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EFFECTS OF CASTRATION UPON THE MORPHOLOGY OF THE ACCESSORY SEX ORGANS OF THE MALE RAT - A SCANNING ELECTRON MICROSCOPY STUDY

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Abstract

A systematic, comparative study of the accessory sex glands of the adult male rat after androgen withdrawal was carried out. The changes were investigated by using scanning electron microscopy at different intervals after surgical castration. The main common signs of epithelial cell involution were flattening of the cell surface, reduction of the size and number of microvilli, some blurring of the cell borders, cessation of secretory activity and diminution of the luminal volume of the glands. Overall, confident signs of atrophy were evident after one week, and complete epithelial involution was reached by the third week. The epithelial cell atrophy was accompanied by a relative stromal hyperplasia. The new observation seems to be that the process of stroma consolidation is progressing for a considerable time subsequent to the completion of the epithelial involution. This phenomenon is particularly evident in the dorsal prostate, the seminal vesicle and the coagulating gland.

Key Words: Prostate, seminal vesicle, coagulating gland, epithelium, stroma, atrophy, castration, rat, scanning electron microscopy, morphology.

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Introduction

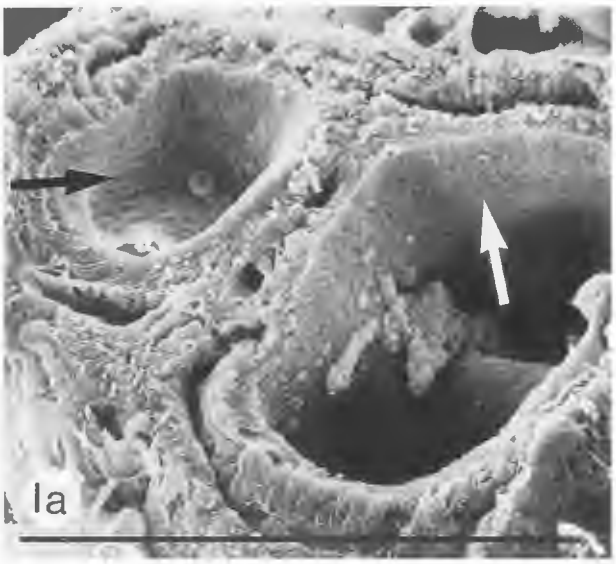
The glandular epithelium of the prostate lobes, the seminal vesicle and the coagulating gland in the rat is dependent on an appropriate amount of circulating androgen to maintain normal structure and function. After withdrawal of the androgen stimulus, a characteristic involution of the epithelium takes place. Such regressive changes are secondary to orchiectomy as well as to various forms of medical castration, as demonstrated by some studies using transmission electron microscopy (TEM) (Dahl and Kjaerheim, 1973; Dahl and Tveter, 1973, 1974; Kjaerheim *et al.*, 1974). These alterations are rapidly reversed by administration of testosterone (Dahl *et al.*, 1974; Thompson and Heidger, 1978). Along with the epithelial involution a relative stromal hyperplasia occurs, as shown by different quantitative methods (Bartsch *et al.*, 1975; De Klerk and Coffey, 1978; Kiplesund *et al.*, 1988; Holterhus *et al.*, 1993).

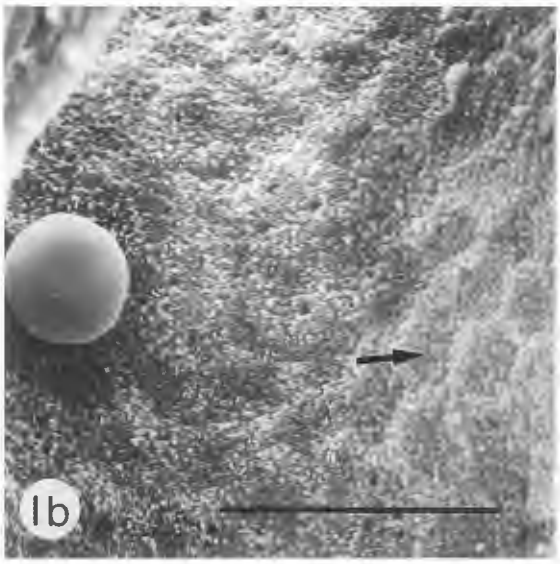
Apparently, there is no previous systematic study of the morphology of all these organs after androgen deprivation using scanning electron microscopy (SEM). In a companion paper, we have described the SEM characteristics of the intact glands of sexually mature animals (Wahlqvist *et al.*, 1996). The present study outlines the architecture of these male pelvic genital organs as shown by SEM at various intervals after bilateral orchiectomy. These findings form the basis of applying SEM as a supplement to TEM in further studies under various forms of hormonal manipulation and during ageing.

