SHORT COMMUNICATIONS

Hormonal Control of Larval Sensitivity to a Nuclear-Polyhedrosis Virus in Noctuids

A nuclear-polyhedrosis virus that infects the Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera, Noctuidae) has been advocated for its biological control (Abul-Nasr, 1959a, b). Unless protection is provided, laboratory breedings are easily infected. However, field populations show generally a rather low mortality which may fairly increase late in the season (Abul-Nasr, 1959a).

Latent virus infections are common in insect caterpillars and render only frank if insects are being exposed to certain stressors (Steinhaus, 1958). It is unknown, however, how this alteration is brought into being.

With *S. littoralis*, it has been experienced that laboratory conditions may sometimes constitute a stressor of such a kind so that some virus diseased larvae develop into morphological intermediates comparable almost to those resulting from allatectomized healthy larvae (El-Ibrashy, 1971). It was tempting, therefore, to think of a possible implication of the hormonal mechanism, that normally controls growth and development (El-Ibrashy and Shehata, 1971), in altering metabolically the tissue sensitivity to latent polyhedrosis virus infection as influenced by the available surrounding conditions.

The virulence of viruses is generally dependent upon the internal milieu of the host and the lipid phase would play an important role in this regard (Revie, 1961). Thus, a knowledge of the state of the lipid associated with the corpus allatum activity in relation to the virulence of polyhedrosis virus in *S. littoralis* would help clarify, if any, a hormonal control of larval sensitivity to the virus multiplication. This, in turn, seems to be fundamentally essential for epidemiologists to manipulate this pathogen to induce epizootics in field populations of this and probably related pests.

MATERIALS AND METHODS

Allatectomy and lipid analysis were performed as previously described (El-Ibrashy, 1971; El-Ibrashy and Boctor, 1970). The hormonally active compounds tested were: a confidential synthetic juvenile hormone analogue (JHA) of a code No. ZR 512 (gifted by Dr. J. Siddall, Zocon Corp., Calif., USA) and the pure insect moulting hormone (MH), Crustecdysone (gifted by Dr. D. H. S. Horn, CSIRO, Melbourne, Australia).

Surgical and chemical treatments were conducted on newly-moulted final instar larvae (of less than 24 hr and of 150–200 mg each) which were obtained from the laboratory culture constantly maintained under salubrious conditions (El-Ibrashy and Chenouda, 1970). After treatment they were kept singly in cups (furnished with daily-renewed castor-oil leaves) and incubated at 30 ± 0.1°C under uncontrolled relative humidity. Each experimental larva was injected with 10 μl of a suitable diluent containing the optimum dose that was almost tolerable at 24 hr of injection (disclosed through an exploratory dose-mortality study using the Abbott's formula (1925) for survival evaluations). Surviving larvae (amounting, at least, to 100 larvae) out of each group were segregated and the resulting healthy spinning larvae were counted so that the per cent deaths due to perpetuated latent virus could be calculated. Lipid extraction was carried out on healthy spinning larvae as well as on morbid virus-infected full-grown larvae that were starved for a time quite enough to empty their alimentary tracts.

RESULTS AND DISCUSSION

Allatectomy almost doubled the per cent of the overt virus-infected larvae (86%) compared either to mock-allatectomized or to untreated ones (43% for either). Larvae treated with MH showed a rather less incidence of death (60%). However, the larvae injected with JHA were the least to show a frank virus infections (27%). The diluents per se were found to be entirely inert. Almost similar results were obtained when Ben-Shaked and Harpaz (1966) tested crude ether extracts (supposed to have JH activity) with larvae of the same species, *Pseudia linare* F. (= *S. littoralis*) that were previously inoculated with polyhedrosis virus.

The virus multiplication in allatectomized larvae has drastically modified the lipid metabolism (Table 1). The total lipid content was greatly reduced comparable to that of mock-allatectomized ones. The iodine value (I. V.) was apparently decreased indicating an increase in the saturation degree of the lipid. Generally, the water content was found to be higher in morbid larvae of each tested group, irrespective of their experimental status, than in the corresponding healthy ones.

The greasy cutworm, *Agrotis ypsilont* Rott.
Table 1. LIPID STATUS AND WATER CONTENT OF *S. litoralis* AND *A. ypsilon* LARVAE IN RELATION TO POLYHYDROSIS INFECTIONS AS INFLUENCED BY CERTAIN TREATMENTS*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Healthy spinning larvae</th>
<th>Morbid full-grown larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total lipid % (dry weight)</td>
<td>I. V. Moisture %</td>
</tr>
<tr>
<td><em>S. litoralis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allatectomy</td>
<td>25.2</td>
<td>95.1</td>
</tr>
<tr>
<td>Mock allatectomy</td>
<td>26.7</td>
<td>88.6</td>
</tr>
<tr>
<td>JHA injections (1 µg/larva)</td>
<td>34.9</td>
<td>83.2</td>
</tr>
<tr>
<td>Pure arachis oil injec. (10 µg/larva)</td>
<td>28.5</td>
<td>79.2</td>
</tr>
<tr>
<td>MH injections (0.1 µg/larva)</td>
<td>28.3</td>
<td>86.6</td>
</tr>
<tr>
<td>10% ethanol injections (10 µl/larva)</td>
<td>29.1</td>
<td>83.6</td>
</tr>
<tr>
<td>Normal (Untreated)</td>
<td>27.1</td>
<td>91.6</td>
</tr>
<tr>
<td><em>A. ypsilon</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allatectomy</td>
<td>26.0</td>
<td>115.6</td>
</tr>
<tr>
<td>Mock allatectomy</td>
<td>25.9</td>
<td>94.2</td>
</tr>
<tr>
<td>Normal (Untreated)</td>
<td>22.1</td>
<td>98.0</td>
</tr>
</tbody>
</table>

