J. Anat. (1992) 180, pp. 1–13, with 15 figures Printed in Great Britain

The orbital glands of the chelonians *Pseudemys scripta* and *Testudo graeca*: comparative histological, histochemical and ultrastructural investigations

G. CHIEFFI BACCARI[1], L. Di MATTEO[2] AND S. MINUCCI[2]

[1] *Istituto di Anatomia Umana Normale and* [2] *Dipartimento di Fisiologia Umana e Funzioni Biologiche Integrate 'F. Bottazzi', Università di Napoli, Italy*

(*Accepted 16 July 1991*)

ABSTRACT

The orbital glands of the chelonians *Pseudemys scripta* and *Testudo graeca* were investigated at the histological, histochemical and ultrastructural levels. Four acinar cell types were seen in the harderian gland of *P. scripta* on the basis of histochemical reactions and ultrastructure. Secretory granules were of 2 types, one showing moderate electron density with an electronlucent core, the other being smaller and more osmiophilic with an electron-dense core. In the harderian gland of *T. graeca* only 2 glandular cell types were found; one type contained secretory granules with a dense core surrounded by a wide zone of lower density. Acinar cells of the anterior lacrimal gland in both species were of 2 types, one being of mucous type. In the harderian gland and in the lacrimal gland of both species, one cell type appeared not to be involved in the secretion of organic material. These cells contained numerous tightly packed mitochondria among which were abundant clumps of glycogen; the cell membrane was specialised at both edges. This cell type was similar ultrastructurally to the 'salt cells' described in the salt-secreting glands of various marine vertebrates, i.e. of the cells involved in transport processes.

These combined histological, histochemical and ultrastructural studies have allowed us to distinguish orbital glands. In the past, the harderian and lacrimal glands in chelonians have often been mistaken for one another.

INTRODUCTION

Reptiles are the first tetrapod group to develop both types of orbital glands, the lacrimal gland and the harderian gland. Despite this they have received little attention at least with regard to their orbital glands. Particularly in chelonians, histological and histochemical investigations appear to be limited to those of Gerzeli (1967), Cowan (1969, 1971, 1973) and Saint-Girons (1985, 1988). With the exception of the studies of Abel & Ellis (1966) and Cowan (1971), there is no documentation on the fine structure of the orbital glands in chelonians. We have used histological, histochemical and electron microscopical techniques to study the orbital glands of *Pseudemys scripta* and *Testudo graeca* and to assess their nomenclature.

MATERIAL AND METHODS

Histology and histochemistry

Adult male (n = 6) and female (n = 6) *Pseudemys scripta* and *Testudo graeca* were examined. Animals supplied by a local dealer during 2 periods of the year (spring and autumn) were decapitated and the glands dissected from the orbits. Anaesthetics were avoided since they may affect the ultrastructure of the gland (Cowan, 1971). The animals were therefore subdued by keeping them at 0 °C for 30 min before killing. Glands were fixed in Bouin's fluid. Paraffin sections (5 μm) were stained with haematoxylin and eosin and/or Mallory's trichrome. The mercury-bromophenol blue method was used for the histochemical detection of proteins. The following histochemical tests for the characterisation of mucosubstances were used: periodic acid–Schiff (PAS) method using diastase digestion for control, PAS after

Correspondence to Dr Gabriella Chieffi Baccari, via Tito Livio 26, 80122 Napoli, Italy.

acetylation, PAS/KOH, alcian-PAS method (AB/PAS), alcian blue 8GX at various pH values (0.2–1–2.5), toluidine blue using hyaluronidase digestion for control, azur A 1 : 5000 buffered at pH 1, 2, 3, 4, 5 for orthochromasia. Sulphation-induced metachromasia was studied using concentrated sulphuric acid followed by 0.01 % azure A in 60 % ethanol. Sialic acid was detected according to Culling's method. For lipid detection, formol-calcium-fixed frozen sections were stained with Sudan black B and Nile blue. Cholesterol and its esters were investigated by Schultz's method.

Two heads of *P. scripta* were immediately immersed in 10 % formalin (24 h). Decalcification was achieved by immersion in 5 % formic acid (8 d). Following decalcification the heads were dehydrated in graded ethanol, and cleared in xylene. After embedding in paraffin, serial sections 10 µm thick of the whole head were prepared.

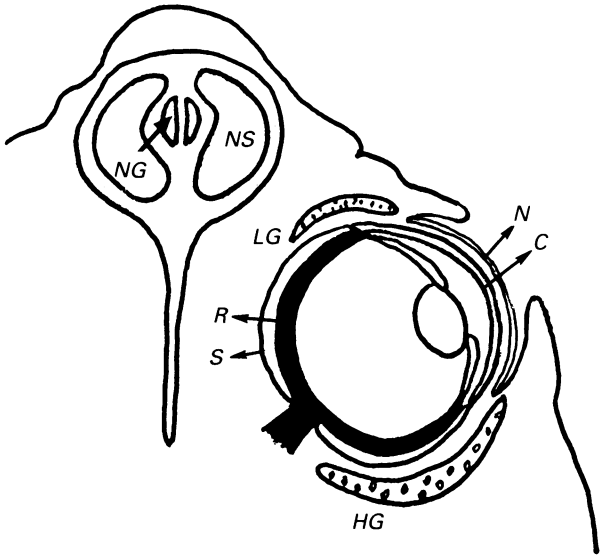


Fig. 1. Schematic representation of the frontal section of the eyeball and associated structures in *Pseudemys scripta* and *Testudo graeca*. *C*, cornea; *HG*, harderian gland; *LG*, lacrimal gland; *N*, nictitating membrane; *NG*, nasal gland; *NS*, nasal sac; *R*, retina; *S*, sclera.

Ultrastructure

Small (n = 1 mm) pieces of tissue were washed and fixed for 2 h at 4 °C in Karnovsky fluid and postfixed for 2 h at 4 °C in 1 % osmium tetroxide. Following fixation, the pieces were dehydrated in a graded ethanol series and embedded in TAAB 812. Grids with thin sections were stained with 4 % uranyl acetate followed by 1 % lead citrate and examined

Table 1. *Histochemical classification of the glandular cells of* Pseudemys scripta *harderian gland*

	Type I cell	Type II cell	Type III cell	Type IV cell	Lumen
MBb	+	+	+	+	+
PAS	++	±	++	+++	+
PAS/diastase	+	−	+	−	+
PAS/acetylation	−	−	−	−	−
PAS/acetylation KOH	++	±	++	+++	−
AB pH 0.2	+	++	+	−	±
AB pH 1.0	+	++	+	−	±
AB pH 2.5	++	+++	+++	−	++
AB/PAS	+++	−	++	−	+++
Azure A orthochromasia					
pH 1.0	−	−	−	−	−
pH 2.0	−	−	−	−	−
pH 3.0	−	−	−	−	−
pH 4.0	−	−	−	−	−
pH 5.0	+	+	+	−	−
Azure A metachromasia					
pH 1.0	−	−	−	−	−
pH 2.0	−	−	−	−	−
pH 3.0	−	−	−	−	−
pH 4.0	−	−	−	−	−
pH 5.0	+	+	+	−	−
Sulphation/azurophil metachromasia	++	+++	++	−	+
Toluidine blue	++	+++	+++	−	+
Toluidine blue/hyaluronidase	−	−	−	−	−
Sialic acid	−	−	−	−	−
Sudan black B	−	−	−	−	−
Nile blue	−	−	−	−	−
Schultz	−	−	−	−	−

The intensity of the reaction is classified into 5 categories from − to +++.

