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Studies on chemotherapy of parasitic helminths (XVIII). Mechanism of spastically paralyzing action of pyrantel in *Angiostrongylus cantonensis*

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Summary. Pyrantel tartrate caused spastic paralysis through stimulating nicotinic cholinoceptors in *Angiostrongylus cantonensis.*

Pyrantel has been used as a broad-spectrum anthelmintic against various nematodal infections including oxyuriasis, ascariasis and ancylostomiasis[1,2]. Regarding the mode and mechanism of antinematodal action of this anthelmintic, however, the only report yet published is that on *Ascaris suum* by Aubry et al.[3].

In the previous papers[4,5], we have selected *Angiostrongylus cantonensis,* the rat lungworm, as an excellent model of a parasitic nematode for detecting and determining the anthelmintic effects of drugs. In contrast to *A. suum,* a traditional model of nematodes in pharmacoligical studies, *A. cantonensis* is easily maintained under laboratory conditions and obtained at need throughout the year, and the whole-worm preparation of this worm is remarkably susceptible to various neuropharmacological agents. The worm was found to be remarkably sensitive to pyrantel; the motility of the worm was affected spastically at concentrations of 10^{-9} M or more[6]. The mechanism of the spastically paralyzing action of pyrantel on the whole worm preparation of *A. cantonensis* was therefore examined, using various neuropharmacological agents.

Materials and methods. Angiostrongylus cantonensis was obtained from rats (Wistar strain) experimentally infected in our laboratory. Female worms (2.5-3.0 cm) were used as whole worm preparations. The worm preparation was suspended in Tyrode's solution in a thermostatically controlled organ bath (7 ml capacity) at 35 °C and gassed slightly with air. Responses of the preparation to drugs were recorded isotonically on a recorder (Toa, EPR-100A) with an isotonic transducer (Nihon Koden, TD-112S), producing a magnification of 15-to 30-fold and exerting a tension of 0.7-0.8 g. Drugs in a single or cumulative dose were given at the points shown by symbols in the figures, and the preparations were kept exposed to drugs until the end of the experiments or until they were washed with Tyrode's solution for about 30 min at times shown by the point W in the figures.

