Microfilaricidal Effect of Fenthion Applied on the Skin of the Dog infected with Dirofilaria immitis

Mem. Fac. Agr. Kagoshima Univ., **16**, 75–82, 1980

Microfilaricidal Effect of Fenthion Applied on the Skin of the Dog infected with *Dirofilaria immitis*[*1]

Tsukasa SAKAMOTO, Tsuyoshi TOGOE[*2], Yasuhiro YAMAMOTO and Yoshio KITANO[*3]

(*Laboratory of Veterinary Pathology*)

Received for Publication September 10, 1979

Introduction

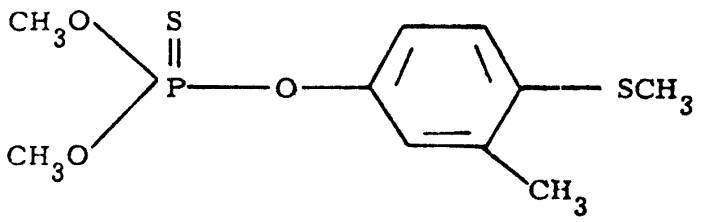
Certain organophosphates were introduced as useful insecticide about three decades ago. Recently, the organophosphates showing a inhibitory action against cholinesterase have been widely used as anthelmintic agents against parasitic nematodes in the intestine of domestic, zoo and laboratory animals too. Several of these cholinesterase inhibitors, such as fenthion[4,7,11], Dursban, metrofonate, coumafos, crufomate, naphthalophos and J8R–381[3] have been, individually, proved to be effective against microfilariae of filarioids[5,6].

The effect of fenthion, originally used in cattle as insecticide, against microfilariae of *Dirofilaria immitis* in a dog was demonstrated by McCarthy[7] and Wallace[11]. Kobayashi et al.[4] injected, once to thrice, a daily dose of 15 mg/kg of body weight of fenthion to the dogs from which adult worms of *D. immitis* burden were eradicated by the intravenous injection of arsenic compound. They proved the microfilaricidal capacity of the drug.

The authors were much interested in the high permeability of fenthion through the skin, and tested the microfilaricidal effect of the drug dripped on the skin of the dog infected with *D. immitis*. In this paper, the results obtained in the investigation are described.

Materials and Methods

The chemical name of fenthion used, is o, o-dimethyl-o-(3-methyl-4-methylthiophenyl)phosphorothioate, and the drug has the following chemical structure. 20% solution of fenthion in dipropylene glycol methyl ether was dripped on to the skin in a dorsal side of the neck of a dog at three doses of 15, 30 and 50 mg/kg body-weight (b.w.). The dogs in which microfilariae were



Structural formula of fenthion

[*1] This work was presented at the 48th Annual Meeting of the Japanese Society of Parasitology.

[*2] Present address: Department of Public Health of Fukuoka City

[*3] Present address: Nansatsu Animal Health Center, Chiran, Kagoshima

detected by blood examination, were selected for the present experiment among many in a dog-pound. Those dogs were mongrel, 2 to 8 years old and 10 to 15 kg in body weight. 5 ml of blood was collected daily at about 15 o'clock. The microfilarial population per ml of blood were counted by Ohishi and other's microfilaria-concentration method[9], using 5% acetone solution containing 0.2 g of sodium citrate and 0.025 g of methylene blue. To estimate, intermediately, the amount of fenthion absorbed through the skin, the activity of serum-cholinesterase was determined by the modified TNB method (Morizono and Kaba[8]), because the drug is known as cholinesterase inhibitor. The level of cholinesterase activity was expressed in the international unit (IU): micromole per ml per minute (μM/ml/min).

Critical anthelmintic trial consisted of one preliminary experiment and three ones.

In the preliminary experiment, 30 mg/kg b.w. of fenthion was applied on to the skins of 2 noninfected dogs. The level of serum-cholinesterase was estimated, daily, for 30 days, after the application.

