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# Ultrastructure of the ductus epididymidis of the echidna, *Tachyglossus aculeatus*

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## INTRODUCTION

Our earlier light microscope studies (Jones & Djakiew, 1978; Djakiew & Jones, 1981) showed that the ductus epididymidis of the echidna is differentiated into only two histologically distinct segments, an initial segment which is structurally similar to the initial segment proper of the epididymis of eutherians (Benoit, 1926; Nicander & Glover, 1973; Fawcett & Hoffer, 1979), and a terminal segment which is involved in considerable apocrine secretion and so is unlike any region in the epididymis of other mammals. These conclusions differ from those of Bedford & Rifkin (1979) who did not mention the apocrine secretion, and considered that the ultrastructure of the epithelium differed between the proximal and distal ends of the initial segment. This report resolves this contention, describes the ultrastructure of the various types of cell in the epithelium lining the ductus epididymidis of the echidna and compares them to the analogous cells in eutherian mammals (Reid & Cleland, 1957; Fawcett & Hoffer, 1979; Flickinger, Howards & English, 1978).

## MATERIALS AND METHODS

Mature male echidnas were collected during the mating season.

Tissue from two animals was fixed by immersion in picric acid–formaldehyde–glutaraldehyde–cacodylate (Jones, 1973) and tissue from two other animals was fixed by perfusion of cacodylate-buffered fixatives through the thoracic aorta (Forssman *et al.* 1977).

The tissue was sampled from four sites. Sites 1 and 2 (proximal and distal ends of the initial segment) corresponded to the sites 3 and the distal end of 4 used by Bedford & Rifkin (1979). Site 3 was at the proximal end of the terminal segment and site 4 was in the middle of the terminal segment.

A buffy coat was prepared from heparinized (20 i.u. per ml) blood by centrifuging at 1000 *g* for 10 minutes at 4 °C. The buffy coat was collected in a Pasteur pipette and dispensed into the picric acid–formaldehyde–glutaraldehyde–cacodylate fixative, fixed for 1 hour and then centrifuged to form a pellet for processing.

The samples for electron microscopy were washed, osmicated, dehydrated in ethanol and embedded in Araldite. Thin sections exhibiting silver and pale gold interference colours were stained with aqueous uranyl acetate (Watson, 1958) at 60 °C, and lead citrate (Reynolds, 1963) and examined under a JEOL 100 CX transmission electron microscope.

The structure of the supranuclear cytoplasm of the principal cells in sites 1 and 2 was compared quantitatively using the stereological methods described by Weibel,

