

# Hypermetabolic Response Induced by Juvenile Hormone Analogues in an Insect

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Some synthetic analogues of insect juvenile hormone cause tremendous rise of respiratory metabolism in the last larval instar of *Dermestes maculatus*. The  $O_2$  consumption values occasionally found in these hypermetabolic larvae (20 ml/g/h) can be classified among the most intensive respiratory rates ever recorded in living organisms. The phenomenon is directly related to the dose of juvenile hormone activity and it is dependent on nutrition. The mechanism of such specific metabolic action of juvenile hormone is based on complete oxidation of the dietary fatty acids, providing metabolic water for larval somatic growth. The excessive amounts of energy created by hypermetabolism are partly converted to heat, suggesting uncoupling of phosphorylation. Some other specific endocrinological features associated with hypermetabolism have been discussed.

## Introduction

Insect ontogeny proceeds as distinctive developmental cycles [1], each cycle being characterized by certain specific pattern in the course of respiratory metabolism [2]. The principal role in the regulation of larval-larval or larval-pupal moulting cycles has been ascribed to juvenile hormone (JH), which is assumed to have indirect effects on metabolism by inducing growth and preventing morphogenesis in the target tissues (for review see ref. [3]). More recently it has been found [4] that some synthetic analogues of JH (juvenoids) can induce an extraordinary rise of respiratory metabolism in larvae of *Dermestes*. Curiously enough, this extensive metabolic change was not associated with any developmental reorganizations among the tissues. Further studies have shown that juvenoids never induce similar hypermetabolic responses in larvae of other species (*Calliphora*, *Gal-leria*, *Spodoptera*), although they do cause some minor metabolic alterations here [5]. It thus appears that hypermetabolism does not represent a general response of insect larvae to juvenoids. It must reflect some specific conditions of hormonal and metabolic regulations that apply only to *Dermestes* and a limited number of other species.

Endocrinological analysis of the developmental regulations in *Dermestes* [6] has indeed revealed a profound deviation from the common schemes known to be valid for most other insect species. We have now renewed the physiological and biochemical analysis of hypermetabolism in the hope to reconcile on this model certain specific effects of JH on insect metabolism, because in other insects these effects are usually masked by simultaneous changes in cellular differentiation.

## Materials and Methods

The larvae of *Dermestes maculatus* DeGeer (= *vulpinus* F.) were reared in glass jars at 27 °C and 18 h photophase, as has been described previously [4]. Juvenoid treatments were made by topical application of the compounds in 1 µl of acetone. Ecdysterone was injected in 2 µl of 10% ethanolic insect Ringer solution.

$O_2$  consumption was measured with a Warburg apparatus at 25 °C using conventional technique [2–4]. The values of  $O_2$  consumption presented in the Figures are averages of 3 to 5 successive readings on several individually measured specimens. For final calculation of the averages we selected only specimens with synchronised development, whose number ( $n$ ) is indicated in the legend. The individual  $O_2$

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consumption curves constructed for each specimen had parallel courses with less than 20% deviation from the mean. Because of several-fold difference between the respiratory rates of hypermetabolic and control larvae, we did not feel obliged to quote all statistical data. In some occasions where simple changes in body weight might modify the course of  $O_2$  consumption curves related to g of weight, we give also the values related to specimen or give changes in body weight.

The following juvenoid compounds were used:

ethyl 11-chloro-3,7,11-trimethyl-2(E)-dodecenoate (I);  
(4E,6E)-5,9,13-trimethyl-4,6-tetradecadien-3-one (II);

4-[(3,7-dimethyl-6,7-epoxy-2-octenyl)oxy]-1-ethyl benzene (III);

11-chloro-3,7,11-trimethyl-2(E)-dodecenoic acid (IV);

*n*-hexyl 7,11-dichloro-3,7,11-trimethyl-2(E)-dodecenoate (V).

The compounds I–V were prepared in the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences. All the ID-50 Morph. units of JH activity (see ref. [7]) whenever used here are exclusively related to topical assays on freshly ec-

dysed pupae of *D. maculatus*. In most experiments we used the compound I which is only medium active in this species (ID-50 Morph. = 5  $\mu$ g per pupa) but it shows long persistence within the body [7].

## Results

There are six larval instars in *D. maculatus*. The 1st to 5th instars undergo rapid growth and ecdyse periodically within 2 to 3 day intervals. The last (6th) instar normally undergoes pupal ecdysis after 9 to 10 days, feeding occurs in the initial 6 days. The 10-day process of larval-pupal transformation occurs even in complete absence of feeding. Moreover, the larvae treated by juvenoids at any time of the last instar never form intermediates or extra-larval stages. Their pupation is delayed and the pupae finally develop into several extra-pupal instars or into adults. The most pronounced hypermetabolic response has been always associated with this inhibition of larval-pupal transformation.

