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The ductuli efferentes of the epididymal region of birds

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INTRODUCTION

The structure of the efferent ductules has been studied in several mammals (Burgos, 1957; Ladman & Young, 1958; Morita, 1966; Martan, Hruban & Slesers, 1967; Ladman, 1967; Yokoyama & Chang, 1971; Hoffer, 1972; Ramos, 1977) and even though their function is not absolutely clear, it is generally agreed that they play a role in fluid absorption and the movement of spermatozoa into the ductus epididymidis.

Only a few reports on the structure of the efferent ductules in birds are available. Hess, Thurston & Biellier (1976) and Hess & Thurston (1977) adopted Tingari's (1971) nomenclature, derived from the domestic fowl, in their studies of the epididymal region of the turkey. Budras & Sauer (1975*a*), however, considered that the efferent ductules of the domestic fowl consisted of two continuous portions: the proximal efferent ductule, (which Tingari (1971) termed the ductuli efferentes) and the distal efferent ductule (which Tingari termed the narrow connecting duct). In his study of the Japanese quail, Aire (1979) adopted the classification proposed by Budras & Sauer (1975*a*); in both segments of the efferent ductules the epithelium consists of ciliated and non-ciliated cells.

Tingari (1972), Budras & Sauer (1975*a*) and Hess & Thurston (1977) studied the fine structure of these cells. Tingari (1972) and Hess & Thurston (1977), however, did not describe certain histological and ultrastructural differences existing between the non-ciliated cells in different parts of the efferent ductules which will be described herein. In their study of the epididymal region of the guinea-fowl Aire, Ayeni & Olowo-Okorun (1979) proposed classifying the non-ciliated cell in the proximal efferent ductule as 'non-ciliated Type I cell' and that in the distal efferent ductule as 'non-ciliated Type II cell'. Tingari (1972) classified only one non-ciliated cell, Type I cell in the 'efferent ductule' of the domestic fowl. The present author has also proposed reclassifying the non-ciliated cell (Tingari's Type II cell) in the connecting ductules, ductus epididymidis and ductus deferens as the 'non-ciliated Type III cell'.

This report examines the histological and ultrastructural characteristics of the efferent ductule as a unit in several different birds, and seeks to clarify certain differences in describing structure and function in earlier reports. It also describes the non-ciliated Type II cell which has only been referred to briefly by Budras & Sauer (1975*a*).

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MATERIALS AND METHODS

Young sexually mature male domestic fowl (*Gallus domesticus*), Japanese quail (*Coturnix coturnix japonica*) and guinea-fowl (*Numida meleagris*) were used in this investigation. All the birds were anaesthetised intraperitoneally with thiopentone. From some, the testis (with the epididymis) was removed immediately and fixed by immersion in 3% glutaraldehyde buffered with 0·067 M sodium cacodylate. Other birds were perfused with the fixative via the descending aorta or (in the case of the Japanese quail) via the left ventricle. Small pieces of tissue from the epididymal regions were cut and immersed in the same fixative overnight or for a longer period before further processing in 2% osmium tetroxide buffered with 0·2 M s-collidine. Dehydration in a graded series of ethanol preceded embedding in Epon.

Semithin sections 1 micron thick were cut and stained in toluidine blue. Thin sections for electron microscopy were cut, stained in uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963), and examined in a Philips EM 201 C or 300 electron microscope.

OBSERVATIONS

For orientation, Figures 1 and 2 show typical histological sections of proximal and distal efferent ductules in the domestic fowl. The histology of the efferent ductules of the Japanese quail (Aire, 1979) is similar to that of the domestic fowl, turkey and guinea-fowl. Only ultrastructural observations will, therefore, be reported here.

Ultrastructural observations

Figures 3 and 4 show survey pictures of the epithelia of the proximal and distal efferent ductules respectively in the domestic fowl.

Non-ciliated Type I cell

The brush border of this cell, which is present in the epithelium of the proximal efferent ductule, possesses long, often branched microvilli which have a central core of microfilaments projecting into the sub-apical cytoplasm to variable levels (Fig. 5). Both the intervillous plasmalemma and that of the microvilli are covered by a granular, slightly fuzzy coat (Fig. 6). A distinct and well-formed terminal web may be observed (Fig. 6). Pinocytotic vesicles are numerous on the brush border and they lead into several tubular canaliculi in the sub-apical cytoplasm (Fig. 7). Large, coated vesicles are also seen in this region of the cell. In well-fixed tissue the lumina of the canaliculi and vesicles are patent, and no difficulty is encountered in differentiating one from the other or from other adjacent organelles.

Several vacuoles with flocculent content are observed in the supranuclear region. These are found amongst the coated vesicles and canaliculi (Fig. 7). Also found among these organelles and as far distally as to the level of the nucleus are numerous dense bodies or globules of varying sizes and configuration (Figs. 3, 8, 9). Two main types of globules are present (Figs. 9, 10). The more common of them have a homogeneous matrix of electron-dense material bounded by a membrane. The other type (heterogeneous bodies) have an irregular form and consist of numerous dense granules, surrounded by lucid areas, and vacuoles. Several intermediate types of dense bodies and lysosome-like structures occur (Fig. 9).

