In all experiments ferritin was found around *Tokophrya* in long fringes (figures 3, 4 and 6) extending above the coat, and also close to the external membrane (figures 4 and 6), as well as in the pits (figure 4) and what is most important inside the organism in flat vesicles measuring 370 × 40 nm (figure 5). The flat vesicles are a structure characteristic of the cytoplasm of *Tokophrya*. They are often assembled in groups of 3–5 (figure 4) or piled up in stacks up to 17 vesicles (figure 5). In previous papers this structure was referred to as a possible primitive Golgi apparatus[5].

No ferritin was ever encountered free in the cytoplasm, or in the epiplasm, or between the membranes covering *Tokophrya*. The tracer was present only in the flattened vesicles, scattered throughout the cytoplasm and often near the pits (figure 6) or sometimes very close to them as if in the process of pinching off (figure 6) strongly suggesting that they derive from the pits. It is assumed that once a vesicle becomes detached from the pit it is carried away and if more are pinched off in succession they remain together forming piles of vesicles.

Since the pits are the only places on the surface of *Tokophrya* covered by a single membrane they are the natural loci for the uptake of ferritin from the medium. When the tracer enters a pit it apparently induces pinocytosis resulting in the formation of the flat vesicles. Thus the pits in conjunction with the flat vesicles are the vehicles transporting ferritin and probably other macromolecules from the medium to the cytoplasm of *Tokophrya*. Both structures perform an important function in Suctoria, organisms deprived of an oral cavity.

Juvenile hormone in larval diapause of the codling moth, *Laspeyresia pomonella* L. (Lepidopterae, Tortricidae)

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Summary. Whereas in last instar larvae of *L. pomonella* kept under long-day-conditions (LD), the JH-titer is temporarily reduced to zero, it stays relatively high in short-day-conditioned (SD) larvae which enter diapause. Application of JH or a juvenoid to LD-larvae results in diapause, if the treated insects are kept under SD-conditions. From these results it is concluded that in *L. pomonella* diapause is initiated by a relatively high titer of JH during the last larval instar.

When larvae of our laboratory strain of the codling moth, *Laspeyresia pomonella*, are reared under short-day-conditions (SD), i.e. with a photophase of less than 14 h per day, they enter diapause. Under long-day-conditions (LD), i.e. with a photophase of more than 16 h per day, the larvae will pupate and produce adults. In several species of Lepidoptera, it has been demonstrated that larval diapause is caused by a high titer of juvenile hormone (JH). In these cases application of high concentrations of JH mimetica not only induced but also maintained larval diapause, even under LD-conditions[1,2]. Besides this proved mechanism, diapause in other species might be caused by a lack of the moulting hormone, as in pupal diapause, which may be broken by injection of ecdysone[3,4]. The object of the present study was to investigate the relationship between JH and the diapause of the codling moth. Therefore the JH titer during the last instar of LD- and SD-conditioned larvae as well as in diapausing insects was determined. Furthermore the influence of topically applied JH was studied in the last instar of LD-conditioned larvae kept under LD- or SD-conditions after the application.

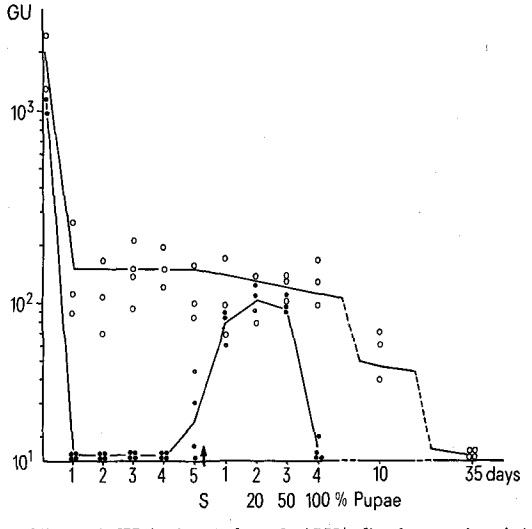

Fig. 1. Titer of JH in haemolymph (GU/ml) of pupating (●) and diapausing (○) *L. pomonella* at different times after last larval moult and spinning of cocoon (S).

Material and methods. The larvae of the codling moth were reared individually at 26 °C on a semisynthetic medium in small plastic boxes[5]; 5 days after the last larval moult, the boxes were opened to allow the larvae to spin their cocoons. Under LD-conditions (continuous light) the larvae pupated 2–4 days later. SD conditions resulting in 100% diapausing larvae were 10 h light to 14 h dark.

The titer of JH in the haemolymph of the codling moth (indicated as Galleria units = GU) was determined by a slightly modified Galleria-Wax Test[6,7]; 100–200 µl haemolymph of 5–12 larvae of a given age were collected and extracted by a 6:1 mixture of ethylacetate and

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ethanol (v:v). The tyrosinase activity was suppressed by a small amount of phenylthiourea[1]. Haemolymph samples of SD- and LD-larvae were collected immediately after the last larval moult and then every 24 h up to pupation of the LD-animals, and in diapausing larvae additionally 10 and 35 days after they had spun their cocoons. Haemolymph of pupae was sampled immediately after the pupal moult.

JH I (50, 100, 500 or 1000 µg/larva) or the JH mimeticum Altosid® (10, 20 or 50 µg/larva) were applied topically 0–12 h after the last larval moult. Up to the treatment, all larvae were kept under LD-conditions; after the application, half of the animals were kept under LD- and SD-conditions respectively. The effects were controlled at intervals of 5 days after the application.

