Ecdysteroid Titres during Autonomous Metamorphosis in a Dermestid Beetle

Jean-Paul Delbecque

Laboratoire de Zoologie, Université de Dijon, Boulevard Gabriel, F-21000 Dijon, France*

and

Karel Sláma

Institute of Entomology, Czechoslovak Academy of Sciences, Praha

Z. Naturforsch. **35 c,** 1066–1080 (1980); received June 19, 1980

Ecdysteroids, Juvenile Hormone, Metamorphosis, *Dermestes maculatus*

The larvae of *Dermestes maculatus* are able to undergo several moults in complete absence of the cephalic and prothoracic neuroendocrine system, showing thus spontaneous metamorphosis. Measurements of ecdysteroid contents by radioimmunoassay methods have revealed distinctive larval, prepupal and pupal ecdysteroid peaks, as found in other species where development requires frequent stimulation by the centrally produced hormones. Mass fragmentographic analysis has indicated that more than 80% of the ecdysteroids detected by the radioimmunoassay during the peaks consists of ecdysterone.

The prothoracic glands (PG) of *D. maculatus* larvae are located in the head capsule and in the cervical region. They can be separated from the body by neck-ligation. Only the larval peak of ecdysterone can be correlated with PG function. The prepupal and pupal peaks of ecdysterone are not produced by the central neuroendocrine system. The isolated abdominal fragments of larvae and pupae positively produce ecdysteroids in the course of their independent development, but this synthesis has been subnormal and occasionally delayed in time. In contrast, the isolated thoracic fragments produce well synchronized peaks of ecdysterone and this does not depend on the PG. It thus appears that some thoracic tissue other than PG is able to maintain the physiological concentration of ecdysterone by means of a concentration-dependent feed-back mechanism.

Due to spontaneous metamorphosis and extremely good survival we have succeeded to induce in this species: a) premature formation of the prepupal cycle of ecdysterone synthesis in the penultimate larval instar by ligation; b) inhibition and delay of the prepupal peak by means of juvenoid treatments; c) reappearance of the prepupal peak in the inhibited "permanent larvae" by exogenous administration of ecdysterone, or; d) modification of the pupal peak by a qualitatively new pupal-pupal one after juvenoid treatments. These experimental transformations of the ecdysterone peaks have suggested that the kind and nature of the peaks are closely related to nature and stage of the ontogenetic development. We therefore believe that the process of larval-pupal reprogramming has occurred in this species long before the increase of ecdysterone titer in the body. Both the prepupal and pupal peaks do not represent a cause but they show to be rather consequences of the developmental programming. Further features associated with possible physiological role of ecdysteroids in insect development have been briefly discussed.

Introduction

Insect ontogeny proceeds as distinctive developmental cycles which are associated with specific peaks in the synthesis of ecdysteroids. The peaks have been found in all developmental stages of insects, including also embryonic and adult stages [1–4]. With regard to hormonal control of insect metamorphosis, considerable attention of insect endocrinologists has been recently attracted to the specific peaks in ecdysteroid content that precede each of the larval, pupal or adult ecdysis. We refer to these peaks as to the larval, prepupal and pupal ecdysteroid peaks, respectively. Their physiological role has been mainly ascribed to morphogenetic reprogramming [5, 6], induction of macromolecular biosynthesis [6, 7], or to secretion of the new cuticle by the epidermal cells [8].

For a long time endocrinologists assumed (see review by Sláma *et al.* [9] that the priviledged source of these hormones were the prothoracic glands (PG). And, as a matter of fact, there are numerous reports in the literature that unequivocally confirm ecdysteroid synthesis within PG of taxonomically quite unrelated species [10–12]. The exact physiolog-

* Equipe de Recherche Associée an C.N.R.S. No. 231.

Reprint requests to Dr. J.-P. Delbecque.

0341-0382/80/1100-1066 $ 01.00/0

ical role of these moulting hormones became a more complex problem, however, when the synthesis of ecdysteroids was found outside the PG, such as in the oenocytes [13, 14], in the isolated abdomens of Lepidoptera [15–18], Diptera [19] and Coleoptera [20–22], as well as in the eggs and ovaries of different species [3, 4, 23, 24].

Among insects that are known to produce ecdysteroids outside PG we can find two Coleopteran species, *Leptinotarsa decemlineata* [20, 21] and *Tenebrio molitor* [14, 22, 25]. Using the sensitive radioimmunoassay techniques we have now investigated some aspects of ecdysteroid synthesis in another Coleopteran species, *Dermestes maculatus*. The major endocrinological feature of this species is that even the small young larval instars are able to undergo spontaneous metamorphosis when deprived of the whole neuroendocrine system. Thus, the perfectly coordinated morphogenesis process of larval-pupal and pupal-adult transformation can here proceed according to rather stereotypic schedule in the isolated larval abdominal or thoracic fragments. We have been very often obliged to quote these and other exceptions related to endocrinology of *D. maculatus*. These data refer mainly to publications by Sláma *et al.* [9] and Sláma [26].

Materials and Methods

The larvae of *Dermestes maculatus* DeGeer (= *vulpinus* F.) were reared in 1 liter jars at 27 °C, 16 h photoperiod. They were fed by dried calf viscera and supplied with a cotton-plugged vial with water to provide moisture. The ligated larvae, isolated body fragments or pupae were kept in 10 cm Petri dishes at 27 °C.

The juvenoid treatments were made by topical application using 1 µl of acetone as a solvent. The compound used in this study was ethyl 11-chloro-3,7,11-trimethyl-2-(E,Z) dodecenoate (juvenoid I), prepared in the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences. Ecdysterone was injected in 1 µl of 10% ethanolic Ringer solution. We used the natural product isolated from *Polypodium vulgare* rhizomes by Dr. J. Jizba of the above Institute.

Extraction of ecdysteroids was made by homogenisation of 100 to 200 mg of the insect material (2 to 6 specimens or body fragments) in 1 ml of methanol (spectrophotometric standard grade, LACHEMA Co., Brno). After 2 h at room temperature the homogenate was centrifuged ($1500 \times g$, 10 min) and the precipitate was twice resuspended and centrifuged again in 1 ml of methanol. The pooled methanolic extracts were partly evaporated under vacuum to 1–2 ml, sealed in glass ampoules and stored at −14 °C until used.

Determination of ecdysteroid content was made by the radioimmunoassay method (RIA) of De Reggi *et al.* [27] as follows: after evaporation of the methanol, the extracts were dissolved in 0.1 M citrate buffer, pH 6.2, and their ecdysteroid content was determined in duplicate assays by competition for antibodies with the radioactive iodinated analogue of ecdysterone. The nature of ecdysteroids involved in the assay was at first investigated by combined thin-layer chromatography (TLC) (Merck precoated plates 20×20 cm, silica gel F_{254}, chloroform/methanol 7/3 as solvent) and RIA on 1 cm scrapped zones. The RIA active zones were subjected to further analysis using mass fragmentography (gas-liquid chromatography coupled with mass spectrometry on LKB 9000 GLC-MS instrument). Prior to the measurements the active TLC-RIA zones were pooled, extracted and finally silylated at 65 °C overnight in pure trimethylsilylimidazole (for more technical details see Delbecque *et al.* [22] and Lafont *et al.* [28].

Each point on the curves in Figs. 1 and 4–10 represents an average of at least 6 determinations (3 samples of 2 to 6 bodies or fragments, each sample analysed in duplicate). This simplified procedure was adopted due to rather accurate results obtained by the RIA method. The range of SEM as indicated in Fig. 1 is more or less valid for all other data obtained by the same technique. During the whole period of constantly low ecdysteroid content (*i.e.* from the 1st to 5th day of last larval instar, *cf.* Fig. 1) we found the total mean value of 14.19 ng/g, the standard deviation was 3.19 and SEM only 0.82.

Results

Ecdysteroid titres during normal development

The postembryonic development of *D. maculatus* is characterized by rapid growth through the five young larval instars; the larvae ecdyse in 2.5 to 3-day intervals at 27 °C. The last (6th) instar larvae

cease to feed after about 5 days. They successively loose the mobility by day 7 and ecdyse into pupae at day 9 or 10. The pupae emerge as adults after 7 days.

