STRUCTURAL AND CYTOCHEMICAL STUDY OF SALIVARY GLANDS IN THE MAGELLANIC PENGUIN *SPHENISCUS MAGELLANICUS* AND THE KELP GULL *LARUS DOMINICANUS*

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It has been reported that salivary gland development of avian species is related to diet (Grasee 1950, Pisanó & Barbieri 1967, Farner & Ziswiller 1972). Hence, granivorous birds that feed on dry food possess better developed salivary glands than do rapacious species. On the other hand, in birds having access to naturally well-lubricated foods, the buccal glands show little development (Forstner 1978, McLelland 1979).

Nevertheless, Avila *et al.* (1989) and Samar *et al.* (1987, 1988, 1993) have demonstrated a considerable development of buccal and lingual glands in the chick embryo and in the adult chicken. The same authors have described in the Lorikeet *Myiopsitta monacha* the presence of buccal glands with a well-structured conformation and intraepithelial acini within the tongue of this species (Samar *et al.* 1992).

To analyse the existence of the buccal salivary glands and their probable functional role, an histological and cytochemical analysis was undertaken on two seabirds, the Magellanic Penguin *Spheniscus magellanicus*, which feeds on fish, crustaceans and cephalopods (Vigil 1973, Yofre *et al.* 1983) and the Kelp Gull *Larus dominicanus*, which is a predatory and scavenging species (Narovsky *et al.* 1984, Ward 1991).

Magellanic Penguins (n=4) and Kelp Gulls (n=5) were collected from the southeast coast of Argentina. The animals were sacrificed in accordance to international protocols for biomedical investigations and samples of salivary glands from the walls of the buccal cavity and tongue removed. Samples were subjected to perfusion fixation and afterwards fixed in 10% formalin at pH 7.4 in a phosphate buffer. The tissues were then dehydrated and embedded in paraffin. Serial sections were cut at 3–4 μm, deparaffinized, hydrated and then subjected to the following procedures (Samar & Avila 1991).

1. Histological staining procedure. *Hematoxylin-eosin*: Routinely used for the general observation of histological structures.

2. Cytochemical staining procedures. These were carried out to identify the chemical products elaborated by the salivary glands' cells.

Periodic Acid – Schiff (PAS): For demonstrating vicinal diol-containing glycoconjugates. A PAS positive reaction produces an intense magenta colour, mainly indicating the presence of glycoproteins and glycogen. *PAS-amylase*: Samples were exposed to enzymatic digestion with salivary amylase. PAS positive substances which disappear after enzymatic action represent glycogen. *Toluidine blue at pH 3.8*: This stain acquires histochemical significance when used at this pH; basophilic (nuclei and ergastoplasm) and metachromatic substances can be identified; sulphated glycosamineglycans (GAG) give a strong alcohol-resistant metachromasia, while non-sulphated GAG and nucleoproteins give a weak methacromasia that is susceptible to alcohol extraction. *Alcian Blue*: was used to study the interaction with tissue polyanions. This polivalent basic dye is selective for the staining of negatively charged macromolecules, and was used at pH 2.5 and 1.0. At pH 1.0 it reacts with sulphated acidic glycosamineglycans and results in a deep blue colour, because of the presence of copper in the molecule.

