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The structure of the Harderian and lacrimal gland ducts of the turkey, fowl and duck. A light microscope study

R. B. BURNS AND M. H. MAXWELL

A.R.C. Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS

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INTRODUCTION

The histology of the Harderian gland has been described in the fowl (Bang & Bang, 1968; Wight, Burns, Rothwell & MacKenzie, 1971) and in the duck (MacLeod, 1880; Brobby, 1972; Kühnel & Beier, 1973), and Burns (1976*a*) has reported on the histology of the lacrimal gland in these two species. Bang & Bang (1968) found large infiltrations of lymphoid cells along the length of the Harderian gland duct in the fowl and pheasant, but no duct tissue was included in the other studies. This is also true of work on immunological aspects of the fowl Harderian gland (Mueller, Sato & Glick, 1971; Sundick, Albini & Wick, 1973; Albini, Wick, Rose & Orlans, 1974; Burns, 1976*b*) where attention was confined to the gland proper, though Burns (1977) has shown that the Harderian gland duct in the fowl might be a possible route of antigen uptake.

The present study investigates the histology and histochemistry of the secretory ducts of the Harderian and lacrimal glands in the domestic fowl, turkey and duck with particular reference to their epithelium and sub-epithelial lymphocyte aggregations.

MATERIALS AND METHODS

Harderian and lacrimal glands and their associated ducts were dissected from turkeys (*Meleagris gallopavo* L.), domestic fowl (*Gallus domesticus* L.) and ducks (*Anas platyrhynchos* L.) at 1, 2, 3, 4, 5, 7, 8, 14, 21, 28, 35 and 42 days after hatching and from adult birds. The glands with their ducts were laid on a piece of card to keep them flat during formol-saline fixation. The tissues were wax-embedded and sectioned at 5 μm. Sections of duct were taken from the proximal, middle and distal regions except for the ducts of the 5 weeks old birds which were serially sectioned, every fifth section being stained with haematoxylin and eosin (H & E) and the intervening sections retained for histochemical study.

Sections were routinely stained with H & E, periodic acid-Schiff (PAS) and Lillie's allochrome (Culling, 1966). Mucopolysaccharides were studied using Hale's dialysed iron (HDI), Steedman's Alcian blue, Alcian blue at pH 1·0 and pH 2·5 (all according to Pearse, 1968), Alcian blue/PAS (AB/PAS), aldehyde fuchsin (AF) and AB/AF (according to Culling, 1966). The presence of glycogen was investigated using Best's carmine, Bauer–Feulgen and PAS with diastase controls (Culling, 1966). Some Carnoy-fixed sections were stained with Kurnick's (1955) methyl green–pyronin-Y technique in order to study plasma cells.

RESULTS

Gross morphology

In the fowl, turkey, and duck a single duct left the anterior tip of the Harderian gland to open into the conjunctival sac at the base of the nictitating membrane. In the fowl the course of this duct could sometimes be followed by a row of pigmented spots on either side, and its single opening located by a circle of spots. In all three species a single duct left the posterior extremity of the lacrimal gland to open onto the deep surface of the lower eyelid.

Histology and histochemistry

The lacrimal and Harderian gland ducts of all three species were surrounded by loose connective tissue and embedded in adipose tissue (Fig. 1). There appeared to be more connective tissue around the ducts of the turkey and duck. In none of the birds was the duct invested by a distinct connective tissue capsule. Blood vessels and nerve fibres penetrated the surrounding tissue and ran into the duct tissues, but their orientation and course did not seem to be the same as that of the duct. Aggregations of lymphocytes were seen near the ducts in all three species. Villiform projections protruded into the lumen of the ducts where the epithelium was folded (Fig. 1). They were most prominent and numerous in the ducts of the fowl, and least evident in the duck.

Epithelium

In all three species the ducts of the Harderian and lacrimal glands were generally lined by a single layer of epithelium, the cells of which varied from cuboidal (Fig. 1) to columnar, though stratified and pseudostratified regions were also seen (cf. Maxwell & Burns, 1979). The cells were subtended by a basement membrane, and flattened cells with a large round nucleus could often be found at the base of the cells.

