# JUVENILE HORMONE ACTION ON THE INTERMEDIARY METABOLISM IN INDIAN MEAL MOTH LARVAE

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ABSTRACT: Juvenile hormone (JH) incubated with mitochondria isolated from Indian meal moth larvae inhibited NAD-mediated oxidations by preventing electron transfer through the nonheme iron component of the respiratory chain. Flavoprotein-mediated oxidations entering the respiratory chain after nonheme iron were not inhibited and for one substrate, succinate, was stimulated. The stimulation of succinate oxidation was most intense in mitochondria isolated from newly molted larvae, declining as they grew to maturity. It is postulated that these JH-induced alterations in mitochondrial metabolism function *in vitro* by diverting metabolites through biosynthetic pathways producing cellular components required in insect growth and development.

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Insect growth and development can be characterized by the marked alterations that occur in respiratory metabolism. For example, the larvae of the Indian meal moth, *Plodia interpunctella*, have a high rate of oxygen consumption at the last larval-larval molt (Fig. 1). After an initial increase, respiration decrease for the remainder of the larval life, reaching a minimum in the midpupal period. Subsequent development of the adult during the last half of the pupal period restores a high respiratory rate by the time of adult eclosion.

These changes in whole insect respiration can be attributed to subcellular changes in mitochondrial metabolism. Mitochondria are the subcellular organelles in which are located the enzymes responsible for trapping energy in the bonds of adenosine triphosphate (ATP) while food is converted to $CO_2$ and $H_2O$. Examination of mitochondria isolated from Indian meal moths at different times during the last larval instar revealed that changes in both the quantity and the oxidative activity of mitochondria contribute to the overall respiratory changes (Fig. 2). Some investigators have linked these changes in respiration and mitochondrial metabolism with endocrine control [1,2], and some have implicated juvenile hormone (JH) as one of the active endocrine agents [3,4,5].

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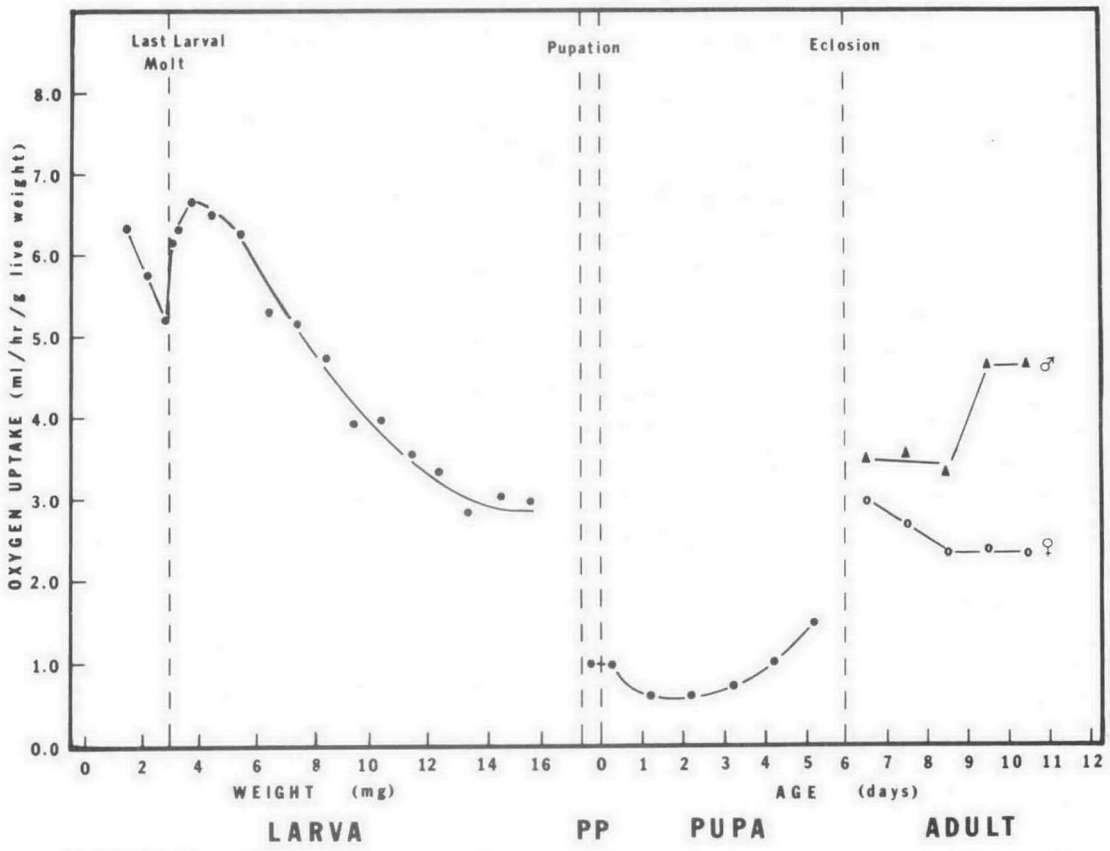


