

Morphology of the Complex Laryngeal Gland in the Atlantic Bottlenose Dolphin, *Tursiops truncatus*

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ABSTRACT

A complex lymphoepithelial gland is a constant feature in the larynx of Atlantic bottlenose dolphins, *Tursiops truncatus*, based on study of 56 animals.

Larynges were removed from fresh, non-decomposed beach-stranded animals for gross examination and histological sampling.

A large lymphoepithelial gland occurs in the rostro-ventral mucosa of the larynx, overlying the cricoid cartilage. It presents as a well-defined, elevated, and heavily trabeculated area. Histological examination reveals a pseudostratified columnar epithelium which branches into the underlying submucosa. The epithelial-lined folds and crypts thus formed are surrounded by aggregations of lymphocytes, which infiltrate this epithelium. Mucous glands are often associated with these lymphoid aggregations.

The histological appearance of the laryngeal gland is remarkably similar to the palatine, or dorsal oropharyngeal tonsils, of *T. truncatus*. It may be analogous to the nasopharyngeal adenoid of terrestrial animals. Age-related involution of the laryngeal gland is not as obvious with increasing animal age (or length) as it is in other mucosa-associated lymphoid tissue. The distribution of this gland among cetaceans is not yet known. We have observed it in individuals of every species we have studied, including *Lagenodelphis hosei*, *Stenella coeruleoalba*, *Stenella attenuata*, *Globicephala macrorhynchus*, *Steno bredanensis*, *Physeter catodon*, *Pseudorca crassidens*, *Mesoplodon europaeus*, and *Kogia breviceps*. Anat Rec 254:98–106, 1999. © 1999 Wiley-Liss, Inc.

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During a detailed study of the lymphoid system of the Atlantic bottlenose dolphin, *Tursiops truncatus*, a thick lymphoglandular structure was observed in the mucosa of the rostro-ventral aspect of the larynx. Despite many studies on the cetacean larynx, most of which are concerned with the vocal apparatus (Reidenberg and Laitman, 1988), very little information is available on this lymphoepithelial organ in this species. Simpson and Gardner (1972) briefly refer to the larynx of *T. truncatus*, and mention the presence of mucous glands and aggregations of lymphoid tissue in the submucosa. Anderson (1878) provides a drawing of what appears to be this structure in the Irriwaddy dolphin (*Orcella brevirostris*), describing it

as “multitudinous sacculations, both forming the floor and anteroinferior laryngeal wall.” Behrmann (1987) describes

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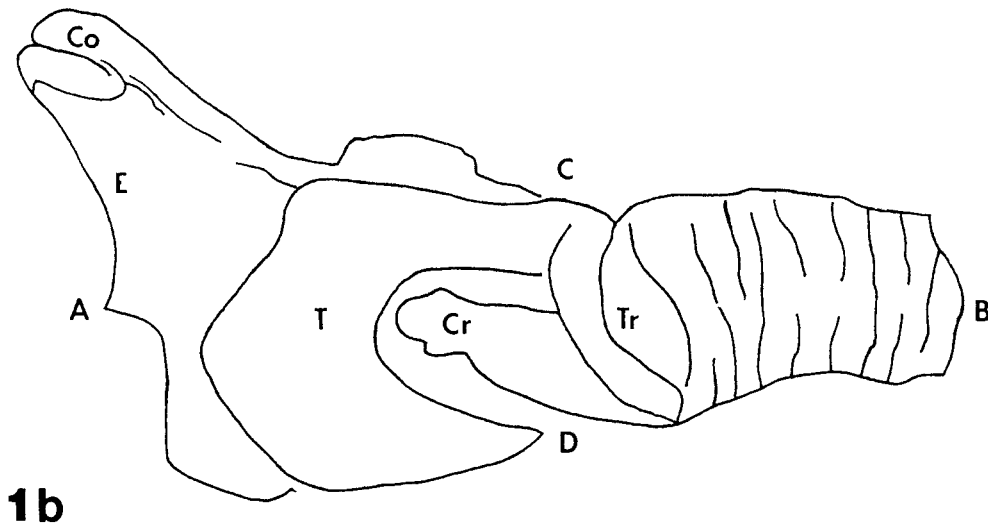


