

Ovarian and Hemolymph Ecdysteroid Titers during Vitellogenesis in *Macrobrachium rosenbergii*

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Changes in ovarian and hemolymph ecdysteroid concentration and composition during vitellogenesis have been investigated in the freshwater prawn *Macrobrachium rosenbergii*. Free ecdysteroids (20-hydroxyecdysone and ecdysone) in hemolymph increased in concentration during vitellogenesis from zero at stage 0 to 1.5 ng/ml at stage I to 7.3 ng/ml in mature, stage IV animals. 20-Hydroxyecdysone (1.2 ng/ml) was detected in the stage IV hemolymph. Ovarian-free ecdysteroid concentration, expressed as nanograms per gram of tissue, fell during vitellogenesis from 83.2 ng/g at stage 0, non-pigmented tissue to 14.2 ng/g at stage IV tissue, being minimal at stage I (6.3 ng/g). However, expression of ovarian free ecdysteroid content as nanograms per ovary revealed a rise from 7.7 ng/ovary at stage 0, nonpigmented tissue to 28.3 ng/ovary at stage IV, again being minimal at stage I (2.0 ng/ovary). 20-Hydroxyecdysone and ecdysone were identified at ovarian stages II-III and stage IV. © 1993 Academic Press, Inc.

It has been proposed that, in addition to neuropeptides (see Charniaux-Cotton and Payen, 1988) and juvenoids (see Laufer and Borst, 1988), ecdysteroids may be involved in the regulation of crustacean vitellogenesis. This proposal is based, in part, on the evident analogy between the endocrine systems of crustaceans and insects. Ecdysteroids are molting hormones in both insects (see Sehna, 1989) and crustaceans (see Chang and O'Connor, 1988) and there is evidence that ecdysteroids are involved in the regulation of yolk protein synthesis in insects (Bownes, 1989).

Ecdysteroids can be detected in the adults of evolutionarily advanced orders which do not molt, indicating other roles for ecdysteroids in the adult (see Hagedorn, 1989). The role of ecdysteroids in insect ovarian development has been studied in detail in *Drosophila melanogaster* and *Aedes aegypti*. In *Aedes*, a blood meal trig-

gers the release of egg-development neurosecretory hormone from the brain, which stimulates ovarian production of 20-hydroxyecdysone to, in turn, stimulate yolk protein synthesis by the fat body (see Hagedorn, 1985, 1989). Similarly, in *Drosophila*, both the fat body and ovary synthesize yolk protein, and the fat body yolk protein gene transcription is stimulated by 20-hydroxyecdysone (see Bownes, 1989). Ovarian and hemolymph ecdysteroid concentrations increase during ovarian development in the majority of insects studied, for example, *Sarcophaga bullata* (Briers and DeLoof, 1980), and four species of blowfly (Briers *et al.*, 1983): *Calliphora vomitoria* (Campan *et al.*, 1985), *Phormia regina* (Yin *et al.*, 1990), *Glossina fuscipes* (Robert *et al.*, 1991), and *Libidura riparia* (Sayah *et al.*, 1991).

There is little information on the role of ecdysteroids in the regulation of vitellogenesis in crustaceans. Several groups have investigated the changes in ovarian and/or hemolymph ecdysteroid concentration during

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vitellogenesis. In the shore crab, *Carcinus maenas*, ovarian and hemolymph ecdysteroid levels markedly increase during vitellogenesis and fall prior to spawning, with levels of 20-hydroxyecdysone and ponasterone A reaching a peak toward the end of vitellogenesis (stage IV) and levels of ecdysone being maximal slightly earlier (Lachaise and Hoffmann, 1977; Lachaise *et al.*, 1981). In *Orchestia gammarellus*, Blanchet *et al.* (1979) observed low ecdysteroid levels in whole body extracts, ovary, and hemolymph during vitellogenesis and a rapid increase in ecdysteroid titers at the end of vitellogenesis, indicating that vitellogenin synthesis is independent of ecdysteroid levels. In *Artemia*, a peak in whole body ecdysone concentration occurs during primary vitellogenesis, but ecdysone levels decrease during secondary vitellogenesis (Walgraeve *et al.*, 1986, 1988). Laufer *et al.* (1988) reported that hemolymph ecdysteroid levels are low and do not fluctuate markedly during vitellogenesis in *Libinia emarginata*, and in *Acanthonyx lunulatus* ovarian ecdysteroid concentrations are high only at the beginning of vitellogenesis and at the end of oocyte maturation (Chaix and DeReggi, 1982).

