Inverse Relationships between Ecdysteroid Titres and Total Body Metabolism in Insects

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In larval and pupal stages of several insect species the changes in total body metabolism appear to be inversely proportional to the course of ecdysteroid titres. The largest peaks of ecdysteroid occur exactly at the time of the lowest metabolic rates. These relationships are consequences of the developmental programming; ecdysteroid has no direct antimetabolic action. The problem of ecdysteroid-metabolic interactions has been discussed in relation to possible homeostatic function of ecdysteroids in insect development.

Introduction

The developmental cycles in insects are always associated with the characteristic cycles in respiratory metabolism. In the group Endopterygota, we can distinguish the larval-larval, larval-pupal and pupal-adult types of the metabolic cycles. Their realization depends on a complex of morphological and physiological changes induced by hormones. Thus, it has been possible to alter the regular sequence of these metabolic cycles by manipulations with the hormonal activity. This has been mainly achieved by removal of the endocrine glands (c. allata) or by administration of the exogenous juvenile hormone or ecdysone to larvae and pupae of several insect species [1–5].

Another prerequisite of the developmental cycles in insects are large peaks in the content of ecdysteroids. The peaks are always located at certain determined ontogenetic positions (larval, prepupal or pupal peaks of ecdysteroids). Their existence has been often related to various biochemical and physiological events like, for example, to apolysis, cell divisions, synthesis of the macromolecules, secretion of new cuticle, etc. (see [6] for review). Among further suggestions for possible physiological role of the ecdysteroid peaks, one ascribed to ecdysteroids the function of homeostatic peripheral tissue factors [7]. There was a prosaic statement [7] that the large exdysteroid peaks occurred at a time of the minimum rate of total metabolism.

In this paper the relationships between ecdysteroid titres and metabolic intensity have been investigated in more details. To this end, I have measured the course of O_2 consumption during the prepupal and pupal periods of some species for which the titres of ecdysteroids have been already known. Moreover, I have used some earlier respirometric data from my protocols (completed eventually by more accurate measurements around the time of pupal ecdysis) so as to make comparisons with the literature data on ecdysteroid contents.

Materials and Methods

The materials and the methods of O_2 consumption measurements in *Galleria mellonella* L. and in *Dermestes maculatus* DeGeer have been previously described [1, 4, 5]. Similar procedures were also employed for the measurements of respiration in *Bombyx mori* L. and *Tenebrio molitor* L. In all these experiments O_2 consumption was measured by the conventional Warburg technique, using respiratory vessels of 10 ml capacity (25 ml for *Bombyx*) and 0.25–0.50 ml of 5% KOH for absorption of CO_2. Each value in Figs. 1 to 3 represents an average of three successive readings of O_2 consumption on at least 10 individually measured specimens at 27 °C. 20-Hydroxyecdysone (natural product isolated from *Polypodium* rhizomes by Dr. J. Jizba) was injected into the last larval instar of *Galleria* in 2 µl of 10% ethanol-Ringer solution to give the final dose of 10 µg · specimen⁻¹. The respirometric data for immobile prepupae and pupae were very constant. The S.E.M. values were generally within the 10%

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limit of the nominal value, but commonly so small that they could not be graphically shown in the Figures.

Results

Fig. 1 gives a comparison of the changes in total metabolism and ecdysteroid content during the nonfeeding metamorphosis stages of the Coleopteran species, *Tenebrio molitor* and *Dermestes maculatus.* It appears that the values of O_2 consumption in these species follow indeed a more or less reciprocal course to that of the ecdysteroid content. Curiously enough, the inflexion points on the metabolic curves, which are indicated by the minimum values of O_2 consumption in each instar, occur exactly at the moments of the prepupal and the pupal peaks of ecdysteroids, *e.g.* at days −3 and +4 in *Tenebrio* or −2 and +3 in *Dermestes.* In addition, quite similar inverse proportionality between the metabolic intensity and ecdysteroid content also exists during the larval- pupal and the pupal-adult metabolic cycles in the Lepidopteran species *Bombyx mori* and *Galleria mellonella,* as shown in Fig. 2. The inflexion points on the metabolic curves have again occurred precisely at a time of the prepupal and pupal peaks of ecdysteroid, *e.g.* days −3 and +4 in *Bombyx* and days −1 and +2 in *Galleria.*

