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Ultrastractural features of the principal cell in the epididymis of the rhesus monkey

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Abstract. The ultrastructural features of the principal cell in the epididymal epithelium of the monkey epididymis are suggestive of the cell carrying out a dual function of absorption and secretion. Both these functions occur on the luminal surface of the cell as well as on the lateral and basal aspects of the cell which face the intercellular spaces. Transmision Electron Microscopic studies of epididymal tissues following their impregnation with lanthanum nitrate indicated that the intercellular spaces are effectively sealed-off from the luminal space by the apically situated tight junctions between adjoining principal cells. The intercellular spaces are contiguous with the perivascular spaces of the subepithelial blood capillaries. It is suggested that the absorptive and secretory functions occuring on the apical surface of cells may be related to the creation of an appropriate intraluminal milieu for the maturation of spermatozoa while the occurrence of these functions in the intercellular spaces may represent an exchange of substances between the principal cells and the subepithelial capillaries.

Keywords. Epididymis; rhesus monkey; ultrastructure; male reproduction; epithelium.

Introduction

Spermatozoa traversing the epididymis undergo a complex process of biochemical, physiological and morphological changes termed 'sperm maturation' which endows them with the ability to fertilize ova (Bedford, 1975). This knowledge has been the basis for considering the epididymis as one of the potential targets whose functions could be extraneously interferred with for purposes of regulating male fertility.

Studies on non-primate species, particularly the rat, have shown that the absorptive and secretory functions of the epithelial cells lining the epididymal lumen are largely responsible for the creation of an intraluminal milieu conducive to sperm maturation (Hoffer *et al.,* 1973; Brandes, 1974; Hamilton, 1975; Wong *et al.,* 1978; Jones *et al.,* 1979). The mammalian epididiymis is known to secrete glycerophosphoryl choline, sialic acid, lipids and protein (Hamilton, 1975).The

scretion of these substances has been demonstrated *in vitro* in the principal and basal cells isolated from the epididymal epithelium (Killian and Chapman, 1980). Similar detailed studies are singularly lacking in primate species although it is fairly well-known that the epididymis in primates is structurally different in certain aspects as compared to that of the rat and other lower mammals (Alsum and Hunter, 1978; Prakash *et al.,* 1979; Ramos and Dym, 1977).

Information on the ultrastructural features of absorption and secretion of the epithelial cells in primate species would be of pertinent relevance for developing and testing such of the fertility regulating agents interfering with epididymal function. In this communication we report certain salient features of the epididymal epithelium in the rhesus monkey with particular reference to the ultrastructural correlates of absorption and secretion. A preliminary report of this study has been presented earlier (Prakash *et al.,* 1980).

Materials and methods

Tissues from the initial, middle and terminal segments of the epididymis (Prakash *et al.,* 1979), were taken from healthy adult rhesus monkeys. The tissues were fixed by vascular perfusion with or by immersion in Karnovsky's fluid containing 1% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4. Post-fixation was carried out in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 h at 4°C. The tissue were further processed for electron microscopy as described (Anand Kumar *et al.,* 1980). Small pieces of tissues were also processed according to the method of Shaklai and Tavassoli (1977) for tracing the contiguity of intercellular spaces by using lanthanum nitrate.

Results

General features

Principal cells are distinguished by the presence of stereocilia (figures 1, 2 and 3) and an extensive, well-developed Golgi apparatus (figure 4 and 5). Differences in the light microscopic features of principal cells situated in different segments of the epididymis have been reported earlier (Prakash *et al.,* 1979). The present ultrastructural studies have revealed that the apical surface of principal cells in the initial segment protrudes into the epidiymal lumen (figure 3). Such cytoplasmic protuberances were found only in the principal cells occuring in the initial segment. The presence of the cytoplasmic protuberances both in material fixed by immersion or by perfusion suggests that these protuberances are not fixation artifacts.

The plasma membranes of adjoining principal cells are attached at their apical end by tight junctional complexes (figure la). At the basal end of adjoining principal cells the plasma membranes form complex interdigitations and the intercellular spaces between the plasma membranes resemble canaliculi (figure 8).

