Difference in the Metabolic Fate of Carbaryl between the
Silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae)
and the Black-tipped Leafhopper, *Bothrogonia*
japonica ISHIHARA (Hemiptera : Tettigellidae)

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Metabolic fate of ^{14}C -carbaryl in silkworm larvae and adults of the black-tipped leafhopper were investigated. Both insects showed different toxic symptoms under topical application with doses of nearly the LD_{50} value. Silkworm larvae rapidly consumed carbaryl until about 4 hr after application, and the substance which was a dominant metabolite appeared simultaneously and reached the maximum level, one thirds the initial dose of the insecticide, at 3 hr after application. Seven hr later it disappeared. Another metabolite was recognized about one hr after application and continued to exist for 8 hr. Water soluble metabolites were also noted in small amounts, as that of the second metabolite. Whereas in the adults of the black-tipped leafhopper no labeled metabolites were found with the exception of water soluble substances, and carbaryl continued to remain almost at 50% of the initial dose even 8 hr after application.

Relationship between the recovery of motility from toxic damage of silkworm larvae and the metabolic fate of carbaryl in the silkworm was discussed.

INTRODUCTION

Carbaryl (1-naphthyl-N-methylcarbamate; Sevin), a representative carbamate insecticide has been widely used, especially on the green rice leafhopper in Japan, because of its acceptable effectiveness as evidenced by a higher toxicity, as much as forty times as that of silkworm larvae which are generally susceptible to the insecticide (KAZANO *et al.*, 1969; KUWANA *et al.*, 1967).

It has been reported in earlier investigations that carbaryl has not been effective on the house fly, *Musca domestica* L. (ELDEFRAWI and HOSKINS, 1961; GEORGHIOU *et al.*, 1961) or the rice weevil, *Sitophilus oriza* L. (STRONG and SBUR, 1961),

whereas it has shown specific insecticidal actions on the large milkweed bug, *Oncopeltus fasciatus* DALLAS (COWAN *et al.*, 1961) or the stable fly, *Stomoxys calcitrans* L. (DRUMMOND, 1962). Studies on the absorption and metabolism of carbaryl in insects pointed that the pattern of the chemical nature of responses of insects to the insecticide differed between species of insects (ELDEFRAWI and HOSKINS, 1961; CAMP and ARTHUR, 1967). Identification of metabolites of carbaryl in insects such as the house fly and cockroaches was tentatively performed for 8 compounds, 5 of which were detected as carbamates (DOROUGH and CASIDA, 1963, 1964).

The present paper is concerned with the metabolic fate of carbaryl in the silkworm larvae and the black-tipped leafhopper, the latter of which is an insect pest on mulberry trees, in relation to the toxic symptoms of the insecticide on the insects with a viewpoint of its selective toxicity.

MATERIALS AND METHODS

Insects

The silkworm, *Bombyx mori*, used was the hybrids between strains Shi 124 and Nichi 124. Those subjected to assay were one day old larvae of the third instar, 32.0 mg of average body weight. The hibernated adults of the black-tipped leafhopper, *Bothrogonia japonica*, were collected from mulberry trees on the fields. A part of those collected was used for the experiment and the remaining for the next generation, was reared on mulberry leaves in a cage covered with lawn. The nymphs thereby obtained were again employed. Average body weight of the adult and the nymph were 58.3 and 21.9 mg, respectively.

Assay for Toxicity of Carbaryl on the Insects

Toxicity of carbaryl to silkworm larvae has already been calculated to be LD₅₀ 12.6 µg/g body weight for the 2nd day of the 4th instar and 15.2 µg/g body weight for the 4th day of the 5th instar with topical application (KUWANA *et al.*, 1967). LD₅₀ value of carbaryl to the black-tipped leafhopper was determined with topical application to be equivalent to 0.99 µg/g body weight for the nymph and 1.48 µg/g body weight for the adult.

Topical Application of ¹⁴C-Carbaryl to the Insects

1-Naphthyl-N-methylcarbamate (carbonyl-¹⁴C; 131 µc/mg, 98% radiochemical purity, purchased from the Radiochemical Centre, Amersham, England) was topically applied on the dorsal skin of silkworm larvae at 0.026 µc (0.217 µg) of carbaryl per individual (6.78 µg/g body weight) through a micropipette involving 1 µl of an acetone solution of the insecticide. Adults of the black-tipped leafhopper, being beforehand anaesthetized with carbon dioxide gas, were topically treated with 0.019 µc (0.141 µg) of ¹⁴C-carbaryl per individual (2.43 µg/g body weight). The ¹⁴C-carbaryl treated insects were deep frozen at definite intervals after application. The number in each batch of experimental insects was five for the silkworm and ten for the black-tipped leafhopper.

