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# The Cells that Secrete the Beaks in Octopods and Squids (Mollusca, Cephalopoda)

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**Summary.** A single layer of cells secretes the hard cephalopod beaks. The beccublasts are tall columnar cells that separate the beak from the surrounding buccal muscles, and must serve to attach these muscles to the beak. Within the cell layer there are three types of cells. The first, and most frequently found contain cell-long fibrils. These fibrils may have contractile and tensile properties. Complex trabeculae extend from the beccublasts into the matrix of the beak. The fibrils are attached to these trabeculae and at the other end of the cells they are anchored near to the beccublast-muscle cell interface, closely associated with the muscles that move the beak.

The second group of cells contain masses of endoplasmic reticulum the cysternae of which are arranged along the long axis of the cell. These cells also contain dense granules and are probably the major source of beak hard tissue. It is probable that each cell secretes its own column of beak hard tissue. The third group of cells contains a mixture of fibrils and secretory tissue.

In the beccublast layer there are changes in the proportion of the three types of cells depending upon the region sampled. In the region where growth is most active there are mostly secretory cells, whereas near the biting and wearing tip there are mainly anchoring type cells.

**Key words:** Beak – Octopods – Squids – Formation – Cell types – Light and electron microscopy.

## Introduction

Within the feeding and digestive apparatus of cephalopods there is "hard" tissue. With X-ray diffraction methods, the oesophagus and stomach cuticle

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of *Loligo* was found to contain $\alpha$ and $\gamma$ chitin respectively, and $\alpha$ chitin in the stomach cuticle of *Eledone* (Rudall, 1962, and personal communication). The oesophageal cuticle of *Loligo* had quite a different diffraction pattern when examined immediately after removal from the animal, and after treatment with potassium hydroxide (Rudall, 1963). A chitin is present in the beaks as well as caustic-soluble substances, since weight-loss occurs after using such a solution as a macerating agent (Nixon, 1968, 1969). The cells responsible for secreting the beaks are confined to their outer surfaces and around the periphery, and are primarily responsible for their growth (Clarke, 1965). The beaks continue to grow throughout the life of the animal (Nixon, 1973) by the deposition of chitin or some precursor.

Secretory ameloblasts are responsible for the secretion of enamel, the hard surface of mammalian teeth. In the upper molar tooth germ of the rat, the secretory ameloblasts have apical and basal terminal webs, the filaments of which resemble myofilaments of smooth muscle at the ultrastructural level (Reith and Ross, 1973). These terminal web filaments display the morphological features of a contractile organelle.

It is known that the enamel of rodent teeth is formed in two major steps. The secretion phase is first, during which an incompletely calcified organic matrix is laid down, and this is followed by a second, maturation phase, during which parts of this matrix and water are removed while additional mineral salts are deposited. Allan (1967) and Reith and Butcher (1967) suggested that the ameloblasts go through a transitional stage in which they change from protein synthesising and secreting cells to ones active in absorptive and transport processes. Kallenbach (1974) showed that fine structural differences occurred in the rat incisor ameloblasts related to the two types activity of these cells.

The hard surface of the tooth breaks up food. The rostrum, or external part, of the beak of the octopus serves a similar function (Altman and Nixon, 1970). It is of interest to see whether the cells which secrete the cephalopod beak, the "beccublasts", have any structural features that can be compared with salient features of the ameloblasts.

## Material and Methods

*Octopus vulgaris* Cuvier, caught in the Catalan Sea, and taken to the Laboratoire Arago, were kept in circulating sea water. One animal, with a dorsal mantle length of 110 mm and approximately 150 g total weight was anaesthetized in 3% ethanol in sea water. An incision was made, between right and left arm 1, to expose the buccal mass which was excised by cutting through the circular lips, the small retractor muscles, the oesophagus and salivary duct. A small piece was cut from the postero-dorsal region of the lateral wall of the upper beak close to the crest (Fig. 1) (Clarke, 1962). This was then put into a cacodylate buffered solution of 2% glutaraldehyde for 7 days. It was dehydrated with graded ethanol, embedded in araldite and sectioned on a Porter Blum ultramicrotome. Some sections were counter stained on the grid using lead citrate (Reynolds, 1963).