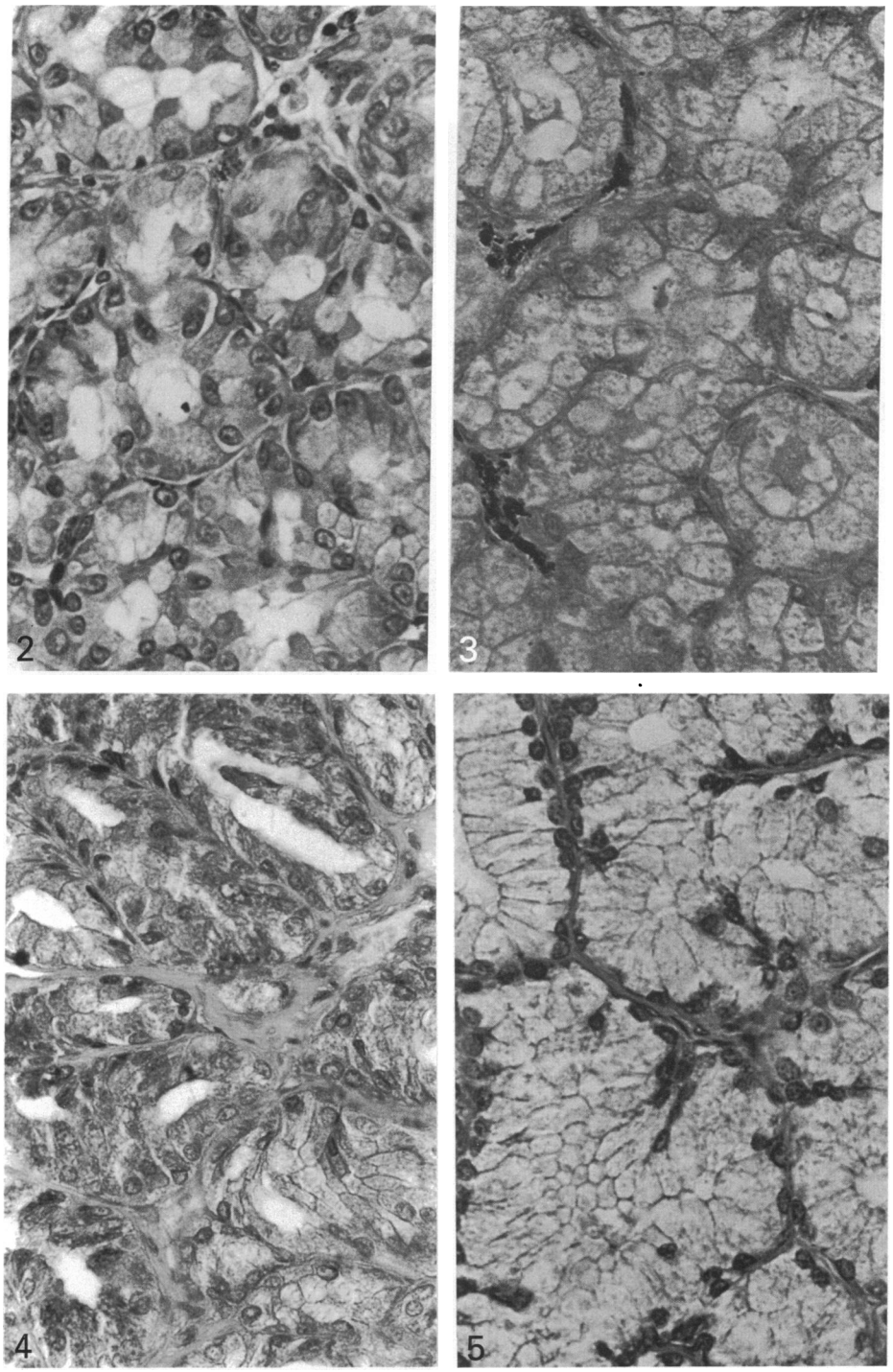


Fig. 2. Light micrograph of the harderian gland of *Pseudemys scripta*. Note the different density of the cytoplasm of the acinar cells. Haematoxylin–eosin. ×250.

Fig. 3. Light micrograph of the lacrimal gland of *Pseudemys scripta*. The cytoplasm of the columnar cells appears to be filled with pale secretory granules. Numerous melanocytes are present in the interstitial tissue. Haematoxylin–eosin. ×250.

Fig. 4. Light micrograph of the harderian gland of *Testudo graeca*. Glandular cells contain numerous secretory granules. Haematoxylin–eosin. ×250.

Fig. 5. Light micrograph of the lacrimal gland of *Testudo graeca*. The columnar cells contain numerous pale secretory granules. Haematoxylin–eosin. ×250.

Table 2. *Histochemical classification of the glandular cells of* Pseudemys scripta *lacrimal gland*

	Type I cell	Type II cell	Lumen
MBb	+	−	±
PAS	+	+++	±
PAS/diastase	+	±	−
PAS/acetylation	−	−	−
PAS/acetylation KOH	+	+++	−
AB pH 0.2	−	−	−
AB pH 1.0	−	−	−
AB pH 2.5	++	−	+
AB/PAS	+	−	−
Azure A orthochromasia			
pH 1.0	−	−	−
pH 2.0	−	−	−
pH 3.0	−	−	−
pH 4.0	−	−	−
pH 5.0	−	−	−
Azure A metachromasia			
pH 1.0	−	−	−
pH 2.0	−	−	−
pH 3.0	−	−	−
pH 4.0	−	−	−
pH 5.0	±	−	−
Sulphation/azurophil metachromasia	+	−	−
Toluidine blue	+	−	−
Toluidine blue/hyaluronidase	±	−	−
Sialic acid	−	−	−
Sudan black B	−	−	−
Nile blue	−	−	−
Schultz	−	−	−

The intensity of the reaction is classified into 5 categories from − to +++.

with a Philips 301 transmission electron microscope.

