Studies on Eupyrene and Apyrene Spermatozoa in the Silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae)

VI. The Factor Related to the Shrinkage of Apyrene Spermatozoa

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As reported in the previous paper (KATSUNO, 1977), apyrene spermatozoa of a curved shape in the seminal vesicle of the male adults shrunk immediately after ejaculation into the copulatory pouch of the female adults.

In the present paper the factor which induced the shrinkage of the apyrene spermatozoa was studied with use of a phase contrast microscope.

The material used in the present study was a hybrid of J 151 x C 131. The seminal vesicle and ejaculatory duct were removed from the unmated males. The seminal vesicle contained eupyrene sperm bundles and apyrene spermatozoa. The ejaculatory duct was divided into three parts, glandula spermatorphae, glandula alba and glandula prostatica, according to ÔMURA (1938). The three kinds of sample were prepared.

a) A very small amount of seminal fluid obtained from the seminal vesicle.

b) Three parts of the crushed ejaculatory duct: i, the middle part of the glandula spermatorphae cut in 3~4 mm; ii, the middle part of the glandula alba cut in 3~4 mm; iii, the middle part of the glandula prostatica cut in 3~4 mm.

c) A very small amount of SØRENSEN'S buffer solution of phosphatic acid (pH 6.5).

Each of three samples was mixed in four kinds of the combination as shown in Table 1 and the state of shrinkage of apyrene spermatozoa were observed by the hanging-drop method. The mixed sample was placed on a cover slip, and the cover slip was inverted over a quartz depression slide and sealed with liquid paraffin. The preparation was observed with a phase contrast microscope at 180 minutes after mixing of samples. Five preparations were prepared for each group and observed under a constant temperature (25°C). From five preparations in each group 500 apyrene spermatozoa were selected at random and the number of the apyrene spermatozoa of a curved and shrunken shape was counted.

As shown in Table 1, when a part of the glandula spermatorphae or glandula alba was present in the mixture (A and B group) the percentage of the shrunken apyrene spermatozoa was 0.8 and 1.2 respectively, and that was almost the same as the control (D group). On the other hand, when a part of the glandula prostatica was present (C group) the percentage was 82.6.

In lepidopterous insects, a description of the shrunken apyrene spermatozoa was only given by SUZUKI and ÔMURA (1951) and those spermatozoa were found in the seminal receptacle of the mated females of the silkworm. In the present paper, it was determined that the factor related to the shrinkage of apyrene spermatozoa lies in the secretory substance of the glandula prostatica. It was considered that the apyrene spermatozoa were activated by the secretory substance and some physiological changes occurred upon in the activation, and as a results, the shrinkage of those spermato-

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**Table 1. Number of Apyrene Spermatozoa which Shrank after Mixing of Samples**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sample</th>
<th>No. of observation of apyrene spermatozoa</th>
<th>Apyrene spermatozoa of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>a curved shape</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>a slightly shrunken shape</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>a shrunken shape</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>A</td>
<td>a</td>
<td>b-i</td>
<td>500</td>
</tr>
<tr>
<td>B</td>
<td>a</td>
<td>b-ii</td>
<td>500</td>
</tr>
<tr>
<td>C</td>
<td>a</td>
<td>b-iii</td>
<td>500</td>
</tr>
<tr>
<td>D</td>
<td>a</td>
<td></td>
<td>500</td>
</tr>
</tbody>
</table>

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Occurrence of Non-Diapause Eggs by Injection of Adenosine-5'-Monophosphate into Bombyx Mori Pupae1,2

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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 volatility 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) (Takahashi 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.

REFERENCES


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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