Effects of juvenile hormone, ecdysterone, actinomycin D, and mitomycin C on the cuticular proteins of *Tenebrio molitor*

By P. ELAINE ROBERTS\(^1\) AND JUDITH H. WILLIS\(^2\)

From the Department of Genetics and Development, University of Illinois

**SUMMARY**

Juvenile hormone (JH), ecdysterone and some antibiotics cause *Tenebrio molitor* to form a second pupa or pupal-adult intermediate. Incorporation of labelled leucine into the cuticular proteins of JH-induced second pupae did not differ from incorporation in normal pupae, and the soluble cuticular proteins from these young second pupae were identical to those extracted from normal pupal exocuticle when analysed by SDS-polyacrylamide gel electrophoresis. However, as these second pupae aged, the major early bands did not undergo a normal decrease in staining intensity, indicating a JH effect on protein insolubilization (sclerotization). The transport of protein into the cuticle may also have been altered by JH; electrophoretic analysis of the new cuticle of treated animals showed intense staining of bands with \(R_F\)'s similar to those of blood proteins.

The new exocuticle produced after treatment of pupae with ecdysterone had soluble proteins which were typical of normal pupae, but extracts from such animals aged prior to cuticle removal yielded bands characteristic of normal adults. Pupae treated with actinomycin D occasionally form new abdominal cuticle with characteristic pupal morphology. These cuticles yielded soluble proteins which, upon analysis, had pupal, pupal and adult, or adult banding patterns. Animals treated with mitomycin C, although retaining vestiges of pupal abdominal characters, had adult cuticular proteins.

**INTRODUCTION**

It is surprising that, despite the significant morphogenetic activity of juvenile hormone (Willis, 1974), very little attention has been paid to those molecules which underlie the morphological characteristics of each stage. Certainly the changes in synthesis and secretion of the cuticular proteins must be viewed as fundamental to an understanding of hormonally controlled changes in cuticular structure and texture. There have been few descriptions of the cuticular proteins of different metamorphic stages, and even fewer investigations of the details of the action of juvenile hormone (JH) in relation to these proteins. This is in sharp

\(^1\) Author's address: Department of Zoology and Entomology, Colorado State University, Fort Collins, Colorado 80523, U.S.A.

\(^2\) Author's address: Department of Genetics & Development, 515 Morrill Hall, University of Illinois, Urbana, Illinois 61801, U.S.A.
contrast to studies on the vitellogenic action of JH in adult insects, for recently there has been a spate of publications concerned with the molecular characterization of the vitellogenins.

Willis (1970) and Willis & Hollowell (1976) have demonstrated that the soluble cuticular proteins of the Cecropia silkmoth are the same when isolated from normal pupae or from second pupae produced following the injection of JH. Anderson, Chase & Willis (1973) showed that the amino acid composition of hydrolysates of unextracted cuticle is the same in both normal and second pupae of Tenebrio. It was the goal of this study to conduct a more thorough analysis of the cuticular proteins produced when a pupa forms a second pupa instead of undergoing the normal pupal–adult transformation.

Such a detailed analysis is especially relevant in the case of Tenebrio, for this species produces second pupae in response to a diverse array of compounds other than JH – antibiotics which are known to interfere with DNA and RNA synthesis, insecticide synergists, ouabain, and even high levels of the insect molting hormone, ecdysterone (Bowers, 1968; Chase, 1967, 1970; Socha & Sehnal, 1972, 1973). Mimicry of JH by mitomycin C, ecdysterone and actinomycin D has not been found in the bug, Oncopeltus fasciatus (Lawrence, 1969; Lawrence & Hayward, 1971; Willis & Hollowell, 1976; and Willis and Rubin, unpublished observations), nor have actinomycin D or mitomycin C been reported to mimic JH in any other species. Chase (1970) questioned whether the JH mimics might be acting by activating the corpora allata, the source of JH, but Socha & Sehnal (1972) eliminated this possibility by demonstrating that JH, ecdysterone, actinomycin D and mitomycin C were all effective on isolated abdomens lacking all known sources of JH. It thus seemed important to examine ‘second pupae’ produced by some of these mimics, applying criteria more stringent than gross morphological ones.

