J. Anat. (1979), **128**, 3, pp. 445–459
With 25 figures
Printed in Great Britain

The ultrastructure of the epithelium of the ducts of the Harderian and lacrimal glands of the turkey, fowl and duck

M. H. MAXWELL AND R. B. BURNS

Agricultural Research Council's Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS

(*Accepted* 9 *March* 1978)

INTRODUCTION

Although extensive studies have been made recently on avian Harderian and lacrimal glands with the light microscope (cf. Burns & Maxwell, 1979), few workers have carried out parallel studies with the electron microscope. Rothwell, Wight, Burns & MacKenzie (1972) studied the fine structure of the Harderian glands of the fowl, and those of the duck, goose, and swan were examined by Kühnel & Beier (1973).

The only ultrastructural study of an orbital gland duct system appears to be that by Alexander, Young & Lennep (1973) on the lacrimal gland duct of the rat. Similar investigations have not been made in birds. The light microscopic structure and histochemistry of the Harderian and lacrimal gland ducts from three species of domestic birds, namely turkey, fowl, and duck, have been reported (Burns & Maxwell, 1979). The present study concerns the fine structure of the duct epithelia of these birds.

MATERIALS AND METHODS

Harderian and lacrimal gland ducts were removed from decapitated fowls (*Gallus domesticus*), ducks (*Anas platyrhynchos*) and turkeys (*Meleagris gallopavo*) of both sexes ranging in age from 1 day to 6 weeks. The ducts were laid on cards and placed in the following fixatives:

Harderian gland duct; 1 % modified Dalton's osmium tetroxide (3 % potassium dichromate; 2·6 % sodium chloride) (pH 7·0 and 330 m-osmoles).

Lacrimal gland duct: 1 % osmium tetroxide in 0·1 M-Millonig's phosphate buffer (pH 7·4 and 340 m-osmoles).

In each case fixation was carried out at 4 °C for 1 hour. The ducts were then dehydrated and embedded in Araldite. Thin sections (30–50 nm) were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined in an electron microscope.

E.M. demonstration of glycogen. Gold-coloured sections were stained with periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-SP) for the specific demonstration of glycogen (Ackerman, 1973). Control ducts that had been placed in 2 % malt diastase (BDH) at 37 °C for 72 hours following fixation were similarly stained.

Light microscopy. 1·0 μm thick resin-removed sections (Maxwell, 1978) were stained with periodic acid–Schiff (PAS), Gomori's aldehyde fuchsin, Alcian blue at pH 1·0 and 2·5 and Hale's colloidal iron to demonstrate goblet cells, or with PAS and Best's carmine for glycogen. Control sections were incubated in 2 % malt diastase at 37 °C for 72 hours prior to staining for glycogen.

Methods of measuring crystal periodicities. The crystalline inclusions present in turkey Harderian and lacrimal gland ducts were measured either directly from the negative image with a micrometer eye-piece, or with an optical diffractometer, using as light source a helium-neon gas laser with a wavelength of 632·8 nm (Chasey, 1971).

RESULTS

Within each species there were no significant differences between the ducts of the Harderian and lacrimal glands. However, differences were observed between the species. Here the ducts are described together and species differences are noted later.

General features of Harderian and lacrimal gland ducts

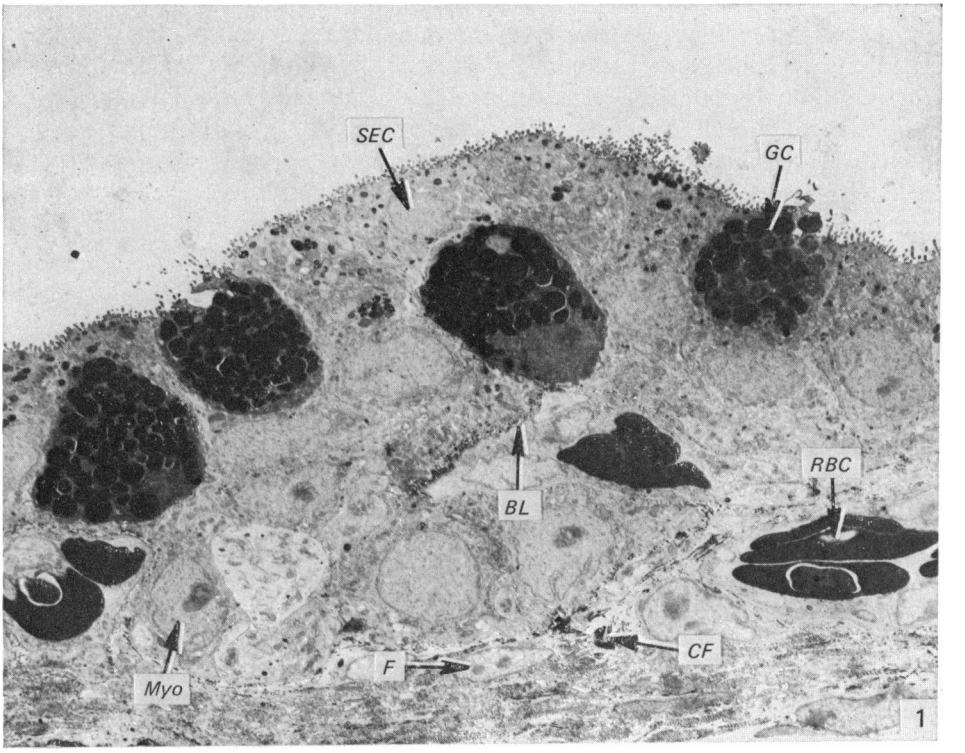
The epithelial cell layer was supported by bundles of collagen fibres interspersed with fibroblasts, elastic fibres and blood capillaries (Fig. 1). The basal lamina was often very irregular and showed many undulations. Between the basal lamina and the epithelial cells there were myo-epithelial cells (Fig. 2). The nuclei of the latter were usually oval and fairly smooth in outline and, although few mitochondria were found in the cytoplasm, many bundles of microfilaments, with single filaments measuring between 6 and 8 nm in diameter (Fig. 2, inset), were seen. Desmosomes linked epithelial and myo-epithelial cells. Tight junctions and other junctional complex elements were found frequently between the apical and lateral plasma membranes of adjacent epithelial cells. Cellular debris was present in the lumen of the ducts.

In all three species some of the epithelium lining the ducts was cuboidal (Fig. 3), some columnar (Fig. 4) and some pseudostratified columnar (Fig. 5). The junction between glandular and proximal duct epithelial cells was indistinct, since glandular epithelial cells classified by Rothwell *et al.* (1972) into stages I and II were frequently found in the proximal part of the ducts. In all cells there were numerous, small, round mitochondria which were frequently surrounded by a network of rough endoplasmic reticulum. Although in some instances the Golgi apparatus present in the epithelial cells was as extensive as some of those seen in glandular stage II cells, often their appearance was less vesicular with narrower cisternae. At the junction of gland and proximal duct the glandular epithelial cells contained many more secretory granules than their adjacent counterparts. Secretory granules in epithelial cells of the duct were entirely filled with secretory product (Fig. 6), whereas they were only partially filled in the gland. The location of the types of epithelial cells within the ducts did not appear to have any direct relationship to species or sex.

The luminal border of the duct epithelial cells bore short and widely spaced microvilli containing microfilaments orientated parallel to their long axis. In birds of different ages the distal regions of the ducts sometimes showed lightly stained degenerating cells and prominent intercellular spacing (Fig. 7), although the general fixation, as judged by the appearance of the mitochondria, was reasonably good. The microvilli on these degenerating cells were rounded and stubby, fewer in number

Fig. 1. Epithelial lining from a 3 days old turkey lacrimal duct showing epithelial cells (*SEC*) and goblet cells (*GC*). Beneath the basal lamina (*BL*), myo-epithelial cells (*Myo*), fibroblasts (*F*), red blood cells (*RBC*) and collagen fibres (*CF*) can be seen. ×2487.