Young *et al.* (1993) recently described the changes in ovarian and hemolymph ecdysteroid titers during vitellogenesis in *Penaeus monodon*. Ovarian and hemolymph free ecdysteroid, predominantly 20-hydroxyecdysone, was maximal in animals with immature (stage 0) ovary, decreased gradually during early vitellogenesis (stage 0 to stage I) and then fell sharply during later vitellogenesis (stage I to stage IV). Polar ecdysteroids, identified as 20-hydroxyecdysone 22-phosphate and 20-hydroxyecdysone 22-oic acid, were detected only in the immature tissue.

Wilder *et al.* (1991) also reported that, in *Macrobrachium rosenbergii*, ovarian ecdysteroid concentration increased during a reproductive molt cycle from 1–2 ng/g tissue during stages C4–D0 to 22.9 ng/g at premolt

stage D₃, compared to 11.4 ng/g at stage D₃ of a nonreproductive molt cycle. Following reproductive molting, peak ecdysteroid concentrations were maintained until spawning. These results indicated that ecdysteroids accumulated in the ovaries during yolk protein uptake.

As part of an investigation into hormonal control of ovarian development in prawns, the changes in titer and identities of ecdysteroids during vitellogenesis in *M. rosenbergii*, have been studied in the ovary and hemolymph.

MATERIALS AND METHODS

Experimental animals. Adult *M. rosenbergii* were maintained in large tanks of constantly circulated fresh water at 23°C and fed daily on minced mussel. The vitellogenic stage of the animals was determined on the basis of the size of the ovary and the degree of lobe development and pigmentation. Stage 0 ovaries occurred in two forms, nonpigmented and pigmented, which were analyzed separately. Hemolymph and ovary samples were stored at –20°C prior to extraction.

Extraction and purification of ecdysteroids. Ovaries and hemolymph from animals at the same stage of vitellogenesis (3–6 animals depending on the stage) were pooled. Crude lipid extracts were obtained and free ecdysteroids, apolar conjugates of ecdysteroids, polar conjugates of ecdysteroids, and ecdysteroid 26-oic acids were purified from the extracts as described previously (Young *et al.*, 1991).

Hydrolysis of ecdysteroid conjugates. Half of each ecdysteroid conjugate fraction was enzymically hydrolyzed and the resulting free ecdysteroids released from the conjugates and any conjugates refractory to hydrolysis were isolated from the incubation mixture on a C₁₈ Sep Pak cartridge (Waters Assoc.) (Young *et al.*, 1991).

Methylation of ecdysteroid 26-oic acids. Ecdysteroid 26-oic acids were methylated using diazomethane in ether as described by Isaac *et al.* (1983).

Radioimmunoassay (RIA). All radioimmunoassays used ecdysone as standard with bound and unbound [23,24-³H] ecdysone (NEN Ltd.) being separated by ammonium sulfate precipitation (Mendis *et al.*, 1983). Two complementary antisera were employed: (A) H-22 (a gift from Professor L. I. Gilbert, Univ. of North Carolina) which was produced on immunization with ecdysone 22-succinylthyroglobulin amide and shows greatest specificity toward the ecdysone nucleus (Warren and Gilbert, 1986); and (B) DHS 1-13.5 (a gift from Professor J. D. O'Connor, Univ. of Cali-

formia) which was produced on immunization with 20-hydroxyecdysone 2-succinylthroglobulin amide and shows greatest specificity for the ecdysteroid side chain (Soumoff *et al.*, 1981).

Reversed-phase high-performance liquid chromatography (HPLC). HPLC was performed as described by Young *et al.* (1991), ecdysteroids being monitored at 254 nm. The following linear gradient (30 min) solvent systems were employed, all at a flow rate of 1 ml/min: system 1, methanol:water (35:65 v/v) increasing to methanol:water (6:4 v/v); system 2, methanol:0.2 M citrate buffer, pH 6.5 (25:75 v/v) increasing to methanol:0.2 M citrate buffer, pH 6.5 (75:25 v/v); system 3, methanol:water (9:1 v/v) increasing to pure methanol.

