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by Alex Willig

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The role of ecdysones in the crustacean molting cycle

by

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Summary

The discovery of insect molting hormones (ecdysones) in crustaceans indicates that molting in crustaceans and, probably, in all arthropods is controlled by ecdysones, a group of non-polar sterols. In crustaceans, the predominant substance appears to be crustecdysone (20-hydroxy-ecdysone). This hormone is biosynthesized from cholesterol or from phytosterols similar to cholesterol. Sterols are essential constituents of the diet of crustaceans, since these animals have been found to be incapable of synthesizing sterols. Except for the conversion of α-ecdysone to crustecdysone, the intermediate steps in crustecdysone biosynthesis are as yet unknown. The site of ecdysone formation appears to be the Y-organs, a pair of glands located in the second antennal or second maxillary segment.

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either
Extirpation of the Y-glands during the intermolt stage or an early premolt stage prevents molting; after re-implantation, the animals undergo molt. The activity of the Y-organs is controlled by neurohormones released by the X-organ sinus gland complex in the crustacean eyestalk. During proecdysis, the molting hormone titer rises and reaches a maximum before ecdysis. During ecdysis, the titer is already decreasing. During proecdysis, a series of biochemical and morphological changes take place, which are probably mediated by the elevated hormone level. Some of these events have been ~~in the experiment~~ experimentally induced by administration of exogenous ecdysones, e.g., stimulation of protein synthesis in the epidermis and hepatopancreas, formation of a new cuticle, formation of gastroliths in freshwater crayfish, yolk formation, and leg regeneration. In these experiments, successful moulting has only occasionally been observed. This may be explained by the fact that injection of ecdysones mimics to an inadequate degree the endogenous hormonal environment during the premolt stages. It may be assumed that a characteristic change of the hormone titer as a function of time is necessary to induce the various biochemical and morphogenetic events in an ordered sequence, and that an integrated progression is a prerequisite for successful ecdysis. Crustecdysone acts not only as hormone but also as pheromone by influencing the sexual behavior of certain male crabs, when it is released by molting females.

1. Crustacean ecdysones

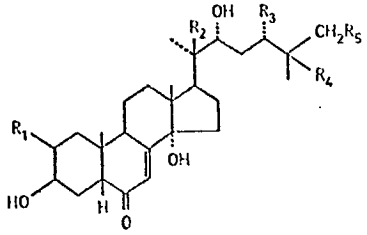
In order to be able to grow, crustaceans, like insects, must molt at periodic intervals, i.e. shed or cast off their exoskeleton and produce a new, larger one. Experimental work carried out by Kopec in 1922 had already demonstrated that the molting process in insects is under hormonal control. Subsequently, both Gabe (1953) and Echalier (1954; 1955) demonstrated that the molt of crustaceans also is induced by a hormone produced in the Y-organs.

The first attempts to isolate the molting hormone of insects were undertaken by Butenandt and Karlson (1954). The latter workers were able to isolate 25 mg of crystalline hormone from 500 kg of dried pupae of the silk moth (Bombyx mori).

This molting hormone was called ecdysone. The process of gradual concentration of the hormone was observed with the aid of a biological test, i.e. the Calliphora test (Fraenkel, 1935; Becker and Plagge, 1939), in which prepupate dipterous larvae are subjected to ligation behind the first third of the body, so that the larvae are able to undergo pupation in the first third of the body. The posterior part of the body, which does not undergo pupation, responds to injection of molting hormone-containing solutions with either complete or partial pupation. The rate of pupation can be related to the quantity of hormone injected. The structure of the molting hormone has been elucidated in its most important aspects by Karlson and his coworkers (for a review, cf. Karlson, 1966). The final structure was determined with the aid of X-ray structural analysis (Huber and Hoppe, 1965) to be 2β,3β,14α,22R,25-penta-hydroxy-5β-cholest-7en-6one. During the processing of their extracts from Bombyx mori, Karlson et al. isolated small quantities of a substance very similar to ecdysone, but somewhat more polar in character, which substance was also active in the Calliphora test. That substance was described as β-ecdysone, and the original substance, as α-ecdysone. Subsequently, β-ecdysone has been isolated by various groups of workers, and has been identified as 20-hydroxy-ecdysone (Hoffmeister, 1966; Hoffmeister and Gruetzmacher, 1966; Hocks and Wiechert, 1966; Hoffmeister et al., 1967; Hocks et al., 1967).

Following isolation of ecdysone from insects, Karlson and his coworkers (Karlson, 1956a; Karlson and Skinner, 1960) found substances in extracts from crustaceans that were active in the Calliphora test. An attempt to isolate the active principle from three tons of the common European shrimp (Crangon vulgaris) was initially without success (Karlson and Schmialek, 1959). Following development of a new extraction and concentration procedure, it became possible to

isolate a small quantity (2 mg) of the pure hormone from one ton of lobster offal (thorax of Jasus lalandei), which hormone was named crustecdysone (Hampshire and Horn, 1966). It turned out that crustecdysone and β-ecdysone represent the same substance (Hoffmeister et al., 1967; Horn et al., 1968). 20-Hydroxyecdysone has been detected furthermore in a series of plants and—independently—was given two additional names, so that six different names are used for this steroid: 20-Hydroxy-ecdysone, β-ecdysone, ecdysterone, crustecdysone, isoinokosterone, and polypodin A. Plants contain additional ecdysone-like substances; no unequivocal experimental results are as yet available regarding their function (for a review, cf. Rees, 1971). The group designation "ecdysones" is used for all ecdysone-like steroids from arthropods and from plants. Apart from crustecdysone, Horn et al., (1966) isolated from Jasus lalandei a somewhat less polar steroid, which initially was regarded as being α-ecdysone. However, isolation of that substance on a greater scale showed that the substance in question was 2-deoxy-crustecdysone (Galbraith et al., 1968). Occurrence of α-ecdysone in crustaceans has hitherto not yet been demonstrated. Faux et al. (1969) found two additional ecdysones in extracts from Callinectes sapidus, viz. callinecdysone A



α-Ecdysone	$R_2 = R_3 = R_5 = H$, $R_1 = R_4 = OH$
Crustecdysone	$R_3 = R_5 = H$, $R_1 = R_2 = R_4 = OH$
2-Deoxycrustecdysone	$R_1 = R_3 = R_5 = H$, $R_2 = R_4 = OH$
Callinecdysone A	$R_3 = R_4 = H$, $R_1 = R_2 = R_5 = OH$
Callinecdysone B	$R_5 = H$, $R_1 = R_2 = R_4 = OH$, $R_3 = CH_3$

and callinecdysone B, which are probably identical with the plant ecdysones inokosterone and makisterone A.

Very little is known at the present time regarding the biosynthesis of the crustacean ecdysones. The crustaceans probably are unable to synthesize the sterol skeleton, and are forced to ingest sterols with their diet. In Astacus, Homarus, Cancer and Lepas, synthesis of sterols from radioactive acetate or from mevalonic acid could not be demonstrated (Zandee, 1962; 1966; 1967; Van den Oord, 1964; Teshima and Kanazawa, 1971). Synthesis of the ecdysones, thus, probably starts with sterols ingested by these animals with their diet, as in the case of insects, where conversion of radiolabelled cholesterol to α-ecdysone and crust-ecdysone has been demonstrated (Karlson and Hoffmeister, 1963; Galbraith et al., 1970; Willig et al., 1971). Intermediate steps of ecdysone biosynthesis have been demonstrated in unequivocal fashion neither in crustaceans nor in insects. King and Siddal (1969) found in both Crangon and Uca conversion of labelled α-ecdysone to crustecdysone, i.e. pprobably the terminal step in crustecdysone biosynthesis. Interestingly, that conversion was more pronounced during molt than during the intermolt period. 57

2. Y-organs

The Y-organs are regarded as the site of synthesis of the molting hormone of crustaceans. These organs are paired groups of epithelial cells located either in the second antennal segment or in the second maxillary segment (Gabe, 1953; 1954). In general, they are regarded as organs corresponding to the prothoracic (or molt) glands of the insects (Passano, 1960; Herman, 1967). Extirpation or destruction of the Y-organs prevents subsequent molting; re-implantation of these glands leads to renewed molting (Echalier, 1954; 1955; 1959; Passano and Jyssum,

1963; Maissiat, 1970a). During proecdysis, the Y-organs exhibit histological changes speaking for increased secretory activity (Gabe, 1956; Matsumoto, 1962). These organs responded with histological changes also to injections of crust-ecdysone or of other steroids (Miyawaki and Taketomi, 1970; 1971; Miyawaki et al., 1971). However, assessment of the different results on the Y-organs is difficult, since, in part, structures have been described as Y-organs, which, without any doubt, have nothing in common with the molt gland, and, in part, the locations of the organs investigated have not been properly described (Sochasky et al., 1972). As yet, there exists no direct evidence indicating that the Y-organs produce ecdysones. As indirect evidence, however, we have the findings reported by Carlisle and Connick (1973)[1], who have been able to extract ecdysones from the Orconectes Y-organs located in the antennal segment; the quantities of these ecdysones varied in dependence on the molt cycle stages.

The secretory activity of the Y-organs is controlled by neurohormones. Removal of the eyestalks, which contain the neurosecretory X-organ-sinus-gland complex, leads in many crustaceans to precocious molting, as does the removal of the sinus gland alone. Re-implantation of the sinus glands delays ecdysis. On the basis of the latter findings, workers have postulated the existence of a molt-inhibiting hormone (MIH) (Brown and Cunningham, 1939; Stephens, 1951; Passano, 1953), representing a peptide hormone, which has already been concentrated and partly characterized (Rao, 1965). The MIH is produced by the neurosecretory cells of the X-organ located in the eyestalk; its release takes place by way of a neurohemal organ, viz. the sinus gland (for review articles, cf.

