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HISTOCHEMICAL AND LECTIN-HISTOCHEMICAL STUDIES OF THE SECRETION FROM THE UROPYGIAL GLAND OF THE ROCK DOVE *Columba livia* (COLUMBIDAE-COLUMBIFORMES)

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ABSTRACT

The uropygial gland of *Columba livia* was studied using standard histochemical and lectin-histochemical methods. Acidic mucins, neutral lipids, glycolipids and phospholipids were normal components of the tubular secretion. The use of lectins showed the distribution of glycoconjugates in the normal secretion. There were no differences between males and females. The uropygial secretion was a mixture of lipid and carbohydrate compounds, the composition of which varied according to the stage of cellular differentiation and secretion formation.

Key words: Histochemistry, lectins, uropygial gland

INTRODUCTION

The uropygial gland is the major sebaceous gland in most birds; but its removal does not affect the survival of passerine birds, hens and pigeons [7,14]. The bilobed gland is enclosed in a capsule of connective tissue and lies at the base of the tail. Each lobe has a central cavity that collects the secretion from tubules arranged radially around the cavity [7]. Wagner and Brood [19] described three zones (I, II and III) based on the length of the tubules corresponding to the peripheral, middle and inner portions of the tubules, respectively. The gland secretion is conveyed to the surface via ducts that, in most birds, open at the top of a papilla [12].

The histological organization is that of a sebaceous gland. The tubular epithelial cells are classified into four well defined layers: a) a germinative layer where cell division occurs and which consists of one or two strata of flat or cuboidal cells with a basophilic cytoplasm and dark nucleus, b) an intermediate layer consisting of 1-5 strata of polygonal cells with a basophilic cytoplasm, c) a secretory layer formed by 1-10 layers of voluminous polygonal cells with secretory granules, d) a degenerative layer characterized by cells with pyknotic nuclei and keratohyaline granules in the cytoplasm. Cell fragments, corneous plates and secretion form the sebum [10].

The construction of the tubule walls is similar in most avian species, with the exception of the Columbiformes. The structure of the lobe is very peculiar in *Columba livia*. The epithelial cells are surrounded by connective tissue septa and do not form tubules that join in a central cavity. At the periphery of the cell bundles there is a small germinative layer in which the intermediate cells and voluminous secretory cells are arranged in a mosaic-like pattern. Degenerating cells are rarely seen [10].

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The uropygial gland is a holocrine gland and its lipid secretion is produced by cells at the bottom of the tubules. Close to the central cavity, epithelial cells degenerate and finally rupture to release their contents into the tubule lumen [12]. The oily material produced by the gland is a variable mixture of compounds composed mainly of esters high molecular weight branched fatty hydroxyacids and long-chain alcohols [6,13,18].

Early studies showed that the secretion served as a water repellent, which prevented the birds from getting wet [11]. However, we have questioned this hypothesis by showing that the size of the gland is not correlated with aquatic/terrestrial nature of the species [16]. The chemical composition of the gland secretion was suggested to be a valuable taxonomic tool [7]. While others have implicated the secretion in pheromone production and antirachitic and antibiotic effects [11]. Piersma *et al*. [17] reported significant seasonal changes in the chemical composition of the gland secretion of the red knot *Calidris canutus*, with a shift from monoester waxes to much higher molecular weight diester waxes. The change in the viscosity of the waxes was considered to facilitate the formation of a more brilliant plumage necessary for courtship.

The histochemical differentiation of lipids and polysaccharide-protein complexes in normal and pathological tissues is important in studies of normal and diseased states [3].

Lectins, which are cell-agglutinating glycoproteins with a specificity for simple or complex carbohydrate groups, are present in a variety of plants and animals. The specificity of lectins for sugar groups makes them useful tools for studying carbohydrate structures on the cell surface [5].

In the present study histochemical and lectin-histochemical techniques were used to study the uropygial glands and secretion of the rock dove, *Columba livia* (Columbidae, Columbiformes).

MATERIAL AND METHODS

Six wild adults (225-423 g) *C. livia* of both sexes (3M, 3F) collected in autumn near La Plata City (Buenos Aires Province, Argentina) were used. The birds were housed at 20-22°C with a natural photoperiod and were provided with a mixture of grains and water *ad libitum* for ten days. The pigeons were sacrificed under mild anesthesia and the uropygial glands were removed as described [15], then fixed in 10% neutral buffered formalin, trimmed, and processed for embedding in paraffin.

