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UPTAKE OF 5-HYDROXYTRYPTAMINE BY *MESOCESTOIDES CORTI* (CESTODA)*

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ABSTRACT: The kinetics of uptake of 5-hydroxytryptamine in tetrathyridia of *Mesocestoides corti* were studied. 5-HT was taken up by 2 saturable systems: a high affinity system with a Michaelis constant (Km) of 1.40×10^{-7} M and a Vmax of 0.54 ng per mg protein per 2 min, and a low affinity system with a Km of 2.50×10^{-6} M and Vmax of 2.56 ng per mg protein per 2 min. Both systems possessed characteristics of active transport which were dependent on temperature, metabolic energy, and stereospecificity.

The presence of 5-hydroxytryptamine, the only biogenic amine in tetrathyridia of *Mesocestoides corti*, was reported previously (Hariri, 1974). Considerable evidence has accumulated during the past decade which suggests that 5-HT may serve as a neurotransmitter in a wide variety of organisms. This contention is supported by the high concentration of this amine within neurons and the presence of enzymes responsible for its synthesis and inactivation (Bogdanski et al., 1957; Garattini and Valzelli, 1965). Sites of norepinephrine uptake in brain slices have been shown to be NA-containing neurons by histochemical studies (Hamberger, 1967). This uptake is shown to be similar in brain slices, synaptosomes, and also in intact brain in vivo by injection of small amounts of ^{3}H-NA into cerebrospinal fluid (Glowinski and Axelrod, 1966). Similarly, when ^{3}H-5-HT is injected into cerebrospinal fluid, the labeled amine is shown by autoradiographic studies to be localized in regions rich in 5-HT terminals (Aghajanian and Bloom, 1967; Fuxe, 1965).

In addition to the presence of 5-HT in *M. corti*, two additional criteria for identification of 5-HT as a putative neurotransmitter in these organisms were examined. One was the occurrence of a synthetic pathway in *M. corti*, and the second the presence of a mechanism by which the effect of the neurotransmitter is terminated through reuptake into the nerve terminal from which it has been released.

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MATERIALS AND METHODS

Materials

The radioactive compounds, ^{14}C-5-hydroxytryptamine (specific activity 27.5 mCi/mmole), DL-3-^{14}C-5-hydroxytryptophan (specific activity 5.21 mCi/mmole), DL-7-^{14}C-norepinephrine (specific activity 55.3 mCi/mmole), 5-hydroxytryptophan, tryptophan, iproniazid phosphate, nialamide, ouabain octahydrate, and p-chlorophenylalanine were purchased from Sigma Chemical Co. (St. Louis, Mo.). Pargyline-HCl was supplied by Abbott Research Laboratories (North Chicago, Illinois). Chlorimipramine was supplied by Ciba-Geigy Corp. (Summit, N. J.). Alpha propyldopacetamide was purchased from Aldrich (Cedar Knolls, N. J.). Chlorometamphetamine, chloromphetamine, and 6-fluorotryptophan were purchased from Regis Chemical Co. (Morton Grove, Ill.). Cyproheptadine was supplied by Merck Sharp and Dohme (Rahway, N. J.). Ninhydrin, Na_2 EDTA, perchloric acid, boric acid, and sodium hydroxide were Analytical Reagents from J. T. Baker Chemical Co. (Phillipsburg, N. J.). Potassium carbonate, potassium phosphate, and sodium phosphate were Analytical Reagents from B & A Laboratories (Morrisontown, N. J.). Sodium chloride was purchased from Mallinckrodt Chemical Works (St. Louis, Mo.). 1-Butanol and n-heptane were certified spectranalyzed from Fisher Scientific Co. (Springfield, N. J.). Protryptiline was supplied by Dr. Ernest Bueding, John Hopkins University (Baltimore, Md.). 8-Beta-carbobenzoxyaminoethyl-1,6-dimethyl-10-alpha-ergotine (MCE) was supplied by Dr. A. H. Glasser, of Farmitalia, Milan, Italy. UML 491 was supplied by Sandoz Ltd. (Switzerland). D-lysergic acid diethylamide (D-LSD), 2-bromo-lysergic acid diethylamide (BOL), and 1-methyl-LSD (MLD) were supplied by Dr. Sidney Roberts, University of California (Los Angeles). Morphine was supplied by Dr. Dermot Taylor, University of California (Los Angeles).

