Calcareous concretions in the skin of toothed whales *(Odontoceti)*

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Abstract

The epidermis of toothed whales contains various forms of calcareous concretions, first described from some toothed whale species by Viale (1979). Concretions have now been found in the skin of other toothed whale species. Their origin was investigated using light and SE-microscopy of fresh and fixed material, and the concretions were also analysed by X-ray energy dispersion.

Kurzfassung

Kalkkonkretionen in der Haut von Zahnwalen (*Odontoceti*)

Die Epidermis der Zahnwale enthält verschiedene Formen von Kalkkonkretionen, die erstmals von einigen Zahnwalarten von Viale (1979) beschrieben wurden. Jetzt wurden in frischen und fixierten Häuten anderer Zahnwalarten ebenfalls Kalkkonkretionen gefunden und deren Entstehung lichtmikroskopisch untersucht. Die separierten Kalkkonkretionen wurden auch mittels Licht- und RE-Mikroskopie untersucht und mit Hilfe der Dispersionsenergie analysiert.

Résumé

Concrétions calcaires dans l 'épidermes des baleines à dents *(Odontocétés)*

L'épiderme des baleines à dents *(Odontocétés)* contient diverses formes de concrétions calcaires qui furent d'abord décrites d'après quelques espèces de baleines par Viale en 1979. On a maintenant également rencontré des concrétions calcaires dans des peaux fraîches et fixées d'autres espèces de baleines à dents et leurs formations ont été étudiées au microscope. Les concrétions calcaires isolées ont été également analysées à l'aide de microscopes radiographiques ainsi que par énergie de dispersion.

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Introduction

The skin *(integumentum commune)* of cetaceans was originally regarded to be mammal-like, elastic, smooth and without any hard substances. In the ninteenth century calcareous concretions and horny substances were discovered by Burmeister (1869) and Kükenthal (1890). Viale (1979) was the first to separate these substances and identify the single forms using electronic microscopy. The origin of the calcareous concretions and their chemical components is still not known.

The present investigation used fresh samples, a simple maceration procedure in warm distilled water, and sophisticated technology including a scanning electron microscope and X-ray microanalyser. This resulted in clear photographs and a better identification of these substances than was hitherto possible.

Material and methods

Fresh samples of the harbour porpoise *Phocoena phocoena* (L., 1758) were fixed in formalin/glutaraldehyd (2% / 2%). Fresh skin segments (5 to 5 centimetres) and fixed samples (4 per cent neutral buffered formalin) of beached toothed whales were also available: One sperm whale *Physeter macrocephalus* L., 1758, two northern bottlenose dolphins *Hyperoodon ampullatus* (Forster, 1770), three killer whales *Orcinus orca* (L., 1758), two bottle-nosed dolphins *Tursiops truncatus* (Montagu, 1821), two common dolphins *Delphinus delphis* L., 1758, and five harbour porpoises *Phocoena phocoena* (L., 1758).

The fixed material was cleaned with fresh water, impregnated with paraffin or resin, cut into histological slices 3 to 8 μm thick, and haematoxilin/eosin stained. The material was identified using phase contrast microscopy and different colour filters. This examination showed that the skin of all examined toothed whales contains hard substances.

After removing the blubber, fresh material was also cut and cleaned using a high pressure water beam. Thereafter the skin was macerated in warm water at 45 °C. The hard material was separated in distilled water by centrifugation, coated in carbon (C) and a qualitative analysis of the elements was made by energy dispersal X-ray micro-analysis (EDAX) in a scanning electron microscope (SEM). These analyses show the relationships of the single elements to each other. The hard substances used for SEM-photography were coated with gold.

During maceration in water all soluble substances were lost. Analyses of the following elements were used to identify individual forms of concretions: Sodium (Na), magnesium (Mg), silicon (Si), phosphorus (P), sulphur (S), chloride (Cl), potassium (K) and calcium (Ca). Carbon (C) used for shadowing of the substances is not considered here.

