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It was reported previously that topical treatments of high doses of a juvenile hormone analogue (JHA) induce prolonged larval duration in the last larval instar: such larvae are called "JH-induced eternal larvae" or "dauer larvae" (Akai and Kobayashi, 1971; Akai *et al.*, 1973). These larvae survived another 15 days or more in the 5 th instar without any signs of spinning or metamorphosis. When the dosage of treatment was reduced, however, larval duration terminated within additional 2 or 3 days as compared with normal development, and the cocoon shell weight was increased (Akai *et al.*, 1971, 1973).

As mentioned in a previous paper (Akai *et al.*, 1988), we noticed a relationship between ecdysteroid titers in the hemolymph and the wandering and spinning behaviors during the last larval instar. Our experiments were successful in inducing spinning or cocooning from the eternal larvae provoked by a high dose of JHA, and these larvae spun enormous cocoons.

The next problem was to elucidate what change of ecdysteroid level in the hemolymph was induced by JHA and ecdysteroid treatments. Kajiura *et al.* (1987) applied JHA to the penultimate instar larvae and revealed that the resultant eternal larvae were characterized by decreased levels of ecdysteroids in the hemolymph. And although they also observed that oral administration of exogenous 20-hydroxyecdysone into the eternal larvae resulted in the dorsal vessel exposure and cocoon spinning, they didn't show the ecdysteroid titers. In this short communication we describe the effctes on ecdysteroid level in the hemolymph by JHA or ecdysteroid treatments in order to ascertain and compensate the previous knowledge.

Matherials and Methods: The larvae of hybrids from a cross between two varieties of *Bombyx mori* (J 02×C 02) were reared on an artificial diet (Kuwanohana) in a culture room at 25-28°C and 12 D : 12 L photoperiod. A high dose of methoprene (150 μg/5 μl acetone) was topically supplied at the 48 th hr after the first feeding of the 5 th instar. Diets containing 20 ppm of 20-hydroxyecdysone (ecdysterone) per dry weight of diet were offered on the 11 th day after the 4 th ecdysis (Fig. 1).

Samples from normal larvae (control) were prepared at 1 day intervals from day 0 to day 15 after the 4 th ecdysis. Samples from JHA-treated larvae were also prepared at 1 day intervals from day 2 after the 4 th ecdysis. In ecdysterone-treated larvae, samples were prepared from day 11 to 19.

For measurments of ecdysteroid level in the hemolymph, 10 μl of hemolymph was collected from each individual and the ecdysteroid titer was determined by radioimmunoassay (Takeda *et al.*, 1986).

Results and Discussion: Changes of ecdysteroid levels from the three types of samples, con-

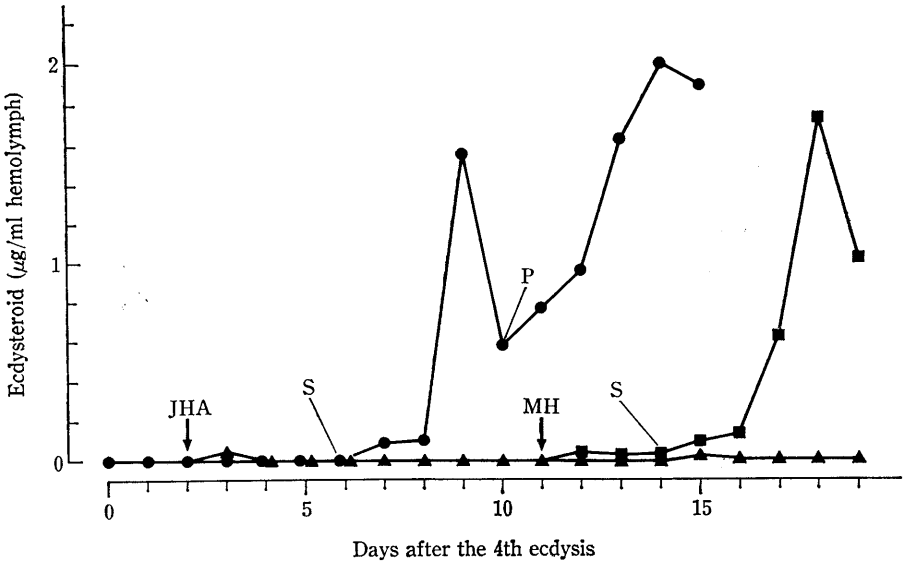

Fig. 1. Ecdysteroid levels in hemolymph of *Bombyx mori* larvae.
● : control, ▲ : methoprene-treated and ■ : ecdysterone-treated.
JHA : methoprene ; MH : ecdysterone ; P : pupation ; S : spinning.

