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Paracloacal glands of *Alligator mississippiensis*: A histological and histochemical study

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The histology of the paracloacal 'musk' glands of adult American alligators (*Alligator mississippiensis*) is described. The gland is a single secretory sac with a single duct and a central lumen partially occluded by a central, cylindrical conglomerate of cells and secretion product. The capsule of the gland consists of an outer layer of smooth muscle and an inner layer of connective tissue containing collagen and elastin fibres. Septa carrying blood vessels radiate from the connective tissue layer of the capsule to the border of the central conglomerate. Parenchymal cells containing lipid droplets enlarge from the periphery to the centre of the gland. Secretions formed by degeneration of cells in the central cylinder are concentrated near the secretory duct. Histochemical tests indicate lipids but not mucopolysaccharides in the glandular exudate.

Contents

Introduction

American alligators (*Alligator mississippiensis*), and presumably all extant crocodilians, possess paired 'musk' glands in the lateral walls of the cloaca. The oily, yellow secretions produced by these glands are thought by many authors to contain sex attractants, evidence for which is critically discussed by Neill (1971). Johnsen & Wellington (1982) have shown that yearling alligators detect conspecific cloacal gland odours in an olfactometer. Whether pheromones are produced, however, is still uncertain.

Several descriptions of the gross anatomy of these crocodilian glands have appeared (see Gerhardt, 1933 for reports prior to 1900), but only a few detailed histological studies exist, two of which are concerned with *A. mississippiensis*. The more extensive report is by Reese (1921) who examined cloacal gland development in alligator embryos. He noted that the glands evaginate from a deep layer of the epidermis and become a solid mass of cells. Three distinct regions

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were observed in the parenchyma of the glands in the more advanced embryos: an outer layer of small, irregular cells with small nuclei; a middle layer of poorly staining, elongate cells with thick membranes; and an inner layer, bordering a central lumen, composed of large spherical or polyhedral cells with nuclei of variable size.

Disselhorst (1904) presented a single drawing of a cross-section of a 'paracloacal musk gland' (Moschusdrüse) from an adult male alligator, along with the only available histological description of these glands in an adult of this species (*A. lucis* = *A. mississippiensis*). The gland contained numerous small spaces filled with fat cells and had a structure Disselhorst likened to that of a mammalian Cowper's gland. Reese (1921), who examined specimens of an undetermined *Caiman* species, Pettit & Geay (1904), who report on a specimen of the Spectacled caiman (*Jacaretinga sclerops* = *Caiman crocodilus*), and Voeltzkow (1899), who examined glands of a Nile crocodile (*Crocodilus madagascariensis* = *Crocodylus niloticus*) provide detailed histological descriptions of cloacal glands in other post-hatchling crocodilians (see also Rathke, cited in Voeltzkow, 1899). These investigators, in contrast to Disselhorst, observed only a single, large, fat- or paste-filled lumen in the glands.

We describe here the microscopic anatomy of the cloacal 'musk' glands of adult American alligators and report the results of histochemical tests on secretions of the glands. We hereafter refer to these glands as paracloacal glands.

Materials and methods

Glands were obtained during August and September 1985 from 6 adult male *A. mississippiensis* (total lengths = 1·7–2·4 m). One specimen was from Grand Chenier, Louisiana; the others were from Port Arthur, Texas. The cloaca of an alligator and the site from which secretions are released from the glands is shown (Plate I a).