The nucleus of the epithelial cells was round to ovoid and was either centrally or basally located. A prominent basophilic nucleolus was often present. The cytoplasm was usually clear and sometimes vacuolated. Small basophilic granules were often present towards the free border of the cells. In the turkey, duck, and older fowls the cells of the duct epithelium reacted weakly with PAS. The PAS staining was much more pronounced in chicks less than one week old (Fig. 6). Epithelial cells stained weakly with HDI, Lillie's allochrome and AB at pH 1·0, but not at all with AF or AB at pH 2·5. In the AB/PAS sequence they showed a weak PAS-reaction and were weakly alcianophilic with AB/AF.

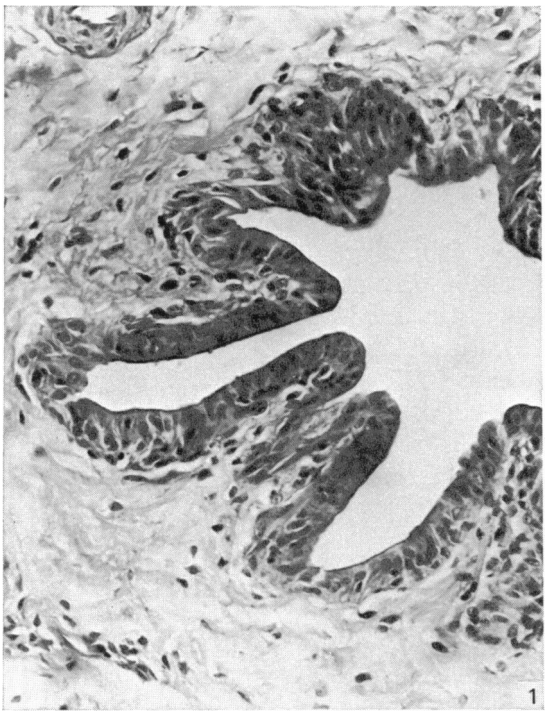
A thin basophilic line often delineated the free edge of the cells (Fig. 1). It was strongly PAS-positive (Fig. 2), and in all ducts it gave a blue colour with HDI and AB at pH 1·0 and pH 2·5, stained purple with AF, was blue in the AB/AF sequence, and alcianophilic with AB/PAS.

Fig. 1. Duck Harderian gland duct. Cuboidal cells are present in the epithelium of the duct. A thin basophilic line delineates the luminal edge. Mid-portion. Adult. H & E. ×400.

Fig. 2. Duck lacrimal gland duct. PAS-positive line at apical edge of epithelium (arrows) and PAS-positive goblet cells. Proximal portion. 4 weeks old. PAS. ×400.

Fig. 3. Fowl Harderian gland duct. A well-defined germinal centre adjoining diffuse lymphoid tissue. Proximal portion. 10 weeks old. H & E. ×400.

