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Effects of PF1022A, a Newly Developed Gabergic Anthelmintic, on Adult Stage of *Angiostrongylus cantonensis* in Rats

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Abstract

To characterize basic properties of antinematode action of PF1022A, a new antinematode drug, effects of the drug against *Angiostrongylus cantonensis* in rats were examined. Rats infected with 20 larvae were treated with PF1022A orally at 10 and 20 mg/kg/day and intraperitoneally at 2.5 mg/kg/day during 11 to 15 days PI. Recovery of adult worms at dissection 49 days PI was almost the same among all treated and non-treated rats. Rats in all groups started larval output in host feces at almost the same day, showing no effects of PF1022A against growth from young adult to mature adult worms and consequently the reproductive activity of the worms. Complete paralysis and restoration by gabergic antagonists and Ca^{2+} were observed *in vitro* in adult worms recovered from rats 24 hr after intraperitoneal treatment with PF1022A at 2.5 mg/kg 9 weeks PI. In addition, the serum collected from the treated rats paralyzed non-treated adult worms. These results suggested that a certain level of PF1022A or some related paralyzing agents occurred in host blood when treated intraperitoneally. Thus, PF1022A seemed not to affect young adults of *A. cantonensis* in the brain due to poor distribution of the drug in the tissue, suggesting involvement of little passage of this compound through the blood-brain barrier. Consequently, the different efficacy of PF1022A between *A. cantonensis* and *A. costaricensis* is also explained from different localization of the worms and drug distribution.

Key words: PF1022A, *Angiostrongylus cantonensis,* young adult, mature adult, different distribution

Introduction

PF1022A is a newly developed antinematode anthelmintic in Japan (Takagi *et al.*, 1991). To know basic characteristics of the new anthelmintic, mechanism of action of PF1022A was studied *in vitro* in previous study using adult worms of *Angiostrongylus cantonensis* (Terada, 1992). It was suggested that the drug caused paralysis synergistically by stimulating the gabergic mechanism and by inhibiting the cholinergic mechanism. PF1022A was also likely to cause a strong inhibition on the utilization of Ca^{2+} and to inhibit the release of acetylcholine from the nerve endings.

For murine angiostrongyliasis costaricensis, a model for the diseases by tissue nematodes, on the other hand, PF1022A was effective on developing larvae at 5 successive doses via any of oral and intraperitoneal routes, although the effects were influenced by formulations of PF1022A (Terada *et al.*, 1993).

The present study aimed to characterize further basic properties of antinematode action of PF1022A, and we examined effects of this new compound on young adult and mature adult stages of *A. cantonensis* parasitizing in different tissues of the different host animal from *A. costaricensis.*

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Materials and Methods

(1) Materials

Female Wistar rats of 5-weeks-old were used

as final host. Each rat was inoculated orally with 20 infective larvae of *A. cantonensis* by the method described by Kino (1984) with a modified concentration of pepsin of 0.04%. PF1022A was donated from Meiji Seika Kaisha as 2.5% formulations of emulsified type designated as the oral cream and solubilized type as the oral solution. Both were diluted to 10 and 40 times with distilled water and sterilized saline for oral and intraperitoneal treatments, respectively, as described by Terada *et al.* (1993).

(2) Experimental design

1) Experiment 1

Effects of PF1022A given orally and intraperitoneally against young adult *A. cantonensis* in rats were examined here. All treated groups consisting of 5 rats were given 5 successive doses from 11 to 15 days PI.

Two groups were treated orally with 10 mg/kg or 20 mg/kg of PF1022A emulsified as the oral cream. One group was treated intraperitoneally with 2.5 mg/kg of PF1022A prepared as the oral solution. Another group received only the vehicle for the oral cream as non-treated control.

Onset of output of the first-stage larvae in rat feces was checked individually every day from 32 days PI. All surviving rats were dissected 49 days PI under over anesthesia with diethyl ether and adult worms were recovered from the lungs and heart. Significance of difference in the mean values was statistically analyzed by Student's *t*-test.

