

Endocrine Activities and Photoperiodic Sensitivity during Prediapause Period in the Phytophagous Lady Beetle, *Epilachna vigintioctopunctata*.

Yoshiaki KONO

*Pesticide Research Laboratories, Takeda Chemical Industries Ltd.
Ichijoji, Sakyo-ku, Kyoto 606, Japan*

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Epilachna vigintioctopunctata ceased to feed and entered diapause after 16 day's vigorous feeding in short-day photoperiods. Diapause could be prevented by the change from a short day to a long day before the 5th day of adult life. If the photoperiodic change occurred later, the beetle entered diapause even in a long day. Therefore, the prediapause period was divided into two stages. The secretory material in the neurosecretory cells of the pars intercerebralis changed in quantity corresponding to these stages. A small amount of the material represented the stage sensitive to a photoperiodic change, the scattering of small aggregates of the material the stage insensitive to a photoperiodic change, and a large amount of the material the early stage of diapause. Throughout these stages, the corpora allata were small in size as compared with those of long-day beetles. These correlations were also found in adults reared on sliced potato which did not enter diapause in spite of their ovarian degeneration in short days. The response of prediapause beetles to a juvenile hormone analogue (ZR-515®) showed that the latter triggered the ovarian development but failed to induce yolk deposition. Another factor, possibly the neurosecretory material from the brain, was necessary for the full ovarian development.

INTRODUCTION

Although many studies have been conducted on the photoperiodic induction of diapause in insects, several problems remain unsolved. In *Antheraea pernyi* (WILLIAMS and ADKISSON, 1964), *Pieris brassicae* (CLARET, 1966), *Leptinotarsa decemlineata* (DE WILDE and DE BOER, 1969), and *Pieris rapae* (KONO, 1975), it has been suggested from brain-implantation and other experiments that photoperiods regulate the release of the neurosecretory (NS) substance. This was confirmed by ultrastructural observations in *Pieris rapae* (KONO, 1975), in which different types of the diurnal rhythm of the NS cell activity are entrained by photoperiod. The activity of NS cells is reduced in a short day.

The ultrastructural features of the NS cells in the pars intercerebralis (PIC) of diapausing pupae of *Pieris*, however, show a much lower activity than in short-day larvae (KONO, 1977). Therefore, the NS activity in *Pieris rapae* changes from the prediapause to the diapause state at a certain stage. Physiological and morphological

comparisons between diapause and non-diapause individuals of *Pieris*, on the other hand, show that a particular physiological condition is established by a short day (CLARET, 1966, 1968; KONO, 1970, 1973). From these observations, the mechanism of diapause induction is supposed to comprise of several steps as follows: Reception of short days→Reduced activity of NS cells in the brain→The prediapause condition→Further decrease in activity of the NS cells→Diapause.

In insects which enter diapause (cessation of feeding and other activities) at the adult stage, such as *Leptinotarsa decemlineata* and *Epilachna vigintioctopunctata*, the prediapause state is easily defined by atrophy of the reproductive organs and development of the fat body. Recently, an interesting phenomenon in relation to the mechanism of diapause induction was found in *Epilachna vigintioctopunctata* (KONO, 1979). The adult of this species does not enter diapause in spite of the degeneration of ovary under short-day condition if they were reared on slices of potato.

In this study, the changes in secretory activity of the NS cells, in size of the corpora allata, and in reaction to a juvenile hormone analogue (JHA) were examined in order to elucidate further details of the steps involved in diapause induction.

MATERIALS AND METHODS

Larvae and adults of *Epilachna vigintioctopunctata* were reared on either food-plant foliage or slices of potato according to the method previously reported (KONO, 1979). The long and short photoperiods were 16L:8D and 10L:14D, respectively at 24–27°C.