Fig. 2. Myo-epithelial cells (Myo) supported on the basal lamina (*BL*) from 8 days old chick Harderian gland duct showing bundles of microfilaments (*MF*) ×7614. Inset. High magnification of microfilaments. ×38880.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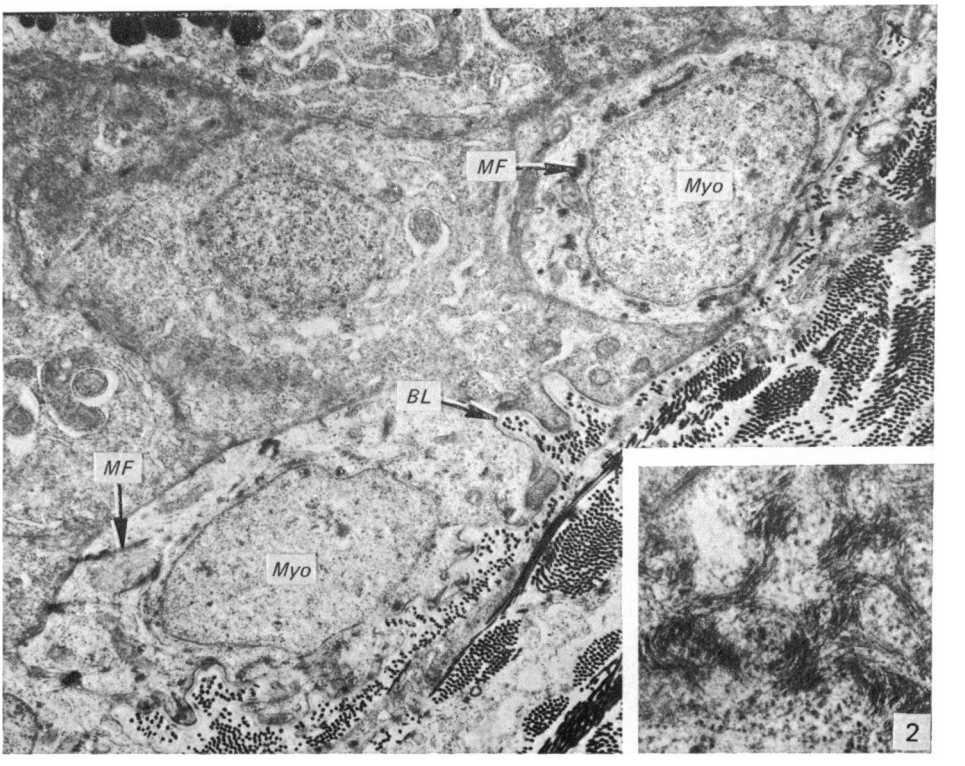
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than on adjacent healthy cells, and in some cases were completely absent. In the distal regions of the ducts, lateral projections and plasma membrane interdigitations between the epithelial cells were more obvious than in other regions. The projections and interdigitations always appeared more complex in the basal parts of the duct epithelium (Fig. 7, inset). Irregular convolutions of epithelium in the form of long outfolds or flaps were frequently seen in younger animals. In the Harderian gland duct of one 2 weeks old turkey, ciliated epithelial cells were seen between cells with microvilli.

Features peculiar to individual species

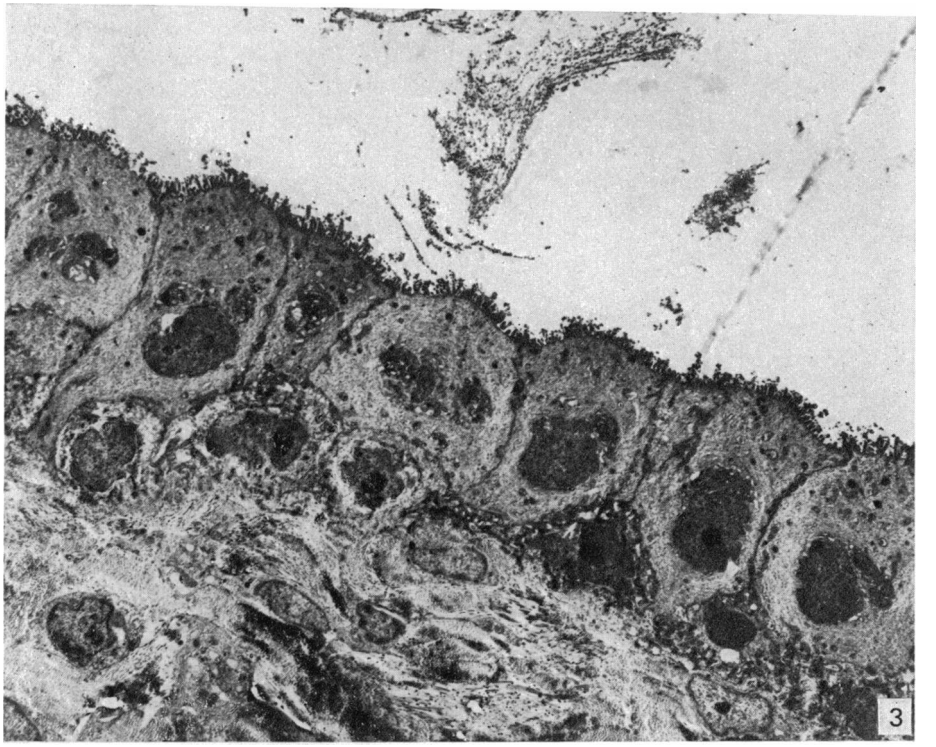
Turkey

The most characteristic feature of the Harderian and lacrimal gland ducts of the turkey was the presence of fibrillary rods (Fig. 8) and/or crystalline inclusions in some epithelial cells (Fig. 9). Although these cells were found at all levels of the epithelium, they were less common in the apical parts. They were difficult to recognize in the birds 1 to 2 days old because the inclusions were both uncommon and often small, measuring only 0·1 μm in diameter in some instances, in contrast to 0·5 μm in later stages. With advancing age, the cells containing these inclusions became more abundant in both ducts, but the inclusions found in the cells of the Harderian gland duct appeared to increase in size more rapidly. In both ducts there was a concomitant increase in the size and incidence of rods and crystals. They were usually distributed basally within the cells.

The rods and crystals were found within the greatly dilated cisternae of the rough endoplasmic reticulum (Fig. 10). At higher magnifications, each rod appeared to be composed of a bundle of microfilaments with individual microfilaments measuring 5 to 6 nm in diameter. The crystals, on the other hand, appeared either as lamellar structures with the lamellae arranged parallel to the long axis, or as a more complex lattice pattern (Fig. 11). Measurements of the repeat patterns of these crystals gave a mean of 7·0 nm. In the more complex crystalline patterns optical diffraction produced a transform of spots that appeared to be arranged in the form of a hexagon (Fig. 11, inset). The rod and crystalline inclusions were not identified in other glandular or visceral organs of the turkey.

In rod- and crystal-containing cells, secretory vesicles, varying in density and number, were found in the epithelial cells of both ducts after about the fourth week of age. The vesicles were membrane-bound and were found in the apical part of the cell just beneath the microvilli. Sometimes these vesicles had discharged their contents into the lumen. The secretory vesicles within the epithelial cells varied in shape and could be either rod-shaped, round, oval, or crescentic (Fig. 12). At higher magnifications these vesicles were generally homogeneous, although sometimes dense inclusions were discerned, but lamellar substructures were never seen. Occasional clusters of lipid-like droplets were found, usually in the opposite pole of the cell to that containing the secretory vesicles.