In the 1st experiment, fenthion was applied, first, at a dose rate of 15 mg/kg b.w. to the skins of the 2 infected dogs, which were male, 2 years old and followed at a dose rate of 30 mg/kg b.w. 8 days after the first application. The microfilaria-population of blood and the level of serum-cholinesterase were observed for 18 days from the first application.

In the 2nd experiment, 7 dogs consisting of 5 males and 2 females, aged 2 to 8 years, weighing 10 to 25 kg and having 320 to 61,620 (average 24,694$\pm$10,050) microfilariae per ml of blood, were used. A dose of 30 mg/kg b.w. of fenthion was applied to the skins of the 7 dogs, and the same dose was given again to 3 of those dogs, two weeks after the first application. The dogs were observed for 20 days in the same way as above.

In the 3rd experiment, a single dose of 50 mg/kg b.w. was applied to 4 infected dogs which were male, 2 to 5 years old and 12 to 21 kg in body weight. In addition to microfilariae and cholinesterase, the levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GOP), alkaline phosphatase (Al-P) and blood urea nitrogen (BUN) in blood and those of protein, glucose, ketone bodies, urobilinogen and hemoglobin in urine were estimated. All of the dogs used were dissected for gross and histological examinations after the experiments.

Results

In the preliminary experiment, 30 mg/kg b.w. of fenthion was applied on to the skins of 2 noninfected dogs. As shown in figure 1, the level of serum-cholinesterase (S-ChE) decreased quickly after the application. The levels of S-ChE of those dogs 6 days after the application decreased to 14.8 and 27.1% of the levels before treatment, at the lowest, respectively. After then, the levels increased gradually, and reached 64.4 and 63.5% of the levels before the treatment, 30 days after the application, respectively.

In the 1st experiment, 15 ml/kg b.w. of fenthion was applied to 2 male dogs having 630 and 7,940 microfilariae per ml of blood, respectively. The microfilaria-population of each of the dogs, decreased to 78.1 and 69.5% of that before the treatment, 8 days after the application, as shown in figure 2. The respective level of S-ChE of the dogs showing 4.29 and 2.81 IU (μM/ml/min) before the treatment, decreased to 50.2 and 59.5% of that before the treatment, 8 days after the application. The dogs were reapplied a dose of 30 mg/kg b.w. of the drug to their skins on the same day. The microfilaria-population of the dogs showed a remarkable decrease, 21.0 and 8.6% of that before the treatment, 5 days after the reapplication. The activity of S-ChE showed the lowest level (1.95

