

Marginal papillae on the tongue of the Harbour porpoise (*Phocoena phocoena*), Bottlenose dolphin (*Tursiops truncatus*) and Commerson's dolphin (*Cephalorhynchus commersonii*)

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Summary

This study shows that in Bottlenose dolphins and in Harbour porpoises the presence of marginal papillae is age related. In Bottlenose dolphins the papillae disappear around the age of 16 years. A 6-year-old Commerson's dolphin also showed remnants of marginal papillae on its tongue. Histological examinations of the papillae of a young Harbour porpoise showed that they are composed of thick flat multi-layer epithelium with probably a high level of cell multiplications. Based on these findings a dual function of the papillae is suggested: the papillae keep the milk from seeping from the buccal cavity during the suckling period, and later in life, when the animal starts eating fish, they aid in feeding. Before swallowing, the water surrounding the fish is squeezed out of the buccal cavity, and the papillae prevent water from re-entering this cavity.

Introduction

When the tongues of an adult, and a one-month-old (so still suckling) Harbour porpoise (*Phocoena phocoena*) became available, conspicuous macro-morphological differences between the two were observed. The tongue of the young animal had marginal papillae and the tongue of the adult had none. What could be the function of these papillae? Do they serve a sensory function such as gustatory or tactile sense, or do they serve an active (muscle) or passive mechanical function?

Donaldson (1977) summarized the data on marginal papillae of dolphin tongues (Caldwell & Caldwell (1972) called them antero-lateral papillae): The marginal papillae at the tip of the tongue are regions of thickened epithelium with a dense underlying dermal core which contains blood vessels and into which long submucosal papillae extend at different angles. Behrmann (1988) showed a longitudinal section through a marginal papilla with a "central cavernous corpus". Some authors (Caldwell & Caldwell, 1972; Donaldson, 1977) suggested that the presence of these papillae might be age-related, in

such a way that they disappear later in life. No potential function of these papillae was suggested.

This study investigates whether the presence of marginal papillae is age-related, and examines the papillae at a histological level. Based on the findings of these investigations, two potential functions of the papillae are suggested.

Materials and Methods

Study animals

General information concerning the animals of which the tongues were used for macroscopic and microscopic examination are listed in Table 1.

Histological investigation

The tongue of a one-month-old Harbour porpoise (PpSH015) was preserved in 4% formalin, soon after death occurred. Scanning electron micrographs (EM) were taken of the surface of the tongue (samples 1 + 2 in Fig. 1). A number of papillae as well as a few samples from the middle of the tongue were excised and used for histological examination. Two samples (not indicated in Fig. 1) were cut on a freezing microtome and used for a first examination; the 20 µm sections were stained with hematoxiline-eosin (HE). The other samples (samples 3-5 in Fig. 1) were embedded in paraffin and 10 µm sections were cut on a Leitz rotary microtome. Sections were stained with HE, with the van Giesson stain or impregnated with silver using the methods of Novotny & Gommer-Novotny (1988) and Sevier & Munger (1965).

Tongue survey

In order to find out if the presence of marginal papillae in dolphins is age-related, a tongue survey was held in a large number of facilities that keep Bottlenose dolphins. The Bottlenose dolphin was chosen because it is the most commonly kept cetacean, so a relatively large sample size could be expected. The facilities were asked to record the (sometimes estimated) birthdate,

Table 1. Animals of which the tongues were examined in this study.

Species	Code	Sex	Estim. age	Origin
<i>Phocoena phocoena</i>	PpSH015	M	1 month	Sylt; W. Germany
<i>Phocoena phocoena</i>	PpSH016	F	adult	North sea, Belgium
<i>Phocoena phocoena</i>	PpSH017	—	adult	North sea, Belgium
<i>Phocoena phocoena</i>	PpSH014	M	6 years	North sea, Holland
<i>Phocoena phocoena</i>	PpSH018	M	6 months	North sea, Holland
<i>Tursiops truncatus</i>	TtAZ001	M	2 weeks	Antwerp Zoo
<i>Tursiops truncatus</i>	TtAZ002	—	14 years	North Atlantic, Antwerp Zoo
<i>Cephalorhynchus commersonii</i>	CcDZ001	—	6 years	Chile, Duisb. Zoo

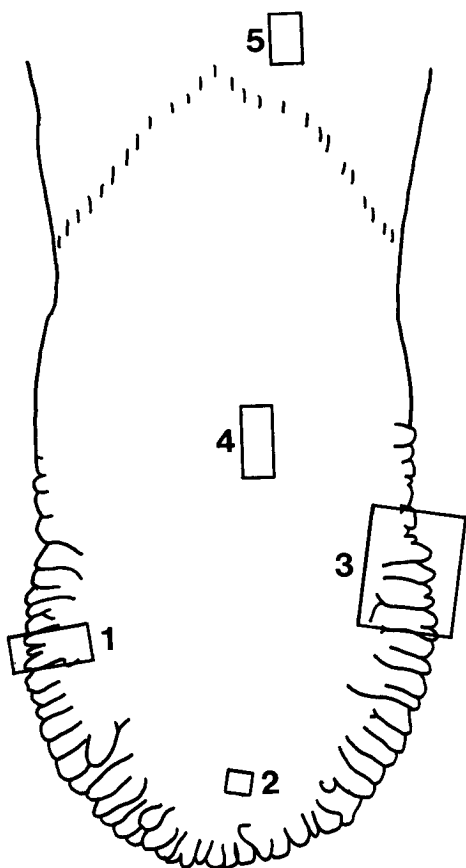


Figure 1. The location of the samples taken from the tongue of a one-month-old Harbour porpoise (PpSH015), used for the scanning electron micrographs (samples 1 and 2) and for histological processing (samples 3, 4 and 5).

and the presence or absence of marginal papillae on the tongues of their animals. Most facilities voluntarily qualified extremely small sized papillae as 'very small'.

