containing enzymes. Our findings listed in Table II indicate that this is the case, but inhibition is rather selective, being limited to 2 of the 5 copper dependent enzyme systems investigated. In one case, ceruloplasmin oxidase, this action is relatively weak with 1.0 m*M* methimazole required to achieve 50% inhibition. On the other hand, the inhibition of tyrosinase is extensive ($I_{50} = 5 \times 10^{-5}$). Relevant to these findings is the discovery by STOLK and HANLON[6] that methimazole depresses the biosynthesis of norepinephrine in rat brain due to the specific inhibition of dopamine-β-hydroxylase, a copper containing enzyme.

The potent inhibition of mushroom tyrosinase activity was examined in some detail. Preincubation of the enzyme with 1.0 m*M* methimazole for 1 h prior to assay showed no more inhibition than what could be accounted for on the basis of dilution into the assay medium. Double reciprocal plots of velocity data for a series of non-saturating concentrations of L-DOPA in the presence of different concentrations of methimazole resulted in changes in both K_m and V_{max} (see Table III). This type of inhibition is qualititively reminiscent of the kind reported for other 2-thiolimidazoles[3] and meets the requirementes for a 'mixed type' inhibition described by FREIDENWALD and MAENGWYN-DAVIES[7]. Appropriate graphical analysis generates an inhibition constant (K_i) which is a weighed value expressing both competitive and non-competitive aspects of inhibition. A second constant, α, which measures the influence of the inhibitor on the dissociation of the enzyme-substrate complex and thereby measures the extent of competitive versus non-competitive inhibition, is also obtained graphically (α = 1.0 for non-competitive inhibitors and infinity for competitive inhibitors). A K_i in the range of 10^{-6} *M* indicates that methimazole is a potent inhibitor of mushroom tyrosinase and an α of 9 to 10 shows that inhibition is chiefly competitive.

Discovery of the copper ion binding capacity of methimazole raises some potentially important points with regard to its therapeutic use. For example, patients manifesting thyrotoxicosis have elevated levels of serum Cu^{++}[8] most of which is probably secondary to an increase in serum ceruloplasmin as is observed in other stress states[9]. One might ask what effect chronic methimazole has on Cu^{++} distribution and metabolism in hyperthyroid individuals. Methimazole therapy is often accompanied by a variety of side effects some of which could be due to abnormalities in Cu^{++} metabolism.

Table II. The effect of methimazole on copper containing metalloenzymes

Enzyme	Source	I_{50}
Ascorbic acid oxidase	Summer squash	No inhibition at 5 m*M* methimazole
Monoamine oxidase	Human blood	No inhibition at 5 m*M* methimazole
Uricase	Porcine liver	No inhibition at 5 m*M* methimazole
Ceruloplasmin (oxidase)	Human blood	1.0 m*M*
Tyrosinase	Mushroom	5×10^{-5} *M*

Experimental conditions are given on Materials and methods. I_{50} = concentration of methimazole required to obtain 50% inhibition under assay conditions employed.

Table III. Kinetic parameters for methimazole inhibition of mushroom tyrosinase

Methimazole $\times 10^6$ *M*	V_{max}[a]	$K_m \times 10^4$ *M*	$K_i \times 10^6$ *M*	α
0	0.463	2.27	–	–
1.0	0.387	5.14	1.00	8.8
2.0	0.346	6.52	1.32	8.9
5.0	0.236	11.0	2.20	10.1

Values were obtained graphically as described in the text. [a] Velocity expressed as Δ 475 nm/min.

Summary. The antithyroid drug, methimazole (1-methyl-2-thiolimidazole), is a powerful chelator of cupric ion. This is reflected in its ability to selectively inhibit certain copper oxidases. Uricase, ascorbic oxidase and monoamine oxidase are not affected. Ceruloplasmin oxidase is slightly inhibited and tyrosinase is markedly inhibited by methimazole.

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Octopamine in the Central Nervous System of an Annelid, *Lumbricus terrestris*

Information about transmitters in the central nervous system of annelids is rather scarce and yet annelids as well as other invertebrates (the nematodes, molluscs and the arthropods) are becoming increasingly important in research into the basic mechanisms of the nervous system. Among the important phylogenetic trends seen in transmitter distributions is the relative importance of the monophenolic amine octopamine in invertebrate nervous systems. Octopamine has been recognized as a normal constituent of adrenergically innervated organs in mammals since the work of MOLINOFF and AXELROD[1]. It occurs endogenously in amounts some 5–10% of that of noradrenaline. MOLINOFF and AXELROD[1] observed that

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octopamine was present in very high concentration in the central nervous system of the lobster, *Homarus*, and since then it has been shown that octopamine occurs normally in large quantities in the other large group of arthropods, the insects[2,3]. Among the *protostomia* octopamine has now been identified in 2 of the major classes of the Arthropoda (Crustacea[1] and insecta[2,3] but not Arachnida or the minor classes). In the Mollusca, it is present in Gastropoda[4,5] and Cephalopoda[6]. The only major phylum of *Protostomia* that has not been investigated is *Annelida*. The purpose of this study therefore was to attempt to establish whether or not the nervous system of an annelid might be a suitable model for an investigation of the metabolism of the phenolic amines and the possible involvement of these substances in neurotransmission processes.

