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The Ultrastructure of Dog Epididymis

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Summary. The ultrastructure of the epididymal duct and ductuli efferentes in the dog has been studied by electron microscopy. The epididymidis can be separated into the classical divisions of caput, corpus and cauda epididymidis on the basis of general morphology and ultrastructure. The ductuli efferentes have a low epithelium with pronounced cilia at the apices of cells and appear to provide primarily a transport role for spermatozoa. In the epididymis proper the caput region is characterized by an extremely large Golgi apparatus with large numbers of lysosomes and nuclear inclusions. Secretory activity appears to be most common in the corpus region. Absorption and secretion are most active in the first two segments while in the cauda epididymidis the long-term storage of spermatozoa in the lumen is associated with many dense crystalline bodies formed in the epithelial cells within the Golgi apparatus and possibly deriving from absorbed macromolecular material from the lumen. The theory of whole sperm cell resorption by the epididymal duct is not supported by this study.

Key words: Dog, Epididymis, Ultrastructure.

INTRODUCTION

The role of the epididymis in reproduction is, despite a large number of investigations (15), not fully understood. Although much is known about the gross physiology of the organ, comparatively little information exists about the part played by the epithelium in the functions of each region. In addition, a great deal of variation has been found in the physiology and structure between species which inhibits the extrapolation of data from one species to another (22). Nevertheless, it is generally accepted that the epididymis is not just a passive transport system for sperm cells but that the spermatozoa undergo several changes in structure and function which give them their full maturity during the epididymal passage (27).

The epithelium of the epididymis influences, if not the spermatozoa directly, at least the composition of the epididymal plasma which forms the environment within which the cells are suspended for various periods before being ejaculated (28). Active absorption and secretion processes of the epididymal epithelium change the chemical composition of the epididymal plasma at different levels of the sperm pathway. These specific activities of the lining cells of the duct appear to be restricted to well defined regions (28). More detailed studies of the regional division on the basis of histological and cytological data have been made for several species (11, 15, 20, 21, 22, 25, 29).

Despite the fact that the dog was one of the first mammals in which conspicuous regional differences of the epididymal epithelium were reported (18), a detailed description of the ultrastructure of the epithelium in the different parts of the organ appears to be lacking. This paper describes detailed ultrastructural characterization of the divisions of the dog epididymis to provide a basis for more clearly elucidating the function of each region.

MATERIALS AND METHODS

Epididymides were removed from 3 healthy mature dogs of no specific breed, during a larger study of the reproductive system, immediately after a lethal dose of pentobarbitone sodium (Euthatal, May & Baker Ltd., Dagenham, Essex). The

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organs were divided into segments comprising the ductuli efferentes, caput, corpus and cauda epididymidis. Pieces of 1 mm^3 size were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 h. The specimens were subsequently washed for 2 h in 0.1 M phosphate buffer (pH 7.4), then post-fixed in 1% osmium tetroxide (Millonig's pH 7.4) for 1 h and further washed in buffer. After dehydration in a graded series of alcohols and immersion in propylene oxide, the tissues were embedded in Araldite epoxy resin. Semi-thin (1 µm) sections were cut, mounted on glass slides and stained with toluidene blue for histological examination. Ultrathin (silver) sections were then cut on an LKB ultramicrotome, collected on unsupported copper grids and stained with uranyl acetate and lead citrate. The sections were examined in an AEI EM6B electron microscope. For histological examination one complete epididymis was pinned to a board and fixed in Bouins, dehydrated, embedded, sectioned longitudinally and stained in the usual way.

RESULTS

Histologically all the epididymides from the three dogs appeared normal and similar. Division of the duct into the four regions including the ductus efferens was obvious and indicated by the abrupt change in epithelial cell height, presence or absence of cilia, and of secretory activity. Spermatozoa were seen in the lumina of each region, the majority occurring in the cauda epididymidis. Histological investigation of the whole organ (Fig. 1) showed that the epididymis could be clearly divided into three segments; the caput, corpus and cauda, corresponding to the initial, middle and terminal segments described by Glover and Nicander (11) for various mammalian species.

a. Ductuli Efferentes

The ductuli efferentes were characterized by relatively short epithelial cells of two main types, some having very prominent cilia and others possessing only microvilli (Fig. 2). In some cells both microvilli and cilia were present (Figs. 3, 4). The cilia had a distinctive root formation deep into the epithelial cytoplasm (Figs. 3, 4) and mitochondria occurred in clusters near to this region. There was very little evidence of absorption from the lumen though micropinocytosis at the apical membrane. In some of these cells there was evidence of a small amount of smooth endoplasmic reticulum occurring mainly in clusters in the apical region (Fig. 4) but there were only scanty short profiles of rough endoplasmic reticulum (Fig. 4). In the supranuclear area a small amount of Golgi apparatus occurred comprising occasional short stacks of cisternae. These gave rise to some small vesicles most of which were smooth surfaced and concentrated towards the apex of the cells. About half of these small vesicles lacked any secretory material while others contained a flocculent precipitate (Fig. 3). There were few multivesicular bodies or coated vesicles. Large numbers of mitochondria occurred, particularly towards the base and apex of the cells (Figs. 2, 3).

The second type of cell (principal cells) with short microvilli possessed lysosomal material with large vacuoles in the apical region (Fig. 2). These cells gave the appearance of performing an absorptive function.

A third type of cell found commonly in this region was the basal cell often containing large quantities of glycogen but otherwise appearing inactive (Fig. 2).

