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A fine structural study of the turkey Harderian gland

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INTRODUCTION

Histological, histochemical and ultrastructural studies of the Harderian gland have been made in the domestic fowl (Wight, Burns, Rothwell & Mackenzie, 1971*a*; Wight Mackenzie, Rothwell & Burns, 1971*b*; Rothwell, Wight, Burns & Mackenzie, 1972; Burns & Mackenzie, 1973; Burns, 1974; Kittner, Oláh & Törö, 1978; Niedorf & Wolters, 1978) in the duck (Ballantyne & Fourman, 1967; Fourman & Ballantyne, 1967; Brobby, 1972; Kühnel & Beier, 1973; Wight & Mackenzie, 1974), in the rook (Burns, 1975) and in the rockhopper penguin (Burns, 1978).

More recently, histological studies (Burns & Maxwell, 1979) and ultrastructural studies (Maxwell & Burns, 1979) were performed on the ducts of the Harderian and lacrimal glands of the turkey, fowl and duck. The lymphoid cells in Harderian glands of a variety of avian species were studied histologically by Burns (1974, 1975) and by Aitken & Survashe (1977) and ultrastructurally by Schramm (1980). Zicca and colleagues (1982) described plasma cell degeneration. Some of the above studies have been reviewed briefly by Sakai (1981).

The only histological studies of the turkey Harderian gland are those by Burns (1974) and by Aitken & Survashe (1977). The present study investigates the general ultrastructure of the turkey Harderian gland.

MATERIALS AND METHODS

Turkeys (*Meleagris meleagris gallopavo*) from a domestic breed stock of varying ages from one day old to four weeks old and some adult birds were used in this study. The Harderian glands were dissected from the orbit following decapitation either as a preliminary to immersion fixation or following perfusion fixation of the head via the caudal artery. Some tissue blocks were fixed in 10% formol saline, and paraffin sections, 4 μm thick, were stained with haematoxylin and eosin or using the periodic acid–Schiff technique (McManus, 1946).

The fixation regime employed for electron microscopy was 2·5% glutaraldehyde in 0·075 M sodium cacodylate buffer (pH 7·4, 360 mosmole) for 2 hours at 4 °C. Pieces of Harderian gland were then washed overnight at 4 °C in 0·075 M sodium cacodylate and 0·2 M sucrose (pH 7·4, 340 mosmole). Postfixation was carried out either in 1% osmium tetroxide in 0·175 M sodium cacodylate (pH 7·4, 350 mosmole) or in 1% modified Dalton's osmium (pH 7·4, 330 mosmole) (Rothwell, 1974) or in 1% osmium tetroxide in 0·175 M sodium cacodylate containing 1·5% potassium ferrocyanide (pH 7·4, 350 mosmole) for 1 hour at 4 °C.

Following fixation, the blocks were dehydrated in ascending concentrations of

ethanol, passed through Inhibisol (Maxwell, 1978) and embedded in Araldite. Ultrathin sections were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined with an electron microscope.

Measurements of the recurrent patterns present in the paracrystalline structures of the granular endoplasmic reticulum were made, either directly from the negative image with a micrometer eyepiece, or with the aid of an optical diffractometer, using a helium–neon gas laser as a light source with a wavelength of 632·8 nm (Chasey, 1971).

Thick sections, of the order of 1·0 μm, were cut from blocks of Harderian glands and stained with 1 % toluidine blue in 1 % borax for 1–2 minutes at 60° C and examined by light microscopy.

RESULTS

The turkey Harderian gland was surrounded by a thin connective tissue capsule and septa from this capsule penetrated into the gland dividing it into lobules of varying size. Acini were seen at the periphery of the lobules and led by short tertiary tubules into secondary tubules, which in turn emptied into a main collecting duct (Fig. 1). The acini, tertiary, secondary and main collecting ducts were lined by a single layer of secretory columnar epithelium (Fig. 2). The epithelial cells tended to be taller in the acini and tertiary tubules but the lumina of the secondary tubules were larger.

The connective tissue capsule was composed of tightly packed layers of fibroblasts and collagen fibres (Fig. 3) and was continuous with the interlobular trabeculae which contained, in addition to fibroblasts and collagen fibres, blood vessels, nerve fibres, amorphous material and below the main and secondary duct epithelia, some myoepithelial cells and accumulations of plasma cells. The nerve fibres in the subepithelial connective tissue were found close to blood vessel endothelium and were generally surrounded by a Schwann cell and contained mitochondria, neurotubules, agranular and granular vesicles 50–80 nm in diameter (Fig. 4).

