Level of ecdysteroids in the hemolymph of the freshwater prawn, *Macrobrachium rosenbergii* (Crustacea: Decapoda) in relation to the phenomenon of cheliped autotomy in males

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Summary. Ecdysteroid titers were determined in the hemolymph of adult freshwater prawn, *Macrobrachium rosenbergii*, during various stages of the molt cycle, using radioimmunoassay. A comparison was made between hormone levels in males which undergo normal molt and dominant males of the population which periodically commit cheliped autotomy. The latter is a recently discovered phenomenon whereby a male voluntarily sheds off both its long claws at molt, once the ratio between body length and combined claw length reaches a ceiling level of about 1:3. Shortly before an autotomizing molt, hormone levels were about three times higher than on the eve of a normal molt.
Key words. Ecdysteroids; cheliped autotomy; *Macrobrachium rosenbergii*.

Voluntary autotomy of the long chelipeds is performed periodically by the hierarchically dominant male of the prawn group once the ratio between body length and paired cheliped length reaches an approximate value of 1:3 as reported by Schmalbach et al.[1]. Since this shedding of both chelae takes place concomitantly with the forthcoming molt, it was suspected that 20-hydroxyecdysone, which has been found to be the sole or predominant molting hormone in extracts or serum of various decapod crustaceans[2], may also be involved in the mechanism of autotomy. Unlike pterygote insects, decapod crustaceans do not cease molting upon reaching maturity.

Hemolymph samples of about 50–100 µl were withdrawn from adult males and females of *M. rosenbergii* at fixed intervals throughout the periods between molts. Since it is known that a few hours difference between sampling different animals could have a tremendous effect upon the results[2], we tried as much as possible to extract samples at the same time. Hemolymph withdrawing was done by using a glass micropipette inserted into an intersegmental membrane at the ventral surface of the abdomen, exercising great care to avoid puncturing of major arteries. Hemolymph sample volumes were about the same for 'normal' and autotomizing males and the animals' weights were similar. It may therefore be presumed that the total volumes of hemolymph in the tested animals were comparable. The tested animals (carapace length 35–65 mm) were maintained in individual 15-l aquaria at a constant temperature of 27 ± 2 °C, as described by Harpaz et al.[3]. The body size of the tested females was similar to that of the males, except for the extremely long chelipeds characteristic of a dominant male. Thus, the time intervals between molts were very close for both sexes. The radioimmunoassay of ecdysteroids was performed according to Porcheron et al.[4] and Porcheron[5]. In this assay, 20-hydroxyecdysone and ecdysone cross-reacted equally well with antiserum; other ecdysteroids were less reactive. Standard S-shaped curves were obtained with 20-hydroxyecdysone and results expressed in picograms of 20-hydroxyecdysone equivalents per microliter of hemolymph.

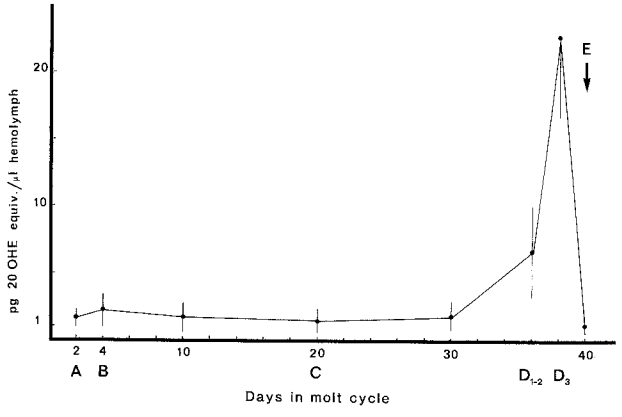
Since only a few males in the culture attain the body dimensions at which autotomy is likely to occur, the number of individuals available for such tests is very limited to begin with. In addition, repeated bleeding as well as handling also causes premature deaths before autotomy is committed. As a result, no more than 5 cases of eventual autotomy could be adequately studied in the course of the 2-year-long experiment.

Figure 1 represents the fluctuations in the hormone concentration in the hemolymph during the entire period between two consecutive regular molts in which no autotomy occurred. The hormone levels comprising this figure are calculated averages for 7 males and 6 females assayed. Since hormone levels for males and females are very similar, as may also be inferred from the unpublished results of E. Derelle and J.J. Meusy (personal communication), both sexes were plotted together on the same graph. The figure shows that at no instance has the hormone concentration in the hemolymph exceeded the level of 32 pg/µl.

On the other hand, in the male prawns that underwent autotomy (table 1), the hormone concentration shortly before the autotomizing molt rises to a level of 56–72 pg/µl which is significantly higher than that recorded on the eve of a regular molt.

The exact mode of action of the autotomy process and the mechanism of its hormonal control are yet to be elucidated. It is therefore quite premature at this stage to suggest that this excessive rise in the hormonal level is the direct trigger for autotomy.

A comparison with other decapod crustaceans, e.g., *Carcinus maenas*[6], *Homarus americanus*[2], *Pachygrapsus crassipes*[7] and *Palaemon serratus*[8] reveals that in general, the molting hormone level in the hemolymph of *M. rosenbergii* is considerably lower. This is evident from the present results as well as from those of Derelle and Meusy (pers. comm.). Only in autotomizing males does the concentration of this hormone rise to a level comparable with the aforementioned crustaceans. It is interesting to point out that in *Pachygrapsus crassipes*[7], a similar 3-fold rise in hormone level was found, but in contrast to the present results, this rise occurred following eyestalk ablation when the hormone titre reached 400 pg/µl as opposed to the normal premolt level of 120 pg/µl.



