HISTO- AND CYTO-PATHOLOGICAL STUDIES ON THE MIDGUT EPITHELIUM OF SILKWORM LARVAE FED *BACILLUS THURINGIENSIS*

Toshihiko IIZUKA

(Laboratory of Sericulture, Faculty of Agriculture
Hokkaido University, Sapporo, Japan)

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Introduction

Histological investigations on insects fed crystal-forming bacteria have been made by TANADA (1953) on *Pieris rapae*, by MARTOURET et al. (1965) on *P. brassicae*, by HEIMPEL and ANGUS (1959) on *Bombyx mori* by HOOPINGER and MATERU (1964) on *Galleria mellonella*, and by SUTTER and RAUN (1967) on *Ostrinia nubilalis*.

There is general agreement that paralysis of the gut is followed closely by break down of the gut epithelium.

Initial work on *Bombyx mori* was reported by HEIMPEL and ANGUS (1959), who were shown histopathological damage to the midgut of silkworm larvae within 60 minutes of feeding on spores and crystals of *Bacillus entomocidus* var. *entomocidus*.

For the primary action of the δ-endotoxin, they postulate that the toxin has a specific substrate and the substrate exists in the cell cementing substances.

SUTTER and RAUN (1967) showed that the cells of the gut epithelium loosened from basement membrane, by intracellular observations lipid like inclusion bodies normally present in healthy cells were disappeared, and microvilli of the goblet cells which caused an apparent vacuolation of these cells were disrupted and the endoplasmic reticulum were destroyed.

The present study was undertaken to observe cytopathologically to the midgut of silkworm larvae which were fed dilute spores and crystals of *Bacillus thuringiensis* var. *thuringiensis*.

Materials and Methods

The strain of silkworm larvae used in this experiments was J124 × C124.

Silkworm larvae were reared on mulberry leaves and on the artificial

diet according to IIZUKA and TAKIZAWA (1969). Early 5th instar larvae were fed with both diet, respectively, treated with *B. thuringiensis* var. *thuringiensis* δ-endotoxin and spores, and the midguts of infectious larvae were dissected at three parts; in the anterior region, central region and posterior region.

Treated larvae were picked at 12 hours intervals for comparison and the dissected parts were fixed in ALLEN's PEA-3 fluid at room temperature for 24 hours before they were placed 70% ethanol. The process of dehydration and paraffin embedding was made by routine methods.

Sections were cut at 5 microns and were stained with DELAFIELD's hematoxylin and eosin.

Results

Infection

HEIMPEL and ANGUS (1959) showed that the histopathological damage of the midgut of silkworm larvae was recognized within 60 minutes of feeding on spores and crystals of *B. entomocidus* var. *entomocidus*, and that the time required to change the epithelium corresponded with the time required to paralyze the gut of silkworm larvae. Also, it is recognized by BURGES (1960) that the variation in the speed of infection is influenced by the dose of spores and crystals of *B. thuringiensis*.

In this experiment, the speed of infection of silkworm larvae was

TABLE 1. Mortality of silkworm larvae by feeding of *Bacillus thuringiensis* var. *thuringiensis*[a)]

Treatment		Time				
		9hr.	12hr.	24hr.	48hr.	72hr.
Reared on treated mulberry leaves						
Experiment 1	5.2×10^8 spores/ml	0[b)]	0	30	—	—
Experiment 2	5.2×10^7	0	0[b)]	5	24	1
Experiment 3	5.2×10^6	0	0	0	0	0
Reared on treated artificial diet						
Experiment 1	4×10^7 spores/ml	0[b)]	0	30	—	—
Experiment 2	4×10^6	0	0[b)]	11	19	—
Experiment 3	4×10^5	0	0	0	0	0

a) The number of larvae tested was 30 individuals of 5th instar larvae in each treatment.

b) General paralysis in larvae after feeding of δ-endotoxin was recognized.

delayed as much as possible by dosing of δ-endotoxin diluted. By the results, appearance of larvae paralyzed (general paralysis) and the speed of degenerations of the cells of epithelium were chronically proceeded. Both larvae reared on mulberry leaves and on the artificial diet were paralyzed at 9 hours after inoculation in experiment 1, and in experiment 2, they were paralyzed at 12 hours after inoculation. The mortality in each experiment was recorded in Table 1.