Reversal of metamorphosis – that is, the production of structures characteristic of an earlier stage – has been described in a limited number of species (Willis, 1974). Tenebrio has been included in this category by Caveney (1970) on the basis of the exocuticular ultrastructure.

Two earlier publications (Roberts & Willis, 1980a, b) describe the electrophoretic banding patterns (see Fig. 1) and patterns of synthesis of the soluble cuticular proteins of Tenebrio. In this paper, the soluble cuticular proteins from animals treated with JH, ecdysterone, actinomycin D (AMD), and mitomycin C were analyzed by SDS-polyacrylamide gel electrophoresis to answer several questions about JH and its putative mimics: (1) Is Caveney’s claim of reversal of metamorphosis of the exocuticle substantiated by the presence of our one larval-specific protein? (2) Are the cuticular proteins of second pupae produced by JH identical to those of normal pupae, which would imply a repetition of the entire program of cuticular protein synthesis, secretion and sclerotization? (3) Does second pupal morphology require the normal pupal protein banding pattern, or is the action of the JH mimics only superficial?
MATERIALS AND METHODS

Tenebrio molitor, yellow mealworms, were reared as described by Roberts & Willis (1980a). Pupae weighing 125 ± 25 mg were anesthetized for 10 min with CO₂ and injected with a 10 µl Hamilton syringe between the last two ventral sclerites. Injections were made 10 min to 4 h after the larval–pupal ecdisis. The following compounds were injected: synthetic C₁₈ juvenile hormone (methyl trans, trans, cis 3,11-dimethyl-7-ethyl-10,11-epoxytrideca-2,6-dienoate) (Eco Control, Inc.), dissolved in light mineral oil; actinomycin D (CalBiochem) dissolved in 10% ethanol; mitomycin C (Sigma) containing NaCl (4 M final concentration) dissolved in distilled water; ecdysterone (Rohto Pharmaceuticals) dissolved in 10% ethanol. Injection volumes varied from 1–3 µl. Control animals were injected with each solvent; none altered the normal banding patterns.

The animals were returned to 28 °C after injection; beginning 96 h later, they were observed daily. Only animals that were unaffected by treatment emerged spontaneously; otherwise the old pupal cuticles were peeled off with forceps as soon as they could be removed easily. The timing of these second pupae was thus much less precise than the timing of normal animals, which was based on the moment of ecdisis. Some animals were frozen immediately after peeling, others were kept at 28 °C for an additional 24 h. The abdominal cuticles were then prepared for electrophoresis as described by Roberts & Willis (1980a). Since most of these new cuticles were very thin, the entire extract (30 µl) was applied in a single well. Only representative results are shown in the figures.

RESULTS

The soluble cuticular proteins of JH-induced second pupae

Banding patterns

The soluble cuticular proteins from 19 animals treated with 10 µg of JH (1 µl of a 34 mM solution) and 7 animals treated with additional injections of 3 or 5 µg of JH on days 2, 4, and 6 after larval-pupal ecdisis, were compared with proteins extracted from normal animals of different stages and ages. (See Fig. 1 for diagram of normal patterns.)

The larval protein banding pattern was not present in any of the extracts of cuticles taken from second pupae induced by JH treatment. Specifically, the early uniquely larval protein at RF 0-53 was never detected, the prominent high-molecular weight band was the pupal 0-21 and not the larval 0-18 and other bands did not form the larval pattern. The second pupal patterns were also unlike those found for adults in that the adult-specific bands 0-68, 0-69, and 0-75 were absent.

Three banding patterns were present in the JH-treated animals:

(i) Three second pupae with soft cuticle (Fig. 2, Ch. 5) had banding patterns identical to normal 1 h pupae. The dominant pupal bands 0-66, 0-71, 0-77 and
Fig. 1. Diagrams of the banding patterns of the soluble cuticular proteins of normal *Tenebrio* drawn from data presented in Roberts & Willis (1980a).

The following symbols are used in the Figures. *L*, Normal larval extract; *P*, normal pupal; *A*, normal adult; subscripts: *e*, 'early' or 1 h after ecdysis, or treated animals with soft cuticle; *l*, 'late' or 24 h after ecdysis. The treatments used on the young pupae prior to formation of new cuticle are designated: *J*, juvenile hormone; *E*, ecdysterone; *D*, actinomycin D; *M*, mitomycin C. Significant positions are labelled with their *R*ₚ's determined from standards run on each gel.
0-88 were present; only a few lightly stained, high-molecular-weight bands were detected.

