Major- and Trace-Element Analyses of a Mermithid Parasite and its Mosquito Host by Proton-induced X-ray Emission

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A few nutritional studies on the mosquito nematode *Romanomermis culicivorax* Ross and Smith (= *Reesimermis nielseni* Tsai and Grundmann, auct., partim.) have been reported by Gordon and Ittycheriah (3). No data are available, however, on the kinds and amounts of major and trace elements utilized or accumulated by this mermithid parasite of mosquito larvae, even though the importance of metals and nonmetals in biological systems has been established (1). Nicholas (6) presented physiological data on the uptake or loss of elements in some nematodes as a function of diet and substrate. This research was done to evaluate the feasibility of utilizing

Received for publication 23 May 1978.

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proton-induced X-ray emission (PIXE) to study the concentration of several major and trace elements of *R. culicivorax* and its host.

Whole-body analyses were performed on the nonfeeding postparasitic stage of *R. culicivorax* since nematode total-body multielemental levels would directly reflect the major and trace elements obtained from feeding in the hemolymph of its mosquito host during the nematode's parasitic phase. In addition, to determine the reliability and sensitivity of PIXE, total-body analyses were conducted on parasitized and unparasitized late 3rd-to-4th-instar larvae of *Culex pipiens quinquefasciatus* Say. *Culex p. quinquefasciatus* were initially obtained from Dr. J. J. Petersen, SEA, USDA, Gulf Coast Mosquito Research Laboratory, Lake Charles, Louisiana, in 1975. Unparasitized and parasitized mosquito larvae and postparasitic nematodes were mass reared in several types of culture trays fabricated from stainless-steel sheets that were soldered with a high-temperature iron. In addition, a sample of parasitized larvae of *C. p. quinquefasciatus* reared in a plastic bucket and frozen ca. 2 months at 0 C prior to these tests was also available for elemental analyses.

A 12:1 preparasite-to-host ratio (60,000 preparasites to 5,000 1st-instar *C. p. quinquefasciatus* larvae) was used in the mass production of *R. culicivorax*. Mosquitoes and nematodes were reared at 25–26 C (ambient) in trays or buckets containing dechlorinated reverse-osmosis water (conductivity = 180 μmhos/cm), and mosquitoes were fed ground rabbit pellets (Jim Dandy Co., Birmingham, Alabama) by the feeding schedule established by Petersen and Willis (7).

Parasitized and unparasitized *C. p. quinquefasciatus* larvae and postparasities were washed several times in distilled water, placed in crucibles, and dried in an oven at 100 C for ca. 18 h. Respective weights of the dried postparasites and parasitized and unparasitized larvae reared in stainless-steel trays and parasitized larvae reared in a plastic bucket were 1.70, 4.76, 1.83, and 2.91 g.

The dried material was then ashed in a muffle furance at 400 C for 24 h, and ca. 100 μg of the ash of each sample was analyzed (two replications) by PIXE using a 3.8-MeV proton beam from the Van de Graaff accelerator of the University of Florida by procedures established by Van Rinsvelt et al. (9).

Spectra of ashed unparasitized and parasitized *C. p. quinquefasciatus* larvae and postparasites of *R. culicivorax* indicate that total-body levels of K, Ca, Mn, Fe, Ni, Cu, Zn, Pb, As, Se, Br, Rb, Sr, Zr, and Sn can be detected in ashed nematode and mosquito larval tissues analyzed by PIXE. Although Rb, As, and Br were present in the X-ray spectra of all tissues analyzed, interference was observed between the X-ray lines of Br and Rb, Pb and Rb, and Pb and As, inhibiting elemental quantification. Low levels (<1 ppm) of Zr, Sn, Ni, and Se presumed to be present in the ashed tissues of several samples were also obscured by background radiation.

Van Rinsvelt et al. (8) and Levy et al. (4, 5) used a similar analytical technique (i.e., ion-induced X-ray fluorescense) to identify major and trace elements in ashed tissues of several insects.