*Alloteuthis subulata* (Lamarck) caught in the English Channel, were taken to The Laboratory, Plymouth, where they were kept in a large tank of circulating sea water. One animal, 124 mm dorsal mantle length, was anaesthetized in 3% ethanol and the buccal mass excised as in *Octopus*. The buccal mass was put into sea water, at room temperature, and left for 24 hours for the tissues

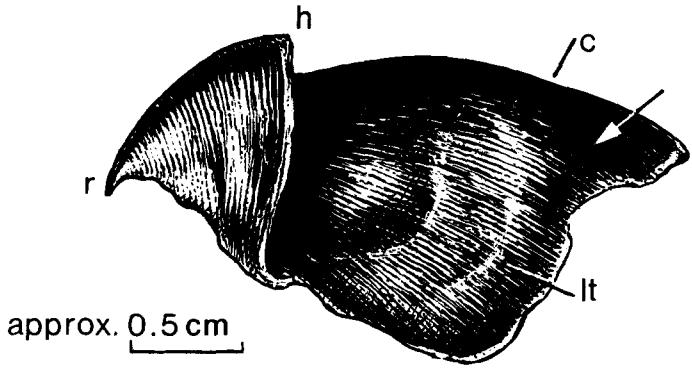


**Fig. 1.** Drawing of the upper beak of *Octopus vulgaris* (body weight 5,028 g), showing the various parts and the region from which material used in this study was taken is indicated with the arrow

*Abbreviations. b*, beccublasts; *bk*, hard tissue of the beak; *c*, crest; *d*, desmosome; *er*, endoplasmic reticulum; *f*, fibrils; *g*, granules; *h*, hood; *l*, lipid droplets; *lt*, lateral wall; *lw*, lipid whorls; *m*, muscle; *n*, nucleus; *nf*, nerve fibre; *os*, oblique striations; *r*, rostrum; *t*, trabecular region. *x*, region of mainly fibrous cells with little cytoplasm; *y*, region of less tall cells with both fibrils and secretory contents the majority of which are fibrous; *z*, tall cells with mainly secretory cells, but with some fibrillar content

to macerate. The beaks were washed in running tapwater and the adhering tissues removed with a fine paint brush; they were then dried in a vaccum dessicator, over sodium hydroxide pellets for 6 days. The beaks were then attached, with clear adhesive (UHU), to an aluminium rivet. The specimens were given evaporative coatings of gold and of carbon (Boyde and Wood, 1969) and the remaining exposed surface of the rivet painted with colloidal silver. A Cambridge Stereoscan S4-10 was used to examine the surface of the beaks.

Material for light microscopy was prepared by fixation in 10% neutral formol in sea water. The specimens were stained using Masson's Trichrome or Cajal's block silver method, and cut at 15 μm intervals (Stephens, 1971).

## Results

In small specimens of *Octopus* taken just before settling, when the animal weighed about 0.5 g, the beccublasts are short cuboidal cells with large nuclei and few fibrils. However, in larger animals, a single layer of columnar cells are found all over the outer and peripheral surfaces of the upper and lower beaks (Fig. 2). These cells, the beccublasts, responsible for the secretion of the material that forms the beaks are very tall (Figs. 3, 4). The cells from the posterodorsal lateral wall close to the crest of the upper beak are about 150 μm long and 5 μm wide. The long axes of these cells run at right angles to the surface of the beak. The ends of the cells distal to the beak are closely associated with the ends of the muscles that move the beak (Fig. 3). Seen in phase contrast the cells contain a mass of longitudinally running fibres that obscure the other cell details (Fig. 4). All the cells have prominent elongated nuclei with dense nucleoli. There are three major types of cells in this layer. All of them are

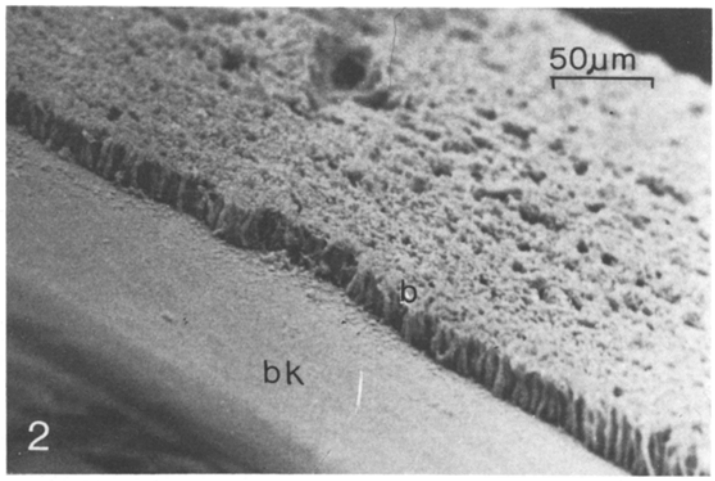


**Fig. 2.** Scanning electron micrograph of the crest of the lower beak of *Eledone* showing the mass of columnar cells upon its outer surface. The cells have been removed from the beak surface in the foreground