Materials and Methods

Fifteen male Wistar rats, 4-6 months old, weighing approximately 300 g were used in the study. The animals were all castrated under thiopental-sodium anaesthesia (50 mg/kg intraperitoneally) through a midline scrotal incision and examined after 3 days, 1, 3 and 6 weeks and 8 months, respectively, with 3 animals at each interval.

The intraaortic perfusion fixation, the dissection of the pelvic genital glands, the preparation of the samples





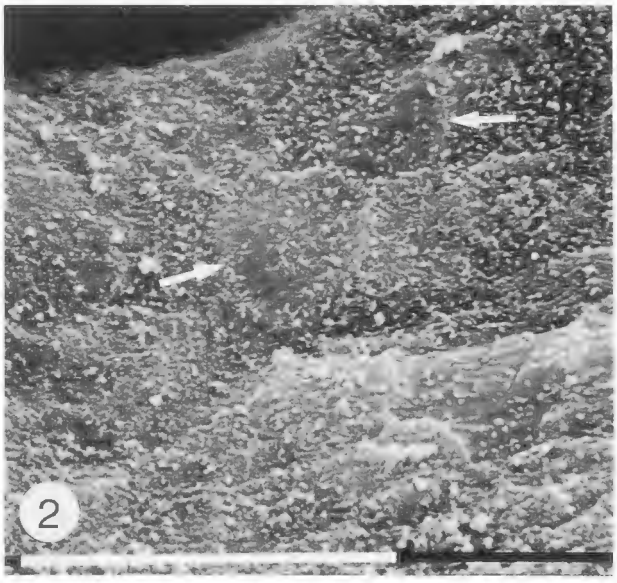


Figure 1. Scanning electron micrographs of the ventral prostate 3 weeks after castration. (a) The normal luminal folded structure has been converted to a rather smooth surface (arrows). Bar = 0.1 mm. (b) The apical cell surface is receded (arrow), and the cell boundaries are partly indistinct. Bar = 10 μm.

Figure 2. Scanning electron micrograph of the luminal cell surface of the lateral prostate 6 weeks after castration. The cells are flat, and the microvilli are reduced in number and length. The cell borders (arrows) are indistinct, making identification of individual cells difficult. Bar = 10 μm.

and the electron microscopic technique have been described in the preceeding paper (Wahlqvist *et al.*, 1996). The preparation was carried out with particular emphasis on visualizing cell surface morphology by dissecting and rinsing off the secretory products from the apical cell surface. To minimize the occurrence of fixation artifacts, the rinsing process was performed subsequent to the perfusion fixation procedure.

Results

All observations in this study are compared with our previous studies of sexually mature, intact animals (Wahlqvist *et al.*, 1996). To avoid a repetitive description of the alterations of the cellular and tissue structures as they appear in each of the five glands, the general features will be described initially.

Grossly, the incipient diminution of the pelvic genital organs is evident after 1 week, and after the third week, there is apparently no further atrophy. No obvious alterations of cell surface structures are observed after 3 days. The cells of the various glands keep their convex-shaped apical membrane populated with microvilli, comparable to the characteristics of the various glands in intact animals. After 7 days, the first signs indicating involution are encountered. Apparently, the morphological alterations of the epithelium of all glands seem to be concluded already after 3 weeks. The apices of the castrate cell appear as quite flat or receded to a level below the cell borders, indicating a reduced volume of the cell. This concave appearance of the cell surface reveals the cell borders very distinctly. Microvilli are reduced in number and length, but these findings are not observed as early as the reduction of the cell volume. All direct and indirect signs of secretory activity are eventually abolished.

