Fine Structure of the Tongue and Lingual Papillae of the Penguin

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Summary. The tongue in four species of penguin was investigated by light microscopy and by scanning electron microscopy, with special reference to the lingual papillae.

1) The middle of the penguin tongue contains a pair of long cartilages and long tendons accompanied by striated muscle bundles.

2) Large, spine-like, and caudally directed lingual papillae (filiform-like papillae) densely cover the dorsal surface of the tongue, apparently serving to catch fishes.

3) By light microscopy, the dorsal lingual epithelium with the lingual papillae are seen as a thick cornified layer, but the lateral and lower surfaces have a thinner cornified layer.

4) The connective tissue core (CTC) under the epithelium of the lingual papilla shows a stereo structure similar to but smaller than the external form of the papilla. The CTC contains some blood vessels and nerve fibers; the lingual glands are found in the submucous layer only in the posterior tongue.

5) Bundles of nerve fibers in the lamina propria of the tongue were immunohistochemically positive for PGP 9.5, and it appeared that Merkel corpuscles were distributed in the connective tissue closely beneath the epithelium of the finger-like papillae, though they were only weakly immunoreactive for PGP 9.5.

6) Numerous fine filaments of elastic fibers are found closely beneath the epithelial cell layer of the dorsal surface of the tongue including the lingual papillae, while there are very few of them on the lateral and lower sides of the tongue.

The penguin is a bird that can not fly and obtain its food in the sea. There are two kinds of bird that can not fly: one lives on land like the ostrich and emu, the other lives both in the sea and on land like the penguin (King and King, 1979). In place of wings, the ostrich and emu have developed strong legs so that they can run fast, while the penguin has modified its wings to flying flippers (Hildebrand, 1974; Bannasch, 1986). The penguin has also adapted to life in severe circumstances such as the frigid temperatures of the Antarctic Ocean (Le Maho et al., 1976).

In our previous studies on the structures of the tongue and lingual papillae of some mammalian species, we have demonstrated that the stereo architecture of the connective tissue cores of lingual papillae represent features characteristic of the animal species (Kobayashi et al., 1992, 1995). We have further pointed out that the development of lingual papillae reflects the food intake habits of the animals (Kobayashi et al., 1997). As far as we know, there is no literature available which deals with the penguin tongue. As the structures of the tongue and lingual papillae of the penguin, a bird adapted to a special environment, was deemed to be of interest, we compared them to those of other birds and some aquatic mammals from a comparative-anatomical point of view.

MATERIALS AND METHODS

Tongues from four species of penguins (Spheniscus demersus, Spheniscus humboldti, Pygoscelis papua, Eudyptes chrysolophus) which were kept at Ueno Zoological Gardens (Tokyo) and died naturally were used in the present study.

The specimens were first fixed in 10% formalin within a few hours after death and photographed. For light microscopic observation, paraffin sections were stained with hematoxylin-eosin (HE), azocarmine, and aldehyde-fuchsine-Masson-Goldner (AF) respectively. Distribution of PGP 9.5 for nerve fibers was visualized immunohistochemically with the avidin-biotin
complex (ABC) method in the paraffin sections of the tongue. The antiserum used in this study was a rabbit anti-PGP 9.5 (anti-protein gene product 9.5) serum purchased from Ultraclone (Cambridge, U. K.). The antigen-antibody reaction was visualized by a Streptavidin-biotin staining kit (Nichirei, Histofine SABPO, Tokyo). Antisera with a working dilution 1:4000 or 1:8000 (incubation time 24 h at 4°C) were examined.

For scanning electron microscopic (SEM) observation, tissues were fixed further in KARNOVSKY's fixative (KARNOVSKY, 1965) and were treated with 3.5N HCl at room temperature (22-25°C) for 2-3 weeks. Some parts of the epithelium were removed from the underlying connective tissue, and both were refixed in 0.5% tannic acid and 1% OsO4, respectively. After dehydration with an ethanol series, they were frozen and dried by the t-butyralcohol method (INOUE and OSATAKE, 1988) and evaporated with platinum-palladium, to be observed with an S-800 scanning electron microscope (Hitachi).

RESULTS

The tongue of the penguin is spindle-shaped; its radix is thick and gradually tapers to a round apex (Figs. 1, 2). The median part of the dorsal surface of the tongue is slightly convex, so that a cross section of the middle part of the tongue looks like a fan (Fig. 3a). Large spine-like lingual papillae are distributed densely on the dorsal surface of the tongue and their tips are inclined in a posterior direction (Figs. 1, 2).