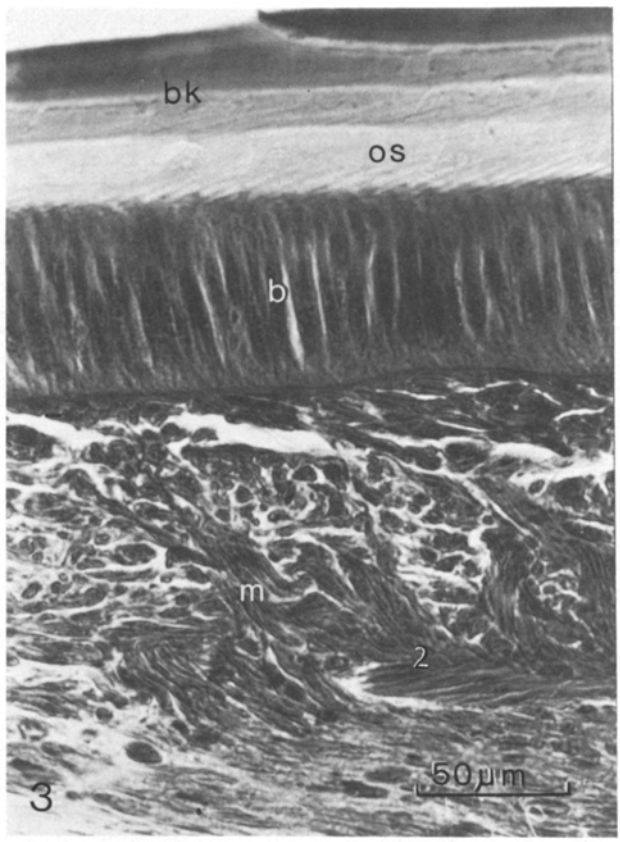


**Fig. 3.** Transverse section of the buccal mass of *Eledone* (Masson's stain) showing the lamellar structure of the beak with its different staining properties. The long beccublast cells are closely associated at one end with the beak and at the other with the muscles that move the beak. There are oblique striations adjacent to the beccublasts within the hard tissue

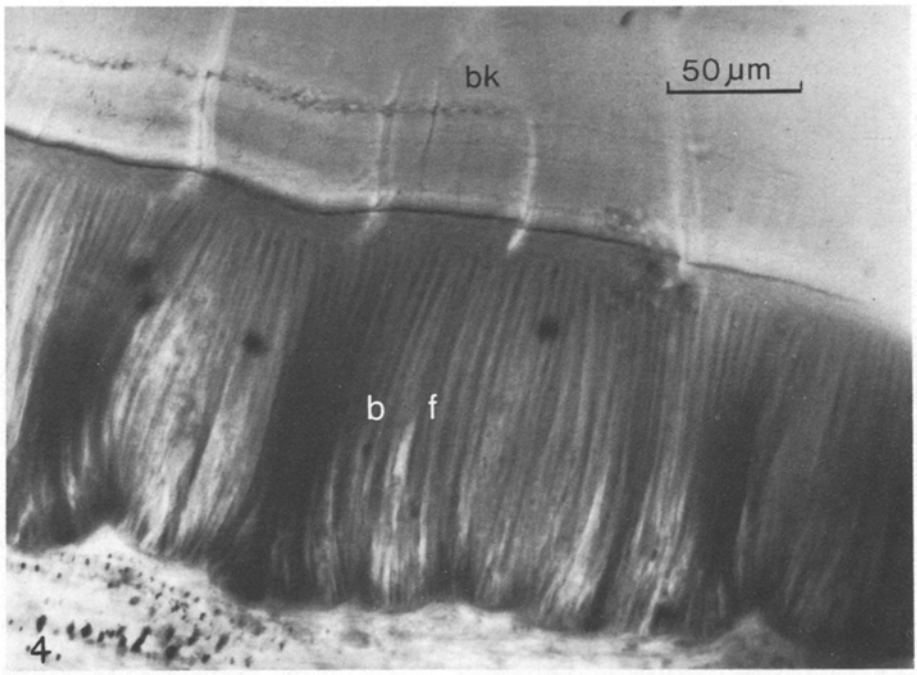


**Fig. 4.** Phase contrast micrograph of a freehand section of part of a plastic embedded osmium fixed postero-dorsal lateral wall region of the beak. The fibrils of the very tall beccublast cells show clearly