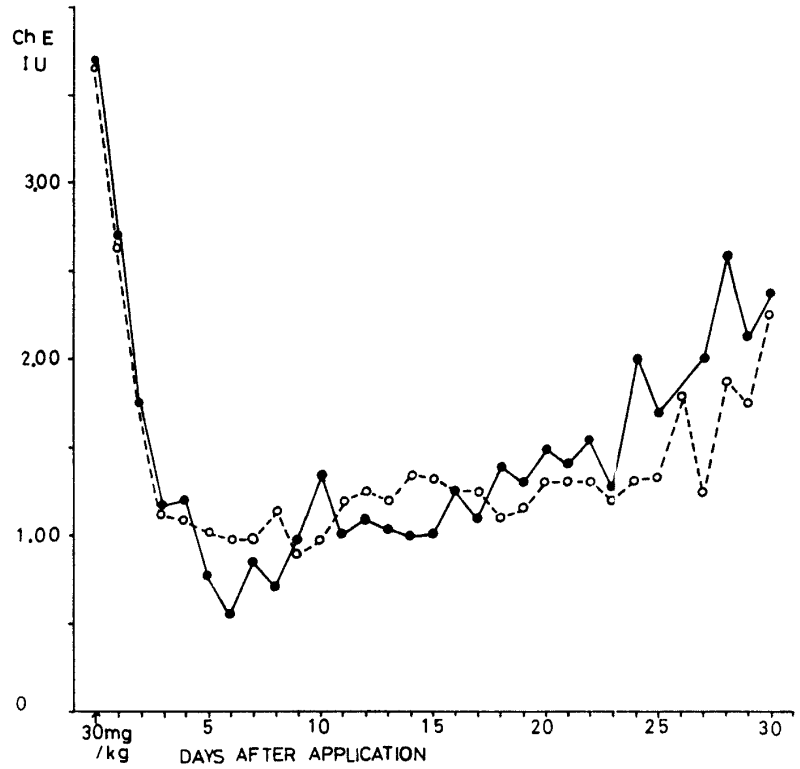


Fig. 1. Effect of cutaneous application of fenthion (30 mg/kg) on serum cholinesterase level of noninfected dogs.

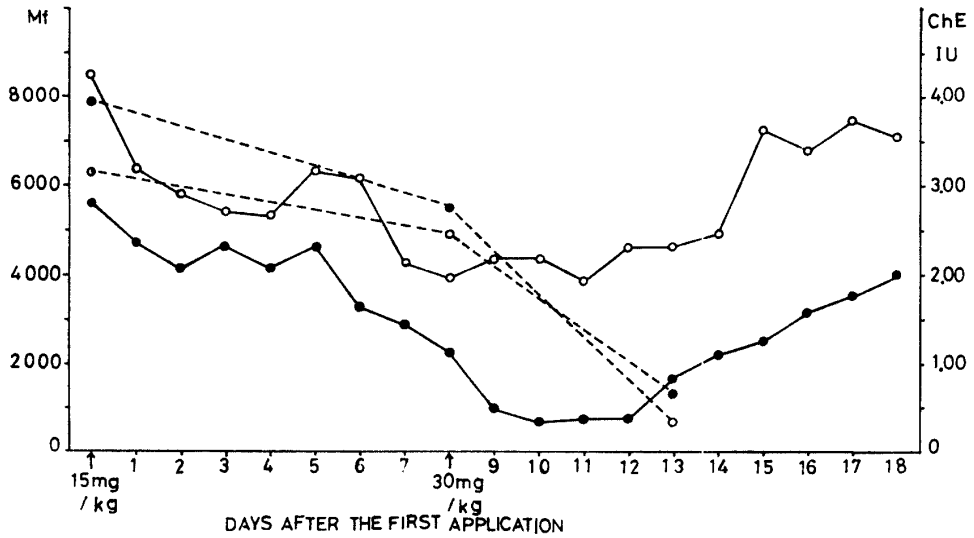


Fig. 2. Effect of two applications of fenthion (15 and 30 mg/kg) on the number of microfilariae (dotted line) and cholinesterase level (solid line).

and 0.38 IU) 3 days after the reapplication. Those levels are equivalent to 45.4 and 12.1% of that before the treatment.

In the 2nd experiment, a single dose of 30 mg/kg b.w. of the drug was applied to the skins of 7 dogs in which 320~61,620 (average: 24,694±10,050) microfilariae per ml were detected in their blood. As shown in figure 3, the microfilaria-population 3, 6 and 9 days after the application were reduced by 0~92 (av.: 42.2) %, 0~88.5 (av.: 31.9) % and 0~51.3 (av.: 23.7) % of that

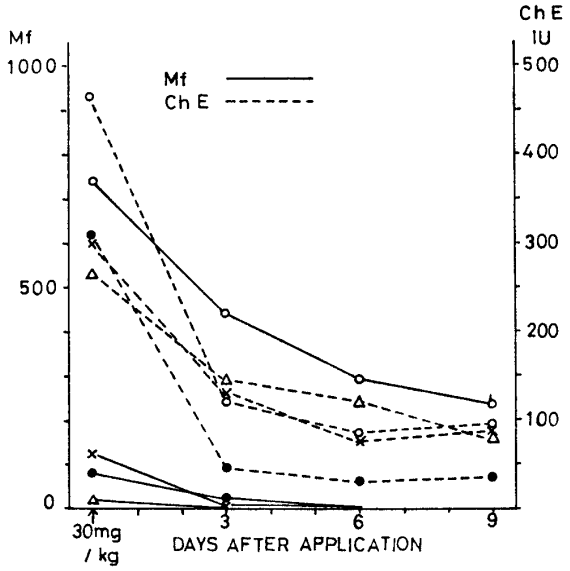


Fig. 3. Effect of cutaneous application of fenthion (30 mg/kg) on the number of microfilariae and cholinesterase level in serum.