[1] Translator's note: See also D.B. Carlisle and F. Knowles, Endocrine Control in Crustaceans, Cambridge University Press, New York, 1959.

Bern, 1962; Scharrer and Weitzman, 1970). Since the removal of the eyestalks occasionally also delays or prevents molting, several authors have postulated 58
the existence of a molt-accelerating neurohormone (MAH) (Carlisle, 1953; Carlisle and Dohrn, 1953; Scheer and Scheer, 1954; McWhinnie and Kirchenberg, 1962; Aiken, 1969; McWhinnie et al., 1969; McWhinnie and Mohrherr, 1970). However, hitherto it has not yet been possible to obtain definite evidence regarding the actual existence of such a hormone. An effect of MAH on the production of ecdysones in the Y-organs also has as yet not been established. According to recent findings reported by McWhinnie et al. (1969; 1972), it appears that MAH controls directly, at least, partial events in the preparation for proecdysis, such as the decalcification of the old exoskeleton.

3. The molt cycle

In the past, the term 'molt' has been used in two different senses in crustaceans and insects, respectively. In part, that term covered only the shedding of the exuvia; in part, already the separation of the epidermis from the old cuticle, i.e. an event taking place considerably earlier than shedding. Already Drach (1939) regarded that separation as the actual molting event. Subsequently, Jenkin (1966) and Jenkin and Hinton (1966) have introduced for all arthropods the concept of apolysis, which covers the separation of the epidermal cells from the old cuticle.

The molt cycle of the crustaceans can be divided into distinct stages. Drach (1939) has developed certain criteria for the differentiation of the individual stages of the molt cycle of brachyura, which will be outlined only briefly in the present paper:

Stage A: The period following immediately after casting-off of the old skeleton (ecdysis, exuviation); the new exoskeleton is still entirely soft.

Stage B: Solidification of the new exoskeleton, due to hardening of the cuticle and incorporation of calcite particles, sets in.

Stage C: Intermolt period. The cuticle hardens further until, finally, the innermost, so-called membranous layer has been produced.

Stage D: Proecdysis starts with apolysis and ends with exuviation (casting-off). Five substages, D_0 to D_4, are distinguished.

Stage E: Molt (ecdysis, exuviation). Casting-off of the old exoskeleton.

For differentiation of the individual stages, we use morphological characteristics like the state of development of new bristles, the thickness of the newly formed cuticle, opening of the dorsal rupturing line in the exoskeleton, the different hardening stages of the new exoskeleton, etc. This division into stages, originally applied only to the crabs, has subsequently been modified and extended to include other crustacean groups. All stages have been divided further into substages (Drach and Tchernigovtzeff, 1967; McWhinnie, 1962; Skinner, 1962; Stevenson, 1961; 1968; Stevenson et al., 1968; Travis, 1965a; 1965b; early literature, in Passano, 1960). Adelung (1967; 1969; 1970) has employed a somewhat different division of the molt cycle for juvenile European shore crabs (Carcinus maenas), which takes into consideration, in particular, the state of development of the walking legs.

The subdivision of Stage D is of particular interest with regard to investigations of the hormonal control of the molt cycle. Since we will discuss further below the morphogenetic and physiological events taking place during the preparatory phase of ecdysis in connection with the molting hormone titer of a crayfish (Orconectes limosus, Willig and Keller, 1973), we wish (in agreement with Stevenson, 1972) to define at this point the substages of Stage D, with particular consideration given to the situation existing in the astacurans. 59

D_0: Apolysis in parts of the integument; appearance of gastroliths; regenerate growth.

D_1: Apolysis in the entire integument; formation of new bristles at the uropods; the new bristles are fully differentiated at the conclusion of this substage; sub-substages D_1' to D_1''' may be distinguished on the basis of the state of bristle development (Drach, 1944; Drach and Tchernigovtzeff, 1967; Stevenson et al., 1968).

D_2: Secretion of the new cuticle on the branchiostegites; at the end of this substage, the new cuticle has attained one half of the thickness exhibited at ecdysis.

D_3: Further growth of both the cuticle and the gastroliths.

D_4: The lower seam of the branchiostegites is distinctly set off from the carapace due to complete decalcification and advanced cuticular reabsorption (molting line).

4. Molting hormone titer and events of the preparatory phase (Figures 1 and 2)

D_0: This stage characterizes the onset of preparation for ecdysis. The sole definite characteristic for the onset of the premolt period is found in the appearance of small gastroliths, which are formed apparently under the influence of a slightly elevated ecdysone level (Willig and Keller, 1973; McWhinnie et al., 1972) (in the following discussions, the term ecdysone will be used as group name or, otherwise, be characterized as α-ecdysone). The Stage D_0 is frequently described as a period of activation, in particular, of the integumentary tissue. That activation, however, has hitherto been defined only to an inadequate extent, since, apart from a certain enlargement of the epidermal cells (Travis, 1955; Dennel, 1960; Skinner, 1962; Stevenson et al., 1968), neither light-microscopical nor electron-microscopical changes have been detected in the integumentary tissue (Keller and Adelung, 1970; Kuemmel et al., 1970); the exoskeleton also appears

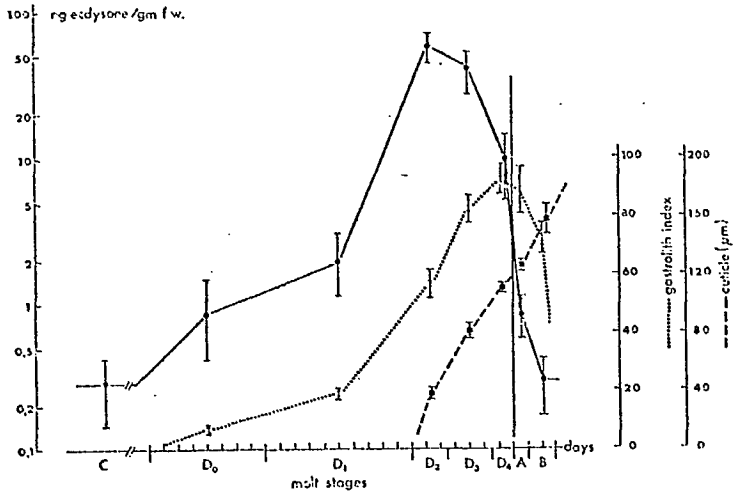


Figure 1 - Molting hormone titer, gastrolith index (mg gastrolith weight/cm carapace length) and cuticle growth in the respective molt stages of the crayfish Orconectes limosus. Mean values and mean errors (±). Molting hormone: Log plot in ecdysone equivalents (from Willig and Keller, 1973).

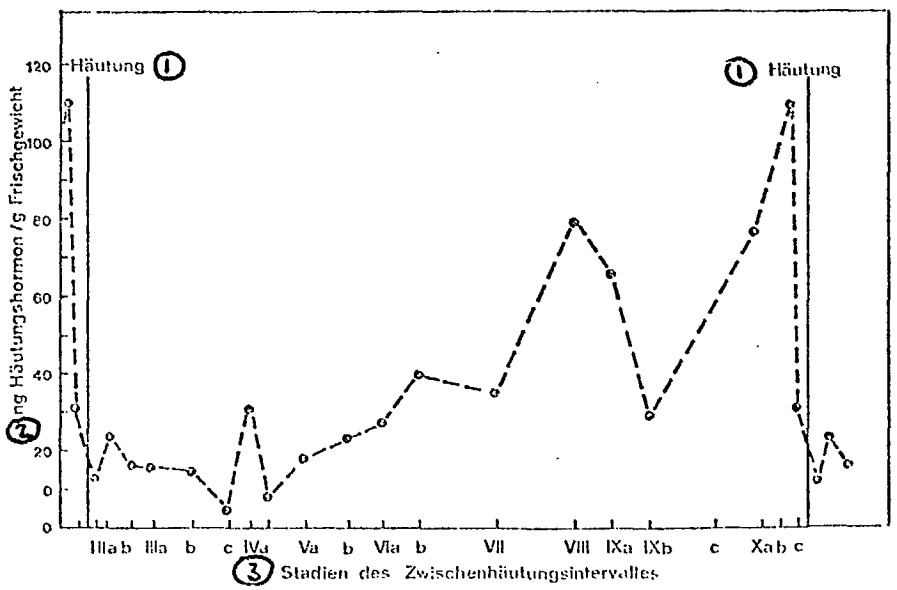


Figure 2 - Molting hormone titer during one intermolt stage of Carcinus maenas (from Adelung, 1969). Key: 1, Molt; 2, ng molting hormone/g fresh weight; 3, Intermolt stages.

to exhibit no changes (Travis, 1965a). The DNA content of the epidermis, related to the fresh weight of the tissue, decreases greatly during this stage; however, it is probable that the total DNA quantity remains constant, i.e. the decreases is only an apparent one due to the enlargement of the cells (Humphreys and Stevenson, 1973). It then is still unclear whether DNA synthesis takes place during D_0, although Tchernigovtzeff (1959) has reported seeing mitoses. The ribosomal RNA is as stable as during the C Stages. The protein synthesis of the epidermal cells is clearly reduced during D_0 compared to the intermolt stages, during which that synthesis remains about constant, if we regard the rate of incorporation of labelled leucine into protein (related to the DNA content) as reliable measure for protein synthesis independent of the water content of the cells (Humphreys and Stevenson, 1973). The oxygen consumption of the integumentary tissue also is reduced compared to the intermolt period (Skinner, 1962). In view of these findings, equalization of Stage D_0 with a period of activation of the epidermal cells appears to be a questionable suggestion. It may be more readily imagined that that stage represents a kind of transition period, during which the growth processes in the integument are arrested, and the biosynthetic apparatus of the cells is redirected toward the approaching morphogenetic events.