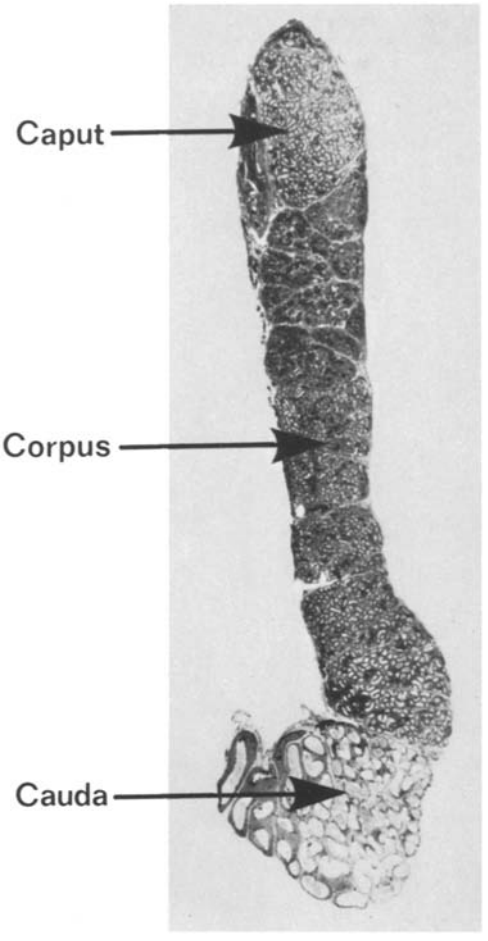
b. Caput Epididymidis

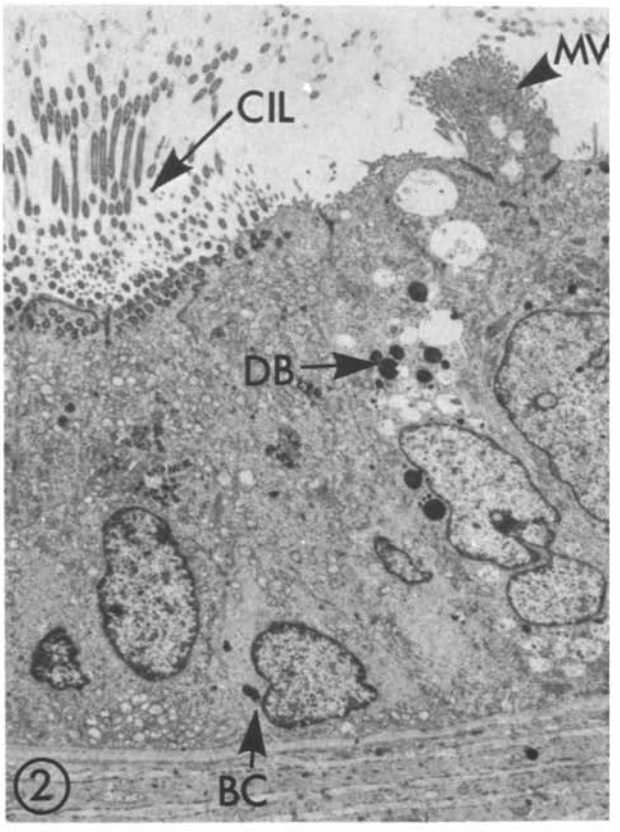
The junction between the ductuli efferentes and the caput epididymidis was distinct and characterized by a sharp increase in epithelial cell height (Fig. 5). The outstanding features of many cells of the caput region were the extensive Golgi activity and the many bizarre nuclear inclusions (Fig. 6). These inclusions took various forms and were membrane bound. Some were highly osmiophilic and had a similar appearance to the many lysosomes in the cytoplasm. The amount of rough ER was quite small but greater in this segment compared with the ductuli efferentes. Stereocilia, being microvilli with a filamentous core, were very long and present at the apex of all epithelial cells (Fig. 5). There was evidence of micropinocytosis at the apical membrane that may have been implicated in absorption. Terminal bars were particularly well formed with many filaments extending from the desmosomes into the apical cytoplasm. The cell membranes were highly interdigitated. In some cells dense breakdown or autophagic products occurred in the supranuclear region or near the nucleus.

At the basement membrane, epithelial cells were interspersed with flat or trilateral basal cells (Fig. 7). These were largely devoid of organelles but there was a frequent occurrence of glycogen, occasional long mitochondria, and bundles of filaments in the cytoplasm. The basal cells appeared quite inactive except for micropinocytosis at the basal membrane.

c. Corpus Epididymidis

An outstanding feature of these cells was the long stereocilia which extended into the lumen, often





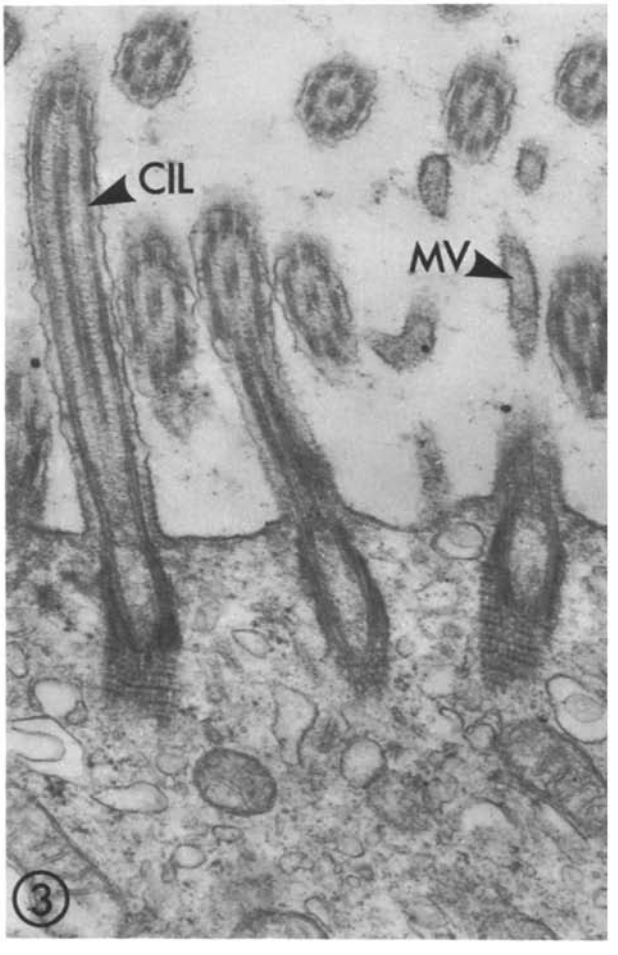


Fig. 1. Longitudinal section through dog epididymis showing the different segments, further separated for ultrastructural examination (x 3.0)

Fig. 2. Ductulus efferens showing two types of epithelium, identified by cilia (CIL) or microvilli (MV). BC = basal cell. DB = dense bodies. (x 2500)

Fig. 3. Cilia (CIL) and microvilli (MV) at the apex of ductulus efferens epithelium. The cilia had a deep root formation into the cytoplasm. A number of small vesicles, some containing flocculent precipitate, gathered near the apical membrane. (60, 000 x)