Fig. 1. **a:** *T. truncatus* larynx with the overlying muscle removed to allow visualization of the laryngeal cartilages. **b:** Schematic drawing of **a**. Co, corniculate cartilage; Cr, cricoid cartilage; E, epiglottis; T, thyroid cartilage; Tr, trachea. The arytenoid and cuneiform cartilages are obscured from view by the thyroid cartilage. A–B, C–D denote planes of dissection for Figures 3 and 4, respectively.

a “laryngeal sac” in this same area, which he interpreted as comparable to the lungs of young mammals or turtles; however, he acknowledges lack of suitable histological material to define the organ further. We are not aware of any other detailed, histologically supported studies of this gland in cetaceans. Two of the most recent studies of the vocal folds and position of the larynx of several species of odontocete illustrate the mucosal surface of this gland complex, but do not describe it histologically (Reidenberg and Laitman, 1987, 1988). Laryngeal glands have been described in several terrestrial mammals, including cats, shrews, and rats, although a lymphoid component of these

glands was not noted (Zaitso, 1976; Spicer et al., 1982; Nakano and Muto, 1990).

The odontocete larynx comprises the same homologous cartilages that are found in terrestrial mammals; epiglottis, corniculate, arytenoid, cuneiform, cricoid, and thyroid (Reidenberg and Laitman, 1987). The rostral extension of the larynx is formed by the epiglottic cartilage, which is caudally apposed by the elongated corniculates. These cartilages together form the laryngeal spout. The corniculates articulate with the dorso-rostral surface of the arytenoid cartilages, which in turn articulate with the dorsal surface of the cricoid cartilage. The cricoid cartilage opens

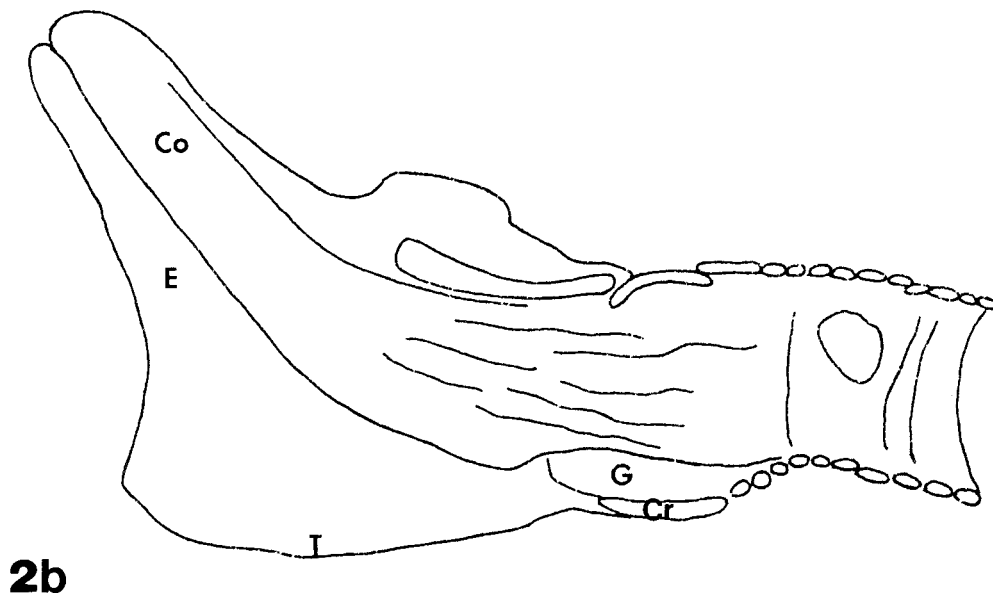


Fig. 2. **a**: Longitudinal section through the larynx of a small adult *T. truncatus*, revealing the laryngeal gland both in section and laterally as trabeculations. **b**: Schematic drawing of **a**. G, laryngeal gland. Refer to Figure 1b for other abbreviations.

ventrally; this opening is partially covered by the thyroid cartilage, which opens dorsally.

Some degree of confusion exists regarding the nomenclature of the paired laryngeal cartilages in cetaceans. This has been addressed and clarified by Reidenberg and Laitman (1987). We have adopted the nomenclature used in their study of the odontocete larynx. Due to the lack of information about the lymphoepithelial gland in cetaceans, our goal is to describe the morphological and histologi-

cal characteristics of this structure in several cetacean species, with emphasis on *T. truncatus*.