Sections 4 µm thick were stained with hematoxylin and eosin. The histochemical and lectin-histochemical methods used have been described elsewhere [4,2]. Sections were stained with alcian blue/PAS at pH 2.5 and PAS methods. For lipid detection, fresh cryostat sections were stained with McManus's Sudan black B, oil red O and Cain's Nile blue sulphate method. Seven biotinylated lectins (Vector Lab., Burlingame Co., CA, USA) were used, as shown in Table 1.

Sections were dewaxed with xylene and rehydrated in graded ethanol solutions. Endogenous peroxidase was blocked with 3% hydrogen peroxide in absolute methanol for 30 min at room temperature. The sections were rinsed several times in 0.01 M phosphate buffered saline (PBS) solution, pH 7.2, and treated with 1% bovine serum albumin in PBS for 15 min before incubating with the biotinylated lectins for 12 h at 4°C. The sections were then incubated with an avidin-peroxidase complex for 45 min at room temperature (ABC, Vector Lab., Burlingame Co., CA, USA). The sections were subsequently rehydrated in PBS and the peroxidase reaction was developed with 0.02% 3,3'-diaminobenzidine (DBA; Sigma Chem. Co., St. Louis, MO, USA) in 0.05 M Tris-HCl (pH 7.6) for 5-10 min at room temperature. Harris' hematoxylin was used as a counterstain.

Table 1. Lectins used for identification of carbohydrate residues in paraffin embedded tissue sections.

Systematic name	Acronym	Specificity	Concentration (µg/ml)
Concanavalin ensiformis	Con A	α D-Man, α D-Glc	30
Ulex europaeus	UEA-1	α L-Fuc	30
Glycine maximus	SBA	α D-GalNac, α D-Gal	30
Dolichus biflorus	DBA	α D-GalNac	30
Arachis hypogaea	PNA	β D-Gal (β 1-3)-DGalNac	30
Ricinus communis	RCA-1	β D-Gal	30
Triticum vulgaris	WGA	β D-GlcNac, NANA	10

Fuc-fucose, Gal-galactose, GalNac-N-acetylgalactosamine, Glc-glucose, GlcNac-N acetylglucosamine, Man-mannose, NANA-N-acetylneuraminic acid.

Lectin binding intensity was graded semi-quantitatively as: negative (-), weak (+), strong (++) or very strong (+++) (Table 2). As controls, the biotinylated lectins were omitted during staining and each lectin was blocked by incubating it with its haptenic sugar for 1 h at room temperature (20-25°C) before incubating with the sections.

RESULTS

The cells of the degenerative layer and the tubule secretion stained lightly with alcian blue (Fig. 1). The tubule secretion also stained positively in the PAS reaction.

The secretion, cell fragments and corneous plates present in the tubule lumen stained black or red with Sudan black B and oil red O stains, respectively. The pattern of staining obtained with Sudan black B showed the presence of lipid compounds, probably phospholipids, in the secretory cells and secretory products (Fig. 2). Staining with oil red O showed that lipofucsins were present in the glandular secretion. The cells of the intermediate, secretory and degenerative layers stained pink with Cain's Nile blue sulphate (Fig. 3).

The lectins reacted with the tubule cells in different intensities. PNA, WGA and RCA-1 showed a moderate to high affinity for cells of the germinative layer (Figs. 4 and 5). Con A (Fig. 6) produced strong staining in cells of the degenerative layer and in the secretion present in the tubule lumen. Cell debris and secretion products in the lumen showed high affinity for WGA. Intermediate and secretory cell layers showed no significant affinity for any of the lectins (Table 2).

There were no histological or histochemical differences between male and female pigeons.

DISCUSSION

Alcian blue staining showed that carboxylated acidic mucins, probably sialidase-sensitive sialomucins, are present in the gland secretion. Since mucins are capable of forming viscous solutions, it is possible that these molecules act as lubricants or protectants on the body surfaces. The positive PAS staining of the tubule secretion products supported the presence of enzyme labile sialomucins and demonstrated that lipid compounds, particularly glycolipids, occur in the secretion.