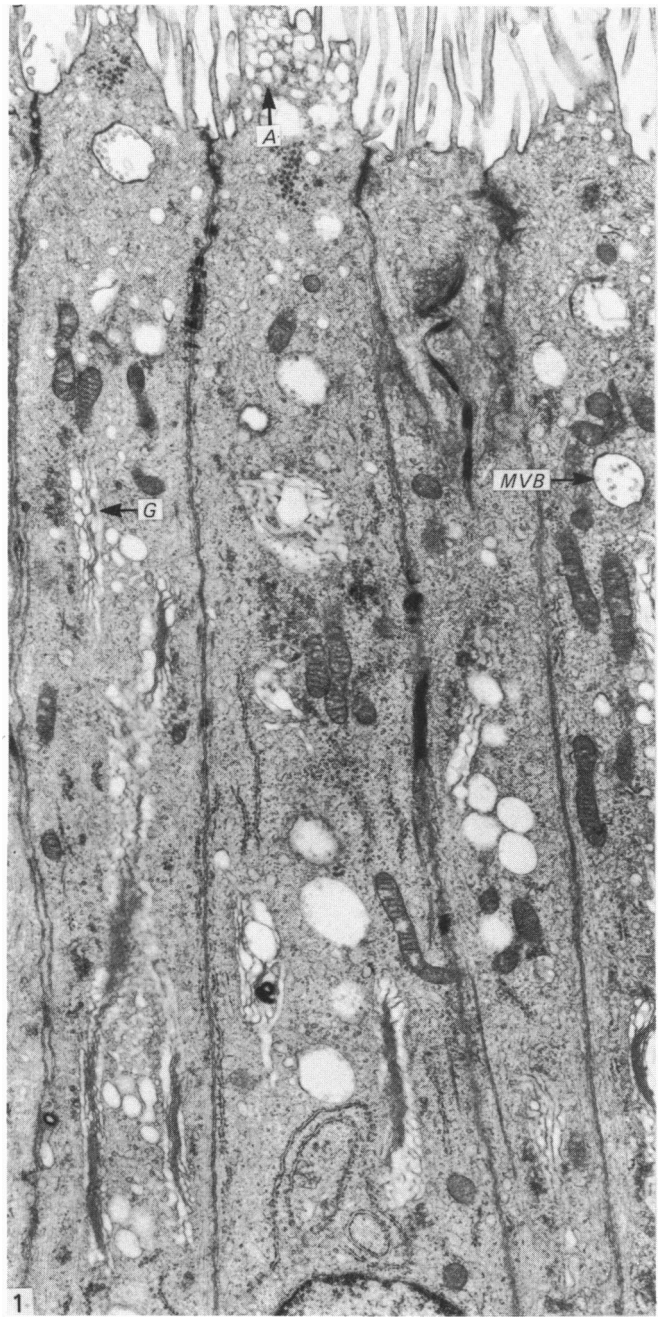



Fig. 1. Electron micrograph of the supranuclear cytoplasm of the principal cells in the initial segment of the ductus epididymidis, showing the apical vesicles (*A*), multivesicular bodies (*MVB*), and the large Golgi apparatus (*G*), which are characteristic of these cells. ×10200.

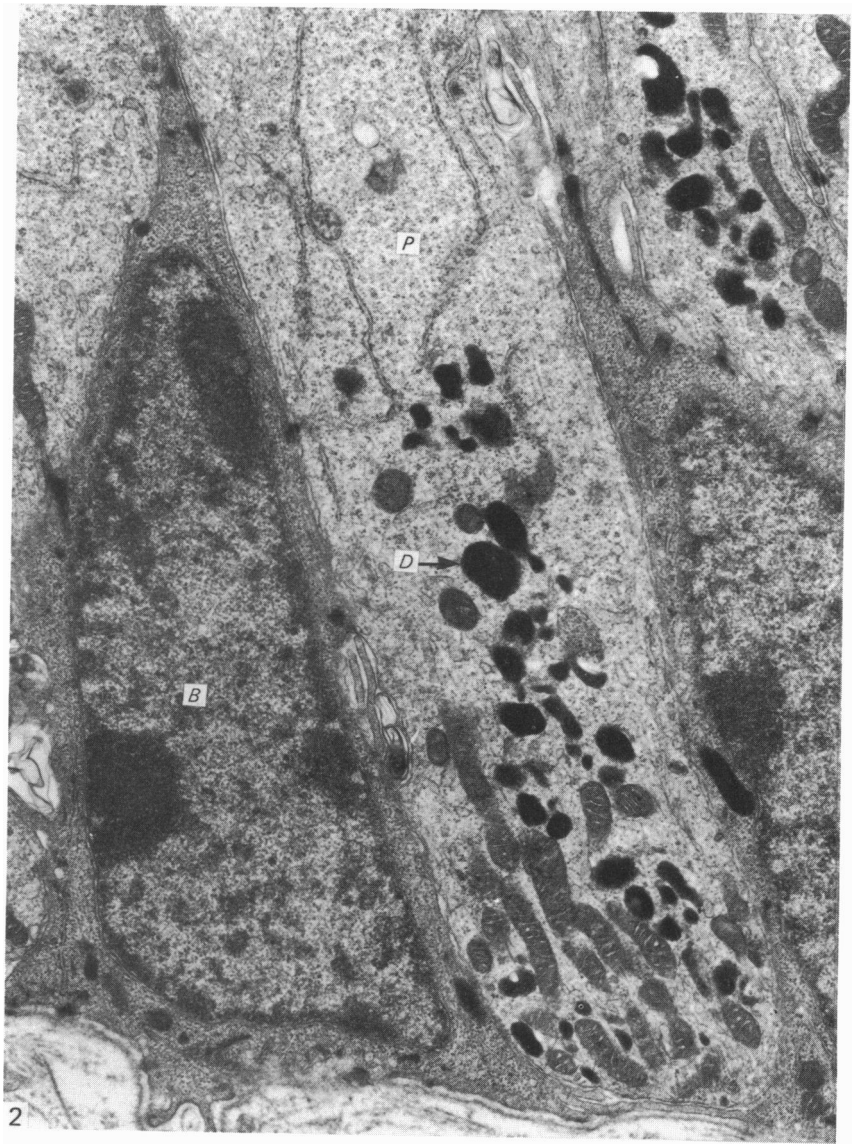



Fig. 2. Electron micrograph of the basal cytoplasm of a principal cell (*P*) containing numerous electron-dense bodies (*D*), and a basal cell (*B*). ×17000.

