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## SCANNING ELECTRON MICROSCOPY OF THE ACCESSORY SEX GLANDS OF THE ADULT MALE RAT

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### Abstract

This study describes the morphology of the accessory sex glands of the adult male rat as observed by scanning electron microscopy (SEM). The purpose was to obtain a systematic and comparative SEM description of these glands and to evaluate different preparation techniques. A common morphological feature is polyhedral delineation of the cells, which exhibited a variable convexity of their apical surface. The cell apices were more or less studded with microvilli. Nevertheless, it was possible to distinguish the glands by their surface morphology. In the ventral prostate, there was a considerable heterogeneity in cell surface appearance. The lateral lobe had a characteristic brush border, and in the dorsal lobe, surface blebbing and intracellular cisternae were observed. The cells of the seminal vesicle were covered by long microvilli, while particularly distinct, elevated cell borders and intracellular cisternae were typical for the coagulating gland. The secretory mechanism was merocrine in the ventral and lateral prostate and the seminal vesicle, and was mainly apocrine in the dorsal prostate. Surprisingly, only merocrine secretion was obvious in the coagulating gland. The most controversial observation, which needs further investigation, was the discovery of large orifices in the apical surface of individual seminal vesicle cells. These orifices may be indicative of an additional apocrine secretion in this gland. In studying this organ system, SEM provides information that adds to previous transmission electron microscopical investigations.

**Key Words:** Scanning electron microscopy, prostate, seminal vesicle, coagulating gland, accessory sex glands, epithelium, secretion, rat, male, morphology.

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### Introduction

The accessory sex organs of the male rat consist of the prostate and the paired seminal vesicles (SV) and the coagulating glands (CG). The prostate is composed of three different lobes, i.e., the ventral (VP), the lateral (LP) and the dorsal (DP) lobes. The fine structure of organelles of these organs has been thoroughly studied by using the transmission electron microscope (TEM) (Dahl *et al.*, 1973). On the other hand, reports on the morphology of the cell surface as seen by scanning electron microscopy (SEM) are scanty. Descriptions of the prostate are restricted to one or two lobes (Stone *et al.*, 1977; Guggenheim *et al.*, 1979). While there are some reports on the surface morphology of the SV in different mammalian species (Hücker and Aumüller, 1976; Murakami *et al.*, 1978; Barham *et al.*, 1979; Wong, 1983; Mifune *et al.*, 1986), there are apparently no SEM studies on the CG in the rat. As far as we are aware, the surface morphology of the CG in the golden hamster has been described only in a single report (Chow, 1988). A systematic study on SEM morphology of this organ system is apparently lacking.

In our laboratory, we investigated the ultrastructure of all these five glands in young, sexually mature rats by using SEM in order to obtain a systematic and comprehensive description of surface morphology of all the accessory sex glands in the male rat. This constitutes a basis for further studies on the effects of various forms of hormonal manipulation of this androgen dependent organ system. The applicability of different preparation techniques will be elucidated as well, and one of these techniques also depicts intracellular morphology.

Another aim of the study was to evaluate how SEM can contribute to our knowledge of the secretory processes in these glands, which have been described by TEM, autoradiography and immunohistochemistry (for a review, see Aumüller and Seitz, 1990).

### Materials and Methods

Seven male Wistar rats 4-6 months old, weighing

250-300 g, were used in this study. Under thiopental-sodium anaesthesia (50 mg/kg intraperitoneally), the aorta was cannulated with a plastic catheter of caliber 3 French proximal to the renal arteries. The tip of the catheter was then positioned just above the aortic bifurcation. The vena cava was opened to create an outlet for the returning perfusion fluid.

Five rats (group I) were initially perfused with 0.9% saline to achieve complete exsanguination of the pelvic organs, and subsequently with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, as fixative. Two rats (group II) were only perfused with 0.9% saline before subsequent preparation. After the perfusion procedure, the various lobes were gently dissected out under the stereomicroscope, cut into thin slices and prepared for SEM. Only the anterior tip of the lateral prostate and the medial part of the dorsal lobe were used, as the intermediate zone may contain a mixture of dorsal and lateral tissue (Gunn and Gould, 1957). The subsequent preparation of groups I and II will be described separately.

The specimens of group I were prepared with emphasis on cell surface structure. It is essential to remove the secretory products from the apical cell surface to obtain optimal SEM preparations. In order to get rid of the surface secretions, it is important that the glutaraldehyde perfusion period lasts for no longer than 3-5 minutes. Otherwise, the secretory products will become too viscous and adhere strongly to the cell surface. In 3 of the 5 animals of group I, the secretions were removed from the prostate lobes by vigorous shaking in distilled water with intermittent drying on a blotting paper. The SV and CG are saccular organs with spacious luminal compartments in contrast to the multilobular parenchymatous prostate lobes. Consequently, the secretory products of SV and CG could be removed by delicate microdissection under the stereomicroscope after opening of the luminal compartments, subsequent to the perfusion fixation. This was combined with rinsing in distilled water, as also applied for the prostate lobes. After rinsing the specimens, they were postfixed with buffered 2.5% glutaraldehyde.

The specimens from the remaining 2 animals of group I underwent no specific rinsing procedure. After the perfusion and postfixation with glutaraldehyde, they were cut by the freeze fracture technique.

In group II, the specimens were prepared for SEM investigation according to the technique described by Tanaka and Naguro (1981). Briefly, this includes initial fixation by buffered 1% osmium tetroxide ( $\text{OsO}_4$ ), treatment in a graded series of dimethylsulfoxide (DMSO) to avoid formation of ice crystals, and then freeze fracturation. The fractured specimens were melted in 50% DMSO and rinsed in buffer. To remove the matrix of

the cytoplasm, the specimens were treated in buffered 0.1%  $\text{OsO}_4$  at 4°C for 56 hours and then in 1%  $\text{OsO}_4$  for one hour.

The specimens from both groups were treated in a graded series of ethanol and critical point dried (Biorad E300, Biorad, Cambridge, MA). They were then sputter coated (Polaron sputter coater E5100, Polaron Equipment, Ltd., Doylestown, PA) with a layer of 30-50 nm platinum. All specimens were investigated in a Philips SEM 515 scanning electron microscope (Philips Electron Optics, Eindhoven, The Netherlands).