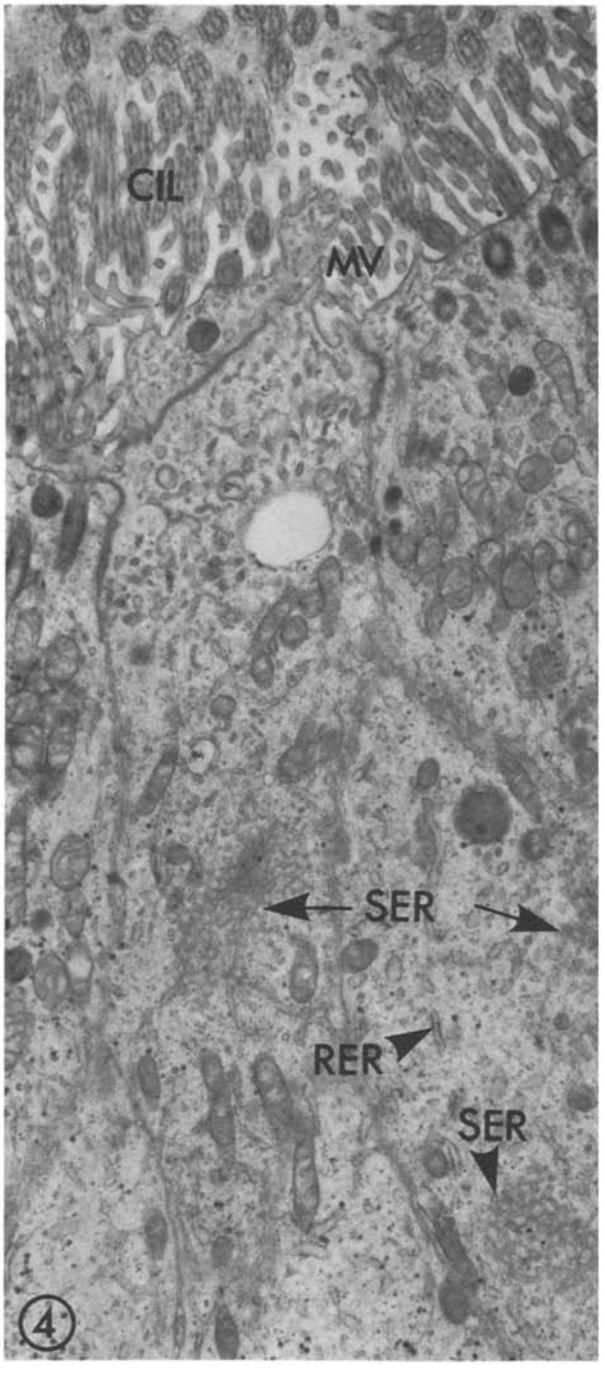


Fig. 4. Apical region of ductulus efferens epithelium showing cilia (CIL) and microvilli (MV) at the apex of the same cell. A moderate amount of smooth endoplasmic reticulum (SER) formed in clusters in the supranuclear region. Only small amounts of rough endoplasmic reticulum (RER) existed in short profiles. (27, 000 x)

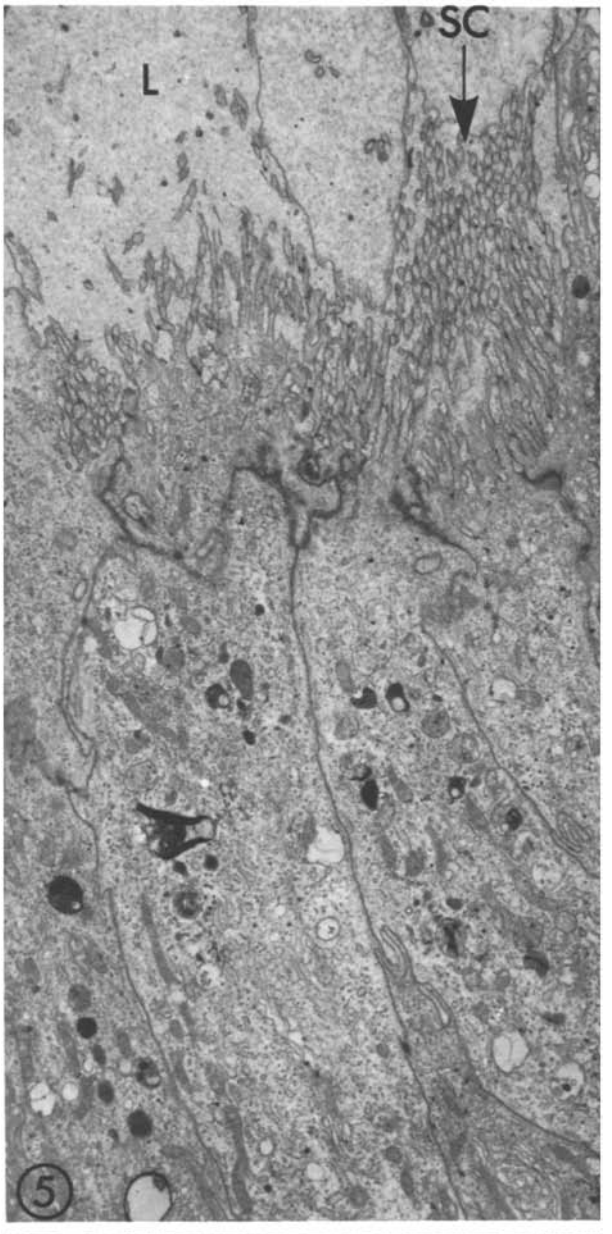


Fig. 5. Caput epididymidis showing long stereocilia (SC) on high epithelial cells. L = Lumen. (6750 x)

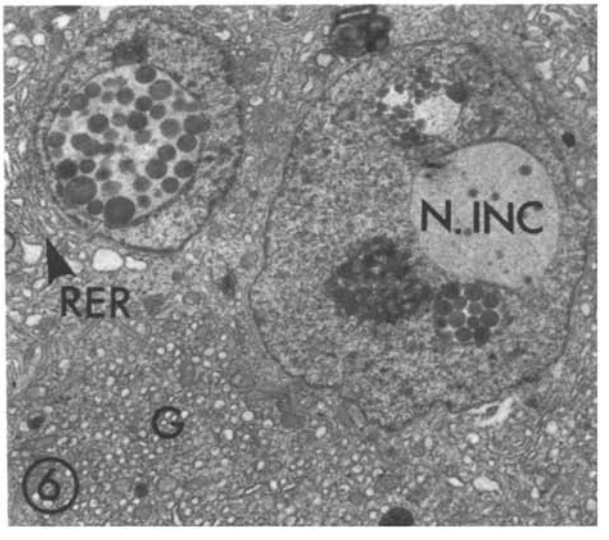


Fig. 6. Nuclear inclusions (N. INCL) were frequently seen in the caput region. Small amounts of rough endoplasmic reticulum (RER) were usually found near the nucleus. Golgi vesicles (G) were abundant throughout the cells. (12, 000 x)

engulfing spermatozoa (Fig. 8) Luminal secretions were mostly filled with flocculent material, probably arising from the many small Golgi vesicles that were distributed throughout the cytoplasm and extended up in between the long stereocilia at the apical membrane. These small vesicles could well have provided an ample means of absorption from, as well as secretion into, the lumen. The Golgi apparatus appeared well organised, as in the caput region, and was generally dilated (Fig. 9). The formation of multivesicular bodies within the Golgi apparatus was obvious in many cases (Fig. 10). There was again little evidence of rough ER and no tubular smooth ER was seen although most of the spherical Golgi vesicles were smooth surfaced.

Many long bundles of fibres occupied the supranuclear region and surrounding the nuclei were large numbers of dense bodies (Figs. 9, 10). These bodies were highly osmiophilic and had the appearance of autophagic breakdown products. Some of these were similar to those in the caput region but were more abundant. Close examination of the dense bodies showed them to have a lamellated structure. Nuclear inclusions were also present in the corpus cells (Fig. 9) but less frequent than in the caput segment.