We must, however, remember that the afore-mentioned findings have been obtained chiefly in the integumentary tissue of the branchiostegites, i.e. the lateral flanks of the carapace. The epidermal activation processes may already be advanced further in the limbs. In the uropods, for instance, we may already see apolysis (Stevenson, 1972); furthermore, regeneration of new limbs starts during D_0; loss of limbs, on the one hand, accelerates the entire preparation for ecdysis (Skinner and Graham, 1970; 1971), while, on the other one, the

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subsequent development of the regenerates is stimulated by the molting hormone (Rao et al., 1971). In-vitro experiments have demonstrated somewhat increased protein synthesis in the midgut gland (Gorell and Gilbert, 1971).

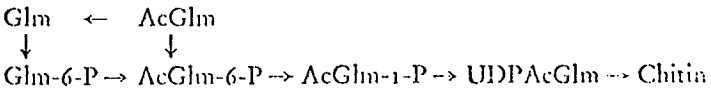
The events taking place during D_0 apparently are induced by an ecdysone 61
titer only slightly elevated; further development requires an increase of the hormone, because development of events is arrested, if either the Y-organs are removed (Echalier, 1954) or the environmental conditions become unfavorable (cf. Passano, 1960).

D_1: The course of molting can still be inhibited during Stage D_1 by removal of the Y-organs. This particular stage is characterized by apolysis in wide parts of the integument, in particular, in the branchiostegites. In the uropods, there appear the new bristles; at the conclusion of this stage, they are fully developed. Subsequently, only the cuticle of the bristles and that of the integument become thicker. During D_1, activation processes in the epidermis are reflected in a somewhat more pronounced development of the rough endoplasmic reticulum; in addition, a pronounced increase of the Golgi fields is in evidence (Kuemmel et al., 1970). The Q_{O_2} of the integumentary tissue increases (Skinner, 1962), as does the turnover of ribosomal RNA (Skinner, 1962). Despite further enlargement of the epidermal cells (corresponding to a weight increase), the DNA content, related to the fresh weight of the integument, increases again. The synthesis of protein, related to the DNA content, increases at the onset of D_1; then decreases again; and finally rises to values, that are greater than those measured during the C Stages (Humphreys and Stevenson, 1973). We are permitted to assume that synthesis of enzymes for breaking down the old cuticle is taking place. In some parts of the integument—in particular, in the limbs, secretion of the new cuticle is already starting. This increase of metabolic activity is preceded by a triplication of the ecdysone titer.

According to findings recently obtained by Stevenson and Tardif (Stevenson, 1972), the increase of protein synthesis is perhaps based on regulatory processes at the translation level. Actinomycin did not inhibit, but stimulate the incorporation of leucine into protein, with that stimulation being greater during D_0 than during D_1; during D_2, however, actinomycin had an inhibitory effect. Possibly, we are dealing here with a RNA-dependent, labile translation repressor for certain proteins, the synthesis of which is inhibited by actinomycin. Apparently, synthesis of this repressor decreases during the natural course of the molt cycle.

D_2: During this stage, during which the new cuticle attains about half the thickness normally exhibited, we are able to discern, already in morphological terms, maximal activation of the integumentary tissue (Keller and Adelung, 1970). That finding corresponds to the peaks reached by the Q_{O_2} (Skinner, 1962) and protein synthesis (Humphreys and Stevenson, 1973), respectively. The turnover of ribosomal RNA increases further (Skinner, 1966). The appearance of the new cuticle is accompanied by a very distinct increase of the incorporation of acetyl glucosamine-^{14}C and glucose-^{14}C into chitin (Hornung and Stevenson, 1971; Lang, 1971; Speck et al., 1972; Stevenson, 1972). Interestingly, the increase in the incorporation of acetyl glucosamine takes place both earlier and more intensively than the incorporation of glucose, which becomes more pronounced only following ecdysis. Speck et al. (1972) have suggested the existence of an intracellular regulatory mechanism, which interferes with the fructose 6-phosphate → glucosamine 6-phosphate reaction and, prior to ecdysis, blocks the formation of chitin from glucose. Gwinn and Stevenson (Stevenson, 1972) have been able to demonstrate an acetyl glucosamine kinase in the epidermis of Orconectes, which returns the acetyl glucosamine liberated from the cuticle, without detour by

way of glucosamine, directly into the newly formed chitin. Synthesis of chitin



is inhibited by actinomycin D, indicating an activation of transcription for 62
new enzymes (Stevenson, 1972). Apart from the enzymes required for the formation of the new cuticle, the cuticular proteins themselves have to be synthesized. These highly distinct synthetic activities are associated with the peak of the molting hormone titer, which, compared to the preceding stages, has increased thirty-fold (Willig and Keller, 1973). During D_2, i.e. following maximal release of ecdysones, removal of the Y-organs no longer prevents ecdysis.

D_3 and D_4: During these stages, during which the pre-exuvial cuticle is produced, we see, at the morphological level, increasing inactivation in the integumentary tissue. The epidermal cells are transformed, due to shrinking, from a cylindrical type into a stilt type (Keller and Adelung, 1970). The Q_{O_2} (Skinner, 1962) and the protein synthesis (Humphreys and Stevenson, 1973) decrease again. In the midgut gland, protein synthesis, which was increased during the early premolt stages, also decreases again (Gorell and Gilbert, 1971). On the other hand, the incorporation of acetyl glucosamine into chitin of the new exoskeleton does not increase compared to D_2. A certain autoradiographic finding (Keller and Adelung, 1970)—according to which the incorporation of uridine into the epidermal nuclei reaches a peak during D_3 and D_4—cannot directly reconciled with the general inactivation being in evidence (apart from the synthesis of chitin). However, it appears possible that this increased incorporation of uridine represents a sign for decreased turnover and accumulation of

stable RNA for the subsequent morphogenetic events and, in particular, for the formation of the endocuticle and the synthesis of chitin following ecdysis. The ecdysone titer decreases strongly in the course of these two stages. The gastroliths have attained their maximal size.

A and B: The stages following ecdysis are distinguished by further inactivation in evidence at the morphological level; the stilt-type character of the epidermal epithelium becomes more pronounced; the incorporation of uridine into the epidermal nuclei decreases (Keller and Adelung, 1970). On the other hand, the incorporation of glucose-^{14}C into chitin increases considerably (Hornung and Stevenson, 1971; Lang, 1971); that incorporation attains its peak during these stages, and decreases only at the end of Stage B, with the final production of the endocuticle. Hohnke (1971) found maximal chitin synthetase activity during Stage A; during Stage B, already a decrease of that activity. In contrast to the premolt stages, the synthesis of chitin is no longer inhibited by actinomycin (Stevenson and Tung, 1971); apparently, no additional RNA is required for that synthesis. On the basis of these findings, we would be permitted to conclude that either the RNA or the enzymes involved in chitin synthesis are extraordinarily stable, because synthesis of chitin continues for two weeks after ecdysis. The titer of the molting hormone decreases steeply during Stage A, and during Stage B reaches the very low values of the intermolt period. The considerable synthetic performances occurring during these two stages, thus, must be independent of the synthesis of both RNA and protein induced by hormonal means.

Comparison of the hormone titer with the metabolic events taking place at the same time permits the conclusion that these events—during the premolt period and Stage D_2—depend on a continuous hormonal action. Each one of these

stages can be shortened by administering exogenous ecdysone (Stevenson and Tschantz, 1973). The possibility that these processes are elicited during an early stage by means of a release of hormone and then continue on independent of the hormone is excluded. Artificial induction by means of injected ecdysone (cf. further below) requires quantities, exceeding by far the maximal endogenous levels measured (Adelung, 1969; Willig and Keller, 1973); this, probably, to ensure more prolonged action. The course of the hormone titer, furthermore, suggests that the ordered sequence of the molt-preparing events depends on an integrated change of the ecdysone titer. For the normal development of the events, it is apparently necessary that a relatively low, only gradually rising ecdysone level be maintained over a relatively long period of time (D_0 to D_1). The very marked activation processes occurring during D_2 require a very high hormonal level, as is indicated by the thirty-fold increase of the molting hormone titer compared to D_1. 63

The molting hormone titer curves of Orconectes (Willig and Keller, 1973) and those of Carcinus (Adelung, 1969) exhibit agreement in several important aspects. In both cases, we find the peak values between D_1 and D_4, with the Carcinus curve, however, exhibiting two maxima in that range—a feature not in evidence in the case of Orconectes. In Carcinus as well as in Orconectes, the hormone titer decreases sharply already prior to ecdysis, i.e. during D_4. In Orconectes, a very low value (0.3 ng/g) is reached during Stage B, while, in Carcinus, the average value is higher (16 ng/g) during the postmolt stages. Apart from species-specific differences, that particular difference might be due to the fact that the intermolt period was much shorter in the juvenile shore crabs employed by Adelung than in Orconectes, since that author had removed seven of the eight walking legs of his animals for the purpose of stimulating

and synchronizing the molting events. Removal of the walking legs can induce precocious molting (Skinner and Graham, 1970; 1972); it is probable that that intervention stimulates the release of ecdysones. It is possible that the normal intermolt stage, with its low hormone titer, is omitted in the case of leg-amputated Carcinus. In correspondence, the rise to the hormone peak is only six-fold in the latter case, while Orconectes exhibited a thirty-fold rise.

