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Gutless Bivalves

Abstract. A new benthic species of the protobranch bivalve genus *Solemya*, from the northeastern Pacific Ocean, lacks a gut. It has no internal digestive enzymatic apparatus; nor is there any provision for the secretion of enzymes into the mantle cavity for extraorganismic digestion. The most likely nutritional mechanism for such an animal is the active absorption of dissolved organic molecules from the environment by the large ctenidial lamellae. These are well provided with blood, cleansed of sediment by cilia, and have phosphatases in their epithelial cells. *Solemya borealis* may also be gutless.

Most bivalve mollusks have a prominent alimentary tract that can easily be discovered by conventional dissection methods. The relatively large stomach is usually surrounded by the digestive diverticula, glandular tissues discernible in the anterodorsal region of the visceral mass without the aid of dissection or microscope. The mouth is often inconspicuous, but the rectum is usually clearly visible next to the posterior adductor muscle. In contrast, members of the protobranch bivalve genus *Solemya* are known for the reduced size of the gut (1, 2). The gut of *Solemya parkinsoni* is so reduced that Owen (2) concluded that the species must have some unusual means of augmenting the processes of digestion and assimilation. He proposed that the mantle cavity might act as an organ of extraorganismic digestion.

We have independently of each other discovered a new species of *Solemya* (3) and have concluded that this species is gutless. A gross anatomical inspection of the largest specimens available to us, with the aid of a dissecting microscope, revealed no esophageal opening behind the depression representing the mouth, no stomach nor digestive diverticula, and no rectum. Earlier investigators had difficulty tracing the alimentary tracts of other species of this genus because of the gut hypotrophy (2, 4). However, further scrutiny of the new species by means of transverse and longitudinal serial histological sections gave no indication whatsoever of any part of an alimentary system. Specimens of shell length 5 mm to 3 cm were sectioned. The largest of these were sexually mature.

In some animals, glandular tissues from the anterior dorsal region and from within the foot had a superficial resemblance to digestive diverticula; these tissues were tested spectrophotometrically for their ability to digest albumin (5). There was no proteolytic activity in the range of hydrogen ion concentrations usually associated with the digestive systems of bivalves (pH 5 to 8) (6). The tissues in question were later discovered to be spent gonad and pedal gland.

The pedal gland is probably the organ that was suggested as a source of enzymes for extraorganismic digestion in *Solemya parkinsoni* (2). This tissue was tested histochemically for esterase and phosphatase activity, α -naphthyl acetate and α -naphthyl phosphate being used as substrates (7). A weak alkaline phosphatase activity was found, but the other results were negative. The cells of the pedal gland were strongly stained by Alcian Blue, which indicates the presence of acid mucopolysaccharide. The large pedal gland opens through a pore to the sole of the foot; we conclude that its function is to secrete the large quantities of mucus with which this organism lines its burrows (8).

Further to the possibility of pallial digestion, whole animals were fixed briefly in cold, neutral Formalin and incubated in esterase and phosphatase mediums (7). A weak phosphatase reaction along the distal edges of the ctenidial lamellae was the only positive reaction over the entire outer surface of the animal. Although we have not exhausted the possibility, we doubt that there is any extraorganismic digestion by endogenous en-

zymes in the mantle cavity and suggest that the means whereby this species obtains its nutriment is by absorbing dissolved organic molecules. The ctenidia, which are large in comparison with those of most other protobranchs and well supplied with blood sinuses, present a suitable surface area for the absorption of organic molecules (8). The presence of phosphatases indicates an active transport mechanism. The foot and mantle may also absorb nutrients.

Symbiotic bacteria in the mantle cavity or in the burrow system could hydrolyze particulate organic material such as wood particles, which are plentiful in the environment. An observation on starch hydrolysis in the mantle cavity of *Solemya parkinsoni* (2) hints at the possibility of digestive symbiosis with bacteria. Another source of useful dissolved nutrients could be facultative anaerobic bacteria in the mantle cavity or burrow system (9). Stanley (10) suggested that bacteria in the burrow of *Solemya velum* might be a source of food.

Although the smallest specimen we sectioned (shell 5 mm long) showed no trace of a gut, the small prodissococonch shell and the size of ova suggest a planktotrophic larva, indicating a functioning gut during early development. The species is benthic and most commonly found burrowing in silt in the vicinity of pulp mills. Whether the pulp mill effluent and wood debris provide food or a congenial environment for symbiotic microorganisms, or whether they simply discourage predators and competitors, remains to be determined.

This new, northeastern Pacific Ocean species of *Solemya* was formerly incorrectly identified with *Solemya panamensis* Dall of the tropical eastern Pacific (3). Hypotrophy of the gut is characteristic of the group, but the degree of reduction of the gut cuts across the present taxonomic arrangement, which is based on shell structure. We have examined other species. *Solemya valvulus* Carpenter and *Solemya velum* Say possess small guts similar to that of *Solemya togata* Poli (1). *Solemya panamensis* Dall and *Solemya* (= *Acharax*) *agassizii* Dall have vestigial guts similar to *Solemya parkinsoni* Smith (2). On the basis of the dissection of a single preserved specimen, *Solemya borealis* Totten appears to be gutless.