Extraction of Carbaryl and Its Metabolites from Insects

Extraction of carbaryl and its metabolites from insects was carried out by the ANDRAWES and DOROUGH (1967) method with some modifications. Whole body of the insect was homogenized with 3 ml of cold acetone and was centrifuged for 10 minutes at 3000 rev/min. The residue obtained was re-extracted with 2 ml of cold acetone and these two extracts were combined. Being evaporated acetone under

reduced pressure, the combined extract was dissolved in 2 ml of acetonitrile. The equal volume of *n*-hexane was added to the acetonitrile solution with shaking vigorously and subsequently the *n*-hexane of the upper layer was discarded. Again, an equal volume of distilled water was added to the acetonitrile layer and was shaken with 3 ml of chloroform to distribute the solutes. Chloroform extraction was repeated to obtain the chloroform soluble metabolites, after removing the solvent under reduced pressure. The aqueous layer was used to immerse the residue that originally resulted in acetone extraction and the water soluble fraction was separated.

Separation and Assay of Metabolites of Carbaryl

Radioactivities of chloroform and water soluble metabolites of carbaryl were determined. Separation of carbaryl and its metabolites from chloroform soluble fractions was performed by thinlayer chromatography on silica gel G plate, 250 μ in thickness, developing with methanol-benzene (9 : 1). After the run, carbaryl and its metabolites were detected with a radiothinlayer chromatoscanner (Aloka TRM-1B) in spots or bands. Rf values of carbaryl and metabolite I and II were 0.58, 0.24 and 0.0, respectively, on the thin-layer chromatogram. Amounts of these compounds were deduced from the total radioactivities of chloroform and water soluble extracts and the relative ratio of autographs of the corresponding spots or bands, respectively.

RESULTS

Change in Toxic Symptoms

Change in toxic symptoms of the insects treated with carbaryl was observed and the outstanding difference between the silkworm and the black-tipped leafhopper was recognized in the process of toxic symptoms. The larvae of the silkworm poisoned with carbaryl showed excitement and vomitted gastric fluids about 15 min after application, and all the larvae became knocked down with the ataxia within

Table 1. RECOVERY OF ^{14}C FOUND IN CARBARYL AND ITS METABOLITES
SEPARATED FROM SILKWORM LARVA

	% of radioactivity to the initial dose at indicated hour after application									
	0.25	0.5	1	2	3	4	5	6	7	8
From of ^{14}C labeled compound recovered from insect body										
Carbaryl	98.7	66.6	32.0	17.2	7.6	0.0	0	0	0	0
Metabolite I	0.0	9.5	20.4	25.5	31.4	31.7	14.0	4.4	0.0	0
Metabolite II	0.0	0.0	4.1	4.0	4.6	5.0	8.1	7.0	6.7	6.1
Water soluble metabolites	1.3	1.6	— ^b	4.3	6.1	7.5	—	—	—	—
Recovery	100	77.7	—	50.7	49.5	44.2	—	—	—	—
Unrecovered and vanished from insect body ^a	0.0	22.3	—	49.3	50.5	55.8	—	—	—	—

Silkworm larva was dosed topically with 0.026 μC of ^{14}C -carbaryl on the first day of the third instar.

^a These figure was not examined and deduced from figures of the recovery of ^{14}C from insect body.

^b not examined.

Fig. 1. Change in appearance of carbaryl and its metabolites in silkworm larvae.

—○— carbaryl, —●— metabolite I,
—⊙— metabolite II,
--□-- water soluble metabolites.

Fig. 2. Change in appearance of carbaryl and its metabolites in black-tipped leafhopper adults.

—○— carbaryl,
--□-- water soluble metabolites.

one hr after application. At 3 hr after application most of the larvae revived from the toxic symptoms of ataxia and some of them began to eat mulberry leaves some 7 hr later. On the other hand, the black-tipped leafhopper that received an application of carbaryl became excited within 30 min and the first signs of "knock down" of adult insects with the ataxia was observed about 3 hr after and almost 30% of the insects were knocked down about 6 hr after application. In about 24 hr after application, the mortality was 60% under the present dose for investigation. Recovery from the toxic symptoms was not observed and the insects with the ataxia died without revival.

