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Juvenile hormone and insect embryogenesis

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Sláma and Williams (1966) first discovered that a crude extract of the juvenile hormone analogue juvabione would prevent hatching of the eggs of the linden bug, *Pyrrhocoris apterus*, when applied either to the female bug or to the freshly laid eggs. The same effect was also found after application of juvenile hormone (JH) and its analogs to the eggs of the wild American silkmoth, *Hyalophora cecropia* (Riddiford and Williams, 1967 ; Riddiford, 1970b). This ovicidal action seems to be one of the most promising means of insect control by juvenile hormone.

The purpose of this paper is to explore the mode of action of JH when applied during embryonic development. Most of the studies were performed on the Cecropia silkworm and utilized a mixture of synthetic JH analogs (Law, Yuan and Williams, 1966), but a synthetic preparation of Cecropia JH (Corey *et al.*, 1968) yielded identical results. Similar general effects have also been seen after application of JH analogs to *Pyrrhocoris apterus* females and eggs (Riddiford, 1969 and unpublished data).

JH Application to the Female

As seen in Table 1, female Cecropia which received high doses of JH or of a mixture of analogs laid eggs which did not hatch. These doses were at least 100- to 1000-fold those necessary to produce pupal-adult intermediates and must be used to counteract the very active breakdown mechanism in the adult female (Riddiford and Williams, 1967).

The hormone was most effective when injected on day 19 of adult development. At this time vitellogenesis is completed in the majority of follicles and the ovaries have entered the terminal phases of oogenesis (Telfer and Anderson, 1968). During the next 2 to 3 days protein-containing refractile bodies are deposited in the cortex of the egg, the egg is hydrated, and the chorion is formed. Figure 1 shows a similar

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TABLE I

Development of Eggs Laid by Female Cecropia *Moths Injected with Juvenile Hormone Materials*

Dosage (mg)		Eggs Oviposited	% Hatched	Developmental Stage of Unhatched Eggs (%)	
Synthetic JH Analog Mixture	Crude Cecropia Oil			Blastoderm	Blasto-kinesis
		Injection on day 19 of Adult Development			
0.5	50	142	8	90	2
0.5	50	300	0	100	0
		Injection before Mating			
—	73	114	91	6	4
1.25	25	237	23	75	2
4.5	90	183	0.5	93	6
5	100	80	0	94	6
		Injection after Mating			
—	50	229	88	1	11
—	200	37	5	92	3
1.25	25	85	36	60	4
2.5 (2)	50	188 (av)	0	100	0
	Inactive Cecropia Oil	*Control Injection*			
—	200 (before mating)	271	79	2	19
—	50 (after mating)	207	98	0.5	1.5

timing of effectiveness of JH with respect to the oviposition cycle of *Pyrrhocoris*. In this species vitellogenesis is completed by day 3 of the cycle (Riddiford, 1969; Enslee, 1971).

After application of extremely large doses of JH to the adult female, embryonic development was blocked in the blastoderm stage, just prior to germ band formation. Occasionally the yolk went on to form yolk cells, and isolated pieces of disorganized tissue were found. But a true germ band was never formed.

The blastoderm is a single layer of cells formed around the periphery of the egg by the migration of the cleavage nuclei into the cortical cytoplasm. Depending upon where the nuclei fall, some will form the germ band, while others form the extra-embryonic membranes. The