Fig. 1 shows a distinctive larval peak in ecdysteroid concentration within the penultimate larval instar, which is followed by constantly low ecdysteroid level during the whole feeding period of the last larval instar. Then, there follows the prepupal peak, culminating at day 8 of the last larval instar and, finally, there is a relatively large pupal peak with the maximum in about the middle of the pupal instar. It is obvious that this pattern of normal ecdysteroid content as shown in Fig. 1 is more or less common to most other Endopterygote insects (see discussion).

The nature of ecdysteroids in D. maculatus

The pooled extracts of 7- to 9-day-old last instar larvae and of 3- to 4-day-old pupae were concentrated and subjected to the combined TLC-RIA analysis. It appeared (Fig. 2) that the far predominating part of the RIA-responsive material cochromatographed with the ecdysterone standard. Moreover, mass fragmentography of the trimethylsilyl derivatives, obtained by silylation of the TLC products from the 6th to 10th zone (*cf.* Fig. 2) revealed that ecdysterone constituted largely predominating ecdysteroid in this species, as documented in Fig. 3. There was very low, if any, ecdysone present, but there was a small amount of another ecdysteroid with the retention time 10′10″, which was probably 20,26-dihydroxyecdysone. The distribution of ecdysteroids during the prepupal peak (not shown in Figs. 2 and 3) was basically the same as that during the pupal peak. Since ecdysterone was mostly responsible for the RIA positive responses in *D. maculatus*, we give the results of our RIA analyses in terms of ecdysterone or ecdysterone equivalents.

The larval peak of ecdysterone

Previous endocrinological studies [26] indicated that the neuroendocrine system of *D. maculatus* (including PG) had lost its essentiality to regulate further development since the middle of the penultimate larval instar. This has been confirmed by more

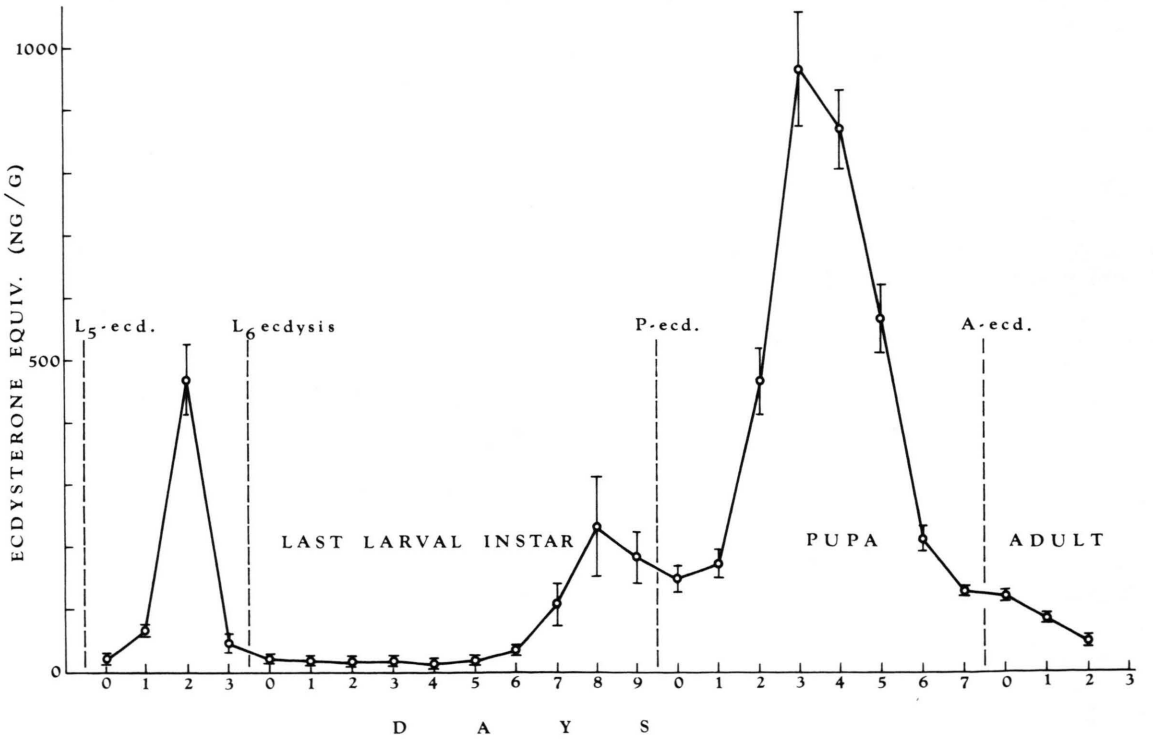

Fig. 1. The content of RIA-positive materials during the course of normal development of *D. maculatus*. The SEM values (vertical lines) have been calculated from 6 determinations in larvae and 12 determinations in pupae and adults.

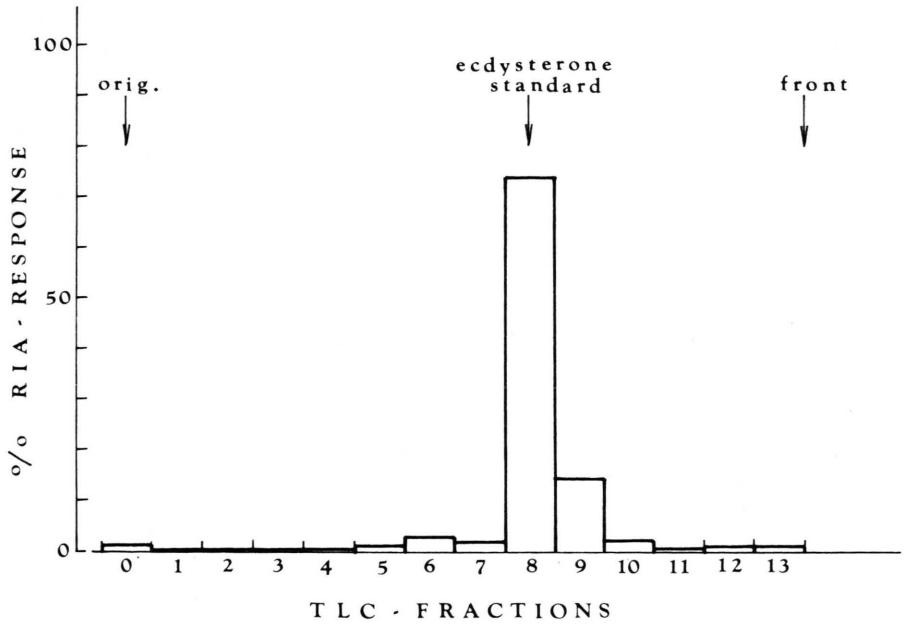

Fig. 2. RIA responses of fractions obtained by thin-layer chromatography (chloroform/methanol 70/30) of the extracts from 3 to 4-day-old pupae of *D. maculatus*.

recent observations [29] which show that the PG of this species indeed exhibit the last secretory cycle in the penultimate instar. The glands are located here in the cervical region, extending into the head capsule. Due to this, ligatures applied behind the head capsule deprive the thoracic part and the rest of the body of all the so far known centralized neuroendocrine glands, including the PG.

The engagement of the neuroendocrine system in the developmental regulations during the penultimate larval instar can be best evidenced by dependence of the moult cycle on nutrition (which is present also in the younger larval instars but not in the last one). Thus, starved larvae of the 5th instar never exhibit the usual 3-day moult cycle unless provided again with food for at least 15 to 20 h. Accordingly, the larval peak of ecdysterone, which normally culminates at 470 ng/g at the 2nd day is postponed; the mean ecdysteroid concentration of the starved larvae has been only 30.4 ng/g at day 2, see Fig. 4.

A somewhat different situation is created when the 5th instar larvae have been deprived of the neuroendocrine system by neck-ligation. Under these circumstances, in the absence of c. allata and PG, the headless body appears to be invariably committed to spontaneous metamorphosis. Thus, when ligated sooner than about 15 h of feeding, the headless body or isolated abdominal fragments develop prematurely into small prothetelic pupae and adults, retaining perfectly all the developmental timings (when isolated after 15 h of feeding the fragments complete at first the larval moult and then perform pupal and adult moults). Our results in Fig. 4 document that the prematurely metamorphosing headless larvae of the 5th instar produce the characteristic 9-day cycle of ecdysterone, including the prepupal peak at the 8th day. The major physiological implication of these results is that some peripheral tissues in the headless body, which had been irreversibly committed to metamorphosis by removal of the endocrine glands, are able to produce the coordinated prepupal peak of ecdysterone without receiving any immediate signal to do so from the central neuroendocrine system.