MATERIALS AND METHODS

Collection and Necropsy

The cetaceans included in this study were collected by the Texas Marine Mammal Stranding Network, under the auspices of the National Marine Fisheries Service. The

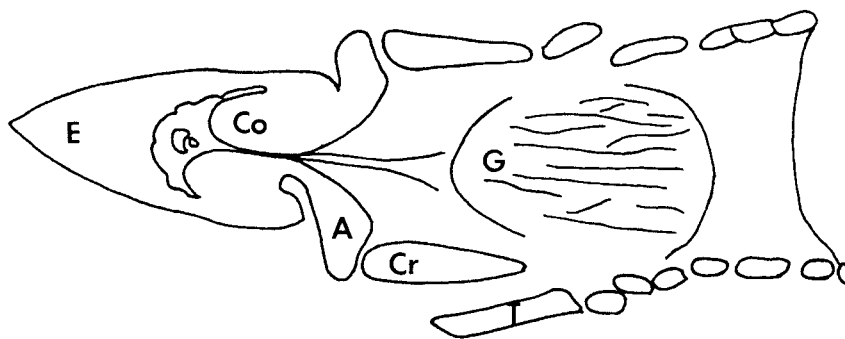
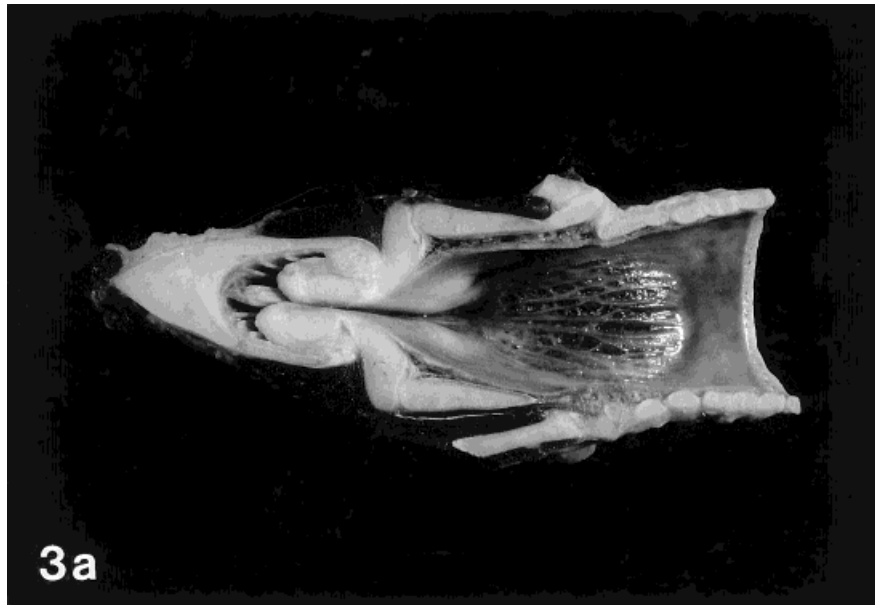


Fig. 3. **a:** Sagittal section of the larynx of a large adult *T. truncatus*, showing the well-defined border of the gland, its elevation above the mucosa of the larynx, and its heavily trabeculated structure. Length of the gland is 4.6 cm, width is 2.4 cm. Plane of section taken along the A–B axis as shown in Figure 1b. **b:** Schematic drawing of a. A, arytenoid cartilages; G, laryngeal gland. Refer to Figure 1b for other abbreviations.

collection area ranged from Brownsville at the Mexican border to Sabine Pass at the Louisiana border, i.e. the entire Texas Gulf coast. Occasionally, animals were also retrieved from adjacent Louisiana.