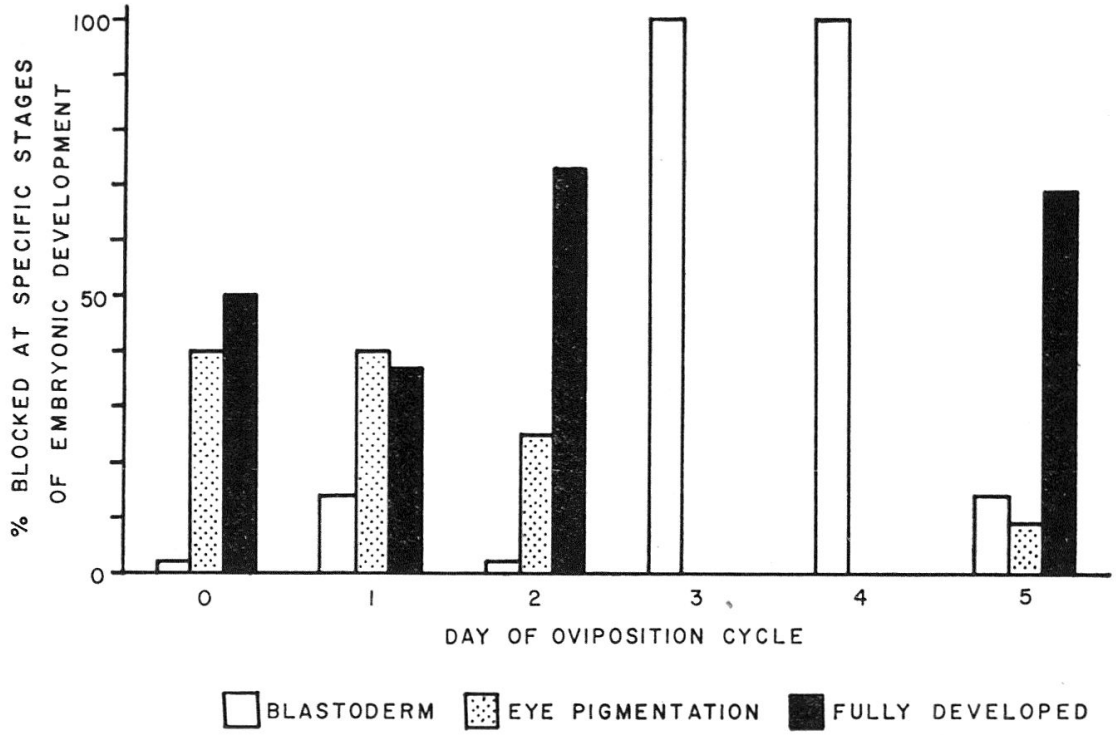

Fig. 1. — The effects of application of 10 μg of the Williams-Law mixture of juvenile hormone analogs to *Pyrrhocoris apterus* females at various times during the oogenesis cycle. Day 0 refers to application to female shortly after a batch of eggs was laid; day 5 application was about 6 hours prior to oviposition. The embryos that developed to the stage of eye pigmentation or beyond had not completed blastokinesis.

beginning of germ band formation is analogous to the beginning of gastrulation in the frog and sea urchin. Also, it can be blocked by actinomycin D (Lockshin, 1966) as can gastrulation (Davidson, 1968). Apparently, the messenger RNA provided by the maternal genome during oogenesis is sufficient for development up to the blastoderm, but no further. Support for this view is also afforded by the formation of the blastoderm but never of an organized germ band in Cecropia eggs subjected to artificial parthenogenesis (Riddiford, 1970b). Therefore, the transition from blastoderm to germ band signals the switch-over of the use of information from the maternal genome to that of the zygotic genome. It is this transition that can be blocked by JH application to the female during oogenesis.

JH Application to the Egg: Effects on Embryonic Development

Once the egg is fertilized and oviposited, JH no longer blocks development at the blastoderm stage, but only later at blastokinesis. Blastokinesis is a series of morphogenetic movements which occurs about midway through embryonic development and signals the beginning of differentiation of the first instar larva. In Cecropia, for instance, a molt

with the formation of the first instar larval cuticle occurs about 1 day after blastokinesis (Mueller, 1962).

When Cecropia eggs were treated either with JH analogs or with authentic Cecropia JH (Corey *et al.*, 1968) immediately after oviposition, hatching was blocked (Riddiford and Williams, 1967; Riddiford, 1970b). The unhatched embryos routinely had not completed blastokinesis, and some did not differentiate beyond this stage. Most, although they had not successfully completed these morphogenetic movements, attained varying more advanced degrees of external differentiation.

After application of JH to early *Pyrrhocoris* embryos, one also finds development blocked in blastokinesis (Enslee, 1971; Matolin, 1970). In these blocked embryos, one very striking internal anomaly occurred in the development of the nervous system. Normally, between 24 and 30 hours after blastokinesis, the abdominal ganglia consolidate and fuse with the thoracic ganglia to form one large ganglionic mass (Seidel, 1924). But in the JH-treated embryos this consolidation did not occur, even though the embryo had subsequently pigmented and shown other signs of external larval differentiation (Enslee and Riddiford, 1970; Enslee, 1971).

Thus, it appears that JH applied to the early embryo or to the female in low doses blocks embryonic development at the embryonic-larval transition. In this respect its action resembles its *status quo* effect on metamorphosis.