Gas chromatography/mass spectrometry (GC/MS). Free ecdysteroids were derivatized to form their trimethylsilyl (TMS) ethers and analyzed by GC/MS with selected ion-monitoring (SIM) as described previously (Evershed *et al.*, 1987; Young *et al.*, 1991).

RESULTS

Ecdysteroids in the Ovary of M. rosenbergii

The amounts of free and conjugated ecdysteroid detected by RIA of the ovary extracts are summarized in Table 1. Free ecdysteroid levels were maximal in stage 0 nonpigmented ovary (81.9 ng/g) and fell during early vitellogenesis to 6.9 ng/g in stage I ovary. Levels then increased gradually to 16.0 ng/g in stage IV tissue. Apolar conjugates of ecdysteroids, estimated both

as the intact compounds (2.2 ng/g) and as the free ecdysteroids released after hydrolysis of the conjugates (0.9 ng/g) were detected only in stage IV tissue. Polar conjugates of ecdysteroids were detected in the more mature (stages II–III and stage IV) tissue.

Portions (ca. 1 ng) of the free ecdysteroid fractions were analyzed by HPLC–RIA (solvent system 1, H-22 antiserum) and, in the case of the extract from stages II–III ovary, a second portion was also chromatographed and analyzed by RIA with the DHS 1-13.5 antiserum. A representative HPLC immunoreactivity profile for stage II–III ovary is shown in Fig. 1. In all cases, except the stage IV tissue extract, analysis with the H-22 antiserum revealed peaks of immunoreactivity corresponding to ecdysone and 20-hydroxyecdysone (Table 1). In the case of the stage IV ovary extract, one peak of immunoreactivity corresponding to 20-hydroxyecdysone was observed. The immunoreactivity profile for the stage II–III extract analyzed by the DHS 1-13.5 antiserum closely agreed with that from the analysis with the H-22 antiserum (Fig. 1), indicating that no ecdysteroids with modifications of the ecdysone nucleus occurred in the ovarian extract.

Further portions (ca. 1 ng with 1 ng mak-

TABLE 1
ECDYSTEROIDS DETECTED BY RIA IN THE OVARIES OF *M. rosenbergii* DURING VITELLOGENESIS

Stage of vitellogenesis	Apparent ecdysteroids in crude fractions [ecdysone equivalents (ng/g wet wt tissue)] ^a							Summary of individual ecdysteroids ^b (ng/g wet wt tissue)			
	1	2	3	4	5	6	7	20E	E	20EOIC	EOIC
0-NP	81.9	—	—	—	—	—	—	64.5	18.7	—	—
0-P	29.9	—	—	—	—	—	—	31.0	4.0	—	—
I	6.9	—	—	—	—	—	—	5.9	0.4	—	—
II–III	8.8	—	—	—	2.8	1.2	1.8	8.1	0.2	1.9	0.1
IV	16.0	2.2	0.9	5.4	1.4	1.3	2.0	14.2	—	4.3	0.7

Note. (—) Not detected; NP, nonpigmented; P, pigmented.

^a 1, free ecdysteroid; 2, apolar conjugates; 3, free ecdysteroid released from apolar conjugates; 4, apolar conjugates refractory to hydrolysis; 5, polar conjugates and ecdysteroid-26 oic acids; 6, free ecdysteroid released from polar conjugates; 7, polar conjugates refractory to hydrolysis and ecdysteroid 26-oic acids.

^b 20E, 20-hydroxyecdysone; E, ecdysone; 20EOIC, 20-hydroxyecdysoneic acid; EOIC, ecdysoneic acid.