Many large goblet cells were found throughout both ducts of the turkey, and at all levels within stratified epithelium. Those with mucous granules of a low density were more common in the 1 to 2 days old birds, whereas denser forms were more frequent after the third day (Fig. 13). Some goblet cells contained both light and dark mucous granules. Fewer goblet cells were found near the exit from the gland than in the remainder of the proximal portion of the duct. Generally, the majority of the cells were found towards the distal ends of both ducts. The luminal surface of the goblet



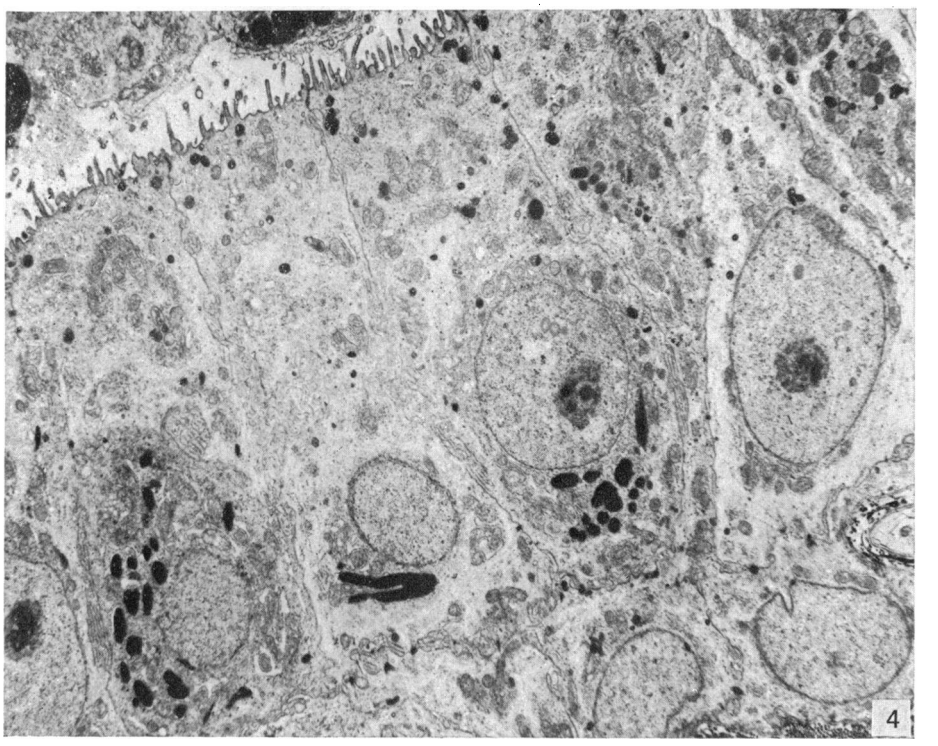
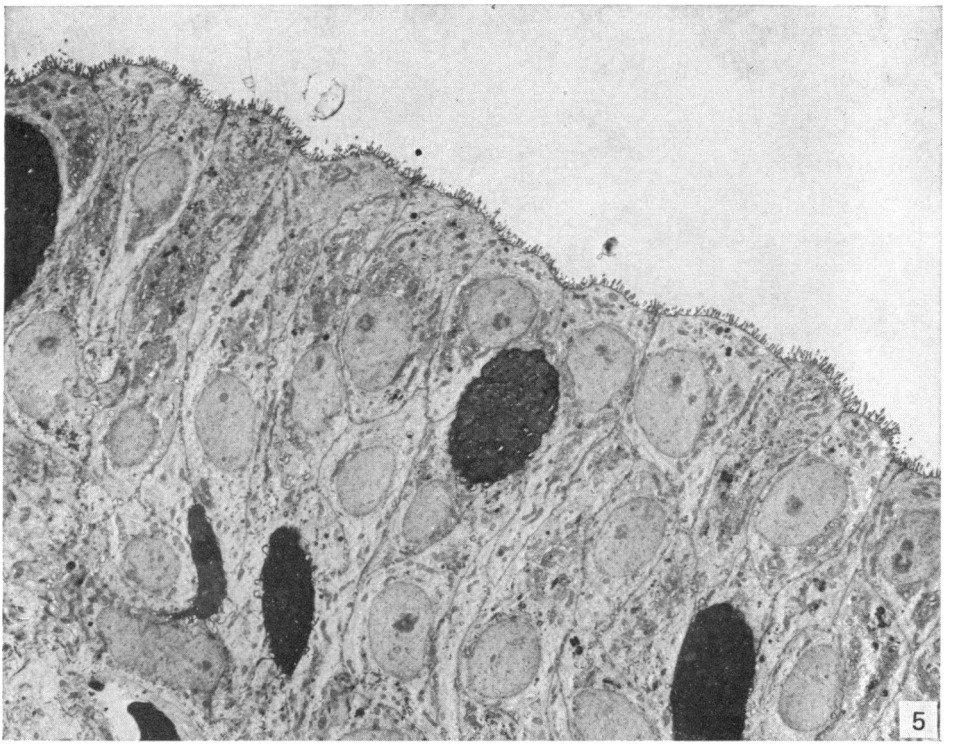


Fig. 3. Cuboidal epithelium from 2 weeks old duck Harderian gland duct. ×2992.

Fig. 4. Columnar epithelium from 4 weeks old turkey lacrimal gland duct. ×4374.



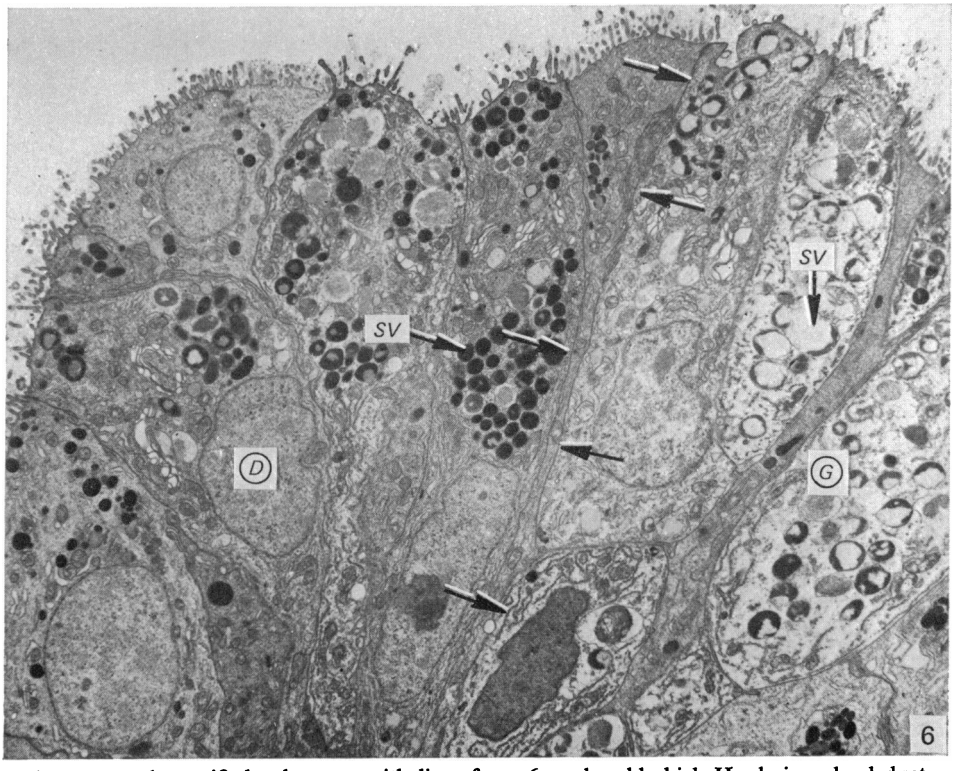


Fig. 5. Pseudostratified columnar epithelium from 6 weeks old chick Harderian gland duct. ×2394.

Fig. 6. Epithelium located at 3 days old turkey Harderian gland and duct junction (arrows). The secretory vesicles (*SV*) in the duct (*D*) appear filled compared to those in the gland (G). ×4374.