associated with many fine trabeculae of beak tissue extending down into the cell cytoplasm at the beak-beccublast interface. The cell membrane probably exists throughout this region and portions of it are thickened (Figs. 5, 6, 7). The thickened regions are generally found alongside the trabeculae where they indent the cell (Fig. 7).

The ends of the beccublast cells in contact with the beak leave their impressions on the surface of isolated beaks (Fig. 8). The impression is in the form of a hexagonal dent with a pitted surface. These polygonal pits are arranged in rows. Each polygon probably represents the interface between a single columnar cell and the newly formed surface of the beak as seen with the transmission electron microscope (Figs. 5, 6). This structure has only been observed in beaks that have neither been boiled in caustic solutions nor taken through graded ethanols. Either treatment probably removes the fine trabeculae that exist at this junction.

There are desmosomes between the cell membranes of the beccublast cells near the beak interface. There are no such thickenings of the cell membrane at the inner ends of the cells adjacent to the muscle interface. Here the cells tend to separate from one another over short distances and the gaps between them are filled with the tissue that is associated with the muscle cells.

Within the hard tissue of the beak there are oblique striations. These striations point forwards and upwards in the lower beak, and can extend throughout

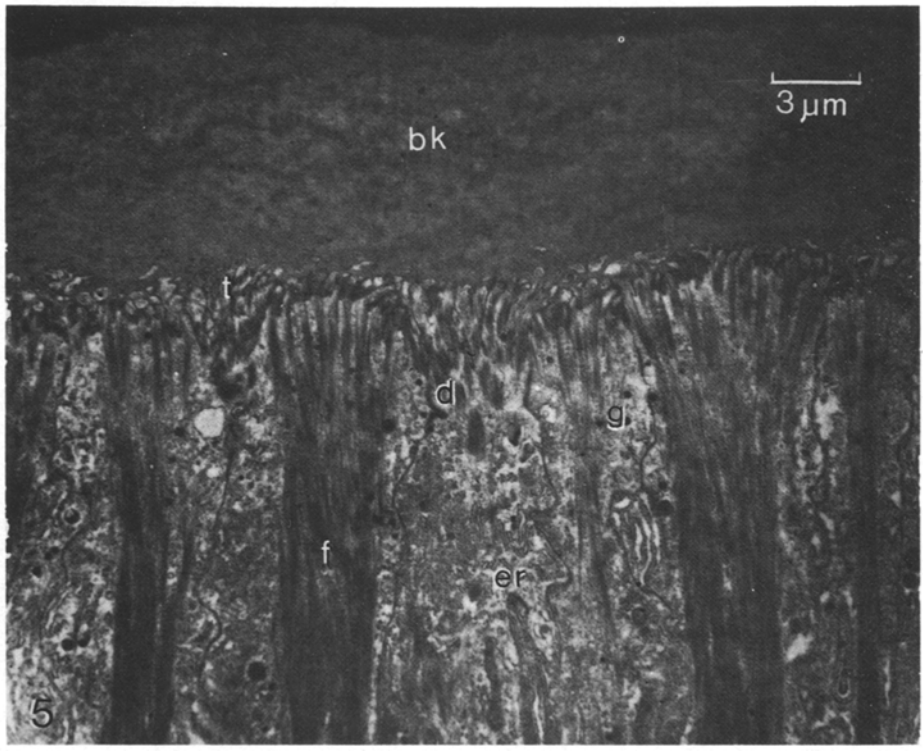


**Fig. 5.** Electron micrograph of the beak-beccublast cell interface showing the long fibres running along the length of many of the cells. Some cells contain electron dense granules

its thickness crossing the zones of differing density (Fig. 3) These striations may occur in the form of splits in the matrix of the beaks or as an apparent fibrillar background within the matrix. Each striation probably represents the material secreted by one beccublast cell. This method of secretion has features in common with the secretion of enamel by ameloblasts.