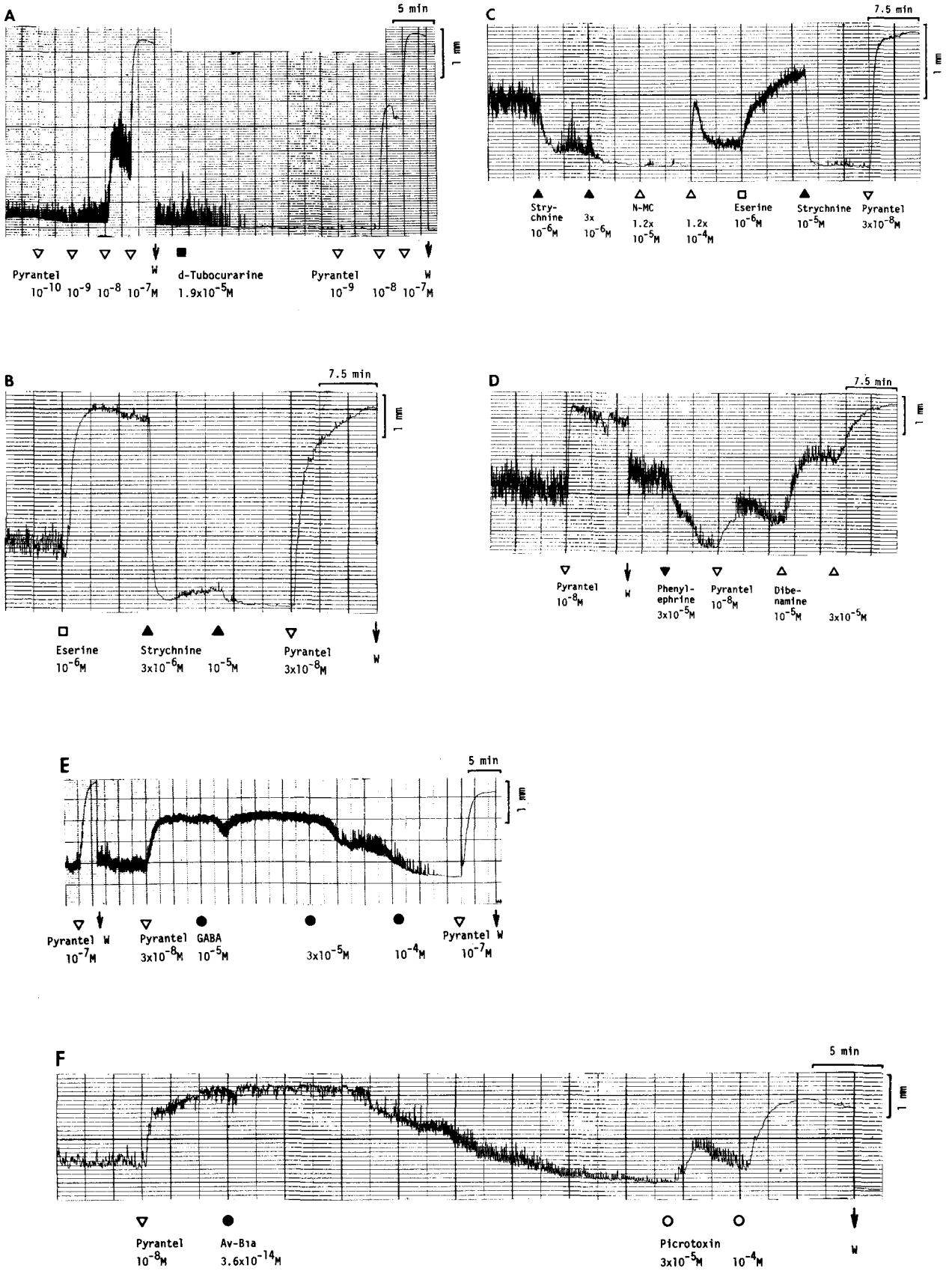
Results and discussion. Using various preparations of *A. suum,* Aubry et al.[3] reported the mode and mechanism of action of pyrantel. For example, when pyrantel in rather higher doses such as 2.5×10^{-5} g/ml (6.4×10^{-5} M) was given to the whole worm preparation of *Ascaris,* a sharp contraction of the worm was seen together with a cessation of spontaneous activity. Mechanism of this action was not defined in this preparation probably because an intact worm or anterior piece of this parasite were little influenced by almost all neuropharmacological agents[7,8]. On the other hand, a prolonged contracture was caused when muscle strips of *Ascaris* were exposed to pyrantel in lower doses such as 1.5×10^{-9} g/ml (3.8×10^{-9} M), and this action was antagonized by the pretreatment with piperazine (10^{-3} g/ml) or d-tubocurarine (2×10^{-5} g/ml, 2.5×10^{-5} M). From these results in *A. suum* together with those on the effects of this anthelmintic in vertebrate systems[3,9], they suggested that pyrantel shows marked persistent nicotinic properties which result in the spastic paralysis of the worm.

A traditional model worm, *A. suum* has been used in pharmacological studies as muscle strips with a longitudinal cut along the lateral line or as eviscerated preparations because of the above mentioned reasons. However, Goodwin[10] described that preparations of this kind give little indication of what may happen to a worm when the anthelmintic reaches it through the alimentary canal of the host in which it lives. Therefore, intact worm preparations should be used if possible for detecting and determining the anthelmintic effects of drugs. When the worm is too large to use as such a preparation, anterior piece preparations should be used because these preparations have the cerebral ganglia and show obvious motility. In contrast to the whole worm preparations of *A. suum,* those of *A. cantonensis* were susceptible to various neuropharmacological agents[4,5]. From the results on these agents, it was suggested that the motility of *A. cantonensis* may be regulated by an excitatory cholinergic mechanism, and inhibitory gabergic and α-adrenergic mechanisms[4,5], and that the cholinoceptors in this worm may be nicotinic in nature[4].

