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Changes in Morphological Characteristics and Ecdysteroids during the Molting Cycle of Tiger Shrimp, *Penaeus monodon* Fabricus

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Ching-Ming Kuo and Wen-Wen Lin (1996) Changes in morphological characteristics and ecdysteroids during the molting cycle of tiger shrimp, *Penaeus monodon* Fabricus. *Zoological Studies* **35**(2): 118-127. The processes and ecdysteroids involved in the molting cycle of tiger shrimp, *Penaeus monodon* Fabricius, were studied. By observing the morphological changes of the setae and the epidermal line in the uropod, the molt cycle was divided into stages A1, A2, B, C, D0, D1′, D1″, D1‴, D2, D3 and E, with stage C being very short (diecdysis). Anti-20-hydroxyecdysone-CMA-BSA rabbit antiserum was prepared, and the qualitative and quantitative changes of hemolymph ecdysteroids during the molting cycle were monitored by using radioimmunoassay (RIA) with or without prior fractionation by high performance liquid chromatography (HPLC). The total amount of hemolymph ecdysteroids was the lowest during stages A, B, and C, followed by a slight elevation during stages D0, D1′ and D1″, a dramatic increase to a prominent peak during stages D1‴ and D2, and then a rapid decline at stage D3. No significant sexual differences throughout the molting cycle were observed, except for stage B ($p < 0.05$). Among the 5 ecdysteroids fractionated by HPLC, 20-hydroxyecdysone was found to be the predominant circulating ecdysteroid in tiger shrimp, and the quantitative changes of these ecdysteroids coincided with that of total ecdysteroids measured. The other ecdysteroids, ecdysone, 5,20-dihydroxyecdysone, 2-deoxy-20-hydroxyecdysterone and 2-deoxyecdysterone were present in minute amounts with a similar profile to that of 20-hydroxyecdysone, and they were more prominent in postmolt (stage A) and intermolt (stage C).

Key words: Molt cycle, Ecdysteroids, Shrimp, Radioimmunoassay (RIA), High performance liquid chromatography (HPLC).

In crustaceans, growth in the life cycle is closely linked to the periodic shedding and reformation of the exoskeleton (ecdysis); the molting cycle is hormonally controlled and associated with environmental cues, such as light and temperature, among others. The molting process, a means of development and growth for an animal with an exoskeleton, dominates the crustacean's life, and most of the physiological processes, especially metabolism, behavior, reproduction, and osmoregulation, are influenced directly or indirectly by the periodic replacement of the integuments and the underlying cycles of metabolite accumulation (O'Connor and Gilbert 1969, Dall and Smith 1978a, b, Muramot 1981, Wassenberg and Hill 1984, Greenaway 1985, Culley and Gray 1987).

The current paradigm concerning the regulation of the crustacean molting cycle suggests that a molting inhibiting hormone (MIH) is synthesized and released from the X-organ sinus gland complex in the eyestalk and this regulates the synthesis and secretion of ecdysone from the Y-organ. MIH and prothoracicotropic hormone (PTTH) are widely regarded as the prime regulators of ecdysteroid secretion in the crustacean Y-organ and insect prothoracic gland, respectively (Spindler et al. 1980, Skinner 1985, Chang 1989, Smith and Sedlmeier 1990). Regulation of Y-organ activity by MIH is reflected in the cyclic changes in the titers of hemolymph ecdysteroids which intimately control the molting process. Dominance of the inhibitory effect of MIH on the Y-organ results in reduced

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titers of hemolymph ecdysteroids, thereby maintaining the organism in an intermolt state. Reduced titers of MIH, occurring naturally in response to environmental cues, permit increased secretion of ecdysone in the Y-organ, and a whole series of molting events is ultimately initiated. It is now well known that the Y-organ of crustaceans secretes ecdysone after biosynthesis from cholesterol; ecdysone is then converted to 20-hydroxyecdysone in hemolymph, testis, hepatopancreas and other tissues (Willig and Keller 1976, McCarthy and Skinner 1977, Chang and O'Connor 1978, McCarthy 1982, Lachaise and Lafont 1984). The initiation of the molting process is characterized by progressive increases in the titers of the circulating molting hormone, particularly 20-hydroxyecdysone (β-ecdysone), which acts on the target tissue, such as the integument, hepatopancreas, muscle and other molting-related tissues, triggering and mediating the consequent changes in the molting processes (Kuppert and Spindle 1982, Traub et al. 1987).

The significance of molting in the physiological processes in crustaceans is undisputed, and comprehension of the molting process and accurate determination of physiological states with reference to the molt cycle appear to be prerequisites to every physiological investigation in these animals. The determination of molt stage and the morphological and histological changes during the molt cycle have been studied in crab, lobster and crayfish (Travis 1955 1957, Skinner 1962, Dall 1965, Stevenson 1972, Aiken 1973, Reaka 1975, Longmuir 1983, Smith 1985), but staging and description of the molt cycle in the tiger shrimp, *Penaeus monodon*, have not been previously attempted. The present study describes the process of the molt cycle, and monitors the changes in the ecdysteroidal profile during this cycle.