Kistler & Sherle (1966). A total of ten electron micrographs per site (approximately equal numbers from each of three animals) of longitudinal sections of the cells was prepared (initial magnification, ×10000; print magnification, ×24000). Each of these was overlaid with a transparent sheet containing a double square lattice (Weibel *et al.* 1966, Fig. 5*c*) and point counts were made of the following organelles in the supranuclear cytoplasm: mitochondria, smooth endoplasmic reticulum, rough endoplasmic reticulum, Golgi apparatus, and lysosomes. The volume fraction of each organelle was calculated as the ratio of the number of test points overlying the organelle to the total number of points within the supranuclear cytoplasm of the cell. In addition, the number of small (diameter less than 0·4 $\mu$m) and large vesicles

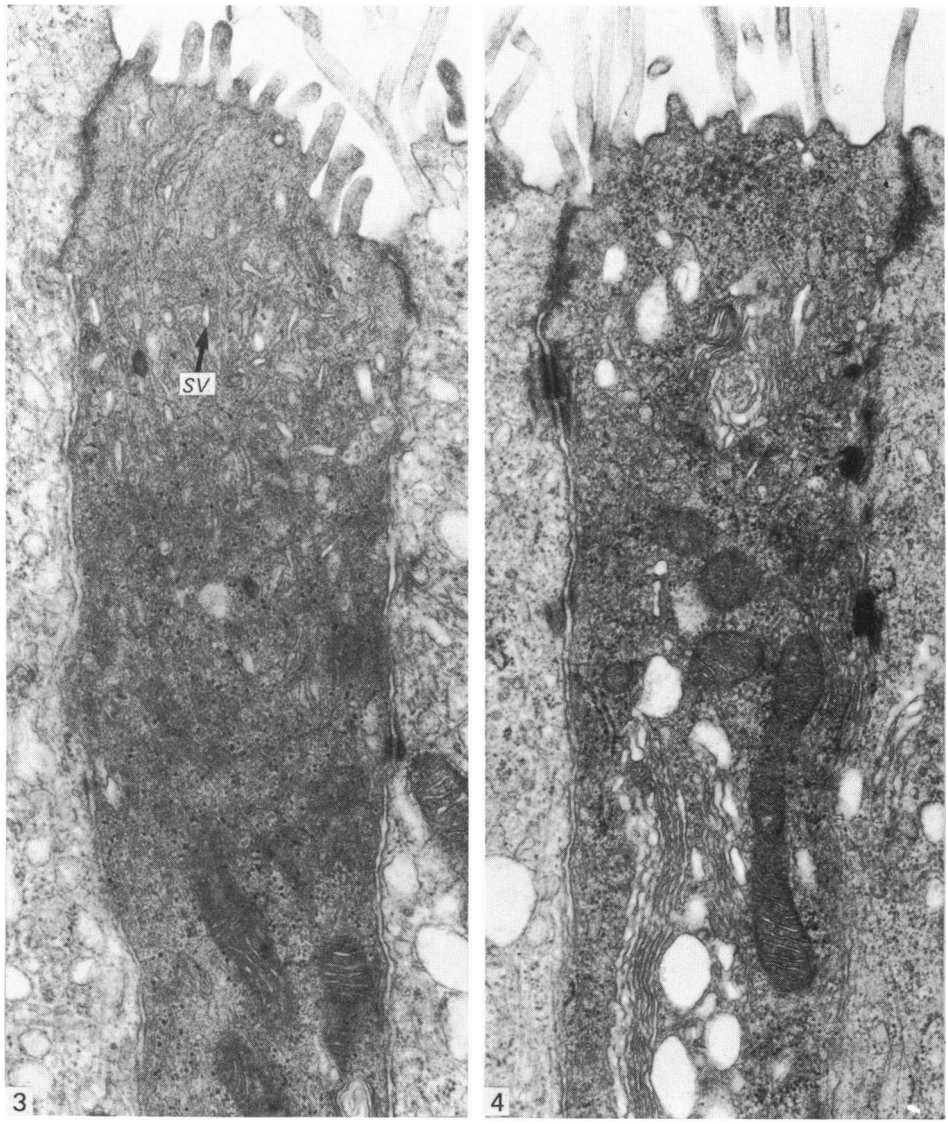



Fig. 3. Electron micrograph of the supranuclear cytoplasm of a narrow cell showing the short microvilli, densely stained cytoplasm and small vesicles (*SV*). ×23700.