### Developmental stages susceptible to hypermetabolism

The most pronounced hypermetabolic responses have been obtained by treatments at the beginning

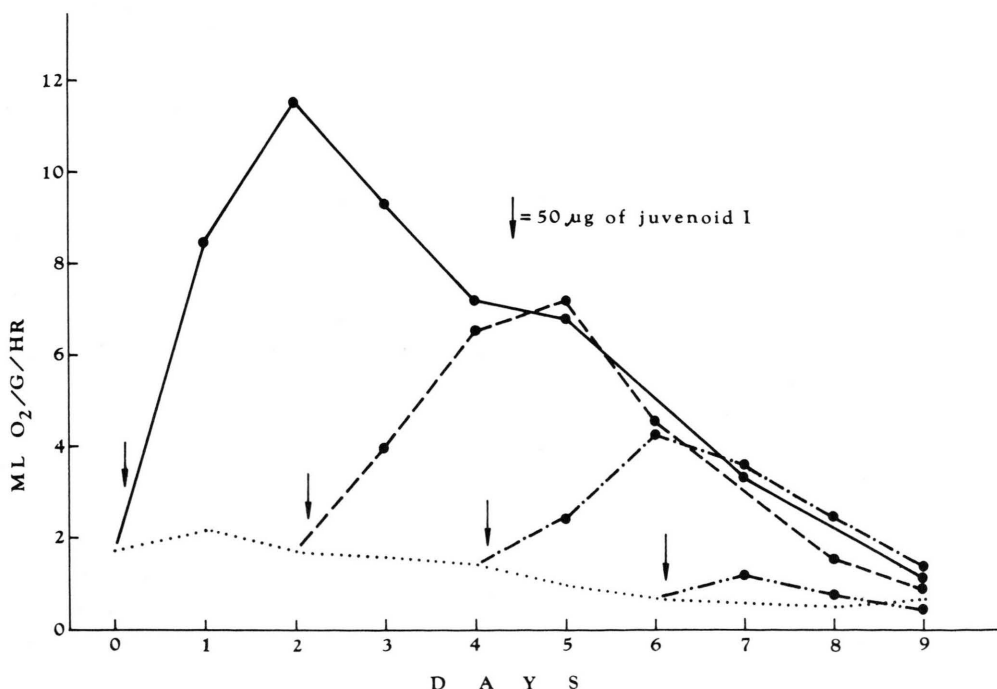
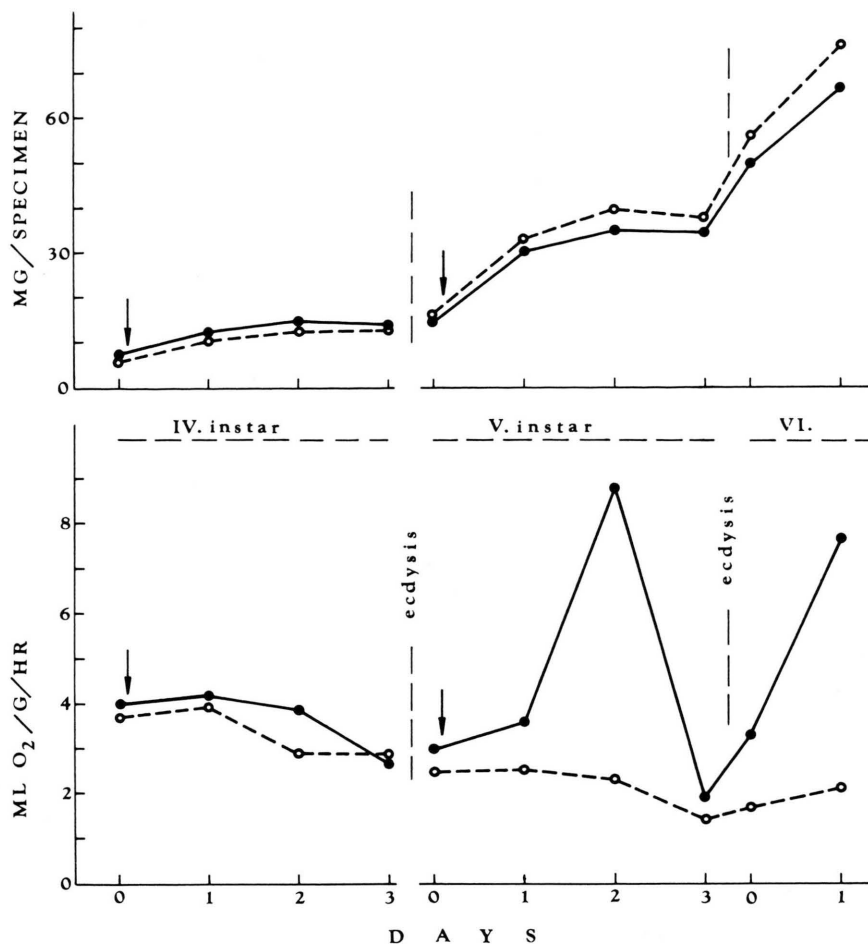


Fig. 1. Effect of juvenoid I on  $O_2$  consumption in the last larval instar of *D. maculatus*. The dotted line shows  $O_2$  consumption of untreated controls. The numbers of individually measured specimens (*n*) in the groups treated at 0, 2, 4 and 6 days after ecdysis were 15, 9, 8 and 13, respectively.