Results

Calcareous corpuscles with a diameter of up to 10 μm originate in skeleroblasts situated in the epidermal prickle cell layer, which are identified by their big nucleus. Two forms of skeleroblasts were discernible. The first mode of calcification by calcium-phosphate

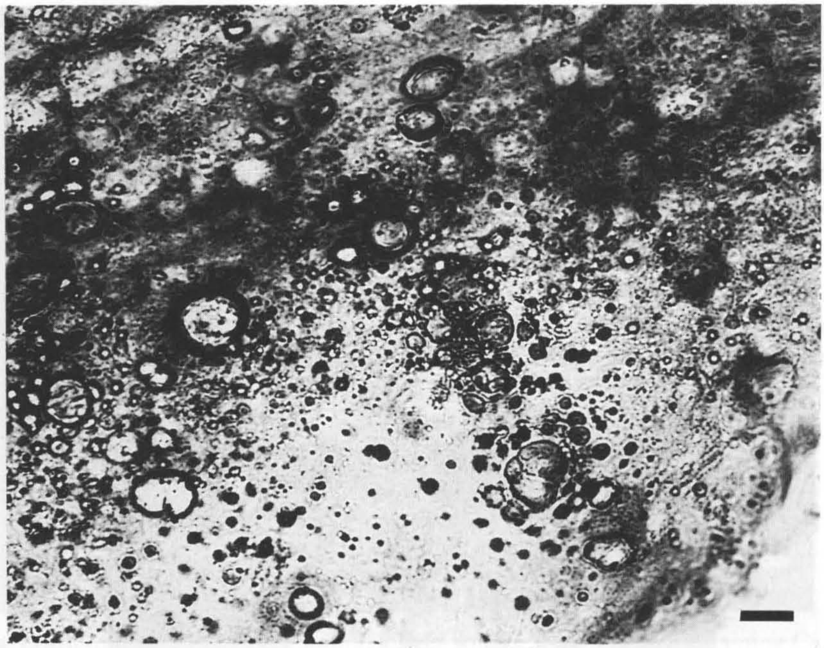

Figure 1: Harbour porpoise: Calcareous spherules (the small dark points) in the parakeratotic layer of the upper lip; unstained 400x. Scale bar = 10 µm.

Figure 2: Common dolphin: A non identified worm (only the tail is visible) surrounded by calcareous concretions, SE photograph. Scale bar = 100 µm.

begins to accumulate at the periphery of the cell leading to the formation of spherules (Figs. 1 and 4). The second mode of calcification begins in the centre of the cell resulting in the formation of the central plate of a chromatophore (Fig. 9).

Small calcareous corpuscles with a diameter of nearly 1 μm originate in the intercellular tissue between the prickle cells. Small and large round calcareous corpuscles combine to form larger calcareous concretions (Figs. 5 and 8) which are stored in the whole integument.

Only a few concretions (Figs. 2, 17 and 18) were located in the prickle cell layer of the epidermis, most hard concretions being located in the upper epidermal layer (parakeratotic layer; Simpson and Gardner 1972). 6000 to 8000 spherical corpuscles with