Electron microscopic observations of tissues impregnated with lanthanum nitrate showed that the tracer had permeated throughout the intercellular spaces as well as the perivascular spaces of the subepithelial blood capillaries. Lanthanum

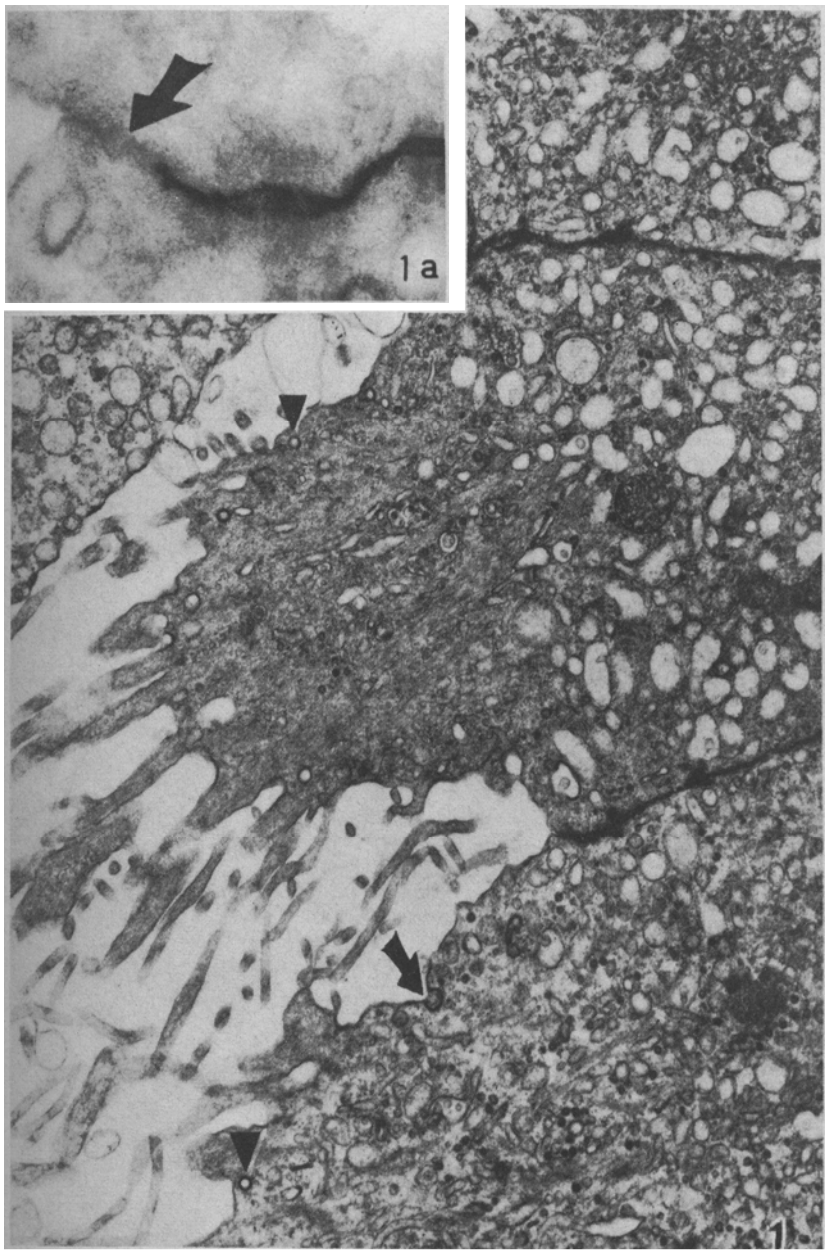
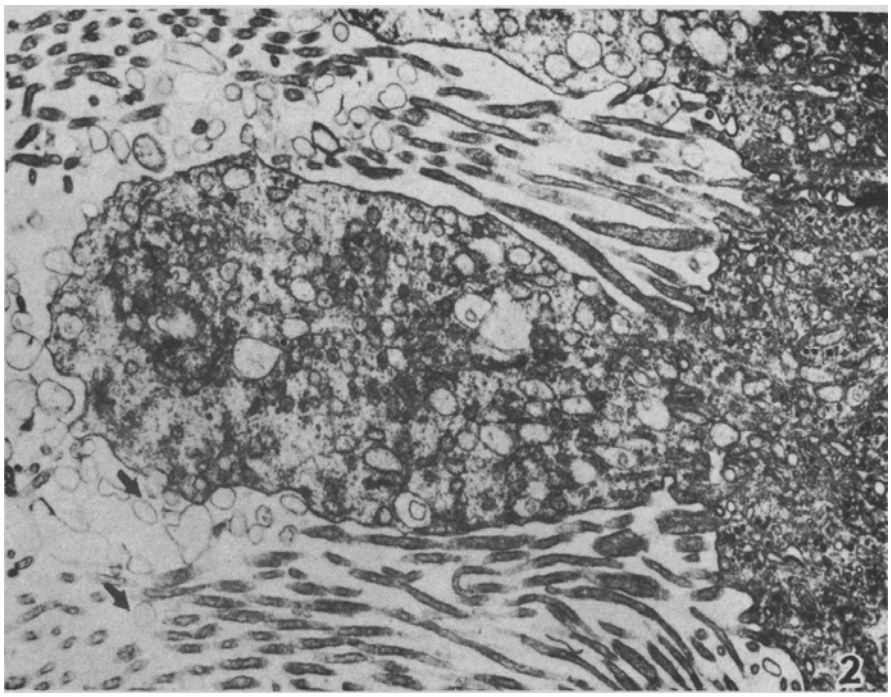


