Pergamon

0022-1910(95)00044-5

J. Insect Physiol. Vol. 41, No. 11, pp. 993–1000, 1995

0022-1910/95 $9.50 + 0.00

Degradation of Juvenile Hormone III *In Vitro* by Non-parasitized and Parasitized *Spodoptera exigua* (Noctuidae) and by the Endoparasitoid *Chelonus inanitus* (Braconidae)

BEATRICE LANZREIN*†‡ BRUCE HAMMOCK*

Received 17 January 1995; revised 17 March 1995

Parasitism of *Spodoptera exigua* by the solitary egg–larval parasitoid *Chelonus inanitus* induces precocious onset of metamorphosis, i.e. parasitized larvae construct a pupal cell and become prepupae in the fifth larval instar while non-parasitized larvae do so in the sixth larval instar. The parasitoid larva emerges as a freshly molted third instar larva from the precocious prepupa. A comparison of the metabolism of racemic ^{3}H-JH III by homogenates of non-parasitized and parasitized larvae at the stage of pupal cell formation revealed that very similar amounts of juvenile hormone III diol, juvenile hormone III acid and juvenile hormone III acid–diol were formed. This shows that the changes in juvenile hormone degradation associated with metamorphosis, which occur in the sixth stadium in non-parasitized larvae, are precociously induced in parasitized larvae in the fifth stadium. In homogenates of parasitoid larvae, juvenile hormone III metabolizing activity was very low during the internal phase of development (first to second instar larvae) and almost excusively juvenile hormone III diol was formed. After emergence from the host, i.e. in third instar larvae, juvenile hormone III metabolic activity increased and esterases became active. In comparison to the host, specific juvenile hormone III degrading activities were always much lower in homogenates of parasitoid larvae than of host larvae. With homogenates of *S. exigua* eggs at a stage 2/3 of embryonic development and shortly before hatching, the pattern of juvenile hormone III metabolism was almost identical whether parasitized or not, and the major metabolite being formed was juvenile hormone III diol. This shows that parasitism by *C. inanitus* does not influence juvenile hormone metabolic activities at these stages.

Juvenile hormone metabolism Parasitoid/host Hymenoptera Chelonus *Spodoptera*

INTRODUCTION

Many hymenopteran endoparasitoids influence the development of their hosts (Vinson and Iwantsch, 1980) and for several parasitoid–host systems it has been documented that such effects are mediated through alterations of host developmental hormones, namely prothoracicotropic hormone (PTTH), ecdysteroids and/or juvenile hormones [reviewed in Beckage (1985) and in Lawrence and Lanzrein (1993)]. Alteration of the host's juvenile hormone (JH) titer by parasitism has been demonstrated in the following parasitoid–host systems: *Apanteles glomeratus–Pieris brassicae* (Schopf, 1984), *Cotesia congregata–Manduca sexta* (Beckage and Templeton, 1986), *Biosteres longicaudatus–Anastrepha suspensa* (Lawrence *et al.*, 1990), *Chelonus* sp.–*Trichoplusia ni* (Grossniklaus-Bürgin and Lanzrein, 1990a; Jones *et al.*, 1990), *Copidosoma floridanum–T. ni* (Strand *et al.*, 1991) and *Glyptapanteles liparidis–Lymantria dispar* (Schopf and Rembold, 1993). It is not yet clear by which mechanisms host JH titers are influenced, but release of JH by the parasitoid and effects of the parasitoid or parasitoid-associated factors on rates of biosynthesis and degradation of JH have been proposed. In fact, JH esterases of host hemolymph have been observed to be altered in most of the above-mentioned as well as additional parasitoid–host systems [reviewed in Lawrence and Lanzrein (1993)]; in *Heliothis virescens* parasitized by *Microplitis croceipes* this effect has been attributed to teratocytes, cells of the parasitoid's serosa released into the host upon hatching (Zhang *et al.*, 1992). Under *in vitro* conditions, hemolymph JH is degraded by JH esterases to

*Department of Entomology, University of California, Davis, CA 95616, U.S.A.

†Present address: Division of Developmental Biology, Zoological Institute, University of Berne, Baltzerstrasse 4, CH-3012 Berne, Switzerland.

‡To whom all correspondence should be addressed.

JH acid while with whole insect or tissue homogenates, JH is degraded to JH acid, JH diol and JH acid–diol, indicating the presence of both, esterases and epoxide hydrolases [reviewed in Hammock (1985)].

Up to now JH metabolism in tissues of parasitized hosts has not been investigated and it is not clear whether parasitization affects JH degradation by host tissues. Furthermore, JH metabolism by parasitoids has not been investigated either. In the two parasitoid species where JH titers have been measured at various stages, namely *Chelonus* sp. (Grossniklaus-Bürgin and Lanzrein, 1990a) and *Glyptapanteles liparidis* (Schopf and Rembold, 1993), both braconid wasps, rather high values of JH III were observed in later stages of larval development. But it is not known whether this reflects high rates of biosynthesis or low rates of degradation of JH or both. For Hymenoptera, only very little information is available on JH metabolism and most of it concerns the honey bee; in the latter rather low JH metabolic activity in both homogenates (Mane and Rembold, 1977) and hemolymph (De Kort *et al.*, 1979) has been observed.

Chelonus inanitus, as well as all other members of the genus *Chelonus*, induce in their lepidopterous host the precocious onset of metamorphosis and developmental arrest in the precocious prepupa (Jones *et al.*, 1981; Grossniklaus-Bürgin *et al.*, 1994). Parasitism of *T. ni* by *Chelonus* sp. was shown to cause premetamorphic changes in JH titer (Grossniklaus-Bürgin and Lanzrein, 1990a) and hemolymph JH esterase (Bühler *et al.*, 1985) already in the penultimate larval instar of the host. Furthermore, the decline in JH titer seen at the end of embryogenesis was delayed in parasitized eggs (Grossniklaus-Bürgin and Lanzrein, 1990a).