Though d-tubocurarine (1.9×10^{-5} M) inhibited the spontaneous motility and also the contractive effects of pyrantel in lower doses (10^{-9} M), this antagonist did not block the contractive effects of pyrantel in higher doses (10^{-8}-10^{-7} M) (fig. A). A similar relationship was observed when eserine was given instead of pyrantel[4]. Therefore, d-tubocurarine probably has a rather low affinity to the cholinoceptors in this worm.

As described in another paper[4], it is suggested that strychnine inhibits the release of acetylcholine (ACh) from the cholinergic nerve ending of *A. cantonensis.* The eserine (10^{-6} M)-induced contraction was completely inhibited by the addition of strychnine (3×10^{-6}-10^{-5} M), whereas pyrantel (3×10^{-8} M) contracted this paralyzed preparation remarkably (fig. B). When the preparation was paralyzed by the lower concentrations of strychnine (10^{-6}-3×10^{-6} M), contraction appeared after adding a higher concentration of N-methylcytisine (N-MC, 1.2×10^{-4} M), a stimulator of the release of ACh from the cholinergic nerve ending of this worm[11], and eserine (10^{-6} M) stimulated this contraction. Then, the contracted preparation was paralyzed again by the addition of a higher concentration of strychnine (10^{-5} M), but pyrantel (3×10^{-8} M) recontracted the paralyzed preparation (fig. C). These results suggest that pyrantel may cause spastic paralysis in *A. cantonensis* through stimulating the nicotinic cholinoceptors rather than through stimulating the release of ACh from the cholinergic nerve ending or inhibiting acetylcholinesterase activity.

It was reported by Aubry et al.[3] that combination therapy with pyrantel and piperazine may well be contraindicated since pyrantel and piperazine by virture of their mechanism of action can be regarded as being potentially mutually antagonistic. Functional antagonism must be also seen in *A. cantonensis* between pyrantel and drugs which stimulate gabergic and/or α-adrenergic mechanisms. Indeed, the contractive effect of pyrantel (10^{-8}-3×10^{-8} M) was antagonized by treatment with α-adrenergic agonists such as phenylephrine (3×10^{-5} M) (fig. D) or gabergic agonists such as γ-aminobutyric acid (GABA, 10^{-5}-10^{-4} M) (fig. E) and avermectin B1a (Av-B1a, 3.6×10^{-14} M), but were reversed by the addition of their antagonists such as dibenamine (10^{-5}-3×10^{-5} M) and picrotoxin (3×10^{-5}-10^{-4} M), respectively (fig. D and F). However, pyrantel in higher doses (10^{-7} M) caused a marked contraction in the prepara-



Interactions between pyrantel and other neuropharmacological agents in *Angiostrongylus cantonensis*. Interactions between pyrantel and d-tubocurarine (A), eserine and strychnine (B), strychnine, N-methylcytisine (N-MC) and eserine (C), phenylephrine and dibenamine (D), γ-aminobutyric acid (GABA) (E), or avermectin B1a and picrotoxin (F) were examined.

tion completely paralyzed by GABA (10^{-4} M) (fig. E). From the relationship between pyrantel and its antagonists such as d-tubocurarine and GABA, it is suggested that this anthelmintic has a high affinity and/or potent intrinsic activity against the cholinoceptors in *A. cantonensis*.
In conclusion, the results in *A. cantonensis* together with those in *A. suum*[3] provide strong evidence that pyrantel acts as an antinematodal anthelmintic through stimulating the nicotinic cholinoceptors in parasitic nematodes.

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Effect of 5,7-dihydroxytryptamine on Auerbach's plexus in the ileum of guinea-pig[1]

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Summary. Action of 100 mg/kg of 5,7-dihydroxytryptamine on Auerbach's plexus in the ileum of the guinea-pig has been studied using Falck and Hillarp's formaldehyde condensation technique. The drug caused partial disappearance of the adrenergic nerve profiles initially but after 10 days of treatment all the lost fibers reappeared.

