International Journal of Invertebrate Reproduction and Development, 12 (1987) 57–72 57
Balaban, Philadelphia/Rehovot

Characterization of the Juvenile Hormone Esterases During Embryogenesis of the House Cricket, *Acheta domesticus*

R. Michael Roe[1], Catherine L. Crawford[1], Craig W. Clifford[2], J.P. Woodring[3], Thomas C. Sparks[3], and Bruce D. Hammock[4]

[1] *Department of Entomology, Box 7613, North Carolina State University, Raleigh, NC 27695-7613,* [2] *Division of Natural Sciences and Mathematics, Northeastern State University, Tahlequah, OK 74464,* [3] *Department of Entomology, Louisiana Agriculture Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, and* [4] *Department of Entomology University of California, Davis, CA 95616, U.S.A.*

Received 11 October 1986; Accepted revised 9 February 1987

Summary

Juvenile hormone (JH) and α-naphthyl acetate (α-NA) esterase activity was measured on a daily basis during embryogenesis of the house cricket, *Acheta domesticus*. In eggs dissected from the lateral oviducts and embryos through blastokinesis, there were elevated levels of nonspecific JH esterase activity. The JH esterase activity could not be resolved from the α-NA esterase activity by gel filtration chromatography and the metabolism of both substrates was inhibited equally by *0,0*-diisopropyl phosphorofluoridate (DFP). From blastokinesis through egg hatch, the JH esterase activity was maintained at relatively low levels and was resolved from the α-NA esterase activity by gel filtration. The α-NA esterase activity was inhibited by DFP while the JH esterase activity was relatively unaffected. Low JH titers in eggs must be maintained through blastokinesis for normal development. Elevated JH esterase activity in eggs during this period appears to have a functional role in the metabolism of maternal JH in the egg.

0168-8170/87/$03.50

house cricket, *Acheta domesticus*, juvenile hormone esterase, embryogenesis

Introduction

Research on the regulatory role of juvenile hormone (JH) during insect embryogenesis has focused on the effects of topical applications of JH, JH mimics and anti-JH's onto adults and developing embryos, and the direct measurement of the JH titer. Sláma and Williams (1966) first discovered that exposure of eggs from the European bug, *Pyrrhocoris apterus*, to juvabione (Bowers et al., 1966) prevented hatching. A number of studies since this time have reported that JH, JH mimics and anti-JH's when applied to adults and eggs at varying times of oogenesis and embryogenesis can block the egg-embryonic-larval transformations, as well as normal larval development and metamorphosis (Sláma and Williams, 1966; Novak, 1969; Riddiford, 1971; Riddiford, 1972; Riddiford and Truman, 1972; Sbrenna-Micciarelli, 1977; Injeyan et al., 1979; Dorn, 1982; Hoffman and Lagueux, 1985). The age- ar dose-dependency of these pharmacological effects indicate a functional role for JH during embryogenesis. This is further supported by measurements of the JH titer. It appears in the migratory locust, *Locusta migratoria*, (Temin et al., 1986) that declining, relatively low levels of JH (probably of maternal origin) occurs through the time of blastokinesis. A titer increase occurs after blastokinesis and JH declines again to low levels by the time of hatching. The declines in JH titer both early and late in embryogenesis suggest a role for JH metabolism in the regulation of embryo development. No JH was found prior to dorsal closure in the cockroach, *Nauphoeta cinerea* but subsequent changes in the JH titer were similar to the locust (Imboden et al., 1978; Lanzrein et al., 1984).

There are a number of potential routes for JH inactivation but ester hydrolysis and epoxide hydration appear to be the primary routes of degradation in insects previously studied (Hammock, 1985). The appearance of JH esterase and epoxide hydrolase activity at specific times of development suggest that JH titer is regulated by a balance between its rate of synthesis and degradation. The selective inhibition of JH esterase *in vivo* maintained abnormally high JH levels and delayed normal last instar development in larval cabbage loopers, *Trichoplusia ni*, providing direct evidence of the functional role of degradation (Prestwich et al., 1984). JH degradation was studied in a number of insect species during larval, pupal and adult development (Hammock, 1985) with no attention given to embryogenesis. Therefore, the goal of thi

study is to determine the route(s) of JH metabolism and its role during embryonic development in the house cricket, *Acheta domesticus.*

Materials and Methods

Enzyme preparation

House crickets, *Acheta domesticus*, were reared on Purina® Cricket Chow at 30± 1°C on a 12L:12D photoregime (Clifford et al., 1977). Day 0 eggs were dissected from the lateral oviducts of females of varying age collected at random from the breeding colony. A 100×15 mm plastic petri dish filled with wet sand was placed into the breeding colony as an ovipositional substrate. Egg dishes were replaced daily and incubated at 35± 1°C (12L:12D) in moisture-tight containers. Eggs were oviposited into the sand continuously throughout the photophase and scotophase and therefore were 0 to 1 day old when transferred to the 35°C incubator. Eggs incubated for 0 to 8 days in one day increments were then separated from the sand by floating the eggs in water, were washed several times, and air-dried. Eggs were weighed and frozen at -85°C until needed for assay. The freeze/thaw cycle had no effect on the metabolic activity being measured in this study. Eggs were homogenized with a Brinkmann Polytron at the rate of 1 mg wet weight per 50μl of sodium phosphate buffer (I=0.2 M, pH 7.4, 0.01% phenylthiourea (PTH), 10% sucrose) for the studies that follow.