Figure 1. The apical part of principal cell in the initial segment is distinguished by the presence of stereocilia and the occurrence of pinocytotic invaginations in the plasma membrane (arrow). The pinocytotic invaginations of vesicles have an electron-dense coat (arrowhead) (×15,000). *Inset.* A representative electron micrograph of an unstained section from a lanthanum nitrate impregnated epididymis taken from the initial segment. The tracer has penetrated the intercellular space only as far as the *zonula occludens (arrow)* which suggests that the epididymal lumen is effectively sealed off from the intercellular space by the *zonula occludens* (× 57,000),



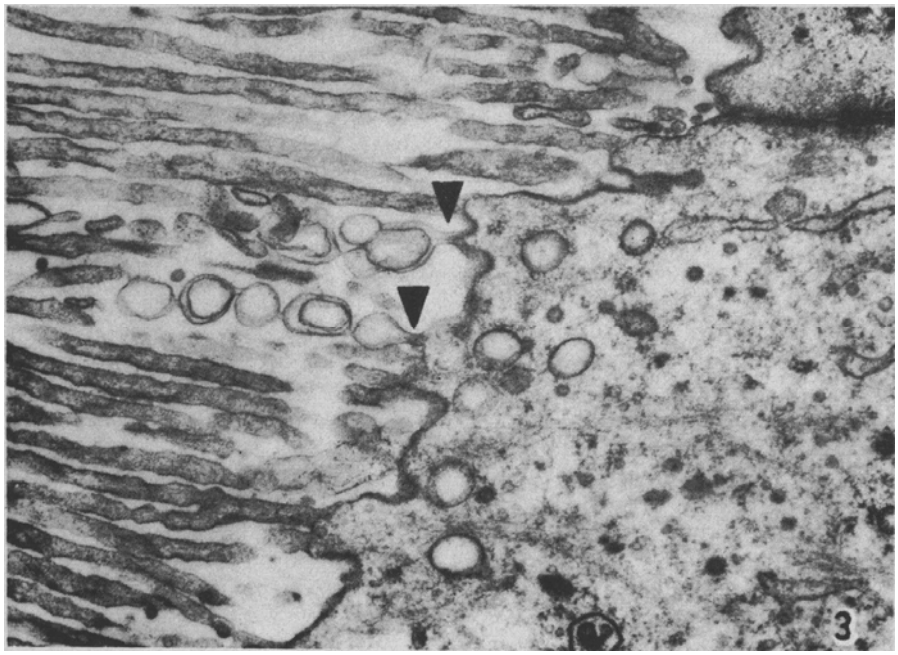


Figure 2. A cytoplasmic protuberance is seen in the apical part of a principal cell of the initial segment. The apical part of the cell includes several smooth surfaced vesicles (arrowheads) (×15,000).

Figure 3. Luminal surface of a principal cell in the middle segment of the epididymis illustrating the possible extrusion (arrowheads) of a smooth surfaced vesicle into the epididymal lumen where they appear as empty spheres as seen in Figure 2 (×27,500).

