mounted and examined in a Zeiss fluorescence microscope equipped with a KP 500 excitation filter and a LP 520 stop filter. SP antiserum preabsorbed with an excess of SP (10 nmoles/ml diluted serum) was used as control serum. After photography, the cover slips were removed, and after rinsing the sections were stained with hematoxylin-eosin for identification of Meissner's corpuscles.

In the dermis and dermal papillae a large number of SP-immunoreactive nerve fibers were observed (fig., A). These fibers seemed to be of different type with regard to morphology and distribution (fig., A–D). Most of the SP positive fibers were free nerve endings in the papillae but a few could also be seen entering the epidermal layer (fig., A). In a few papillae SP-positive endings were seen in Meissner's corpuscles (fig., B), whereas other papillae contained beaded SP-positive endings in the cupular portion, without obvious association to Meissner's corpuscles (fig., C,D). In the deeper layers some SP immunoreactive nerve fibers were found in close contact to sweat gland ducts (fig., E) and in relation to blood vessels. No fluorescent nerve fibers were observed when the control serum was used. In the epidermis a population of cell bodies exhibited a green fluorescence (fig., A and D). This fluorescence was regarded as unspecific, since it occurred also on consecutive sections incubated with the control serum.

The present results give strong evidence for a SP-like peptide in free nerve endings and in Meissner's corpuscles of the human digital skin. No definite evidence for occurrence of SP-positive nerves in Merkel's discs was obtained. The distribution and morphology of the SP immunoreactive nerve endings resemble the distribution and morphology of small diameter nerve fibers described earlier by the use of light and electron microscopical techniques[17–19], and suggest that they are sensory in nature. The finding of SP-like immunoreactivity in free nerve endings in human digital skin is consistent with earlier observations in cat[7] and rat[9].

Free nerve endings have been suggested to be involved in nociception[20] in contrast to the Meissner's corpuscles, which have been associated with transmission of mechanical stimuli[20]. Thus, SP-like immunoreactivity is observed in nerve endings with apparently different physiological properties. Cauna[17] has, however, reported that a few Meissner's corpuscles contain a 2nd type of nerve endings, in addition to the large diameter fibers reacting to mechanical stimuli. These fibers were thin and resembled the free nerve endings but had a winding course, similar to that of the large diameter nerves, within the corpuscle. It was suggested that these fibers were associated with pain transmission rather than non-noxious stimuli[17]. Although the SP-immunoreactive nerve fibers in some Meissner's corpuscles most likely represent the type of nerves associated with pain mediation, it cannot be excluded that there exists a second population of SP containing sensory neurons reacting to mechanical stimuli (see also Henry[10]).

Free nerve endings within the dermal papillae were often observed in close connection with capillary loops[18]. Such arrangements may be the morphological background for the neurogenic inflammatory processes, where SP mediated vasodilatation and plasma extravasation occurs after antidromic stimulation of sensory nerves[12]. These mechanisms have been suggested to take part via axon collaterals of SP-containing primary sensory neurons[12,21].

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Studies on chemotherapy of parasitic helminths (XVII). Effects of pyrantel on the motility of various parasitic helminths and isolated host tissues

