

Occurrence and Levels of 5-Hydroxytryptamine in *Schistosoma mansoni*

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SUMMARY

The presence of 5-hydroxytryptamine has been demonstrated in the parasitic trematode *Schistosoma mansoni*. The concentration of this biogenic amine is more than 10 times that of mammalian brain. The localization of 5-hydroxytryptamine is not confined to the central nervous system, and consideration should be given to its possible function as a central or peripheral neurotransmitter.

Previous studies have provided evidence for the role of acetylcholine as an inhibitory transmitter in *Schistosoma mansoni* (1). Whereas the parasite's motor activity was reduced by cholinomimetic agents and acetylcholinesterase inhibitors, it was markedly increased when the worms were exposed to cholinergic blocking agents such as atropine, mecamylamine, or pempidine. This suggested the presence in these helminths of an excitatory transmitter whose action opposes that of acetylcholine and becomes unmasked when the cholinergic receptors are blocked (1). Since 5-hydroxytryptamine markedly enhances the motor activity of *S. mansoni*, its possible role as an excitatory neurotransmitter in this parasite is currently being investigated. In this communication,

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the occurrence and concentration of 5-hydroxytryptamine in *S. mansoni* are reported.

Adult schistosomes were obtained from mice which had been exposed 7-10 weeks previously to cercariae of *S. mansoni* (Puerto Rican strain) by tail immersion (100 cercariae were used per mouse). The worms were placed in 75% horse serum (diluent, distilled water), washed in a buffered glucose-containing salt medium, blotted, and frozen in a container immersed in liquid nitrogen. The frozen worms were weighed and homogenized in 0.3 M perchloric acid (1.5 ml/100 worms) at 2-4°. The homogenate was centrifuged at $8000 \times g$ for 15 min at 2°. Unless otherwise stated, 5-hydroxytryptamine was determined in the supernatant according to Snyder *et al.* (2). In a number of experiments, the protein content of the residue was determined by the method of Lowry *et al.* (3).

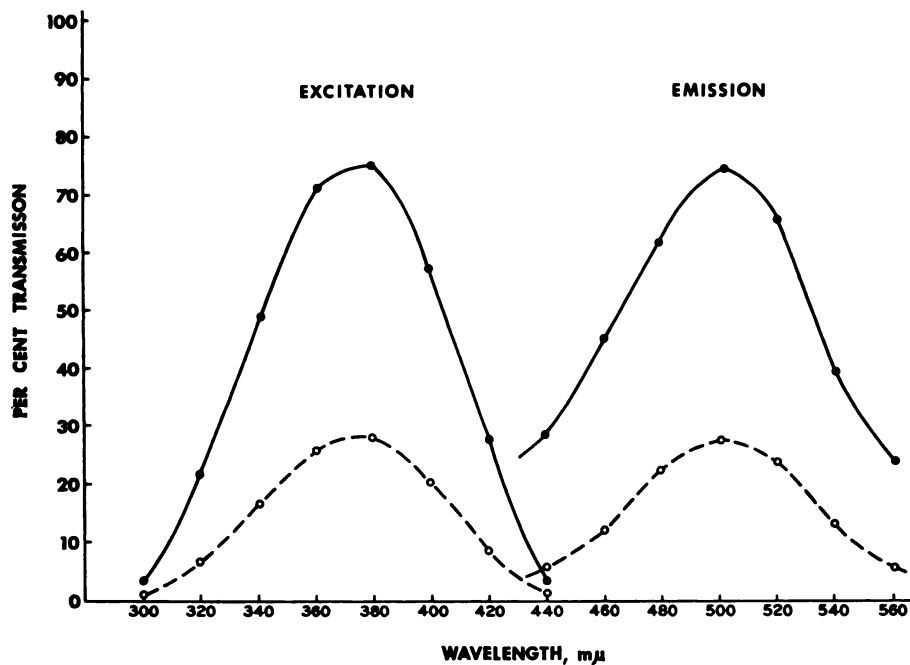


FIG. 1. Excitation and emission spectra of the ninhydrin reaction products of extracts of *S. mansoni* (upper curves) and of a solution of 5-hydroxytryptamine (lower curves)

For the excitation spectra the emission wavelength was set at 500 mμ, and for the emission spectra the excitation wavelength was set at 385 mμ.