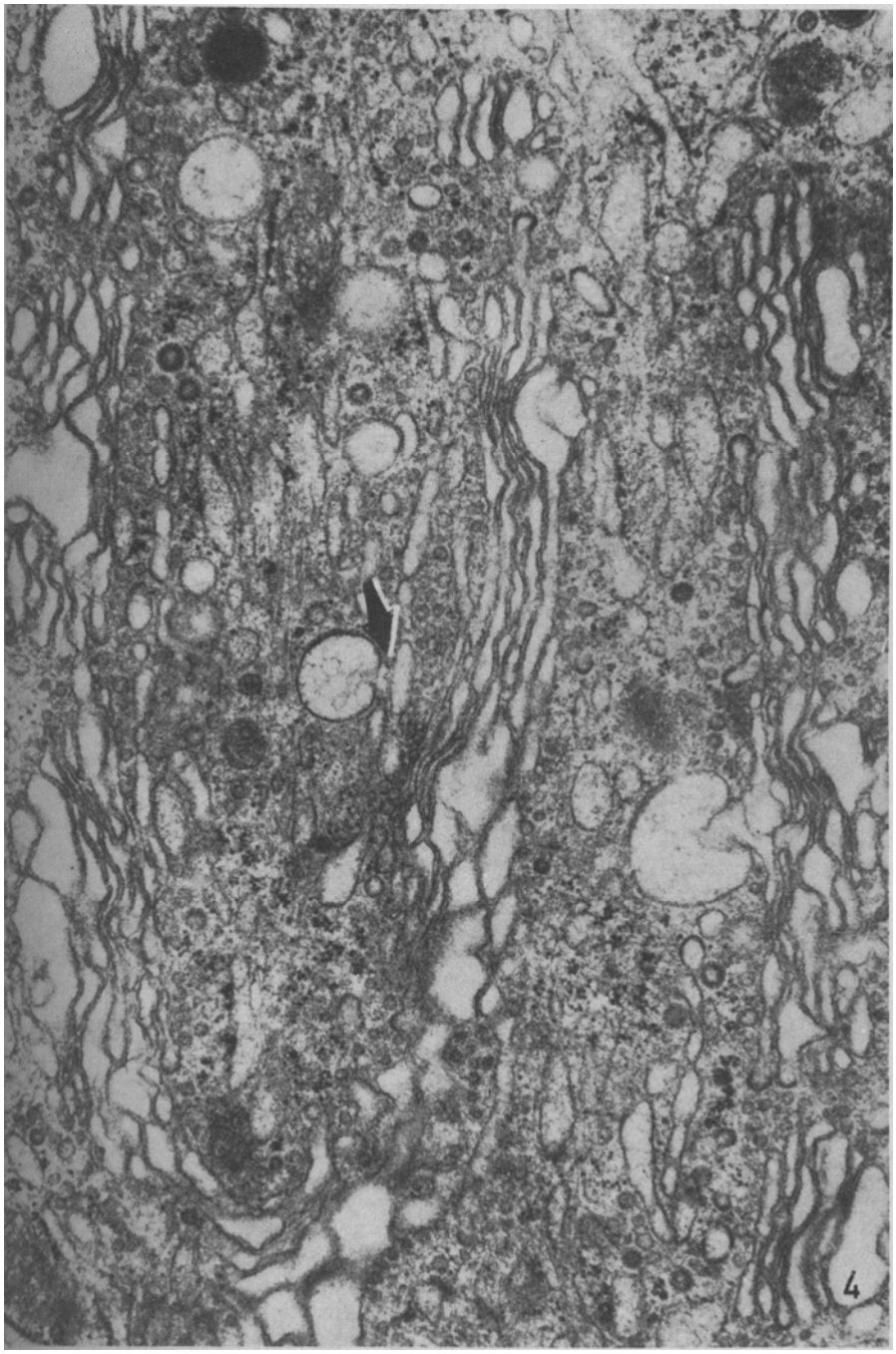


Figure 4. Golgi apparatus and the associated smooth surfaced vesicle(s) in the principal cell. One-such vesicle is associated (arrow) with the 'trans' face of the Golgi cistern (×27,000).