MATERIALS AND METHODS

Tiger shrimp *Penaeus monodon* of both sexes, 16-20 g in body weight, were reared in 350-l tanks in which shrimp were individually housed in 1 000-cm^3 compartments to avoid losses from cannibalism. The shrimp were acclimated in duplicate under conditions of ambient photoperiod, 27 ± 1 °C and 25 ppt salinity, and were alternately fed daily with squid, shrimp and artificial feed.

Molt stages were determined by the sequential changes in the setae and epidermal line in the uropod according to the morphological criteria of *Penaeus esculentus* described by Smith and Dall (1985).

20-Hydroxyecdysone antiserum preparations

The 7-ketone group of 20-hydroxyecdysone was reacted with the amino group of carboxymethoxyamine (CMA) to form a carboxymethyloxime (Borst and O'Connor 1974). This reactive group was then reacted with the primary amino groups of albumin to form a stable conjugate (Erlanger et al. 1957). Accordingly, 20-hydroxyecdysone was first derivatized in a 4% CMA solution in pyridine at 40 °C for 24 h. The resultant oxime derivatives (20-hydroxytecdysone-CMA or 20-hydroxyecdysone 7-O-carboxymethyloxime) were purified by use of preparative TLC plates in solvent containing methanol:chloroform (2:3 v/v), and the band corresponding to the derivative was eluted with ethanol. 20-Hydroxyecdysone-CMA was then conjugated to bovine serum albumin (BSA) using the method developed for testosterone-CMA-BSA conjugation (Thorell and Larson 1978).

Aliquots of 18 mg 20-hydroxyecdysone 7-O-carboxymethyloxime and 7.5 μl tri-n-butylamine was dissolved in 0.5 ml dioxane, and 4 μl isobutyl chlorocarbonate was added after the solution was chilled to 11 °C. The mixture, first incubated at 4 °C for 20 min, was added to mix with 42 mg BSA solution in 2 ml water-dioxane mixture (1:1 v/v, pH 9.0). The reaction was continued at 4 °C for another 5 h. The mixture was finally dialyzed against double distilled water for 24 h, and then adjusted to pH 4.5 with HCl; precipitation of 20-hydroxyecdysone-BSA conjugate was performed at 4 °C for 4 days.

Anti-20-hydroxyecdysone-CMA-BSA rabbit antiserum was prepared by subcutaneous and intracutaneous injection of the antigen into 3 New Zealand rabbits. The rabbits were given booster shots and antibody titers were monitored every week after the 1st injection. At the end of twelve weeks, rabbits were exsanguinated and the serum collected from the jugular artery was lyophilized and stored as described by Thorell and Larson (1978).

Extraction and quantification of ecdysteroids

Hemolymph samples were collected during each molt stage via syringe puncture of the arthropodial membranes of the pleopods. Serum was separated from the clotted hemolymph by centrifugation at 5 000 g, 4 °C for 15 min. Serum samples

at 100 μl were mixed with 1.3 ml 80% methanol and then centrifuged at 5 000 g for 15 min. The extraction procedure was performed twice. These supernatants were pooled and then evaporated to dryness under nitrogen and stored at −20 °C until use. The recovery efficiency of the extraction method employed was 99%, which was determined by addition of 11 000 dpm tritiated ecdysone.

Radioimmunoassay of ecdysteroids

The hemolymph ecdysteroid concentrations at each molt stage were determined in duplicate by radioimmunoassay. Both unknown samples and 20-hydroxyecdysteroid standards (122 pg/ml to 500 ng/ml) were dissolved in 200 μl 10 mM phosphate buffer saline geletin (PBSG, pH 7.4), in which 100 μl [^{3}H]-cdysone (22 000 dpm) and 100 μl antiserum were added in sequence. The mixture was vortexed and incubated at 4 °C overnight, or settled at room temperature for 40 min and then at 4 °C for 30 min. The free tritiated ecdysteroids were removed by adding 200 μl dextran T-70 coated activated charcoal solution, and the mixture was then centrifuged at 5 000 g, 4 °C for 15 min. The radioactivity of each supernatant was counted by use of a liquid scintillation counter (Beckman, LS5801), and the concentrations were calculated from the standard curve established. The counting efficiency was approximately 40%.

Ecdysteroid fractionation by HPLC and quantification by RIA

An aliquot of 4 ml hemolymph pooled from 10 shrimp at the same molt stage was extracted twice with 10 ml absolute methanol. The supernatant was dried under reduced pressure by SpeedVac. Extraction efficiency was 92%. The extracts were then partially purified by using a Sep-Pak C18 cartridge (Waters Associates). The cartridge was first loaded with 5 ml 30% methanol. The extract dissolved in 1.2 ml 30% methanol was then loaded into the Sep-Pak C18 cartridge. Fraction 1 was eluted from the Sep-Pak with 2 ml 30% methanol and discarded; while fraction 2, which contained the ecdysteroids, as eluted from the cartridge with 2 ml 100% methanol, collected, filtered with 0.4 μm Millipore filters and then evaporated to dryness.