2) Experiment 2

In this experiment, we examined effects of PF1022A on the motility of mature adults of *A. cantonensis* to assess whether PF1022A or related factors occurred in blood of host rats after intraperitoneal treatment. Nine weeks PI with 20 infective larvae, two groups of 2 rats were treated intraperitoneally with 2.5 mg/kg of PF1022A prepared as the oral solution and the vehicle for the oral solution, respectively. Twenty-four hours after treatment, adult worms were recovered and the motility of the worms was examined *in vitro* by the visual observation and isotonic transducer methods (Terada *et al.*, 1987a). Additionally, effects of the serum collected from the treated and control rats on the motility of worms recovered from the non-treated rats were examined.

Results

1. Experimental 1

The results are summarized in Table 1. One rat in the non-treated group died in the 6th week of infection, but the data were excluded from the results because dissection to collect worms was not able to be performed. The mean recovery was 18.0 worms in the non-treated group. The recovery in any treated groups was not significantly different from the control. The first-stage larvae were first detected in rat feces between 37.0 and 39.2 days PI in all groups.

Table 1 Effects of PF1022A given orally and intraperitoneally on the number of worms recovered and onset of larval output in feces of rats infected with *A. cantonensis*

Experimental group	No. of rats examined	No. of worms recovered			Onset of larval output (days PI)
		Female	Male	Total	
Non-treated control	4	10.3 ± 2.5	7.8 ± 1.0	18.0 ± 1.6	37.5 ± 0.6
10.0 mg/kg, p.o.	5	9.0 ± 2.0	8.8 ± 2.0	17.8 ± 1.8	37.0 ± 0.0
20.0 mg/kg, p.o.	5	8.2 ± 2.4	9.6 ± 2.6	17.8 ± 0.8	37.2 ± 0.5
2.5 mg/kg, i.p.	5	9.8 ± 2.6	6.3 ± 1.7	16.6 ± 2.3	39.2 ± 1.5

All rats were inoculated 20 infective larvae of *A. cantonensis*, and treated 11 to 15 days PI.
Results are represented as mean ± SD.

2. Experiment 2

When observed visually, all female and male worms from the rats treated intraperitoneally kept being paralyzed for 1 day or more in Tyrode's solution at 37°C after removal, though they could respond against mechanical stimuli. Remarkable paralysis was also detected by means of the isotonic transducer method in the treated worms but not in the control worms (Fig. 1). The paralysis of the worms from the treated rats was restored by adding bicuculline and picrotoxin, gabergic antagonists with Ca^{2+} (Fig. 2),

