Hormonal Control of the Body-colour Change in Larvae of the Larger Pellucid Hawk Moth, *Cephonodes hylas* L. (1). Hajime Ikemoto (Tokyo Prefectural Isotope Research Station, Fukasawa, Setagaya, Tokyo), Received January 27, 1975. *Botyu-Kagaku* 40, 59, (1975).

10. オオスカシバの体色変化に関するホルモン的制御 (1)

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オオスカシバの摂食時幼虫は緑色をしめすが, 終令末期になると暗赤色に変わる. しばらくすると赤色色素は次第に消失し, 淡緑色の前蛹になる. このような退色は脱皮ホルモンによって誘起され, 幼若ホルモンによって抑制される. 退色に対する脱皮ホルモンの臨界期は営繭の中期あたりのように思われる.

The integument of larvae of the larger pellucid hawk moth, *Cephonodes hylas* at the feeding age is green in colour, but before cocoon formation the colour turns in deep dark red owing to the production of ommochromes. After a short time they become into the pale green prepupae, red pigments being vanished gradually.

The present report deals with hormonal control of the depigmentation phenomenon which was observed with the progress of prepupation in the mature larvae of the larger pellucid hawk moth.

Body-colour change in the larvae of *Cephonodes hylas*

The last larval instar up to the larval-pupal molt is divided into several phases, corresponding to the developmental steps of metamorphosis.

During the first four days after the last larval molt, the integument of the larvae is bright green in colour and they feed vigorously. The larval haemolymph in this stage is greenish blue in colour.

On the fifth day after the last larval molt the colour of the integument of the larvae turns into deep dark red, and the haemolymph changes its colour from greenish blue to yellowish green, keeping the same appearance to the stage of pupal molt. The deep dark red larvae cease to feed and come down from a trunk in nature and they wander restlessly around in a feeding container (wandering stage).

Then the larvae begin to spin a dark red cocoon 12 hours after the body-colour changed into deep dark red. It takes a few hours to build their cocoon in which they spend the remaining part of larval stage, prepupal, and pupal stages.

The gut content begins to turn red and red pigment begins to vanish from the epidermal cells of the integument, within 12 hours after the cocoon formation was completed. Further 12 hours later, they become into the pale green prepupae, the colour in the dorsal region from metathorax to terminal abdomen showing dark green.

Prepupae molt to pupae 1-2 days after prepupation.

Material and methods

Insect material: Eggs or young instar larvae of *Cephonodes hylas* were collected from Cape jasmine in Tokyo prefecture area, and they were individually reared under the constant range of temperature of 24-26°C in the laboratory. The last instar larvae were used for the present experiments.

Isolated abdomens: Ligature was made at the back of the thorax of the last instar larvae and the anterior part of the body was cut off immediately after ligation.

Topical application of juvenile hormone (JH) and its analogues: The following compounds kindly supplied by the late Prof. M. Fukaya were used.

1) *Cecropia* JH: methyl-12, 14-dihomojuvenate (Mori *et al.* 1971)

2) ZR 512: ethyl-3, 7, 11-trimethyl-2, 4-dodecadienoate (purity 75 %) (Zoëcon)

3) CT 5: 1-(3, 4-methylenedioxyphenyl)-7-epoxy-4, 8-dimethyl-nona-1, 3-diene (purity 70%) (Chang

and Tamura 1971)

These compounds, mixed isomers, dissolved in 1 μl of acetone were applied to dorsal integument of the thorax of the larvae. To the control 1 μl of acetone alone was applied.

Injection of molting hormone: Effect of molting hormone on the depigmentation as before mentioned was examined by injecting β-ecdysone into the isolated abdomens. β-Ecdysone dissolved in 2 μl of distilled water was injected into an isolated abdomen in proper concentration through the 4th abdominal leg. An equal amount of distilled water alone was injected to the control.

Results

1) *Effects of injection of β-ecdysone on the developmental process*: In order to ascertain the effects of various titers, β-ecdysone solution was injected into isolated abdomens of the deep dark red larvae.