HPLC separation

Partially purified ecdysteroid extracts were fractionated by reverse phase HPLC with a Merck LichroCART manufix column (3 μm; 12.5 cm × 4 mm), and eluted with an isopropanol/methanol/water mixture. The proportion of these 3 solvents in the solvent mixture was changed from 8:0:92 (v/v/v) at the beginning to 15:20:65 at the end of 60 min by a linear gradient program. Flow rate (1 ml/min) remained constant, and the ecdysteroid peaks were detected by a UV detector at 250 nm. According to the standard elution time of the 5 ecdysteroids, 5,20-dihydroxyecdysone, 20-hydroxyecdystone, ecdystone, 2-deoxy-20-hydroxyecdystone and 2-deoxyecdystone were collected respectively, at the elution times of 13.5 to 15.5 min, 15.5 to 18.5 min, 28.5 to 31.5 min, 31.5 to 34.5 min and 48.5 to 51.5 min (Fig. 1). These ecdysteroid fractions were then dried by SpeedVac.

The dried fractions of the 5 ecdysteroids recovered for each molt stage were quantified by RIA as described previously. Each fraction was assayed by using its respective ecdysteroid as a standard.

RESULTS

Molt cycle

The molt cycle is divided into 11 stages, namely A1, A2, B, C, D0, D1′, D1″, D1‴, D2, D3 and E, following the morphological changes of the epidermal line and setal development (Fig. 2). The morphological criteria and duration of each molt

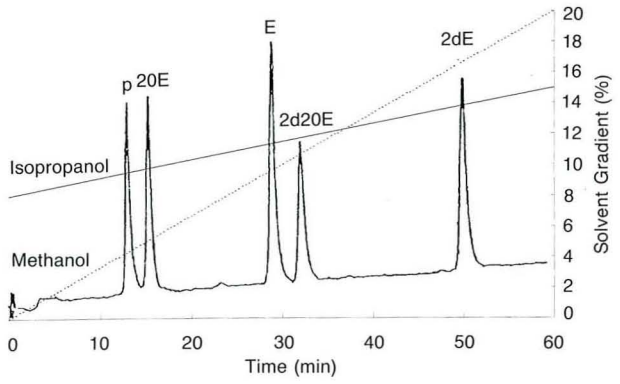

Fig. 1. Chromatogram of ecdysteriod standards, fractionated by reverse HPLC. Column: Merck LiChroCart (3 μm; 4 mm ϕ × 12.5 cm). Mobile phase: Isopropanol, methanol and water; flow rate: 1 ml/min; detector; UV detector at 250 nm. P: polypodine B (5,20-Dihydroxyecdysone); 20E: 20-Hydroxyecdysone; E: Ecdysone; 2d20E: 2-Deoxy-20-hydroxyecdysone; and 2dE: 2-Deoxyecdysone.

stage are summarized in Table 1. The intermolt stage (C stage) in *P. monodon* was found to be rather short, lasting only 1 to 2 days, and the molt cycle of this species is categorized in the diecdysis group.

Specificity of β-ecdysone antiserum

The production of antibodies in the 3 immunized rabbits was monitored by measurement of [^{3}H]-ecdysone binding. The highest antibody titer of the 3 rabbits was 7 250, so a 1:9 060 dilution of the antiserum was used to obtain 40% binding of the antiserum. The cross-reactivity of the 20-hydroxyecdysone antiserum to various ecdysteroids is summarized in Table 2. Ecdysone and 2-deoxyecdysone structurally differ from 20 hydroxyecdysone in lacking the C-20 hydroxyl group and both the C-20 and C-2 hydroxy groups, respectively. These 2 compounds were 238% and 143%, respectively, more efficient and sensitive than other competitors for the antibody binding sites. The standard curve of 20-hydroxyecdysone binding to this antiserum is shown in Fig. 3.