Results

Macro morphological findings

– Harbour porpoise: The marginal papillae on the tongue were the most prominent in the one-month-old, still suckling, Harbour porpoise (Fig. 2a) and were still fairly large in a 6-month-old animal (Fig. 2b). They were reduced in size in adult animals (Fig. 2c and 2d), and were completely absent in a very old animal (Fig. 2e).

– Bottlenose dolphin: A 2-week-old (so still suckling) Bottlenose dolphin had marginal papillae on its tongue (Fig. 3a), which were much bigger than those found on the tongue of a 14-year-old animal (Fig. 3b). – Commerson's dolphin: Only one tongue was available; that of a 6-year-old animal. This tongue carried remnants of marginal papillae (Fig. 4).

The macroscopic shapes of the tongues of the 1-month-old Harbour porpoise and the 2-week-old Bottlenose dolphin are only slightly different (compare Figs. 2a and 3a). However, more conspicuous are the differences of shape of their marginal papillae (Figs. 5a and 5b). Those of the Harbour porpoise are often bilobed, finger-like protrusions with a basal fold between papilla and tongue. This fold is absent in the Bottlenose dolphin; the papillae have a more flat appearance and are generally undivided with only occasional small side-lobes.

Histological findings

The scanning EM photographs (Fig. 6) show a normal epithelial surface without any special structures

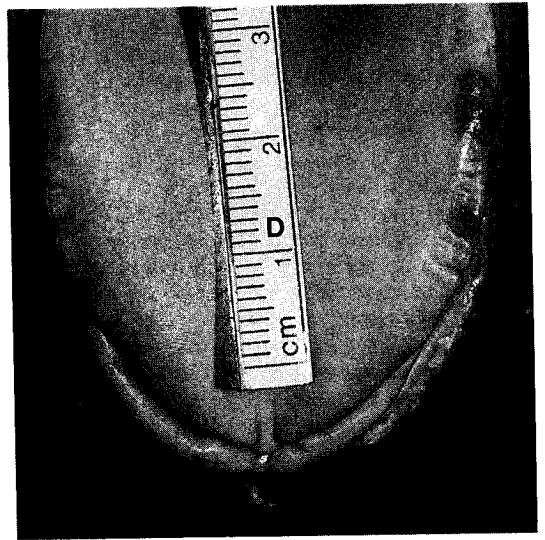
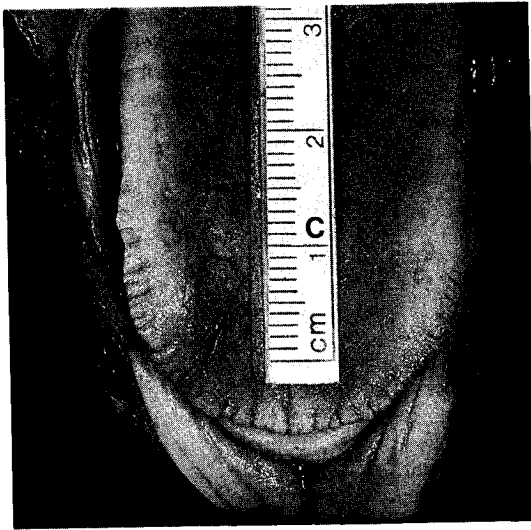
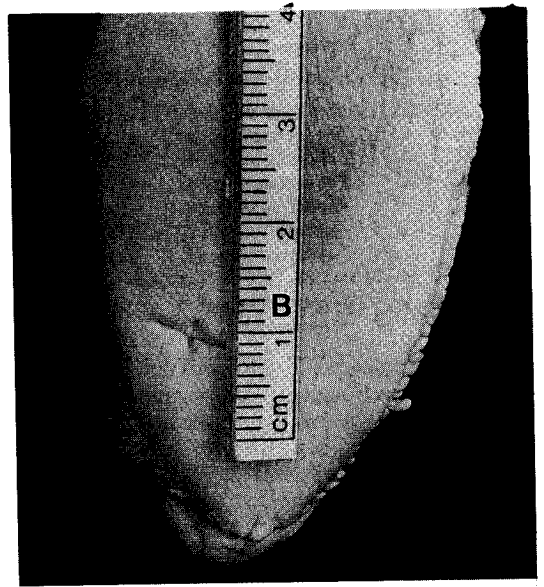


Figure 2. (A-D).



Figure 2. (c)

Figure 2. The marginal papillae on the tongues of Harbour porpoises of different ages: A, a 1-month-old animal (PpSH015), B, a 6-month-old animal (PpSH018), C, an adult animal (PpSH017), D, a 6-year-old animal (PpSH014) and E. a very old animal (PpSH016).

such as pores of taste buds. The frozen and paraffin embedded sections provide a reasonable picture of the normal histology of this tongue. The HE stained sections show that the samples of the skin of the tongue and those of the papillae always consist of a multilayered keratinized epithelial epidermis with a dermis of dense irregular connective tissue (Fig. 7). Bundles of muscle fibres are abundantly present below the dermis in the middle of the tongue, but not in the marginal papillae. The dermis has many protrusions into the overlying epidermis. Very large, elongated and richly vascularized protrusions penetrate the marginal papillae and bear many branching shoots. The epidermal matrix is strongly folded around these branches (Figs. 8 and 9). The effect is that the epidermis of the papillae seems to be considerably thicker than that of the dorsal skin of the tongue. The outer keratinizing layer of the epidermis lacks this folded character. The combination of this feature and the strong vascularization of the papillae suggests a high cell division rate and a heavy wear of the keratinized layer. In spite of the presence of many small vessels, the dermal papillae do not have the appearance of cavernous tissue (as opposed to Behrmann, 1988), (Fig. 11).

