The influence of juvenile hormone on the feeding behaviour of last instar larvae of the codling moth, *Laspeyresia pomonella* (Lep., Tortricidae), reared under different photoperiods

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Summary. Duration of the feeding stage and corresponding weight increase during the last larval instar of the codling moth, *Laspeyresia pomonella,* are controlled by JH. Larvae reared under short day conditions have a relatively high titer of JH during the last larval instar and enter diapause as mature larvae. They feed longer and become heavier than larvae reared under long day conditions, which have no JH during the last larval instar and pupate when mature. By application of the JH mimetic Altosid® during the first 2 or 3 days of the last larval instar, the duration of feeding activity of larvae reared under respectively long and short day conditions was prolongated and the larvae became significantly heavier. The feeding behaviour could only be influenced by the juvenoid as long as the feeding activity of the larvae had not yet ceased.

Diapause is a strategy of insects to survive adverse conditions. Although metabolism and consequently the utilisation of reserve materials is much reduced during diapause, the body weight decreases as a result of fat, glycogen, and protein catabolism[1,2]. It is therefore to be expected that insects with larval or pupal diapause have more metabolic reserves at the beginning of the dormancy period than insects of a comparable stage which continue morphogenesis.

Interactions between JH and metabolism are known from a number of insects, as reviewed by Slama et al.[3], and in some Lepidoptera an influence of JH on spinning behaviour[4,5] and the duration of the feeding period[6] has been demonstrated. However, information is scarce about hormones regulating the intake of food, the deposition of metabolic reserves, and its expression in body weight.

The present study shows that cessation of feeding before moulting or diapause in the last larval instar of *Laspeyresia pomonella* is influenced by the titer of naturally occurring or topically applied JH. Larvae of the codling moth have been used because of their advantages for such experiments: (a) The larvae can be easily reared on an artificial medium[7], which excludes all influence of varying food quality. (b) Depending on the duration of the photoperiod it is possible to simultaneously rear larvae going into diapause and larvae which pupate at the end of the instar. (c) For both developmental types the JH titer during the last larval instar is known[8].

Previous observations suggested that mature larvae reared under a short day photoperiod (SD), i.e. diapause induced larvae (LD larvae), are larger and leave the rearing medium later than mature larvae reared under a long day photoperiod (LD) and which are ready for pupation (= LD larvae). The difference in the titer of JH in last instar larvae reared under different photoperiods suggests that JH may play an important role not only in regulating development both with and without diapause but also behaviour and other corresponding parameters.

Material and methods. Codling moth larvae were reared individually at 26 °C on a semisynthetic medium in small boxes[7]. Diapause is induced by SD (10 h light/14 h dark). Mature larvae, reared under SD spin a dense cocoon and enter diapause without further moulting. LD conditions (continuous light) induce mature larvae to pupate 2-4 days after they have spun their cocoon. In order to avoid temperature deviations at different light regimes, all larvae were reared in the same climatic chamber with continuous light. Diapause was induced by putting half of the larvae into a light impermeable photopaper bag for 14 h every day. Under these conditions larval development is com-