The subepithelial plasma cells had a large nucleus with condensed heterochromatin arranged in clumps adjacent to the nuclear membrane (Fig. 5). Their cytoplasm contained mitochondria with moderately dense matrices, ribosomes and Golgi elements. In some plasma cells, most of the cytoplasm was occupied by dilated profiles of granular endoplasmic reticulum containing moderately dense homogeneous material (Fig. 6). Plasma cells containing Russell bodies (Mott cells) were seen infrequently. The number of plasma cells increased with the age of the bird.

The secretory epithelium was uniform in appearance at all ages. The most characteristic appearance was of a bipolar columnar epithelial cell, often possessing two quite distinct accumulations of vesicles, one in the basal region and another towards the apical border of the cell (Fig. 7). Between these vesicles, there was a basally situated circular to ovoid nucleus with an even, coarse condensation of chromatin

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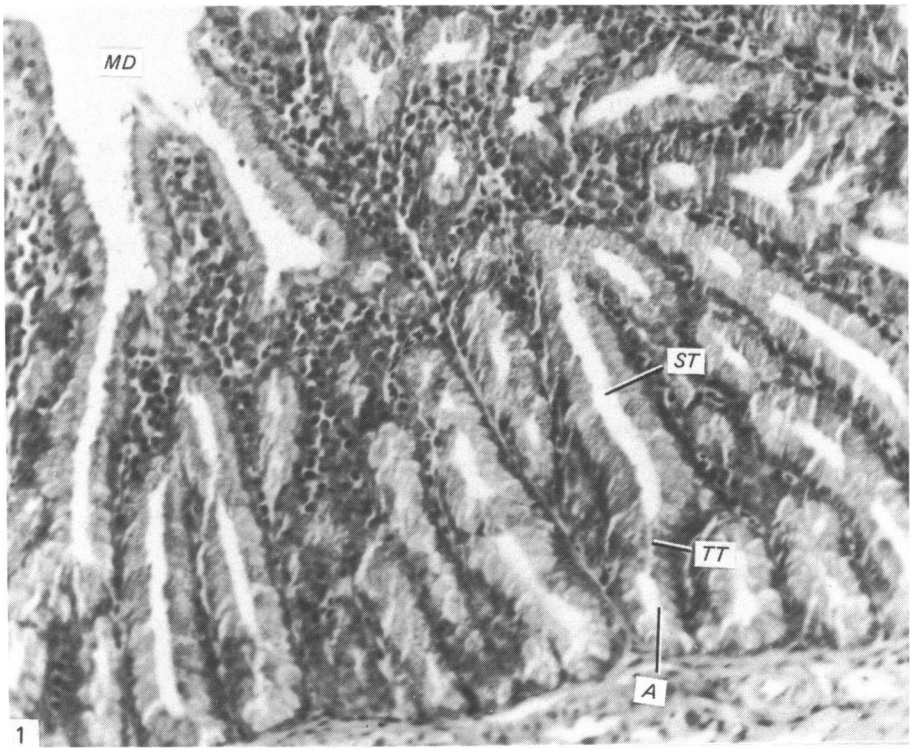
Fig. 1. Low magnification light micrograph of turkey Harderian gland lobule containing acini (*A*), tertiary tubules (*TT*), secondary tubules (*ST*) and main duct (*MD*). × 190.

Fig. 2. Low magnification electron micrograph of turkey Harderian gland acini lined by a single layer of secretory columnar epithelium. × 4250.

Fig. 3. Connective tissue capsule composed of tightly packed layers of fibroblasts (*F*) and collagen (*C*) fibres. × 8200.



