PROBOSCIS REGENERATION OF *CHICOREUS VIRGINEUS* AND *RAPANA RAPIFORMIS* (PROSOBRANCHIA: NEOGASTROPODA)

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ABSTRACT

The pleuroembolic proboscis measures 16-23 mm in *Chicoreus virgineus* and 30-35 mm in *Rapana rapiformis*. After amputation of 10 mm length of the proboscis, *C. virgineus* regenerated faster (107 days) than *R. rapiformis* (132 days). After feeding resumed, it took *C. virgineus* 17 h, and *R. rapiformis* 21 h to drill a complete borehole in the bivalve prey *Meretrix meretrix*. The time to drill a hole prior to amputation was 12 h and 14 h respectively. Regeneration of proboscis in relation to the variation of biochemical constituents was studied in the two muricid species till they resumed normal feeding activity under laboratory conditions. During regeneration, the concentrations of protein, carbohydrate, lipid and glycogen decreased markedly in the foot, mantle and adductor muscle of both species.

INTRODUCTION

Boring of prey shells by muricid gastropods involves close interaction of the proboscis, propodium and the accessory boring organ (ABO) in a predictable cycle which repeats itself continuously throughout the process of boring. Predators can make a borehole in the shells of prey and extend the proboscis deep into the prey to feed within a wide radius of soft tissue. However, the proboscis may accidentally be amputated when pinched between the valves of the prey. Loss of this important organ is not fatal, as the snail possesses enough metabolic reserves to survive until the proboscis is regenerated (Carriker 1981).

Early studies on aspects of shell penetration by boring gastropods were conducted by Oswald (1894), Herrick (1906), and Carriker (1943). Day (1969) investigated the feeding of the cymatiid gastropod *Argobuccinum argus* in relation to the structure of proboscis and secretions of the proboscis gland. The anatomical functions in the buccal mass of prosobranch and amphineuran molluscs were analysed by Graham (1973). Recently, Miller (1989) investigated the structure and functions of the toxoglossan proboscis.

Literature regarding the study of proboscisectomy of predatory gastropods is very scanty. Demoran & Gunther (1956) investigated amputation of proboscis of *Thais haemostoma*. Carriker & Van Zandt (1972), and Carriker *et al.* (1972) stated that the radula, being a relatively hard structure, provides a readily measurable parameter for quantitative determination of proboscis regeneration. Recently, Muthiah & Sampath (1994) observed the regeneration of proboscis of *Cymatium pileare* in Indian waters.

The present study was conducted to describe the general morphology and histological features of the proboscis and also to determine the rate of its regeneration after amputation in two muricid gastropods *Chicoreus virgineus* and *Rapana rapiformis* under laboratory conditions.

MATERIALS AND METHODS

The snails were collected from Porto Novo coastal waters (11°29' N; 79°47' E), the Bay of Bengal, and acclimatized in the laboratory in filtered sea water for one month prior to proboscisectomy. *Chicoreus virgineus* (total length 6.0-6.5 cm) and *Rapana rapiformis* (total length 5.9-6.6 cm) were selected for the present study. The soft body

was removed by cracking the shell. The proboscis was dissected free in clean sea water. For histological studies, the proboscis was fixed in Zenker's fluid. Sections of 3 mm thickness were stained in Delafield haematoxylin and counterstained with aqueous eosin. The stained sections were dehydrated through ascending grades of alcohol, cleaned in xylene, and mounted with DPX.

Both species of muricids were actively feeding on bivalves, *Meretrix meretrix*, in the early hours of the day. The snails as well as the prey were removed from the tank. The valves of *M. meretrix* were pressed tightly together to avoid withdrawal of the proboscis. Proboscisectomy was performed in quick succession on 40 individuals of each species of *C. virgineus* and *R. rapiformis*. The amputated portion of proboscis, including the complex buccal mass, was taken out, measured, and the number of transverse rows in the radula counted. The proboscisectomized snails were kept individually in perforated plastic tubs of 5 litre capacity. *M. meretrix* (length 3.3-3.5 cm, width 2.8-3.0 cm) were examined daily, while changing the sea water, to check whether they had been attacked. Resumption of feeding by the proboscisectomized snails indicated that regeneration of the proboscis had occurred. Biochemical constituents of foot, mantle and adductor muscle were estimated every 10 days in two individuals of each species till they resumed normal feeding activity. Methods were applied according to Raymont *et al.* (1964) (protein), Dubois *et al.* (1956), (carbohydrate), Folch *et al.* (1960) (lipid), and Carrol *et al.* (1956) (glycogen).