JH Application to the Egg: Delayed Effects on Postembryonic Life

As the germ band forms, the eggs become progressively less sensitive to JH (Riddiford and Williams, 1967). Those individuals which hatched after JH treatment appeared as perfectly normal first instar larvae, but later in postembryonic life development was often blocked (Riddiford, 1970b). The Cecropia silkworm proved to be an ideal insect for investigating these delayed effects because each larval instar has its own characteristic coloration and tubercle morphology. This so-called larval heteromorphosis has been attributed to a declining titer of JH (Williams, 1961).

When JH was applied to Cecropia eggs before blastokinesis, the animals which hatched showed delayed effects during larval life (Fig. 2). Many larvae molted to intermediate types, showing some characteristics of the proper instar and reforming some characteristics of the preceding instar as illustrated in Figure 3. Others died during the molting process or immediately thereafter. Several instances of a total repeat of an instar were seen in such individuals which died in the molt.

When JH was applied after blastokinesis, i.e., during the larval differentiation phase of embryonic development, most individuals

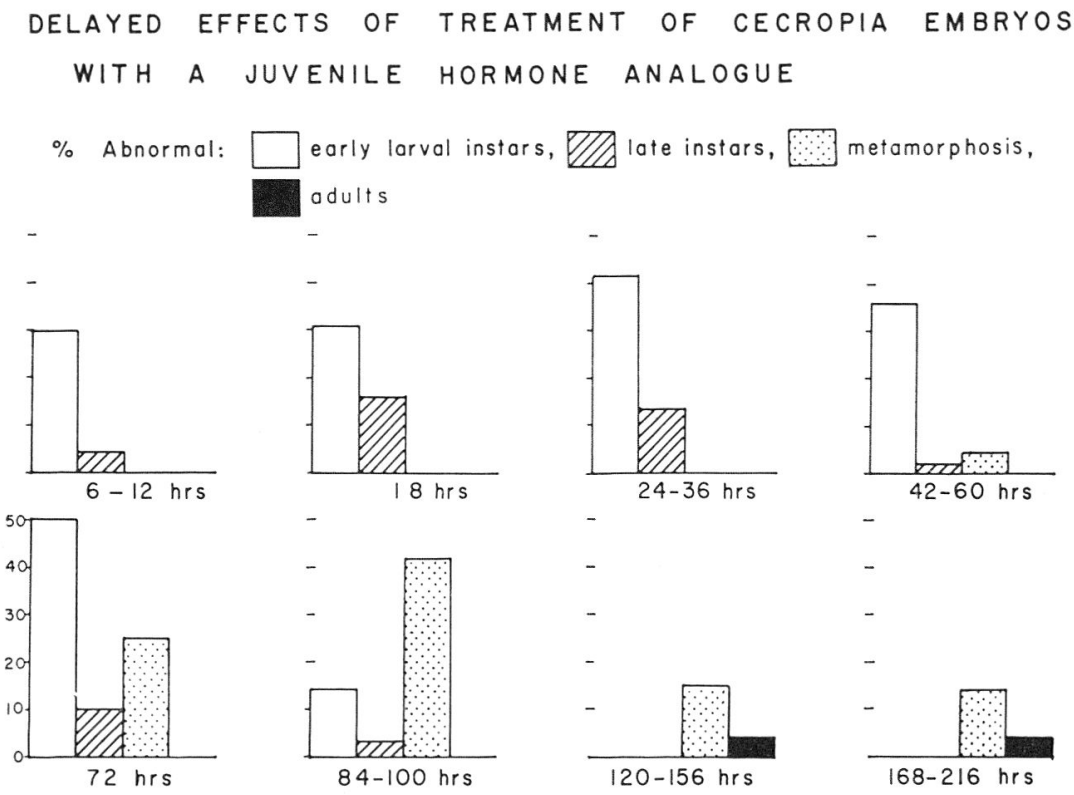

Fig. 2. — Effects of application of the Williams-Law mixture of juvenile hormone analogs at various times during embryonic development of *Hyalophora cecropia*. The treatment produced abnormalities which were manifest during post-embryonic life. "Early larval instars" are the first to third stages; "late instars" are the fourth and fifth stages; "metamorphosis" refers to the larval-pupal transformation; and "adults" include individuals which either did not initiate or complete adult development or were sterile adults. The timing of the stages of embryonic development are as follows: blastoderm formation at 18 hours; beginning of dorsal closure at 72 hours, and blastokinesis from 96–108 hours. The data are taken from Riddiford, 1970b.