Tissue preparation

Tetrathyridia of *M. corti* were maintained in Swiss albino mice. The animals were killed by

cervical dislocation and tetrathyridia were transferred from body cavity into petri dishes containing physiological saline solution (0.85% NaCl). Worms were washed several times in this saline and 0.2 ml of sedimented tetrathyridia (600 to 800 individuals, equivalent to 2.5 to 3 mg protein) were used throughout the experiments.

Determination of 5-hydroxytryptamine

Worms were prepared as described above and dried by means of a Millipore filter. Organisms were homogenized in 2.5 ml of ice-cold 0.4 N perchloric acid containing 0.02% ascorbic acid, 0.2% Na_2 EDTA, and 0.029% pargyline HCl (a monoamine oxidase inhibitor) at 2 to 4 C as described by Andén and Magnusson (1967). This solution was prepared freshly every 24 hr. Homogenates were centrifuged at 10,000 *g* for 15 min at 2 C. The supernatant was transferred into a glass-stoppered centrifuge tube and the pH was adjusted to 9 to 9.5 with 5 N potassium carbonate at 2 to 4 C. Thereafter, the assay procedure of Snyder et al. (1965) for 5-HT was used. Each tube received 0.5 ml of 0.5 M borate buffer (pH 9.3), 1.5 g of sodium chloride, and 15 ml of 1-butanol. The tubes were shaken for 10 min, centrifuged, and the aqueous phase was removed by aspiration. The organic phase was shaken for 3 min with 2 ml of 0.1 M borate buffer (pH 9.3) which had been previously saturated with sodium chloride. The tubes were again centrifuged and 10 ml of the organic phase transferred to a glass-stoppered centrifuge tube containing 1.4 ml of 0.05 M phosphate buffer (pH 7.0) and 15 ml of n-heptane. Tubes were shaken for 2 min, centrifuged, and the organic phase was aspirated. The aqueous phase was analyzed for 5-HT.

Oxidation and fluorometric estimation of 5-HT

The fluorometric determination was carried out in an Aminco-Bowman spectrophotofluorometer.

5-HT fluorescence was developed by reacting a sample of the final aqueous phase (0.05 M phosphate buffer, pH 7.0) with ninhydrin as described by Snyder et al. (1965). Covered tubes were heated for 30 min at 75 C and subsequently placed in ice. The ninhydrin product was activated at 385 mμ and resulting fluorescence determined at 495 mμ.

In vitro incubation of worms

Organisms were obtained from mice and washed as described above. All incubations were carried out in GIBCO medium NCTC 135. The organisms were preincubated in the latter for at least 10 min before any drug was added. Total of 0.2 ml of sedimented tetrathyridia were used per 10 ml of medium for an incubation period of 5 hr. In all incubations lasting more than 5 hr, the worms were transferred to fresh medium at 5-hr intervals. All incubations, regardless of temperature, were carried out in a Dubnoff metabolic shaking incubator.

Determination of labeled compound uptake

Organisms were preincubated at temperature used for experiment for 10 min, for equilibration, before a solution of labeled compound was added. After the appropriate incubation time, the worms were removed from the incubation tube, by means of a Pasteur pipette, and transferred on a 0.45-mμ Millipore membrane filter under vacuum. The worms, on the filter, were flushed with 6 ml of NCTC 135 to wash off labeled material adhering to the surface of the worms. The latter were then removed from the filter and homogenized in a glass homogenizer (kept in ice) containing 2.5 ml of homogenizing medium described above. The homogenate was centrifuged at 9,000 *g* for 15 min. After centrifugation, 1 ml of the tetrathyridia supernatant was placed into a vial containing 15 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) for determination of radioactivity in a Beckman liquid scintillation spectrometer (Model LS-330). Total uptake was calculated for 2.5 ml of homogenization medium. Uptake at 0 C was considered to be a measure of the amine entering the organisms by passive diffusion and nonspecific binding. For kinetic studies, active transport was taken as the difference between the uptake measured at 37 and 0 C. In all other experiments, the uptake value was not corrected for this latter component, which represents only about 4 to 5% of the total uptake at 37 C for low 5-HT concentrations. Linear regressions for determining kinetic constants were fitted by eye, and at least three independent experiments were performed for each value.

