21. The Control of Growth and Development in *Bombyx mori*. XXXVII

Some Aspects on a Hormone Controlling Adult Eclosion

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Some aspects on a hormone controlling adult eclosion are described in this paper. This hormone seems to be organized in brain neurosecretory cells.

Materials and methods. The eggs of hybrids, J. NO. 106 X Daizo, were incubated at 25°C in the light condition. The larvae developed from those eggs were reared at 25°C with a relative humidity of 75% under 12 hours of the daily illumination. After pupation, the pupae were maintained at 25°C in the light condition for 16 hours and in the darkness for 8 hours (16L:8D). Pupal days needed for adult eclosion were estimated as about 10 ± 1 days in this hybrid. For the assessment of an eclosion hormone pharate *Bombyx mori* moths were used. For at least one week prior to their use, the developing pupae were exposed to a defined photoperiod (photoperiod phase from 13.00 to 5.00). Under these conditions, the pupae emerged after 10 days at 25°C. At the 9th day of the pupal stage, the pupae were assayed for the eclosion hormone. One can recognize the presence of the eclosion hormone activity by eclosion within 1.0 to 3.0 hours after the hormone-injection. If the pupae did not emerge until 3.5 hours after injection, the assay was scored negative.

The extraction of the eclosion hormone used for the injection was prepared as follows: 1) The male adult or pupal heads (1000) were collected. 2) Acetone powder of the heads was prepared, and 2% NaCl was used for extraction of the eclosion hormone. 3) After heat treatment (90°C, 20 min) the solution was evaporated in reduced pressure and the residues were rinsed with chloroform / methanol (2:1). 4) Methanol fraction which was separated by centrifugation was evaporated, and the residues were re-extracted with 2% NaCl. The crude substance thus extracted was used for assessment.

Results. 1) *Effect of photoperiod on adult eclosion.* When the pupae were exposed to a defined photoperiod (16L:8D), adult eclosion was taken place during 9 to 11 days (Fig. 1). As shown in

Fig. 1

Fig. 2

Fig. 1. Patterns of eclosion under a 16L: 8D photoperiod. Ordinate: number of emerged individuals. Abscissa: hour. Black: darkness. White: light. A: 9-day-old pupae. B: 10-day-old pupae. C: 11-day-old pupae.

Fig. 2. Patterns of eclosion after light on under a 16L: 8D photoperiod. Ordinate: number of emerged individuals. Abscissa: minute after light on. ○: male. ●: female.

Fig. 2, adult eclosion was taken place within 3 hours after light on, while it did not appear until next light on. The maximum peak appeared at 45 minutes in the male, while at 60 minutes in the female.

2) *Effect of injection of crude extracts on eclosion.* When crude extracts were injected into the 9-day-old pupae, a large number of the test animals promoted their eclosion (Fig. 3). Percentage of positive response was 68–80 in the male and 64–84 in the female (Table I), showing a maximum peak at 60 minutes (Fig. 4).

3) *Relationship between eclosion and several substances.* Both 2% NaCl and ecdysterone showed no activity for *Bombyx* eclosion hormone assay as shown in Table I.

Discussion. Truman (1973), working on eclosion of the Chinese oak silkworm, stated that the eclosion hormone produced in the brain and transported to the corpus cardiacum was a key role of eclosion. According to him, the eclosion hormone may be present in the holometabolous of insects. It remains unknown whether our crude extracts are of similar nature, or not, to the eclosion hormone extracted by Truman with insect ringer solution. Since brainless pupae

Fig. 3

Fig. 4

Fig. 3. Patterns of eclosion after injection of crude extracts. Ordinate: number of emerged individuals. Abscissa: hour after injection. A: injection of 2% NaCl (control). B: injection of crude extracts.
 Fig. 4. Pattern of eclosion after injection of crude extracts. Ordinate: number of emerged individuals. Abscissa: minute after injection.

Table I. Effect of injection of 2% NaCl, ecdysterone and crude extracts on adult eclosion

Substances		No. of assay	Injected dose	No. of eclosion	% of positive response
2% NaCl	♀	100	10 μ l	0	0
		25	1 μ g	0	0
Ecdysterone	♀	25	5 μ g	0	0
		25	7.5 μ g	0	0
		25	10 μ g	0	0
		25	10 μ l	18	72
Crude extracts	♂	25	10 μ l	17	68
		25	10 μ l	20	80
		25	10 μ l	17	68
		25	10 μ l	16	64
	♀	25	10 μ l	19	76
		25	10 μ l	21	84
		25	10 μ l	19	76
		25	10 μ l	19	76

emerged at random (Oshiki, personal communication), and no adult eclosion was taken place with 3 hours after light on under the ecdysterone-injection. The titer of the molting hormone releasing from the prothoracic glands during the pupal stage is high in the first half, while very low in the second half (Hanaoka *et al.* 1974). Accordingly, the molting hormone has no effect on adult eclosion. It must be determined in the near future whether this eclosion

hormone is the same as the brain hormone activating the prothoracic glands or not.

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