The prepupal peak of ecdysterone

As soon as the larvae of *D. maculatus* reach the last larval instar they obligatorily undergo a stereotypic 9-day larval-pupal transformation, irrespective of starvation or any other environmental signal. Under experimental conditions, this 9-day developmental stereotype becomes successively realized in the headless body or even in the isolated abdomen that has been deprived of the intestine, Malpighian

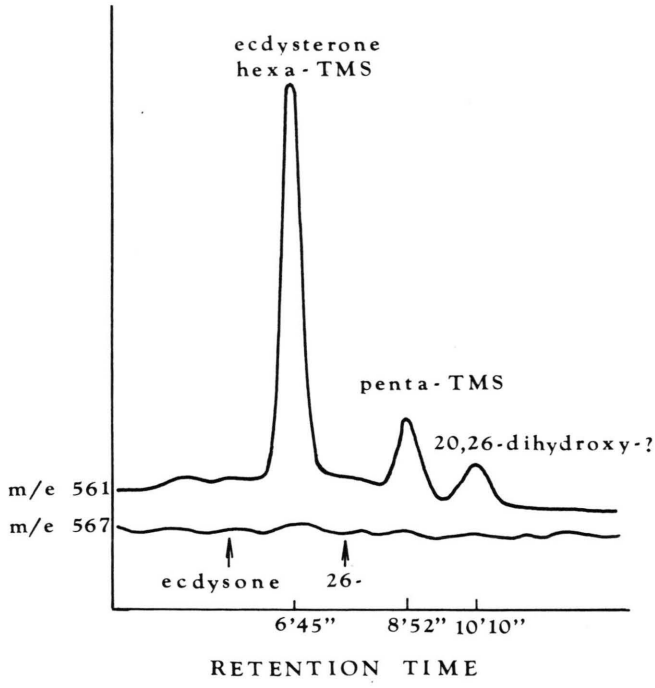

Fig. 3. Mass fragmentogram of the purified fractions (TLC zones 6 to 10) from 3 to 4-day-old pupae. Monitoring at *m/e* 561 allows analysis of the silylated derivatives of 20-hydroxyecdysteroids (hexa TMS derivative of ecdysterone, penta-TMS derivative of ecdysterone and probably hepta-TMS derivative of 20,26-dihydroxyecdysone). Ecdysone and 26-hydroxyecdysone derivatives, which can be monitored at *m/e* 567 have not been detected (arrows indicate their respective retention times).

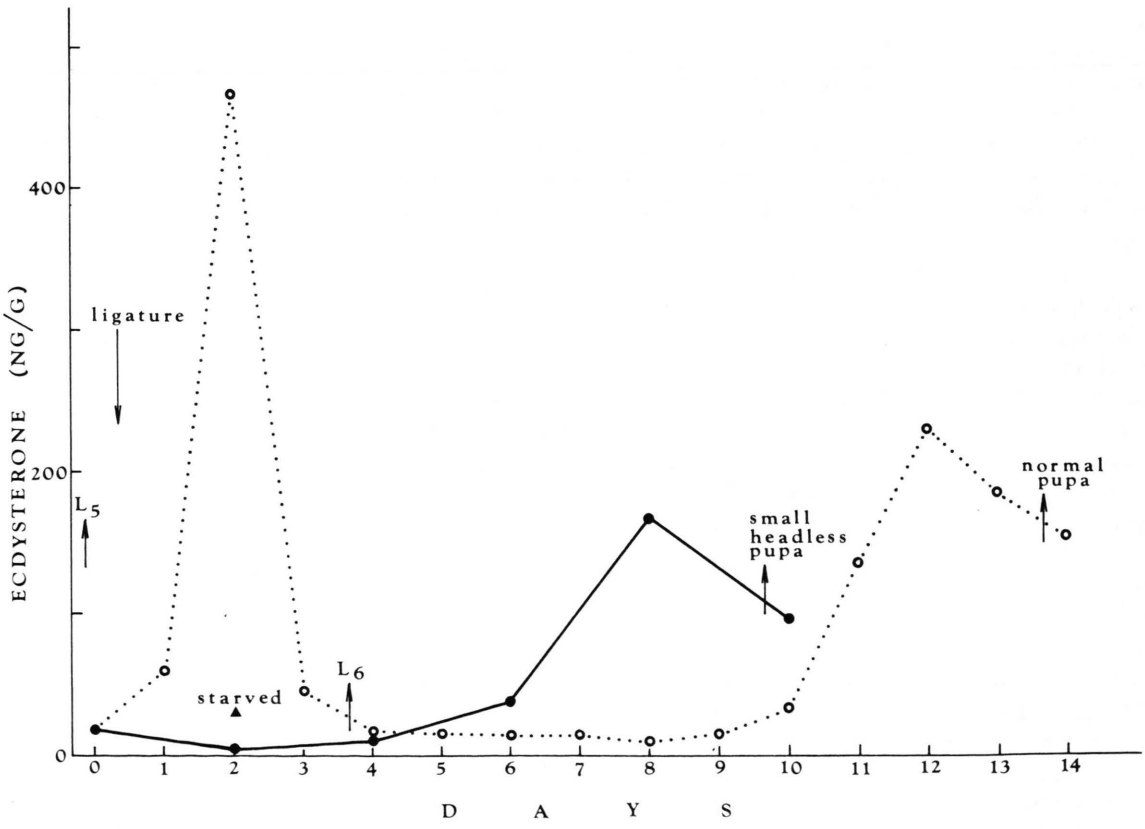

Fig. 4. Ecdysteroid concentration in the neck-ligated larvae of the penultimate (5th) instar (full line). The dotted line is for normal untreated controls. Triangle indicates ecdysteroid content in the starved 2-day-old 5th instar larvae.

tubules, gonads and of considerable part of the fat body [26]. The data in Fig. 5 provide evidence that the headless larvae of the last instar (lacking PG) are also able to produce the regular peak of ecdysterone with the maximum around the 8th day. The abdominal fragments that had been isolated soon after ecdysis were equally able to produce ecdysterone, although in smaller concentrations (see Fig. 5). In spite of this, however, the abdominal epidermal cells were able to form a perfect pupal cuticle after a slight occasional delay in time (about 24 h in average).

In the next experimental series (Fig. 6) we have isolated both the thoracic and abdominal fragments of the 6-day-old last instar larvae, leaving them to develop separately the pupal structures. The larger abdominal as well as the smaller thoracic fragments contained about equal amounts of ecdysterone (see Fig. 6 A), both showing a distinct peak at the 8th day. However, the concentration of ecdysterone per unit of weight (Fig. 6 B) was much smaller in the abdominal fragment while it was maintained at almost normal control level in the thoracic one. There are several physiological implications of these results in Fig. 6: a) Some larval abdominal tissues are capable to synthesize ecdysterone in the course of their spontaneous formation of the pupal structures; b) Metamorphosis in this species does not depend on any localized abdominal source of ecdysterone, since the isolated thoracic part also develops independently, and; c) The larval thoracic fragment, though void of PG, does not only produce ecdysterone but it can actually maintain the same concentration as found in the normally developing intact body.

