ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS
Vol. 278, No. 2, May 1, pp. 461–466, 1990

Regulation of Juvenile Hormone Esterase Gene Expression in the Tobacco Budworm (*Heliothis virescens*)[1]

Victor J. Wroblewski,[2] Lawrence G. Harshman, Terry N. Hanzlik, and Bruce D. Hammock[3]
Departments of Entomology and Environmental Toxicology, University of California, Davis, California 95616

Received August 28, 1989, and in revised form December 29, 1989

The tissue distribution, developmental control, and induction of juvenile hormone esterase (JHE) mRNA was examined in *Heliothis virescens* using an 800-base pair fragment of a JHE cDNA clone. Northern hybridization analysis of poly(A)$^+$RNA from fat body and integument of fifth stadium larvae indicated the presence of a single JHE mRNA species having an estimated length of 3 kilobases. On Day 2 of the fifth stadium (L5D2), basal JHE mRNA levels were 3-fold higher in the integument than the fat body, which correlated with the higher specific activity of the enzyme in the integument at this time. However, JHE mRNA levels in the fat body on Day 4 of the fifth stadium were 9-fold higher than on Day 2, while mRNA levels in the integument remained the same. This endogenous increase in JHE mRNA and activity in the fat body occurred at the time of peak hemolymph JHE activity. JHE mRNA was not detected in third stadium larvae which have very low levels of JHE activity. Treatment of L5D2 larvae with the juvenile hormone mimic epofenonane resulted in a 7- and 14-fold increase in the level of JHE mRNA in the integument and fat body, respectively. The mRNA induced in both tissues was of the same estimated length as the constitutively expressed message. The data indicate that the developmental regulation and induction of JHE can occur at the level of mRNA. There is evidence that the fat body secretes more JHE than does the integument and could be the major source of hemolymph JHE. © 1990 Academic Press, Inc.

Insect metamorphosis is known to be regulated by at least two classes of epithelial hormones, the ecdysones and the juvenile hormones (1, 2). Juvenile hormone (JH)[4] is responsible for the maintenance of larval characteristics and its levels are intimately related with the determination of the nature of a molt (3). It has become increasingly evident that in addition to decreased biosynthesis, the degradation of JH is important for controlling the circulating hormone titer (4). In the lepidopteran larvae examined, ester hydrolysis by juvenile hormone esterase (JHE) is a major route of JH metabolism (5). Earlier investigations have indicated that the function of JHE is critical for proper insect development, and that the appearance of this low abundance, very active enzyme in the hemolymph is temporally controlled (6, 7). Increase in the level of JHE activity during the final stadium in larval development is one of the first known biochemical events in a series of changes that lead to pupation. Therefore, studying the regulation of JHE expression can provide a more basic understanding of insect metamorphosis and may allow identification of additional factors which are fundamental to these developmental changes.

The biochemistry of juvenile hormone esterase(s) has been well studied in several lepidopteran species including the important agricultural pest *Heliothis virescens* (tobacco budworm) (8–10). Although the enzyme(s) from the different species appear to differ in some respects, there is a consistent pattern of JHE titer in most lepidopteran larvae (11). Nevertheless, the activity of the enzyme can be increased by treatment with juvenoids during the ultimate larval stadium of several lepidopteran species (12, 13). These findings suggest that control of JHE expression may be more important than differences in the nature of the enzyme. The basis for

[1] This research was supported by NSF (DCB-8518697), USDA (85-CRCR-1-11715), Burroughs Wellcome Toxicology Scholar Award (B.D.H.), and NIEHS Postdoctoral Fellowship ES05426 (V.J.W.).

[2] Present address: Department of Drug Metabolism and Disposition, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285.

[3] To whom reprint requests should be addressed.

[4] Abbreviations used: JH, juvenile hormone; JHE, juvenile hormone esterase; 10× SSC, 1.5 M NaCl, 0.15 M Na citrate (pH 7).