Both titer curves exhibit a steep drop shortly before ecdysis, which drop appears to be very essential as an event, since normal ecdysis and normal cuticle formation do not occur without that drop; that has been demonstrated in experimental work, in which the hormone level was artificially increased shortly before ecdysis by administration of exogenous ecdysone (cf. further below). Recently, Carlisle and Connick (1973) have published a hormone titer curve for Orconectes, which deviates significantly from the results reported by Adelung (1969) and by Willig and Keller (1973) to the extent that it shows strongly elevated hormone values also after ecdysis. Comparison of the respective findings, however, is difficult, since several methodical details—for instance, the reference basis for the hormone values—have not been reported [by Carlisle and Connick, 1973]. Faux et al. (1969) have also found high hormone levels in Callinectes sapidus after ecdysis. During the early premolt stage, these blue crabs contain small quantities of callinecdysone A; shortly before ecdysis, greater quantities of that hormone, and, at the same time, a little crustecdysone; and after ecdysis, high concentrations of crustecdysone, in addition to a little callinecdysone B. The high crustecdysone titer after ecdysis has been associated with the hardening of the new cuticle, as is the case in insects, where Karlson and Sekeris (1962) have been able to demonstrate an induction of the synthesis of tanning quinones by ecdysone. However, hardening of the cuticle apparently

does not depend on the presence of this hormone in both Orconectes and Carcinus. It is probable that the latter event is controlled by neurohormones produced in the eyestalks (Fingerman and Yamamoto, 1964).

5. Action of exogenous ecdysones

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Soon after the isolation of the insect molting hormone, viz. ecdysone, ecdysones were isolated also from crustaceans. The suggestion that ecdysones controlled the molting events also in that arthropod group arose. As soon as synthetic ecdysones or natural ecdysones of adequate purity had become available, workers went ahead and attempted to induce ecdysis in crayfish [and related animals] with the aid of these substances. Furthermore, it had become possible by that time to investigate the action of the ecdysones on individual metabolic processes involved in the ecdysial event.

5.1. Moltings

The most important question, of course, was whether ecdysones actually would induce molting in crustaceans, i.e. whether administration of exogenous ecdysone would lead to a reduction of the duration of the intermolt period. The first evidence in that direction was provided by Carlisle (1957), who reported that ecdysis was induced in Carcinus maenas by means of injecting Y-organ extracts. Once methods for extraction and concentration of ecdysones had been developed, Carlisle (1965) was able to induce ecdysis in juvenile shore crabs by means of repeated injections of extracts from a series of crustaceans (copepods, euphausiaceans, mysidaceans, Carcinus) and from the migratory desert locust (Schistocerca). The hromone content of these extracts is not known. The Y-organs of the shore crabs employed had been destroyed prior to injection, in

order to prevent molting hormone production by the experimental animals. However, in shore crabs still possessing their Y-organs, Adelung (1967) was able to determine no reduction of the molt cycle following administration of about 0.2 µg ecdysone; Skinner and Graham (1970) obtained a similar negative result in Gecarcinus lateralis. Lowe et al. (1968) also were unable to induce ecdysis in intact Procambarus simulans using crustecdysone doses of 0.025 to 0.250 µg/g. Following injection of similar doses into animals whose eyestalks—i.e. the source of the molt-inhibiting hormone—had been removed, the animals underwent molt more rapidly than did control animals whose eyestalks had also been removed. It would then appear that the hormone dose administered was inadequate in the case of the animals still possessing their eyestalks, although that dose—as we know today—was very high compared to the natural hormone level prevailing prior to ecdysis. Using still higher crustecdysone doses of 3 to 25 µg/g, Krishnakumaran and Schneiderman (1968; 1969; 1970) were able to induce ecdysis or preparation for ecdysis, respectively, in intact Armadillidium, Procambarus and Uca. Similar findings have been obtained by Balesdent (1971) in Asellus aquaticus; Maissiat (1970b) and Maissiat and Legrand (1970) in Ligia oceanica; Reidenbach (1971) in Idotea balthica; Blanchet and Charniaux-Cotton (1971) in Orchestia gammarella; Graf (1972) in Orchestia cavimana; Kurata (1968) in Penaeus japonicus; Fukutake et al. (1969)[1], Hubschman and Armstrong (1972) in Palaemonetes kadiakensis; Rao et al. (1973) in Homarus americanus; Warner et al. (1969) and Warner and Stevenson (1972) in Orconectes obscurus; McWhinnie et al. (1972) in Orconectes virilis; and Smiley (1971) in both Procambarus clarkii and Cambarus sciotensis. We may mention in this connection that ecdysis by means of exogenous ecdysones has

[1] Quoted after Miyawaki and Taketomi (1971).

been induced also in chelicerata (Limulus, Araneus, Dugesiella) (Krishnakumaran and Schneiderman, 1970; Jegla and Costlow, 1970; Jegla et al., 1972; Herman, 1972).

In the majority of all these investigations, complete, normally proceeding 65
moltings have been observed only occasionally, while molt-preparing events—like apolysis, production of a new cuticle, formation of new bristles, regenerate growth, and gastrolith formation—have been induced in reliable fashion. The early premolt stages up to Stage D_2 can be shortened by administering exogenous ecdysones (Stevenson and Tschantz, 1973). The finding showing that complete molting can be induced only with difficulty, no doubt, may be explained by the fact that the hormonal environment characteristic for the natural premolt period can be recreated only to an inadequate degree by administering this hormone. We must assume that gradually increasing release of the hormone as a function of time is necessary to induce the various biochemical and morphogenetic events in the different tisues in an ordered sequence, and that such an integrated progression is a prerequisite for successful molting. Injection of exogenous ecdysone, in fact, will induce molt-preparing processes, but these processes do not take place in ordered sequence in time. The integrated progression of the molt-preparing events depends on the hormone titer changing in a highly defined fashion. It is important not only that the titer rises at the proper point in time, but also that it again drops shortly before acdysis. Blanchet (1972) has been able to demonstrate in Orchestia that injection of crustecdysone toward the end of Stage D_2 impedes and delays ecdysis due to supernormal thickening of the new cuticle. Retardation of ecdysis following crustecdysone injection has been observed also by Graf (1972). Following administration of high crustecdysone doses, Krishnakumaran and Scheiderman (1970) found in Procambarus, Uca and various

chelirata that abnormal cuticles and deformed bristles had been produced. These findings demonstrate that hyperstimulation is possible—a phenomenon observed also in insects, and described by Williams (1968) as hyperecdysonism. In the natural course of the molting events, that state is avoided by the hormone titer drop occurring at the proper time. Following ecdysis, i.e. during Stages A and B, the tissues appear not to respond to ecdysones, since findings obtained by Laissiat and Legrand (1970) in Porcellio and by Rao et al. (1973) in Homarus americanus have shown that administration of exogenous ecdysone during these stages does not result in stimulation of cuticle growth. The tissues respond again to this hormone only during Stage C.

5.2. Different actions of α-ecdysone and crustecdysone

In both Uca pugilator and Homarus americanus, high doses of crustecdysone, in fact, have led to induction of preparation for ecdysis, but the animals died in most cases before completion of ecdysis (Rao et al., 1972; 1973). The percentage of successful moltings was greater following treatment of the animals with α-ecdysone. An explanation for the lower mortality following administration of α-ecdysone may be found in the conversion of α-ecdysone to crustecdysone, which has been demonstrated by King and Siddall (1969). Gradual conversion could bring about that a relatively low crustecdysone level is maintained over a relatively long period of time, which level imitates the natural situation better does than one single high crustecdysone dose. That explanation requires that crustecdysone is the actual, active hormone, and that α-ecdysone is merely an inactive biosynthetic precursor, whose presence even in high concentrations is innocuous. However, in insects, it has been found that both ecdysones exert their specific actions, with an additional metabolite (3-dehydro-ecdysone) playing a

certain role (Karlson; cf. pages 23 to 33 of the present Volume). A further explanation for the higher percentage of successful moltings following adminstration of α-ecdysone is found in a result reported by Adelung (1967) in Carcinus maenas. Injected radioactive α-ecdysone was almost completely broken down within two hours. Extracts from animals having received injections of α-ecdysone, in correspondence, contain only very little molting hormone activity after a few hours; however, the hormone titer of the animals rises again to a considerable extent several hours after the hormone injection, and then drops only slowly. It would then appear that administration of exogenous hormone stimulates the production of endogenous hormone in the Y-organs. Due to the existence of this positive feedback mechanism, a greater quantity of hormone reaches the target organs than has actually been injected, and an elevated hormone level is maintained for a longer period of time. However, it is not known whether α-ecdysone alone exerts this effect; it is possible that crustecdysone, too, stimulates the hormone production in the Y-organs, since that hormone brings about histological changes in these organs, which point toward increased activity in the tissues (Miyawaki and Taketomi, 1970). 66

5.3. Regenerate growth

Numerous crustaceans are able to regenerate lost limbs. The lost limb (leg) is initially replaced by a soft regenerate, which requires several molts for developing into the complete limb. Bliss (1956) distinguished two phases of regenerate growth, viz. basal growth, which takes place during the intermolt period, and a second growth step, which is observed only during the premolt stage. It had to be assumed that the second growth phase of regenerating limbs is stimulated by the elevated ecdysone level prevailing during that stage. Following

injection of ecdysone, Rao et al. (1971; 1972) observed accelerated regenerate growth in Uca. It was found that not only the first growth phase, but also the second one can be accelerated by administration of exogenous ecdysone. In both phases, the rate of growth of the regenerates was higher following administration of high ecdysone doses than following administration of low doses.