Histopathology

HEIMPEL and ANGUS (1959) reported that the primary site of the action of the δ-endotoxin is the anterior midgut region of silkworm larvae. As histopathological investigations of anterior, central and post-midgut region in this experiment also corresponded with their results, figures were microscopically shown only central midgut region.

In experiment 1, the epithelium of the midgut of larvae paralyzed for 9 hours after inoculation were microscopically observed. Pictures 1 and 2 of Plate I showed changes in the central midgut epithelium after 9 hours feeding the toxin, respectively. At this time, it was already found that there were many vacuoles resulted from the disruption of the cytoplasms in the goblet cells and that both nuclei of the goblet cells and cylindrical cells were hypertrophied.

Pictures 3 and 4 of Plate I (24 hours) showed extensive separation of cells from basement membrane.

The speed of infection in experiment 2 was more delayed. Pictures 1 and 2 of Plate II showed vacuolations of the cytoplasms in the goblet cells at 12 hours after feeding the inoculum diluted than experiment 1. In this experiment, vacuolations of the cytoplasms in the goblet cells were not enough proceeding. Pictures 3 and 4 of Plate II were found the separation of cells from the basement membrane and spherical body which seemed to result from disruption of the cytoplasm in the goblet cell. The spherical body of the cytoplasms in the goblet cells was also found in silkworm larvae infected with infectious flacherie by IWASHITA (1965) and in silkworm larvae infected with *Streptococcus faecalis* AD-4 by IIZUKA (1972). However, morphology and stainability of this spherical body were not the same as the spherical body recognized by them. Pictures 1 and 2 of Plate III (48 hours) showed a spongy like epithelium resulted from vacuolations of the cytoplasms of the goblet cells and from disruption of the cylindrical cells.

As the histopathological investigations of the larvae reared on the

artificial diet were fundamentally obtained as the same results with larvae reared on mulberry leaves, only pictures of the epithelium in experiment 1 were shown. Pictures 1 and 2 of Plate IV (12 hours) showed the midgut epithelium of larvae reared on the artificial diet. Vacuolations of the cytoplasms in the goblet cells were also apparent. Pictures 3 and 4 of Plate IV (24 hours) also showed a spongy like epithelium.

In microscopically observations by using crushing method to the peritrophic membranes dissected, it was recognized that spores within peritrophic membrane after dosing to silkworm larvae with *B. thuringiensis* germinated at immediately before death and vegetative cells were found in the midgut and in the hemolymph after death.

Discussion

Silkworm larvae are so strongly affected by δ-endotoxin of *B. thuringiensis* (Type I insect), but when the slight doses of the toxin are inoculated into larvae, the speed of infection is delayed (BURGES, 1960).

The histopathological changes from 15 minutes to 60 minutes after feeding on the δ-endotoxin of *B. entomocidus* were observed by HEIMPEL and ANGUS (1959), and they postulated that the toxin had a specific substrate and the substrate existed in the cell cementing substances. However, the results obtained in this experiment showed that the initial histo-pathological changes had begun from the cytoplasms of the goblet cells in the midgut epithelium after 9 hours feeding the toxin. And then, the most remarkable cytopathological changes were vacuolations of the cytoplasms in the goblet cells. Vacuolations of the cytoplasms were corresponded with the observations by SUTTER and RAUN (1967). The separations of cells from the basement membrane were not always observed in all individuals, but finally, a spongy like epithelium resulted from vacuolations of the cytoplasms in the goblet cells and from disruption of the cylindrical cells were found.

From the results obtained in this experiments, it is postulated that the initial site of action of the δ-endotoxin is the cytoplasms of the goblet cells in the midgut epithelium and vacuolations of them disappear the action on the permeability control of midgut epithelium.