0003-9861/90 $3.00
Copyright © 1990 by Academic Press, Inc.
All rights of reproduction in any form reserved.

the developmental changes in JHE expression or the 'induction' of JHE activity is not known. Processing of precursor proteins or, alternatively, their rate of secretion, degradation, or synthesis could be altered. An altered rate of JHE abundance could, in turn, be a consequence of a change in the synthesis or turnover of JHE mRNA. The objectives of the present work were to study the tissue distribution of JHE mRNA and to determine whether mRNA levels are altered during development and after juvenoid treatment in *H. virescens*.

MATERIALS AND METHODS

Animals. Larvae of *H. virescens* (tobacco budworm) were provided by Dow Chemical Co. (Walnut Creek, CA), and reared on an artificial diet at 27°C with a 16L:8D photoperiod. Animals were synchronized by grouping larvae which ecdysed from the 4th to 5th stadium within an 8-h period and discarding animals which were in the upper or lower 10% of the body weight sample on Day 1 of the fifth stadium. The experiments described were performed using groups of 20 to 80 animals.

JHE cDNA probe. A 3000-base pair cDNA of *H. virescens* fat body JHE has been cloned and sequenced in this laboratory (14). An 800-base pair fragment of this cDNA insert was obtained by restriction digestion using BAM H1. This fragment was subcloned into M13, sequenced using ^{32}P and the dideoxy chain termination method (15), and found to correspond to the coding region of JHE representing the first 30 N-terminal amino acids of the purified hemolymph enzyme from *H. virescens*. The BAM H1 fragment of the JHE cDNA was separated from the plasmid on a 0.8% agarose gel and electroeluted from the gel. Random priming was used to label the fragment with ^{32}P.

Analysis of RNA. Total RNA was isolated from larvae or larval tissues by a guanidinium thiocyanate extraction procedure (16). Poly(A)$^+$RNA was prepared by oligo(dT)cellulose chromatography (17) and accounted for 2.5–3% of the total isolated RNA. The integrity of poly(A)$^+$RNA was assured by analysis of translation products in a rabbit reticulocyte lysate (18). For Northern hydridization analysis, poly(A)$^+$RNA was separated by electrophoresis in 1% agarose gels containing 2.2 M formaldehyde and transferred to nitrocellulose filters (19). Post-transfer gels were routinely stained with ethidium bromide to ensure quantitative transfer of RNA. Poly(A)$^+$RNA was then hybridized to the ^{32}P-labeled BAM H1 JHE fragment. Filters were hybridized for 24 h with the cDNA probe (2×10^8 cpm/μg, 1×10^6 cpm/ml) in a solution of 50% formamide, 0.2% polyvinyl-pyrrolidone (MW 40,000), 0.2% bovine serum albumin, 0.2% ficoll (MW 400,000), 0.05 M Tris-HCl (pH 7.5), 1 M NaCl, 0.1% Na pyrophosphate, 1% $NaDodSO_4$, 10% dextran sulfate, and denatured salmon sperm DNA (100 μg/ml) at 42°C. Filters were washed twice for 30 min each under three different conditions: 2× SSC at room temperature, 2× SSC with 1% $NaDodSO_4$ at 65°C, and 0.1× SSC at room temperature. Autoradiography was at room temperature with exposure times of 18–36 h. Transcript size was estimated from the mobility of RNA molecular size markers (Bethesda Research Laboratories, BRL, Gaithersburg, MD). For quantitative analysis, RNA was denatured by heating at 68°C for 15 min in 10× SSC with 2.2 M formaldehyde and bound to nitrocellulose filters using a slot-blot apparatus (BRL). Hybridization conditions were as described above. The amount of JHE mRNA in slot-blots was quantified by laser densitometry.