Basal cells (Fig. 9) were again present between epithelial cells and showed the same characteristics as before.

d. Cauda Epididymidis

The lumen of the cauda epididymidis was filled with spermatozoa and debris of the seminal fluid. This debris was comprised of degraded cells, cytoplasmic droplets from the spermatozoa and general flocculent material in the semen. Stereocilia at the apex of the cells again extended into the lumen and engulfed the cellular debris (Fig. 11).

The mean height of the epithelial cells was less than that in the corpus region. The principal feature of the cells was the very great quantity of dense 'break-down' products which occurred mainly in the apical region (Figs. 11-13). Some of these bodies, on first examination, and especially at the histological level, had the appearance of residual spermatozoa that might have been resorbed from the lumen. Closer examination, however, showed that these products were synthesised by the Golgi apparatus and had a lamellated or crystalline structure (Fig. 13).

The Golgi apparatus appeared extensively as large stacks of parallel cisternae but remained well organised and there were far less Golgi vesicles in these cells than in those of the caput and corpus regions. What small vesicles there were extended up to the apical membrane but again there was less evidence of active absorption or secretion than in the previous two seg-

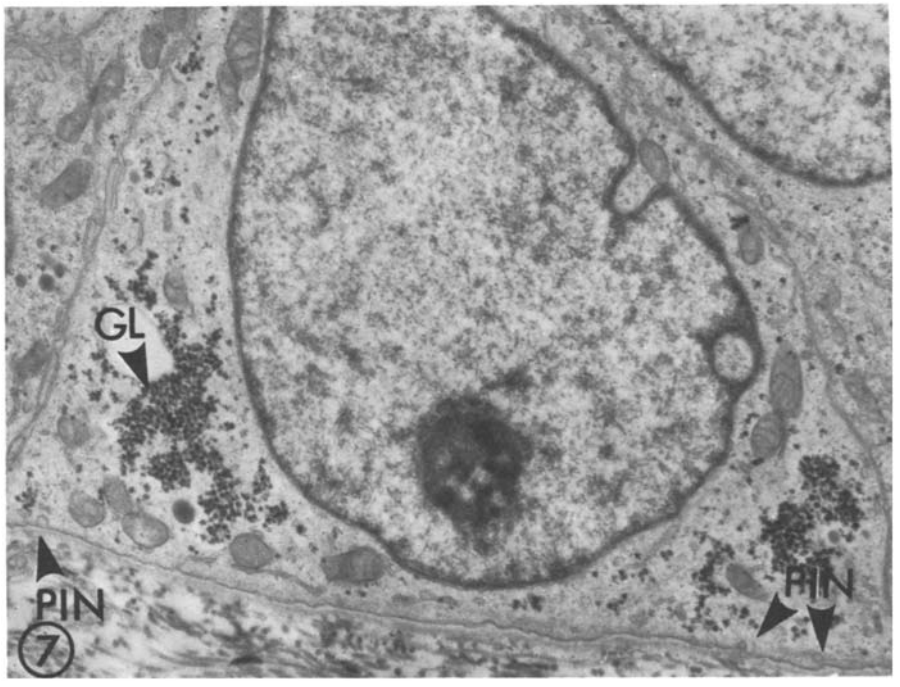
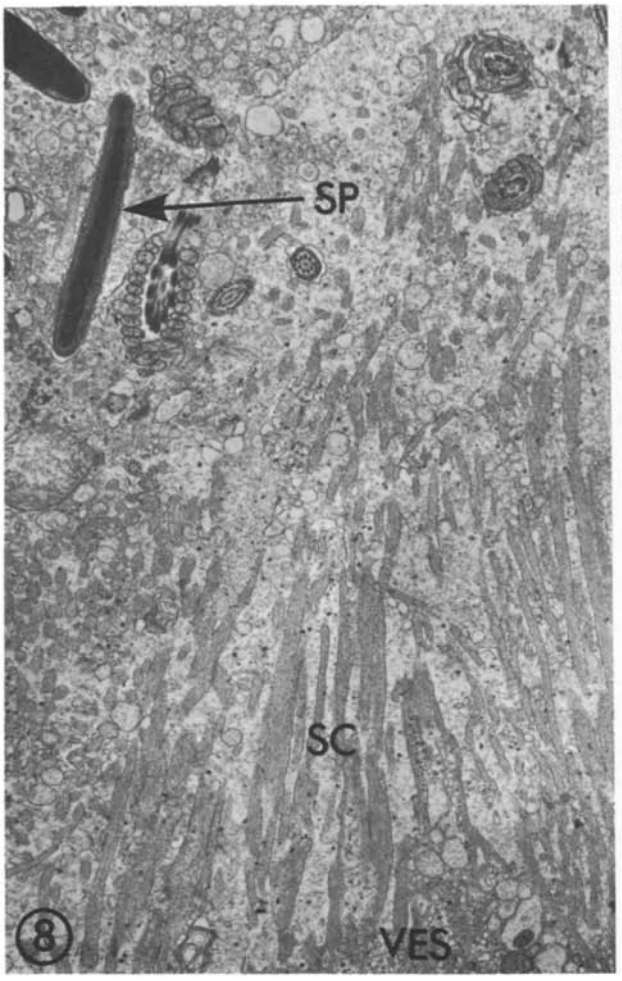


Fig. 7. Basal cell of the ductus epididymidis interspersed between epithelial cells. These basal cells appeared dormant except for pinocytotic activity (PIN) at the basement membrane. Large stores of glycogen (GL) were frequently seen. (40, 000 x)



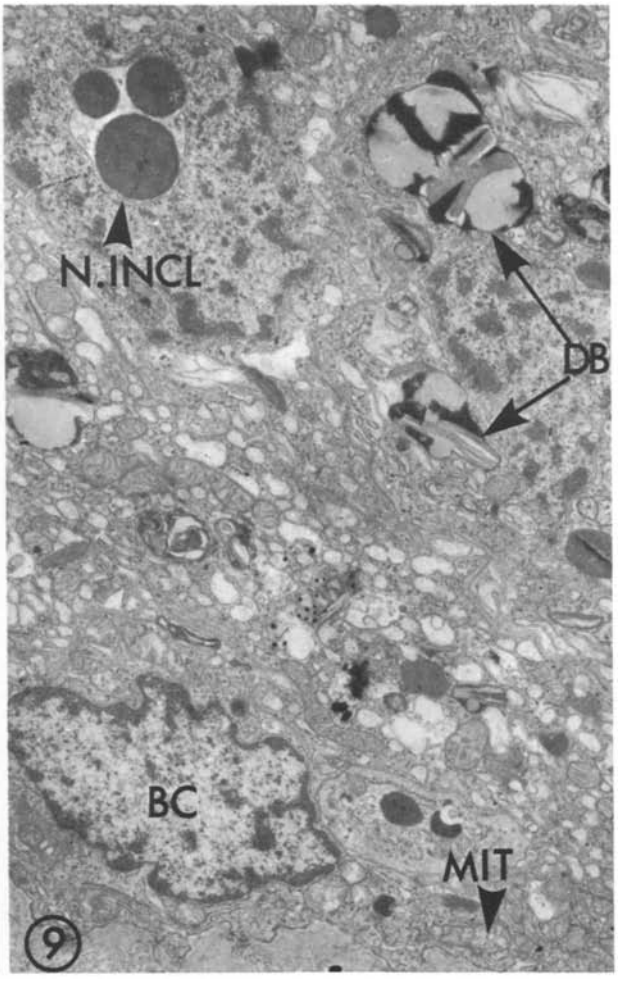


Fig. 8. Corpus epididymidis - apical region of epithelial cell. Long stereocilia (SC) extended into the lumen and engulfed spermatozoa (SP). Small vesicles (VES) were found at the base of the stereocilia. (8000 x)