The pink colour obtained with Cain's Nile blue sulphate confirmed the presence of simple lipids, specially neutral lipids (palmitic or stearic acid derivatives), in the secretory cells and other components of the tubule secretion. These findings agree with those reported by Jacob and Zeman [9] for *Columba palumbus*. It is interesting that this similarity in the concentration of particular fatty acids was found in taxonomically related species. A previous study [6] reported the detailed fatty acid composition of lipids from the uropygial gland of *C. livia* and showed that 57% of the neutral fatty acids were by palmitic and stearic acids.

The lectin binding pattern of the germinative cells showed that these cells had N-acetylglucosamine/galactose components, whereas cells of the intermediate and secretory

Table 2. Lectin binding pattern in the uropygial gland of *Columba livia*.

Lectin	Cellular layer				Secretory product
	Germinative	Intermediate	Secretory	Degenerative	
PNA	++	-	-	-	-
WGA	+++	-	-	-	+++
RCA-1	+++	-	-	-	-
UEA-1	-	-	-	-	-
SBA	-	-	-	-	-
DBA	-	-	-	-	-
Con A	-	-	-	+++	+++

Intensity of binding: (-) negative, (+) weak, (++) strong, (+++) very strong. For lectin abreviations, see Table 1.

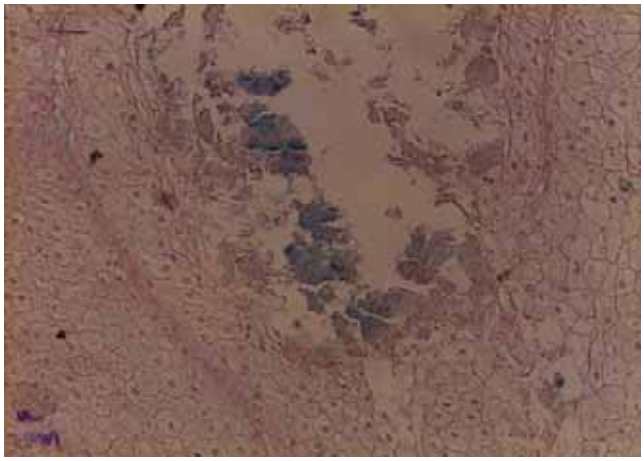

Figure 1. Alcian blue staining of degenerative layer and tubule secretion. Bar = 25 µm.

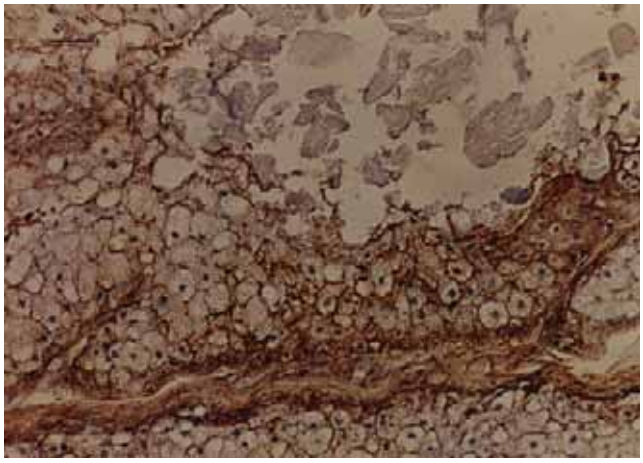

Figure 4. Binding pattern of RCA-1 to cells of the germinative layer. Bar = 25 µm .

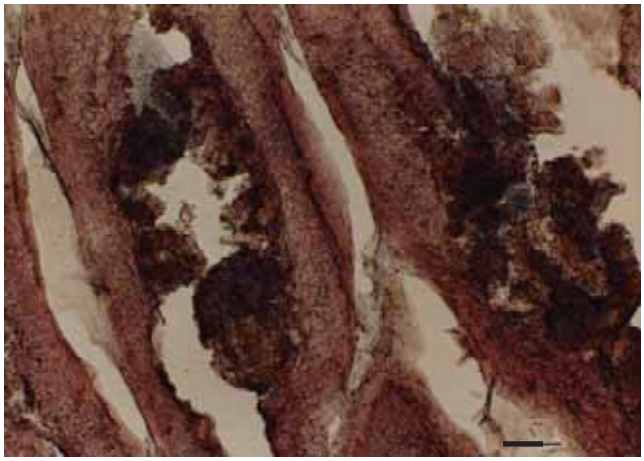

Figure 2. Sudan black B staining of secretory and degenerative cells. Bar = 50 µm.