The relationships outlined in Figs. 1 and 2 obviously apply only to the situations of low metabolism and high ecdysteroid content, which is typical for the nonfeeding metamorphosis stages. It thus appeared essential to test a possibility that the extremely high metabolic rates (as found in the feeding

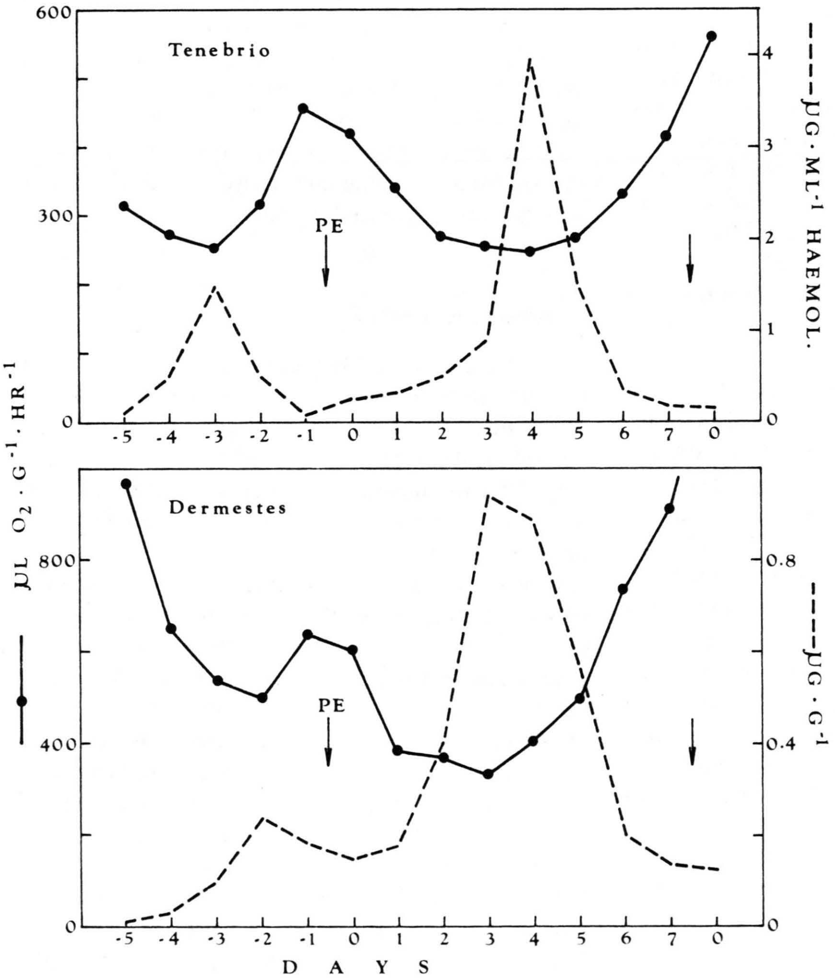

Fig. 1. Changes in respiratory metabolism (full line) and ecdysteroid content (broken line) during prepupal and pupal development of *Tenebrio molitor* and *Dermestes maculatus.* Arrows indicate ecdyses, PE is for pupal ecdysis. Ecdysteroid content in ml of haemolymph or g of fresh weight has been reconstructed from the data given in [12] and [14].