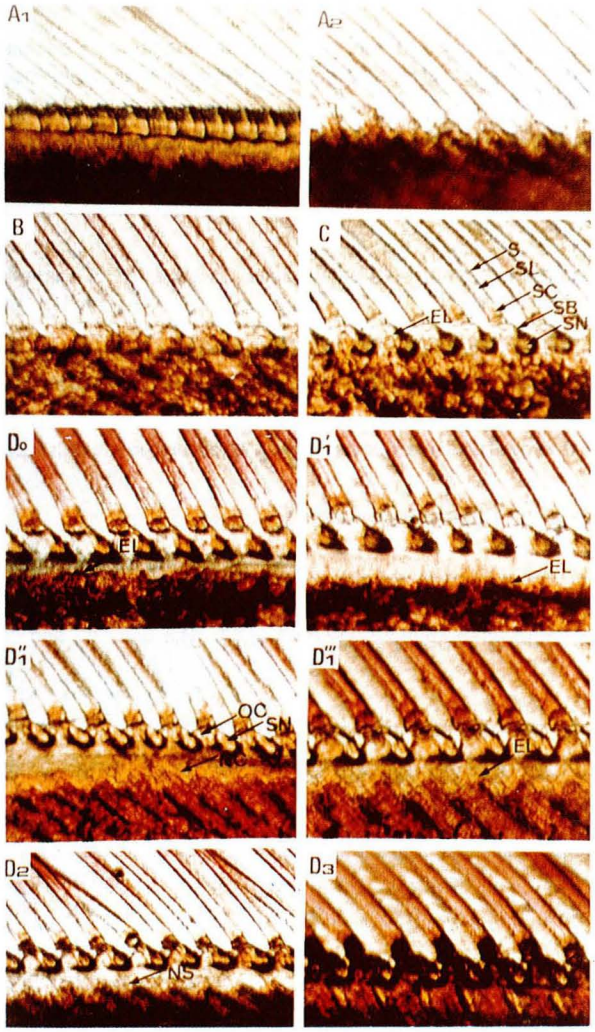

Fig. 2. Morphology of uropod edge in tiger shrimp, *Penaeus monodon*. S: setal shaft; SL: setal lumen; SC: setal cone; SB: setal base; SN: setal node; EL: epidermal line; OC: old cuticle; NC: new cuticle; NS: new setae.

Total hemolymph ecdysteroids during the molt cycle

The sexual differences in the total hemolymph ecdysteroids during the molting cycle are compared in Table 3. The patterns of hemolymph ecdysteroid contents throughout the molting cycle in both sexes are similar, and the differences in the ecdysteroid contents between the sexes at most molt stages are insignificant ($p < 0.05$), except a minor sexual difference observed at stage B. The pooled data indicate that the total hemolymph ecdysteroid contents were the lowest at the post-molt phase (stages A and B) and ranged 3.25 to 5.04 ng/ml. The ecdysteroid contents were slightly elevated from the intermolt phase (8.12 ± 0.73 ng/ml) towards the early premolt phase (6.23 ± 0.50, 9.23 ± 0.88 ng/ml hemolymph at stages D0 and D1′, respectively), followed by a notable increase in stage D1″ (44.24 ng ecdysteroids/ml hemolymph). An ecdysteroid surge in the hemolymph was observed in stages D1‴ and D2, measured at 201.73 ± 19.50 ng/ml and 227.86 ± 20.99 ng/ml, respectively. A drastic decline in the ecdysteroid level to 43.76 ± 9.15 ng/ml was observed toward the end of the premolt period (stage D3). The ecdysteroid levels at the molting phase were unfortunately not obtainable due to difficulties in hemolymph sampling during ecdysis. The trend of ecdysteroid content changes in the entire molting cycle reveals that the changes in the ecdysteroid levels among the molt stages are statistically significant ($p < 0.001$), except those between stages D1″ and D3, and stages D1‴ and D2.

Fractionation and quantification of ecdysteroids

The hemolymph ecdysteroids and their precursors were fractionated by reverse HPLC. Each fraction was collected according to its respective elution time and then quantified by RIA. 20-Hydroxyecdysone was the dominant ecdysteroid circu lating in the hemolymph, ranging 62.95% to

97.87% of total hemolymph ecdysteroids throughout the molt cycle. The levels at the post-molt and intermolt stages were lowest ranging 1.27 to 1.97 ng/ml hemolymph, followed by a notable elevation of hemolymph 20-hydroxyecdysone during the premolt stage. Hemolymph 20-hydroxyecdysone significantly increased from 10.93 ng/ml at stage D0 to 169.76 ng/ml and 213.12 ng/ml at staged D1 and D2, respectively. This was followed by a sharp decline in the hemolymph 20-hydroxyecdysone concentration at stage D3, measured at 66.28 ng/ml (Fig. 4, Table 4). The percent of 20-hydroxyecdysone at each stage of the molt cycle varied, starting at 62.95% to 65.46% at post-molt, and 64.38% at intermolt. The hemolymph 20-hydroxyecdysone levels ranged 86.54 % to 97.87% in the premolt phase (Table 4).