6-Hydroxydopamine causes long lasting depletion of noradrenaline from the peripheral tissues, but adrenergic ganglia are significantly less sensitive to its action[2-4]. However, little is known about the action of 5,7-dihydroxytryptamine on the peripheral tissues[5,6]. In the present investigation the effect of this drug on Auerbach's plexus in the ileum of the guinea-pig has been studied.
Guineapigs weighing 250–300 g were used in the present investigation. 100 mg/kg of 5,7-dihydroxytryptamine, dissolved in 0.9% saline containing 0.1 mg/ml of ascorbic acid was injected i.p. into 6 guinea-pigs. The rest of the animals were given the same amount of saline, containing ascorbic acid only. Guinea-pigs were killed by cervical dislocation 24 h, 48 h and 10 days after the drug administration. One control animal was also sacrificed at each interval of the treatment. Small pieces of the ileum were dissected out. Their longitudinal smooth muscle layer was carefully separated from the wall of the intestine, with Auerbach's plexus attached, and the pieces stretched on slides. The stretch preparations were dried over phosphorus pentaoxide and exposed to paraformaldehyde vapor at 80 °C for 1 h. The preparations were mounted in liquid paraffin and examined using a Leitz Orthomat Fluorescence Microscope using routine filters (excitation filter BG 12 and barrier filter 530/nm). For comparing the effects of 5,7-DHT with 6-hydroxydopamine(6-OHDA) 100 mg/kg of this drug was given to a few guinea-pigs in the same way as described above, and samples treated similarly.
Auerbach's plexus lies between the longitudinal and circular smooth muscle layers and is made up of small ganglia

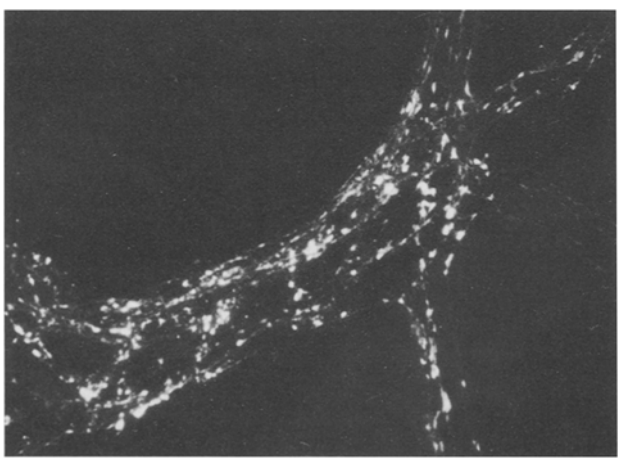

Figure 1. Photomicrograph of Auerbach's plexus in the ileum of an untreated guinea-pig exhibiting a network of varicose adrenergic nerve fibers. Intramural nerve cell bodies can be seen as black oval patches in between the adrenergic nerve fibers. × 15.

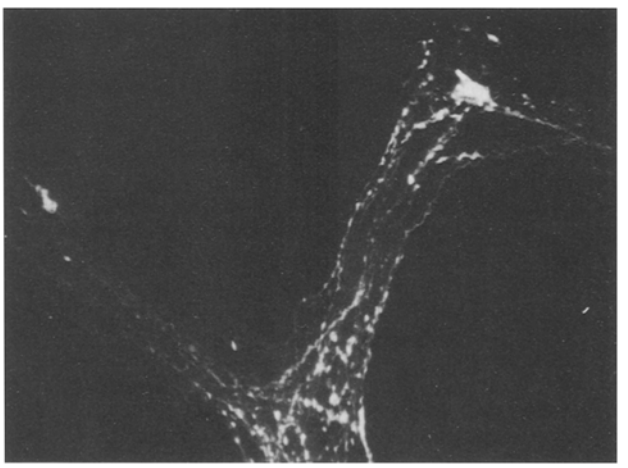

Figure 2. Photomicrograph of the stretch preparation of Auerbach's plexus 24 h after 5,7-DHT treatment. A few fibers are very swollen and some of the fibers have disappeared. × 11.