The ligature was performed on green larvae about 6 hours before the onset of dark reddening in order to obtain deep dark red isolated abdomens. Preliminary experiment showed that the ligature performed at this time caused the whole body to keep deep dark reddening, and abdomens of the ligatured animals survived at least 2 weeks with no signs of depigmentation and pupation.

As shown in Table 1, a small dose (1-3μg) caused to vanish red pigment from the integument without pupal molt. As dose increased, the pupation of an isolated abdomen was apt to be proceeded. A large dose (20 μg) caused a pupal molt, being not accompanied by colour change in appearance.

It is concluded therefore that the depigmentation and pupal molt are induced by the same kind of molting hormone, a concentration at which the hormone is effective to the two processes being different.

2) *Effect of time of ligation on the depigmentation*: The ligation was made at various times during the period from the onset of dark reddening stage to shortly after completion of cocoon formation in order to determine the critical period for the production of molting hormone effective to the depigmentation.

Results of ligation experiments showed that the depigmentation in the deep dark red larvae was controlled by molting hormone which was produced until the middle stage of cocoon formation (Table 2).

All of the isolated abdomens were unable to pupate. Only in a few specimens ligatured shortly after cocoon formation the pupal integument was observed partially at the dorsal region.

3) *Effect of juvenile hormone and its analogues on the depigmentation*: The effect of *Cecropia* JH and its analogues on the depigmentation was tested by topical application of the chemicals to the deep dark red larvae over several stages from wandering to shortly after cocoon formation.

As shown in Table 3, both *Cecropia* JH and

Table 1. Effects of β-ecdysone injected into isolated deep dark red abdomens*.

Dose μg	No. of isoloted abdomens used	Response to β-ecdysone**			No. of isolated abdomens which did not response
		Depigmentation	Prepupation	Pupation	
20	5	0	3	5	0
10	5 (1)	1	1	0	0
	5 (4)	1	4	4	0
5	5 (1)	0	0	1	0
	5 (4)	3	3	1	1
3	5	4	3	0	1
1	5	3	0	0	2
Control	5	0	0	0	5

* Received β-ecdysone 2-3 days after ligation.

** Evaluation was made 5 days after the injection.

Table 2. Effect of time of ligation on depigmentation.

Time of ligation	No. of larvae used	Response		Change of mean* colour
		No depigmentation	Depigmentation	
Onset of dark reddening stage	7	7	0	4.9→4.9
Wandering stage	9	8	1	5.0→4.7 (3)**
Cocoon building stage	7	2	5	5.0→4.1 (3.7)
Shortly after formation of cocoon building	13	1	12	5.0→1.5 (1.1)

* Type 1: Pale green with darkening dorsal region.
Type 5: Deep dark red.
Type 2-4: Indermediate between 1 and 5.
** The number in parentheses indicates mean value in depigmentated individuals only.
Evaluation was made 3 days after ligation.

Table 3. Effect of JH and its analogues on the depigmentation in the deep dark red larvae.

Compound		No. of larvae used	Mean colour just after prepupation*
CT 5	50μg	7	1.7
ZR 512	20μg	8	3.1
	10	3	3.3
Cecropia JH	20μg	4	3.3
	10	6	2.5
Control		10	1.6

* Symbols as the same in Table 2.

ZR 512 inhibited the depigmentation in the deep dark red larvae of *Cephonodes hylas*, not only at the treated part of the integument but also at the other part of the integument, when tested at 10-20 μg level per individual. CT 5 was inactive up to 50 μg level.

These treated larvae pupated normally.

Discussion

The larvae of *Cerura vinula* (Lepidoptera, Notodontidae) exhibit chromatic change similar to that of *Cephonodes* larvae. Red pigmentation in the larval integument of *Cerura vinula* is brought about by a small increase of ecdysone and is inhibited by JH and its analogues (Bückman 1954, Hintze-Podufal and Fricke 1971). However, hormonal mechanism as to the disappearance of red pigment from epidermal cells of the integument of the deep red mature larvae, eventually raising pale green prepupae, has not been studied in this species.