For observations of NS cells in the PIC and corpus allatum (CA), adults were sacrificed at intervals and cut heads were fixed in formaldehyde 5–7 hr after light-on in order to avoid possible biases due to the daily fluctuation of endocrine activity. The secretory material in the NS cells was stained *in situ* by aldehyde-fuchsine (DOGRA and TANDAN, 1964). At least 5 brains were observed by a light microscope in each ex-

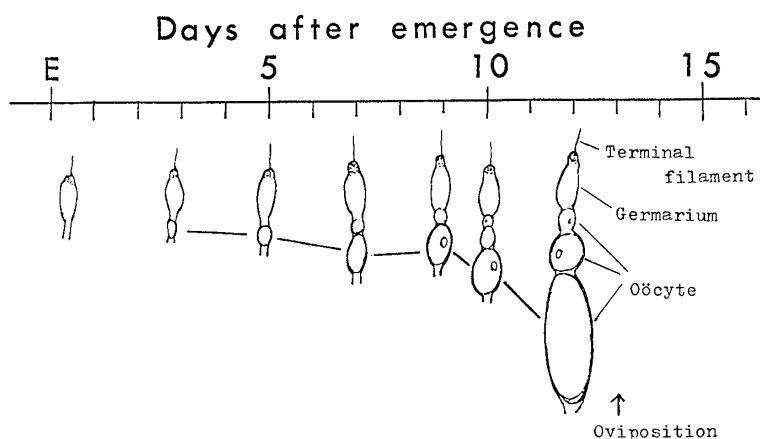


Fig. 1. Development of oöcytes in the ovariole when the adult beetles were reared on slices of potato under long-day conditions (16L-8D, 25°C). The developmental stage is denoted by the number of days after emergence under these conditions; for example, stage 1 (day 1)—no oöcyte in vitellarium; stage 12 (day 12)—matured oöcyte; stage 13 (day 13)—oviposition.

perimental group. The diameter of CA was measured by an eye piece micrometer along the long axis. Ovaries were observed under a dissecting microscope and their developmental stages were scored according to the ovarian development when reared on sliced potato in long days (Fig. 1).

One μ l per individual of acetone solution containing JHA (0.5 or 4 μ g of ZR-515®) was applied topically on the ventral surface of the abdomen of beetles.

RESULTS

1. Changes in secretory activity of the NS cells of PIC

When adults were reared on foliage of the food-plant in short days, their responsiveness to transfer to the long day was retained until the 5th day of adult life (Fig. 4-IV). If continuously kept in the short day, they ceased feeding on about the 16th day. The NS cells were therefore, observed on the 5th, 12th and 18th days in the short day. Those of 5-day-old adults in the long day were also observed for comparison.

On the 5th day, the NS material aggregated to form larger masses in the short day than in the long day (Fig. 2). On the 12th day of adult life, many small aggregates of the NS material appeared in the short day. After the cessation of feeding on the 18th day, the secretory material increased and formed large masses which include vacuoles.

2. Change in diameter of CA

Under the long day, the CA grew larger in size till oviposition. After the transfer to the short day, the CA diameter decreased. When the adults were kept in the short day from emergence, their CA remained small in size (Fig. 3). The CA of diapausing

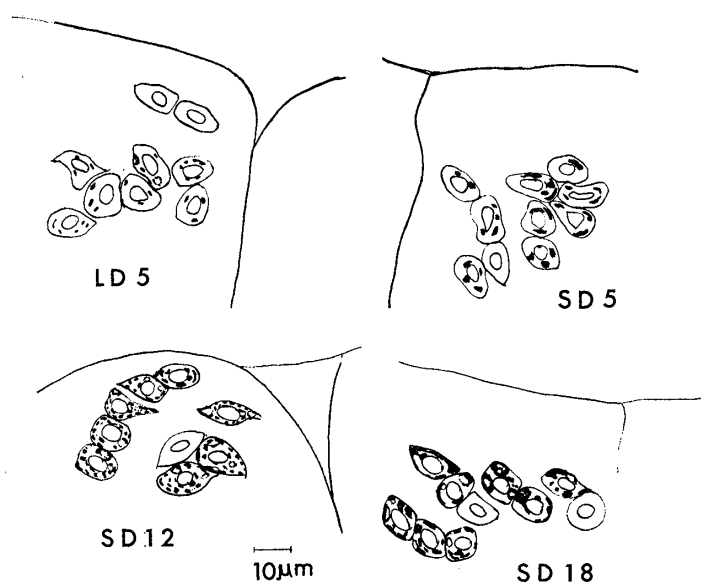


Fig. 2. Accumulation of the secretory material stained with paraldehyde-fuchsin in the NS cells of PIC. LD5: 5-day-old adults in the long day, SD5, SD12, SD18: 5-, 12- and 18-day-old adults in the short day.