Levels of JHE mRNA in development. For the study of developmental changes, RNA was isolated from whole body homogenates of third stadium and Day 4–5 fifth stadium larva. RNA was also isolated from fat body and integument (cuticle and epidermis) of fifth stadium larva at 2000 h of Day 2 and 1500–2000 h of Day 4, times when hemolymph JHE activity are relatively low (Day 2) and peaking (Day 4). In studies requiring tissue dissection, the tissues were quickly frozen in a dry ice/ethanol bath and stored in liquid N_2 prior to RNA isolation.

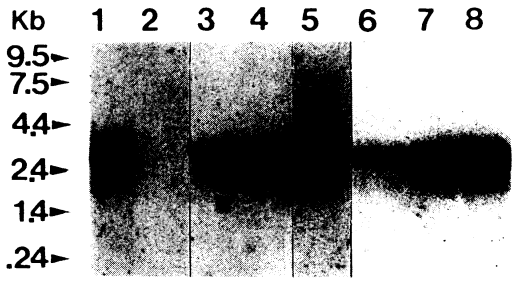

FIG. 1. Northern blot analysis of JHE mRNA in poly(A)$^+$RNA of *H. virescens*. Poly(A)$^+$RNA (5 μg per lane) was separated by electrophoresis in 1% agarose gels containing 2.2 M formaldehyde, transferred to nitrocellulose, and hybridized with cDNA labeled by random priming. Position of RNA size markers are indicated by arrows. Lane 1, fifth stadium (Day 4–5) larvae; lane 2, third stadium larvae; lane 3, fifth stadium Day 2 (L5D2) integument; lane 4, L5D4 integument; lane 5, L5D2 integument (after 9 μg/epofenonane/larva, 2×); lane 6, L5D2 fat body; lane 7, L5D4 fat body; lane 8, L5D2 fat body (after 9 μg epofenonane/larva, 2×). This figure is a composite of reactions done at different times using the same conditions. All experiments were repeated several times with different preparations.

Induction of JHE mRNA. RNA was isolated from the fat body and integument of fifth stadium larvae at 2000 h of Day 2, after topical application of epofenonane (9 or 50 μg/larva) at 200 h of Day 1 and 800 h of Day 2. JHE mRNA in the developmental and induction experiments was qualitatively and quantitatively evaluated by Northern and slot-blot hybridizations.

Species comparison of JHE mRNA. Poly(A)$^+$RNA was also isolated from the fat body of fifth stadium (Day 4–5) *H. zea* and analyzed by blot hybridization with the cDNA probe to *H. virescens* JHE as described above. These heliothine species are closely related, but biochemical evidence indicates the presence of one form of hemolymph JHE in *H. virescens* and two forms in *H. zea* (10).

Analysis of JHE activity and protein. Hemolymph and tissues from the same groups of animals used for RNA isolation were assayed for JHE activity with a partition method (20) modified for use in tissues containing several hydrolyzing enzyme systems by performing assays in the presence and absence of the JHE inhibitor 3-octylthio-1,1,1-trifluoropropan-2-one at 10^{-4} M (21). Tissues were homogenized with a Brinkman Polytron and activities measured after pelleting cellular debris at 2000*g* for 5 min. Relative levels of JHE protein in the tissue homogenates were also evaluated by Western blotting (22). Proteins were fractionated by $NaDodSO_4$-PAGE using a 10% gel (1.5 mm thick), transferred to nitrocellulose, and detected using a polyclonal antibody to hemolymph JHE of *H. virescens* purified by affinity chromatography (10). Total protein levels were determined by the method of Bradford (23).