Blocking (methylation) and saponification (demethylation) reactions: Used to confirm the presence of glycosamineglycans with sulphated and carboxyle groups stained with Alcian blue. Methylation at 37°C esterifies carboxylic groups and blocks the alcianophilia of mucosubstances containing these groups; substances having sulphate groups remain unaffected. Methylation at 60°C blocks alcianophilia of carboxyle groups and hydrolizes
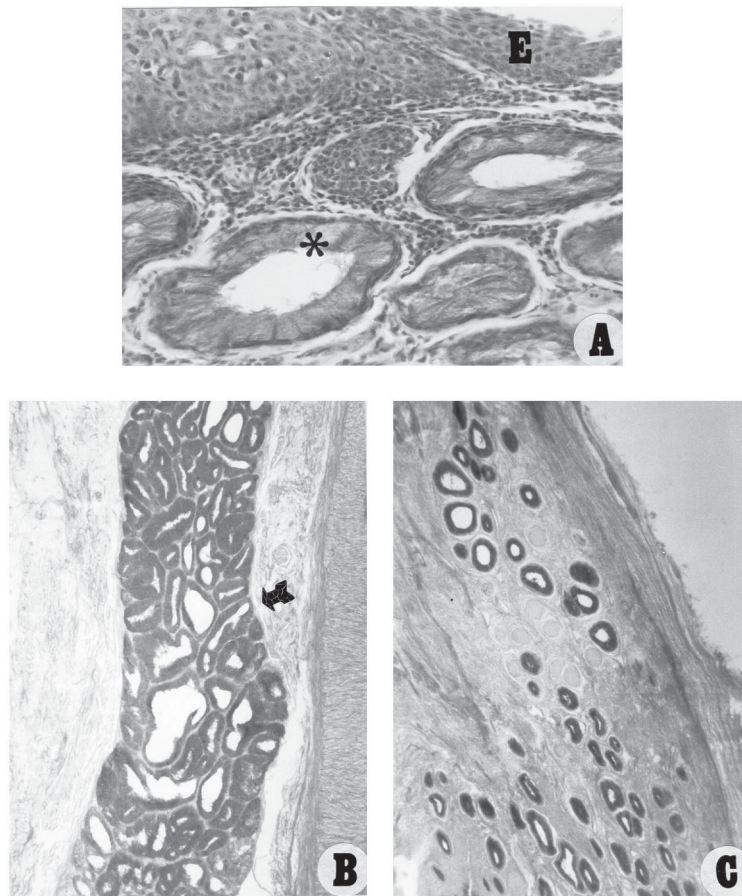
Figure 1. *Magellanic Penguin* Spheniscus magellanicus:

a: *Palate alveolar salivary glands. The secretory epithelium consists exclusively of mucous cells (asterisk). Superficial epithelium (E). Hematoxylin and Eosin stain. 400X.*
b: *Alveolar salivary glands reveal a strong PAS reaction which is resistent to α-amylase digestion (arrow). PAS stain. 250X.*
c: *Alveolar salivary glands. The cells present an intense metachromasia at the floor of the mouth cavity. Toluidine blue pH 3.8 stain. 250X.*

sulphate groups, which are lost to the medium. Demethylation made after methylation at 37°C unblocks carboxylic groups, and alcianophilia is reestablished. Demethylation after methylation at 60°C restores alcianophilia of carboxylic groups but not of sulphate groups, which are hydrolysed and lost to the medium.

Digestion with neuraminidase (sialidase): Selective enzymatic remotion of sialic acid with neuraminidase is used to identify sialic acid residues of glycoconjugates sialoglycoproteins and sialoglycans. The difference in staining between control and neuraminidase-treated samples after staining with PAS and Alcian blue at pH 2.5 indicates the existence of accessible sialic acid.

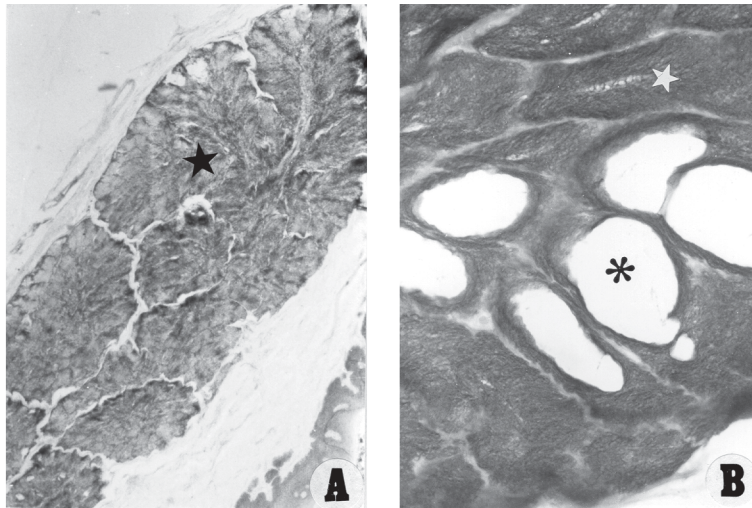Salivary gland development distributed in the wall of the