The region of the beak in apposition to the beccublast cells is pale and not very electron dense. Some 20 µm from the cell/beak interface there is a sudden transformation to a much more dense material (Fig. 3). This presumably marks the site along which some chemical change has occurred upon the previously secreted matrix. Although there is much dense material between this transition zone and the cell/beak interface, it seems likely that this transformation is the result of some activity of the beccublast cells.

The first major group of beccublast layer cells are the secretory cells that contain masses of endoplasmic reticulum and contain few if any fibrils. Sparsely distributed throughout their cytoplasm are spherical electron dense bodies. Their density is similar to that of the beak (Figs. 5, 6). Some of them are much more dense than the inner layer of the beak and resemble the density of the outer layers. It is probable that these electron dense bodies also contribute to the beak formation. The granules vary in size between 0.2 µm and 1 µm

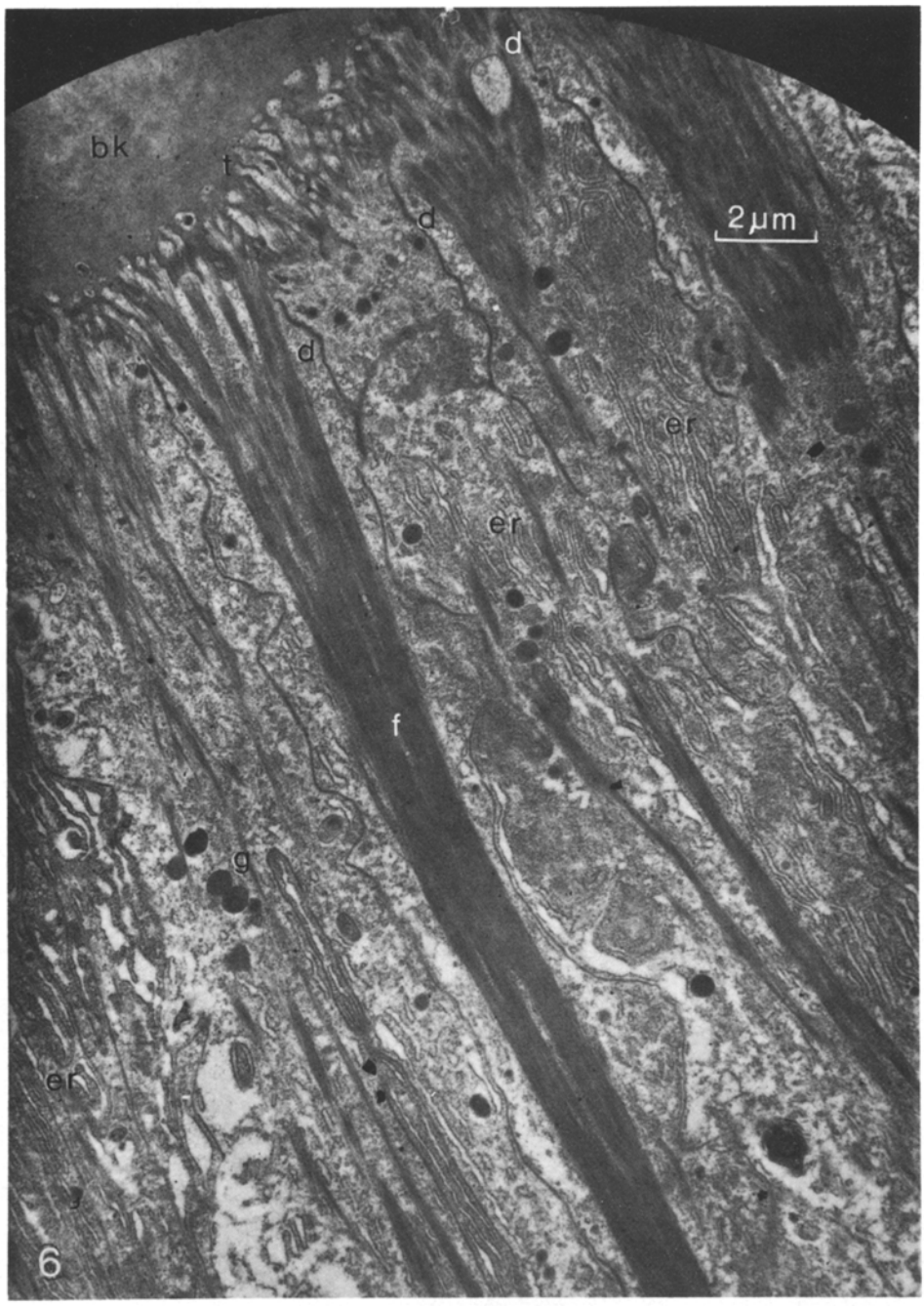


**Fig. 6.** Electron micrograph of several of the beccublast cells at the beak interface that have contents of longitudinally arranged endoplasmic reticulum, and a number of electron dense granules