## Results

Although ultrastructural similarities exist between single glands, there are comparatively few morphological characteristics that are common for all these five male accessory genital glands. Nevertheless, the three prostatic lobes reveal some common features with regard to the survey topography. They are composed of acini separated by a relatively thin layer of loose supportive tissue; it is almost impossible by SEM to distinguish between smooth muscle and connective tissue cells. The luminal surface of each acinus displays an irregular pattern of folds lined by columnar epithelial cells. Structures resembling branching excretory ducts are occasionally observed. In contrast, the seminal vesicle and the coagulating gland appear principally as more saccular organs with spacious luminal compartments. Common for all five glands are the polyhedral outline and the convex apical surface of the luminal epithelial cells.

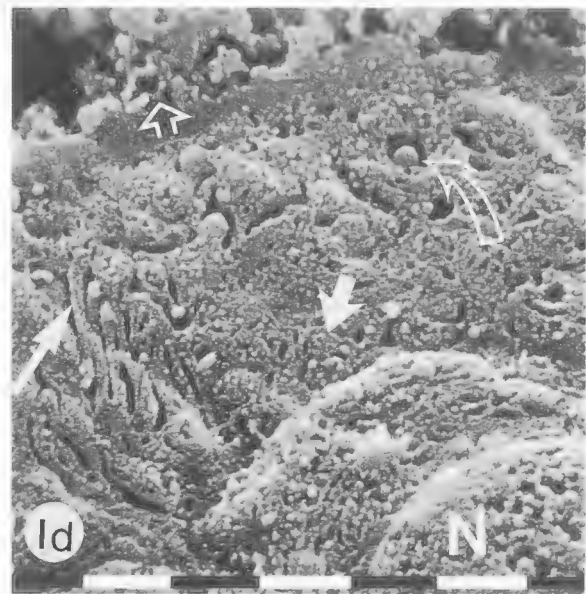
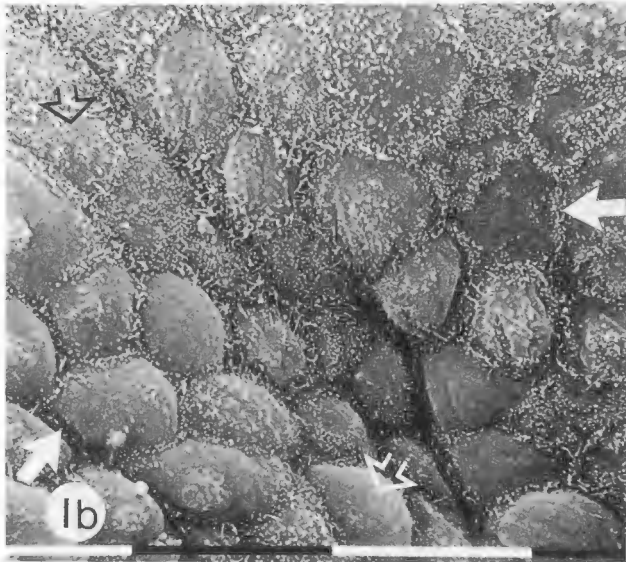
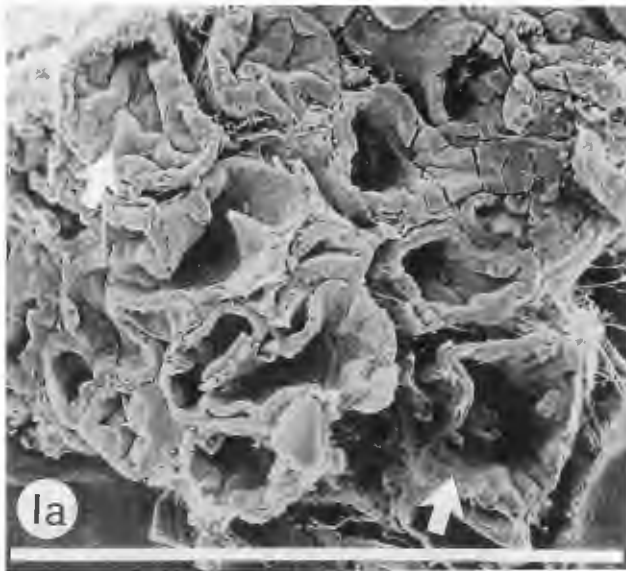
By combining the three different preparation techniques, various aspects of morphology are elucidated. The specimens depleted of secretory products give a useful survey impression of the architecture of the glands, as well as details of the cell surface. The specimens prepared by the freeze fracture technique only, show some intracellular secretory vacuoles. By this technique, observations of details of cell surface morphology are more difficult and occasional due to adhesion of the secretory product to the cell surface. The Tanaka/Naguro technique, comprising osmium maceration of the cytoplasm, reveals additional intracellular details and some surface characteristics as well. However, the surface observations made by this technique, must be interpreted cautiously.

The following discussion will describe the various glands separately. For the prostate lobes, the description mainly refers to acinar morphology.

### Ventral prostate (VP)

The most prominent survey feature of the acinar luminal surface of VP is a rather shallow infolded structure (Fig. 1a). Branching excretory ducts without

