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### Effects of Insecticides on Spiracle Movements in Insect

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### **ABSTRACT**

We studied the effects of permethrin and DDVP on thoracic spiracle movements of the male cockroach. Permethrin increased, rather than decreased, the average open time of thoracic valve 1 in low or lethal doses, with no large change in the frequency of the opening and closing. In thoracic spiracle 2, the average open time was reduced by 1/3 of the control, but the opening frequency was markedly increased. DDVP had a similar effect on thoracic spiracle 1. The action of DDVP was only transiently and partially antagonized by atropine.

These results suggest that the thoracic spiracle 1 valve tends to open, probably by a peripheral control mechanism, rather than to close, during permethrin or DDVP poisoning in insects.

#### INTRODUCTION

The potent insecticides permethrin, a pyrethroid, and DDVP, an organophosphate, both produce severe convulsions and paralysis eventually causing death in insects. In mammals, death may occure by a simple disfunction of respiratory movement. In adult insects, spiracles accurately regulate the respiration and water retention (MILLER, 1966), but no information on the effects of insecticides on spiracle movement appears to be available.

In these experiments, we studied the effects of permethrin and DDVP on thoracic spiracle movements of the cockroach.

### **MATERIALS and METHODS**

Male adult American cockroaches *Periplaneta americana* L. were used. The insects were anaesthetized in a test tube by cooling with ice, and then stuck on their backs on slide glasses with "Aron alpha" (Toagoseikagaku, Tokyo, Japan). The slide glass was set on its side as shown in Fig.1, and an other slide glass placed in front of the insect so it could put its feet on the glass which reduced struggling.

## Recording of spiracle mevements.

Under a dissection microscope, spiracle movements could be observed in cockroaches restrained in this way (Fig.2). When the spiracle valve was closed, the observer pushed a button connected to a magnetic lever on a pen recorder, and released it when the valve was open. From these records, an average open time (%) and the frequency in closing and opening of the spiracle valve were determined for every five-minute period.

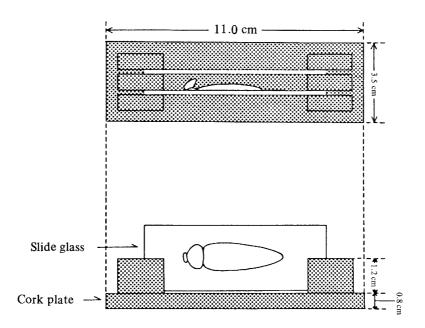


Fig. 1 Setup for immobilization of cockroach.

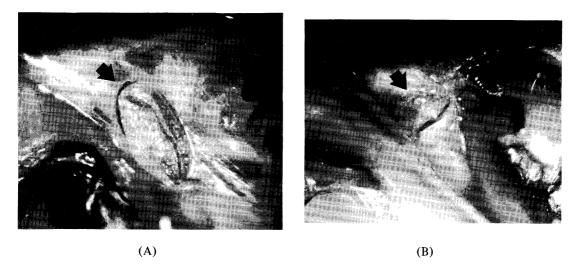


Fig. 2 Thoracic spiracle 1 (A) and 2 (B) under dissection microscope.

### Insecticides and drugs.

Permethrin (31.13 mg) (Sumitomo Chemicals, Osaka) was dissolved in 1 ml of ethanol, for use as stock solution. This was diluted with an insect saline solution (1.3 % sodium chloride solution) before use. Injection was into the abdomen of the insect.

DDVP (Nihon Noyaku K. K., Tokyo) was dissolved in ethanol at 50 % as the other stock solution. One microliter of the solution was administered orally.

Atropine sulfate (Wako Pure Chemicals, Osaka) was dissolved in an insect saline solution at  $10^{-2}$  g/ml. Five microliter of the solution was injected into the abdomen of the insect.