Porphyrins. Porphyrins were detected under microscopic examination using ultraviolet light and a Wood's ultraviolet filter (360 nm) which produces a red fluorescence in porphyrin pigments.

RESULTS

Gross anatomy

Pseudemys scripta and *Testudo graeca* have 2 main orbital glands. The harderian gland is located on the posterior aspect of the eyeball (Fig. 1). It is oval in shape and 3 mm in length in *Pseudemys scripta*, and about 5 mm in *Testudo graeca*. Its secretion drains through a horizontal row of ducts along the lateral surface of the nictitating membrane. The lacrimal gland lies flattened medially in the orbit and empties through ducts into the conjunctival space along the medial surface of the nictitating membrane (Fig. 1). It is about half the length of the harderian gland, in both species. These glands appear pinkish-grey to the naked eye due to the considerable amount of pigment contained in the connective tissue capsule. Chelonians lack nasolacrimal ducts.

Histology and histochemistry

The 2 orbital glands are surrounded by a thin connective capsule containing blood vessels and nerve fibres. Thin strands of connective tissue with numerous melanocytes and several mast cells penetrate between the acini and ducts of the glands. Various myoepithelial cells are observed between the glandular cells and the basal lamina. Structural and cytological differences can be observed between the 2 orbital glands.

Pseudemys scripta

(i) *Harderian gland.* The gland consists of acini. The glandular cells are columnar or pyramidal, having a single basally located nucleus. The secretory granules accumulate between the nucleus and the free surface of the cell. They are basophilic and appear more dense in some cells than in others (Fig. 2).

The ducts of the gland are lined by a low cuboidal epithelium. In the larger ducts it becomes columnar.

The results of the histochemical tests of the harderian gland are summarised in Table 1. It is possible to distinguish in the epithelium of the acini 4 types of cells. The more numerous cells (type I) have granules with a PAS-positive central core surrounded by an AB/PAS-positive ring. Type II cells show granules only AB-positive (pH 0.2–1–2.5). Type III cells contain both types of granules. All 3 cell types are weakly positive to the PAS reaction and stain metachromatically with toluidine blue and with azur A after sulphation. Type IV cells are represented by cells strongly positive only to the PAS reaction. The intensity of the PAS reaction decreases after digestion with diastase, indicating the presence of glycogen.

Myoepithelial cells are infrequent and are located peripherally in the acini. These cells lie flat against the basement membrane of the acini and are negative with the histochemical tests for mucosubstances.

The mercury–bromophenol blue reaction is weakly positive in all 4 cell types. In the acinar lumina the secretory product is strongly AB/PAS-positive.

The ducts of this gland are lined by pale cells which do not stain with the methods used, except for the apical cytoplasm, which stains intensely with the AB/PAS method. Occasionally, strongly AB/PAS-positive cells are easily distinguished between the pale cells. Neither lipids nor porphyrins were detected by histochemical methods.

Table 3. *Histochemical classification of the glandular cells of* Testudo graeca *harderian and lacrimal glands*

	Harderian gland			Lacrimal gland		
	Type I cell	Type II cell	Lumen	Type I cell	Type II cell	Lumen
MBb	++	±	++	±	−	±
PAS	++	+++	++	+	+++	+
PAS/diastase	+	−	±	±	−	−
PAS/acetylation	−	−	+	−	−	−
PAS/acetylation KOH	++	+++	+	+	+++	−
AB pH 0.2	+	−	+	−	−	−
AB pH 1.0	+	−	+	−	−	−
AB pH 2.5	++	−	++	+++	−	+++
AB/PAS	+	−	+	++	−	+
Azure A orthochromasia						
pH 1.0	−	−	−	−	−	−
pH 2.0	−	−	−	−	−	−
pH 3.0	−	−	−	−	−	−
pH 4.0	±	−	−	−	−	−
pH 5.0	±	−	−	−	−	−
Azure A metachromasia						
pH 1.0	−	−	−	−	−	−
pH 2.0	−	−	−	−	−	−
pH 3.0	−	−	−	−	−	−
pH 4.0	−	−	−	−	−	−
pH 5.0	+++	−	+	−	−	−
Sulphation/azurophil metachromasia	+++	−	++	+	−	+
Toluidine blue	+++	−	+	−	−	−
Toluidine blue/hyaluronidase	−	−	−	−	−	−
Sialic acid	−	−	−	−	−	−
Sudan black B	−	−	−	−	−	−
Nile blue	−	−	−	−	−	−
Schultz	−	−	−	−	−	−

The intensity of the reaction is classified into 5 categories from − to +++.

(ii) *Lacrimal gland.* This is composed of acini containing only one type of columnar cells, which stain pale with haematoxylin and eosin and Mallory's trichrome (Fig. 3). Two cell types may be characterised by histochemical tests (Table 2). Type I cells show basally located nuclei and their cytoplasm is filled with secretory granules that are AB- (pH 2.5) and PAS-positive. Rare PAS-positive cells (type II) are found scattered in the acinar epithelium. In these cells the PAS positivity disappears after diastase digestion.

The acinar lumen is narrow and usually filled with secretory material positive to AB at pH 2.5 (Table 2). The ducts are lined with a single layer of columnar cells whose apical cytoplasm is AB-positive at pH 2.5. Occasionally AB/PAS-positive cells are found scattered between them. Neither lipids nor porphyrins were detected.

No differences were observed between male and female orbital glands.

Testudo graeca

(i) *Harderian gland.* This consists of tubulo-acini with large lumina which are lined by single cuboidal epithelium. A thin sleeve of connective tissue rich in mast cells and melanocytes encloses each tubule and several secretory ducts (Fig. 4).

The secretory cells contain a large nucleus and their cytoplasm may appear either pale or filled with small basophilic secretory granules.

As a result of histochemical tests, 2 distinct cell types may be identified (Table 3). The tubulo-acini are paved with secretory cells, the cytoplasm of which is packed with granules which react with AB (pH 0.1–2–2.5), PAS and with mercury–bromophenol blue. Moreover, these cells stain metachromatically with toluidine blue, with azur A at pH 5, and with azur A after sulphation. Intermingled among these cells are type II cells that are strongly PAS-positive. In both cell types the PAS reaction decreases after diastase digestion.

In the lumina, the secretory product is mercury–bromophenol blue and AB/PAS-positive and the apical portions of columnar duct cells are also AB/PAS-positive. Neither lipids nor porphyrins were detected with the histochemical methods used.

