Invertebrate Reproduction and Development, 16 (1989) 33–42
Balaban, Philadelphia/Rehovot
0168-8170/89 /$03.50 © 1989 Balaban

The mysid *Siriella armata*, a biological model for the study of hormonal control of molt and reproduction in crustaceans: a review

J. CUZIN-ROUDY[1] AND A.S.M. SALEUDDIN[8]
[1]*Universite P et M. Curie,CNRS (URA 716) CEROV, 06230, Villefranche sur mer, France and* [2]*Department of Biology, York University, North York, M3J 1P3, Ontario, Canada*

Summary

The mysid *Siriella armata* provides a new biological model for investigations on the molting and reproductive physiology in crustaceans. The main endocrine centres (Y-organ, mandibular organ, androgenic gland, X-organ and sinus gland) have been described and are available for experimentation. Experimental cautery of Medulla Interna-Medulla Externa-X-organ-sinus gland complex (MI-ME-X-organ-SG) of the eyestalk inhibited molt and brood production demonstrating that the complex plays a role in regulation, at least *via* a positive action upon the circulating ecdysteroids. In the present paper, the results already obtained are reviewed and the perspectives offered by this biological model discussed in reference to other crustaceans.

Key words: mysids, molt-and reproduction-regulation, neurosecretion.

Introduction

In crustaceans, the main biological processes are cyclical, particularly those involved in growth through molting and reproduction. Several decades of research have shown that these processes are regulated by a complex endocrine system (reviews by Carlisle and Knowles, 1959; Fingermann, 1987). Environmental inputs are integrated by the central nervous system. Neurotransmitters and neuromodulators command the release of neuropeptides which govern the production of hormones by endocrine glands (Quackenbuch, 1986). Molting is controlled by molting hormones (MH), which are known to be ecdysteroids, and by neurosecretions from the central nervous system accumulated and released by a neurohemal organ, the sinus gland (SG). Investigations on the physiology of molt and reproduction in crustaceans have been carried out on benthic or terrestrial malacostracans, namely decapods, isopods and amphipods (review by Spindler et al. 1980, 1984).

We have suggested the use of the mysid *Siriella armata* as a new biological subject for neuroendocrine control studies (Cuzin-Roudy and Saleuddin, 1985). Concerning the anatomical features of the main neurosecretory centres and the SG, which are situated in the eyestalk, the shrimp-like mysids resemble decapods. However, they are more like amphipods and isopods because the pattern of sexual differentiation in juveniles and reproduction which is strictly linked to molt cycle (Cuzin-Roudy et al. 1981). These aspects of developmental and reproductive biology are easy to investigate in *S. armata* under laboratory conditions. Here we will review the principal results already obtained, adding new data concerning localization of endocrine centres and the various responses of the

epidermis to molt inhibiting factors. The perspectives gained by investigations on this organism will be presented here with reference to other malacostracan models, with the aim of providing new information for the comprehension of integrated control of molt and reproduction in crustaceans.

Siriella armata as an Experimental Organism

Swarms of the mysid *Siriella armata* inhabit the neritic zone of the bay of Villefranche-sur-mer. The maximum size of adults is only 24 mm, but swarms of several hundreds can be caught easily by a diver with a hand net. Maintained in the laboratory, these mysids keep their swarming behavior, molt and reproduce.

Mysids molt, grow and reproduce throughout their adult life. The duration of the juvenile development and of the adult molt cycle depends upon temperature. In *S. armata*, the adult molt cycle varies from 10 to 34 days in females, and from 7 to 21 days in males, in the temperature range between 23°C and 13°C. Under laboratory conditions (temperature: 20°C; photoperiod: 16 h light/8 h dark; food: *Artemia nauplii*), the development of the young, from their expulsion from the marsupium to full sexual maturity and gamete production, takes 36 days for males and 57 days for females. The duration of the molt cycle increases progressively during successive juvenile stages and is longer for adult reproducing females (Cuzin-Roudy et al. 1981). It lasts 9 days for adult males, and 12 days for adult females which mature a brood in their marsupium and prepare a new batch of eggs during every successive molt cycle (Cuzin-Roudy and Tchernigovtzeff, 1985).

Molt Cycle

A precise timing of the molt cycle was established for both sexes using Drach's method modified for mysids where the integument is transparent and poorly calcified (Cuzin-Roudy and Tchernigovtzeff, 1985). The criteria concerning the development of epidermis and cuticle used for staging are given in Table 1. The succession of events is different from what was observed in *Natantia* (Freeman and Bartell, 1975), especially because of the lack of formation of cones at the setal bases. The development of the new setae inside the cuticle of the old setae is similar to that of euphausiids (Buchholz, 1982) and crab larvae (Anger, 1983). In *S. armata*, apolysis is synchronous throughout the integument. Ecdysis is instantaneous in that carapace lifts up as whole and the mysid slides out from the old cuticle while swimming.

Molt staging was facilitated in females by the existing synchrony between the development of the integument during molt preparation, the development of the brood in the marsupium and the maturation of a new batch of eggs in the ovary. Molt staging could be attained by examination of live female under a stereomicroscope (see Cuzin-Roudy and Tchernigovtzeff, 1985).