The papillae were uniform in shape, being roughly conical with a sharp tip. Their size, however, varied according to location. Those distributed on the apex were small and became larger in the posterior direction; at the most posterior end of the tongue they became somewhat smaller. These papillae were aligned regularly in a longitudinal direction. In the middle part of the dorsal tongue five lines of the papillae could be counted in Spheniscus demersus (Fig. 1a), Pygoscelis papua (Fig. 1c) and Spheniscus humboldti (Fig. 1e) and seven lines in Eudyptes chrysolophus (Fig. 1g). The papillae on the middle line inclined uniformly to the posterior direction, whereas the lateral ones inclined to the latero-posterior direction on the dorsal surface of the anterior half, and to the medio-posterior direction on the posterior half (Figs. 1a, c, e, g, 2).

In light microscopic preparations, the dorsal lingual epithelium, including that of the papillae, was considerably thick, being covered with a thick cornified layer (Figs. 3a, b, 4a, 6a). The lateral and lower surfaces of the tongue possessed a thinner epithelium and cornified layer (Figs. 3a, 6c).

At the base of the lingual papillae, coarse kerato-hyaline granules were found in the granular layer of the epithelium. None of the lingual glands could be found in the anterior 4/5 of the tongue, but large masses of the glands were recognized in the submucous layer of the posterior 1/5.

A thick plate of cartilage formed the longitudinal axis of the tongue. In the tongue, two thick columnar bundles of striated muscle ran parallel closely beneath the cartilage (Figs. 3a, 5). A pair of tendons were located at each dorso-lateral corner of the two muscle strings as shown in Figures 3a and 5, and extended from the postero-lateral region of the tongue to the tip as a pair of long slender strings. Also found were cross sections of elastic fibers which appeared as numerous dots arranged in a line closely beneath the basal cells of the epithelium (Fig. 4a) when the papilla was cut crosswise.

Under the SEM, the surface of the CTC of the papilla revealed crape-like fine parallel striations running along the long axis of the papilla when the epithelial cell layer was removed (Figs. 3c, 4b). These fine parallel striations found on the CTC of the papillae by SEM seemed at least partially to correspond to the elastic fibers found in the light microscope preparations. Bundles of numerous collagenous fibers were distributed in the connective tissue layer under the epithelial cell layer.

Elastic fibers were distributed among these collagenous fibers; they branched into many fine filaments and ran almost vertically to the basal cells of the interpapillary epithelium (Fig. 6a). SEM revealed numerous branched fine filaments reaching the basal area of the epithelium (Fig. 6b). The ends of these fine filaments of elastic fibers apparently were directly connected to the basement membrane of the epithelium. Fine filaments branching from thick elastic fibers were distributed in the lamina propria towards each basal cell. On the lateral and ventral side of the tongue, elastic fibers were sparse, in contrast with those on the dorsal side of the tongue. Figure 6c shows the scarce occurrence of elastic filaments closely beneath the epithelium on the ventral side of the tongue. SEM images showed small numbers of fine filaments intermingled with thick collagenous bundles (Fig. 6d).

The immunoreactivity for PGP 9.5 was demonstrated in nerve bundles distributed in the submucosa and in sparsely distributed nerve fibers in the lamina propria closely beneath the basal cells of the epithelium (micrograph not shown). PGP positive cell
masses composed of several rounded cells were frequently found closely beneath the epithelial cell layer at the base of the filiform-like papillae. These Merkel corpuscle like structures showed only a weakly positive reaction for PGP 9.5 (micrograph not shown).

**DISCUSSION**

It is known that penguins are phylogenetically most closely related to loons, albatrosses, herons, and grebes on the basis of the quantitative immunological comparisons of three avian proteins: transferrin, ovalbumin and penalbumin. These data support the theory that the ancestors of penguins were flying oceanic birds, and that flightlessness in penguins has evolved independently from that in ratites (Ho et al., 1976).

The tongue of birds is a highly diverse organ showing considerable variability in its size, form and structure, much of which can be closely related to feeding habits (Schumacher, 1927; McLelland, 1979). Harrison (1964) divided the modes of adaptation of the avian tongue into three main categories: 1) adaptations for collecting food, 2) adaptations for manipulating food, and 3) adaptations for swallowing.

1) Tongues adapted to collect food are all protrusible organs and function primarily as probes, spears, brushes and capillary tubes. The protrusibility is due to an exceptionally well-developed hyobranchial apparatus (apparatus hyobranchialis).