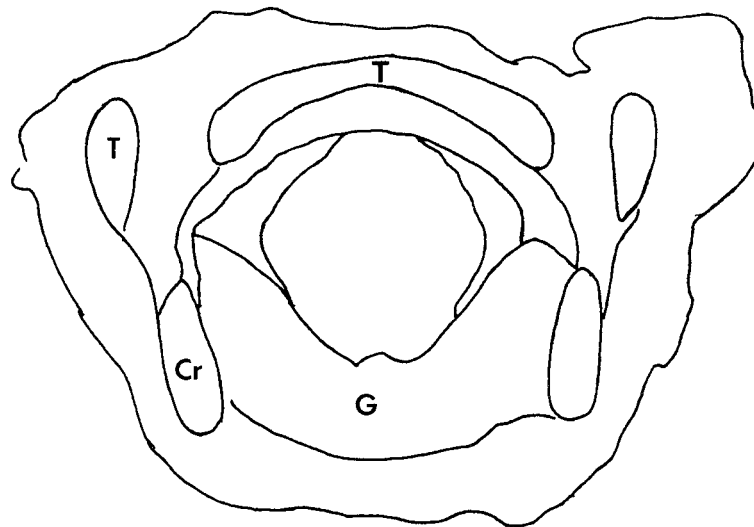
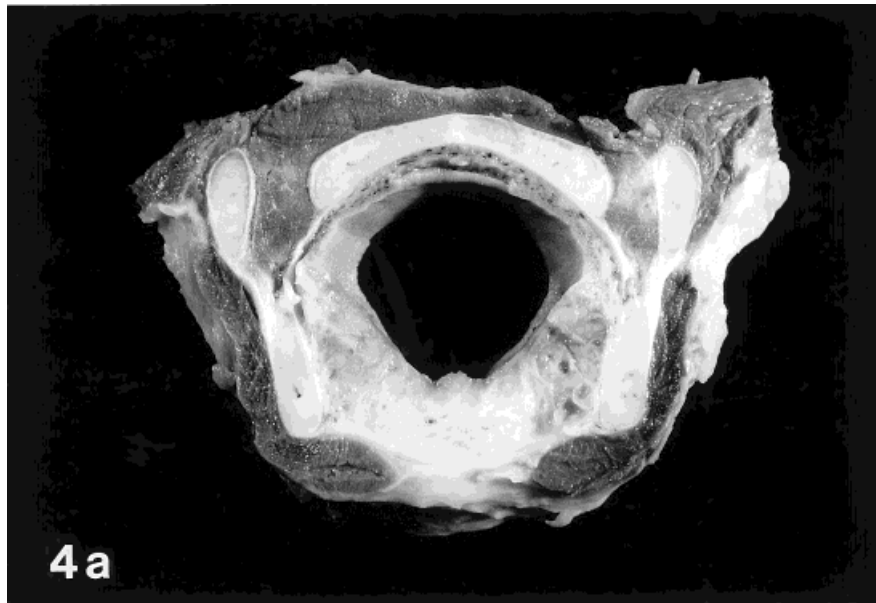
We examined 66 fresh stranded, non-decayed cetaceans, including 56 Atlantic bottlenose dolphins (*Tursiops truncatus*), one Fraser's dolphin (*Lagenodelphis hosei*), one striped dolphin (*Stenella coeruleoalba*), two spotted dolphins (*Stenella attenuata*), one short-finned pilot whale (*Globicephala macrorhynchus*), one rough-toothed dolphin (*Steno bredanensis*), one infant sperm whale (*Physeter catodon*), one false killer whale (*Pseudorca crassidens*), one Antillean beaked whale (*Mesoplodon europaeus*), and one pygmy sperm whale (*Kogia breviceps*).

These animals were taken to a central laboratory at Texas A&M University at Galveston for necropsy, which included gross examination and systematic histological sampling of all tissues and lesions. The animals had been dead approximately 2–20 hr before necropsy. The postmor-

tem interval was rarely ascertained with certainty because most animals were found dead. Suitability for this anatomical study was based on lack of evidence for putrefaction. Tissue preservation ranged from fair to excellent, except for the intestinal mucosae, which were usually somewhat autolyzed. Dissection was complete, with examination of all organs. During the course of necropsy, all viscera were removed, and all soft tissues were dissected from the skeleton. Tissues were collected in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin (H&E), or hematoxylin, phloxine and saffron (HPS), a trichrome stain.

Age Estimation

Sexual maturity of the animals in this study was determined by histological examination of the testes or ovaries. Males with mature spermatogenic cells and sper-



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Fig. 4. **a:** Cross-section of the larynx of a large adult *T. truncatus*, revealing the association between the gland and various cartilages. Maximum thickness of the gland is 1.4 cm. **b:** Schematic drawing of a. G, laryngeal gland. Plane of section taken along the C–D axis as shown in Figure 1b. Refer to the same figure for other abbreviations.

matozoa in the seminiferous tubules were considered sexually mature. Females with mature ovarian follicles, corpus luteum, or corpus albicans were considered sexually mature. Ages were determined for 30 of the animals by the method described by Hohn et al. (1989) in which the repeating patterns of dentinal laminations, or growth layer groups (GLG), were counted. Sexual maturity has been determined in *Tursiops* spp. to be associated with 8–12 GLG in females and 10–15 GLG in males (Mead and Potter, 1990). One GLG represents 1 year of age (Hohn et al., 1989).