The pellet, obtained at 9,000 *g* for 15 min, was suspended in an aliquot of 0.1 N NaOH and protein was measured by the method of Lowry et al. (1951). For determination of labeled compound in medium, 1 ml of the latter was also placed in a vial and 15 ml of Aquasol were added for counting of radioactivity. The vials were counted for 10 min and counts were corrected for background. Tissue-to-medium (T/M) ratios were calculated according to Shaskan and Snyder (1970).

RESULTS AND DISCUSSION

The occurrence and concentration of 5-HT in *M. corti* was reported previously (Hariri, 1974). Indirect attempts have been made to demonstrate tryptophan hydroxylase and aromatic L-amino acid decarboxylase activities in these organisms. Biosynthesis of 5-HT in mammalian brain has been well established (Gal et al., 1963, 1964; Gal and Marshall, 1964; Levin et al., 1960; Udenfriend, 1959). No evidence could be found indicating hydroxylation of tryptophan in *M. corti*. Incubation of the organisms in a modified NCTC medium, containing 1×10^{-4} M tryptophan,

TABLE I. *5-HT levels of* Mesocestoides corti *following incubation at 37 C in medium NCTC 135 with or without tryptophan (1×10^{-4} M), monoamine oxidase inhibitor (1×10^{-3} M), or tryptophan hydroxylase inhibitor (1×10^{-4}M).*

Addition	Incubation time (hr)	ng 5-HT × mg protein^{-1}
None	6	36 ± 01 (3)
Tryptophan + pargyline	6	38 ± 01 (6)
Tryptophan + iproniazid	6	36 ± 01 (3)
Tryptophan + nialamide	6	39 ± 01 (3)
None	48	23 ± 01 (2)
Tryptophan	48	22 ± 00 (2)
Tryptophan + nialamide	48	21 ± 00 (2)
Tryptophan + iproniazid	48	26 ± 00 (3)
Alpha propyldopacetamide	48	25 ± 00 (3)
6 Fluorotryptophan	48	24 ± 00 (2)

Figures are mean values SD; number of experiments in parentheses.

failed to produce any significant increase in concentration of 5-HT or motor activity of the worms (Table I). By contrast, stimulation of motor activity of *M. corti* is readily observable when organisms are incubated in a very low concentration of 5-HT for a very short period of incubation. Since 5-HT is chiefly degraded by monoamine oxidase (Weissbach et al., 1957; Neff and Tozer, 1968), several inhibitors of this enzyme (pargyline, iproniazid, and nialamide) were added to the incubation medium for up to 2 days. This again revealed no difference in 5-HT level. The lack of an increase in 5-HT after prolonged incubation with tryptophan and with a monoamine oxidase inhibitor might merely reflect saturation of tryptophan hydroxylase with endogenous tryptophan. One also cannot rule out the possibility of occurrence of some other types of monoamine oxidase that can use 5-HT as a substrate but yet are not inhibited by the inhibitors used in this study. In the mouse, there are at least three types of mitochondrial monoamine oxidases capable of deaminating kynuramine, one type being harmine-sensitive and another (containing two forms) being harmine-resistant (Squires, 1968). Studies in *S. mansoni* have also failed to demonstrate hydroxylation of tryptophan, while the presence of 5-HT in these organisms has been established (Bennett et al., 1969, 1971, 1973; Chou et al., 1972). Hydroxylation of tryptophan in the 5 position is the first and the rate limiting reaction in the formation of serotonin (Lovenberg et al., 1967). The activity of this enzyme could be inhibited specifically by alpha propyldopacetamide (Corrodi et al., 1967) or 6-fluorotryptophan (Peters, 1971). Incubation of organisms in the presence of either of these inhibitors at concentration of 1×10^{-4} M for 2 days produced no change in the total amount of 5-HT (Table I). Therefore, the evidence merely suggests, but does not rule out, the lack of hydroxylation of tryptophan in these organisms.