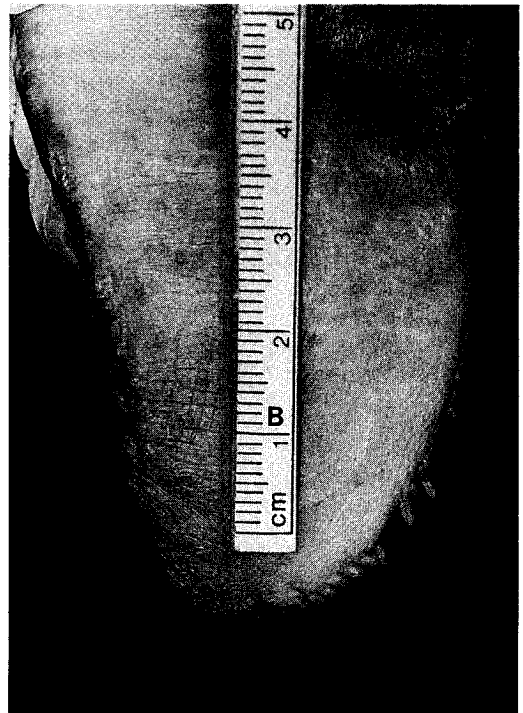
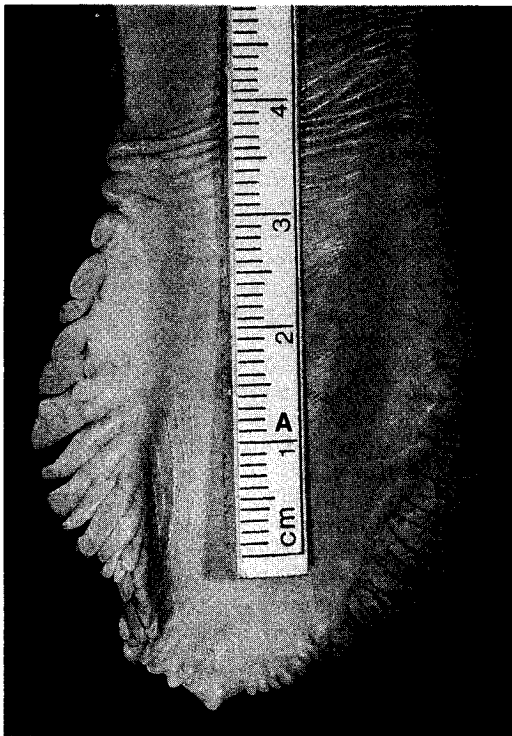


Figure 3. The marginal papillae on the tongues of Bottlenose dolphins of 2 different ages: A, a 2-week-old animal (TtAZ001) and B, a 14-year-old animal (TtAZ002).

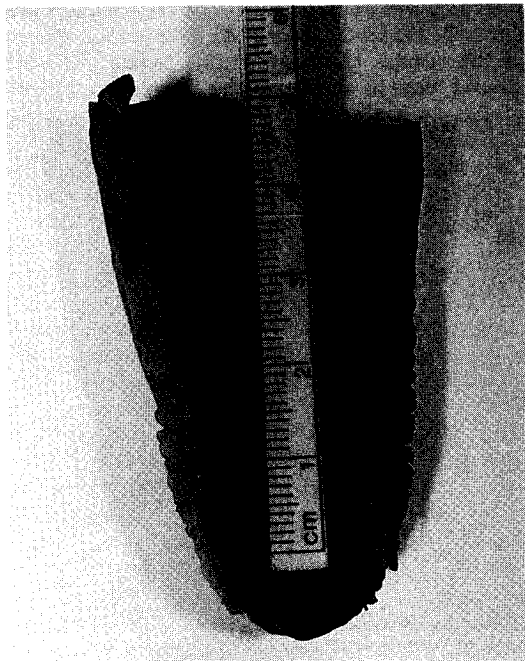


Figure 4. The marginal papillae on the tongue of a 6-year-old Commerson's dolphin (CcDZ001).

Our material was not very suitable to stain nervous elements. It was possible to recognize the Golgi-Manzoni (laminated) corpuscles (Fig. 10), particularly in sample 5 of Fig. 1. These corpuscles are mechano-receptors. Quite often two or three of these corpuscles were surrounded by a common capsule of connective tissue. In the other samples (samples 3 and 4 in Fig. 1) it was very hard to identify such corpuscles; this suggests a very low density.

Tongue survey

The tongue survey shows, that at least in the Bottlenose dolphin, the presence of the marginal papillae is age-related (Fig. 11). All the animals in the survey had papillae on their tongues until the age of 13, and all animals older than 28 had no papillae left. Between the age of 12 and 28 some of the animals still had the papillae although, if present, they were very small. The average age of disappearance is probably around 16, although data about animals of this age are scarce.