2) Tongues adapted to manipulate food are non-protrusible structures which generally closely conform to the shape of the mandible and function basically to hold and manipulate food. The tongues of many piscivorous species are adapted to hold the...
Fig. 2. SEM figure of the dorsal surface of the tongue of a young penguin, *Spheniscus humboldti*. Note numerous large filiform-like lingual papillae. a. Anterior half of the tongue. b. Posterior half of the tongue and pharyngeal region with aditus laryngis (*asterisk*). Bar: 500 μm
Fig. 3 a. Light micrograph showing a cross section of the middle part of a penguin’s tongue of *Sphenicus demersus*. p Cross section of a filiform-like papilla (see Fig. 4a), d interpapillar region of the mucous membrane of the tongue (see Fig. 6a), v ventral region of the mucous membrane of the tongue (see Fig. 6c). ca cartilage, m muscle, arrows tendon. Azocarmin staining. Bar: 500 μm. b. Light micrograph showing a longitudinal section of a filiform-like papilla (p) stained by azocarmin. co Connective tissue core of a filiform-like papilla, h epithelial cell layer with thick cornified red layer, s stratum spinosum of the epithelium. Bar: 100 μm. c. SEM figure of the lingual papillae of *Spheniscus demersus*. The epithelium of a lingual papilla has been partially removed and the connective tissue surface exposed (arrow). Note fine parallel striations running along the long axis of the papilla. Bar: 300 μm.
slippery prey by means of numerous stiff, sharp, caudally-directed papillae.

3) Tongues adapted to swallowing are seen in most species including those with major adaptations for procuring food or eating. Usually the papillae of these tongues are distributed in a transverse row at the root of the tongue, but may also be scattered along the lateral margins and on the dorsal surface.

The penguin has numerous large spine-like papillae on the dorsal surface of the tongue. Though there is some difference in size, their external form is uniform, and shows a conical shape with a sharp tip pointed in the posterior direction. These papillae are presumed to correspond to the filiform papillae of mammalian species; the penguin thus possesses a single type of lingual papillae on the dorsum of the tongue. Equipped with a sharp tip covered by a very strong cornified layer, the filiform papillae of the penguin, almost as large as 1 cm in length, form a structure dangerous to the touch.

Based on the structure characterized by the numerous stiff, sharp, caudally-directed papillae adapted to hold the slippery prey, the tongue of the penguin comes under the second type of HARRISON's classification of the avian tongue. However, the presence of a long cartilaginous plate and thick striated muscle bundles accompanied by slender tendons is in common with HARRISON's first type, characterized by a well-developed hyobranchial apparatus. The detailed structure of the hyoid bone in other birds was described by BRADLEY (1960a), PORTMANN (1965) and McLELLAND (1975). Though the actual movement of penguin's tongue has not yet been recorded clearly, it is probable that it moves powerfully, especially in an antero-posterior direction, judging from the above mentioned structure.

Our previous comparative microscopic studies have suggested that the degree of the morphological differentiation of the lingual papillae may reflect in some cases not so much the animal order as the food intake habits of the animal (KOBAYASHI et al., 1995, 1997). It has been demonstrated that carnivorous mammals (dogs and cats) have the strongest filiform papillae with a sharp tip, containing a CTC of one large, thick posterior protrusion besides numerous lateral and anterior short rod-shaped protrusions (KOBAYASHI, 1992). On the other hand, plant eating animals such as cattle and goats possess large, rod-shaped filiform papillae with a rounded tip containing a CTC consisting of a bundle of extremely numerous
slender protrusions (ASAMI et al., 1995). The external form of the filiform papillae in omnivorous mammals such as the human and crab-eating monkey consists of numerous spearhead-like protrusions extending from the margin of a basal columnar bulge. The CTC of the filiform papillae in the human and crab-eating monkey has secondary rod-shaped processes issuing from the top of the round primary process (KOBAYASHI et al., 1989, 1994). The epithelium of the filiform papillae in omnivorous animals is thinner than that in meat eating and plant eating animals, and the degree of its cornification is lowest in omnivorous animals, among the three. After removal of the epithelium, the CTC reveals a shape different from the external form of the filiform papilla in usual mammalian species.