The enzyme which converts 5-hydroxytryptophan (5-HTP) to 5-HT, aromatic L-amino acid decarboxylase, has been described in a variety of mammalian tissues, and perhaps is one of the most ubiquitous enzymes occurring in a variety of tissues. There is evidence for decarboxylation of 5-HTP and 1-dihydroxyphenylalanine in *S. mansoni* (Bennett and Bueding, 1971, 1973). The 5-HT level of *M. corti* following incubation at 37 C in medium NCTC with 5-HTP (1×10^{-4} M) was as high as 0.074 μg/mg protein after 18 hr of incubation (Table II). This level began to decrease as incubation time proceeded, pos-

TABLE II. *5-HT levels of* M. corti *following incubation at 37 C in medium NCTC 135 with or without 5-HTP (1×10^{-4}M).*

Incubation medium	Incubation time (hr)	ng 5-HT × mg protein^{-1}
NCTC 135	6	38 ± 02 (7)
NCTC 135 + 5-HTP	6	46 ± 01 (3)
NCTC 135	12	36 ± 02 (3)
NCTC 135 + 5-HTP	12	55 ± 01 (3)
NCTC 135	18	36 ± 01 (2)
NCTC 135 + 5-HTP	18	74 ± 00 (2)
NCTC 135	24	35 ± 03 (3)
NCTC 135 + 5-HTP	24	66 ± 00 (2)

Figures are mean values SD; number of experiments in parentheses.

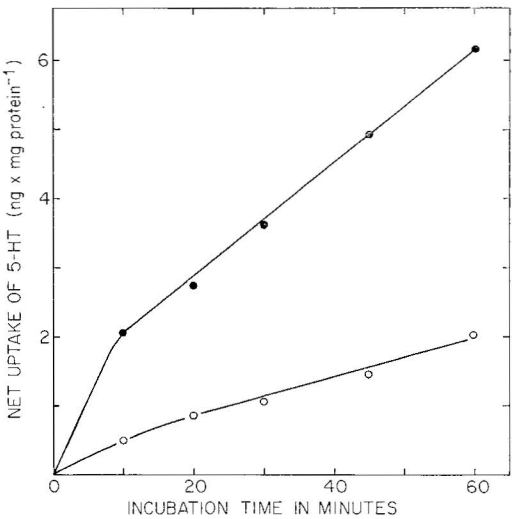

FIGURE 1. Time course of 5-HT uptake following incubation in 1×10^{-6} M 5-HT (●—●) and 2×10^{-7} M 5-HT (○—○) at 37 C.

sibly because of 5-HT destruction by monoamine oxidase and/or leakage of excessive 5-HT from the organisms (see below).

The time course of 5-HT uptake by *M. corti* at 37 C is shown in Figure 1. Uptake at 37 C was initially rapid and it appeared that organisms had the ability to accumulate 5-HT against a concentration gradient. The 5-HT concentration in the worms did not reach a constant level after 60 min, suggesting that the uptake of 5-HT in these organisms might not be mediated by a saturable membrane transport system similar to the uptake of NE by brain slices and cat heart described by Dengler et al. (1962), uptake of 5-HT in *S. mansoni* (Bennett and Bueding, 1973), and Kilejian (1966) for the uptake of L-proline in *H. diminuta*. However, it should be pointed out that 5-HT could have reached a constant level if the incubation time had been prolonged.

TABLE III. *Velocity* of ^{14}C-5-HT uptake in* M. corti *measured at two temperatures, two concentrations, and four time periods.*

Incubation time (min)	Concentration	Temperature	
		21 C	37 C
1	2×10^{-7} M	0.37 (3)	0.65 (3)
2	2×10^{-7} M	0.28 (2)	0.36 (2)
3	2×10^{-7} M	0.25 (2)	0.34 (2)
5	2×10^{-7} M	0.39 (2)	0.43 (2)
1	1×10^{-6} M	1.35 (2)	1.51 (2)
2	1×10^{-6} M	1.03 (2)	1.05 (2)
3	1×10^{-6} M	0.38 (4)	0.88 (3)
5	1×10^{-6} M	1.15 (2)	1.40 (2)

* Velocity is expressed in nanograms of 5-HT taken up per mg protein per min; number of experiments is shown in parentheses.

TABLE IV. *Effect of temperature (0 C) on the uptake of ^{14}C-5-HT by* M. corti.