The effect of exogenous juvenile hormone activity

In contrast to many other insects, the most potent juvenoids (including those which are very active on pupae of *D. maculatus*) completely failed to cause any supernumerary moults in the larvae of this species. Instead of this, however, the larval treat-

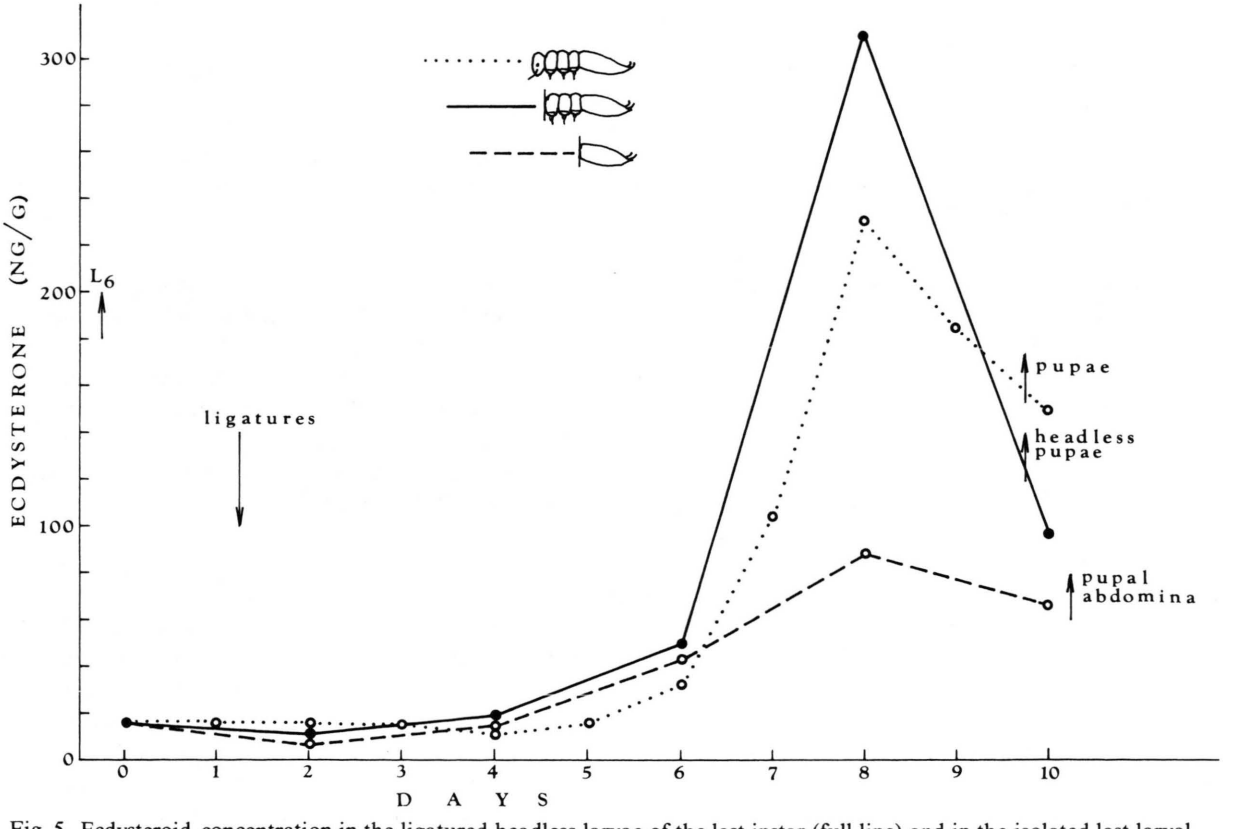

Fig. 5. Ecdysteroid concentration in the ligatured headless larvae of the last instar (full line) and in the isolated last larval abdomens (broken line). Dotted line is for normal last instar larvae.

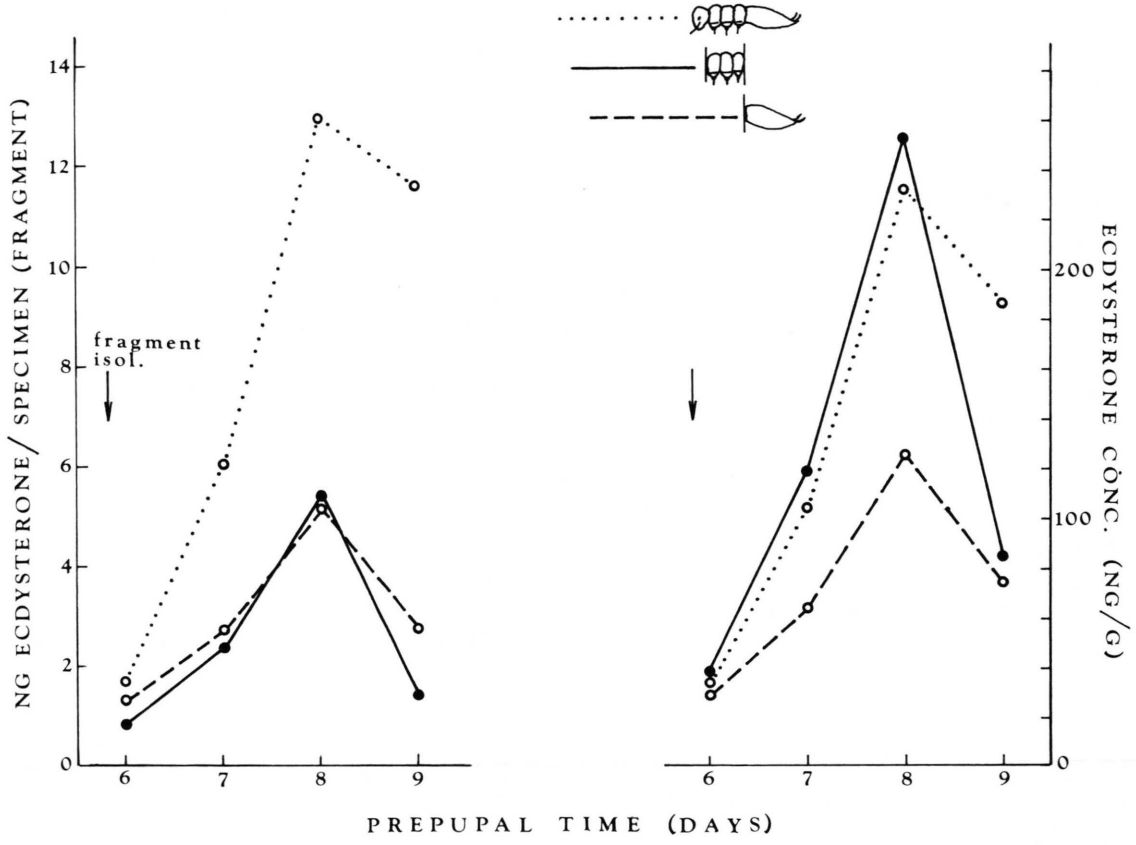

Fig. 6. Ecdysteroid content (A) and concentration (B) in the isolated thoracic compartments (full line) and abdominal compartments (broken line), prepared from 6-day-old last instar larvae. Dotted line is for normal intact controls. All parts made perfect pupal cuticles at days 9 and 10.

ments have been always followed by an immediate arrest of the stereotypic larval-pupal morphogenetic programme. When performed during the feeding period of the last larval instar, the juvenoid treatment induces enormous hypermetabolic response [30, 31]. In the headless body or in the isolated abdomen the juvenoid treatment produced long lasting and dose-dependent inhibition of metamorphosis ("permanent larvae") without hypermetabolic action [9, 30].

The results in Fig. 7 provide evidence that the hypermetabolic larvae, as well as the headless "permanent larvae" treated by the juvenoid I, contain a constantly low amount of ecdysterone in their bodies. The process of morphogenetic reprogramming and the resulting prepupal peak of ecdysterone have been postponed and conserved for later use, as has been demonstrated below. This shows that the prepupal peak of ecdysterone is tightly bound to certain determined stage of the morphogenetic process and, therefore, it can be prevented or delayed hand-in-hand with the physiological inhibition of this process. As some of these results have been obtained on specimens lacking the central neuroendocrine system, it is obvious that the described interactions between juvenoid and ecdysterone must be realized at the level of the peripheral tissues.

The effect of exogenous ecdysterone

In the previous experiment (Fig. 7) we have determined that the internal morphogenetic "clock" (which makes the tissue competent to form the prepupal peak of ecdysterone exactly after 8 days since the end of the previous larval-larval one) can be completely stopped by juvenile hormone activity. Now we have explored a possibility to restore again performance of the "clock" by creating an artificial