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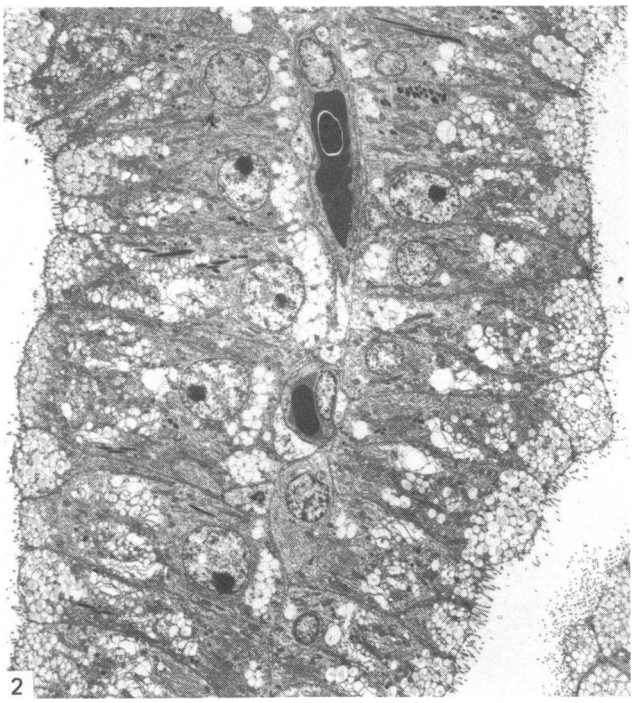

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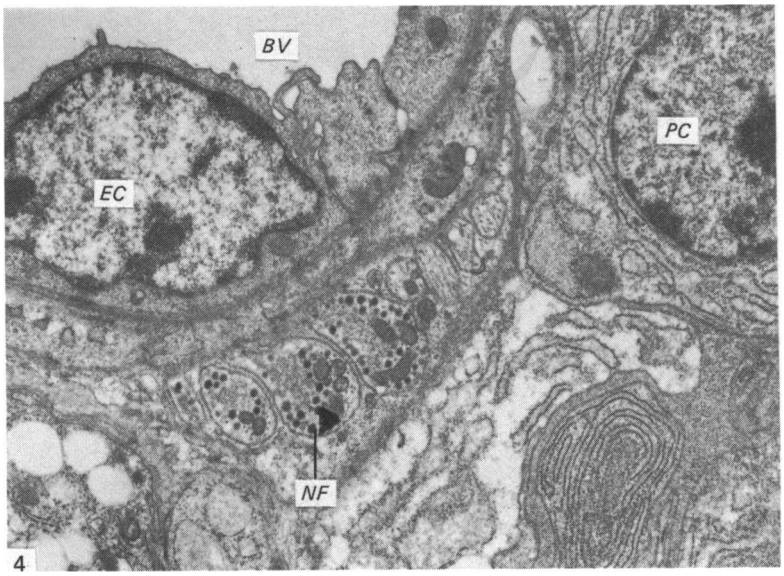
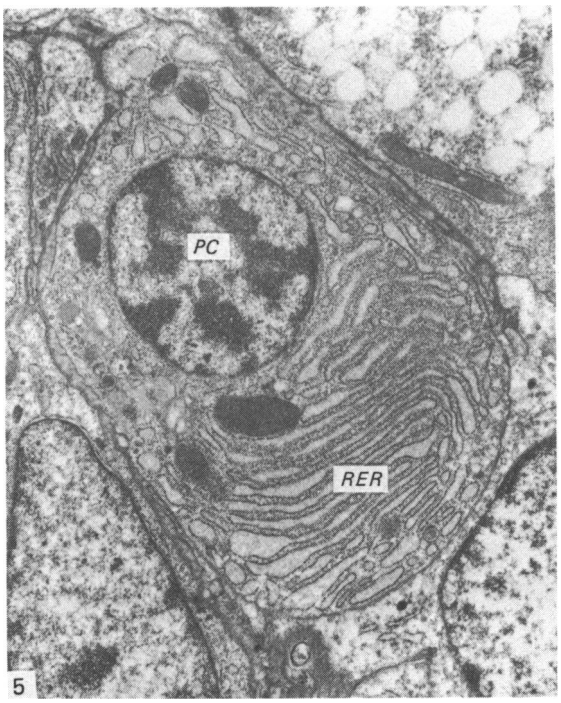