Assays

Metabolites of juvenile hormone III were identified tentatively by the methods of Hammock and Roe (1985). Homogenates of day 2 and 9 post-ovipositional eggs were incubated at 30°C with [10–^{3}H] JH III (NEN) mixed with unlabeled JH III at a final substrate concentration of 5×10^{-6}M. The reaction was quenched with NaCl to saturation and extracted 3 times with ethyl acetate. Metabolites extracted in ethyl acetate (>99% of the total radioactivity) were co-chromatographed with [10–^{3}H] JH, JH acid, JH diol and JH diol-acid standards on Brinkmann Polygram® SILG, 0.25 mm thick plastic TLC plates developed in hexane:ethyl acetate:glacial acetic acid (66:33:1). Each lane was cut into 4 mm fractions and assayed by liquid scintillation counting. Percent recovery of activity from the plate was >99%. JH acid standard was synthesized enzymatically from the labeled JH III substrate described above, using plasma JH esterase from prepupal cabbage loopers (*T. ni*). JH diol was synthesized chemically from JH III as described by Mumby and Hammock (1979) (~85% pure) and enzymatically (>99% pure) with egg homogenates of the tobacco hornworm (*Manduca sexta*) treated with 10^{-3}M

0,0-diisopropyl phosphorofluoridate (DFP) and 3-octylthio-1,1,1-trifluoro-2-propanone (OTFP) to inhibit the JH esterase activity. Inhibitors were preincubated 10 min at 30°C with homogenate before the addition of JH substrate. JH acid and JH diol standards were metabolized to JH diol-acid (with an R_f consistent with published values) (Hammock et al., 1975), using 4th instar larval mosquito epoxide hydrolase and JH esterase, respectively. JH acid standard incubated in sodium acetate buffer (0.5 M, pH 4.0, 25μg/ml 2,6-di-*t*-butyl-4-methylphenol, 0.5% ethanol) as described by Mumby and Hammock (1979) also produced JH diol-acid with an identical R_f.

JH esterase activity in the remaining studies was assayed by the partition assay method described by Hammock and Roe (1985). Alpha-naphthyl acetate (α-NA) esterase was assayed according to the procedure of Sparks et al. (1979). Assay incubation temperature in these studies was 30°C. The substrate concentrations were 5×10^{-6}M for the JH esterase assay and 2.3×10^{-4}M for the α-NA esterase assay. Final homogenate dilutions and incubation times were chosen to yield a linear hydrolysis rate with tim Protein concentration was measured with the BioRad protein assay (Bio-Rad Laboratories, 1977) using bovine serum albumin (fraction V, Sigma) as a standard. Daily changes in JH esterase activity, α-NA esterase activity, and protein during embryogenesis were measured on crude egg homogenates. JH and α-NA esterase assays were run in triplicate and then averaged for each replicate.

Gel filtration

Undiluted day 1, 4 and 8 egg homogenate was centrifuged at 12,000 ×**g** for 30 min and filtered through glass wool. An aliquot of 2.5 ml was loaded on a 1.5 × 100 cm (i.d.) Sephacryl S-200 (Pharmacia) column and eluted with sodium phosphate buffer (I = 0.2 M, pH 7.4, 0.01% PTU, 10% sucrose) at a flow rate of 10 ml/h. Fractions of 2.2 ml were assayed for enzyme activity immediately after the column run was finished. The molecular weight was estimated from a comparison of the peak elution volume for JH and α-NA esterase activity with that for the following standards: (a) blue dextran, (b) β-amylase from sweet potato, (c) aldolase from rabbit muscle, (d) bovine serum albumin (Fraction V), (e) ovalbumin, (f) carbonic anhydrase from bovine erythrocyte, (g) α-chymotrypsinogen from bovine pancreas, and (h) ribonuclease A from bovine pancreas (a–g from Sigma and h from Serva).

Inhibition of esterase activity

Inhibitors dissolved in ethanol were preincubated 10 min at 30°C with enzyme (final ethanol concentration 1%), substrate added and JH and α-NA esterase activity compared with ethanol controls. One percent ethanol had no significant effect on esterase activity. The I_{50} is the inhibitor concentration at which the esterase activity was reduced by one half and was determined from final inhibitor concentrations of 1×10^{-3} to 1×10^{-10}M using least-squares regression formulas. The inhibitor *0,0*-diisopropyl phosphorofluoridate (DFP) was from Aldrich. This DFP in our hands did not inhibit prepupal JH esterase activity from *T. ni* at 10^{-4}M but inhibited 89% of the α-NA esterase activity as previously described by Sparks and Hammock (1979). DFP from other sources inhibited *T. ni* JH esterase activity under these same conditions. The inhibitor 3-octylthio-1,1,1-trifluoro-2-propanone (OTFP) was synthesized by techniques published previously (Hammock et al., 1984). I_{50}'s were determined for day 1 and 4 esterases separated by gel tration (no dilution).