RESULTS

Northern blot analysis of JHE mRNA transcript. Hybridization to a ^{32}P-labeled cDNA probe allowed a comparison of JHE mRNA transcript(s) present in two larval stages of *H. virescens*, in tissues during periods of low and high JHE activity in the fifth stadium and after treatment with the JH mimic, epofenonane (Fig. 1). A single JHE mRNA species having an estimated size

TABLE I

Juvenile Hormone Esterase Activity in *H. virescens*[a]

	Integument	Fat body	Gut	Hemolymph
	(nmol JH-III hydrolyzed/min/mg)[b]			
L5D2[c]	0.30 ± 0.06	0.13 ± 0.04	0.03 ± 0.02	0.61 ± 0.11 (27 ± 4.5)[d]
L5D4	0.43 ± 0.11	0.84 ± 0.28	0.04 ± 0.02	5.35 ± 0.98 (319 ± 63)[d]
L5D2 (ep 9)[e]	5.31 ± 2.45	0.64 ± 0.21	0.03 ± 0.01	3.12± 0.88 (138 ± 36)[d]
L5D2 (ep 50)[e]	4.74 ± 2.66	0.71 ± 0.37	0.04 ± 0.03	3.52 ± 1.09 (155 ± 53)[d]
	Larval homogenates			
3rd stadium	0.03 ± 0.01			
5th stadium	2.38 ± 0.95			

[a] JHE activity was determined using a partition assay (21) in the presence and absence of 10^{-4} M 3-octylthio-1,1,1-trifluoropropan-2-one.

[b] Data are the means ± SD of 3 or 4 groups of animals (15–40 animals/group).

[c] Day 2 of fifth larval instar.

[d] Nanomoles JH-III hydrolyzed/min/ml.

[e] After treatment with two doses of epofenonane (9 or 50 μg/larva).

of 3.0 kb was detected in all poly(A)$^+$RNA samples examined except those from third stadium larvae. The relative JHE mRNA signal intensity in the preparations corresponds to differences in JHE activity (Table I). Third stadium larvae possess very low levels of JHE activity and no signal was detected in poly(A)$^+$RNA from this source even after prolonged exposure times of the autoradiograph. Comparison of mRNA from two heliothine species indicated the presence of a single transcript in *H. zea* having the same length as the transcript in *H. virescens* (Fig. 2). This is interesting since the multiple forms of hemolymph JHE reported to be present in *H. zea* (10) are not apparent as multiple transcripts. However, one of these two forms is glycosylated. The cDNA probe did not produce a hybridization signal in poly(A)$^+$RNA from mouse liver, indicating that under the hybridization conditions the probe does not recognize unrelated sequences (not shown).

Developmental changes in JHE mRNA. The specific activity of JHE and the level of its message in the fat body and integument differed considerably when measured on Day 2 (low hemolymph activity) or Day 4 (peak activity) of the fifth stadium. On Day 2, integument contained the highest JHE activity (0.3 nmol/min/mg), which was 2.3-fold higher than the activity in the fat body (Table I). This difference was reflected in the levels of JHE mRNA detected in these tissues by slot-blot analysis. JHE mRNA levels were 3-fold higher on Day 2 in the integument than in the fat body. However, from Day 2 to Day 4, the specific activity of JHE increased by 6.5-fold in the fat body (0.84 nmoles/min/mg) but was unchanged in the integument. Similarly, the levels of JHE mRNA in the fat body during this time increased by 9-fold, reflecting the increase in activity in this tissue and hemolymph, while mRNA levels in the integument were similar on Day 2 and Day 4 (Fig. 3). Gut contained very low levels of JHE activity on Day 2 and Day 4.

Induction of JHE mRNA by epofenonane. Treatment of L5D2 larvae with epofenonane (9 μg/larva, 2×) resulted in a 4.8-, 5-, and 18-fold increase in the activity of JHE in the hemolymph, fat body, and integument, respectively, 12 h after the second application (Table I). Administration of a higher dose of epofenonane (50 μg/larva, 2×) did not produce a further elevation of JHE activity. Also, the increases after 12 h were higher than those observed when the tissues were assayed 24 h after the second dose (data not shown). Treatment with epofenonane (9 μg/larva, 2×) also resulted in an increase in the level of JHE protein in the hemolymph, fat body, and integument of L5D2 as determined by reaction with antibodies to purified hemolymph JHE of *H. virescens* (Fig. 4). Due to the extended reaction necessary to maximize detection of this very low abundance enzyme, other proteins also showed a reaction to a much lesser extent. However, the major signal had approximately the same molecular weight as the purified hemolymph enzyme. There is an indication that the hemolymph enzyme is