Fig. 4. Duck Harderian gland duct. Diffuse lymphoid tissue separated from the lumen by a layer of low epithelial cells. Proximal portion. 4 weeks old. H & E. ×640.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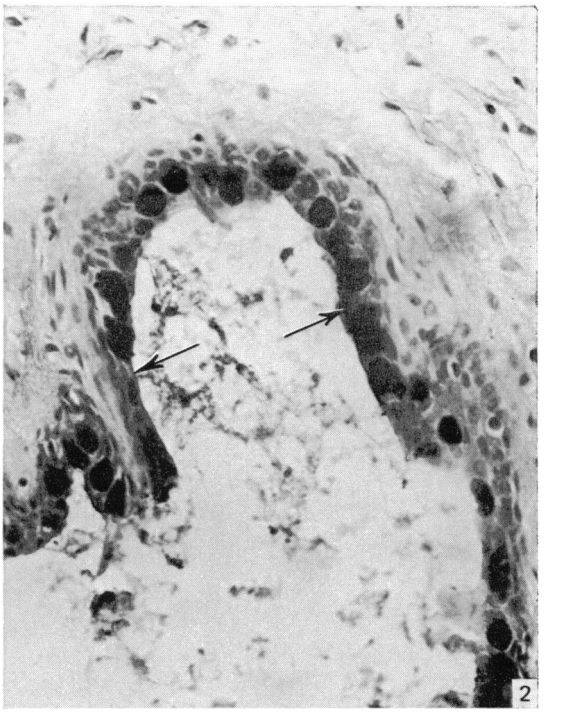
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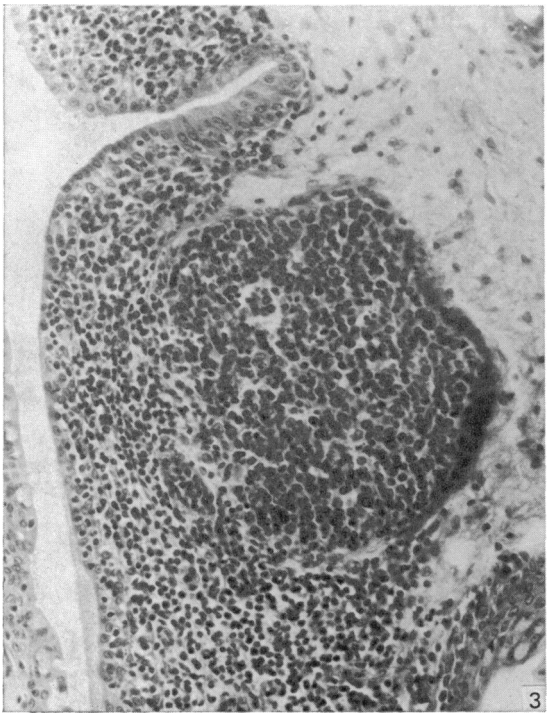
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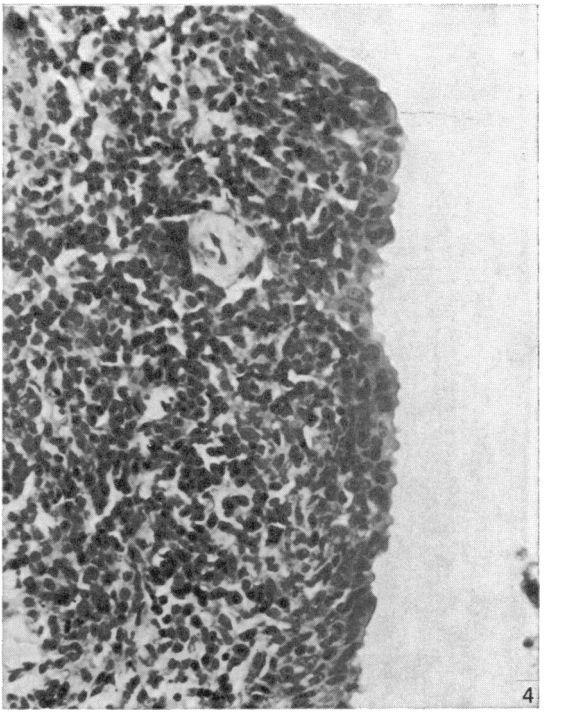
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Goblet cells

Interspersed among the epithelial cells of all ducts there were varying numbers of goblet cells (Fig. 2). Counts made on serial sections of ducts of 5 weeks old birds showed that the mid-portions had fewer goblet cells than either the distal or proximal regions. The AB/PAS sequence distinguished three types of goblet cell on the basis of their colour reactions. They were red, purple and blue respectively, and are designated here as types A, B and C.

Type A goblet cells (stained red following AB/PAS) were the most numerous. Their colour was more intense with AB at pH 1·0 than at pH 2·5; they were PAS-positive, fuchsinophilic with AB/AF, but did not stain with HDI.

Type B goblet cells (stained purple with AB/PAS) were fuchsinophilic with AB/AF, PAS-positive and light blue following HDI, and they tended to give a stronger reaction with AB at pH 2·5 than at pH 1·0.

Type C goblet cells were least numerous in all ducts. They gave a blue colour in the AB/PAS sequence, were alcianophilic in the AB/AF reaction, stained more intensely with AB at pH 2·5 than at pH 1·0, gave an intense blue colour with HDI and were only weakly PAS-positive.