Discussion and conclusions

The characteristic from which mammals derive their name is the fact that they are fed milk from mammae during the first period of their lives. The milk transfer in terrestrial mammals occurs as follows: A young

grasps the swollen nipple between tongue and hard palate. Alternate suction and compression, caused by these parts of the mouth, help pass the milk to the pharynx. Suction is created when the tongue is withdrawn from the hard palate, and compression by its reposition to that surface. The contact of the nipple against the palate and posterior tongue elicits suckling or 'milking'. This suckling reflex is a process of squeezing the sinuses of the areola rather than simply suction on the nipple. The young can breathe during suckling since continuity of the respiratory tract is not interrupted. As the young swallows, there is a sudden elevation of the posterior part of its tongue, which forces the contents of the posterior pharynx into the oesophagus while the epiglottis simultaneously closes the entry to the larynx, and the muscles of the soft palate block the entry to the nose. The peristaltic movement of the oesophagus further transports the milk to the stomach (Davidson, 1972; Nelson, 1987).

How do terrestrial mammals keep milk from leaking out of their mouths? Young herbivores are usually suckled when the mother is standing. That way gravity is helping in directing the flow of milk. Some species of terrestrial mammals, for instance primates, have cheeks which enclose the buccal cavity and thus prevent milk from seeping from the buccal cavity. A group of terrestrial mammals with long snouts and sometimes no cheeks, that suckle their pups while lying down, are carnivores and pigs. In these species the tongues of young animals (during their suckling period) differ morphologically from those of adults. Still suckling carnivores and pigs have marginal papillae (papillae marginales: I.C.V.A.N., 1983) on their tongues, which persist only some weeks after birth. Dyce *et al.* (1987) describe the marginal papillae of a cat and suggest that these papillae assist in suckling from the teat. It seems likely that these papillae prevent milk from seeping from the cavity between tongue and palate, when the tongue is moving the milk towards the pharynx.

Toothed whales are marine mammals which, like terrestrial mammals, suckle their offspring for a certain period after birth. The difference however, is that cetaceans suckle their young underwater. In toothed whales the milk-gland exits are usually hidden in the mammary slits, but the nipples protrude quite noticeably during the period of suckling (Green, 1972). It seems likely that the suckling technique is basically the same as in terrestrial mammals. In odontocetes the oesophagus and tracheae are completely separated (Green, 1972), so suction in the buccal cavity can never be created by the lungs. The movement of the tongue has to transport the milk further after it is injected into the buccal cavity by the mother. Like many terrestrial carnivores, dolphins have a long rostrum and no cheek pads. They also

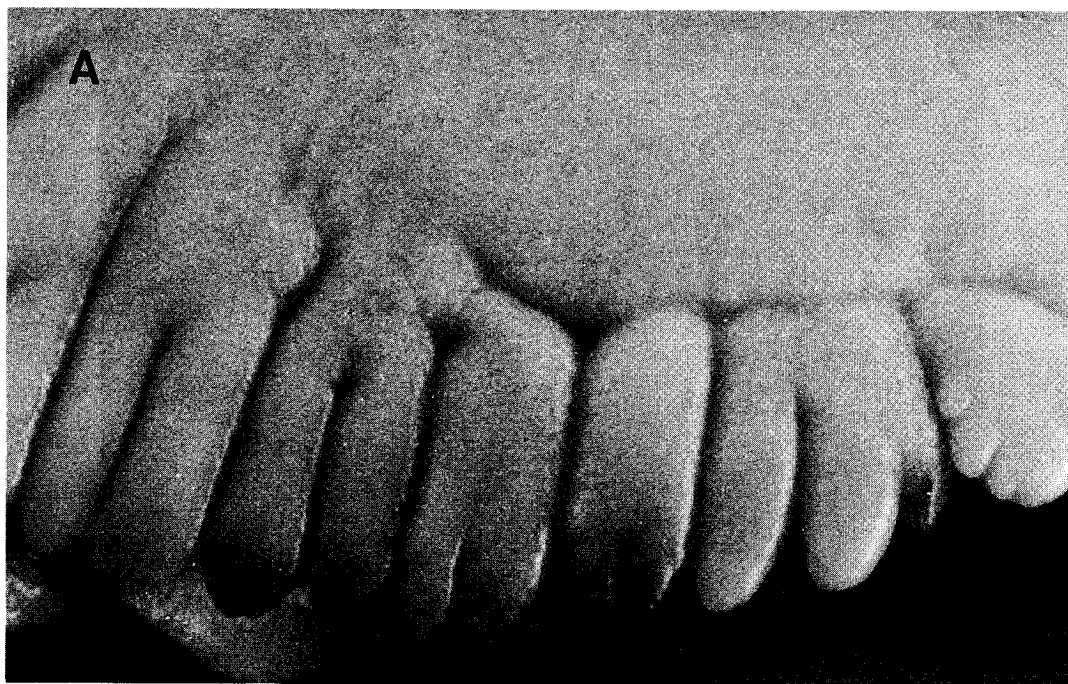


Figure 5. A, A close-up of the marginal papillae of a 1-month-old Harbour porpoise (PpSH015), and B, A close-up of the marginal papillae of a 2-week-old Bottlenose dolphin (TtAZ001).