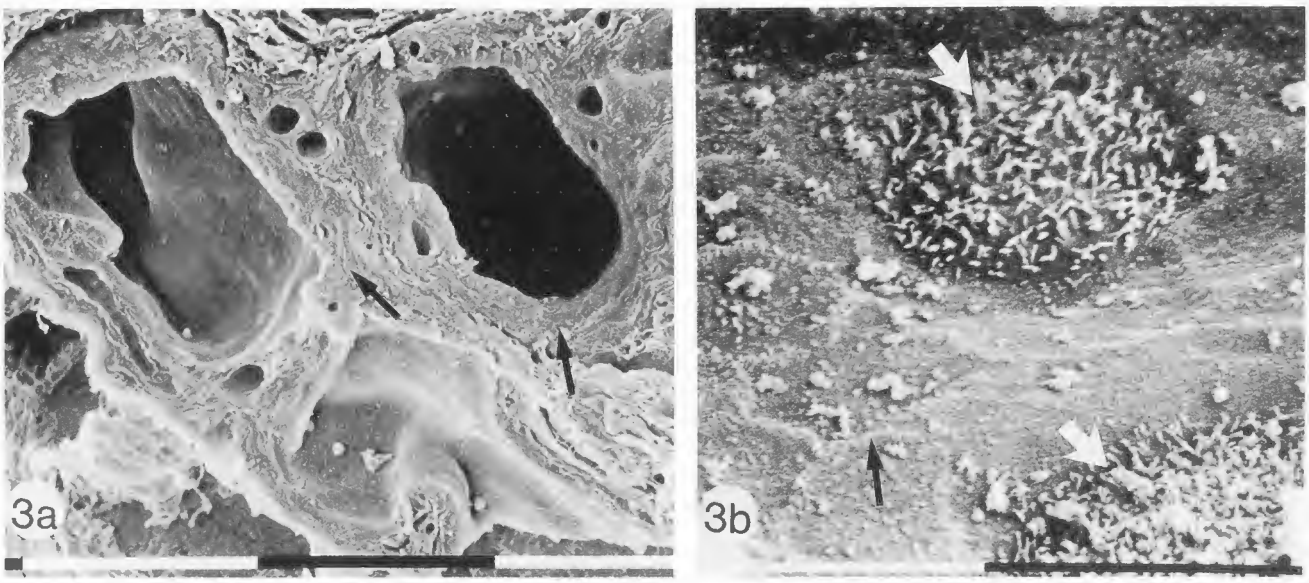


Figure 3. Scanning electron micrographs of the dorsal prostate 6 weeks after castration. (a) Survey micrograph of acini. Particularly the stroma circumscribing the acini has been increasingly consolidated throughout the postcastration period (arrows). Bar = 0.1 mm. (b) The luminal cell surface is flat, and the cell borders are indistinct (black arrow). Scattered cells still keep their microvilli (white arrows). Bar = 10 μm.

Parallel to the reduction of the cell volume, the lumina of all five glands become considerably shrunken. This reduction of the luminal volume, together with the impression of increased viscosity of the residual secretory material, makes it even more difficult to rinse off the secretory products from the apical cell surface.

A gradual, relative increase of the interstitial supportive tissue (stroma) occurs during the early postcastration period. As the size of the prostate lobes is reduced, there is a shift in favor of the stroma compared to the epithelial tissue. Among the prostate lobes, the stromal hyperplasia is particularly obvious in the dorsal lobe, where the individual acini are circumscribed by a broad rim of densely packed fibers with a pronounced capillary network. In the seminal vesicle and the coagulating gland, their fibrous walls show a relative increase in thickness.

Although the general features are outlined above, some few additional remarks will be made on the individual glands.

Ventral prostate (VP)

After 3 days, the configuration of the acini and the stroma of the VP is similar to that of intact animals. The characteristic normal feature of great variation in density of apical microvilli between individual cells within the same acinus still remains. After 7 days, the cell apices look more uniform. All cells have short microvilli, and the apical membrane has lost its convex shape, creating more prominent luminal cell borders.

