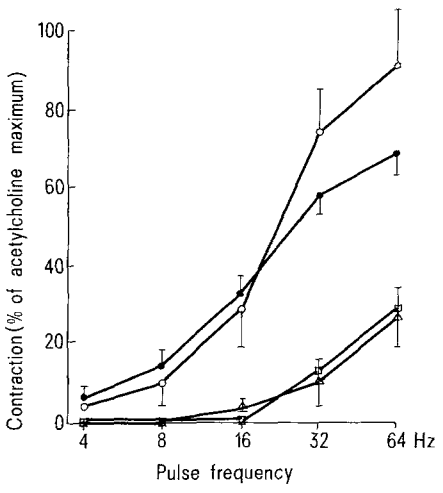
those to oxytocin unaffected. Propranolol antagonized inhibitions produced by isoprenaline. Previous experiments[2] showed that this concentration of propranolol did not affect aminophylline inhibitions of the rat uterine horn. The cervix contracted to high concentrations of noradrenaline (1 and 4×10^{-5} moles/l) but not in a concentration related manner. Significantly greater responses were seen to noradrenaline and at lower concentrations in the presence of propranolol. These contractions were abolished by phentolamine. The latter results suggest that noradrenaline produces inhibition via β-adrenoceptors at low concentrations and contractions via α-adrenoceptors at higher concentrations.

Histochemical evidence suggests a reduction in cervical cholinergic innervation density towards the end of pregnancy. This is supported by the higher frequencies of field stimulation required to elicit responses in the cervix of the term pregnant rat compared to the cervix of the non-pregnant rat[3]. However, the ability to produce cholinergic and noradrenergic responses by field stimulation of intrinsic neurones in the cervix suggest that a functional innervation may be present. These may have a role in retaining the fetuses before term and aiding their delivery at term.



Contractile responses of isolated cervix of day-22 pregnant rat to field stimulation in controls (●; n=37) or in the presence of hyoscine (△; 1×10^{-8} moles/l; n=8) or tetrodotoxin (□; 3.1×10^{-7} moles/l; n=8) or propranolol (○; 1×10^{-8} moles/l; n=8). Stimulation parameters: 0.5 msec pulses, 60 V, 10 sec trains every 100 sec. Means±SEM are shown. Repeat-controls did not differ from controls. The responses in the presence of hyoscine and tetrodotoxin (at 16, 32 and 64 Hz) and in propranolol (at 32 and 64 Hz) differed significantly ($2\ p<0.05$) from their respective controls.

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Tryptophan (Trp), serotonin (5-HT), monoamino oxidase (MAO) and 5-hydroxyindole acetic acid (5-HIAA) in brain and subesophagic ganglions of earthworms. Effects of Parathion*

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Summary. Tryptophan, 5-HT, MAO and 5-HIAA were determined in the first 5 segments of earthworms (Oligochaetae) where the brain and subesophagic ganglions are located. Tranylcypromine (IMAO) decreased MAO activity increasing 5-HT and decreasing 5-HIAA. Motility and survival of worms were disturbed. In *Allolobophora* species (young worms), parathion fumigation decreased acetylcholinesterase (AChE) activity and increased Trp, 5-HT and 5-HIAA. Motility was diminished after 24 h: it worsened after 72, but returned to normal levels 40/50 days later.

The presence of catecholamines[2], serotonin[3], and, more recently, octopamine[4] in the brain ganglion of annelids, was demonstrated in the Lumbricidae family, *Lumbricus terrestris*, Oligochaetae class.

The earthworm performs an important function in soil fertilization, augmenting nitrogen, magnesium, phosphorus and potassium content, derived from organic matter. Normally, during agricultural procedures, these soils are fumigated with organophosphorous insecticides, which could affect these useful annelids.

The aim of this paper is to study the relationship between treatment of soil with Parathion (an organophosphorous insecticide) and the following biochemical parameters in the earthworm: content of Trp, 5-HT and 5-HIAA, as well as 2 enzymatic systems: MAO and AChE.

Studies started in March 1977 (autumn) using non-classified earthworms (±6 cm in length) with non-developed clitellum which were obtained from hens' droppings.

Earthworms, in their own soil, were housed in a big box from where they were moved to circular boxes (25 cm diameter×9 cm height). The earth, free of earthworms belonging to the lot of treated ones, was dried in a stove at 90 °C, and the water lost was returned with a 1 mg/ml solution of tranylcypromine. They were maintained at 24 °C (light from 07.00 h to 19.00 h).

Controls and treated earthworms were removed from the deep zone 24 and 72 h later. After cleaning the remaining earth with tap water, the first (±) 5 rings containing the brain and subesophagic ganglions were dissected on Petri dishes with ice and under lens.

