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Effect of ecdysterone on the moulting, cuticle and calcium metabolism of Orchestia cavimana Heller (Crustacea, Amphipoda, Talitridae)

by Francois Graf

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EXPERIMENTAL BIOLOGY - Effect of ecdysterone on the moulting, cuticle and calcium metabolism of Orchestia cavimana Heller (Crustacea, Amphipoda, Talitridae). Paper (*) by Francois Graf, presented by Pierre-Paul Grasse.

In the case of Orchestia cavimana, ecdysterone may cause the occurrence of phenomena preparatory to moulting when it is injected during the A, B or C periods, but it will delay exuviation when injected on the days preceding moulting (end of D). The moult hormone must be absent or totally inhibited during exuviation and its presence or absence controls the calcium metabolism of the posterior caeca. Under certain experimental conditions, exuviation becomes impossible, although after attempts at ecdysis, the Amphipod is physiologically in a post-exuvial period as it has two cuticles.

INTRODUCTION - The work of Drach ((1), 2)) on the cycles taking place between moults in the case of Decapods has identified 4 periods, valid for all Crustaceans: Period A - immediately after moulting; the skeleton is soft. Period B - first consolidation of the skeleton. Period C - the skeleton has reached its final hardness but continues to thicken during part of this period. Period D - the future skeleton progressively builds up under the existing one. E - exuviation. For the Amphipods, these periods were identified by Charniaux-Cotton (3) and Graf (4). For O. cavimana, the total duration of the interval between moults is approximately 23 days in the case of animals 13 mm long and from 40 to 50 days for more mature animals (20 mm).

It is to be noted also that the Orchestia stocks up calcium before moulting (4); during the pre-exuvial D period, the posterior caeca form calcareous concretions in the lumenas which will be reabsorbed immediately after exuviation, this large calcium deposit being used to consolidate the new exoskeleton. There is then at moulting time a reversal of the calcium secretion process in the epithelium of the posterior caeca; during the D period, the calcium crosses the caecal

epithelium from base to apex, it crosses back from apex to base in periods A and B. In both directions the calcium basically flows outside the cells, that is within the more or less expanded space between cells (5).

It was necessary to study the effect of synthetic ecdysone on the calcium metabolism as caecum homografting experiments (6) had shown that concretions are formed in the transplanted caecum at the same time as in the caecum of the host, which would lead to believe that these concretions are formed as a result of hormonal activity (4).

The effect of the synthetic ecdysone has already been tested on Peracarida. In the case of the Amphipod Orchestia gammarella (7), the introduction of an ecdysterone crystal in sexually-inactive females and males in periods B or C gives rise in twelve hours to the phenomena preparatory to moulting (phase D_o) and the D period is quite abbreviated. In Isopods, ecdysone causes moulting in Ligia (8), Idothea (9) and Asellus (10) while restoring the moulting stage in Ligia under experimental anecdysis following removal of the moult glands (11). Although the effect of ecdysone is highly demonstrative in periods B and C, it is not so during the other periods. In the case of Ligia, ecdysone injected at the end of D or during A or B remains without effect, which suggests that it is rapidly destroyed or eliminated (8). In Asellus (10), the hormone does not act during D, but perturbs the exuviation process at the end of D and thereby extends the interval between moults.

Our experiments were carried out exclusively on male adults of the species Orchestia cavimana (size 13 to 22 mm) in order to avoid possible interaction with vitellogenesis (7). An ecdysterone crystal (12) (average size: 0.1 x o.4 mm) was

introduced between tergites 7 and 8 in the dorsal compartment and dissolved within a few hours in the hemolymph.

EXPERIMENT 1 - The introduction of ecdysterone at the end of B or C induces period D_o some twelve hours afterwards and moulting follows within the next 6 or 7 days, which confirms results obtained for O. gammarella (7). Moreover, our observations show that this 6- or 7-day period has nothing to do with the size of the specimen and the quantity of hormone injected (more than one crystal).

EXPERIMENT 2 - Injection of ecdysterone during D_1'', D_2 or D_3 causes a lengthening of D period, and moulting does not occur until 5 or 6 days, regardless of size (in 15 cases, only one young, 14-mm male that had received ecdysone in D_3 moulted after four days). The lengthening of the interval between moults is not the only effect of introducing ecdysterone at the end of the D period; in animals treated at that time or in D_3, the calcium accumulation is much larger than in control specimens and the pre-exuvial layer of the cuticle is thicker. Moreover, although exuviation is normal for specimens treated in D_1'', in those treated in D_3 it is difficult and often incomplete for young specimens and will not occur at all in the more mature (there are many attempts at moulting, appendices are retracted within the old cuticle, air infiltrates between the two cuticles, dehiscence slits do not form; it would appear that the resorption process of the old cuticle is perturbed). These mature animals which cannot moult will die, but in the hours following the attempts at exuviation and preceding death, reabsorption of calcium within the caeca begins.