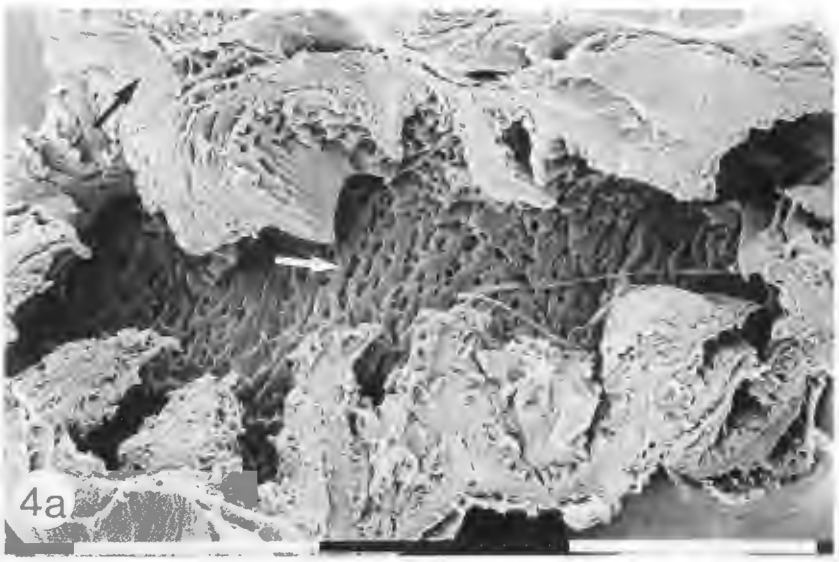
After 3 weeks, the luminal folds of the acini are less developed (Fig. 1a), the cell apices are concave, and the microvilli are more densely packed than in normal animals (Fig. 1b). The cell apices do not reveal any significant further alterations when examined after 6 weeks and 8 months.

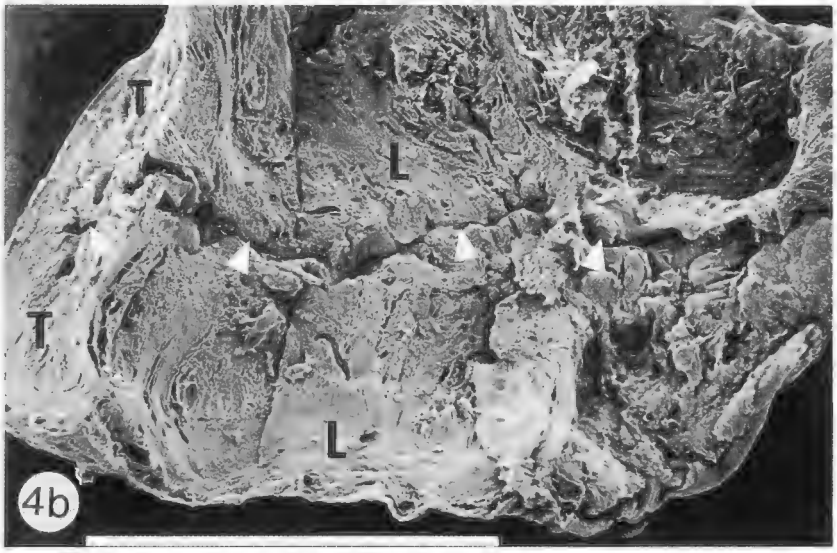
Lateral prostate (LP)

After 7 days, the cell apices of the LP have receded, giving a concave appearance. The microvilli still form a brush border, but after 6 weeks, the cells are flat with a substantial decrease in the density and the length of the microvilli. The luminal cell borders are slightly elevated and rather indistinct (Fig. 2). This lobe appears to keep the folded pattern of the acini more than the ventral lobe. On the other hand, the diminution of the luminal volume is comparable to the alterations of the other prostate lobes. The stromal hyperplasia is not as developed as in the other accessory sex glands.