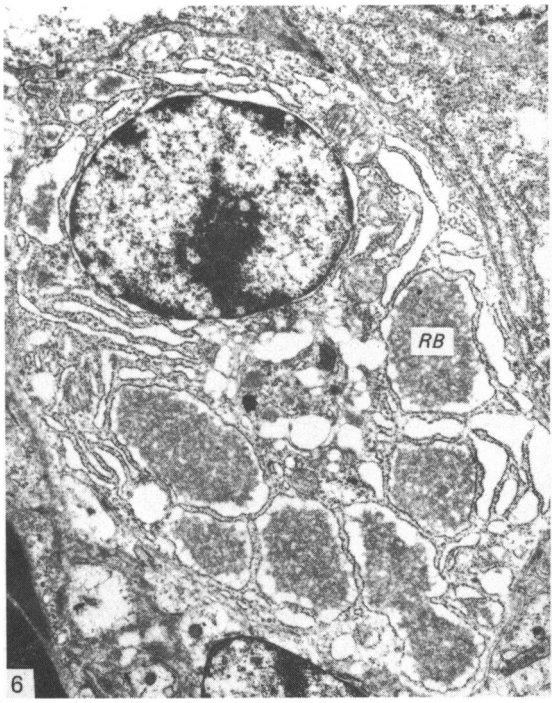
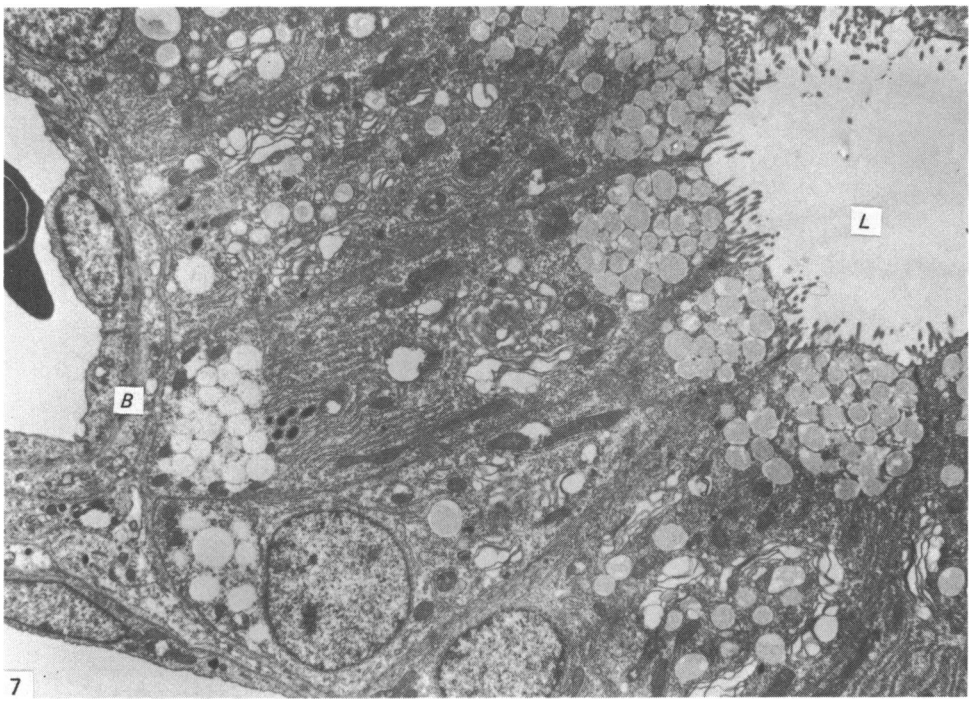


Fig. 4. Nerve fibres (*NF*) in subepithelial connective tissue adjacent to a blood vessel (*BV*) which is bordered by an endothelial cell (*EC*). Plasma cells (*PC*) are also present. × 11158.

Fig. 5. Subepithelial plasma cell (*PC*) with abundant granular endoplasmic reticulum (*RER*). × 13680.

Fig. 6. Plasma cell with dilated profiles of granular endoplasmic reticulum. Russell bodies (*RB*) are present in this cell. × 21280.