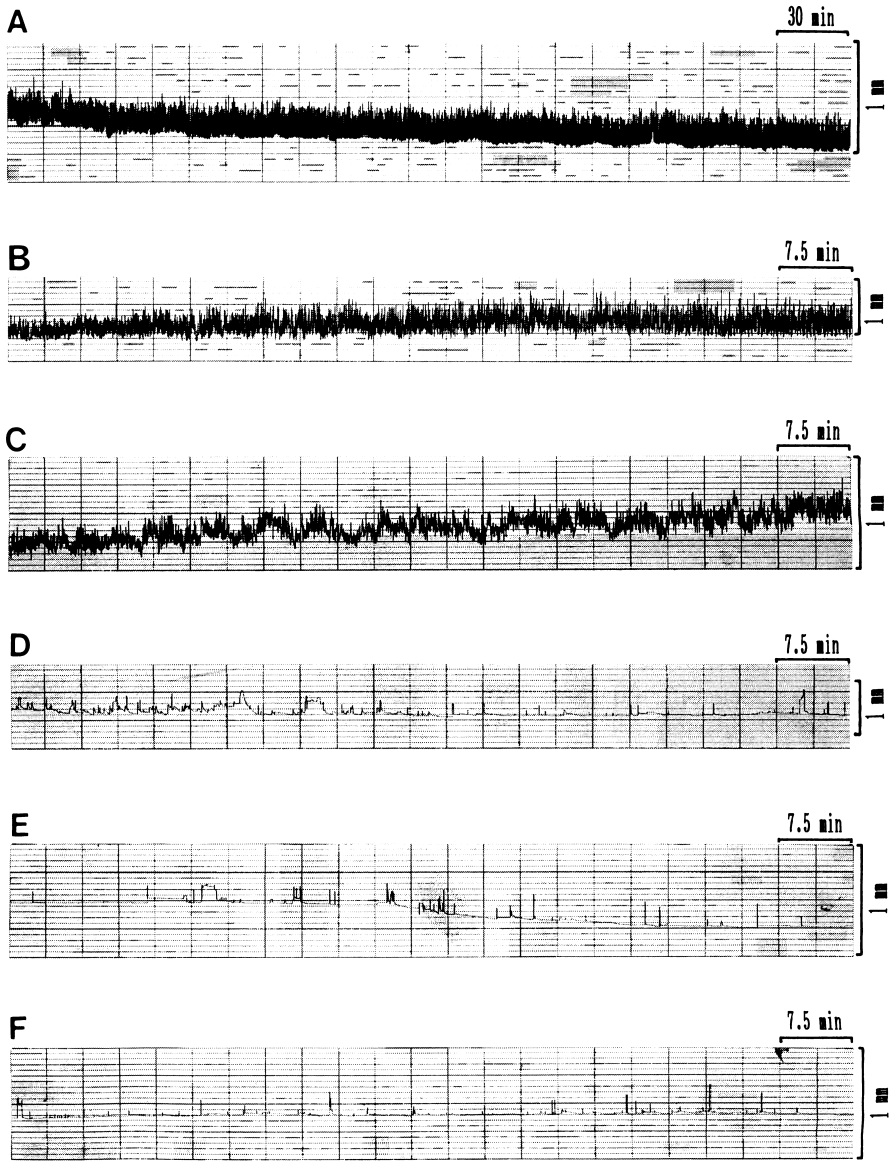

Fig. 1 Motility of the adult females recovered from non-treated control rats (A–C) and from intraperitoneally treated rats with PF1022A at a dose of 2.5 mg/kg (D–F). The worm was suspended with a tension of 0.8 g.