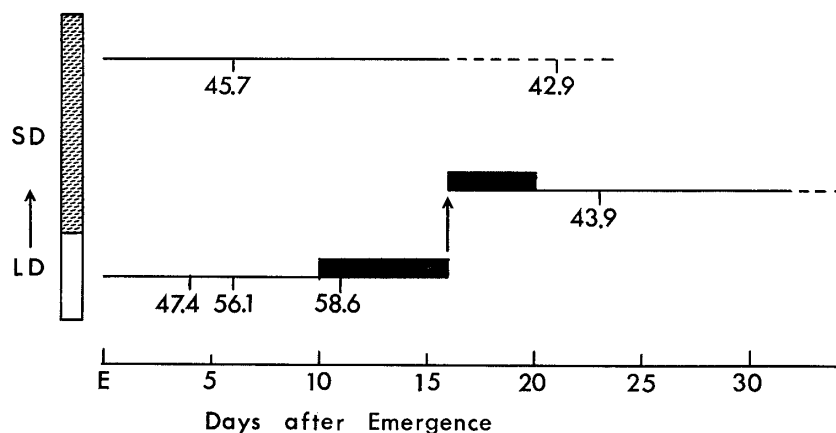


Fig. 3. Change in diameter (μm) of the CA in the adult *Epilachna*. SD : short day ; LD : long day ; solid and dotted lines : feeding and not feeding, respectively ; black bar : oviposition.

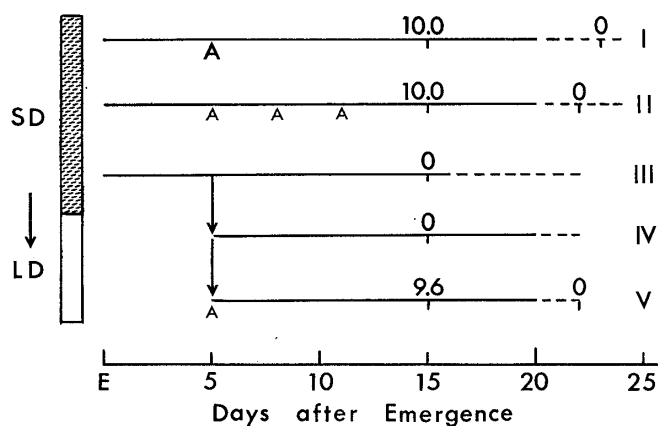


Fig. 4. Effect of JHA application on the ovarian development in adults reared on food-plant foliage. Small and large A show 0.5 and 4 $\mu\text{g}/\text{insect}$ topical application, respectively. SD : short day ; LD : long day ; solid line : feeding ; dotted line : not feeding ; numerals along the line : the developmental stage of ovary, ↓ : change of photoperiod. Each group consisted of 10 females and 10 males.

adults were the smallest, being about 70% in diameter of those of ovipositing adults.

3. Effect of JHA application

(1) *Adults reared on foliage.* In the short day, the ovaries did not develop in adults reared on foliage. When 0.5 or 4 μg of JHA per insect was applied to them, ovaries develop to Stage 10 (Fig. 1), but no further development occurred. The adults entered diapause after the degeneration of the oöcytes in spite of JHA application (Fig. 4-I). The same result was obtained with a three times application of 0.5 $\mu\text{g}/\text{insect}$ JHA (Fig. 4-II). Application of JHA to adults transferred to the long day after 5 short days resulted in incomplete development of ovaries (Fig. 4-V).

(2) *Adults reared on potato.* When reared on slices of potato, adults never entered

diapause even in the short day in spite of the ovarian degeneration (Fig. 5-VI). JHA did not induce complete development of their ovaries regardless of the time of application (Fig. 5-IV, V).

JHA application failed to attain the last stage of yolk deposition in adults reared either on foliage or on sliced potato. In some cases, 4 oöcytes were found in each ovariole, a situation never observed in normal females. Application of JHA to the adults, whose ovaries had already begun to develop in the long day, could not bring about the full maturation of oöcyte when they were subsequently transferred to the short day (fig. 5-III). Therefore, application of JHA did not seem to substitute for the long-day effect.