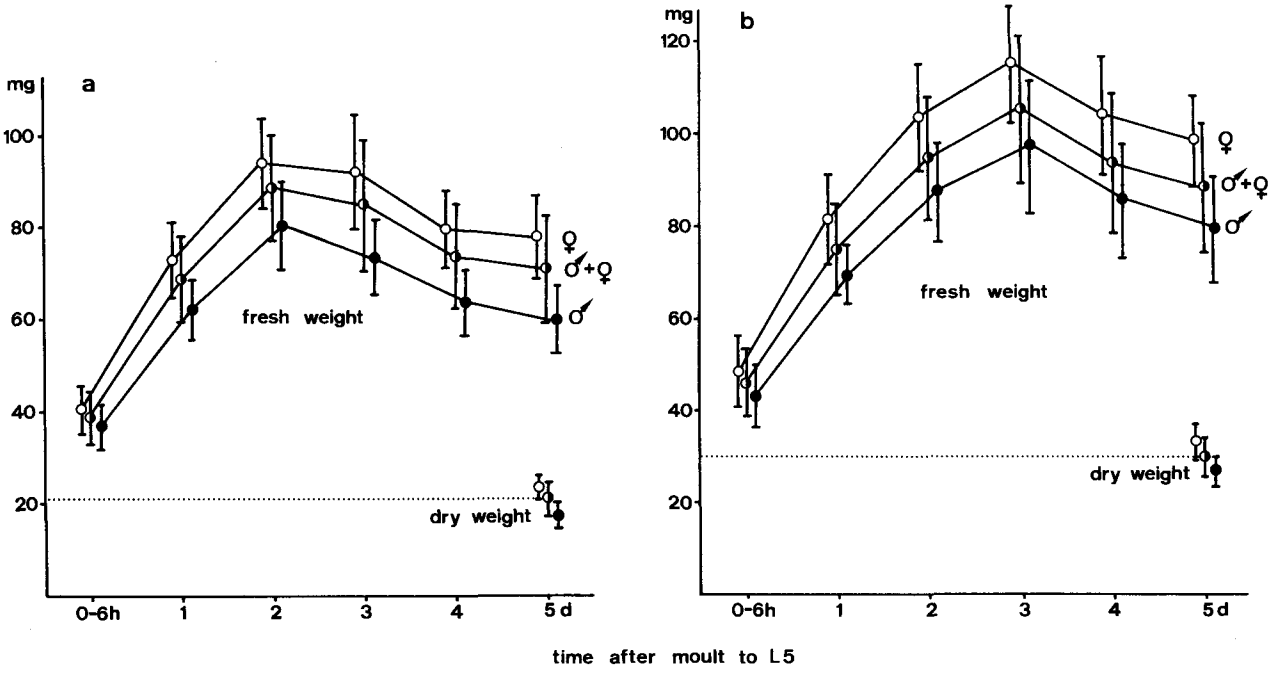

Fig. 1. Different average weights and standard deviations of males and/or females under long day (a) and short day (b) conditions.

pleted in 16±1 days. The time for 50% of the insects to reach the last larval instar is 10 days under LD and 11 days under SD conditions. Freshly moulted last instar larvae were separated every 6 h, weighed immediately on an analytic balance, and reweighed at intervals of 24 h until the larvae ceased feeding. They were frozen at −20 °C immediately after the last weighing, lyophilized, and weighed again when fully dry.

To investigate the duration of the feeding period, larvae were transferred every 12 h into boxes containing new medium. By the feeding traces on the surface of the fresh medium it could be seen whether or not the larvae were still feeding. Male larvae could be distinguished from female larvae by the dark pigmented testicles shining through the integument of their back.

The effect of additional JH was tested by topical application of the juvenoid Altosid®, given by Zoëcon Inc., Palo Alto. Larvae were treated with 25 µg of the juvenoid in 1 µl of acetone immediately after the larvae had been weighed. Half of the treated larvae were further kept under LD, whereas the other half was reared under SD. Each experimental group consisted of at least 40 insects and the results were subjected to Student's t-test.

Results and discussion. Comparison of the weights of larvae reared under SD and LD showed that already 0–6 h after the last larval moult, SD larvae are significantly heavier (45.5 mg) than LD larvae (39.1 mg, see figure 1). On the first 2 days, the increase of weight takes a parallel course in LD and SD larvae. On the 3rd day the fresh weight of the LD larvae begins to decrease continuously (figure 1, a), whereas the weight of the SD larvae still increases (figure 1, b). After the 3rd day, the average weight of LD and SD larvae decreases again in a parallel course. The average weight difference of 6.4 mg at the beginning was more than doubled in the course of the last larval instar (15.9 mg). These differences in fresh weight are paralleled by significant differences in dry weight, indicating that the increase in fresh weight of diapause induced larvae is not only due to water retention.

The weight differences mentioned above are similarly expressed in males and females (figure 1), though the latter are always significantly heavier, independent of age and light regime ($p \leq 0.01$).