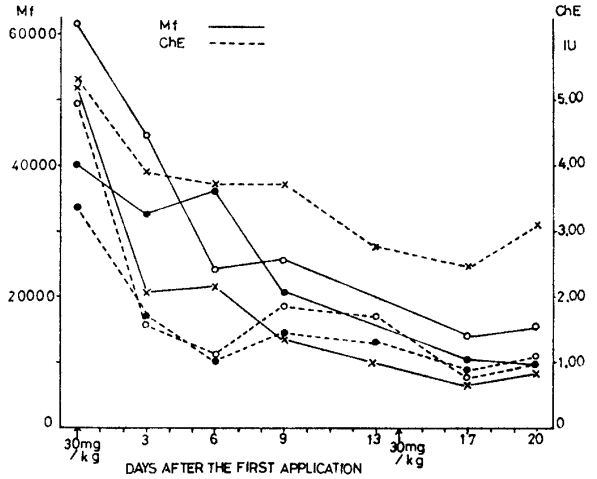


Fig. 4. Effect of two applications of fenthion (30 mg/kg) on the number of microfilariae and cholinesterase level in serum.

before the treatment, respectively. The levels of S–ChE 3, 6 and 9 days after the treatment decreased from 2.63～5.30 (av.: 3.86±0.40) IU to 0.45～3.83 (av.: 1.62) IU, 0.31～3.71 (av.: 1.32) IU and 0.37～3.72 (av.: 1.54) IU, respectively. The average of those levels 3, 6 and 9 days after the treatment corresponded to 41.9, 34.2 and 39.9% of that before the treatment, respectively. Three of those dogs were reapplied the same dose 2 weeks after the first application. As shown in figure 4, the microfilaria-population in the blood of the dogs decreased to 12.3～26.5 (av.: 20.57) % and 17.3～25.4 (av.: 22.3) % 3 and 6 days after the reapplication, respectively. The level of S–ChE decreased to 45.14～88.19 (av.: 66.28) % 3 days after the reapplication.

In the 3rd experiment, a single dose of 50 mg/kg b.w. of fenthion was applied to the skins of 4 dogs in which 520～13,440 (av.: 5,944) microfilariae per ml of their blood were detected. The number of microfilariae showed a general tendency to decrease with the lapse of time. As shown in figure 5, however, a considerable irregularity in the decrease of microfilaria-population was seen among the individuals of those dogs. The levels of S–ChE decreased conspicuously as shown in figure 6. The dogs diminished slightly their appetite. The average level of GOT decreased 35.5% of that before the treatment 8 days after the application, as shown in figure 7. As shown in figure 8, on the contrary, the level of GPT increased conspicuously in general, and reached 2.5 times the level before the treatment, 6 days after the application. The level of activity of Al–P decreased obviously after the application as shown in figure 9, and reached the lowest one, 1.9～6.8 (av.: 4.13) IU, 4 days after the application. The level of BUN varied unstably through the application. The temporal appearance of protein in urine was detected in 3 of the dogs, 6 or 8 days after the application.

All of the dogs used were held in an autopsy after the experiments. Adults of *D. immitis* living in heart and pulmonary aorta were still survived. No lesions caused by the drug, were seen in histological examination.