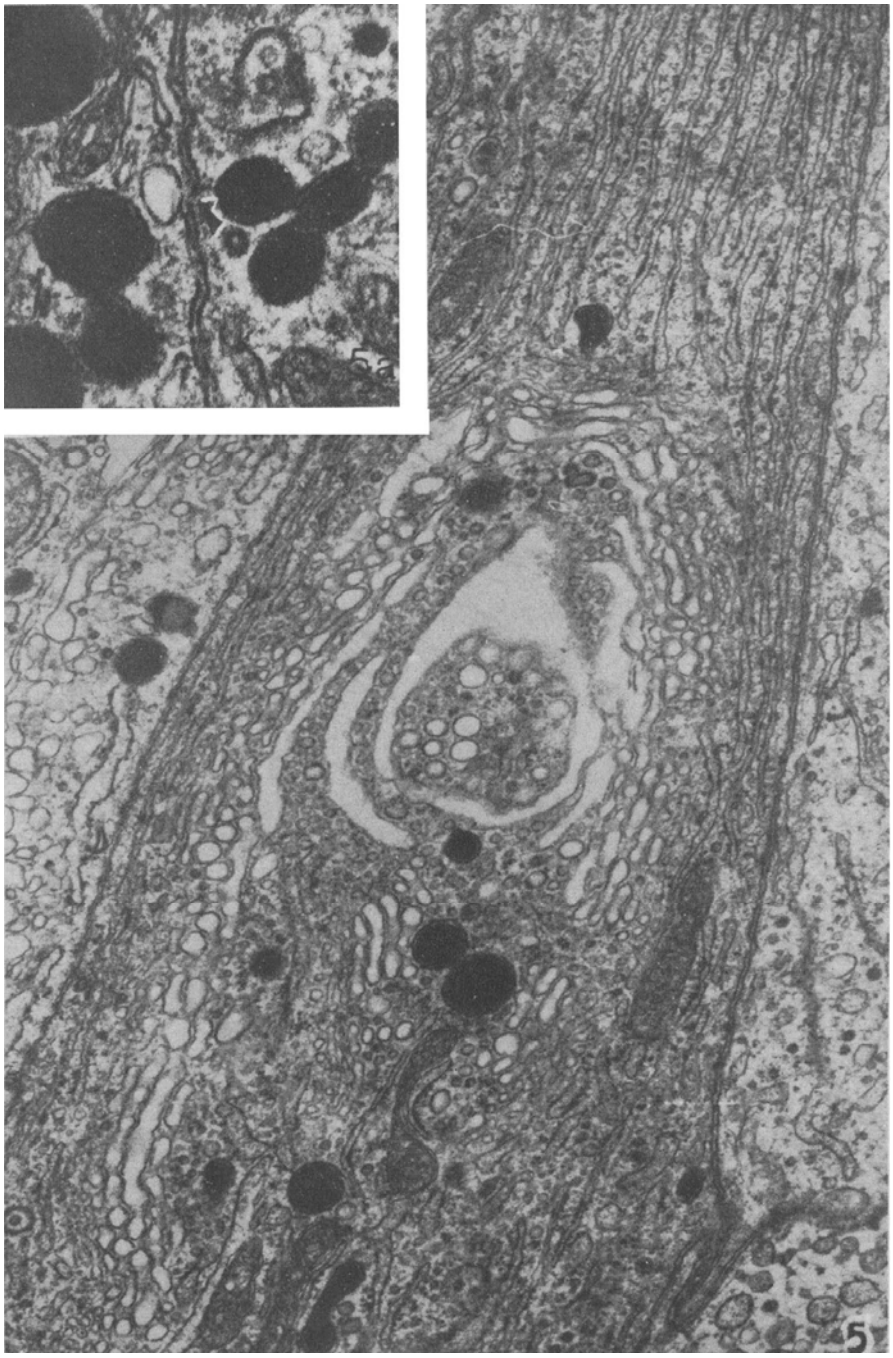


Figure 5. Electron-dense granules are also found within the Golgi appartus of the principal cell (r 31,000). *Inset.* Illustrates a cluster of four secretory granules formed by the coalescence of their outer bounding membranes. This particular cluster of secretory granules is attached to the plasma membrane by a tubular stalk (*arrow*) which opens into the intercellular space. In an adjacent cell, an empty vesicle is attached to the plasma membrane. This vesicle has presumably discharged its electron-dense contents (×69,000).

nitrate did not penetrate the apically situated tight junctional complexes (figure 1 a). These findings suggest that the intercellular spaces are contiguous with the perivascular spaces but are effectively sealed-off from the epididymal lumen.