The results of the present experiments demonstrate that such depigmentation as in the deep dark red larvae of *Cephonodes hylas* is induced by a considerable amount of molting hormone and inhibited by JH and its analogues. It seems likely that the depigmentation in the deep red larvae of *Cerura vinula* may be induced by molting hormone and inhibited by JH and its analogues as in the case of *Cephonodes* larvae.

The change in colour from green to dark red in the larvae of *Cephonodes hylas* at the end of the last instar is induced by molting hormone and inhibited by JH and its analogues (Ikemoto, in press and unpublished data). It is concluded that dark reddening, depigmentation and pupal molt in the last instar larvae of *Cephonodes hylas* are induced by the same kind of hormone, ecdysone, an effective concentration of that hormone being different with each step of metamorphosis, and are inhibited by JH and its analogues. Molting hormone increases gradually as normal course of event.

In some lepidopterous larvae, green larvae turn to brown or red in colour when winter is coming. It may be supposed that the body-colour change as described above is induced by considerable titer of ecdysone to such extent that pupation is not induced, and thereafter ecdysone titer decreases and JH titer increases so that red colouration may remaine unchanged for a long period of

winter.

Summary

Green larvae of *Cephonoded hylas* turn to deep dark red in colour 5 days after the last larval molt, 24 hours later vanishment of red pigments from the larval epidermal cells of the integument, and further 12 hours later pale green prepupae are raised.

Such depigmentation as mentioned above was induced by molting hormone and inhibited by juvenile hormone. It was also shown that the critical period during which the production of molting hormone necessary for depigmentation occurred at the middle stage of cocoon formation.

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A Stimulant of the American Cockroach, *Periplaneta americana* L. (Orthoptera: Blattidae), Occurring in *Solidago altissima* L. (Compositae). Chikao Nishino and Keiko Tsuzuki (Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo, Japan) Received Jan. 27, 1975. *Botyu-Kagaku*, 40, 62, 1975.

11. セイタカアワダチソウ (*Solidago altissima* L.) に含まれるワモンゴキブリ (*Periplaneta americana* L.) の興奮物質

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セイタカアワダチソウ (*Solidago altissima* L.) よりワモンゴキブリ (*Periplaneta americana* L.) の興奮物質を単離した．この物質 0.05mg を与えると，雄雌ともに活性試験を受けたゴキブリの10～20%が，5分以内に特徴ある行動で興奮した．この物質の構造は，化学的あるいはスペクトルによる知見からセスキテルペンアルコールである α-カジノールまたは T-カジノールのいずれかであることが判明した．

Since the insect moulting hormone, ecdysones, were isolated from some plants[1,2], many chemists have given their attentions to plant products possessing pheromonal or hormonal activity for insects.

Recently, Bowers and Bodenstein[3] reported that few plant-derived compounds, D-bornyl acetate, α-santalol and β-santalol, caused sexual excitement in the male American cockroach (*Periplaneta americana* L.). They also found that the male American cockroach shows typical sexual behaviour to the hexane or methanol extracts of 18 flowering plants. However, the structure of sex pheromone mimics still remains undetermined.

We also have been interested in active constituents occurring in plants for noxious insects, especially, cockroaches. As the first target, our investigations were directed to stimulants for the American cockroach in plants of the *Compositae* family found widely in Japan. By examining 12 species of them, the methanol extracts of 6 species produced strong response from the male cockroach (Table 1). Particularly, the cockroach exposured to the methanol extract of the leaf of *S. altissima* L. (*Seitaka-awadachi-so*) displayed an interesting behaviour which is different from the characteristic sexual excitment.

We have been carried out studies on the isolation and the structure of the active constituent of *S. altissima* which is the most heinous weed and distributed throughout Japan. The spectral and chemical evidences born out that the stimulant, $C_{15}H_{26}O$, should be α-cadinol or T-cadinol.

Cockroach used in bioassay and bioassay