Fig. 5. **a:** Cross-section through the laryngeal gland of an immature male *T. truncatus*. The arrows point to the large aggregations of lymphocytes which surround and permeate the epithelial-lined crypts. Mucous glands are not apparent in this section. Laryngeal cartilage at bottom, lumen at top. Magnification 10 \times , HPS stain. **b:** Wall of a mucosal infolding indicated by arrows in a, showing dense aggregation of lymphocytes (L), the lymphocyte-permeated epithelium (E), and intraluminal secretion (S) Magnification 160 \times , HPS stain. **c:** Non-ciliated pseudostratified columnar surface epithelium. Magnification 160 \times , HPS stain.

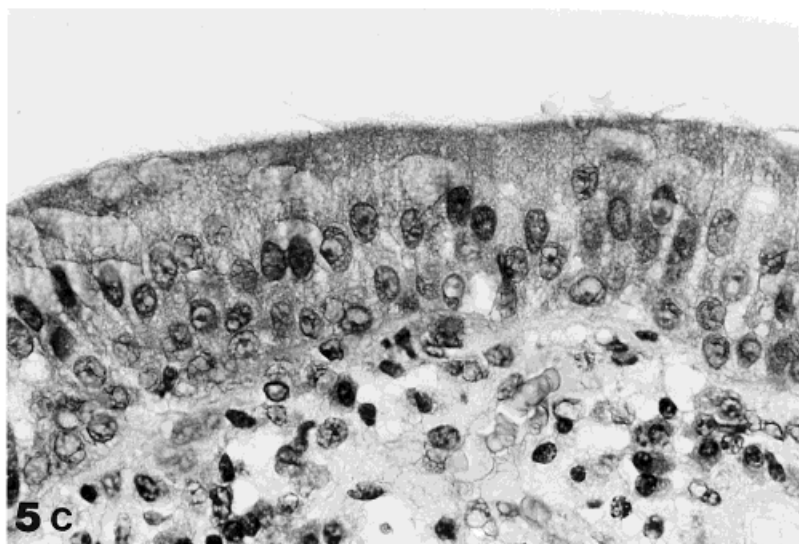
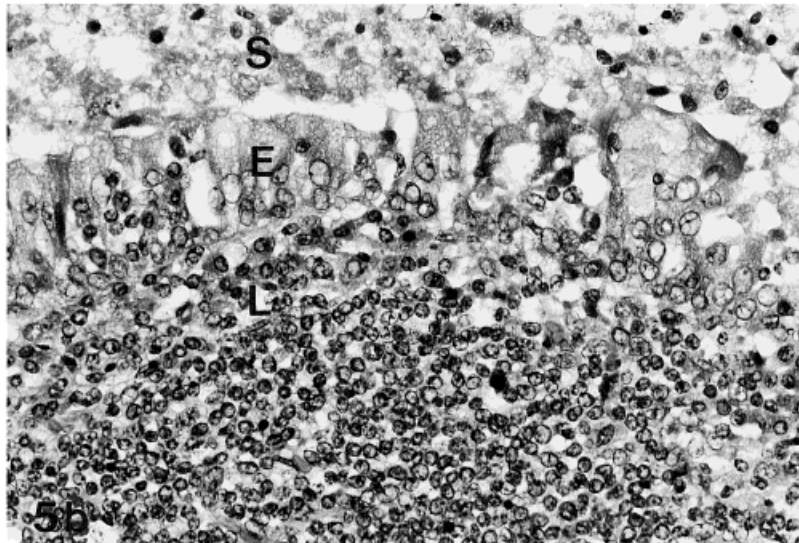
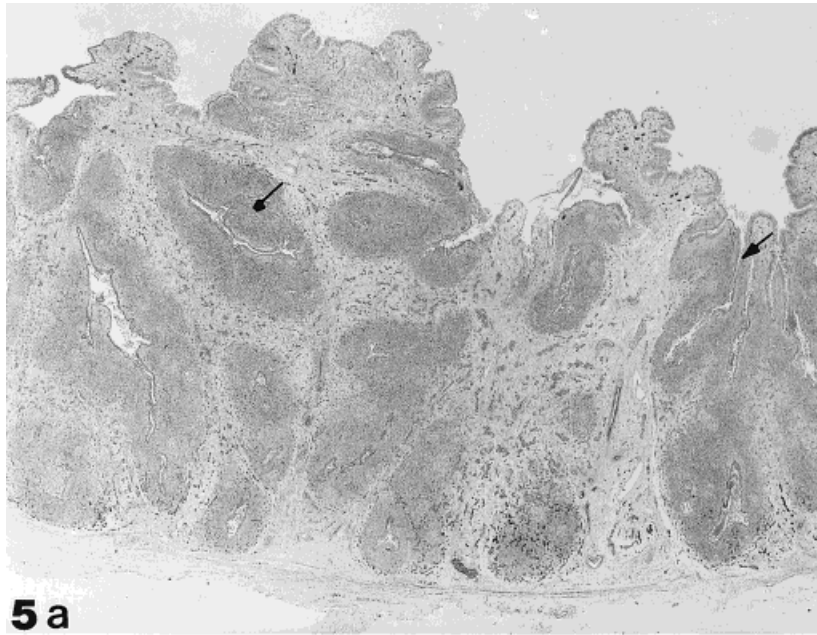