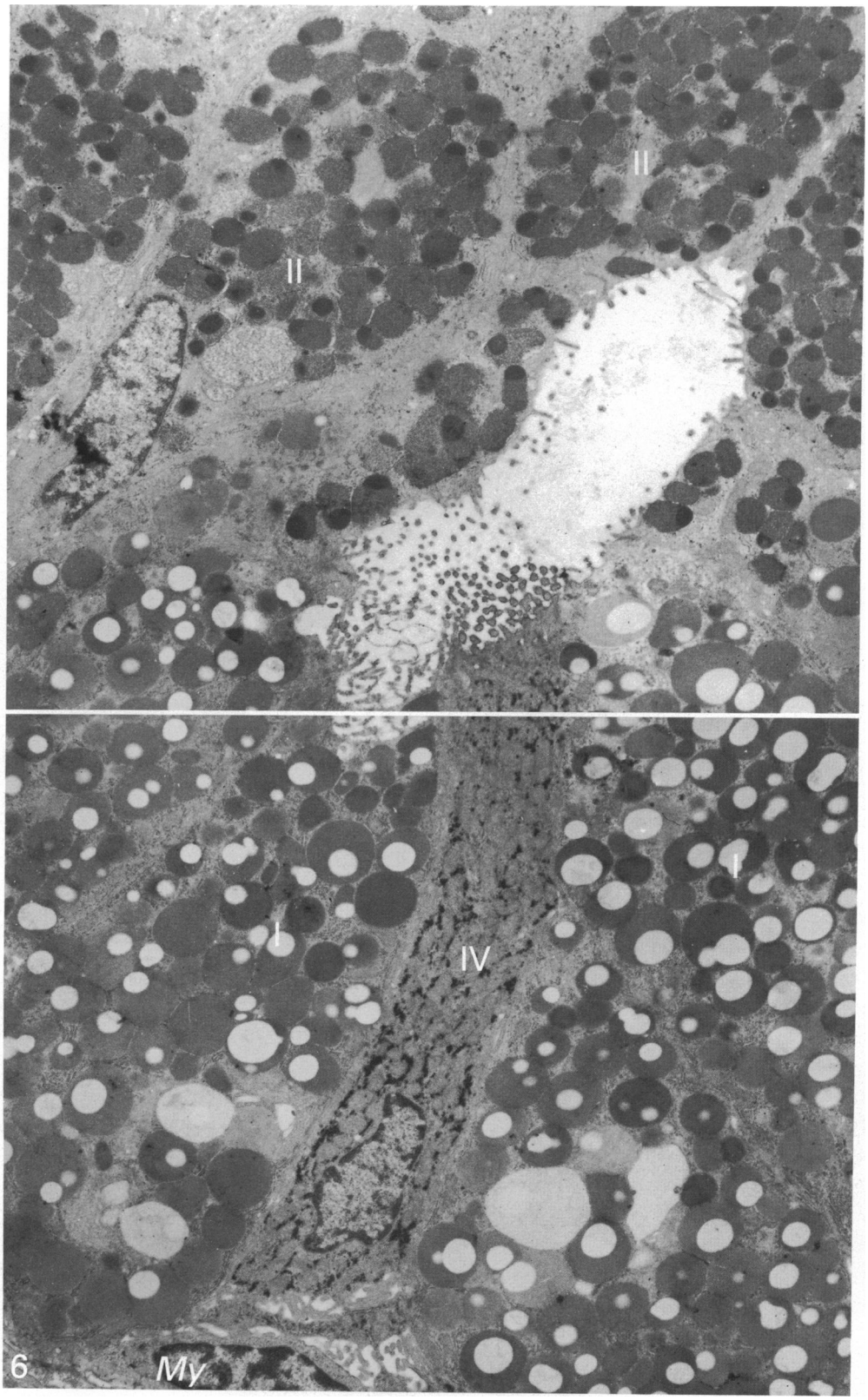


Fig. 6. Electron micrograph of the harderian gland of *Pseudemys scripta*, showing different acinar cell types. The core of some secretory granules appears as the negative of others. Among glandular cells, a salt cell can be identified by the presence of numerous mitochondria and glycogen accumulations. Numerous interdigitations are present at the basal pole of the type IV cell. I, type I cell; II, type II cell; *My*, myoepithelial cell. ×3000.