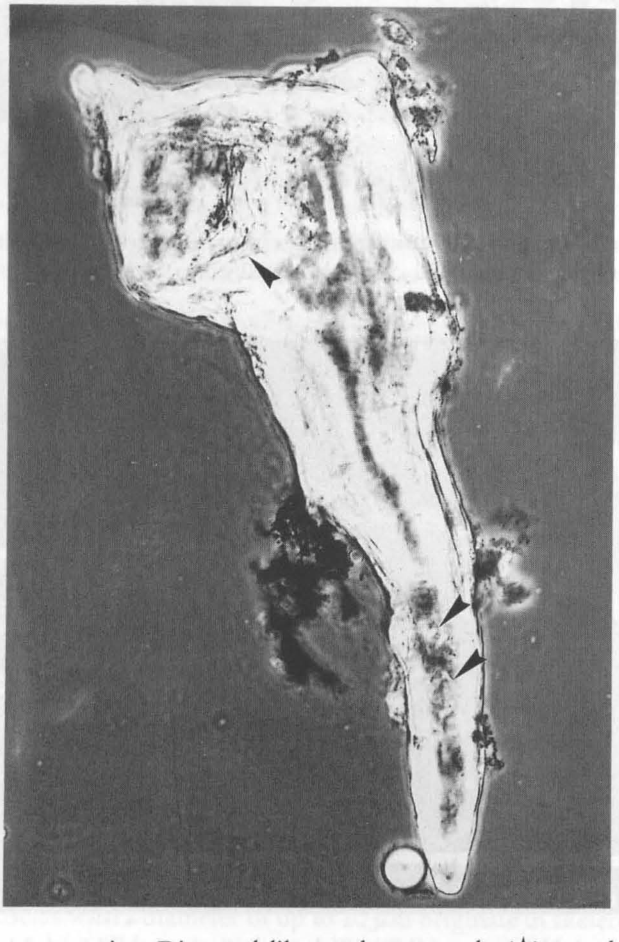

Figure 3: Harbour porpoise: Diamond-like oxalate crystals (▲) are the germs of this calcareous concretion, separated in fresh water; length = 270 μm.

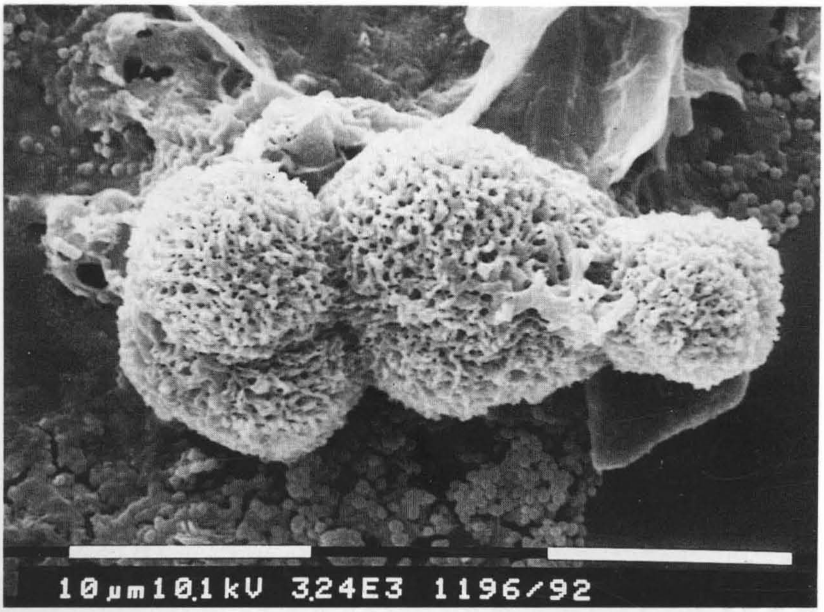

Figure 4: Bottle-nosed dolphin: Foraminifera-like spherical corpuscles containing Ca, Mg, S, and Si, SE-photograph. Scale bar = 10 µm.