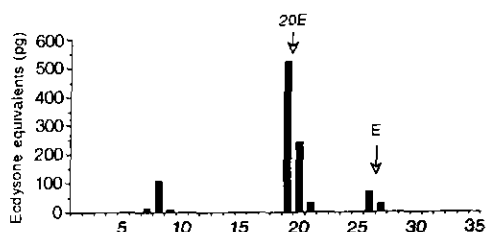


FIG. 1. Immunoreactivity profiles for free ecdysteroids from stages II-III ovary of *M. rosenbergii*, separated on HPLC system 1, and assayed with the H-22 antiserum. Positions of elution of authentic ecdysteroids are shown as E (ecdysone) and 20E (20-hydroxyecdysone).

isterone A added as internal standard) of the free ecdysteroid fractions were analyzed by GC/MS(SIM). On analysis of the extracts from stages 0, 1, and II-III samples, peaks were observed in the m/z 561 and m/z 567 mass chromatograms which co-chromatographed with 20-hydroxyecdysone and ecdysone, respectively. On analysis of the stage IV ovarian extract, a peak in the m/z 561 mass chromatogram with a retention time (RT) consistent with 20-hydroxyecdysone was observed, but no ecdysone was detected. Representative GC/MS data are shown in Fig. 2. The 20-hydroxyecdysone and ecdysone were quantified (A) directly from the RIA data

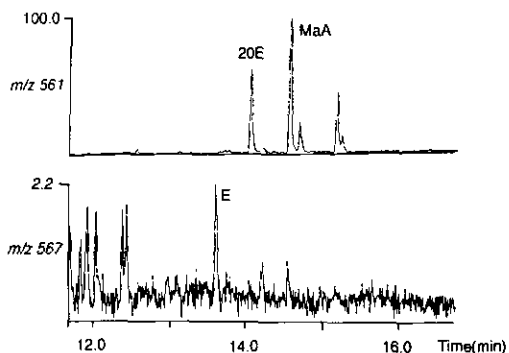


FIG. 2. Mass chromatograms of m/z 561 and m/z 567 ions from the GC/MS(SIM) analysis of TMS ethers of free ecdysteroids from stage 0 (nonpigmented) ovary of *M. rosenbergii*. Retention times of authentic ecdysteroid TMS ethers are shown as E (ecdysone), 20E (20-hydroxyecdysone), and MaA (makisterone A, internal standard).

taking into account the cross-reactivity of 20-hydroxyecdysone with the H-22 antiserum, and (B) from the GC/MS data by reference to the internal standard. There was agreement between the values for ovarian free ecdysteroid concentration determined by the two techniques.

Portions of the intact polar conjugates from stages II-III and stage IV ovary were analyzed by HPLC-RIA (solvent system 2, H-22 antiserum). A representative immunoreactivity profile is given in Fig. 3A. In both

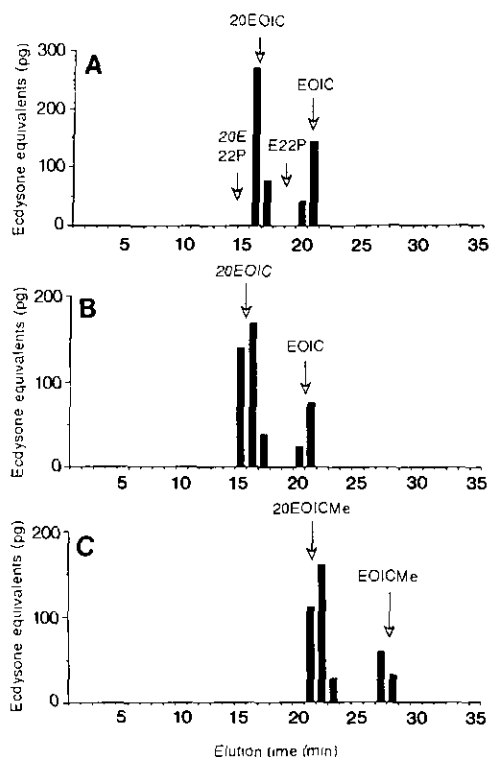


FIG. 3. Immunoreactivity profiles for (A) intact polar conjugates and ecdysteroid 26-oic acids, (B) ecdysteroid 26-oic acids and any polar conjugates refractory to hydrolysis, and (C) methylated ecdysteroid 26-oic acids, from stages II-III ovary of *M. rosenbergii*. All extracts were separated on HPLC system 2 and assayed with the H-22 antiserum. Positions of elution of authentic ecdysteroids are shown as 20E22P (20-hydroxyecdysone 22-phosphate), E22P (ecdysone 22-phosphate), 20EOIC (20-hydroxyecdysone 26-oic acid), EOIC (ecdysone 26-oic acid), 20EOICMe (20-hydroxyecdysone 26-oic acid methyl ester), and EOICMe (ecdysone 26-oic acid methyl ester).