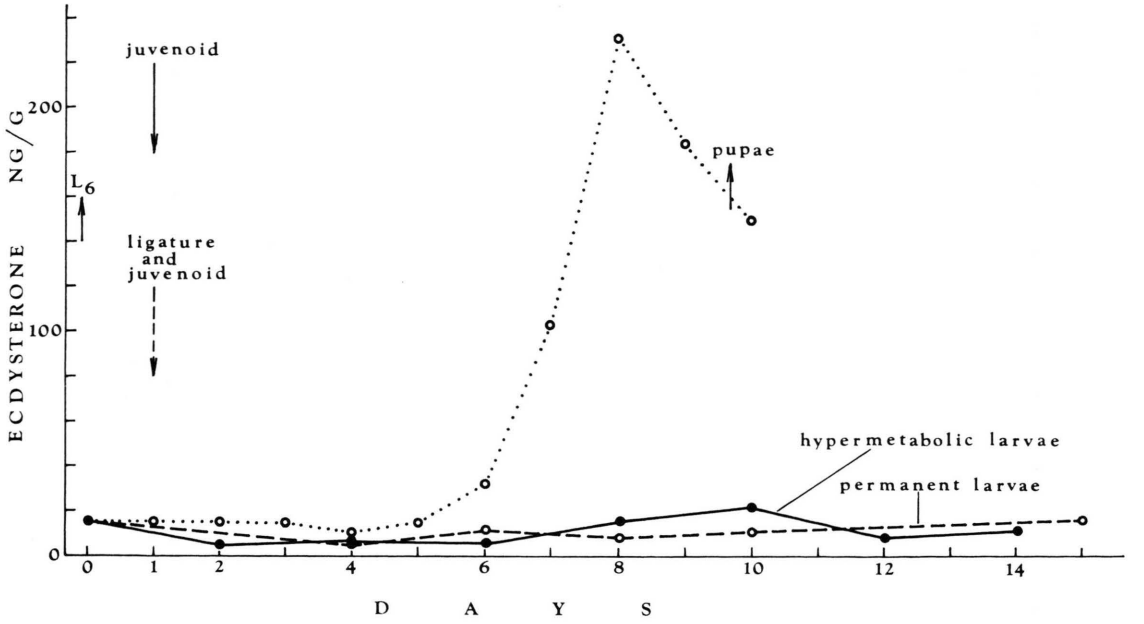

Fig. 7. The effect of juvenoid I (50 µg per specimen) on ecdysteroid concentration of normal (full line) and headless (broken line) larvae of the last instar. The dotted line is for untreated controls.

peak of ecdysterone. The most difficult problem in these experiments was concerned with determination of the right, physiologically effective dose of ecdysterone. Previous studies have shown that a relatively large dose of ecdysterone (0.5 to 10 µg/specimen) cause in this system one or more rapid extra-larval moults in 2.5 to 3-day intervals (see Sláma *et al.* [9], p. 351). In contrast, lower doses (0.01 to 0.2 µg/specimen) could restrain the inhibition imposed by juvenile hormone and have reinduced the morphogenetic process with normal timing of developmental events [26].

Finally, as in the experiments outlined in Fig. 8, we used the "permanent larvae" after 7 days of their developmental arrest by juvenoid I. The larvae were now injected with 0.2 µg of ecdysterone per specimen, which gave the initial calculated concentration of 3773 ng per g of body weight. The data in Fig. 8 show that the RIA method detected the actual concentration 3520 ng/g of ecdysterone about 30 min after the injection. The bulk of exogenous ecdysterone rapidly disappeared from the system, leaving two days later only 774 ng/g. Since this time, however, there was only rather small and relatively constant degradation of ecdysterone, the rate of which was approx. 37.7 ng/g/day. It is most important that around the 8th day after the injection we have indeed found a distinctive increase of the ecdysterone concentration. Assuming that the rate of degradation of the injected ecdysterone did not change profoundly, the described increase could be very well equivalent to the expected endogenous prepupal peak of ecdysterone (amounting about 250 to 280 ng/g). And, as a matter of fact, morphological inspections revealed that the experimental animals were immobile prepupae at the 8th day after the injection. A few of them that were left aside became headless pupae in the next two days. The noninjected control group of "permanent larvae" remained in the inhibited larval stage for several weeks. From the endocrinological points of view it is also important that the headless pupae produced by ecdysterone injections never develop into adults. Due to a constant presence of juvenoid residues in the body they almost invariably performed one or more extra-pupal moults. Exact reasons why juvenoid does not affect the ecdysterone induced larval-pupal reprogramming while strongly affecting the later pupal-adult one remain obscure.

The major physiological implication of the above results is that a peak of ecdysterone can serve as a signal to set in motion the autonomous self-regu-

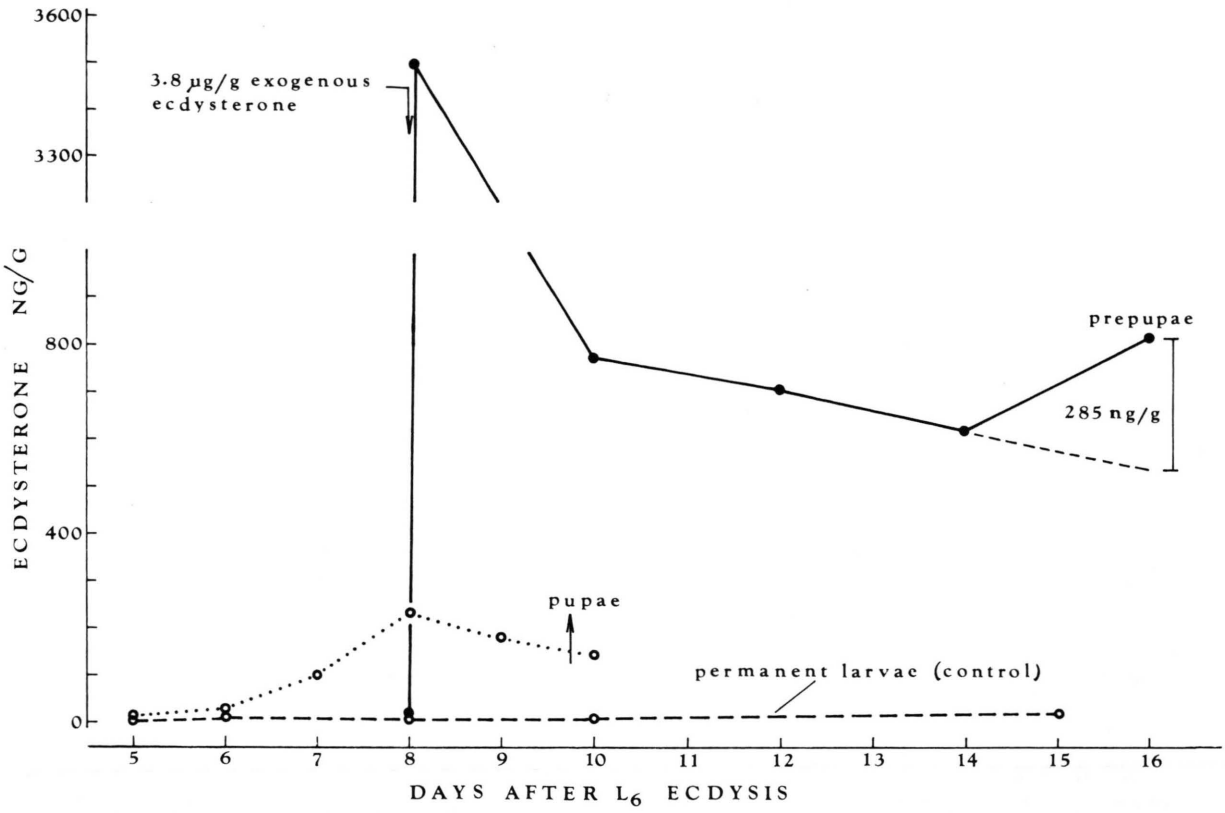

Fig. 8. Effect of exogenous ecdysterone injections (200 ng/specimen, = 3.8 µg/g) on ecdysteroid concentration in the "permanent larvae" (ligated last instar larvae treated with 100 µg of juvenoid I one day after ecdysis). Full line shows injected larvae, broken line noninjected "permanent larvae" and dotted line is for intact controls. The injected larvae became headless pupae 9 to 10 days after the injection.

lating morphogenetic programme, which has been previously arrested by juvenile hormone. Reinduction of the morphogenetic programme by exogenous ecdysterone has been associated with the built-in message for the prepupal synthesis of endogenous ecdysterone. And, since the stimulus to form the prepupal peak of ecdysterone came as long as 8 days in advance, it is quite realistic to assume that the endogenous peaks do not represent a cause (as generally believed) but rather a consequence of certain determined stage on the morphogenetic programme.

The pupal peak of ecdysterone

The pupal-adult morphogenesis of *D. maculatus* differs from the previous larval-pupal one by absence of the start/stop code. Therefore, any tissue which has once attained the pupal ontogenetic stage must immediately proceed towards adult differentiation. Alternatively, as in the presence of exogenous juvenile hormone, the tissues are obliged to perform one or more intercallated extra-pupal moults. In the case of the first alternative we have found (see Fig. 9) that the male and female pupae exhibit relatively large pupal peak of ecdysterone, which has almost identical course in both sexes (*cf.* Fig. 1 and 9). This suggests that the pupal peak is not directly associated with the ovarian production of ecdysterone, as may be the case in some other species. Fig. 9 also shows that there have not been any more conspicuous changes in the ecdysterone content at the beginning of adult life.