trol, JHA-treated eternal larvae, and ecdysterone-administrated eternal larvae, were measured by radioimmunoassay (Fig. 1).

In the control larvae the ecdysteroid level remained at a very low level from day 0 to day 6 after the 4 th ecdysis, and then increased in conjunction with spinning. The ecdysteroid peak before pupation showed 1.6 μg/m*l* hemolymph on day 9 after the 4 th ecdysis, and decreased to 0.6 μg/m*l* hemolymph at pupation (day 10). After pupation, the ecdysteroid level again increased, and attained 2 μg/m*l* hemolymph on day 4 after pupation. Similar patterens of the ecdysteroid level in the hemolymph have been reported previously (Calvez *et al.*, 1976 ; Kiguchi *et al.*, 1985).

In the experiment with JHA-invoked eternal larvae, all of the test animals continued feeding 9 more days after the control larvae began to spin, and became huge eternal larvae, 11 to 14 grams in body weight. The ecdysteroid level from these eternal larvae increased slighty (0.05 μg/m*l* hemolymph) only on day 1 after the JHA treatment (Fig. 1), but the level returned to zero the next day and remained low until day 12 after the JHA-treatment. These results suggested that the methoprene treatment repressed the ecdysteroid titer in the hemolymph, although it is not yet clear whether it was inhibiting JH secretion from corpus allatum cells or not. This finding will be of great value in the manipulation of larval development from the view point of hormonal control (Akai *et al.*, 1988).

The ecdysteroid treatment on day 9 after the JHA-treatment induced a slight increase in ecdysteroid level (0.04 μg/ml hemolymph) the next day, and also induced the start of spinning on day 3 and an ecdysteroid peak on day 7 after the ecdysteroid treatment (Fig. 1). No evidence was obtained, however, of whether the increase in ecdysteroid level originated from the diet or from prothoracic gland cells stimulated by the

ecdysterone treatment. In some insects such as *Mamestra brassicae*, ecdysone activates secretion of prothoracicotropic hormone (PTTH) in the brain (Agui and Hiruma, 1977). It is possible that ecdysteroid derived from the diet activates PTTH secretion in the brain, which, in turn, stimulates prothoracic gland cells.

In conclusion, the JHA treatment inhibited the augmentation of the ecdysteroid level in the hemolymph, and the ecdysteroid treatment of the eternal larvae increased the ecdysteroid level slowly in the hemolymph to cause the recovery of spinning ability.

References

AGUI, N. and HIRUMA, K. (1977): Gen. Comp. Endocrinol. **33**,, 467-472.

AKAI, H. and KOBAYASHI, M. (1971): Appl. Ent. Zool., **6**, 138-139.

AKAI, H., KIGUCHI, K. and Mori, K. (1971): Appl. Ent. Zool., **6**, 218-220.

AKAI, H., KIGUCHI, K. and MORI, K. (1973): Bull. Seric. Exp. Sta., **25**, 287-305.

AKAI, H., TAKABAYACHI, K. and KIUCHI, M. (1988): J. Seric. Sci. Jpn., **57**, 341-344.

Calvez, B., Hirn, M. and de Reggi, M. (1976): FEBS Lett., **71**, 57-61.

KAJIURA, Z., KADONO-OKUDA, K. and YAMASHITA, O. (1987): J. Seric. Sci. Jpn., **56**, 398-406.

KIGUCHI, K., AGUI, N., KAWASAKI, H. and KOBAYASHI, M. (1985): Bull. Seric. Exp. Sta., **30**, 83-100.

TAKEDA, S., KIUCHI, M. and UEDA, S. (1986): Bull. Seric. Exp. Stat., **30**, 361-374.