FIGURE 1. Oxygen consumption during the development of *Plodia interpunctella*.

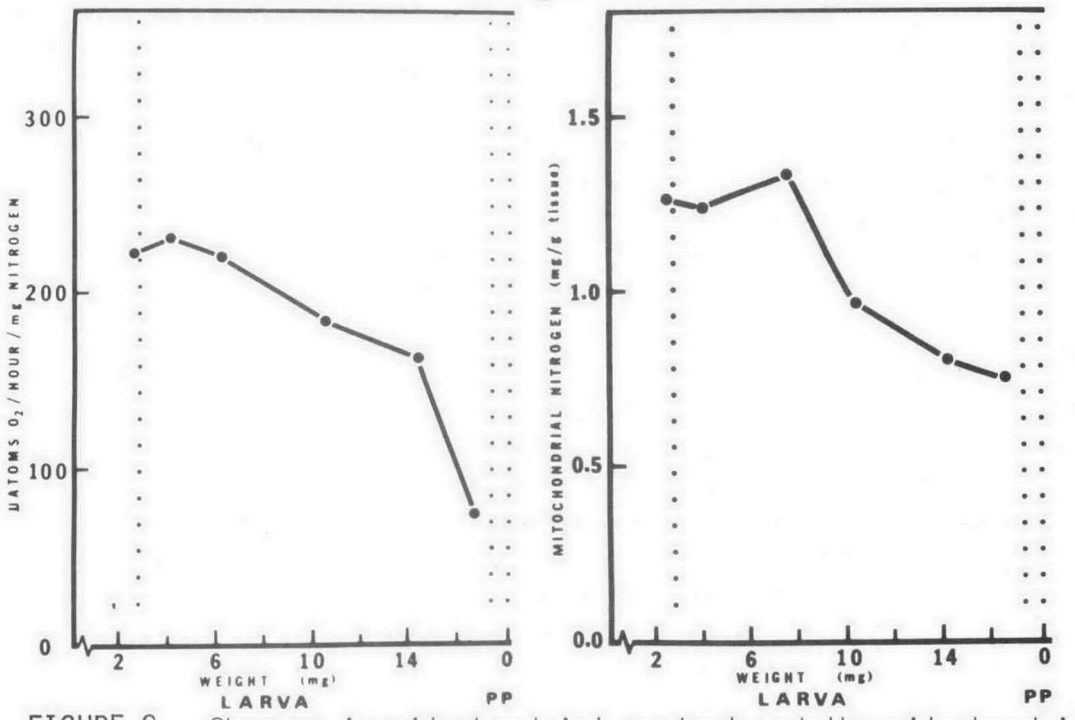


FIGURE 2. Changes in mitochondrial content and the mitochondrial oxidative activity (Pyruvate-malate oxidation) during the last larval instar.

Recently, we were able to demonstrate that synthetic *Hyalophora cecropia* JH [6] had a direct effect on the metabolism of mitochondria isolated from larvae of the Indian meal moth [7]. We found that JH inhibited mitochondrial electron transport in the nonheme iron region between NADH dehydrogenase and ubiquinone (Fig. 2). This inhibition of electron transport prevents the re-oxidation of NADH, which in turn inhibits the NAD-linked oxidations in the citric acid cycle.