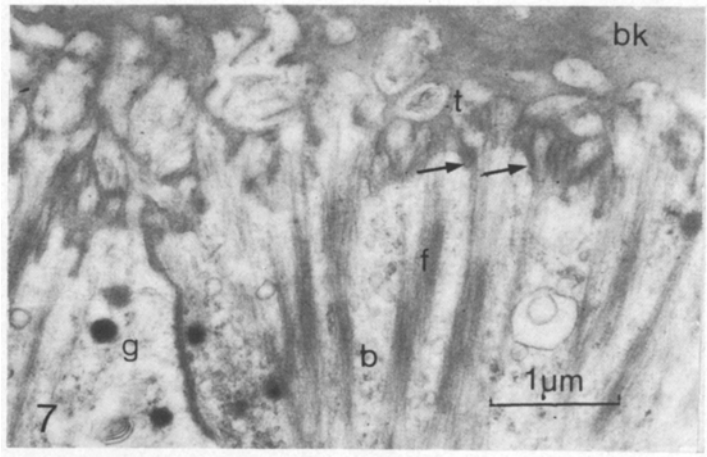


**Fig. 7.** High power electron micrograph of the beccublast/beak interface showing the trabeculae of the cell membranes. The arrows indicate the thickened membranes at the interfaces with the myofibrils. There is a possible light/dark banding. The fibres have dimensions similar to those of smooth muscle fibrils

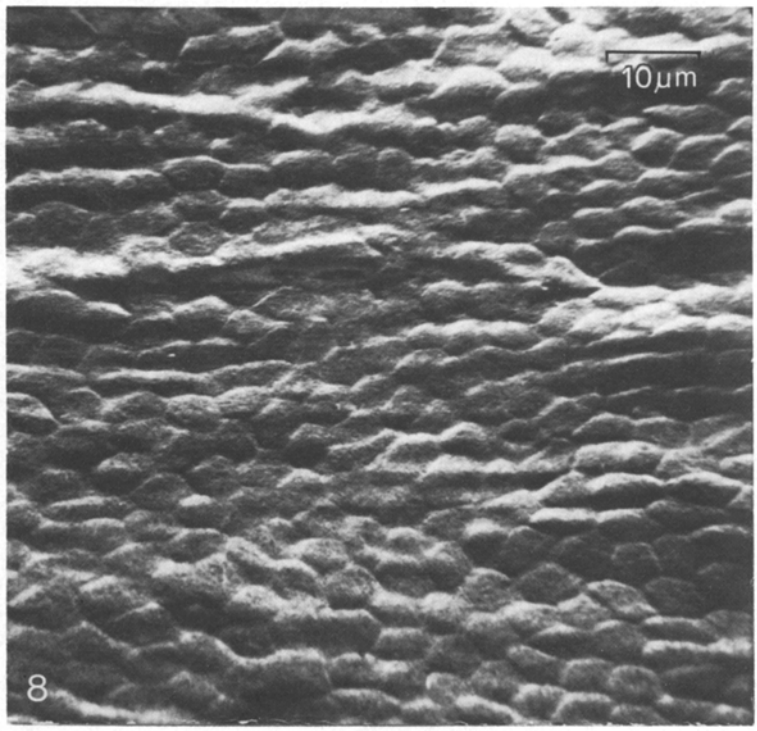


**Fig. 8.** Scanning electron micrograph of the surface of the upper beak, in the crest region, of *Alloteuthis* showing the aligned polygonal imprints of the beccublast cells