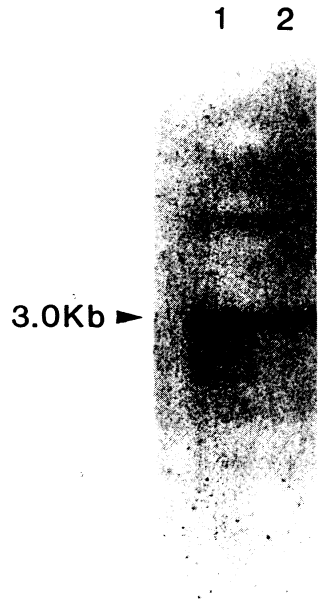

FIG. 2. Comparison of JHE mRNA in fat body from fifth stadium (Day 4–5) heliothine larva. Poly(A)$^+$RNA (2.5 μg/lane) was blot hybridized as described in Fig. 1. The size of the JHE transcript was estimated using RNA molecular weight standards. Lane 1, *H. virescens* fat body; lane 2, *H. zea* fat body.

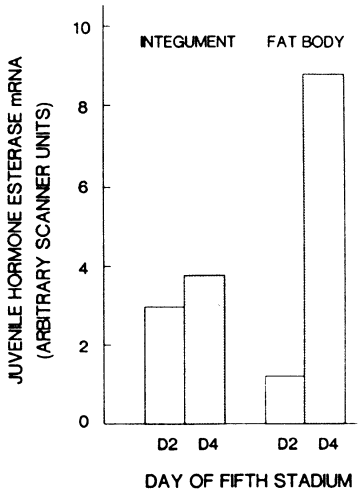

FIG. 3. Developmental changes in JHE mRNA levels in tissues of *H. virescens*. Poly(A)$^+$RNA was isolated from the fat body and integument during the fifth stadium on Day 2 (L5D2) or Day 4 (L5D4). The RNA was denatured and 100- and 200-ng samples applied to nitrocellulose filters with a slot-blot apparatus. Filters were hybridized with a cDNA probe labeled with ^{32}P and autoradiographic signal intensity quantified by laser densitometry. Results are expressed in arbitrary scanner units with values representing the mean from two groups of animals for each day in development (40–80 animals/group).

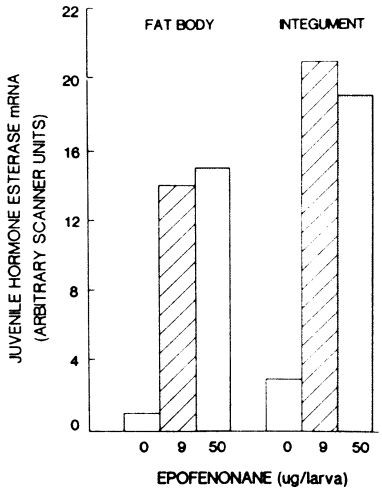

FIG. 5. Induction of JHE mRNA levels by epofenonane treatment. Poly(A)$^+$RNA was isolated from the fat body and integument of L5D2 larvae before and after treatment with two doses of epofenonane (9 or 50 μg/larva). JHE mRNA was quantified by laser-densitometry of the slot-blot hybridization as in Fig. 3. Data are expressed in arbitrary scanner units with values representing the mean of two groups of animals for each treatment (40–80 animals/group).

slightly smaller (Fig. 4, lanes 2 and 3), which could be a function of processing. JHE mRNA levels were also elevated in both the fat body and the integument, with no difference observed between doses of 9 and 50 μg/larva (Fig. 5). These increases (7- and 14-fold for the integument and fat body), however, did not directly correspond to the magnitude of the changes in JHE activity. JHE activity was higher in integument and lower in fat body than expected from the changes in mRNA (Fig. 5, Table I).