Time (min) incubated in 2×10^{-7} M 5-HT	Net amount of 5-HT taken up (ng × mg protein^{-1}) at two time intervals
10	0.25 (2)
60	0.26 (2)

Number of experiments in parentheses.

In an attempt to demonstrate the initial rates of 5-HT uptake which would follow the classical Michaelis–Menten equation for saturable-substrate interactions, the worms were incubated in various concentrations of ^{14}C-5-HT (Table III). The velocity of 5-HT uptake between 1 and 5 min was not proportional, namely, it declined for the first 3 min and increased at 5 min. Initial rates of 5-HT uptake had a similar pattern when incubation temperature was lowered to 21 C.

The amount of nonspecific binding of 5-HT was determined by incubation of the worms at

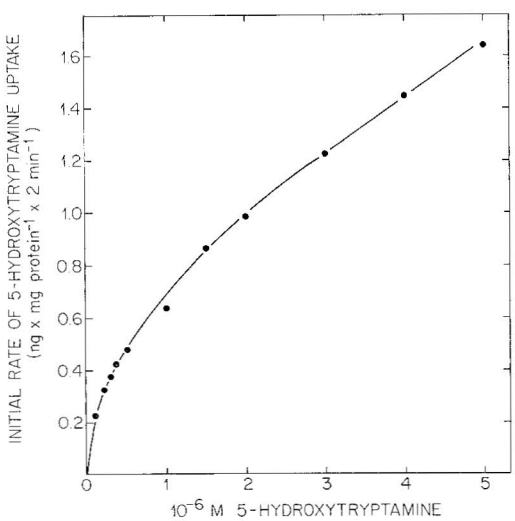

FIGURE 2. Effect of external substrate concentration on the initial rate of 5-HT uptake by *M. corti* at 37 C. Specific uptake was taken as the difference between the uptake measured at 37 and 0 C.

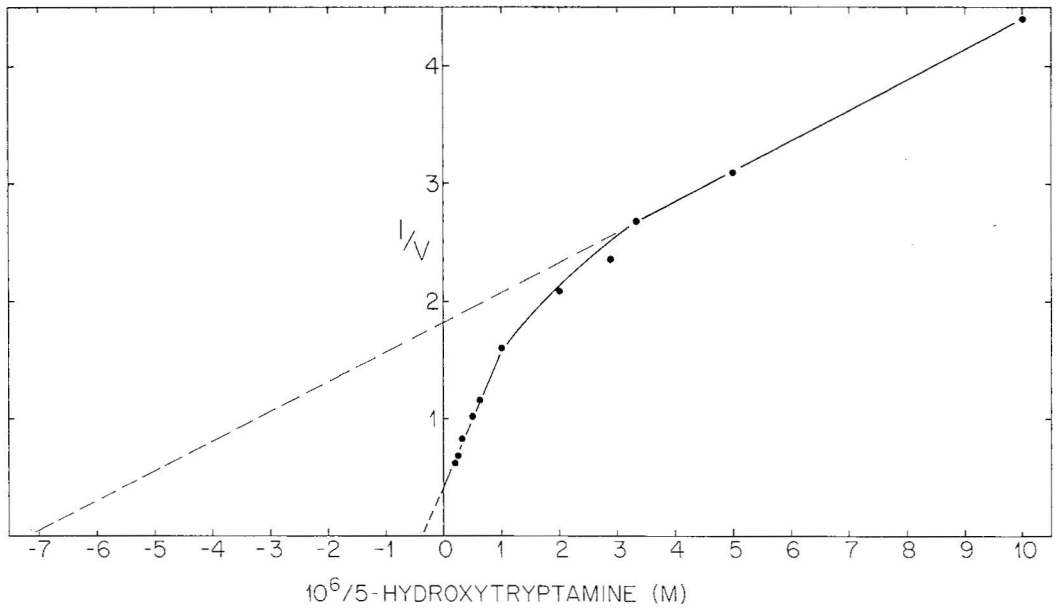

FIGURE 3. Lineweaver–Burk plot of 5-HT uptake in 2-min incubations. Incubation was carried out at 37 C.