Lymphoid cells

Along the length of all Harderian and lacrimal gland ducts scattered clusters of lymphoid cells were aggregated either as germinal centres (Fig. 3) with a well defined cortex and medulla, or were present as diffuse lymphoid tissue (Fig. 4): the latter were more common in all three species. They were usually seen near the lumen of the duct, from which they were separated by a layer of low cuboidal cells (Fig. 4). Germinal centres tended to be most numerous along the ducts of the fowl, and least along those of the duck, and they were often more deeply seated in the adipose and connective tissue surrounding the duct than were the diffuse lymphoid aggregates.

Small lymphocytes predominated in the diffuse lymphoid tissue (Fig. 4), although large lymphocytes and pyroninophilic plasma cells also occurred. The cells in the germinal centres were mostly large lymphocytes and plasmablasts, with some mature plasma cells in the medulla; small lymphocytes were also present.

Occasionally single plasma cells and Russell body-containing plasma cells were observed along the duct just below the epithelial cells (Fig. 5).

Glycogen

Best's carmine-, PAS- and Bauer–Feulgen-positive material was seen in the epithelial cells of the ducts of the fowl up to seven days of age. Diastase control

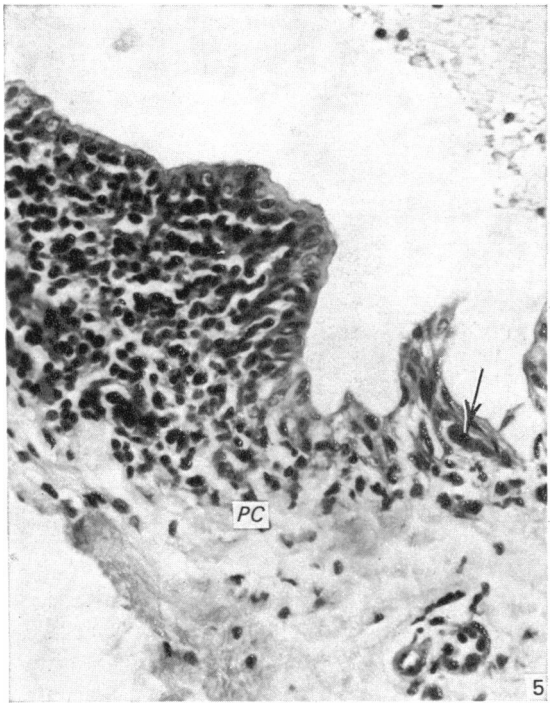
Fig. 5. Fowl Harderian gland duct. A Russell body-containing plasma cell (arrow) and diffuse lymphoid tissue with plasma cells (*PC*) at the periphery. Mid-portion. 6 weeks old. H & E. × 640.

Fig. 6. Fowl Harderian gland duct. Glycogen occurs as a perinuclear deposit (arrows), cytoplasmic granules and in the lumen. Proximal portion. 1 day old. PAS. × 1580.

Fig. 7. Turkey lacrimal gland duct. Diastase control section for Fig. 8. Glycogen has been removed. Distal portion. 2 days old. Bauer–Feulgen. × 400.

Fig. 8. Turkey lacrimal gland duct. Positively stained material (glycogen) in the lumen and along the luminal edge. Distal portion. 2 days old. Bauer–Feulgen. × 400.