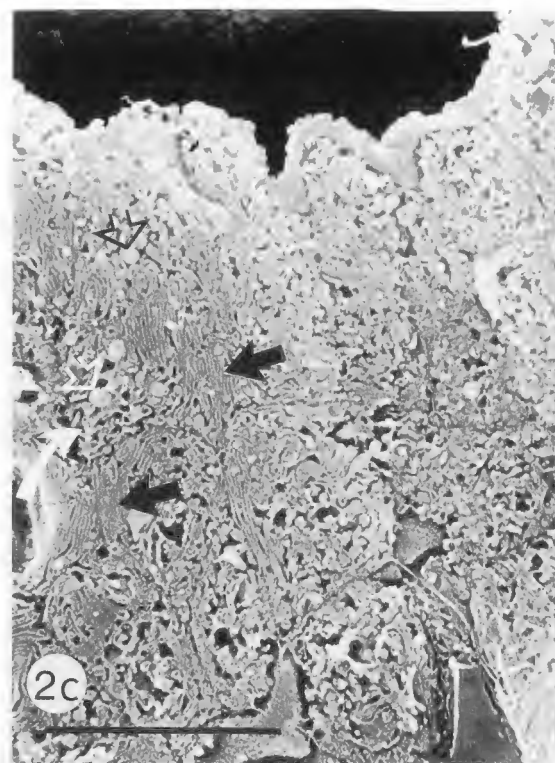
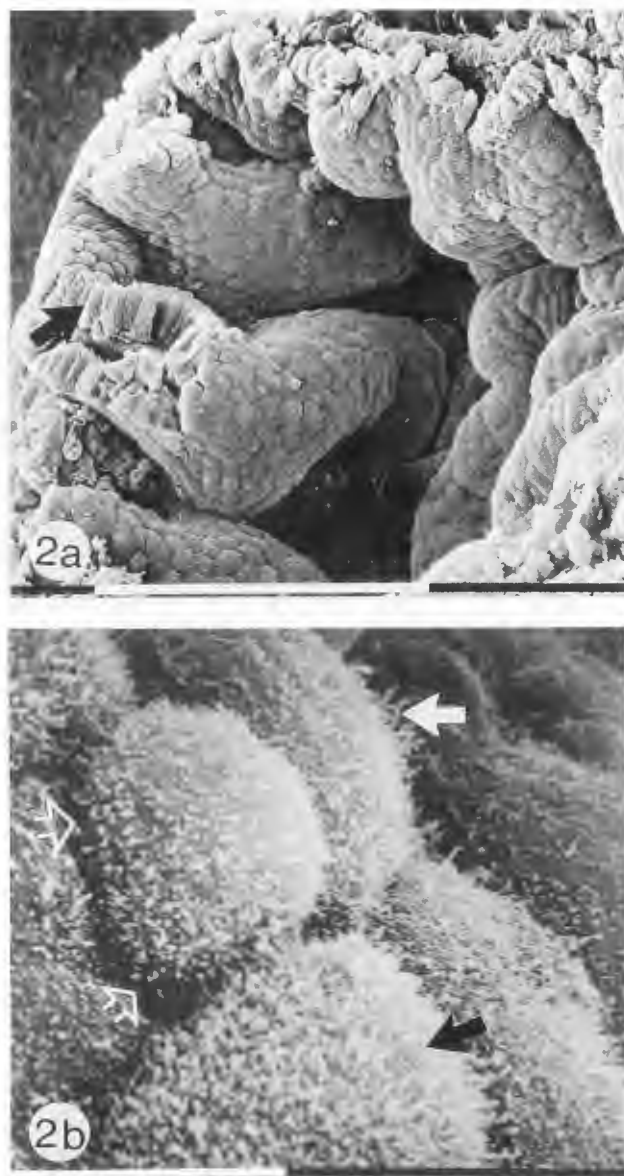




**Figure 1.** Scanning electron micrographs of the ventral prostate. (a) Survey micrograph of the acini lying close together with a thin intervening stroma. The acini exhibit a varying degree of luminal folded structure (arrows). Bar = 1 mm. (b) Luminal surface of cells within an acinus. The cell borders are distinct (closed arrows). The luminal prominence and the amount of microvilli are variable among individual cells (open arrows). Bar = 10  $\mu$ m. (c) Local prominence of the apical cell surface (blebbing) sporadically observed in osmium macerated specimens (arrow). Bar = 1  $\mu$ m. (d) Osmium macerated specimen showing the subcellular structure. The nucleus (N), RER (long, white arrow), Golgi area (short, white arrow), secretion vacuoles (curved, open arrow) and the apical surface (straight, open arrow) may be depicted. Bar = 1  $\mu$ m.

foldings are occasionally observed. A typical feature is the great variability of surface characteristics between neighbouring luminal cells (Fig. 1b). Generally, the cell apices are protruding into the lumen, while flat cells are relatively rare. The degree of the apical convexity is independent of whether the cell is situated in the concave bottom or on the convex top of the foldings. The majority of the cell apices are only slightly dome-shaped. On





**Figure 2.** Scanning electron micrographs of the lateral prostate. (a) Survey micrograph of an acinus with well developed luminal foldings lined by columnar epithelial cells (arrow). Bar = 0.1 mm. (b) Acinar convex cell surface densely populated with long microvilli, constituting a brush border (closed arrows). Apical cell boundaries are visualized as deep grooves between the cells (open arrows). Bar = 10  $\mu\text{m}$ . (c) An osmium macerated specimen showing subcellular structures. The typical feature is an enormous amount of RER (closed, straight arrows) producing secretion granules (open arrows). The granules are incorporated in vacuoles by the Golgi apparatus (curved arrow). Bar = 10  $\mu\text{m}$ .

