The alteration of juvenile hormone titre in *Spodoptera litura* (F.) due to a baculovirus infection

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Summary. The haemolymph juvenile hormone levels of *Spodoptera litura* were remarkably low (540 Galleria units (GU)/ml) at the last larval moult as well as prior to pupation (194 GU/ml). During the last intermoult period this was 2600 GU/ml for a 24 h-period. On the other hand, the JH level in the haemolymph of NPV-infected last instar larvae was initially 1740 GU/ml but was maintained at 2400–2600 GU/ml during the next 48 h. Finally, the JH titre fell to 1393 GU/ml, but only prior to death. The failure of the diseased larvae to undergo the larval pupal moult is ascribed to the alteration of the JH titre in the haemolymph.

Failure to metamorphose is a common occurrence in the larvae of *Spodoptera litura* afflicted with nuclear polyhedrosis. However, such individuals continue to live as oversized larvae for 2–3 additional days. Our studies indicate that virus infection leads to an inhibition of larval moulting and pupation which is, however, dependent on the dose of the inoculum and larval age. Incomplete tanning of pupae is occasionally noticed if they are infected in the last instar. These observations suggest the interference of nuclear polyhedrosis with the hormonal balance of the host. This paper deals with the change in the JH titre in the haemolymph of *S. litura* during the course of nuclear polyhedrosis, and its influence on metamorphosis.

Materials and methods. Haemolymph samples (200 µl each) from healthy larvae of *S. litura* were collected from the 8th day (5th instar) to the 12th day (9–12th day = 6th instar). Haemolymph samples from diseased larvae, which were fed with a concentration of 557×10^6PIB/ml on the 8th day, were collected from the 10th to the 14th day (1 day prior to death). The levels of circulating JH in the haemolymph were determined by bioassay of the lipid extracts against 0–24 h-old *Galleria mellonella* pupae according to de Wilde et al.[1], with the following modifications: 1. The haemolymph extracts were diluted in 200 µl of olive oil; 2. 3 doses of test material were collected (0.22, 0.29 and 0.37 µl/pupa) as drop on a siliconised glass plate with the aid of a microapplicator. Each dose of test material was mixed with a small amount of melting paraplast (m.p. 52 °C) before sealing the wound made on the mesonotum of the test pupae; 3. As the size of the pupal patch formed varied, the response was scored according to the size of the pupal patch at the wound site. The scores adopted were 1, 0.5, 0.25 and 0 for a large, clear pupal patch, medium-sized dark brown patch, a small wound area devoid of scales, and no response, respectively. The combined scores from 6 test pupae were used to calculate the JH titre in Galleria units ml of haemolymph (GU/ml). The JH titre in terms of dl juvenile hormone[2] was derived as 1 GU is equivalent to 5×10^{-6} µg of this hormone[1].

Results and discussion. The level of JH in the haemolymph on the 8th day, 1 day before the last moult, was 540 GU/ml with a marginal increase (569 GU/ml) immediately after the last larval moult. On the 10th and 11th day the JH titre was 2713 and 2602 GU/ml respectively. The JH titre sharply decreased to 194 GU/ml on the 12th day, a day prior to the larval pupal moult. On the other hand, the JH titre in the haemolymph of diseased larvae was 1740 GU/ml on the 10th day and was maintained around 2400–2600 GU/ml during 11 to 13 days, followed by a decrease on the 14th day to 1393 GU/ml. The following day the diseased larvae died with pronounced symptoms of polyhedrosis.