Fig. 9. Corpus epididymidis - basal region showing basal cells (BC), dense lamellated bodies (DB) and nuclear inclusions (N. INCL). Many small mitochondria (MIT) were frequently found near the basement membrane. (8000 x)

Fig. 10. Corpus epididymidis, showing the formation of multivesicular bodies (MVB) within the Golgi apparatus (G). The dense bodies had a structural appearance. Lysosomes (LYS) were also found within the Golgi apparatus. (25, 000 x)

Fig. 11. Cauda epididymidis showing stereocilia (SC) and dense bodies (DB) in the apical cytoplasm. (6, 500 x)

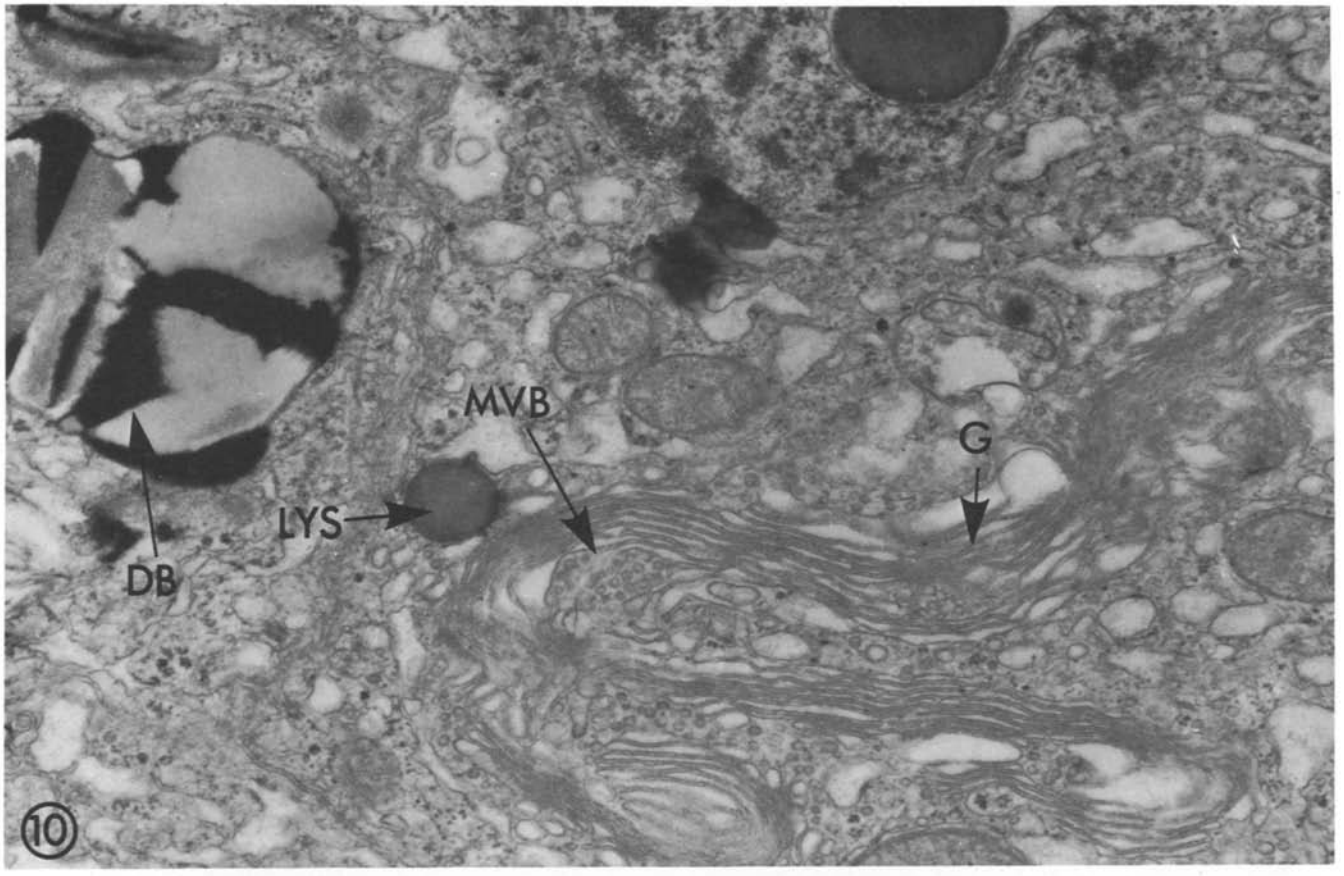
ments (Fig. 12). There was, however, far more evidence of protein synthesis within the cauda epithelial cells as shown by the increased amount of rough endoplasmic reticulum. This occurred near to the nucleus and was generally in small groups of parallel or curved cisternae (Fig. 12) occasionally appearing dilated. It was occasionally seen towards the cell extremities as pinched off fragments but was never very extensive. Smooth endoplasmic reticulum was never seen.

Throughout the cauda region, as with the other segments, there was never any evidence of resorption of whole spermatozoa into the epithelium.

Basal cells appeared similar to those in other regions of the organ.

e. Stroma

In all regions of the organ the epithelial cells were surrounded at the basement membrane by a thick, well defined basal lamina. Micropinocytosis only occurred at the base of the basal cells.