Table 1: Principal criteria used for staging the molt cycle of *Siriella armata* from *in vivo* observation of external ramus of uropod with light microscopy

Molt stage	Observation of the integument
E	Ecdysis
A1	Cuticle thin and soft. Blood lacunae with haemocytes in setae and spines
A2	Cuticle thin but rigid. Lacunae and haemocytes in setae and spines
B	Cuticle thickens. Lacunae condense progressively in one main blood lacuna, central to the appendage
C	Cuticle has attained maximal thickness. Limits of main blood lacuna are conspicuous at bases of setae and spines, which exhibit close-textured tissues
Apolysis	Epidermis starts to retract from cuticle
D0	Progressive retraction of epidermis
D1 (early)	Splits appear between basal matrix-cells of setae and spines
D1 (middle)	Matrices of new setae and spines half split
D1 (terminal)	Splitting is complete, attaining 1/3 of length of old setae
D2 (early)	Deposition of new cuticle is visible as refringent layer on surface of epidermis
D2 (terminal)	Barbules are conspicuous on cuticle of new setae

Reproductive Cycle

Embryonic and post-embryonic development of the young occur in the marsupim of the female. Juveniles are liberated immediately before ecdysis of the female, shortly after which she lays a new batch of eggs in the marsupim. A secondary vitellogenic cycle starts for a new batch of oocytes on the second day (stage B) of the female molt cycle. Secondary vitellogenesis is not only cyclic as in other crustaceans (Charniaux-Cotton, 1985) but also strictly linked to the molt cycle, offering an example of the type-2 pattern for the regulation of simultaneous gonadal and somatic growth in crustaceans (Adiyodi and Subramoniam, 1983; Charniaux-Cotton, 1985).

During juvenile development, gonads and gonoducts differentiate before the appearance of secondary sexual

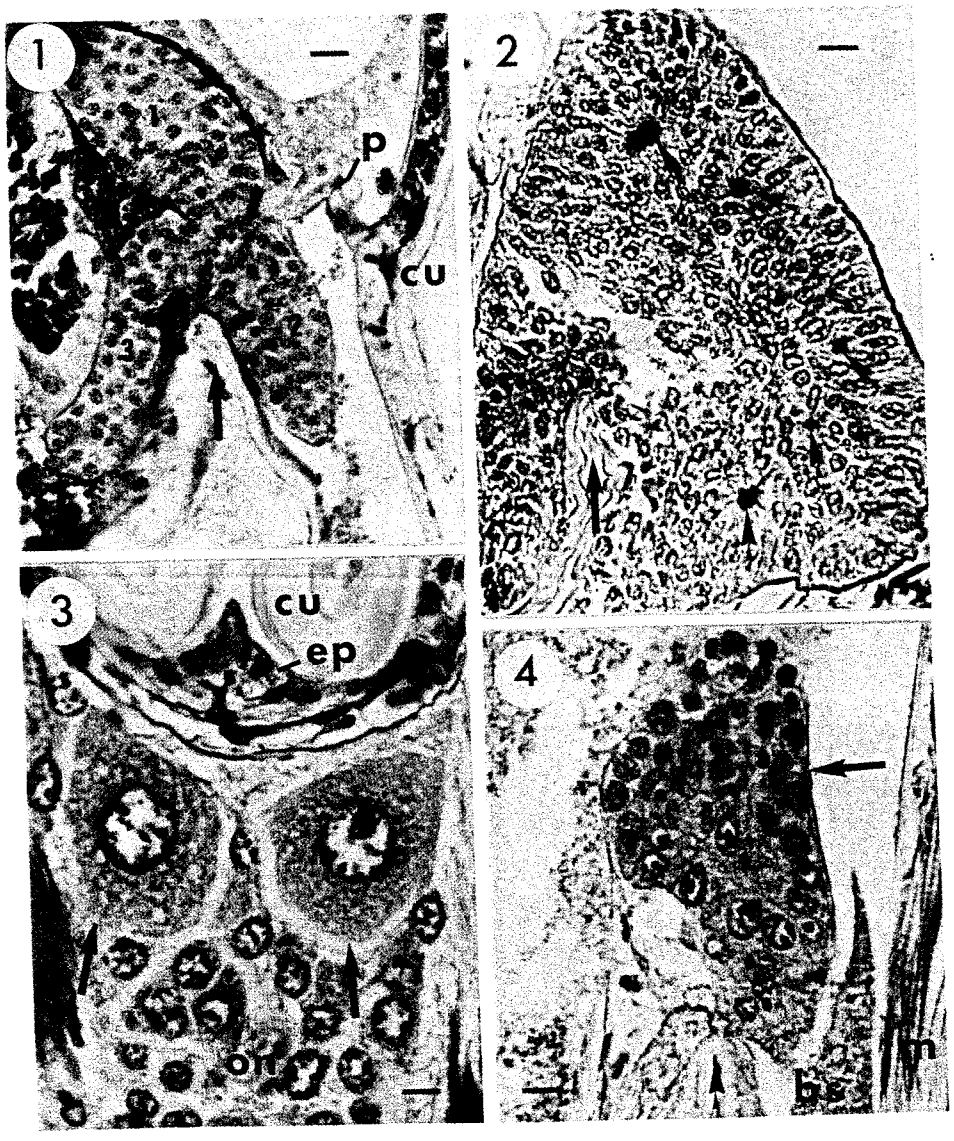


Fig. 1. *Siriella armata*: Histological section of the Y-organ of a female (molt stage C: diecdysis) showing its three lobes (1,2,3) cu: cuticle; p: peduncle formed by an invagination of the external integument; arrow indicates the pleural invagination of the maxillular segment. Bar = 15 µm. Staining: paraldehyde fuchsine and Halmi's triple stain.

Fig. 2. *Siriella armata*: Section of the Y-organ of a female totally inhibited following MI-ME X-organ destruction. Small arrows show the accumulation of a product in the lumen of the acini of the gland; large arrow: as in Fig. 1. Bar = 20 µm. Staining as in Fig. 1.

Fig. 3. *Siriella armata*: Two giant neurones (arrows) of the anterior protocerebron of a female (molt stage C: diecdysis). Note their superficial situation under the frontal integument. cu: cuticle; ep: epidermis; on: ordinary neurones; Bar = 10 µm. Staining as in Fig. 1.

Fig. 4. *Siriella armata*: The mandibular organ (arrow) of a female (molt stage C: diecdysis). bs: blood sinus; m: ventral longitudinal muscle. Bar = 10 µm. Staining as in Fig. 1.

characters as in other peracarids (Cuzin-Roudy et al. 1981) and as in euphausiids (Cuzin-Roudy, 1987), whilst the reverse is true in brachyurans (Payen, 1986).