The cyclic changes in hemolymph ecdysone

Table 1. Morphological changes during the molting cycle of tiger shrimp, *Penaeus monodon*

Period	Stage	Duration	Morphological appearances
Postmolt	A1	1 h	The stage immediately follows ecdysis; cuticle is soft and membraneous in form; setal bases are evenly aligned and fully filled with cellular matrix.
	A2	8 h	Cuticle is still soft and membranaceous; retraction of cellular matrix from distal ends of the setae begins.
	B	2 d	Cuticle shows parchment-like consistency and becomes more rigid; setal node becomes visible; epidermal cells appear to be loosely packed; constrictions in setal lumen begin and plugs consequently formed.
Intermolt	C	1-2 d	Exoskeleton attains maximal rigidity; setal cone visible in most of the setae; epidermis fills setal base with narrow translucent fringe.
Premolt	D0	3-4 d	Retraction of epidermis from setal bases begins and epidermis eventually forms a straight line under setal bases.
	D1′	3 d	The epidermis of uropod withdraws further from setal bases, invagination of epidermis begins and epidermal line becomes wavy.
	D1″	2 d	The epidermal line of uropod becomes moderately scalloped.
	D1‴	2 d	Scalloping of the epidermal line of uropod is uniform and maximal in depth; setae reach maximum invagination.
	D2	2 d	Formation of new setae visible; uniform edge of scalloping epidermal line is disrupted by extrusion of new setae.
	D3	12 h	Exoskeleton becomes increasingly brittle and delicate.
Molting	E		Process of ecdysis.

The molt cycle of tiger shrimps at the size of 16-20 gm in body weight, was monitored under temperatures of 27 ± 1 °C and a salinity of 25 ppt. The duration of each molting stage is presented on the basis of 16 to 18 day molt cycle. The unit "h" and "d" represent hour and day, respectively.

Table 2. Cross-reactivities of antiserum obtained from 20-hydroxyecdysone-CMA BSA conjugate

Ecdysteroids	Amount (ng) required for 50% inhibition of [^{3}H]-ecdysone binding	Cross-reaction[a] (%)
Ecdysone	0.21	238
20 Hydroxyecdysone	0.50	100
2-Deoxyecdysone	0.35	143
2-Deoxy-20-Hydroxyecdysone	0.98	51
5,20-Dihydroxyecdysone	0.69	72
Cholesterol	ND[b]	ND[b]

[a]Defined as X ÷ Y×100, where X is the mass of unlabeled 20-hydroxyecdysone and Y the mass of the heterologous compound required for 50% inhibition of [^{3}H]-ecdysone binding.
[b]ND: Inhibition was not detected at the concentration up to 1 000 ng of competitive inhibitor.

concentrations during the molt cycle were found to be similar to those of 20-hydroxyecdysone, but at a much lower level, 0.17 to 0.24 ng/ml and 0.2 ng/ml in the postmolt and intermolt phases, respectively, followed by an increase up to 1.02 ng/ml at stage D1″ and peaking in stages D1‴ and D2 (3.12 and 3.91 ng/ml, respectively). In contrast, the percent of ecdysone to total hemolymph ecdysteroid or to total 20-hydroxyecdysone decreased from 8.63% to 8.76% or 13.39% to 13.71%, respectively, in the postmolt phase to the lowest level of 0.69% to 1.77% or 0.71% to1.84%, respectively, in the late stages of the premolt phase, right before the molting phase (Fig. 4).

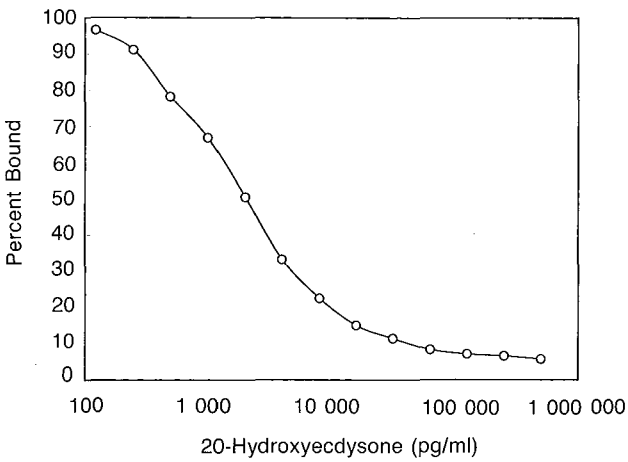

Fig. 3. Standard curve of 20-hydroxyecdysone radioimmunoassay.

The cyclic changes in hemolymph 2-deoxy-20-hydroxyecdysone concentration were found to parallel those of ecdysone. Concentrations increased from 0.11 ng/ml in postmolt stage to 0.44 to 1.14 ng/ml in the premolt stage (D1″ to D1‴), and peaked in stages D1‴ and D2 (1.52 and 1.7 ng/ml, respectively). The concentration showed a notable drop in stage D3 (0.39 ng/ml). The percent of this ecdysteroid to 20-hydroxyecdysone was highest (17.26%) at intermolt, followed by a gradual decline from 4.76% (D0 stage) to 0.59% (D3 stage) in the premolt phase (Fig. 4). The hemolymph 2-deoxyecdysone concentration remained rather constant at 0.09 ng/ml to 0.21 ng/ml, except in stage D2 during which the concentration peaked at 1.04 ng/ml. The changes in the percent of 2-deoxyecdysone to 20-hydroxyecdysone was similar to that of 2-deoxy-20-hydroxyecdysone, but at a lower level (5.07% at stage C and 0.14% at stage D3) (Fig. 4).