The glands were excised within 1 h of being killed, and one of each pair was placed into either 10% neutral buffered formalin or Carson's modified Millonig's phosphate-buffered formalin (Sheehan & Hrapchak, 1980). Glands examined by light microscopy were sectioned in longitudinal and cross-section 4–7 μm and routinely stained with haematoxylin (H) and eosin (E). Formalin-fixed sections were also stained using a modified alcian blue technique for acid mucopolysaccharide (Thompson, 1966); an ammoniacal silver carbonate technique for reticulum, collagen and elastin (Humason, 1962); and a modified Feulgen

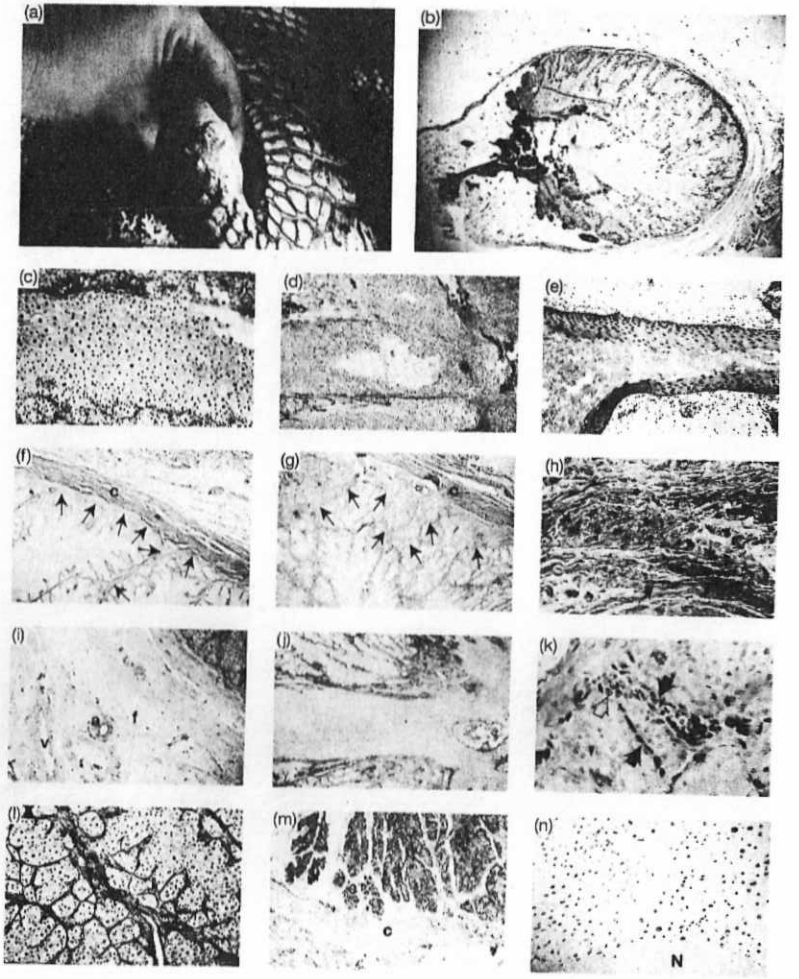

PLATE I. (a) Cloaca of alligator showing region from which paracloacal glands were excised. (b) Longitudinal section through paracloacal gland at region of secretory duct (left). (×7). (c) H & E stained longitudinal section of cells at central cylinder of paracloacal gland. (×67). (d) H & E section of central region of paracloacal gland with degenerative region (right). (×16). (e) H & E section of secretory duct (left) exiting gland. (×65). (f) Alcian blue section of capsule taken from an area of the gland opposite the duct. Light area at tip of arrows stained blue. The remainder of the gland stained magenta. c = capsule. (×20). (g) Alcian blue stained section of region near secretory duct. Arrows indicate blue region. c = capsule. (×25). (h) Ammoniacal silver carbonate stain of capsule in region of secretory duct. Dark regions, as indicated by arrows, are brilliant red, indicating elastin. The remainder is blue, indicating collagen. (×65). (i) Connective tissue components—veins (v), arteries (a) and adispose tissue (f)—surround the gland proper. H & E. (×25). (j) Alcian blue section of central cylinder and necrotic region. Note abrupt termination of septa near these regions. (×16). (k) Section demonstrating blood cells within septa. Closed arrow head = erythrocytes; open arrow head = leucocytes. Alcian blue. (×175). (l) Ammoniacal silver carbonate stain demonstrating cell clusters separated by connective tissue septa near the periphery of the gland. (×65). (m) Section stained with Oil Red O demonstrating lipid nature of parenchyma. c = capsule. (×16). (n) Fuelgen stain demonstrating dark, flat nuclei near necrotic area (N) and the abrupt loss of cell nuclei. (×67).

reaction for DNA (Troyer, 1980). Frozen cryostat sections were stained using an Oil Red O method for lipids (Sheehan & Hrapchak, 1980). Each histochemical technique was performed on each gland obtained.