SD larvae reach the last instar 1 day later than larvae, which may account for the distinctly greater weight of the former. A 2nd increase of weight difference is caused by the prolongation of the feeding period of diapause induced L_5, as shown in figure 2. These larvae feed approximately 1 day longer than LD larvae. Differences in the duration of the feeding periods are also responsible for the differences in the weights of males and females under the same light regime. Females feed nearly half a day longer than males (figure 2).

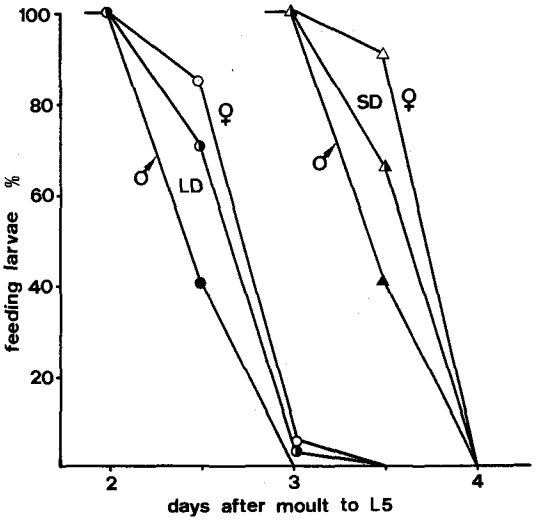

Fig. 2. Influence of the photoperiod on the duration of the feeding period of males and/or females in the last larval instar. LD: long day, SD: short day conditions.

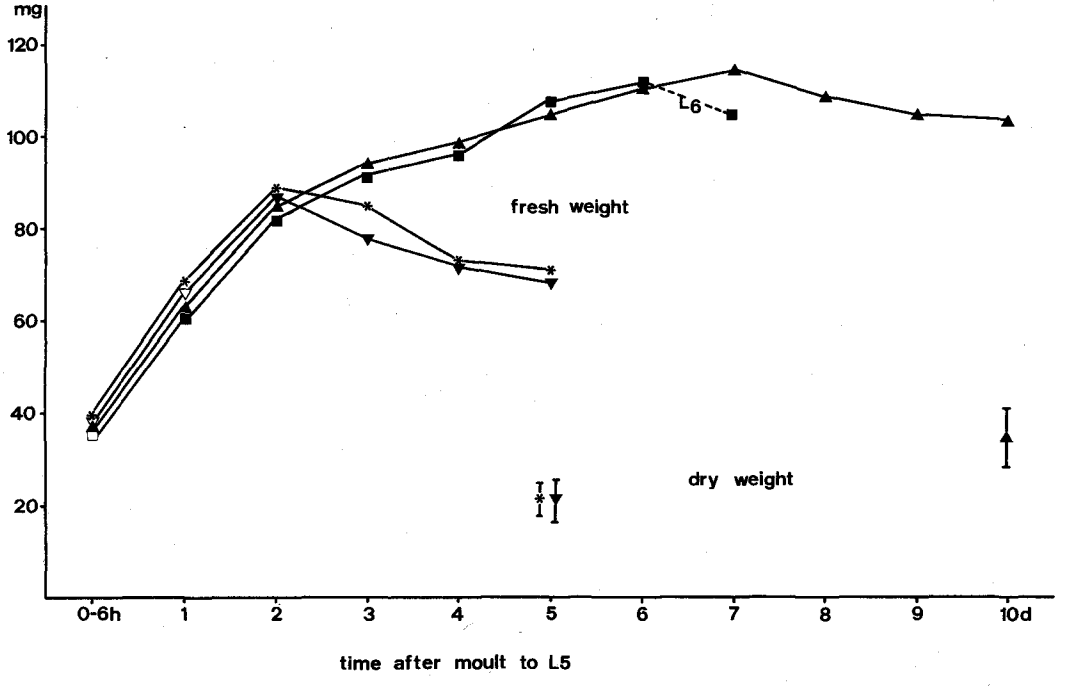

Fig. 3. Influence of 25 µg of Altosid®, applied at days 0 (▲), 1 (□ ■), and 2 (▽ ▼) after the last larval moult on the average weight of larvae, reared under *long day* conditions. Open signs = untreated, solid signs = treated larvae. Untreated controls = *.