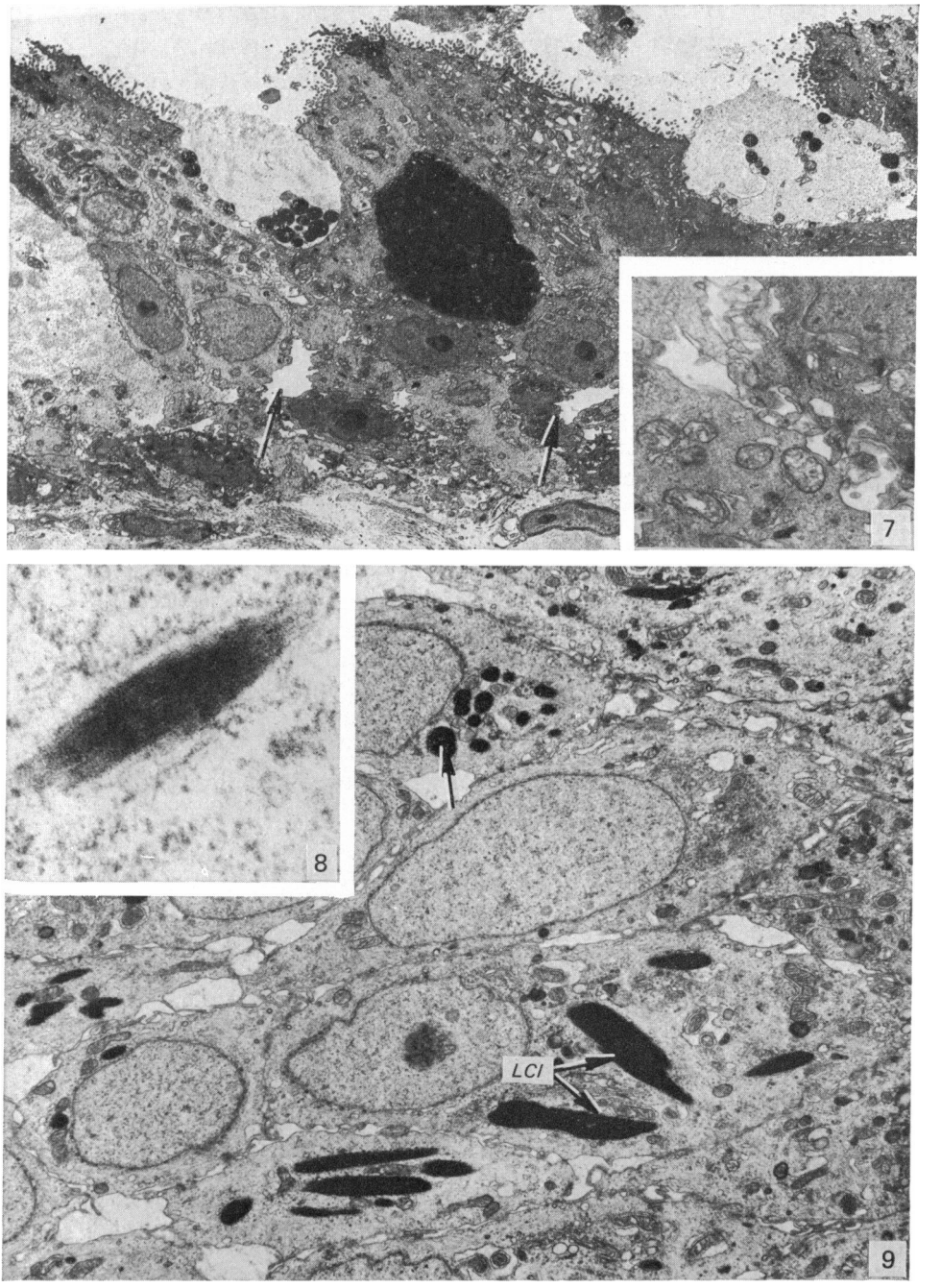


Fig. 7. Epithelium from the distal end of a 5 weeks old turkey Harderian gland duct showing intercellular spaces (arrows). × 2378. Inset: Cellular interdigitations. × 10150.

Fig. 8. An obliquely cut portion of a fibrillary rod seen in the epithelial cell from a 1 day old turkey lacrimal gland duct. × 64800.

Fig. 9. Columnar epithelial cells from a 6 weeks old turkey Harderian gland duct. Longitudinal crystalline inclusions (*LCI*) and those seen in transverse section (arrow) are indicated. × 6156.

cells usually contained a few microvilli. Often these cells were seen discharging their mucous granules into the lumen (Fig. 14), particularly at the distal end of the duct, and only the glycocalyx and a few mucous granules remained (Fig. 15).

Although glycogen particles were not seen within the cytoplasm of the epithelial cells they were frequently found attached, or adjacent to, the microvillous border. Some aggregates of particles were occasionally found in the intercellular spaces during intermediate duct growth, and these aggregates extended into the lumen.

Chick

The most prominent feature in both secretory ducts of young chicks was the presence of large quantities of α and β-glycogen particles throughout the entire cytoplasm of the surface epithelial cells. The presence of glycogen was shown by PAS (Fig. 16*a*, *b*) and Best's carmine, when applied to 1 to 3 μm thick sections. PA-TCH-SP treatment of ultrathin sections was used to confirm the presence of glycogen because this technique is specific for glycogen at the electron microscope level; tissue blocks pre-treated with diastase before dehydration and embedding were devoid of glycogen.

The accumulations of glycogen were at their maximum on the first day of hatching, although sometimes similar amounts could still be demonstrated in birds 2 days old (Fig. 17). The glycogen deposits diminished in quantity after the third day and by the seventh day it was rare to find much glycogen within the epithelial cells. There was less evidence of glycogen in the basal regions of the epithelium although particles were occasionally found in the myo-epithelial cells of young chicks. After the first week there was little evidence of single β-glycogen particles in the cell cytoplasm, although β and γ-glycogen dissociation was conspicuous on and between the surface microvilli (Fig. 18). There appeared to be more glycogen present in the lacrimal than in the Harderian gland duct.

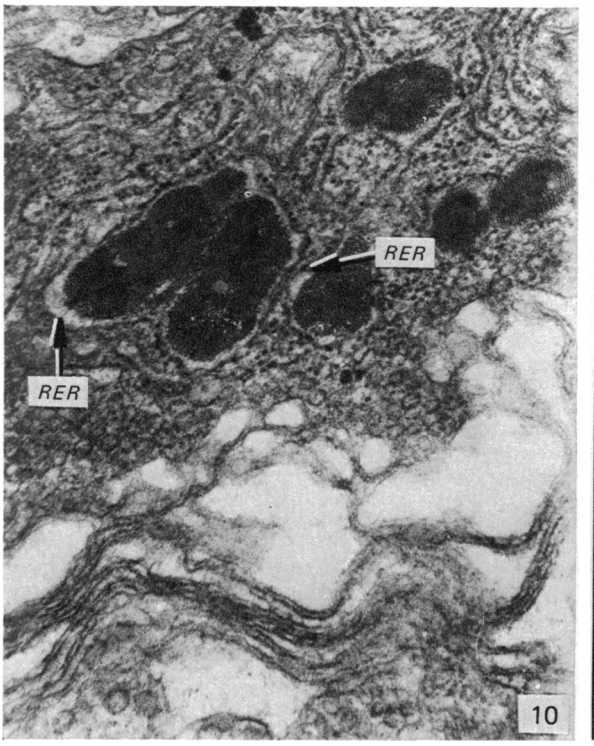
The number and distribution of secretory vesicles in chick Harderian and lacrimal gland ducts differed from that in the turkey. Generally there was more vesicular activity in the Harderian gland duct than the lacrimal duct, but considerably less than in these ducts in the turkey. In many cases the vesicles were found within various parts of the cytoplasm (Fig. 19) and not, as in the turkey, arranged apically. Fewer secretory vesicles were present in the ducts after the seventh day. Goblet cells appeared to be more common in the Harderian than lacrimal gland ducts. In the very young chicks they were less numerous and lighter in density (Fig. 20). Goblet cells were never as abundant as in the turkey. Frequently they contained both light and dark mucous granules, although darker goblet cells predominated after two weeks of age.

Fig. 10. Transverse sections of crystalline inclusions are seen within distended rough endoplasmic reticulum (RER) cisternae: 9 days old turkey lacrimal gland duct. × 48600.