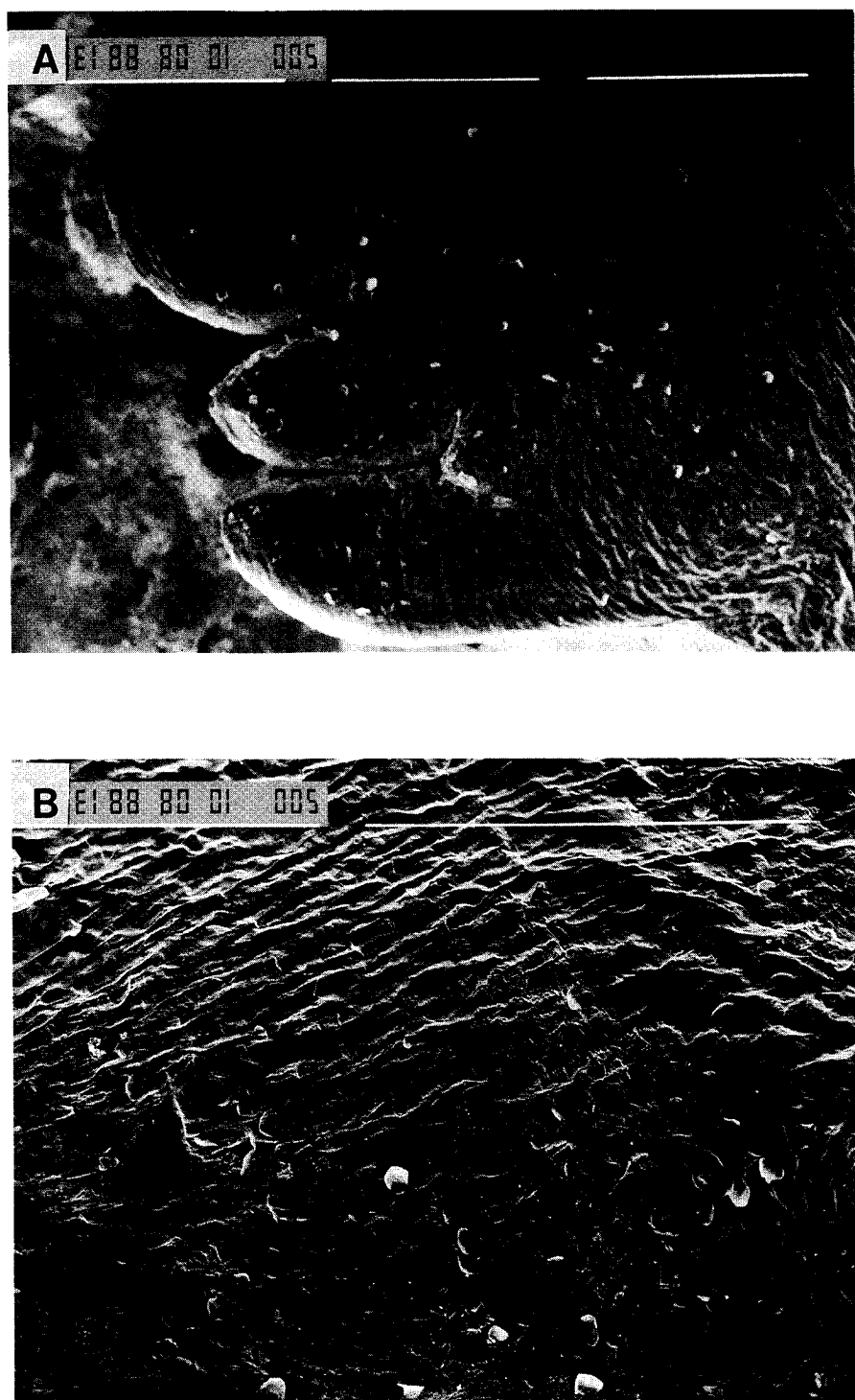


Figure 6. Low power scanning EM photographs of the epidermis of the tongue of the Harbour porpoise 015. A, Marginal papillae (area 1 in Fig. 1) and B, Surface of the tongue at the base of the papillae (area 2 in Fig. 1).

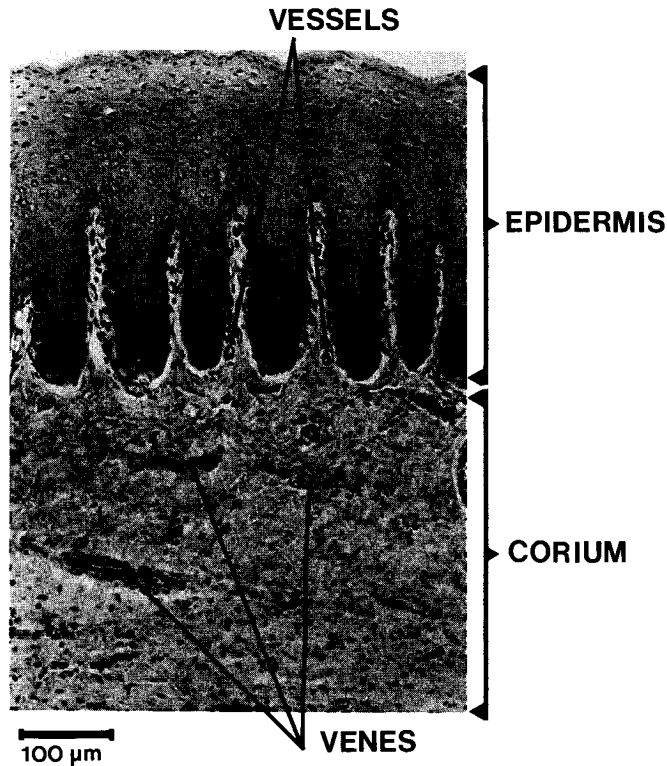


Figure 7. Section through skin of upper surface of the tongue of Harbour porpoise 015 (sample 4 in Fig. 1).

have papillae on the margins of their tongue during the suckling period. However, they retain these papillae for many years after suckling has stopped.