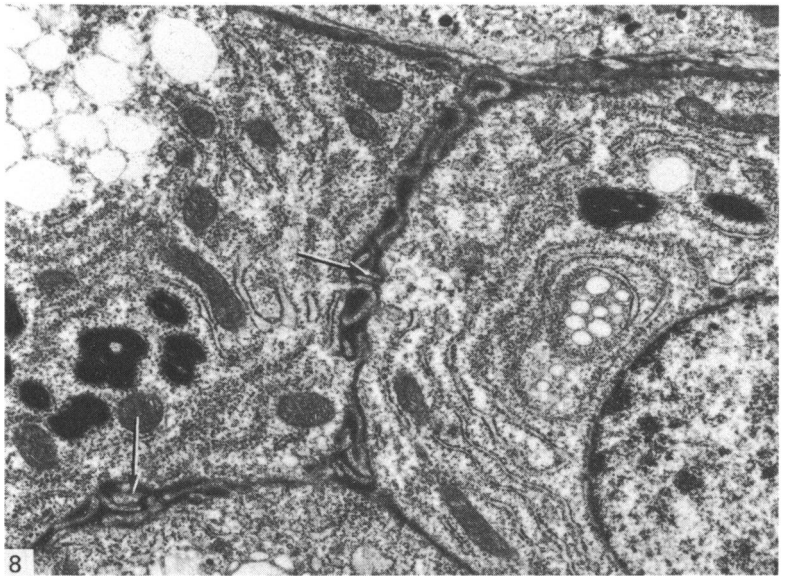
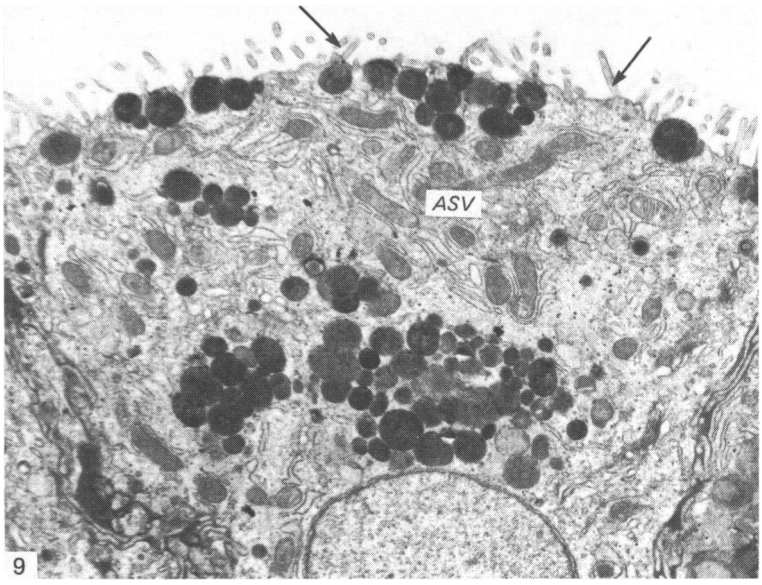


Fig. 7. Bipolar columnar epithelial cells with vesicles near the tubule lumen (*L*) and also at the base (*B*) of the cell. × 7750.

Fig. 8. Prominent complex membranous interdigitations can be seen between epithelial cells (arrows). × 11714.



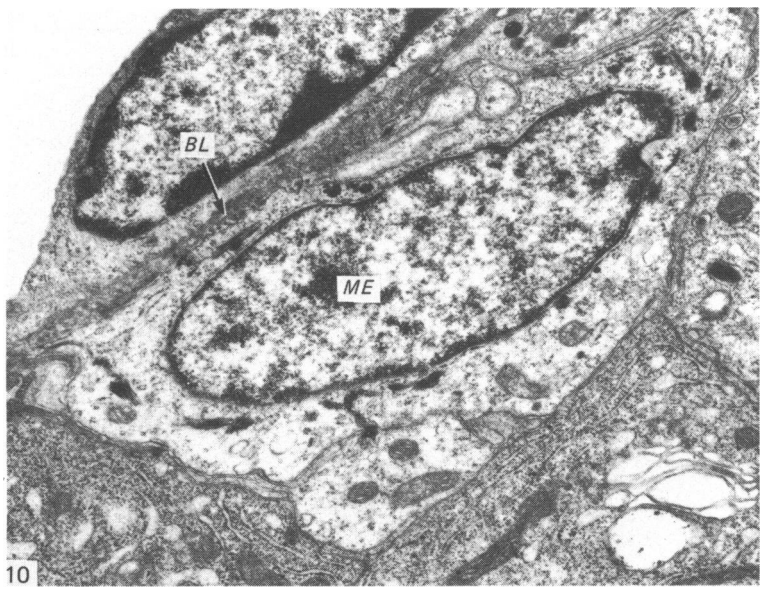


Fig. 9. Epithelial cells containing apical secretory vesicles (*ASV*) adjacent to microvillous projections (arrows). × 8748.