The second developmental alternative was investigated in pupae exposed to juvenile hormone activity (topical treatment by 50 µg of juvenoid I per specimen, applied 1–2 days before pupal ecdysis). These pupae formed an extra-pupal instar after 4 to

5 days. This could be easily recognized according to the formation and pigmentation of the characteristic pupal epidermal tubercles on the pronotum and mesonotum. The extra-pupal instars are unable to ecdyse spontaneously; they remain telescoped within the previous cuticles. The results of RIA analysis (Fig. 9) revealed that the induced switchover from the pupal-adult to the stationary pupal-pupal developmental programme was associated with a profound reduction and abbreviation of the original pupal peak of ecdysterone. Thus, in exact proportions to the accelerated secretion and pigmentation of the extra-pupal cuticle, the newly induced pupal-pupal peak of ecdysterone declined to the low pre-ecdysial levels of 200 ng/g correspondingly sooner than in the control pharate adults. In this experiment we did not expect repeated formation of the second and further extra-pupal instars. Nevertheless, these findings are again in favour of the conclusion that the endogenous peaks of ecdysterone do specifically reflect the type of developmental changes that take place in the body. In addition, the substantially different action of the juvenile hormone analogue on larvae and on pupae point out that this hormone may affect ecdysterone synthesis in a different way when introduced at various ontogenetic stages.

The problem of ecdysterone synthesis in pupae

Due to anatomical changes in tracheation at the late pharate pupal period, the disintegrating remnants of the PG move from the head capsule and from cervical region into the prothorax of pupae. Therefore, it is no more possible to separate PG from the pupal thorax by applying neck-ligation, as in larvae. The pupal PG show high degree of disintegration just after pupal ecdysis. Their cytological appearance and their complete degeneration by the middle of the pupal instar exclude a possibility that these cells could have contributed by ecdysterone

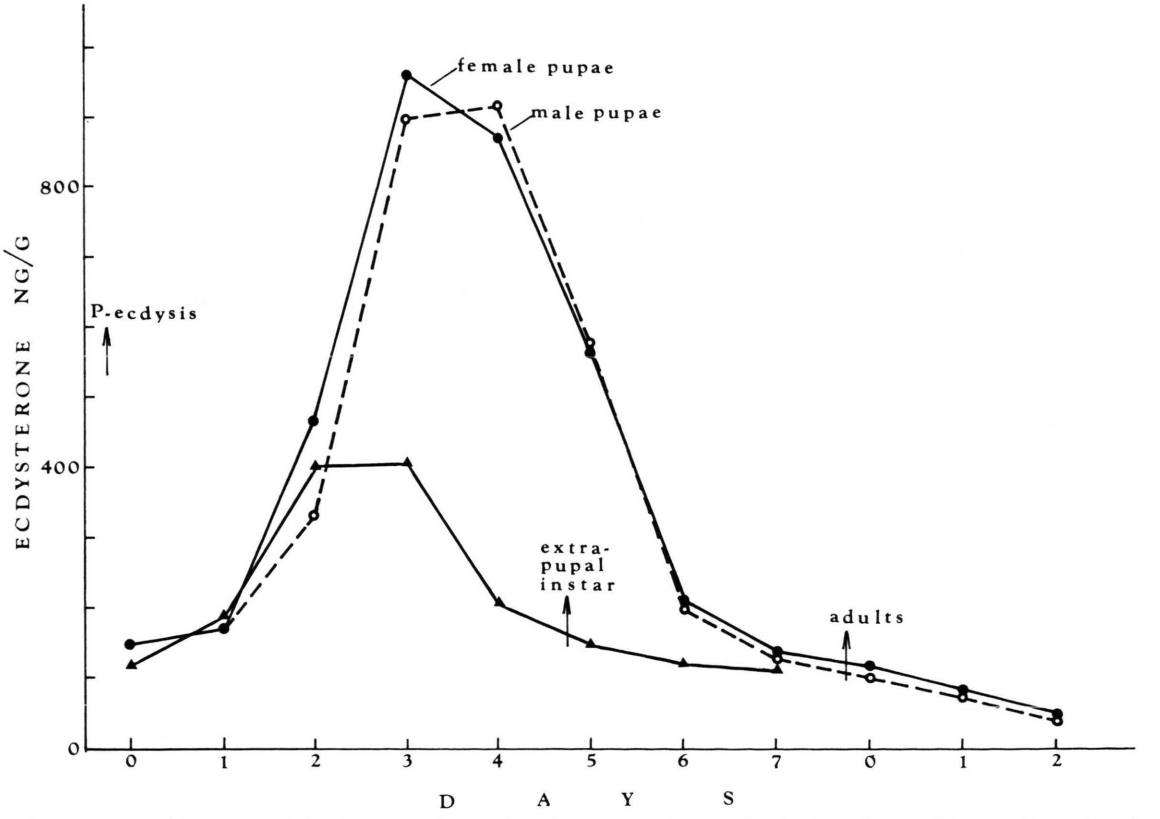

Fig. 9. Ecdysteroid concentration in the male and female pupae and at the beginning of adult life. The line with triangles shows ecdysteroid concentration associated with development of an extra-pupal instar (50 µg of juvenoid I per specimen, applied 1 to 2 days before pupal ecdysis).