0 C. The nonspecific binding remained constant within 1 hr of incubation (Table IV). For kinetic studies, the organisms were incubated at the various concentrations of 5-HT for 2 min and from the amount taken up were subtracted the corresponding values obtained at 0 C for 2 min incubation.

The initial rate of 5-HT uptake by tetrathyridia of *M. corti* at varying concentrations of 5-HT is shown in Figure 2. The organisms did not show a complete saturation phenomenon. At low external concentrations of 5-HT, the rate of uptake of 5-HT increased rapidly with increasing external concentration. This rapid increase started to diminish when external concentration of 5-HT was approximately 1×10^{-6} M and beyond this level any increase in external concentration resulted in a slow but linear increase (Fig. 2).

The biphasic nature of saturation curves obtained at various external concentrations indicates that 5-HT uptake was a complex process, and was due to at least two distinct transport systems. At low concentrations an active transport system saturable at higher concentrations of 5-HT was operative, whereas at higher external concentration (greater than 5×10^{-7} M) 5-HT was probably entering the organisms by a multiple transport system, simple diffusion plus an active transport saturable system. The presence of at least two distinct processes was indicated even more clearly when the uptake values were plotted according to the method of Lineweaver and Burk (1934) (Fig. 3). By extrapolating the two linear portions of the curve two distinct Km were obtained, indicating that 5-HT was taken up by two saturable systems. The high affinity had a Km of 1.40×10^{-7} M and Vmax of 0.54 ng per mg protein per 2 min; the low affinity system had a Km of 2.50×10^{-6} M and Vmax 2.56 ng per mg protein per 2 min.

An important factor to be considered is the data obtained from the discontinuity in the Lineweaver–Burk plot. By extrapolating the two linear portions of the curve, one can obtain two Km. Such simple extrapolating, however, cannot distinguish between saturable and diffusion-controlled processes. Therefore, a method of analysis of these data, described originally by Akedo and Christensen (1962) and subsequently developed by Reid et al. (1970), should be adopted. Although such an analysis was not carried out in this study, other experiments were performed to answer the question of active transport.

TABLE V.—*Inhibition of 5-HT uptake of* M. corti.

Drug	Concentration molar	5-HT Molar	Net uptake ng × mg protein^{-1}	Inhibition of uptake %
–	–	1×10^{-6}	6.25 (4)[a]	–
Chlorimipramine	1×10^{-5}	1×10^{-6}	5.10 (2)	19
	5×10^{-5}	1×10^{-6}	3.95 (2)	37
Protryptiline	1×10^{-5}	1×10^{-6}	4.35 (2)	31
	5×10^{-5}	1×10^{-6}	3.55 (2)	43
Ouabain	5×10^{-5}	1×10^{-6}	4.00 (2)	36
Chlorometamphetamine	5×10^{-5}	1×10^{-6}	4.8 (2)	23
Chloroamphetamine	5×10^{-5}	1×10^{-6}	4.3 (2)	31
LSD[b]	1×10^{-5}	1×10^{-6}	5.58 (2)	11
	5×10^{-5}	1×10^{-6}	4.95 (2)	21
BOL[b]	1×10^{-5}	1×10^{-6}	5.65 (2)	10
	5×10^{-5}	1×10^{-6}	5.15 (2)	17
	1×10^{-6}	1×10^{-6}	6.35 (1)	0
UML[b]	1×10^{-5}	1×10^{-6}	6.0 (2)	4
	5×10^{-5}	1×10^{-6}	5.75 (2)	8
	1×10^{-6}	1×10^{-6}	6.3 (1)	0
MLD[b]	1×10^{-5}	1×10^{-6}	5.93 (2)	5
	5×10^{-5}	1×10^{-6}	5.68 (2)	9
Morphine	5×10^{-5}	1×10^{-6}	4.85 (2)	23
	1×10^{-6}	1×10^{-6}	5.53 (2)	12
MCE[b]	1×10^{-5}	1×10^{-6}	3.85 (2)	39
	5×10^{-5}	1×10^{-6}	3.05 (2)	51
Cyproheptadine	1×10^{-5}	1×10^{-6}	5.25 (2)	17
	5×10^{-5}	1×10^{-6}	3.30 (2)	47
–	–	2×10^{-7}	2.12 (3)	–
Cyproheptadine	1×10^{-5}	2×10^{-7}	1.75 (2)	17
MCE[b]	1×10^{-5}	2×10^{-7}	1.63 (2)	23
–	–	2×10^{-5}	103.50 (2)	–
Cyproheptadine	1×10^{-4}	2×10^{-5}	49.58 (2)	52
MCE[b]	1×10^{-4}	2×10^{-5}	25.95 (2)	75