Results

The duration of embryogenesis at 35°C was 9.5 days. No eggs hatched prior to 9 days or after 10 days. The major route of JH metabolism during embryogenesis was ester hydrolysis. JH III substrate incubated at 30°C for 15 min with day 2 homogenate diluted to a final concentration of 1 mg wet weight per 800μl buffer or for 45 min with 1/400 diluted day 9 egg homogenate was metabolized to 36 and 49% JH acid (R_f 0.54), respectively (Fig. 1). No other metabolites were detected using these conditions. When JH III substrate was incubated with heat killed homogenate, no metabolism was observed.

Protein concentration and α-naphthyl acetate (α-NA) esterase activity per mg wet weight remained relatively constant throughout embryogenesis and were similar to that for day 0 eggs dissected from the oviducts (Fig. 2). JH esterase activity per mg wet weight, however, was highest in day 0 and day 1 eggs and declined to one-third of its peak activity by the fifth day of embryogenesis. From day 5 through day 9, JH esterase activity remained unchanged. The same developmental pattern of change for JH esterase occurred relative to the total protein concentration. α-NA esterase activity in nMoles/min per mg wet weight for dissected and newly layed eggs was 100 times that for JH esterase activity. There was no significant change in the overall α-NA esterase activity during the time period between day 1 and day 5 when JH

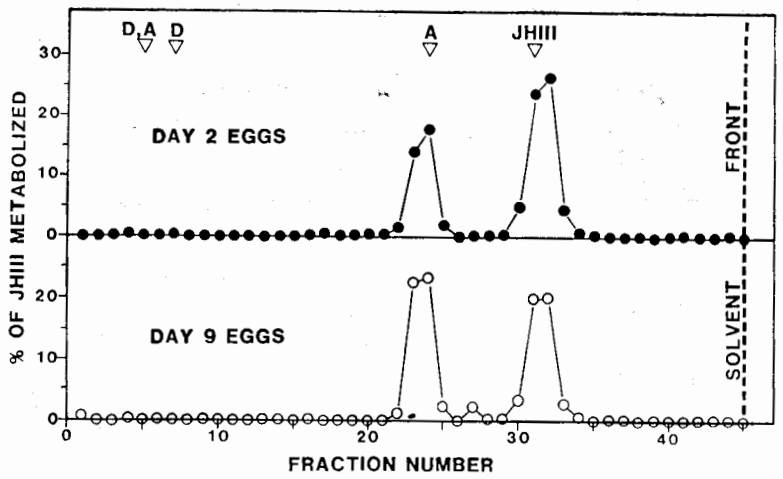

Fig. 1. Route of JH III metabolism in day 2 (•) and day 9 (○) eggs of the house cricke Homogenates of day 2 eggs were diluted to a final concentration of 1 mg wet weight p 800μl of buffer and day 9 eggs 1/400, and incubated with ^{3}H-JH III (final conc. 5×10^{-6}M) for 15 and 45 min, respectively, at 30°C. The only metabolite identified by TLC was JH-acid. Fraction 1 is at the origin and subsequent fractions are in 4 mm increments from the origin. The R_f's for JH diol-acid (D,A), JH diol (D), JH acid (A), and JH (III) are 0.12, 0.16, 0.54 and 0.69, respectively. These results were repeated three times with different homogenates.

esterase activity was declining.

The gel filtration profile for homogenates of day 1 post-ovipositional eggs clarified by centrifugation at 12,000 ×g and filtered through glass wool, resolved two peaks of activity at fractions 27 and 34 (Fig. 3). The first peak eluted as part of the exclusion volume with an apparent molecular weight >200,000 and the molecular weight of the second peak was 130,000. Reported molecular weights for insect hemolymph and tissue JH esterases range from 34,000 to >300,000 (Sparks and Hammock, 1979; Whitmore et al., 1974; Sanburg et al., 1975; Kramer and Childs, 1977; Rudnicka et al., 1979; Klages and Emmerich, 1979; Peter et al., 1979; McCaleb et al., 1980; Mane and Chippendale, 1981; Coudron et al., 1981; Yuhas et al., 1983; Roe et al., 1983; Bean et al., 1983; Lessman and Herman, 1984). Two peaks of α-NA esterase activity were also eluted with corresponding apparent molecular weights to that for JH esterase (Fig. 3). DFP inhibited the first and second peak of JH and α-NA esterase activity (Fig. 3) only at high concentrations (I_{50}'s 9×10^{-5} to 2×10^{-4}M, Table I). There was little difference in the inhibitor

