

カイコ蛹へのアデノシン5-リン酸注射による非休眠卵出現について

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zoa was induced.

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Occurrence of Non-Diapause Eggs by Injection of Adenosine-5'-Monophosphate into *Bombyx Mori* Pupae^{1,2}

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Since the extraction of the diapause hormone responsible for arrested embryonal development of the silkworm *Bombyx mori* was achieved by HASEGAWA (1957), the properties of the hormone have been clarified (ISOBE *et al.*, 1976). The voltinism of this insect, however, is changed by exogenous chemicals. For example, non-diapause eggs are produced in diapause egg producers injected with uranyl nitrate (HASEGAWA, 1943), 2,4-dinitrophenol (DNP) (TAKEI and NAGASHIMA, 1970), and ouabain (TAKEDA and HASEGAWA, 1975). The reverse case was demonstrated by injections of potassium chloride (YOSHITAKE, 1954) and ouabain (TAKEDA and HASEGAWA, 1976).

Recently, the properties of fructose-1,6-diphosphatase (FDPase), which is a key enzyme in the pathway of gluconeogenesis in mammals, were surveyed in pharate adult ovaries of *Bombyx mori* (SUZUKI *et al.*, 1973). This enzyme activity *in vitro* is inhibited by adenosine-5'-monophosphate (AMP), but not by adenosine-5'-triphosphate (ATP). During the course of our study on the role of FDPase *in vivo*, we have found that AMP functions in part to alter the diapause nature of this insect. The preliminary results are described in this paper.

Silkworm pupae used here were Daizo (bivoltine race), which produce diapause eggs by exposure to heat of 27°C and light during their embryonic life. In addition, Akajuku and Shuko × Ryuhaku of univoltine silkworm races were used.

Nucleotides to be tested were dissolved in distilled water or 50 mM Tris-maleate buffer (pH 7). Ten to 30 µl of these solutions were injected into female pupae and pharate adults of different ages through the tergal intersegment membrane between the 4th and 5th abdominal segments, avoiding the dorsal vessel. The doses were adjusted so as to be expressed as final concentrations *in vivo*. To consider the *in vivo* concentration of nucleotides injected, the water content of the silkworm pharate adults used here was tentatively estimated from the volume of supernatant fluid after centrifugation of homogenates to be about 60% of body weight.

To survey the effect of chemicals on diapause alternation, diapause eggs and non-diapause egg shells in each batch were counted about 20 days after oviposition, when larvae have already hatched from non-diapause eggs.

Nucleotides used were obtained from Boehringer Mannheim Corp.; AMP (disodium salt), ATP (disodium salt).

First, nucleotides were tested on pharate adults of univoltine races (Akajuku and a hybrid of Shuko × Ryuhaku) at different stages of pupal-adult development. The injections of various amounts of ATP and AMP did not significantly alter the diapause nature of the resultant eggs. Although all eggs showed a characteristic color for diapause eggs after AMP injections, a few larvae hatched from the eggs 20 days after oviposition. For example, 2 of the injected 5 adults of the Akajuku race laid mixed eggs of colored diapause and colored

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Table 1. OCCURRENCE OF NON-DIAPAUSE EGGS IN DIAPAUSE EGG PRODUCERS (DAIZO RACE) INJECTED WITH AMP

Nucleotide	Dose injected ^a (μ mole)	Final conc. <i>in vivo</i> (mM)	No. of pupae injected	No. of adults laying non-diapause eggs and their occurrence in each bath			
				Adult	Diapause egg	Non-diapause egg	Non-diapause %
AMP	0.8	1	5 (4) ^c	0			0
	4	5	6 (6)	1	372	49	11.6
	8	10	6 (6)	2	96	274	74.1
					0	124	100
					95	1	1
	16 ^b	20	10 (8)	4	287	59	17.1
					191	94	33
160					249	60.9	
24	30	5 (1)	1	0	111	100	
— ^d			(48)	1	251	93	27

^a Nucleotide was injected into 4-day-old female pupae except 16 μ moles of AMP^b where 16 μ mole AMP dissolved in 30 μ l were injected dividedly into same pupa at 2, 3 and 4 days of age with 10 μ each.

Numerals in the parentheses denoted the number of adults laying eggs.

