

JUVENILE HORMONE IN RELATION TO THE LARVAL-PUPAL TRANSFORMATION OF THE CECROPIA SILKWORM

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Juvenile hormone (JH) has long been known to oppose or prevent the metamorphosis of insects (Wigglesworth, 1934). In the Lepidoptera its most familiar effects are on the pupal-adult transformation where one observes the formation of a second pupa or pupal-adult intermediates (Piepho, 1952; Williams, 1961). Its effects on the larval-pupal transformation of Lepidoptera were first demonstrated by Piepho (1939, 1942) by the implantation of active corpora allata into final instar larvae of the wax moth *Galleria mellonella*. At the subsequent molt either a supernumerary larva or a larval-pupal intermediate was produced. In experiments on *Galleria* Sehnael (1968) confirmed the effects of implanted corpora allata and duplicated them by the application of Cecropia-JH to final instar larvae (Sehnael and Meyer, 1968).

It has been difficult or impossible to cause these effects on other species of Lepidoptera (Bounhiol, 1938; Piepho, Böden, and Holz, 1960; Williams, 1961). Fukuda (1944) was able to produce supernumerary *Bombyx mori* larvae but only by implanting both active corpora allata and active prothoracic glands into final stage larvae. Williams (1961) could not affect the metamorphosis of last instar *Hyalophora cecropia* larvae by the implantation of active corpora allata. Staal (1967) found that implantation of active glands into a larval tubercle caused the retention of only that tubercle in an otherwise normal pupa. Finally, Willis (1969) has reported the retention of larval tubercles on certain Cecropia pupae thought to be infected with *Nosema*, a microsporidian from which a material with JH activity has been extracted (Finlayson and Walters, 1957; Fisher and Sanborn, 1962, 1964).

Several years ago, when potent JH analogues became available, it seemed likely that application of high doses at the proper time might be able to block the larval-pupal transformation of the Cecropia silkworm. My preliminary studies (Riddiford, 1968b) showed that high daily doses of these materials during the fifth larval instar failed to provoke an extra larval instar although in many individuals certain larval characters were preserved. Even more surprising was the finding that JH application to mature larvae which had completed spinning prevented diapause and interfered with the larval-pupal transformation of the internal organs without affecting the metamorphosis of the integument.

The present paper reports the details of a study of the effects of exogenous JH on the larval-pupal metamorphosis of the Cecropia silkworm.

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MATERIALS AND METHODS

Experimental animals

Cecropia were reared on wild cherry trees (Telfer, 1967) and in the laboratory either on an artificial diet (Riddiford, 1968a) or on cherry leaves sprayed with an antibiotic mixture (Riddiford, 1967). For the duration of the fifth instar and the prepupal period, the animals were reared at 25°–26° C under 17 hours of daily illumination.

Juvenile hormone applications

A mixture of synthetic juvenile hormone analogues was prepared by bubbling hydrogen chloride gas through farnesenic acid dissolved in ethanol (Vinson and Williams, 1967); this mixture (JH-A) was used in most experiments. When injected into *Polyphemus* pupae (Williams, 1961), 10 μ g gave a + 3 pupal-adult intermediate; 20 μ g of a second preparation of the same mixture was necessary for a + 3 score. Therefore, in the presentation of the data a dose of the second preparation was equated to half that of the first preparation.

In certain experiments, I also made use of a synthetic Cecropia C18-JH (JH-C) synthesized by Corey, Katzenellenbogen, Gilman, Roman, and Erickson (1968). In the *Polyphemus* pupal assay 0.05 μ g of this compound gave a + 3 score.

The hormonal materials in 1 to 5 μ l of acetone ("Nanograde," Mallinckrodt) were applied topically along the dorsal midline of the meso- and metathorax and, less often, to the abdomen of fifth instar larvae or prepupae. Daily applications were always made at the same time of day, usually in the late morning, and were continued until the larva stopped feeding and emptied its gut.

Surgical procedures

The techniques of brain removal and implantation were as previously described (Williams, 1946, 1952; Schneiderman, 1967). After surgery, the pupae were placed at 25° C and observed at biweekly intervals to detect the initiation of adult development.

RESULTS

(1.) Topical application of juvenile hormone at successive stages in the fifth larval instar

(a) *External morphology of pupae obtained from treated larvae.* Fifth instar Cecropia larvae exposed to JH formed pupae which often retained certain external larval characters. The scoring system outlined in Table I was based on 67 individuals which had been treated with specific doses of JH-A and JH-C during the fifth instar. The dorsal tubercles, particularly in the region where the hormone was administered, proved to be most sensitive to topical application (Fig. 1A). With increasing dosage, the spiracles and patches of dorsal epidermis were next affected, followed by the thoracic legs, the labrum, and the larval prolegs. Finally, the highest dose of 20 μ g JH-A daily (higher doses were lethal) produced a larval-

TABLE I

Scoring system for evaluating external larval characteristics reformed on Cecropia pupae after juvenile hormone treatment of larvae

0	Normal pupa
+1	Traces of dorsal and supraspiracular tubercles retained near site of application on meso- and/or metathoracic tergites and often extending posteriorly to first or second abdominal segments.
+2	Partial to complete reformation of dorsal and supraspiracular tubercles on thorax and anterior abdominal segments; occasional patches of larval cuticle on tergites; many spiracles of larval type.
+3	Complete reformation of dorsal and supraspiracular and sometimes of infra-spiracular tubercles on thorax and on at least the 3 anterior abdominal segments; extensive patches of larval cuticle on meso- and metathoracic tergites; larval-type spiracles; larval claws retained on thoracic legs.
+4	All or nearly all tubercles retained; patches of larval cuticle on tergum and at sites of larval prolegs; spiracles and labrum of larval type; tips on pupal antennae and on thoracic legs larval.
+5	Head and thorax with larval characters throughout; abdominal tubercles and prolegs retained. Remainder of abdomen with pupal cuticle.

pupal intermediate which retained a larval head and thorax and larval tubercles on a pupal abdomen.