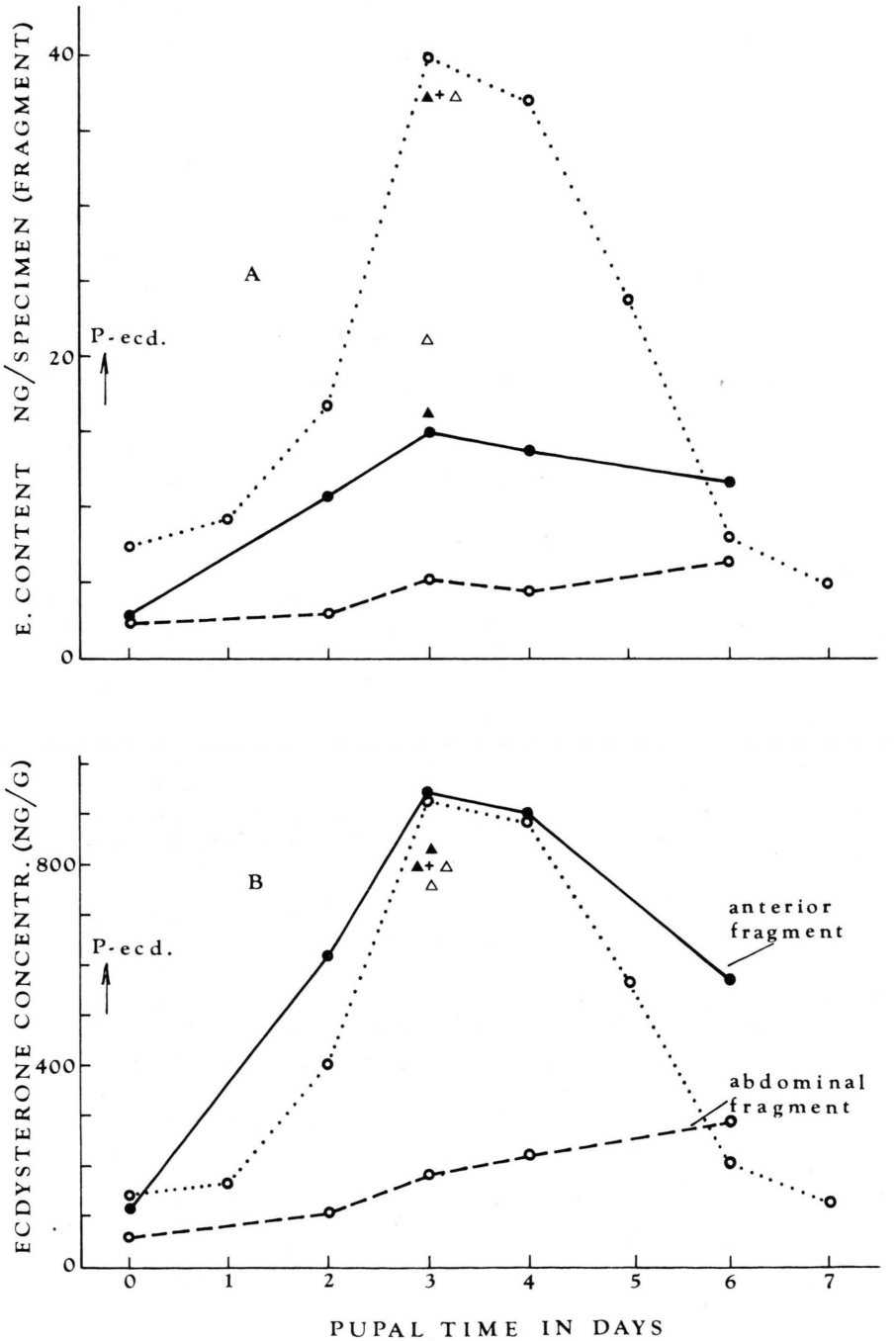

Fig. 10. The content (A) and concentration (B) of ecdysteroids in the isolated anterior fragments (head + thorax, full line) and in the pupal abdomens (broken line). The dotted line is for intact pupal body. Triangles indicate amounts of ecdysterone in the anterior (black triangles) and abdominal (open triangles) compartment of the normal 3-day-old pupae.

synthesis during the pupal peak (*cf.* Sláma [29]). Moreover, the whole cephalic neuroendocrine system is not essential for adult differentiation, because the headless pupae as well as the isolated pupal abdomens all develop perfect adult epidermal structures. For these reasons we have decided to measure separately ecdysterone production in the fairly well surviving anterior fragments (head and thorax) and in the abdominal fragments.

In the control experiment we have at first determined the distribution of ecdysterone in the anterior anterior and posterior compartment of the normal 3-day-old pupae. We have found that the larger abdominal part contained proportionally more ecdysterone, but the concentration per g of weight was almost identical in both the fragments (see triangles in Fig. 10). This indicates that in normally developing pupae ecdysterone is distributed in equal concentrations throughout the anterior and posterior parts.

Fig. 10 A shows that the relatively small anterior fragment developing in isolation contained considerably more ecdysterone than the larger abdominal one. There was a clear maximum at the third day when the anterior fragment contained about the same amount of ecdysterone as the fragment of the equally old normal pupa. Accordingly, see Fig. 10 B, the concentration of ecdysterone in the isolated anterior fragment has followed more or less similar course with that of the intact body. In contrast, the concentration in the abdominal fragment has been low, non synchronized with the usual peak, though having a constantly increasing tendency. Comparison of the data in Fig. 10 A and 10 B provides evidence that the successive rise of ecdysterone concentration in the isolated abdomens was not solely due to the loss of weight. However, the delay in the abdominal production of ecdysterone may be related to the occasional delay in the formation and pigmentation of the adult cuticle by the isolated pupal abdomens.

Comparison of the data in Figs. 5 and 6 with Fig. 10 provides evidence that these results obtained on pupal fragments are basically similar to those obtained on the larval fragments. They point out again the principal physiological role of the anterior body compartments for regulation of ecdysterone production. With regard to the degenerated pupal PG and absolute unessentiality of the head structures for adult development, we may conclude that (similarly like in the larval stage) some thoracic tissues other than PG take the responsibility for these regulations also in the pupal stage. Assuming that some thoracic tissues supply most of ecdysterone to the whole body, then one might theoretically expect accumulation of ecdysterone in the thorax after removal of the abdominal recipiental part. Our results have clearly shown, however, that the concentration of ecdysterone in the isolated larval thorax or in the anterior pupal compartment does not surpass the limits determined by the concentration of ecdysterone in the intact whole body. Among other possibilities there exists one attractive physiological explanation of these relationships, *i.e.* that some thoracic tissues other than PG are capable to regulate production of ecdysterone by means of a concentration dependent feed-back mechanism.

Discussion

The described findings on *Dermestes* are creating a serious problem relative to the physiological function and source of ecdysteroids, which are generally assumed to be produced by the PG for stimulation of the moult cycles. The previous endocrinological studies in this species (Sláma *et al.* [9, 26, 30, 31]) provided clear experimental evidence that the c. allata and PG are used here only for the control of the larval-larval moult cycles, while the whole process of metamorphosis proceeds spontaneously according to a stereotypic programme. At present we still cannot satisfactorily explain why should this spontaneously metamorphosing species with nonessential neuroendocrine system exhibit the common larval, prepupal and pupal peaks of ecdysterone; and why are these peaks located at exactly the same ontogenetic positions as in many other insects, whose development seems to require an obligatory stimulus from the central neuroendocrine system. The striking similarity in the location of the peaks as well as in the nature of the ecdysteroids produced can be found especially between *Dermestes* and other Coleopteran species studied, *i.e. Tenebrio* [8, 32] and *Leptinotarsa* [21].

Apart from general similarities there are also some minor physiological differences in the ecdysteroid titres between *Dermestes* and other species. For example, it has been observed in several insects, such as in *Manduca* [33], *Pieris* [7] or *Tenebrio* [8, 32] that the prepupal peak may be preceded by a small initial peak, which has been assumed to play an im-

portant role in the reprogramming of larval-pupal development [5]. In *Locusta* even a third peak has been evidenced [11]. In all these species the small initial peaks can be correlated with the critical periods of PG activity. They also correlate with an increased secretion by the PG *in vitro* [11, 33]. In *Dermestes* such a small initial peak has not been evidenced, although we have measured ecdysterone concentration in several independent experimental series of the last larval instar. Provided that the small initial peak really does not exist here, its absence could be related to the lack of any critical period for PG action.

There are also taxonomical differences related to the role of PG during insect metamorphosis. The gland is generally believed to be essential for stimulation of metamorphosis and for the production of ecdysteroid peaks in Lepidoptera. In *Pieris*, however, the isolated larval abdomens did not produce ecdysteroids [34], while the pupal abdomens did [17]. In Coleoptera (*Tenebrio*), the larval and at least a part of the prepupal peaks of ecdysterone could be correlated to PG activity, whereas the large pupal peak occurred at a time when the PG cells already showed considerable degree of degeneration [22, 25]. In *Dermestes* only the larval-larval peak of ecdysterone coincides with the secretory cycle in the PG [29], while the prepupal and pupal peaks are consequences of the spontaneous metamorphosis being unrelated to PG functions.

In this paper we have presented a cross-linked evidence that the spontaneously developing larval or pupal abdomens of *D. maculatus* are certainly able to synthesize ecdysterone. Our results are in many respects similar to the findings on *Leptinotarsa* as described by Hsiao *et al.* [20, 21]. The independent abdominal synthesis of ecdysteroids has been actually found in a number of other species; *Mamestra* [15], *Bombyx* [16, 18], *Pieris* [17], *Musca* [19], *Leptinotarsa* [20, 21] and *Tenebrio* [22]. In certain species, as for example in *Bombyx* [18] or in *Galleria* [35], this abdominal synthesis could be due to the ecdysteroid production in the ovaries. In *Tenebrio* [22] as well as in *Dermestes* the production of ecdysteroids outside the PG is not linked to ovarian growth. Neither it is localized to some abdominal or thoracic organ, because both the abdominal and thoracic fragments produce ecdysterone.

Owing to the presence of larval PG within the head capsule and due to the inactivity of PG in the last larval and pupal instars of *Dermestes* [29], we have been able for the first time to recognize the regulatory role of some thoracic tissues (other than PG) in the ecdysteroid production of the whole body. Realization that some other thoracic tissue than PG may be engaged in the independent regulation of ecdysteroid concentration strongly suggests that all previous literature data, which axiomatically ascribed any functions of the whole thoracic segments to PG, should be reinvestigated. The existence of a feed-back mechanism by means of which some peripheral thoracic tissues might regulate the concentration of ecdysterone in the body seems to merit further attention. Experimental evidence is needed in order to exclude a possibility of an artifact which could be caused by a more intensive degradation of ecdysterone in the abdominal compartments. However, the generally small rate of exogenous ecdysterone degradation (37.7 ng/g/day) as has been found in the headless body (see Fig. 8) does not substantiate fully this argument. On the other hand, there are some physiological reasons that may favour existence of the described feed-back mechanism. For example, the thoracic segments contain most of the extensively growing epidermal structures (legs, wings) whose perfect formation is most critical for successfull ecdysis and consequently for survival. Whether the above mechanism exists or not, our results make clear that in the spontaneously developing system of *Dermestes* some peripheral tissue must take over the control of ecdysterone production in the body.