Here we compared the JH metabolism between homogenates of non-parasitized and parasitized *Spodoptera exigua* and of the parasitoid *C. inanitus*. We namely investigated patterns and rate of JH metabolism in the course of larval development of the parasitoid as well as in non-parasitized and parasitized eggs and larvae of *S. exigua*.

MATERIALS AND METHODS

Insects

Non-parasitized and parasitized *Spodoptera exigua* were reared on an artifical diet at 27°C and a 16 h light:8 h dark photoperiod. Under these conditions non-parasitized larvae normally pass through six larval instars and parasitized larvae enter metamorphosis precociously in the fifth instar. The larvae used for experimental purposes were always staged by measuring their head capsule width and weight, and parasitization was always verified by dissection. For rearing the solitary egg–larval parasitoid *Chelonus inanitus* female wasps were allowed to oviposit into 27–32 h old host eggs. Under these conditions, the parasitoid first instar larvae hatch approx. 24 h later while the host is still in the embryonic stage. One day later the host larvae hatch and then pass through four larval instars; during this time, the parasitoid larva remains in the first instar but grows and passes through signifcant morphological changes [for details of the development of *C. inaitus* see Grossniklaus-Bürgin *et al.* (1994)]. One day after the host has molted into the fifth instar the parasitoid molts into its second instar. The host then precociously enters metamorphosis, constructs a pupal cell and becomes a prepupa. At this stage the parasitoid molts into the third instar, emerges from the precocious prepupal host and feeds on it. The parasitoid larva then spins a cocoon within the pupal cell of the host and pupates.

Chemicals and reference compounds

Tritiated racemic JH III [10 – ^{3}H (N)] was purchased from New England Nuclear Research Products (NEN, Boston, MA) and had a specific activity of 17.4 Ci/mmol. As reference compounds we used JH III, which had been synthesized by the method of Anderson *et al.* (1972), and JH III acid, JH III diol and JH III acid–diol. The latter compounds were prepared as described (Meyer and Lanzrein, 1989). Each compound showed a single spot on TLC analysis.

Assay for measuring degradation of racemic juvenile hormone III in tissue homogenates and hemolymph

Precisely staged non-parasitized and parasitized eggs or larvae (containing the parasitoid) or parasitoids removed from hosts or emerged from hosts were homogenized at 4°C in phosphate buffered saline (PBS), pH 7.2, containing 0.1% phenylthiourea (PTU, Aldrich) in a 25–50:1 volume to tissue weight ratio. Debris was removed by centrifugation at 6000 ***g*** for 10 min. A portion of the supernatant was used for measurement of the protein concentration in the BCA assay (Pierce Company, Rochard, IL) which is based on the dye binding method of Bradford (1976). Bovine serum albumine (BSA) (Fraction V, Sigma) was used as a protein standard. For determination of JH metabolism 10 μl of ethanol containing 3–5 pmol ^{3}H-JH III was first added to 10 × 75 mm glass tubes, then 100 μl PBS/PTU (=control) or various quantities of homogenate supernatant in PBS/PTU (final volume 100 μl) were added and thoroughly mixed with the labeled JH. Our substrate concentration resembles physiological conditions and we therefore usually tested three dilutions per homogenate in order to have among them concentrations where more than 50% of the ^{3}H-JH III was still intact at the end of the incubation. After a short centrifugation to concentrate the mixture in the bottom of the tube it was incubated for 30 min at 30°C. Thereafter it was extracted with 700 μl acetonitrile containing triethylamine (two drops per 100 ml) to prevent hydrolysis of epoxide by acidic impurities. After centrifugation the supernatant was collected and the precipitate washed with another 500 μl acetonitrile. The solvent was thereafter evaporated in a Speedvac Concentrator and the extract taken up in either 100 μl of acetonitrile, when extracts were analysed by thin layer chromatography (TLC), or in 100 μl Hepes

buffer:acetonitrile (7:3) when extracts were analysed by high pressure liquid chromatography (HPLC).

HPLC and TLC

For HPLC analyses the method described by Halarnkar and Schooley (1990) was applied. We used a polymer column (PLRP-S, 100 Å, 5 μm, 50 × 4.6 mm) and a guard column from Polymer Laboratories Inc., Amherst, MA, and a Hepes buffer/acetonitrile gradient. Unlabeled JH III as well as its metabolites JH III acid, JH III diol and JH III acid–diol were coinjected as references, and radioactivity was monitored with a β-RAM Flow Monitor (in Flow-BD, IN/US Service Comp., Fairfield, NJ). For TLC analyses we either used LK5DF silicagel plates (80 Å, 5 × 20 cm, thickness 250 μm) from Whatman or T-6145 polyester silicagel sheets (254 nm, 20 × 20 cm, 250 μm) from Sigma. The extract, dissolved in acetonitrile, was spotted onto the plate together with unlabeled reference compounds, and as solvent we used ethylacetate/hexane (4:7, v/v). After chromatography the positions of the standards were marked under u.v. light. The polyester sheets were automatically counted with a TLC scanner (System 200 Imaging Scanner, Bioscan, WA). Each lane was counted 20 min. Peaks were tentatively identified by co-chromatography with the reference compounds and were quantified by integration with the scanner software package (NSCAN). Metabolites were expressed as a percentage of the total radioactivity present in all the peaks. Because the counting efficiency is very low in the scanning system using ^{3}H we often used Whatman normal phase silicagel TLC and measured the radioactivty by scraping zones of 0.5 cm and counting them by liquid scintillation counting using Tru-Count scintillation fluid (Trulab Supply Company, Libertyville, IL). For calculation of specific activities (pmol JH III degraded per min and per mg protein) we only used data from incubations where more than 50% of JH III was still intact at the end of the incubation.