Most of the treated larvae formed diapausing pupae which, after 3 or more months of chilling at 5° C, formed essentially normal adults when placed at room temperature. It was of considerable interest that the sites which retained larval cuticle in the pupa were covered with pupal cuticle in the moth (Fig. 1B).

(b) *Daily applications.* As seen in Table II, daily applications of either JH-A or JH-C from the beginning of the fifth instar caused the formation of pupae which showed the retention of certain larval characters. The higher the dose, the more affected were the pupae. Of the 21 treated animals, 10 did not live to adult

TABLE II

Effects on pupae of daily applications of juvenile hormone materials to Cecropia larvae throughout the fifth instar

Daily dosage (in 1 μ l acetone)	Number treated	Days before spinning	Number pupae	JH effects*
0 [Controls]	3	13-15	3	0
Juvenile Hormone Analogue Mixture				
1 μ g	3	14-17	3	0, 0, +0.5
5 μ g	3	16-20	3	+2, +2, +3
10 μ g	7	13-16	7	+2(2), +3(5)
20 μ g	2	15-17	2	+4, +5
Cecropia C-18 Juvenile Hormone				
0.5 μ g	2	17	1	+2.5
1 μ g	4	12-21	3	+1, +1, +4

* System for scoring of external characters is presented in Table I.

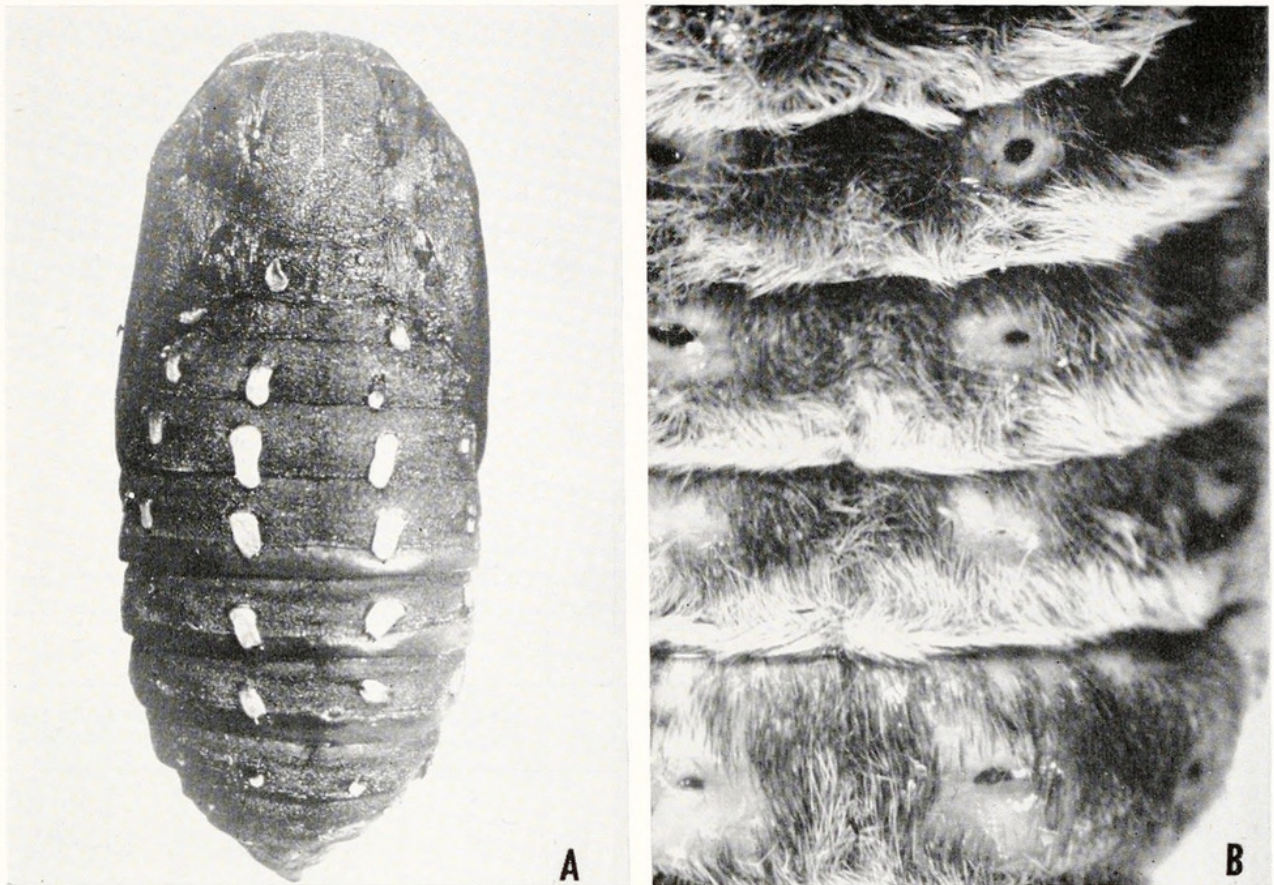


FIGURE 1. (A) *Cecropia* pupa showing retention of larval tubercles on metathoracic and abdominal tergites after application of 10 μg of a mixture of juvenile hormone analogues daily during the fifth instar; (B) A portion of the abdominal tergites of the adult moth which developed from the pupa in Figure 1A. Note the spots of pupal cuticle at the sites where larval tubercles had been on the pupa.

emergence. Three other pupae (two receiving 5 μg JH-A and one 0.5 μg JH-C) did not enter diapause and emerged as adults 30 to 50 days after pupation.