cases, peaks of immunoreactivity corresponding to 20-hydroxyecdysone and ecdysone were observed. GC/MS analysis of the free ecdysteroid released on hydrolysis of the polar conjugates did not detect any ecdysteroid. The presence of the acids was further confirmed by HPLC-RIA (solvent system 2, H-22 antiserum) of portions (2×1 ng) of the polar conjugate refractory to hydrolysis and ecdysteroid 26-oic acids fraction, one portion of which had been methylated. In the non-methylated portion, peaks of immunoreactivity corresponding to 20-hydroxyecdysone and ecdysone were observed (Fig. 3B) and this was confirmed by detection of peaks of immunoreactivity corresponding to the methylated acids in the methylated portion of the extract (Fig. 3C). The ecdysteroid acids were quantified directly from the RIA data, taking into account their cross-reactivities with the H-22 antiserum (Warren and Gilbert, 1986).

HPLC-RIA analysis (solvent system 3, H-22 antiserum) of the intact apolar conjugates from the stage IV ovary failed to detect any immunoreactivity with a retention time (RT) consistent with that of ecdysteroid fatty acyl esters. It is probable that the high level of lipid contaminants in this extract had interfered with the RIA of the crude fraction, giving a misleadingly high figure for ecdysteroid content.

The levels of 20-hydroxyecdysone, ecdysone, and their corresponding 26-oic acids detected in the ovaries of *M. rosenbergii* during vitellogenesis are summarized in Table 1.

Ecdysteroids in the Hemolymph of M. rosenbergii

The amounts of free and conjugated ecdysteroid detected by RIA of the hemolymph extracts are summarized in Table 2. Immunoreactivity was not observed in any of the ecdysteroid-containing fractions from stage 0 hemolymph. The low levels of free ecdysteroids detected in stage I (1.3 ng/ml) and stages II-III (1.2 ng/ml) hemolymph, increased rapidly to 11.0 ng/ml in stage IV hemolymph. Ecdysteroid apolar conjugates (2.0 ng/ml) and polar conjugates (5.6 ng/ml) were only observed in stage IV hemolymph.

Portions (ca. 1 ng) of the free ecdysteroid fractions were analyzed by HPLC-RIA (solvent system 1, H-22 antiserum) and, in the case of the stage IV hemolymph extract, a second portion was also chromatographed and analyzed by RIA with the DHS 1-13.5 antiserum. In all cases, peaks of immunoreactivity cochromatographing with both ecdysone and 20-hydroxyecdysone were detected. Portions (ca. 1 ng plus 1 ng makisterone A as internal standard)

TABLE 2
ECDYSTEROIDS DETECTED BY RIA IN THE HEMOLYMPH OF *M. rosenbergii* DURING VITELLOGENESIS

Stage of vitellogenesis	Apparent ecdysteroids in crude fractions [ecdysone equivalents (ng/ml)] ^a							Summary of individual ecdysteroids (ng/ml) ^b		
	1	2	3	4	5	6	7	20E	E	20EOIC
0	—	—	—	—	—	—	—	—	—	—
I	1.3	—	—	—	—	—	—	0.6	0.9	—
II-III	1.2	—	—	—	—	—	—	0.7	1.4	—
IV	11.0	2.0	0.8	0.8	5.6	10.2	1.8	6.6	0.7	1.2

Note. (—) Not detected.

^a 1, free ecdysteroid; 2, apolar conjugates; 3, free ecdysteroid released from apolar conjugates; 4, apolar conjugates refractory to hydrolysis; 5, polar conjugates and ecdysteroid 26-oic acids; 6, free ecdysteroid released from polar conjugates; 7, polar conjugates refractory to hydrolysis and ecdysteroid 26-oic acids.

