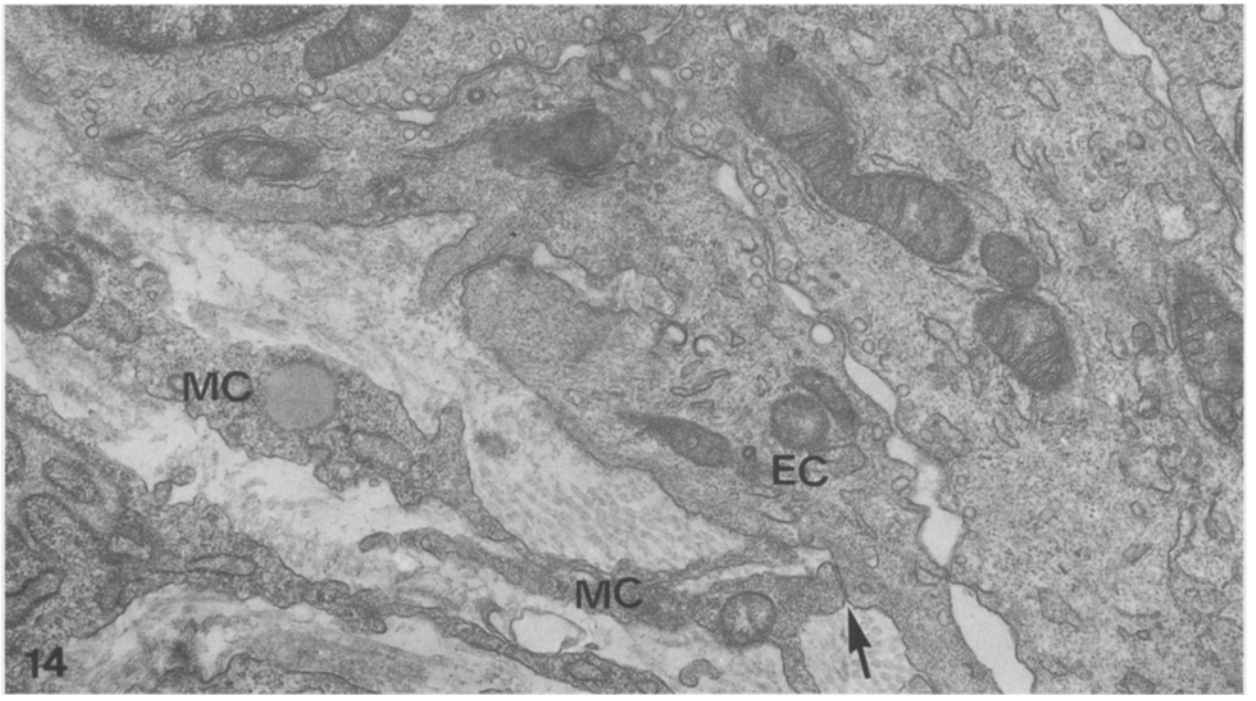


Fig. 12. The very folded multilaminar basal lamina (BL) of a 13-week old control fetus with adjacent mesenchymal cells (MC). Collagen fibres are seen in intercellular spaces (CF). x 6000

Fig. 13. The amount of granular endoplasmic reticulum and intercellular collagen fibres has increased in the dihydrotestosterone-treated fetus at the age of 13 weeks when compared to the control of the same age (Fig. 12). BL: basal lamina; EC: epithelial cell; GR: granular endoplasmic reticulum; MC: mesenchymal cell. x 6000

Fig. 14. Basal lamina has partly disappeared in the dihydrotestosterone-treated explant (a 13-week old fetus). The arrow indicates a direct cellular contact between an epithelial cell (EC) and a mesenchymal cell (MC). x 20.000