[a] Numbers of experiments shown in parentheses.
[b] The abbreviations used are: LSD, d-lysergic acid diethylamide; BOL, 2-bromo-LSD; UML, methysergide; MLD, 1-methyl-LSD; 8β-carbobenzyloxyaminomethyl-1,6-dimethyl-10α-ergoline.

The effect of drugs on the uptake of 5-HT was tested by incubation of organisms with the drug for a period of ½ hr and subsequent additional hour of incubation after ^{14}C-5-HT was added to the incubation medium. 5-HT uptake was inhibited when the worms were incubated with derivatives of imipramine, chlorimipramine, and protryptiline (Table V). P-chloroamphetamine and p-chlorometamphetamine, two known selective 5-HT depletors (Miller et al., 1970), and ouabain also inhibited the uptake of 5-HT. D-lysergic acid diethylamide (LSD) and 2-bromo-LSD were much less potent. Gaddum and Picarelli (1957) showed that tryptamine receptors in guinea-pig ileum could be blocked by morphine. In this study, morphine blocked the uptake of 5-HT in *M. corti*. Methysergide (UML) and 1-methyl-LSD (MLD) had virtually no inhibitory effect on 5-HT uptake, while two other 5-HT blocking agents, 8β-carbobenzyloxyaminomethyl-1,6-dimethyl-10α-ergoline (MCE) and cyproheptadine, proved to have equally strong inhibitory effects on 5-HT uptake. MCE has been shown to be a very powerful 5-HT blocking compound in S. *mansoni* (Bennett and Bueding, 1973), *F. hepatica* (Beretta and Locatelli, 1968), and mammalian tissues (Beretta et al., 1965; Ferrini and Glasser, 1965).

The specificity of the worm's uptake mechanism was tested by incubating the worms with labeled ^{14}C-5-HT for 5 min and comparing the amount of label taken up with that of worms which were incubated in ^{14}C-labeled 5-HTP and dl-NE. The results are summarized in Table VI. The presence of a carboxy group on the indole-ethylamine molecule (5-HTP) markedly decreased the rate of uptake. NE was taken up by the worms but in considerably lesser amounts than 5-HT. However, it should be pointed out that in rat tissue, uptake process is stereochemically selective, having much higher affinity for naturally occurring (–)-NE

TABLE VI. *Uptake of ^{14}C-labeled 5-HT, 5-hydroxytryptophan, and norepinephrine by* M. corti *during 5-min incubation at 37 C.*

Compound	Concentration	Tissue-to-medium ratio	Label taken up compared with 5-HT
	molar	cpm/cpm	%
5-HT	1×10^{-6}	9.23 (3)*	100
5-hydroxytryptophan	1×10^{-6}	2.58 (2)	28
dl-norepinephrine	1×10^{-6}	3.62 (2)	39

* Numbers of experiments shown in parentheses.

TABLE VIII. *Effect of two different temperatures on the high affinity uptake of ^{14}C-5-HT by* M. corti.

Time (min) incubated in 2×10^{-7} M 5-HT	Net amount of 5-HT taken up (ng × mg protein^{-1})	
	21 C	37 C
10	–	0.48 (2)
20	–	0.85 (2)
30	0.275 (2)	0.90 (3)
45	0.33 (3)	1.25 (2)
60	0.50 (2)	2.12 (2)

Number of experiments in parentheses.

than that for the (+)-enantiomer (Iversen et al., 1971; Hendley and Snyder, 1972), although guinea pig and rabbit appear to lack such stereochemical specificity (Jarrott, 1970). Presuming that the organisms have a stereospecificity for isomers of NE, then only the levo-isomer would be taken up by the organisms, and therefore only one-half of external NE concentration should be considered. Therefore, if this is the case, T/M ratio for NE is twice as high, which is close to the T/M ratio obtained with 5-HT.

