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STUDIES ON THE ENDOCRINOLOGY OF ISOPOD CRUSTACEANS. MOULTING IN *LIGIA OCEANICA* (L.)

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(Text-fig. 1)

Experiments performed upon decapod crustaceans have produced much information on the hormonal control of moulting in this group. So far as I am aware, however, no one has tried to discover how moulting in other groups of Crustacea is controlled. This note represents the beginning of an attempt to investigate this aspect of moulting in the Isopoda.

Observations on the Moulting Process in *Ligia oceanica*

It is well known that isopods, unlike most Malacostraca, moult in two portions, shedding the old cuticle of the posterior part of the body sometime before that of the front end. This seems to have been noticed first in *Porcellio* by Schöbl (1879). A detailed account of moulting in some Oniscoidea is given by Herold (1913); Numanoi (1934) describes the process in *Ligia exotica*; Tait (1917) describes it in *L. oceanica*. Briefly, in this species, after a premoult period of 6 or 7 days, a complete separation takes place between the integument of the fourth and fifth thoracic segments; the tergites of the fifth, sixth and seventh segments split longitudinally at the sides (dorso-laterally), and the animal extracts itself from the hinder part of the cuticle by a series of writhing movements. This process takes 10–12 min. The animal walks away using only the anterior walking legs since the posterior ones are not stiff enough to use for at least 12 h. In my experience moulting usually takes place during the hours of daylight. After a variable period (Tait states 4 days at a temperature of 10·5–14.5° C) the anterior thoracic segments split dorso-laterally. Once more the animal extracts itself from the old integument by writhing movements, taking about 30–40 min. I have found that the anterior integument is usually cast in one piece except in underfed animals which have been for sometime in the laboratory. Tait, however, found that this part of the integument was most usually torn into fragments, while the dorsal portions of segments 2, 3 and 4 often remained attached to the animals for some days. This condition I have only seen in animals which moulted in difficult conditions after a period of shortage of food in the laboratory. Indeed, it seems likely that most of Tait's animals were underfed and observed after some

lengthy period of captivity, for he states: 'It is a curious fact that in *Ligia*, subsequent to posterior and prior to anterior moult, no increase in the girth of the posterior part of the body can be detected with the eye.... Even when moult is complete *Ligia* is not obviously larger than before'. I find this is only true of starved animals.

The interval between posterior and anterior moult is not nearly so constant as Tait implies. He states that at a temperature of 10·5–14·5° C the interval is 4 days; he makes no mention of any variability. I have found that at a temperature of 15·7–17·4° C the interval is usually 2 or 3 days, but on occasion it may be only 1 day, in either large or small individuals, while periods of 4, 5 or 6 days between the two halves of the moult are not unusual. In five of the animals which I have observed anterior moult has been delayed to the day immediately before the succeeding posterior moult, while in two it has been delayed even beyond this, so that a posterior moult has been followed within 3 days by two anterior moults, one corresponding to the posterior moult of some weeks previously, and one corresponding to the more recent posterior moult.

The average intermoult period at a temperature of 15·7–17·4° C was 47·44 days, corresponding closely with the periods recorded by Nicholls (1931).

The Effect of Extracts of *Leander* Eyestalks on Moulting Rate in *Ligia oceanica*

In these experiments about 340 specimens of *Ligia*, freshly collected from Drake's Island, Plymouth Sound, in December 1953, were placed singly in numbered 450 ml. containers in the laboratory each with a piece of *Fucus vesiculosus* for food. Each day the containers were rinsed out with sea water and the weed damped. All moults, deaths and escapes were recorded daily. After a preliminary observation period of 1 week one-third of the animals were injected with an extract of whole eyestalks of female *Leander serratus*. These eyestalks had been excised in August, at a period when they contain a high titre of moult-accelerating hormone, and stored in the refrigerator in absolute acetone. The extract was prepared by draining off the acetone, evaporating the remainder at room temperature, until there was no longer any perceptible aroma of acetone, and grinding the eyestalks with distilled water, acidified to pH 3·8 with hydrochloric acid, in a mortar with washed sand as an abrasive. The extract was filtered and the residue washed with a small measured quantity of acidified water. The total filtrate represented forty eyestalks per millilitre. Each *Ligia* that was injected received 0·05 ml., i.e. two eyestalk equivalents. The injection was made with a tuberculin syringe fitted with a no. 28 needle, inserted in a forward direction between the tergites of the sixth and seventh free thoracic segments into the lateral musculature. No animals died immediately after the injection, but some were found dead 24–48 h later;

these are recorded in Table I. One-third of the animals received no injection; one-third received an injection of acidified distilled water. All animals which had moulted either half of the body during the preliminary observation period were rejected from the experiment.

TABLE I. THE NUMBERS OF DEAD AND OF MOULTS IN EACH GROUP

Days after injection 0	Group 1. Injected eyestalk extract 96 alive		Group 2. Not injected 92 alive		Group 3. Injected distilled water 94 alive	
	Dead	Moults	Dead	Moults	Dead	Moults
1	5	3	1	3	6	2
2	11	13	0	2	8	2
3	0	4	0	2	1	2
4	0	5	0	2	0	3
5	0	2	0	2	0	2
	16	27	1	12	15	11

The dead recorded are those which died without initiating posterior moulting; the moults are the posterior moults which were at least begun, even if the animals died before completion of the moult. The numbers are given only for the 5 days after injection, when there was a significant difference in moult rates; Fig. 1 illustrates the moult rate over a longer period of time.