Microtubules are seen running mostly parallel to the cell axis especially in the supranuclear zone of the cell where they are most numerous (Fig. 11). The Golgi

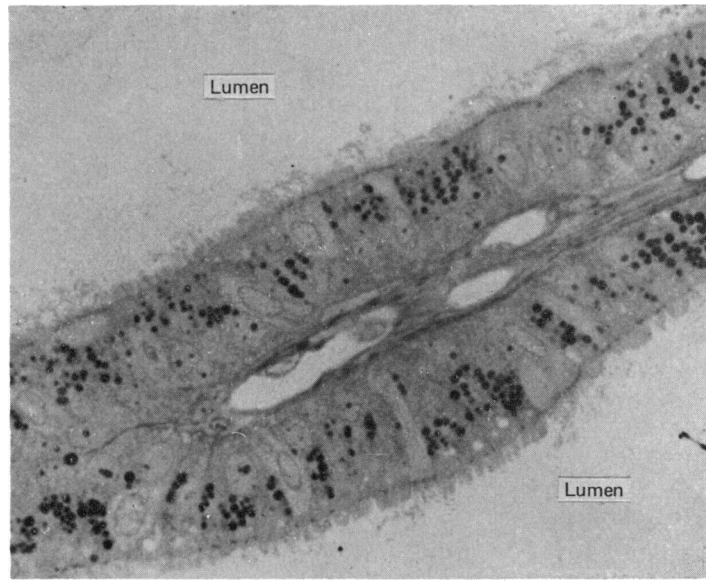

Fig. 1. Histological section of the proximal efferent ductule epithelium of the fowl. Note the presence of dense bodies and apical vacuoles in the non-ciliated Type I cells. The ciliated cells are lighter staining. Also note that the tissue was well perfused as judged from the empty blood vessels in the periductular connective tissue. No blebs appear on the apical surfaces of any of the cells. Toluidine blue. × 1280.

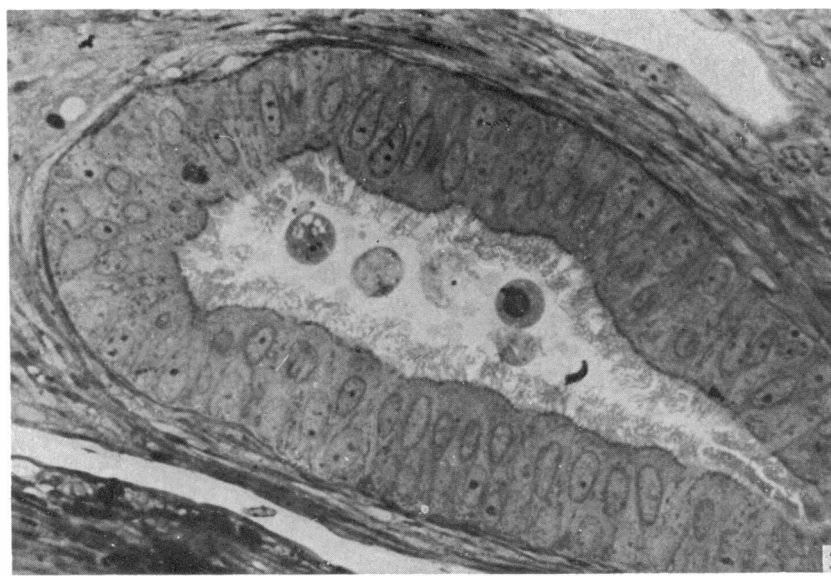

Fig. 2. Histological cross section of the distal efferent ductule of the fowl. Note that neither globules nor vacuoles are seen in the cells. Note also the stratification of the epithelium; the upper layer of nuclei belongs to the ciliated cells and the lower, more circular nuclei to the non-ciliated Type II cells. Ciliated cells are more numerous in this ductule than in the proximal one. Toluidine blue. × 1280.

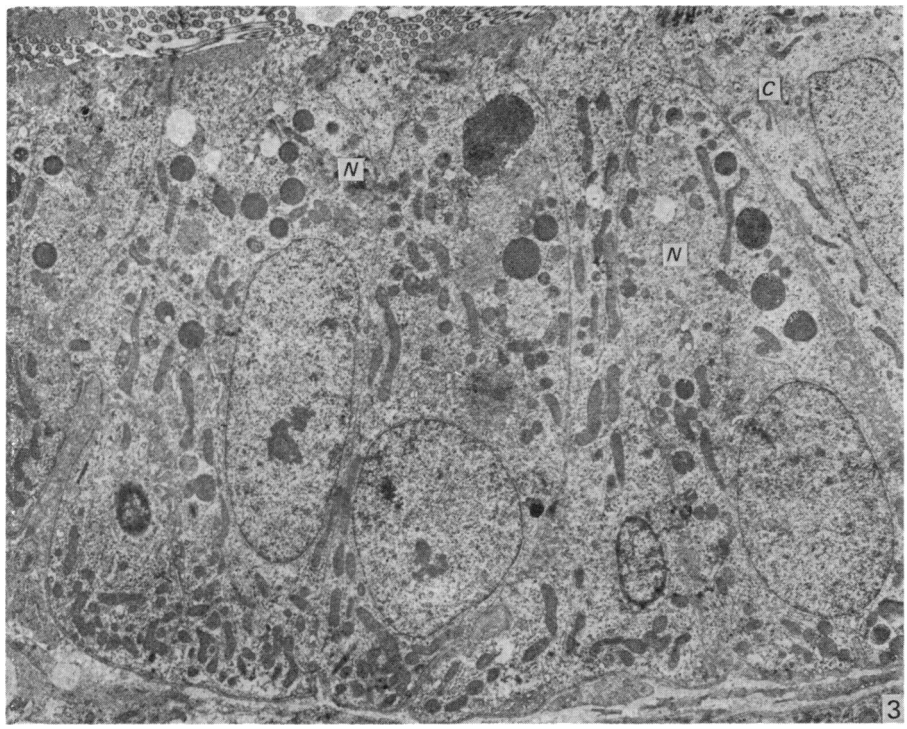

Fig. 3. Survey electron micrograph of proximal efferent ductule epithelium of the fowl showing the non-ciliated cell Type I (*N*) with its dense bodies and vacuoles, and the lighter ciliated cell (*C*). ×4032.

apparatus is conspicuous, is moderately developed and located in the supranuclear region of the cell (Fig. 9). Stacks of cisternae which bud off small, coated vesicles and vacuoles from their often dilated ends make up the Golgi apparatus. The numerous mitochondria appear in various shapes ranging from oval to very elongated and are seen throughout the cytoplasm except in the apical web region where organelles are normally absent (Figs. 8, 9, 11). They are remarkably numerous in the immediate supranuclear and especially in the infranuclear regions where they are tightly packed together in several cells (Fig. 12). Long, tubular strands of rough endoplasmic reticulum (RER) may be seen anywhere in the cytoplasm, but some of them may be seen running alongside or surrounding mitochondria (Fig. 8). The RER strands may be branched and often have dilated lumina filled with amorphous slightly electron-dense material. Free ribosomes and polyribosomes are also seen scattered throughout the cell cytoplasm.