Fig. 2. Body weight and  $O_2$  consumption of the control (broken line) and juvenoid treated larvae during 4th and 5th instar. Arrows indicate topical treatment by  $100 \mu\text{g}$  of juvenoid I ( $n=7$  to 10 specimens in each group).



of the last larval instar, as shown in Fig. 1. Later treatments are gradually less effective while treatments of the pharate pupae (after day 6) are virtually ineffective. The proportions of  $O_2$  consumption curves in Fig. 1 indicate that there exists a well expressed developmental limitation in the intensity of hypermetabolic response. The limitation is also present when excessive doses of more than  $100 \mu\text{g}$  of juvenoid I have been used.

We did not find hypermetabolic response to juvenoid I in the 4th larval instar (Fig. 2), which is expected to contain endogenous JH. The appearance of the first, quite distinct though temporary, response has been localized to the second-half period of the penultimate (5th) instar. The treated 5th instar larvae again resumed hypermetabolic activity after ecdysis into the 6th instar. Additional measurements (not included in Fig. 2) also revealed that larvae re-

ceiving a continuous treatment by excessive doses of juvenoid I since the beginning of 3rd instar develop the first characteristic symptoms of hypermetabolism only after traversing the middle of the 5th instar. According to this, the hypermetabolic responses are localized to the period beginning at the middle of the penultimate instar and terminating at the end of the feeding period of the last instar.

#### *Dose-response relationships*

Using treatments in the most sensitive period at the beginning of the last larval instar, we have found apparent correlations between the administered dose of juvenoid I and the degree of hypermetabolic response, see Fig. 3. The increase in the sum of  $Q O_2$  values over the control level, calculated for the whole 9 day period, is directly proportional to the degree of JH activity expressed in the pupal ID-50 equiva-

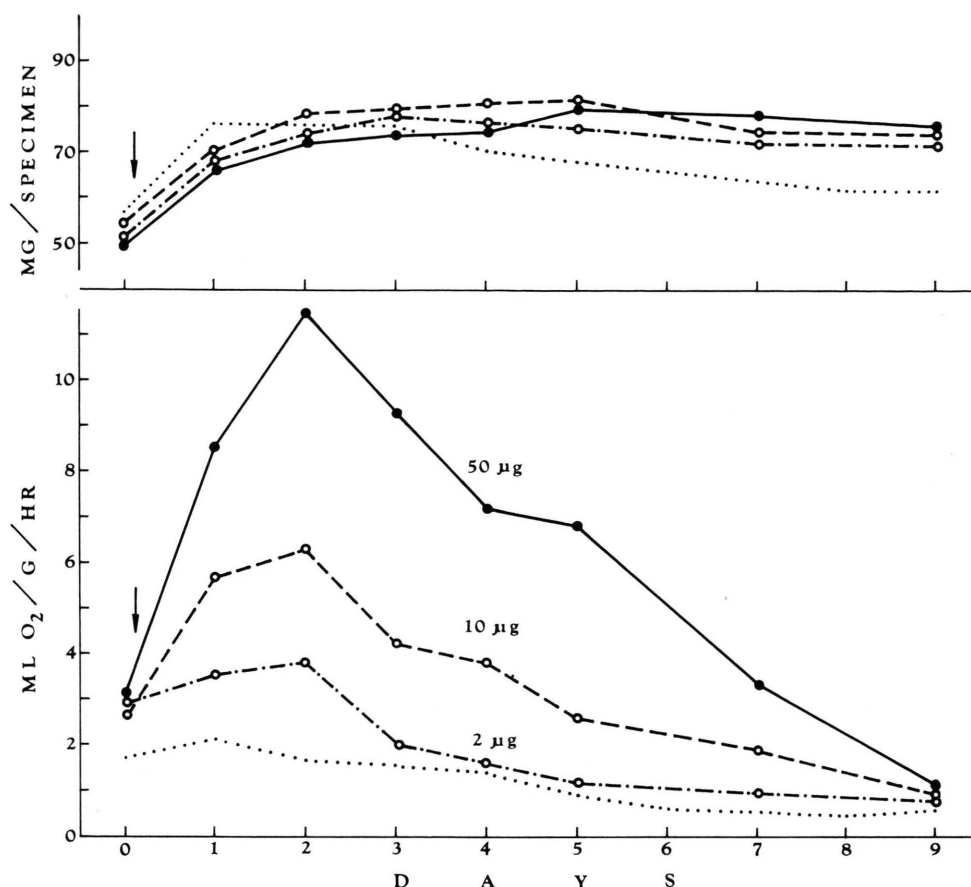


Fig. 3. Body weight and  $O_2$  consumption after treatment of last instar larvae with various doses of juvenoid I. Dotted line is for untreated controls ( $n = 15$  for  $50 \mu g$  dose, 6 for  $10 \mu g$ , and 5 for  $2 \mu g$ ).

lents (the doses of 2, 10 and  $50 \mu g$  of juvenoid I as used in Fig. 3 are equivalent to 0.4, 2 and 10 ID-50 units, respectively).

