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Mechanisms of Insecticidal Action of Deoxypodophyllotoxin (Anthricin). II.[1] Histopathological Studies on Tissues of Silkworm Larvae Intoxicated by Deoxypodophyllotoxin

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It was previously reported that deoxypodophyllotoxin (I) shows strong insecticidal activity against several kinds of insects, including the 5th instar larvae of silkworm, *B. mori*. In the present work, in order to clarify the mechanisms of insecticidal action of I, histopathological examination of the fat body, the skin, the Malpighian tubules, and the ventriculus of intoxicated silkworm larvae was carried out and destruction of the epidermal cells was noted. No abnormality of the Malpighian tubules or the ventriculus in the treated larvae was found.

Although the fat body cells were also destroyed, the extent of destruction was almost the same both in the administered group and in a fasted group. Therefore, it is unlikely that the primary site of action of I is the fat body cells.

Keywords——deoxypodophyllotoxin; lignan; *Anthriscus sylvestris*; Umbelliferae; histopathological examination; epidermal cell; silkworm larvae; insecticidal mechanism

Deoxypodophyllotoxin (I)[2-4] is a kind of lignan which is widely distributed in higher plants. Kozawa *et al.*[5] reported that I shows relatively strong insecticidal activity against insect species. In order to clarify the mechanisms of insecticidal action of I, we previously investigated the distribution of I into the tissues of the 5th instar larvae of silkworm, *B. mori*, and it was found that large amounts of I accumulated in the fat body, the skin, the Malpighian tubules and the ventriculus of the larvae for a long time.[6] At the same time, destruction of the cell structure was observed visually in the skin and the fat body.

In the present study, histopathological examination of the epidermal cells, the fat body cells, the ventriculus and the Malpighian tubules of larvae intoxicated with I was carried out.

Materials and Methods

Chemical——Deoxypodophyllotoxin (I)[2] isolated from the root of *Anthriscus sylvestris* (Umbelliferae) was used.

Insect——The 5th instar larvae of silkworm (body weight 2.5—3.1 g) were used.

Treatment of Larvae——The leaf-dipping method[7] was used. Acetone solution of I was prepared at a concentration of 1000 ppm. Fresh mulberry leaves were dipped into the acetone solution and dried in a room. The larvae were released into a Petri dish containing the treated leaves. For the control group, mulberry leaves treated with acetone alone were used. Another group of larvae was kept in a Petri dish without leaves for examination of the effect of fasting.

Histopathological Examination——After the treatment, larvae of each group were anesthetized with CO_2 at the indicated time and dissected to obtain the skin, the fat body, the Malpighian tubules and the ventriculus. The tissues

were washed with 0.85% NaCl solution and fixed in Bouin's solution (saturated picric acid 75 ml, formalin 25 ml and glacial acetic acid 5 ml) for 24 h. The fixed tissues were cut into slices of 5—6 μm thickness after being embedded in paraffin. They were stained with hematoxylin-eosin solution, and examined through an optical microscope with a magnification of 400. The thicknesses of the epidermal cells and cuticle were measured with a micrometer.

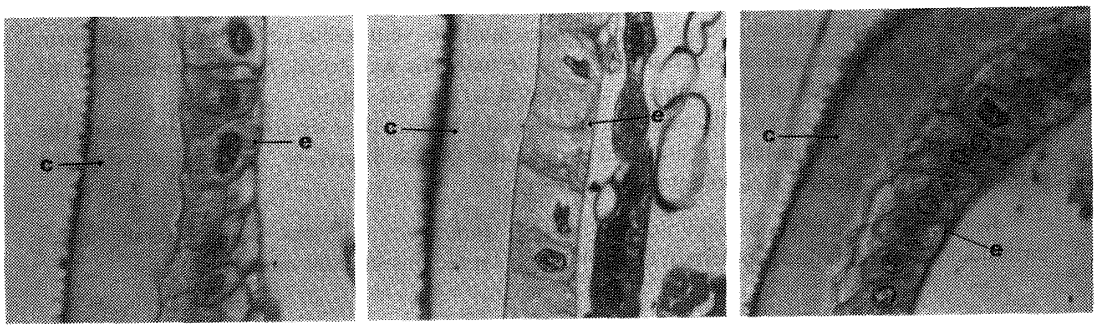
Temperature——Every experiment was carried out at 23.5±1.5 °C.

Results

After administration of I to the larvae, severe destruction of the skin and the fat body were observed with the naked eye as the toxic symptoms increased. The results of microscopic examination are shown in Figs. 1—4.

As shown in Fig. 1, up to 24 h the histopathological figures of both epidermal cells and the overlying cuticle from the administered and fasted larvae were normal compared to those of the control group. The thicknesses of these tissues were also generally similar among the three groups, though those of the epidermal cells in the administered and fasted groups were somewhat less than in the control group (Table I).