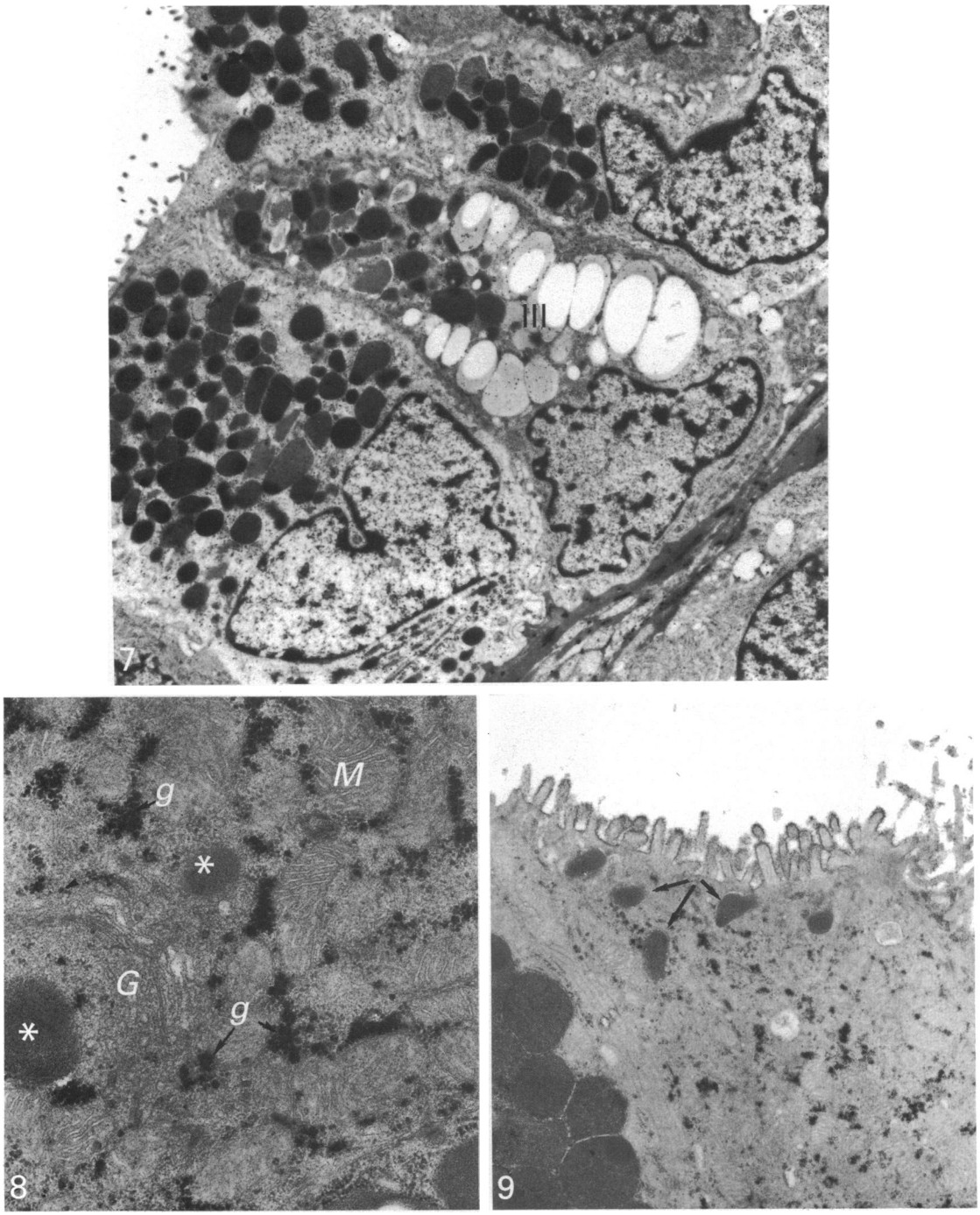


Fig. 7. Electron micrograph of the harderian gland type III acinar cells of *Pseudemys scripta* filled with 2 types of secretory granules in different cell compartments. ×4600.

Fig. 8. High magnification of the cytoplasm of type IV cell of the harderian gland of *Pseudemys scripta*. Note the presence of dense bodies (asterisks) close to the Golgi complex. *M*, mitochondria; *G*, Golgi apparatus; *g*, glycogen. ×12000.

Fig. 9. High magnification of the free border of the type IV cell of the harderian gland of *Pseudemys scripta*. Dense bodies (arrows) are visible at the apical pole. The finger-like microvilli are covered by a thick glycocalyx. ×12000.

(ii) *Lacrimal gland.* This gland is acinar in type (Fig. 5). Most of the acinar cells are AB- (pH 2.5) and PAS-positive (type I cell). In the lacrimal gland of *Testudo graeca* infrequent PAS-positive cells (type II cells) are also seen between the glandular cells (Table 3). In the acinar lumen the secretory product is AB-positive at pH 2.5. Numerous goblet cells are seen in the several ducts of the gland. Neither lipids nor porphyrins have been found.

Also in *Testudo graeca* no differences were observed between male and female orbital glands.

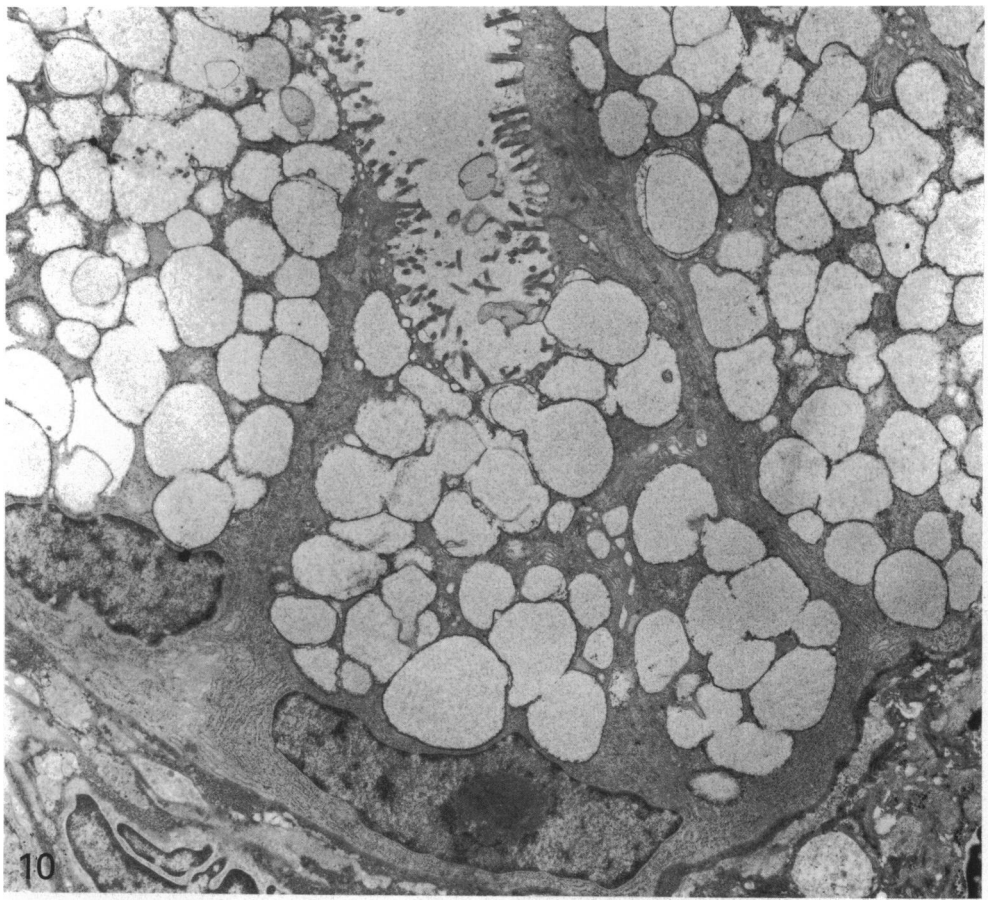


Fig. 10. Electron micrograph of the glandular cells of the lacrimal gland of *Pseudemys scripta* filled with clear and homogeneous secretory granules. The secretory granules are secreted by exocytosis. ×4400.