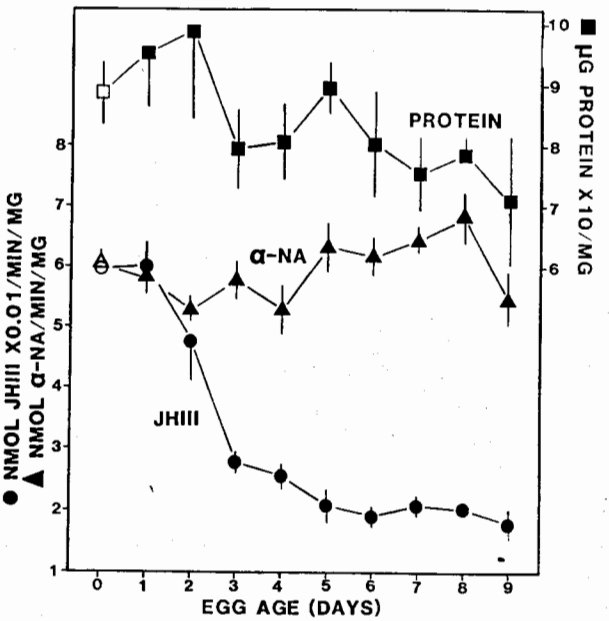

Fig. 2. Protein concentration (■) and juvenile hormone III (●, JH III) and α-naphthyl acetate (▲, α-NA) metabolized per mg wet weight of eggs dissected from the lateral oviducts (open symbols) and post-ovipositional (closed symbols) eggs. Day 1 post-ovipositional eggs are actually 0 to 1 day old, day 2 actually 1 to 2 day old, etc. Each replicate consists of approximately 130 eggs homogenized 1 mg wet weight per 50µl of buffer. Each point plotted represents the mean for assays on at least six homogenates diluted to obtain a linear hydrolysis rate with time at 30°C. The JH III and α-NA substrate concentration was 5×10^{-6} and 2.3×10^{-4}M, respectively. The vertical lines are ± two standard errors of the mean which in some cases do not exceed the size of the symbol.

potency between JH and α-NA esterase activity for both peaks. OTFP was a more potent inhibitor of JH and α-NA esterase activity (I_{50}'s 1×10^{-9} to 3×10^{-8}M, Table I) but inhibition varied only slightly between JH and α-NA esterase. The I_{50} for OTFP averaged lower for α-NA esterase in contrast to previous research with hemolymph of the cabbage looper, *T. ni*, where the I_{50} of OTFP was three orders of magnitude lower for JH than α-NA esterase (Hammock et al., 1984).

For day 4 and 8 eggs analyzed by gel filtration chromatography, there was a major peak of JH esterase activity in fractions 31 and 33, respectively (apparent molecular weights of 200,000 and 150,000, Figs. 4 and 5). There was a single α-NA esterase peak in fraction 39 (apparent molecular weight

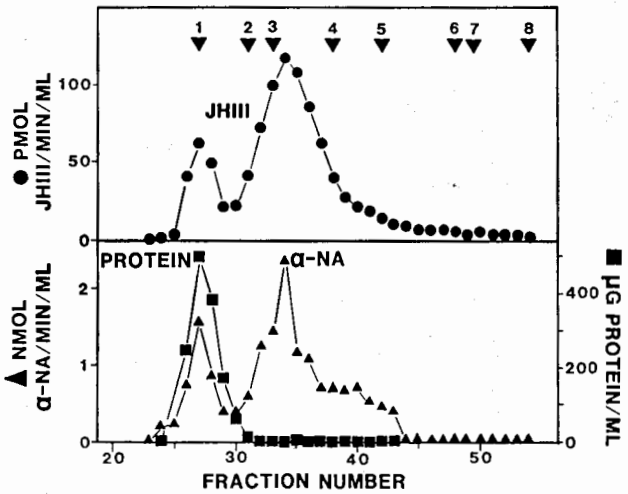

Fig. 3. Gel filtration pattern for protein (■) and α-naphthyl acetate (▲, α-NA) and juvenile hormone III (●, JH III) esterase metabolism in day 1 eggs. Undiluted homogenate was centrifuged and filtered; 2.5 ml were chromatographed on a 1.5 × 100 cm Sephacryl S-200 column (10 ml/h, 2.2 ml fractions). The standards are (1) blue dextran (2,000,000 in molecular weight), (2) β-amylase (200,000), (3) aldolase (158,000), (4) bovine serum albumin (68,000), (5) ovalbumin (45,000), (6) carbonic anhydrase (29,000), (7) α-chymotrypsinogen (24,500), and (8) ribonuclease A (13,700). Two peaks of JH and α-NA esterase activity were eluted (fractions 27 and 34). These results were duplicated from aliquots of a single homogenate.

of 65,000, Figs. 4 and 5) which correlated with a minor JH esterase peak for both day 4 and 8 eggs. The inhibition profile of day 4 JH and α-NA esterase activity was different from that of day 1 eggs. α-NA esterase (fraction 39, Table I) was inhibited by low concentrations of DFP ($I_{50} = 4 \times 10^{-6}$M) while JH esterase was relatively insensitive ($I_{50} = 2 \times 10^{-4}$M). OTFP, on the other hand, was a more potent inhibitor of JH esterase (fraction 31, $I_{50} = 8 \times 10^{-8}$M) than of α-NA esterase ($I_{50} = 3 \times 10^{-7}$M).