The gutless condition of another non-parasitic invertebrate group, the Pogonophora, is well known (11). On the basis of their biology, zoologists have been inclined to associate gutlessness with small sessile animals with low energy requirements and large ratios of surface area to

volume. However, this *Solemya* sp. is a relatively large animal that can burrow actively and sustain swimming by water-jet propulsion for periods of as long as 1½ minutes (8). Its gutless condition might have been surprising were it not for recent discoveries of several groups of gutless Pogonophora much larger than those found originally. These include large specimens of Vestimentifera (12) and giant tube worms collected from the volcanic vent regions of the Galápagos Rift (13). Jones (14) believes that the latter tube worms are related to the Pogonophora and confirms that they are gutless. Southward *et al.* (15), reporting on the value of dissolved organic molecules as food for the pogonophoran *Siboglinum fiordicum*, discussed the importance of association with microorganisms and reviewed the spectrum of physiological relationships with microorganisms that might be useful to benthic aerobes. In such symbioses, a burrow or a tube seems to be necessary to contain and confine the related organisms and prevent the dissipation of useful solutes. In comparing the biology of the *Solemya* sp. with *Siboglinum* and the Galápagos Rift tube worms, we point out a common feature: high densities of these gutless animals are found in the vicinity of extraordinary energy sources. Dense populations of *Siboglinum* are found near a commercial fish farm (15); the Galápagos

Rift tube worms are found around volcanic vents in association with chemosynthetic bacteria (13); and *Solemya* sp. forms dense populations near pulp mills (8). This may provide a clue to the evolutionary ecology of such organisms.

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Neurotransmitter Receptor Binding in Bovine Cerebral Microvessels

Abstract. Purified preparations of microvessels from bovine cerebral cortex contain substantial levels of alpha-adrenergic, beta-adrenergic, and histamine 1 receptor binding sites but only negligible serotonin, muscarinic cholinergic, opiate, and benzodiazepine receptor binding. Norepinephrine and histamine may be endogenous regulators of the cerebral microcirculation at the observed receptors.

Cerebral blood vessels are dynamically regulated to respond to variations in cerebral metabolic activity. Chemical factors such as carbon dioxide, potassium, calcium ions, and adenosine nucleotides are thought to accumulate within the perivascular space to couple brain blood flow and energy metabolism (1). Neuronal projections to cerebral blood vessels may also provide a mechanism by which the brain regulates its own blood flow, blood volume, and capillary area (2). The superior cervical ganglia and locus ceruleus are thought to send axonal projections that innervate small blood vessels in the forebrain (3). These vessels contain norepinephrine and related enzymes (4) as well as a beta-ad-

renergic-sensitive adenylate cyclase (5). Serotonin-containing neurons with cell bodies in the raphe nuclei also appear to project to cerebral microvessels (6), as may neurons containing vasoactive intestinal peptide and substance P (7). To clarify how transmitters might regulate vessel caliber, we measured receptor binding for a variety of neurotransmitters and drugs in purified preparations of small cerebral blood vessels. We report that microvessels from bovine cerebral cortex contain specific alpha-adrenergic, beta-adrenergic, and histamine 1 receptor binding sites.

Brain microvessel preparations were made from calf brains (8). To separate the microvessels from surrounding pa-

renchyma, pia and arachnoid membranes were removed with forceps under 3-diopter magnification (6). For each preparation, 50 g of cortical gray matter was used. Approximately 10-g lots of gray matter were removed and homogenized with two to three volumes of cold (4°C) 0.9 percent NaCl in a loosely fitting (0.1-mm clearance) Potter-Elvehjem homogenizer. The homogenates were centrifuged at 1500g for 15 minutes, the supernatant fractions discarded, and the pellets washed one more time. The pellets were resuspended in 60 to 80 ml of 0.25M sucrose, layered over gradients of 1.5 and 1.0M sucrose, and centrifuged in an SW 27 rotor at 58,000g for 45 minutes. The resulting microvessel fraction was resuspended in 100 ml of cold saline, adsorbed onto small glass beads, and washed extensively with cold buffer. The vessels were then trapped in a nylon filter (80-µm mesh), again washed extensively, and frozen until assay. Thus approximately 500 mg of microvessels were obtained from 50 g of bovine cortical gray matter. Both the microvessel preparation and frozen calf cortex were homogenized in 80 volumes of tris-HCl (pH 7.7 at 25°C). The tissue suspensions were sieved through 500-µm nylon mesh and immediately assayed for receptor binding.

Substantial levels of alpha-adrenergic, beta-adrenergic, and histamine 1 receptor binding were detected in the preparations (Table 1). Binding of *p*-[³H]aminoclonidine and [³H]WB-4101 to alpha-adrenergic receptors in the microvessels was almost half that found in bovine cortical homogenates. [³H]Dihydroalprenolol binding to beta-adrenergic receptors and [³H]mepyramine labeling of histamine 1 receptors were about 30 and 65 percent, respectively, of binding levels in neuronal membrane preparations. By contrast, only very low levels of binding were detected for [³H]serotonin, [³H]spiroperidol, and D-[³H]lysergic acid diethylamide (labeling of serotonin receptors); for ³H-labeled D-Ala-Leu-enkephalin and [³H]naloxone (labeling of opiate receptors); for [³H]flunitrazepam (labeling of benzodiazepine receptors); and for [³H]quinuclidinyl benzilate (labeling of muscarinic cholinergic receptors). Interestingly, the microvessel percentage of brain benzodiazepine binding was low despite the fact that the absolute levels of binding were higher in microvessels than for most other ³H-labeled ligands. In preliminary saturation experiments, the various levels of binding of ³H-labeled ligands in microvessels and brain membranes reflected differences in the maximum number of binding sites,