Endocrine glands

In males of *S. armata*, an androgenic gland (AG) lies on the vas deferens (Cuzin-Roudy et al. 1981). This gland is well developed in males at juvenile stage VI and is likely the source of an androgenic hormone (AH) responsible for the development and the maintenance of male characters and production of gametes, as demonstrated in other peracarids (Charniaux-Cotton, 1959) and in decapods (Payen, 1980).

We report here the localization of a mandibular organ and Y-organ in the ventral region of the thorax. The mandibular organ of a young adult female at stage C is shown in Fig. 4 and it lies in the mandibular segment, beside a longitudinal muscle and a blood sinus. The histological feature is similar to that described by Le Roux (1974) for the mandibular organ of adult *Homarus americanus*. This gland in *S. armata* might be the source of methylfarnesoate, a product similar to the juvenile hormone of insects. Laufer et al. (1987) have shown that methylfarnesoate from the mandibular gland stimulates ovarian development in the crab *Libinia emarginata*.

The presence of a Y-organ has been reported in mysids by Gabe (1956, 1967). In *S. armata*, the Y-organ is situated at the top of the pleural invagination of the maxillulary segment, and is attached to the external epidermis as described for euphausiids and the decapod *Natantia* by Le Roux (1974). In *S. armata*, the Y-organ is composed of 3 lobes connected to the integument by a cuticular peduncle (Fig. 1). At stage C (diecdysis), it is formed of regularly packed cells, with relatively poor cytoplasm, spherical nuclei with scarce chromatin and one or two conspicious nucleoli. The cycle of activity of the Y-organ during the normal molt cycle has not yet been investigated, but the Y-organ of a female totally inhibited for molt and brood maturation is shown in Fig. 2. The structural features are the same as in Fig. 1, but a paraldehyde fuchsin (PAF) positive product fills the lumen of the acini of the gland.

The sinus gland (SG) and X-organ have been described in mysids (Gabe, 1967; Hogstad, 1969; Kulakovskii, 1970), which differ anatomically from those in the eyestalk of decapods. A more detailed study of *S. armata* has shown that several types of neurons (G1, G2, G3, G4 types and a Giant cell) are more or less scattered among ordinary neurons on the neuropil of the X-organ (Cuzin-Roudy and Saleuddin, 1985). Only the G1 cells are grouped in a conspicuous MI-ME X-organ.

An ultrastructural study of the MI-ME X organ-SG complex (Cuzin-Roudy, Ashton and Saleuddin, 1988) has shown that the secretory activity of the G1 cells is cyclical, reaching maximum at stage D0, when neurosecretory granules accumulate in the cell bodies. Granules are transported in the axons of the G1-cells and accumulate in type-2 swellings and terminals of the SG. The neurosecretion from the G1 cells is then released by exocytosis in the main blood sinus of the eyestalk.

G1-cells of *S. armata* appear structurally similar to type-1 cells of crayfish (Shivers, 1967; Andrew and Saleuddin, 1978; Andrew et al. 1978) and to the alpha-cells forming the MEX in *Palaemon serratus* (Bellon-Humbert et al. 1981), but differ in that the G1-cells are the exclusive constituents of the MI-ME X-organ.

The SG of crustaceans is a complex neurohemal structure (for review see Skinner, 1985). In *S. armata*, we have found four different types of neurosecretory granules in the axonal terminals where exocytotic profiles are commonly seen. Type-2 granules originating from the G1 cells, are extruded during the end of stage C and during DO, but a few do at D2 and A1.

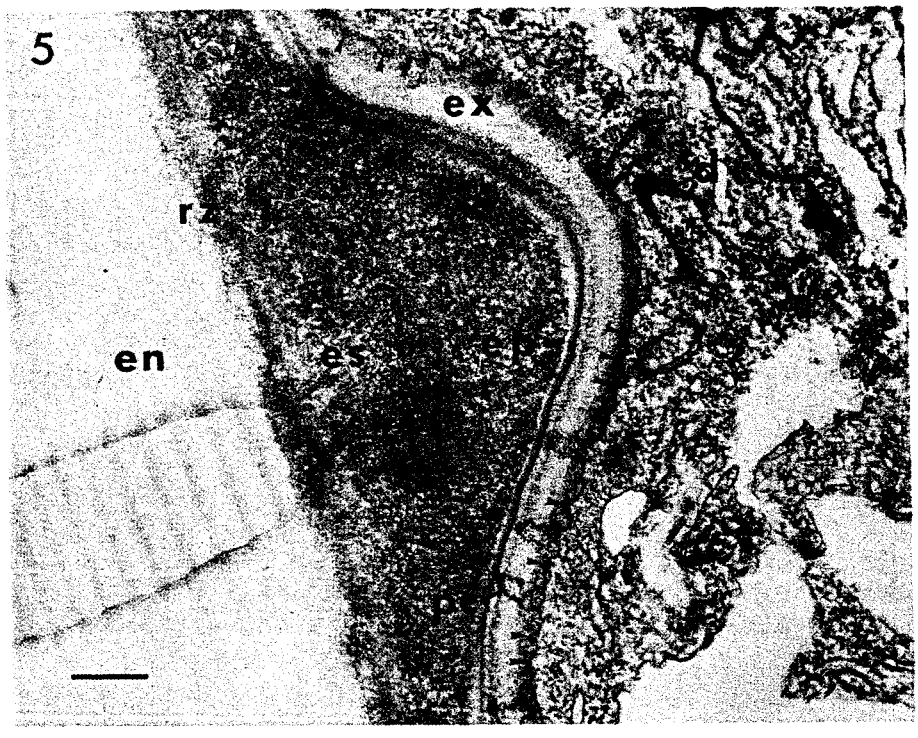
Other cell types identified in *S. armata* eyestalk are G2, G3, G4-cells and the Giant cell. The structural relationship of these cells with the SG has not yet been ascertained.

Neurosecretory neurons each measuring 40 μm in diameter, are present in the anterior part of the protocerebron (Fig. 3). However, their structure has not yet been studied by electron microscopy, but they might possibly be the source of the larger type-1 granules (diameter: 187.3±12.2 nm) seen in the basal part of the SG (Cuzin-Roudy, Ashton, Saleuddin, 1988).