This series of experiments shows that injection of ecdysterone in animals about to moult delays moulting and abnormally prolongs the stocking up of calcium and the secretion of a new cuticle, which means that in normal animals in D_3-E

either the moulting hormone concentration is low or the hormone is inhibited. One argument in favor of the first is that no matter what the size of the specimen or the time of injection (experiments 1 and 2), moulting occurs or is delayed six days after introduction of the crystal, the synthetic hormone persisting or being active only during these six days.

EXPERIMENT 3 - Injection of ecdysterone during A or B (0 to 36 hours after moulting) induces a new moulting. When the injection is given immediately after moulting (that is in an animal whose cuticle has only the pre-exuvial layer and posterior caecal epithelium of which has begun to re-absorb the calcium) the calcium of the caeca is absorbed more and more slowly during the first 24 hours (average), after which time secretions are reversed and calcium is again deposited on the old concretions where reabsorption was incomplete. During the very first hours, a thin post-exuvial layer forms on the cuticle.

Twenty-four to forty-eight hours after injection, period D begins but will last only 5 to 7 days, (rarely 4). During this period a large amount of calcium accumulates in the caeca and a new cuticle is formed under the old, unfinished one. Setae and the nails of this new skeleton are quite short (half the length of those on the old cuticle). The animal is abnormally soft and seems prostrate, but shows life when disturbed. In D3 -E, the animal makes unsuccessful attempts at moulting. Exuviation does not occur but reabsorption of caecal calcium begins rapidly in a way which is particularly strong and the "post-exuvial" layer of the new cuticle is formed. All specimens in this experiment were destroyed within the three days following attempts at exuviation but given their apparent good condition, they probably could have survived longer with their two cuticles.

This experiment shows that the calcium cycle of the posterior caeca is controlled by the moult hormone: concretions are formed in its presence, reabsorption occurs in its absence, which confirms that the moulting hormone is no longer present upon moulting or attempts at moulting. Finally, the fact that in this experiment the moulting hormone lasts from 6 to 7 days only, should be insisted upon.

EXPERIMENT 4 - Two specimens were injected with ecdysterone as they were about to moult. Moulting occurred within three hours. One specimen was destroyed 24 hours after moulting: reabsorption of calcium in the caeca is normal (it was slowed down or stopped in specimens in experiment 3). The second specimen moulted a second time after a normal interval (36 hours after the second moult, calcium reabsorption was normal).

This experiment remains to be developed, but the two results obtained suggest that under experimental conditions an anti-hormone factor exists at exuviation as in both cases the synthetic hormone was totally inhibited. In this respect, we may return to experiment 3. If ecdysterone is injected shortly after moulting, calcium reabsorption stops only twenty-four hours later, while injection of the hormone 24 hours after moulting stops reabsorption within 5 hours, which means that the effect of the synthetic hormone is not as strong or is momentarily inhibited in the hours following the moult.

DISCUSSION - The experiments show that while inducing phenomena preparatory to moulting, (exp. 1,3) the synthetic hormone also delays exuviation (exp. 2) and its effect seems constant during the 6 or 7 days following injection. On the other hand, the effect of ecdysterone is totally inhibited (exp. 4) or simply delayed (exp. 3) if it is injected immediately before or after exuviation. The results would lead to the formulation of the following hypotheses concerning Orchestia under natural conditions:

1. The moult hormone is produced only during a relatively short time and moulting occurs only when the hormone concentration is low or nil and as a result of an exuviation factor.
2. The moult hormone is produced during a longer period and inhibited upon the approach of moulting by an exuviation factor which acts directly or indirectly as an anti-hormone factor.

The choice between these two hypotheses will certainly be made easier by experimenting with smaller doses of ecdysterone (the ideal dose being that which causes a normal D period, from the duration standpoint) and particularly by trying to identify the hypothetical exuviation factor.

(*) Session of Feb. 28, 1972.

Diagram 1 -

Control specimens

posterior caecum days

Exp. 1

Exp. 2

ecdysterone

Exp. 3

exuviation impossible (2 cuticles)

but

physiologically in A then B

Exp. 4