(ii) One second pupa with soft cuticle (Fig. 3, Ch. 2) had cuticular proteins which banded like those from normal 2 to 4 h pupae, but it also had many bands with molecular weights greater than 25000 daltons ($R_F = 0.50$). Such high-molecular-weight proteins normally are extracted only from cuticle several hours past ecdysis. Four of these bands (A–D) correspond in mobility to four of the blood proteins; but their presence in this extract does not seem to be due to contamination since other prominent blood bands were not detected. (See fig. 1, Roberts & Willis, 1980a, for electrophoretic pattern of blood proteins.)

(iii) The third pattern was present in cuticle extracted from 15 animals (Fig. 4) that had been maintained at 28 °C for 24 h after removal of the old pupal cuticle. This pattern was unlike that of any normal pupa. The pattern was basic-
ally late pupal (Fig. 4, Ch. 1), but with these exceptions: (a) bands A-D were much darker; (b) in eight samples the region between $R_F$'s 0.50 and 0.65 had the early pupal pattern of three widely spaced bands, while the other seven had either an intermediate pattern consisting of an upper band, two pairs and the lower band of the third pair, or the late pattern of three pairs of bands; (c) band 0.66 persisted as a major band; (d) bands 0.71 and 0.77 were the most intensely stained bands, a situation normally encountered in extracts from early pupae; (e) band 0.82 which was present in normal late pupae was absent from four of these samples.

Multiple injections of JH were delivered to seven animals; their new cuticle showed banding patterns identical to those of other pupae treated but once with JH.
Synthetic patterns

Synthesis of the soluble cuticular proteins was studied by injecting second pupae with [14C]leucine, either when the old pupal cuticle could first be removed (early animals) or after 24 h of maintenance at 28 °C (late animals). One early and two late animals were analyzed. The pattern of synthesis of the early animal (Fig. 5, top) was indistinguishable from that of normal pupae injected immediately after ecdysis (fig. 2 of Roberts & Willis, 1980b). This pattern had slight labelling in several bands with mobilities slower than 0.56, the incorporation in 0.56 was greater than that in 0.60 and the bands 0.66, 0.71, 0.77, 0.80, 0.86, 0.88 were labelled. Of the bands with mobilities like the blood bands, in this sample A was not detectable; B, C, and D were unusually lightly stained; and C and D were slightly labelled. (The labelling of the other high-molecular-
weight bands was similar to that found in normal pupae injected 2 to 24 hr after ecdysis."

The two late second pupae (Fig. 5, bottom) yielded cuticular proteins that showed a pattern of leucine incorporation like that of the normal 48 h pupa (fig. 2 of Roberts & Willis, 1980b). Late normal pupae and late second pupae differed from early normal pupae in having several high-molecular-weight bands, each of which incorporated 0.5–1% of the total counts in their peaks; band 0.60 had relatively more counts than band 0.56; bands 0.77 and 0.88 were labelled; and bands 0.66 and 0.71 were no longer labelled. These latter two bands, which in normal late pupae were very weak, thus persisted in the second pupae as stained bands in the absence of concurrent synthesis. The band at 0.21 (C) which is conspicuously labelled in normal late pupae, was only slightly labelled in these second pupae.
Juvenile hormone and cuticular proteins

Soluble cuticular proteins of second pupae and pupal-adult intermediates produced by compounds which 'mimic' the action of JH

Ecdysterone

We analyzed the cuticles of 14 *Tenebrio* which were injected with 32 µl of ecdysterone (2 µl of a 68 mM solution). New cuticles formed 4 days after injection, and the old cuticle was peeled off and discarded. We extracted cuticles from such fresh animals and also from some which had been maintained at 28 °C for 24 h after peeling. The abdominal cuticle from these animals was morphologically identical to that from normal pupae. The lateral phalanges had well developed gin traps, and urogomphi were present at the tip of the abdomen. The cuticles of some animals which had been maintained at 28 °C for an additional day appeared to have several layers, as if multiple cuticles had been formed rather than a single thick cuticle. Since these cuticles adhered rather closely they were analyzed together in cases where the inner layer was thick enough to be freed of muscle.