The following findings have led to a hypothesis on the function of the marginal papillae in dolphins:

– The survey in captive Bottlenose dolphins showed that they keep the papillae until they are approximately 16 years old. In older animals the papillae are reduced or absent. However, the disappearance of the papillae in time may not effect many animals since, in the wild, many Bottlenose dolphins do not reach the age of 16 years (Sergeant *et al.* 1973; Bryden, 1986; Perrin and Myrick, 1980).

– The histological data suggest a high level of cell division in the epithelium of the marginal papillae; which may be necessary to replace the cells broken down by wear and tear of the papillae against the palate. The fact that the marginal papillae disappear later in the dolphin's life, may be an effect of ageing. The irregular shape of the papillae of older animals (Figs. 2c, 2d, and 3b) seems to support the idea of disappearance due to wear.

– Compared to other areas on the tongue, the marginal papillae of the Harbour porpoise contained

a low number of taste and tactile receptors. This agrees with findings of Behrmann (1988) in *Phocoena phocoena* and with observations by Caldwell & Caldwell (1972) in *Tursiops truncatus*. So, although dolphins have taste receptors on their tongue, (Nachtigall & Hall, 1984; Nachtigall, 1986) they are not, or only scarcely, present in the marginal papillae.

– Dolphins do not drink much sea water (Ridgway, 1972). To minimize the swallowing of water when eating underwater, a water-tight barrier must exist between the buccal cavity and the pharynx. The lumen of the oropharynx is small and, with the tongue retracted, its entrance is completely occluded. The dolphin can swim with its mouth open, without water entering the pharynx (Donaldson, 1977).

Based on these findings a dual function of the marginal papillae in dolphins can be proposed: during the suckling period they prevent milk from seeping from the cavity between the tongue and palate, and later in life they act as a barrier between the aquatic environment and the same cavity when, after the water is squeezed out, a fish is swallowed.

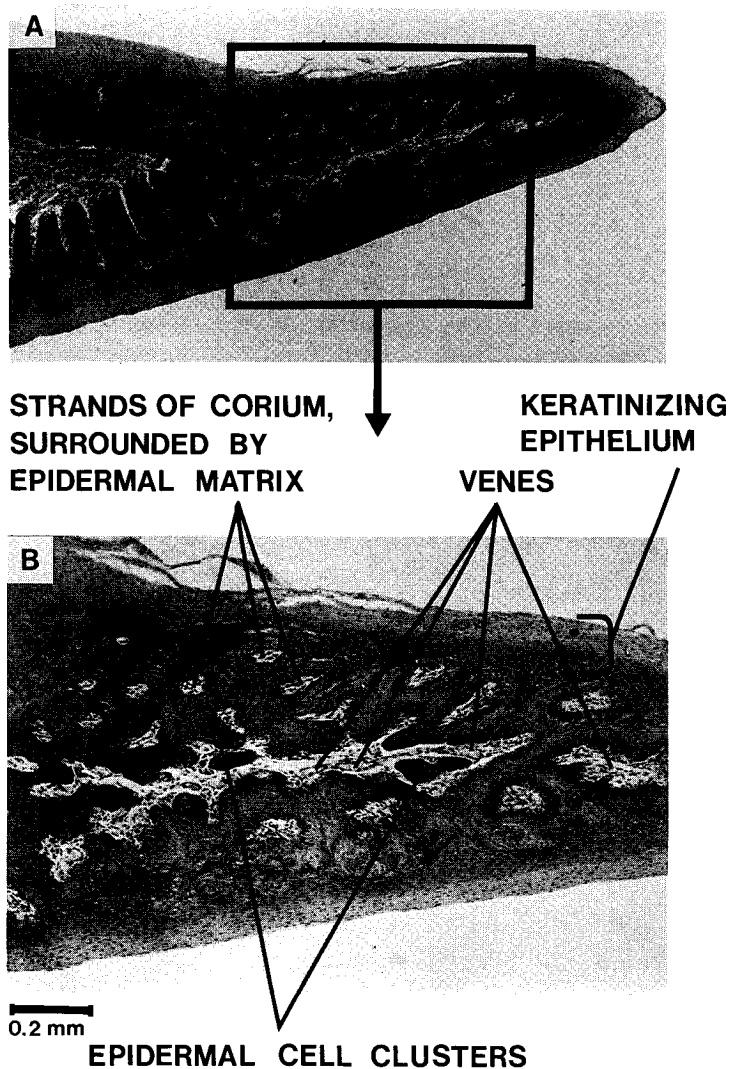


Figure 8. A, Length wise section through a marginal papilla of Harbour porpoise 015 (sample 3 in Fig. 1). B, Detail of the length wise section through a marginal papilla. Note the many branching strands of corium protruding into the epidermis.

The marginal papillae probably do not create the water tight barrier alone. Donaldson (1977) suggests that mucous secretion in the mouth may have a protective function against passage of water since it is immiscible with water. The mucous does not seem to have the same function as in terrestrial mammals. It is not necessary for lubrication (or mastication) of the already slippery fish that is swallowed whole.