Discussion

JH esterase activity was highest in eggs dissected from the oviducts and on day 1 of embryogenesis. Cleavage, the formation of the blastoderm and germ band, and the differentiation of the germ band into a protocephalon and protocorm in the house cricket occurs during the time period of maximum JH esterase activity (or by day 1 as derived from Lauga (1969)). During blastokinesis, JH esterase activity declines reaching its lowest level at the period of dorsal closure (day 5). Activity remains unchanged through subsequent

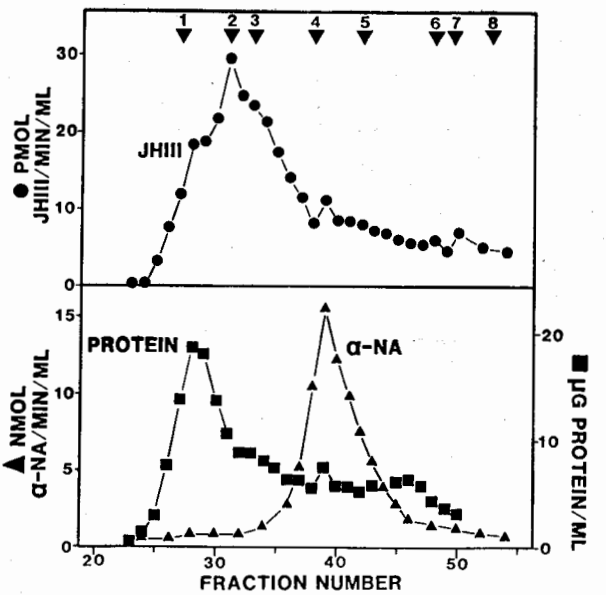

Fig. 4. Gel filtration pattern for protein (▪) and α-naphthyl acetate (▲, α-NA) and juvenile hormone III (●, JH III) esterase metabolism in day 4 eggs. The procedures and standards are described in Fig. 3. The major JH esterase peak was in fraction 31 and a single α-NA esterase peak eluted in fraction 39. These results were duplicated from aliquots of a single homogenate.

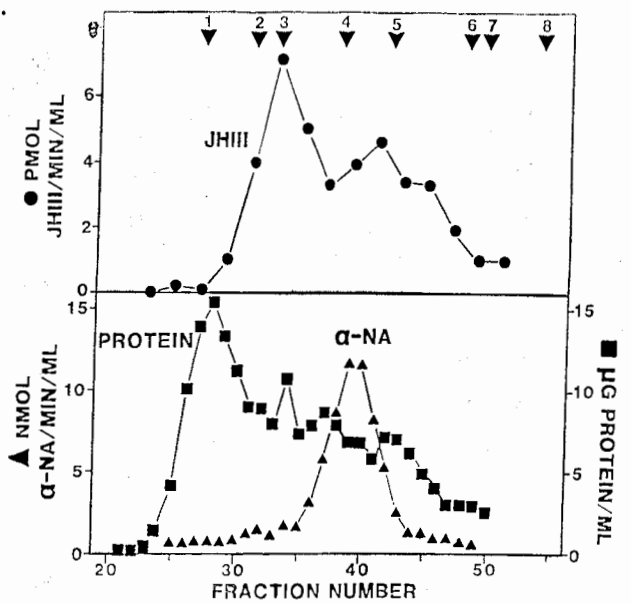

Fig. 5. Gel filtration pattern for protein (▪) and α-naphthyl acetate (▲, α-NA) and juvenile hormone III (●, JH III) esterase metabolism in day 8 eggs. The procedures and standards are described in Fig. 3. The major JH esterase activity peak was in fraction 33 and single α-NA esterase peak eluted in fraction 39. These results were duplicated from aliquots of a single homogenate.

TABLE I.
Inhibition of α-naphthyl acetate and juvenile hormone esterase activity from homogenates of day 1 and 4 eggs partially purified by gel filtration*

Egg Age:	Day 1		Day 4	
Peak Fraction No.:	27	34	31	39
α-NAE Inhibition (I_{50}, M)+				
DFP	1×10^{-4}	2×10^{-4}	—	4×10^{-6}
OTFP	1×10^{-9}	5×10^{-9}	—	3×10^{-7}
JHE Inhibition (I_{50}, M)+				
DFP	9×10^{-5}	1×10^{-4}	2×10^{-4}	—
OTFP	4×10^{-9}	3×10^{-8}	8×10^{-8}	—

* The peak fractions (Figs. 3 and 4) and one fraction before and after the peak were pooled and assays were performed without further dilution at least in duplicate and replicated twice.

+I_{50} is the inhibitor concentration at which the esterase activity was reduced by one hal The I_{50} was determined from inhibitor concentrations of 1×10^{-3} to 1×10^{-10}M using least squares regression formulas. The r^2 values were at least 0.94. Inhibitors dissolved in ethanol were preincubated for 10 min at 30°C with enzyme, substrate added, and esterase activity compared with ethanol controls. DFP, *0,0*-diisopropyl phosphorofluoridate; OTFP, 3-octylthio-1,1,1-trifluoro-2-propanone.

development up to the time of hatching.