In poorly fixed tissue, by immersion or perfusion, the apical cytoplasm may protrude into the tubular lumen to form blebs already described by several authors (Tingari, 1971; Budras & Sauer, 1975*a*; Hess, Thurston & Biellier, 1976; Hess & Thurston, 1977). These blebs may contain such dense bodies, mitochondria, apical canaliculi and vesicles as already described for the non-ciliated Type I cell. The microvilli are also seen to swell and become bulbous. Accompanying these changes may be extremely exaggerated intercellular spaces.

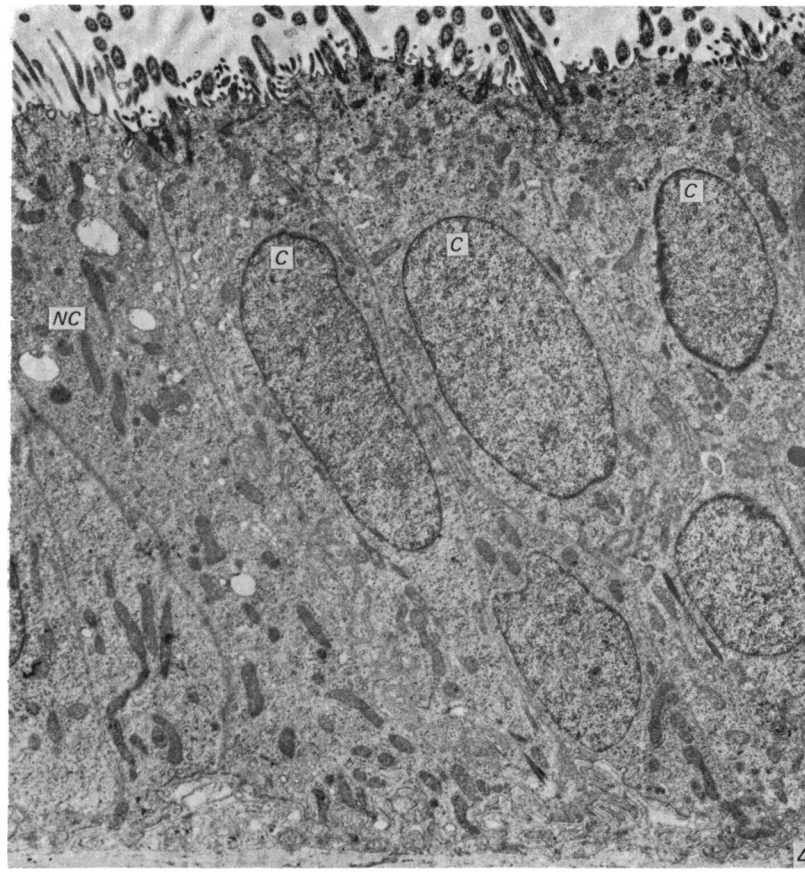

Fig. 4. Survey electron micrograph of the distal efferent ductule epithelium of the fowl. Note that dense globular bodies are not observed in the non-ciliated Type II cells (*NC*). There are more ciliated cells (*C*), in the ratio of 5:1, than non-ciliated cells. × 6272.

Non-ciliated Type II cell

This cell, with the ciliated cell, forms the epithelial lining of the distal efferent ductule (Fig. 13).

Coated vesicles in the sub-apical region of the cell are present as in the Type I cell, but they are, however, fewer and smaller in size (Fig. 14). The tubular canaliculi which seem to bud off or connect to coated vesicles in the Type I cell are hardly observed in the Type II cell. Vacuoles, if present, are small and few, but have a content similar to those of Type I cells.

The most striking characteristic of the Type II as compared to the Type I cell is the typical absence of dense bodies or globules in the former cell (Fig. 13). Fewer mitochondria than in the non-ciliated Type I cell, moderately distended RER, Golgi apparatus and a few vacuoles filled with densely-staining material, probably lysosomes are found in the supranuclear region of the cell (Fig. 13). Very few organelles are found in the infranuclear zone of the cell.

Ciliated cell

The usually electron-lucent cell bears typical cilia and a few short microvilli (Figs. 15, 17). Between the microvilli may be found small coated micropinocytic

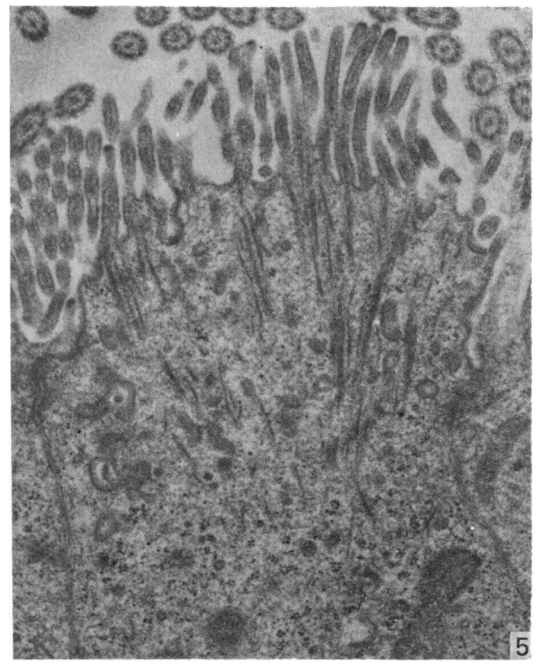

Fig. 5. Apical portion of Type I cell of the guinea-fowl showing microvilli with a central core of microfilaments projecting into the cytoplasm of the cell. ×17280.