The JH secreted by the corpus allatum (CA) controls many physiological events of morphogenesis[3,4]. This hormone determines the character of the moult by suppressing the adult differentiation in favour of larval structures[5]. The level of circulating JH in the haemolymph provides information about the equilibrium existing between the rate of CA secretion, tissue uptake and haemolymph enzyme deactivation. The secretory activity of CA of *S. litura* observed in the present investigation is similar to that of *Hyalaphora cecropia*[6,7]. A low level of JH was observed in the haemolymph of *S. litura* prior to the larval pupal moult as in the case of other Lepidopterans like *Diatraea grandiosella*[8] and *Pieris brassicae*[9]. It is well established that metamorphosis proceeds in the presence of a low level of JH[5]. Thus, in *S. litura* also, metamorphosis is a consequence of falling JH titre. On the other hand, the JH level in the haemolymph of diseased larvae increased to a maximum on the 11th day and was maintained on the 12th and 13th days, with a fall on the 14th day. This indicates a delay in the production of JH in the case of diseased larvae. The final reduction in the JH titre is much lower than in the case of healthy larvae. The larval pupal moult is thus inhibited due to the mainte-

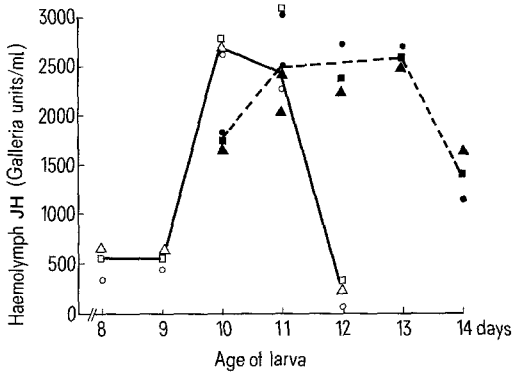

Fig. 1. Juvenile hormone titre in the haemolymph of 6th instar larvae of *Spodoptera litura* —— normal - - - diseased. Dose: ● ○ – 0.22; ■ □ – 0.29; ▲ △ – 0.37 µl.

Juvenile hormone titre in the haemolymph of *Spodoptera litura*

	5th instar	6th instar								
Day	8	9	10		11		12		13	14
	N	N	N	D	N	D	N	D	D	D
GU/ml	540	569	2713	1740	2602	2503	194	2461	2595	1393
dl JH $\times 10^{-3}$µg	2.7	2.84	13.56	8.7	13.01	12.51	0.97	12.3	12.97	6.96

N: normal, D: diseased.

nance of a high level of JH, which is a consequence of virus multiplication. The high JH titre in the diseased larvae could be ascribed to a) the CA remaining active during the course of polyhedrosis as indicated by the ultrastructure (unpublished observation) and b) the fact that as the fat body is one of the major sites of multiplication of virus leading to its degradation, it is likely that the lipid soluble JH would not have been stored in large quantities but have remained in an active state in the haemolymph. Apart from this, a low activity of the haemolymph enzymes that degrade JH and the inefficient excretion of JH through Malpighian tubules might be the other possibilities.

Prothetely due to nuclear polyhedrosis virus (NPV) infection in *Lambdina fiscellaria somniaria* and *Orgyia pseudotsugata*[10], and metathetely due to entomopox virus infection in *Choristoneura biennis*[11], were observed earlier, In the case of prothetely it was suggested that virus multiplication might have inhibited synthesis/release of JH, whereas in the case of metathetely, the effects were similar to those caused by excess JH. The reduction in JH titre to a very low level, which is essential for pupation, fails to occur due to NPV infection; this eventually prevents pupation in *S. litura*. This report presents direct evidence of a hormonal imbalance which occurs due to NPV infection.

1 J. de Wilde, G.B. Staal, C.A.D. de Kort, A. de Loof and G. Baard, Proc. K. ned. Akad. Wet. *71*, 321 (1968).
2 K.H. Dahm, B.N. Trost and H. Roller, J. Am. chem. Soc. *89*, 5292 (1967).
3 V.B. Wigglesworth, Q. Jl microsc. Sci. *77*, 191 (1936).
4 V.B. Wigglesworth, Q. Jl microsc. Sci. *79*, 91 (1936).
5 W.W. Doane, in: Developmental systems: Insect Vol. 2, p. 291. Ed. S.J. Counce and C.H. Waddington. Academic Press, London 1973.
6 C.M. Williams, Biol. Bull. mar. biol. Lab., Woods Hole *121*, 572 (1961).
7 L.I. Gilbert and H.A. Schneiderman, Gen. comp. Endocr. *1*, 453 (1961).
8 C.-M. Yin and G.M. Chippendale, J. exp. Biol. *64*, 303 (1976).
9 C. Varjas, P. Pagui and J. de Wilde, Experientia *32*, 249 (1976).
10 O.N. Morris, J. invertebr. Path. *16*, 173 (1970).
11 A. Retnakaran and F.T. Bird, J. invertebr. Path. *20*, 358 (1972).

