

Hormonal Control of Insect Metamorphosis

The hormonal control of insect metamorphosis was shown by the dramatic experiments of Wigglesworth (1934), who studied *Rhodnius prolixus*, a blood-sucking bug that has five instars before undergoing a striking metamorphosis. When a first-instar larva of *Rhodnius* was decapitated and fused to a molting fifth-instar larva, the minute first instar developed the cuticle, body structure, and genitalia of the adult. This showed that blood-borne hormones are responsible for the induction of metamorphosis.

Wigglesworth also showed that the corpora allata, near the insect brain, produces a hormone that counteracts this tendency to undergo metamorphosis. If the corpora allata was removed from a third-instar larva, the next molt turned the larva into a precocious adult. Conversely, if the corpora allata from fourth-instar larvae were implanted into fifth-instar larvae, these larvae would molt into extremely large "sixth-instar" larvae rather than into adults.

Transplantation of insect tissues carried out in several laboratories eventually generated an integrated account of how metamorphosis takes place. Although the detailed mechanisms of metamorphosis differ between species, the general pattern of hormone action is usually very similar. Like amphibian metamorphosis, the metamorphosis of insects appears to be regulated by effector hormones controlled by neurosecretory peptide hormones in the brain. The molting process is initiated in the brain, where neurosecretory cells release prothoracicotropic hormone (PTTH) in response to neural, hormonal, or environmental factors. PTTH is a family of peptide hormones with a molecular weight of approximately 40,000, and it stimulates the production of ecdysone by the prothoracic gland. Ecdysone, however, is not an active hormone, but a prohormone that must be converted into an active form. This conversion is accomplished by a heme-containing oxidase in the mitochondria and microsomes of peripheral tissues such as the fat body. Here the ecdysone is changed to the active hormone 20-hydroxyecdysone.

Each molt is occasioned by one or more pulses of 20-hydroxyecdysone. For a molt from a larva, the first pulse produces a small rise in the hydroxyecdysone concentration in the larval hemolymph (blood) and elicits a change in cellular commitment. The second, large pulse of hydroxyecdysone initiates the differentiation events associated with molting. The hydroxyecdysone produced by these pulses commits and stimulates the epidermal cells to synthesize enzymes that digest and recycle the components of the cuticle. In some cases, environmental conditions can control molting, as in the case of the silkworm moth *Hyalophora cecropia*. Here, PTTH secretion ceases after the pupa has formed. The pupa remains in this suspended state, called diapause, throughout the winter. If not exposed to cold weather, diapause lasts indefinitely. Once exposed to two weeks of cold, however, the pupa can molt when returned to a warmer temperature (Williams, 1952, 1956).

The second major effector hormone in insect development is juvenile hormone (JH). JH is secreted by the corpora allata. The secretory cells of the corpora allata are active during larval molts but are inactive during the metamorphic molt. This hormone is responsible for preventing metamorphosis. As long as JH is present, the hydroxyecdysone-stimulated molts result in a new larval instar. In the last larval instar, the medial nerve from the brain to the corpora allata inhibits the gland from producing juvenile hormone, and there is a simultaneous increase in the body's ability to degrade existing JH (Safranek and Williams 1989). Both these mechanisms cause JH levels to drop below a

critical threshold value. This triggers the release of PTTH from the brain (Nijhout and Williams 1974; Rountree and Bollenbacher 1986). PTTH, in turn, stimulates the prothoracic glands to secrete a small amount of ecdysone. The resulting hydroxyecdysone, in the absence of JH, commits the cells to pupal development. Larval-specific mRNAs are not replaced, and new mRNAs are synthesized whose protein products inhibit the transcription of the larval messages. After the second ecdysone pulse, new pupal-specific gene products are synthesized (Riddiford 1982), and the subsequent molt shifts the organism from larva to pupa. It appears, then, that the first ecdysone pulse during the last larval instar triggers the processes that inactivate the larva-specific genes and prepare the pupa-specific genes to be transcribed. The second ecdysone pulse transcribes the pupa-specific genes and initiates the molt (Nijhout 1994).

From the 1950s until recently, it had been thought that the type of molt was determined by the juvenile hormone titre at the time of the ecdysone pulses. High levels of JH induced larvae, intermediate levels of JH produced pupae, while low levels of JH produced adults (see Piepho 1951). However, when the titre of JH could actually be determined, it was found that it fluctuated during the final instar period, having specific peaks and troughs. Metamorphosis is not correlated with or caused by a progressive decline in JH activity. The control of metamorphosis appears more complex (Figure 1).