Dorsal prostate (DP)

Already after 1 week, the characteristic well outlined spots denuded of microvilli, are still present in the DP, as in intact animals. The luminal blebs, however, are less numerous, smaller and more collapsed. Later on, most cells show a flat apical surface, creating more distinct luminal cell borders (Fig. 3b). The microvilli are significantly reduced both in size and number. However, a few moderately convex-shaped cell apices





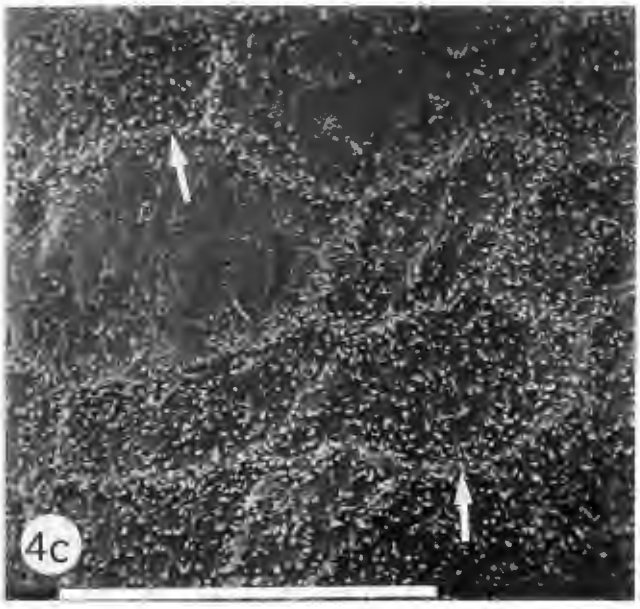


Figure 4. Scanning electron micrographs of the seminal vesicle following castration. (a) Longi-tudinal section 3 weeks after castration. The luminal volume is reduced, and the luminal ridges (white arrow) are closer together than normally. The relative thick-ness of the wall (black arrow) has increased. Bar = 1 mm. (b) Longitudinal (L) and transverse (T) sections 8 months after castration. The lumen is nearly completely obliterated (arrows). Bar = 1 mm. (c) The luminal cell surface is flat 3 weeks after castration. The cell borders are seen as ridges (arrows) rather than the grooves observed in normal animals. Bar = 10 μm.

heavily studded with microvilli, are occasionally observed as long as 6 weeks after castration (Fig. 3b). Although the acini are shrunken, they keep their normal luminal topography with a rather smooth, slightly folded surface. In this lobe, there is a particularly dense consolidation of the stroma underlying the epithelium (lamina propria).

Seminal vesicle (SV)

Already after 3 days, the number of apical orifices of the cell membrane, presumably resulting from an active secretion process, is significantly reduced in the SV. After 7 days, there are no surface signs of secretion, but the microvilli are comparatively well preserved. By the third week, the cell apices are flat or slightly concave with short microvilli distributed with highly variable density (Fig. 4c).

The survey topography of the luminal surface shows striking alterations. The typical transverse ridges are

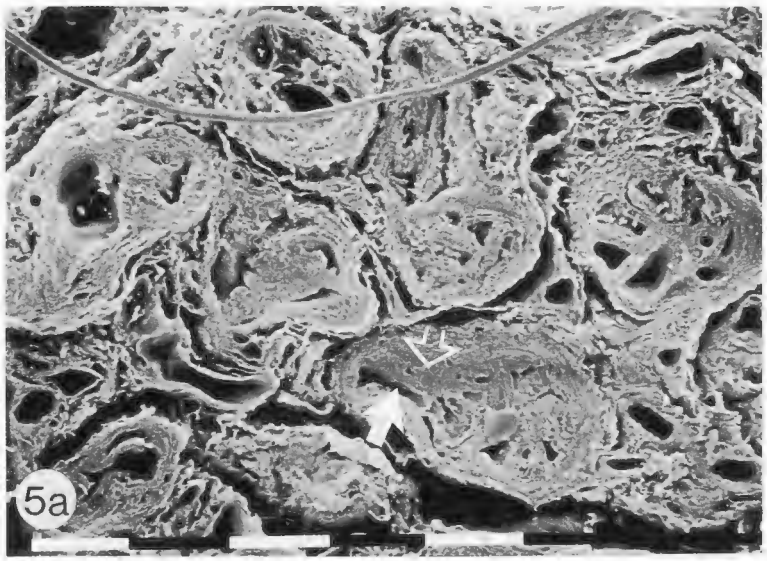


Figure 5. Scanning electron micrographs of the coagulating gland after castration. **(a)** Survey micrograph 8 months after castra-tion. The luminal volume has been considerably shrunken (closed arrow). The surrounding stroma is increasingly condensed (open arrow). Bar = 0.1 mm. **(b)** The luminal cell surface (white arrow) is only slightly flatter 3 weeks after castration compared to intact animals. The cell borders (black arrow) are even more distinct than normal. Bar = 10 μm.