Figure 5.

Teeth were not available from 36 animals, and therefore these were placed in age classes and identified as neonatal, sexually immature, or sexually mature. Since involutional changes in the lymphoid system appear to take place around or before sexual maturity, teeth aging of mature animals was not considered essential for this study.

Causes of Stranding

The causes of stranding of the animals included in this study were highly variable, and included both disease and trauma. It was not clear how the physiological or pathological conditions associated with stranding may have affected the laryngeal gland; however, no effect was apparent.

RESULTS

In all 66 cetaceans examined, a lymphoglandular structure or complex was observed as a constant feature in the mucosa at or near the base of the laryngeal spout. The gland size appeared to be proportional to the size of the larynx; i.e., large larynges had large glands, small larynges small glands.

A lateral, external view of the larynx of an adult *T. truncatus* is presented in Figure 1, with the cartilages named according to the scheme of Reidenberg and Laitman (1987). The gland is present at the ventral opening of the cricoid cartilages of the larynx, occasionally extending caudally into the trachea. In *T. truncatus* there was no discernable difference in the gland between males and females. For all other species, not enough specimens were available to permit this judgment. In the bottlenose dolphin it maintained a fairly constant size relative to the larynx in which it is found. It was readily recognized as an oval, heavily trabeculated, well-defined thickening of the mucosa, with its long axis paralleling the long axis of the larynx. In adult animals it measured 3–4.5 cm long and 2–3 cm wide. The margins may extend laterally to about half the circumference of the larynx. Commonly the gland ranged up to 1.5 cm thick in large animals. The surface was thrown into longitudinal, interconnecting folds typically 1–1.5 mm wide, separated by pits and crevices about 1 mm wide. The gross appearance and extent of the gland is shown by sectioning in the sagittal (Fig. 2), horizontal (Fig. 3) and transverse (Fig. 4) planes.

Histologically, the laryngeal gland presented as a complex lymphoglandular structure with the general organization of a tonsil (Figs. 5, 6). The superficial invaginations of the overlying non-ciliated pseudostratified columnar epithelium (Fig. 5c) into the submucosa resembled the human nasopharyngeal adenoid, while the deeper, ramifying epithelial-lined crypts resembled the human palatine tonsil (Burkitt et al., 1993).

The prominence of mucous glands within the laryngeal gland appeared to relate inversely to the presence of lymphoid aggregations. In immature *T. truncatus*, large aggregations of lymphocytes surrounded and permeated the epithelial-lined crypts (Fig. 5b), while mucous glands appeared sparse. Conversely, the glands of the mature animals, having undergone moderate involution, displayed fewer lymphocyte aggregations with a greater prominence of mucous glands (Fig. 6b). In mature *T. truncatus*, the onset of involution appeared to be associated with dilation of the epithelial-lined crypts which tended to accumulate mucoid secretions.