As multiple JH metabolic pathways were expected with homogenates we did not use partition assays for JH esterase or JH epoxide hydrolase which would allow much higher sample throughput. We preferred to use chromatography-based techniques which allow defining pathways with greater confidence and higher sensitivity and which would allow the detection of new and unexpected metabolites. HPLC and TLC analyses each offer advantages in terms of speed and sample throughput in different situations. The high resolution of the HPLC method of Halarnkar and Schooley (1990) offers advantages in metabolite identification while the ability to process large numbers of samples in parallel by TLC also offers advantages (Hammock and Roe, 1985). Here a combination of HPLC and TLC was used: we initially analyzed hosts and parasitoids at all stages and with several dilutions by HPLC and then accumulated additional quantitative data using TLC.

RESULTS

To investigate whether the parasitism induced precocious metamorphosis is characterized by the same premetamorphic pattern of JH metabolism as seen in normal metamorphosis we compared the JH III metabolism between homogenates of non-parasitized sixth instar larvae and parasitized fifth instar larvae (containing the parasitoid) at the stage of pupal cell formation. In addition, JH metabolism by homogenates of parasitoids dissected out of such larvae was analyzed. A typical example of the results of such an experiment is shown in Fig. 1 which gives the relative proportions of JH III and its metabolites formed after incubation of homogenates with ^{3}H-JH III during 30 min. The results obtained with 11.84 μg BSA equiv. of non-parasitized sixth instar *S. exigua* larvae are given in the top panel and those obtained with 9.24 μg BSA equiv. of fifth instar parasitized *S. exigua* (which precociously entered metamorphosis) in the middle. In both cases the major metabolites being formed were JH III diol, JH III acid and JH III acid–diol and their relative proportions were very similar for non-parasitized sixth instar and parasitized fifth instar larvae. This was confirmed by several other analyses. Measurements of the specific JH III metabolizing activity in fifth and sixth instar non-parasitized and fifth instar parasitized larvae revealed that JH metabolizing activity was very low in fifth instar non-parasitized larvae and reached values in the range of 40–60 pmol/min/mg BSA equiv. for non-parasitized sixth instar larvae and parasitized fifth instar larvae at the stage of digging and of pupal cell formation (data not shown) indicating precocious premetamorphic modification of JH metabolism by parasitism. With homogenates (90 μg BSA equiv.) of second instar parasitoid larvae (a stage when they consume hemolymph of their host) dissected out from pupal cell forming host larvae JH III was degraded much less efficiently than with host homogenates and almost exclusively JH III diol was formed (Fig. 1, bottom). This was confirmed by additional analyses and the specific JH III metabolizing activity was always between 0.3 and 0.6 pmol/min/mg BSA equiv. for second instar parasitoids. The tailing of the radioactivity peaks seen in all three panels seems to be due to mixing effects in the chamber where the scintillation fluid is added.

Having observed a low JH III metabolizing activity and the almost exclusive formation of JH III diol in second instar parasitoids which live internally in the host, we then investigated whether JH III metabolic activity changes with the emergence from the host. A typical example of results obtained with homogenates of external third instar parasitoid larvae is shown in Fig. 2. With the lower concentration (25.5 μg BSA equiv.) JH III diol and JH III acid were the major metabolites and were formed in equal proportions. With the higher concentration (51 μg BSA equiv.) JH III acid was the predominant metabolite, and together with JH III diol also traces of JH III acid–diol were found. These results show that after emergence from the host, JH III metabolism increased in the parasitoid

larva and esterases became active. The different ratios of metabolites obtained with the two concentrations of protein could be due either to the fact that with the higher protein concentration metabolism was slightly out of the linear region and/or to different apparent affinities of esterase and epoxide hydrolase to racemic JH III and/or to different apparent affinities to the two enantiomers.

The specific JH III metabolic activity was then investigated in the course of parasitoid development (from late first instar larvae to late third instar larvae) and is shown in Fig. 3. The results show an extremely low acitivty in first instar larvae, a low activity throughout the second larval instar and an increase after emergence from the host in the third larval instar.

As earlier JH determinations with the parasitoid/host system *C. sp./T ni* had indicated a higher JH titer in parasitized eggs than in non-parasitized eggs 42–50 h after parasitization (Grossniklaus-Bürgin and Lanzrein, 1990a) we investigated whether parasitism affects JH III metabolism shortly after parasitization in the related system *C. inanitus/S. exigua*. A typical example of such an analysis is shown in Fig. 4. The data revealed no