ULTRASTRUCTURE

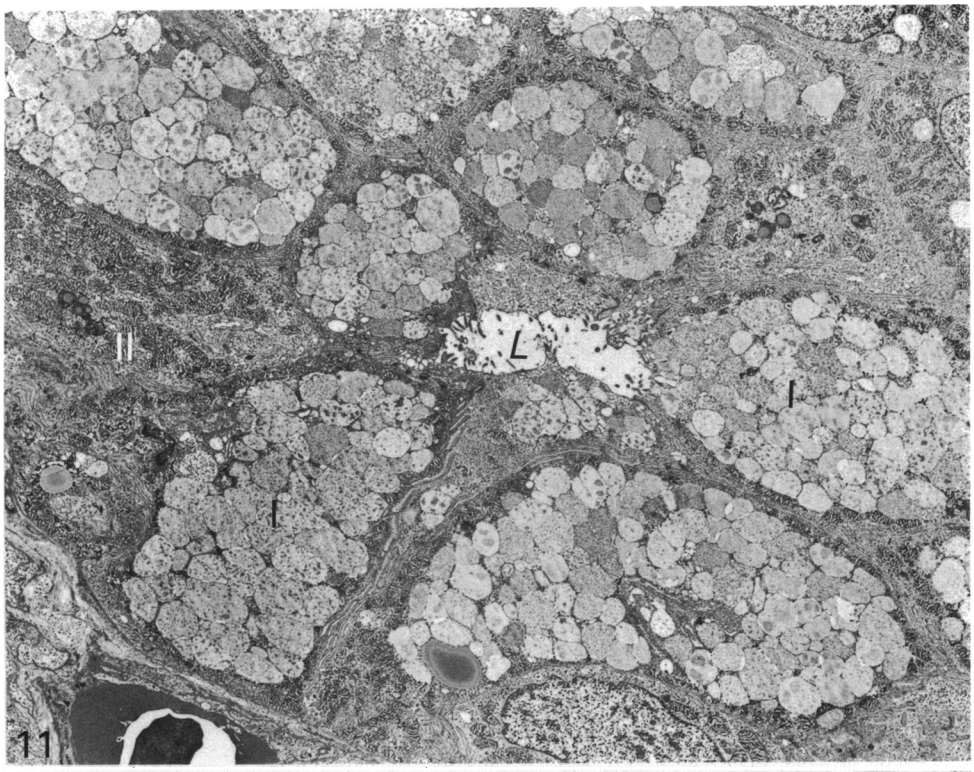
Pseudemys scripta

(i) *Harderian gland.* By electron microscopy, 4 types of cells can be recognised in the secretory epithelium of the harderian gland (Fig. 6). They correspond to the 4 types of glandular cells identified by histochemical methods. Type I cells are characterised by a large number of secretory granules occupying most of the cytoplasm. These granules have a moderate electron density with an electronlucent core (Fig. 6). Type II cells contain smaller and more osmiophilic granules, which show an electron-dense core surrounded by a zone of lower density (Fig. 6). These granules, like previous ones, are bounded by a unit membrane.

Type III cells are scattered between the above 2 cell types: they contain both of the above-mentioned secretory granules, in different cell compartments (Fig. 7).

In all 3 types of secretory cell, the nucleus is basal and irregularly shaped. Rare mitochondria with lamellar cristae, cisternae of rough endoplasmic reticulum (RER) and variable numbers of granules of glycogen are seen among the secretory droplets (Fig. 6). The cell membrane shows numerous microvilli at the apical surface (Fig. 6). The secretory granules are secreted by exocytosis.

The type IV cell, columnar in shape, appears wedged between the secretory cells (Fig. 6). This cell type is less frequent than the first 3 cell types and is not involved in the secretion of organic materials. The most prominent feature of the cytoplasm of these cells is the number of tightly packed mitochondria (Fig. 8). They are usually oriented with the long axis of the cell and have lamellar cristae that are oriented more or less parallel to the short axis of the mitochondrion. Another prominent feature of this cell type is that it is rich in glycogen. Clumps of glycogen are abundant among the mitochondria (Fig. 8). Some dense bodies (presumably lysosomes) are located close to the Golgi complex (Fig. 8) and accumulate at the apical pole of the cell (Fig. 9). The nuclei are basal and oval in shape and do not show nucleoli. The Golgi complex, represented by small lamellae randomly distributed



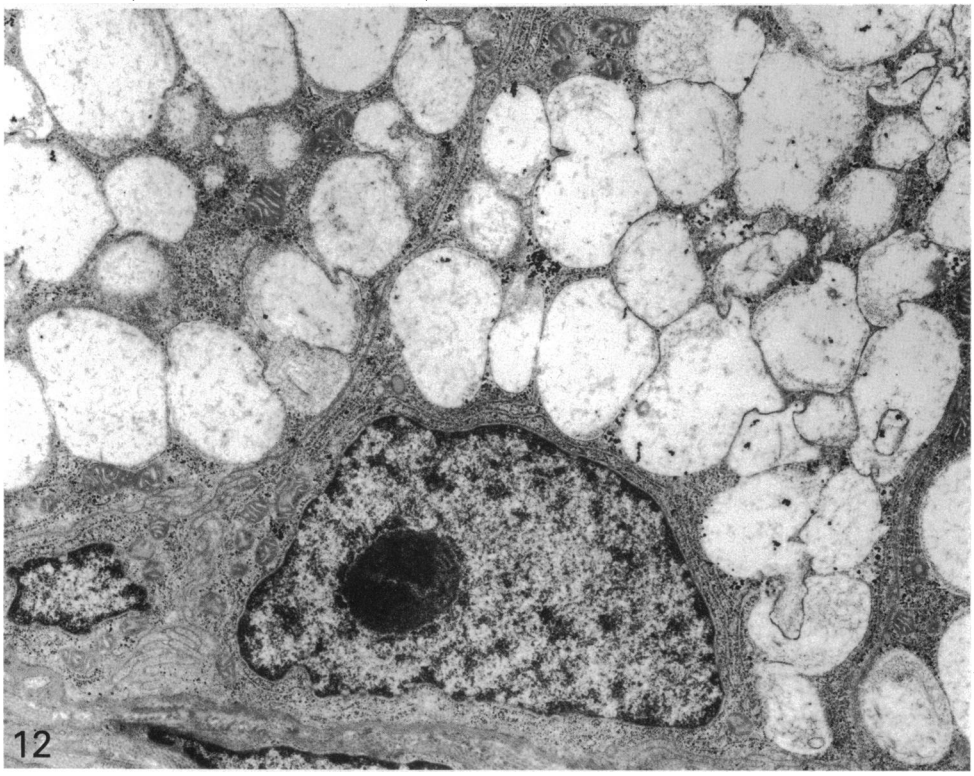
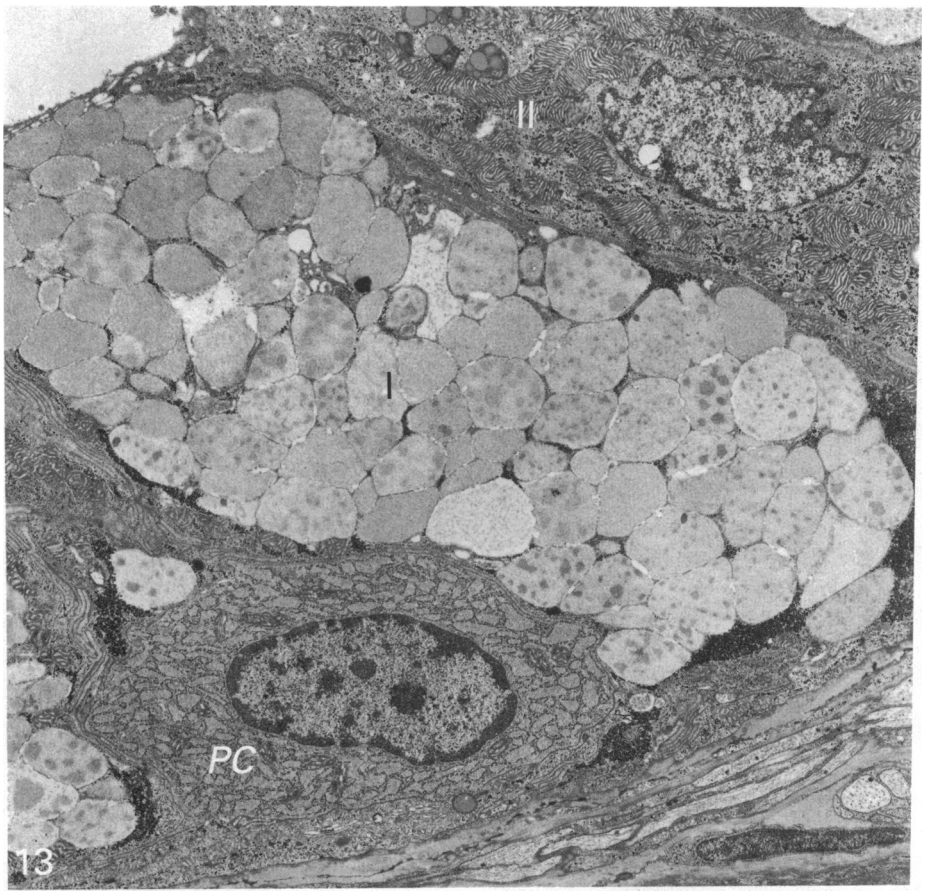
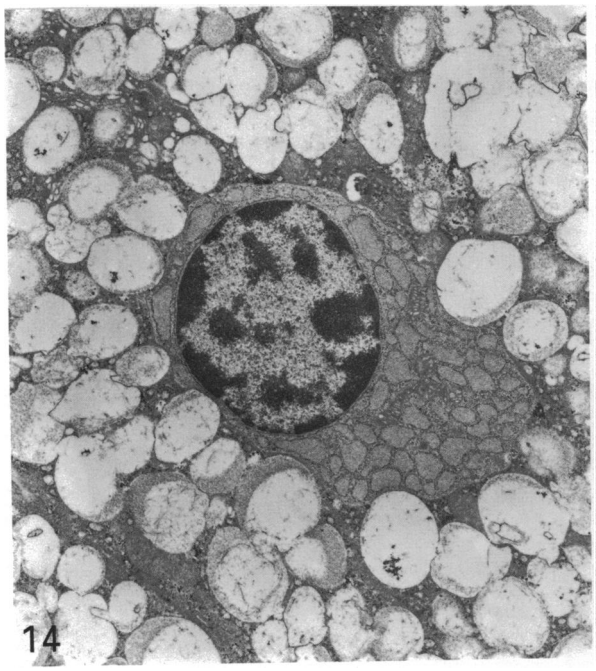