Amputation of several walking legs results in both Carcinus (Adelung, 1969) and Gecarcinus (Skinner and Graham, 1970; 1972) in a shortening of the molt cycle. On the basis of that finding, we are permitted to conclude that the loss of limbs stimulates both the production and the release of ecdysones either directly in the Y-organs or indirectly by way of a lowering of the MIH level. Hormone release accelerates regenerate growth, and leads to precocious ecdysis, after which event the animal again possesses a complete set of limbs. It appears probable that the high ecdysone titer and the bimodal character of the titer curve found in leg-amputated shore crabs (Adelung, 1969) may be explained by this stimulation of hormone release.

5.4. Calcium metabolism

During the preparatory phase, or proecdysis, numerous crustaceans produce gastroliths in the anterior division of the stomach, which concretions consist of calcium carbonate. A part of the calcium released from the old exoskeleton is stored in the gastroliths. During the concluding phase of ecdysis (postecdysis), the gastroliths are again dissolved, and the calcium liberated is incorporated into the new exoskeleton. In the case of crayfish dwelling in freshwater containing little calcium, the advantage of that mechanism is found in a saving of calcium (McWhinnie, 1962). Marine crustaceans also retain and store a part of the calcium liberated from the old exoskeleton; this, however, not in the form

of gastroliths, but in dissolved form in the hemolymph. The calcium content of the hemolymph of Carcinus increases during proecdysis by about 80 per cent, 67
and decreases again during postecdysis (Adelung, 1970). However, the absolute quantity of calcium stored in the hemolymph is very small; supply of calcium for the new exoskeleton represents no problem for marine shore crabs.

The close connection existing between calcium metabolism and the molting events suggests that that metabolism may be controlled by the molting hormone. However, due to the low degree of similarity existing between the titer curves of molting hormone and of hemolymph calcium during the molt cycle, Adelung (1970) believed that regulation of the calcium level by crustecdysone is improbable. On the other hand, injection of Y-organ extract reportedly results in Carcinus in a three-fold rise of the blood calcium level (Carlisle, 1957). By means of injecting crustecdysone, Krishnakumaran and Schneiderman (1970) were able to induce gastrolith formation in Procambarus species; Smiley (1971), in both Procambarus clarkii and Cambarus sciontensis; and McWhinnie et al. (1972), in Orconectes virilis. In Orchestia, crustecdysone promotes calcium storage in the caecum (Graf, 1972). Various reports agree that the gastroliths are better developed following administration of low crustecdysone doses than following administration of high doses. Using X-ray photography in intact animals, McWhinnie et al. (1972) have been able to determine exactly the time of appearance of gastroliths following hormone administration. Following repeated injections of 1 µg/g, these gastric concretions appeared considerably more rapidly than following injections of 8 µg/g. It would then appear that a low hormone level is required for the coordinated formation of gastroliths. A relatively low ecdysone titer actually does exist during the early premolt stages, i.e. at the time of gastrolith formation.

During preparation for ecdysis, the calcium metabolism, however, is not controlled solely by ecdysone. It appears that neurohormones are also involved, since injection of eyestalk extract produced in Orconectes a decrease of the calcium content of the branchiostegites, while crustecdysone brought about no changes (McWhinnie et al., 1972). In correspondence, it has been found that the calcium level in the hemolymph is increased by eyestalk hormones (Charmantier Daures and Vernet, 1970). Eyestalk hormones control the calcium liberation from the old exoskeleton, while ecdysone promotes the formation of gastroliths.

5.5. Protein metabolism

The quantity of organic substances is lower in many crustaceans during the preparatory phase (proecdysis) than during the C Stages (cf. Passano, 1960). The increased contents of free amino acids (FAA) of the hepatopancreas, the hypodermis, the muscles and the hemolymph point toward a breakdown of the organic reserves (McWhinnie et al., 1972). Since it was possible to induce that increase by means of removing the eyestalks, there was evidence for hormonal control of amino acid mobilization, which takes place either by way of the eyestalk neurohormones or, indirectly, by way of ecdysone production in the Y-organs. That question was resolved by injecting crustecdysone, which caused the content of FAA to increase (McWhinnie et al., 1972).

Either simultaneously with this amino acid mobilization or a little later, there occurs a marked increase of the de-novo synthesis of proteins in the epidermis (Skinner, 1965; McWhinnie and Mohrherr, 1970; Stevenson, 1972), which reaches its peak during D_2 at the same time as the maximal release of the molting hormone. That increase is controlled by the molting hormone. Gorell and Gilbert (1969) observed increased incorporation of leucine into proteins of the

hepatopancreas following treatment with crustecdysone *in vitro*; following administration of crustecdysone, McWhinnie et al. (1972) found also *in vivo* increased leucine incorporation into both the hepatopancreas and the epidermis. In that particular experiment, the epidermis responded more rapidly to the hormone with a significant increase of protein synthesis, *viz.* within 24 to 36 hurs, while the midgut gland responded only after 48 hours. These particular results were obtained following one or two injections of the hormone; it is probable that these effects would be even more marked under continuous influence of the hormone. 68

The finding showing that protein synthesis in the midgut gland is stimulated by crustecdysone treatment characterizes that organ as a site of attack of the hormone (Gorell and Gilbert, 1969). Following treatment of the midgut gland with tritiated α-ecdysone both *in vivo* and *in vitro*, respectively, Gorell et al. (1972) found that certain proteins were labeled, *viz.* a 6.35 S protein and a 11.5 S protein; the light protein probably represents a subunit of the heavy one. Analysis of the radioactive, bound material surprisingly revealed that it was neither α-ecdysone nor crustecdysone, but a metabolite still not identified, which is somewhat less polar than α-ecdysone. The view that this metabolite actually represents the active form of the hormone (Gorell et al., 1972) is supported by the fact that there exists a specifically binding protein; that would be unusual in the case of an inactive product of degradation.

The findings presently available underline the influence exerted by ecdysones on the mechanism of protein sysnthesis; however, adequate investigations regarding the induction of formation of specific RNA individuals under the influence of ecdysone(s) are still missing in crustaceans. Following removal of the eyestalks, Fingerman et al. (1967) observed a decrease of the RNA content of the midgut gland, while Skinner (1966) found a prolonged rise of the content of

ribosomal RNA in both the midgut gland and the hypodermis. However, following eyestalk removal, we might also be dealing with elimination of neurohormones (Kurup and Scheer, 1966), which elimination cannot be distinguished from specific ecdysone effects, if the Y-organs have not also been removed.

5.6. Vitellogenesis

Removal of the eyestalks led in Leander to precocious yolk formation in the ovaries; implantation of eyestalks prevented vitellogenesis (Panouse, 1943). It would appear that vitellogenesis is controlled by neurohormones produced in the eyestalks. However, that control possibly takes place only by indirect means, i.e. it could take place by way of the ecdysone production in the Y-organs controlled by the eyestalk hormone (MIH). In fact, it has been found that yolk formation in Porcellio is inhibited following extirpation of the Y-organs (Besse and Maissiat, 1971). Injection of α-ecdysone induces precocious yolk formation in Asellus (Reidenbach, 1971); in Idotea, α-ecdysone stimulates growth of the endocrine follicular tissue, vitellogenesis, and growth of the oostegites (Balesdent, 1971). In Orchestia, however, crustecdysone did not affect the gonads, and vitellogenesis was not stimulated (Blanchet, 1972). These findings may be due to either species-specfic differences or different modes of action of the two ecdysones.

6. Pheromonal action of crustecdysone

Already one hundred years ago, Louis Agassiz described the characteristic sexual behavior of the males of certain brachyuran species. They search for females about to undergo molting, and grasp and hold them then for several days 69
until molt occurs. Copulation takes place right after the old exoskeleton has been cast off. Before the males grasp the females, they run about on the tips of the first three pairs of walking legs, with the cephalothorax raised high.

That behavior is exhibited by Portunas sanguinolentus males kept in isolation, is water is led into their aquarium, in which water premolt females has been kept first. The female then must excrete a pheromone (Ryan, 1966). That pheromone is excreted with the urine, and the males do not respond, if the excretory pores of the females have been sealed with paraffin. A pheromone has also been demonstrated in the case of Homarus americanus, which is discharged by the female and elicites sexual behavior in the male. However, in the case of that species, the pheromone is excreted by freshly molted females (Atema and Engstrom, 1971).

The pheromone excreted by portunid females has been concentrated by Kittredge and Takahashi (1972), with the sexual behavior of the males being used as biological test [for the degree of concentration attained]. It was found that this pheromone was a highly polar steroid. The suggestion that the pheromone represented excreted molting hormone arose, since it is excreted by premolt females. Actually, it has been possible to elicit typical sexual behavior in males with the aid of crustecdysone, with concentrations as low as 10^{-13} M being effective. Cancer anthonyi and Cancer antennarius also responded to crustecdysone. Since the males exhibited (no) sexual behavior in the presence of other premolt males, juvenile females of their own species or adult females of closely related species (Ryan, 1966), they must have available additional information regarding species relationship, sex and physiological state, which, without doubt, cannot be transferred by way of crustecdysone. However, it has as yet not been investigated whether that information may be transferred also by chemical means.