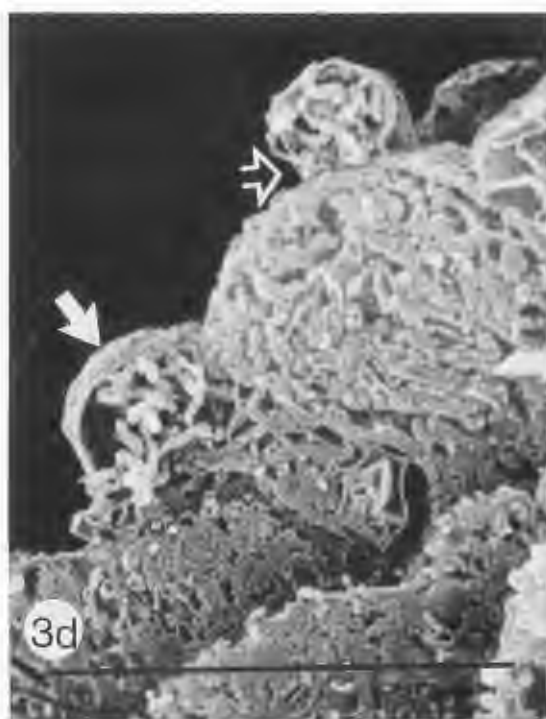
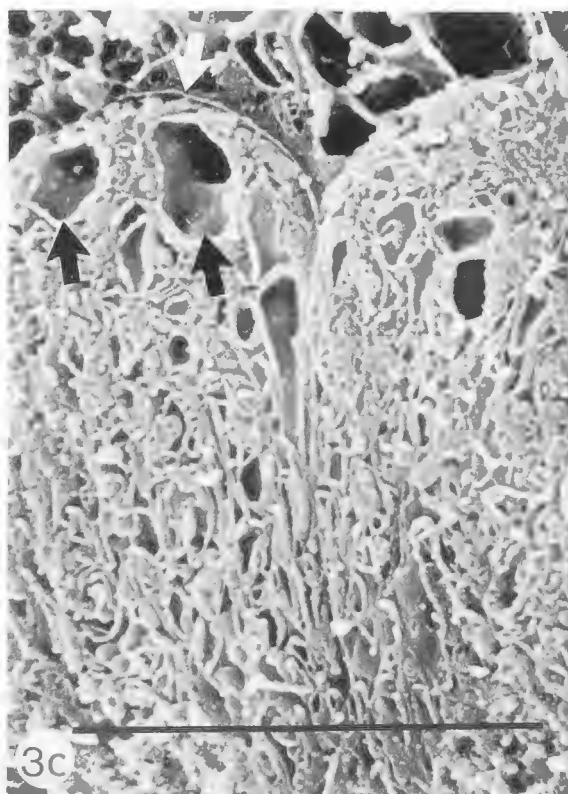
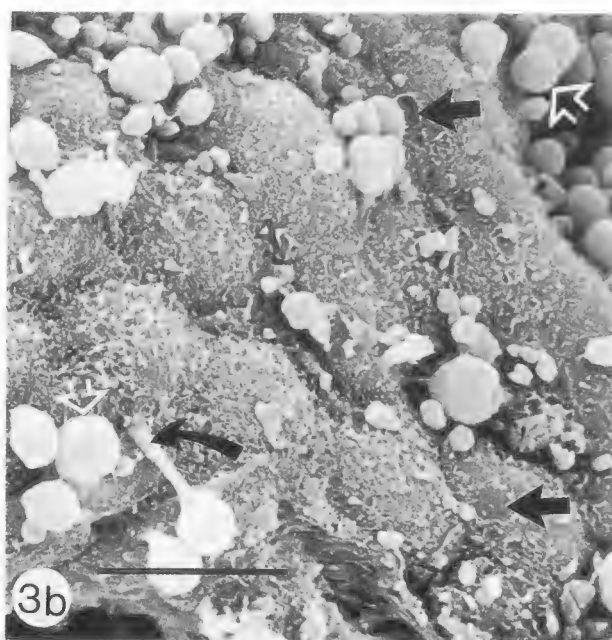
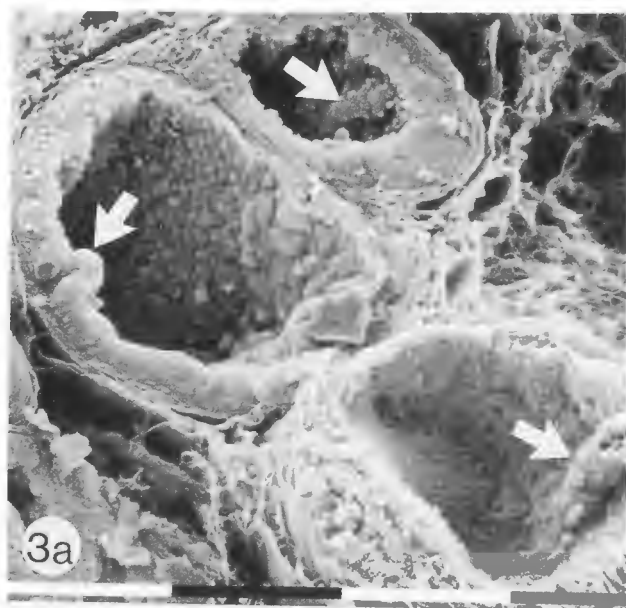
the other hand, the density of the microvilli on the surface is highly variable between individual cells. The diameter of the microvilli is constantly 0.2  $\mu\text{m}$ , but their length varies. When few microvilli are present, they tend to be located adjacent to the cell border. In some regions, neighbouring cells have a more uniform appearance with a convex cell surface evenly covered by short microvilli.

All the cells of the excretory ducts look similar with a convex surface populated with short microvilli and with no evidence of secretory activity. The longest diameter of the ovoid cells is parallel to the direction of the duct, similar to the lateral lobe.

The specimens prepared by the simple freeze fracture technique demonstrate secretory vacuoles containing different types of secretory products. These vacuoles are located apically. The typical secretory product con-

sists of homogeneous, dense spherical vacuoles. Occasionally, these vacuoles harbor single granules with a variable density. Some vacuoles contain a fine granular material.

The Tanaka/Naguro preparation technique demonstrates a round, basally located nucleus with a distinct nucleolus. The most obvious finding is a well developed rough endoplasmic reticulum (RER) mainly located apically to the nucleus (Fig. 1c). The RER is generally arranged as straight or slightly curved parallel membranes. The Golgi apparatus is located apically and occupies only a small part of the cytoplasm. This technique demonstrates that the secretion granules located within the secretion vacuoles are emptied into the lumen



**Figure 3.** Scanning electron micrographs of the dorsal prostate. (a) Survey micrograph of typical acini with a comparatively smooth luminal surface. Only low crests (arrows) are protruding into the lumen. Bar = 0.1 mm. (b) Luminal surface of acinar cells. Convex cell surfaces with distinctly outlined spots devoid of microvilli (closed arrows). Generous amount of luminal blebs (open arrows). A single bleb is about to be pinched off at its constricting base (curved arrow). The base is probably artificially elongated during the preparation procedure. Bar = 10  $\mu$ m. (c) Osmium macerated specimen visualizing the interior of the cell. Cisternae (black arrows) are located in the apical part of the cell

as well as just beneath the cell surface (white arrow). Bar = 10  $\mu$ m. (d) A developing apical bleb (closed arrow). Another bleb is about to be pinched off at its base (open arrow). Bar = 10  $\mu$ m.



by rupture of the vacuolar membrane through the apical cell membrane (merocrine secretion).

Blebbing is regarded as the morphological expression of an apocrine form of secretion. On the luminal surface, blebbing starts by an increasing prominence of the cell apices (Fig. 1d). In the VP, blebbing is an exceptional finding. When present, it is limited to only a part of the cell surface area, and the phenomenon is observed solely in the specimens prepared by the Tanaka/Naguro technique.

#### Lateral prostate (LP)

The luminal acinar surface of the lateral prostate is composed of an irregular pattern of anastomosing folds resembling the ventral lobe. However, the folds lie closer together than in the VP, thus creating a greater surface area (Fig. 2a). It is particularly difficult to remove the secretory product from the cell surface of this gland. When cleaned, the cell surface shows a rather regular pattern with nearly no variations within the same acinus. The polyhedral outlined apical cell surface protrudes substantially into the lumen (Fig. 2b). The cell borders are visualized as deep grooves. In the branching excretory ducts, the cell apices are similar to those of the acini, but their delineation is more ellipsoid.

The most characteristic surface feature of the LP are numerous, densely packed microvilli covering the apices of all cells with minimal variation between individual cells (Fig. 2b). The LP is easily recognized by this carpet of microvilli, known from other organs as a brush border.