JH-A prevented the complete evacuation of the gut (the normal prelude to spinning) in at least 5 of the 15 treated individuals. All these larvae subsequently spun cocoons of which five were very thin with abnormally large valves. Though the cessation of feeding and a partial evacuation of the gut occurred at the normal time in larvae treated with JH-C, the onset of spinning was delayed by 8 to 10 days except for one individual that had received 1 μg . Furthermore, the time between spinning and pupal ecdysis was usually 1.5 to 2 times the normal 10 days. In an extreme case, a larva which received daily applications of 1 μg JH-C failed to empty its gut or to spin. It lived 42 days after the cessation of feeding, but showed no signs of pupal differentiation. These findings strongly argue that gut evacuation and the initiation of spinning can be delayed or even blocked by JH.

Retention of larval integumentary characters was also seen in eight pupae formed from larvae that received daily treatment with 10 or 20 μg JH-A beginning on day 5 or 10 of the fifth instar and continuing until spinning. Application of 20 μg JH-A for three consecutive days prior to spinning was sufficient to cause a +2 to +3 reaction in the resulting pupa.

Although JH-C produced the same type of results as did JH-A (Table II), much higher doses were necessary in terms of the activities of the two materials in

the *Polyphemus* pupal assay. This difference is presumably due to a more rapid inactivation of JH-C by mature larvae.

(c) *Single applications.* A single dose of JH-A was administered to a series of 41 individuals. These larvae at the time of treatment were at successively later stages in the fifth instar, ranging from pharate fifth stage (−1 day) to the mature fifth stage at the time of gut evacuation (11.5 to 14th day). The results summarized in Table III show that the dorsal tubercles were sensitive to the hormone up until the emptying of the gut. The time of maximum sensitivity seemed to be about 2 to 4 days prior to gut evacuation. Larvae treated with 150 μ g JH-A on day 8 and 9 emptied their gut on day 11 to 12 and began spinning one to two

TABLE III
Effects on pupae of a single dose of a mixture of juvenile hormone analogues applied to fifth instar Cecropia larvae

Day of 5th instar	15 μ g		60 μ g		150 μ g		250 μ g	
	Number treated	JH effects*	Number treated	JH effects*	Number treated	JH effects*	Number treated	JH effects*
−1							2	0,0
0							2	+1,**
1							2	**
5	1	0			2	0, +1		
6	2	0, +1			2	+1, +1		
7	2	0, 0	2	0, 0	2	+2, +2		
8	2	0, 0	1	+2	2	+4, +4		
9	1	0			1	+4	1	+4
10	2	0, 0	1	+2	1	+3		
11					1	+4		
12§	1	+1	1	+3	2	+2, +3		
13§			1	+2				
14			2	+1, 0	2	0, +1		

*System for scoring external larval characters reformed on the pupa is presented in Table I.

** Death before pupation, usually at time of gut evacuation.

§ Although normal time for gut evacuation at 26° C is day 11.5 to 14, these individuals had not emptied their guts at the time of application.

days later. Applications before this time were much less effective, presumably because of the inactivation of the hormone.

When JH-A was applied on days 8, 9, or 10 of the fifth instar, occasional individuals spun thin cocoons or flat pads of silk. These anomalies were usually associated with a 1 to 3 day delay between gut evacuation and the onset of spinning. I found that the time of evacuation can be predicted from the weight of the larva. Thus, during the fifth instar there is a steady increase, then a plateau. Two days after the beginning of the plateau, evacuation occurs. When 150 μ g JH-A was applied one day after the beginning of the plateau, evacuation was delayed for 1 to 4 days. When the hormone was applied to larvae in the process of gut evacuation, there was no delay in the initiation of spinning and normal cocoons were spun.

(2.) *Topical application of juvenile hormone to spinning larvae*

Once a larva has emptied its gut and initiated spinning, the larval integument becomes very resistant to JH. Thus, after the application of 250 or 500 μg JH-A on the first day of spinning, only an occasional individual formed a pupa which retained one or two dorsal tubercles near the site of application. After the first day even these structures could no longer be affected. All the pupae given JH as spinning larvae entered diapause and after several months of preliminary chilling developed into normal moths when placed at room temperature.