Fig. 4. Electron micrograph of the supranuclear cytoplasm of an apical cell showing long microvilli and densely stained cytoplasm containing an abundance of cell organelles. ×23600.

in the supranuclear cytoplasm of each cell was counted. The statistical significance of differences between sampling sites was tested in analyses of variance (volumetric proportions were transformed to arcsine angles before analysis). The standard errors shown in Table 1 were calculated from the variance between animals.

## RESULTS

A narrow isthmus separated the head and tail of the epididymis of the echidna and also delineated the two histologically distinct regions of the ductus epididymidis, the initial and terminal segments. The whole duct was lined by a pseudostratified columnar epithelium.

Table 1. *The volumetric proportion of mitochondria, smooth endoplasmic reticulum (S.E.R.), rough endoplasmic reticulum (R.E.R.), Golgi apparatus (Golgi) and lysosomes, and the mean number of small and large vesicles in the supranuclear cytoplasm of principal cells from the initial segment of the ductus epididymidis of the echidna*

| | Percentage volume of supranuclear cytoplasm | | | | | Mean number of vesicles | |
|---|---|---|---|---|---|---|---|
| | Mitochondria | S.E.R. | R.E.R. | Golgi | Lysosomes | Small | Large |
| Site 1 | 4·878±0·846 | 5·378±0·705 | 1·153±0·205 | 6·555±1·127 | 0·155±0·074 | 27·095±4·943 | 1·657±0·495 |
| Site 2 | 4·757±0·910 | 5·140±0·602 | 1·083±0·196 | 6·824±0·473 | 0·269±0·143 | 23·749±3·123 | 1·845±0·261 |

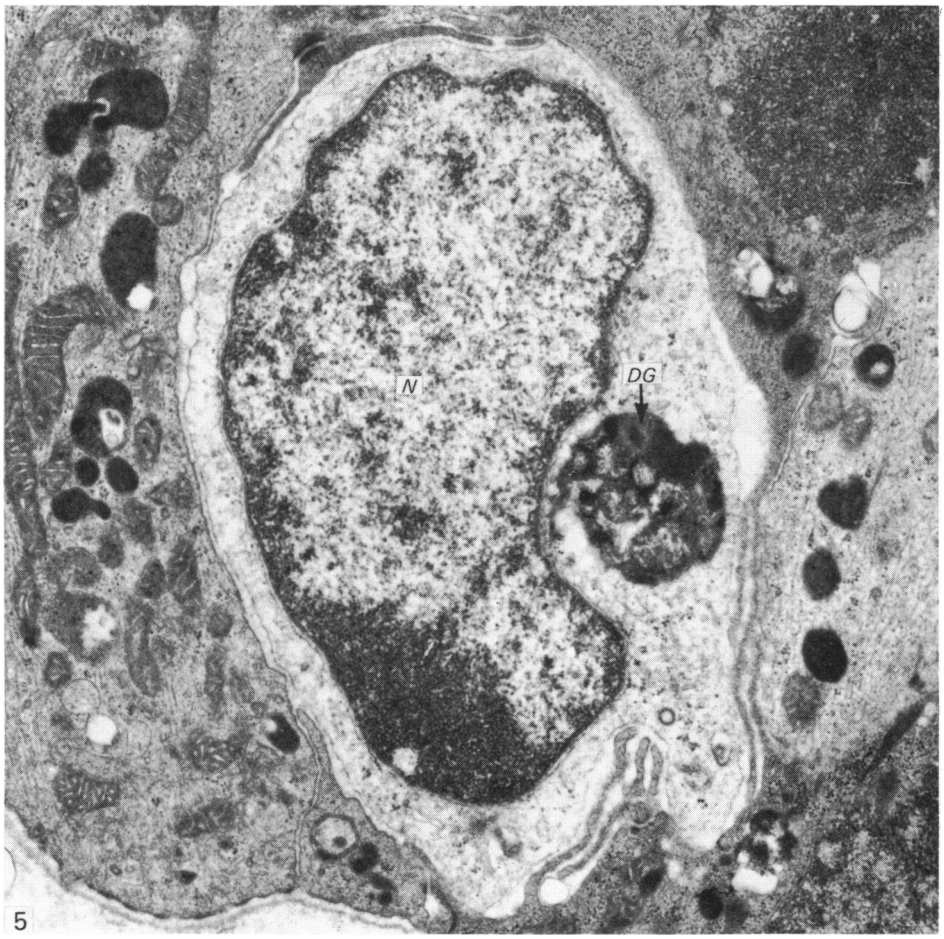
The epithelium lining the initial segment was very tall (*ca.* 54 $\mu$m) and was composed of principal, basal, apical, narrow and halo cells. The principal cells (Fig. 1) made up about 85 % of the volume of the epithelium. They contained predominantly euchromatic nuclei which contained regions of condensed heterochromatin; nucleoli lay adjacent to the nuclear membrane. The majority of cells contained one nucleus, but several binuclear cells were observed. The cells had long stereocilia alternating with deep invaginations of the membrane into the apical cytoplasm. The base of these invaginations appeared to be involved in the production of pinocytotic vesicles. The apical cytoplasm contained numerous vesicles, paired centrioles, complexes of fibrogranular material and occasional electron-dense bodies. The supranuclear cytoplasm was characterized by large vesicles, multivesicular bodies, smooth endoplasmic reticulum and cisternae of Golgi lamellae. Mitochondria, rough endoplasmic reticulum, polysomes and microtubules occurred throughout the cytoplasm of the cell, but electron-dense bodies occurred mainly in the basal cytoplasm (Fig. 2).