In the specimens prepared by the etching technique of Tanaka/Naguro, the most prominent finding is a developed RER arranged as narrow parallel membranes (Fig. 2c). Scattered cells with cisternae like those observed in the DP are encountered as well. Only one type of secretory vacuoles containing a single granule is observed. These secretory granules are emptied into the lumen by rupture of the vacuolar membrane through the apical plasmalemma as typical for a merocrine secretion.

#### Dorsal prostate (DP)

The DP has fewer folds of the acinar surface compared to the other two prostate lobes. The acini are oval or circular with low crests protruding into the lumen rather than forming deeper indentations (Fig. 3a). Contrary to the VP and similar to the LP, the apical surface has a rather uniform appearance within the acini. The apical surface with its polyhedral outline is slightly convex and is populated by relatively few, short microvilli. Characteristically, small, sharply outlined areas lacking microvilli, are observed on the cell surface.

The most prominent surface characteristic of the DP are a large number of round, apparently membrane-bound secretory bodies of variable size (blebs). Such

blebs are regularly seen on the surface in a variable amount in the specimens prepared with the rinsing procedure. They are obviously detached from the cell by having their base being pinched off (Figs. 3b and 3d).

The Tanaka/Naguro technique demonstrates cisternae located in the apical compartment of the cytoplasm (Fig. 3c). The cisternae are outlined by a single-layered granular membrane. Their interior surface reveals holes leading into papillary projections of the cisternae, resembling the coagulating gland. An abundant RER mainly apical to the nucleus, oriented as curved lamellae lying closely together, is also encountered. On the other hand, the number of membrane-bound secretory granules in the cytoplasm is generally low.

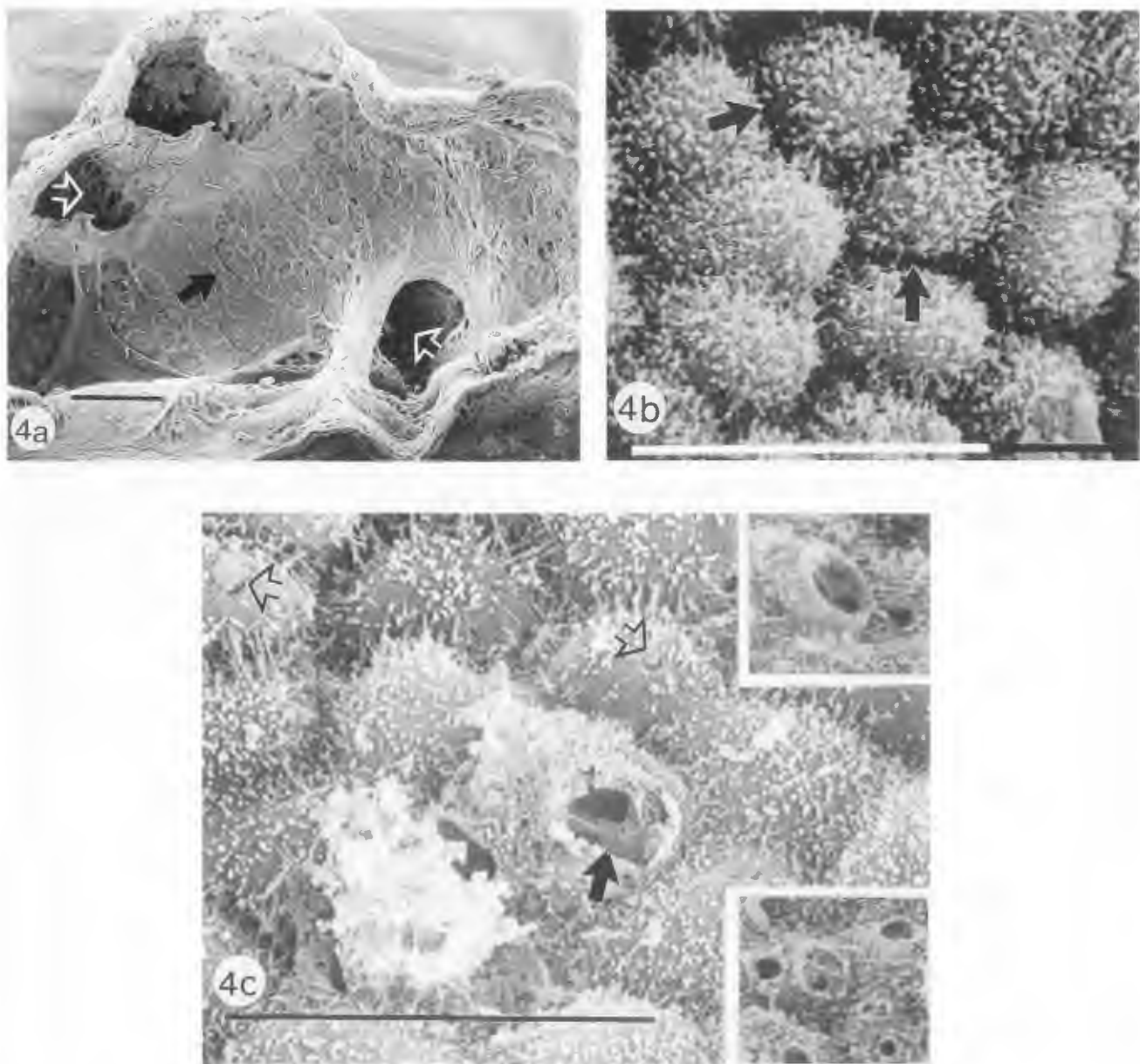
#### Seminal Vesicle (SV)

The survey appearance of the luminal surface of this saccular gland reveals a system of anastomosing ridges oriented in various directions (Fig. 4a). The principal direction is perpendicular to the long axis of the gland, at least in the central part of it. In the peripheral pouches forming the undulating outer surface of the organ, the ridges are oriented more longitudinally. The intermediate clefts are deeper in the peripheral pouches than centrally in the gland. In addition to these clefts, round holes submerge into underlying crypts.

The apical cell surface has a polyhedral (hexagonal) and convex shape. A prominent characteristic is a large number of microvilli densely and evenly populating the apical cell membrane (Fig. 4b). Their width is consistently 0.12  $\mu\text{m}$ , but their length varies between 0.2 and 0.7  $\mu\text{m}$ . In some specimens, numerous secretion granules are found on the luminal surface (Fig. 5a). Smaller granules fusing into larger ones are occasionally observed.