A comparison of figures 2 and 1 shows that the onset of weight decrease of the L_5 corresponds to the time when the insects cease feeding and prepare for migration to a spinning site. This preparation includes complete emptying of the gut, which is probably the most important cause of the weight losses.

LD larvae stop feeding at 2-3 days after the L_5-moult (figure 2). When treated at different times with the juvenoid Altosid®, the feeding behaviour of these larvae could only be influenced if the juvenoid was applied at 0-24 h after the L_5-moult. The early treated larvae continued feeding for 4-5 days or longer after untreated or later treated larvae had stopped feeding. The weight of larvae treated at 0-6 h increased up to 7 days after the L_5-moult and then dereased slowly. The final fresh and dry weights of these larvae were significantly higher than those of control larvae (figure 3). Although most larvae treated at 0-6 h stopped feeding 7 days after the L_5-moult, some of them, especially female larvae, continued feeding and reached weights of more than 170 mg at 10 days. If the juvenoid was applied 1 day after the L_5-moult, the larvae fed for 4-5 more days and then moulted to an additional larval instar (figure 3: L_6). The same would have happened later on with the larvae treated at 0-6 h, had they not been lyophilized after 10 days[9].

In SD larvae the feeding period could be influenced up to 2 days after the last larval moult (figure 4). Application of the juvenoid to 3-day-old L_5 gave only in some larvae a weight increase up to 7 days after moulting, whereas other larvae behaved like untreated insects. Again, the fresh and dry weights of larvae treated at 0-2 days after the L_5-moult are significantly higher than those of the controls and of larvae treated at 3-4 days after the L_5-moult. The weights of the latter do not differ significantly from those of the controls. Changing the photoperiod after treating the larvae with the juvenoid had neither an influence on the feeding behaviour nor on fresh nor dry weights.

Repeated application of doses of 25 µg of Altosid®, started at different days after the last larval moult up to the time when the control larvae spun their cocoons, gave the same results as if the juvenoid had been applied only once, on the day of the 1st treatment.

The results show that in *L. pomonella* a high titer of a topically applied juvenoid during the first 24 h of the last larval instar will prolongate the feeding stage and consequently raise the insects' weight. In the case of diapause induced L_5 the titer of endogenous JH is naturally high[8]. Although it falls from more than 1000 *Galleria* units (GU) per ml of haemolymph to $^1/_{10}$ of this concentration within 24 h after moulting, the level of about 100 GU is maintained throughout the instar. Contrary to this the JH titer of normal L_5 drops to zero within less than 24 h after the last larval moult. Since diapause induced L_5 feed longer and become heavier than L_5 which are ready to pupate, it is probable that the natural high titer of JH in the former is responsible for these differences. Thus a natural high titer of endogenous JH during the first 24 h of the last larval instar or of a topically applied juvenoid during the same period will not only induce diapause, as shown earlier[8], but also cause the preparatory behaviour leading to greater metabolic reserves vital for diapausing insects. The fact that the topically applied juvenoid also causes a considerable prolongation of the already longer feeding period of diapause induced L_5, concommitant with a corresponding weight increase, and that the treatment with the juvenoid may produce this effect even when applied as late as 3 days after the last larval moult, indicates that maintenance of feeding activity beyond the 2 days of L_5 preparing for pupation is determined by the continuity of a high titer of JH. It becomes then at least partly clear why the titer of endogenous JH in diapause induced L_5 stays high during the whole instar, although induction of diapause is possible within the first 24 h only[8].