Fig. 9. Turkey lacrimal gland duct. PAS-positive structures (arrows) in epithelial cells. Goblet cells are present. Proximal portion. 5 weeks old. PAS. × 1580.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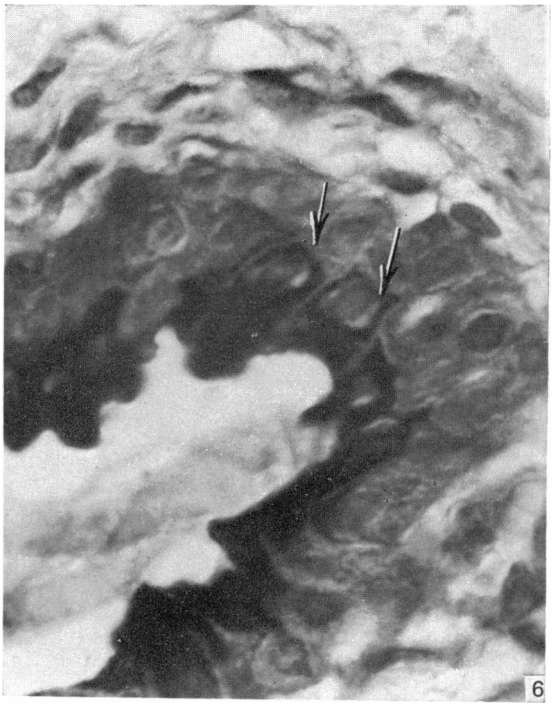
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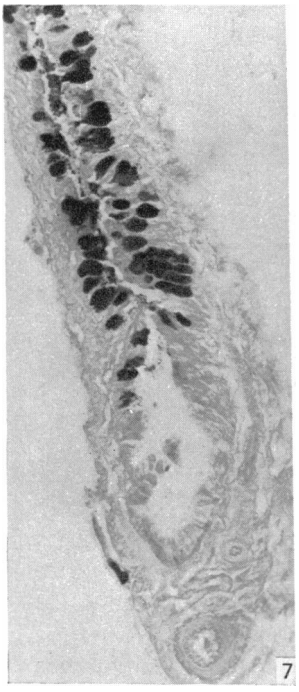
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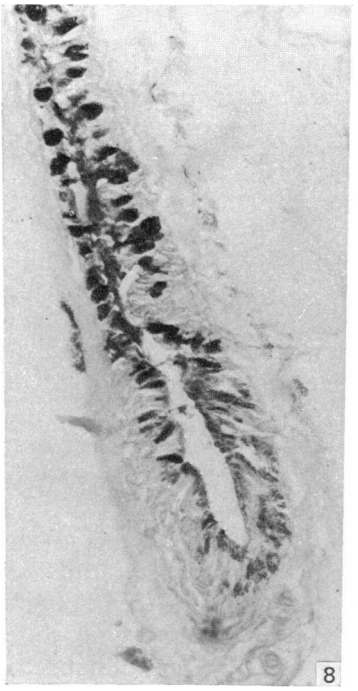
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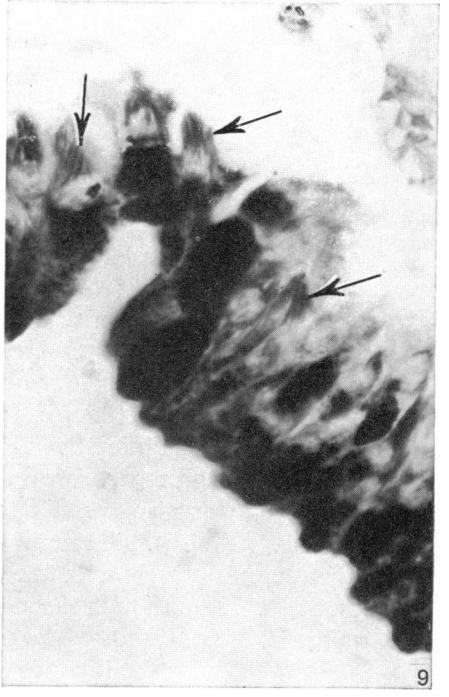
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sections did not show this material. It occurred as cytoplasmic granules towards the free border of the cells, and also as a perinuclear deposit (Fig. 6). It was not seen in the cells of either duck or turkey ducts, though it did occur lining the edge of the lumen, and also possibly between epithelial cells, in all three species (Figs. 7, 8), being most pronounced in younger birds.

Other remarks

Lymphocytes and a few plasma cells were seen in the lumina of all ducts. Extrusion of lymphoid cells between epithelial cells into the lumen from diffuse lymphoid tissue was a feature common to all ducts.

The ducts of the turkey differed from those of the fowl and duck in that cells with PAS-positive streaks were found only in the epithelia of the former (Fig. 9).

More germinal centres were found in the ducts of the fowl, as well as a generally greater population of lymphocytes. The ducts of the turkey had more goblet cells than those of the fowl or duck.