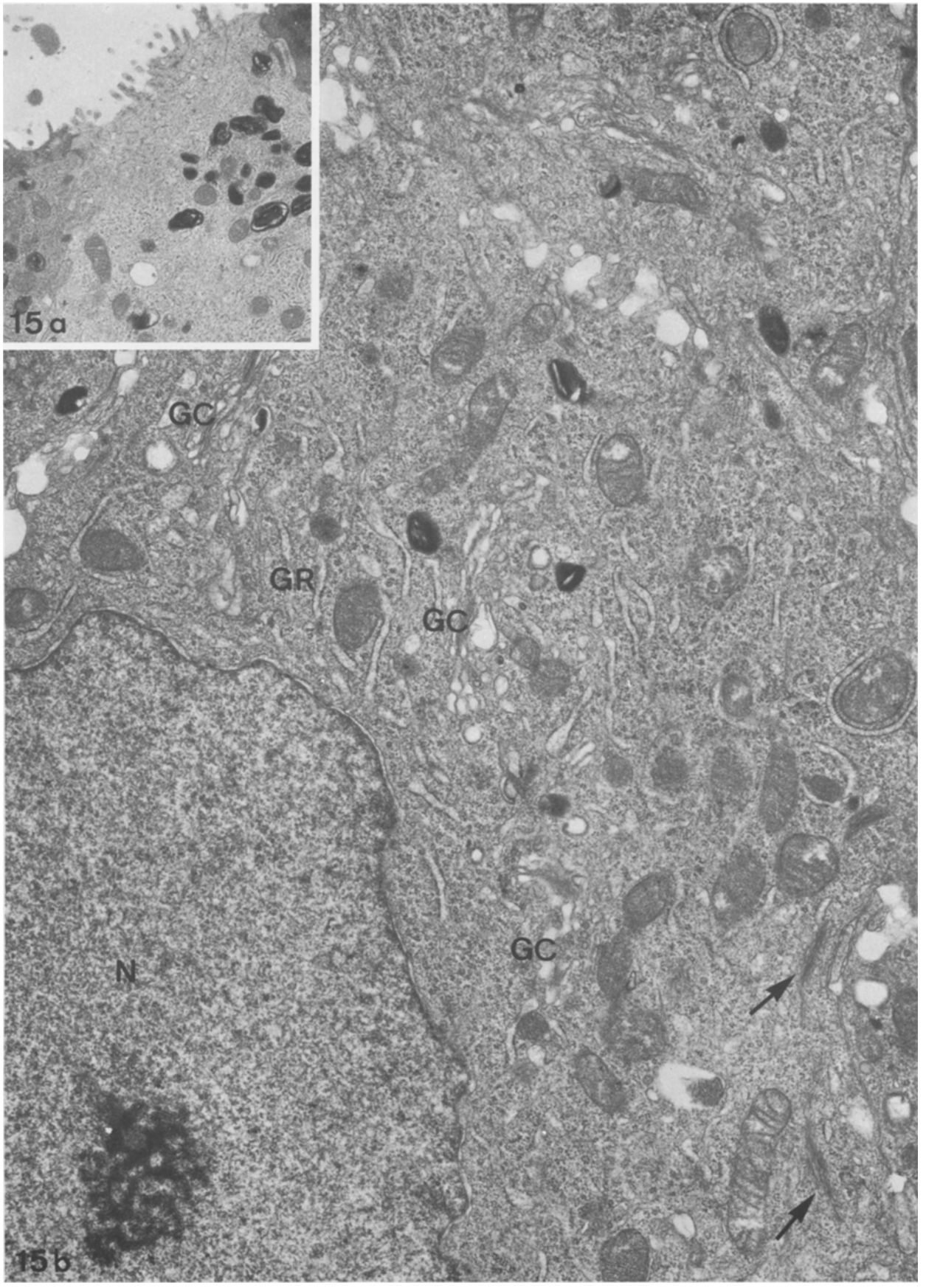


Fig. 15. Apical part of the epithelium (a) and peri- and supranuclear region of an epithelial cell (b) of a testosterone-treated 11-week old fetus with large Golgi complexes (GC) and numerous granules with membranous content. The arrow points at bundles of intermediate filaments; GR: granular endoplasmic reticulum; N: nucleus. (a) x 6400, (b) x 16.500

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