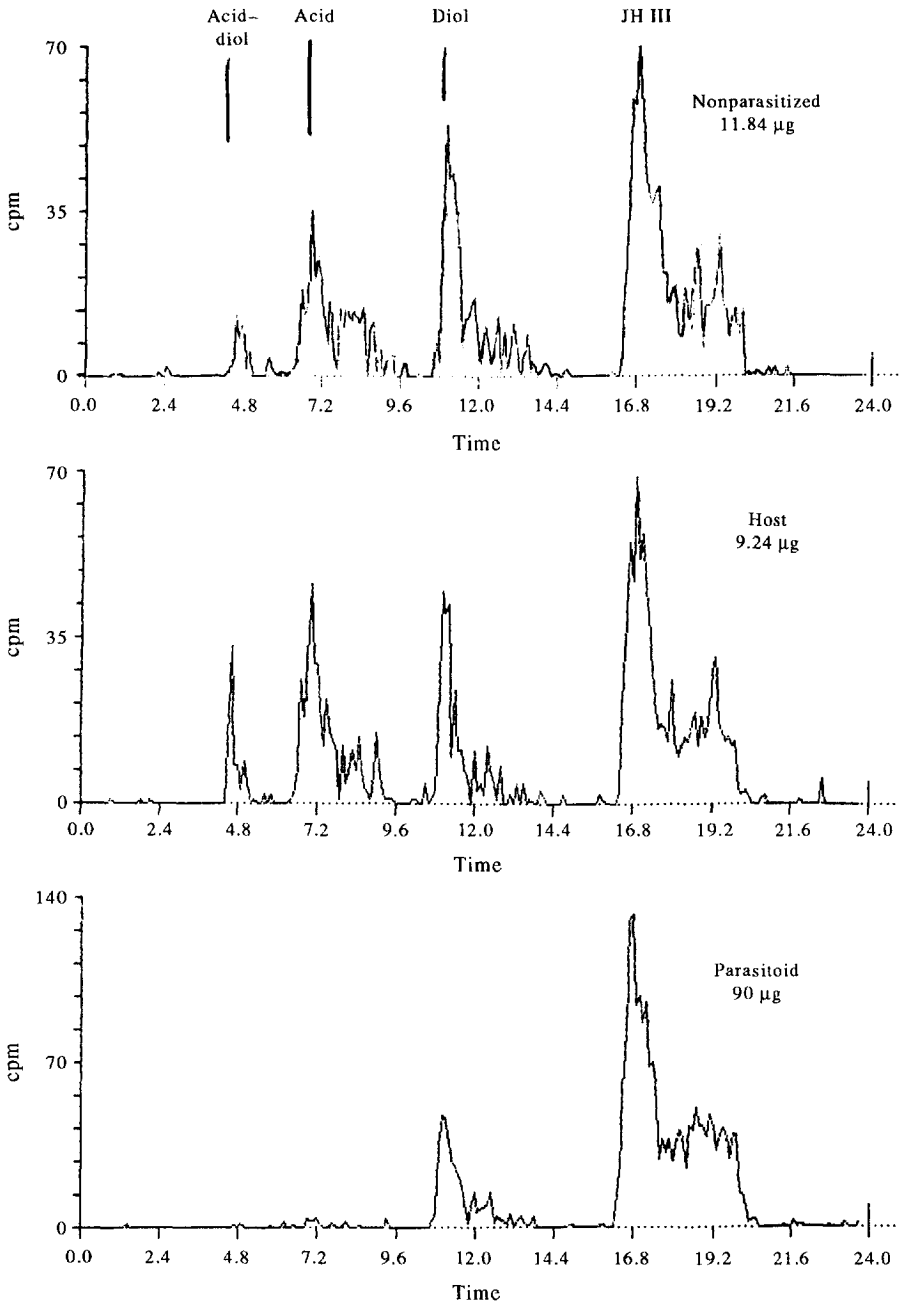

FIGURE 1. HPLC-radiochromatogram of JH III and metabolites observed after incubation of ^{3}H-JH III with homogenates of non-parasitized (top) and parasitized (middle) *S. exigua* at the stage of pupal cell formation and of second instar *C. inanitus* larvae dissected out of the latter (bottom). The retention times of coinjected reference substances JH III, JH III diol, JH III acid and JH III acid–diol are indicated on the top. From each homogenate three dilutions were tested in the assay and we show only the result of one dilution; the protein concentration in µg BSA equiv. is indicated in each panel.