DISCUSSION

Walls (1942) has said that the avian lacrimal gland has a single duct. This has been confirmed in the fowl and duck by Nicolescu (1971) and Burns (1976*a*), and now in the turkey. Mammalian lacrimal glands can have many ducts (Walls, 1942) although the camel is exceptional in having only one (Awkati & Al-Bagdadi, 1971). Burns (1974) and Survashe (1976) have shown that the avian Harderian gland has a single duct, as does the gland in mammals, snakes, lizards and turtles, but in crocodiles many ducts are found (Walls, 1942).

The lacrimal and Harderian glands have a common embryonic and phylogenetic origin (Prince, 1956), so that some similarities in the ducts might be expected as they fulfil basically similar functions. The present study has shown a general morphological and histochemical similarity between the Harderian gland duct and the lacrimal gland duct of the same species, and also between different species of bird, even though their Harderian and lacrimal glands may differ (cf. MacLeod, 1880; Bang & Bang, 1968; Wight *et al.*, 1971; Wight & MacKenzie, 1976; Burns, 1976*a*).

The light staining of the epithelial cells of the ducts induced by PAS indicated the presence of mucopolysaccharides, which were probably acidic mucosubstances because of the blue colour obtained with HDI and the light colouring with AB at pH 1·0 and pH 2·5 (Pearse, 1968).

The intense staining by PAS in the epithelial cells of young chicks was due to the presence of glycogen, proved by its absence in diastase-treated control sections. Glycogen was also seen in the lumen of the ducts of all three species, especially in younger birds. The perinuclear situation (Fig. 6) of glycogen in young chicks corresponds closely with the picture seen at the ultrastructural level (Maxwell & Burns, 1979), although the amount of glycogen seen lining the lumina of the ducts (Figs. 7, 8) appeared to be greater at the light microscope level.

In both ducts the line of PAS-positive material lining the edge of the lumen was shown by HDI, AB at pH 1·0 and pH 2·5 to consist of acidic mucosubstances.

Histochemical reactions within the goblet cells indicated that type A cells, the most numerous in all ducts, contained neutral mucopolysaccharides, since they stained red in the AB/PAS sequence and were PAS-positive. This was confirmed by a more

intense reaction with AB at pH 1·0 than at pH 2·5, fuchsinophilia in the AB/AF reaction and no colour with HDI. Type B goblet cells were PAS-positive and stained purple with AB/PAS, which is suggestive of mixed acid and sulphated mucosubstances. This was supported by the results obtained with the other mucosubstance stains used. Type C goblet cells, the least numerous, gave a blue colour with AB/PAS and with AB/AF, indicating a predominance of acid mucins which was affirmed with the other stains.

The presence of lymphocytes in the fowl Harderian gland duct has been reported previously by Bang & Bang (1968) and by Survashe (1976), although only the latter mentioned the occurrence of germinal centres. The present study has shown that lymphocytes are found in both the lacrimal and Harderian gland ducts of the fowl, turkey, and duck either as diffuse lymphoid tissue or as discrete germinal centres. Payne (1971) has said that there is convincing evidence to show that diffuse lymphoid tissue is thymus-dependent, whereas germinal centres and plasma cells are bursa-dependent. The fowl Harderian gland has been shown to have a mainly bursa-dependent population of plasma cells (Sundick *et al.* 1973), and to be capable of antibody formation against particulate (Meuller *et al.* 1971) and soluble antigens (Burns, 1976*b*). Thus the presence of two distinct populations of lymphoid cells in the draining duct might be important in T and B cell co-operation in immune reactions along the ducts, especially as the fowl Harderian gland duct has been shown to be the possible route of antigen uptake (Burns, 1977). The lacrimal gland duct of the camel resembles that of the bird in that it is ringed by lymphocytes both as germinal centres and as diffuse aggregates (Awkati & Al-Bagdadi, 1971).

The PAS-positive structures found in epithelial cells of turkey ducts (Fig. 9) probably correspond to the crystalline inclusions reported elsewhere (Maxwell & Burns, 1979).