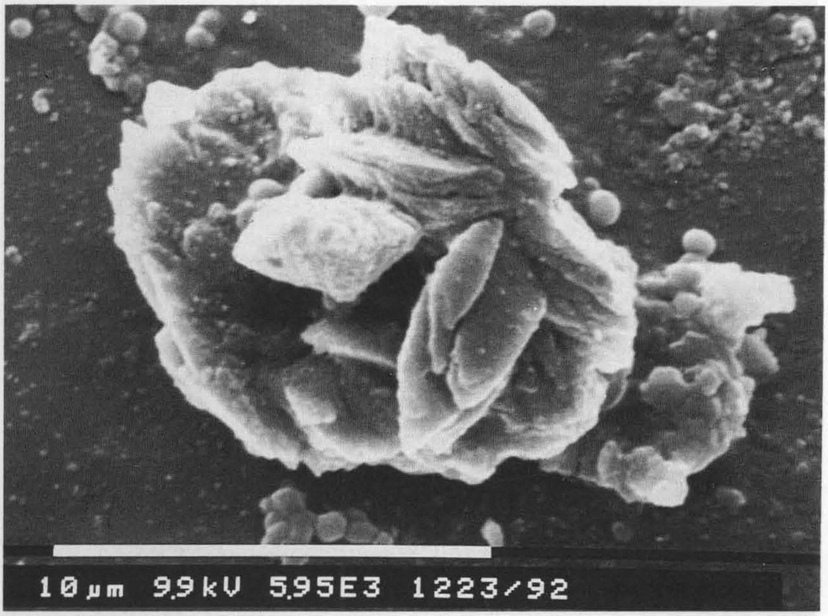

Figure 5: Harbour porpoise: Solid calcareous concretions containing elements (Fig. 10 C) nearly comparable to hard limestones, and are also very hard, SE photograph. Scale bar = 10 µm.

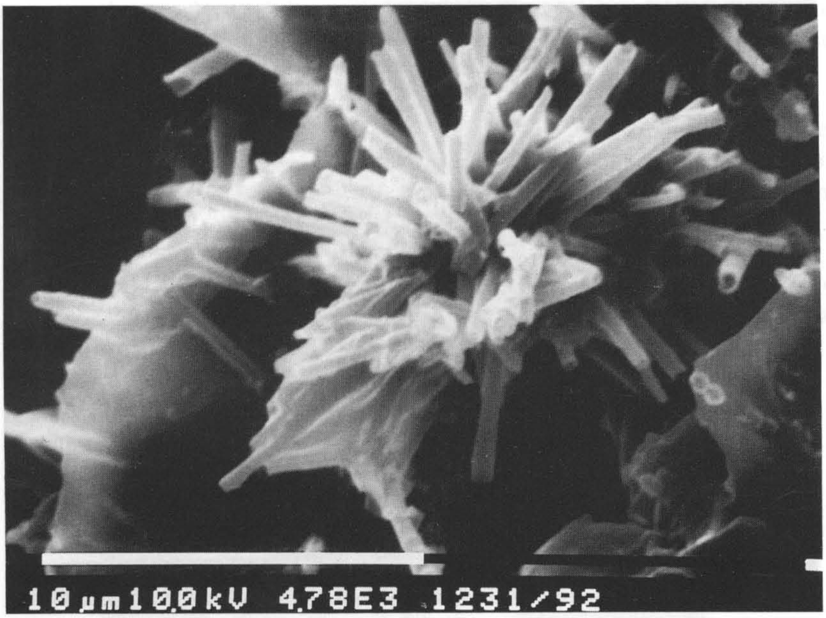

Figure 6: Common dolphin: Yellow pigments stored between the spiny concretions and only seen in unfixed samples using a light microscope. SE photograph. Scale bar = 10 µm.

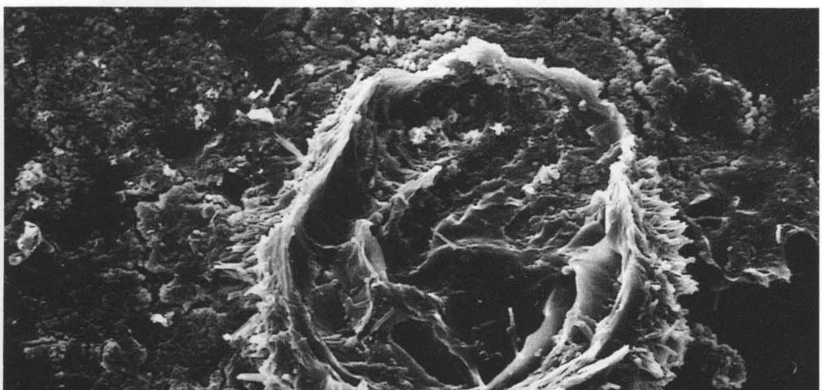

Figure 7: Harbour porpoise: Centre of the rosette-like concretion, SE photograph. Scale bar = 100 µm.