* All treatments were replicated 4–5 times and a replicate consisted of 6–7 individuals.
* The diluent of the confidential juvenile hormone analogue.
* The diluent of the pure insect moulding hormone.
* The larvae were perorally inoculated with a crude polyhedrosis inocula obtained from moribund *S. litoralis* larvae.

(Another noctuid) is comparatively harder than *S. litoralis* to be spontaneously infected with polyhedrosis virus. Therefore, allatectomy was performed using larvae perorally inoculated with polyhedrosis derived from moribund *S. litoralis* larvae that was uniformly sprayed onto castor-oil leaves on which *A. ypsilon* larvae were being fed. Allatectomized *A. ypsilon* larvae were only the experimental animals of this species which showed an overt virus infection (40%). These findings (Table 1) clearly conform to those of *S. litoralis* and this would keep up the validity of the study.

It is, then concluded that the virus multiplication to a pathological level is only attained whenever the physiological state of the host renders suitable for such a process to proceed. This includes a change in the lipid status altogether with the moisture content which, in turn, are apparently regulated by the hormonal balance indispensable generally for growth and development of both species (El-Ibrashy and Shehata, 1971).

Therefore, a discovery of agents that inhibit or intervene with normal activity of the corpus allatum should be of great economic importance as they would potentiate the virulence of polyhedrosis under a prevailing natural conditions.

REFERENCES


The egg diapause of the silkworm, *Bombyx mori* L., is known to be predetermined at the pupal stage by the action of a hormone, called either the diapause factor (Fukuda, 1951) or the diapause hormone (Hasegawa, 1951, 1957), which originates from the suboesophageal ganglion. Further, it has been proved that this hormone accelerates the accumulation of 3-hydroxykynurenine in the ovaries of silkworm pupae so that the eggs affected by the hormone become brown in color in a few days after oviposition due to ommochromic formation from 3-hydroxykynurenine (Yamashita and Hasegawa, 1964; Sonobe and Ohnishi, 1971). According to Osanai and Koga (1966), the ommochromes formed in the diapause eggs of the silkworm are xanthomatinic and ommine. Recently, it was revealed that in the common armyworm, *Leucania separata* Walker, some hormonal factor(s) released from both the brain-ganglia complex and the suboesophageal ganglion cause(s) black pigmentation of melanin and reddish brown pigmentation, which may be of xanthomatinic, in the integument of the larvae (Ono and Saito, 1972).

Thus, in the silkworm the suboesophageal ganglion is responsible for diapause induction and coloration of the eggs and in the common armyworm the brain-ganglia complex and the suboesophageal ganglion are connected with the coloration of the larval integument. Therefore, it is of interest to search for the possibility as to whether the hormonal factor(s) concerning larval coloration in the common armyworm is(are) related to the diapause induction of the silkworm eggs.

In this experiment, the effects of implantation of various ganglia, taken from the common armyworm, into the female silkworm pupae on the embryonic diapause in the silkworm were investigated. For this purpose, fifty *Leucania* larvae per plastic case (9 cm in diameter and 200 ml in content) were reared on maize leaves. The eggs of polyvoltine race N4 of *B. mori* incubated at 15°C were kindly provided by the Sericultural Laboratory, Faculty of Agriculture, Nagoya University and the larvae were reared on mulberry leaves at 24—25°C.

The 5th instar common armyworm larvae, about 24 hr before molting, were dissected in sterilized 0.9% NaCl solution and the brain-ganglia complex (br—cc—ca) of *B. mori* pupae were removed. These ganglia were rinsed three times with a few drops of 0.9% NaCl solution placed on slide glasses and then implanted into the abdomens of female silkworm pupae 4 days after pupation. As control, two pieces of glass tube (1 mm in length and 0.5 mm in diameter) containing 0.9% NaCl solution were implanted into the abdomens of pupae. As shown in Table 1, most moths emerging from the pupae which had received either Br—CC—CA and SG laid egg masses mixed with diapause eggs (brown in color), semi-diapause eggs (light brown) and non-diapause eggs (light yellow), while, the majority of eggs deposited by the moths which had received the 1st TG were non-diapause similar to eggs of the control. This result shows that both Br—CC—CA and SG of the 5th instar common armyworm larvae about 24 hr before their next larval molting release some hormonal factor(s) which induce(s) embryonic diapause in the silkworm. The term "semi-diapause eggs" used here temporarily signifies the light brown eggs, the embryos of which visibly developed till 20 days after oviposition and most of them died before hatching. A detailed description of the semi-diapause eggs has been presented by

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