The appearance of the apical cell surface is in other respects variable and heterogeneous. Relatively large holes of the surface membrane are conspicuous, but smaller, well circumscribed openings on the surface are encountered as well (Fig. 4c). Through the latter, single secretory granules are emptied into the lumen. The larger holes that are quite characteristic for this gland, are partial defects of the surface. The bottom of these holes appears as a fenestrated membrane. Other cells may show a local prominence of an intact surface membrane.

The simple freeze fracture technique demonstrates numerous secretory vacuoles located apically in the cell. Vacuoles with an eccentrically located small secretion granule are rather numerous. These granules are emptied into the lumen as typical for a merocrine secretion process, and they are also demonstrated by the Tanaka/Naguro preparation technique (Fig. 5a). Moreover, other details of the apical cytoplasm can only be studied by this latter technique. The ovoid nucleus is located



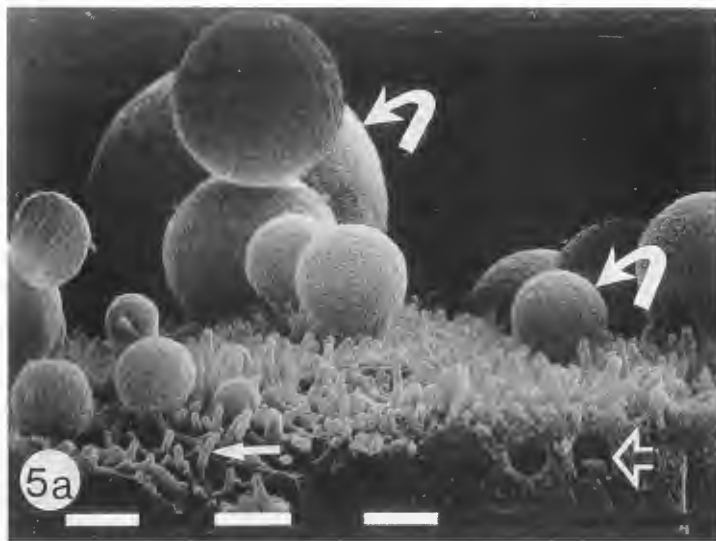
**Figure 4.** Scanning electron micrographs of the seminal vesicle. (a) Survey micrograph of the thin-walled gland cut longitudinally. The lumen is constituted by a central cavity with ridges oriented transversely (closed arrows) in addition to peripheral pouches (open arrows). Bar = 1 mm. (b) Luminal convex cell surface heavily populated with microvilli. The cell boundaries reveal as deep grooves (arrows). Bar = 10  $\mu$ m. (c) Wide orifices of the cell surface, possibly indicative of an apocrine secretion (closed arrow). Bar = 10  $\mu$ m. The inserts show subsequent stages of the restoration of the cell membrane in the orifices. In the **upper** insert, the orifice has a fenestrated bottom. Centrally, in the **lower** insert, the cell membrane is nearly completely restored, with only the circular delineation of the orifice left. Secretion granules in small, sharply outlined orifices of the apical membrane indicate merocrine secretion (open arrows).

basally. The RER is rather extensive and is oriented as parallel, narrow cisternae often in a curved fashion. The supranuclear Golgi area is comparatively large, and double membrane enveloping of the secretion granules occurs in this region (Fig. 5b).

#### Coagulating gland (CG)

The survey architecture of this gland is characterized by anastomosing folds arranged distinctly different from those of the seminal vesicle. At low magnification, the gland is composed of ovoid units. Each unit





**Figure 5.** Scanning electron micrographs of osmium macerated specimens of the seminal vesicle. (a) Secretion vacuole (open arrow) beneath the apical cell membrane, which is carpeted by long microvilli (closed arrow). Luminal secretion granules (curved arrows) are variably sized. (b) A particularly abundant Golgi area (closed arrow) located apical to the nucleus (curved arrow). A double membrane enveloping a secretion granule is observed (open arrow). Bars = 10  $\mu$ m.

## Discussion

shows an irregular pattern of anastomosing folds with indentations of variable depths (Fig. 6a). This gives the organ a vast luminal surface area.

The apical surface of the epithelial cell is slightly convex. The number of microvilli populating each cell is significantly less than in the LP and the SV. They are also shorter than in those glands and tend to concentrate particularly along the cell borders (Fig. 6b). Due to this distribution, the cell borders are very distinct in the CG. Convincing signs of luminal blebbing of the apical cytoplasm are not observed. The cell surface apparently reveals no holes or craters in contrast to the SV.