TABLE IV

Effects on the incidence of diapause and on adult differentiation of a single application of 150 μg of a mixture of juvenile hormone analogues to Cecropia larvae and prepupae between spinning and pupal ecdysis

Time of application (days after gut evacuation)	Number treated	Number failing to diapause	Juvenile characters in resulting moths†						
			External*	Internal**					
				Thorax	Fat body	Gut	Gonads	Access- sory glands	Ab- dominal interseg- mental muscles
0	3	1	0	2§	5	3	4	1	2
1	3	0	0	0	0	0	0	0	0
2	7	4	0	2	3.8	2.6	3	2.2	2.3§
3	3	2	0	2.8	4	2	3.5	1	1.5
4	2	2	0.5	4	4	3§	4.5	2.8	5
Ocellar retraction									
5	2	2	0.2	3§	4	3.5§	4	3	4§
6	2	2	1.3	3.5	5	4.5	4.5	3.8	5
8	1	1	0.5	2.5	3	1	3	3	1
White prepupa									
9	2	2	0	2	4	1	3	1	2

† When there is more than one treated individual, an average score is used. This average is based only on the scores for nondiapausing individuals.

* Scoring of external characteristics based on pupal assay for juvenile hormone, Williams (1961).

** Scoring of internal characteristics based on Figure 2.

§ At least one individual showed retention of larval characters.

(3.) *Topical application of JH to post-spinning larvae and to prepupae*

A single application of JH at any time from the cessation of spinning until pupal ecdysis had no effect on the integument of the resulting pupa. A most surprising finding was that many of these pupae failed to diapause so that moths were formed 24 to 30 days after pupal ecdysis. Furthermore, dissections of 2-day-old pupae which had been treated with 150 μg JH-A early in the prepupal period revealed that many internal organs retained larval characteristics. For example, the hindgut retained the larval diverticula, the gonads were much smaller than in normal pupae, the larval thoracic and abdominal muscles were mostly intact, and

there were 7 instead of the usual 6 abdominal ganglia. In normal pupae at the time of pupal ecdysis, the morphology of the viscera is pupal except for a small amount of degenerating larval musculature and the subsequent fusion of abdominal ganglia to reduce the number from 6 to 5. Consequently, it is clear that after JH treatment of the early prepupa, the metamorphic processes of the viscera had been severely retarded with little or no effects on the pupation of the integument.

(a) *Prevention of diapause.* A single dose of 1, 5, 10, 25, 50, 100, 150, or 300 μg JH-A was applied to each of 91 individuals at various times ranging from gut evacuation until pupal ecdysis. Table IV summarizes the effects of 150 μg JH-A as a function of the time of application.

In the first three columns of Table IV we see that during the period of gut evacuation and spinning, the larvae were very insensitive to JH-A. Most of the resulting pupae diapaused and, after several months of preliminary chilling, de-

TABLE V

*Endocrine activity of brains of freshly pupated individuals after juvenile hormone treatment during the prepupal period
(Tested by implantation into brainless diapausing Cecropia pupae)*

Time of brain removal after pupal ecdysis	Number implants	% Donor pupae developing	% Host pupae developing	Days for initiation of development of host pupae
Juvenile hormone-treated prepupae				
4-12 hours	15	40	47	19, 33, 38, 111, 141, 150, 151
14-18 hours	5	60	20	160
24-26 hours	9	67	22	9, 190
30-36 hours	4	75	25	9
Untreated prepupae				
4-12 hours	3*	0	0	—

* For a more extensive control series, see Table III in Williams (1952).

veloped into normal adults when returned to 25° C. However, when the application was delayed until after spinning, the resulting pupae failed to diapause. This same result was observed when 50 μg JH-A was applied on day 3 or subsequently up to 12 hours after pupal ecdysis.

The obligatory diapause of the *Cecropia* pupa is due to the cessation of secretion of the brain's prothoracicotropic hormone prior to pupal ecdysis (Williams, 1952). Therefore, it was of importance to determine if the prevention of diapause by JH was due to an interference with this programmed shutdown of the brain or to a prothoracicotropic effect of the applied JH (Gilbert and Schneiderman, 1959; Williams, 1959). To test their endocrine competence, brains from fresh pupae which had been treated with JH-A at various times during the prepupal period were implanted into brainless diapausing *Cecropia* pupae. As seen in Table V, brains removed within 12 hours after the pupal ecdysis stimulated development of

about 50% of the brainless hosts. As the time between ecdysis and brain removal increased, the number of developing hosts declined, thereby signaling a decline in the hormonal activity of the brain. This decrease was matched by an increasing percentage of development among the brainless donors, indicative of release of the prothoracicotropic hormone prior to the brain's excision.

The non-diapausing pupae formed pharate moths which were apparently normal to external examination, the only self-evident abnormality being their failure to undergo ecdysis. Upon dissection under Ringer's solution, all these individuals revealed the preservation of many internal pupal characters.

(b) *Internal morphology of adults obtained from exposure of prepupae to JH.* Figure 2 diagrams the scoring system for the internal organs of the "adults" which developed from the treated prepupae. Zero signifies full adult development, whereas