RESULTS

The pleuroembolic proboscis of *C. virgineus* and *R. rapiformis* is characterised by a terminal buccal mass and a non-permanent rhynchodium (proboscis sac) which is formed only when the proboscis is retracted. When a proboscis is fully everted, the mouth lies at the tip, and the proboscis sac disappears. The proboscis of *C. virgineus* measured 16-23 mm and *R. rapiformis* 30-35 mm. It consists of a much elongated tube or sheath (laminated muscle) enclosing the buccal mass which opens anteriorly into the mouth, and dorsally into the oesophagus. The distal tip of the proboscis bears a projected, circular, tactile peristomal rim inside which the true mouth lies. The mouth opens into the buccal cavity, and the middorsal wall of the buccal cavity opens into the oesophagus. The odontophore lies in the posterior half of the buccal mass and it is the fleshy tongue-like protuberance supporting the active part of the radula. The dorsoventrally compressed oval body of the odontophore is covered by dorsal and ventral odontophoral protractor muscles. The odontophore is overlaid by several layers of muscles. The odontophoral cartilages consist of two laterally flattened, cylindrical bodies connected ventrally by transverse muscles. In both species studied, the radula is located in the radular sac surrounded by a sub radular membrane. The accessory salivary glands, placed ventrally on the proboscis, are shaped somewhat like a kidney bean, externally covered by a tough epithelium. The proboscis of both species comprises tall columnar epithelial cells arranged in a single layer which contains a basal nucleus, and closely packed mitochondria in a distal portion.

Generally, the muricids first try to insert their proboscis prior to drilling by holding apart the valves of the prey with the help of the labial spine. The proboscisectomy was undertaken only when the animals were feeding without involving the drilling process. In consequence, the amputated length of the proboscis varied in size, depending upon how far the proboscis was inserted between the valves at the time of proboscisectomy. The amputated lengths were categorised into lengths from 3 mm to 10 mm.

The shape of boreholes made on the valves by snails, and the time spent on drilling were examined soon after complete regeneration of the proboscis.

A steep fall in protein, carbohydrate, lipid and glycogen were recorded until complete recuperation of proboscis. All proboscisectomized snails survived till they resumed feeding activity.

DISCUSSION

The ability of muricid gastropods to penetrate the shell of prey allows them to feed on otherwise inaccessible organism much larger than themselves. However, after penetration of the shell, snails risk to lose the proboscis in two ways while feeding: (a) amputation by small crabs and fish when the proboscis is extended into the mantle cavity through the borehole, and (b) pinching and subsequent loss while the proboscis is inserted between the valves. Amputation by both means may occur in nature, though the frequency of occurrence is unknown.

The accessory salivary glands are placed ventrally on either side of the proboscis, similar to findings in other stenoglossans: *Oliva* (Küttler 1913), *Nucella* and *Ocenebra* (Graham 1941), and *Urosalpinx* (Carriker 1943). Accessory salivary glands secrete digestive enzymes, such as amylase and protease. During the course of digestive processes the food particles are partly digested in the buccal cavity by amylo and proteolytic activities and then sent through the oesophagus for further digestion.

Molluscs can efficiently regenerate various parts of the soft body (Hyman 1967). In this study, two species of muricids regenerated the proboscis within a short period, in accordance with findings in *Thais haemostoma* (Demoran & Gunther 1956). Carriker *et al.* (1972) measured a rapid rate of increase in length of radulae of *Urosalpinx* and *Eupleura*. In the present study the time of onset of boring of prey, following proboscisectomy, varied over a period of 23 days in different individuals. We also found considerable variation, depending on the lengths of lost proboscis. *C. virgineus* resumed feeding after 17-107 days and *R. rapiformis* after 28-132 days.

Feeding is only possible when the radula is completely regenerated. In general, the earliest regenerating teeth, the median or rachidian teeth, increased most rapidly in size. Isarankura & Runham (1968) reported a very rapid rate of replacement of the radula in newly hatched prosobranchs and pulmonates. The present muricids spent more time on feeding when they started to eat again after regeneration. This may be due to incomplete regeneration of radula teeth. The median teeth regenerated faster than the marginal teeth, and the marginal teeth were underdeveloped.

Concentrations of biochemical constituents, including glycogen, decreased in the mantle, foot, and adductor muscle during regeneration of the amputated proboscis. However, drastic changes were noticed only in mantle tissue of both species. In comparison, the snail *Lymnaea stagnalis* has vesicular connective tissue cells for glycogen storage and these cells occur in large numbers in the mantle edge (Sminia 1972). Hence, in view of the glycogen storage capacity of *L. stagnalis*, the presence of high glycogen content in mantle tissue of the muricids may be understood. The steep drop in glycogen concentration of the tested muricids may indicate that they depend on glycogen for energy production during starvation following proboscisectomy.

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ISSN 0858-3633