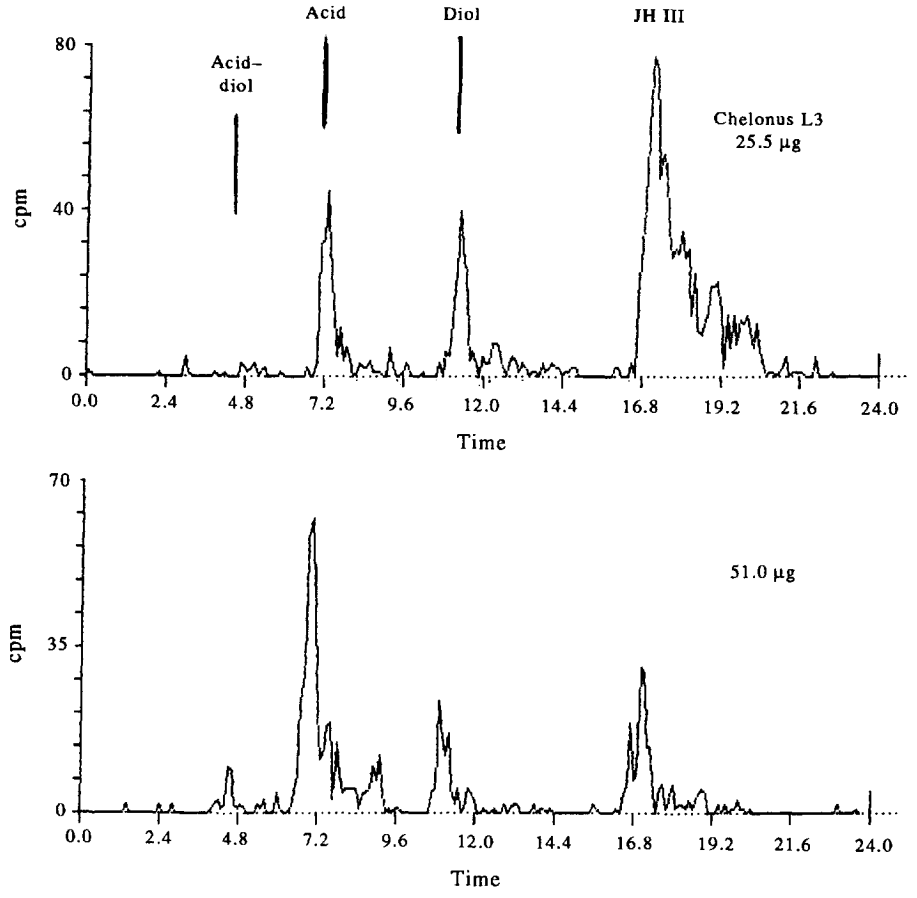

FIGURE 2. HPLC-radiochromatogram of JH III and metabolites observed after incubation of ^{3}H-JH III with homogenate of external third instar parasitoid larva (fresh weight of 17.7 mg). The results of two concentrations, namely 25.5 and 51 µg BSA equiv. are shown. The retention times of coinjected references are indicated on the top.

difference between non-parasitized and parasitized eggs 24 h after parasitization (host embryos are at 2/3 of embryonic development) and 48 h after parasitization (host embryos are shortly before harching); JH III diol was the major metabolite being formed. The comparison between the stage 24 and 48 h after parasitization shows that JH III was more efficiently metabolized in the former. This and additional analyses gave specific JH III metabolizing activites of 20–22 pmol/min/mg BSA equiv. for the stage 24 h after parasitization and 6–7 pmol/min/mg BSA equiv. for the stage 48 h after parasitization. Also in newly hatched first instar larvae no difference between parasitized and non-parasitized larvae was seen (data not shown) and specific JH III metabolizing activity was 12–14 pmol/min/mg BSA equiv. for both.

DISCUSSION

After incubation of racemic ^{3}H-JH III in close to physiological concentrations with homogenates of non-parasitized or parasitized larvae of *S. exigua* JH III acid, JH III diol and JH III acid–diol were the major metabolites being formed (Fig. 1), and with hemolymph only JH III acid was formed (data not shown). This reflects the presence of JH esterase and JH epoxide hydrolase in tissues and the presence of only JH esterase in hemolymph. Similar observations have also been made with other species [reviewed in Hammock (1985)] and Slade and Zibitt (1972) were the first to show the co-occurrence of JH esterase and JH epoxide hydrolase in tissues. With homogenates of *C. inanitus* almost exclusively JH III diol was formed during the internal phase of larval development (Fig. 1 and data not shown), but after emergence from the host JH III acid, JH III diol and JH III acid–diol were formed (Fig. 2 and data not shown). Also, other *in vitro* investigations with whole body or tissue homogenates of various species revealed that the relative importance of the two enzyme systems JH esterase and JH epoxide hydrolase varies extensively between species and developmental stages (Slade and Zibitt, 1972; Ajami and Riddiford, 1973; Slade and Wilkinson, 1974; Slade *et al.*, 1976; Wing *et al.*, 1981; Hammock, 1985; Share *et al.*, 1988; Jesudason *et al.*, 1992).

We are aware that co-chromatography with authentic standards never proves chemical identity. However, co-chromatography on a reversed phase HPLC system and co-chromatography on normal phase TLC systems gives us confidence in our tentative assignments of the JH metabolites.

With homogenates of eggs of *S. exigua* JH III diol was

by far the predominant metabolite being formed (Fig. 4); similar observations were made with homogenates of eggs of *Manduca sexta* (Share *et al.*, 1988). In *S. exigua*, rates of JH III degradation were much higher at the stage 2/3 of embryonic development than shortly before hatching of the first instar larvae (Fig. 4 and data not shown). The quality and quantities of JHs have not been determined in *S. exigua* but in the closely related species, *S. littoralis*, JH II and JH I together with small quantities of JH III were found in eggs and their titers were seen to be highest at 1/2 to 2/3 of embryonic development and to drop towards hatching of the first instar larvae (Lanzrein and Rembold, personal communication). A similar fluctuation in JH titer was seen in eggs of *Heliothis virescens* and *M. sexta*, where the natural JHs are JH I, JH II and JH 0 and JH 0, iso-JH 0, JH I and JH II respectively (Bergot *et al.*, 1981) and also in eggs of *T. ni* where JH I and JH II were tentatively identified (Grossniklaus-Bürgin and Lanzrein, 1990b). Thus, there is similarity with respect to titer fluctuations in eggs of these various lepidopterous species. However, with respect to changes in rates of JH degradation by egg homogenates the results obtained with *S. exigua* and *M. sexta* are dissimilar. In *M. sexta*, rates of JH degradation remained at a similar level until hatching (Share *et al.*, 1988) but the rates declined in *S. exigua* (Fig. 4).

It is not clear to what extent *in vitro* measurements of JH metabolism by homogenates allow extrapolation to the *in vivo* situation. Up to now only a few *in vivo* studies have been carried out; recent results obtained with last instar larvae of *M. sexta* indicate that a diol–phosphate conjugate represents a major metabolite *in vivo* (Halarnkar *et al.*, 1993). This is in addition to JH III acid and JH III acid–diol, the major metabolites found after *in vitro* incubation [reviewed in Hammock (1985)]. In the short *in vitro* incubations with *S. exigua* homogenates there was no indication of conjugate formation (Fig. 1). As measurements of recovery in acetonitrile supernatant and pellet showed that only very little radioactivity remained with the pellet and that more than 95% was in the supernatant we assume to have analyzed all major metabolites by HPLC or TLC. It is also not clear to what extent the use of the natural JH (10R-JH II for the host, 10R-JH III for the parasitoid) as substrate would influence the outcome of the experiments. In a cockroach differential degradation of racemic and 10R-JH III has been observed (Lanzrein *et al.*, 1993).