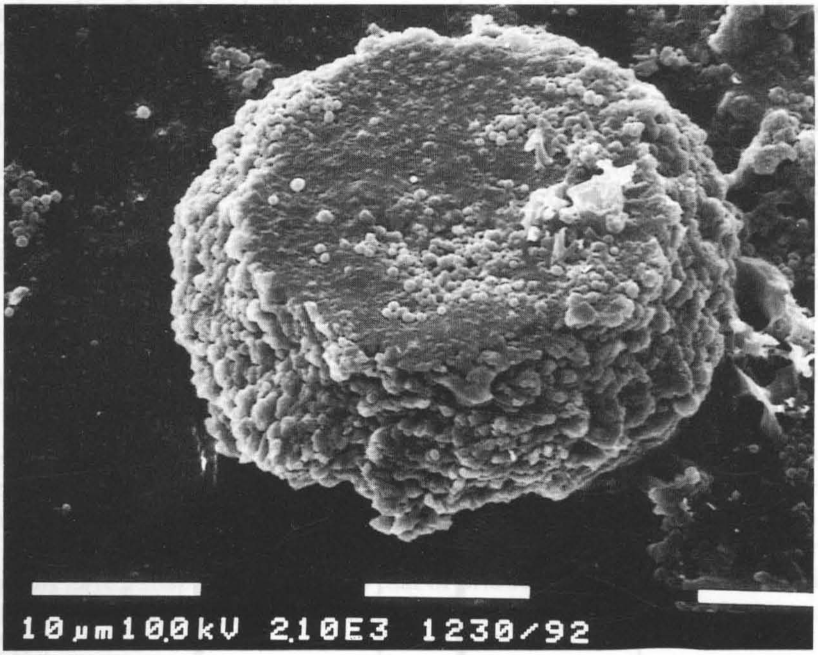

Figure 8: Harbour porpoise: Mushroom-like concretion, SE photograph. Scale bar = 10 µm.

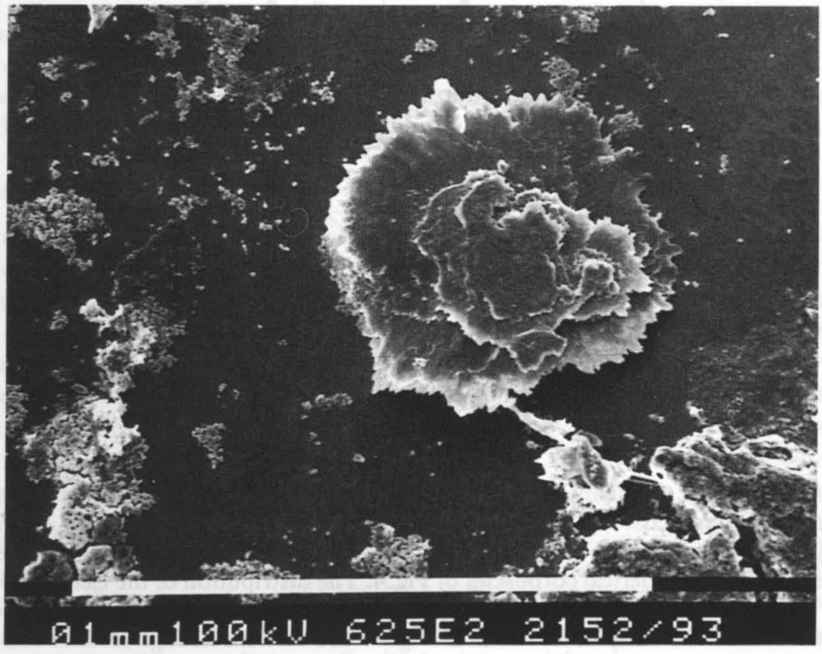

Figure 9: Killer whale: Calcareous disk of a melanophore, SE photograph. Scale bar = 100 µm.

diameters less than 10 μm can be found per square centimetre of the parakeratotic layer (Fig. 1). Using colour filters the concretions could clearly be distinguished from lipid droplets, horny substances and chromatophores.