LD larvae treated with the juvenoid at the beginning of the last larval instar became as heavy as diapause induced untreated L_5, although the former would not enter diapause if kept under LD, but eventually moult to an additional larval instar. This, and the fact that JH prolongates the

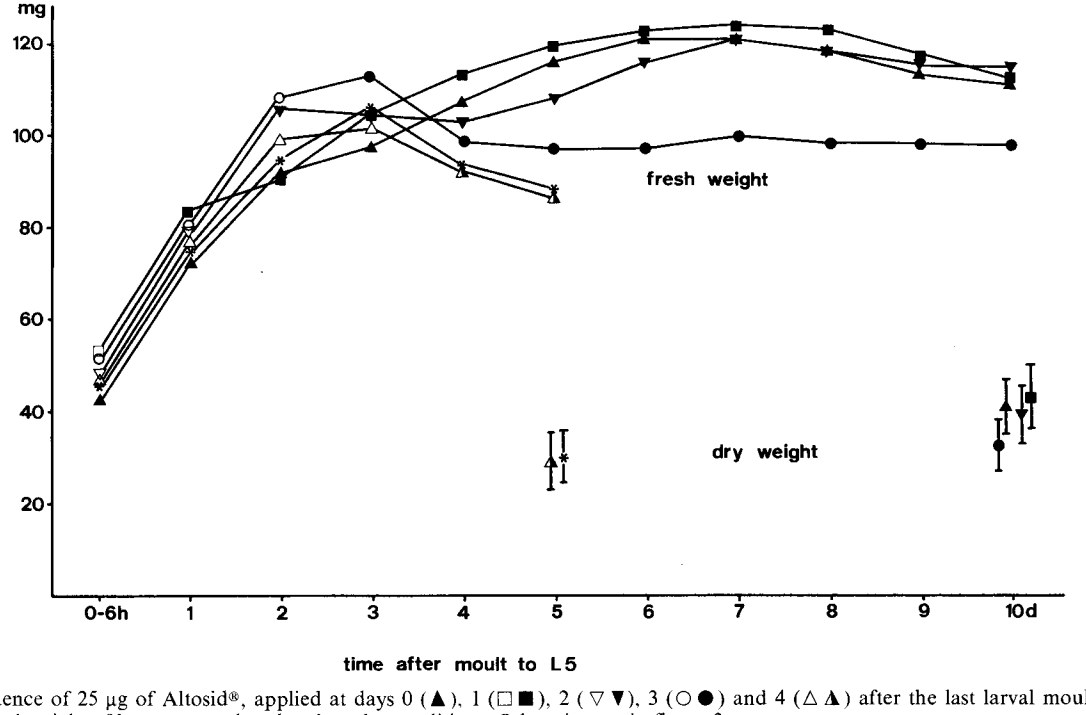

Fig. 4. Influence of 25 µg of Altosid®, applied at days 0 (▲), 1 (□ ■), 2 (▽ ▼), 3 (○ ●) and 4 (△ ▲) after the last larval moult on the average larval weight of larvae, reared under *short day* conditions. Other signs as in figure 3.

feeding period also in diapause conditioned larvae indicates that induction of diapause and prolongated feeding activity is regulated by different titers of JH which, in addition, may act on different and perhaps even independent systems. The fact that both effects serve the same purpose of surviving the adverse conditions of winter does not speak against this hypothesis.

The results also indicate that prolongation of the feeding period can only be induced by JH when the insects have not yet ceased their feeding activity. Obviously a gate, limiting responsiveness of the system to JH, must exist at a certain time ahead of the end of the feeding period. After this gate has been passed neither single nor repeated application of a juvenoid can prolongate feeding. This result suggests that feeding behaviour and development in last instar larvae of *L. pomonella* are closely linked and thus unidirectional, i.e. not reversible like the spinning behaviour of prepupal *Pieris brassicae*[5].

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Prolactin and growth hormone releasing activity of [D-Met^2, Pro^5]-enkephalinamide in the rat after systemic administration

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Summary. The growth hormone (GH) and prolactin releasing (PRL) activity of [D-Met^2, Pro^5]-enkephalinamide ($EKNH_2$), an opioid peptide analog with higher opiate agonist activity that morphine, was compared in the unanesthetized male rat to those of equimolar doses of morphine upon systemic injection. $EKNH_2$ proved to be a higher PRL, but not GH, releaser than the opiate alkaloid.