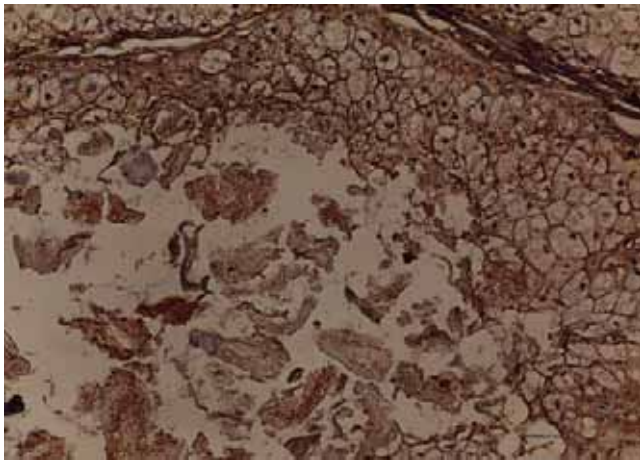

Figure 5. Binding pattern of WGA to cells of the germinative layer and secretory products. Bar = 25 µm.

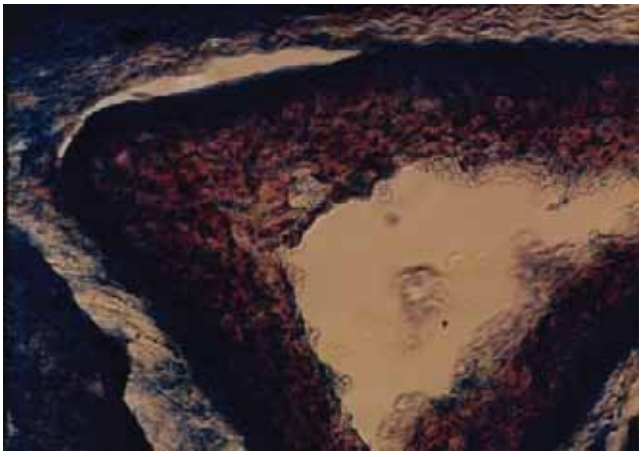

Figure 3. Cain's Nile blue sulphate staining of cells of the intermediate, secretory and degenerative layers. Bar = 50 µm.

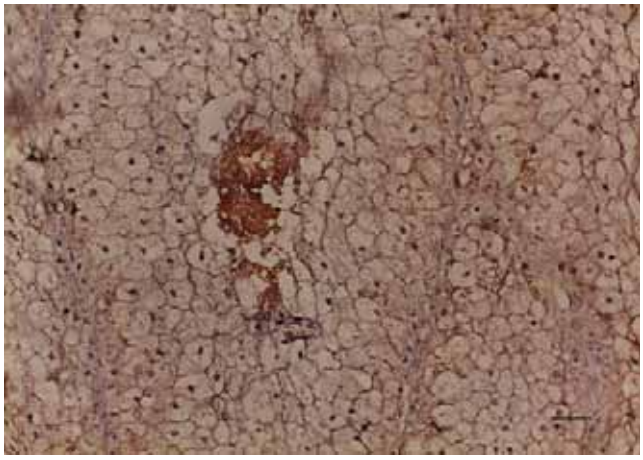

Figure 6. Binding pattern of Con A to cells of the degenerative layer and secretory products. Bar = 25 µm.

layers did not react to lectins. Degenerative cells and tubule secretion showed positive responses for N-acetyl glucosamine and glucose/manose groups. Asnani and Ramachandran [1] showed that the activity of the uropygial gland is greater during the breeding season. Our results were obtained with birds collected in the non-breeding season. Consequently our findings may represent the basal state of the gland. In contrast to our data, Jacob *et al*. [8] demonstrated sexual variation in the chemical composition of the gland secretion in domestic ducks during the reproductive season.

In conclusion, the composition of the uropygial gland secretion of *C. livia* is a complex mixture of lipid and carbohydrate compounds, the composition of which varies according to the stage of cell differentiation and secretion production. This secretion may be involved in protecting the body surface of the birds from the environment.

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