hatched. Larval life proceeded normally, but metamorphosis was blocked (Fig. 2). After JH application to the late Cecropia embryo, postembryonic development often was blocked at the beginning of metamorphosis, i.e. at the time that the larva empties its gut and spins a cocoon. Some larvae were able to spin a normal cocoon but then were not able to pupate. Still others became pupae which reformed the fifth larval instar tubercles and occasionally other parts of the larval integument as seen in Figure 4. Although usually this first stage of metamorphosis — the larval-pupal transformation — was blocked, occasionally an individual from a treated egg became a normal pupa, but later either did not complete adult development or formed a sterile adult.

All these delayed effects can also be obtained by the contemporaneous application of juvenile hormone. But the doses required are at least tenfold higher than that applied to the embryo to obtain the same

effect. For instance, daily doses throughout larval life of 5 μg of the Williams-Law mixture of JH analogs was necessary to produce larval intermediates, whereas 0.16 μg applied to germ band embryos was

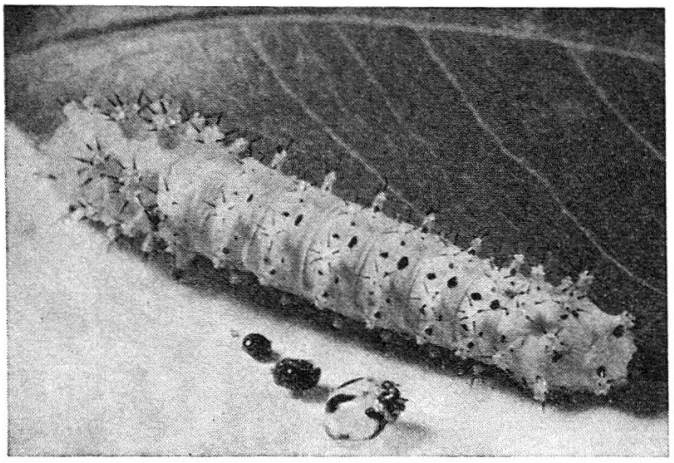

Fig. 3. — A larval intermediate of the Cecropia silkworm produced by treatment of the embryo with 0.16 μg of the Williams-Law mixture of juvenile hormone analogs during the formation of the germ band. This larva which has molted three times has thoracic tubercles typical of the fourth instar but has reformed the dorsal and lateral abdominal spots and the spots on the genae typical of the third stage larva.

sufficient (Riddiford, 1970b). Also, to obtain pupae with reformed larval tubercles (Fig. 4), at least 150 μg of the JH analog mixture must be applied 2 to 3 days before emptying of the gut (Riddiford, 1968). Similar animals were obtained after only 1.6 μg was applied to the late egg (Riddiford, 1970b).

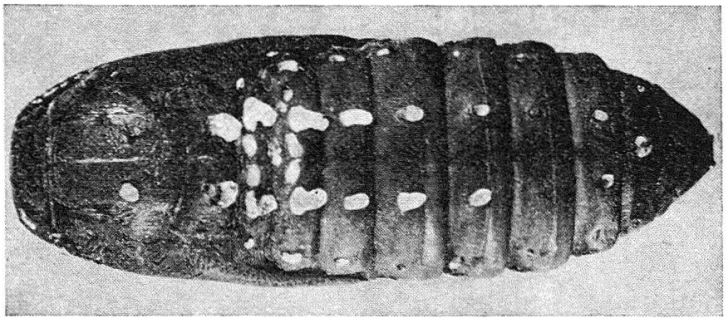

Fig. 4. — A Cecropia pupa which has reformed larval tubercles and patches of larval integument. This individual hatched from an egg treated with 1.6 μg of a Williams-Law mixture of juvenile hormone analogs after blastokinesis.

The disparity of the respective doses clearly shows that the delayed effects cannot be due to the persistence of applied hormone in the insect throughout larval life. Rather, the applied JH must have a direct effect on the embryos, either on the programming of the cells *per se* or on the programming of the corpora allata as first suggested by Willis (1969).

