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Brain Hormone in the Head of *Bombyx* Moth (Lepidoptera : Bombycidae)¹

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Since the first active extract of the brain hormone from the pupal brain of the silkworm, *Bombyx mori* was prepared by KOBAYASHI and KIRIMURA in 1958, many papers on the chemical characteristics of an active ingredient of the brain hormone were clarified (GERSCH and STÜRZEBECHER, 1968; ICHIKAWA and ISHIZAKI, 1963; ISHIZAKI, 1969; ISHIZAKI and ICHIKAWA, 1967; KOBAYASHI and YAMAZAKI, 1966, 1974; NISHITSUTSUJI-UWO, 1972; NISHITSUTSUJI-UWO and NISHIMURA, 1975; SUZUKI *et al.*, 1975; WILLIAMS, 1967; YAMAZAKI and KOBAYASHI, 1969). Although several investigators obtained brain hormone materials from brains of insects, they could not obtain enough materials to work on its isolation owing to limitation of the collection of insect brains and little brain hormone content of the material.

Then we have investigated the brain hormone material besides pupal brains of the silkworm and it was found that the brain hormone was easily extracted from imaginal head in which the brain hormone was contained as much as that in the pupal brain. Therefore the heads of *Bombyx* moths

are a suitable material for brain hormone extraction. In 1969, ISHIZAKI has investigated changes in titer of the brain hormone during development of the silkworm and reported that the head of newly emerged moth contained high titer of the brain hormone. But it is not confirmed whether or not the brain hormone activity in the head extract is originated from the secretion of the brain. The experiment was performed in order to confirm this problem.

The present paper deals with the results of the investigation of the brain hormone in the head of *Bombyx* moth.

Collection of the head of brainless *Bombyx* moth: By the injection of ecdysone, the dauer pupae (artificially diapausing, debrained pupae) of *Bombyx mori* became brainless moths by progress of imaginal development and the heads of the resulting moths were cut off with a razor. The heads collected were acetone-dried and stored at -20°C until the experiment. Also as the control, the heads of the normal moths were collected soon after emergence and stored under the same condition mentioned above.

Extraction of the brain hormone from the head: Each acetone-dried heads obtained from brainless moth and normal one was homogenized in 2 percent. NaCl solution and the supernatant was obtained by centrifuging the homogenate. The supernatant was heated at 95°C for 5 min. The test solution as the brain hormone was obtained by the removal of the precipitate. From one hundred of the heads, 10 ml of the test solution was obtained.

Bioassay for brain hormone activity: The dauer pupae of the silkworm were used as test ob-

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Table 1. THE BRAIN HORMONE ACTIVITY OF THE EXTRACT FROM HEADS OF *Bombyx* MOTHS

Samples	Dose ^a (μ l)	No. of dauer pupae	No. of moths emerged (%)	No. of pupae dead
Normal	10	10	4 (40)	1
<i>Bombyx</i> moth	20	10	10 (100) ^b	0
	50	10	9 (90) ^b	1
	100	10	8 (80) ^b	2
Brainless	10	10	0 (0)	0
<i>Bombyx</i> moth	20	10	0 (0)	0
induced by injection of ecdysone	50	10	1 (10)	0
	100	10	1 (10)	0

^a Ten ml of the extract (test solution as the brain hormone) was obtained from one hundred of the heads of each sample.

^b Positive.

ject for the bioassay of the brain hormone as previously described (KOBAYASHI and KIRIMURA, 1958).

Each test solution was injected into test objects for the bioassay and tests were considered positive on experience with the bioassay, when 50% or more of the test objects pupated. The results obtained were presented in Table 1.

From the results, it was found that the brain hormone was not contained within the head of brainless moth induced from the dauer pupa by the injection of ecdysone, even though the hormone was contained within the head of the normal moth. This fact suggests that the brain hormone in the head of newly emerged, normal moth is secreted from neurosecretory cells in the brain during the imaginal development.

Then, with the extract of the heads in normal *Bombyx* moth, the nature of the active ingredient was investigated and it was found that the active ingredient was precipitated with 40 to 70 percent. saturation of ammonium sulfate, 35 to 55 percent. (v/v) of acetone. By digestion with Pronase or trypsin it was inactivated. From the results, the nature of the active ingredient in the head of *Bombyx* moth is seemed to be the same as that in the brain of *Bombyx* pupae (YAMAZAKI and KOBAYASHI, 1969).

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