MVB
G
LYS
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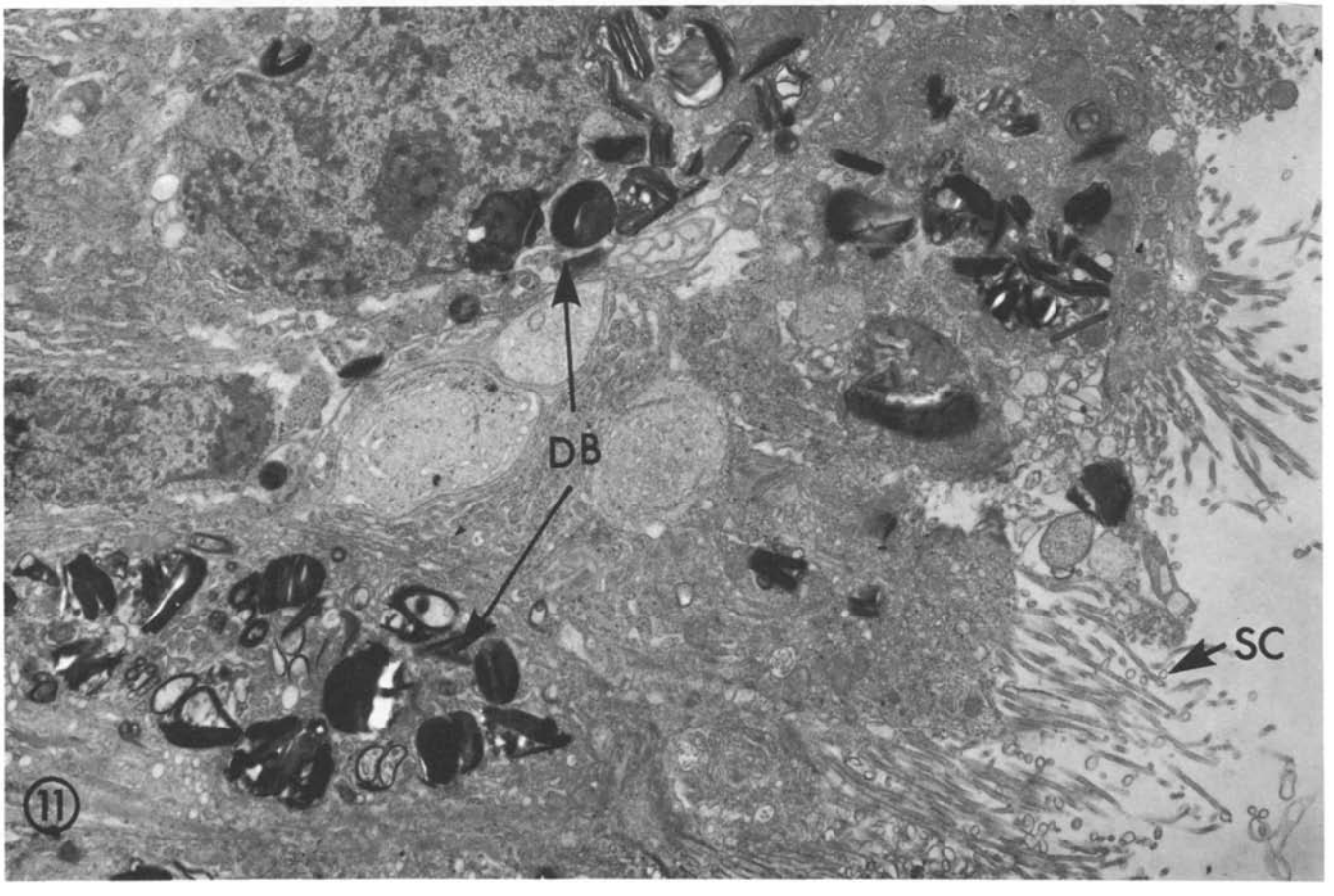
DB
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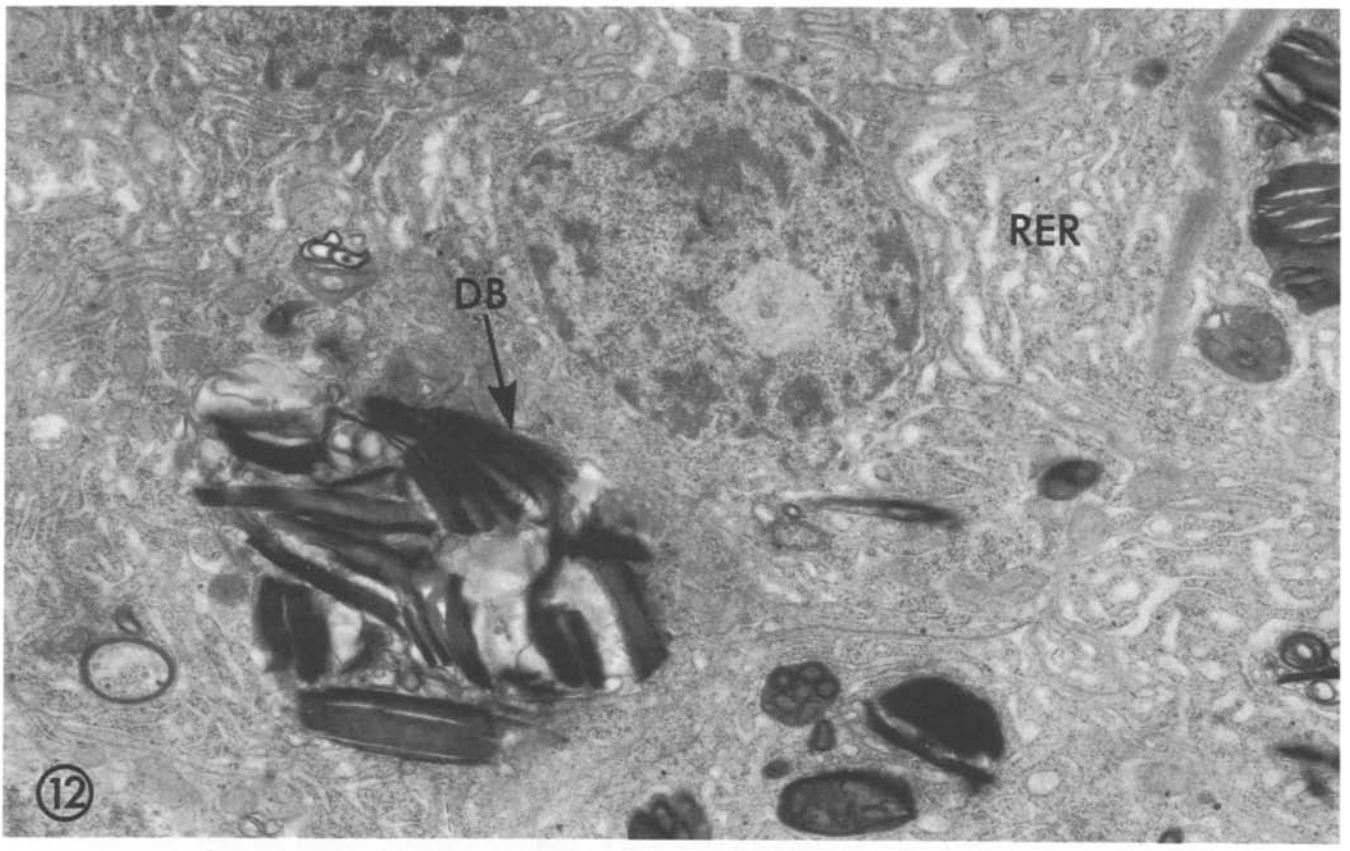
RER
DB
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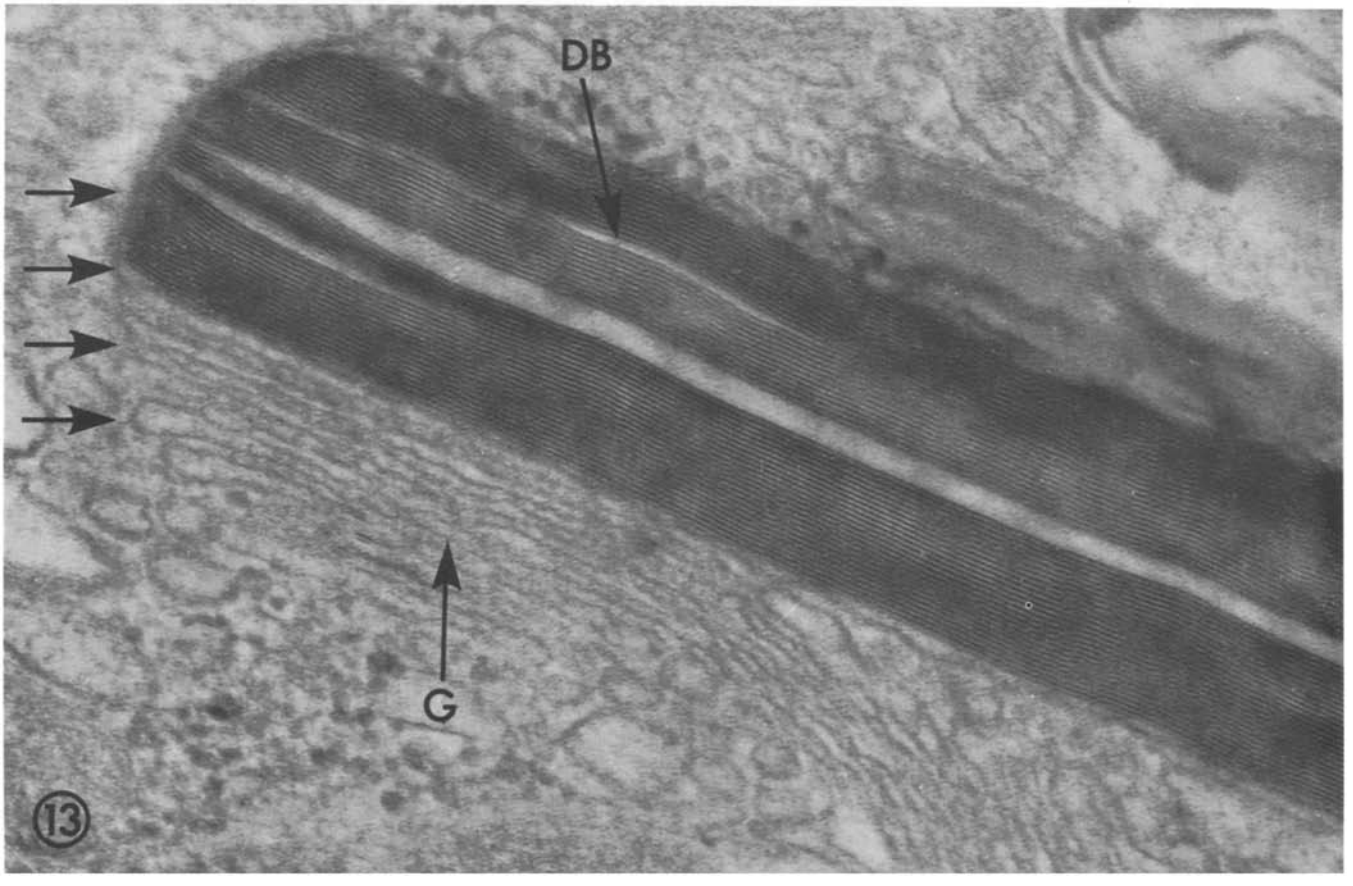
DB
G
13