At 48 h after administration, a few granules stained rather faintly with hematoxylin appeared in the endocuticle of the control group. According to Ito,[8] these granules represent the dissociation and reabsorption figures of endocuticular materials due to the activity of epidermal cells, which produce and replace the cuticle at each molt. On the other hand, such



administered group fasted group control group

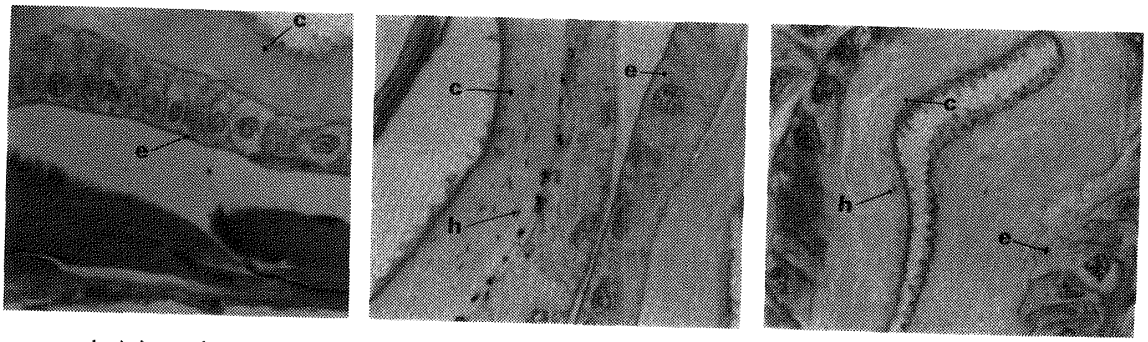
Fig. 1. Photographs of the Epidermis of the 5th Instar Larvae of Silkworm, *B. mori*, at 24 h after Administration of Deoxypodophyllotoxin (I)

Hematoxylin-eosin staining ×400×1/2: c, cuticle; e, epidermal cells.

TABLE I. The Changes of the Epidermal Cells and the Cuticle in Silkworm Larvae Treated with Deoxypodophyllotoxin (I)

Tissue	Group	Time (h) after administration				
		12	24	48	72	96
Epidermal cells	Administered group	17.29±4.24	20.17±5.79	15.75± 5.63	7.88± 2.69	6.66±2.59
	Fasted group	20.72±5.79	23.57±1.69	17.74± 7.38	17.53± 4.17	17.41±4.47
	Control group	17.81±4.10	26.42±5.60	30.93±10.02	35.15±11.2	30.71±9.58
Cuticle	Administered group	23.97±5.07	24.44±3.64	22.52± 3.47	21.94± 3.75	21.01±3.47
	Fasted group	23.24±2.96	24.51±4.65	28.76± 6.62	28.09± 3.65	24.18±3.71
	Control group	22.60±3.55	25.38±2.36	36.15± 5.61	37.04± 4.77	39.55±6.20

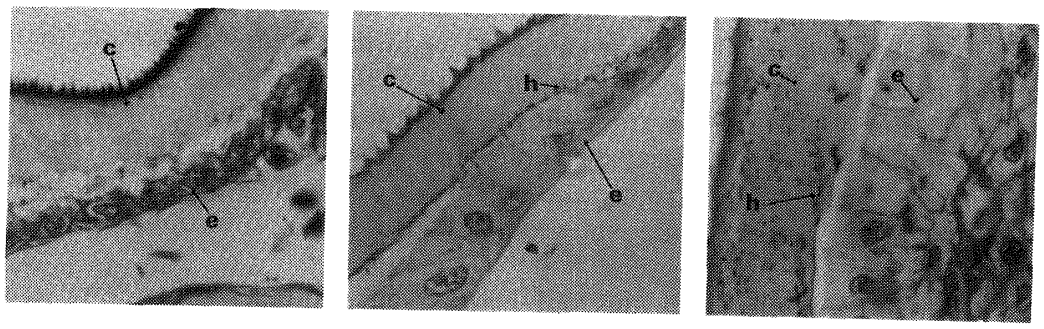
Thickness unit: μm.
Dose: 1000 ppm.
Method of treatment: Leaf-dipping method.
Each value represents the mean±S.D. of cells ($n=17$).



administered group fasted group control group

Fig. 2. Photographs of the Epidermis of the 5th Instar Larvae of Silkworm, *B. mori*, at 48 h after Administration of Deoxypodophyllotoxin (I)

Hematoxylin-eosin staining ×400×1/2: c, cuticle; e, epidermal cells; h, hematoxylin-stainable granules.



administered group fasted group control group

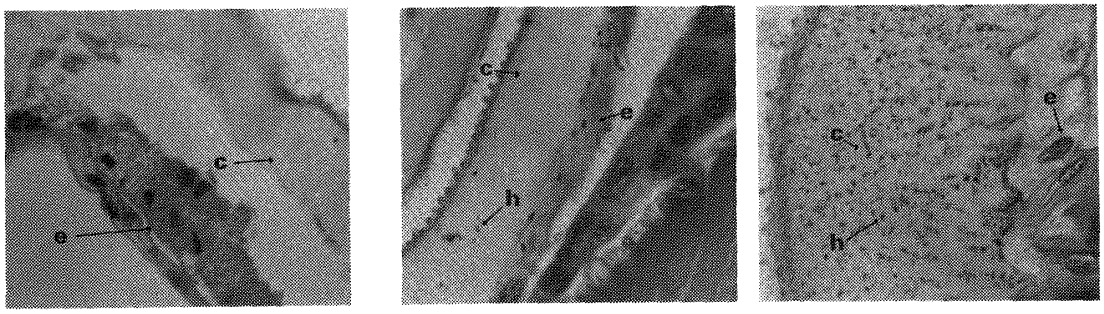
Fig. 3. Photographs of the Epidermis of the 5th Instar Larvae of Silkworm, *B. mori*, at 72 h after Administration of Deoxypodophyllotoxin (I)

