LARVAL DEVELOPMENT FOLLOWING JUVENILE HORMONE ANALOGUE TREATMENT OF EGGS OF THE LESSER MILKWEED BUG, *LYGAEUS KALMII*

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Abstract—When juvenile hormone analogues were applied to developing embryos of *Lygaeus kalmii*, at the approximate time of blastokinesis, morphological effects were observed in larval life as well as at the time of metamorphosis. Of particular interest was the finding that the distinctive colour pattern characteristic of early larval instars was retained in later instars. A hypothesis is presented which explains these results and the different results reported in the literature for other insects.

INTRODUCTION

AMIDST the ever-increasing literature on the juvenile hormone (JH) of insects, a particularly intriguing area of investigation in recent years has been the study of the effect of JH or its analogues (JH-A) when applied during embryonic development (SLÁMA and WILLIAMS, 1965, 1966; RIDDIFORD and WILLIAMS, 1967; RIDDIFORD, 1970). To date, studies on susceptible species have shown that JH applied before blastokinesis causes abnormal embryos or early larvae, whereas application after blastokinesis has delayed effects—'normal' larvae followed by developmental abnormalities at or after metamorphosis. In the present study, *Lygaeus kalmii* embryos were treated with JH-A. This treatment not only affects them at metamorphosis, but also causes morphological modifications during the larval instars.

MATERIALS AND METHODS

Eggs of *Lygaeus kalmii* were obtained from laboratory-reared cultures originally derived from locally collected populations. The individual eggs from each clutch (14–69 eggs/clutch) were separated and placed in 100×20 mm plastic Petri dishes lined with Whatman No. 1 filter paper. Half the eggs in each clutch served as controls. All animals were reared on milkweed seeds and water at 25°C under a 14 hr daily photoperiod (14 : 10).

Eggs were treated with the Williams–Law mixture (LAW *et al.*, 1966) of synthetic JH-A as supplied commercially (Calbiochem). Each egg received 0·25 or

2·5 μg of JH-A dissolved in 0·05 μl of acetone (Baker's Reagent Grade), applied topically via a Hamilton microlitre syringe. Controls received acetone only or no treatment. The appearance of pigment within the egg served as a developmental marker indicating the time of blastokinesis. Eggs were treated within 5 hr after observation of pigmentation. Subsequent observations were made daily. Non-developing eggs and cast skins were removed from the culture dishes.

RESULTS

Embryonic development

The normal time span between oviposition and hatching in laboratory-reared *L. kalmii* at 25°C is 7 to 8 days, the variation apparently being caused by retention of the fertilized eggs by the female. In order to standardize the time of hormone application, the onset of pigment formation was used as a marker. Hatching occurred simultaneously within any given clutch of eggs, regardless of the treatment given. In both the hormonally treated groups and the controls, 47 per cent of the eggs hatched.

Larval development: effects of JH-A on larval morphology

As often occurs in insects, the larval instars of *L. kalmii* have distinctive colour patterns. The first three instars are deep orange with a light yellow longitudinal stripe on either side of the dorsal midline of the abdomen. These stripes are absent in 99 per cent of the normal fourth and fifth instar larvae, which are solid orange. However, about 1 per cent of larvae (figure based on observation of laboratory stocks) retain the striped colour pattern throughout larval life. Mature larvae with this striped pattern undergo normal metamorphosis.

There was no difference in the development of those controls which received acetone treatment and those which received no treatment. In all cases, the data from these two groups were pooled and are presented simply as the control group.

The effect of JH-A on the development of colour pattern is shown in Table 1. The higher dose of JH-A appears to be causing the retention of the early larval colour pattern in the later instars. The striking effect of JH-A on colour pattern was totally unexpected inasmuch as no previous researcher has reported any larval effect of JH-A treatment immediately following blastokinesis. There was no effect of JH-A upon the time of larval ecdyses, which was identical in control and experimental insects.

JH-A effects upon metamorphosis

The metamorphosis of *Lygaeus* normally follows the fifth instar. The effects of JH-A are shown in Table 2. The JH-A interferes with normal metamorphosis, causing adultoids or supernumerary instars. These results correspond with those reported for a variety of other insects after similar treatment (RIDDIFORD and WILLIAMS, 1967; RIDDIFORD, 1970; RIDDIFORD and TRUMAN, 1972).

TABLE 1—EFFECTS OF EMBRYONIC TREATMENT WITH JH-A ON THE DEVELOPMENT OF LARVAL
COLOUR PATTERN IN *L. kalmii*

| | Instars 1–3 | | | Instars 4–5 | | |
| | Colour pattern | | | Colour pattern | | |
JH-A treatment	No. normal	No. abnormal	Percentage abnormal	No. normal	No. abnormal*	Percentage abnormal
0·25 μg	73	0	0	63	10	~16
2·50 μg	52	0	0	7	45	~90
Control†	108	0	0	107	1	~1

    * Abnormal, maintenance of a colour pattern which normally occurs only in instars 1 to 3.
    † There was no difference in the development of controls treated with acetone and those receiving no treatment. The data from both groups are combined under the heading 'Control'.

TABLE 2—EFFECTS OF EMBRYONIC TREATMENT WITH JH-A ON METAMORPHOSIS IN *L. kalmii*

| | Most advanced developmental stage attained* | | | | | | |
JH-A treatment	Dead 5th	P 6th	6th	P 7th	PA	Adultoids	Adults
0·25 μg	3	20	1	0	44	3	2
2·50 μg	5	31	13	3	0	0	0
Control†	7	0	0	0	0	0	101

    * P, pharate; PA, pharate adultoid.
    † Control as in Table 1.