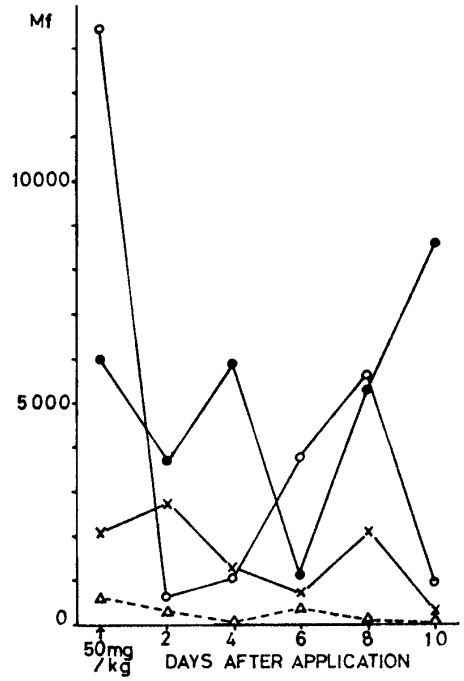


Fig. 5. Effect of cutaneous application of fenthion at a high dose rate on the number of microfilariae.

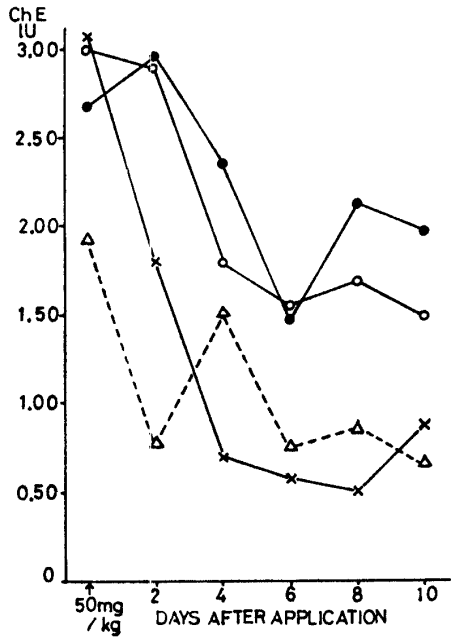


Fig. 6. Effect of cutaneous application of fenthion at a high dose rate on serum cholinesterase level.

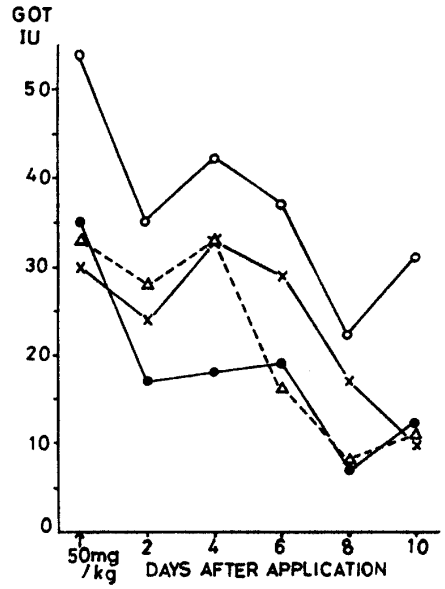


Fig. 7. Effect of cutaneous application of fenthion at a high dose rate on GOT level.

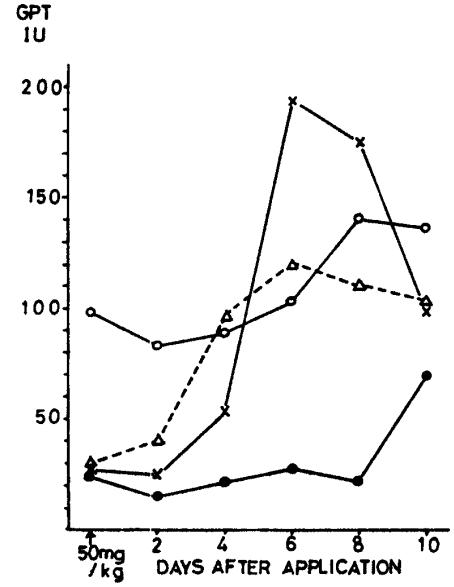


Fig. 8. Effect of cutaneous application of fenthion at a high dose rate on GPT level.