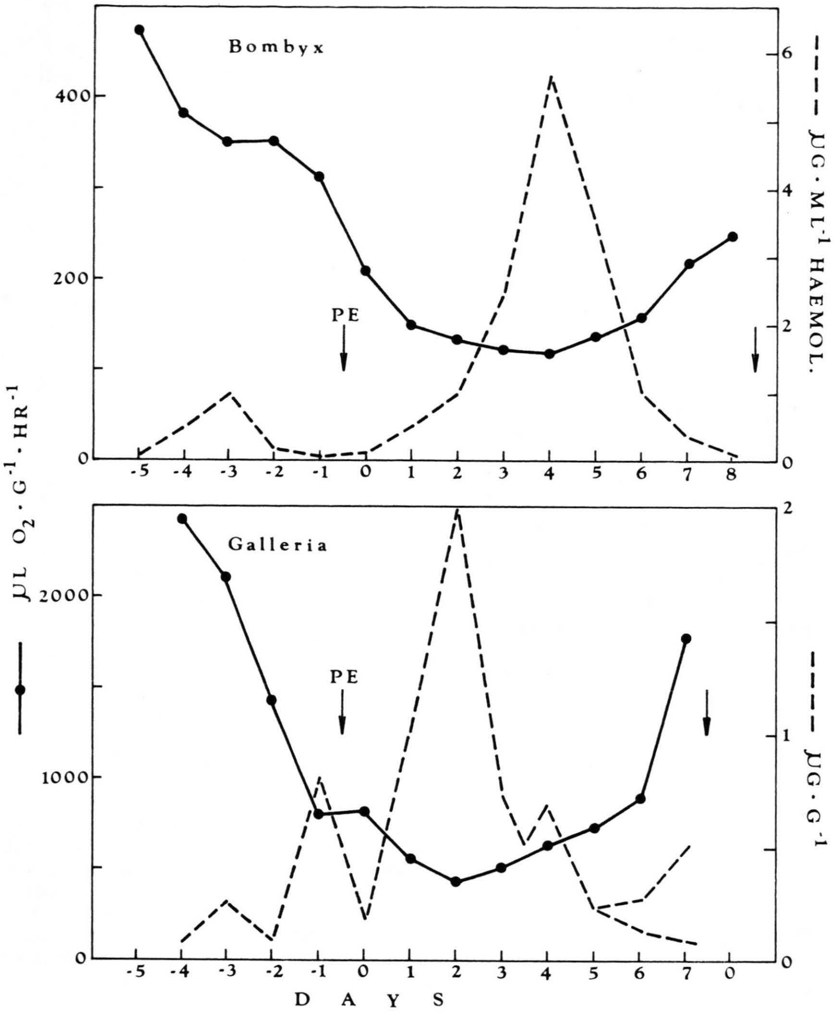

Fig. 2. Changes in respiratory metabolism (full line) and ecdysteroid content (broken line) during prepupal and pupal development of *Bombyx mori* and *Galleria mellonella.* Arrows indicate ecdyses, PE is for pupal ecdysis. Ecdysteroid content in ml of haemolymph or g of fresh weight has been reconstructed from the data in [15] for *Bombyx* and [8, 9] for *Galleria.*

periods of the larval instars) would be also, correspondingly, related to low ecdysteroid titres. Such eventuality that the relationship applies not only to low but also to high metabolic rates has been experimentally tested in the last instar larvae of *Galleria.* Here the minimum metabolic intensity (450 μl $O_2 \cdot g^{-1} \cdot h^{-1}$) of the whole postembryonic development occurs at day-2 after pupal ecdysis (see Fig. 2), when the concentration of ecdysteroid reaches the maximum (2000 ng · g^{-1}; see [9]). During the feeding period of the last larval instar, the metabolic maximum (6600 μl $O_2 \cdot g^{-1} \cdot h^{-1}$) always occurs close to the first day after the last larval ecdysis (see the dotted line in Fig. 3). And, as a matter of fact, this is exactly the period of the lowest ecdysteroid content in the body (less than 50 ng · g^{-1}; se [8, 9]). These results indicate clearly that the inverse proportionality between the total metabolism and ecdysteroid concentration is not limited to the nonfeeding metamorphosis stages but exists in the feeding larval stages as well.

According to what has been shown, the periods of high metabolism-low ecdysteroid content are regularly alternating with the periods of low metabolism – high ecdysteroid content. This recognition may bring some new aspects into our study of the hormonal control of ontogenetic development in insect organism. The peaks of ecdysteroids, although occurring at the minimum metabolic rates, may stimulate the progressive rise of total body metabolism which is observed later during the pharate pupal or pharate adult periods. It has been previously observed [4, 5]