Materials and methods. Large earthworms (3–4 g) were obtained from Carolina Biological Supplies. (Burlington, N.C., USA) Portions of the central nervous system were dissected out and frozen immediately on dry ice. Portions of muscle and the gizzard were taken as tissue controls. Octopamine was assayed using an enzymatic-isotopic method based on that of SAAVEDRA[7]. Frozen tissues were homogenized in volumes of ice cold 0.02 *M Tris*-HCl buffer, pH 8.6 containing 1 m*M* pargyline (Abbott Laboratories), heated to 95 °C for 3 min to precipitate protein and centrifuged at 10,000 × *g* for 5 min in a Beckman microfuge. 50 µl aliquots of the clear supernatant were incubated for 30 min at 37 °C after the addition of 20 µl of a mixture containing 200 µl of partially purified phenylethanolamine-N-methyl transferase (Miles Laboratories), 30 µl of ^{3}H-s-adenosyl methionine (S.A. 12.1 Ci/mmol; 1 mCi/ml) and 420 µl of 0.05 *M Tris*-HCl, pH 8.6. Internal standards were prepared by adding 1 ng of authentic DL-octopamine to 50 µl aliquots of tissue extract. Blanks were prepared from 50 µl aliquots of the buffer used for homogenization. The reaction was stopped by the addition of 70 µl of 0.5 *M* borate buffer, pH 10 containing 0.2 mg/ml authentic synephrine as carrier. The mixture was then extracted with 1.2 ml of toluene : isoamyl alcohol (3 : 2 v/v). After centrifugation (1000 × *g*) 1.0 ml of the organic phase was transferred to a second centrifuge tube containing 0.5 ml of the borate buffer and extracted a second time. After centrifugation (1000 × *g*), 800 µl of the toluene-isoamyl alcohol extract was transferred to counting vials containing 800 µl of toluene-isoamyl alcohol and dried overnight at 80 °C in a chromatography oven. The radioactivity content was determined by liquid scintillation counting following the addition of 1.0 ml of ethanol and 10 ml of Aquasol (New England Nuclear). Samples were also taken up in 10 µl of ethanol and applied to the origin zones of thin layers of silica gel (Eastman Kodak #6061) on which authentic samples of synephrine, N-methylphenylethanolamine and N,N-dimethyloctopamine had also been applied. The chromatograms were developed in 4 different solvent systems: a) butanol saturated with 1 *N* HCl (uppers phase); b) butanol – formic acid (90:10 v/v); c) butanol-acetic acid-water (4–1–1 v/v); d) t-amyl alcohol-methylamine-water (80:10:10 v/v). The chromatograms, after removal from the tank, were air dried and sprayed with Pauly's reagent[8] in order to visualize the various amine zones. The chromatograms were then cut into 1 cm pieces. The amount of radioactive label was determined by liquid scintillation counting following the addition of 1 ml of ethanol and 10 ml of Aquasol as described above.

Results. A partial distribution of octopamine in the earthworm is shown in the Table. The highest levels were found in the cerebral ganglia (or brain) and subpharyngeal ganglia with lesser amounts in the ventral nerve cord and gizzard. There was no detectable amount in the somatic muscle. Considering to sensitivity of the assay, this means there was less than 33 pg/mg.

Octopamine in the Earthworm

Tissue	Octopamine (pg/mg)[a]
Cerebral ganglia	7390 ± 390 (7)
Subpharyngeal ganglia	8110 ± 640 (7)
Ventral nerve cord (anterior portion)	5340 ± 480 (7)
Gut (gizzard)	1060 ± 160 (3)
Muscle	< 33 (4)

[a] ± S.E.M., number of determinations in parentheses.

The identity of the ^{3}H-synephrine formed in the enzymatic assay was confirmed following separation on thin layers of silica gel in the 4 solvent systems mentioned above. In each system only a single radioactive peak, isographic with authentic synephrine, was located. No significant amounts of radioactivity were found to be associated with N,N-dimethyloctopamine or N-methylphenylethanolamine.

Discussion. The levels of octopamine in the central nervous system of the earthworm are considerably higher than any previously reported for nervous tissue. This suggests that this monophenolic amine may play an important role in the nervous system of the earthworm and other annelids. Whilst there appeared to be little if any octopamine in the somatic muscle, substantial amounts were found in the gizzard. This suggests that octopamine may function as a transmitter in the peripheral nervous system of the earthworm. Recent work suggests that octopamine may also be a peripheral transmitter at the light organ in the firefly[9], in the lobster[10] and, conceiveably, in *Aplysia*, where stimulation of an octopamine-containing cell[11] (L7) in the abdominal ganglion leads to excitation of gill and siphon muscle[12]. In the past, efforts to identify monoaminergic transmitters in annelids have centered around the catecholamines and 5-HT. The noradrenaline level in the earthworm is reported to be 1 µg/g for the nerve cord and 2 µg/g for the brain[13]. This latter group[13] also claim that the nervous system of the earthworm does not contain dopamine. 5-Hydroxytryptamine (5-HT) is present in earthworm ganglia at a concentration of 10.4 µg/g[14] and it has been shown recently