JH Application to Late Embryos: Action on the Corpus Allatum

The delayed action of juvenile hormone on metamorphosis after application to the late embryo was also seen in *Pyrrhocoris* (Riddiford, 1970a). Normally during the molt to the fifth instar the corpus allatum

stops secreting JH, and the larva molts to an adult 7 days later (Williams and Sláma, 1966). If, however, active corpora allata are implanted (Sláma, 1964) or JH is applied at the outset of the fifth instar, then the individual molts after 5 days to a supernumerary sixth instar larva (Williams and Sláma, 1966). A supernumerary seventh instar larva may also be produced by similar treatment of the sixth instar larva. Application of JH to the *Pyrrhocoris* embryo after blastokinesis also produced sixth instar larvae (Riddiford, 1970a). Some of these giant larvae molted again to seventh instar larvae, but they never metamorphosed.

To test the hypothesis that the JH application somehow affected the programming of the corpus allatum the following experiments were performed (Riddiford and Truman, 1972). Fertile *Pyrrhocoris* eggs 24 to 36 hours before hatching were treated with JH. Then 24 hours after the molt of the resulting larvae to the fifth instar, the corpus allatum was removed and implanted into a normal host of the same age. As seen in Table 2 and Figure 5, the allatectomized treated bugs nearly

TABLE 2

Application of Juvenile Hormone Analogs to Late Pyrrhocoris *Embryos: Effects on the Corpus Allatum*

	Number	% Normal Adults	% Adultoids				
			+1	+2	+3	+4	+5
Treated	36	14	8	16	19	33	6
Treated allatectomized . .	23	87	13	0	0	0	0
Treated allatum implanted into normal host .	40	47	31	22	0	0	0
Treated allatum removed and reimplanted .	5	40	20	20	20	0	0
Normal allatum implanted into normal host . . .	42	100	0	0	0	0	0

all molted to normal adults. By contrast, many of the normal hosts which had received the corpus allatum implants showed marked JH effects. Table 2 also shows that such effects were not seen when the corpus allatum from untreated fifth instars was implanted into a normal host. The fact that adultoids produced by the implants of treated

corpora allatum did not show as pronounced effects as seen in the donors is apparently due to a large variation in the functioning of implanted allata. A case in point was a series of control implants of the normally functional corpora allata from freshly molted fourth instar larvae into freshly molted fifth instar larvae. Upon the next molt, the hosts showed effects ranging from supernumerary sixth instar larvae to normal adults.

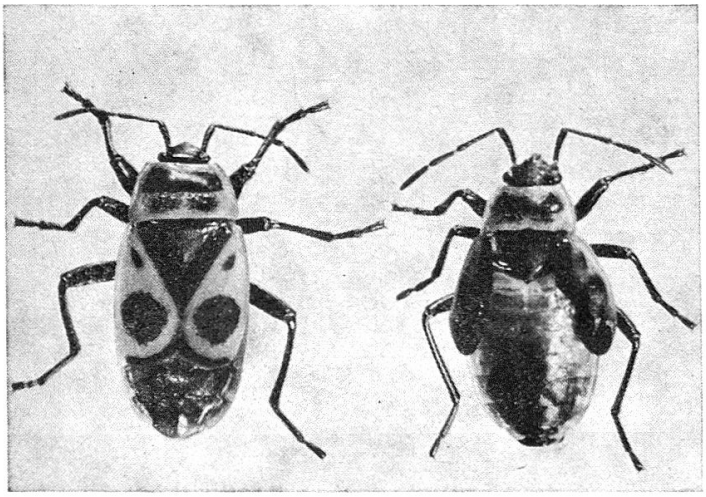

Fig. 5. — Left: A normal *Pyrrhocoris* adult which had been treated with the Williams-Law mixture of juvenile hormone analogs as a late embryo, then was allatectomized as a freshly molted fifth instar. Individuals receiving the same treatment as embryos, but not allatectomized formed larval-adult intermediates (Type III adultoids; Williams and Sláma, 1966). Right: An untreated individual which received the corpus allatum from the one on the left at the outset of the fifth instar. The larval type wings and the red patches of larval cuticle on the ventral side of the abdomen class it as a type II adultoid.

From these data, it is clear that juvenile hormone applied to the late embryo somehow affects the corpus allatum so that it does not shut off normally at the beginning of the fifth instar. Also, the applied JH was not still present in the bug at the outset of the fifth instar as suggested by Willis and Lawrence (1970), since the allatectomized treated bug formed a perfectly normal adult.

Summary

Figure 6 summarizes the effects of JH when applied to Cecropia at various stages during its life history.