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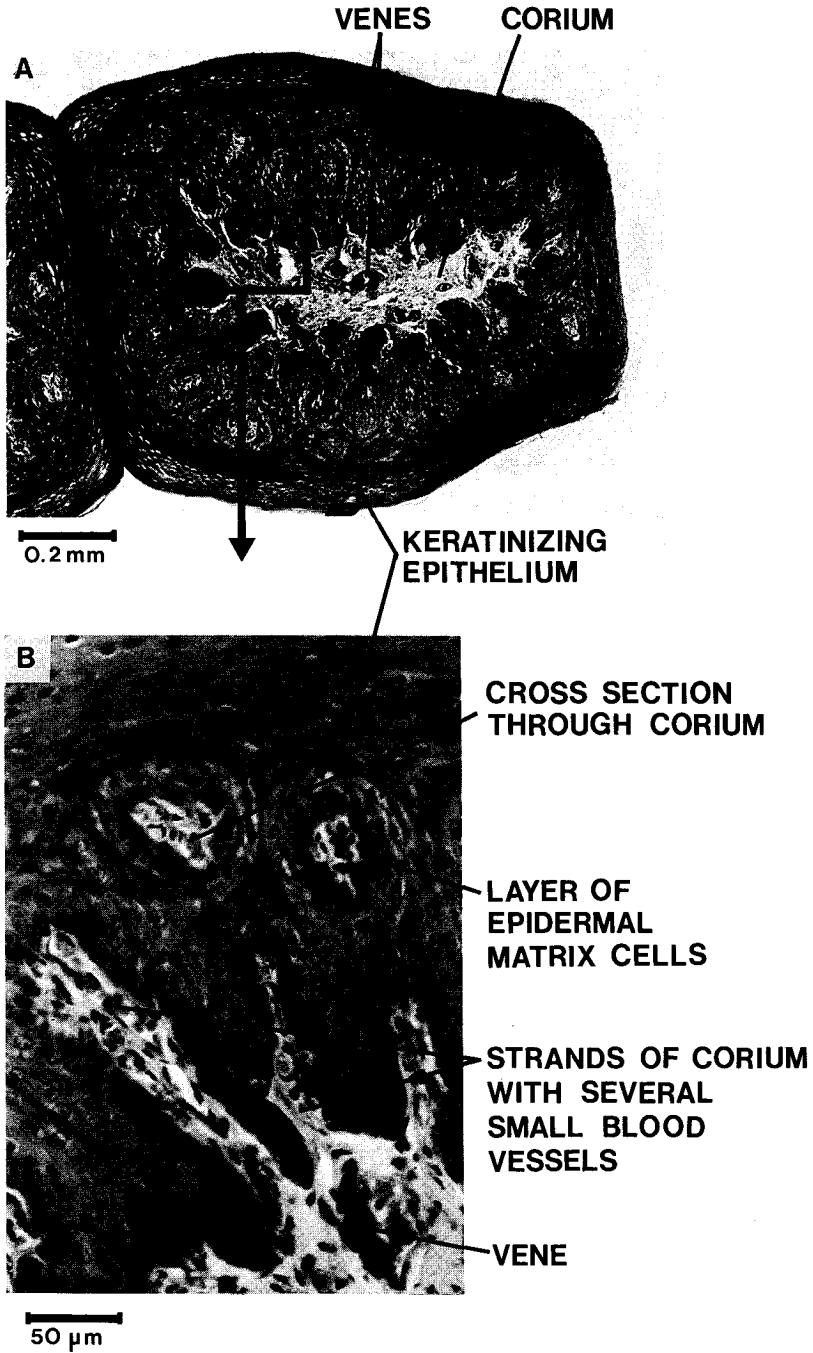


Figure 9. A, Cross section through neighbouring papillae of Harbour porpoise 015 (sample 3 in Fig. 1). Note the many venes in the corium and the very strongly folded character of the epidermal matrix around the many strands of corium. B, Detail of the cross section in high magnification showing part of the strongly folded epidermis.

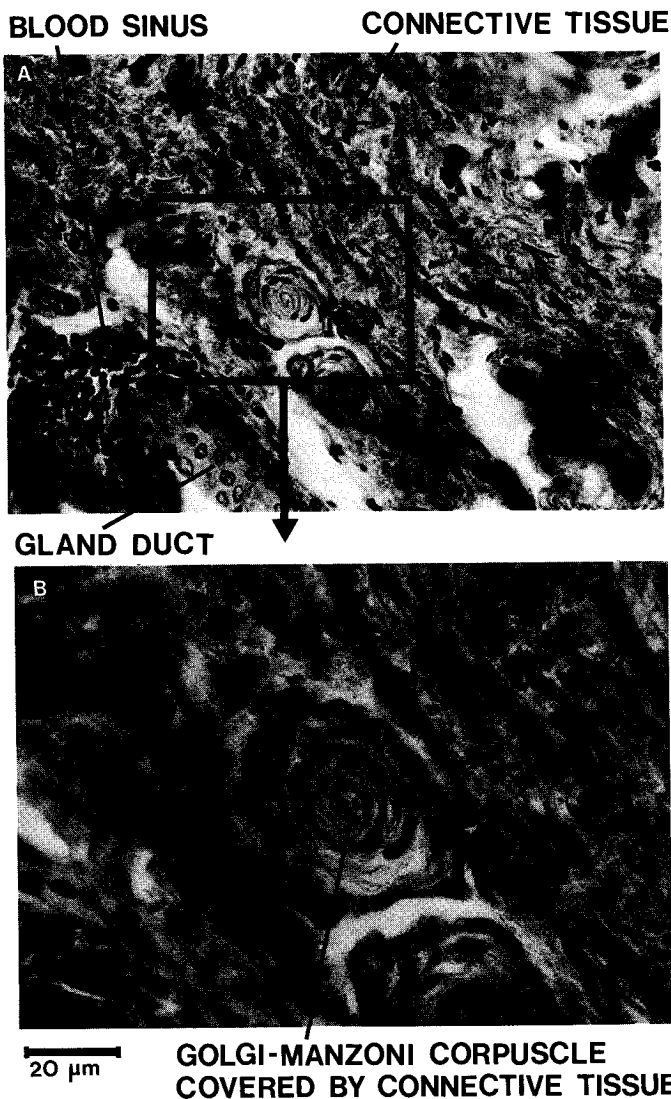


Figure 10. A, Two Golgi-Manzoni corpuscles lying rather deep in the corium near a blood sinus (sample 5 in Fig. 1). B, Detail of a Golgi-Manzoni corpuscle with dense surrounding connective tissue capsule.