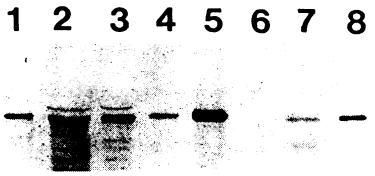

FIG. 4. Induction of JHE protein levels in tissues of *H. virescens*. Hemolymph, fat body, and integument homogenates were obtained from L5D2 larvae before and after treatment with two doses of epofenonane (9 μg/larva). Proteins (150 μg/lane) were fractionated by NaDodSO$_4$-PAGE on a 10% gel (1.5 mm thick), electrophoretically transferred to nitrocellulose, and JHE was immunochemically detected using polyclonal antibodies to purified hemolymph JHE from *H. virescens*. Lanes 1 and 8, 250 ng of purified hemolymph JHE from *H. virescens*; lane 2, control hemolymph; lane 3, treated hemolymph; lane 4, control integument; lane 5, treated integument; lane 6, control fat body; lane 7, treated fat body.

DISCUSSION

The degradation of JH by juvenile hormone esterase is believed to be important in modulating hormone titer at critical periods in larval insect development. Since the level of JHE activity is thought to be intimately involved with proper development of lepidopteran larvae (4–6), it seems reasonable that its expression would be under tight regulatory control. However, the basis for the changes in the level of JHE activity during development, the tissue distribution, and source of the enzyme in the hemolymph have not been clearly defined. We addressed these questions by examining JHE control at the mRNA level with the use of a cDNA probe.

The observation that JHE activity in the fat body increases at times of higher hemolymph JHE titer has led to the assumption the protein is synthesized and secreted by the fat body *in vivo* (7, 24). Our work indicates that the JHE gene is constitutively expressed in both the fat body and the integument during the fifth stadium. However, the regulation of JHE expression during this developmental period in *H. virescens* is apparently tissue specific. In the present study there was a differential increase in constitutive JHE expression in the fat body and integument from Day 2 to Day 4 of the fifth stadium, a period when hemolymph titer of the enzyme is increasing. During this period, JHE mRNA in the fat body increased 9-fold, while the level of JHE mRNA in the integument remained constant (Fig. 3). These temporal changes in JHE mRNA levels during the ultimate larval stadium suggest that JHE is synthesized and secreted by

the fat body, while there is a constant expression of the enzyme in a prime JH target tissue, the integument. At this juncture, the factors which modulate the developmental expression JHE have not been well characterized. Studies on the induction of JHE have led to the hypothesis that JH, either alone or in concert with neural–humoral factors from the head, acts to control JHE expression (12, 13, 25). Our work also indicates that exogenously applied juvenoids can regulate the level of JHE mRNA in both the fat body and integument, but suggest that there is no clear relationship between JHE expression and JH titer during larval development (26). A factor which may influence the developmental pattern of JHE expression is the tissue-specific expression of transcriptional activators other than JH. Such a mechanism has been shown to regulate the developmental expression of several mammalian genes (27–29). Alternatively, the different expression patterns in the fat body and integument may be due to factors that influence the steady state mRNA level. Candidates include promoter elements which regulate the rate of transcription or sequences within the RNA transcript that influence mRNA processing or stability of the mature transcript in the cytoplasm.