Glands placed in Carson's fixative were post-fixed in osmium tetroxide for electrom microscopy. They were dehydrated in graded series of ethanol and embedded in Spurr's low-viscosity resin for ultramicrotomy. Sections exhibiting a gold to silver interference colour were viewed on a Philips 420 and a Hitachi H-500 electron microscope.

Results

Examination of the gland by light microscopy reveals a large, oval, encapsulated structure with a single duct (Plate I b, e). The gland contains large polyhedral cells that progressively degenerate as they approach the secretory end (Plate I c, d, j). Cells at the periphery of the gland are relatively small, but they enlarge toward a central cylinder of coagulated cells that runs parallel to the long axis of the gland. The central cylinder terminates in a necrotic area that empties into a lumen leading to a secretory duct (Plate I e).

The capsule has a thin layer of connective tissue surrounded by a thicker layer of smooth muscle (Plate I f). The connective tissue layer stains faintly blue with ammoniacal silver and brightly blue with alcian blue (Plate I f, g). This layer becomes thicker in the region of the secretory duct (Plate I g) where elastin and collagen fibres become prominent (Plate I h). Loose connective tissue and adipose tissue containing arteries, veins and nerves surround the smooth muscle layer (Plate I i; Plate II b).

Projections or septa arising from the connective tissue portion of the capsule extend into the substance of the gland to the region of the central cylinder (Plate I b, f, j). These septa, in which blood cells are observed (Plate I k; Plate II b), divide the glands into progressively smaller

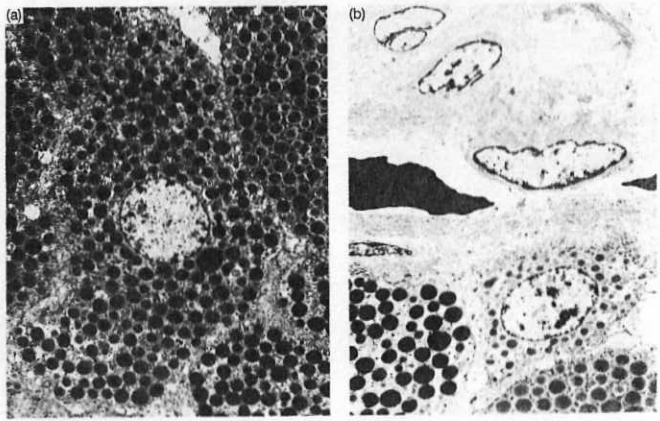

PLATE II. (a) Electron micrograph of parenchymal cell demonstrating abundance of lipid droplets. (×3000). (b) Electron micrograph of septal area with marginal parenchymal cells at bottom of figure. (×3,300).

subdivisions at the periphery (Plate I l). Septa are absent at the secretory end (Plate I j) and do not extend into the region of the central cylinder (Plate I c, d, j). Ammoniacal silver stain reveals that the larger septa contain collagen and elastin fibres (Plate I h); the smaller partitions stain black for reticular fibres (Plate I l).