DISCUSSION

The cetacean laryngeal gland we have described is not the same as the poche gutturale described in *Mesoplodon* by Anthony and Coupin (1930), which by their description and illustration is actually the pterygoid sinus complex. They also point out a diverticule médian at the base of the epiglottis (their Fig. 19), which we did not find in *T. truncatus*. However, they illustrate by line drawing the interior of the *Mesoplodon* larynx showing linear grooving of the mucosa in the same location as the laryngeal gland we have described in this report. The cetacean laryngeal gland appears to be the same as the honey-combed glandular structure described by Anderson (1878) in *Orcella*, making allowances for species differences. It is less clear which structure is being referred to by Behrmann (1987). His illustration of a "laryngeal organ or laryngeal sac" in the larynx of the harbor porpoise, *Phocoena phocoena*, is in the location of the laryngeal gland we describe, but his drawings do not seem comparable to our histological preparations. Behrmann also states that in the harbor porpoise laryngeal organ, mucous glands are sparse; however, they are plentiful in the laryngeal gland we describe in *T. truncatus*. In addition, we did not see a resemblance to lung tissue as suggested by Behrmann (1987).

The unusual location of the cetacean laryngeal gland exposes the animal to hazard. Located within and at the base of the larynx, it occupies space at the expense of the diameter of the airway, a truly critical location. Any involvement of the gland increasing its bulk, such as infection and subsequent inflammation and edema, would be at the expense of the airway. However, in the 66 cetaceans examined, we did not observe any significant occlusion of the laryngeal lumen by the gland, even in individuals with greatly enlarged lymph nodes associated with infection. This suggests that the immune responsiveness of the laryngeal gland and the lymph node system are in some way segregated. Nevertheless, considering the extreme consequences that could result from an increase in gland size, it is logical to assume that the gland has a critical function, one sufficient to justify the risk of its location.

Although the adaptive function of the cetacean laryngeal gland remains unknown, some speculations to its function can be inferred from its unique position within the respiratory tract. In cetaceans, all breathing occurs through the blowhole (Schenkkan, 1973; Mead, 1975) and unlike adult humans, cetaceans lack an enlarged oropharynx due to the intranarial position of the larynx (Reidenberg and Laitman, 1987). Because of the extensive separation of the digestive and respiratory tracts in these animals, inspired air would bypass typical oropharyngeal lymphoid defenses like those found in man and other mammals, and is conducted directly into the larynx, trachea, and lungs. The usual first point of contact of inspired antigens in the human adenoid and other components of Waldeyer's ring (Clemente, 1985) is lacking in the dolphin. One function of the cetacean laryngeal gland, therefore, could be to act as the respiratory equivalent of an oropharyngeal tonsil, screening inspired air for foreign antigens. In essence, it is the adenoid, relocated from the nasopharynx to the larynx. Thus it is not homologous to the laryngeal glands of the cat, shrew, and rat. The cetacean gland's highly convoluted surface might also serve as a mechanical trap for inhaled foreign particulate matter, holding it in a position to be