Experimental inhibition of molt and brood-preparation

Destruction of the MI-ME X-organ is performed by electrocauterization of the dorsal part of the eyestalk where it lies superficially under the transparent integument (Cuzin-Roudy and Saleuddin, 1985). There is no bleeding and this treatment ensures a better survival than complete eyestalk deprivation. It is also more precise as other parts of the eyestalk are left intact.

A total molt inhibition is obtained when electrocauterization of MI-ME X-organ is performed before stage C. The inhibition is clearly visible in a live preparation of a uropod, at the level of formation of



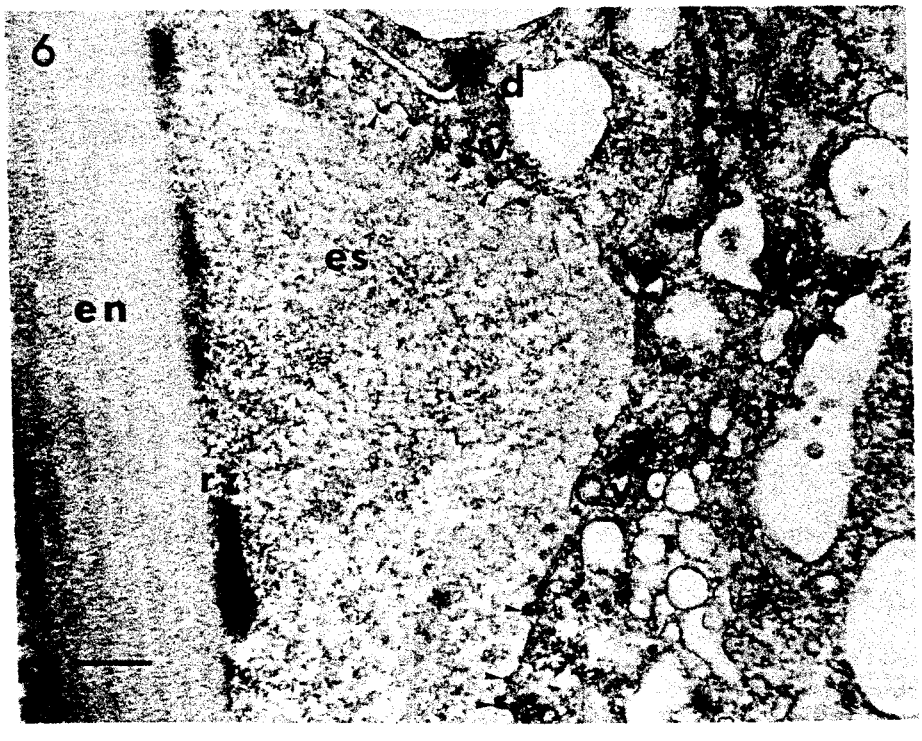


Fig. 5. *Siriella armata*: Ultrastructure of the integument of a female at stage D2 (day 12 of the normal cycle), taken at the base of the eyestalk; d: desmosome between neighbouring epidermic cells; en: endocuticle of the old cuticle; ep: epicuticle of the new cuticle; es: exuvial space; ex: exocuticle of the new cuticle; pc: pore canal; rz: resorption zone; arrow heads show the apical plates of the microvilli of epidermic cells. Bar = 0.3 μm. × 34,000

Fig. 6. *Siriella armata:* The same part of the integument in a totally inhibited female, following MI-ME X-organ electrocauterization, also fixed on day 12. Note the lack of deposition of a new cuticle. v: vesicle; others: as in fig. 5. Bar 0.3 μm. × 34,000

new setae. The splitting of the setal matrices is always arrested at its beginning (Cuzin-Roudy and Saleuddin, 1985).

We report here that inhibition of epidermal activity is not limited to setae-bearing appendices but affects the whole integument. Fig. 5 shows the ultrastructure of the integument from the base of the eyestalk of a normal female fixed at D2 (day 12 of the molt cycle) as compared to the integument of a totally inhibited female, also fixed at day 12 (Fig. 6). In both, the epidermis is folded inside the old cuticle, indicating that cell-multiplication was not suppressed in the inhibited animal. The ecdysial space similarly is unaffected, indicating that apolysis and retraction of epidermis were also normal. Microvilli and their membrane-plaques are conspicuous on the apical membrane of epidermal cells. Coated vesicles are present in the cytoplasm near the apical membrane, and may fuse with it, as described by Powell and Halcrow (1985) during epicuticle secretion in *Idothea baltica*. In the normal female the epicuticle formation is completed. The exocuticle with pore canals is in the course of deposition (Fig. 5). In the inhibited integument, only a faint dense substance is discernable along the apical membrane and no structured epicuticle is deposited (Fig. 6). The situation is similar for males. Therefore, following the destruction of the MI-ME X-organ epidermal cell activity is blocked after apolysis and during resorption of the old cuticle, but before deposition of the new epicuticle.

Concurrent with the arrest of the molt cycle is the arrest of secondary vitellogenesis (Cuzin-Roudy and Saleuddin, 1985). A similar observation of arrest of secondary vitellogenesis, following the MEX extirpation has been described by Faure et al. (1981) in *Palaemon serratus*.

In *S. armata*, when destruction of the MI-ME X-organ is incomplete or performed during stage C, a partial inhibition can be obtained. In that case, the final process of ecdysis only is affected. Ovary development may appear normal, but egg-laying does not occur when ecdysis is inhibited (Cuzin-Roudy and Saleuddin, 1985).

Regulation of Molt Preparation and Ecdysis

Molt regulation in crustaceans is believed to involve ecdysteroids (molting hormone: MH) secreted by the Y-organ and acting upon target tissues, and a neuropeptide molt inhibiting hormone: MIH, which has not been fully characterized. The presence of a molting accelerating hormone (MAH) has also been suggested in decapods (Carlisle and Knowles, 1959) and isopods (Martin et al. 1980).