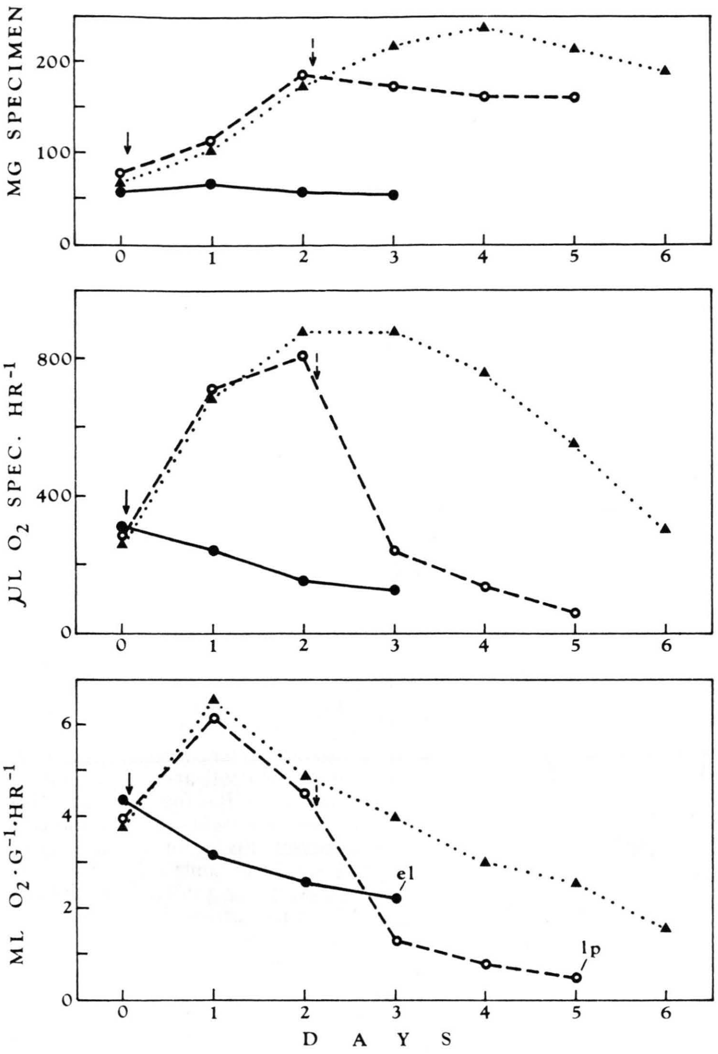

Fig. 3. Effect of 20-hydroxyecdysone injections (10 μg · specimen^{-1}) on growth and O_2-consumption in the feeding last larval instar or *Galleria mellonella*. Arrows indicate the moment of injection; el-formation of extra-larval instars, lp-formation of larval-pupal intermediates. The dotted line is for controls injected by the solvent at day 0.

that an extra supply of exogenous 20-hydroxyecdysone is almost ineffective on low respiratory rates of the immobile metamorphosis stages. Now I have studied what happens when the concentration of ecdysteroid is artificially increased in the feeding larvae with extremely high metabolic rates. These experiments were performed in the feeding last instar larvae of *Galleria*, which were known to have a very small endogenous ecdysteroid content (approx. 50 ng · g^{-1}; see [8, 9]). The artificial ecdysteroid peak was induced by injections of 10 μg of 20-hydroxyecdysone, either before (day 0) or after (day 2) the larvae attained the metabolic maximum (see Fig. 3). The injected specimens formed extra-larval instars that failed to ecdyse spontaneously (group injected at day 0), or formed larval-pupal intermediates (group injected at day 2) with common symptoms of "hyperecdysonism".

The results in Fig. 3 show that the presence of relatively large amounts of 20-hydroxyecdysone results in considerable reduction of O_2 consumption in the feeding larvae. It has to be noted, however, that all the treated larvae invariably ceased to feed and seriously reduced their locomotory movements. Injections of smaller doses (1 μg, 0.5 μg) did not induce the precocious extra-larval ecdysis nor the described metabolic inhibition (20-hydroxyecdysone is rapidly metabolized and excreted, both the morphological and metabolic effects were very variable in contrast to the results described in Fig. 3). Because the results in Fig. 3 were quite similar to the situation previously found in larvae of *Dermestes* [5], I measured the effects of starvation and immobilization and found the same reduction of the respiratory rates in these larvae of *Galleria* as in the 20-hydroxyecdysone treated ones. In addition, injections of 10 µg of 20-hydroxyecdysone into the 2-day-old pupae of *Galleria* did not inhibit respiratory metabolism, which was in contrast to the results obtained on larvae. It is most likely, therefore, that the metabolic inhibition described in Fig. 3 has been only a side-effect caused by starvation and immobilization. Irrespective of whether it was a direct or indirect action, the results in Fig. 3 have shown anyhow that the presence of relatively large ecdysteroid concentrations in the body would not be compatible with the extremely high metabolic rates of the feeding and rapidly growing larvae.