The smallest calcareous grains, with a diameter of nearly 1 μm, surrounded foreign particles, which became fully integrated into the calcareous grains forming a non-vulnerable scale (Figs. 2 and 3). Up to 24 of these calcareous concretions of various forms – with lengths of up to 300 μm – can be found per square centimetre of the ventral epidermis. The elemental composition of these scales is somewhat similar to that of skull bones, although they contain less phosphorus (Fig. 10 A).

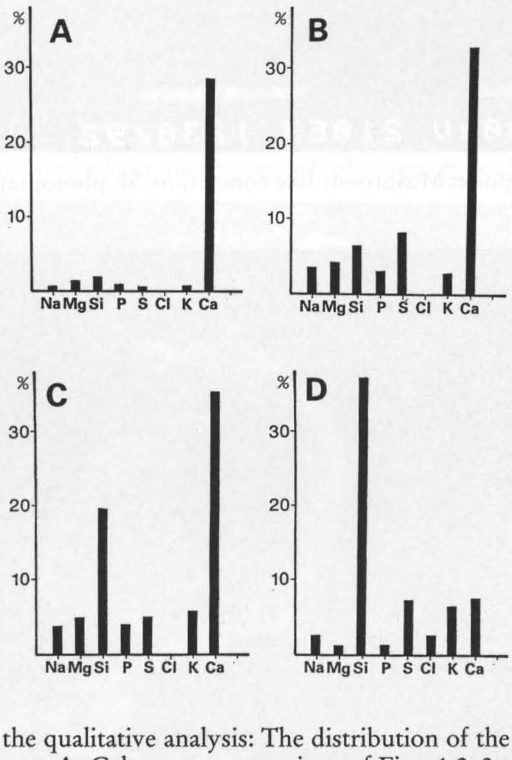

Figure 10: Results of the qualitative analysis: The distribution of the elements expressed in per cent in the total mass. **A:** Calcareous concretions of Figs. 1,2, 3 and 8. The distribution of the elements is comparable with the beginning of an ossification in the skin of vertebrates. **B:** Foraminifera-like spherical corpuscles of Fig. 4. The distribution of the elements is bone-like. **C:** The distribution of the elements in the solid concretion of Fig. 5 show a limestone-like consistence. **D:** Prickly concretions of Figs. 6 and 7. With its high level of silicon, the spines of the spiny concretions have a hair-like structure.

Besides the spherules and scale-like concretions some other substances are located in the skin of odontocetes. Foraminifera-like spherical corpuscles (Fig. 4) with a diameter up to 15 µm are frequently found in the epidermis. Some of these globular corpuscles form clusters or arrow-like forms. In these substances (Fig. 10 B) there was a high degree of calcium associated with keratotineous elements (Mg, Si, P, S and K). Solid (Fig. 5) and foliated scales were rare. Some scales grew together forming concretions with an extension of more than 0.5 mm². There was a high portion of calcium combined with less keratotineous substances (Si, S, and P) (Fig. 10 C) in both types of concretions. The functions of the solid and foliated scales are still unknown.

With a high accumulation of silicon and less calcium (Fig. 10 D), the spiny concretions (Fig. 6) have a keratotineous character. On examining unfixed skin particles of the common dolphin their function became clearly visible. Between the hollow spines bright yellow pigments are laid down.