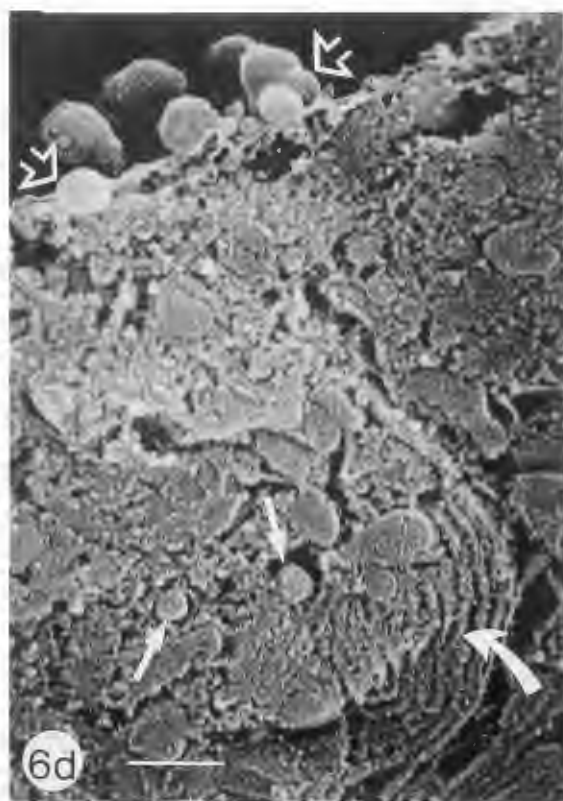
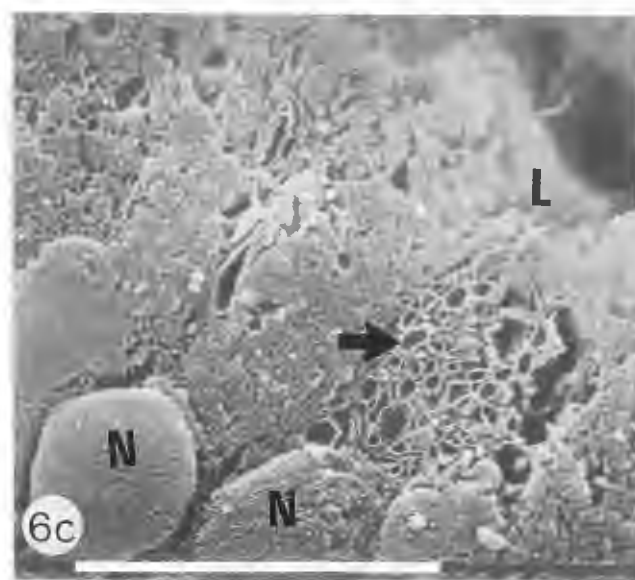
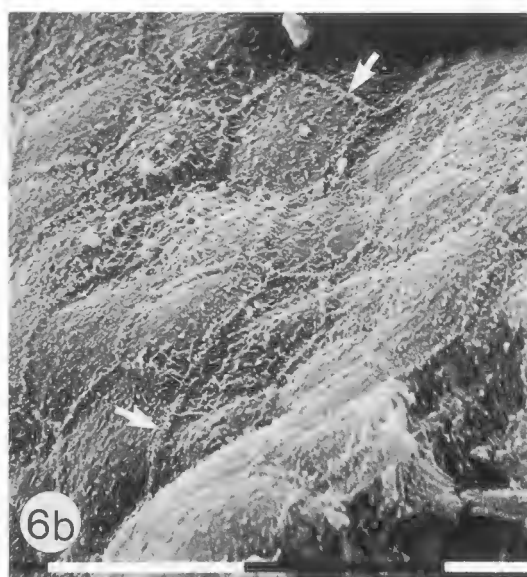
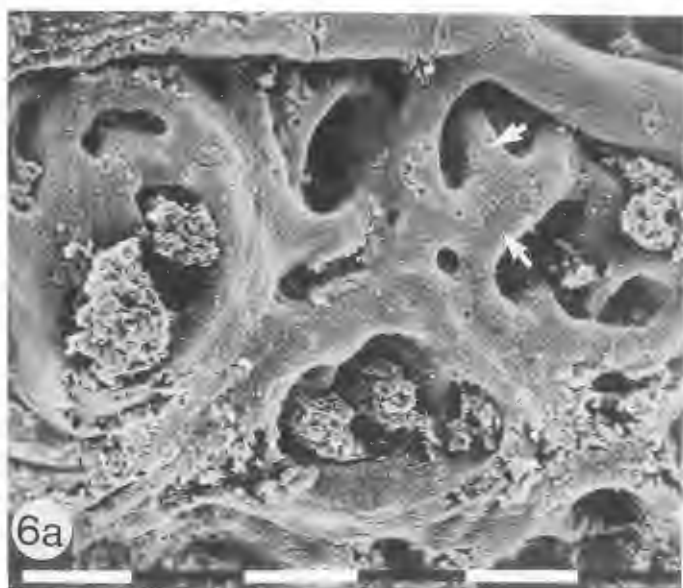
The Tanaka/Naguro technique shows an abundant RER oriented as narrow, parallel and slightly curved lamellae. Apically, there are some membrane-bound vacuoles with eccentrically located secretion granules (Fig. 6d). Apparently, these secretion granules are released from the cell by rupture of the enveloping membrane through the apical plasmalemma. This is consistent with a merocrine secretion process.

A typical observation in the epithelium of this gland is the great number of cisternae with varying degrees of filling. They are located from the nuclear region to the apical border and are easily demonstrated by the Tanaka/Naguro etching technique (Fig. 6c).

The present paper is, to the best of our knowledge, the first comprehensive SEM report on the accessory sex glands of the male rat concentrating on a comparative description of the SEM morphology of this organ system. A secondary aim is to focus on the secretory process and the utility of different preparation techniques.

To evaluate the applicability of the SEM technique, different preparation techniques were tested. In order to obtain a reliable visualization of the surface structures, it is mandatory to remove the secretions by a specific rinsing procedure. Otherwise, the surface is hidden by the secretions, and no studies of surface areas can be performed. Therefore, the freeze fracture technique without any rinsing procedure only rarely reveals the cell surface. Neither can the different intracellular details be examined by this technique, except for the secretory vacuoles demonstrated in the VP and the SV. Consequently, this preparation technique has been abandoned in our subsequent studies.

A process of rinsing the secretory product away from the surface subsequent to perfusion fixation gave the best visualization of the surface morphology. Applying this preparation technique to all glands simultaneously and uniformly, also makes it easier to rule out fixation artifacts for two reasons: first, the glands are fixed



**Figure 6.** Scanning electron micrographs of the coagulating gland. (a) Survey micrograph of the anastomosing ridges (arrows). Bar = 0.1 mm. (b) Typical apical cell surface area with a comparatively scarce amount of microvilli and particularly distinct, elevated cell borders (arrows). Bar = 10  $\mu$ m. (c) Osmium macerated specimen showing cells containing a variable amount of cisternae (arrow) in the apical region. Nuclei (N) and lumen (L) are landmarks for orientation. Bar = 10  $\mu$ m. (d) Osmium macerated specimen showing the apical part of a single cell with secretion granules located in vacuoles (closed arrows) and in the lumen (open arrows). The RER (curved arrow) is prominent. Bar = 1  $\mu$ m.

before any manipulation. Second, observations, as potential fixation artifacts, ought to be present in all glands, and not only in one or two glands.

To our knowledge, application of the Tanaka/Naguro technique with etching of the matrix of the cytoplasm by osmium maceration has previously not been described to any extent in the accessory male sex glands of the rat. The advantage of this technique is that it depicts intracellular as well as surface structures. The



nucleus, Golgi area, RER and the secretion vacuoles with their granules are well preserved. However, the limitation of this technique seems to be the interpretation of the observations of surface morphology, which should be undertaken cautiously. Loss of intracinar tension caused by removal of the luminal secretory product prior to the fixation procedure and alterations in osmolarity, may produce artifacts as the blebbing occasionally observed in the VP.

Although differences exist, some common morphological features are revealed in this genital organ complex: the luminal surface shows a folded structure lined by columnar epithelial cells with a convex apical surface and a polyhedral (hexagonal) delineation. The apical plasmalemma is populated with microvilli, and all glands have an abundant capacity of synthesis and secretion of proteinaceous material. Despite the existing similarities, TEM studies have shown that the various accessory sex glands have specific intracellular characteristics, and it is possible to subgroup these glands (Dahl *et al.*, 1973). Correspondingly, the present SEM study indicates that the same glands can be identified by specific features of their epithelial cell surface as well; this concept is summarized below.