4. Influences of rearing on potato and JHA application on the endocrine system

The NS cells and CA size in adults reared on slices of potato and in those given

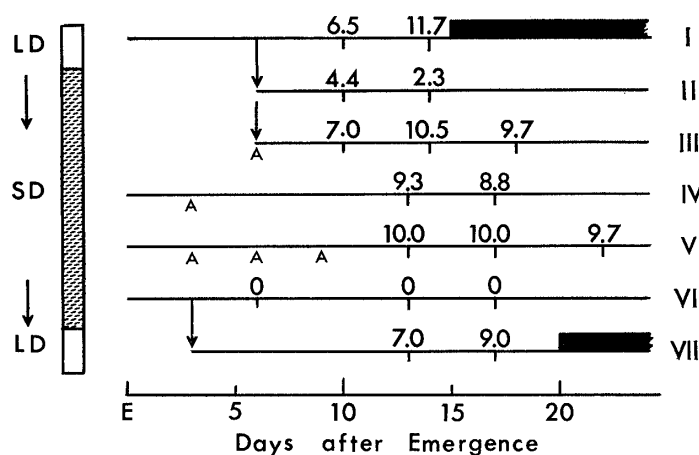


Fig. 5. Effect of JHA application on the ovarian development in adults reared on slices of potato. Black bar : oviposition. Other symbols are the same as in Fig. 4.

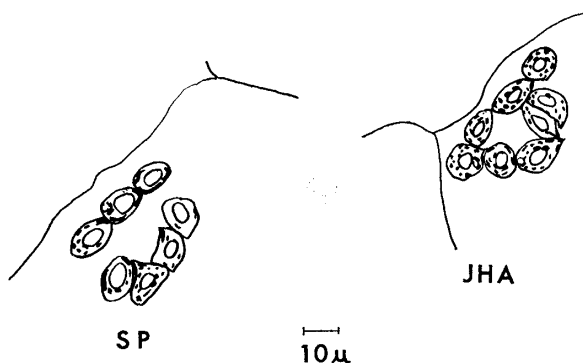


Fig. 6. Appearance of the NS cells in PIC in adults reared on sliced potato or treated with JHA. SP : the 12th day of adult life on sliced potato ; JHA : the 12th day treated with JHA and reared on the foliage.

Table 1. CA DIAMETER OF 12-DAY-OLD ADULTS REARED ON SLICED POTATO OR TREATED WITH JHA UNDER SHORT-DAY CONDITIONS

| Treatment | CA diameter |
|--|--------------------|
| Fed on sliced potato | 48.2 μm |
| JHA treatment ^a and reared on the foliage | 40.5 μm |

^a JHA was applied on the 5th day of adult life (0.5 $\mu\text{g}/\text{insect}$).

0.5 μg JHA on the 5th day of short-day treatment were observed 12 days after adult emergence. In the former, the NS material was not so much accumulated in the cytoplasm, but the CA remained in a small size. In the latter, many small aggregates of the secretory material scattered in the NS cells. The general appearance was the same as that in 12-day-old adults kept in the short day without JHA application (Fig. 6). The CA were small, being not influenced by the JHA application (Table 1).

DISCUSSION

1. *Diapause termination by JHA*

Termination of adult diapause by JH or JHA has been reported in several coleopterous insects, such as *Hypera postica* (BOWERS and BLICKENSTAFF, 1966), *Oulema melanopus* (CONNIN et al., 1967) and *Semiadalia undecemnotata* (HODEK et al., 1973). In the last species, however, a large dose of JHA is necessary to terminate diapause and induce oviposition; for example, about 100 $\mu\text{g}/\text{insect}$ topical application of ZR-515® is required to resume activity, although a much lower dose of the same chemical (0.04 $\mu\text{g}/\text{insect}$) inhibits the pupal-adult metamorphosis (SEHNAL, 1976).