Recent reports have suggested participation of morphinomimetic compounds, i.e. enkephalins and endorphins, in the control of prolactin (PRL) and growth hormone (GH) secretion in the rat[1-4].

The magnitude of the neuroendocrine and behavioral responses elicited by these peptides appears to be related to their ability to resist enzymatic destruction. Thus, the greater activity of β-endorphin than [Met^5]-enkephalin, both as GH and PRL releaser[5] and in producing analgesia[6], was ascribed to better protection of the labile Tyr-Gly bond of the enkephalin by the remaining part of the peptide chain of β-endorphin, which has the [Met^5]-enkephalin sequence at its N terminus[5]. To obtain enzyme-resistant analogs, both terminals of [Met^5]-enkephalin were blocked with N-methyl and C-amide groups[7] or the Tyr-Gly bond was protected by replacement of glycine by D-alanine in position 2[8,9].

Recently Bajusz and associates[10] have replaced in the enkephalin pentapeptide the methionine^5 moiety by proline-amide and the glycine^2 by D-methionine. The new synthetic derivative obtained [D-Met^2, Pro^5]-enkephalinamide ($EKNH_2$) showed upon systemic, as well as intracerebroventricular (i.v.t.) administration, an higher opiate agonist activity both in vitro and in vivo rat preparations than either β-endorphin or morphine[11].

In the work to be reported, the GH and PRL-releasing activity of this pentapeptide were tested in the unanesthetized male rat following systemic injection and were compared to those of equimolar doses of morphine.

Materials and methods. Male Sprague-Dawley rats (300 g b.wt) were kept at 22±2 °C and exposed to 14 h of light each day (06.00-20.00 h). Laboratory chow and water were available ad libitum. Experiments were started 3 days after the insertion of indwelling jugular cannulae into the right atrium. On the day of the experiment, in order to minimize stress effects, animals were placed in a sound-proof and temperature-controlled room, 2 h before the beginning of blood sampling.

Blood samples (0.3 ml) were taken, at different time intervals (see legend to figures) and were immediately replaced with the same amount of heparinized saline. Plasma GH and PRL levels were determined by a double antibody radioimmunoassay, using the methods of Schalch and Reichlin[12] and Niswender et al.[13] for GH and PRL, respectively. All results were expressed in ng/ml in terms of the NIH standard rat GH-RP-1, whose potency is 0.6 IU/mg and rat PRL RP-1, whose potency is 11 IU/mg. The sensitivity of the GH and PRL assays is 1.0 ng/ml; intra-assay variability was 5% for both GH and PRL while repeated assay of a reference plasma showed an interassay variation, of 12% and 6%, respectively. Doses up to 50 ng/ml of GH and PRL standard did not cross react in the reciprocal assay. To avoid possible interassay variations in each experiment all samples were assayed in a single assay. Significance of differences between groups was calculated by the Student's t-test.

[D-Met^2, Pro^5]-enkephalinamide acetate-trihydrate (mol.wt 726) was synthesized in our laboratories according to a previously described procedure[10]. Morphine hydrochloride (mol.wt 321) was obtained from Carlo Erba, Milan.

Results and discussion. As appears from figure 1, $EKNH_2$ induced a significant PRL rise at 0.2 and 1.0 mg/kg i.v. at 5 and 10 min ($p < 0.001$) and at 5 min, 10 min ($p < 0.005$), 20 min ($p < 0.001$) and 40 min ($p < 0.02$), respectively.

Only the higher dose of morphine (0.5 mg/kg i.v.) was able significantly to increase PRL levels ($p < 0.025$ at 5 and 10 min), while the lower dose (0.1 mg/kg) was completely ineffective in this sense. Analyses of the areas under each 40-min secretory profile by planimetry showed that the PRL releasing activity of each dose of $EKNH_2$ was significantly greater than that evoked by the corresponding dose of morphine (2990±297 vs 1357±410, $p < 0.01$ and