^d One of 48 non-injected pupae laid mixed eggs of diapause and non-diapause. In other controls, all producers injected with distilled water or buffer did not lay non-diapause eggs.

non-diapause eggs, the mean percentage of the non-diapause types being about 3.1.

The same kind of experiment was further carried out in a bivoltine race, Daizo, with AMP which was injected at different stages of pupal-adult development, 1 to 7 days. AMP injection caused production of non-diapause eggs in varying degrees and the production depended upon the developmental stages, middle stages being most sensitive to AMP injection.

The dose-response of AMP was then examined; the results are summarized in Table 1. Non-diapause eggs were produced by AMP injections at a dosage of more than 0.8 μ moles, and the percentage increased with increased dosages. It should be noted here that numbers of moths which laid non-diapause eggs were small in all experimental groups. To improve the response to AMP, a total of 16 μ moles of AMP was divided into 3 parts and injected successively into the same animals at 2, 3 and 4 days (see footnotes in Table 1). Such an application appeared to more effectively induce non-diapause egg producers but the percentage of non-diapause eggs was not so promoted. In another series of experiments done with the same producers, almost the same results were obtained as shown here. Therefore, it is conceivably that AMP may not directly affect the alternation

of the diapause nature of silkworms.

Endogenous AMP concentrations in some insects are reported to fall within the range of 0.1 to 1.0 mM, but this differs by tissue and developmental stage (CAREY and WYATT, 1963; SACKTOR and HURLBURT, 1966; CROMPTON and BIRT, 1967). Assuming that these values are applicable to the AMP concentration in silkworms, the concentration required for induction of non-diapause eggs is 20-fold higher than that of endogenous AMP. Further, the effective concentration is also higher than that for inhibition of fructose-1,6-diphosphatase activity (SUZUKI et al., 1973). ATP showed no effect on non-diapause egg production in the present study. However, TAKEI and NAGASHIMA (1970) have shown that ATP can fortify non-diapause egg production induced by dinitrophenol injections. Accordingly, further studies are required to consider AMP action in the control mechanism of the diapause nature of silkworms, but inhibition of FDPase by AMP may contribute in part to the reduced glycogen accumulation in ovaries which results induction of non-diapause eggs in silkworms (YAMASHITA and HASEGAWA, 1965).

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Preliminary Observations on the Mating Behaviors of the White-spotted Tussock Moth, *Orgyia thyellina* (BUTLER) (Lepidoptera : Lymantridae)^{1,2}

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Some observations on the mating behaviors of the tussock moth *Orgyia* spp. have been reported in relation to their ecology and sex pheromones (RERCY et al., 1971; ODERA, 1972; GRANT, 1975; GRANT et al., 1975). However, no attention has been given to morphological differences in their wings. The author assumed that differences in flying ability are closely associated with the mating behaviors. Preliminary observations were conducted on the mating behaviors of an insect species which shows a dimorphism in the wing form.

The insect used was the white-spotted tussock moth *Orgyia thyellina*. The wing dimorphism of this species appears in only the female and it was found to be controlled by the photoperiod during the larval stage (KIMURA and MASAKI, 1977; SATO,

1977). The material was collected from the field of the Fruit Tree Research Station at Hiratsuka, and the larvae were reared on an artificial diet of the silkworm, *Bombyx mori*, by the same method previously reported by Sato (SATO, 1977). Pupae obtained were placed in a pair of transparent ice-cream cups (6 cm in diam. and 5.5 cm in height). Rearing and observation were made at 25°C. The photoperiod was set for a longday (LDC; light-on, 6 : 00; light-off, 22 : 00) or a short-day condition (SDC; light-on, 6 : 00; light-off, 18 : 00). Observation of the adult emergence and mating was conducted at 30 minute intervals. In the dark period, a red electric lamp (5 w) was used to facilitate the observation. Attraction tests with virgin females in the field were carried out twice, once in late September, 1975 and again in late June, 1976. The females used in these tests were reared under natural photoperiodic conditions in the laboratory. Under the natural conditions, almost all females appear in brachypterous form in autumn and macropterous form in summer (KIMURA and MASAKI, 1977; SATO, 1977). The number of males trapped was surveyed at three hour intervals.

Emergence time of the adult moths. The results of observation of the emergence time are shown in Fig. 1. The adults under the LDC emerged mainly between 7 hr and 13 hr after light-on, showing a

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