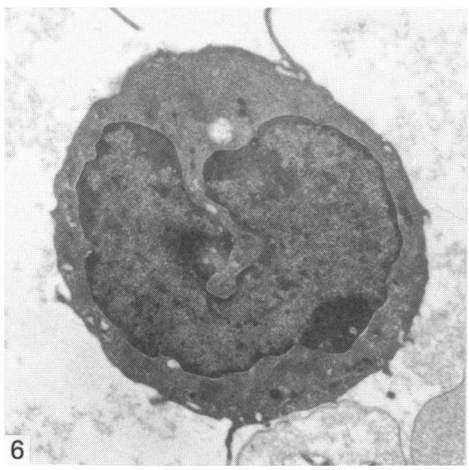
Table 1 shows that there was no difference in the size or occurrence of organelles in the principal cells sampled from the proximal and distal ends of the initial segment.

Basal cells were usually triangular shaped in sections and lay on the basal lamina. They stained more intensely than principal cells (Fig. 2). They contained a large heterochromatic nucleus and sparse cytoplasm which contained some electron-dense bodies, mitochondria, rough endoplasmic reticulum and free ribosomes.

The nuclei and most of the cytoplasm of the apical and narrow cells were located above the row of principal cell nuclei. Their cytoplasm stained more intensely than the cytoplasm in the principal cells. The apical cells (Fig. 4) were characterized by long stereocilia, a predominantly euchromatic nucleus containing patches of heterochromatin, and an abundance of cellular organelles. The apical cytoplasm contained both small and large vesicles, centrioles, and complexes of fibrogranular material. The supranuclear cytoplasm contained a large Golgi apparatus, and smooth and rough endoplasmic reticulum; mitochondria and polysomes occurred throughout the cytoplasm of the cell. The narrow cells (Fig. 3; Sun & Flickinger, 1980) were characterized by very short stereocilia, a nucleus containing a heavy margin of heterochromatin and, relative to the principal and apical cells, a paucity of cellular organelles. They contained elongated or cup-shaped vesicles in the apical cytoplasm (some appeared to be budding from the apical plasmalemma), and smooth and rough endoplasmic reticulum, mitochondria and numerous polysomes throughout the cytoplasm of the cell.