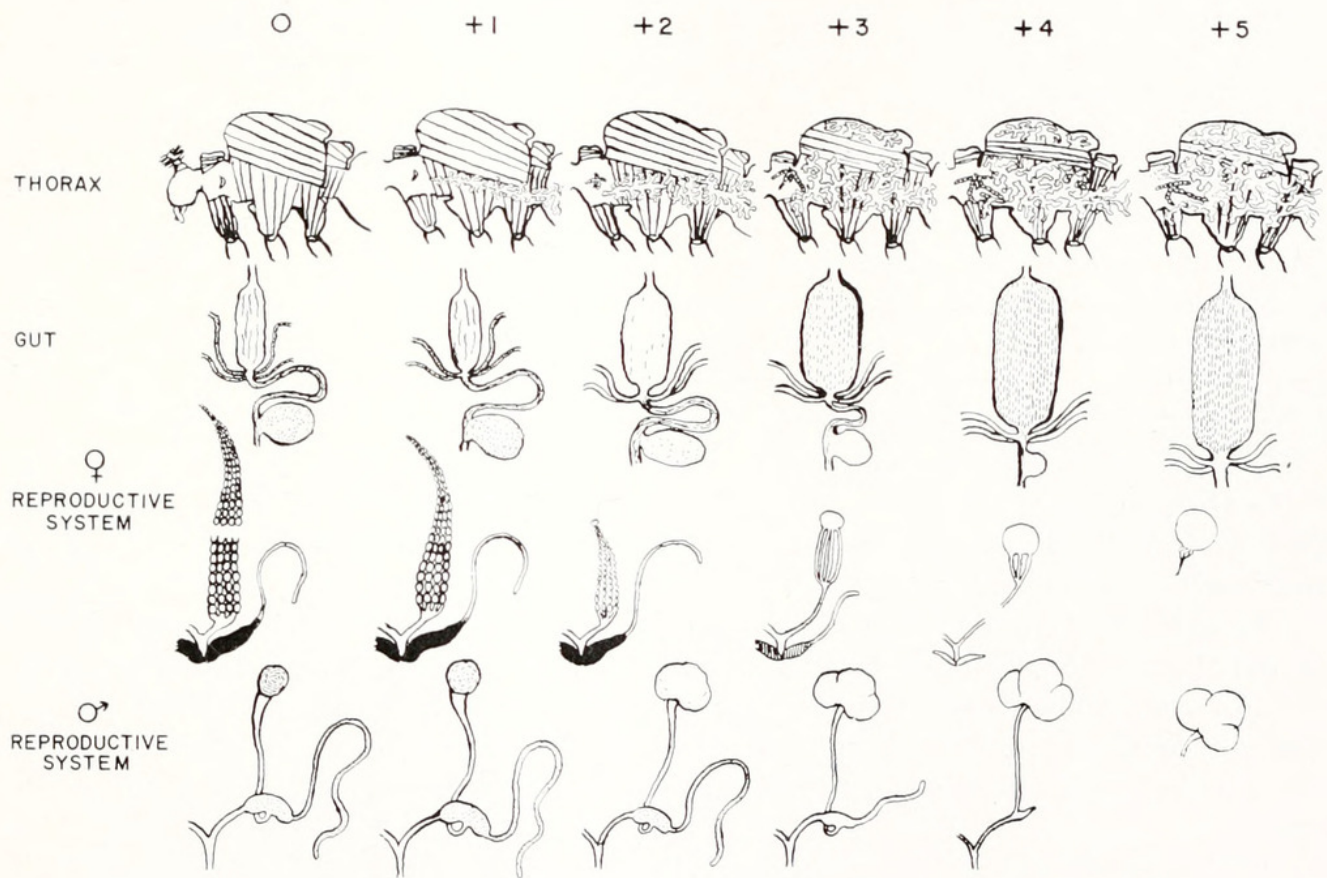


FIGURE 2. A diagrammatic representation of a scoring system for the juvenile hormone effects on the metamorphosis of the internal organs; 0 signifies the adult form; +5 designates the pupal form.

organs scored as +5 remained completely pupal. A total of 62 individuals were scored in this manner.

Of the three regions of the body, the organs within the head were least affected. During adult development, the brain normally undergoes great enlargement of the optic lobe area due to the axonal ingrowth from the developing compound eyes. Since the eyes always underwent full development in treated animals, the brain showed the same gross appearance as in normal adults.

Most of the moths formed from the JH-treated prepupae failed to emerge. Upon dissection they showed faulty development of the flight muscles. The tergosternal muscles were most affected, followed by the ventral longitudinal and, finally, the dorsal longitudinal muscles. Except in +5 individuals the leg musculature was usually present but reduced in volume. Concomitant with a decrease in the thoracic muscles was an increased quantity of fat body in the thoracic cavity. Juvenile hormone treatment also caused the retention of the prothoracic glands. In +3 individuals these glands were approximately half the size of the pupal organs.

In normal *Cecropia* moths the status of the abdominal organs is as follows (see left hand column in Fig. 2): The midgut is short and thin-walled, and communicates with a long coiled hindgut which culminates in a large rectal pouch and associated caecum (Judy and Gilbert, 1969). The Malpighian tubules empty into the gut at the juncture of the mid- and hindgut. In the moth these tubules are thin, straight-sided, coiled, and full of chalky tan meconium. The adult fat body is arranged in long, unbranched strips, each of which has a small trachea as a mid-rib. In normal female moths, most of the abdomen is occupied by the ovaries. The ovarioles contain about 250–300 mature chorionated eggs as well as numerous other oocytes in various stages of maturation (Telfer and Rutberg, 1960). The female accessory glands are well-developed and contain a dark reddish-brown glue. In normal male moths, the testes are small, bright yellow, and contain a small number of mature sperm. The vas deferens leads from each testis to the paired seminal vesicles which contain most of the sperm and receive the contents of the long tubular accessory glands. Ducts from the seminal vesicles come together to form the short median ejaculatory duct.

The abdomen of normal moths contains four ganglia. These innervate the three wide bands of the intersegmental muscles which line each side of segments 4 through 6. Within 48 hours of adult emergence, these muscles ordinarily are eliminated (Finlayson, 1956; Lockshin and Williams, 1965).