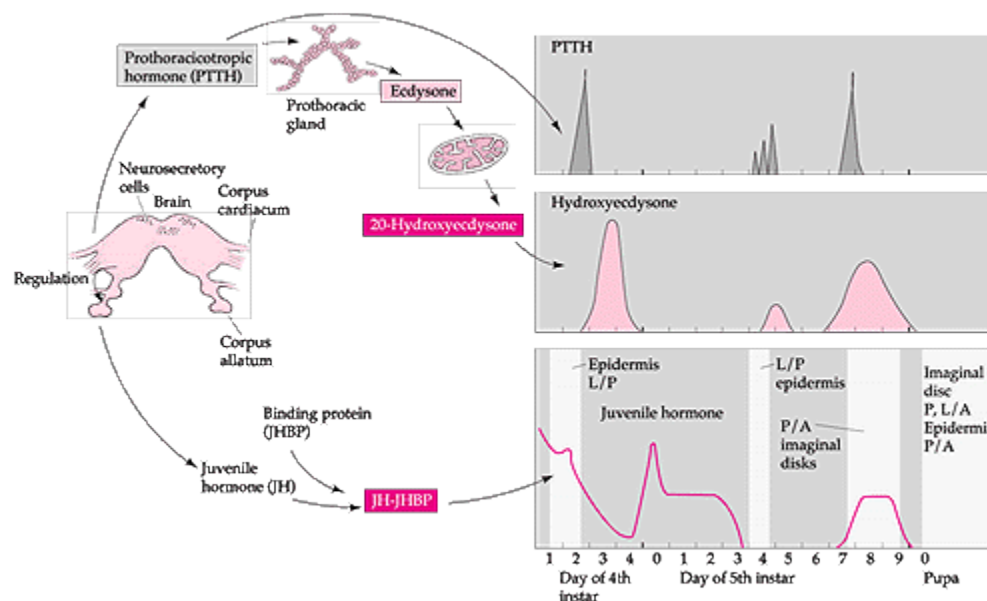


Figure 1 Schematic diagram illustrating the control of molting and metamorphosis in the tobacco hornworm moth. There appear to be critical sensitive periods when the presence or absence of JH determines whether a tissue is retained at the same stage or changes to a more mature state. Different tissues have different sensitive periods. (After Nijhout 1994.)

As shown in Figure 1, in the tobacco hornworm moth *Manduca sexta*, there are specific times when different cells are sensitive to juvenile hormone. As a general rule, if JH is present during a JH-sensitive period, the current developmental state is maintained, whereas if JH is absent during that period, this tissue will progress to a more mature developmental state. The onset and duration of the JH-sensitive period appears to be an autonomous state of the cell and is not controlled by hormones (Nijhout, 1994). (It has been hypothesized that this may be a time when JH receptors are available in these tissues). In each larval instar, there is a period where the presence of JH prevents the larval epidermis from transforming into pupal epidermis. If JH is present, the epidermis continues to be pupal, if JH is absent, it becomes pupal. During the penultimate instar larva, JH titres are able to retain the epidermis in its larval condition. During the last instar, there are two windows of JH sensitivity. The first is for the epidermis. At this time, though, ecdysone levels have dropped

significantly. Thus, the epidermis will be transformed from larval epidermis to pupal epidermis. The second JH sensitive period concerns the imaginal disc tissue. At this time, however, the JH titre has risen again, so that the imaginal discs are not instructed to evert and differentiate. The molt transforms the larva into a pupa (Nijhout and Wheeler, 1982). The next time the ecdysone pulses occur, no JH is seen during the critical periods. The epidermis transforms from pupal to adult, and the imaginal discs are allowed to evert and differentiate. Injection of JH into the pupa at this time can cause it to molt again into a second pupa (Williams, 1959).

For a retrospective on the early work in insect metamorphosis, see Wigglesworth (1954).

The molt timer: critical weight as a checkpoint for metamorphosis

In *Manduca* (and presumably many other arthropods, as well), there is a critical weight during the last larval stage. Once this weight is reached, there is an endocrine cascade that initiates metamorphosis (Nijhout and Williams 1974). Feeding ends at this time, and the insect's body has to have stored all the food it needs to undergo these major changes. At the metamorphic critical weight, JH is removed from the hemolymph both by the suppression of JH in the corpora allata and by the appearance of a JH-specific esterase in the fluid. If the diet is poor, however, the larval period is extended to ensure that the critical weight is reached. However larvae in their last instar that have the corpora allata removed (so that they do not have any JH) show no adjustment to starvation. Rather, they initiate metamorphosis on day 4, whether or not they have attained the critical weight. (And when they metamorphose before the critical weight, they typically fail to pupate and die in the process.) Thus, in the last instar, the continuing presence of JH can delay the initiation of metamorphosis, but the removal of JH, alone, is insufficient to allow the larva to enter metamorphosis. This constant period between the onset of feeding and the initiation of metamorphosis suggests there is not only a minimum weight, but also a minimum time for starting metamorphosis (Suzuki et al. 2013). Thus, there seems to be a positive temporal "molt" signal working with a negative "but not until you weigh this much" signal. This interaction is probably able to be modified such that insect metamorphosis accommodates the ecological needs of the different insects.

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