During the time period of elevated JH esterase activity in the house cricket (up to dorsal closure), JH concentration is relatively low in the eggs of other insect species. In *L. migratoria* for example the JH III titer in eggs is low at ovulation (approximately one-third that of peak levels found later in embryogenesis) and declines to undetectable levels by late blastokinesis and during dorsal closure (Temin et al., 1986). The cockroach, *N. cinerea*, has no detectable egg JH II during the same time period (Imboden et al., 1978). Earlier studies by Gilbert and Schneiderman (1961) demonstrated that eggs of allatectomized females from the American silkmoth, *Hyalophora cecropia*, contained no JH-active material, in contrast to eggs produced by normal females, suggesting that JH produced by the mother was transferred to the egg. It was also concluded by Temin et al. (1986) that the JH III found in developing eggs of *L. migratoria* prior to dorsal closure was also of maternal origin as it was found in eggs at ovulation and since it was previously established that embryonic corpora allata do not differentiate until later during embryogenesis. The JH concentration in the hemolymph of the adult female locust was 50 times that found in newly laid eggs.

JH levels in adult female crickets are relatively high during the early stages of oogenesis to promote yolk deposition (Woodring and Sparks, unpublished; Renucci and Strambi, 1983) and it is likely based on the above discussion that this maternal JH is transferred passively to the egg. A number of studies have also indicated that abnormally high JH levels late in oogenesis and early in embryogenesis will disrupt normal development. JH applied in large doses to adult female bugs, *Pyrrhocoris apterus* or to *H. cecropia* after the completion of vitellogenesis blocks germ band formation (Riddiford, 1971, 1972). Applications to the freshly laid eggs of several species blocks the embryonic-larval transformation (Riddiford, 1971, 1972; Injeyan et al., 1979; Riddiford and Williams, 1967; Matolin, 1970; Riddiford, 1970). A working hypothesis in the house cricket is that low JH titer is critical to normal development early in embryogenesis and that high JH esterase activity during this period plays a functional role in the metabolism of maternal JH. It is well established for other developmental stages that ester hydrolysis plays a critical role in the regulation of JH titer (Hammock, 1985; Prestwich et al., 1984; Hammock et al., 1984). Declining JH esterase activity during the time when α-NA esterase activity remains unchanged also supports a changing role for JH metabolism during embryogenesis. JH metabolism was lowest in the house cricket after dorsal closure at the time period in *L. migratoria* and *N. cinerea* when there was an increase in JH titer (Temin et al., 1986; Imboden et al., 1978).

The JH esterases in day 1 eggs are likely synthesized during oogenesis, as it is present with equivalent metabolic activity to eggs dissected from the oviduct. Two JH esterases were resolved that were poorly inhibited by DFP, effectively inhibited by low concentrations of OTFP, and also metabolized α-NA. The JH esterase of this developmental age is either non-specific, in that it also metabolizes α-NA, or the α-NA esterase activity was not successfully resolved from the JH esterase activity. The similarity in the inhibition profiles for JH and α-NA esterase activity also supports the presence of a non-specific JH esterase. The major JH esterase peak resolved on day 4 appears to have more specific activity for JH than α-NA as compared to that for day 0. DFP inhibits α-NA esterase activity at a lower I_{50} than that for JH esterase activity and the reverse is true for OTFP. These characteristics are consistent with previous reports for specific JH esterase from other species (Hammock, 1985; Hammock et al., 1984). The retention times differ for JH versus α-NA esterase activity for day 4 and for JH esterase on day 4 versus day 0. The decline in JH esterase activity from day 2 through 5 may not be simply a

reduction in non-specific JH esterase concentration or activity after day 1 but possibly the substitution of new JH esterase(s).

The major route of JH metabolism in cricket eggs was ester hydrolysis but in newly layed eggs of the tobacco hornworm (*M. sexta*), the tobacco budworm (*Heliothis virescens*), the bollworm (*H. zea*) and the cabbage looper (*T. ni*), there were two routes of metabolism, epoxide hydration and ester hydrolysis (Roe and Crawford, unpublished). JH esterase and epoxide hydrolase activity was present in fat body, brain and nerve cord homogenates of female house crickets (Renucci, 1986) but was not detected in the whole body homogenates of cricket embryos.

In preovipositional eggs and developing embryos of the house cricket prior to dorsal closure, there are elevated levels of nonspecific JH esterase activity during the time in other insect species when JH levels are declining or not detectable. JH specific esterase activity appears by the time of blastokinesis and is maintained at relatively low levels through hatching. The JH titer in adult female crickets is relatively high during the early stages of oogenesis to promote yolk deposition and is likely transferred passively to the ovary and developing oocytes. It also appears that low JH titers in eggs must be maintained through the period of germ band formation and blastokinesis for normal development to occur. Elevated JH esterase activity in eggs dissected from the oviducts and in developing embryos through blastokinesis appears to have a functional role in the metabolism of maternal JH.

Acknowledgements

This material is based upon work supported by the U.S. Department of Agriculture, the Competitive Research Grants Program on Plant Stress, under Agreement No. 85-CRCR-1-1661. This research was also funded by the NC Board of Science and Technology (85-5007), the NC Biotechnology Center (85-G-00701), a Biomedial Research Support Grant No. RR07071, the NCSU Agriculture Foundation, and a Department of Health and Human Services, National Service Award F32 GM09223-02 from the National Institute of General Services. Dr. B.D. Hammock was supported by grants from NIEHS, NSF, and USDA (ES01710-06, DCB-8518697, and 85-CRCR-1-1715, respectively).