As is evident from Figure 2, JH application to prepupae had pronounced effects on the adult differentiation of all abdominal organs. Effects on the gut ranged from the full retention of pupal characteristics, with a large midgut and short straight hindgut, to the normal adult condition. Also, the Malpighian tubules varied from the knobby pupal type which were filled with meconium to tubules of the adult type.

In the most affected males (+5), the testes were completely pupal. Each gonad was divided into four thin-walled compartments which were filled with germinal cysts showing no elongation or spermatogenesis. A +3 male testis was intermediate in size and contained both round immature cysts and elongate cysts with maturing spermatocytes but only occasional bundles of mature sperm. The +3 male accessory glands were small and contained little or no secretions. Similarly, the most affected females (+5) retained pupal ovaries. More common was the +3 condition in which the ovarioles were well-formed but contained a few immature oocytes and no mature chorionated eggs. The +3 glue glands were small and filled with a yellowish fluid. In both sexes the development of the gonads was usually more retarded than that of the accessory glands.

Effects on the fat body were scored as +5 when this tissue was arranged in flattened branching ribbons which lacked a central trachea. The score +3

indicates that approximately half the cells were dissociated as is typical of early adult development. In animals scored +3 to +5, five separate abdominal ganglia were found, signaling a failure of the normal fusion. The nerve cords of these same individuals also lacked the heavy tracheation normally seen in adults. The inter-segmental muscles were retained in *Cecropia* scored as +3 or higher.

Occasionally, as noted in Table IV, certain internal organs retained larval characteristics. Among these were the following: part of the musculature of the midgut walls, the diverticula of the larval hindgut, persistence of large portions of the silk glands and of the intersegmental muscles in the anterior abdomen and occasionally in the thorax, and retention of labial glands of the larval type.

(c) *Prevention of internal metamorphosis.* Those treated *Cecropia* which underwent pupal diapause invariably developed into normal adults after several months of preliminary chilling. By contrast, when JH treatment caused immediate development without pupal diapause, the resulting moths ordinarily showed the juvenile internal characteristics listed in the last 6 columns of Table IV. The metamorphosis of the viscera was severely affected by hormone application on day 4 or 5 after gut evacuation. In many of the resulting moths, larval viscera were often preserved. Late in the prepupal period the internal organs were less affected, and no larval structures remained in the adults. The same experiments testing the lower dose of 50 μg JH-A on 31 individuals showed essentially similar results. The effects were nearly as pronounced as after 150 μg except on day 6 and 7 when the molting fluid presented a barrier to the penetration of topically applied hormone (Williams and Sláma, 1966).

Ocellar retraction signals the onset of the prepupal period, whereas the final phase of this period is accompanied by loss of green integumentary pigment ("white prepupa"). The effects of graded doses of JH-A were examined at these two particular stages which are designated as "early" and "late" prepupae. The results summarized in Table VI reveal that most of the viscera were generally more affected after early application. Also, the prepupae treated early showed a more consistent dose-response relationship than did those treated late. Yet the late application of JH-A was more effective in the prevention of diapause. When JH was applied to white prepupae, larval structures were not retained since by this stage most larval tissues were in a stage of advanced histolysis or had nearly completed their pupal transformation.

As noted above, normal pupal-adult transformation of the viscera occurred in those individuals which diapaused after JH treatment. Similar results were obtained with five *Cecropia* treated with 50 μg JH-A as early prepupae and forced to diapause by removal of their brains immediately after pupal ecdysis. All developed into normal moths after an injection of 20 μg α -ecdysone 3 months later. Thus, the effect of JH on the internal organs decayed in time, suggesting that the only action of the hormone might be on the brain to avert the normal onset of pupal diapause.

This hypothesis was tested on a series of 10 normal *Cecropia* pupae by the injection of 20 μg α -ecdysone on 4 successive days beginning 12 hours after pupal ecdysis. The pupae promptly initiated development and emerged about 26 to 30 days later. All were normal adults both externally and internally. Thus, in the JH-treated animals, the effects on the viscera cannot be accounted for merely by the precocious onset of adult development. As has already been noted, JH applica-

TABLE VI

Sensitivity of early and late prepupae to a single application of a mixture of juvenile hormone analogues

Dose (μ g)	Number treated	Number failing to diapause	Juvenile characters in resulting moths†						
			External*	Internal**					
				Thorax	Fat body	Gut	Gonads	Access- sory glands	Ab- dominal interseg- mental muscles
Application at time of ocellar retraction (days 4–5)									
5	2	0	0	0	0	0	0	0	0
10	9	3	0	1.8	2.7	2.3§	2	1.7	2
50	12	11	0.4	3.3	4.2	2.9§	3.9	2.6	4.2§
150	4	4	0.4	3.5	4.6	3.2§	4.2	3	4.3§
300	2	2	2	4.5	5	5	5	4.5	5§
Application to white prepupa (days 8–9)									
1	2	0	0	0	0	0	0	0	0
5	6	4	0	1.5	2.8	2	2.2	1.5	0.8
10	5	3	0	1.7	2.0	0.3	2.2	1	0
50	5	4	0	2.5	4.2	3	3.6	2.7	3
100	3	3	0.3	1.8	3.7	2.5	3.3	2	3
150	3	3	0.2	2	3.7	1	2.7	1.7	1.7

† When there is more than one treated individual, an average score is used. This average is based only on the scores for nondiapausing individuals.