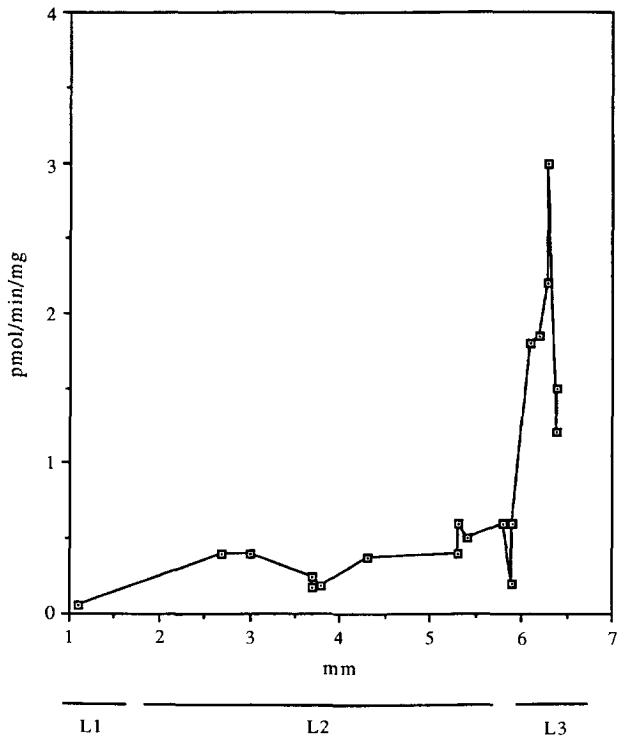

FIGURE 3. Specific JH III degrading activity (pmol/min/mg protein) of homogenates of *C. inanitus* late first (L1), second (L2) and third (L3) instar larvae. Abscissa: length of parasitoid in mm. Shortly after the molt into the third instar the parasitoids emerge from the host.

The pattern of JH III metabolism was almost identical in homogenates of non-parasitized sixth instar larvae and of parasitized fifth instar larvae of *S. exigua* at the stage of pupal cell formation (Fig. 1). In non-parasitized penultimate (=fifth) instar larvae JH degradation was very low (data not shown). This shows that precocious metamorphosis induced by parasitism by *C. inanitus* is associated with the same premetamorphic changes in JH metabolism as seen in normal metamorphosis. There was also great similarity with respect to JH and ecdysteroid titer fluctuations between parasitized precocious last instar larvae and non-parasitized last instar larvae in the *T. ni*/*Chelonus* sp. system (Grossniklaus-Bürgin and Lanzrein, 1990a) and the same holds true for hemolymph JH esterase fluctuations (Bühler *et al.*, 1985). All these observations suggest that parasitism by wasps of the genus *Chelonus* causes premetamorphic changes of the host's endocrine system through manipulation of a central step in metamorphosis induction. The fact that JH III metabolism was almost identical in homogenates of non-parasitized and parasitized eggs at 2/3 of embryonic development and shortly before hatching of the first instar larvae (Fig. 4) and also in newly hatched first instar larvae (data not shown) indicates that parasitism does not influence JH metabolism in homogenates either qualitatively nor quantitiatively at these stages.

In homogenates of parasitoid larvae JH III metabolizing activity was very low during the internal phase of development (Fig. 1 and Fig. 3) and the almost exclusive metabolite formed was JH III diol (Fig. 1). *Chelonus inanitus* was seen to contain exclusively JH III and its titer is high in second instar larvae and reaches values of 5–6 ng/g (Steiner, Lanzrein and Rembold, personal communication). Also in *Chelonus* sp. JH III was seen to reach high levels in second instar larvae (Grossniklaus-Bürgin and Lanzrein, 1990a). After emergence from the host JH III metabolic activity of homogenates increased and esterases became active (Fig. 2 and Fig. 3). A comparison between parasitoid and host revealed that specific JH III degrading activities were always much lower in homogenates of parasitoid larvae (0.06–0.6 pmol/min/mg BSA equiv.) than in homogenates of hosts (6–14