Use of trade names in this publication does not imply endorsement of the products named or criticism of similar ones not mentioned. Paper No. 10767 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695, U.S.A.

References

1 Bean, D.W., Goodman, W.G. and Beck, S.D., Regulation of juvenile hormone esterase activity in the European corn borer, *Ostrinia nubilalis*, J. Insect Physiol., 29 (1983) 877–883.

2 Bio-Rad Laboratories, Bio-Rad protein assay, Tech. Bull. 1051 (1977).

3 Bowers, W.S., Fales, H.M., Thompson, M.J. and Uebel, E.C., Juvenile hormone: identification of an active compound from balsam fir, Science, 154 (1966) 1020–1021.

4 Clifford, C.W., Roe, R.M. and Woodring, J.P., Rearing methods for obtaining house crickets, *Acheta domesticus*, of known age, sex, and instar, Ann. Ent. Soc. Am., 70 (1977) 69–74.

5 Coudron, T.A., Dunn, P.E., Seballos, H.L., Wharen, R.E., Sanburg, L.L. and Law, J.H., Preparation of homogeneous juvenile hormone specific esterase from the haemolymph of the tobacco hornworm, *Manduca sexta*, Insect Biochem., 11 (1981) 453–461.

6 Dorn, A., Precocene-induced effects and possible role of juvenile hormone during embryogenesis of the milkweed bug *Oncopeltus fasciatus*, Gen. comp. Endocrinol., 46 (1982) 42–52.

7 Gilbert, L.I. and Schneiderman, H.A., The content of juvenile hormone and lipid in Lepidoptera: sexual differences and developmental changes, Gen. Comp. Endocrinol., 1 (1961) 453–472.

8 Hammock, B., Nowock, J., Goodman, W., Stamoudis, V. and Gilbert, L.I., The influence of hemolymph-binding protein on juvenile hormone stability and distribution in *Manduca sexta* fat body and imaginal discs *in vitro*, Mol. Cell. Endocrinol., 3 (1975) 167–184.

9 Hammock, B.D., Abdel-Aal, Y.A.I., Mullin, C.A., Hanzlik,T.N. and Roe, R.M., Substituted thiotrifluoropropanones as potent selective inhibitors of juvenile hormone esterase, Pestic. Biochem. Physiol., 22 (1984) 209–223.

10 Hammock, B.D., Regulation of juvenile hormone titer: degradation. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, G.A. Kerkut and L.I. Gilbert, eds., Pergamon Press, New York, 7 (1985) 431–472.

11 Hammock, B.D. and Roe, R.M., Analysis of juvenile hormone esterase activity, Meth. Enzymol., 111 (1985) 487–494.

12 Hoffman, J.A. and Lagueux, M., Endocrine aspects of embryonic development in insects. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology, G.A. Kerkut and L.I. Gilbert, eds., Pergamon Press, New York, 1 (1985) 435–460.

13 Imboden, H., Lanzrein, B., Delbecque, J.P. and Lüscher, M., Ecdysteroids and juvenile hormone during embryogenesis in the ovoviviparous cockroach *Nauphoeta cinerea*, Gen. Comp. Endocrinol., 36 (1978) 628–635.

14 Injeyan, H.S., Tobe, S.S. and Rapport, E., The effects of exogenous juvenile hormone treatment on embryogenesis in *Schistocerca gregaria*, Can. J. Zool., 57 (1979) 838–845.

15 Klages, G. and Emmerich, H., Juvenile hormone metabolism and juvenile hormone esterase titer in hemolymph and peripheral tissues of *Drosophila hydei*, J. Comp. Physiol., 132 (1979) 319–325.

16 Kramer, K.J. and Childs, C.N., Interaction of juvenile hormone with carrier proteins and hydrolases from insect haemolymph, Insect Biochem., 7 (1977) 397–403.

17 Lanzrein, B., Imboden, H., Bürgin, C., Brüning, E. and Gfeller, H., On titers, origin, and functions of juvenile hormone III, methylfarnesoate, and ecdysteroids in embryonic development of the ovoviviparous cockroach *Nauphoeta cinerea*. In: Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, J.A. Hoffman and M. Prochet, eds., Springer-Verlag, Heidelberg, 1984, pp. 454–465.

18 Lauga, P.J., Table chronologique du développement embryonnaire de *Acheta domesticus* L. (Orthoptère, Gryllidae), a 25°C., Ann. Sci. Natl., Zool., Paris, No. 11 (1969) 483–504.

19 Lessman, C.A. and Herman, W.S., Juvenile hormone metabolism, binding and esterase activities in the haemolymph of the adult monarch butterfly (*Danaus P. plexippus* L.: Lepidoptera), Insect Biochem., 14 (1984) 445–452.

20 Mane, S.D. and Chippendale, G.M., Hydrolysis of juvenile hormone in diapausing and non-diapausing larvae of the southwestern corn borer, *Diatraea grandiosella*, J. Comp. Physiol., 144 (1981) 205–214.

21 Matolin, S., Effects of a juvenile hormone analogue on embryogenesis in *Pyrrhocoris apterus* L., Acta Ent. Bohemoslov., 67 (1970) 9–12.