Fate of Carbaryl in Silkworm Larvae

Recovery of ^{14}C found in carbaryl and its metabolites in silkworm larvae treated with topical application is as shown in Table 1 and changes in the appearance of the metabolite is presented in Fig. 1. The amount of carbaryl rapidly decreased and

disappeared within 4 hr after application. Metabolite I, a major metabolite of carbaryl, appeared in about 30 min and reached a maximum, one third the initial dose of the carbaryl, about 3 to 4 hr later, and then disappeared in about 7 hr after application. Metabolite II appeared one hour after application and continued to be noted even 8 hr after. The maximum amount of metabolite II was 8.1% of the carbaryl applied initially. Water soluble metabolites of carbaryl were recognized and amounted to almost the same as metabolite II. The same patterns of transition of carbaryl in the chloroform soluble fraction were obtained when the chemical was applied orally (SUGIYAMA *et al.*, unpublished; reported at the 40th Annual Meeting of the Japanese Society of Sericulture (1970)).

Table 2. RECOVERY OF ^{14}C FOUND IN CARBARYL AND ITS METABOLITES
SEPARATED FROM BLACK-TIPPED LEAFHOPPER ADULT

	% of radioactivity to the initial dose at indicated hour after application									
	0.25	0.5	1	2	3	4	5	6	7	8
Form of ^{14}C labeled compound recovered from insect body										
Carbaryl	98.2	84.1	96.6	86.4	67.1	70.7	66.1	73.7	58.0	52.4
Water soluble metabolites	1.8	3.2	3.8	6.3	8.2	8.7	7.4	9.8	6.9	6.9
Recovery	100	87.3	100.4	92.7	75.3	79.4	73.5	83.4	65.0	59.3
Unrecovered and vanished from insect body ^a	0.0	12.7	0	7.3	24.7	20.6	26.5	16.6	35.0	40.7

Black-tipped leafhopper was dosed topically with $0.019\ \mu\text{C}$ of ^{14}C -carbaryl on the hibernated adult.

^a These figure was not examined and deduced from figures of the recovery of ^{14}C from insect body.

Fate of Carbaryl in the Black-tipped Leafhopper Adults

The pattern of metabolism of carbaryl in the black-tipped leafhopper was considerably different from that in the silkworm and no metabolite of carbaryl that was soluble in chloroform was found. In Table 2 and Fig. 2, it may be noted that carbaryl in the adults diminished slowly, and about 50% of the initial amount of carbaryl remained 8 hr after application. Water soluble metabolites reached maximum levels 4 to 6 hr after application, but the amounts of those were almost the same as that transformed in the silkworm. Carbaryl was more toxic to the nymphs of the leafhopper than to the adults but the insecticide in the nymph probably changes in the same way as in the adults.

DISCUSSION

It has been pointed that the metabolism of carbaryl differs among species of insects. The large milkweed bug formed only one polar product, the house fly formed 3 metabolites and cockroach formed 6 metabolites (ELDEFRAWI and HOSKINS, 1961). Since then, DOROUGH and CASIDA (1964) identified 8 metabolites of carbaryl in both the house fly and the cockroach.

In our present and unpublished studies, metabolites of carbaryl in silkworm larvae were separated to at least 3 compounds, which were named temporarily as metabolite I, being dominant, metabolite II, and the water soluble substance. Toxicity

of metabolite I was several hundred times less toxic than that of carbaryl (our unpublished data). In another of the experiments concerned with chemical assay, metabolite I has not been identified as any hydrolyzed substances of carbaryl, such as 1-naphthol or derivatives of 1-naphthol in silkworm larvae. The response of the black-tipped leafhopper to carbaryl seems to be close to the large milkweed bug, which metabolizes carbaryl to only water soluble substances. These relative high toxicity of carbaryl to the black-tipped leafhopper and the milkweed bug as compared to the house fly, the German cockroach and the silkworm may be related to the high rate of detoxication, which appeared as consumption of carbaryl, as pointed by CASIDA (1963). Specific toxicity of carbaryl, closely related phylogenetically to insect species, may be represented by the specificity in the metabolism, absorption and excretion of carbaryl in insects, and the peculiar biological revival from toxic symptoms found in silkworm larvae treated with carbaryl should be explained by rapid release from the blocked state of the concerned enzymes, after the rapid transformation of carbaryl to less toxic metabolites.

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