Bibliography

1. ADELUNG, D.: Die Wirkung von Ecdyson bei *Carcinus maenas* L. und der Crustecdysontiter während eines Häutungszyklus. Zool. Anz. Suppl. *30*, 264–272 (1967).
2. – Die Ausschüttung und Funktion von Häutungshormon während eines Zwischenhäutungs-Intervalles bei der Strandkrabbe *Carcinus maenas* L. Z. Naturforsch. *24b*, 1447–1455 (1969).
3. – Die Veränderungen des Kalziumgehaltes in der Kutikula und der Hämolymphe der Strandkrabbe *Carcinus maenas* L. während eines Häutungsintervalles. Zool. Anz. Suppl. *33*, 244–248 (1970).

AIKEN, D. E.: Photoperiod, endocrinology and the crustacean molt cycle. Science *164*, 149–155 (1969).

ATEMA, J. und D. G. ENGSTROM: Sex pheromone in the lobster, *Homarus americanus*. Nature, Lond. *232*, 621–263 (1971).

BAUDIENT, M.-L.: Action d'une ecdysone de synthèse sur la mue et sur la sexualité du crustacé isopode femelle *Asellus aquaticus* L. C. R. Acad. Sci. Paris, Ser. D *273*, 1972–1974 (1971).

4. BECKER, E. und E. PLAGGE: Über das Puppariumbildung auslösende Hormon der Fliegen. Biol. Zb. *59*, 326–341 (1939).

BERN, H. A.: The properties of neurosecretory cells. Gen. comp. Endocr., Suppl. *1*, 117–132 (1962).

BIESE, G. und J. MAISSIAT: Action de la glande de mue sur la vitellogenèse du Crustacé Isopode *Porcellio dilatatus*. C. R. Acad. Sci. Paris, Ser. D *273*, 1975–1978 (1971).

BLANCHET, M.-F.: Effets sur la mue et sur la vitellogenèse de la β-ecdysone introduite aux étapes A et D_2 du cycle d'intermue chez *Orchestia gammarella* Pallas (Crustacé Amphipode). Comparaison avec de la β- et de la α-ecdysone aux autres étapes de l'intermue. C. R. Acad. Sci. Paris, Ser. D *274*, 3015–3018 (1972).

BLANCHET, M.-F. und H. CHARNIAUX-COTTON: Contrôle du déclenchement et de la durée de la période D du cycle d'intermue par l'ecdysterone, chez le Crustacé Amphipode *Orchestia gammarella* (Pallas); interaction avec la vitellogenèse. C. R. Acad. Sci. Paris, Ser. D *272*, 307–310 (1971).

BLISS, D. E.: Neurosecretion and the control of growth in a decapod crustacean. In: (K. G. Wingstrand, ed.) Bertil Hanström, Zoological papers in honour of his sixty-fifth birthday November 20th 1956, 56–75. Lund, Sweden: Zoological Institute (1956).

BROWN, F. A. und O. CUNNINGHAM: Influence of the sinus gland of crustaceans on normal viability and ecdysis. Biol. Bull. Woods Hole *77*, 104–114 (1939).

5. BUTENANDT, A. und P. KARLSON: Über die Isolierung eines Metamorphose-Hormons der Insekten in kristalliner Form. Z. Naturforsch. *96*, 389–391 (1954).

CARLISLE, D. B.: Studies on *Lysmata seticaudata* Rossi (Crustacea: Decapoda) - III. On the activity of moult accelerating principle(s) when administered by the oral route. IV. On the site of origin of the moult-accelerating principle – experimental evidence. Pubbl. Sta. Zool. Napoli *24*, 278–291 (1953).

– On the hormonal inhibition of moulting in decapod crustacea. II. The terminal anecdysis in crabs. J. Mar. Biol. Ass. U.K. *36*, 291–307 (1957).

– The effects of crustacean and locust ecdysones on moulting and proecdysis in juvenile shore crabs. Gen. comp. Endocr. *5*, 366–372 (1965).

CARLISLE, D. B. und P. F. R. DOHRN: Studies on *Lysmata seticaudata* Rossi (Crustacea: Decapoda) – II. Experimental evidence for a growth- and moult-accelerating factor obtainable from eyestalks. Pubbl. Sta. Zool. Napoli *24*, 69–83 (1953).

CARLISLE, D. B. und R. O. CONNICK: Sur l'organe Y, la glande de la mue chez les Crustacés décapodes macroures. C. R. Acad. Sci. Paris, Ser. D *276*, 45–47 (1973).

CHARMANTIER DAURES, M. C. und G. VERNET: Effets de l'ablation des pédoncules oculaires sur la variation de la concentration en calcium dans l'hémolymphe de *Pachygrapsus marmoratus* Fabricius au cours du cycle d'intermue. C. R. Acad. Sci. Paris, Ser. D *271*, 1646–1649 (1970).

DENNELL, R.: Integument and exoskeleton. In: «The Physiology of Crustacea» (T. H. Waterman, ed.) vol. 1, 449–472. New York: Academic Press 1960.

DRACH, P.: Mue et cycle d'intermue chez les crustacés décapodes. Ann. Inst. Oceanogr. Monaco *19*, 103–391 (1939).

– Etude préliminaire sur le cycle d'intermue et son conditionnement hormonal chez *Leander serratus* (Pennant). Bull. Biol. Fr. Belg. *78*, 40–82 (1944).

DRACH, P. und C. TCHERNIGOVTZEFF: Sur la méthode de détermination des stades d'intermue et son application générale aux crustacés. Vie Milieu Ser. A: Biol. Mar. *18*, 595–610 (1967).

ECHALIER, G.: Recherches expérimentales sur le rôle de «l'organe Y» dans la mue de *Carcinus maenas* (L.) Crustacé Décapode. C. R. Acad. Sci. Paris *238*, 523–525 (1954).

– Rôle de l'organe Y dans le déterminisme de la mue de *Carcinus maenas* (L.). Expériences d'implantation. C. R. Acad. Sci. Paris *240*, 1581–1583 (1955).

– L'organe Y et le déterminisme de la croissance et de la mue chez *Carcinus maenas* (L.), Crustacé Décapode. Ann. Sci. Nat. Ser. 12, Zool. Biol. Anim. *1*, 1–57 (1959).

FAUX, A., D. H. S. HORN, E. J. MIDDLETON, H. M. FALES und M. E. LOWE: Moulting hormones of a crab during ecdysis. Chem. Comm. *1969*, 175–176 (1969).

FINGERMAN, M. und Y. YAMAMOTO: Endocrine control of tanning in the crayfish exoskeleton. Science *144*, 1462 (1964).

FINGERMAN, M. T., T. DOMINICZAK, M. MIYAWAKI, C. OGURO und Y. YAMAMOTO: Neuroendocrine control of the hepatopancreas in the crayfish *Procambarus clarkii*. Physiol. Zool. *40*, 23–30 (1967).

FRAENKEL, G.: A hormone causing pupation in the blowfly *Calliphora erythrocephala*. Proc. R. Soc. (B) *118*, 1–12 (1935).

GABE, M.: Sur l'existence, chez quelques Crustacés Malacostracés, d'un organe comparable à la glande de la mue des insectes. C. R. Acad. Sci. Paris *237*, 1111–1113 (1953).

– Particularités morphologiques de l'organe Y (glande de la mue) des Crustacés Malacostracés. Bull. Soc. Zool. Fr. *79*, 166 (1954).

GABE, M.: Histologie comparée de la glande de mue (organe Y) des Crustacés Malacostracés. Ann. Sci. Nat. Ser. *11*, Zool. Biol. Anim. *18*, 145–152 (1956).

GALBRAITH, M. N., D. H. S. HORN, E. J. MIDDLETON und R. J. HACKNEY: Structure of deoxycrustecdysone, a second crustacean moulting hormone. Chem. Comm. *1968*, 83–85 (1968).

GALBRAITH, M. N., D. H. S. HORN, E. J. MIDDLETON und J. A. THOMSON: The biosynthesis of crustecdysone in the blowfly *Calliphora stygia*. Chem. Comm. 1970, 179–180 (1970).

GORELL, T. A. und L. I. GILBERT: Stimulation of protein and RNA synthesis in the crayfish hepatopancreas by crustecdysone. Gen. comp. Endocr. *13*, 308–310 (1969).

– Protein and RNA synthesis in the premolt crayfish *Orconectes virilis*. Z. vergl. Physiol. *73*, 345–356 (1971).

GORELL, T. A., L. I. GILBERT und J. B. SIDDALL: Binding proteins for an ecdysone metabolite in the crustacean hepatopancreas. Proc. Nat. Acad. Sci. USA *69*, 812–815 (1972).

GRAF, M. F.: Action de l'ecdysterone sur la mue, la cuticle et le métabolisme du calcium chez *Orchestia cavimana* Heller (Crustacé Amphipode Talitridé). C. R. Acad. Sci. Paris, Ser. D *274*, 1731–1734 (1972).

HAMPSHIRE, F. und D. H. S. HORN: Structure of crustecdysone, a crustacean moulting hormone. Chem. Comm. *1966*, 37–38 (1966).

HERMAN, W. S.: The ecdysial glands of arthropods. In: «International Review of Cytology» (G. H. Bourne und J. F. Danielli, eds.) vol. 22, 269–347. New York: Academic Press (1967).

– Molt initiation in response to phytoecdysones and low doses of animal ecdysones in the arthropod, *Limulus polyphemus*. Gen. comp. Endocr. *18*, 301–305 (1972).

5a. HOCKS, P. und R. WIECHERT: 20-Hydroxy-Ecdyson, isoliert aus Insekten. Tetrahedron Lett. *1966*, 2989 (1966).