Fig. 11. Electron micrograph of a harderian gland acinus of *Testudo graeca*. The glandular cells are filled with secretory granules in different maturation phases (I). I, type I cell; II, type II cell; *L*, acinar lumen. ×2200.

Fig. 12. Electron micrograph of the glandular cell of *Testudo graeca* lacrimal gland. Note the abundant vesicular RER around the nucleus. ×6000.





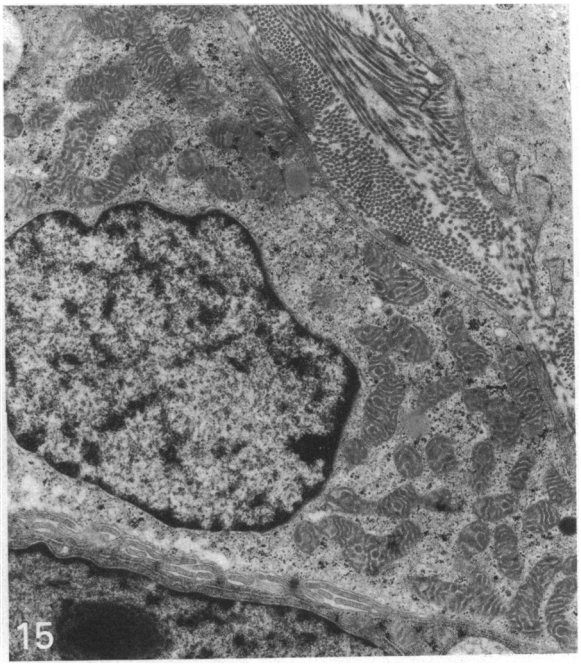


Fig. 13. Electron micrograph of *Testudo graeca* harderian gland. Note the presence of a plasma cell (*PC*) among glandular cells. I, type I cell; II, type II cell. ×2600.

Fig. 14. Electron micrograph of a plasma cell of *Testudo graeca* lacrimal gland. ×6000.

Fig. 15. Electron micrograph of the 'salt cell' of *Testudo graeca* lacrimal gland. Note the abundance of mitochondria and the numerous lateral sheets of the cytoplasm. ×8000.

throughout the cytoplasm, is well developed (Fig. 8). The cell membrane is modified at the apical and basal surfaces of the cell. It shows numerous processes at its base (Fig. 6), while at the free border it develops short finger-like microvilli covered by a thick glycocalyx that stains intensely with osmium tetroxide owing to its sialic acid content (Fig. 9).

Rare myoepithelial cells lie flat against the basement membrane of the acinus. Their large nuclei, which occupy more than half of the cell volume, are surrounded by a thin rim of cytoplasm (Fig. 6). A few plasma cells were identified among the glandular acini (not shown).

(ii) *Lacrimal gland.* Ultrastructural observations reveal the presence of only 2 cell types in the acini of the lacrimal gland. The more numerous cells contain clear and homogeneous secretory granules which fill three quarters of the cell (Fig. 10). These large granules are surrounded by a unit membrane which may fuse with apical cell membrane, suggesting that their content is released into the lumina by exocytosis (Fig. 10). Numerous microvilli are seen on the surface of these cells. The nuclei are irregular in shape and confined to the basal cytoplasm. Mitochondria with lamellar cristae are rare while the RER is well developed between the secretory granules.

The 2nd cell type is far less frequent and shows the same ultrastructure as described for the type IV cell found in the harderian gland. These cells have a basal nucleus, numerous mitochondria with lamellar cristae, very abundant glycogen, a well developed Golgi complex and a thick glycocalyx at the apical surface of the cell (not shown).

Testudo graeca

(i) *Harderian gland.* At the ultrastructural level, the harderian gland contains glandular cells which are filled by a large number of secretory granules (Fig. 11). These closely packed granules possess a unit membrane and are generally in the form of a moderately dense homogeneous material. They sometimes show a dense core surrounded by a wide zone of lower density. Glycogen in the form of coarse rosettes appears throughout the cytoplasm (Fig. 13). These cells correspond to the type I cell identified by histochemical methods. Elongated mitochondria show very well developed lamellar cristae (Fig. 11).