* Scoring of external characteristics based on pupal assay for juvenile hormone, Williams (1961).

** Scoring of internal characteristics based on Figure 2.

§ At least one individual showed retention of larval characters.

tion to the early prepupa retards the pupal transformation of the internal organs. Consequently, in response to the secretion of ecdysone immediately after pupal ecdysis, these larval or larval-pupal viscera can only become pupal or pupal-adult.

DISCUSSION

Juvenile hormone and the larval-pupal transformation

The activity of the corpora allata of the *Cecropia* larva declines throughout the fifth instar and prepupal period and becomes zero by the time of pupal ecdysis (Williams, 1961). The titer of extractable JH from a closely related, non-diapausing species, *Samia cynthia ricini*, also shows these same changes (Patel and Madhavan, 1969). The declining titer of JH is thought to be the reason why pupation rather than another larval molt occurs at the conclusion of the fifth larval stage. Yet, as seen in Tables II and III, neither single nor multiple doses of JH were able to cause a normal fifth instar to molt into a sixth instar larva.

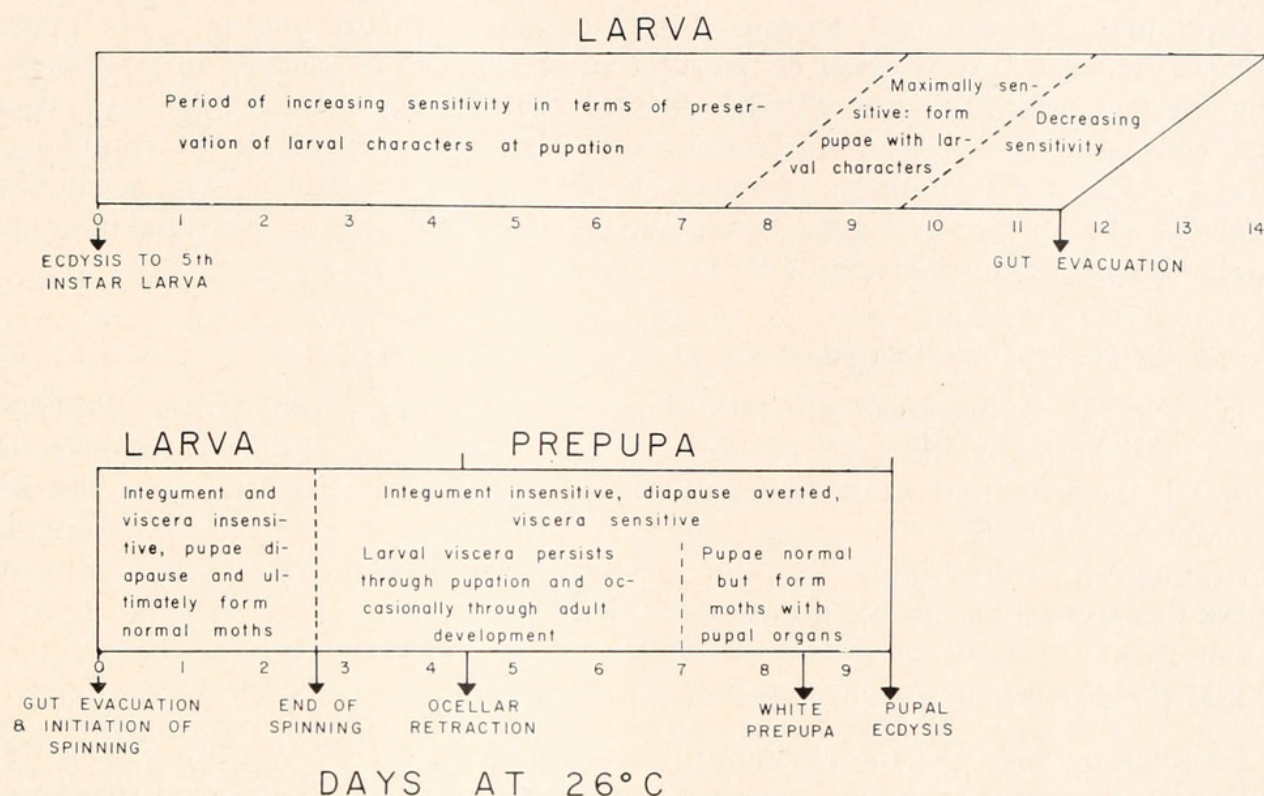


FIGURE 3. Time-table of developmental events at 26° C during the fifth larval instar and prepupal stage. The changing sensitivities to topically applied JH are indicated. The slanted lines indicate the variation in the timing of the recorded events in normal, untreated individuals.

On the basis of the experiments reported here, we now see that certain epidermal structures of fifth instar larvae remain sensitive to JH, especially when the latter is applied 4 days before the initiation of spinning. The larval tubercles were found to be most sensitive, followed by the tergal epidermis, the mouthparts, and finally the thoracic legs and abdominal prolegs. Nearly all of these larvae diapaused after pupation; after preliminary chilling they developed into normal adults, except that the tubercles and other sites where larval characters had been retained underwent pupation instead of adult differentiation (Figs. 1A and B). This result is the same as that reported by Willis (1969) for *Cecropia* infected with *Nosema*.