6. HOCKS, P., G. SCHULZ, E. WATZKE und P. KARLSON: Die Struktur des β-Ecdysons (1). Naturwissenschaften *54*, 44–45 (1967).

7. HOFFMEISTER, H.: Ecdysteron, ein neues Häutungshormon der Insekten. Angew. Chem. *78*, 269–270 (1966).

8. HOFFMEISTER, H. und H. F. GRÜTZMACHER: Zur Chemie des Ecdysterons. Tetrahedron Lett. *1966*, 4017–4023 (1966).

9. HOFFMEISTER, H., H. F. GRÜTZMACHER und K. DÜNNEBEIL: Untersuchungen über Struktur und biochemische Wirkung von Ecdysteron. Z. Naturforsch. *22b*, 66–70 (1967).

HOHNER, L. A.: Enzymes of chitin metabolism in the decapod, *Hemigrapsus nudus*. Comp. Biochem. Physiol. *40*, 757–779 (1971).

HORN, D. H. S., E. J. MIDDLETON, J. A. WUNDERLICH und F. HAMPSHIRE: Identity of the moulting hormones of insects and crustaceans. Chem. Comm. *1966*, 339–341 (1966).

HORN, D. H. S., S. FARRI, F. HAMPSHIRE und M. E. LOWE: Isolation of crustecdysone (20R-hydroxyecdysone) from a crayfish (*Jasus lalandei* H. Milne-Edwards). Biochem. J. *109*, 399–406 (1968).

HORNUNG, D. E. und J. R. STEVENSON: Changes in the rate of chitin synthesis during the crayfish molting cycle. Comp. Biochem. Physiol. *40*, 341–346 (1971).

10. Huber, R. und W. Hoppe: Zur Chemie des Ecdysons. VII. Die Kristall- und Molekülstrukturanalyse des Insektenverpuppungshormons Ecdyson mit der automatischen Faltmolekülanalyse. Chem. Ber. *98*, 2403–2424 (1965).

Hubschman, J. H. und P. W. Armstrong: Influence of ecdysterone on molting in *Palaemonetes*. Gen. comp. Endocr. *18*, 435–438 (1972).

Humphreys, C. R. und J. R. Stevenson: Changes in epidermal DNA, protein and protein synthesis during the molt cycle of the crayfish *Orconectes sanborni* (Faxon). Comp. Biochem. Physiol. *44*, 1121–1128 (1973).

Jegla, T. C. und J. D. Costlow: Induction of molting in horseshoe crab larvae by polyhydroxy steroids. Gen. comp. Endocr. *14*, 295–302 (1970).

Jegla, T. C., J. D. Costlow und J. Alspaugh: Effects of ecdysones and some synthetic analogs on horseshoe crab larvae. Gen. comp. Endocr. *19*, 159–166 (1972).

Jenkin, P. M.: Apolysis and hormones in the moulting cycles of Arthropoda. Ann. Endocr. Paris *27*, 331–341 (1966).

Jenkin, P. M. und H. E. Hinton: Apolysis and arthropod moulting cycles. Nature, Lond. *211*, 871 (1966).

Karlson, P.: Biochemical studies on insect hormones. In: «Vitamins and Hormones» (K. V. Thimann, ed.) vol. 14, 228. New York: Academic Press (1956a).

11. – Chemische Untersuchungen über die Metamorphosehormone der Insekten. Ann. Sc. Nat., Zool. Biol. Anim., Ser. II, *18*, 125–138 (1956b).

12. – Ecdyson, das Häutungshormon der Insekten. Naturwissenschaften *53*, 445–453 (1966).

13. Karlson, P. und P. Schmialek: Notiz über die Isolierung von Malonsäure-amid-β-phenyläthylamid aus Garneelen. Hoppe-Seyler's Z. Physiol. Chem. *316*, 83–87 (1959).

Karlson, P. and D. M. Skinner: Attempted extraction of crustacean moulting hormone from isolated Y-Organ. Nature, Lond. *185*, 543 (1960).

14. Karlson, P. und C. E. Sekeris: Zum Tyrosinstoffwechsel der Insekten. IX. Kontrolle des Tyrosinstoffwechsels durch Ecdyson. Biochim. Biophys. Acta *63*, 485–495 (1962).

15. Karlson, P. und H. Hoffmeister: Zur Biogenese des Ecdysons. I. Umwandlung von Cholesterin in Ecdyson. Hoppe-Seyler's Z. Physiol. Chem. *331*, 398–300 (1963).

16. Keller, R. und D. Adelung: Vergleichend morphologische und physiologische Untersuchungen des Integumentgewebes und des Häutungshormongehaltes beim Flußkrebs *Orconectes limosus* während eines Häutungszyklus. Wilhelm Roux' Archiv *164*, 209–221 (1970).

King, D. S. und J. B. Siddall: Conversion of α-ecdysone to β-ecdysone by crustaceans and insects. Nature, Lond. *221*, 955–956 (1969).

Kittredge, J. S. und F. T. Takahashi: The evolution of sex pheromone communication in arthropoda. J. theor. Biol. *35*, 467–471 (1972).

Kopeč, S.: Studies on the necessity of the brain for the inception of insect metamorphosis. Biol. Bull. Woods Hole *42*, 323–342 (1922).

Krishnakumaran, A. und H. A. Schneiderman: Chemical control of moulting in arthropods. Nature, Lond. *220*, 601–603 (1968).

– Induction of molting in crustacea by an insect molting hormone. Gen. comp. Endocr. *12*, 515–518 (1969).

– Control of molting in mandibulate and chelicerate arthropods by ecdysones. Biol. Bull. Woods Hole *139*, 520–538 (1970).

17. Kümmel, G., H. Claassen und R. Keller: Zur Feinstruktur von Cuticula und Epidermis beim Flußkrebs *Orconectes limosus* während eines Häutungszyklus. Z. Zellforsch. *109*, 517–551 (1970).

Kurata, H.: Induction of molting in a prawn, *Penaeus japonicus*, by inokosterone injection. Bull. Jap. Soc. Sci. Fish. *34*, 909–914 (1968).

Kurup, N. G. und B. T. Scheer: Control of protein synthesis in an anomuran crustacean. Comp. Biochem. Physiol. *18*, 971 (1966).

18. Lang, R.: Chitinsynthese bei dem Flußkrebs *Orconectes limosus:* Aktivität der Phosphoglucosamin-isomerase und Einbau von Glucose-U-^{14}C in Chitin. Z. vergl. Physiol. *73*, 305–316 (1971).

Lowe, M. E., D. H. S. Horn und M. N. Galbraith: The role of crustecdysone in the moulting crayfish. Experientia *24*, 518–519 (1968).

Lowe, M. E. und D. H. S. Horn: The role of crustecdysone in molting and gastrolith formation in the crayfish *Procambarus simulans*. Physiol. Syst. Semiarid Environ., Semin. 1967, 155–163 (1969).