Between the secretory cells, several cells containing only numerous mitochondria and abundant glycogen are seen. They correspond to the type II cell identified histochemically, and are very similar to the type IV cell described in the harderian gland of *P. scripta* (Fig. 13).

Numerous cells with a nucleus containing cartwheel chromatin and a vacuolated ER were found among glandular cells. These cells were identified as plasma cells by their ultrastructural features (Fig. 13).

(ii) *Lacrimal gland.* Ultrastructure of the lacrimal gland is similar to that described in *P. scripta* (Fig. 12). The secretory granules, surrounded by a unit membrane, contain homogeneous materials of low density. The nucleus is often surrounded by an abundant vesicular RER (Figs 12, 14). Among the glandular cells, several cells contain numerous mitochondria and abundant glycogen. The cell membrane shows considerable complexity, with adjacent cell membranes exhibiting many processes. The membranes generally follow a sinusoidal path of varying height and depth. This correlates well with the extremely irregular outline of the cell which is seen also by the light microscope. Lateral sheets of cytoplasm form an interleafing association with the cytoplasmic sheets of the adjacent cells (Fig. 15). Some plasma cells have been identified intermingled with lacrimal cells (Fig. 14).

DISCUSSION

Our combined histological, histochemical and ultrastructural studies on the orbital glands of *Pseudemys scripta* and *Testudo graeca* indicate that so far the lacrimal gland and the harderian gland have been mistaken for one another in chelonians. In fact, the sparse literature is rather confusing (for reviews, see Bellairs & Boyd, 1947, 1949; Cowan, 1973; Saint-Girons, 1985, 1988, 1989). To date, authors have stated, mainly on the basis of histological and histochemical studies, that chelonians lack an anterior lacrimal gland. They located the harderian gland at the medial corner of the orbit and a lacrimal gland at the posterior aspect of the eyeball. We have arrived at the opposite conclusion on the basis of the following considerations. (1) The gland lying at the medial corner of the orbit is a true mucous gland, both histochemically and ultrastructurally; the secretory granules are of the mucous type and by EM they contain clear and homogeneous material. (2) The gland located at the posterior aspect of the eyeball is seromucous, as shown both histochemically and ultrastructurally. On EM the secretory granules are heterogeneous and more dense, common for the proteinaceous secretion of the harderian gland.

The assumption of Cowan (1969) that the secretion

of the harderian gland and lacrimal gland of *Pseudemys scripta* is respectively serous and seromucous is based only on limited histochemical observations. The concept of 'serous' and 'mucous' is at present based both on histochemical properties and electron density of the secretory granules (for review, see Sakai & Yohro, 1987). According to our observations, although the PAS and AB-PAS reactions are positive in both glands, the electron density of the secretory granules is high in the harderian gland (the lacrimal gland according to Cowan) and very low in the lacrimal gland (the harderian gland according to Cowan).

We therefore believe it is justified to identify as the 'harderian gland' what previously has been called the posterior lacrimal gland by Cowan and Saint-Girons, and similarly we will refer to as the 'anterior lacrimal gland' what has previously been considered to be the harderian gland. This criticism can probably be extended to all the chelonians so far studied.

The secretion of the lacrimal gland in both species is uniform. According to histochemical tests, it secretes predominantly acidic sulphated mucosubstances. The harderian gland shows more variability in this respect. In *P. scripta* 2 types of secretory granules are produced either by different cells or by the same cells. The secretory granules of type I cells contain, predominantly, strongly acidic sulphated mucosubstances, while those of type II cells are more proteinaceous. Type III cells are intermediate between the first 2 cell types.

The harderian gland of *T. graeca* has only one type of glandular cell, the secretion of which is mainly glycoproteins and strongly acidic mucosubstances.

Some cells of the harderian gland (type IV cell of *P. scripta* and type II cell of *T. graeca*) and of the lacrimal gland (type II cell in both species) are not involved in the secretion of organic material. Based on their ultrastructure, these cells are similar to the 'salt cells' described in the salt-secreting glands of various marine vertebrates, i.e. of those cells involved in transport processes. Various structures have been associated with the function of salt secretion such as the rectal gland of elasmobranchs (Doyle, 1962; Bulger, 1963) and the nasal gland of marine birds (Komnick, 1963; Ellis & Abel, 1964). All these organs share in general the structures, both fine and coarse, of electrolyte-separating organs. The salt cells show special membrane adaptations at both the basal and apical surfaces. The thickening of the glycocalyx of the short finger-like microvilli at the free border is reminiscent of the cellular processes of the podocytes covering the renal glomerular capillaries in mammals (Rhodin, 1958). Bennett (1963) has proposed that the anionic charge of the glycocalyx could produce an effective means of attracting, trapping and concentrating cations. In fact some mucopolysaccharides do have the capacity for binding cations and can function as ion-exchange resins (Farber, 1950). The abundant mucopolysaccharides associated with the absorptive surface of salt-secreting cells in the orbital glands of chelonians, in the nasal gland of birds and in the rectal gland of elasmobranchs support the hypothesis that the glycocalyx is linked with electrolyte transport. Also, at the basal pole of the cells numerous processes appear to contact the underlying connective tissue. This type of architecture results in a direct communication of the intercellular space with the basement membrane. These specialisations are associated with the richness of glycogen and mitochondria oriented parallel to the long axis of the cell. Since in the salt glands the transport is in the opposite directions, mitochondria are distributed throughout the cytoplasm.

Among reptiles, the presence of salt cells in the orbital glands has been observed so far only in chelonians (Abel & Ellis, 1966). Also the types of secretory granules seen in chelonians appear different from those described in other reptiles so far investigated. The 'special secretory granules' described by Chieffi Baccari et al. (1990) in the lizard *Podarcis s. sicula* show a composite structure. The 3 components of these granules are sharply separated. A similar structure has been found in a species of the same order, the snake *Coluber viridiflavus* (S. Minucci, unpublished observations).

The uniformity of the secretory product by the lacrimal gland in the chelonians leads us to suppose that its main function is the lubrication of the eyeball. As for the harderian gland, the variety of secretory granules observed at the ultrastructural level leads us to hypothesise other possible functions besides the obvious lubrication of the eyeball and osmoregulation, such as a source of pheromones (for review, see Olcese & Wesche, 1989).

The presence of plasma cells among the glandular cells of the chelonian orbital glands strongly supports the possible role of both orbital glands in immunoresponses, as suggested in mammals and birds (for review, see Burns & Maxwell, 1979).

ACKNOWLEDGEMENTS

This work was supported by the MURST (Grants 40% and 60%) and CNR (Grant no. 90.032227.CT04). We thank Mr G. Falcone for his excellent technical assistance.

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