The application of JH mimics to fifth stadium lepidopteran larvae has been shown to result in an "induction" of hemolymph JHE activity (12, 13). This response has not been well documented in tissues other than the hemolymph and fat body. Earlier studies which provided support for this observation, however, mistakenly assumed that JHE activity could be accurately monitored with the use of the general carboxylesterase substrate α-naphthyl acetate, and left unestablished the mechanism underlying the increase in activity (30, 31). As in other lepidopteran species, treatment with epofenonane caused an elevation of JHE activity in the hemolymph and fat body of L5D2 *H. virescens* (Table 1). Induction of JHE activity also occurred in the integument. Significant levels of JHE activity have been reported to be present in the integument of other lepidoptera, but this is the first indication that the activity is inducible in this tissue (24, 32). The evidence presented demonstrates that the increase in tissue activity is due at least in part to an increase in mRNA levels and that the induced message is apparently the same as that found endogenously (Figs. 1 and 5). It is uncertain whether the increased mRNA levels are solely the result of increased transcriptional activity or if post-transcriptional effects mediated by the hormone are also involved. The results from this paper indicate that the levels of JHE mRNA and activity in the integument are higher than the fat body on L5D2 both endogenously and after induction. However, the relative increase in mRNA levels after epofenonane treatment is greater in the fat body. The induced changes in the level of JHE mRNA in the fat body and integument do not correlate well with changes in the activity of the enzyme in these tissues. However, the data are consistent with the enzyme being retained in the integument and secreted from the fat body. This may result from tissue differences in the rates of translation, post-translational processing, or secretion of JHE which may perturb the proportionality between mRNA and protein levels. In this regard, the sequence of the JHE cDNA from *H. virescens* indicates the presence of a signal peptide on the initial translation product (14). As has been suggested for other proteins (33) the signal peptide probably serves a regulatory function with differences in this region or in the cleavage of this region related to differential secretion of the enzyme from tissues.

Biochemical studies have indicated that there are one or possibly two very similar forms of JHE present in the hemolymph of *H. virescens* (10, 14). The present work provides evidence that a single JHE mRNA species exists both endogenously and after induction in the fat body and integument of *H. virescens*. These mRNAs are indistinguishable on Northern blots (Fig. 1). Similarly, a comparison of fat body mRNA from *H. virescens* and *H. zea* indicated the presence of one transcript in *H. zea* having the same length as the message from *H. virescens* (Fig. 2). The detection of *H. zea* JHE mRNA with the cDNA probe to the message from *H. virescens* indicates a high degree of homology between the JHE transcript in these two heliothine species. In contrast under high stringency hybridization conditions, no hybridization signal was observed when the probe was used to examine poly(A)$^+$RNA samples from the fat body of another lepidopteran species (*Manduca sexta,* not shown). Purified hemolymph JHE from *H. zea* has been shown to exist as two forms with one form being glycosylated (10). The results of the present work suggest that the two enzymes in *H. zea* could arise from post-transcriptional modification.

In summary, JHE mRNA is expressed in both the fat body and the integument of *H. virescens* larvae. It appears that changes in the levels of expression of this mRNA are tissue specific, and under complex regulatory control. Further studies of JHE expression may allow identification of factors which are fundamental to the regulation of gene expression during insect development. Advances in the understanding of insect metamorphosis may then lead to the design of specific compounds for insect control based upon disruption of this developmental process.

ACKNOWLEDGMENTS

We thank Janet Stuckey of Dow Chemical Co. for supplying *H. virescens* and *H. zea*. Thanks also go to Eva Kurz, Genetics Department (UCD), for many helpful discussions.