The endothelial cells of the sub-epithelial blood capillaries contain many pinocytotic vesicles of the type described by Palade (1960) (figure 7).

Pinocytosis

Two types of pinocytotic invaginations were observed in the plasma membrane of principal cells. Pinocytotic invaginations having an electron-dense coat were present on the luminal, lateral and basal surfaces of the cell (figure 1, 6). These invaginations probably give rise to the coated vesicles observed within the principal cell (figure 1). The second type of pinocytotic invagination has a smooth surface and such invaginations occur mostly on the lateral and basal parts of the cell (figure 6). These invaginations perhaps give rise to the smooth-surfaced pinocytotic vesicles found within the cell.

Intracellular inclusion

Smooth surfaced vesicles containing a flocculent material and membrane-bound, electron-dense granules are amongst the distinguishing cytoplasmic inclusions to be found in the principal cell. The smooth-surface vesicles (measuring *ca.* 500nm) occur mostly in the apical region of the cell (figure 2) while the electron-dense granules (measuring *ca.* 300 nm) are concentrated in the basal part (figure 7). The ultrastructure of the Golgi region suggests that the smooth surfaced vesicles are formed on the 'trans' face (Palade 1975) of the Golgi apparatus (figure 4) while the electron-dense granules occur at the terminal ends of the Golgi cisterns (figures 4, 5).

The smooth-surfaced vesicles appear to be extruded from the apical surface of the principal cells into the epididymal lumen where they appear as hollow sphere (figure 2, 3).

The membrane-bound electron-dense granules found in the principal cells closely resemble the secretory granules found in certain endocrine organs such as the adenohypophysis or the pancreas. The granules in the principal cell occur either individually or in clusters formed by the coalescence of their bounding membranes (figure 5). Some of the electron-dense granules were found attached to the plasma membrane by tubular extensions of their surrounding membrane (figure 5a). In a fortuous plane of sectioning (figure 5a) or in section observed by tilting the goniometer stage, one can visualise the opening of the tubular extensions into the intercellular spaces. In a few instances, empty membrane-bound vesicles were also found attached to the plasma membrane (figure 5a, inset). These ultrastructural features suggest that the contents of the electron-dense granules are perhaps discharged into the intercellular space; however, the actual process of such a discharge is yet to be observed.

The ultrastructural features of the principal cell observed in several electron micrographs obtained during the present study are illustrated diagrammatically in figure 8.