Ecdysteroid levels, as well as the pattern of the different ecdysteroids present in the hemolymph have been investigated in a limited number of crustacean species and related to the regulation of the molting process and to cyclic maturation of the ovary (review by Spindler et al., 1980, 1984). The variation of titer of ecdysteroids during the molt cycle also offers an indirect assay for MIH, because MIH regulates the secretion of ecdysteroids by the Y-organ (Quackenbush, 1986).

Variations of the hemolymph titers of ecdysteroids have been investigated during the molt cycle of *S. armata*, for males, females and inhibited animals following the destruction of MI-ME X-organ (Cuzin-Roudy et al. 1989). Ecdysteroid blood titers are low during postmolt and before ecdysis, and there is only one sharp peak during premolt. The titer in males of *S. armata* are considerably higher than these in the shrimp *Palaemon serratus* (Van Vormhoudt et al. 1985). They are similar to the concentrations found in amphipods (Graf and Delbecque, 1987) but higher than in isopods (Girard and Maissiat, 1983; Hoarau and Hirn, 1978). The position of the peak is late D1 for males of *S. armata*.

In *S. armata* the titers were also higher in females than those observed in vitellogenic females of the amphipod *O. gammarellus* (Blanchet et al., 1979) and the isopod *H. brevivornis* (Hoarau and Hirn, 1978). The peak occurred at D2 for the females of these three species.

In crustaceans, as in other arthropods, a relationship is postulated between the increase of ecdysteroid titer in the hemolymph with the occurrence of apolysis and with the activity of the cuticle secreting epidermis. Maissiat and Graf (1973) have induced apolysis, or inhibited ecdysis in isopods, depending on the time of the experimental application of 20-hydroxyecdysone. Graf and Delbecque (1987) postulated that the peak occurring at D1 in *Orchestia cavimana* might trigger the deposition of epicuticle. In males of *S. armata*, the peak occurs a few hours before the onset of epicuticle secretion. Once started, cuticle secretion proceeds with low ecdysteroid titers in males, during the end of premolt as well as during postmolt. The fact that in females titers are still very high at D2 indicates that chitin synthesis and deposition are largely independent of absolute ecdysteroid titers.

Our results with molt inhibited *S. armata* yield further data about the start of epidermal activity and the succession of processes involved during molt-preparation. In case of total molt-inhibition, the process of formation of the new setae and spines stops at a precise step, the beginning of the splitting between epidermal cells, resulting in the reorganization of the setal matrices. The same step in the normal cycle corresponds to the ascending part of the peak of D1 (day

7 in males, and 9 in females) and is more advanced from the onset of epicuticle deposition (day 8 and 11, respectively), corresponding to the high concentrations followed by a significant decrease in males. Epicuticle deposition never begins in totally inhibited animals where the ecdysteroid concentrations never increase over the baseline. The conclusion follows that the first step of epidermal reorganization might be triggered by the increase of titers over a threshold, at D0 or onset of D1, while epicuticle secretion and deposition appear linked to the higher titer of ecdysteroids of late D1. These hypotheses are consistent with the discrepancies found in previous reports (reviewed by Spindler et al. 1980) which can be largely explained by a lack of precision in the distinction between the successive steps of molt preparation.

The necessity of relatively high levels of ecdysteroids for the achievement of cell movements insuring the complete splitting of the setal matrices may be compared to the morphological effect obtained in insect cell-culture by treatment by ecdysteroids, resulting in a change of shape through an action upon the extension of the microtubular network (Koolman and Spindler, 1983). In *Palaemon serratus*, Tchernigovtzeff (1976) has shown an important microtubular structure in cells forming the setal matrices. It may be also interesting to point out that exceptionally high titers of ecdysteroids were related to the autotomizing molt of dominant males in *Macrobrachium rosenbergii* (Harpaz et al. 1987).

Freeman and Costlow (1984) have stimulated apolysis in larvae of *Rhithropanopeus harrisii* with 20–0H ecdysone *in vivo* and *in vitro*. In *S. armata*, no significant increase in ecdysteroid concentration was observed during postmolt or during intermolt. Apolysis occurs normally in molt-inhibited animals, with titers remaining low. This is an indication that the movements involved in epidermal retraction during apolysis are of a different nature than movements involved by cell reorganization in the epidermis before cuticule formation. Moreover, apolysis does not seem to be affected directly nor indirectly by neurosecretions of the MI-ME X-organ.

Ecdysteroid titers in partially inhibited males were low at D1 and high at the time of ecdysis. We have related the inhibition of ecdysis to the abnormal high titers because the epidermis and the new cuticle seem to have normal structure (Cuzin-Roudy et al. 1989). Therefore, low levels of ecdysteroids are necessary for normal ecdysis in *S. armata*, as has also been demonstrated by Blanchet (1972) for *Orchestia gammarella*.

Abundance of type-2 granules in the G1-cells and SG at D0 coincides with the elevation of the titers of ecdysteroids in *S. armata*. Destruction of the MI-ME X-organ prevents this elevation. Therefore, the neurosectretion of the G1-cells appears involved in the regulation of several processes of molt preparation, including a positive action upon ecdysteroid titers.

Regulation of Brood Preparation

The relationship between molting and reproduction is diverse throughout the crustacean phylum (Adiyodi and Subramoniam, 1983) and their integration is regulated by complex, and yet largely unknown, endocrine controls (Quackenbush, 1986). In *S. armata*, secondary vitellogenesis starts at the beginning of the molt cycle, when ecdysteroid titers are relatively low, which is in agreement with previous observations in other crustaceans presenting a synchrony of secondary vitellogenesis with the molt cycle (review by Charniaux-Cotton, 1985).

In *S. armata*, it was striking to observe that circulating ecdysteroids were ten times higher in females than in males but the response of the epidermis during molt preparation was the same in both sexes (Cuzin-Roudy et al. 1989). It is then likely that the processes leading to molt are controlled by ecdysteroids and that the higher titers in females are involved in oocytes development.