A second effect of JH on citric acid cycle oxidations was a 2- to 3-fold stimulation of the oxidation of succinate [6]. Our studies, which are not yet completed, indicate a complex mechanism whereby JH activates the succinic dehydrogenase enzyme complex without affecting the permeability characteristics of the mitochondrial membrane.

Mitochondria isolated from last-instar larvae of different ages indicated that insect age did not affect the inhibition of NADH oxidation by JH. On the other hand, JH stimulation of succinate oxidation was maximal with mitochondria from last-instar larvae that had just undergone the last larval-larval molt but was totally absent with mitochondria from larvae approaching the larval-pupal molt.

Now, the question was, what would be the purpose of these alterations in mitochondrial metabolism? Extrapolating our *in vitro* observations to the metabolism of the insect during the early part of the last larval instar when JH titer is high would provide the following hypothetical picture (Fig. 3). The complete oxidation of food components would be restricted because of the inhibited oxidation of NADH in mitochondria. However, carbohydrates would still be oxidized to pyruvate anaerobically *via* glycolysis coupled to the aerobic regeneration of NAD *via* the α-glycerophosphate shuttle [8]. Some of the accumulated pyruvate could be utilized in the cytosol for the synthesis of malate. Malate could enter the mitochondria and, proceeding through the activated succinic dehydrogenase, form the precursors for synthesis of porphyrin, which when combined with the appropriate protein, would form additional cytochrome.

The overall effect of JH would be to increase the respiratory capacity of the larvae by stimulating the formation of additional mitochondrial oxidative units during a short, finite period early in the instar when JH titer is highest. The utilization of these newly synthesized oxidative units would occur during the remainder of the instar when JH titer is declining. Such a pattern of mitochondrial synthesis and utilization during a larval instar would account for the changes we observed in larval respiration (Fig. 1).

To test this hypothesis we compared the mitochondrial cytochrome contents of larvae treated continuously with JH after the middle of the 4th instar with those not treated with JH. We found that JH treatment did not affect the amount of cytochrome from mitochondria until after the larvae molted to the last larval instar (Fig. 4). During the last larval instar JH treatment