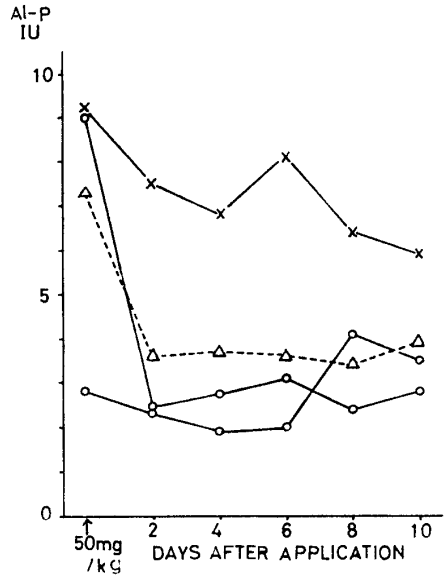


Fig. 9. Effect of cutaneous application of fenthion at a high dose rate on alkaline phosphatase level.

Discussion

Fenthion, a water insoluble organic thiophosphate, was originally used in cattle, as insecticide against warble fly larvae. McCarthy[7] and Wallace[11] reported that the administration of the drug in a single intramuscular dose of 15 mg/kg b.w. showed high activity against microfilariae of *Dirofilaria immitis*. Kobayashi et al.[4] also stated that the subcutaneous injection of fenthion in 1 to 3 doses of 15 mg/kg b.w. was highly effective against microfilariae in dogs treated previously with arsenic compound for killing the adult worm of *D. immitis*.

In the present experiment, the authors observed that the decrease of microfilaria-population was not so remarkably shown in the dogs in which a dose of 15 mg/kg b.w. of the drug was applied on to their skin. The cutaneous application of the drug at a dose rate of 30 mg/kg b.w. showed a remarkable decrease of microfilaria-population. The decrease of microfilaria-population in the dogs given a dose of 50 mg/kg b.w. of the drug, was quite little in proportion to the case in which high dose was given. In addition to the decrease of S-ChE, the signs of side effects such as decrease of appetite, proteinuria, the decrease of GOT- and Al-P-levels, and the increase of GPT-level were seen in some part of those dogs. From the facts mentioned above, it was considered that the cutaneous application of fenthion at a dose rate of 30 mg/kg b.w. is suitable for eliminating microfilariae from the infected dogs.

On the other hand, Garlick et al.[1,2] reported that new principals of heartworm disease therapy in which the microfilaricide, dithiazanine iodide was used first, was followed by the adulticide, thiacetarsamide sodium. They explained that the toxic effects of the adulticide could be almost totally prevented by firstly eliminating the microfilariae, using the microfilaricide, because prior elimination of the accumulated microfilaria-population improved pulmonary arterial circulation, and brought forth the effective resorption of dead adult *D. immitis*, following the administration of arsenic. Also, they stated that only fenthion of the cholinesterase inhibiting organophosphate killed both microfilariae and the migrating later stage larvae. In the present experiment, the cutaneous application of fenthion revealed conspicuously microfilaricidal effect. Sakamoto et al.[10]

experienced that dithiazanine iodide, a kind of cyanine dye, was shown to be toxic to both parasites and their host. Accordingly, the present authors considered it to be significant that the application of fenthion to the skin should be examined as the prior elemination together with adulticide.

Summary

20% solution of fenthion in dipropylene glycol methyl ether was applied to the skins in the dorsal sides of necks of the noninfected and infected dogs. The effects of the drug against microfilaria of *Dirofilaria immitis* and serum-cholinesterase were tested. The results obtained are summarized as follows.