In contrast to some results obtained on other systems (mostly Lepidopteran [5]), we have found that the increased concentrations of ecdysterone are not needed for developmental reprogramming in *Dermestes*. There is ample evidence to show [9, 26] that the larval-pupal process of developmental reprogramming proceeds here slowly, step by step, during the initial 5-days of the last larval instar, when concentration of endogenous ecdysterone is kept at the minimal level (see Fig. 1). By the time when the concentrations begins to rise (day 6) the reprogramming is just finished. In addition, the pupal-adult reprogramming proceeds in *D. maculatus* just during the initial 24 h after pupal ecdysis, when endogenous concentration of ecdysterone is again relatively low. By the experiments illustrated in Fig. 8 we have determined, however, that when morphogenesis had been preliminarily inhibited by

juvenile hormone, the peak of ecdysterone can serve as a stimulus for the whole genetically programmed differentiation process, including not only one but several phases of the developmental re-programming.

The inhibitory action of juvenile hormone analogue on the prepupal peak of ecdysterone (Fig. 7) is not a unique feature. It has been also found in *Blatella* by Masner *et al.* [36]. We have to realize, however, that this inhibitory action does not reflect a general action of juvenile hormone on the ecdysteroid peaks. Depending on developmental programme, juvenile hormone can modify the course of ecdysteroid peaks as has been described in the pupal stage. It is quite important to consider that the interactions between juvenile hormone and ecdysteroids we have described in *Dermestes* are not mediated by the central neuroendocrine system. They have been clearly realized at the peripheral tissue level.

Due to extremely good survival and special features of the spontaneously metamorphosing model of *Dermestes* we could use certain endocrinological manoeuvres which would hardly be possible in other species. Thus we have managed to induce, for instance, the premature prothetelic formation of the prepupal peak in the penultimate larval instar. We also managed to induce a qualitatively new, stationary pupal-pupal peak of ecdysterone or postponed the prepupal peak by juvenoid treatments. Moreover, we have succeeded to reinduce the prepupal peak of ecdysterone in the JH-inhibited larval body by exogenous ecdysterone. These artificial transformations of the ecdysteroid patterns provide evidence that, irrespective of the absolute age or size of the body, the kind of ecdysterone production always reflects the nature and ontogenetic stage of the current developmental programme. This special model enabled us to recognize that some peaks of ecdysteroids can be produced by the peripheral tissues as a consequence of the inherited programme, without interference of the central neuroendocrine system. This is in conflict with some current interpretations concerning physiological role of ecdysteroids in other insects, where the peaks are assumed to be produced by the central neuroendocrine system. We are conscious that our interpretations based on rather special developmental model are very challenging. They have been discussed in more details elsewhere [29].

Acknowledgements

We are indebted to Prof. B. F. Maume and Prof. P. Padieu of Dijon University for enabling us to use the LKB-9000 GLC-MS instrument, to Mrs. J. Pichlová for technical assistance and to M. De Reggi, M. Hirn, and M. Delaage for the kind supply of RIA antigens and antibodies.

[1] E. D. Morgan and C. F. Poole, Comp. Biochem. Physiol. **57 (B),** 99–109 (1977).
[2] H. Emmerich, Verh. Dtsch. Zool. Ges. **70,** 184–201 (1977).
[3] M. Gersch, Zool. Jb. Physiol. **82,** 171–184 (1978).
[4] J. A. Hoffmann, M. Lagueux, C. Hetru, M. Charlet, and F. Goltzene, in: Progress in Ecdysone Research (J. A. Hoffmann, ed.), Elsevier, Amsterdam, p. 431–465 (1980).
[5] L. M. Riddiford, Gen. Comp. Endocrinol. **34,** 438–446 (1978).
[6] J. J. Wielgus, W. E. Bollenbacher, and L. I. Gilbert, J. Insect Physiol. **25,** 9–16 (1979).
[7] R. Lafont, B. Mauchamp, J. L. Pennetier, P. Tarroux, L. De Hys, and J. P. Delbecque, Experientia **31,** 1241–1242 (1975).
[8] J. P. Delbecque, M. Hirn, J. Delachambre, and M. De Reggi, Develop. Biol. **64,** 11–30 (1978).
[9] K. Sláma, M. Romaňuk, and F. Šorm, Insect. Hormones and Bioanalogues. Springer Verlag, Wien, New York 1974.
[10] W. E. Bollenbacher, W. Goodman, W. V. Vedeckis, and L. I. Gilbert, Steroids **27,** 309–324 (1976).
[11] M. Hirn, C. Hetru, M. Lagueux, and J. A. Hoffmann, J. Insect. Physiol. **25,** 255–261 (1979).
[12] S. L. Smith, W. E. Bollenbacher, and L. I. Gilbert in: Progress in Ecdysone Research (J. A. Hoffmann, ed.), Elsevier, Amsterdam, p. 139–162 (1980).
[13] F. Romer, Naturwissenschaften **58,** 324–325 (1971).
[14] F. Romer, H. Emmerich, and J. Nowock, J. Insect Physiol. **20,** 1975–1988 (1974).
[15] M. Gersch and J. Stürzebecher, Experientia **27,** 1475–1476 (1971).
[16] K. Nakanishi, H. Moriyama, T. Okauchi, S. Fujioka, and M. Koreeda, Science **176,** 51–52 (1972).
[17] J. Claret, P. Porcheron, and F. Dray, Experientia **33,** 1389–1390 (1977).
[18] E. Ohnishi and F. Chatani, Develop. Growth Differ. (Japan) **19,** 67–70 (1977).
[19] G. Studinger and A. Willig, J. Insect Physiol. **21,** 1793–1798 (1975).
[20] T. H. Hsiao, C. Hsiao, and J. De Wilde, Nature **255,** 727–728 (1975).
[21] T. H. Hsiao, C. Hsiao, and J. De Wilde, J. Insect Physiol. **22,** 1257–1261 (1976).
[22] J. P. Delbecque, J. Delachambre, M. Hirn, and M. De Reggi, Gen. Comp. Endocrinol. **35,** 436–444 (1978).

[23] H. H. Hagedorn, J. D. O'Connor, M. Fuchs, B. Sage, D. A. Schlaeger, and M. K. Bohm, Proc. Nat. Acad. Sci. (US) **72,** 3255–3259 (1975).
[24] M. Lagueux, M. Hirn, and J. A. Hoffmann, J. Insect Physiol. **23,** 109–120 (1977).
[25] I. Glitho, J. P. Delbecque, and J. Delachambre, J. Insect Physiol. **25,**187–191 (1979).
[26] K. Sláma, Coll. Int. C.N.R.S. **251,** 499–506 (1976).
[27] M. L. De Reggi, M. H. Hirn, and M. A. Delaage, Biochem. Biophys. Res. Commun. **66,** 1307–1315 (1975).
[28] R. Lafont, G. Somme-Martin, B. Mauchamp, B. F. Maume, and J. P. Delbecque, in: Progress in Ecdysone Research (J. A. Hoffmann, ed.), Elsevier, Amsterdam, p. 45–68 (1980).
[29] K. Sláma, Acta Ent. Bohemoslov. **77,** 145–168 (1980).
[30] K. Sláma and M. Hodková, Biol. Bull. (Woods Hole) **148,** 320–332 (1975).
[31] K. Sláma and I. Kryspin-Sørensen, Z. Naturforsch. **34 c,** 599–607 (1979).
[32] J. P. Delbecque, M. Prost, B. F. Maume, J. Delachambre, R. Lafont, and B. Mauchamp, C. R. Acad. Sci. D. **281,** 309–312, (1975).
[33] W. E. Bollenbacher, W. V. Vedeckis, L. I. Gilbert, and J. D. O'Connor, Develop. Biol. **44,** 46–53 (1975).
[34] R. Lafont, B. Mauchamp, J. L. Pennetier, and M. De Reggi, Experientia **33,** 1662–1663 (1977).
[35] T. H. Hsiao and C. Hsiao, J. Insect Physiol. **25,** 45–52 (1979).
[36] P. Masner, W. Hangartner, and M. Suchý, J. Insect Physiol. **21,** 1755–1762 (1975).