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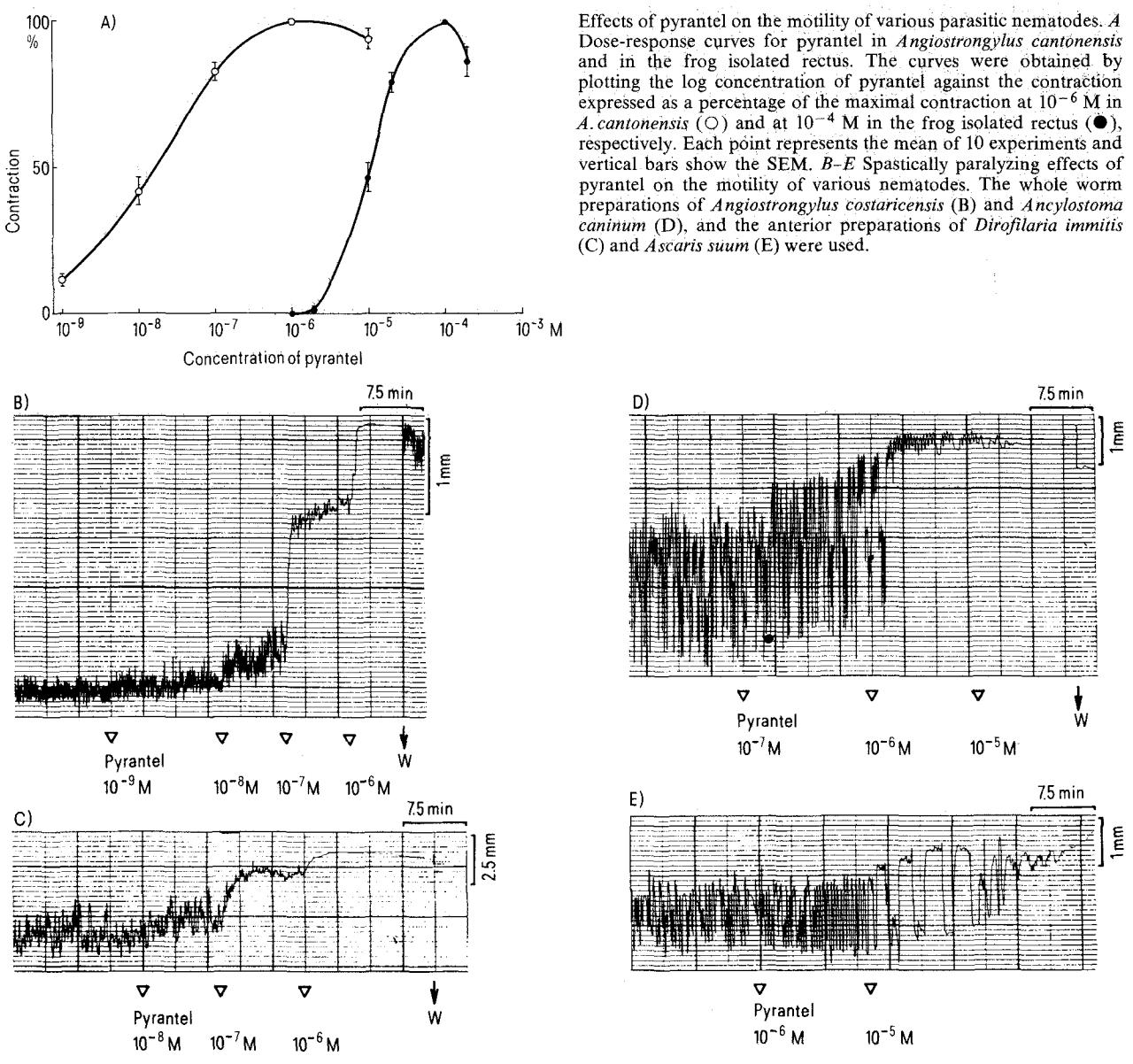
Summary. Pyrantel tartrate caused spastic paralysis in various parasitic nematodes, but not in cestodes and trematodes.

Since Austin et al.[1] discovered the anthelminitic effects of pyrantel tartrate (pyrantel) from laboratory assays using *Nematospiroides dubius* in mice and *Nippostrongylus muris* in rats, there have been many reports regarding the in vivo efficacy of this drug in animals[1,2] and man[3,4]. It was reported that pyrantel is effective against various intestinal



Effects of pyrantel on the motility of various parasitic nematodes. *A* Dose-response curves for pyrantel in *Angiostrongylus cantonensis* and in the frog isolated rectus. The curves were obtained by plotting the log concentration of pyrantel against the contraction expressed as a percentage of the maximal contraction at 10^{-6} M in *A. cantonensis* (○) and at 10^{-4} M in the frog isolated rectus (●), respectively. Each point represents the mean of 10 experiments and vertical bars show the SEM. *B–E* Spastically paralyzing effects of pyrantel on the motility of various nematodes. The whole worm preparations of *Angiostrongylus costaricensis* (B) and *Ancylostoma caninum* (D), and the anterior preparations of *Dirofilaria immitis* (C) and *Ascaris suum* (E) were used.

nematodes in animals such as sheep and dogs, including ascarids and ancylostomes[1,2]. It was also reported that the drug is effective in the treatment of various helminthiases in man such as oxyuriasis and ascariasis[3,4]. However, the drug has little effect against *Trichuris* spp. in sheep, dogs and man, and also against cestodes in dogs[1,2,4]. On the other hand, there have been few reports regarding the in vitro effects of pyrantel except a report describing the mode and mechanism of action of this drug in *Ascaris suum*[5]. Thus, we now report in vitro effects of pyrantel against various helminths including nematodes, cestodes and trematodes, and against isolated host tissues.

Materials and methods. Pyrantel tartrate was kindly offered from Pfeizer Taito Co. Ltd. Worms were obtained from animals sacrificed at the Hamamatsu Slaughterhause and the Shizuoka Prefectural Dog Center, or from animals experimentally infected in our laboratory. The isotonic transducer and visual observation methods previously described were used[6,7].

Results and discussion. It was shown that effects of pyrantel in our in vitro experiment agreed well with in vivo effects reported[1–4]. Pyrantel caused contraction and finally spastic paralysis in various nematodes except *Trichuris vulpis*. Among nematodes examined, *Angiostrongylus cantonensis* and *A. costaricensis* were most susceptible to the drug and contracted at the concentrations of 10^{-9} M and higher (fig., A). Other worms were less sensitive and contracted at 10^{-8} M and higher *(Dirofilaria immitis)*, at 10^{-7} M and higher *(Ancylostoma caninum)*, or at 10^{-6} M and higher *(Toxocara canis* and *A. suum)* (fig., B-E). In these experiments, after exposure to pyrantel, the preparations were washed repeatedly with Tyrode's solution for about 30 min. Though the preparations exposed to higher concentrations of pyrantel (10^{-6} to 10^{-5} M) were incapable of restoring resting tone (fig., B-E), those exposed to lower concentrations (10^{-8}–10^{-7} M) were rather easily reversed by washing, as shown previously[8].