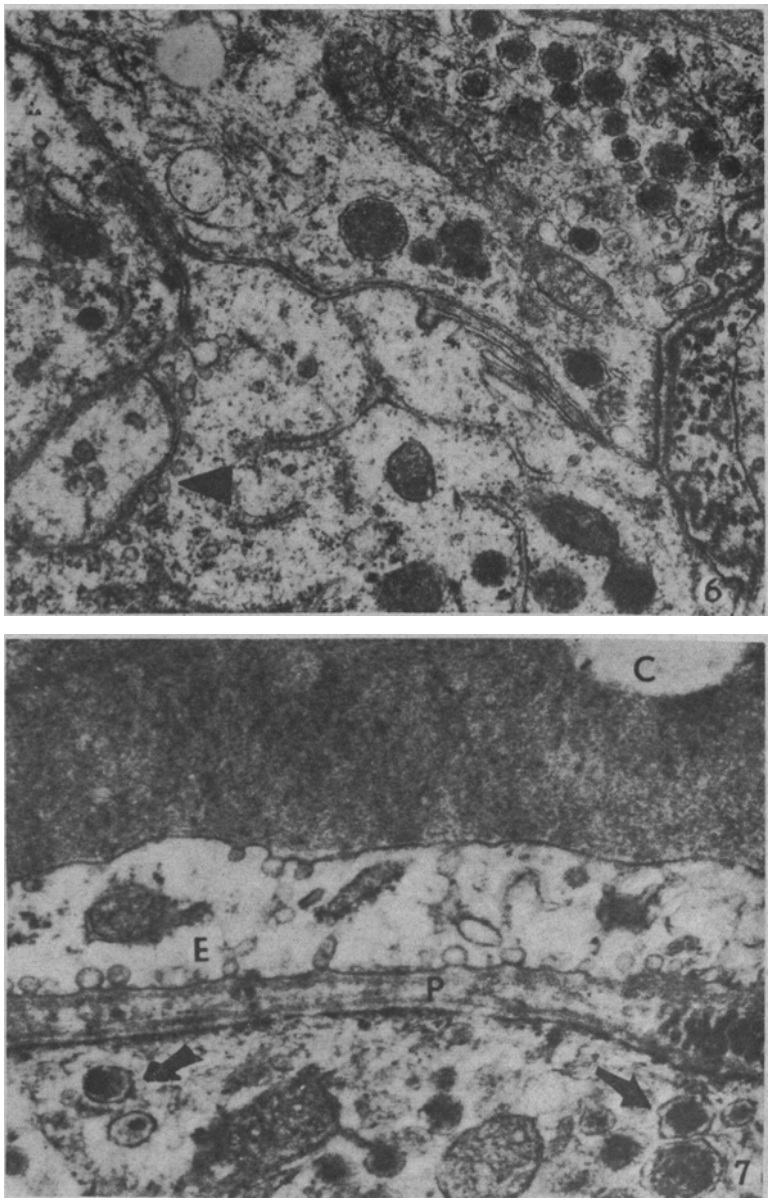


Figure 6. Illustrates the basal parts (towards the right side of the picture) of two adjoining principal cells in the middle segment of the epididymis showing the interdigitation of the plasma membranes which results in the intercellular spaces appearing as a canaliculi-like strucutre (*arrow*). Smooth surfaced pinocytotic invaginations and smooth surfaced pinocytotic vesicles (*arrowheads*) occur on the canaliculi-like structure. Pinocytotic invaginations with an electron-dense coat occur on the lateral' and basal surfaces of the cell (r 31,500).

Figure 7. Membrane-bound, electron-dense secretory granules occur mostly towards the basal part of the principal cell (*arrow*).The endothelial cell (E) of the sub-epithelial blood capillary contains many pinocytotic vesicles some of which are found on the luminal surface of the sub-epithelial blood capillary (C) and others occur towards the perivascular space (P) (r 45,000).