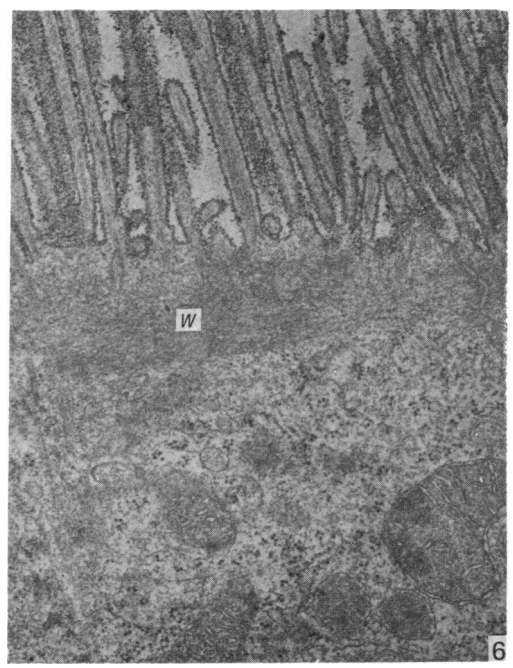

Fig. 6. Apical portion of Type I cell of the fowl showing a terminal web (*W*). Note fuzzy covering of microvilli and intervillous plasma membrane. Organelles are normally absent in the terminal web region. ×22400.

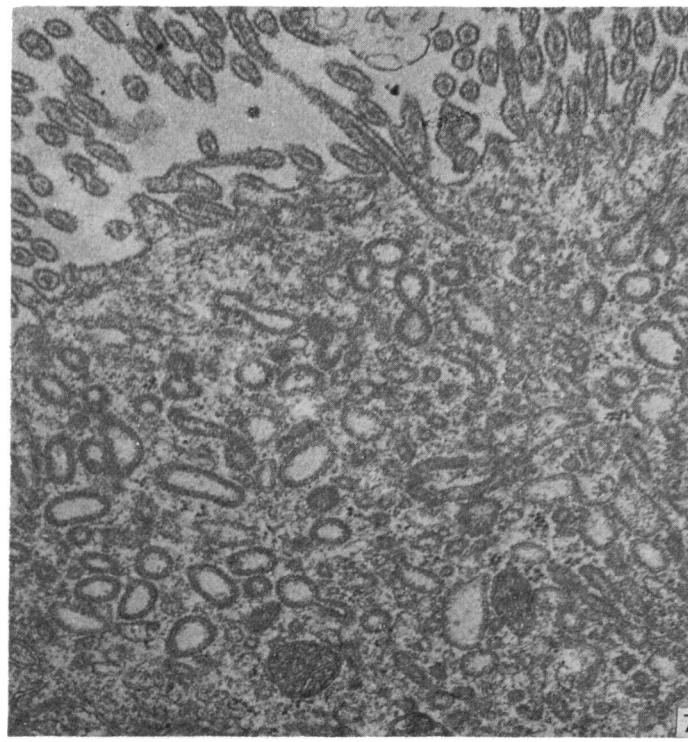

Fig. 7. Apical region of Type I cell of the fowl showing a dense pack of apical canaliculi and vesicles. Note that their lumina are patent. ×22400.

invaginations of the cell surface. Neither tubular invaginations nor canaliculi are present in this cell. One or more large vacuoles with flocculent content or smaller vesicles may, however, be present.

The nucleus is large and often irregularly shaped (Figs. 15, 17). The Golgi apparatus is moderately developed and located in the supranuclear region of the cell (Figs. 15, 17). Rough endoplasmic reticulum appears in a few short or long strands or coils and scattered throughout the cell. Polyribosomes are also seen scattered throughout the cytoplasm (Figs. 16, 17).

Oval and, less commonly, elongate forms of mitochondria are concentrated in the supranuclear region (Figs. 15, 17), but a large number of them may also be seen in the infranuclear zone of the cell.

Microfibrillar bundles may be seen around the nucleus or in the subapical region or running parallel to the length of the basal portion of the cell (Figs. 16, 17). Microtubules are also seen.

Only a few, small, dense globular bodies and lysosome-like bodies are present in the cytoplasm of this cell.

Intraepithelial lymphocytes

Cells regarded as lymphocytes are occasionally seen in the epithelium especially towards the basal lamina (Fig. 18). Their nuclei are heterochromatic and few organelles are found in the relatively electron-lucent cytoplasm. No junctional complexes are found between this cell and adjacent epithelial cells. Intraepithelial

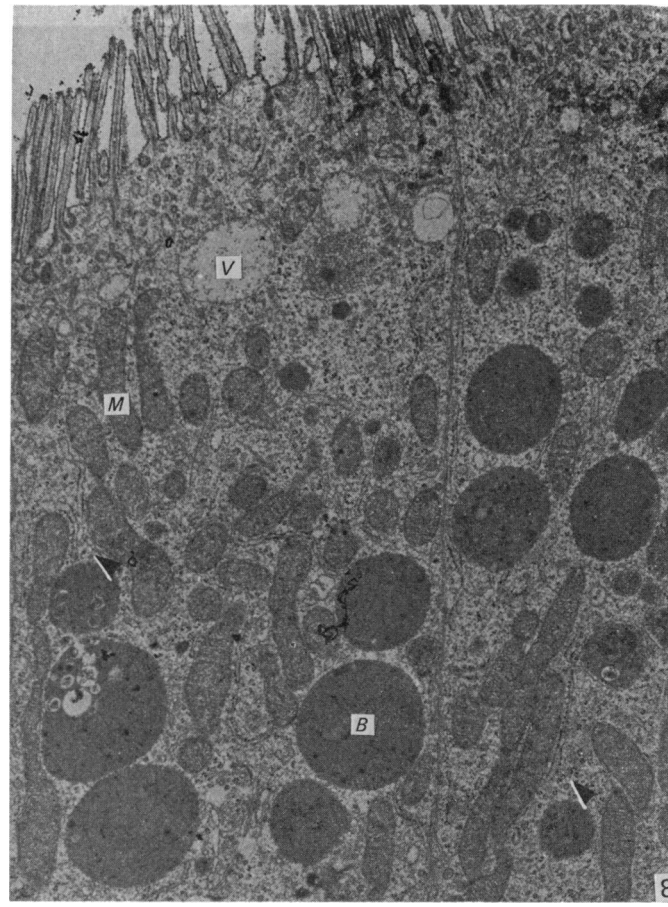

Fig. 8. Non-ciliated Type I cells of the guinea-fowl. Note the general arrangement of the organelles: vacuole (*V*); numerous mitochondria (*M*), dense bodies (*B*) and strands of RER (arrowheads) closely associated with mitochondria. × 10080.