Summary

Histo- and cyto-pathological studies were made on the midgut epithelium after feeding by diluted doses on *B. thuringiensis* var. *thuringiensis* in silkworm larvae reared on mulberry leaves and the artificial diet. The results obtained were summarized as follows:

1. In silkworm larvae fed *B. thuringiensis*, the histological changes of epithelium occurred at first in the cytoplasms of the goblet cells after 9 hours (general paralysis) dosing *B. thuringiensis*, and vacuolations resulted from the disruption of the cytoplasm were found.

2. At immediately before death of silkworm larvae, a spongy like epithelium resulted from vacuolations of the cytoplasms of goblet cells and from disruption of the cylindrical cells were observed.

3. Spores within peritrophic membrane germinated at immediately before death and vegetative cells were found in the midgut and in the hemolymph after death.

4. Differences between larvae reared on mulberry leaves and larvae reared on the artificial diet were not recognized.

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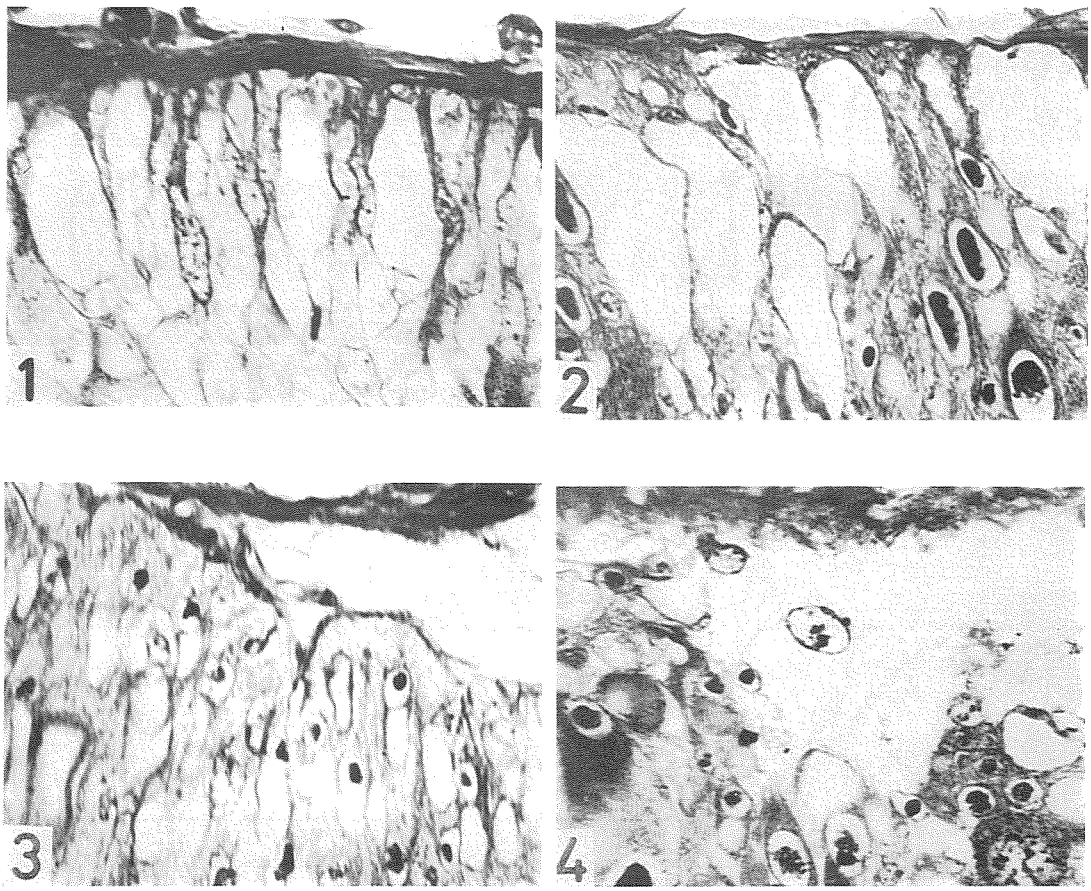

Plate I

The midgut epithelium of silkworm larvae reared on treated mulberry leaves. × 600.

1 and 2. Vacuolations of the cytoplasms in the goblet cells after 9 hours in experiment 1.