Figure 2. *Kelp Gull* Larus dominicanus:

a: Gland lobes composed by PAS reactive acini (star). PAS stain. 250X.
b: Acini (star) and alveoli (asterisk) glands with PAS positive glycoproteins. PAS stain. 400X.

mouth cavity was observed in both species. In the Magellanic Penguin the mouth cavity salivary glands were mucous with alveoli having a large lumen. In the floor of the mouth cavity some glands were located within the epithelium, but others were in the subepithelial layer. Palate areas showed abundant glands surrounded by mononuclear cells (Fig. 1a–c). Lingual glands appeared in the ventral region and were predominantly mucous and alveolar. In comparison, glands of the Kelp Gull showed an acinar structure, but they had a mucous appearance like that of the Magellanic Penguin (Fig. 2a–c). In both species, PAS-positive, alcianophilic and metachromatic mucosubstances were located in the mucous cells of glands and in the lumen of acini and alveoli. The cells were strongly reactive with PAS and their cytoplasm was filled with numerous amylase-resistant, bright purple granules. The lumen of acini and alveoli was also filled with PAS-positive material. Alcianophilia, as revealed by a deep blue coloration with Alcian blue at pH 2.5 and 1.0, indicated a strong reaction in the mucous-secreting units and in the lumen. When Toluidine blue stain was carried out at pH 3.8 an intense alcohol-resistant metachromasia could be observed.

Neuraminidase produced a decrease of PAS and Alcian blue at pH 2.5 reactions, but the α-amylase did not exert any effect on PAS positive substances in glands of both birds, indicating the presence of glycoproteins. Blocking reactions showed that the sulphated glycosamineglycans were increased in relation to the nonsulphated molecules in the glands of the two species.

The comparative morphology of salivary glands has been studied by many investigators for more than a century (e.g. Reichel 1883, Greschik 1913, Bock 1961, Foelix 1970) and the adaptation of these glands according to feeding habits, has been thoroughly described by Antony 1920 (cited by Ziswiller & Farner 1972).

It is generally agreed that, in fish-eating birds, whose intake is composed of wet food, salivary glands are poorly developed (Grasee 1950, Pisano & Barbieri 1967, Farner & Ziswiller 1972). However, from the results of the present work, it is evident that salivary gland development is important and that glycoprotein and glycosamineglycan secretions are abundant in the two seabirds studied, despite the fact that both seabirds feed on moist food (Yofre *et al* 1983, Ward 1991). We

can not offer a ready explanation to account for this apparent discrepancy. Moreover, penguins have long, pointed tongues, the upper surface being covered by numerous conical sharp, horny papillae pointing backwards to manipulate and direct slippery food towards the the esophagus. By comparison, gulls have tongues which do not appear to be specially adapted either for collection, manipulation or swallowing of food (McLelland 1979). In any event, it has to be kept in mind that the feeding habits of the Kelp Gull differ markedly from those of the Magellanic Penguin.

The oldest known function of salivary glands is to supply lubricatory molecules (Heidrich 1908, Chodnik 1948, McCaillon & Aitken 1953). These molecules not only coat the food, but the oral soft tissues as well, thus exerting a protective action on the mucosal surface (Young & Van Lennep 1978, Mandel 1987). Furthermore, salivary mucins possess properties (low solubility, high viscosity, adhesiveness), which enable them to concentrate on buccal mucosal surfaces, where they provide an effective barrier against dessication, while glycoproteins may exert a protective role against enzymatic acidic elements in contact with the mucosa. Additionally, sulphated glycosamineglicans may inhibit pathogens in the buccal cavity. It has been demonstrated that the sialic acid glycoconjugates conditions the hydrophilic environment to maintain the hydration of the mucosal surface, providing an effective barrier against bacterial activity (Farner 1978, Tabak *et al* 1982, Supraset *et al*. 1986).

It is possible that glycoconjugates secreted by the epithelium of buccal glands are necessary not only to protect mucous membrane integrity, but also to perform other functions, especially coating and softening food, facilating transit towards the stomach (Sharon 1981, Bee de Speroni & Chikilian 1983).

Further biochemical and histochemical studies of mucosubstances in the salivary glands of the Magellanic Penguin and Kelp Gull species are required to understand the relationship between ingested food and gland secretion.

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