^b 20E, 20-hydroxyecdysone; E, ecdysone; 20EOIC, 20-hydroxyecdysone acid.

were also analyzed by GC/MS(SIM). Again, peaks were observed in the m/z 561 and m/z 567 mass chromatograms with RTs consistent with those of authentic 20-hydroxyecdysone and ecdysone, respectively. Comparison of ecdysteroid levels by HPLC-RIA and GC/MS(SIM) for hemolymph samples were in agreement.

A portion (ca. 1 ng) of the intact polar conjugate and ecdysteroid acid fraction from stage IV hemolymph was analyzed by HPLC-RIA (solvent system 2, H-22 antiserum). One peak of immunoreactivity was observed that cochromatographed with 20-hydroxyecdysone. GC/MS(SIM) of the corresponding free ecdysteroid released on hydrolysis of the polar conjugate fraction failed to detect any ecdysteroid, indicating that the polar ecdysteroid fraction did not contain any ecdysteroid conjugates. The presence of 20-hydroxyecdysone in stage IV hemolymph was further confirmed by HPLC-RIA analysis (solvent system 2, H-22 antiserum) of two portions (2×1 ng) of the polar conjugate refractory to hydrolysis and ecdysteroid acids fractions, one of which had been methylated. In the nonmethylated portion, one peak of immunoreactivity consistent with authentic 20-hydroxyecdysone was observed, and in the methylated fraction one peak of immunoreactivity corresponding to the methyl ester of 20-hydroxyecdysone was detected. The 20-hydroxyecdysone in stage IV hemolymph was quantified directly from the RIA data, taking into account its cross-reactivity with the H-22 antiserum (Warren and Gilbert, 1986).

A portion (ca. 1 ng) of the intact apolar conjugates fraction was analyzed by HPLC-RIA (solvent system 3, H-22 antiserum); however, no immunoreactivity was observed which corresponded to ecdysteroid fatty acyl esters. As was the case in the ovary, it appears that the high level of lipid contaminants in this extract may have interfered with the RIA to give a misleadingly high value for ecdysteroid content.

The levels of 20-hydroxyecdysone, ecdysone, and 20-hydroxyecdysone acid detected in the hemolymph of *M. rosenbergii* are summarized in Table 2.

DISCUSSION

Quite different trends in ecdysteroid concentration were observed in the ovary and hemolymph of *M. rosenbergii* during vitellogenesis. The free ecdysteroid concentrations were generally much higher in ovaries than in hemolymph (cf. Tables 1 and 2). In the ovary, levels of 20-hydroxyecdysone fell from 64.5 ng/g in stage 0 (nonpigmented) tissue to 14.2 ng/g in stage IV ovary (Table 1). Similarly, levels of ecdysone fell from 18.7 ng/g in the immature ovary to 0 ng/g in stage IV ovary. The situation was reversed in the hemolymph, where both 20-hydroxyecdysone and ecdysone could not be detected in stage 0 tissue, but rose to 6.6 ng/ml and 0.7 ng/ml, respectively, in stage IV tissue (Table 2), perhaps suggesting that the ovarian ecdysteroid present at stage 0 had either been synthesized in the ovary, or had been generated by hydrolysis of ovarian conjugates sequestered at a different stage of the cycle.

20-Hydroxyecdysone and ecdysone were detected at relatively low levels in stages II-III and stage IV ovary, and the former was also observed in stage IV hemolymph. It is possible that the ob-

TABLE 3
ECDYSTEROID CONTENT PER OVARY DURING
VITELLOGENESIS IN *M. rosenbergii*

Stage of vitellogenesis	Ecdysteroid (ng/ovary) ^a			
	20E	E	20EOIC	EOIC
0-NP	6.0	1.7	—	—
0-P	3.5	0.4	—	—
I	1.9	0.1	—	—
II-III	8.7	0.2	2.0	0.1
IV	28.3	—	8.6	1.4

Note. (—) Not detected.

^a 20E, 20-hydroxyecdysone; E, ecdysone; 20EOIC, 20-hydroxyecdysone acid; EOIC, ecdysone acid.

served decline in ovarian free ecdysteroids may, at least in part, result from their conversion into acids and subsequent release into the hemolymph for excretion.

