On the Maintenance of *Deontostoma californicum*

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Nematode species selected for physiological, developmental, and biochemical studies should be reasonably representative of the majority of nematodes and as large as possible so morphological structure can be utilized as an experimental unit. Animal parasitic nematodes are among the largest but usually are not routinely obtainable in abundance. Those easily obtained *e.g. Ascaris lumbricoides* are not typical nematodes and cannot be artificially cultured through successive life cycles. Moderate-sized facultative insect-parasitic neoaplectanid nematodes are easily reared *in vitro* and have often been used. Plant parasitic and soil nematodes are exceedingly small. For a basic nematode study the complications of parasitic specialization should be avoided. Among the moderate-sized free-living marine nematodes (10–30 mm in length) found along the California Pacific coast, *Deontostoma californicum* appears to be a suitable experimental animal. For this reason several of its ecological parameters were explored.

Deontostoma californicum is found in the intertidal zone and can be collected from the holdfasts of *Egregia laevigata* and *Laminaria digitata* (4). Its preferred habitat appears to be the matrix of sand, detritus and partially decayed organic matter found in a holdfast. It is the matrix which is important: well established *Ulva lactuca* also forms a similar matrix on the rock that can support good populations of *D. californicum*. *Deontostoma californicum* occupies this habitat with other nematodes, annelids, crustaceans and other forms of fauna as well as microflora. At Dillon Beach, California, *D. californicum* occurs in greater density in matrices exposed to the incoming sea.

The gut contents of this marine nematode are normally a dark reddish-brown or, on occasion, may be red or black. The red appears most often in nematodes in close proximity to large red fungal mats growing near the holdfast and may be caused by ingestion of free-floating bits of mycelium. The black usually occurs in nematodes found in an extensively decomposed holdfast. Usually the more putrified holdfasts yield but few nematodes. The gut contains diatoms, algae and faunal fragments; evidently *D. californicum* ingestion is nonselective. In experiments various colored plastic or colored grease pencil particles were incorporated into seawater agar. Overnight the intestinal contents of *D. californicum* on these media became colored by whatever pigmented particles were present. Chitwood's (2) cornmeal-calcium carbonate-seawater medium for other marine nematodes was unsatisfactory; *D. californicum* placed overnight on the medium in petri dishes crawled up to the undersurface of the petri plate cover. Sugar-seawater agar medium alone or inoculated with fragments of raw holdfast matrix was also unsuitable; *D. californicum* placed on either medium were quickly overcome by contaminating microorganisms.

In other experiments *Aphelenchus avenae*, a phytophagous soil nematode reared on the fungus *Pyrenochaeta terrestris* on potato dextrose agar medium, was washed, concentrated and allowed to osmoregulate in seawater, then placed with *D. californicum* in a seawater-sand habitat (1). Both species coexisted for several months with no reproduction or change in numbers. It has as yet not been possible to rear *D. californicum* axen-

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TABLE 1. Molar composition of selected balanced salt solutions and nematode activity.

	Puck's[a] Saline G	Puck's Saline F	Dulbecco	Gey	Hank	Earl	Spinner	Seawater
NaCl	0.14	0.13	0.14	0.14	0.14	0.12	1.2	0.80
KCl	0.005	0.004	0.003	0.005	0.005	0.005	0.05	0.009
$MgSO_4$	0.0006	0.0006			0.0004	0.0008	0.008	
$CaCl_2$	0.002	0.002	0.0009	0.003	0.001	0.002		0.01
KH_2PO_4	0.001	0.0006	0.002	0.0002	0.004			
Na_2HPO_4	0.001	0.001	0.008	0.008	0.0003			
$NaHCO_3$		0.01				0.03		
$MgCl_2$			0.0005	0.001	0.0005			0.05
Na_2SO_4								0.03
NaH_2PO_4						0.0009	0.08	
Glucose	0.006	0.006		0.001	0.006	0.006	0.06	
Nematode Activity (9 days)	—†	—	—	+	+	—	—	+++

[a] Seawater salt concentrations were calculated from data in "Sea Shore Life," J. W. Hedgpeth, University of California Press, 136 pp 1964. All other salt concentrations were calculated from data in Gibco Reference Manual, Grand Island Biological Company, 3175 Staley Road, Grand Island, New York 14072.
† (+++) = high activity; (+) = sluggishness; (−) = no activity.

ically, probably because of the toxicity of the axenizing treatment (5).

Freshly-collected nematodes, placed in selected, balanced salt solutions (Table 1) used for excised organ culture, maintained the nematode less satisfactorily than seawater. An aliquant of nematodes was removed daily from each salt solution and allowed to recover overnight in seawater. Only in salt solutions of Gey and Hank were the nematodes alive, though very sluggish, at the end of nine days. In Spinner's solution they appeared somewhat shrunken and wrinkled, whereas in the other salt solutions they remained quite turgid. It is evident from a comparison of the molar composition of the balanced salt solutions with seawater (Table 1) that the nematode turgidity was explicable in terms of the osmotic pressures of the solutions, all of which were hypotonic to seawater, except for Spinner's which was hypertonic. The longer survival in Gey's and Hank's salt solutions suggest that *D. californicum* is not tolerant of higher concentrations of either bicarbonate or dibasic and monobasic phosphates. This probably follows from the known osmotic relations of this nematode (3). The nematodes survived several week's storage in seawater at 4 C in reasonably good condition provided the water was exchanged and aerated daily. Nevertheless, approximately 50% of the eggs mechanically forced from the uteri of gravid females and incubated in seawater in B.P.I. dishes sealed with petroleum jelly under a cover slide at 15 C, embryonated into normal juveniles after 30 days.

Freshly collected nematodes are in a highly-irritated state (writhing vigorously) and when placed together in a small beaker

of seawater, clump together by intertwining and by the stickiness of the secretion produced by the caudal spinneret. It is not unusual to observe an isolated nematode with its movement restricted by loops of spinneret secretion around its body. In the first day or so after collection, irritated nematodes may produce copious amounts of spinneret secretion; within a few days this is much reduced. X-ray diffraction analysis reveals that the spinneret thread is amorphous. With increased storage time the irritability of the nematode decreases. A freshly collected cluster of nematodes disentangle and become inactive when left undisturbed in darkness; however, when disturbed they reclump. After extended storage time this response diminishes and can be mildly effected only after prolonged aeration.

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