MAISSIAT, J.: Etude expérimentale du rôle de «l'organe Y» dans le déterminisme endocrine de la mue chez l'Isopode Oniscoide *Porcellio dilatatus* Brandt. C. R. Acad. Sci. Paris *270*, 2573–2574 (1970a).
– Anecdysis expérimentale provoquée chez l'Oniscoide *Ligia oceanica* L. et rétablissement de la mue par injection d'ecdysone ou réimplantation de glande maxillaire. C. R. Soc. Biol. *164*, 1607–1609 (1970b).
MAISSIAT, J. und J. J. LEGRAND: Contribution à l'étude expérimentale du contrôle hormonal du cycle de mue chez les Oniscoides *Porcellio dilatatus* et *Ligia oceanica*. C. R. Soc. Biol. *164*, 359–362 (1970).
MATSUMOTO, K.: Experimental studies of the neurosecretory activities of the thoracic ganglion of a crab, *Hemigrapsus*. Gen. comp. Endocr. *2*, 4–11 (1962).
MCWHINNIE, M. A.: Gastrolith growth and calcium shifts in the freshwater crayfish, *Orconectes virilis*. Comp. Biochem. Physiol. *7*, 1–14 (1962).
MCWHINNIE, M. A. und R. J. KIRCHENBERG: Crayfish hepatopancreas metabolism and the intermolt cycle. Comp. Biochem. Physiol. *6*, 117–128 (1962).
MCWHINNIE, M. A., M. O. CAHOON und R. JOHANNECK: Hormonal effects on calcium metabolism in Crustacea. Am. Zool. *9*, 841–855 (1969).
MCWHINNIE, M. A. und C. J. MOHRHERR: Influence of eyestalk factors, intermolt cycle and season upon ^{14}C-leucine incorporation into protein in the crayfish *(Orconectes virilis)*. Comp. Biochem. Physiol. *34*, 415–437 (1970).
MCWHINNIE, M. A., R. J. KIRCHENBERG, R. J. URBANSKI und J. E. SCHWARZ: Crusteedysone mediated changes in crayfish. Am. Zool. *12*, 357–372 (1972).
MIYAWAKI, M. und Y. TAKETOMI: Structural changes induced in the cell of Y gland of crayfishes by an administration of ecdysterone. Dobutsugaku Zasshi (Zool. Mag.) *79*, 150–155 (1970).
– Changes of Y gland cell structure of the crayfish, *Procambarus clarkii*, during the molt cycle and in some experimental conditions. Kumamoto J. Sci., Biol. *10*, 55–67 (1971).
MIYAWAKI, M., Y. TAKETOMI und E. F. COUCH: The susceptibility of Y-gland cells of the crayfish, *Procambarus clarkii*, to exogenous steroids. Annot. Zool. Japon. *44*, 42–46 (1971).
PANOUSE, J. B.: Influence de l'ablation du pédoncule oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus*. C. R. Acad. Sci. Paris *217*, 553–555 (1943).
PASSANO, L. M.: Neurosecretory controle of molting in crabs by the X-organs sinus gland complex. Physiol. Comp. Oecol. *3*, 155–189 (1953).
– Molting and its control. In: «The Physiology of Crustacea» (T. H. Waterman, ed.) vol. 1, 473–536. New York: Academic Press 1960.
PASSANO, L. M. und S. JYSSUM: The role of the Y-organ in crab proecdysis and limb regeneration. Comp. Biochem. Physiol. *9*, 195–213 (1963).
RAO, R. K.: Isolation and partial characterisation of the moult-inhibiting hormone of the crustacean eyestalk. Experientia *21*, 593–594 (1965).
RAO, R. K., M. T. FINGERMAN und C. HAYS: Effects of α-ecdysone and 20-hydroxyecdysone on limb regeneration and molting in the fiddler crab, *Uca pugilator*. Am. Zool. *11*, 106 (1971).
– Comparison of the abilities of α-ecdysone and 20-hydroxyecdysone to induce precocious proecdysis and ecdysis in the fiddler crab, *Uca pugilator*. Z. vergl. Physiol. *76*, 270–284 (1972).
RAO, R. K. S. W. FINGERMAN und M. FINGERMAN: Effects of exogenous ecdysones on the molt cycles of fourth and fifth stage American lobsters, *Homarus americanus*. Comp. Biochem. Physiol. *44*, 1105–1120 (1973).
REES, H. H.: Ecdysones. In: «Aspects of Terpenoid Chemistry and Biochemistry» (T. W. Goodwin, ed.) London: Academic Press 1971.
RLIDENBACH, J.-M.: Action d'une ecdysone de synthèse sur la mue et le fonctionnement ovarien chez le Crustacé Isopode *Idotea baltbica* (Pallas). C. R. Acad. Sci. Paris, Ser. D *273*, 1614–1617 (1971).
RYAN, E. P.: Pheromone: evidence in a decapod crustacean. Science *151*, 340–341 (1966).
SCHARRER, B. und M. WEITZMAN: Current problems in invertebrate neurosecretion. In: «Aspects of Neuroendocrinology» (W. Bargmann und B. Scharrer, eds.) 1–23. New York: Springer Verlag 1970.
SCHEER, B. T. und M. A. R. SCHEER: The hormonal regulation of metabolism in crustaceans. Pubbl. Sta. Zool. Napoli *25*, 397–418 (1954).
SKINNER, D. M.: The structure and metabolism of a crustacean integumentary tissue during a molt cycle. Biol. Bull. Woods Hole *123*, 635–647 (1962).
Amino acid incorporation into protein during the molt cycle of the land crab, *Gecarinus lateralis*. J. Exp. Zool. *160*, 226–233 (1965).

– Macromolecular changes associated with the growth of crustacean tissues. Am. Zool. *6*, 235–242 (1966).

SKINNER, D. M. und D. E. GRAHAM: Molting in land crabs: stimulation by leg removal. Science *169*, 383–385 (1970).

SKINNER, D. M. und D. E. GRAHAM: Loss of limbs as a stimulus to ecdysis in Brachyura (true crabs). Biol. Bull. Woods Hole *143*, 222–233 (1972).

SMILEY, J. W.: Induction of gastrolith formation by exogenous ecdysterone. Ass. Southeastern Biologists Bull. *18*, 56 (1971).

SOCHASKY, J. B., D. E. AIKEN und N. H. F. WATSON: Y-Organ, molting gland, and mandibular organ: a problem in decapod Crustacea. Can. J. Zool. *50*, 993–997 (1972).

19. SPECK, U., K. URICH und U. HERZ-HÜBNER: Nachweis einer Regulation der Glucosaminbildung bei dem Flußkrebs *Orconectes limosus* zur Zeit der Häutung. Z. vergl. Physiol. *76*, 341–346 (1972).

STEPHENS, G. C.: A molt-inhibiting factor in the central nervous system of the crayfish, *Cambarus* sp. Anat. Rec. *111*, 572–573 (1951).

STEVENSON, J. R.: Polyphenol oxidase in the tegumental glands in relation to the molting cycle of the isopod crustacean *Armadillidium vulgare*. Biol. Bull. Woods Hole *121*, 554–560 (1961).

– Metecdysial molt staging and changes in the cuticle in the crayfish *Orconectes sanborni* (Faxon). Crustaceana *14*, 169–177 (1968).

– Changing activities of the crustacean epidermis during the molting cycle. Am. Zool. *12*, 373–380 (1972).

STEVENSON, J. R., R. H. GUCKERT und J. D. COHEN: Lack of correlation of some processes in the crayfish. Biol. Bull. Woods Hole *134*, 160–175 (1968).

STEVENSON, J. R. und D. A. TUNG: Inhibition by actinomycin D of the initiation of chitin biosynthesis in the crayfish. Comp. Biochem. Physiol. *39*, 559–567 (1971).

STEVENSON, J. R. und J. A. TSCHANTZ: Ecdysterone – presence necessary to sustain the moulting process in crayfish. Nature, Lond. *242*, 133–134 (1973).

TCHERNIGOVTZEFF, C.: Croissance épithéliale et cycle d'intermue chez *Leander serratus*. C. R. Acad. Sci. Paris, Ser. D *248*, 600–602 (1959).

TESHIMA, S. and A. KANAZAWA: Biosynthesis of sterols in the lobster, *Panulirus japonica*, the prawn, *Penaeus japonicus*, and the crab, *Portunus trituberculatus*. Comp. Biochem. Physiol. *38* B, 597–602 (1971).

TRAVIS, D. S.: The molting cycle of the spiny lobster, *Panulirus argus* Latreille. II. Postecdysial histological and histochemical changes in the hepatopancreas and integumental tissue. Biol. Bull. Woods Hole *108*, 88–112 (1955).

– The deposition of skeletal structures in the Crustacea, 1. The histology of the gastrolith skeletal tissue complex and the gastrolith in the crayfish, *Orconectes (Cambarus) virilis* Hagen – Decapoda. Biol. Bull. Woods Hole *118*, 137–149 (1965a).

– The deposition of skeletal structures in the Crustacea, 5. The histomorphological and histochemical changes associated with the development and calcification of the branchial exoskeleton in the crayfish, *Orconectes virilis* Hagen. Acta histochem. *20*, 193–222 (1965b).

VAN DEN OORD, A.: The absence of cholesterol synthesis in the crab, *Cancer pagurus* L. Comp. Biochem. Physiol. *13*, 461–467 (1964).

WARNER, A. C., J. R. STEVENSON und C. CHURCH: The initiation of precocious premolt in *Orconectes obscurus* with injection of α-ecdysone and crustecdysone. Am. Zool. *9*, 1085 (1969).

WARNER, A. C. und J. R. STEVENSON: The influence of ecdysones and eyestalk removal on the molt cycle of the crayfish *Orconectes obscurus*. Gen. comp. Endor. *18*, 454–462 (1972).

WILLIAMS, C. M.: Ecdysone and ecdysone-analogues: their assay and action on diapausing pupae of the *Cynthia* silkworm. Biol. Bull. Woods Hole *134*, 344–355 (1968).

WILLIG, A., H. H. RIES und T. W. GOODWIN: Biosynthesis of insect moulting hormones in isolated ring glands and whole larvae of *Calliphora*. J. Insect Physiol. *17*, 2317–2326 (1971).

WILLIG, A. und R. KELLER: Molting hormone content, cuticle growth and gastrolith growth in the molt cycle of the crayfish *Orconectes limosus*. Z. vergl. Physiol. (im Druck).

ZANDEE, D. I.: Lipid metabolism in *Astacus astacus* L. Nature, Lond. *195*, 814–815 (1962).

– Metabolism in the crayfish *Astacus astacus* (L.). III. Absence of cholesterol synthesis. Arch. Int. Physiol. Biochem. *74*, 435–441 (1966).

– Absence of cholesterol synthesis as contrasted with the presence of fatty acid synthesis in some arthropods. Comp. Biochem. Physiol. *20*, 811–822 (1967).

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Translations of foreign-language titles

1. Action of ecdysone in Carcinus maenas L. and the crustecdysone titer during the molt cycle.
2. Release and function of the molting hormone during one intermolt stage of the European shore crab, Carcinus maenas.
3. Changes in the calcium content of the cuticle and of the hemolymph of the European shore crab, Carcinus maenas, during one molt
4. The hormone causing puparium formation in flies.
5. Isolation of the metamorphosis hormone of insects in crystalline form.

5a. 20-Hydroxy-ecdysone isolated from insects.

6. Structure of β-ecdysone.
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8. Chemistry of ecdysterone.
9. Studies of the structure and biochemical action of ecdysterone.
10. Chemistry of ecdysone. 7. Crystal and molecular structure analysis of the insect pupating hormone, ecdysone, with the aid of automatic convolution molecule analysis.
11. Chemical investigations of the insect metamorphosis hormone.
12. Ecdysone, the insect molting hormone.
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14. Tyrosine metabolism of insects. 9. Control of tyrosine metabolism by ecdysone.
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18. Chitin synthesis in the crayfish Orconectes limosus: Activity of phosphoglucose amine isomerase and incorporation of glucose-U-^{14}C into chitin.
19. Demonstration of a regulatory mechanism in glucosamine formation in the crayfish Orconectes limosus during the time of molt.