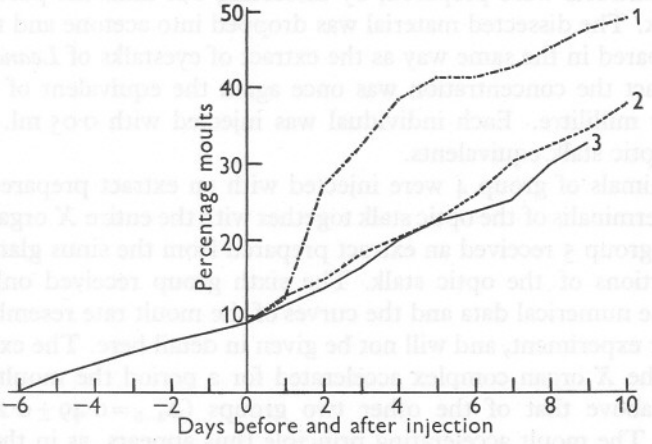

Fig. 1. The percentage moult-rates in the three groups of animals of the first experiment. For explanation see text.

The numbers which died without first moulting and the numbers which initiated the moult of the posterior half of the body are recorded in Table I; some of these latter died without completing the moult; the criterion adopted was simply the initiation of the moult process and the partial withdrawal from the exoskeleton of the posterior half of the body. Parallel probit lines were computed for the moulting rate in the three groups of animals by the method described by Carlisle & Dohrn (1953). The value of Δ_{1-2} was $0{\cdot}57 \pm 0{\cdot}23$,

which, with 180 degrees of freedom, gave $P < 0{\cdot}01$. The rate of moulting in the group injected with eye-stalk extract was thus significantly greater at the 1% level of probability from that in the uninjected control group and also from that in the control group injected with distilled water. The percentage moulting rate is illustrated graphically in Fig. 1, where it will be seen that after an initial period of accelerated moulting the moult rate in the injected group drops back to normal again, giving a line parallel to that of the controls.

The Effect of Extracts of the Optic Stalks of *Ligia* upon Moulting Rate in *Ligia oceanica*

I have previously shown (Carlisle, 1953) that the moult-accelerating hormone of Natantia is produced in the eyestalks, within the *X* organ complex. Isopoda, however, are sessile-eyed Crustacea and lack eyestalks. The elements of the *X* organ complex are located instead in the optic stalks of the brain within the head, where also is found the sinus gland. Experiments using extracts of the optic stalks of *Ligia oceanica* were therefore made. They followed the same plan as those of the previous section. Three groups of animals were used, each group consisting of about ninety individuals. Two different extracts were prepared, by dissecting out different portions of the optic stalk. The dissected material was dropped into acetone and the extracts were prepared in the same way as the extract of eyestalks of *Leander*. In the final extract the concentration was once again the equivalent of forty optic stalks per millilitre. Each individual was injected with 0·05 ml. of extract, i.e. two optic stalk equivalents.

The animals of group 4 were injected with an extract prepared from the medulla terminalis of the optic stalk together with the entire *X* organ complex. Those of group 5 received an extract prepared from the sinus glands and the distal portions of the optic stalk. The sixth group received only acidified water. The numerical data and the curves of the moult rate resemble those of the earlier experiment, and will not be given in detail here. The extract of the parts of the *X* organ complex accelerated for a period the moulting rate of group 4 above that of the other two groups ($\Delta_{4-5} = 0{\cdot}49 \pm 0{\cdot}21$, whence $P < 0{\cdot}01$). The moult accelerating principle thus appears, as in the Natantia, to reside in the *X* organ complex and to be absent from the sinus gland.

Discussion

The hormonal control of colour change in Isopoda is similar in many respects to that of Natantia (Kleinholz, 1937; Ståhl, 1938*a*, *b*; Okay, 1945; Suneson, 1947; see also Knowles & Carlisle, 1956). It appears from the experiments reported here that this resemblance extends also to some aspects at least of the hormonal control of moulting. Thus the moult-accelerating principle of the

eyestalk of *Leander*, which is known to be active also on the natantian *Lysmata* (Carlisle & Dohrn, 1953) has a similar effect in accelerating the processes of the premoult in *Ligia*. Moreover, extracts of the *X* organ complex of *Ligia* have had the same effect, whereas the sinus-gland extract has had no effect on the moult rate. This agrees well with the conclusion that the moult-accelerating principle in *Lysmata* is present in both the ganglionic and the sensory papilla *X* organs and absent from the sinus gland (Carlisle, 1953). It seems probable then that essentially the same factor is present in the *X* organ complex both of Isopoda and Natantia and is responsible for the control of the course of proecdysis in both.

I have found no evidence that any of my experimental procedures have interfered at all with the interval between the two halves of the moult. It is reasonable to suppose that this somewhat variable interval is under some kind of hormonal control, but if so this is entirely separate from the control exerted by the moult-accelerating principle of the *X* organ complex.

SUMMARY

The moulting process of *Ligia oceanica* is briefly described, and mention is made of the great variability of the period between the two halves of the moult.

The moulting rate of *Ligia* is increased temporarily by a single injection of eyestalk extract of female *Leander*.

The moulting rate is similarly increased by a single injection of extract of the *X* organ complex of *Ligia*, but is unaffected by an extract of the sinus gland and the distal positions of the optic stalk, when this is injected at equivalent concentrations.

The moult accelerating principle of the *X* organ complex of the optic stalk is probably responsible for the control of the course of proecdysis in *Ligia*.

No evidence has been found that eyestalk or optic stalk extracts of the kind used interfere in any way with the length of the interval between the two halves of the moult.

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