The evidence that hypermetabolism was not just a result of some nonspecific side effects of compound I was finally provided by testing other structural types of juvenoids. Thus, as documented in Fig. 4 A, the hypermetabolic response can be obtained with juvenoid II. This compound has ID-50 of  $0.01 \mu g$  per pupa. It is a dienolic ketone lacking the 11-chloro substituent as well as the important alkyloxycarbonyl group, which are essential structural features of I. Moreover, Fig. 4 B shows that hypermetabolism can be induced even with compound III, which belongs to a structurally different group of aromatic juvenoids related to 4-alkyl substituted geranyloxybenzenes (ID-50 is  $0.001 \mu g$ ). In large contrast to this, juvenoids IV and V, which are structurally related to I but have only slight or no JH activity in *D. ma-*

*culatus* (ID-50  $\geq 200 \mu g$ ), did not induce hypermetabolic responses (Fig. 4 B).

#### Dependence of hypermetabolism on nutrition

The hypermetabolic larvae have extremely good appetite. They consume more food and produce more excrements than controls. The relatively small increase of their body weight is not adequate to the amount of food they consume. Because all specimens were individually measured, we could easily observe and measure the effects of a constant amount of food on respiratory metabolism. We have found that consumption of equal amounts of food of equal quality is always followed by several-fold higher increase of  $O_2$  consumption in the treated larvae. This provides evidence that JH analogues cause qualitative changes in the intermediary metabolism of these larvae.

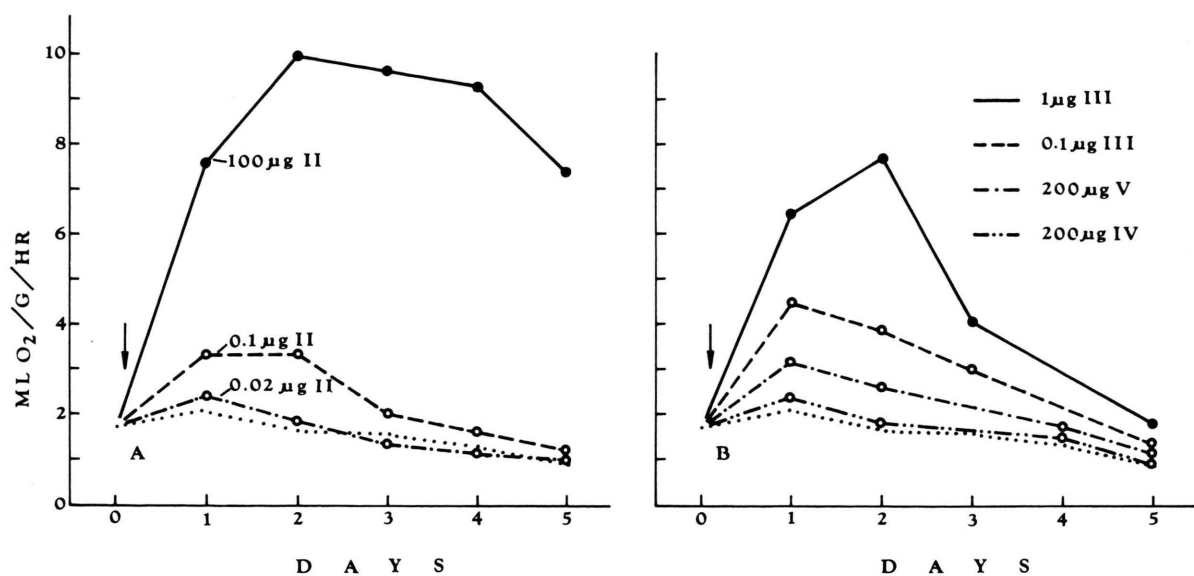


Fig. 4. The effect of juvenoids II to V on  $O_2$  consumption in the last larval instar. Dotted line is for untreated controls ( $n=5$  to 10 specimens in each group).