The presence in females of much higher levels of ecdysone, 20-hydroxyecdysone and an important fraction of high polarity products (HPP) seems to be linked to the storage of ecdysteroids in oocytes during secondary vitellogenesis (Lachaise and Hoffman, 1977; Lachaise et al. 1981; Chaix and de Reggi, 1982. Oocytes, eggs and embryos of different crustaceans have been shown to accumulate ecdysteroids (review by Spindler et al. 1984; 1987) and Chaix and de Reggi (1982) demonstrated that HPP were passed on from the ovary to the eggs in the crab *Acanthonyx lunulatus*.

Neurofactors are known to act on the control of secondary vitellogenesis in crustaceans. A vitellogenesis inhibiting hormone (VIH), has been reported as a product of the SG, at least in seasonally reproducing decapods (Payen, 1986), in isopods(Legrand et al. 1982) and recently in the brain of the amphipod *Orchestia gammarella* (Blanchet-Tournier, 1987). In *Palaemon serratus*, where a GIH is postulated, secondary vitellogenesis is nevertheless blocked, in a similar way as in *S. armata* after MI-ME X-organ destruction, but by an extirpation of the MEX (Faure, Bellon-Humbert and Charniaux-Cotton, 1981). Presence of vitellogenesis stimulating, or activating, neurohormones (VSH or VAH) have been reported in crabs and isopods (review in Payen, 1986). In *Orchestia*, in addition to MH the presence of a

protocerebral factor is required, for the onset of secondary vitellogenesis and yolk accumulation (Blanchet-Tournier, 1982). According to Gupta et al. (1987), a stimulating factor released from the eyestalk is necessary to ensure normal vitellogenesis in the crab *Paratelphusa hydrodromus*, as well as synchronous development of the oocytes in the ovary.

To summarize, the G1-cells of the MI-ME X-organ might be the source of a neurohormone regulating the course of secondary vitellogenesis, at least by way of a positive action upon the hemolymphatic ecdysteroids involved in ovarian activity during brood preparation.

Conclusions and Perspectives

The mysid *S. armata* offers a good biological model where molt-inhibition can be induced experimentally at different successive steps in the molt cycle and this led us to propose a more refined definition of molt inhibition.

Apolysis, the first sign of premolt, was never inhibited by MI-ME X-organ destruction. Therefore, it appears independent from a direct action of the hormone(s) of the G1-cells. It is also independent from the variations of concentration of ecdysteroids, at least from the large ones leading to the peak at premolt. We were also able to define a new step subject to inhibition during molt-preparation, namely the structural reorganization of epidermal cells at early D1, which is related to increasing concentrations of ecdysteroids. Epicuticle secretion is more precisely related to the ecdysteroid peak.

The results of our studies upon the MI-ME X-organ and hemolymph ecdysteroids titers, demonstrate that the events of premolt, as well as those concerned with brood maturation, respond to variations in ecdysteroid titer in the hemolymph and also to neurosecretion from the G1 cells of the MI-ME X-organ.

The main results reported here show that 1) low ecdysteroid titers are necessary for ecdysis; 2) an elevation of the concentration of ecdysteroids is necessary for the reorganization of the epidermal cells following apolysis and for subsequent cuticular secretion; 3) the peak of ecdysteroid titer immediately precedes the onset of cuticle secretion; 4) the titers of ecdysteroids are controlled by the neurosecretion from the G1 cells of the MI-ME X-organ.

The neurosecretory factor from G1 cells would be functionally comparable to the MAH of isopods and amphipods (review in Martin et al. 1980) and to the prothoracicotropic hormone of insects (Bollenbacher and Gilbert, 1982).

The elucidation of the precise role played by the MI-ME X-organ in *S. armata* awaits further investigations. Although more than one neurohormone may be secreted by the G1 cells our results suggest one neurohormone mediating indirect control of crucial steps of molt- and brood-preparation through an action on circulating ecdysteroids.

In isopods, Martin et al. (1980), have postulated an antagonistic and alternative action of two neurohormones, the MIH and the MAH, for the control of molt. In *S. armata*, the MIH would be produced during post molt, resulting in low ecdysteroid titers. Identification of MIH has been sought for several decades now and was said to be elusive, mainly because of the lack of a simple biological assay for molt inhibition (Quackenbush, 1986).

Finally, *S. armata* offers a model were the MI-ME X-organ is constituted by one type of neurosecretory cells, the G1 cells, contributing to the SG type 2 neurosecretory granules. Even if we are aware that one type of granule might contain the precursor of two neuropeptides, as demonstrated for vertebrates and molluscs, this feature renders the experimental model provided by *S. armata* considerably simpler than the ones offered by the SG of decapods, which is likely to contain up to 18 neurosecretory factors (Cooke and Sullivan, 1982). To date, *S. armata* is the first biological model among stalked-eyed crustaceans presenting an X-organ-sinus gland structure clearly involved in the regulation of molt and reproduction.

References

Adiyodi, R.G. and Subramoniam, T., Arthropoda-Crustacea. In: Reproductive Biology of Invertebrates. Vol. 1, Oogenesis, Oviposition and Oosorption, K.G. and R.G. Adiyodi, eds., John Wiley and Sons, Ltd., 1983, pp. 443–495.

Andrew, R.D., Saleuddin, A.S.M., Structure and innervation of a crustacean neurosectory cell. Can. J. Zool. 56 (1978) 423–443.

Andrew, R.D., Orchard, I., Saleuddin, A.S.M., Structural reevaluation of the neurosecretory system in the crayfish eyestalk. Cell Tissue Res., 190 (1978) 235–246.

Anger, K., Moult cycle and morphogenesis in *Hyas araneus* larvae (Decapoda Majidae) reared in the laboratory. Helgolander Meeresuntersuchungen-Helgolander Meeresunters, 36 (1983) 285–302.