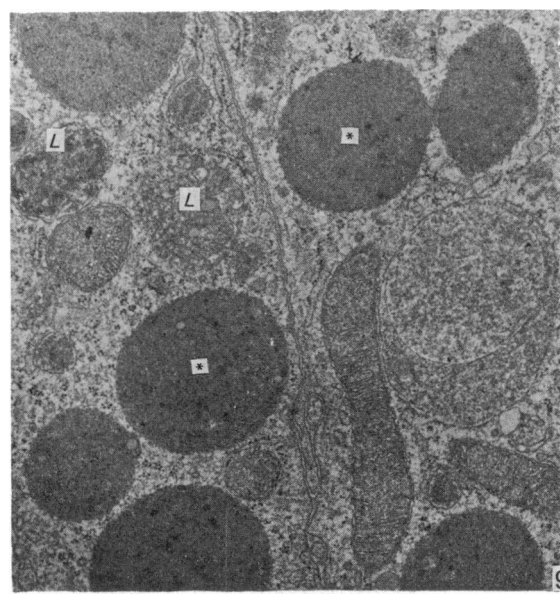

Fig. 9. Middle portion of Type I cell of the fowl. Lysosome-like bodies (*L*);

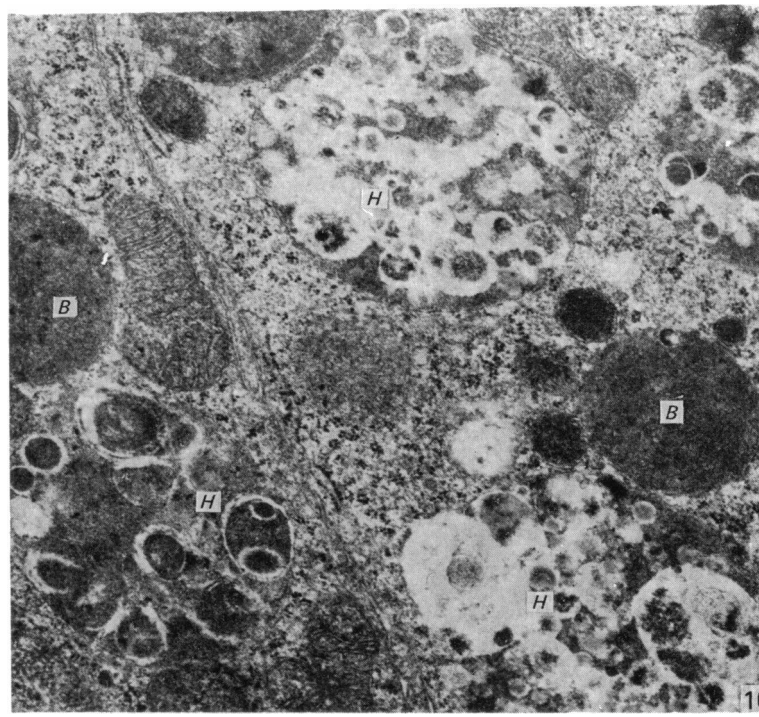

Fig. 10. Other forms of dense bodies (*B*) and heterogeneous bodies (*H*) in the cytoplasm of Type I cells. Fowl. ×20160.

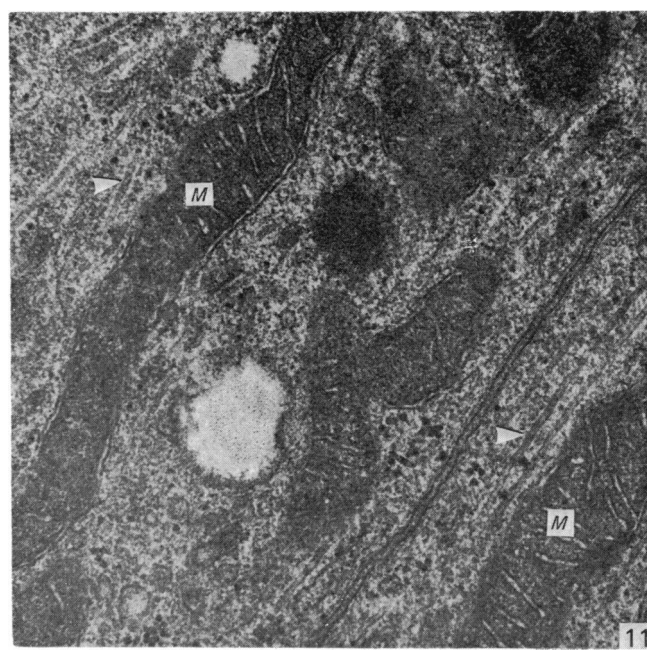

Fig. 11. Microtubules (arrowheads) in Type I cell of the Japanese quail, also long mitochondria (*M*). ×40320.