Fig. 11. Transverse section of crystalline inclusion from 6 weeks old turkey lacrimal gland duct. The periodicity measurements of this hexagonal lattice structure are 8·3, 6·8, 8·3 nm. × 15540. Inset. Diffraction pattern showing hexagonal arrangement of spots representing the marked area of the crystal.

Fig. 12. Pleiomorphic forms of secretory vesicles (*SV*) in a 4 days old turkey Harderian gland duct epithelial cell. × 4104.

Fig. 13. A portion of epithelium from 9 days old turkey lacrimal gland duct showing light goblet cells (*LGC*) and dark goblet cells (*DGC*). × 1618.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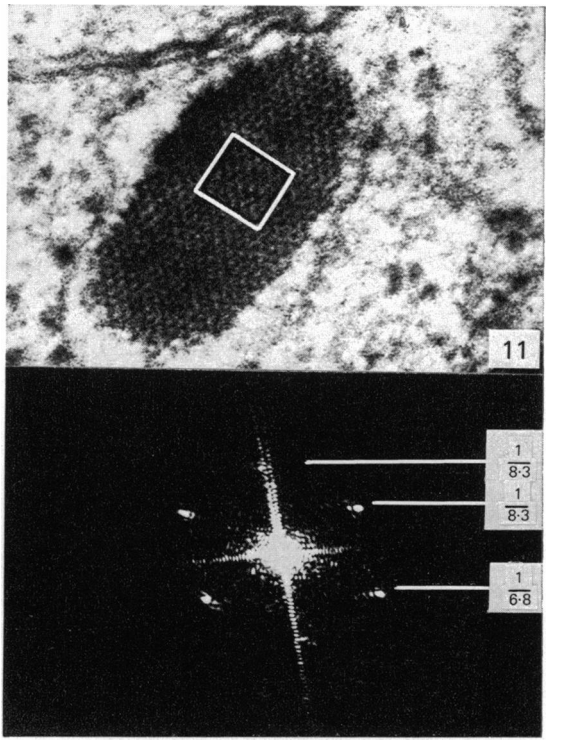
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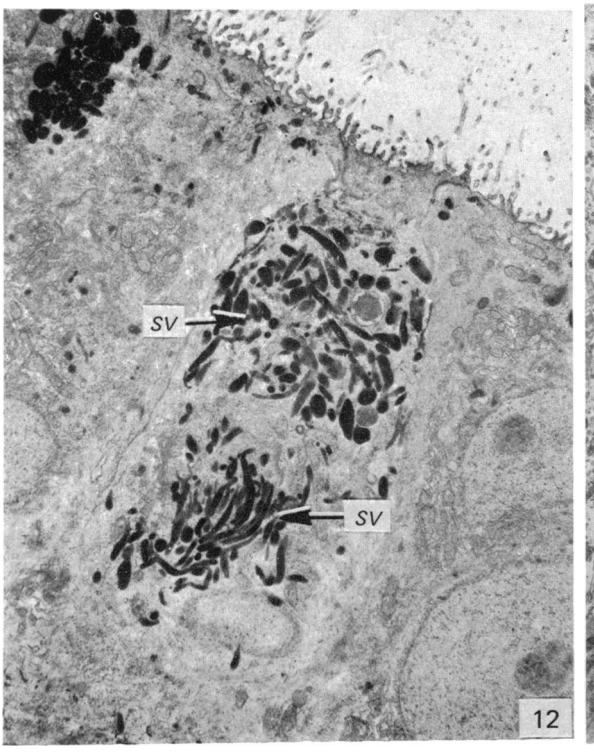
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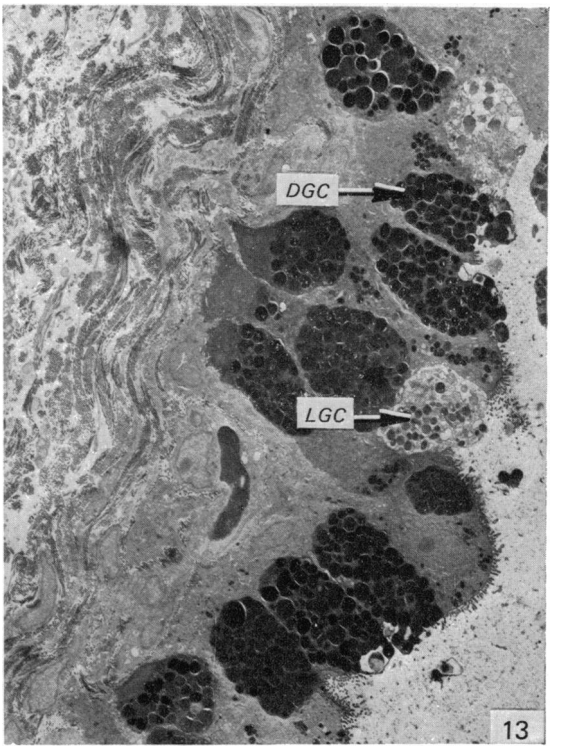
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Duck

In many ducks up to about 2 weeks old, the epithelium of the Harderian and lacrimal gland ducts was deeply invaginated. Ducts from all ages of birds contained many secretory vesicles in the apical regions of their surface epithelial cells (Fig. 21). Many of these vesicles had 'fingerprint-like whorls' which were seen discharging their contents into the lumen of the ducts (Fig. 22). In some instances this discharge appeared greater in the Harderian than lacrimal gland duct.

Goblet cells were present at all stages of Harderian and lacrimal gland duct development. Many of the goblet cells were pale (Fig. 23) and contained sparse mucous granules. Often the glycocalyx was densely stained. Other goblet cells contained mucous granules that were similar to myelin figures; a few cells were very dense. In the basal parts of some goblet cells densely stained and irregular lipid-like droplets were also found.

A cell peculiar to the duck, and present in both ducts, was found to contain numerous mitochondria (Fig. 24). These mitochondria-rich cells were fairly plentiful, normally had a high nucleus/cytoplasm ratio, were found at all levels of the epithelium, and in some cases were seen to abut onto the luminal surface (Fig. 25). In the early age groups the cells were often dense in appearance, but as the bird developed they became less so, making the mitochondria appear more prominent. When some of the cells were seen in the distal end of the ducts their nuclei appeared pyknotic.

Generally there was very little evidence of inter- or intracellular β-glycogen deposition and only very rarely were a few epithelial cells seen containing aggregates of α-glycogen. No particles were found in association with the microvillous border. A frequent observation was the presence of multivesicular bodies in duct epithelia of all age groups.

DISCUSSION

The results of the present study indicate that the epithelia lining the ducts of the Harderian and lacrimal glands of the turkey, fowl and duck are basically similar in morphology, and confirm the findings of light microscopy (Burns & Maxwell, 1979).

All three species of bird contained numerous secretory vesicles in their duct epithelial cells. The presence of these vesicles is in accord with similar organelles described by Alexander *et al.* (1973) in lacrimal ducts of rats. However, several of the vesicles were seen to discharge their contents into the lumen, particularly in the duck, but exocytosis was not recorded by Alexander *et al.* although they considered that the vesicles were involved in the secretory process. The appearance of the secretions of both ducts in the duck were quite unlike those found in the fowl or the turkey, as

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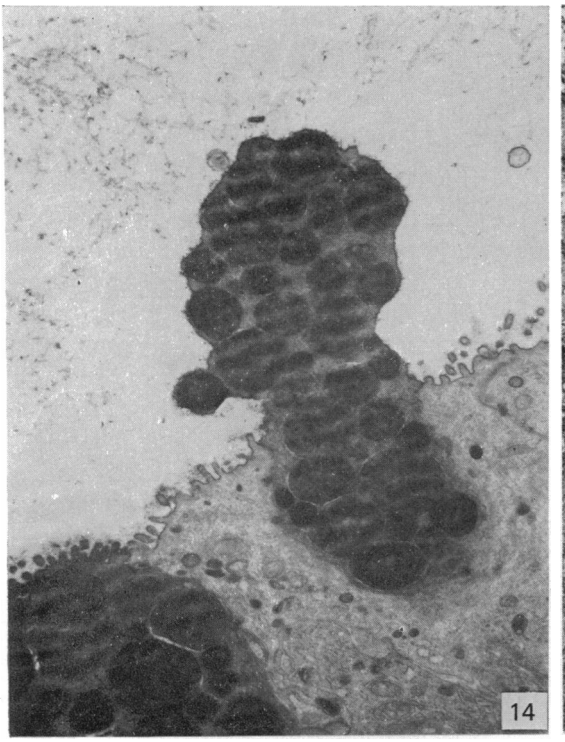
Fig. 14. A dark goblet cell seen extruding its mucus granules into the lumen of the duct: 3 days old turkey lacrimal gland duct. ×9396.