Bellon-Humbert, C., Van Herp, F., Strolenberg, G.E.C.M., Denuce, J.M., Histological and physioligical aspects of the *medulla externa* X-organ, a neurosecretory cell group in the eyestalk of *Palaemon serratus* Pennant (Crustacea, Decapoda, Natantia). Biol. Bull. 160 (1981) 11–30.

Blanchèt, F., Effets sur la mue et sur la vitellogenèse de la beta-ecdysone introduite aux étapes A et D2 du cycle d'intermue chez *Orchestia gammarella* Pallas (Crustacé, Amphipode). Comparaison avec les effets de la beta- et

de l'alpha-ecdysone aux autres étapes de l'intermue. C.R. Acad. Sc., Paris, sér. D. 274 (1972) 3015–3018.

Blanchet-Tournier, M.F., Quelques aspects des interactions hormonales entre la mue et la vitellogenèse chez le Crustacé Amphipode *Orchestia gammarellus* (Pallas). Reprod. Nutr. Develop. 22 (1982) 325–344.

Blanchet-Tournier, M.F., Mise en évidence d'une activité neurohormonale inhibitrice de la vitellogenèse chez l'amphipode *Orchestia gammarella*, Can. J. Zool. 65 (1987) 1917–1922.

Blanchet-Tournier, M.F., Porcheron, P., Dray, F., Variations du taux des ecdystéroïdes au cours du cycle de mue et de vitellogenèse chez le crustacé Amphipode *Orchestia gammarellus*. Int. J. Invertebr. Reprod. 1(1979) 133–139.

Bolenbacher, W.E. and Gilbert, L.I., Neuroendocrine control of postembryonic development in insects: the prothoracicotropic hormone. In: Neurosecretion: molecules, cells, systems, D.S. Farner, K. Lederis, eds. Plenum Press, New York, 1982, pp. 361–370.

Buchholz, F., Drach's molt staging system adapted for euphausiids. Mar. Biol. 66 (1982) 301–305.

Carlisle, D.B and Knowles, F., Endocrine control in custaceans. Cambridge University Press, New York, 1959, pp. 1–120.

Chaix, J.C. and de Reggi, M., Ecdysteroid levels during ovarian development and embryogenesis in the spider crab *Acanthonyx lunulatus*. Gen. Comp. Endocr. 47 (1982) 7–14.

Charniaux-Cotton, H., Etude comparée du développement post-embryonnaire de l'appareil génital et de la glande androgène chez *Orchestia gammarella* et *Orchestia mediterranea* (Crustacé, Amphipode) — Autodifférenciation ovarienne. Bull. Soc. Zool. France, 84 (1959) 10–115.

Charniaux-Cotton, H., Vitellogenesis and its control in malacostracan crustacea. Amer. Zool. 25 (1985) 197–206.

Cooke, I.M., Sullivan, R.E., Hormones and neurosecretion. In: The Biology of Crustacea, Vol. 3, D.E. Bliss, H.L. Atwood, D.C. Sandeman, eds. Academic Press, New York, 1982, pp. 205–290.

Cuzin-Roudy, J., Gonad differentiation in the Antarctic krill, *Euphausia superba* Dana (Crustacea: Euphausiacea). J. Crustacean Biol. 7 (1987) 518–524.

Cuzin-Roudy, J., Berreur-Bonnenfant, J. and Fried-Montaufier, M.C., Chronology of post-embryonic development in *Siriella armata* (M.Edw.) (Crustacea: Mysidacea) reared in the laboratory: growth and sexual differentiation. Int. J. Invert. Reprod. 4 (1981) 193–208.

Cuzin-Roudy, J., Saleuddin, A.S.M., A study of the neurosecretory centres of the eyestalk in *Siriella armata* M.Edw. (Crustacea: Mysidacea): their involvement in molting and reproduction. Can. J. Zool. 63 (1985) 2783–2788.

Cuzin-Roudy, J., Tchernigovtzeff, C., Chronology of the female molt-cycle in *Sirielia armata* M.Edw. (Crustacea: Mysidacea) based on marsupial development. J. Crustacean Biol. 5 (1985) 1–14.

Cuzin-Roudy, J., Ashton, M.L. and Saleuddin, A.S.M., Neurosecretory centres from the eyestalk of *Siriella armata* M.Edw. (Crustacea: Mysidacea): Ultra-structural variations during normal and experimentally inhibited molt and reproductive cycle. Cell. Tiss. Res. (1988) 254:381–391.

Cuzin-Roudy, J., Strambi, C., Strambi, A., Delbecque, J.P., Hemolymph ecdysteroids and molt cycle in males and females of *Siriella armata* M.Edw. (Crustacea: Mysidacea): possible control by the MI-ME X-organ of the eyestalk. Gen. Comp. Endocrin. (1989) 74:96–109.

Faure, Y., Bellon-Humbert, C. and Charniaux-Cotton, H., Folliculogenèse et vitellogenèse secondaire chez la crevette *Palaemon serratus* (Pennant): contrôle par les pédoncules oculaires et l'organe X de la medulla externa. C.R. Acad. Sci., Paris, sér.D, 293 (1981) 461–466.

Fingermann, M., The endocrine mechanisms of crustaceans. J. Crustacean Biol. 7 (1987) 1–24.

Freeman, J.A. and Bartell, C.K., Characterization of the molt cycle and its hormonal control in *Palaemonetes pugio* (Decapoda: Caridea). Gen. Comp. Endocrinol. 25 (1975) 517–528.

Freeman, J.F. and Costlow, J.D., Endocrine control of apolysis in *Rhithropanopeus harrisii* larvae. J. Crustacean Biol. 4 (1984) 1–6.

Gabe, M., Neurosecretion. Gauthier-Villars, Paris, 1967, pp. 1091.

Gabe, M., Histologie comparée de la glande de mue (organe Y) des Crustacés Malacostracés. Ann. Sc. Nat., Zool. (Paris) 11 (1956) 145–152.