The survey topography of the acini of the prostate lobes is principally not very dissimilar, in contrast to aspects of apical cellular morphology. The VP is characterized by a marked variation in density of microvilli within the same acinus as previously described in the LP by TEM (Schrodt, 1961; Flickinger, 1971). Typical for the LP is an enormous amount of long microvilli, constituting a prominent brush border. Similar to the proximal tubular cell of the kidney, the prominent microvilli may be the location of specific membrane-bound enzymatic processes and transport mechanisms of low molecular weight substances. In the DP, a dominant observation is an abundant amount of luminal blebs. The SV shows a characteristic saccular, ovoid appearance with mucosal folds oriented principally transversely to the long axis of the gland in contrast to the longitudinal orientation reported by Hücker and Aumüller (1976). The luminal cell surface appearance is heterogeneous. Some cells are evenly covered with long microvilli, while other cells reveal distinctly outlined fenestrated orifices of the apical plasmalemma. The CG has typical anastomosing spacious luminal compartments, and the cell boundaries are particularly distinct.

In the osmium macerated specimens, the cisternae of the DP and CG are located apical to the nucleus. In TEM micrographs, on the contrary, the cisternae are essentially observed in the basal area of the cell (Dahl *et al.*, 1973). We suggest that this finding should be regarded as a fixation artifact, as the nucleus itself seems to be displaced to an even more basal location in

our osmium macerated SEM specimens than in the specimens prepared for TEM by perfusion fixation.

With regard to the secretory mechanisms, our findings support, as well as contrast, previous reports. The heterogeneity of microvilli distribution observed particularly in the VP, may represent initial surface signs of secretion. Focal clearance of microvilli has been observed by Kachar and da Silva (1980) to precede both merocrine and apocrine secretion, being an incipient stage of these secretory processes. On the other hand, apical blebs arise from the microvilli themselves in the DP and the CG (Hawkins and Geuze, 1977; Aumüller and Adler, 1979; Chow and Pang, 1989).

Morphological signs of merocrine secretion were clearly demonstrated in the VP, the LP and the SV. This is in accordance with previous TEM reports (Helminen and Ericsson, 1970; Dahl *et al.*, 1973). Our observation of merocrine secretion in the CG may, according to the literature, account for only a part of the secretion from this gland. Two pathways of protein secretion are suggested by Barlett *et al.* (1984) in the DP and the CG of the rat. One slow route, taking hours, is mediated through the Golgi apparatus, while a rapid route is bypassing the Golgi area, leading to secretion within 40 minutes. Samuel and Flickinger (1986a) have detected immunoreactive proteins in epithelial secretory vacuoles of the mouse CG. These are secreted in the merocrine way, probably representing the slow secretory route (Samuel and Flickinger, 1986b). On the other hand, Seitz *et al.* (1990) have by immunohistochemistry observed apocrine extrusion of the enzyme transglutaminase in the DP and CG, presumably being responsible for a rapid mode of secretion. Steinhoff *et al.* (1994) have shown that the merocrine and apocrine mechanisms in the CG secrete proteins are different in structure. Surprisingly, we were unable by the SEM technique to confirm apocrine secretion in the CG, although we regard its operation as confirmed in the literature.

Apical blebbing in the DP has been described by TEM in the rat (Dahl *et al.*, 1973; Aumüller and Adler, 1979; Seitz *et al.*, 1990) and in the hamster (Toma and Buzzel, 1988). The present study shows the ability of SEM to confirm the presence of apocrine secretion in the DP. The surface signs are apical local prominence of the cell surface, apical blebs being pinched off at a constricting base and multiple round bodies of variable sizes in the acinar lumina. Moreover, there were relatively few membrane bound vacuoles of the cytoplasm in the osmium macerated specimens, considered by Aumüller and Adler (1979) as one of the criteria necessary for an apocrine secretion. On the other hand, we were unable to recognize dilation of the tip of the microvilli, considered to initiate the apocrine secretion process (Nicander *et al.*, 1974; Wong and Tse, 1981; Toma and

Buzzel, 1988).

The most controversial finding in the present study is probably the observation of rather wide apical orifices of some seminal vesicle cells. We regard these as a sign of a hormonally dependent secretory process rather than artifacts, as they are infrequently observed in castrated animals (our own observations). Moreover, we have seen the same type of apical orifices to an even greater extent during the initial stimulatory phase following administration of a gonadotropin-releasing hormone agonist (currently, unpublished results). Whether these observations represent merocrine or apocrine secretion, is not settled by the present study. Clermont *et al.* (1992) have shown one large and one small type of secretory vacuoles in the apical cytoplasm of seminal vesicle epithelial cells. The observed orifices of the apical plasmalemma may represent a merocrine release of the large granules.

Alternatively, apocrine secretion may operate in addition to merocrine secretion. Apical blebs denuded of microvilli and with a diameter comparable with that of the large orifices, are indicative of apocrine secretion. In bovine seminal vesicle bleb-like structures are common (Agrawal and Vanha-Perttula, 1987), while Mifune *et al.* (1986) regard the large orifices of the surface to be a sign of cell degeneration. Supposing that apocrine secretion is present in the SV, our findings indicate that the detachment of the blebs from the cell is different from that in the DP. In the SV, the bleb release is probably accomplished through rupture along the broad base of the bleb, leaving a temporary orifice, instead of being pinched off at a constricting base, as observed in the DP. The former mechanism of bleb release was clearly observed by Messelt (1982) in the rat submandibular gland. Messelt (1982) also observed microvilli in the bottom of the apical orifices, while the orifices in our specimens were revealed as a fenestrated membrane. We interpret these observations as different stages in the restoration process toward a mature cell membrane following the apocrine release of the secretory material. However, more evidence has to be gathered before an apocrine mode of secretion can be confirmed in the SV.

In conclusion, the present study confirms previous TEM observations that each male accessory sex gland has its own ultrastructural characteristics, also when investigated by SEM. Despite its limitations, SEM appears to be a valuable supplement to TEM, particularly when elucidating the secretory process. Additionally, SEM has the capacity of visualizing wider areas, thus detecting morphological dissimilarities within the individual glands. The principal secretory mechanism is merocrine in the VP, the LP and the SV, while apocrine secretion predominates in the DP. In the CG merocrine,

secretion works as a supplement to the apocrine process reported in the literature. Finally, we can not exclude apocrine secretion as an additional process in the SV.

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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.