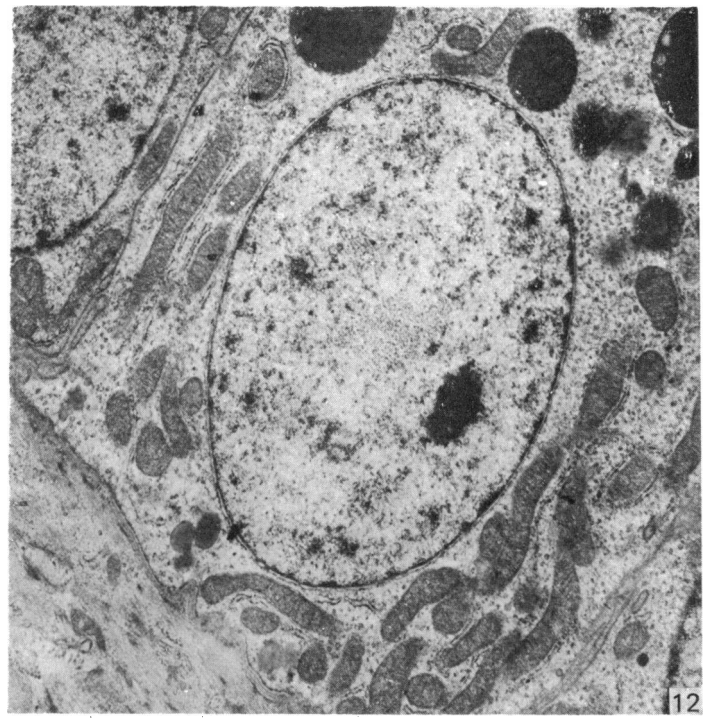

Fig. 12. Basal portion of Type I cell of the Japanese quail. Numerous and large mitochondria are present at the base of the cell. × 10080.

lymphocytes may be found at any level in the epithelium. This cell has been reported more elaborately somewhere else (Aire & Malmqvist, 1979).

DISCUSSION

Ladman & Young (1958) described differences in the structure of the non-ciliated cells in the efferent ducts of the guinea-pig and came to the conclusion that a proximal and distal segment of the ducts existed. Reid & Cleland (1957) also observed an initial zone and a terminal zone in the efferent ducts of the rat. In the birds studied here it is clear that the efferent ductules consist of both a proximal and distal segment. Both segments are lined by ciliated and non-ciliated cells, but the chief differences existing between the segments are to be found in the greater ductular diameters and epithelial folding of the proximal, as compared to the distal, segment and the structural differences between the non-ciliated Type I and II cells. Some confusion about the cell types found in the two segments of the efferent ductules, i.e. the proximal efferent ductule (Tingari's efferent ductule) and distal efferent ductule (Tingari's narrow connecting ductule) arises from observations made by Tingari (1972) in the fowl and Hess & Thurston (1977) in the turkey. Tingari did not consider that there was a different non-ciliated cell type in the distal efferent ductule (i.e. Tingari's narrow connecting ductule) and Hess & Thurston, employing Tingari's (1972) classifications described one cell type, non-ciliated Type I cell, for the proximal and distal efferent ductules as well as the connecting ductules. Morita (1966) showed that three types of non-ciliated cells

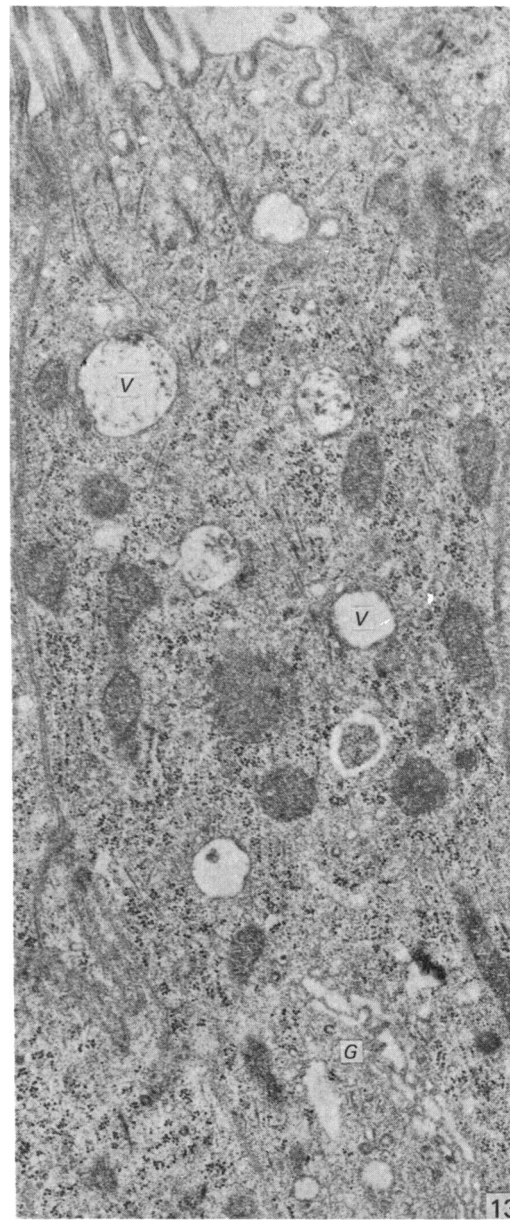

Fig. 13. Non-ciliated Type II cell in the distal efferent ductule of the fowl; Golgi complex (*G*) and vacuoles (*V*). ×17280.

occurred in the efferent ducts of the human, and ascribed specific functions of absorption or secretion to them.

Tingari (1972) regarded the non-ciliated cells in the efferent ductules to be less electron-opaque than the ciliated cells. Our observations and those of Hess & Thurston (1977) convey a contrary opinion.