Halo cells (Reid & Cleland, 1957) occurred between adjacent principal cells in the



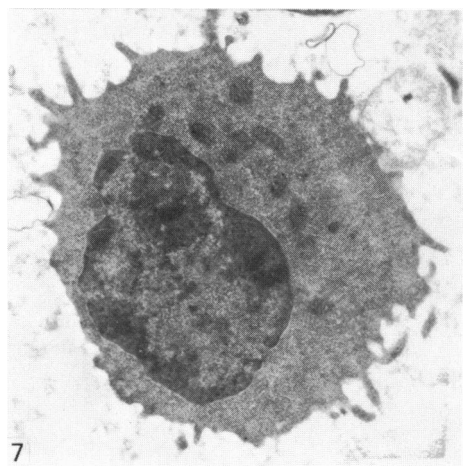
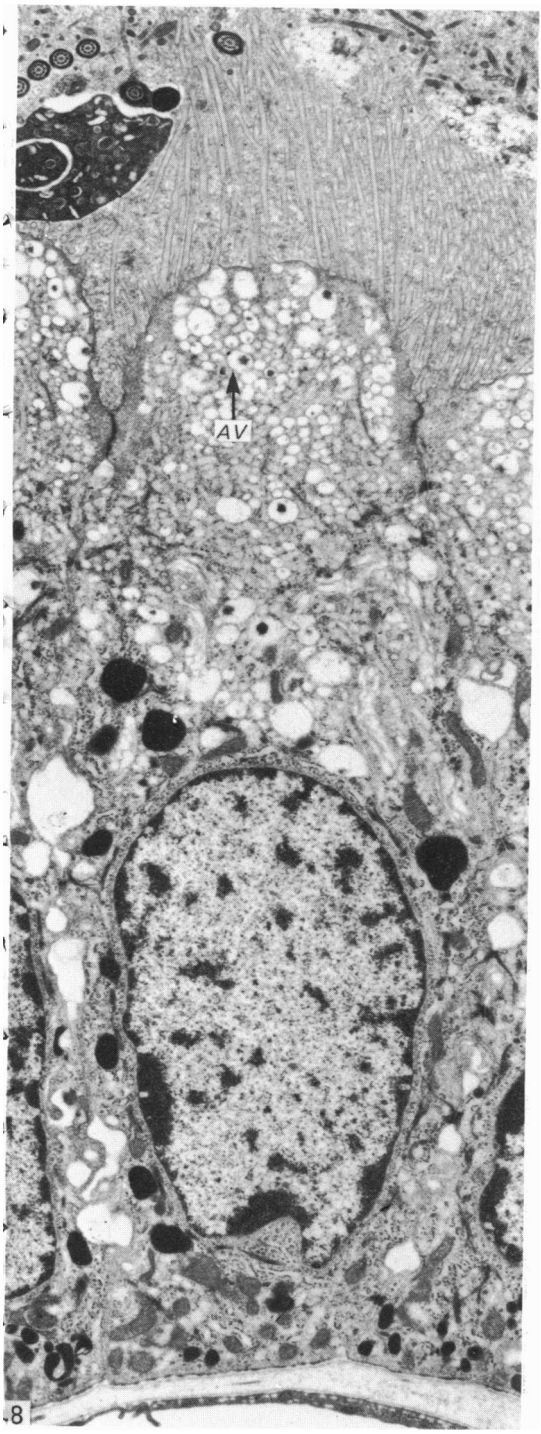




Fig. 5. Electron micrograph of an intraepithelial leucocyte showing tne large regularly shaped nucleus (*N*) and an electron-dense granule (*DG*) in the cytoplasm. × 18500.

Fig. 6. Electron micrograph of a neutrophil prepared from a buffy coat of echidna blood, showing its large, multilobulated nucleus. × 8300.

Fig. 7. Electron micrograph of a lymphocyte prepared from a buffy coat of echidna blood, showing its oval nucleus. × 10000.