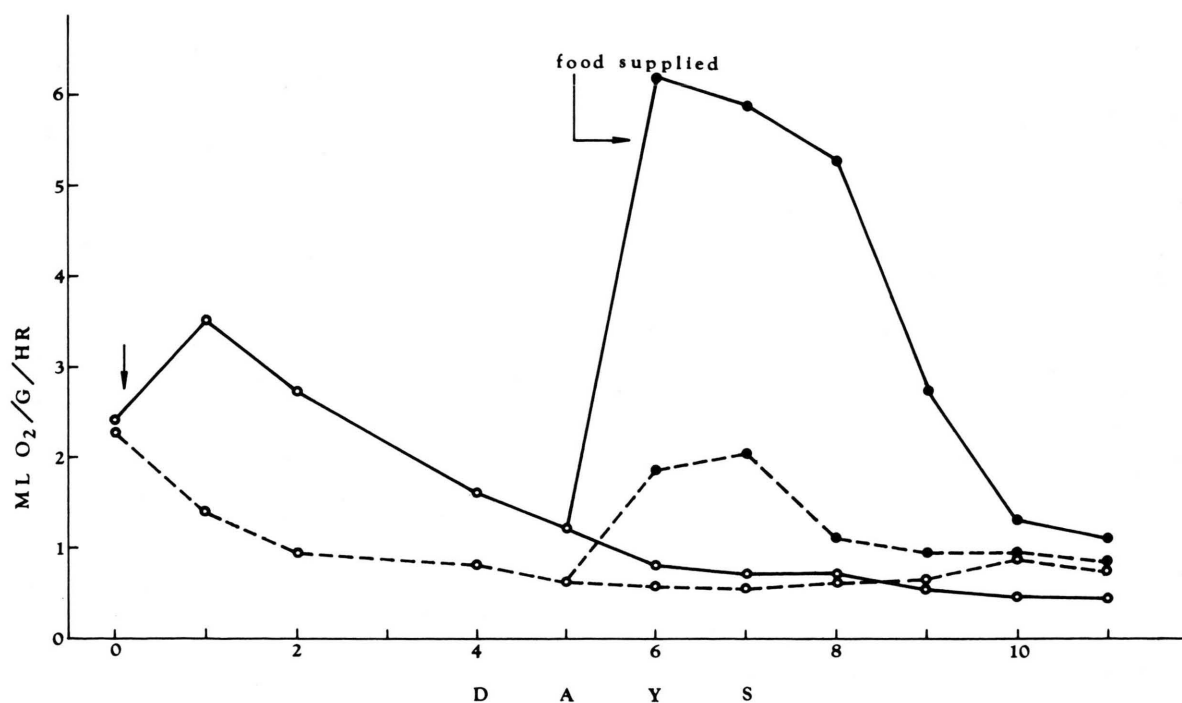


Fig. 5.  $O_2$  consumption of the treated (full line) and untreated (broken line) larvae of the last instar. The larvae were starved as indicated by open circles or fed after 5th day, as indicated by full circles. Arrow indicates topical treatment by 100  $\mu$ g of juvenoid I ( $n=10$  specimens in each group until day 5, and 5 specimens after day 6).

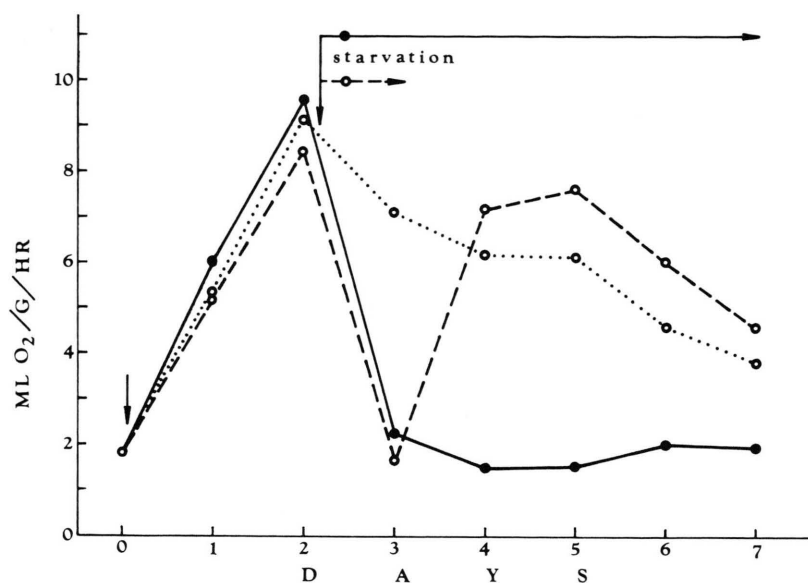


Fig. 6. O<sub>2</sub> consumption of the last instar larvae treated by 100 µg of juvenoid I (arrow). The larvae were: a) deprived of food after day 2 (full line,  $n=8$ ); b) deprived of food temporarily between days 2 and 3 (broken line,  $n=8$ ); or, c) kept constantly on food (dotted line,  $n=7$ ).