Implantation of active CA does not evoke oviposition in the diapausing beetle of *Leptinotarsa decemlineata*, although the same treatment is effective in allatectomized females (DE WILDE and DE BOER, 1969). ZR-515®, which inhibits pupal-adult metamorphosis with a dose of 0.001 $\mu\text{g}/\text{insect}$, does not terminate diapause in *Leptinotarsa* even with a higher dose (SEHNAL, 1976). In *Epilachna* at the prediapause stage, 0.5 μg ZR-515®/insect was enough to stimulate the ovarian development, but neither a three times treatment with 0.5 $\mu\text{g}/\text{insect}$ nor a single one with 4 $\mu\text{g}/\text{insect}$ completed the full yolk deposition in the ovary (Fig. 4). In these species, therefore, some other factor, probably the neurosecretion from the brain, as inferred in *Calliphora* (THOMSEN, 1952), *Schistocerca* (HIGHNAM, 1962), and *Tenebrio* (LAVERDURE, 1972), must be necessary along with JH for the complete development of ovaries.

The NS cells remained inactive after application of JHA in *Epilachna* (Fig. 4 and 5). In *Pterostichus nigrita* also, the neurosecretion in addition to JH has been suggested to be necessary for the yolk deposition based on histological observations (HOFFMANN, 1970).

It can be concluded that the reproductive diapause in these insects is not caused and maintained only by inactivation of CA as a result of shortage of the neurosecretion from the brain.

2. *Photoperiodic sensitivity and endocrine features*

The prediapause period should be divided into two stages in *Epilachna* reared on

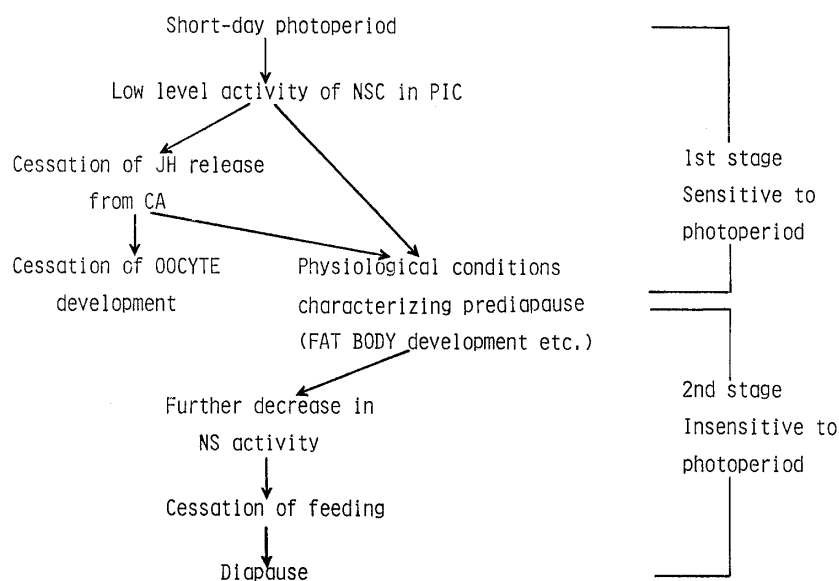


Fig. 7. Mechanism of the photoperiodic induction of diapause in *Epilachna*.

food-plant foliage. The first stage is from the adult emergence to the 5th day, during which the adult maintains the sensitivity to the change from a short day to a long day. The second stage is from the 6th day to the cessation of feeding; the adult loses the sensitivity to a photoperiodic change, but continues feeding. The adult then ceases to feed and enters diapause.

The appearance of NS cells represents the two prediapause and early diapause stages. The NS material increases in the cytoplasm in the successive stages. It seems that the reaction to a change from short day to long day conditions occurs only when the NS material is not accumulated in the cytoplasm, because the 12th-day-old adults reared on sliced potato retains its photoperiodic sensitivity and has only a small amount of the secretory material in their NS cells.

For the diapause incidence, the accumulation of a large amount of the material in the NS cells of PIC must be necessary, and this does not seem to be triggered directly by a short photoperiod, since adults reared on sliced potato never enter diapause in spite of ovarian degeneration caused by JH shortage. Scattered aggregates of secretory material in the NS cells are always accompanied by a small size of CA that might indicate decreased activity. In *Epilachna* as in *Leptinotarsa decemlineata* (DE WILDE and DE BOER, 1969), the activity of NS cells of PIC seems to be decreased by short days, and consequently the secretory activity of CA is suppressed.

In the second stage of prediapause, fat bodies develop markedly in adults reared on food-plant foliage (KONO, unpublished data). The physiological state characterized by such fat body development seems to be related to the further accumulation of the secretory material in the NSC cytoplasm for the entrance of diapause. The adults reared on sliced potato could not attain to this physiological state because of the poor fat body development due to nutritional deficiency.