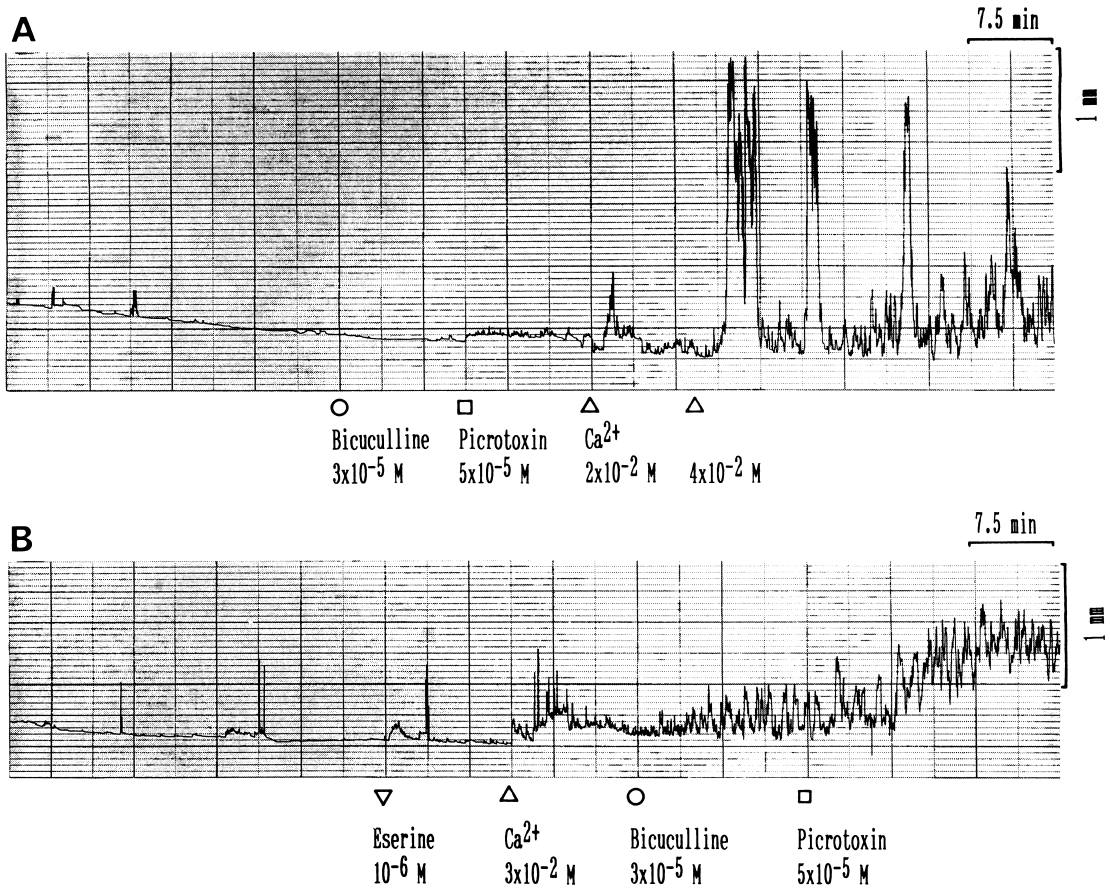

Fig. 2 Effects of some neuropharmacologic agents and Ca^{2+} on the paralyzed female *A. cantonensis* recovered from rats 24 hr after intraperitoneal treatment with PF1022A at a dose of 2.5 mg/kg. In Figs. 2 and 3, drugs in a single or cumulative dose were given successively at the points shown by the symbols. Each figure shows the representative of 5 similar tracings.

Effects of the serum of the treated and non-treated rats are shown in Fig. 3. When the serum of the non-treated rats was given against worms from the non-treated rats at the final concentration of 1:40, little effects were observed except small bubbles which appeared probably due to protein components contained in the serum and disappeared by adding 35 μl of ethanol (final concentration of 0.5%). On the other hand, paralysis was elicited when the serum of the treated rats was added at the same concentration, and it was antagonized by the gabergic antagonists with Ca^{2+} or eserine.

Discussion

It is known that most of larval *A. cantonensis* arrive in the central nervous system of the host rats within 3 days PI (Wallace and Rosen, 1969; Jindrak, 1970) and stay there till at least 26 days PI. During the period in the brain the last molt occurs 10 to 11 days PI and worms develop to young adult stage (Alicata and Jindrak, 1970). Therefore, when the host rat was treated from 11 to 15 days PI, the developmental stage of the parasite in the brain must have corresponded to the young adult.