Figure 3 summarizes the effects of exogenous JH from the outset of the fifth instar until the ecdysis of the pupa 22 to 24 days later. The metamorphosis of the internal organs was unaffected when JH was applied prior to the onset of spinning. By contrast, during the entire prepupal period, JH had pronounced effects on the viscera but little or no effects on the metamorphosis of the epidermis. These findings imply that at the time of spinning the epidermis becomes insensitive to JH, whereas the viscera become sensitive. These results are in marked contrast to those obtained with *Galleria* in which both the integument and the internal organs are sensitive to JH before, but not after spinning (Sehnal, 1968; Sehnal and Meyer, 1968).

The difference between the two species almost certainly hinges on the duration of the pupal stage. *Galleria* initiates adult development within 24 hours after pupal ecdysis; that being so, it is necessary for the internal organs of *Galleria* to synchronize their metamorphosis with that of the integument. By contrast,

Cecropia undergoes a pupal diapause which persists for many months. Therefore, the larval-pupal transformation of the internal organs can be delayed or postponed. Schneiderman and Williams (1953) showed that during the first 10 days after pupal ecdysis, the respiration of Cecropia declines to the diapause level. Similarly, in this period RNA synthesis continues in the internal organs as well as in the epidermis which is depositing the entire pupal endocuticle (Berry, Krishnakumaran, Oberlander, and Schneiderman, 1967).

JH and the prevention of diapause

JH applied to prepupae prevents diapause by blocking the normal inactivation of the brain at the time of pupal ecdysis (Williams, 1952). As seen in Table VI, the sensitivity of the brain to JH is maximal at precisely the time when it normally is shut-off. The exogenous JH prevents the complete larval-pupal transformation of the brain. The net result is that the brain retains its ability to secrete the prothoracicotropic hormone (Table V).

JH and the disruption of metamorphosis of the internal organs

As seen in Table IV, the metamorphosis of internal organs was affected by JH application to prepupae, but only when diapause was averted. The larval structures which occasionally persisted throughout metamorphosis were in virtually all cases tissues which normally break down prior to pupal ecdysis. JH apparently blocked this process so that they persisted into the adult.

Those organs which undergo a distinct metamorphosis rather than dissolution seemed to respond to JH by a retardation in metamorphosis rather than a complete cessation of change. Therefore, in treated individuals in which diapause was averted, the initiation of adult development caused those organs which were still larval to become pupal and those which were partway through the larval-pupal transformation to become pupal-adult. Preliminary experiments suggest that when either the gonads or fat body are transplanted from treated individuals just after pupal ecdysis and are caused to develop immediately by implanting them into normal chilled pupae, they transform into pupal or pupal-adult organs just as they do *in situ*. By contrast, when the treated prepupae underwent diapause, apparently the internal organs "caught up" and subsequently developed into normal adult organs.

The mechanism of JH action in the larval-pupal transformation

According to Williams (1961), juvenile hormone must be present at the outset of adult development to exert its *status quo* action. When JH injection was delayed until the 3.5 day after apolysis, it had little or no effect on adult development (Williams, 1968). Apparently, the pupal epidermis is most sensitive to JH when it is undergoing the early rounds of DNA synthesis just after apolysis (Bowers and Williams, 1964; Krishnakumaran, Berry, Oberlander, and Schneiderman, 1967; Schneiderman, Krishnakumaran, Bryant, and Sehnal, 1969). Similarly, the final stage larval epidermis of *Galleria* becomes insensitive to JH once the cells have replicated their DNA (Sehnal, 1969; Sehnal and Novak, 1969; Schneiderman *et al.*, 1969).

In Cecropia, Krishnakumaran *et al.* (1967) have shown that DNA synthesis begins at the base of the tubercles on the first day of spinning and in the general epidermis the next day. To exert its *status quo* effect on the integument, JH had to be applied at least one and optimally two to three days before the onset of this DNA synthesis.

By contrast, in the viscera sensitivity to JH persists throughout the prepupal period. Yet only in the brain and hemocytes does DNA synthesis continue until after the pupal ecdysis (Krishnakumaran *et al.*, 1967). In all the other tissues and organs such as the midgut, fat body, and thoracic musculature, DNA synthesis shuts off at least 3 days before the pupal ecdysis although extensive histolysis and reorganization continue. Thus, JH can act on the viscera even after DNA synthesis has ceased, apparently by interfering with degradation and reorganization of the tissues.

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SUMMARY

1. Pupae retaining larval integumentary structures were formed after topical applications of Cecropia juvenile hormone or a mixture of juvenile hormone analogues to fifth instar Cecropia larvae prior to the initiation of spinning. Even daily applications of high doses did not result in a supernumerary larval molt.

2. The integument was most sensitive to juvenile hormone (JH) two to four days prior to spinning.

3. During the period of cocoon spinning, both the larval integument and viscera were insensitive to exogenous JH.

4. During the prepupal period, the integument remained insensitive but the larval-pupal transformation of the viscera was retarded by topical application of JH.

5. Juvenile hormone applied during the prepupal period prevented the normal "shutting-off" of the prothoracicotropic activity of the brain prior to pupal ecdysis. Consequently, the pupae did not diapause but initiated adult development within 5 days. The subsequent adults were normal externally but retained pupal or pupal-adult viscera with occasional retention of larval characters.

6. Unlike the epidermis, the viscera are sensitive to JH not only at the reported time of DNA synthesis, but also during the following period of tissue histolysis and reorganization.

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