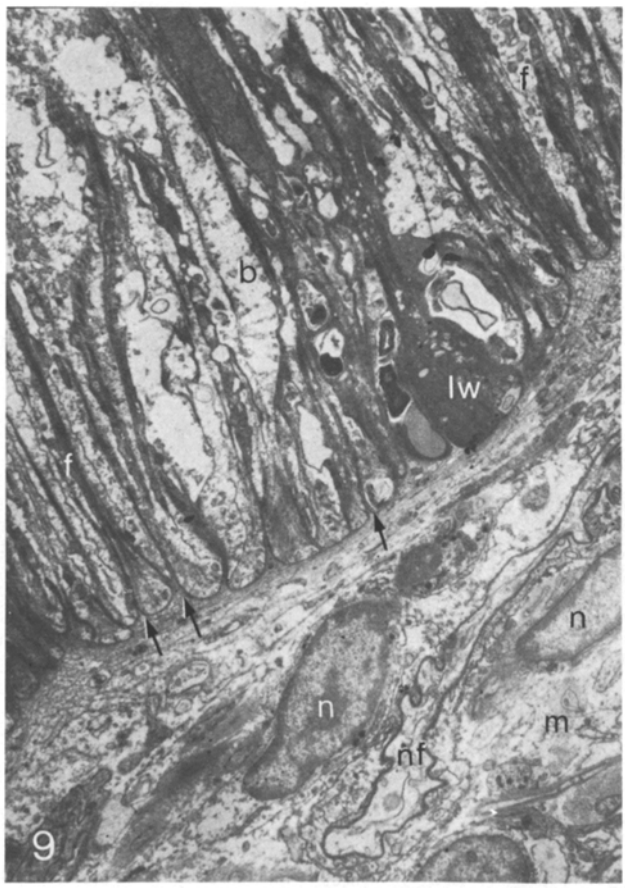


**Fig. 9.** Electron micrograph of the beccublast cells, at their junction with the muscles of the beak, showing the large lipid droplets. The intercellular extensions of the basement lamella (arrows) can be seen and a nerve fibre just outside the beccublast cell layer

and occur in all three types of cells. The granules are membrane bound, and are usually found near the regions of endoplasmic reticulum and in those regions of the cell nearest to the beaks. They are also seen in cells containing few myofibrils but much endoplasmic reticulum as well as in the solely endoplasmic reticulum containing cells.

The second major group of cells contains masses of fibrils that extend the whole length of the cells (Figs. 4, 5 and 6). These fibrils are arranged in bundles. Each bundle contains many fibrils. The bundles show a light dark periodicity in osmium fixed material. The period is about 1 μm. The bundles of fibrils spread out near the beak-beccublast interface to occupy the whole area of the interface, whereas throughout the rest of the cell, they are more concentrated and there are regions of clear cytoplasm between them. The fibrils themselves show many features similar to smooth muscle myofilaments (Figs. 6, 7). The myofi-

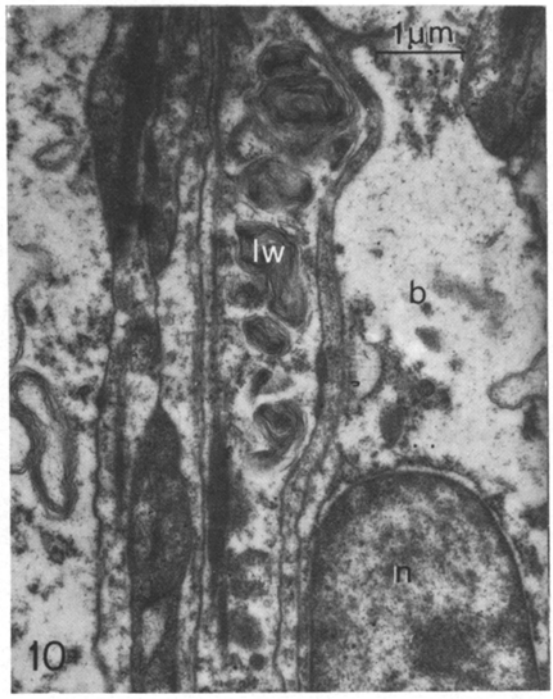


**Fig. 10.** Section from the middle region of a beccublast cell containing lipid whorls

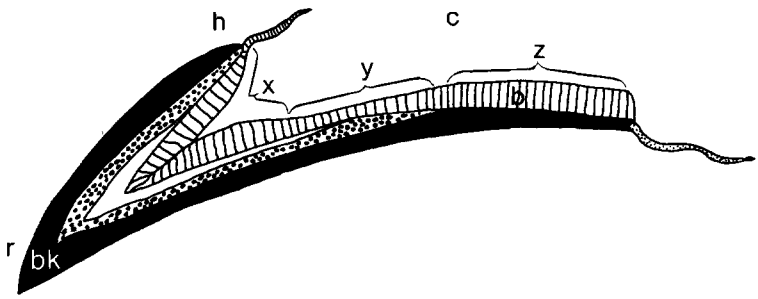


**Fig. 11.** Diagram of a beak to show the various regions containing a majority of one of the three major types of beccublast cells. *x y z*

brils at the beak beccublast interface often end against the thickenings of the cell membrane that are associated with trabecular intrusions (Fig. 7).