The parenchymal cells near the periphery of the gland vary in shape from flat to cuboid, are relatively small, and appear to be arranged in small clusters (Plate I l). As the cells approach the central cylinder they become larger, measuring up to 75 μm in length, and polyhedral in shape (Plate I c). These cells have a prominent oval nucleus and a clear cytoplasm. They do not stain with alcian blue except for the plasma membrane and nucleus which are magenta. They stain strongly positive with Oil Red O (Plate I m), and electron microscopy reveals that their cytoplasm is filled with electron dense droplets of liquid uniformly dispersed throughout the cytoplasm (Plate II a). The nuclei of these cells are centrally located and contain sparse heterochromatin, indicating that they are transcriptionally active. Mitochondria and other cell organelles are observed only occasionally.

As the parenchymal cells approach the secretory pole their cytoplasm becomes more translucent and their plasma membranes appear to thicken. The membranes of these cells are basophilic, but at the secretory pole they abruptly become acidophilic. The nuclei of the cells at the region of abrupt transition become pycnotic and karyolyse (Plate I n). The cells degenerate into an acidophilic secretion that passes into the lumen of the duct (Plate I b, d, e, j). This secretion stains strongly positive with Oil Red O for lipids.

The single, large duct of the gland is lined with stratified squamous epithelium and is surrounded by loose connective tissue (Plate I c).

Discussion

Reptiles, overall, possess an array of glands in the cloacal region. Whiting (1969) recognized as many as 11 such glands among the amphisbaenians, lizards and snakes that she surveyed. As a result of Whiting's study and that of Gabe & Saint Girons (1965), the histology of reptile cloacal glands is best known in the squamates. In crocodilians, the only macroscopic glands in the cloacal region of which we are aware are the paracloacal glands. The evolutionary relationships between these glands and those of other reptile orders is uncertain. It seems best, as Quay (1972) suggested, to regard them as histogenetically related but non-homologous secretory structures.

A distinctive feature of the alligator paracloacal glands is the presence of extensive septa radiating from the connective tissue layer of the capsule to a central, cylindrical conglomerate of cells. A similar organization has been described in the glands of *Caiman* (Pettit & Geay, 1904; Reese, 1921) and *Crocodylus* (Voeltzkow, 1899). Disselhorst (1904), on the other hand, mentioned neither septa nor a central cylinder in the alligator gland he examined. Moreover, there are no indications in our study, or in others, of the small scattered spaces Disselhorst depicted in the periphery of the gland. As Disselhorst himself acknowledged, part of some other gland (not excised by him) may have been examined.

Based apparently on information provided by Reese (1921), Madison (1977) suggested that paracloacal glands exhibit either holocrine or apocrine secretion. Our observations indicate that they, like many other reptilian skin glands (Quay, 1972), are holocrine: cells at the periphery of the gland move toward the central cylinder, abruptly degenerate near the secretory pole, and liberate their contents. The positive reaction obtained for lipids in alligator secretions is consistent

with the identification of alcohols, including citronellol, and other lipids in *Caiman* paracloacal glands by Fester & Bertuzzi (1934) and Fester, Bertuzzi & Pucci (1937).

The conglomerate of cells that comprises the central cylinder is most likely formed as a result of hypoxia and lack of nutrients since the septa, in which we have found evidence of blood channels, do not extend into this region. Pettit & Geay (1904) reported that an extensive capillary bed is present in all regions of the Spectacled caiman paracloacal glands, except in a desquamate area within the gland. Voeltzkow (1899) also observed blood vessels and 'lymphatic bodies' in the gland of a Nile crocodile.

The neural mechanisms by which secretions are discharged from any of the array of reptile cloacal glands have not been investigated. Even the effector organs that force secretions from the glands are not known in many cases (cf. Whiting, 1969). Paracloacal glands probably discharge their secretions by contracting the smooth muscle in the outermost layer of the capsule, but other muscles also may participate. Gadow (1887), for example, described a sphincter muscle in *Alligator* and *Crocodylus* that, when contracted, everts the gland. He also mentioned a non-striated 'cord' that extends, in the male at least, from the smooth muscle of the gland capsule to the crura penis. The effects of the contraction of these muscles in the eversion of the paracloacal glands or on the expulsion of their secretions remain to be examined.

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