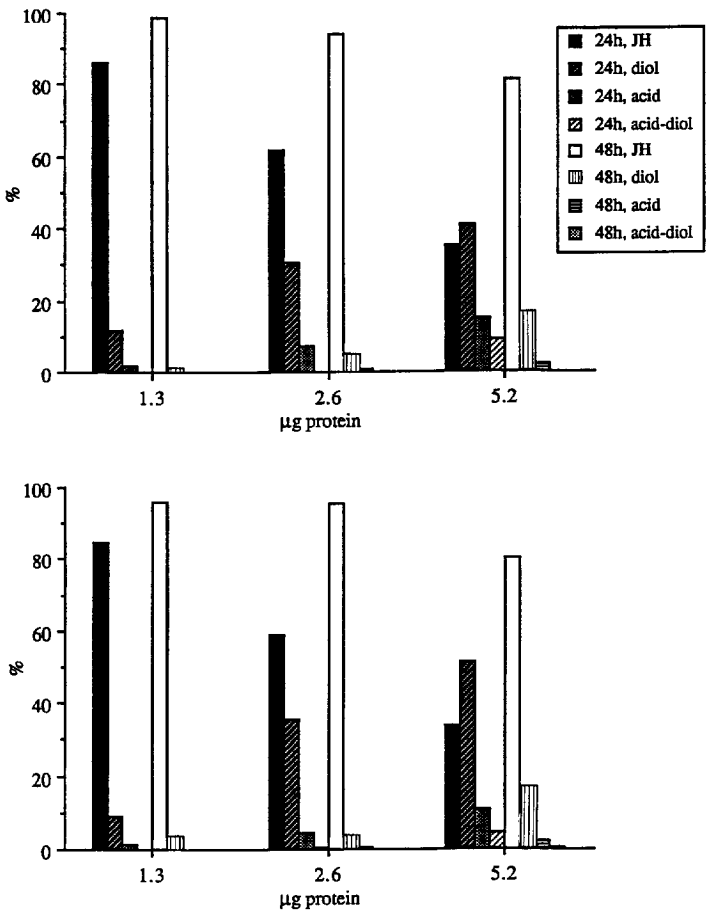

FIGURE 4. JH III and metabolites observed after incubation of ^{3}H-JH III with homogenates of non-parasitized (top) and parasitized (bottom) eggs of *S. exigua* 24 and 48 h after parasitization. Bars represent percentage of substrate and of metabolites found after incubation with three different concentrations of homogenate which are indicated in µg protein below the bars.

pmol/min/mg BSA equiv.) (Fig. 1 and Fig. 3 and data not shown). In the honey bee *Apis mellifera*, the only other hymenopteran where JH degradation studies have been carried out, JH I degrading activity was undetectable in hemolymph of third and fourth instar larvae and very low in fifth instar larvae and pupae; homogenate specific degrading activities between 0.1 and 0.8 pmol/min/mg fresh weight were determined for second instar larvae to pupae (Mane and Rembold, 1977). Calculation on a per mg protein basis yields approximate values between 0.02 and 0.16 pmol/min/mg protein which are similar to the ones obtained with internal stages of *C. inanitus* (Fig. 3). Other investigations with the honey bee also indicated very low JH I degrading activities both in hemolymph (De Kort *et al.*, 1979) and *in vivo* where formation of JH I acid, JH I diol and JH I acid–diol was observed (De Kort *et al.*, 1977). These observations suggest that JH degradation is generally low in larval Hymenoptera regardless of whether they are free-living or endoparasitic.

REFERENCES

Ajami A. M. and Riddiford L. M. (1973) Comparative metabolism of the Cecropia juvenile hormone. *J. Insect Physiol.* **19,** 635–645.

Anderson R. J., Henrich C. A., Siddall J. B. and Zurflüh R. (1972) Stereoselective synthesis of the racemic C-17 juvenile hormone of Cecropia. *J. Am. Chem. Soc.* **94,** 5379–5386.

Beckage N. E. (1985) Endocrine interactions between endoparasitic insects and their hosts. *A. Rev. Ent.* **30,** 371–413.

Beckage N. E. and Templeton T. J. (1986) Physiological effects of parasitism by *Apanteles congregatus* in terminal-stage tobacco hornworm larvae. *J. Insect. Physiol.* **32,** 299–314.

Bergot B. J., Baker F. C., Cerf D. C., Jamieson G. and Schooley D. A. (1981) Qualitative and quantitative aspects of juvenile hormone titers in developing embryos of several insect species: discovery of a new JH-like substance extracted from eggs of *Manduca sexta*. In *Juvenile Hormone Biochemistry* (Eds Pratt G. E. and Brooks G. T.), pp. 33–45. Elsevier/North–Holland Biomedical Press, Amsterdam.

Bradford M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72,** 248–254.

Bühler A., Hanzlik T. N. and Hammock B. D. (1985) Effects of parasitization of *Trichoplusia ni* by *Chelonus* sp. *Physiol. Ent.* **10,** 383–394.

De Kort C. A. D., Wieten M. and Kramer S. J. (1979) The occurrence of juvenile hormone specific esterases in insects. A comparative study. *Proc. Konin. Ned. Akad.* **82,** 325–331.

De Kort C. A. D., Wieten M., Kramer S. J. and Goewie E. (1977) Juvenile hormone degradation and carrier proteins in honey bee larvae. *Proc. Konin. Ned. Akad.* **80,** 297–301.

Grossniklaus-Bürgin C. and Lanzrein B. (1990a) Endocrine interrelationship between the parasitoid *Chelonus* sp. and its host *Trichoplusia ni*. *Arch. Insect Biochem. Physiol.* **14,** 201–216.

Grossniklaus-Bürgin C. and Lanzrein B. (1990b) Qualitative and quantitative analysis of juvenile hormone and ecdysteroids from the

egg to the pupal molt in *Trichoplusi ni*. *Arch. Insect Biochem. Physiol.* **14,** 13–30.

Grossniklaus-Bürgin C., Wyler T., Pfister-Wilhelm R. and Lanzrein B. (1994) Biology and morphology of the parasitoid *Chelonus inanitus* (Braconidae, Hymenoptera) and effects on the development of its host *Spodoptera littoralis* (Noctuidae, Lepidoptera). *Invert. Reprod. Dev.* **25,** 143–158.

Halarnkar P. P., Jackson G. P., Straub K. M. and Schooley D. A. (1993) Juvenile hormone catabolism in *Manduca sexta*: homologue selectivity of catabolism and identification of a diol–phosphate conjugate as a major end product. *Experientia* **49,** 988–994.

Halarnkar P. P. and Schooley D. A. (1990) Reversed-phase liquid chromatographic separation of juvenile hormone and its metabolites, and its application for an *in vivo* juvenile hormone metabolism study in *Manduca sexta*. *Analyt. Biochem.* **188,** 394–397.