DISCUSSION

The molt cycle of crustaceans is generally categorized into postmolt, intermolt, premolt and molt (ecdysis) phases, though the molt stages in each phase vary with the species concerned. In decapods, the molt cycle is mainly divided into 5 stages, namely stages A and B (postmolt), stage C (intermolt), stage D (premolt) and stage E (molt, ecdysis) (Drach 1939). The methodologies used to

Table 3. Total hemolymph ecdysteroid titers (ng/ml) during the molt cycle of tiger shrimp, *Penaeus monodon*

Stage	Total Ecdysteroid Titers in Hemolymph			*t*-value
	Males	Females	Pooled	
A	5.1 ± 0.7 (10)	5.0 ± 0.6 (13)	5.04 ± 0.43 (23)	0.07
B	4.1 ± 0.4 (20)	2.6 ± 0.4 (29)	3.25 ± 0.30 (49)	2.54*
C	5.5 ± 0.5 (21)	6.8 ± 0.8 (29)	6.23 ± 0.50 (50)	−1.27
D0	7.3 ± 0.5 (33)	8.6 ± 1.1 (53)	8.12 ± 0.73 (86)	−0.87
D1′	10.2 ± 1.4 (24)	8.1 ± 1.0 (20)	9.23 ± 0.88 (44)	1.20
D1″	55.9 ± 16.5 (8)	34.9 ± 6.9 (10)	44.24 ± 8.37 (18)	1.18
D1‴	223.2 ± 19.6 (4)	184.6 ± 21.2 (5)	201.73 ± 19.50 (9)	0.63
D2	236.9 ± 23.2 (8)	209.7 ± 26.5 (4)	227.86 ± 20.99 (12)	1.37
D3	52.9 ± 11.6 (6)	32.8 ± 6.2 (5)	43.76 ± 9.15 (11)	0.70

Values given are Mean ± SEM; values in parentheses are the number of samples.
*represents that the sexual differences in hemolymph ecdysteroids titers are statistically significant at the 5% level.

Table 4. Changes in hemolymph ecdysteroid contents in tiger shrimp, *Penaeus monodon*, during the molting cycle

Molting		20-Hydroxyecdysone		Ecdysone		2-Deoxy,20-Hydroxyecdysone		2-Deoxyecdysone		5,20-Dihydroxyecdysone		Total
Period	Stage	(ng/ml)	(%)	(ng/ml)	(%)	(ng/ml)	(%)	(ng/ml)	(%)	(ng/ml)	(%)	(ng/ml)
Post-molt	A	1.27	65.46	0.17	8.76	0.11	5.67	0.17	8.76	0.22	11.34	1.94
Post-molt	B	1.75	62.95	0.24	8.63	0.31	11.15	0.10	3.60	0.38	13.67	2.78
Intermolt	C	1.97	64.38	0.20	6.54	0.34	11.11	0.10	3.27	0.45	14.71	3.06
Premolt	D0	10.93	86.54	0.54	4.28	0.52	4.12	0.13	1.03	0.51	4.04	12.63
Premolt	D1′	15.44	91.96	0.28	1.67	0.44	2.62	0.21	1.25	0.42	2.50	16.79
Premolt	D1″	28.53	90.43	1.02	3.23	1.14	3.61	0.09	0.29	0.77	2.44	31.55
Premolt	D1‴	169.76	96.36	3.12	1.77	1.52	0.86	0.09	0.05	1.68	0.95	176.17
Premolt	D2	213.12	96.37	3.91	1.77	1.70	0.77	1.04	0.47	1.38	0.62	221.15
Premolt	D3	66.28	97.87	0.47	0.69	0.39	0.58	0.09	0.13	0.49	0.72	67.72

determine the molt stages in crustaceans primarily include histological examination of the integument and determination of the state of setogenesis on appendages. Advantages of using setogenesis include its rapidity, simplicity, and sampling replicability. This approach has been widely used as a criterion for molt staging in a number of decapods, including natantians (Scheer 1960, Kamiguchi 1968), anomurans (Kurup 1964) and macrurans (Aiken 1973). Among the penaeids, criteria for assessing molt stages are described for *Penaeus duorarum* (Schafer 1968), *P. merguiensis* (Longmuir 1983), *P. esculentus* (Smith and Dall 1985), *P. stylirostris* (Huner and Colvin 1979, Robertson et al. 1987), *P. setiferus* (Robertson et al. 1987), *P. vannamei* (Chan et al. 1988), and *P. japonica* (Okamura et al. 1989). The classification of the molt cycle in *P. monodon* in this study is primarly derived from the system of molt stage based on the sequential morphological changes of the epidermal line and setal development in *P. esculentus* as described by Smith and Dall (1985). Knowles and Carlisle (1956) defined the molt cycle with a long intermolt phase as anecdysis, and that with a relative short intermolt phase as diecdysis. Crustaceans of the suborder Reptantia, such as crabs, lobsters and crayfish, are categorized in the anecdysis group, while those of the suborder Nantantia including prawns and shrimp in the diecdysis group. With a molt cycle of 17 to 19 days as in tiger shrimp and an intermolt phase lasting only 1 to 2 days, the molt cycle of tiger shrimp is categorized in the diecdysis group.