Mean concentration of 20-hydroxyecdysone in the hemolymph of adult *M. rosenbergii* prawns during the intervening period between two normal molts. Results are expressed in picograms of 20-hydroxyecdysone equivalents per microliter of hemolymph. Each point of the curve represents the mean concentration calculated from 13 different results, vertical bars represent standard deviations. Capital letters = stages of the molt cycle; E = Ecdysis; 20-OHE = 20-hydroxyecdysone.

Case histories of 5 adult *M. rosenbergii* males that underwent autotomy

Animal	1	2	3	4	5
2 days after molting	2.2	1.9	1.4	2.5	2.4
Mid cycle (intermolt)	3.8	1.5	2.7	2.3	1.6
4 days prior to molting	13.2	4.5	4.1	9.5	8.9
2 days prior to molting	56.0	61.3	72.0	68.0	63.4

Results expressed in picograms of 20-hydroxyecdysone equivalent per µl hemolymph.

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Effect of the juvenoid methoprene on the hemolymph composition of the cabbage maggot *Delia radicum* (Diptera: Anthomyiidae)

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Summary. Treatment of post-feeding larvae of the cabbage maggot *Delia radicum* with methoprene did not affect the capacity of the insect to pupate, but suppressed eclosion to the adult stage. The concentration of hemolymph trehalose was significantly decreased by methoprene treatment, although hemolymph protein and amino nitrogen levels were unaffected.
Key words. Amino nitrogen; *Delia radicum;* eclosion; hemolymph; methoprene; protein; trehalose.

A variety of compounds with juvenile hormone activity offer considerable promise as a replacement for or adjunct to existing methods for controlling insects[2]. Under laboratory conditions, synthetic cecropia juvenile hormone and a juvenile hormone analog possessed ovicidal activity against eggs of the cabbage maggot *Delia radicum* and prevented adult emergence from puparia[3]. Field trials, using the juvenoid, showed promising control potential[4]. More recently, it was found that eggs of *D. radicum* hatched subsequent to treatment with the juvenoid methoprene (isopropyl (2E,4E)-11-methoxy-3,7-11-trimethyl-2,4-dodecadienoate), but pupae that eventually developed failed to undergo eclosion to adults[5]. No information is available concerning the physiological effects of juvenoids on *D. radicum,* although such a knowledge would provide insight into their mode of action and prove helpful in identifying side effects on non-target invertebrates[6]. In view of the potential usefulness of insect growth regulators for controlling populations of *D. radicum,* a major pest of root and stem crucifers in North America and Europe, this study was done to determine the effect(s) of methoprene treatment on the hemolymph composition of post-feeding larvae.

Delia radicum larvae were obtained from a stock colony of the insect, maintained in the greenhouse[5]. To demonstrate that the post-feeding larval stage was sensitive to methoprene, insects were removed with forceps from the soil in which infested rutabagas had been grown, within 24 h after they had left the host plant, rinsed in distilled water and placed on filter paper until needed. Larvae were held anteriorly with forceps and, using a micropipet, topically treated over the entire thoracic and abdominal regions with 1 µg methoprene in 1 µl dimethylsulfoxide (DMSO), or with 1 µl of DMSO (controls). Treated larvae (three replicates of 10 insects per treatment) were reared (20 °C; 16-h photoperiod) using a small-scale petri dish rearing system[5] and percent pupation and adult eclosion recorded.

For experiments on hemolymph composition, methoprene or DMSO-treated larvae were maintained (36 h) in pupation units made from 9-cm-diameter petri dish bases[5], prior to hemolymph extraction. Hemolymph was not collected from larvae that pupated during the 36-h incubation period. Surface-dried larvae were held with forceps under a stereomicroscope and punctured on the ventral surface, immediately posterior to the larval mouth hooks, with a fine insect pin. The fluid which exuded was drawn to fill a 1-µl micropipet, then applied to the base-line of an activated thin-layer chromatography plate for carbohydrate analysis, or blown into 5 ml of trichloracetic acid (TCA) for amino nitrogen and protein quantification. A total of 5 µl of pooled hemolymph was required for each carbohydrate determination and 10 µl for each amino nitrogen/protein replicate. Since only a very small volume of clear hemolymph (1–2 µl) could be obtained from each cabbage maggot, each carbohydrate value was based on hemolymph taken from 3–5 insects, while each amino nitrogen/protein determination involved hemolymph taken from 5–10 larvae. Carbohydrates were separated by TLC and fractions detected using an α-naphthol-sulfuric acid reagent spray[7]. To quantify separated carbohydrates, plates were developed containing hemolymph samples spotted alongside standards, but only the standards were subsequently sprayed with the detection reagent. Areas of the unsprayed plate coating (2 × 2.5 cm) corresponding in R_f values to the standards were scraped into centrifuge tubes. Anthrone reagent (3 ml) was added to each sample, which was colorimetrically assayed for carbohydrate[8], values being calculated by reference to a calibration curve prepared from the requisite standard. Hemolymph aliquots blown into TCA were assayed colorimetrically for amino nitrogen[9] and protein[10] concentration. Amino nitrogen concentration of the hemolymph was calculated by reference to a glycine calibration curve and protein concentration was expressed relative to a bovine serum albumin standard. Three replicates of