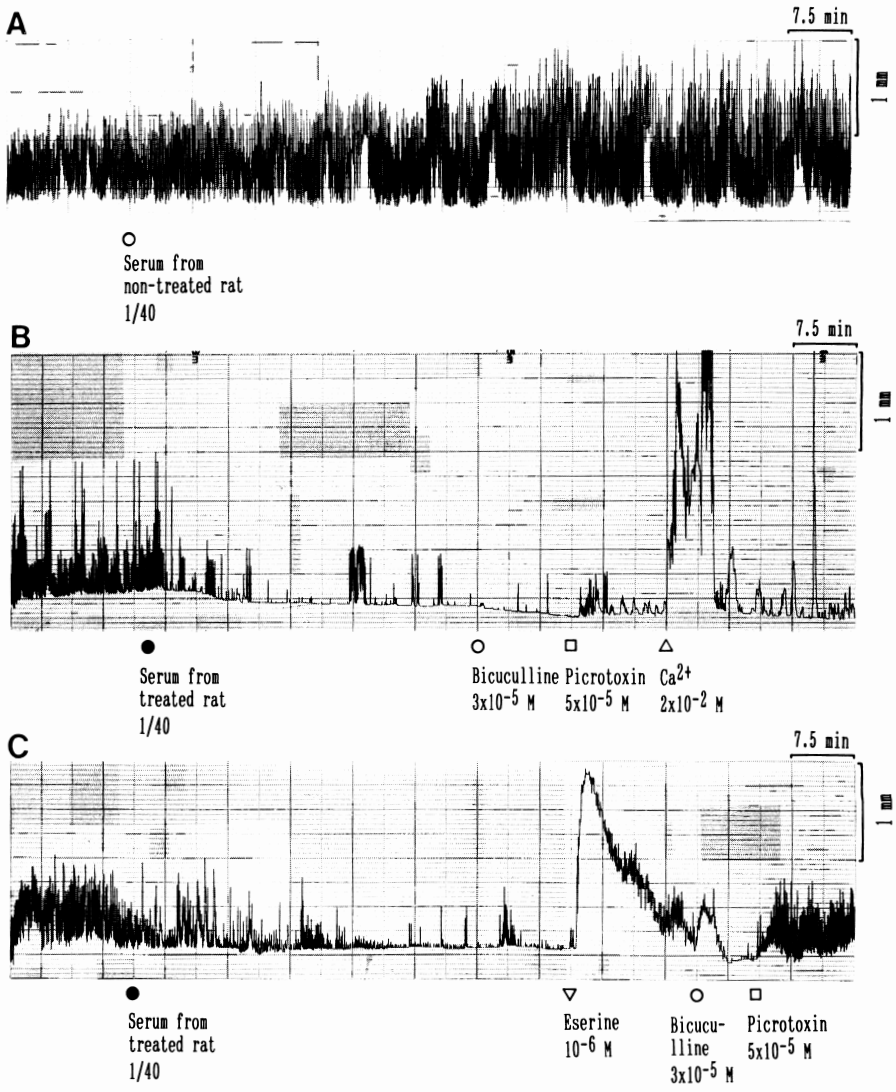

Fig. 3 Effects of the serum collected from non-treated (A) or treated (B, C) rats on the motility of adult *A. cantonensis* removed from non-treated rats. The serum was collected 24 hr after treatment as in Fig. 1.

In infections with *A. cantonensis* in rats, larval output in host feces began before 40 days PI with a tendency to become earlier in heavier infections (Kino, 1984). The present results of simultaneous onset of larval output implied no decrease in the number of mature worms and no delay in maturation. The worm recovery without any differences among the groups also indicated no worm death related to the treatment. Thus it was concluded that PF1022A given from 11 to 15 days PI had no effects against growth of the young adults to mature worms and consequently the reproductive activity of the grown-up *A. cantonensis* regardless of the route of treatment. On the other hand, PF1022A given orally as well as intraperitoneally killed developing larvae of *A.*

costaricensis parasitizing in the mesenteric arteries (Terada *et al.*, 1993). Despite the doses of PF1022A were lower than those in the present study, nearly complete larvicidal effects were observed at an intraperitoneal dose of 0.625 mg/kg and an oral dose of 10 mg/kg. The different results between these different but closely related species must be implying involvement of intervention of some factors related to the action of PF1022A.

Different absorption of PF1022A from the intestine of different host animals may be considered: i.e., the drug can be absorbed from mouse intestine, but not from rat intestine. In the present experiment, however, little effects were also caused by intraperitoneal treatment, suggesting no relation of the absorption to the different effects of PF1022A. The adult worms of *A. cantonensis* treated intraperitoneally were completely paralyzed and restored by adding gabergic antagonists with Ca^{2+} similarly to the results on the paralyzing action of PF1022A in non-treated worms (Terada, 1992). In addition, non-treated worms were paralyzed by the serum collected from the treated rats. These results suggest that even during the period when young adult worms stayed in the brain PF1022A or some related paralyzing factors occurred at a certain level in host blood. Why did not the effective component affect the worms in the brain?

Some differences in susceptibility to drugs may be also considered between young and grown-up adults of *A. cantonensis*. It has been reported, however, that many anthelmintics including diethylcarbamazine (Hawking, 1979), mebendazole (Hayashi *et al.*, 1982) and milbemycin D (Terada *et al.*, 1987a, b) were shown to have killing efficacy against any stages of nematodes and larval or young adult worms were more susceptible than mature worms. This is probably true also for PF1022A and it is most probable that this new anthelmintic did not affect young adults in the brain not due to less susceptibility of them but to poor distribution of the drug in the tissue. Since *A. costaricensis* does not migrate to the host brain in its life cycle, the different efficacy between *A. cantonensis* and *A. costaricensis* was probably attributable to poor distribution of the drug in the rat brain, by blockage at the blood-brain barrier.

There have been some reports, on the contrary, describing killing action against larval or young adult worms of *A. cantonensis* in rats by the gabergic anthelmintics such as avermectin B_{1a} (Ishii *et al.*, 1983), ivermectin (Ishii *et al.*, 1985) and milbemycin D (Terada *et al.*, 1987a). In addition, avermectin B_{1a} previously administered to mice enhanced some of the pharmacologic actions of diazepam affecting in the brain (Williams and Yarbrough, 1979). Campbell (1987) detected a higher level of ivermectin than usual concentrations in the brain of collie dogs that showed most severe toxic signs after treatment. All of these reports suggest penetration of these gabergic anthelmintics into the central nervous system through the blood-brain barrier. The difference in the penetration ability through the barrier may be related to chemical structure of the compounds. Although factors determining the extent of penetration of drugs through the blood-brain barrier have not been revealed, involvement of some factors such as lipid solubility was suggested (Goldstein and Betz, 1986). Since PF1022A has a unique chemical structure different from all other gabergic anthelmintics (Takagi *et al.*, 1991), some factors derived from the structure may be related to the poor penetration through the barrier.