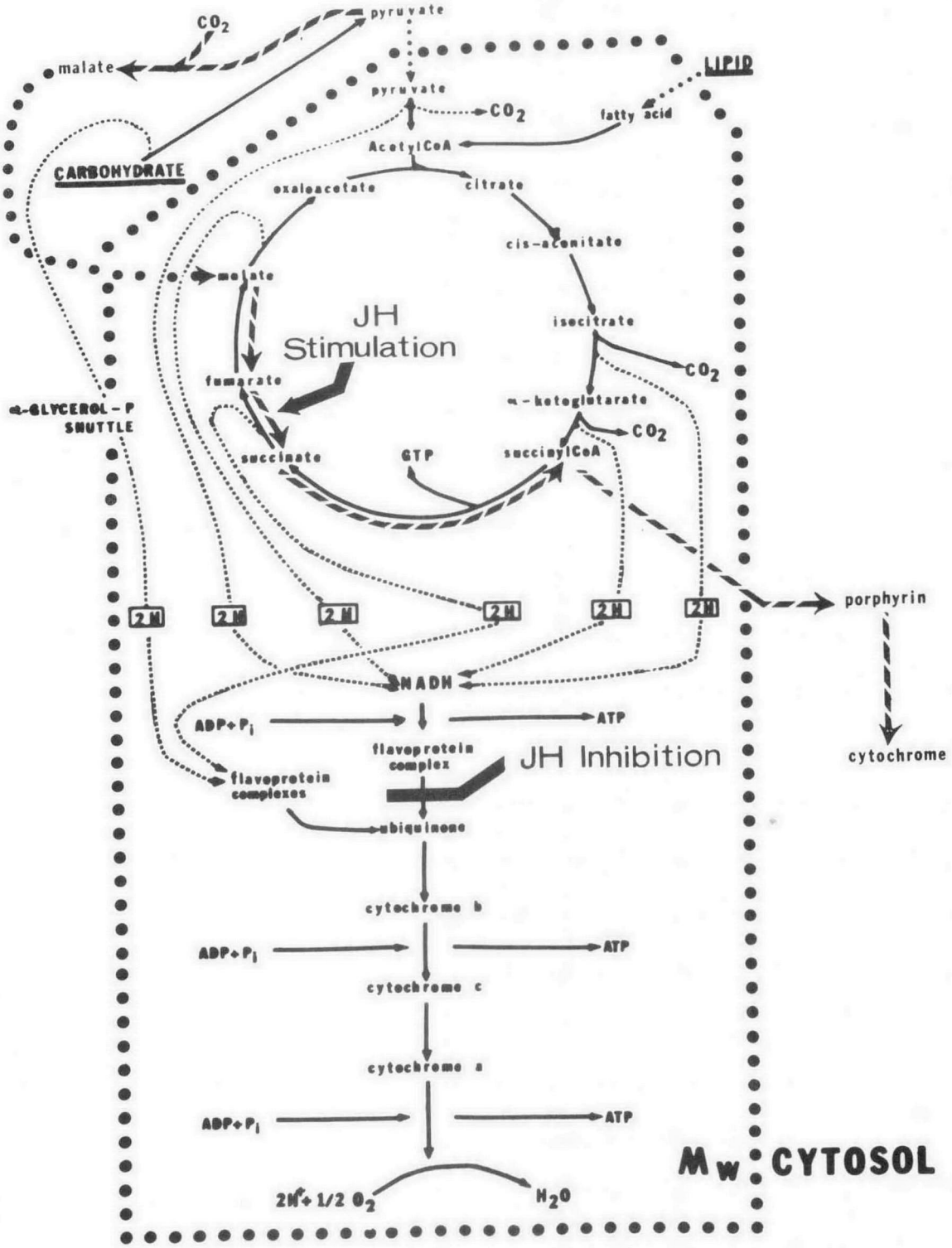


FIGURE 3. Effect of high JH titer on mitochondrial metabolism of *Plodia* larvae. The heavy broken arrows indicate postulated pathway for cytochrome synthesis which is favored by high JH titer.