3 and 4. Separations of the cells from basement membrane after 24 hours in experiment 1.

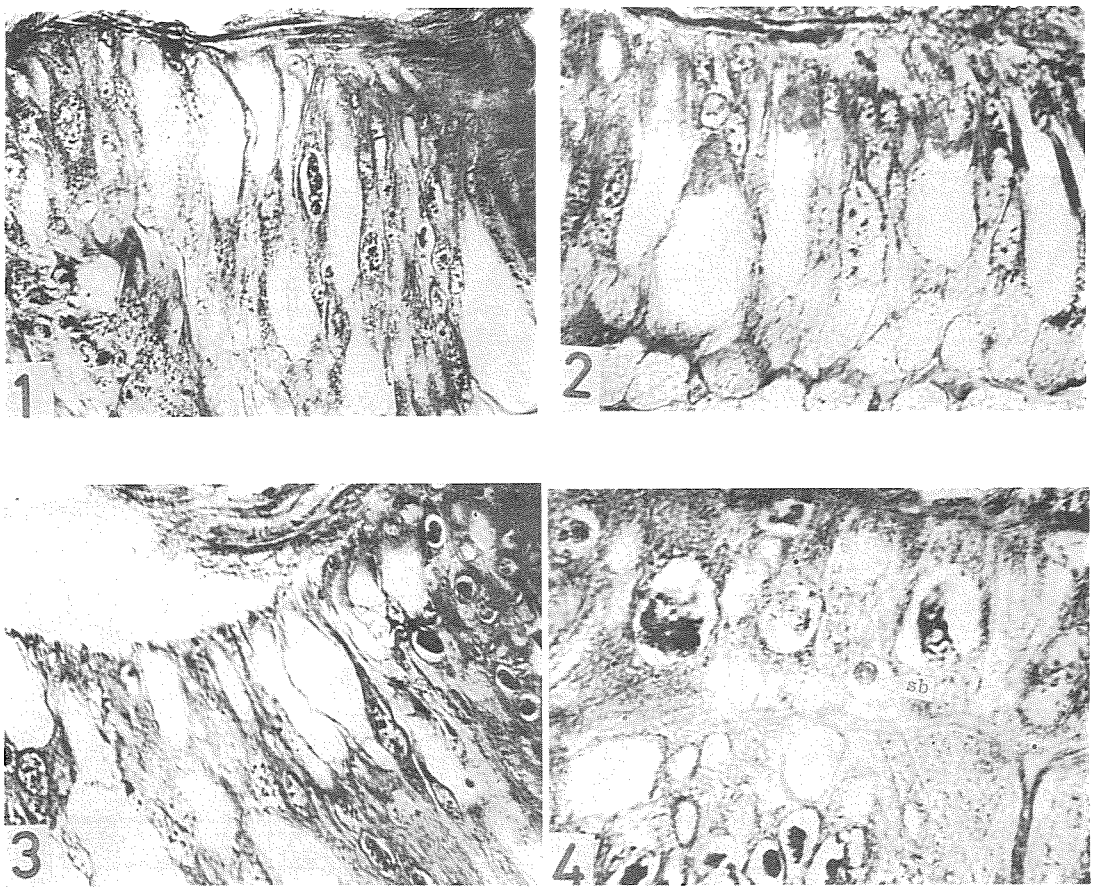

Plate II

The midgut epithelium of silkworm larvae reared on treated mulberry leaves. × 600.

1 and 2. Vacuolations of the cytoplasms in the goblet cells after 12 hours in experiment 2.

3. Separations of the cells from basement membrane after 24 hours in experiment 2.

4. Appearance of spherical body (sb) after 24 hours in experiment 2.

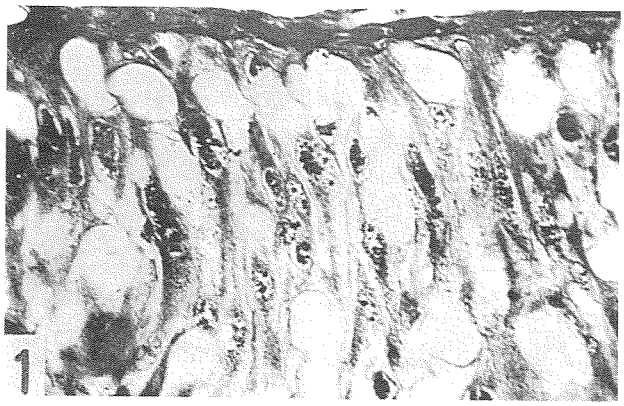

Plate III

The midgut epithelium of silkworm larvae reared on treated mulberry leaves. × 600.