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

Hammock B. D. and Roe R. M. (1985) Analysis of juvenile hormone esterase activity. In *Methods in Enzymology, Vol. III, Steroids and Isoprenoids, Part B* (Eds Law J. H. and Rilling H. C.), pp. 487–494. Academic Press, Orlando, FL.

Jesudason P., Anspaugh D. D. and Roe R. M. (1992) Juvenile hormone metabolism in the plasma, integument, midgut, fat body, and brain during the last instar of the tobacco hornworm, *Manduca sexta* (L.). *Arch. Insect Biochem. Physiol.* **20,** 87–105.

Jones D., Jones G. and Hammock B. D. (1981) Developmental and behavioral responses of larval *Trichoplusia ni* to parasitization by an imported braconid parasite *Chelonus* sp. *Physiol. Ent.* **6,** 386–394.

Jones G., Hanzlik T., Hammock B. D., Schooley D. A., Miller C. A., Tsai L. W. and Baker F. C. (1990) The juvenile hormone titre during the penultimate and ultimate larval stadia of *Trichoplusia ni*. *J. Insect Physiol.* **36,** 77–83.

Lanzrein B., Wilhelm R. and Riechsteiner R. (1993) Differential degradation of racemic and 10R-juvenile hormone III by cockroach (*Nauphoeta cinerea*) haemolymph and the use of lipophorin for long-term culturing of corpora allata. *J. Insect Physiol.* **39,** 53–63.

Lawrence P. O., Baker F. C., Tsai L. W., Miller C. A., Schooley D. A. and Geddes L. G. (1990) JH III levels in larvae and pharate pupae of *Anastrepha suspensa* (Diptera: Tephritidae) and in larvae of the parasitic wasp *Biosteres longicaudatus* (Hymenoptera: Braconidae). *Arch. Insect Biochem. Physiol.* **13,** 53–62.

Lawrence P. O. and Lanzrein B. (1993) Hormonal interactions between insect endoparasites and their host insects. In *Parasites and Pathogens of Insects* (Eds Beckage N. E., Thompson S. N. and Federici B. A.), Vol. 1, pp. 59–86. Academic Press, San Diego, CA.

Mane S. D. and Rembold H. (1977) Developmental kinetics of juvenile hormone inactivation in queen and worker castes of the honey bee, *Apis mellifera*. *Insect Biochem.* **7,** 463–467.

Meyer W. R. and Lanzrein B. (1989) Degradation of juvenile hormone and methylation of juvenile hormone acid by corpora cardiaca–corpora allata of the cockroach, *Nauphoeta cinerea*: I. Biochemical aspects. *Arch. Insect Biochem. Physiol.* **10,** 303–316.

Schopf A. (1984) Endokrinologische Untersuchungen an dem Wirt–Parasit-System: *Pieris brassicae–Apanteles glomeratus*. *Ent. exp. Appl.* **36,** 265–272.

Schopf A. and Rembold H. (1993) Changes in juvenile hormone titer of gypsy moth larvae by parasitism of *Glyptapanteles liparidis*. *Naturwiss.* **80,** 527–528.

Share M. R., Venkatesh K., Jesudason P. and Roe R. M. (1988) Juvenile hormone metabolism during embryogenesis in the tobacco hornworm *Manduca sexta*. *Arch. Insect Biochem. Physiol.* **8,** 173–186.

Slade M., Hetnarski H. K. and Wilkinson C. F. (1976) Epoxide hydrase activity and its relationship to development in the southern armyworm *Prodenia eridania*. *J. Insect Physiol.* **22,** 619–622.

Slade M. and Wilkinson C. F. (1974) Degradation and conjugation of Cecropia juvenile hormone by the southern armyworm (*Prodenia eridania*). *Comp. Biochem. Physiol. B* **49,** 99–104.

Slade M. and Zibitt C. H. (1972) Metabolism of Cecropia juvenile hormone in insects and mammals. In *Insect Juvenile Hormones: Chemistry and Action* (Eds Menn J. J. and Beroza M.), pp. 155–176. Academic Press, New York.

Strand M. R., Goodman W. G. and Baehrecke E. H. (1991) The juvenile hormone titer of *Trichoplusia ni* and its potential role in embryogenesis of the polyembryonic wasp *Copidosoma floridanum*. *Insect Biochem.* **21,** 205–214.

Vinson S. B. and Iwantsch G. F. (1980) Host regulation by insect parasitoids. *Q. Rev. Biol.* **55,** 143–165.

Wing K. D., Sparks T. C., Lovell V. M., Levinson S. O. and Hammock B. D. (1981) The distribution of juvenile hormone esterase and its interrelationship with other proteins influencing juvenile hormone metabolism in the cabbage looper, *Trichoplusia ni*. *Insect Biochem.* **11,** 473–485.

Zhang D., Dahlman D. L. and Gelman D. B. (1992) Juvenile hormone esterase activity and ecdysteroid titer in *Heliothis virescens* larvae injected with *Microplitis croceipes* teratocytes. *Arch. Insect Biochem. Physiol.* **20,** 231–242.

Acknowledgements—We would like to express our thanks to Mrs Wan Yuan for help in culturing *S. exigua* and Dr Jaffar Nourooz-Zadeh for technical help and stimulating discussions. Financial support by the Swiss National Science Foundation (grants 31-27723.89 and 31-32329.91) to B. L. and by the U.S. National Science Foundation (grant DCB 91-19332) and U.S. Department of Agriculture (grant 91-37302-6185) to B. H. is gratefully acknowledged.