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that the ventral nerve cord of another annelid, the leech, contains both 5-HT and catecholamine-containing cells (as revealed by fluorescence microscopy)[15]. Moreover, both ^{14}C-octopamine and ^{14}C-dopamine are formed during incubation (of leech nervous tissue) in media containing ^{14}C-tyrosine[15]. There was no evidence of production of noradrenaline[15]. If it can indeed be confirmed that little if any noradrenaline is present in annelid nervous systems, such an observation would conform well to the established pattern that invertebrate central nervous systems contain more dopamine than noradrenaline[16]. Another most important emerging pattern is the importance in invertebrate nervous systems of octopamine. While octopamine is found only in small amounts in vertebrate central nervous systems[1], it occurs in relatively large amounts in all the invertebrate groups so far examined. This now appears to be true for all the major groups of the *Protostomia*, i.e. the molluscs, the arthropods and the annelids. The high levels of octopamine in the *Protostomia* suggests that this amine plays an important role in these groups. Furthermore, the differences in octopamine content, in connection with the already-noted predominance of dopamine over noradrenaline in invertebrate nervous systems[16], may reflect a fundamental biochemical dichotomy between the nervous systems of *Protostomia* and *Deuterostomia*.

Summary. Octopamine has been found in very high concentrations in cerebral and subpharyngeal ganglia of the earthworm *Lumbricus terrestris* and may function as a neurotransmitter in the peripheral nervous system.

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[17] I thank Drs. A. BOULTON, A. V. JUORIO and P. H. WU for helpful discussion and Dr. B. DAVIS for a sample of N,N-dimethyloctopamine. Support in the form of a M.R.C. of Canada fellowship is acknowledged.

Presence of a Specific Uridine 5'-Monophosphate Pyrophosphorylase in Baker's Yeast

Uracil is readily incorporated into pyrimidine nucleotides and nucleic acids by many bacteria, apparently via its reaction with 5-phosphorybosyl 1-pyrophosphate (PRPP), catalyzed by UMP pyrophosphorylase[1-3].

GRENSON[4] showed that in yeast UMP liberates uracil in two steps, catalyzed by a phosphatase and by an uridine ribohydrolase respectively. In addition the same author observed that uracil could be converted to UMP by a *Saccharomyces cerevisiae* mutant lacking uridine kinase activity. These observations prompted us to check the possible presence of UMP pyrophosphorylase in yeast. The data reported in this communication show the presence of such activity in baker's yeast and contribute to the elucidation of its fundamental role in the UMP recycling.

In addition, aim of this report is also to furnish the suitable assay conditions, found for the first time on partially purified preparation, for a more detailed study of the enzyme.

Materials and methods [8-^{14}C] Adenine (50 mCi/mmol), [2-^{14}C] Uracil (50 mCi/mmol), [8-^{14}C] Hypoxanthine (50 mCi/mmol), [2-^{14}C] Cytosine (50 mCi/mmol) and [2-^{14}C] Thymine (50 mCi/mmol) were purchased from the Radiochemical Center, Amersham, England. PRPP as tetrasodium salt, bases, nucleotides, orotic acid, $MgCl_2$, orotidine 5'-monophosphate decarboxylase, orotidine 5'-monophosphate pyrophosphorylase and all other chemicals were purchased from Sigma Chemical Co. The actual concentration of PRPP was determined by its capacity to convert orotate to orotidine-5'-monophosphate[5].

The standard assay for the enzyme activity was performed as follows: 10 nmoles of PRPP, 0.6 μmoles $MgCl_2$, 8 nmoles of 2-^{14}C uracil, 10 μmoles of phosphate buffer (pH 7.8) were mixed with an appropriate amount of enzyme solution in a final volume of 0.2 ml. After incubation at 37 °C for 30 min 25 μl of 50% trichloroacetic acid were added.

After centrifugation, 10 μl of the supernatant were spotted onto cellulose thin layer (Eastman Kodak) together with an appropriate amount of UMP and uracil chromatographed for 30 min in a solvent system composed by butanol, water and acetic acid in the ratio 4:2:1 (v/v). After development of the chromatogram, it was dried and the spots, visualized by fluorescence, corresponding to uracil and UMP, were cut out and placed in scintilla-

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Table I. Purification of uridine 5'-monophosphate pyrophosphorylase

Step	Volume (ml)	Total proteins (mg)	Total activity (units)	Specific activity (units(mg)	Purification (fold)	Yield (%)
1 Crude extract	150	1395.0	5370.7	3.85	—	100
2 Ammonium sulfate fractionation (50–85% saturation)	6	242.8	5365.9	22.1	5.94	99.9
3 Agarose A 1.5	17	24.14	2710.9	112.3	29.17	50.5

Results are the average of 4 preparations each starting with 150 g of baker's yeast. Assay mixtures and conditions were as described under 'Materials and methods'.