Collet of the Musée Oceanographique in La Rochelle and Vivian de Buffrenil of the Museum National d'Histoire, Paris for the age determination of a tooth. We thank Evert de Graaf for the dissection of some of the tongues, Merijn de Bakker for the preparation of the tissue samples, Henk Merjenburgh for taking the macroscopic pictures, Brigitte Slingerland for drawing the graphs and Nancy Vaughan for her comments on the text. We thank John Hagendoorn

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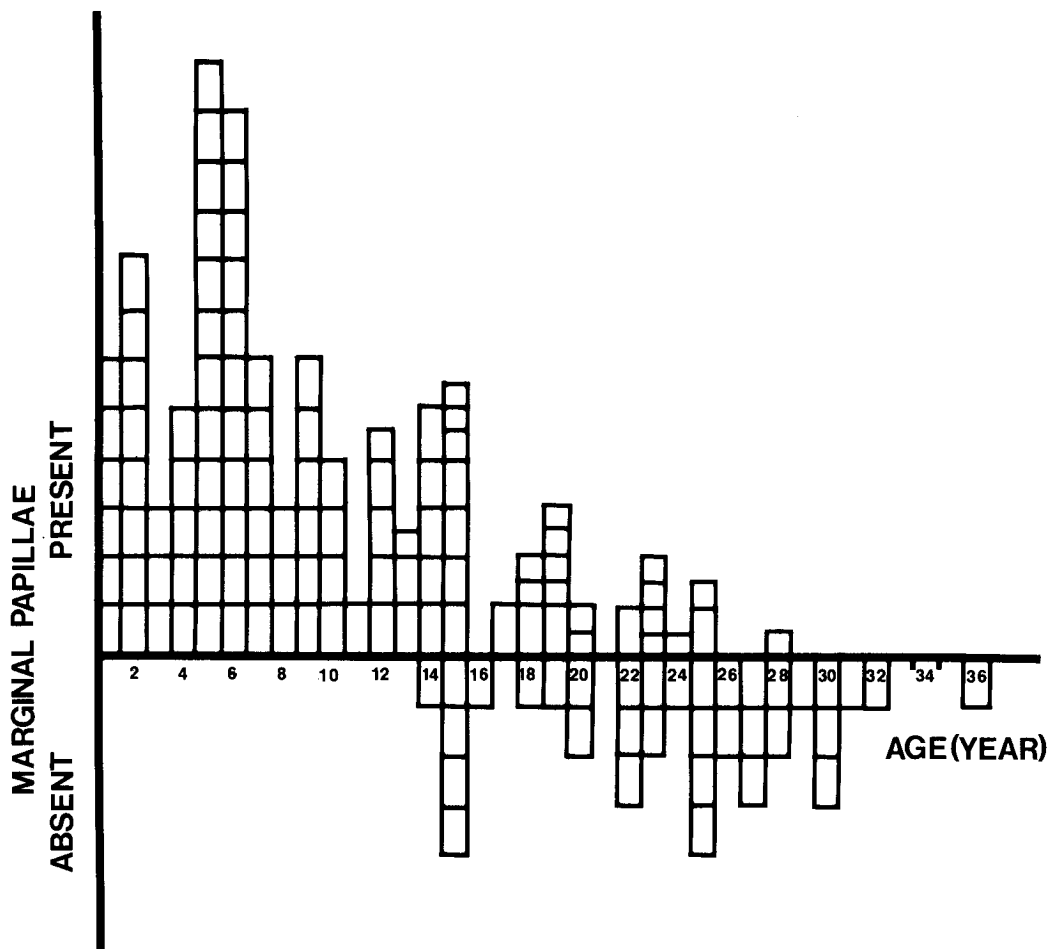


Figure 11. The presence and absence of marginal papillae on the tongues of Bottlenose dolphins of different ages in marine mammal facilities. The large boxes indicate normal sized papillae and the small boxes indicate small papillae. Not all facilities made qualitative remarks.

Zoologic de Barcelona; Bernhard Neurohr, Duisburg Zoo; M. Fothergill, Sea World Durban; Claus Hagenbeck, Carl Hagenbeck Tierpark, Hamburg; Giuseppe Caniglia, Delphinarium Riccione; Martin Huigen, Delphinarium Münster; Luc Tolpe, Brugge Dolphinarium; Paul van de Sande, Antwerp Zoo; Randy Brill, Brookfield Zoo; A. Gauckler, Nurnberg Zoo; Michelle Magee, NOCS, Hawaii; John Braithwaite, Morecambe Marineland; Joanne Lowenstein-Whaley, Marineland of Florida; Alan Eastcott, Brighton Dolphinarium.

Hopefully, this cooperation between marine mammal facilities from all over the world will continue to help in the gathering of more information on the animals whose well-being we are responsible for.

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