Fig. 15. An evacuating goblet cell with some mucus granules remaining; 5 weeks old turkey Harderian gland duct. ×4374.

Fig. 16. 1·0 μm thick resin-removed section demonstrating glycogen deposition in surface epithelial cells of 1 day old chick lacrimal gland duct. PAS technique without nuclear counterstaining. ×250. Inset ×780.

Fig. 17. Glycogen (*G*) deposits in epithelial cells of 1 day old chick lacrimal gland duct stained by the PA-TCH-SP technique. No lead or uranyl counterstaining. ×2916.



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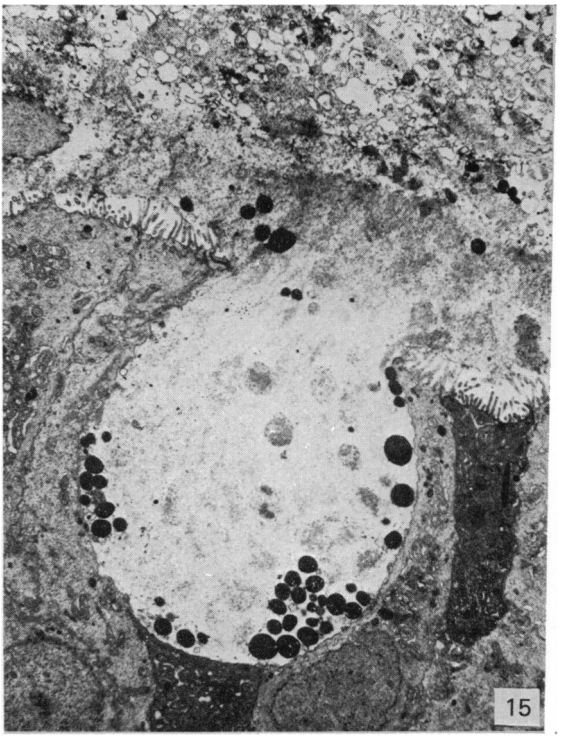

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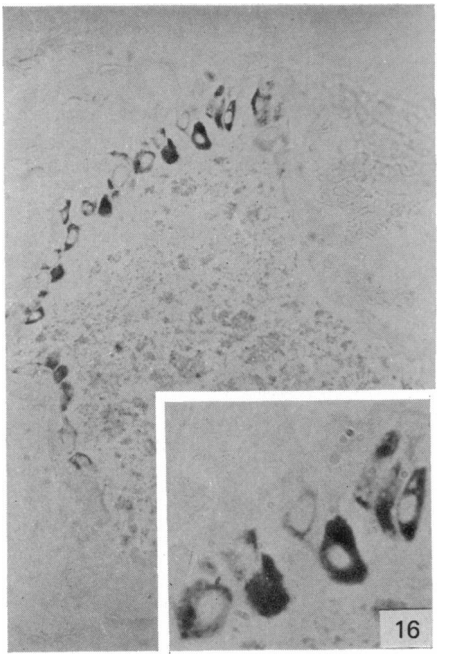

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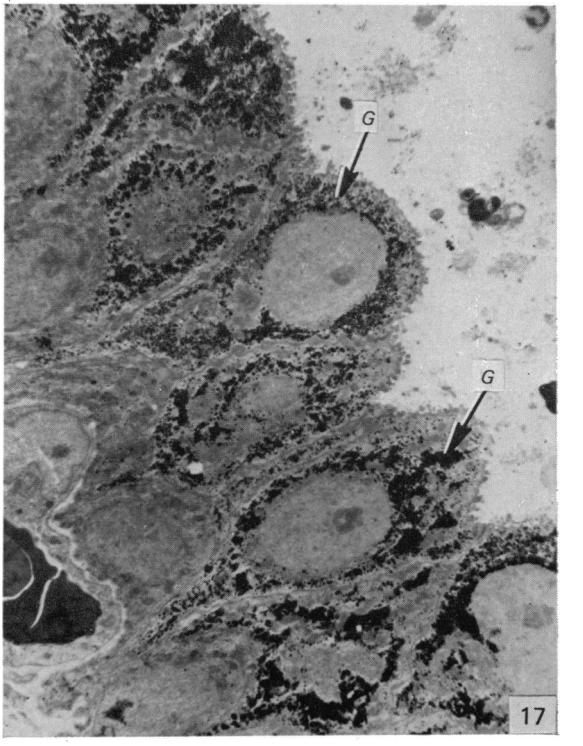

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they were of a lower electron density, and were composed of 'finger-print-like' patterns. This morphological difference might be connected in some way to the observations of Burns (1976) that the predominant type of secretion in the lacrimal gland of the duck was a sialomucin, as compared to the acidic sulphated muco-substance in both glands of the fowl (Wight, MacKenzie, Rothwell & Burns, 1971; Burns, 1976).

Goblet cells were found in similar numbers in both ducts in the duck and the turkey, but in the fowl there was a tendency for more to occur in the Harderian than in the lacrimal gland duct. With the light microscope (Burns & Maxwell, 1979) the goblet cells were seen to be less numerous in the middle parts of the ducts, although this reduction in number did not appear to be influenced by the type of epithelium present. From the number of goblet cells reported in this study, and the copious supply of mucus obtained from the ductal secretory vesicles and also from the gland (Rothwell *et al.* 1972) it is clear that birds produce much mucoid secretion. Since it is known that the Harderian gland is involved with immunity (Mueller, Salo & Glick, 1971), it may be speculated that this secretion, as well as being a lubricant, may have some immunological significance.

In turkeys, fibrillary rods and crystalline inclusions were found in some of the epithelial cells of both ducts. At the light microscope level the PAS streaks (Burns & Maxwell, 1979) frequently seen were considered to be analogous with the rods and crystals. These organelles were found within the cisternae of the rough endoplasmic reticulum, and were shorter than the crystals observed by Rothwell & Maxwell (1979) in the turkey Harderian gland. The presence of two types of structures in ductal cells could be explained by the fact that the protein molecules in the fibrillary material may orientate under certain physiological conditions to form a crystal, as described by Rothwell & Maxwell (1979). Further work is required to understand the composition and function of these crystals.

The large accumulations of α-glycogen particles which appeared at days 1 to 3 in the cytoplasm of the surface epithelial cells of both ducts of the fowl were not evident in the turkey or the duck. It is of interest that only the ducts should contain glycogen, and not the glands (Rothwell *et al.* 1972); and, furthermore, the question is raised whether the duck and the turkey utilize their glycogen resources earlier than the fowl, since less glycogen was found at comparable times in these species. Freeman (1965) assumed that in the fowl the mobilization of glycogen was required as an energy source during the stressful efforts of hatching.