In view of the substantial increase in ovary mass during vitellogenesis, ovarian values were also expressed as ng ecdysteroid/ovary (Table 3). When expressed in this way, an overall increase in ovarian 20-hydroxyecdysone content was observed from 6.0 ng/ovary in stage 0 (nonpigmented) tissue to 28.3 ng/ovary in stage IV tissue, being minimal in stage I tissue (1.9 ng/ovary). Ecdysone levels, however, fell from 1.7 ng/ovary in stage 0 (nonpigmented tissue) to 0 ng/g in stage IV tissue.

These results exhibit differences from those reported for the same species by Wilder *et al.* (1991), who observed an increase in ovarian free ecdysteroid content from 1–2 ng/g to 22.9 ng/g during vitellogenesis. In the present study, an increase in ovarian ecdysteroid content was only observed when the data were expressed as nanograms per ovary. Our observations, however, show similarities to those made in other species. In *O. gammarellus*, hemolymph ecdysteroid titers increase rapidly at the end of vitellogenesis (Blanchet *et al.*, 1979), as was observed in *M. rosenbergii*. The trends observed in the ovary of *M. rosenbergii* resemble those reported for *A. lunulatus*, where a decrease in ovarian ecdysteroid titer (expressed as pmol/mg) was observed during vitellogenesis (Chaix and DeReggi, 1982). However, in this species, a large increase in ecdysteroid titer was detected at the end of vitellogenesis, whereas 20-hydroxyecdysone levels only increased slightly. In *C. maenas*, ovarian ecdysteroid levels, expressed as either picomoles per gram or picomoles per ovary, rose gradually during vitellogenesis (Lachaise and Hoffmann, 1977; Lachaise *et al.*, 1981). In *M. rosenbergii*, however, an increase in ovarian ecdysteroid was observed only if the data were expressed as nanograms per ovary.

The trends observed in *M. rosenbergii* differ markedly from those recently reported for *P. monodon* (Young *et al.*, 1993). In the ovary of *P. monodon*, an overall decline in ovarian ecdysteroid content, when expressed both as nanograms per gram of tissue and nanograms per ovary, was observed. This may indicate that the roles, if any, of ecdysteroids in vitellogenesis in the two species differ. It may be that, in *M. rosenbergii*, where ovarian ecdysteroid levels (expressed as nanograms per ovary) increase during vitellogenesis, ecdysteroids are involved in the stimulation of vitellogenesis, whereas in *P. monodon*, in which ovarian ecdysteroid falls, they are not involved in vitellogenesis.

An analogous situation, where ecdysteroids stimulate vitellogenesis in some but not all related species, exists in the insects. Ecdysteroids do not stimulate vitellogenesis in a number of insect orders and it is thought that the use of ecdysteroids in the regulation of vitellogenesis has evolved only recently (Lanot *et al.*, 1989). There is some evidence that the hormonal mechanisms which regulate vitellogenesis in *P. monodon* and *M. rosenbergii* may differ, as *P. monodon*, in contrast to *Macrobrachium*, does not readily undergo vitellogenesis in captivity. Moreover, a second analogy between insects and crustaceans can be drawn, as in both groups there is evidence that ecdysteroids are involved in meiotic reinitiation (Lanot and Cledon, 1989).

Obviously, a great deal more work will be required to determine the roles of ecdysteroids in vitellogenesis in crustaceans. If the analogy between insects and crustaceans is to be pursued, then an investigation of juvenoids in crustaceans will be important. In mosquitoes, it is proposed that, in response to egg development neurosecretory hormone (EDNH), the ovary produces ecdysteroids which stimulate vitellogenin synthesis by the fat body. Juvenoids render the ovary more competent to respond to EDNH and the fat body more

competent to respond to ecdysteroids (see Bownes, 1989; Hagedorn, 1989). There is evidence that the crustacean juvenoid (methyl farnesoate) is involved in the regulation of vitellogenesis (see Laufer and Borst, 1988). Thus, in crustaceans, it will be important to study the occurrence of both methyl farnesoate and ecdysteroids, and their changes in concentration, during vitellogenesis.

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