Fig. 10. Myoepithelial cell (*ME*) beneath columnar epithelium and adjacent to basal lamina (*BL*). × 12685.

and a prominent nucleolus, surrounded by lamellae of granular endoplasmic reticulum and, in the supranuclear region, large areas of expanded Golgi saccules.

The basal plasma membrane generally followed a smooth contour displaced only to accommodate features of the underlying trabeculae. The plasma membranes apically formed 'zonulae occludentes' and occasionally desmosomes were evident along their length. More characteristic was the development to a greater or lesser extent of complex membranous interdigitations between cells (Fig. 8). The apical plasma membrane showed a generally convex contour when apical secretory vesicles were present or it was thrown into a series of small microvillous projections. Coated vesicles were often seen in the process of formation at the base of the microvilli or complete in the apical cell cytoplasm (Fig. 9).

The basally situated vesicles were generally smooth in contour measuring up to 1·0 μm in diameter and contained homogeneous electron-lucent material. In some preparations, the material in these vesicles was lipid-like in appearance. The vesicles were always closely associated with elements of granular endoplasmic reticulum and mitochondria were often present in varying numbers within the cells. There was no evidence of secretory release of contents.

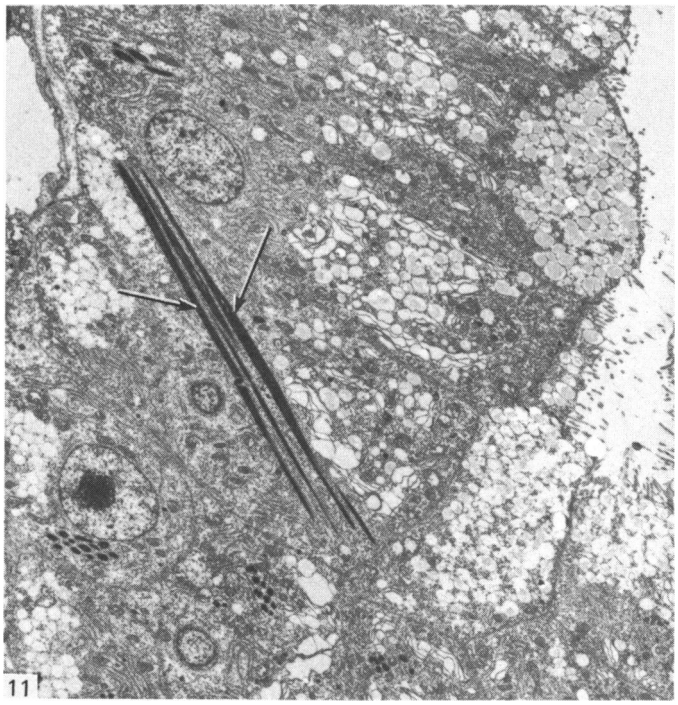
The granular endoplasmic reticulum was well developed in these cells and was found extensively in the basal third of the cell adjacent to the nucleus, arranged in lamellar arrays. The interlamellar spaces were frequently dilated and contained an homogeneous moderately dense material. As well as organised lamellae, isolated dilated profiles of granular endoplasmic reticulum were more generally distributed. Free ribosomes were also distributed throughout the cell.

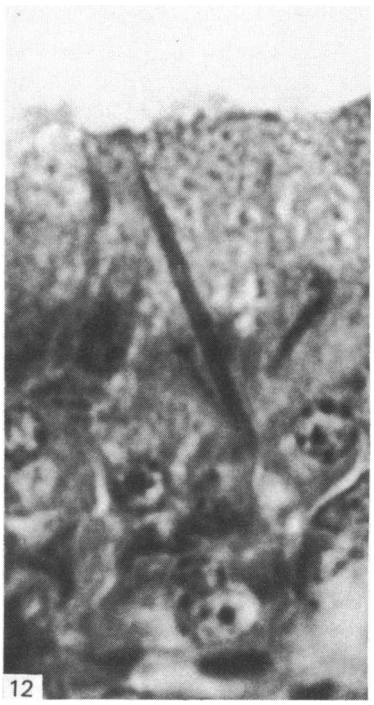
The Golgi apparatus was well developed in the supranuclear and apical regions of the cell. There were several accumulations of dilated, stacked cisternae together with an array of variably sized vesicles, giving this region of the cell a lacework appearance, possibly contributing to the difficulties of fixation of the material. However, this appearance of the Golgi apparatus was found with a variety of fixation regimens and was therefore not considered to be an artefact of fixation.