Hemolymph ecdysteroid titers during the molt cycle in various crustacean species have often been quantified by RIA because of its sensitivity to the picogram level (Carlisle and Connick 1973, Willig and Keller 1973, McCarthy and Skinner 1977, Chang and O'Connor 1978, Stevenson et al. 1979, Hopkins 1983 1986, Soumoff and Skinner 1983). However, the antiserum prepared from 20-hydroxyecdysone-CMA BSA conjugate showed varying

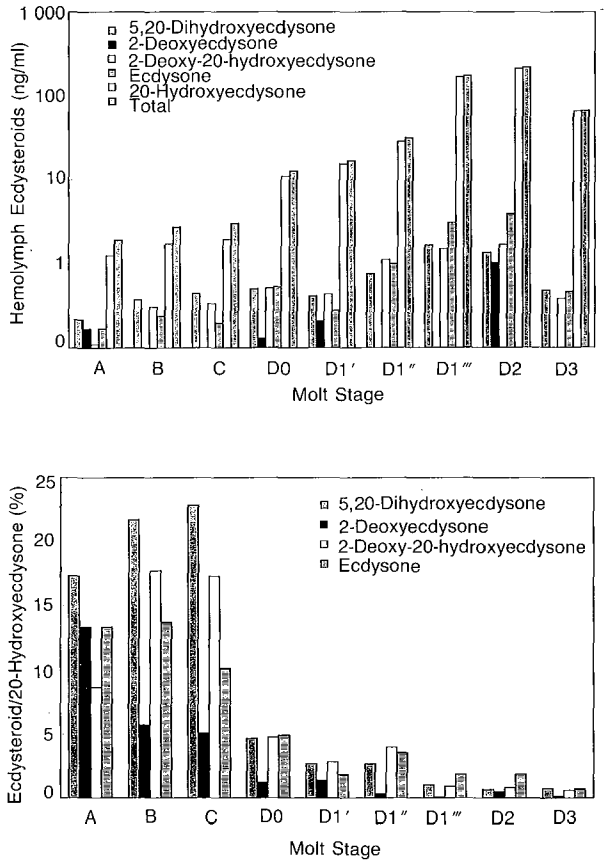

Fig. 4. Changes in total hemolymph ecdysteroid level, and 5 HPLC-fractionated ecdysteroid titer (Upper panel) and the percent of various ecdysteroids to 20-Hydroxyecdysone (Lower panel) in the hemolymph of tiger shrimp, *Penaeus monodon*.

sensitivity toward different ecdysteroids. It was 2.4- and 1.43-fold more sensitive to ecdysone and 2-deoxyecdysone than to 20-hydroxyecdysone, while its affinity toward all other ecdysteroids tested was less than that of 20-hydroxyecdysone. Similar observations have been reported elsewhere (Soumoff et al. 1981). Accordingly, quantification of ecdysteroids by RIA was not specific and may cause some difficulty in interpretation. Fractionation of ecdysone and 20-hydroxyecdysone is necessary prior to RIA, when separate quantifications of ecdysone and 20-hydroxyecdysone are required. However, total hemolymph levels of unspecified ecdysteroids measured by RIA and those assayed after HPLC fractionation were highly correlated with the same order of magnitude, though minor differences in ecdysteroid quantification were noted.

No sexual differences in hemolymph ecdysteroid titers throughout the molt cycle were detected. The changes observed here in the circulating concentrations in hemolymph of tiger shrimp followed patterns generally found in other crustaceans: shore crab, *Carcinus maenas*, (Adelung 1971); crayfish, *Orconectes propinquus* (Carlisle and Connick 1973), *Astacus leptodactylus* (Chang and O'Connor 1978, Durliat et al. 1988), *Orconectes limosus* (Willig and Keller 1973), and *O. Sanborni* (Stevenson et al. 1979, Spindler et al. 1980); land crab, *Gecarcinus lateralis* (McCarthy and Skinner 1977); blue crab, *Callinectes sapidus* (Soumoff and Skinner 1983); and fiddler crab, *Uca pugilator* (Hopkins 1983 1986). In the tiger shrimp, *P. monodon*, low ecdysteroid titers were detected in the hemolymph of postmolt, intermolt and early premolt specimens (3.25 to 5.04 ng/ml, 6.23 ng/ml and 8.12 to 44.24 ng/ml, respectively). The major peak (201.73 to 227.86 ng/ml) was seen in stages D1‴ and D2, during which the new cuticle is formed by the epidermis. A similar pattern of changes in hemolymph ecdysteroid titers was reported in *P. vannamei*, but the titers were found to be much higher than those in *P. monodon*, i.e., 30 ng/ml at premolt, 13 to 50 ng/ml at intermolt, and 220 ng/ml at stage D1″. At stage D3, during which the old cuticle is reabsorbed, hemolymph titers rapidly dropped until ecdysis.