The cuticular proteins which were extracted immediately after the animals were peeled were similar to those from normal early pupae, with one exception which was adult-like. All of the normal heavy early bands were present, but in six of nine samples they were more intensely stained than in normal pupae.

Second pupae which had been aged for 24 h yielded an unexpected result. Despite their superficial pupal morphology, the banding pattern of their cuticular proteins (Fig. 3, Ch. 1; Fig. 6, Chs. 1, 2) was like that of the normal adult 4–8 hr after ecdysis (see fig. 3, Roberts & Willis, 1980a). There was some variation among individuals, including relative intensities of some high-molecular-weight bands. Band 0-68 which is unique to early adults was present in only two of the six extracts (Fig. 3, Ch. 1; Fig. 6, Ch.2). This band was absent in 1 of 23 normal early adults analyzed for our background study. Another band found only in early adults, 0-69, was present in all six samples, while a third uniquely adult band, 0-75, was found in four of these second pupae, although it was light, as is normal in young adults. In addition, there were some highly stained bands which migrated like the major blood bands, A–D.

Figure 3, Chs. 1,2, illustrates the distinctive differences between the soluble cuticular proteins produced in response to treatment with ecdysterone as opposed to JH. The pupal bands 0-66 and 0-71 are very light, or are absent from the extracts of the ecdysterone-treated animal, but are prominent in the extracts of the JH-induced second pupa. Additional bands, 0-68, 0-69, unique to the adult, are distinct only in extracts from cuticles formed after ecdysterone injection.

Actinomycin D

Of 120 animals which were injected with AMD (at 0–4 h after the larval-pupal ecdysis) (1–3 µl of a 120 µM solution), 75, which received doses of 0.3 µg or more, died without secreting new cuticle on the abdomen. Eighty percent of the
Table 1. **Characteristics of intermediates formed after Actinomycin D treatment**

<table>
<thead>
<tr>
<th>Fig./Ch.</th>
<th>Estimated relative age</th>
<th>Lateral phalanges*</th>
<th>Cuticular sculpturing†</th>
<th>Protein banding‡</th>
<th>Presence of bands, A,B,C,D</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/3</td>
<td>Early</td>
<td>+</td>
<td>pp</td>
<td>P</td>
<td>+</td>
</tr>
<tr>
<td>2/4</td>
<td>Early</td>
<td>+</td>
<td>u</td>
<td>P+Ad</td>
<td>+</td>
</tr>
<tr>
<td>4/4</td>
<td>24 + h</td>
<td>+</td>
<td>u</td>
<td>P+Ad</td>
<td>+</td>
</tr>
<tr>
<td>6/4</td>
<td>Early</td>
<td>–</td>
<td>pp</td>
<td>Ad</td>
<td>+</td>
</tr>
<tr>
<td>2/1</td>
<td>24 + h</td>
<td>–</td>
<td>u</td>
<td>P+Ad</td>
<td>+</td>
</tr>
<tr>
<td>2/2</td>
<td>24 + h</td>
<td>–</td>
<td>u</td>
<td>Ad</td>
<td>+</td>
</tr>
<tr>
<td>6/6</td>
<td>4-20 h</td>
<td>–</td>
<td>up</td>
<td>Ad</td>
<td>+</td>
</tr>
<tr>
<td>6/5</td>
<td>4-20 h</td>
<td>–</td>
<td>up</td>
<td>Ad</td>
<td>+</td>
</tr>
</tbody>
</table>

* +, Present; −, absent.
† u, unsculptured; pp, patches of pupal cuticle in unsculptured cuticle; up, patches of unsculptured cuticle in adult-like cuticle.
‡ Ad, Adult specific proteins, 0·69, 0·75; P, early pupal bands 0·66, 0·71.

animals which received 0·2 or 0·25 μg of AMD formed a new abdominal cuticle between 10 and 13 days after injection. By the time the abdominal cuticle was formed, the new pronotum had begun to tan. The new cuticles were isolated on the first day that the old abdominal cuticle had obviously become thin due to digestion, except for one sample (Fig. 4, Ch. 4), which was aged an additional 24 h. Since these animals did not ecdyse spontaneously, their approximate ages were estimated by other criteria. One cue to approximate age was the extent of tanning of the pronotum compared to normal adults; thickness and flexibility of the abdominal cuticle were also considered. The results of these estimates are listed in Table 1.