The rosette-like concretions (Fig. 7) are garnished with short spines. In the skin of the common dolphin yellow-red pigment granules are also found between the spines. These concretions with spines, called "micro-ursins" by Viale (1979), are found garnished with bright crystals in the white skin regions of the harbour porpoise and the killer whale. The crystals have a shape comparable to that of uric acid. The qualitative analysis of the centrally situated substance is very similar to the analysis of the spiny concretions (Fig. 10 D).

Big mushroom-shaped concretions were mostly situated in the prickle cell layer (Fig. 8). They are conglomerates of the small calcareous concretions.

Together with the central basal plate of chromatophores (Fig. 9) and iridophores, up to 12 000 concretions per square centimetre of the epidermis of various types can be stored. The basal plates and corpuscles of the chromatophores up to a diameter of 50 µm contain a high level of calcium.

Discussion

In contrast to terrestrial mammals, the skin of the examined toothed whales possessed many very small calcareous concretions. Using colour filters they were clearly visible under the light microscope, and they evidently differed from keratotineous substances. Using scanning electron microscopy together with X-ray microanalysis the form and structure of the concretions became clearly visible. The possibility to make X-ray microanalyses of the substances smaller than one micrometre permits the separation of calcareous concretions from other substances also located in the skin. Together with the examinations of Viale (1979) the shapes of calcareous concretions of nine odontocete species are now known.

At nearly 270 µm long (Fig. 3) the compact calcareous scales which enclose foreign particles such as nematodes, sand, sponge clerites or alcionaria, are very large. Particularly in the harbour porpoises many of these scales were found in the ventral skin. The isolation of foreign particles is best known from bivalves during pearl formation, in contrast the skin of mammals normally discharges particles.

The calcareous concretions of solid (Fig. 5) and foliated concretions are strengthened by keratotineous substances. Comparable concretions were found by Viale (1979) in the skin of Cuvier's beaked whale *Ziphius cavirostris* Cuvier 1823. The spiny concretions (Fig. 6) termed "micro-ursins" by Viale (1979), were also found in Cuvier's beaked whale. The hair-like spines and spaces between the spines of the "rosette-like" concretions (Fig. 7) are also strengthened by keratotineous substrates. The accumulation of crystals between the spines indicates that these concretions are newly developed forms of chromatophores containing different pigments. Between the spines in spiny concretions in the skin of harbour porpoises and the sperm whale brown pigments are laid down, whereas the spiny concretions of the common dolphins (Fig. 6) contain yellow pigments. The rosette-like concretions are white in harbour porpoises and killer whales, and brown-red in the bottle-nosed dolphins.

Foraminifera-like spherical corpuscles (Fig. 4), first described in odontocetes by Viale (1979), were also found in all examined toothed whales, although their function remains unknown. Some of these concretions are located in the centre of horny scales, and it may be that the spherical concretions are remnants of a reptilian skin. However, similar spherical corpuscles are also found in invertebrates and plants (Watabe *et al.* 1976).

The accumulation of calcareous spherules (Fig. 1) with a diameter less than 1 μm, in the parakeratotic layer indicates that they originate in the skin. The structure of larger calcareous concretions (Fig. 8) shows that these are built up from the small spherules. The small calcareous concretions may be the germs of the reptilian-like scales which have been discovered by Burmeister (1869) and Kükenthal (1890).

Beside the calcareous concretions many kinds of metabolites were found in the skin of toothed whales, which were similar to those stored in the human skin (Harrison 1957). But by quantitative analysis of the elements the calcareous concretions are clearly different from uric acid crystals.

An accumulation of calcareous concretions in epidermal layers of mammals similar to the beginning of an ossification, is not recorded. The skin skeleton of armadillos (Xenarthra) is situated in deeper layers of the integument. The existence of chromatophores (Behrmann 1993) in the skin of mammals is also not recorded. Calcareous concretions and chromatophores are known from reptiles, amphibians and fishes. The skin of cetaceans contains horny substrates and hairs as well as these concretions.

The integument of cetaceans is not comparable in structure to the skin of other vertebrates; cetaceans possess an own skin structure, and contain remnants from their ancestors.

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