

probably related to the lens. But the hypothesis, following which the paracrystals of the eyes could be attuned to some parameter of the bioluminescent emission would not explain the existence of a similar lens in nonluminous species. Nevertheless, it remains puzzling to observe, in the same animals, an unusual organelle exceptionally developed in the 2 organs which have to do with light.

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### Protein digestion in the sea gooseberry *Pleurobranchia bachei* A. Agassiz (Ctenophora: Tentaculata)<sup>1,2</sup>

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**Summary.** Peaks of proteolytic activity of pharyngeal juice occur at pH 5.75 and pH 7.5. The proteases responsible for digestion include a tryptic alkaline protease and a thiol-activated acid protease which is probably cathepsin B. Levels of proteolytic activity parallel those of other carnivorous invertebrates which feed on zooplankton.

*Pleurobranchia bachei* is a small, ovoid, comb jelly which preys on a wide range of marine zooplankton by means of bizarre sticky papillae borne on its tentacles called colloblasts or 'lasso cells'. Food-laden tentacles are typically thrust into the comb jelly's mouth where pharyngeal juices elicit prey release followed by rapid digestion.

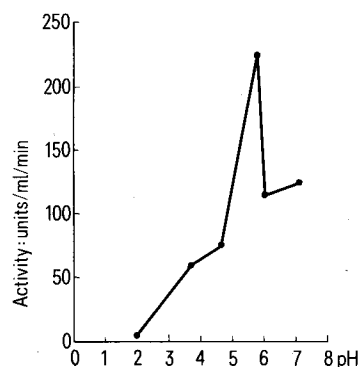
There is little information available on protein digestion in the Ctenophora<sup>4</sup>, and nothing is known about the proteases involved. In this report, we have examined proteolytic action on the pharyngeal juice of *P. bachei* and obtained a preliminary characterization of the enzymes involved. Our studies in protein digestion have necessarily emphasized acid proteases. This is due to a) the very limited quantity of digestive juice available to us for analysis and b) its acid range pH (about pH 5.3).

Several hundred *P. bachei* were obtained by plankton net at depths of 1–20 m from Indian Arm, Vancouver, British Columbia. Pharyngeal juices were carefully extracted by inserting a 3-mm length of 0.38 mm inner diameter by 1.09 mm outside diameter Intramedic polyethylene tubing into the comb jelly's foregut and applying vacuum using a  $\mu$ l syringe. Typical yields of digestive juice per individual were 2–5  $\mu$ l. Samples were pooled and immediately quenched in liquid nitrogen followed by freezer storage at  $-30^{\circ}\text{C}$ .

Proteolytic activity of 2 0.3-ml lots of pharyngeal juice was estimated<sup>5</sup> employing serum albumin as a substrate. Digests were run at  $37^{\circ}\text{C}$ . Freed amino acids were determined at 280 nm in microcuvettes in a Hitachi-Perkin-Elmer spectrophotometer<sup>6</sup>. A pH profile was obtained over a range of pH 2–8, using universal phosphate buffer<sup>7</sup>. The following activators and inhibitors were applied to the extracts to obtain a partial characterization of the proteases involved: 0.008 M cysteine (cathepsin B and C activator); 0.001 M iodoacetamide (cathepsin B inhibitor); 1% trypsin egg-white inhibitor; 0.23% L-1-tosylamide-2-phenyl-ethylchloromethyl ketone (TPCK: chymotrypsin inhibitor); 0.23%

N-a-p-tosyl-L-lysine chloromethyl ketone (TLCK: trypsin inhibitor).

The pH profile of the 1st sample (figure) shows a prominent peak between pH 5 and 6: 225 units at pH 5.75 and the indication of a 2nd peak above pH 7: 125 units per ml at pH 7.1. The 2nd sample which was tested showed greater activity: 420 units/ml at pH 5.6 and 416 units/ml at pH 7.5. TPCK chymotrypsin inhibitor had no inhibitory effect at pH 7.5. TLCK trypsin inhibitor caused a 70% reduction in activity at pH 7.5. Trypsin egg-white inhibitor caused a 98% reduction in activity at pH 7.5. Cathepsin B and C activator cysteine caused a 90% increase in activity at pH 5.6. Iodoacetamide caused a 60% reduction in activity at pH 5.6. These results indicate the presence of a tryptic protease and a thiol-activated acid protease which appears to have cathepsin B properties. Chymotrypsin, the enzyme which has been found in Cnidaria<sup>8</sup> does not appear to be present on the basis of the effect of chymotrypsin inhibitor. In-



Effect of pH on proteolytic activity of *Pleurobranchia* pharyngeal juice.

terestingly, proteolytic activity of the pharyngeal juice of *P. bachei* reaches similar levels of activity to the gastric juice of zooplankton-feeding septibranch bivalves<sup>9</sup>. Though the occurrence of acid proteases has been described as rare<sup>10</sup>,

this is probably a reflection of the rarity of investigations and the predilections of comparative enzymologists to concentrate upon the more familiar vertebrate enzyme homologues.

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### Self-suturing by a synaptid sea cucumber (Holothuroidea: Echinodermata)<sup>1,2</sup>

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**Summary.** The synaptid sea cucumber *Opheodesoma spectabilis* responds uniquely to wounding by closing the wound channel via its own 'sticky' anchor ossicles.

The Hawaiian sea cucumber *Opheodesoma spectabilis* Fisher is a conspicuous inhabitant in the quiet protected waters of Kaneohe Bay, Oahu Island, where it browses upon the surfaces of the brown alga *Sargassum echinocarpus*<sup>3</sup> and coral rubble. Lacking tube feet, *O. spectabilis* adheres to the substratum by means of thousands of minute, calcareous ossicles which protrude from the integument of its lengthy body<sup>4,5</sup>. *O. spectabilis* lives openly on the reef flat and

would appear vulnerable to predation by fishes, and indeed, attacks do occur<sup>3</sup>. Interestingly, *O. spectabilis* responds uniquely to wounding by rapid closure and sealing of the wound channel by means of 'sticky' dermal ossicles until healing can occur.

Anchor ossicles are a characteristic feature of synaptid sea cucumbers<sup>5</sup> and give the body surface its unpleasant, sticky feeling when handled. In *O. spectabilis*, anchor ossicles arise

SEM preparation of the outer body wall of *Opheodesoma spectabilis*. A thin 300 µm diameter nodular cap of epithelium has been dissected away to expose a single anchor ossicle in its natural posture (white point). Inset. Isolated button ossicle and anchor ossicle. During self-suturing, the pointed hooks of the anchor ossicles are protruded through the thin epithelium to stick together the edges of the wound channel (white bar equals 100 µm).