The apical secretory vesicles were closely packed, membrane-bound and of size varying from 300 to 900 nm in diameter; they contained a coarse, often fibrillar secretion product. Developmental stages of these vesicles were frequently seen emanating from the Golgi elements. As the cell became filled with these apical vesicles, there was a tendency for them to coalesce. This was particularly so prior to release of the vesicular content which was by fusion of the vesicle membrane with the apical plasma membrane and subsequent rupture.

Mitochondria were typically elongate with a dense matrix and transverse cristae. They were found scattered throughout the cell, often next to dilated profiles of granular endoplasmic reticulum or to basal vesicles. In favourable sections, myoepithelial cells were found beneath the columnar secretory epithelium and close to the basal lamina (Fig. 10). They had an ovoid nucleus occupying the larger part of the cell; in the juxtanuclear region, cytoplasmic organelles were sparse. These comprised circular to ovoid mitochondria, irregular tubules and vesicles of agranular endoplasmic reticulum, Golgi elements and short cisternae of granular endoplasmic reticulum. The characteristic myofilaments were found in small clusters throughout the cytoplasm.

Typically, the cytoplasm of the secretory cells of the main and secondary ducts contained dense osmiophilic rods or crystalline structures (Fig. 11), which reacted positively with the periodic acid–Schiff technique (Fig. 12). With the electron micro-





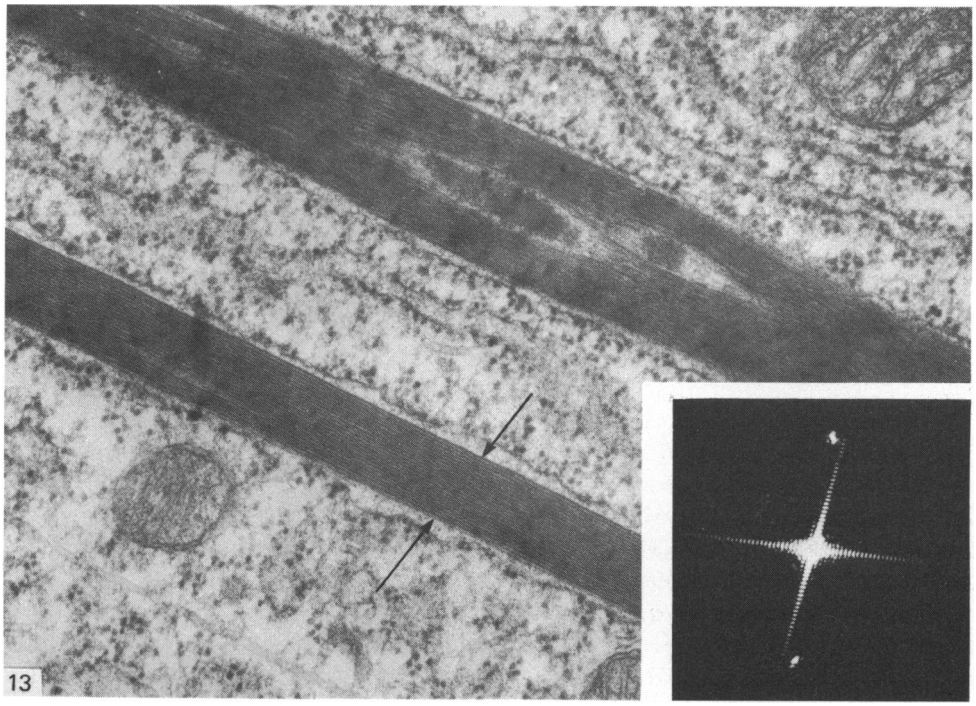


Fig. 11. Dense osmiophilic rods or crystalline structures can be seen in this epithelial cell (arrows). × 7440.

Fig. 12. Rod-like structures in epithelial cells. Periodic acid–Schiff technique. × 2000.

Fig. 13. The rods in this epithelial cell display a fibrillar or crystalline substructure (arrows). × 41000. Inset: The optical diffraction pattern represents a periodicity of 8·5 nm.

scope, they occurred in a variety of sectional planes sometimes extending the length of the cell (10 μm) and they were always found issuing from the basal regions of the cell juxta-apposed to the nucleus within the elements of the granular endoplasmic reticulum. In some instances the rods displayed a fibrillar or crystalline substructure (Fig. 13) with mean repeat pattern measurements of 7·0 nm.