22 McCaleb, D.C., Reddy, G. and Kumaran, A.K., Some properties of the haemolymph juvenile hormone esterases in *Galleria mellonella* larvae and *Tenebrio molitor* pupae, Insect Biochem., 10 (1980) 273–277.

23 Mumby, S.M. and Hammock, B.D., A partition assay for epoxide hydrases acti on insect juvenile hormone and an epoxide-containing juvenoid, Anal. Biochem., 92 (1979) 16–21.

24 Novak, V.J.A., Morphogenetic analysis of the effects of juvenile hormone analogues and other morphogenetically active substances on embryos of *Schistocerca gregaria* (Forskål), J. Embryol. Exp. Morph., 21 (1969) 1–21.

25 Peter, M.G., Gunawan, S., Gellissen, G. and Emmerich, H., Differences in hydrolysis and binding of homologous juvenile hormones in *Locusta migratoria* hemolymph, Z. Naturforsch., 34c (1979) 588–598.

26 Prestwich, G.D., Eng., W.-S., Roe, R.M. and Hammock, B.D., Synthesis and bioassay of isoprenoid 3-alkylthio-1,1,1-trifluoro-2-propanones: potent, selective inhibitors of juvenile hormone esterase, Arch. Biochem. Biophys., 228 (1984) 639–645.

27 Renucci, M., Juvenile hormone degradation in nerve tissues and fat body of female *Acheta domesticus* (Insecta, Orthoptera), Comp. Biochem. Physiol., 84A (1986) 101–106.

28 Renucci, M. and Strambi, C., Juvenile hormone levels, vitellogenin and ovarian development in *Acheta domesticus*, Experientia, 39 (1983) 618–620.

29 Riddiford, L.M., Effects of juvenile hormone on the programming of postembryonic development in eggs of the silkworm, *Hyalophora cecropia*, Develop. Biol., 22 (1970) 249–263.

30 Riddiford, L.M., Juvenile hormone and insect embryogenesis, Mitt. Schweiz. Entomol. Ges., 44 (1971) 177–186.

31 Riddiford, L.M., Juvenile hormone and insect embryonic development: its potential role as an ovicide. In: Insect Juvenile Hormones. Chemistry and Action, J.J. Menn and M. Beroza, eds., Academic Press, New York, 1972, pp. 95–111.

32 Riddiford, L.M. and Truman, J.W., Delayed effects of juvenile hormone on insect metamorphosis are mediated by the corpus allatum, Nature (London), 237 (1972) 458.

33 Riddiford, L.M. and Williams, C.M., The effects of juvenile hormone analogues on the embryonic development of silkworms, Proc. National Academy of Sciences (U.S.A.), 57 (1967) 595–601.

34 Roe, R.M., Hammond, A.M. Jr. and Sparks, T.C., Characterization of the Plasma juvenile hormone esterase in synchronous last stadium female larvae of the sugar cane borer, *Diatraea saccharalis* (F.), Insect Biochem., 13 (1983) 163–170.

35 Rudnicka, M., Sehnal, F., Jarolím, V. and Kochman, M., Hydrolysis and binding of the juvenile hormone in the hemolymph of *Galleria mellonella*, Insect Biochem., 9 (1979) 569–575.

36 Sanburg, L.L., Kramer, K.J., Kézdy, F.J. and Law, J.H., Juvenile hormone-specific esterases in the haemolymph of the tobacco hornworm, *Manduca sexta*, J. Insect Physiol., 21 (1975) 873–877.

37 Sbrenna-Micciarelli, A., Effects of farnesyl methyl ether on embryos of *Schistocerca gregaria* (Orthoptera), Acta Embryol. Exp., 155 (1977) 295–303.

38 Sláma, K. and Williams, C.M., 'Paper factor' as an inhibitor of the embryonic development of the European bug, *Pyrrhocoris apterus*, Nature (London), 210 (1966) 329–330.

39 Sparks, T.C. and Hammock, B.D., A comparison of the induced and naturally occurring juvenile hormone esterases from last instar larvae of *Trichoplusia ni*, Insect Biochem., 9 (1979) 411–421.

40 Sparks, T.C., Willis,W.S., Shorey, H.H. and Hammock, B.D., Haemolymph juvenile hormone esterase activity in synchronous last instar larvae of the cabbage looper, *Trichoplusia ni*, J. Insect Physiol., 25 (1979) 125–132.

41 Temin, G., Zander, M. and Roussel, J.-P., Physico-chemical (GC-MS) measurements of juvenile hormone III titres during embryogenesis of *Locusta migratoria*, Intl. J. Invertebr. Rep. Dev., 9 (1986) 105–112.

42 Whitmore, D. Jr., Gilbert, L.I. and Ittycheriah, P.I., The origin of hemolymph carboxylesterases 'induced' by the insect juvenile hormone, Mol. Cell. Endocrinol., 1 (1974) 37–54.

43 Yuhas, D.A., Roe, R.M., Sparks, T.C. and Hammond, A.M. Jr., Purification and kinetics of juvenile hormone esterase from the cabbage looper, *Trichoplusia ni* (Hübner), Insect Biochem., 13 (1983) 129–136.