Measurements of circulating ecdysteroids are more variable between species than are whole-animal titers. However, all species exhibit a similar trend of increasing ecdysteroid levels during premolt to a maximum prior to ecdysis, followed by a decline to basal intermolt levels as determined, when the hemolymph ecdysteroids were fractionated by HPLC and then quantitated by RIA. The ecdysteroid titers during the molt cycle in tiger shrimp resemble the general trend of ecdysteroid concentrations reported in the other crustaceans, though the values of ecdysteroid measurements vary by species to some extent. The range of ecdysteroids in *P. monodon* hemolymph, 1.94 to 2.78 ng/ml at postmolt, 3.06 ng/ml at intermolt, 12.63 to 31.55 ng/ml at early premolt to 221.15 ng/ml in late premolt, is comparable to hemolymph titers of the Kuruma prawn *P. japonica* with values of 13.1 ± 2.6 ng/ml. 16.5 ± 2.1 ng/ml, 37.1 ± 10.6 ng/ml and 223 ± 75 ng/ml for the respective stages (Okumura et al. 1989).

Interspecific variation in ecdysteroids in the hemolymph of crab species was also noted: ecdysteroid titers ranged from 5 ng/ml (intermolt) to 44 ng/ml (late premolt) in blue crab, *Callinectus sapidus* (Soumoff and Skinner 1983); nearly zero to 120 ng/ml in *Pachygrapsus crassipes* (Chang and O'Connor 1978); 10 to 150 ng/ml in *Gecarcinus lateralis* (Soumoff and Skinner 1983); and 62 to 470 ng/ml in *C. maenas* (Lachaise et al. 1976). Extraordinarily high ecdysteroid titers of *C. maenas* (30 ng/ml at intermolt up to 15 000 ng/ml) was once reported (Andrieux et al. 1976). The crayfish *Orconectes samborni*, has a minimal titer of 4 ng/ml at intermolt and a maximum of 30 ng/ml at late premolt (Stevenson et al. 1979). Juvenile lobsters, *Homarus americanus*, exhibited basal levels of ecdysteroids of less than 35 ng/ml and peak titers of 350 ng/ml (Chang and Bruce 1980). These variations may reflect actual differences existing among species, with precise patterns being species specific (Stevenson et al. 1979, Chang and Bruce 1980). However, the possibility exists that the discrepancy in the determination of molt stages, as well the specificity of ecdysteroid antiserum prepared, might also be the causes of the detected interspecific variation, since all the data presented were quantified by the RIA method. Despite considerable differences in the maximum titers of ecdysteroids in different species, a general molt cycle-correlated pattern of changes does exist, at least for ecdysteroids in hemolymph. In most species, a major peak in ecdysteroids occurs at stage D2 of proecdysis, and the ranges of ecdysteroid levels reported in the literature are generally comparable among crustaceans.

The ecdysteroid contents in hemolymph measured by RIA on the samples with or without HPLC fractionation were compared, and the ecdysteroid measurements coincided closely. The extraction and quantitative methodologies employed in the

present study are valid, and the choice of methodology depends upon the objectives of investigation.

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草蝦脫殼週期中形態特徵，脫殼激素含量及類型之變化

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甲殼類成長、生殖均與週期性脫殼作用息息相關，它也直接或間接影響到其他生理作用之正常進行，因此，脫殼期鑑定在甲殼類生理研究上至爲重要。本文探討草蝦脫殼週期之形態特徵，並以放射免疫法及高效液相層析法，測定血淋巴液中脫殼激素含量及類型變化，藉以瞭解脫殼激素類型與各脫殼期間之相關性。就尾扇剛毛新生及表皮線之形態變化(A_1, A_2, B期)，脫殼中期(C期)，脫殼前期(D_0, D_1', D_1'', D_1''', D_2, D_3等期)及脫殼期(E期)等階段。

脫殼激素總量在脫殼後A, B, C期處於低濃度之狀態(3.25-6.23 ng/ml間)，自 D_0, D_1', D_1'' 期逐漸升高，D_1''' 及 D_2 期遽增而達到高峰(213.12 ng/ml)，隨後後呈現在脫殼前 D_3 期明顯下降之勢。

血淋巴經以HPLC分離鑑定α型及β型脫殼激素及其類似物，包括5,20-Dihydroxyecdysone, 2-Deoxyecdyoone及2-Deoxy-20hydroxyecdysone等，以R1A加以定量，結果顯示，在整個脫殼週期中β型脫殼激素爲最重要之成份，含量比例最高佔脫殼激素總量之62.95%至97.87%，其在脫殼週期中之含量變化，亦與總量之變化一致。

關鍵詞：脫殼週期, 脫殼激素, 草蝦, 放射免疫法, 高效液相層析法。

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