Ultrastructurally, both the Type I and II cells described in the present study possessed structures in the apical region of the cell which are consistent with fluid

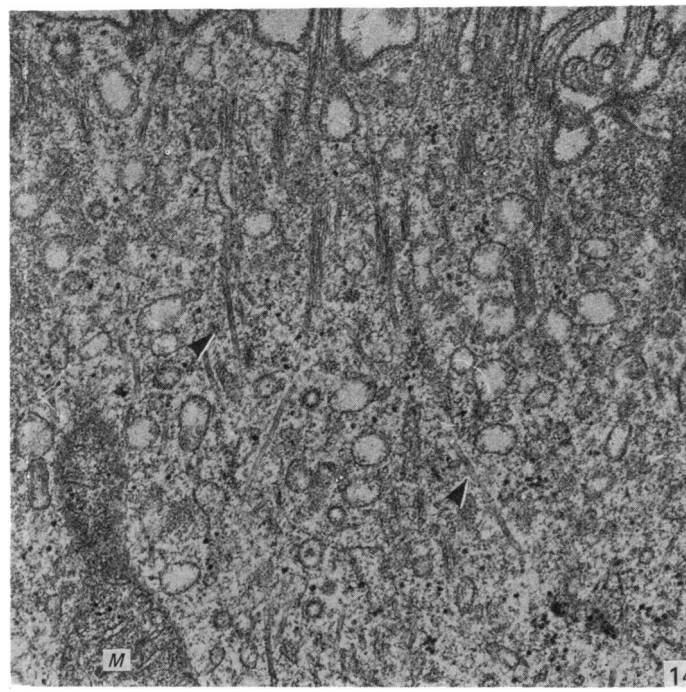

Fig. 14. Cranial portion of non-ciliated Type II cell of the fowl showing microvilli and their microfilaments projecting into the cytoplasm. Note the presence of small vesicles, microtubules (arrowheads) and mitochondrion (*M*). × 38400.

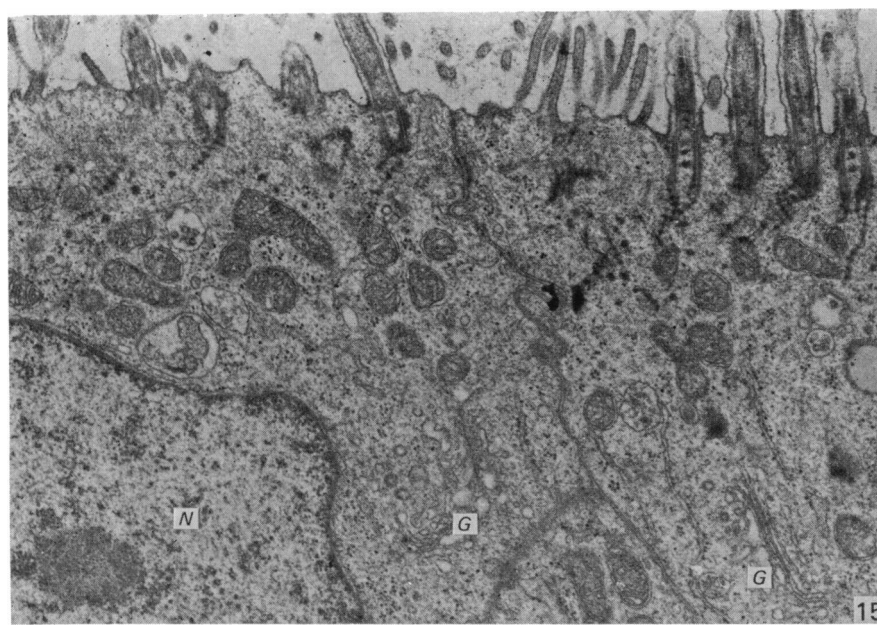

Fig. 15. Ciliated cell in the distal efferent ductule of the fowl. Note the sections of the cilia and their roots, numerous oval mitochondria in the supranuclear cytoplasm, Golgi complex (*G*) and nucleus (*N*). × 19200.

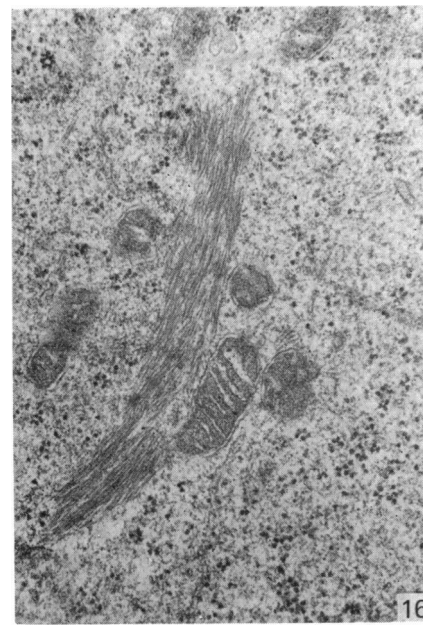

Fig. 16. Infranuclear region of ciliated cell. Note presence of central bundle of microfibrils, long RER and numerous polyribosomes. Guinea-fowl. ×33600.

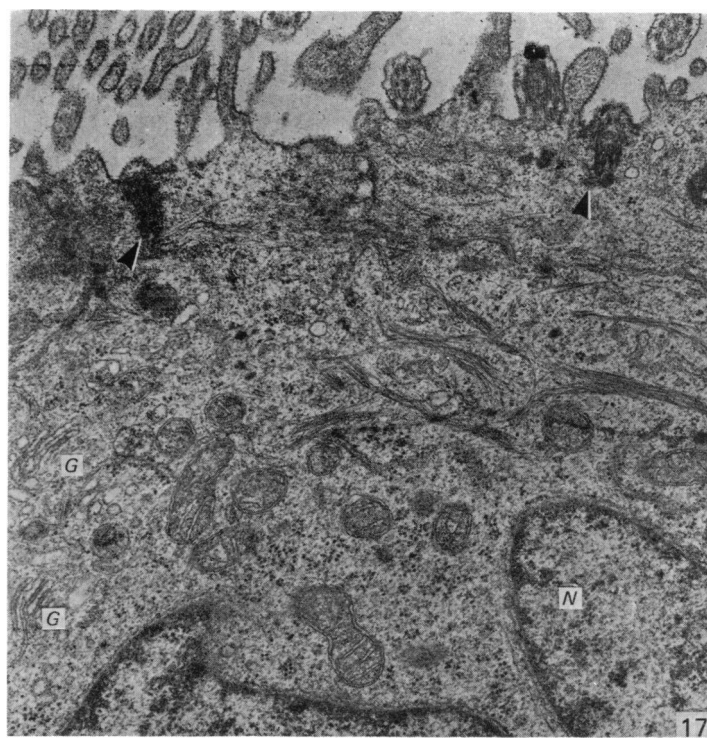

Fig. 17. Apical portion of ciliated cell showing bundles of microfibrils, Golgi complex (*G*) and irregular nucleus (*N*). Note also basal bodies of cilia (arrowheads) and some microvilli projecting from the apical surface. Japanese quail. ×24000.