The concentration of 5-hydroxytryptamine in schistosomes varied between 4.4 and 5.9 μg/g (wet weight) (Table 1). Both the excitation and fluorescence

curves as a function of wavelength of the ninhydrin reaction product with the worm extracts were identical with those of a known sample of 5-hydroxytryptamine

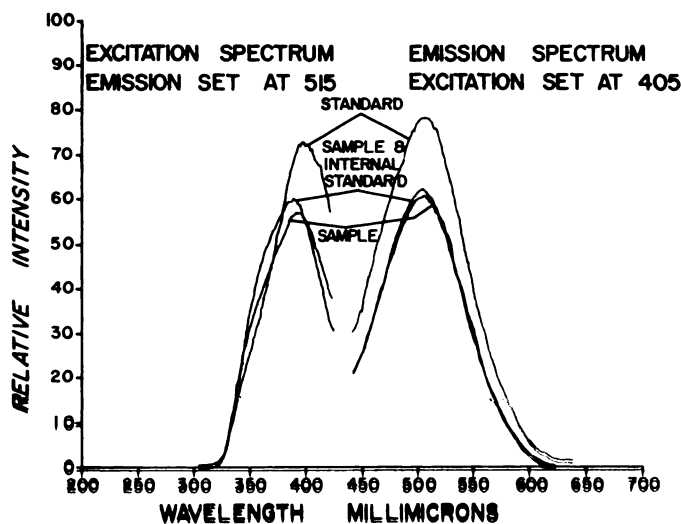


FIG. 2. Excitation and emission spectra of norepinephrine, determined by the method of Shore and Olin (4.) of an extract of *S. mansoni*, and of a mixture of both

TABLE 1
Concentrations of 5-hydroxytryptamine
in *S. mansoni*

Preparation of parasite	5-Hydroxytryptamine content	
	$\mu\text{g/g}$, fresh wt.	$\mu\text{g/g}$ protein
Pairs	5.0	
	5.4	
	5.9	29.6
	4.4	
	5.8	31.3
	5.6	34.6
Males	5.15	
	4.65	33.2
	6.0	31.6
Females	5.6	
	5.5	
	6.3	35.5
	5.2	33.5
Heads (males)	8.0	66.2
Heads		53.7
Decapitated males	4.0	28.5
	4.25	30.5
	4.7	28.6

extracted in the same manner (Fig. 1). Similarly, when 5-hydroxytryptamine was extracted from schistosomes and determined by a different method (induced fluorescence in strong acid) (4), the excitation and fluorescence curves were in close agreement with those of authentic 5-hydroxytryptamine.

Male and female worms had approximately the same 5-hydroxytryptamine concentrations. While male heads (cut off below the acetabulum) contained more 5-hydroxytryptamine than the remaining portions of the worms, the 5-hydroxytryptamine concentration of the headless bodies was also considerable, indicating that the localization of this substance is not limited to the central nervous system of the parasite. This was in agreement with the observed distribution of yellow fluorescent material throughout many areas of the parenchyma of the worm, detectable by the use of a modification of the histo-

chemical fluorescence method of Falck (5).

In addition to 5-hydroxytryptamine, the worms also contained catecholamine-like material as determined by the method of Shore and Olin (4). The fluorescence excitation and emission peaks of the material extracted and treated according to the method of Shore and Olin were essentially identical with those for authentic norepinephrine carried through the same procedure (Fig. 2). This suggests that the original material in the schistosomes was a catecholamine. The concentration of this amine (or amines) in *S. mansoni* was estimated to be less than 10 times than that of 5-hydroxytryptamine (i.e., approximately $0.4 \mu\text{g/g}$, wet weight). The presence of catecholamines was also suggested by the finding of green fluorescent material in both the head and the remaining portions of the worms when the histochemical fluorescence method (5) was used. However, the yellow fluorescence was much more intense.

It is noteworthy that the concentration of 5-hydroxytryptamine in *S. mansoni* is more than 10 times that of mammalian brain and 10–50 times higher than that reported for three other trematodes (6, 7). In a fourth trematode, *Cerebratulus lacteus*, high levels of 5-hydroxytryptamine ($2.3 \mu\text{g/g}$) are limited to the head region (6). By contrast, in *S. mansoni*, concentrations in the neighborhood of $4 \mu\text{g/g}$ were found in parts of the worm from which the head region had been removed. Only a small portion of the total weight (or protein content) of the parasite can be accounted for by its nervous system alone. Therefore, in addition to a role as a neurotransmitter, the possibility of other functions for 5-hydroxytryptamine in *S. mansoni* should be considered.

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