1 and 2. Spongy like epithelium after 48 hours in experiment 2.

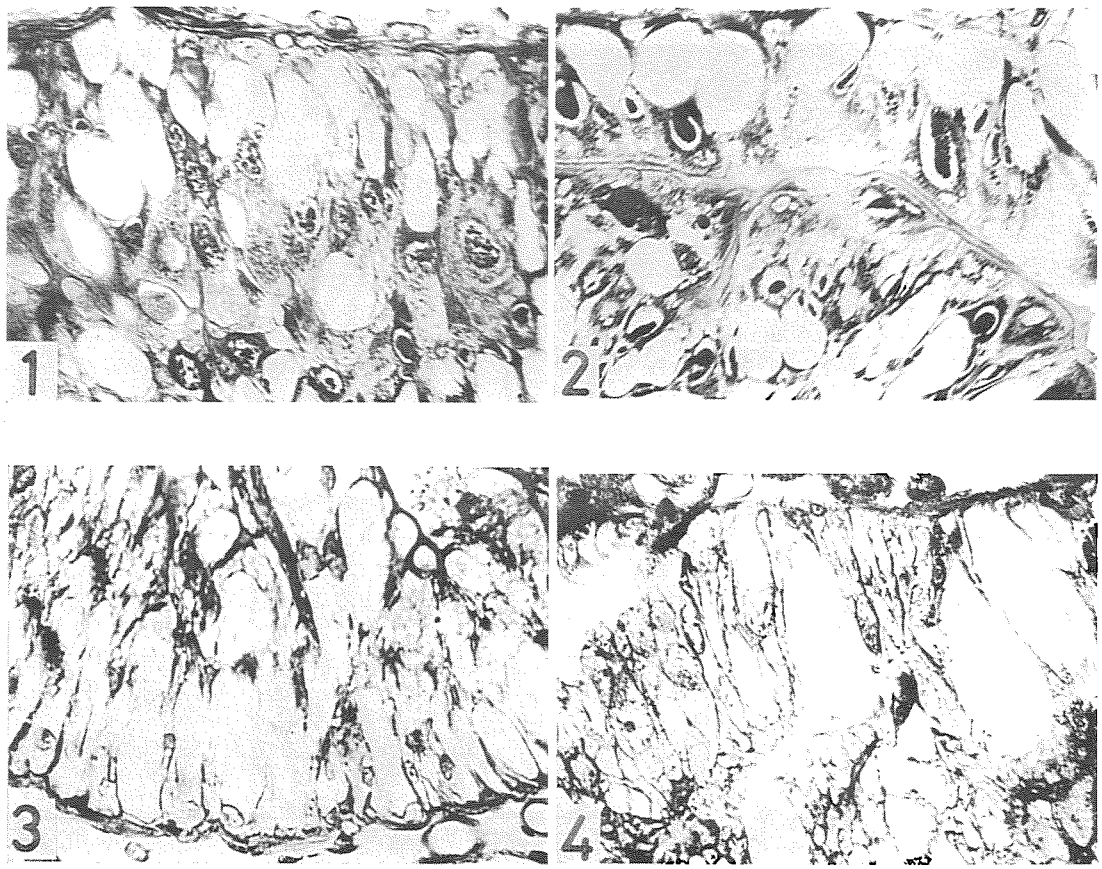

Plate IV

The midgut epithelium of silkworm larvae reared on the treated artificial diet. × 600.

1 and 2. Vacuolations of the cytoplasms in the goblet cells after 12 hours in experiment 2.

3 and 4. Spongy like epithelium after 24 hours in experiment 1.