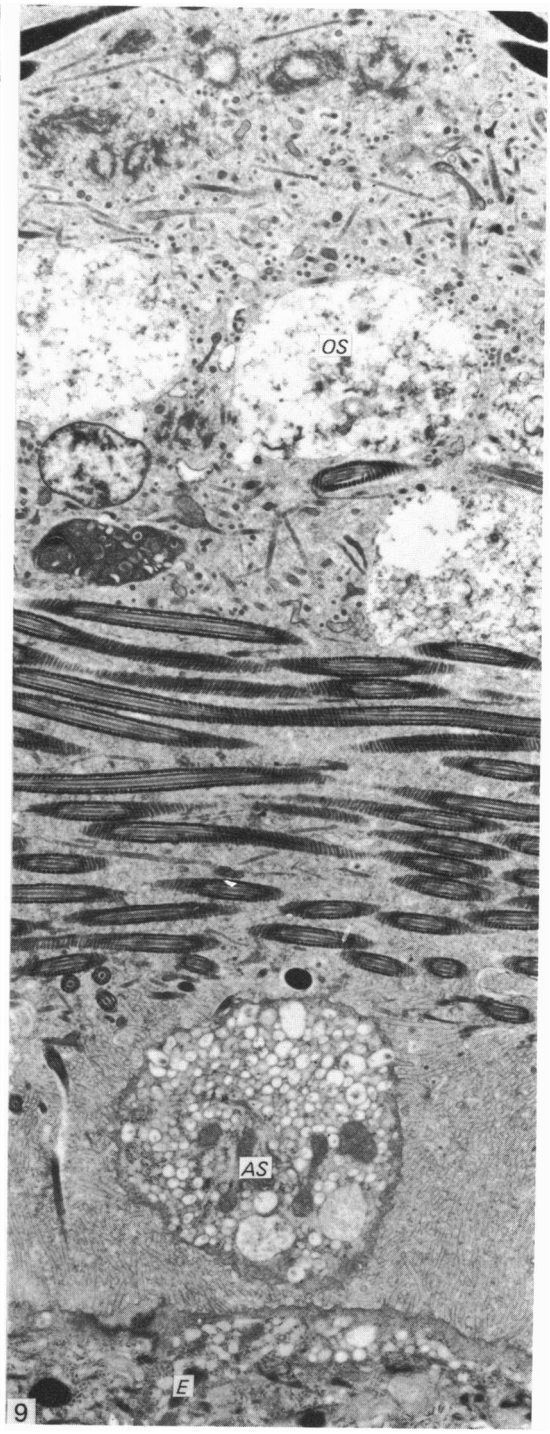




Fig. 8. Electron micrograph of principal cells in the terminal segment of the ductus epididymidis. Note the abundance of supranuclear vesicles (*AV*). ×5700.

Fig. 9. Electron micrograph of the lumen adjacent to the epithelium (*E*) lining the terminal segment of the ductus epididymidis. Newly formed apocrine secretions (*AS*) appear similar to the apical cytoplasm of principal cells. Within the lumen of the duct the secretions degenerate (*OS*) and contain flocculent electron-dense material. ×7500.

middle or basal region of the epithelium (Fig. 5). They were characterized by a fairly oval heterochromatic nucleus surrounded by cytoplasm with a lower electron density than that of the principal cells. The cytoplasm contained some rough endoplasmic reticulum, ribosomes and large, electron-dense granules. From the examination of the leucocytes in the buffy coat preparation of blood, it was considered that (based mainly on their nuclear form) the halo cells were lymphocytes (Fig. 7). The latter were characterized by an oval, heterochromatic nucleus surrounded by cytoplasm containing few mitochondria and some rough endoplasmic reticulum. In contrast, neutrophils (Fig. 6) were the other type of leucocyte present in echidna blood (Bolliger & Backhouse, 1960). They were characterized by a multilobulated, predominantly euchromatic nucleus; their cytoplasm contained a few electron-dense bodies and mitochondria, and some rough endoplasmic reticulum.

The epithelium lining the terminal segment of the ductus epididymidis was low (*ca.* 17 $\mu$m). It contained no apical or narrow cells and fewer basal and halo cells than the initial segment. The principal cells (Fig. 8) were stereociliated and their nuclei were predominantly euchromatic, with a few heterochromatic regions throughout and adjacent to the nuclear envelope. The apical cytoplasm contained numerous vesicles, extensive rough endoplasmic reticulum and a few mitochondria. The vesicles varied in size and their contents varied from electron-transparent to either a diffuse, electron-dense material, or a granular, flocculent electron-dense material. The supranuclear cytoplasm contained large vesicles, a Golgi apparatus, numerous mitochondria, extensive dilated rough endoplasmic reticulum and free ribosomes. The perinuclear and basal cytoplasm was occupied by electron-dense bodies, mitochondria, rough endoplasmic reticulum and polysomes.