SUMMARY

The secretory ducts of the Harderian and lacrimal glands of the turkey, fowl and duck are similar in both structure and function. The single duct of each gland is lined by mucus-secreting epithelium. The epithelial cells vary from cuboidal to columnar, and goblet cells of three types are found. Type A, the most numerous, contain neutral mucopolysaccharides, type B have mixed acid and sulphated mucosubstances and type C, the least numerous, contain acid mucins. In both ducts glycogen is present in the lumen, but it occurs intracellularly only in the fowl up to seven days after hatching. Lymphocytes, either as diffuse tissue or as germinal centres, are found scattered along the length of the ducts. Some plasma cells and plasmablasts are also present.

REFERENCES

ALBINI, B., WICK, G., ROSE, M. E. & ORLANS, E. (1974). Immunoglobulin production in chicken Harderian glands. *International Archives of Allergy and Applied Immunology* **47**, 23–34.

AWKATI, A. & AL-BAGDADI, F. (1971). Lacrimal gland of the camel (*Camelus dromedarius*). *American Journal of Veterinary Research* **32**, 505–510.

BANG, B. G. & BANG, F. B. (1968). Localised lymphoid tissue and plasma cells in paraocular and paranasal organ systems in chickens. *American Journal of Pathology* **53**, 735–751.

BROBBY, G. W. Snr. (1972). On the Harderian gland of the domestic duck (*Anas platyrhynchos*). Morphological and histochemical investigations. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **133**, 223–230.

BURNS, R. B. (1974). The Harderian gland in birds. M.Phil. Thesis, University of London.

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

BURNS, R. B. (1976*b*). Specific antibody production against a soluble antigen in the Harderian gland of the domestic fowl. *Clinical and Experimental Immunology* **26**, 371–374.

BURNS, R. B. (1977). Possible route of antigen uptake by the Harderian gland of the domestic fowl. *British Poultry Science* **18**, 407–409.

CULLING, C. F. A. (1966). *Handbook of Histopathological Techniques*. London: Butterworth & Co. Ltd.

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

KURNICK, N. B. (1955). Pyronin Y in the methyl green-pyronin histological stain. *Stain Technology* **30**, 213–230.

MACLEOD, J. (1880). Sur la structure de la gland de Harder du canard domestique. *Archives de Biologie* **1**, 45–57.

MAXWELL, M. H. & BURNS, R. B. (1979). The ultrastructure of the epithelium of the ducts of the Harderian and lacrimal glands of the turkey, fowl and duck. *Journal of Anatomy* **128**. (In Press).

MEULLER, 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. *Cellular Immunology* **2**, 140–152.

NICOLESCU, V. (1971). Cercetări anatomice asupra inervării si irigării arterio-venoase a glandei lacrimale si a glandei lui Harder la păsările domesticia. *Lucrări Stiintifice Seria C. Medicină Veterinarià* **14**, 21–33

PAYNE, L. N. (1971). Fowl lymphoid system. In *Physiology and Biochemistry of the Domestic Fowl*, vol. 2 (ed. D. J. Bell & B. M. Freeman). London: Academic Press.

PEARSE, A. G. E. (1968). *Histochemistry, Theoretical and Applied*, 3rd ed., vol. 1. London: Churchill.

PRINCE, J. R. (1956). *Comparative Anatomy of the Eye*. Illinois: Springfield.

SUNDICK, R. S., ALBINI, B. & WICK, G. (1973). Chicken Harder's gland: evidence for a relatively pure bursa-dependent lymphoid cell population. *Cellular Immunology* **7**, 332–335.

SURVASHE, B. D. (1976). Immunological aspects of avian paraocular glands with particular reference to the Harderian gland of the domestic fowl. Ph.D. Thesis, University of Liverpool.

WALLS, G. L. (1942). The vertebrate eye and its adaptive radiation. *Cranbrook Institute of Science Bulletin* **19**.

WIGHT, P. A. L., BURNS, R. B., ROTHWELL, B. & MACKENZIE, G. M. (1971). The Harderian gland of the domestic fowl. I. Histology, with reference to the genesis of plasma cells and Russell bodies. *Journal of Anatomy* **110**, 307–315.

WIGHT, P. A. L. & MACENKZIE, G. M. (1976). Mucosubstances in the Harderian gland of the domestic duck. *Research in Veterinary Science* **17**, 114–121.