The time course of the appearance of contraction was dependent on the concentration of pyrantel and/or on the worm preparations used; immediate contractions were elicited after adding the drug in lower concentrations such as 10^{-7} M (fig., B) and gradual contractions appeared after adding the drug in higher concentrations, such as 10^{-5} M (fig., E). This difference in onset and/or potency of drug

action is probably related to the varying abilities of the drug to penetrate the cuticle of the worms[3] and to reach the receptive sites or to differences in the susceptibility of receptive sites.

As reported previously regarding the sensitivity of various neuropharmacological agents[8], it may be likely that blood nematodes are more susceptible to various drugs than intestinal nematodes. The difference may arise from the fact that the habitat of blood nematodes is highly homeostatic compared to that of intestinal nematodes. Since an intact worm or an anterior piece of *A. suum* was little influenced by neuropharmacological agents[8-10], this worm was used in the form of muscle strips with a longitudinal cut along the lateral line or as eviscerated preparations. It was reported that in vitro, pyrantel immediately caused contracture in muscle strips of *A. suum* at lower doses (10^{-9}–8×10^{-9} g/ml), compared with rather slow spastic paralysis in the whole worm preparation of this worm at higher doses (2.5×10^{-5} g/ml)[5].

T. vulpis was not sensitive to pyrantel at concentrations of 10^{-5} M or less, and this result agrees well with those in chemotherapy of trichuriasis in animals and man[1,2,4]. Since *T. vulpis* was remarkably less sensitive to various drugs examined in our laboratory, it may be that the worm is less permeable and/or less susceptible to drugs. Pyrantel at the concentrations of 10^{-5} M or less had little effect against cestodes such as *Dipylidium caninum, Diplogonoporus grandis* and plerocercoids of *Diphyllobothrium erinacei,* and trematodes such as *Metagonimus yokogawai, Paragonimus westermani, P. miyazakii, Fasciola hepatica* and *Schistosoma japonicum.*

Aubry et al.[5] suggested that pyrantel acts by stimulating nicotinic receptors in *A. suum,* and recently we have reported that this anthelmintic may act similarly in another nematode, *A. cantonensis*[11]. The cholinergic mechanism in nematodes such as *A. suum*[12,13] and *A. cantonensis*[8] is suggested to be nicotinic from results on both cholinergic agonists and antagonists. In cestodes such as *D. caninum*[14] and trematodes such as *Schistosoma mansoni*[15] and *F. hepatica*[15], however, the cholinergic mechanism was suggested to be neither nicotinic nor muscarinic. Thus, differences between the nature of the cholinoceptors of nemathelminths and plathelminths may give an explanation for the lower susceptibility of plathelminths to drugs such as pyrantel which affects nematodes through its nicotinic actions.

Isolated host tissues such as the mouse ileum and the frog rectus were also sensitive to pyrantel at the concentrations of 10^{-6} M or more (fig., A). The effects of pyrantel against both skeletal and smooth muscles have been reported in experiments in vivo as well as in vitro[5,16]. It was reported that these effects on the host tissues are also caused by stimulating the nicotinic receptors in autonomic ganglia and skeletal neuromuscular junctions[5,16]. However, no particular clinical syndrome of toxicity has been reported for pyrantel[16,17]. This discrepancy may be due to the fact that pyrantel is always given orally and is poorly absorbed from intestine[16].

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Inhibition of host phenoloxidase activity by parasitoid hymenoptera

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Summary. Partial or complete inhibition of hemolymph phenoloxidase activity occurred in host species attacked by some parasitoid wasps. In one system, inhibition of enzyme activity could be achieved by injection of a virus purified from parasitoid ovaries.

The most common response of insect larvae to the presence of large foreign objects is the formation of a multicellular capsule of hemocytes[1]. Such encapsulation reactions are often (but not necessarily) associated with the deposition of melanin[1-4], and several workers have accordingly suggested that melanization per se might play an important role in insect immunity[1,5]. In support of this view, recent studies have shown that phenoloxidases responsible for the formation of melanin can be activated by cell wall components of parasitic fungi[6] and bacteria[7]. Furthermore, it has been suggested that phenoloxidases might act as opsonins[8], and indeed it seems clear that active enzyme is deposited on some foreign surfaces[6]. Thus it might seem reasonable to suppose that some parasitoids might have evolved mechanisms to inhibit the activation and/or activity of phenoloxidase (PO). Earlier studies have in this regard provided equivocal results[9]. We now report that at least in some systems, PO activity in the hemolymph of parasitized insect larvae is suppressed.

Materials and methods. The parasitoid used for most ex-