lying closer together already after 1 week. This evolution becomes even more evident during the next weeks (Fig. 4a). The luminal volume of the gland is gradually reduced during the early postcastration period. When examined after 8 months, the lumen is practically totally obliterated (Fig. 4b). Simultaneously, there is a substantial relative increase of the subepithelial stroma, making a thick and dense wall of the gland. Contrary to the alterations of the epithelial cells, the relative increment of the stroma of the SV appears to progress as time passes by the initial three weeks following castration. The relative stromal growth does not cause an increased volume of the gland, but rather entails an increasing consolidation of the stroma by heavy packing of its fibrous components. The ratio between the thickness of the wall and the cross sectional diameter of the lumen is 1:3 and 15:1 three weeks and eight months following castration, respectively, compared to a ratio of 1:7 in intact animals.

Coagulating gland (CG)

The most characteristic finding of the cell apices of the CG is a relatively early reduction in number and length of the microvilli, thus leaving wide areas of the plasmalemma as a smooth, slightly undulating membrane (Fig. 5b). Contrary to the other glands, the apical membrane is not concave even as long as 8 months after castration. The luminal cell borders are slightly elevated and thus very distinct, as in intact animals, throughout all the postcastration period.

The survey topography of the luminal folded structure is comparatively well preserved. Nevertheless, there is an increasing element of fibrous subepithelial

tissue, creating a thicker wall around the glandular lumina, comparable to the observations in the DP (Fig. 5a). As in the DP, the relative stromal hyperplasia is particularly well developed within the lamina propria.

Discussion

To our knowledge, the present paper reports the first comparative study on morphological alterations of the male pelvic sex glands in the rat following castration by using scanning electron microscopy.

All five lobes of the pelvic genital glands revealed involutive phenomena which alter the three-dimensional apical pattern of the epithelium, as well as the luminal and subepithelial morphology. Principally, all glands underwent the same type of regressive alterations, but the timing and the degree of these changes were somewhat different in the individual glands. The apical surface of the epithelial cells became flat or concave with easily detectable cellular boundaries. The microvilli were significantly reduced in length and number. Direct and indirect evidence of secretory activity were almost instantly abolished. Mucosal folds were less elaborate, and acinar luminal volume was considerably shrunken. Eventually, atrophy of the epithelium was accompanied by a remarkable relative increase in the amount of stromal tissue when compared to the amount of glandular tissue.

The flattening of the normal appearing convex apical plasmalemma is a surface manifestation of mass loss within the epithelium. It reflects a reduction of the organelles responsible for synthesizing and enveloping the secretory products. In a morphometric study by Kiplesund *et al.* (1988), the greatest reduction of epithelial height 1 week after castration was observed in the VP (45%). In the LP, DP and CG, the reduction was in the order of 30-39%. By TEM, a reorganization of the rough endoplasmic reticulum (RER) and a general reduction of cytoplasm has been observed as early as 2 days following castration (Dahl *et al.*, 1973). Barham *et al.* (1979) found surface alterations in the SV of guinea pig to occur before significant intracellular ultrastructural changes were detected by TEM. Our finding is the opposite. In the CG, it takes a longer time for this flattening of the surface to occur, and it does not appear to the same extent as in the other lobes, as the basis is a less convex surface (Wahlqvist *et al.*, 1996). That is consistent with the TEM findings of a certain preservation of the voluminous RER-cisternae in this lobe during the first 2 to 3 weeks after castration (Dahl and Kjaerheim, 1973; Dahl and Tveter, 1973) and after as long as 12 weeks of estrogen treatment (Holterhus *et al.*, 1993).

A most prominent observation was the decrease of luminal volume in all lobes. In a study by Holterhus *et al.* (1993), the relative volume of the glandular lumen in the CG was reduced from about 65 vol% in intact animals to about 10 vol% twelve weeks after castration. Kiplesund *et al.* (1988) have shown that the reduction of the luminal volume by the first week after castration is greater in the VP and LP than in the DP and CG. However, the difference between the lobes at this early interval may be effected by differences in the timing of the involution process.