#### RESULTS and DISCUSSION

Fig.3 (a) and (b) show the average open time and frequency of thoracic spiracle movements of the male cockroach with no chemicals. The open-time value was different between thoracic spiracles 1 and 2, probably due to different neuronal control. Since ethanol was used as the vehicle for the insecticides, its effect on spiracle movement was examined. Administration by injection of ethanol (5  $\mu$ l) slightly stimulated spontaneous movement, but it produced no marked changes in spiracle movement (Fig.3 (c) and (d)). The effects of permethrin on spiracle movement were examined at low and lethal doses (Fig.4). In thoracic spiracle 1, the open time was increased, rather than decreased, with

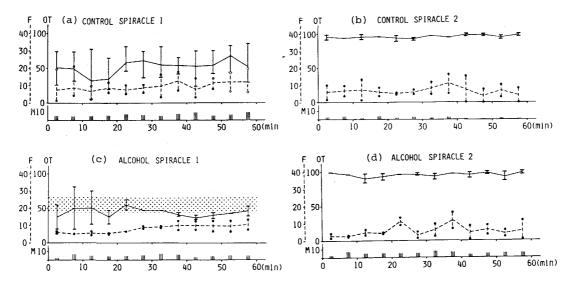


Fig. 3 The average open time (%) and frequency in closing and opening of thoracic spiracles 1 and 2, and the effect of ethanol. At 0 min, ethanol (5  $\mu$ l) was injected. OT, average open time ( - ): F, frequency of spiracle movements (---): M, frequency of struggle movements. Vertical bar, range of minimum and maximum (n = 3). Shaded area, range of control experiments.

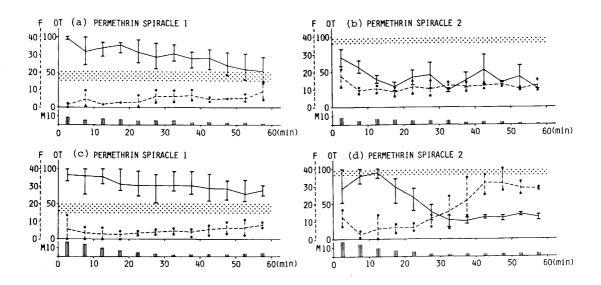


Fig. 4 Effect of permethrin on spiracle movements. At 0 min, permethrin was injected with a low  $(1.17 \mu g, (a) \text{ and (b)})$  or a lethal dose (58.5  $\mu g, (c)$  and (d)). See footnot of Fig.3 for details.

no large change in the frequency in closing and opening, at both doses of permethrin. In thoracic spiracle 2, the open time was reduced to a half to one-third of the control level, whereas the frequency was markedly increased, with lethal doses of permethrin.

In the experiments with a lethal dose of DDVP, we only observed thoracic spiracle 1. The open time markedly increased, but the frequency decreased greatly (Fig.5). The action of DDVP was antagonized transiently and partially by atropine (0.05 mg/insect), which slightly suppressed spiracle movement. Thus the valve of thoracic spiracle 1 tended to open rather than close during the severe convulsions induced by both insecticides, although results were different from those for spiracle 2. Under these conditions,

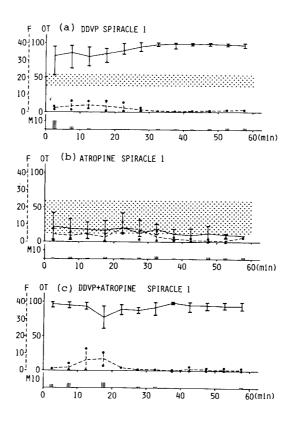


Fig. 5 Effects of DDVP on spiracle movements, and antagonism by atropine. At 0 min, 1 ul of 50 % DDVP ethanol solution was administered orally and 0.5 mg of atropine was injected into the abdomen. See footnote of Fig.3 for details.

normal respiratory movements would be interrupted, but a simple diffusion mechanism may operate to supply oxygen. Spiracle movements are either controlled separately and sometimes peripherally at a certain  $CO_2$  tension or by central oscillators in the central nervous system (MILLER, 1966: KAMMER, 1976). Both of the insecticides studied stimulate the insect CNS, but have no marked effect on the frequency in closing and opening of the spiracle valve except in a few cases. These results suggest that spiracle movements are predominantly under peripheral control in convulsive insectiside poisoning.

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