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Biogenic Amines and their Metabolites in Homogenates of the Vinegar Eelworm, *Turbatrix aceti*[1]

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Biogenic amines serve as neurotransmitters in vertebrates and insects, and their occurrence has been reported in a number of other species of invertebrates. Among helminths, they have been isolated from the liver fluke *Fasciola hepatica* (9) and the nematodes *Ascaris lumbricoides* (10), *Ascaridia galli* (10), *Nippostrongylus brasiliensis* (1), *Litomosoides carinii* (12), *Caenorhabditis elegans* (8), and *Trichostrongylus colubriformis* (6,7). Adults of *T. colubriformis* exhibited ovipositional and ingestive responses to histamine, dopamine, octopamine, γ-aminobutyric acid (GABA), and serotonin in vitro (2,3). Ward et al. (14) reported chemotactic responses by males of *N. brasiliensis* to several biogenic amines during bioassays, and Sulston et al. (13) identified dopaminergic neurons in *C. elegans* and *Ascaris lumbricoides* cytochemically via formaldehyde fluorescence. These compounds may have functions in addition to their putative ones as neurotransmitters, such as the triggering and regulation of cell divisions in early ontogenesis (5). Our interest in them is associated with research on nematode behavior and the environmental stimuli to which parasitic nematodes respond in order to complete their life cycles.

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Turbatrix aceti were cultured in *Caenorhabditis briggsae*-maintenance medium (4) containing 4% acetic acid, cholesterol, β-sitosterol, ergosterol, and hemoglobin (11). After harvesting they were washed thoroughly in Tyrode's solution, homogenized, and processed for the identification of biogenic amines and their metabolites; high-performance liquid chromatography (HPLC), as described previously for *Trichostrongylus colubriformis* (6), was used. The identity of the compounds was confirmed through comparison of the elution profiles with those of authentic standards processed identically.

The mean amounts of biogenic amines and their metabolites recovered from three or more lots (batches) of homogenized mixed sexes and juveniles of *T. aceti* ranged from 6.57 to 1,851.72 μg/g tissue (Table 1). In addition, epinephrine (3.11 μg/g tissue) and synephrine (6.50 μg/g tissue) were identified in one batch of homogenate, and N-acetyldopamine, 0.12 and 5.93 μg/g tissue, was identified in two batches. Although 5-hydroxyindoleacetic acid (HIAA) and 3-methoxytyrosine (O-methyl-DOPA) have been found in homogenates of *T. colubriformis* (6), neither of these compounds were present in detectable amounts in homogenates of *T. aceti*. In organisms where its metabolism is known, serotonin is converted to HIAA by monoamine oxidase. Confirmation, through further investigations, of the absence of HIAA in *T. aceti* in vivo would be intriguing because it would suggest that if these worms metabolize serotonin, they do it via an uncommon pathway.

The wide ranges and high variances for

TABLE 1. Biogenic amines and their metabolites in *Turbatrix aceti* expressed as μg/gram wet tissue.

Compound	Mean	1 SEM†	Range		n†
			Minimum	Maximum	
Dihydroxyphenylalanine	1,851.72	355.68	597.73	2,490.26	5
Dopamine	13.42	5.92	0.24	35.46	6
Epinine	13.74	5.56	2.79	33.96	5
Hydroxymethylphenylglycol	153.36	26.95	79.11	208.12	4
Metanephrine	30.40	24.06	0.72	78.04	3
Norepinephrine	41.96	25.40	0.13	107.81	4
Normetanephrine	57.26	15.07	9.92	104.58	5
Octopamine	17.00	7.26	3.37	39.03	5
p-Hydroxymandelaldehyde	1,409.22	279.98	500.81	2,152.16	5
Serotonin	6.57	1.22	0.26	10.59	8
Vanillylmandelic acid	1,036.92	183.09	444.39	1,590.85	8

† SEM = standard error of the mean; n = number of batches of homogenate assayed.

each of these compounds may be caused by several factors. Each of the batches of homogenate were prepared from cultures with different, and unknown, proportions of males, females, juveniles, and eggs. Also, the cultures were not of the same age and population density. In addition, differences in handling the cultures during their storage and preparation may have produced different behavioral responses in the nematodes which may be reflected in the levels of neurotransmitters.

The mean levels of dihydroxyphenylalanine (DOPA), p-hydroxymandelaldehyde, and vanillylmandelic acid were unexpectedly high. In vertebrates and insects, DOPA is a principal metabolite of tyrosine, which, in turn, is decarboxylated to dopamine. In the adult hermaphrodites and juveniles of *Caenorhabditis elegans,* dopamine is the transmitter in the cephalic and deirid neurons; other dopaminergic neurons have been reported from the caudal region of male *C. elegans* and from *Ascaris* (13). Consequently, a neurotransmitter function for dopamine in *T. aceti* may be responsible for the high levels of its precursor, DOPA. Vanillylmandelic acid is one of the final products of catecholamine metabolism in vertebrates, being secreted in the urine, and its formation from epinephrine and norepinephrine has been described in nematodes (7). Perhaps such final products of catecholamine metabolism in nematodes will be found to serve as pheromone-like agents of chemical communication between these organisms.

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