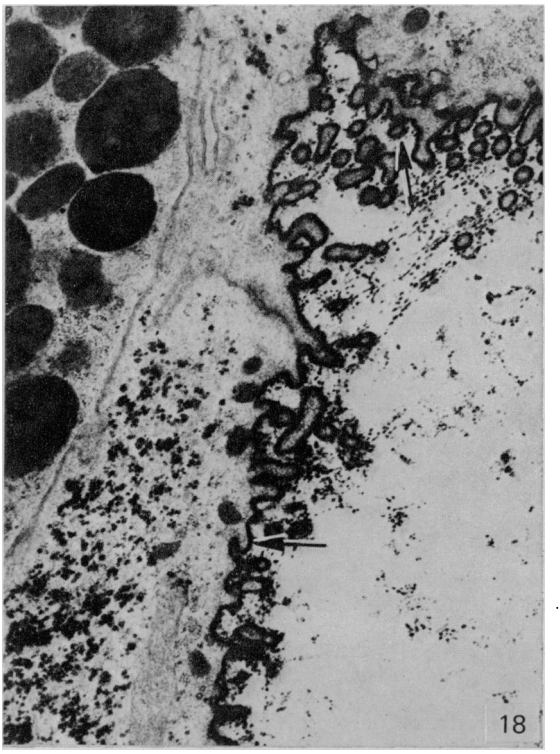
The significance of the mitochondria-rich epithelial cells found in both secretory ducts of the duck is unknown. Their life cycle was such that they appeared dense in young birds and were less dense as the bird matured, thereby revealing the mitochondria in greater detail. Similar cells were neither mentioned nor illustrated by Kühnel & Beier (1973) in the study by these authors of the Harderian glands of Anatidae. Thus it may be assumed that they are peculiar to the ducts. The 'clear'

Fig. 18. Glycogen particles are conspicuous on and between the surface microvilli as well as within the cytoplasm (arrows) stained with uranyl acetate and lead citrate; 1 day old chick lacrimal gland duct. × 21 600.

Fig. 19. A wide distribution of secretory vesicles (*SV*) is seen in this area of 2 days old chick Harderian gland duct. × 4374.

Fig. 20. Light goblet cells (*LGC*) are seen in this 1 week old chick Harderian gland duct. × 2736.

Fig. 21. Secretory vesicles (*SV*) are seen in an apical position in this 2 weeks old duck lacrimal gland duct. × 6264.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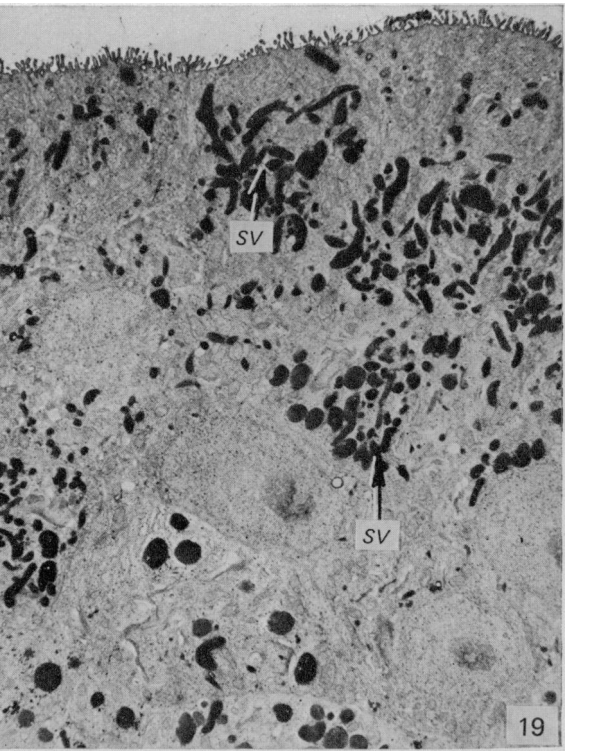
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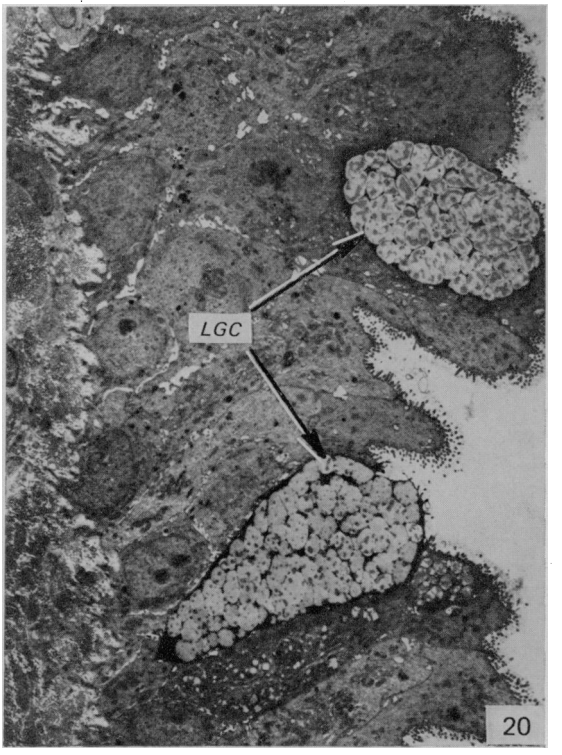
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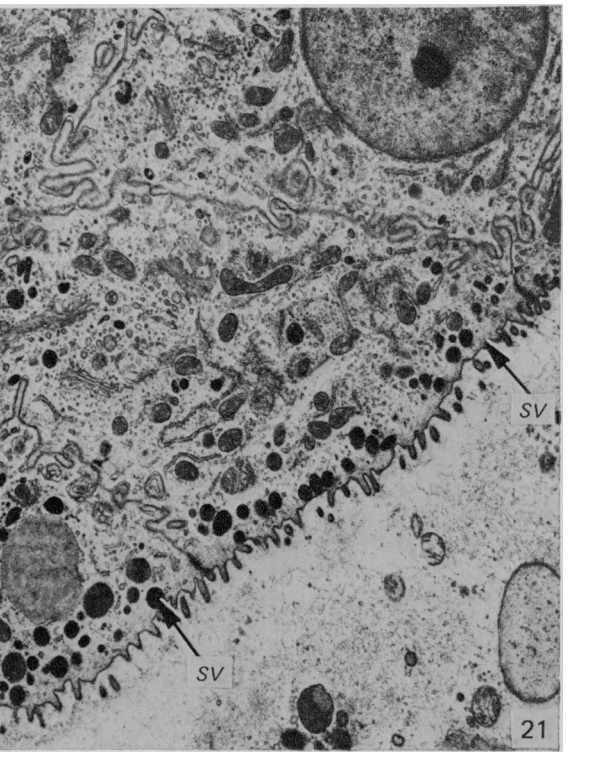
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cells described by Alexander *et al.* (1973) also contained numerous mitochondria, and may have a similar, albeit unknown, function to the mitochondria-rich cells described in this study. Although myoepithelial cells were found in both studies, the cell types II and IV reported by Alexander *et al.* (1973) were not encountered here.

SUMMARY

The ultrastructure of the epithelium in the Harderian and lacrimal gland ducts of the turkey, fowl and duck has been investigated. The findings establish that the ducts are fundamentally similar in morphology, although species differences occur.

The duct epithelia are composed of cuboidal, columnar or pseudostratified columnar cells with many secretory vesicles as well as goblet cells. PAS-positive fibrillary rods and crystalline inclusions occupied the cisternae of the rough endoplasmic reticulum of the epithelial cells of turkeys. The crystals, which were thought to evolve from the rods, had a mean periodicity of 7·0 nm. In chicks between 1 and 3 days of age, but not in poults and ducklings, the duct epithelia displayed large intracytoplasmic deposits of glycogen which disappeared by the seventh day.

REFERENCES

ACKERMAN, G. A. (1973). Ultrastructural localization of glycogen in erythrocytes and developing erythrocytic cells in normal human bone marrow. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **140**, 433–444.

ALEXANDER, J. H., YOUNG, J. A. & LENNEP, E. W. VAN (1973). The ultrastructure of the duct system in the rat extraorbital lacrimal gland. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **144**, 453–466.

BURNS, R. B. (1976). The structure of the lacrimal glands of the domestic fowl and of the duck. *Research in Veterinary Science* **21**, 292–299.

BURNS, R. B. & MAXWELL, M. H. (1979). The structure of the Harderian and lacrimal gland ducts of the turkey, fowl and duck. A light microscope study. *Journal of Anatomy* **128**, 285–292.