Various pupal characters can be recognized on the abdomen. Only three animals had lateral phalanges and these lacked gin traps and bristles; urogomphi were also lacking. The abdomens of the other five animals were all adult-like except for the sculpturing of their cuticles. Some had unsculptured sternal cuticle (such smooth cuticle was not found in any untreated animal at any stage). In two individuals this cuticle had pupal patches, consisting of untanned cuticle with a somewhat rough surface bordered by smooth tanned cuticle. One animal had cuticle with adult sculpturing, interrupted by unsculptured patches (Table 1).

Extracts of cuticle from eight AMD-treated animals which had formed new cuticle with both pupal and adult morphological characteristics were analyzed. There was considerable qualitative and quantitative variation among these animals in banding patterns, but this variation was not related to the degree of retention of pupal characters (Table 1). All extracts had the high-molecular-weight bands A–D at concentrations exceeding those found in extracts of normal cuticles. Extracts from one animal which had formed small lateral phalanges with an unsculptured cuticle containing pupal patches showed a perfect early
pupal banding pattern (Fig. 6, Ch. 3). The soluble cuticular proteins from a second animal with lateral phalanges included bands 0·66, 0·71, and 0·77, which are found in early pupae, but also the uniquely adult bands 0·68 and 0·69 (Fig. 2, Ch. 4). The third animal with lateral phalanges also yielded a banding pattern with characteristics of both pupal and adult cuticle, namely pupal band 0·71 and adult band 0·75, but not bands 0·66, 0·68, and 0·69 (Fig. 4, Ch. 4).

Four additional animals lacked lateral phalanges and had predominantly un-sculptured cuticle. One had pupal patches in this cuticle, but its cuticular proteins had an adult banding pattern (Fig. 6, Ch. 4). Another had no morphological evidence of pupal cuticle, but its proteins contained both pupal and adult bands (Fig. 2, Ch. 1). The other two animals, plus one which had adult-like cuticle with unsculptured patches, had bands 0·69 and 0·75 which are characteristic of adult extracts taken 4–14 hr after ecdysis. In summary, the AMD-treated animals had
secreted cuticles whose soluble cuticular proteins formed patterns which ranged from the early pupal pattern, through pupal–adult intermediates, to normal adult patterns, and all had additional high-molecular-weight bands.

**Mitomycin C**

Mitomycin C also caused the formation of pupal–adult intermediates, or the pupae died without forming a new cuticle. Those which received 5–10 μg (0.5–1 μl of a 2-9 mM solution) showed no retention of pupal characteristics, while those injected with more than 15 μg died without forming new cuticle. Of those which received 10–15 μg, 11 died, 24 formed perfect adult abdominal cuticle, and 13 pupal–adult intermediates were formed. The latter were adult-like in the head and legs, while the pronota and abdomens were abnormal. Four animals retained small vestiges of lateral phalanges lacking all traces of gin traps or bristles, and urogomphi were uniformly absent. The ventral abdominal cuticles of these intermediate animals either were smooth, lacking the normal pitted appearance of the adult, or the pits were very shallow with extensive smooth areas between them. The color ranged from cream to a tan which was darker than that of normal 24 h adults. Without exception, all 13 animals whose cuticles were analyzed showed the adult banding pattern. Samples in Fig. 3, Chs. 3–5, were from animals sacrificed as soon as the new cuticle was formed. We also examined cuticular extracts from comparable animals which had been maintained for an additional 24 h at 28 °C.

All patterns were comparable to those obtained from adults 4–8 h after ecdysis, since bands 0-68 and 0-69 were detected as well as band 0-75. The bands which migrated with mobilities of the major blood bands (A–D) were not as deeply stained as were the ones from second pupae induced by JH or ecdysterone, or in the intermediates derived from AMD treatment; the intensity was closer to that in normal adult extracts. Band A was detected in only 3 of the mitomycin C-treated animals.