The pools, consisting of 10-15 heads each, weighed about 60-90 mg. From them, the determination (table 1) of 5-HT,

Table 1. Effect of tranylcypromine on 5-HT, MAO and 5-HIAA upon unclassified Oligochaetae

	IMAO 24 h Controls	Treated	72 h Controls	Treated
5-HT, µg/g fresh tissue (60–90 mg)	1.06 ±0.11 (3)	1.43 ±0.02 (4)	1.00 ±0.02 (3)	1.25 ±0.02 (4)
	$p<0.05$		$p<0.005$	
5-HIAA, µg/g fresh tissue (60–90 mg)	0.38 ±0.03 (3)	0.27 ±0.02 (3)	0.34 ±0.05 (3)	0.14 ±0.03 (3)
			$p<0.02$	
MAO, µmoles 4-HOQ/g fresh tissue · min (50–60 mg)	0.041±0.003 (3)	0.010±0.001 (3)	0.046±0.002 (2)	0.014±0.001 (4)
	$p<0.001$		$p<0.001$	

In brackets: number of pools. Values are mean ± SEM. Two-tailed Student's t-test.

Table 2. The effect of Parathion on Trp, 5-HT, 5-HIAA, MAO and AchE in Oligochaetae of the Lumbricidae family: *Allolobophora*

	Controls	Treated with Parathion (24 h)
Trp, µg/g fresh tissue (70–140 mg)	13.9 ±2.0 (3)	23.7 ±1.4 (2)
	$p<0.05$	
5-HT, µg/g fresh tissue (82–94 mg)	0.69 ±0.08 (3)	2.8 ±0.12 (4)
	$p<0.001$	
5-HIAA, µg/g fresh tissue (82–94 mg)	0.10 ±0.02 (3)	0.18 ±0.02 (4)
	$p<0.02$	
MAO, µmoles 4-HOQ/g fresh tissue · min (45–75 mg)	0.050±0.006 (3)	0.044±0.02 (4)
AchE µmoles substrate/g fresh tissue · min (30–50 mg)	5.3 ±0.4 (6)	3.4 ±0.2 (3)
	$p<0.005$	

In brackets: number of animals. Values are mean ± SEM. Two-tailed Student's t-test.

5-HIAA[9] and MAO[10] was made. As a substrate of MAO, kynuramine dihydrobromide was used and its activity was expressed in µmoles of product (4-hydroxyquinoline)/g of fresh tissue/min.

Other experiments were carried out using young earthworms classified as *Allolobophora* with non-fully developed clitellum (±4 cm) and obtained from another source (garden soil) and at another time (November, end of spring, 1977); they were housed in rectangular plastic boxes with holes in the floor and earth up to 25 cm in length, 18 cm width and 2.5 cm height. The earth containing earthworms was fumigated with 25 ml Parathion solution (Folidol, 0.5 mg/ml) which inhibited AChE in Oligochaetae[11].

Trp[10], 5-HT, 5-HIAA, MAO and AChE (kit Boehringer, Mannheim GHBH) were determined in the first 5 cephalic ring pools, 24 h later than in the previous experiment (table 2). AChE activity was expressed in µmoles of substrate (acethylthiocholine)/g fresh tissue/min. Earthworms showed a reduced motility and a spiral winding position maintaining head upright in most of the cases.

Inspite of the effects caused by Parathion, the earthworm survived for 40 days. 5 weeks after Parathion fumigation, the values in 3 pools (with 10–15 heads each) were determined: AChE, 3.1 µmoles/min · µg fresh tissue (f.t.); 5-HT, 0.56 µg/g fresh tissue and 5-HIAA, 0.06 µg/g fresh tissue. These values tended to return to normal levels and motility recovered. Parathion, used in these doses, did not affect earthworms' life.

As in the case of Myhrberg[2], these findings confirmed the presence of 5-HT in brain ganglion of Oligochaetae. Welsh and Moorhead[3] obtained 10.4 µg/g fresh tissue of 5-HT in nerve cords of *Lumbricus terrestris*. The differences obtained in the content are likely to be due to variation in the species used, as well as experimental conditions.

As the Trp amount increased at the same time as 5-HT, this 5-HT could probably have its origin there. This fact suggested the hypothesis of the existence of 5-hydroxylase and decarboxylase in earthworms. The presence of MAO was also shown, which responded to a classic inhibitor, such as tranylcypromine, and of 5-HIAA, which probably would not be the principal metabolite of 5-HT.

The AChE inhibition caused by Parathion would increase the amount of acetylcholine (as is admitted) and this would determine the imbalance of Trp, 5-HT and 5-HIAA levels.

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