REFERENCES

1. Schneiderman, H. A., and Gilbert, L. I. (1964) *Science* **143**, 325–333.

2. Sehnal, F. (1985) *in* Comprehensive Insect Physiology, Biochemistry and Pharmacology (Kerkut, G. A., and Gilbert, L. I., Eds.), Vol. 2, pp. 17–102, Pergamon Press, Oxford.
3. Willis, J. H. (1974) *Annu. Rev. Entomol.* **19,** 97–115.
4. Hammock, B. D., Abdel-Aal, Y. A. I., Hanzlik, T., Jones, D., Jones, G., Roe, R. M., Rudnicka, M., Sparks, T. C., and Wing, K. D. (1984) *in* Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones (Hoffman, J., and Porchet, M., Eds.), Springer-Verlag, Berlin/Heidelberg.
5. Hammock, B. D. (1985) *in* Comprehensive Insect Physiology, Biochemistry and Pharmacology (Kerkut, G. A., and Gilbert, L. I., Eds.), Vol. 12, pp. 431–472, Pergamon Press, Oxford.
6. Kramer, S. J., Weiten, M., and De Kort, C. A. D. (1977) *Insect Biochem.* **7,** 231–236.
7. Wing, K. K., Sparks, T. C., Lovell, V. M., Levinson, S. O., and Hammock, B. D. (1981) *Insect Biochem.* **11,** 473–485.
8. Abdel-Aal, Y. A. I., and Hammock, B. D. (1986) *Science* **233,** 1073–1076.
9. Hanzlik, T. N., and Hammock, B. D. (1987) *J. Biol. Chem.* **262,** 13,584–13,591.
10. Abdel-Aal, Y. A. I., Hanzlik, T. N., Hammock, B. D., Harshman, L. G., and Prestwich, G. (1988) *Comp. Biochem. Physiol.* **90B,** 117–124.
11. Jones, G., Jones, D., Wing, K. D., Rudnicka, M., and Hammock, B. D. (1981) *J. Comp. Physiol.* **148,** 1–10.
12. Jones, G., and Hammock, B. D. (1983) *J. Insect Physiol.* **29,** 471–475.
13. Jones, G., Jones, D., and Hiremath, S. (1987) *Insect Biochem.* **17,** 897 904.
14. Hanzlik, T. N., Abdel-Aal, Y. A. I., Harshman, L. G., and Hammock, B. D. (1989) *J. Biol. Chem.* **264,** 12,419–12,425.
15. Sanger, F., Nicklen, F., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
17. Aviv, H., and Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
18. Jackson, R. J., and Hunt, T. (1983) *in* Methods in Enzymology (Fleischer, S., and Fleischer, B., Eds.), Vol. 96, pp. 50–74, Academic Press, San Diego.
19. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
20. Hammock, B. D., and Roe, R. M. (1985) *in* Methods in Enzymology (Law, J. H., and Rilling, H. C., Eds.), Vol. 111, pp. 487–494, Academic Press, San Diego.
21. Share, M. R., and Roe, R. M. (1988) *Anal. Biochem.* **169,** 81–88.
22. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
23. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
24. Vince, R. K., and Gilbert, L. I. (1977) *Insect Biochem.* **7,** 115–120.
25. Jones, G., Wing, K. D., Jones, D., and Hammock, B. D. (1981) *J. Insect Physiol.* **27,** 85–91.
26. Schooley, D. A., Judy, K. J., Bergot, B. J., Hall, M. S., and Jennings, R. C. (1976) *in* The Juvenile Hormones (Gilbert, L. I., Ed.), pp. 101–117, Plenum Press, New York.
27. Brand, S. J., and Fuller, P. J. (1988) *J. Biol. Chem.* **263,** 5341–5347.
28. Logsdon, C. D., Perot, K. J., and McDonald, A. R. (1987) *J. Biol. Chem.* **262,** 15,765–15,769.
29. Boulet, A. M., Erwin, C. R., and Rutter, W. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3599–3603.
30. Whitmore, D., Whitmore, E., and Gilbert, L. I. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1592–1595.
31. Whitmore, D., Gilbert, L., and Ittycheriah, P. I. (1974) *Mol. Cell. Endocr.* **1,** 37–54.
32. Mitsui, T., Riddiford, L. M., and Bellamy, G. (1979) *Insect Biochem.* **9,** 637–643.
33. Briggs, M. S., and Gierasch, L. M. (1986) *in* Advances in Protein Chemistry (Anfinson, C. B., Edsall, J. T., and Richards, F. M., Eds.), Vol. 38, pp. 109–180, Academic Press, San Diego.