**DISCUSSION**

*Is reversal of metamorphosis of the exocuticle substantiated by the presence of the larval-specific protein?*

The soluble proteins from the exocuticle of second pupae induced by treatment with JH did not reflect the reversal of metamorphosis claimed for this part of the cuticle on the basis of the spacing of the lamellae (Caveney, 1970). The early, heavily stained, larval band 0-53 was absent from all treated animals examined, and the diagnostic protein banding pattern was distinctly pupal. While the lamellae of the exocuticle appear most like those of larval cuticle, Caveney (personal communication) now gives a more conservative interpretation to his morphological data and suggests that he may have observed a modification of normal pupal spacing.
Are the cuticular proteins of JH-induced second pupae identical to those of normal pupae?

Second pupae, formed in response to treatment of _Tenebrio_ with high levels of JH at the beginning of the pupal stage, were anatomically identical to normal pupae, but the cuticle was more transparent and within 24 h became very brittle. The banding pattern of the cuticular proteins extracted from JH-induced second pupae with soft cuticle was identical to that of normal pupae sacrificed immediately or 1 h after ecdysis. Thus, initially, the pupal proteins of the exocuticle were precisely duplicated.

However, the banding pattern of extracts taken from animals which had aged an additional 24 h was unlike that of normal pupae in two respects. The dominant early bands, which normally diminished in relative intensity, remained as major proteins in late second pupae. In addition, high-molecular-weight bands which migrate with the same mobility as the major blood bands were present in higher quantities than were seen at any time during the normal pupal state. While there is considerable evidence suggesting that proteins are transported from the blood into the cuticle (Fox, Seed & Mills, 1972; Koepp & Gilbert, 1973; Phillips & Loughton, 1976a, b), conclusive proof is lacking (Ruh & Willis, 1974). If the source of these high-molecular-weight proteins is the blood, their increased concentration suggests that their uptake is increased in epidermis which has been exposed to JH.

Study of isotopically labelled leucine incorporated into second pupae resulting from treatment with JH revealed that the second pupa repeats the synthetic sequence of the normal pupa. Labelling in the early experimental animals was like that in normal 2-4 h pupae, while the proteins extracted after isotope was injected into late animals were labelled like those of normal 48 h pupae. The ‘blood’ bands which contribute to the staining pattern of second pupae were not conspicuously labelled. Significantly, the ‘young’ pupal bands (R_p’s 0.66 and 0.71) that persist in old second pupae had not incorporated labelled leucine administered to second pupae that had been aged for 24 h. We thus conclude that the persistence of these early bands in late second pupae reflects diminished sclerotization rather than persistence of synthesis.

These studies thus give preliminary indications of two hitherto undocumented actions of JH – increase of epidermal permeability and decreased cuticular sclerotization. The former is intriguing in view of JH’s well-documented role in increasing vitellogenin accumulation in the oocytes of many adult insects (Bell & Barth, 1971). Yet it must be remembered that cuticles from animals treated with ecdysterone and actinomycin D also had ‘blood’ bands.
Do JH mimics cause secretion of the same soluble cuticular proteins as are found in JH-induced second pupae?

**Ecdysterone**

The abdomens of second pupae produced in response to high doses of ecdysterone were identical in morphology to those of second pupae produced following an injection of JH. Furthermore, the soluble cuticular proteins of very young second pupae produced by ecdysterone were like those of normal early pupae or JH-induced second pupae, so we can conclude that exocuticle formation is the same in normal pupae and ecdysterone-induced second pupae. But later, the systems diverge. The extracts from the ecdysterone-treated animals had bands which are unique to the early (0·68 and 0·69) and late (0·75) adult, while the pupal bands (0·56, 0·60, 0·66, and 0·77) that dominated the JH-induced second pupal pattern were absent or reduced in their staining intensities. The high-molecular-weight bands that migrate like the blood bands were even more intensely stained in these animals than in the JH-treated second pupae.

How do we interpret these results? First, the modification of permeability to blood proteins previously noted following JH treatment was present here, too; thus it is not a uniquely JH effect. Second, it appears that the pupal program for exocuticular proteins is used for a second time in these animals, but when the moment arrives for endocuticle deposition, they switch almost exclusively to the adult pattern. Caveney (1970) reported a similar finding in his studies of cuticular ultrastructure after JH treatment. He observed that the epidermal cells bordering a patch of second pupal cuticle first make pupal and then adult endocuticle.