PRO EXPERIMENTIS

A colloidal gold prepared with ultrasonics

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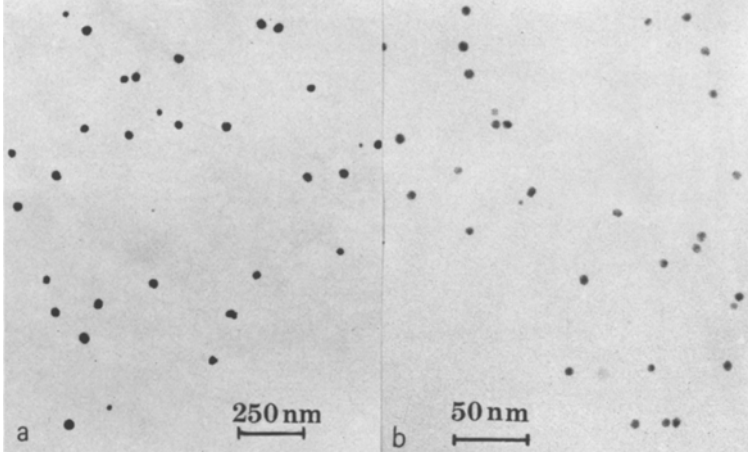
Summary. With the application of ultrasonic energy a colloidal gold may be easily prepared with a particle diameter of less than 10 nm and suitable for use as an immunological marker for TEM-studies. This new approach replaces the use of phosphorus, traditionally used for producing gold sols of the smallest diameter.

Traditionally, the most commonly used methods for the preparation of colloidal gold are based on the reduction of gold chloride with a variety of substances such as phosphorus[1,2], formaldehyde[3], ethyl alcohol[4], tannic acid[4] and, more recently, sodium citrate[5], usually in association with heat. Such reducing agents produce colloids varying considerably in particle size but only the use of a solution of phosphorus in ether[6] results in diameters averaging less than 10 nm, and suitable as an immunological marker for our studies at the ultrastructural level. It was therefore decided to investigate the possibility of making a similar gold sol using a less hazardous substance by substituting ultrasonics for the conventional energy source.

The basic technique chosen for investigation with ultrasonics was that of Oswald[4] in which ethyl alcohol is used as the reducing agent. A neutral solution of gold chloride was prepared as described in the legend. Half of the solution was heated prior to the addition of ethyl alcohol and the



Random electron micrographs of heat (a) and ultrasonically (b) induced gold colloids showing degree of dispersion. Preparation: 0.2 ml of a 1% $H(AuCl_4)$ solution was diluted to 100 ml and made neutral with 0.2 M K_2CO_3. 50 ml was heated to 75 °C and 0.5 ml ethyl alcohol was added. After approximately 5 min a reddish pink colour reached a maximum in intensity with an absorption at 540 nm (a). To the remaining 50 ml, 0.5 ml ethyl alcohol was added. Sonication was carried out at 20 kc and 125 W by immersing a flat ended probe approximately 1 cm under the surface. After 2 min a pinkish colour reached a maximum in intensity with an absorption at 520 nm (b). For electron microscopy the grids were dipped into a 0.1% aqueous solution of poly-L-lysin, washed twice in aqua dest. and after being submerged into the respective sols for approximately 2 min were again washed and finally dried on filter paper.