Although no clear range of cell types was identified, there were many cytological variations from the images just described. These variations depended on the degree of development of the organelles and cytological features. In particular, the dense crystal-containing rods of the granular endoplasmic reticulum tended not to be present in the acinar epithelium. There was no evidence of porphyrin pigment in the turkey Harderian gland.

DISCUSSION

Burns (1974) described the histology of the turkey Harderian gland as a lobulated, compound tubulo-acinar Type 1 gland with a predominantly merocrine secretion similar in most respects to that of the fowl (Wight *et al.* 1971*a*). The present ultrastructural evidence supports this description. There was no indication of apocrine or holocrine secretions since debris was not recognised in tubule lumina.

The secretory epithelial cells in the Harderian gland of the turkey appear to be bipolar in nature, composed of apical secretions of mucus-containing vesicles and basal aggregations of non-secreting lipid-like droplets. This mucoid secretion is characteristic of birds, in contrast to mammals where the secretion is lipoidal, and compared with reptiles where the secretion is serous/seromucoidal (Paule & Hayes, 1958). The nine-banded armadillo, on the other hand, has a mixed, compound tubulo-acinar Harderian gland with mucus-secretory acini in the proximal part of the gland whilst the major part (distal) has lipid-secreting acini (Weaker, 1981). Porphyrin pigments have not been reported in the armadillo and their absence was noted by Wight *et al.* (1971*b*) in the fowl as in the present study. Concretions of porphyrins have been reported in the Harderian glands of some rodents (female hamsters, mice, rats) and possibly the gland has functions other than moistening and nourishing the cornea (Claybough & Norvell, 1974). Indeed, in addition to its known immunological participation (Burns, 1976), the gland in rodents may secrete porphyrin pigments in a thermo-regulatory role (Thiessen, Pendergrass & Harriman, 1982).

Extensions of the autonomic nervous system have a close relationship with exocrine gland cells (Kühnel, 1971, 1972) and this has been demonstrated in the Harderian gland of Anatidae (Kühnel & Beier, 1973) and in the present study. Fourman & Ballantyne (1967) considered that, in view of the abundance of acetylcholinesterase-positive innervation in the Harderian glands of ducks, nervous tissue influences the gland secretions. Kühnel & Beier (1973) upheld this view and the demonstration of similar innervation in the fowl (Burns & Mackenzie, 1973) and turkey in the present study lends further support.

A striking feature of the columnar epithelial cells is the occurrence of fibrillar or crystalline osmiophilic rods measuring in some cases 10·0 μm in length. Although these Schiff-positive structures are recorded by Burns & Maxwell (1979) and Maxwell & Burns (1979) in both Harderian and lacrimal gland ducts of turkeys, in those studies they rarely extended beyond 0·5 μm in length compared with the rod-shaped structures of the glands seen in the present study. Their periodicities, however, are similar (about 7·0 nm) and the crystals are considered to evolve from the fibrillary

structures (Maxwell & Burns, 1979). Crystalline structures have been reported in the lacrimal glands of dogs (Radnót, 1972), but they appear to be quite different to those of the present study, and repeat pattern measurements were not made. Crystalline inclusions within granular endoplasmic reticulum have been widely documented (Ghadially, 1982). Their formation is due to the volume of synthesised protein exceeding the volume which the Golgi complex can package or which the cell can utilise, consequently accumulation and crystallisation takes place.

SUMMARY

The ultrastructure of the turkey Harderian gland is described and the findings support previous histological descriptions of the gland.

The gland is a compound tubulo-acinar structure composed of characteristic bipolar epithelial cells providing a predominantly merocrine secretion to the lumina. In the basal aspect of the cell, aggregations of non-secretory lipid-like droplets were evident and apically, the secretion was mucoid. The cells had abundant mitochondria, granular endoplasmic reticulum, ribosomes and a complex network of Golgi elements. In the subepithelial regions, myoepithelial cells and large numbers of plasma cells were seen.

Within the granular endoplasmic reticulum cisternae of the epithelial cells, fibrillary or crystalline rods in some instances measuring up to 10 μm in length, with a 7·0 nm repeat pattern, were frequently seen.

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