Table 1 presents quantitative multielemental analyses of *R. culicivorax* postparasites and parasitized and unparasitized hosts. The data indicate that parasitized *C. p. quinquefasciatus* larvae reared in stainless-steel trays contained a greater multi-elemental concentration than parasitized larvae reared in plastic buckets. This suggests that an inert plastic container is a better rearing chamber for PIXE analyses because quantitative error can result from the uptake and accumulation of various elements as contaminants in the tissues of mosquito larvae and nematodes reared in stainless-steel trays.

When analyzed by PIXE, similarities were seen in the concentration of some major and trace elements in postparasitic *R. culicivorax* reared in paraffin-coated galvanized trays at the Gulf Coast Mosquito Research Laboratory, SEA, USDA, Lake Charles, Louisiana (Table 1). The whole-body levels of some of the elements, however, differed significantly from data for postparasites reared in stainless-steel trays, attributed to metallic contaminants from the trays, differences in host and subsequent nematode diet and water-quality

TABLE 1. Multi-elemental whole-body analyses of postparasites of *R. culicivorax* and parasitized and unparasitized *C. p. quinquefasciatus* larvae.

Element	Mean concentration (ppm ash weight)				Unparasitized larvae
	Postparasites		Parasitized larvae		
	Stainless-steel tray	Galvanized tray[a]	Plastic bucket	Stainless-steel tray	Stainless-steel tray
K	13,086.9	—	19,639.8	26,530.2	25,779.6
Ca	9,363.9	7,752.5	5,083.0	7,398.3	12,634.7
Mn	40.0	56.5	25.7	44.7	55.8
Fe	102.3	228.7	477.4	709.4	1,345.5
Ni	1.2	18.8	<1.0	<1.0	<1.0
Cu	90.3	103.2	95.0	138.8	207.4
Zn	760.3	878.1	2,067.9	3,660.2	4,041.3
Pb+As+Rb[b]	48.4	19.0	1,091.2	1,597.0	180.1
Se	2.1	4.1	3.1	6.8	<1.0
Br+Rb[c]	13.8	9.8	14.8	28.8	8.8
Sr	9.3	20.8	24.6	51.6	135.6
Zr	<1.0	—	28.7	45.1	<1.0
Sn	<1.0	—	195.0	200.0	55.0

[a]No quantitative data were generated for K, Zr, Sn although spectra indicated high concentrations of K and extremely low concentrations of Zr and Sn.
[b]Concentration mainly represents lead.
[c]Concentration mainly represents bromine.

differences in the rearing systems between the 2 laboratories (7).

In general, our data from stainless-steel trays indicated that parasitized larvae had a greater concentration of K, Pb, Se, Br, Zr, and Sn and lower levels of Ca, Mn, Fe, Cu, Zn, and Sr than unparasitized larvae (Table 1). Since all environmental factors were identical it is assumed that quantitative differences between parasitized and unparasitized larvae reared in stainless-steel trays were a result of *R. culicivorax*, even though the effect of *R. culicivorax* on host uptake, utilization, or loss of various elements is not known.

Postparasites reared in stainless-steel trays contained lower levels of all elements (except Ni and Br) than did unparasitized hosts. The accumulation of these elements by postparasites is presumed to be due mainly to the nutritional relationship between a parasite and its host.

Although the significance of major and trace elements in the growth of and development of *R. culicivorax* is not known, our data suggest that PIXE, an extremely sensitive analytical technique, may be useful in elucidating important physiological and nutritional relations between *R. culicivorax* and its host, e.g., in determining elemental co-factors necessary in the formulation of an insect tissue-culture medium for successful *in vitro* culturing of *R. culicivorax* (2). Techniques for analyzing the host hemolymph and preparasitic stage of *R. culicivorax* by PIXE are being investigated.

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