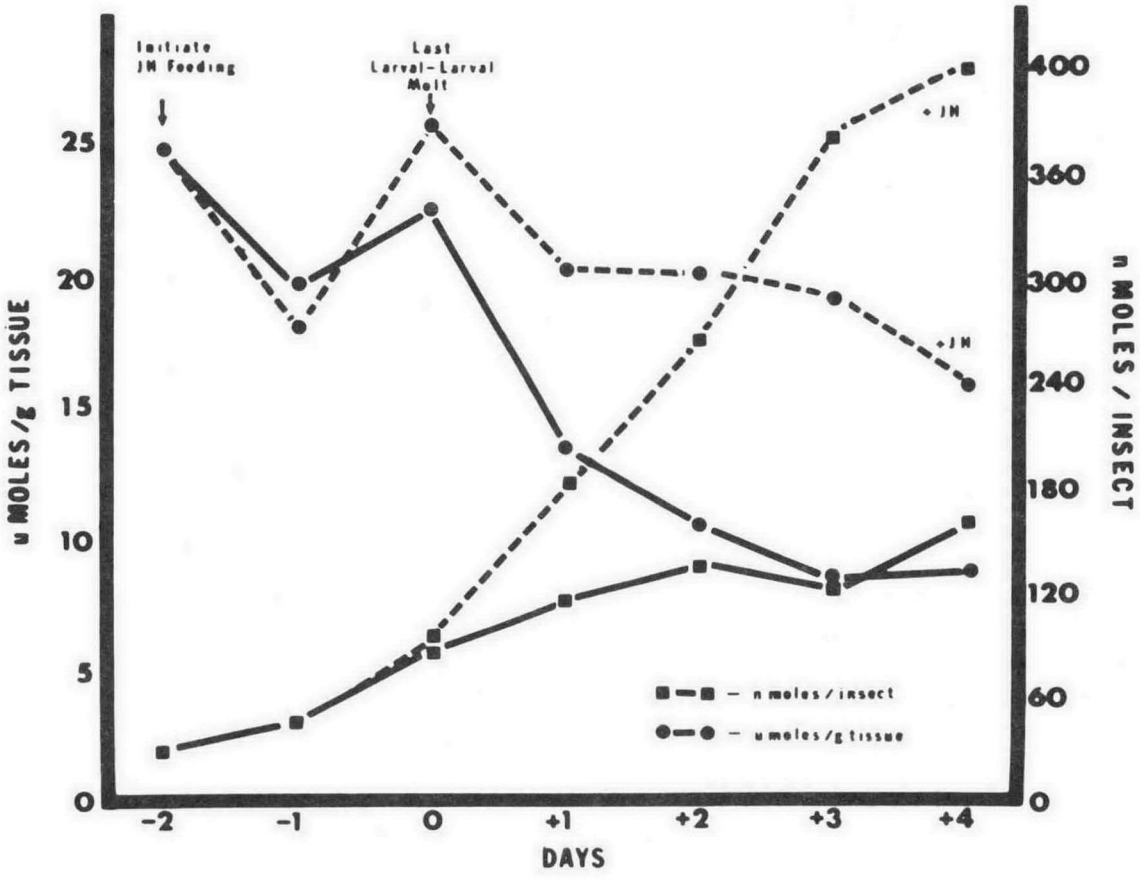


FIGURE 4. Mitochondrial cytochrome content during development of Indian meal moth larvae.

increased the amount of cytochrome from mitochondria 2- to 3-fold and maintained the elevated level for several days before declining. Comparisons of the amounts of mitochondrial cytochrome per insect clearly show that JH accelerated the rate of cytochrome synthesis (Fig. 4). If the JH treatment resulted in a supernumerary molt, cytochrome content was again elevated at the molt.

These observations indicated that JH treatment did indeed result in the predicted increase in cytochrome synthesis; however, it is also apparent that the full expression of JH on cytochrome synthesis relied upon the intervention of ecdysone at the molt. Classical interpretation of these data would be that JH makes available the genetic information for the synthesis of one or more key proteins in the cytochrome synthetic pathway. Ecdysone would act nonspecifically by turning on the protein synthetic machinery [9], accelerating the formation of those proteins dictated by the available genetic information.

The exact mechanism of how JH makes available the genetic information is not clear. A number of hypotheses have been advanced which implicate the transcriptional or translational levels of protein synthesis as the primary site of JH action [10]. However, it is evident from our studies that JH control at these levels

does not adequately describe a mechanism for our observations on mitochondrial metabolism. Our *in vitro* studies indicated that JH has a direct and immediate affect on the mitochondrial metabolic pathways associated with porphyrin and cytochrome synthesis. Furthermore, we have found that the immediate and full stimulation of cytochrome synthesis *in vivo* can be induced by JH, but only when the JH treatment was initiated shortly after the last larval-larval ecdysis.

Three possible mechanisms of JH action can be suggested from our observations up to this point: (1) JH simultaneously alters mitochondrial metabolism and makes available the necessary genetic information for the complete synthesis of cytochrome when protein synthesis is stimulated by ecdysone. (2) JH alters mitochondrial metabolism causing the accumulation of porphyrin (or heme) or one of its precursors which acts as an inducer in the classical Jacob-Monod [11] scheme of genetic regulation. Ecdysone stimulation of protein synthesis would then form the proteins necessary for completing cytochrome synthesis. (3) Finally, all components of the protein synthesizing system for cytochromes may be available at the time of ecdysone secretion, but the extent of cytochrome-related protein synthesis would depend upon the amount of porphyrin (or heme) made available by the JH-induced alteration in mitochondrial metabolism.

In studies now underway we are trying to determine which of these mechanisms is the one responsible for the stimulation of cytochrome synthesis by JH.

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