Beyond the basal lamina there was a well organised arrangement of collagen, fibroblasts and smooth muscle cells in several layers (2 in the caput, up to 6 in the cauda), close packed and lying parallel to the basement membrane. Further from these layers the interductal spaces were occupied by a relatively loose arrangement of collagen and fibroblasts. Blood capillaries were seen quite frequently and occurred near to the basal cells.

DISCUSSION

Although the general histological appearance of the dog epididymis has several features in common with other species (15), at the ultrastructural level there are distinct differences which relate to the possible cellular function of each part of the gland. Cellular differences in the separate segments of the ductus epididymidis and the ductuli efferentes were very well defined, the change from one region to another occurring quite abruptly. The organ could be clearly separated into the caput, corpus and cauda regions corresponding to the initial, middle and terminal segments described by Glover and Nicander (11).

Cilia occurred only in the ductuli efferentes. This feature indicated that these cells may be primarily concerned with transport of spermatozoa into the epididymal tract and the principal cells with absorption of testicular fluid. Throughout the epididymis the protein synthesising rough endoplasmic reticulum was very small, contrary to that found in the rat (14), rabbit (24) and hamster (25). The greatest concentration was found in the cells of the cauda region while it was extremely scarce in the ductuli efferentes. Smooth endoplasmic reticulum, however, was recognised, in a tubular form, in only small amounts in the caput epididymidis and ductuli efferentes. This is interesting in view of the very large quantities of this organelle (17) and of steroid biosynthesis (14) in the epididymis of the mouse. Other species, however, vary in the smooth membrane content of the epididymal tracts and in their capacity for synthesising steroids. The appearance of smooth ER also varies from one species to another and even within one species between different segments. The presence of smooth reticulum does not necessarily only indicate steroid synthesising activity, however. Apart from its role of harbouring enzymes for steroid biosynthesis it may act to sequester cholesterol as a precursor for steroid production (7). Thus the very large numbers of small, spherical, smooth membraned vesicles seen throughout the epididymis, but particularly in the caput region, may represent such a function even though they lack the normal elongate appearances of conventional smooth endoplasmic reticulum. It seems more likely, however, that these vesicles derive from the very extensive Golgi apparatus and are concerned with the absorptive or secretory role of the epithelial cells (see below).

Other work from this Institute (Younes & Pierrepoint unpublished data) has demonstrated active steroid metabolising enzymes in the three major regions of the canine epididymis. Earlier studies had shown the incorporation of $|1\text{-}^{14}C|$ acetate into cholesterol and testosterone in the epididymis of the mouse (17), rat (16), rabbit, ram and hamster (14). In these species steroid synthesis was found to be greatest in the upper segments of the epididymis. It was shown by Hamilton and Fawcett (16), however, that incorporation of the acetate into testosterone was greater in the vas deferens than in the caput epididymidis for both the rat and the mouse even though there was apparently more smooth endoplasmic reticulum in the caput region. Hamilton (14) concluded from this that large amounts of smooth endoplasmic reticulum in a cell are not necessarily a good morphological criterion for steroid synthesising activity beyond the metabolism of cholesterol. It may, rather, indicate the sequestration of cholesterol which, besides being a precursor of other steroids, is also a major component of cellular membranes and may be stored in those regions of the duct where membrane turnover is greatest. In the caput and corpus regions of the epididymis, in particular, the absorptive and secretory activity of the cells requires a very large turnover of membrane and the presence of the very many small spherical vesicles in the cytoplasm may reflect this sequestration. It seems most likely that, since steroids bind to spermatozoa (6) and depress sperm metabolism (31) one function of the epididymis may be to ensure a steady secretion of steroids into the lumen as the spermatozoa travel towards the cauda region. The steroid secretion may not only derive from the epithelial cells since much of it could come from peripheral blood, the epididymal cells acting as a modulator of supply.