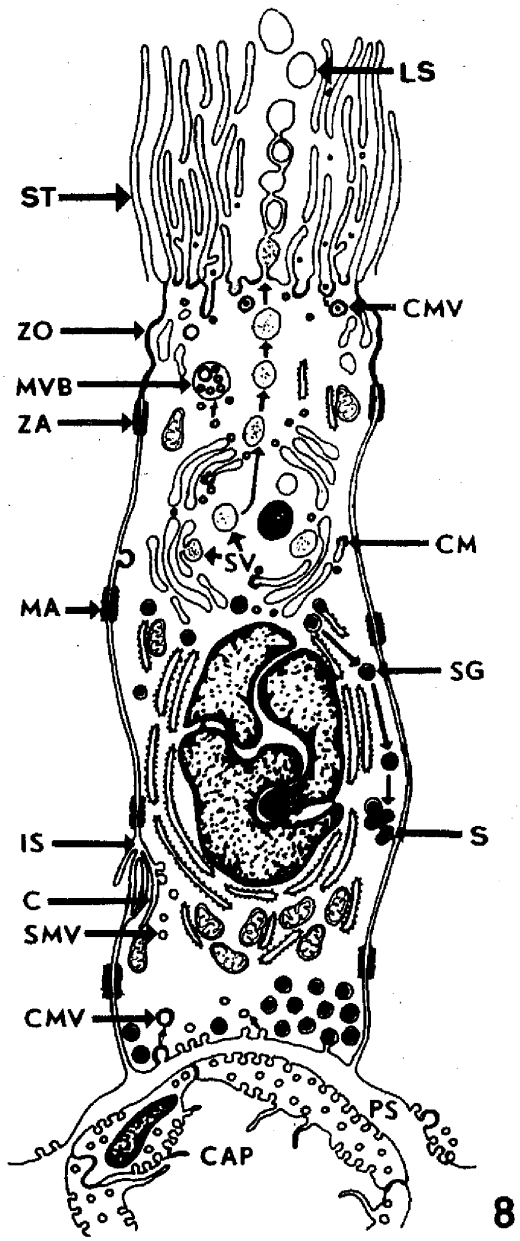


Figure 8. A composite diagram of the ultrastructural features of the principal cell as illustrated in the electron micrographs figures 1-7. The possible sequence of events related to the secretory function of the cell, as interpreted from the electron micrographs, is shown. Abbreviations: C: Canaliculi-like structures formed by the intercellular adjoining principal cells. CAP: Subepithelial blood capillary. CMV: Coated pinocytotic vesicle. CM: Coated microvesicle. IS: Intercellular space. LS: Luminal sphere. MA: *Macula adherens.* MVB: Multivesicular body. PS: Perivascular space. S: Site at which the electron-dense secretory granules are attached to the plasma membrane by a tubular stalk opening into the intercellular space. SG: Electron-dense secretory granules. SMV: Smooth surfaced pinocytotic vesicles. ST: Stereocilia. SV: Smooth surfaced secretory vesicle which are extruded into the epididymal lumen. ZA: *Zonula adherens.* ZO: *Zonula occludens.*

Discussion

The ultrastructural features of the principal cell indicate that the cell carries out a dual function of absorption and secretion. The absorptive processes are carried out by the formation of coated and smooth-surfaced pinocytotic invaginations. It is interesting to note that the coated pinocytotic invaginations are found on all aspects of the cell whereas the smooth-surfaced pinocytotic invaginations occur only at the lateral and basal regions of the principal cell. It remains to be shown whether the formation of two types of pinocytotic invaginations are indicative of the cell incorporating qualitatively different types of substances. Other studies have shown that coated pinocytotic vesicles incorporate protein-rich fluids (Roth and Porter, 1964; Friend and Farquhar, 1967) while the smooth-surfaced pinocytotic vesicles have been associated with the transcellular transport of electrolytes (Palade, 1960; Kurosumi, 1961),

The secretory products of the principal cells are formed in the Golgi apparatus and they are of two types. The smooth-surfaced vesicles are found mostly in the apical part of the cell while the electron-dense, membrane-bound granules are found more towards the basal part of the cell. Polarization of secretory products within the cell could perhaps indicate the sites at which they are secreted. The smooth-surfaced vesicles are extruded into the epididymal lumen while the products contained in the electron-dense granules are perhaps discharged into the intercellular spaces. Ultrastructural evidence indicative of secretory activity has also been reported in the initial segment of a number of mammals (Nicander and Malmqvist, 1977) and in the principal cells of vas deferens in man (Hoffer, 1976).

Ultrastructural studies of lanthanum-nitrate impregnated epididymis clearly indicate that the intercellular spaces are effectively sealed-off from the epididymal lumen by the apically situated tight junctions of the principal cells. A similar conclusion was arrived at by other investigators studying the rat (Friend and Gilula, 1972) and mice (Suzuki and Nagano, 1978) epididymis. The intercellular spaces, however, appear to be contiguous with the perivascular spaces of the sub-epithelial capillaries. The occurrence of large number of pinocytotic vesicles in the endothelial cells of the sub-epithelial capillaries strongly indicates the possibility of an exchange of substances between the intercellular spaces and the blood capillaries. Thus, it would appear that the epididymis contains two spatially separated compartments *viz.,* the luminal compartment and the intraepithelial compartment comprising the intercellular spaces.

The precise functional significance of the process of absorption and secretion carried out by the principal cells is not known. It is possible that the occurrence of both these activities on the apical surface of the principl cell contribute to the creation of an appropriate luminal milieu for the maturation of spermatozoa as has been suggested by other investigators (Nicander and Malmqvist, 1977; Wong *et al.,* 1978). The process of absorption and secretion occuring in the intraepithelial compartment may well represent an exchange of substances between the intercellular spaces and the subepithelial capillaries. If this is indeed so then it is most likely that the products secreted by the electron-dense granules into the

intercellular spaces may find their way into the sub-epithelial blood capillaries and they may thus have an endocrine function.

While most of the previous studies on the mammalian epididymis have focused their attention on the absorptive and secretory activities occurring on the luminal surface of the epididymal epithelium, the present studies have drawn attention to similar activities occurring in the intraepithelial compartment. The functional significance of these activities occurring in the intraepithelial compartment needs to be further elucidated.

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