Discussion

The described results are in favour of the earlier suggestion [7] that the titre of ecdysteroid in immature insects is inversely proportional to the metabolic rate. This relationship has been found in the taxonomically distant orders Coleoptera and Lepidoptera so that its presence in other insect groups may be anticipated. A major problem in such estimations is that the respirometric data are mostly available for species that have not been hitherto assayed for the content of ecdysteroids and vice versa. However, in some representatives of Diptera-Cyclorrhapha and Hymenoptera, the large peaks of ecdysteroids occur quite conspicuously at the identical developmental periods where other species of the group exhibit the minimum metabolic rates. And, even in the group of Exopterygote insects, there is an U-shaped metabolic depression at the end of the last larval instar [10], which again coincides with the occurrence of the most pronounced ecdysteroid peaks (*cf.* [11]). These observations favour an assumption that the inverse proportionality of the metabolic-ecdysteroid interactions may thus be a rather widespread feature.

When combined with a careful analysis of the earlier observations [13], the present results demonstrate that the largest peaks of ecdysteroids occur just at the bottom of the U-shaped metabolic curve, which is prerequisite for metamorphosis of all the Pterygote insects [10]. Provided that this was indeed so, one could perhaps predict location of the largest ecdysteroid peaks in insect metamorphosis according to the previously known respirometric data.

In addition to the normal development (see Fig. 1 and 2), ecdysteroid titers remain related to metabolic changes even under seriously aberrant experimental conditions. This is documented by comparisons of the respirometric data [4] with the ecdysteroid content [12] in *Dermestes*. Here the "hypermetabolic" responses induced by juvenile hormone, or transpositions of the normal pupal-adult ecdysteroid pattern into the stationary pupal-pupal one, occurred hand-in-hand with the corresponding changes in respiratory metabolism [4, 12]. Further recollection of the previous data on hormonal control of respiratory metabolism in insects (see [1, 4, 10, 13] for review) and their confrontation with some recent data on regulation of ecdysteroid titres (see [11] for review) revealed additional physiological consequences. It comes out, for instance, that the largest peaks of ecdysteroid in the life cycle are indeed always bound to the lowest metabolic rates. By contrast, the alternative combination of high metabolism and low ecdysteroid content is not obligatory. In normal development we find many exceptions in this respect, mainly related to larval or pupal diapause, which is characterized by generally low metabolism and equally low ecdysteroid content [11, 13]. Similar exceptions have been experimentally induced by application of juvenile hormone to larvae of *Dermestes* (*cf.* [4, 5, 12]).

In the course of normal development the large ecdysteroid peaks never occur at the beginning of larval instars, which is the period of most intensive somatic growth. The results in Fig. 3 and the previous results in larvae of *Dermestes* [5] suggest the occurrence of serious pathophysiological interventions caused by ecdysteroids in the feeding larvae. Previous studies [5] also provided evidence that these effects on metabolism were indirect. This is further supported by findings [4, 5] that 20-hydroxyecdysone does not affect respiratory metabolism of the immobile metamorphosis stages. It is thus clear that ecdysteroids have no direct pharmacological antimetabolic action. This conclusion is consistent with the long time advocated view suggesting indirect metabolic action for insect growth hormones in general [1].

We finally arrive to the conclusion that ecdysteroid neither stimulates nor inhibits the total metabolism, yet the peaks are for some unknown reasons firmly bound to the periods when the body is at relative metabolic rests. A possibility that such inverse proportionality would be just a simple outcome of the metabolic inactivation of ecdysteroid might be true for the feeding larvae but it is not relevant to the metamorphosis stages or to diapause. According to a recent view [7], which still awaits further elucidation, the physiological function of ecdysteroids in insects is not restricted to that of a centrally produced hormone. According to these conclusions, the large peaks of ecdysteroids during metamorphosis are not produced by the prothoracic glands. They are made by some peripheral tissues for mutual correction and homeostatic synchronization of their respective developmental programmes. The large peaks of ecdysteroid are thus believed to be consequences of the determined stages that have been reached in the autonomic morphogenetic programme [7, 12]. These stages are among other features characterized by intensive cellular divisions and large proliferative growth, especially among the epidermal and other epithelial tissues. When adopting these views, the coincidental appearance of low metabolic rates at the time of ecdysteroid peaks may obtain some physiological reasoning. Namely, that a dividing cell or proliferating tissue cannot simultaneously perform extensive biochemical functions (digestion and nutrition, chemical synthesis of the reserve materials, storage of energy and other anabolic functions) which have large demands for energy obtained from biological oxidations. Insect endocrinology provides ample evidence to show that these functions are mostly executed in the periods between the peaks of ecdysteroids, when the cells are generally in the interphase state with fully functional mitochondria and other cytoplasmic structures.

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