The model of 'two-steps-determination of diapause' as discussed above (Fig. 7) seems to explain the nutritional alteration of diapause determination in insects.

REFERENCES

- BOWERS, W. S. and C. C. BLICKENSTAFF (1968) Hormonal termination of diapause in the alfalfa weevil. *Science*. **154** : 1673-1674.
- CLARET, J. (1966) Mise en évidence du rôle photorecepteur du cerveau dans l'induction de la diapause, chez *Pieris brassicae*. *Annales d'Endocrinologie* **27** : 311-300.
- CLARET, J. (1968) Modifications physiologiques provoqués par la photoperiode pendant la prédiapause chez la chenille de *Pieris brassicae*. *C. r. Acad. Sci. Paris (D)* **266** : 1156-1159.
- CONNIN, R. V., O. K. JANTZ and W. S. BOWERS (1967) Termination of diapause in the cereal leaf beetle by hormones. *J. econ. Entomol.* **60** : 1752-1753.
- DOGRA, G. S. and B. K. TANDAN (1964) Adaptation of certain histological techniques for in situ demonstration of the neuroendocrine system of insects and other animals. *Quart. J. micr. Sci.* **105** : 455-466.
- HIGHNAM, K. C. (1962) Neurosecretory control of ovarian development in *Schistocerca gregaria*. *Quart. J. micr. Sci.* **103** : 57-72.
- HODEK, I., Z. RUZICKA and F. SEHNAL (1973) Termination of diapause by juvenoids in two species of ladybirds. *Experientia* **29** : 1146-1147.
- HOFFMANN, H. J. (1970) Neuro-endocrine control of diapause and oöcyte maturation in the beetle, *Pterostichus nigrita*. *J. Insect Physiol.* **16** : 629-642.
- KONO, Y. (1970) Photoperiodic induction of diapause in *Pieris rapae crucivora* Boisduval. *Appl. Ent. Zool.* **5** : 213-224.
- KONO, Y. (1973) Difference of cuticular surface between diapause and non-diapause pupae of *Pieris rapae crucivora*. *Appl. Ent. Zool.* **8** : 50-52.
- KONO, Y. (1975) Photoperiodic sensitivity of the implanted brain of *Pieris rapae crucivora* and ultrastructural changes of its neurosecretory cells. *Jap. J. appl. Ent. Zool.* **17** : 203-209.
- KONO, Y. (1975) Daily changes of neurosecretory type-II cell structure of *Pieris* larvae entrained by short and long days. *J. Insect Physiol.* **21** : 249-264.
- KONO, Y. (1977) Ultrastructural changes of neurosecretory cells in the pars intercerebralis during diapause development in *Pieris rapae*. *J. Insect Physiol.* **23** : 1461-1473.
- KONO, Y. (1979) Abnormal photoperiodic and phototactic reactions of the beetle, *Epilachna vigintioctopunctata*, reared on sliced potatoes. *Appl. Ent. Zool.* **14** : 185-192.
- LAVERDURE, A-M. (1972) L'évolution de l'ovaire chez la femelle adulte de *Tenebrio molitor*. La prévitellogenèse. *J. Insect Physiol.* **18** : 1477-1491.
- SEHNAL, F. (1976) Action of juvenoids on different groups of insects. In : *The Juvenile Hormones* (ed. by L. I. GILBERT). New York and London : Plenum Press, pp. 301-322.
- THOMSEN, E. (1952) Functional significance of the neurosecretory brain cells and corpus cardiacum in the female blow-fly, *Calliphora erythrocephala*. *J. exp. Biol.* **29** : 137-172.
- DE WILDE, J. and J. A. DE BOER (1969) Humoral and nervous pathways in photoperiodic induction of diapause in *Leptinotarsa decemlineata*. *J. Insect Physiol.* **15** : 661-675.
- WILLIAMS, C. M. and P. L. ADKISSON (1964) Physiology of insect diapause, XIV. An endocrine mechanism for the photoperiodic control of pupal diapause in the oak silkworm, *Antheraea pernyi*. *Biol. Bull.* **127** : 511-525.