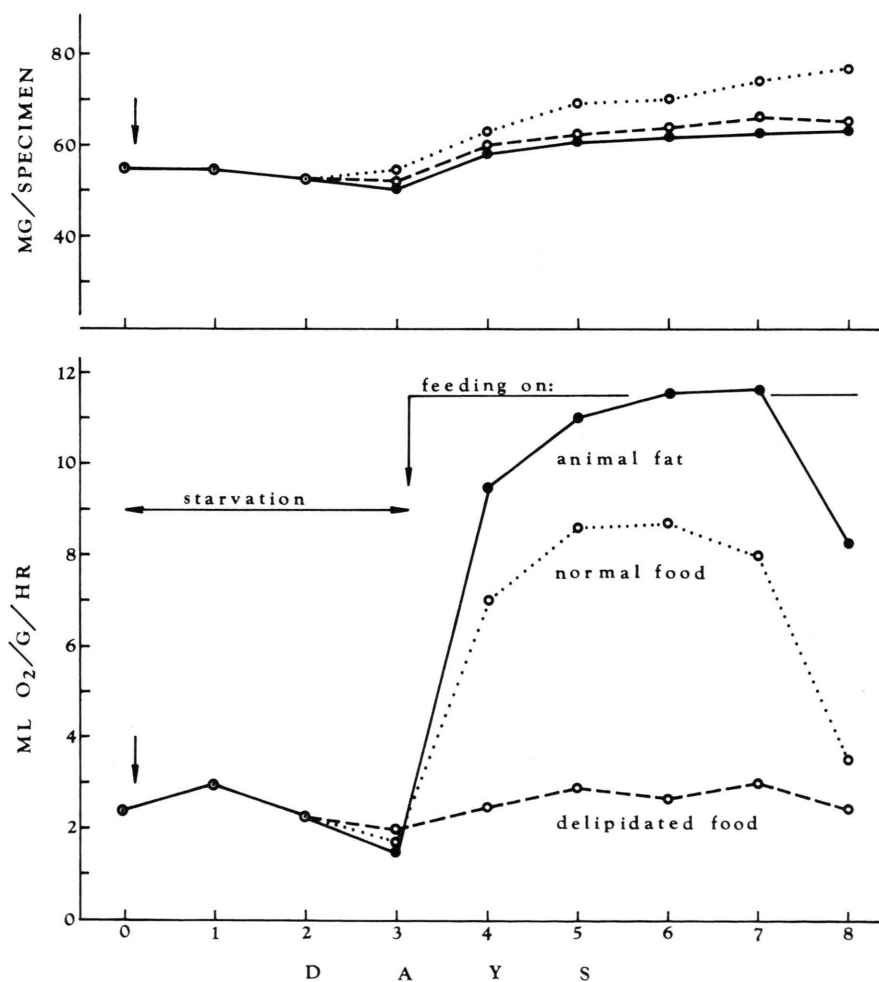


Fig. 7. Body weight and O<sub>2</sub> consumption of the last instar larvae treated by 100 µg of juvenoid I per specimen (arrow). The larvae were starved until day 3 and then received food of different quality, as indicated ( $n=6$  to 8 specimens in each group following day 2).

When completely deprived of food, the larvae show relatively small but significant elevation of respiration after juvenoid treatments (Fig. 5).

However, the typical hypermetabolic response in their  $O_2$  consumption can occur only after they resume feeding. Further experiments involving interrupted feeding (Fig. 6) have shown that the hypermetabolic larvae again rapidly reduce the rate of  $O_2$  consumption within a 24 h period of fasting. We have also observed that this interrupted hypermetabolic activity can be once more restored by feeding, provided that the larvae are not older than 6 to 7 days after ecdysis (Fig. 6).

#### Hypermetabolism and the quality of food

The above results indicate that the substrates used as a fuel for hypermetabolic energy involve principally some materials ingested as food. In order to get some information concerning the nature of these nutritional substrates, we performed similar series of experiments as in Fig. 5, except that we offered the

treated larvae food of different quality. We have found (Fig. 7) that feeding treated larvae on a delipidated food (dried calf viscera extracted by chloroform-ethanol) did not produce hypermetabolic response. In striking contrast to this, however, feeding on pure calf suet, containing excess of animal triglycerides, produced enormous hypermetabolic reaction. The main, and perhaps exclusive, nutritional substrate catabolized during hypermetabolism appears thus to be the dietary lipid, not the protein nor carbohydrate (glycogen).