DISCUSSION

Juvenile hormone plays a major rôle in controlling the postembryonic development of insects. It is also involved in regulating the maturation of oöcytes in certain insects. SLÁMA and WILLIAMS (1965) discovered that topical application of JH or its synthetic analogues to insect embryos would interfere with postembryonic development, just as similar treatment of larvae had been shown to interfere with metamorphosis. This finding stimulated much interest in the question of the mechanism of the action of JH. The following is a summary of the experimental evidence concerning postembryonic effects of JH applied during embryonic life.

When eggs of *Hyalophora cecropia* (RIDDIFORD and WILLIAMS, 1967; WILLIS, 1969), *Oncopeltus fasciatus* (RIDDIFORD, 1970), and *Pyrrhocoris apterus* (RIDDIFORD, 1970) are treated with JH-A after blastokinesis, the resulting larvae appear normal. However, at the time of maturation, normal metamorphosis is (a) interfered with

or (b) blocked by the production of supernumerary instars. This pattern of events is now well established and is highly reproducible. It must be pointed out, however, that all insects are not susceptible to this type of JH treatment. *Pieris brassicae*, for example, is reported to have shown no such interference with maturation (BENZ, 1971).

The species currently under study shows effects similar to those listed above. There is, however, one very important difference: in contrast to other species so far studied, *L. kalmii* larvae also show a morphological effect of embryonic treatment with JH-A. The characteristic colour pattern of the normal fourth and fifth instar larvae is replaced by a striped pattern like that of the earlier instars. This discovery constitutes evidence that the JH-A treatment affects the entire post-embryonic period, not merely metamorphosis.

In 1970 RIDDIFORD used ^{3}H-juvabione to show that no detectable concentration of this hormone analogue was present in third instar *Pyrrhocoris* larvae following treatment of the same insects as embryos. RIDDIFORD and TRUMAN (1972) have recently shown that removal of the corpus allatum from embryonically treated fifth instar larvae results in normal metamorphosis and, conversely, that implantation of glands from treated embryos into controls can interfere with metamorphosis. These experiments are cited as further evidence that residual analogue is not the causative agent and as support for the hypothesis, originally suggested by WILLIS (1969), that embryonic treatment with JH-A selectively interfered 'with the embryonic programming of the corpus allatum such that it does not cease secretion as a prelude to metamorphosis' (RIDDIFORD and TRUMAN, 1972). Thus, this hypothesis predicts an undisturbed pattern of 'normal' JH secretion throughout larval development with an abnormal continuation of hormone production at the time of metamorphosis.

As previously stated, the results of the current study on *Lygaeus* contradict the generality of this hypothesis. Effects are observable during larval development which correlate directly with the JH-A treatment during embryonic life. In explanation of these apparently contradictory results, the following hypothesis is suggested.

Inherent in the genetic instructions of the insect embryo is a pattern of development, i.e. embryo, larval stage 1, larval stage 2, etc., pre-maturation stage, adult. This sequence is set up so that each advance in the development of each individual part of the organism is initiated by a certain titre of JH (review by WIGGLESWORTH, 1970). In order to maintain developmental synchrony, e.g. to ensure that all parts of the organism achieve first instar development before the second instar is initiated, it is assumed that for any given developmental stage the threshold of the least sensitive part of the system is below the threshold which initiates the *next* developmental stage in the *most* sensitive part. The range of JH output may or may not be the same for each larval instar, but it always drops significantly prior to metamorphosis. The flexibility required by such a model can be viewed as evolutionarily advantageous since it allows for minor fluctuations in absolute hormone output without affecting the synchrony of the total system.

Assuming all of this as the normal situation, how might the addition of extra JH, assumed to be prior to the production of endogenous hormone, affect this system ? The crucial supposition is that the amount of hormone initially produced, or present during this initial phase of activity, determines the level of subsequent hormone production. Then the effect of topical application of JH-A would be to alter the corpus allatum's output of JH throughout larval development, though not interfering with the temporal pattern of its secretion. A system with many parallels to the one just described is known to exist in the vertebrates (BARRACLOUGH, 1967). Treatment of mammalian embryos with androgen or oestrogen prior to the onset of endogenous secretion of these hormones causes major changes in the quantity and pattern of their secretion later on in development (FLERKÓ, 1971).

Such an hypothesis allows us to make several predictions. First, the added JH-A must be present during a critical period just prior to or simultaneous with the production of endogenous hormone. Earlier application would disrupt the synchrony of early embryonic events causing abnormalities and possibly death of the embryo. This is exactly the finding reported by RIDDIFORD and WILLIAMS (1967). Application after the onset of endogenous production could have immediate effects, but not a permanent one, at least not via this pathway. Implantation of corpora allata into fifth instar *Rhodnius* produces supernumerary larval instars, followed by giant adults (WIGGLESWORTH, 1970).

An additional attractive feature of this model is that it allows us to explain all three of the types of responses to JH-A thus far observed after treatment of embryos. An insect with a tightly synchronized system might show no larval effects of the extra JH, but would show maturation effects, as *Oncopeltus* does. A system with less synchrony of thresholds among its individual parts might show some effects of JH: for example, the production of an adultoid rather than an extra larval instar or the maintenance of a larval colour pattern in an otherwise 'normal' *Lygaeus* larva. Still other insects, such as *Pieris*, may have a 'maturation threshold' which allows a significant amount of JH to be present without interfering with metamorphosis. Another equally possible explanation for lack of effect is that the time of application was after the sensitive period or that topically applied JH-A are differentially and more effectively degraded than endogenous hormone in these insects.

The hypothesis is also directly testable. For example, application of an appropriate concentration of JH-A to third instar *Lygaeus* should produce fourth instar larvae which retain the juvenile colour pattern.

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