Following castration, the secretory activity of the epithelial cells of all lobes is drastically and almost instantly reduced. The blebs of the DP are small and shrunken in shape, and the orifices of individual cell apices of the SV are no longer observed after three days. This reduced secretory activity is consistent with the SEM observations in the SV of guinea pig by Barham *et al.* (1979) and Wong (1983) and with TEM observations in the rat CG (Holterhus *et al.*, 1993). In the DP and CG, the effect upon the secretion appears to be slightly different, whether the androgen deprivation is effected through orchiectomy or estrogen treatment (Steinhoff *et al.*, 1994). In surgically castrated animals, immunoreactivity for the enzyme transglutaminase (TGase) and apical bleb formation were practically brought to cessation during the second week. By the twelfth week, immunoreactivity for TGase was still preserved in estrogen treated animals. Moreover, apical protrusion of the cytoplasm was observed, but no real apical blebs were formed.

There is obviously some discrepancy in the literature concerning reduction of cell number after androgen deprivation. In their morphological studies Barham *et al.* (1980) and Wong (1983) found a reduction of the epithelial cell number in the SV after castration. We were not able to demonstrate morphological signs of such a reduction, as Wong (1983) describes as cell rupture and extrusion of the cell content into the lumen. Landström *et al.* (1990) in their morphometric studies of the Dunning rat prostate adenocarcinoma, were also unable to demonstrate a drop in cell number 6 weeks after castration. However, they found a significant reduction of the cell number when estrogen was added to orchiectomy, although the mechanism of action for this is not yet clarified. On the other hand, Kiplesund *et al.* (1988) calculated the numerical reduction of epithelial cells in the VP and LP to be in the remarkable order of 70%, one week after castration. The reduction was less in the DP and CG. On that basis, they rank the lobes with respect to androgen sensitivity, claiming that the VP and LP are more androgen dependent than the DP and CG.

The relative stromal hyperplasia accompanying the atrophy of the glandular epithelium was a consistent observation. As also noted by Holterhus *et al.* (1993) in

the rat CG, the stromal hyperplasia in the DP was particularly evident within the lamina propria. Thompson *et al.* (1979) observed by a morphological investigation that increased amount of collagen bundles and filaments in the rat VP were characteristic of both untreated castrated and estrogen-treated castrated animals. However, smooth muscle cells were more frequently observed within the prostatic stroma of estrogen treated animals. Subsequent investigations in the prostate of dog (Rohr *et al.*, 1981) and in the rodent CG (Andersson and Tisell, 1982; Holterhus *et al.*, 1993) have also indicated that estrogen treatment seems to be necessary for a significant smooth cell activation to occur. However, the relative increase of the smooth muscle cell volume in estrogen treated animals may be effected by a rearrangement of elongated smooth muscle cells as much as by an effectively increased cell number (Holterhus *et al.*, 1993).

The timing of the stromal alterations appears to differ from that of the epithelial involution. According to our present observations, the relative stromal growth and consolidation appear to proceed far beyond the time point for completion of the epithelial regression. Holterhus *et al.* (1993) found the stromal alterations in the CG to be completed 6 weeks after castration. In the SV, we observed a continuation of the relative stromal increment also after the sixth week. The extremely well developed stromal condensation observed in the SV after 8 months might be assumed to be fortified by a process of ageing. However, our SEM studies of unmanipulated aged rats rather show a reduction of the stromal tissue with increasing age (currently, unpublished results). Consequently, we regard our present finding of long-term relative stromal hyperplasia a consequence of androgen deprivation rather than aging.

In conclusion, SEM reveals characteristic alterations of the luminal surface morphology and the overall glandular architecture in the accessory sex glands of the male rat after castration. Involution of epithelial cell surface, diminution of luminal volume and a relative stromal hyperplasia are obvious features. The degree of these alterations and the timing by which they are effected are variable in the various lobes.

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Editor's Note: All of the reviewer's concerns were appropriately addressed by text changes, hence there is no **Discussion with Reviewers**.