Hematoxylin-eosin staining ×400×1/2: c, cuticle; e, epidermal cells; h, hematoxylin-eosin-stainable granules.

granules did not appear in the cuticle of the administered group (Fig. 2). The thicknesses of the epidermal cells in the administered and fasted groups showed some decrease as compared to the control group and even to the 24 h values of the same groups. Similarly the cuticular thickness in the control was markedly increased at 48 h after administration, while those in the administered and fasted groups did not increase or even decreased somewhat (Table I).

About 72 h after administration, as shown in Fig. 3, the epidermal cells of the administered group were severely damaged compared with those of the control group. Their alignment was disordered, and chromatin granules in the nuclei showed pycnosis. The thicknesses of the epidermal cells and the cuticle of the administered group were markedly decreased as compared to the control group or even to the preceding stage of the same group. On the other hand, the epidermal cells and the cuticle of the fasted group were thinner than those of the control group, but they showed no abnormality (Fig. 3 and Table I). The number of hematoxylin-stained granules in the cuticle of the control group increased markedly, while in the administered group they did not appear at all. Those in the fasted group increased less markedly than in the control group.

About 96 h after administration, the nuclei and the basement membrane of the epidermal cells of the administered group were disordered to a greater extent than after 72 h (Fig. 4). As shown in Table I, the epidermal cells and the cuticle were thinner than at 72 h. Hematoxylin-stained granules were not found. The coagulation of chromatin in the nuclei was greater than



administered group fasted group control group

Fig. 4. Photographs of the Epidermis of the 5th Instar Larvae of Silkworm, *B. mori*, at 96 h after Administration of Deoxypodophyllotoxin (I)

Hematoxylin-eosin staining ×400×1/2: c, cuticle; e, epidermal cells; h, hematoxylin-stainable granules.

at 72 h, and the basement membrane was thinner as compared to the control group. On the other hand, the epidermal cells and the cuticle of the fasted group were thinner than those of the control group, but they showed no abnormality (Table I).

Although the photographs are not shown, from 48 h after administration the fat body cells of the administered group became atrophied, and the numbers of oil droplets decreased. After 72 h there was a marked atrophy and only a few oil droplets were found. However, the photographs of the fasted group showed the same kind of atrophy as those of the administered group.

On the other hand, the ventriculus and the Malpighian tubules were normal in all groups throughout the observation period.

Discussion

The present results show that at least a part of the insecticidal action of I is due to its destructive action on the epidermal cells (Figs. 2—4).

This is in accordance with the fact[6] that the color of the skin of the larvae became brown as the toxic symptoms progressed. The onset of the intoxication by I was at about 48 h after administration. The main symptoms associated with the early stage of the intoxication by I have already been reported in the previous paper[6]; 1) greatly decreased ingestion of food and 2) greatly decreased excretion of feces. The phenomena observed here are consistent with the view that I acted on the epidermal cells rather than on the nervous system. In the present study the results of the histopathological investigation confirmed this: namely, from 72 h to 96 h after administration, 1) the thicknesses of the epidermal cells and the cuticle decreased rapidly (Figs. 3 and 4, and Table I), 2) the cuticular hematoxylin-stained granules which represent normal activity of the epidermal cells failed to appear, and 3) the chromatin granules in the nuclei in the epidermal cells showed pycnosis and the basement membranes became detached from epidermal cells. The first two phenomena suggest that normal activity in the epidermal cells of the administered group is blocked at the 72 h period. The third observation is consistent with reports that I has antitumor activities against human epidermoid carcinoma[9] and acute lymphocytic leukemia L-1210 in mice.[10] Further work is planned, including a study of the interaction of I with DNA. At the same time, it was observed that the thicknesses of the epidermal cells and the cuticle of the fasted group decreased as compared to the control group, but showed no abnormality (Table I and Figs. 3 and 4). The decrease seems to be simply due to the undernourishment.

In an investigation of the distribution of I into the tissues of silkworm larvae,[6] it was

found that 1) a large amount of I accumulated in the fat body for a long time, and 2) changes in the tissues could be observed visually. In the present study the fat body showed abnormal features in the treated group. However, in the fasted group they showed similar abnormalities. Therefore, it is unlikely that the toxicity of I is related to the destructive action of I on the fat body cells. In fact, it is already well known that the fat body of insects is easily damaged by physiological changes, such as starvation. On the other hand, the ventriculus and the Malpighian tubules did not show any abnormalities.

It seems reasonable to conclude that the cause of the delayed insecticidal action of I is a strong destructive effect of I on the epidermal cells.

References and Notes

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