In the penguin, in contrast, the CTC of the papilla closely resembles the external form of the filiform papilla, showing only a smaller size. The tongue of the chick has stiff, sharp, caudally directed papillae which are distributed in a few transverse rows only on the root of the tongue. Thus, the chick's tongue comes under the type 3 of HARRISON's classification. The filiform-like papilla of the chick is similar to that of penguins both in external form (BRADLEY, 1960b) and in stereo structure of the CTC after removal of the epithelium (KOBAYASHI et al., 1992). The surface of the CTC in both the penguin and chick has numerous fine striations running parallel along the long axis of the papilla. The characteristic adaptation in the penguin's tongue can be thus visualized by compari-

![Fig. 5. Scanning electron micrograph of a cross-sectioned middle part of the tongue of Spheniscus demersus. ca Cartilage, m striated muscle, t tendon. Bar: 500 μm](image-url)
Fig. 6 a. Light micrograph of the upper region of the tongue of *Spheniscus demersus*. Note numerous elastic fibers (purple in color) in the lamina propria (LP) closely beneath the epithelium (EP). Aldehyde fuchsin staining. Bar: 50 μm. b. SEM figure of connective tissue fibers found at the boundary closely beneath the epithelium (EP) on the upper region of the tongue of *Spheniscus demersus*. LP lamina propria. Bar: 2 μm. c. Light micrograph of a ventral region of the tongue of *Spheniscus demersus*. Elastic fibers are very scarce in the lamina propria closely beneath the epithelium (EP). Aldehyde-fuchsin staining. Bar: 50 μm. d. SEM figure of connective tissue fibers distributed closely beneath the epithelium (EP) on the ventral side of the tongue of *Spheniscus demersus*. LP lamina propria. Bar: 2 μm
son with that in the chick.

Among some flightless birds that are given the name ratites (ostrich, rheas, emus, kiwis etc), the penguins (Spheniscidae) constitute an exceptional category since their wings are used for propulsion through the water instead of flying (KING and KING, 1979). The wings are flattened and the bones much widened, forming a rigid, powerful flipper (HILDEBRAND, 1974). There seems to be little information about the structure of the tongue of the ratites, and we can not, at present, depict a more precise relationship among these birds including penguins with regard to their lingual morphology.

Böck (1971) found unmyelinated and a few myelinated axons in the lamina propria of the filiform papillae of the guinea pig. On the basis of the findings of unmyelinated axons showing striking swellings caused by an accumulation of mitochondria, he proposed that these structures may represent the receptive sites of nerves. The present study demonstrated the presence of PGP 9.5 positive nerve fibers in the lamina propria and submucosa of the filiform papillae. The suggested occurrence of Merkel-like cells composed of several PGP 9.5 positive cells closely beneath the base of the filiform-like papillae may indicate a possibility that these papillae of the penguin are also involved in mechano-reception in the mouth. It is interesting in this context that Merkel corpuscles were found exclusively in the connective tissue papillae closely beneath the base of the filiform-like papillae. The suggested occurrence of Merkel-like cells closely beneath the filiform-like papillae may indicate a possibility that these papillae of the penguin are also involved in mechano-reception in the mouth. It is interesting in this context that Merkel corpuscles were found exclusively in the connective tissue papillae closely beneath the base of the finch, *Lonchura striata* (TOYOSHIMA, 1989; TOYOSHIMA and SHIMAMURA, 1991).

The taste buds in birds have shown great variability in distribution and structure between different species. This may arise because birds in different habits often eat different foods and have different mechanisms of food intake. Avian taste buds have been recognized at the basal posterior part of the tongue, around and in the proximal part of the pharynx, and in the distal palatal mucosa (ANDREW and HICKMAN, 1974; REUTTER and WITT, 1993). In the present study we could not find taste buds in the penguin’s tongue. However, it could not be excluded that they might occur in the pharyngeal or palatal mucosa which we were unable to examine in the present study.

Dolphins, which are mammals living in the sea, have a spearhead-like tongue with a flat surface that lacks any lingual papillae (YOSHIMURA and KOBAYASHI 1997). On the other hand, the Stellar sea lion, which is also a mammal living at the seaside, has many large lingual papillae (KOBAYASHI et al., 1994). These are long, finger-like and relatively soft structures; the cornification of the epithelium is remark-

able weak. This weak cornification in the Stellar sea lion may represent its adaptation to the seaside environment of land animals. In contrast, the penguin developed sharp lingual papillae maintaining the characteristics of hard cornification of many other birds. The existence of different types of lingual papillae of animals must be considered both from the animal order and from the dietary habits of the animals concerned.

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REFERENCES