#### Effects of ecdysterone on hypermetabolism

The normal or juvenoid treated last instar larvae of *D. maculatus* undergo rapid, larval-larval like moults after injections of 1 or more  $\mu\text{g}$  of ecdysterone [6]. We have now induced these rapid moults in hypermetabolic larvae to see their effect on respiratory metabolism. We have found a drastic decrease of hypermetabolism within 24 h after ecdysterone injections into the feeding larvae (Fig. 8 A). Unfor-

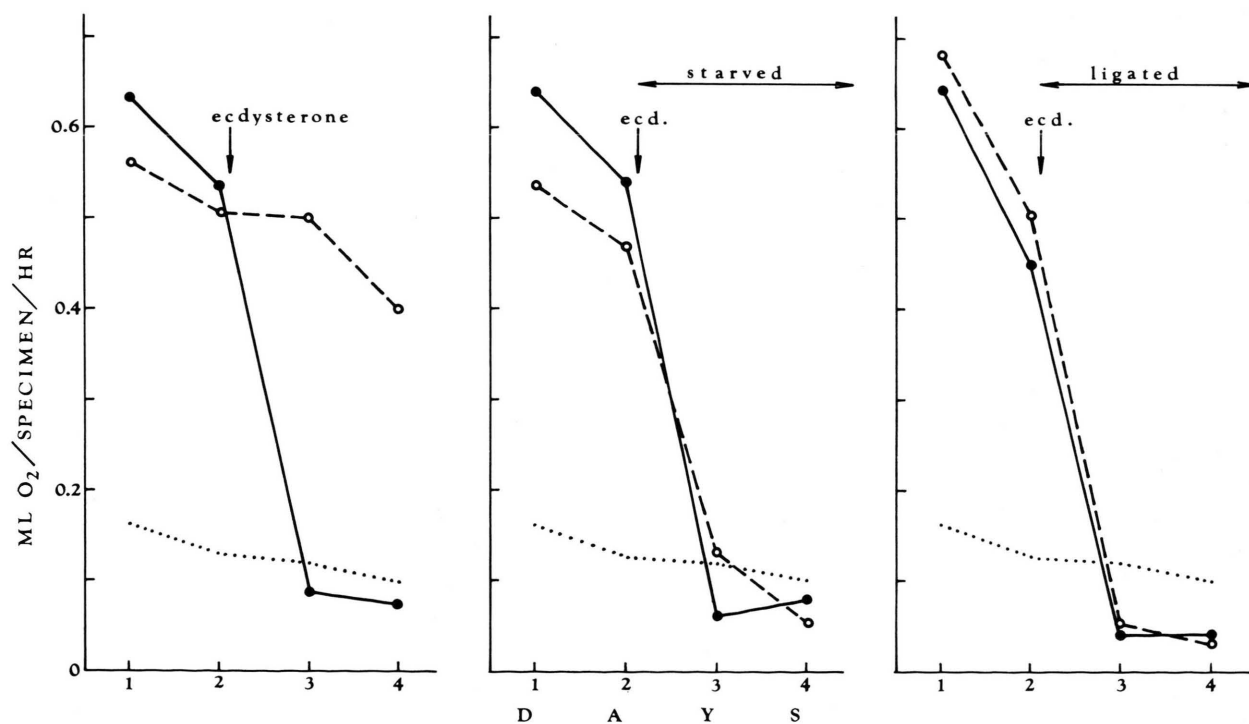


Fig. 8. The effects of ecdysterone injections ( $20\ \mu\text{g}$  per specimen, indicated by full lines) on  $O_2$  consumption of 2-day-old hypermetabolic larvae. Hypermetabolic response was induced by  $100\ \mu\text{g}$  of juvenoid I at day 0. Ecdysterone injections after day 2 are indicated by arrow. The control larvae (broken lines) received injections of Ringer. The dotted line is for untreated fed controls ( $n=8$  specimens in each group).



tunately, the injected larvae that underwent accelerated moult simultaneously arrested feeding. Therefore, in further experiments with ecdysterone we were obliged to eliminate feeding interferences by using starved or ligatured specimens. In this case (see Fig. 8 B, 8 C) ecdysterone injections proved to be ineffective, suggesting that the effects of ecdysterone on hypermetabolic larvae were indirectly caused by arrested nutrition.

#### *CO<sub>2</sub> output in hypermetabolic larvae*

After realizing that triglyceride is the main substrate for hypermetabolism, we have calculated some stoichiometric relationships associated with a complete metabolic combustion of trilinoleate. According to this, a complete oxidation of 1 mg of this material would require 1.97 ml of O<sub>2</sub>, yielding 1.46 ml of CO<sub>2</sub> and 0.95 mg of water. The respiratory quotient should thus correspond to 0.74. Using indirect Warburg method we have measured the respiratory quotient of several dozens of the 2-day-old hypermetabolic last instar larvae. The results consistently revealed the values of RQ between 0.73 and 0.74, with the mean of 0.734 and standard deviation less than 0.02. These values of RQ exactly correspond to the above calculated ones for metabolism of triglycerides. The untreated larvae of equal age had more variable RQ of 0.75 to 0.85. The production of physiological amounts of CO<sub>2</sub> during hypermetabolism also shows that the substrates are properly metabolized and not simply converted to higher oxidation products.