Results and discussion. The JH-levels of SD- and LD-animals are shown in figure 1. In LD-conditioned last instar larvae, the concentration of JH decreased rapidly during the first day after moulting. From the 2nd to the 4th day, no JH could be detected. Subsequently the JH-titer increased to a second maximum when 20% of the larvae had pupated. Then the concentration of JH decreased again until none, or traces only, could be found in freshly moulted pupae. These results with LD reared *L. pomonella* agree well with those described from *Pieris brassicae* and *Mamestra brassicae*[8].

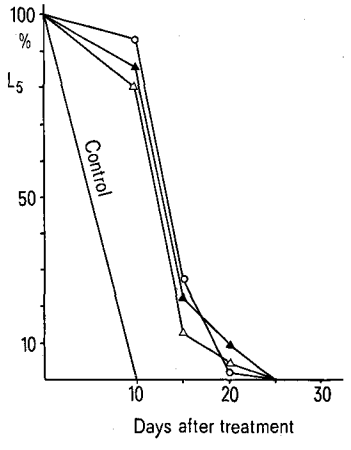

Fig. 2. Delayed pupation of L_5 treated with different doses of Altosid® under LD-conditions (▲ = 10 µg, △ = 20 µg, ○ = 50 µg).

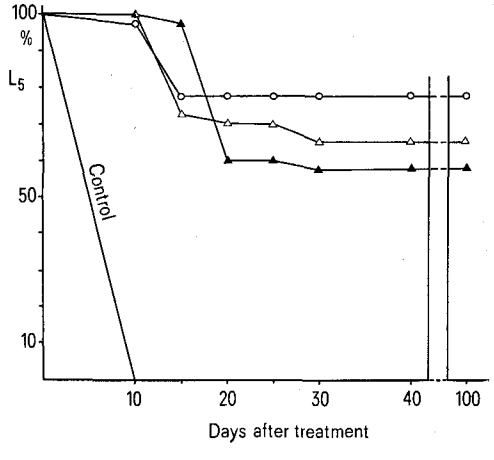

Fig. 3. Diapausing larvae after treatment of LD-conditioned insects with different doses of Altosid® reared subsequently under SD-conditions (▲ = 10 µg, △ = 20 µg, ○ = 50 µg).

In diapause induced larvae (SD), the concentration decreased too, but only to a level of about 100 GU/ml of haemolymph. This level stayed fairly constant until the larvae had spun their cocoons. After this the JH-titer began to sink slowly, and 35 days later no JH could be detected.

Application of JH 0–12 h after the last larval moult of LD- conditioned insects resulted in 4 types of reactions: 1. no reaction, larvae pupated normally, 2. larvae moulted to larval-pupal intermediate forms, 3. larvae moulted to an additional larval instar (L_6) which continued feeding and 4. larvae stopped feeding, spun cocoon and entered diapause.

At 10 days after the last larval moult, all control larvae which were not treated and kept under LD-conditions had pupated. Compared with these, the treated larvae which were kept under LD-conditions moulted delayed, showing type 1, 2 or 3 reactions (figure 2). Treatment with Altosid® or JH I gave very similar results. The first moulting larvae gave mainly supernumery larvae, whereas the late moulting larvae produced mainly pupae. Intermediate forms (type 2) were produced by early and late moulting insects. Diapausing larvae (type 4) were obtained only when the treated larvae were kept under SD-conditions after the JH had been applied (figure 3). Since the untreated larvae under the same light regime pupated normally within 10 days after the last larval moult, it can be concluded that diapause in the JH-trated larvae was induced by the hormone. If, on the other hand, larvae were reared under SD-conditions and changed to LD immediately before or after the last larval moult, the larvae still entered diapause, indicating that at this moment diapause is already programmed and cannot be changed anymore by another light regime.

Whereas the titer of JH in last instar larvae of *L. pomonella* is temporarily reduced to zero, if the insects have been conditioned for pupation, it remains relatively high in larvae in which diapause has been induced by a short day regime. This may indicate that diapause is caused by a high titer of JH, which suppresses either the production or the action of the moulting hormone ecdysone. This hypothesis is strenghened by the fact that diapause can be induced in LD-conditioned larvae by JH or a JH mimetic, applied topically 0–12 h after the last larval moult, if, after the treatment, the insects are kept at a light regime with a short photoperiod. On the other hand, since all JH-treated larvae moulted under LD-conditions, it can be concluded that under this light regime a single dose of JH cannot prevent the production of the moulting hormone. However, it seems that ecdysone is only produced after the titer of the JH has been reduced below a critical level. This is supported by the fact that even under LD-conditions moulting is delayed by a high JH-titer, indicating a genuine antagonism between the 2 hormones, as described in other lepidopterous species in which JH may produce dauerlarvae[9,10]. In addition, unpublished experiments indicate that treatment with JH followed by SD-conditions cannot prevent moulting if the hormone is applied later than 12 h after the last larval moult, at a time when the production of ecdysone has probably already been initiated.

The results thus indicate that diapause in *L. pomonella* is initiated by a relatively high titer of JH. However, the fact that the hormone disappears in the diapausing larvae within 35 days, indicates that JH is not necessary for the maintenance of the diapause.

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