

methoxyflurane 0.5%, enflurane gives only 16% inhibition and halothane effects are scarce and not statistically significant. The different inhibition of RNA-synthesis can be ascribed to higher blood levels of inorganic fluoride after methoxyflurane than after enflurane or halothane treatment in rats<sup>8-10</sup>.

The data reported in figure 2 indicate that sodium fluoride parallels to a certain extent the methoxyflurane effect causing an early inhibition of RNA-synthesis, but the pattern of this inhibition differs in some features from that caused by the anesthetic: methoxyflurane does not alter the orotic acid uptake into kidney cells and the inhibition by

fluoride, that is marked at 2 and 3 h, is no longer evident at 4 h after NaF administration on the basis of corrected values.

The data presented in this paper suggest that in kidneys an early event after methoxyflurane or NaF administration is the inhibition of RNA-synthesis, which may be responsible of the degenerative changes described in kidney proximal tubules after methoxyflurane or fluoride treatment<sup>4</sup>. The inhibited synthesis of RNA may be a result of other derangements in cellular metabolism, although no alteration in subcellular structures of the kidney cells was detected<sup>4</sup> immediately after methoxyflurane anesthesia.

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## Mechanical responses of the isolated cervix of the day-22 pregnant rat to field stimulation<sup>1</sup>

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**Summary.** Field stimulation of isolated, spirally-cut cervix from day-22 pregnant rats produced contractions which could be inhibited by tetrodotoxin or hyoscine and potentiated by propranolol. The rat cervix would appear to receive both cholinergic and noradrenergic innervations whose transmitters activate muscarinic cholinceptors and  $\beta$ -adrenoceptors respectively.

Transmural stimulation of isolated uterine horns of non-pregnant rats demonstrated cholinergic contractions, noradrenergic inhibitions and possibly the presence of at least a 3rd neurotransmitter<sup>2</sup>. Cholinergic contractions of isolated, luminally perfused cervix of non-pregnant rats have also been produced by transmural stimulation<sup>3</sup>; inhibitory responses to transmural stimulation were not studied. Histochemical studies also suggest cholinergic and noradrenergic myometrial and cervical innervations in the rat<sup>3,4</sup> whose density decreases during pregnancy<sup>4,5</sup>. Near term the density of noradrenergic innervation of the guinea-pig cervix<sup>6</sup> and the cholinergic innervation of the rat cervix<sup>5</sup> appear greater than those of the respective myometria. In the present study, field stimulation and antagonist drugs have been used to detect the presence and the likely nature of the neurotransmitters of any cervical innervation in the late pregnant rat.

**Materials and methods.** Spirally-cut cervical preparations from Sprague-Dawley rats on day 22 of gestation (day of finding copulation plug = day 1) were set up in modified Krebs at 37°C as described previously<sup>7</sup>. A Grass S8 stimulator was used to apply field stimulation to the cervix via a pair of parallel stainless steel ring electrodes, 1 cm apart, with pulses of 0.5 msec and of supra-maximal voltage (60 V). After obtaining constant contractions to an acetylcholine maximum, stimuli were applied in ascending frequencies from 1 to 64 Hz using 10-sec trains every 100 sec. Tissues were incubated in solutions containing modifying drugs for 30 min before agonist drugs and stimulation were

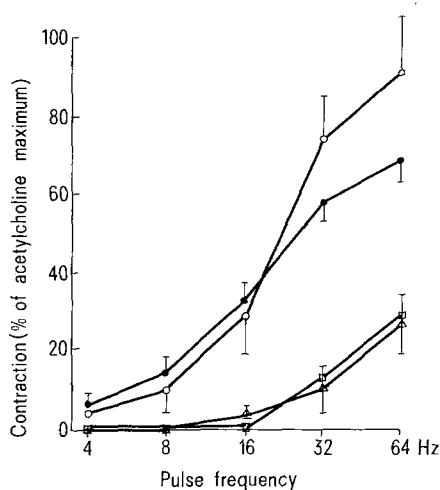
repeated. The Wilcoxon matched pair signed rank test<sup>8</sup> was used to test the significance of differences.

**Results and discussion.** Field stimulation produced single phasic contractions of the cervix with initial responses seen to 4 or 8 Hz, depending on the tissue, and amplitude increasing with frequency. The maximal response of the cervix to field stimulation was  $68.8 \pm 5.6\%$  (mean  $\pm$  SEM;  $n=37$ ) of that of an acetylcholine maximum.

Tetrodotoxin ( $3.1 \times 10^{-7}$  moles/l) clearly antagonized the contractions to field stimulation at low-pulse width (figure) suggesting that the responses were nerve-mediated. Field stimulation of spirally-cut uterine horns from the same animals did not consistently produce contractions and these were not significantly antagonized by tetrodotoxin. This could be due to the much greater spontaneous contractility of the uterine horns or their lesser density of innervation compared to the cervix. Hyoscine ( $1 \times 10^{-8}$  moles/l) antagonized responses to field stimulation suggesting that the cervix possesses a cholinergic innervation whose transmitter acts on muscarinic cholinceptors. Responses at 32 and 64 Hz were potentiated by propranolol ( $1 \times 10^{-8}$  moles/l) but not modified further by addition of phentolamine ( $1 \times 10^{-7}$  moles/l) to the propranolol. This indirectly suggests some noradrenergic innervation whose transmitter acts solely on  $\beta$ -adrenoceptors to produce relaxation.

The antagonistic properties of the modifying drugs were tested by measuring their effects on the responses to various agonists. Hyoscine was selective in that it produced an 83-fold antagonism of acetylcholine contractions but left

those to oxytocin unaffected. Propranolol antagonized inhibitions produced by isoprenaline. Previous experiments<sup>2</sup> showed that this concentration of propranolol did not affect aminophylline inhibitions of the rat uterine horn. The



Contractile responses of isolated cervix of day-22 pregnant rat to field stimulation in controls (●;  $n=37$ ) or in the presence of hyoscine (Δ;  $1 \times 10^{-8}$  moles/l;  $n=8$ ) or tetrodotoxin (□;  $3.1 \times 10^{-7}$  moles/l;  $n=8$ ) or propranolol (○;  $1 \times 10^{-8}$  moles/l;  $n=8$ ). Stimulation parameters: 0.5 msec pulses, 60 V, 10 sec trains every 100 sec. Means  $\pm$  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.

cervix contracted to high concentrations of noradrenaline ( $1$  and  $4 \times 10^{-5}$  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  $\beta$ -adrenoceptors at low concentrations and contractions via  $\alpha$ -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<sup>3</sup>. 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.

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## Tryptophan (Trp), serotonin (5-HT), monoamino oxidase (MAO) and 5-hydroxyindole acetic acid (5-HIAA) in brain and subesophageic 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 subesophageic ganglions are located. Tranlycypromine (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<sup>2</sup>, serotonin<sup>3</sup>, and, more recently, octopamine<sup>4</sup> 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-classi-

fied earthworms ( $\pm 6$  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  $\times$  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 tranlycypromine. 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 ( $\pm$ ) 5 rings containing the brain and subesophageic 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,