At the ends of the cells distal to the beak-beccublast interface the myofibrils tend to fuse both with the cell membrane and the invaginations of cell membrane and associated basement lamella between adjacent beccublast cells (Figs. 9, 10). Another common feature in beccublast cells is found in this distal region. Here there are large accumulations of masses of lipid-like membranous whorls and

droplets (Figs. 9, 10). These whorls are found in other parts of the cells but they are most common in the basal regions of the cells that contain the masses of endoplasmic reticulum.

The third major group of cells contains both fibrils and endoplasmic reticulum in varying amounts.

The cells of the beccublast layer are in three major regions. Those nearest the crest are tall and columnar, the majority of them having mainly secretory cytoplasmic contents but some fibrils, those of the intermediate group are less tall with the proportions of fibrillar and secretory tissues reversed, whereas those nearest the hood and rostrum of the beak look dead or dying with mainly fibrillar contents (Fig. 11). Suggesting that the major secretory activity takes place over the crest, and that the other cells are less concerned with secretion, and more with anchorage.

Silver staining reveals that there are nerve fibres closely associated with the inner ends of the beccublast cells, but so far we have been unable to find any myoneuronal junctions on these cells.

## Discussion

Ameloblasts are known to move relative to one another while the enamel matrix of the rat incisor is formed (Butcher, 1956; Boyde, 1964) and the direction of movement is lateral to the long axes of the cells. Reith and Ross (1973) found morphological evidence of contractile elements in the secretory ameloblasts of the rat. While there was no reason to suppose that the mechanism of enamel secretion and that of chitin formation should have any features in common, the morphological similarities within their blast cells are inescapable. The beaks of *Octopus* are secreted by tall columnar cells and these cells too have contractile elements, although they are arranged along the long axes of the secretory cells and not across them as in the rat ameloblasts. The myoid-like elements presumably provide the cells with the facility of movement which may be of importance for the growth (Clarke, 1962, 1965; Nixon, 1973) and modelling of the beaks. It is of interest to speculate whether these fibrils have any further functions such as increasing the intracellular pressure of secretory cells in specific areas in response to stresses, or if they just provide a mechanical anchor for the extrinsic muscles of the buccal mass that move the upper and lower beaks during feeding. The interdigitation between the beccublast cell and the beaks provides via the beccublast cell and its myofibrils an excellent mechanism for the anchorage of the extrinsic muscles of the buccal mass. This must be an important feature for such soft-bodied animals with few skeletal elements.

There are some parallels which may be drawn between the beccublast cells and the ameloblast. The large, irregular bodies with "lipid-like" membranes appear to correspond to the electron dense phagosomes of the ameloblast (Kallenbach, 1974). The close association between the beccublast and the secreted tissue is also seen in the secretory phase of the rat ameloblast (Kallenbach,

1974). We have not found any region where there is a gap between the beccublasts and the beak as is found in the maturation phase of ameloblasts.

The trabecular region is the place where the hard tissue of the beak is first deposited. Here the contents of the beccublasts are somehow transformed into the hard tissue.

There are electron dense granules within some cells and these may be the source of the hard tissue but we have seen no evidence of granules passing through the membrane. There are no structures like the membrane coating granules such as are found in keratinizing oral epithelium by Hayward and Hackemann (1973). Presumably either these electron dense granules have little to do with beak formation, or they are transformed into some electron lucent material before being converted into the electron opaque hard tissue of the beak.

The apparent similarity between the cells that form the enamel in rats and those that secrete the beaks in cephalopods is surprising. However, it probably indicates that the basic mechanism of hard tissue secretion has a long evolutionary history and has been utilized in secreting two very different hard tissues. Interestingly it is of note that recent observations suggest that the radula is secreted in a different manner (Dilly and Nixon, in preparation).

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