The vast Golgi complex found throughout the epididymis of the dog, as with other species, also lends support to the theory of an absorptive

Fig. 12. Dense bodies (DB) in the cauda cytoplasm having a lamellated appearance. Rough endoplasmic reticulum (RER) occurred near to the nucleus. (15, 000 x)

Fig. 13. Synthesis (arrowed) of lamellate dense body (DB) by the Golgi apparatus (G) in the cauda epididymidis. (125, 000 x)

as well as secretory role for the epithelial cells. The caput and corpus regions were found to be richest in Golgi apparatus and had the greatest quantity of small vesicles, both smooth and coated, near the apices of the cells, Experiments with the vas deferens of the rat (8) using horseradish peroxidase injected into the lumen have indicated the mechanism by which proteinaceous material is taken up by the Golgi apparatus across the apical membrane. Similar observations have been made within the epididymis by Burgos (1), Nicander (24) and Nicander et al. (26). This mechanism involves pinocytosis of the apical membrane, the formation of large coated vesicles which then become smooth surfaced, and the eventual fusing with multivesicular bodies. The multivesicular bodies are considered to be lysosomes and derive from the Golgi apparatus (Fig. 11), although Hamilton (15) has suggested they are active in fluid transport across the cell. Smaller coated vesicles originate either from the Golgi apparatus where they possibly transport acid hydrolases to the multivesicular bodies, or from pinocytotic invagination of the apical membrane during absorption from the lumen. The similarities between the vas deferens and the epididymis also strongly suggest a similar mechanism of absorption in the epithelium of the latter organ.

Large, dense-bodies were found in all three regions of the epididymis with increasing frequency and size towards the lower segments. Although these had, at first glance, the appearance of phagocytosed spermatozoa, it was clear upon further examination that they were products of synthesis within the epithelial cells. In the caput region they were amorphous and appeared as secondary lysosomes or autophagic vacuoles. In the corpus and cauda region, however, the bodies had a distinct crystalline or lamellated appearance. In the cauda epididymidis the synthesis of these lamellates was clearly seen within the Golgi apparatus, and they often occupied the major part of the supranuclear cytoplasm. There was no evidence to suggest that these highly osmiophilic bodies derived from spermatozoa even though their overall density was similar, after osmium fixation, to the nuclei of luminal sperm. It is thought by some (5) that these "zebra bodies" are lysosomal in nature and may be formed as a result of the accumulation of undigested material such as cholesterol within the cytoplasm.

After careful examination in all three animals used, not a single spermatozoon was found within the epithelial cells of any of the four regions studied. This is contrary to the observations by Nicander (24) of epithelial spermiophagy in the rabbit. It thus seems most likely that if resorption by the epithelium does occur, then it follows degeneration of whole cells in the lumen and pinocytosis of macromolecules at the apical membrane (30). This would also accommodate the increased number of cytoplasmic droplets found in the lumen of the cauda and shed by the spermatozoa during their epididymal passage and maturation. The theory of resorption of whole spermatozoa is controversial and may be species-dependent. Whereas some workers have shown evidence for whole sperm cell resorption (19, 13, 3) there is a greater weight of evidence against this view (10). The observations here of epididymal ultrastructure do not support the theory of sperm cell resorption in the dog.

Basal cells were found throughout the ductus epididymidis. They had a similar appearance in each segment except that those in the caput region had, like the epithelial cells, very invaginated lateral membranes. A characteristic feature of the basal cells was the extensive micropinocytosis which occurred at the basement membrane, this feature being absent in all the high prismatic epithelial cells. The basal cells would thus seem to have the capacity for uptake or transfer of macromolecular material to or from the stroma.

The nuclear inclusions seen primarily in the caput region but occasionally in other segments are an enigma (23). They were invariably membrane bound and had an appearance somewhat similar to secondary lysosomes. It is perhaps significant that they occurred most frequently in that segment having a large number of cytoplasmic lysosomes. Nuclear inclusions are most commonly found in tumours and tissues with a high rate of cellular mitosis (9). Such phenomena are generally thought, however, to arise from pseudoinclusions dependent upon irregularity of nuclear shape. In the epididymis this method of formation seems quite unlikely, especially in view of the regular appearance of the nuclear envelope. The inclusions generally took the form of one or more highly osmiophilic spherical bodies, often enclosed within a larger pale vacuole in some cases accounting for up to 50% of the nuclear volume. The largest of the dense spherical bodies were similar in size and density to the cytoplasmic lysosomes. Nuclear inclusions, similar in some respects to those seen here, have been observed in kidney and liver cells following lead poisoning and have, by autoradiography, been seen to contain lead (4). The same type of inclusions have been isolated (12) and shown to contain lead bound to protein in a relatively constant ratio. The inclusion bodies may function as a store or depot for intracellular elements in addition to lead. The epididymis is known, for example, to contain high concentrations of zinc (2), this element being an essential component of spermatozoa. A study of intranuclear inclusions in the epididymis of dog by Nicander (23) indicated their eosinophilic nature and their lack of acid phosphatase activity, nucleoproteins or lipids. Although commonly observed in a number

of species their function in the epididymis remains an enigma. Work is now in hand to analyse the nuclear inclusions of the epithelial cells in the analytical electron microscope to determine their composition and possible subcellular function.

CONCLUSION

The different segments of the ductus epididymidis and ductuli efferentes are well defined morphologically. The ductuli efferentes appear to provide a transport role for the spermatozoa as they pass into the epididymal tract and an absorptive role for testicular fluid. Within the epididymis the caput, corpus and cauda regions appear to serve different functions according to their ultrastructural chracteristics. The very large Golgi apparatus found in the caput and corpus segments indicate a high level of absorption and secretion. In addition, the presence of smooth endoplasmic reticulum in the ductuli efferentes and caput regions suggests a steroid biosynthetic activity. Resorption of whole spermatozoa does not appear at any level, but macromolecular material taken up at the apical membrane may well be incorporated into the large lamellated bodies seen within the Golgi region, especially in the cauda segment.

The dog epididymis, while basically similar to that described for other domestic animals, has unique features of morphology and ultrastructure that allow a functional interpretation in each segment of the gland.

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