Girard, P. and Maissiat, R., Variations du taux des ecdystéroïdes hémolymphatiques chez le mâle le *Ligia oceanica* (L) (Crustacea, Isopoda, Oniscoidea) en fonction du cycle de mue et des modifications structurales de l'organe Y. Can. J. Zool. 61 (1983) 534–538.

Graf, F. and Delbecque, J.P., Ecdysteroid titers during the molt cycle of *Orchestia cavimana* (Crustacea, Amphipoda). Gen. Comp. Endocrinol. 65 (1987) 23–33.

Gupta, N.V.S., Kurup, K.N.P., Adiyodi, R.G. and Adiyodi, K.G., The antagonism between somatic growth and ovarian growth during different phases in intermoult (stage C4) in sexually mature freshwater crab, *Paratelphusa hydrodromus*. Int. J. Invert. Reprod. Dev. 12 (1987) 307–318.

Harpaz, S., Kahan, D., Morinière, M. and Porcheron, P., Level of ecdysteroids in the hemolymph of the freshwater prawn *Macrobrachium rosenbergii* (Crustacea: Decapoda) in relation to the phenomenon of cheliped autotomy in males. Experientia, 43 (1987) 901–902.

Hoarau, F. and Hirn, M., Evolution des taux d'ecdystéroïdes au cours du cycle de mue chez *Helleria brevicornis* (Isopode Terrestre). C.R. Acad. Sci., Paris, sér. D, 286 (1978) 1443–1446.

Hogstadt, A.M., The X organ and sinus gland of *Mysis relicta* Loven (Crustacea: Mysidacea). Nytt. Mag. Zool., 17 (1969) 105–109.

Koolman, J. and Spindler, K.D., Mechanisms of action of ecdysteroids. In: Endocrinology of Insects, R. Downer, H. Laufer, eds., Alen R. Liss, New York, 1983, pp. 179–201.

Kulakovskii, E.E., Neurosecretory system of *Mysis oculata*. Dok.1 Ad. S.S.S.R., 192 (1970) 226–228.

Lachaise, F. and Hoffmann, J., Ecdysone et

développement ovarien chez un décapode, *Carcinus maenas*. C.R. Acad. Sci., Paris, sér. D, 285 (1977) 701–704.

Lachaise, F., Goudeau, M., Hetru, C., Kappler, C., Hoffmann, J.A., Ecdysteroids and ovarian development in the shore crab *Carcinus maenas*. Hoope-Seyler's Z. Physiol. Chem. 362 (1981) 521–529.

Laufer, H., Borst, D., Baker, F.C., Carrasco, D., Sinkus, M., Reuter, C.C., Tsai, W. and Schooley, D.A., Identification of a juvenile hormone-like compound in a crustacean. Science 235 (1987) 202–205.

Legrand, J.J., Martin, G., Juchault, P., Besse, G., Contrôle neuroendocrine de la reproduction chez les Crustacés. J. Physiol. 78 (1982) 543–552.

Le Roux, A., Mise au point à propos de la distinction entre l'organe Y et l'organe mandibulaire chez les Crustacés Eucarides. C.R. Acad. Sc. Paris, sér. D 278 (1974) 1261–1264.

Maissiat, J. and Graf, F., Action de l'ecdystérone sur l'apolysis et l'ecdysis de divers crustacés isopodes. J. Insect. Physiol. 19 (1973) 1265–1276.

Martin, G., Besse, G. and Mocquart, J. P., Contrôle neurohumoral du cycle de mue chez les mâles de l'Oniscoide *Porcellio dilatatus* Brandt. Bull. Soc. Zool. France, 105 (1980) 73–81.

Payen, G., Experimental studies of repoduction in Malacostraca crustaceans. Endocrine control of spermatogenetic activity. In: Advances in Invertebrate Reproduction, W. Clark and T.S. Adams, eds. Elsevier, Amsterdam, 1980, pp. 187–196.

Payen, G., Endocrine regulation of male and female genital activity in crustaceans, a retrospect and perspectives. In: Advances in Invertebrate Reproduction, 4, M. Porchet, J.C. Andries and A. Dhainaut, eds., 1986, pp. 125–134.

Powell, C.V.L. and Halcrow, K., Formation of the epicuticle in a marine isopod, *Idothea baltica* (Pallas). J. Crustacean Biol. 5 (1985) 439–448.

Quackenbush, L.S., Crustacean endocrinology, a review. Can. J. Fish Aquat. Sci. 43 (1986) 2271–2282.

Shivers, R.R., Fine structure of crayfish optic ganglia. Univ. Kansas Sci. Bull. 47 (1967) 677–733.

Skinner, D.E., Molting and regeneration. In: The Biology of Crustacea, Vol. 9, D.E. Bliss and L.H. Mantel, eds., Academic Press, New York, 1985, pp. 43–145.

Spindler, K.D., Dinan, L. and Londershausen, M., On the mode of action of ecdysteroids in crustaceans. In: Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, J. Hoffmann and A. Porchet, eds., Springer-Verlag, Berlin, 1984, pp. 255–264.

Spindler, K.D., Keller, R. and O'Connor, J.D., The role of ecdysteroids in the crustacean molting cycle. In: Progress in Ecdysone Research, J.A. Hoffmann, ed., Elsevier, Amsterdam, 1980, pp. 247–280.

Spindler, K.D., Van Vormhoudt, A., Sellos, D. and Spindler-Barth, M., Ecdysteroid levels during embryogenesis in the shrimp, *Palaemon serratus* (Crustacea Decapoda): quantitative and qualitative changes. Gen. Comp. Endocrinol. 66 (1987) 116–122.

Tchernigovtzeff, C., Sur l'organisation des matrices sétigères chez deux crustacés malacostracés étudiée au microscope électronique. C.R. Acad. Sc. Paris, sér. D, 282 (1976) 727–729.

Van Vormhoudt, A., Porcheron, P., Le Roux, A., Ecdystéroïdes et synthèses protéiques dans l'hépatopancréas de *Palaemon serratus* (Crustacea, Decapoda) au cours de cycle d'intermue. Bull. Soc. Zool. Fr. 110 (1985) 191–204.