1. In the noninfected dogs applied fenthion at a dose rate of 30 mg/kg body weight (b.w.), the level of serum-cholinesterase decreased quickly, reaching the lowest level 6 days after the application, and then increased gradually again.
2. In the 2 infected dogs given 15 mg/kg b.w. of fenthion, their microfilaria-population decreased to 78.1% and 69.5% of that before the treatment 8 days after the treatment. 30 mg/kg b.w. of the drug was reapplied to the dogs. And their microfilaria-population decreased to 21.0% and 8.6% of that before the treatment, 5 days after the reapplication.
3. A dose of 30 mg/kg b.w. of fenthion was applied to 7 dogs, and the same dose was reapplied to 3 of the dogs 2 week after the first application. Their microfilaria-population decreased remarkably after each application.
4. In 4 dogs given a dose of 50 mg/kg b.w. of the drug, the decrease of microfilaria-population was little in proportion to the high amount of dose. In addition to the decrease of serum-cholinesterase, the signs of side effects, such as decrease of appetite, proteinuria, decrease of glutamic oxaloacetic transaminase and alkaline phosphatase, and increase of glutamic pyruvic transaminase were in some part of those dogs.

From the above results, it was considered that the effective dose of fenthion should be 30 mg/kg b.w. for the dermal application.

Acknowledgement

The authors wish to express their cordial thanks to Prof. I. Kono and Mr. N. Yasuda of this laboratory and Miss T. Iwatsuki of Tohbu Meat-inspection Center, Sueyoshi, Kagoshima for their kind advices and helps in this study.

References

1) Garlick, N. L.: The management of canine dirofilariasis. *Canine Practice*, **2**, 22–27 (1975)
2) Garlick, N. L.: Canine dirofilariasis: 547 clinical cases. Treated first with dithiazanine iodide then with thiacetarsamide sodium. *Ibid.*, **3**, 44–47 (1976)
3) Kobayashi, S., Ohishi, I. and Kume, S.: Evaluation of a low toxic preparation of organic phosphorous (JSR–381) for removal of canine filariae. *Jap. J. vet. Ass.*, **18**, 64–67 (1965)
4) Kobayashi, S., Kakinuma, K., Ohtake, K., Hayashi, M., Ohishi, I. and Kume, S.: Effects of fenthion against microfilaria of *Dirofilaria immitis*. *Jap. J. vet. Sci.*, **34** (Suppl.) 177 (1972) (in Japanese)
5) Lämmeler, G., Herzog, H. and Grüner, D.: Experimental chemotherapy of filariasis. in Marois, M. (ed.), Development of chemotherapeutic agents for parasitic diseases. p. 157–175, North Holland Publishing Co., Amsterdam-W. (1975)

6) Lämmeler, G. and Grüner, D.: Chemotherapeutic studies on *Litomosoides carinii* infection of *Mastomys natalensis* 6. The filaricidal activity of organophosphorous compounds. *Tropenmed. Parasit.*, **26**, 359–369 (1975)
7) McCarthy, J.: In Bradeley, R. E. (ed.), Canine heartworm disease, a discussion of the current knowledge. University of Florida, Gainsville (1970)
8) Morizono, M. and Kaba, H.: Clinical studies on serum-cholinesterase in domestic animals I. Measuring method of serum-cholinesterase activity in domestic animals. *Bull. Fac. Agr. Kagoshima Univ.*, No. 27, 115–121 (1977) (in Japanese with English summary)
9) Ohishi, I., Kobayashi, S. and Kume, S.: Studies on diagnosis of canine filariasis III. Concentration of microfilariae in the test blood. *J. Jap. vet. med. Ass.*, **12**, 149–153 (1959) (in Japanese with English summary)
10) Sakamoto, T., Takahashi, H. and Nakamata, M.: Studies on strongyloidiasis of the peacock I. The anthelmintic action of phenothiazine, kamala and dithiazanine iodide against parasites in the peacock. *Jap. J. vet. Res.*, **12**, 39–46 (1964)
11) Wallace, C. R.: In Bradeley, R. E. (ed.), Canine heartworm disease, a discussion of the current knowledge. University of Florida, Gainsville (1970)