The principal cells were involved in considerable apocrine secretion (Fig. 9). The structure of the newly formed apocrine secretions was similar to the apical cytoplasm of the principal cells. Within the lumen of the duct the secretions appeared to degenerate, initially to form membrane-bound, vacuolated structures containing flocculent electron-dense material. Ultimately, the membrane might disrupt, so that the flocculent material formed the matrix of the luminal contents.

## DISCUSSION

It was concluded from these studies that the epithelium lining the initial segment of the ductus epididymidis of the echidna is structurally similar along its length and is analogous to the initial segment proper of other mammals (Benoit, 1926; Glover & Nicander, 1971; Nicander & Glover, 1973; Cummins, 1976; Flickinger *et al.* 1978; Fawcett & Hoffer, 1979). Bedford & Rifkin (1979) proposed that the initial segment in the ductus epididymidis of the echidna should be classified into two parts on the basis of differences in the regularity of the luminal border of the epithelium, the size of the Golgi apparatus and the concentration of spermatozoa in the lumen of the duct. Our findings indicate that any swelling or blebbing of the apical cytoplasm is due to technical problems of fixation and does not vary systematically along the duct. Further, Table 1 indicates that the Golgi apparatus is about the same size at either end of the segment. An increase in concentration of luminal spermatozoa certainly does occur along the duct (Bedford & Rifkin, 1979; Djakiew & Jones, 1981). However, this also occurs in the initial segment of other animals (Crabo, 1965; Jones, 1980; Hinton, White & Setchell, 1980) as a consequence of fluid re-

absorption, and so it is not an appropriate criterion for classifying the segment into two subdivisions.

The occurrence and structure of the basal, apical and narrow cells in the initial segment of the ductus epididymidis of the echidna is consistent with findings in other mammals except that, in the echidna, the apical cells are more electron-dense than the principal cells, whereas in the rat and hamster the electron density of these cells is much the same (Nicander & Glover, 1973; Moore & Bedford, 1979). Although there is some variation in structure of the halo cells between the echidna and rat (Hoffer, Hamilton & Fawcett, 1973) the conclusion that they are migratory lymphocytes is the same for both species.

It is concluded from these studies that the terminal segment of the ductus epididymidis is involved in considerable apocrine secretion. This function is quite different from the function of the epididymal epithelium of other mammals which have been studied. The secretions may be involved in the maturation of spermatozoa: the latter form clumps, lose their cytoplasmic droplets and become motile when they enter the terminal segment (Djakiew & Jones, 1981, unpublished data); the secretions may function physically to separate spermatozoa, to avoid the consequence of overcrowding, or they may provide a metabolic substrate for spermatozoa.

The confirmation, in these studies, that the ductus epididymidis of the echidna is structurally differentiated into only two segments (Jones & Djakiew, 1978; Djakiew & Jones, 1981), is of considerable interest to comparative reproductive biologists. Because the epididymis of the platypus seems to be similar to that of the echidna (Temple-Smith, 1973) it is proposed that the evolution of the epididymis in therian mammals has involved the development of new, structurally distinct, middle segments of the ductus (Glover & Nicander, 1971), and modification of the terminal segment. These segments were presumably evolved to allow, respectively, additional post-testicular development (Bedford & Rifkin, 1979; Bedford, 1979) and storage of spermatozoa (Jones & Djakiew, 1978; Djakiew & Jones, 1981). Further, the ocurrence of only two segments in the ductus epididymidis of protherian mammals indicates that mammals such as the elephant (Jones, 1980; Holt, Jones & Skinner, 1980; Jones & Brosnan, 1981) and European mole (Suzuki & Racey, 1976), which have a middle segment in their ductus epididymidis, have reproductive tracts which are more advanced than has previously been appreciated (Eckstein & Zuckerman, 1960).

SUMMARY

It is concluded that the ductus epididymidis of the echidna is divided into only two structurally distinct segments which are each homogeneous along their length. The initial segment in the echidna is structurally very similar to the initial segment proper in the epididymis of all other mammals which have been studied, whereas the terminal segment is structurally quite different from the terminal segment in other mammals. The terminal segment in the echidna is involved in considerable apocrine secretion of highly membranous material. The secretions gradually degenerate and occupy a large proportion of the lumen of the duct.

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