Is it significant that the ecdysterone was injected in young pupae of *Tenebrio* before they had begun to secrete their own endocuticle? It would certainly be instructive to see if either JH or ecdysterone prevents the completion of the pupa's normal developmental program for endocuticle formation once it has begun. But it must be remembered that JH is fully effective in causing second pupae in the Cecropia silkmoth, even when injected into pupae which have been in diapause for over a year, long after completion of both their exo- and endocuticles. Conversely, ecdysterone fails to mimic JH in *Oncopeltus* even when administered immediately after ecdysis in last instar larvae, prior to initiation of endocuticle formation (Willis, unpublished observations). Given all this, it would be premature to speculate on the stability of *Tenebrio*'s messenger RNAs, or other types of stable information for exocuticle synthesis.

**Actinomycin D**

AMD is well known and widely used as an inhibitor of RNA synthesis, although it certainly has other actions as well (Leinwand & Ruddle, 1977). To date it has been reported as a mimic of JH only in *Tenebrio* (Chase, 1967, 1970; Socha & Sehnal, 1972). These findings are in accord with ours: only a small
proportion of treated pupae form second pupal abdomens, and metamorphosis of the anterior regions (head, thorax, and legs) occurs whenever any cuticle is formed. None of the animals used in our study had a perfect pupal abdomen, yet they all showed various degrees of retention of pupal characters. In one of the eight abdomens whose cuticles were analyzed, AMD mimicked JH in producing an early pupal banding pattern of soluble cuticular proteins; however, four had adult patterns, and three had both pupal and adult cuticular proteins. The simplest explanation of these findings is that AMD may only temporarily suppress the adult program. Andersen et al. (1973) found that the amino acid composition of hydrolysates of abdominal cuticle from AMD-treated animals resulted in glycine/alanine ratios and apolar amino acid contents similar to those of the adult, while the ratios of serine to the sum of glutamic acid plus glutamine were larval–pupal in nature. This suggests that there was a mixture of pupal and adult proteins in these cuticles. The effect of AMD would then be in accord with the sequential synthesis of pupal and adult proteins which we have documented in the case of ecdysterone.

Mitomycin C

While mitomycin C was capable of causing re-formation of pupal characters, including lateral phalanges, and formation of intermediate characters, including patches of pupal or unsculptured cuticle, the cuticular banding patterns consistently included only the adult proteins. Either mitomycin C did not cause the production and/or deposition of pupal proteins, or these proteins constituted too small a portion of the total protein extracted to be detectable. The latter is more likely, considering the pupal morphology. It was surprising to find that inhibition of DNA synthesis was effective in preserving an area destined for cell death – the lateral phalanges. Unfortunately, it is not known whether DNA synthesis precedes this involution. The blood-like bands were very lightly stained in all of the mitomycin C-treated animals, similar to those of normal adults.

Interpretation of the action of mimics

These studies reveal that ecdysterone and actinomycin D are effective in causing Tenebrio to secrete a new abdominal exocuticle containing the proteins which characterize normal pupal exocuticles. Thus, it is not surprising that the morphology of animals treated with these mimics was pupal, for it is the superficial layer of the cuticle which creates its external form and texture. Neither of these mimics suppressed secretion of adult endocuticular proteins. The latter finding suggests that these mimics work at a different level from that of JH, or that they have ceased to be effective, possibly by being metabolized, by the time endocuticular secretion begins. We did not examine whether the pupal exocuticular proteins found in animals treated with these mimics were synthesized de novo after their administration, nor did we determine the time of synthesis of
the mRNA templates for cuticular proteins. Since the ability of ecdysterone, actinomycin D and a host of other agents to mimic JH is peculiar to Tenebrio (See Introduction), detailed analyses of the action of these mimics at the level of transcription or translation are more apt to elucidate the peculiarities of cuticle formation in Tenebrio than to reveal details of the general mode of action of juvenile hormone.

We thank Prof. Stanley Friedman for his cogent comments throughout the course of this work and David P. Eisenman for his help in the preparation of the manuscript. We also thank Prof. G. R. Wyatt for comments on the manuscript. This paper is based upon a thesis submitted by P.E.R. for the doctoral degree at the University of Illinois at Urbana-Champaign. P.E.R. received support from a Cell Biology Training Grant to the School of Life Sciences. Additional support came from grant AG-00248 from the National Institutes of Health.

REFERENCES


(Received 31 July 1979, revised 19 October 1979)