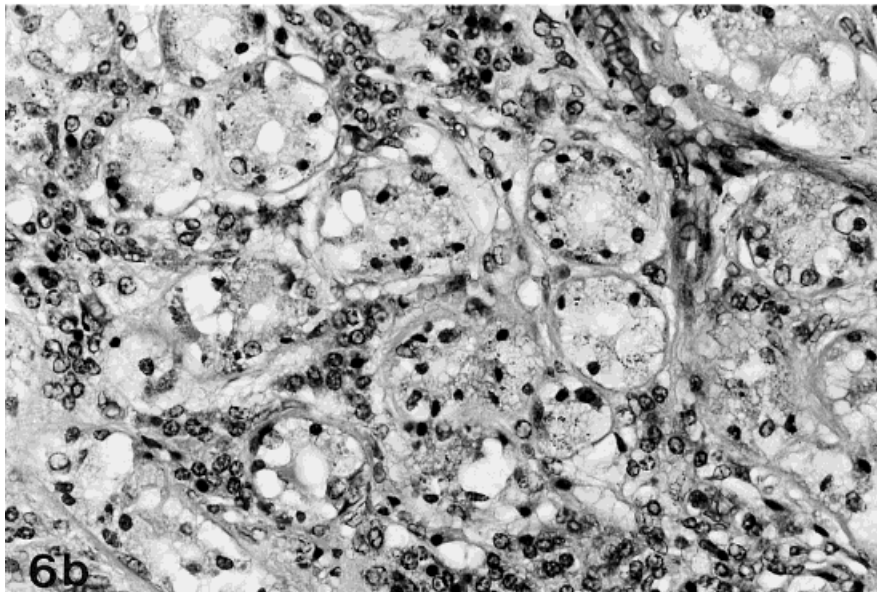
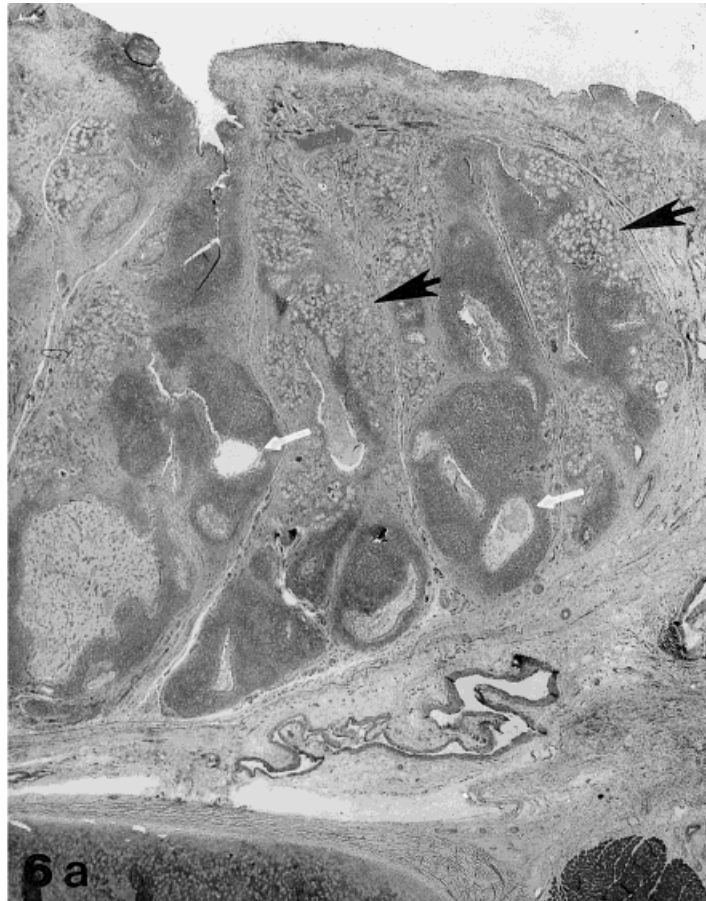


Fig. 6. **a:** Longitudinal section through the laryngeal gland of a mature male *T. truncatus*. This gland has undergone involution. Lymphocytes are depleted. Mucous glands have become more apparent (black arrows). The epithelial-lined crypts are also dilated and accumulating mucoid secretions (white arrows). Laryngeal lumen at top. Magnification 10 \times , HPS stain. **b:** Mucous glands with associated lymphocytes. Magnification 100 \times , HPS stain.

expelled on the next exhalation. We have observed ova of a fluke (probably *Nasitrema* sp.) which is commonly found inhabiting the pterygoid sinus, entrapped in the folds of the laryngeal gland.

In *T. truncatus*, the laryngeal gland is present in every animal, with no apparent histological difference between males and females. It appears to undergo a gradual process of involution with age, but not to the degree of other mucosa-associated lymphoid tissue, which normally evidences a more pronounced loss of lymphocytes and crypt dilation by the onset of sexual maturity (Cowan and Smith, 1998). This process of involution, whether in the laryngeal gland or other mucosa-associated lymphoid tissue, suggests that the contribution to immune function of the affected structure is diminished over time (Romano et al., 1993; Cowan and Smith, 1998).

Although currently the distribution of the laryngeal gland among all cetaceans is not known, we have observed this gland in all nine species we have examined. Furthermore, the Reidenberg and Laitman (1988) study on the existence of vocal folds in odontocetes illustrates the interior of the larynx in several different species, including *Delphinapterus*, *Phocoena*, and *Lagenorhynchus*. These photographs display trabeculated areas at the base of the larynx similar to those observed in *T. truncatus*. Their study also describes areas of trabeculation in *Globicephala* and *Delphinus* lying at the ventral opening of the cricoid cartilage. Due to autolysis, the authors were unable to perform extensive histological examination of these tissues. Given such observations, it is likely that the laryngeal gland exists in most, if not all, species of odontocetes. However, the presence of this gland can only be accurately confirmed through histological study.

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