Final calculations have shown that a gram of hypermetabolic *D. maculatus* larvae, exhibiting O<sub>2</sub> consumption of 10 ml per hour, would metabolize each hour approximately 5 mg of a triglyceride converting it into 7.25 ml of CO<sub>2</sub> and 4.75 mg of water. Physiological implication of these data is that the larvae can gain in one day the amounts of metabolic water which is equivalent to 10% or more of their body weight.

#### **Discussion**

The described results allow to characterize hypermetabolism as a special type of JH-dependent digestive metabolism which is used by *D. maculatus* larvae to obtain water and energy from extensive oxidation of the dietary lipids. The intensity of respira-

tion, occasionally surpassing the values of 20 ml of O<sub>2</sub> consumed per g per h, may be classified among the highest rates of respiratory metabolism ever encountered in any living organism.

The larvae of *D. maculatus* are adapted to feed on the completely dry animal tissues. The diet is rich in lipid, carbohydrate and protein, but the limiting factor for somatic growth is represented by water that has to be completely supplied by metabolism. Our results suggest that this rather specific metabolic function, *i. e.* to provide water for growth, has been closely linked to the usual morphogenetic action of JH in this species. Special concern for metabolic water may help to explain the findings [5] that JH analogues do not induce hypermetabolism in larvae of other insect species, which mostly receive enough water by feeding or drinking.

Physiological action of JH is closely related to somatic growth in all insects [1]. Indeed, we can hardly find a case in which corpora allata would produce JH in a nonfeeding stage [7]. A physiological lack of JH (like in the last larval instars) or an artificial lack produced by allatectomy (in adult females), generally result in hypertrophy of the fat body. This is accompanied by accumulation of the neutral lipid, glycogen and protein [7]. Similar accumulation of the nutritional reserves occurs also in the body of normal last instar larvae of *D. maculatus*. By contrast, the hypermetabolic larvae do not deposit but utilize most of the nutritional lipid and accumulate water. According to these physiological measures, the hypermetabolic response caused by JH analogues may be considered as a reciprocal case to allatectomy.

The metabolic formation of water by oxidation generates large amounts of free energy which is transformed to macroergic phosphate bonds and partly converted to heat [8]. Assuming normal coupling of oxidation to oxidative phosphorylation, the excessive energy created by hypermetabolism would have to cause a rapid shift towards formation of ATP, whose accumulation might then become a rate limiting factor of further oxidations. We expect, therefore, that the hypermetabolic larvae should contain uncoupled oxidative phosphorylation. This assumption is substantiated by recent findings of Chefurka [9] who shows that juvenoids cause uncoupling of phosphorylation in the mitochondria of various animals. In addition, we have found that the hypermetabolic larvae have elevated body temperature in compari-



son to the untreated controls. Although we have only initiated exact measurements of the amounts of heat produced, it is clear that at least some part of the hypermetabolic energy is converted to heat.

The unusual metabolic effects of JH analogues may be connected with some endocrinological exceptions found in the development of *D. maculatus* [6]. For example, secretion of the hormones from corpora allata as well as from prothoracic glands terminates in this species already by the middle of the penultimate larval instar. Since this time on, all further development including the whole metamorphosis proceeds spontaneously according to rather stereotypic morphogenetic programme. When being set in motion, this programme is automatically realized without dependence on nutrition and endocrine glands (note that even the isolated larval abdomens undergo normal metamorphosis). Further characteristics of this system are that an exogenous source of JH activity may temporarily inhibit the initial phases of the programme and, eventually, cause the hypermetabolic response in the last larval instar. Later administration of JH leads to continuous repetitions of the pupal instars [6].

It was generally believed [7] that the action of JH on insect larvae may become manifested only by simultaneous action of ecdysone. The above endocrinological data reveal, however, that during hypermetabolism the JH analogues must necessarily act alone without interactions of ecdysone. Under these circumstances and in the absence of functional prothoracic glands the larval cells cannot traverse the

initial phases of the morphogenetic programme, but they can still retain and perform some specifically "larval" metabolic functions of JH, such as is to generate water for larval somatic growth. According to this simple principle, JH analogues may cause retention of the existing "larval" type of the intermediary metabolism, which includes among other things also production of metabolic water from the nutritionally supplied fatty acids. Alternatively, in absence of JH activity, these fatty acids will be esterified and deposited in the fat body cells for their later use during metamorphosis.

We believe that the above outlined principle is also operating under physiological conditions of endogenous JH to provide metabolic water for growth of the young larval instars. We have found transitory peaks of very high intensity of  $O_2$  consumption (8 ml/g/h) in the middle of the normal 2nd larval instar. This is equivalent to hypermetabolism in the last larval instar. It would be unrealistic, however, to expect in young larvae a similarly large hypermetabolic response as found in the last instar. The young larvae contain functional prothoracic glands inducing rapid sequence of moults in 2 to 3-day intervals. This is associated with periodic interruptions of feeding and metabolism, as has been exemplified by the experiments with ecdysterone in Fig. 8.

#### Acknowledgements

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