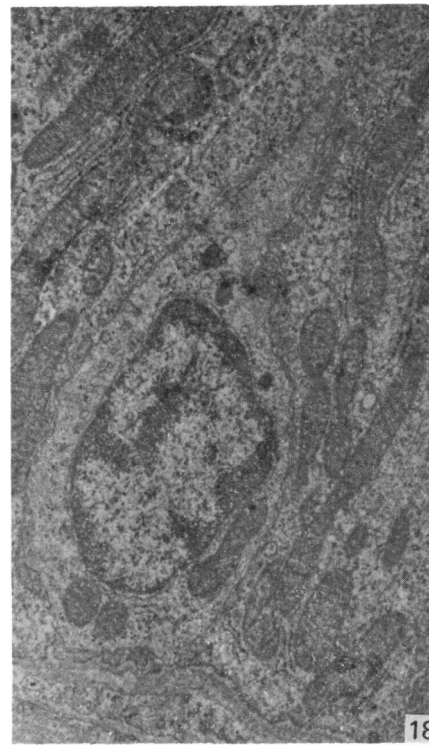

Fig. 18. An intraepithelial lymphocyte wedged between non-ciliated cells of the fowl. Note lighter cytoplasm, and heterochromatic nucleus of the lymphocyte. A few organelles are present. Junctional complexes between the lymphocyte and adjacent cells are not present. ×13440.

absorption property. But both the apical canaliculi and coated vesicles were better developed in the Type I cell. Indeed, coated vesicles were predominantly observed in the Type II cell. It is not known if this has any influence on fluid reabsorption in both cell types, but Hoffer (1972) noted that whereas apical canaliculi were present in the non-ciliated cells of the efferent ducts of the mouse, they were absent in the rat which, however, possessed coated vesicles. It is these canaliculi and coated vesicles which Tingari (1972) and Hess & Thurston (1977) described elaborately as 'worm-like', 'rod' and 'bar-bell' shaped structures located in the apical cytoplasm of the non-ciliated Type I cells. Various speculations concerning their structure and functions were made. The present author observed similar collapsed structures in poorly-fixed avian epididymal tissue, and Ericsson (1964) showed that poor fixation by immersion produced a similar picture in homologous cells of the rat kidney, which may be artefacts.

The presence of large numbers of dense globules or bodies as well as heterogeneous bodies in the non-ciliated Type I cell, and the relative absence of these in the Type II cell is interesting. These bodies seem to correspond to lysosomes and residual bodies. The Type I cell seems to fit the description by de Duve & Wattiaux (1966) of a cell suffering from 'chronic constipation'. It is apparent that the Type II cell functions less in absorption of fluid and broken-down germ cells than the Type I cell which shows an elaborate fluid conducting system, lysosomes and residual bodies. The larger lumen, as well as the folded epithelium of the proximal efferent

ductule, and the concentrated cellular plug of spermatozoa in the distal efferent ductule support the absorptive role of the proximal segment.

Secretory capabilities have been attributed to the efferent ductules in various species (Morita, 1966; Martan *et al.* 1967; Yokoyama & Chang, 1971; Hoffer, 1972; Tingari, 1972; Ramos, 1977). Limited secretory activity is suspected in the non-ciliated Type I and II cells because of their possession of moderately distended RER often surrounding or accompanying large mitochondria, and the presence of numerous bristle-coated vesicles in the region of the well developed Golgi apparatus. Secretion granules have not been observed in these cells, but Palade (1975) postulates that the concentration of secretory material in many secretory cells is omitted; thus secretion granules of usual appearance are absent. Tingari (1973) and Budras & Sauer (1975*b*) have shown that hormone synthesis occurs in the epididymal region of the sexually mature cockerel especially in the proximal efferent ductules. The presence of numerous, well formed microtubules may be involved in the transport of the secretory products within the cells. Microtubules have been implicated in intracellular transfer of secretory products (Gomez-Acebo & Hermida, 1973; Gemmell & Stacey, 1977).

Budras & Sauer (1975*a*) and Hess & Thurston (1977) consider the apical protrusion of the non-ciliated Type I cell as evidence of apocrine secretion in this cell. The present author observed the presence of these blebs in poorly fixed tissue and their absence in well fixed, perfused epididymal tissue. Similar blebs seen in mammalian epididymal tissue fixed by immersion have also been regarded as apocrine secretory activity (Morita, 1966; Yokoyama & Chang, 1971). But Nicander (1970) and Hamilton (1975) consider these as artefacts resulting from poor fixation. Ericsson (1964) made similar observations concerning poorly fixed homologous cells of the kidney.

Intraepithelial lymphocytes in the efferent ducts were occasionally seen only in the domestic fowl. They were not observed in sections of the efferent ductules of the Japanese quail and guinea-fowl. Whether or not they are always absent in the latter two birds is not known. The origin and possible role of these cells in the excurrent ducts of the testis of the domestic fowl have already been discussed (Aire & Malmqvist, 1979).

SUMMARY

Ultrastructural studies were undertaken on the efferent ductules of the testis of the domestic fowl (*Gallus domesticus*), Japanese quail (*Coturnix coturnix japonica*) and guinea-fowl (*Numida meleagris*). Four cell types were identified and described: ciliated cells which were found in the epithelium of both the proximal and distal segments of the efferent ductules, non-ciliated Type I cell which, together with the ciliated cell, formed the epithelium of the proximal efferent ductule and the non-ciliated Type II cell which, together with the ciliated cell, formed the epithelium of the distal efferent ductule. Intraepithelial lymphocytes were the fourth cell type found in the epithelium of both segments of the efferent ductule of the fowl only.

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