Embryonic development can be thought of as a progressive utilization of genetic information. In the insect embryo two major critical steps are the switching on of zygotic genome at blastoderm, then the larval genome at blastokinesis. Just as in postembryonic development where JH is thought to prevent the derepression of new information (Williams, 1963; Williams, 1971), so it seems that it has a similar action in embryonic development. Application of JH to the female blocks her eggs at the first step — the activation of the zygotic genome. Once the egg is fertilized and laid, JH can no longer block the formation of the germ band, but only later at the embryonic-larval transition.

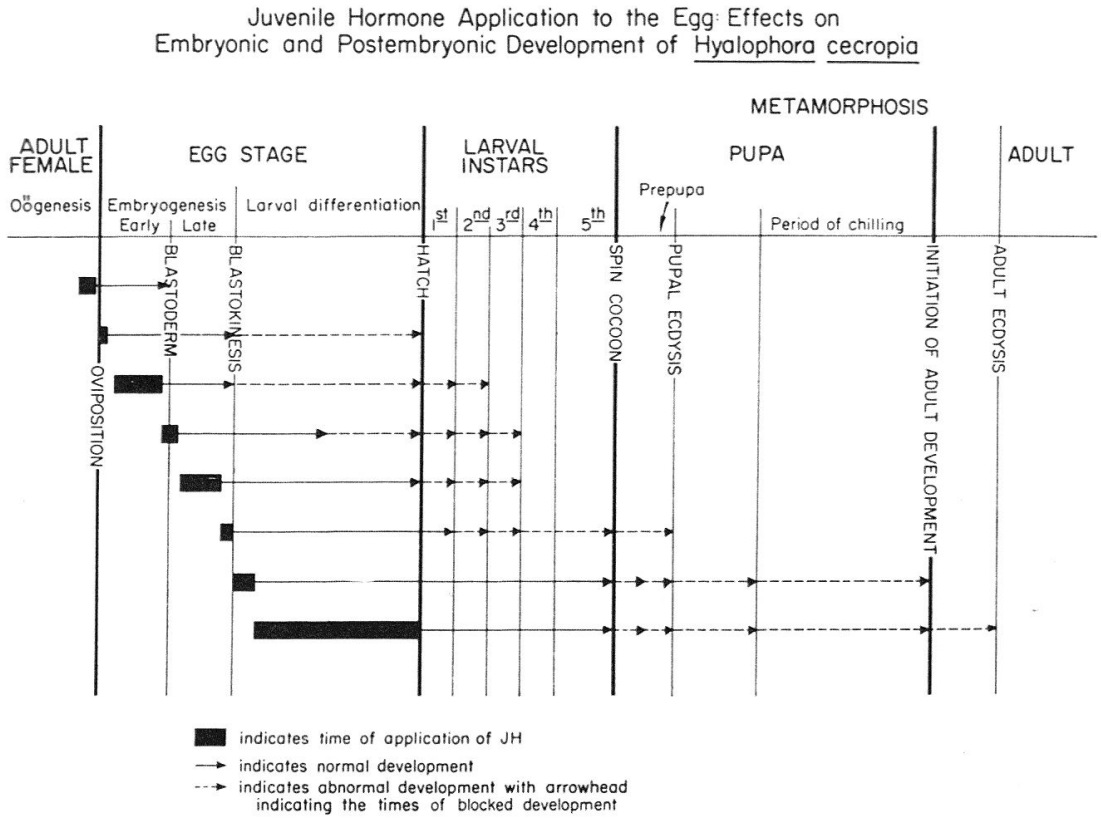

Fig. 6. — Juvenile hormone application to the egg: effects on embryonic and post-embryonic development of *Hyalophora cecropia*. From Riddiford, 1970b.

It is interesting to note that JH can exert these effects on embryonic development, even though it normally plays no role in these events. Also, unlike in postembryonic life, it acts in the absence of ecdysone.

When JH is applied after germ band formation (i.e., after the turning-on of the embryonic genome), it has progressively less effect on embryonic development and more effect much later in postembryonic life. This delayed action appears to be due to its action on the developing corpus allatum such that its secretions do not ebb and flow in the normal manner.

As a practical means of insect control by JH, the immediate ovicidal effects are the more important. But the delayed effects mean that although some larvae hatch after treatment, their chances of survival to reproductive maturity are slim.

* * *

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