Superiority of PF1022A as anthelmintic has been suggested by a synergistic mechanism of action and highly selective toxicity (Terada, 1992). If PF1022A does not truly pass the blood-brain barrier, it will not cause any severe adverse effects related to the passage into the brain and consequently become an excellent anthelmintic available to various types of nematode infections except the infection in the brain.

References

1) Alicata, J. E. and Jindrak, K. (1970): Angiostrongylosis in the Pacific and Southeast Asia. Charles C Thomas Publisher, Springfield, Illinois.
2) Campbell, W. C. (1987): Ivermectin and heartworm. Seminars in Veterinary Medicine and Surgery (Small Animals), 2, 48–55.

3) Goldstein, G. W. and Betz, A. L. (1986): The blood-brain-barrier. Scientific Amer., 255 (9), 70–79.
4) Hawking, F. (1979): Diethylcarbamazine and new compounds for the treatment of filariasis. Ad. Pharmacol., 16, 129–194.
5) Hayashi, M., Terada, M., Ishii, A. I., Kino, H. and Sano, M. (1982): Studies on chemotherapy of parasitic helminths (XIV). Anthelmintic effect of mebendazole on *Angiostrongylus cantonensis* in rats. Jpn. J. Parasitol., 31, 575–580.
6) Ishii, A. I., Terada, M., Kino, H. and Sano, M. (1983): Studies on chemotherapy of parasitic helminths: Effects of avermectin B_{1a} on *Angiostrongylus cantonensis* in rats. Intern. J. Parasitol., 13, 491–498.
7) Ishii, A. I., Terada, M. and Sano, M. (1985): Studies on chemotherapy of parasitic helminths (XXIII). Effects of ivermectin on *Angiostrongylus cantonensis* in rats. Jpn. J. Parasitol., 34, 411–417.
8) Jindrak, K. (1970): The pathology of intracranial angiostrongylosis in rats. J. Comp. Path., 80, 287–297.
9) Kino, H. (1984): Parasite density and the fecundity of *Angiostrongylus cantonensis*. Parasitology, 89, 275–285.
10) Takagi, M., Sasaki, T., Yaguchi, T., Kodama, Y., Okada, T., Miyadoh, S. and Koyama, M. (1991): On a new cyclic depsipeptide, PF1022A with anthelmintic effects. Nippon Nogeikagaku Kaishi, 65, 326. (in Japanese)
11) Terada, M., Ishii, A. I., Dharejo, A. M., Hayashi, M. and Sano, M. (1987a): Studies on chemotherapy of parasitic helminths (XXVIII). *In vivo* efficacy of milbemycin D against larval stages of *Angiostrongylus cantonensis* and *A. costaricensis*. Jpn. J. Parasitol., 36, 24–29.
12) Terada, M., Dharejo, A. M., Ishii, A. I., Nacapunchai, D. and Sano, M. (1987b): Effects of milbemycin D on adult *Angiostrongylus cantonensis* in rats. Parasitol. Res., 73, 375–380.
13) Terada, M. (1992): Neuropharmacological mechanism of action of PF1022A, an antinematode anthelmintic with a new structure of cyclic depsipeptide, on *Angiostrongylus cantonensis* and isolated frog rectus. Jpn. J. Parasitol., 41, 108–117.
14) Terada, M., Ishih, A., Tungtrongchitr, A., Sano, M. and Shomura, T. (1993): Effects of PF1022A on developing larvae of *Angiostrongylus costaricensis* in mice. Jpn. J. Parasitol., 42, (3) in press.
15) Wallace, G. and Rosen, L. (1969): Studies on eosinophilic meningitis. VI. Experimental infection of rats and other homoiothermic vertebrates with *Angiostrongylus cantonensis*. Am. J. Epidem., 89, 331–344.
16) Williams, M. and Yarbrough, G. G. (1979): Enhancement of *in vitro* binding and some of the pharmacological properties of diazepam by a novel anthelmintic agent, avermectin B_{1a}. Eurpen. J. Pharmacol., 56, 273–276.