CHASEY, D. (1971). *Fine structure and macromolecular studies on cilia and flagella.* Ph.D. thesis, University of London.

FREEMAN, B. M. (1965). The importance of glycogen at the termination of the embryonic existence of *Gallus domesticus*. *Comparative Biochemistry and Physiology* **14**, 217–222.

KÜHNEL, W. & BEIER, H. M. (1973). Morphologie und Cytochemie der Harderschen Druse von Anatiden. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **141**, 255–283.

MAXWELL, M. H. (1978). Two rapid and simple methods used for the removal of resins from 1·0 μm thick epoxy-embedded sections. *Journal of Microscopy* **112**, 253–255.

MUELLER, A. P., SATO, K. & GLICK, B. (1971). The chicken lacrimal gland, gland of Harder, caecal tonsil and accessory spleen as sources of antibody producing cells. *Cell Immunology* **2**, 140–152.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology* **17**, 208–212.

ROTHWELL, B. & MAXWELL, M. H. (1979). Crystalline inclusions in two avian orbital glands. *Experientia* (In Press).

ROTHWELL, B., WIGHT, P. A. L., BURNS, R. B. & MACKENZIE, G. M. (1972). The Harderian glands of the domestic fowl. III. Ultrastructure. *Journal of Anatomy* **112**, 233–250.

WATSON, M. H. (1958). Staining of tissue sections for electron microscopy with heavy metals. *Journal of Biophysical and Biochemical Cytology* **4**, 475–478.

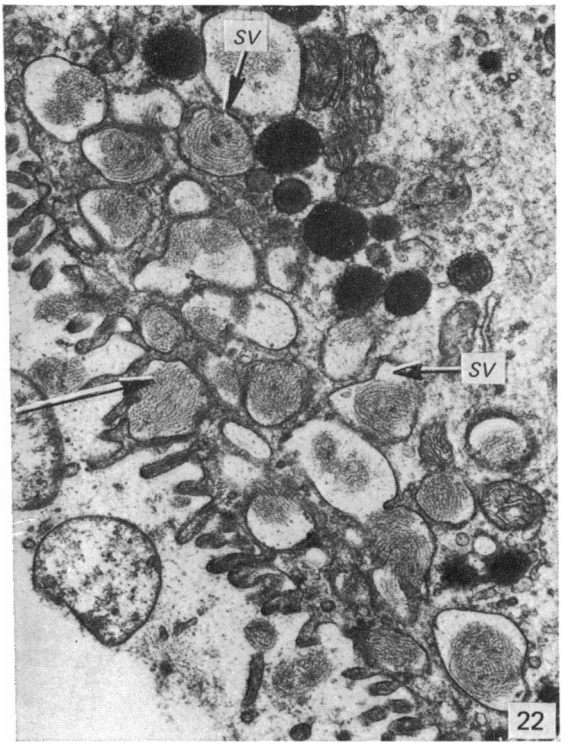
WIGHT, P. A. L., MACKENZIE, G. M., ROTHWELL, B. & BURNS, R. B. (1971). The Harderian glands of the domestic fowl. II. Histochemistry. *Journal of Anatomy* **110**, 323–333.

Fig. 22. Secretory vesicles (*SV*), some with 'finger-print-like' whorls are seen discharging their contents into the lumen of the duct (arrow); 6 weeks old duck Harderian gland duct. × 23000.

Fig. 23. Goblet cell containing mucus granules with little density; 6 weeks old duck Harderian gland duct. × 5832.

Fig. 24. Pale mitochondria-rich cells (arrows) are seen in the epithelium of this 6 weeks old duck Harderian gland duct. × 2394.

Fig. 25. A mitochondria-rich cell (arrow) seen abutting onto the luminal surface; 2 weeks old duck Harderian gland duct. × 5832.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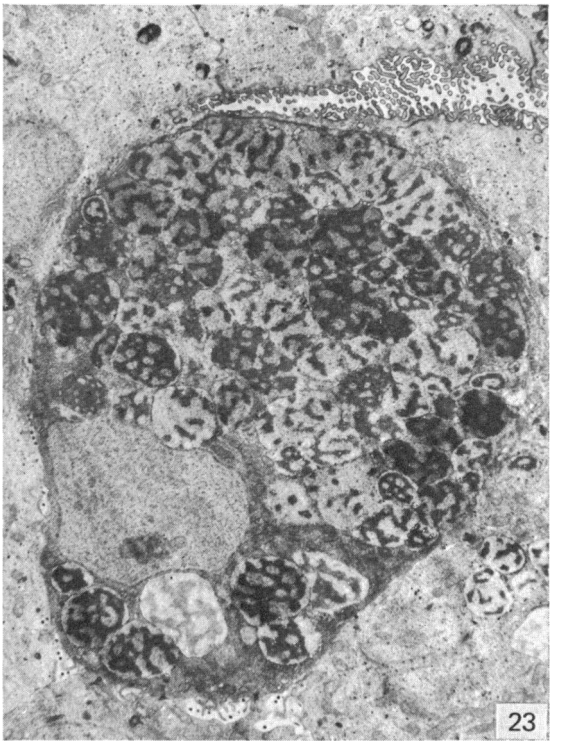
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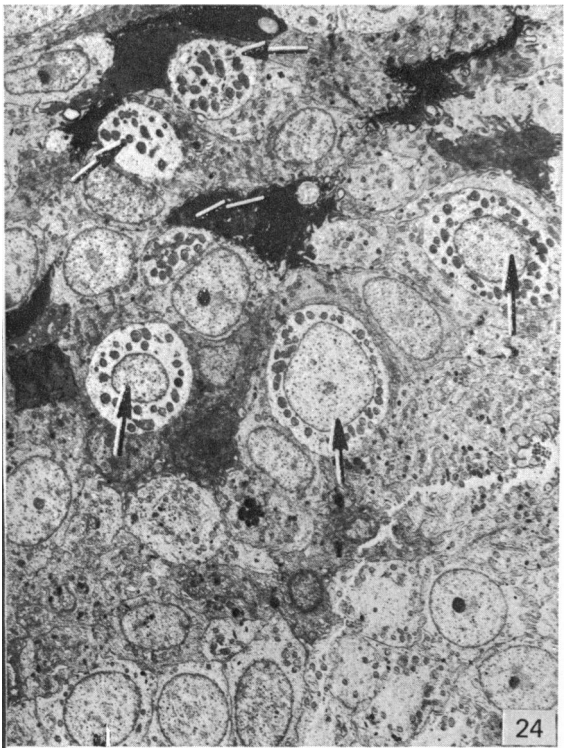
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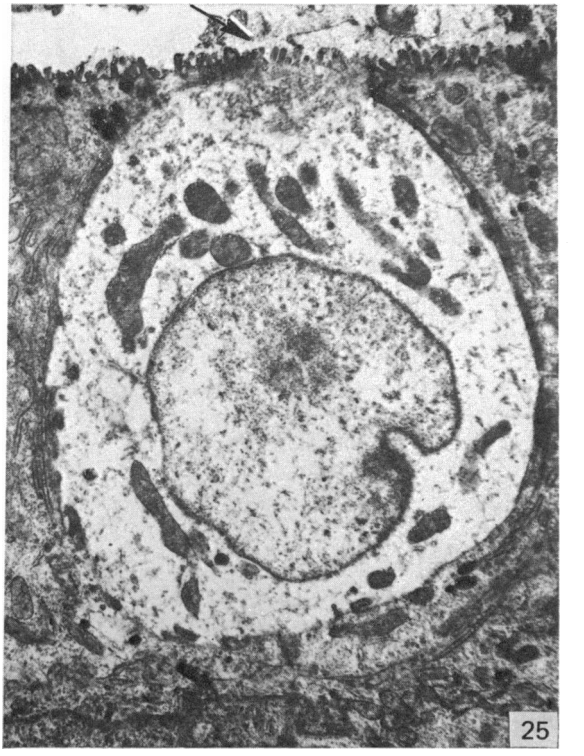
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