The reuptake of a neurotransmitter into the nerve terminal from which it has been released is a mechanism that serves as a rapid and economical way for terminating the actions of released amine (Iversen and Kravitz, 1966). In mammalian central and peripheral nervous systems, this reuptake is operating against a concentration gradient, namely tissue-to-medium (T/M) ratio is greater than one (Iversen and Kravitz, 1966; Iversen, 1970). The reuptake is stereospecific, requires sodium ion, is reduced by a decrease in temperature, and is saturable. When tetrathyridia of *M. corti* were incubated at 37 C in medium NCTC 135 containing varying concentrations of 5-HT (2×10^{-7}–5×10^{-5} M) Tables VI, VII), it was found that the parasites had the ability to accumulate 5-HT against a concentration gradient. The T/M ratios were 2.12 or greater, depending on the concentration of 5-HT in medium and time of incubation.

The effect of temperature on 5-HT transport showed temperature-dependent sensitivity in the organisms. The total uptake was considerably lowered at 21 C (Tables VIII, IX). The uptake at 37 C as well as 21 C was initially rapid and reached a maximum level after 60 min.

The ability of the worms to retain 5-HT was determined by measuring the rate at which the organisms lost 5-HT following incubation of organisms with this amine. Worms were incubated at 37 C for 2 hr in 1×10^{-6} M and 1×10^{-7} M 5-HT. They were then transferred to an incubation medium containing no 5-HT for 30, 60, and 180 min (Fig. 4). The release of 5-HT apparently is temperature-dependent since there was no release when the temperature of the medium was lowered to 0 C.

The presence of a low concentration of 5-HT in *M. corti* (Hariri, 1974) and the lack of tryptophan hydroxylase indicates that these organisms must obtain their 5-HT from their host. The normal habitat of these organisms is

TABLE VII. *Uptake of ^{14}C-5-HT by* M. corti *in various time intervals and concentrations at 37 C.*

Concentration	Incubation time	Tissue-to-medium ratio
molar	min	cpm/cpm
1×10^{-6}	120	18.9
1×10^{-5}	120	17.3
5×10^{-5}	120	13.4
5×10^{-6}	2	2.12
2×10^{-7}	10	7.68

TABLE IX. *Effect of two different temperatures on the low affinity uptake of ^{14}C-5-HT by* M. corti.

Time (min) incubated in 1×10^{-6} M 5-HT	Net amount of 5-HT taken up (ng × mg protein^{-1})	
	21 C	37 C
10	0.37 (2)	2.10 (2)
20	0.50 (2)	2.70 (1)
30	0.65 (3)	3.60 (2)
45	0.99 (2)	4.80 (2)
60	–	6.20 (2)

Number of experiments in parentheses.

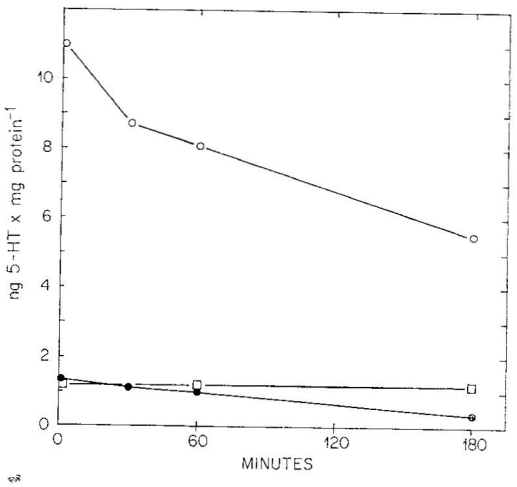

FIGURE 4. Release of 5-HT by *M. corti* following incubation for 2 hr at 37 C in 1×10^{-6} M 5-HT (○—○) and 1×10^{-7} M 5-HT (●—●) and at 0 C in 1×10^{-7} M 5-HT (□—□).

in the peritoneal cavity. Aside from the central nervous system, enterochromaffin cells are the major site of 5-HT synthesis in mammals (Erspamer and Testini, 1959). It is conceivable that the release of 5-HT from these cells could provide a source of 5-HT for these organisms. The high affinity 5-HT uptake mechanism of *M. corti* can provide an effective means of supplying the worms with this neurotransmitter which it may not be able to synthesize.

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